E3S Web of Conferences **271**, 04012 (2021) *ICEPE 2021*

Root morphological, Cd accumulation and tolerance characteristics of 2 *Dianthus caryophyllus* cultivars under Cd stress

Jiong Wu^{1, a, †}, Changdi Ke^{1,b,†}, Yanqun Zu^{1*}, Yu Din^{1,c} and Taiting Li^{1,d}

¹Department of Ecological Environment, College of Resources and Environment, Yunnan Agricultural University, Kunming, Yunnan, China

[†]Changdi Ke and Jiong Wu contributed equally to this work and should be considered co-first authors

Abstract: For studying the physiological response of two different Dianthus caryophyllus cultivars on Cd stress, pot experiment was carried out to measure proline and glutathione in leaves, five types of organic acids in root exudates (oxalic acid, malic acid, acetic acid, tartaric acid, citric acid), soluble sugars and free amino acids, root length, root surface area, root volume, root projected area and Cadmium content in soil, plant roots and aboveground. According to the effects of cadmium stress on the physiological and biochemical characteristics of two Dianthus caryophyllus, the results showed that: the growth of two cultivars are affected, "Master" and "Xiao Yan" are manifested as plant height, leaf, flower buds and biomass decreased, but the "Master" by the stronger inhibitory effect. The root length, total root surface area, total root projected area, and average root diameter of the "Master" increased under cadmium stress, but the root volume decreased. However, the root length, total root surface area, root volume, and root projected area of the "Xiao Yan" under cadmium stress decreased, while the average root diameter increased. The glutathione in the leaves of the two cultivars decreased, the proline content of the leaves of the "Xiao Yan" increased, while that of the "Master" decreased. In the root exudates, the free amino acid content of the two cultivars are reduced, and the secretion of organic acids is also inhibited (except for the citric acid secreted in the "Master"), while the soluble sugar content in the root exudates is expressed as "Xiao Yan" increased, and the "Master" decreased. According to the physiological response to the two cultivars under cadmium stress, the "Xiao Yan" is more suitable for soil restoration in the mining area of Lanping area.

1 Introduction

The situation of soil Cd pollution is severe in China. In 2014, the "National Survey Bulletin of Soil Pollution Status" published by China showed that the total over-standard rate was 16.1%. From the perspective of pollution distribution, soil pollution in the southern area is heavier than northern area, and the over-standard rate of farming locations is 19.4 %. The type of pollution is mainly inorganic, and the excessive rate of heavy metal cadmium is as high as 7.0%^[1]. On May 31, 2016, the State Council officially issued the "Soil Pollution Prevention and Control Action Plan", which pointed out that the inferior quality rate of cultivated land in my country was 27.9%^[2]. Huang et al. compared 336 articles published in my country from 2005 to 2017. It was found that 8 kinds of heavy metals (Cd, Cr, Hg, Pb, As, Cu, Zn, Ni) compared with the background value, the content of Cd and Hg increased, However, the content of the other 6 elements did not accumulate significantly ^[3]. Yang et al. analyzed the content of heavy metals in soil of 402

industrial lands and 1041 agricultural lands in my country, and based on the evaluation results, determined that Cd, Pb and As are priority heavy metals^[4]. According to the results of the detailed survey of agricultural land soil pollution in 2019, the soil environment of agricultural land across the country is generally stable. The main pollutant affecting the soil environmental quality of agricultural land is heavy metals, of which cadmium is the primary pollutant.

The enrichment of Cd in soil comes from natural and man-made aspects ^[5]. Geological weathering is the main natural source of Cd in the soil ^[6]. Cd naturally exists in the earth's crust and is mainly related to Zinc sulfide ore ^[7]. There are many anthropogenic sources of Cd in the soil, and pollution mainly comes from wastewater, waste gas and solid waste discharged by agriculture, mining and smelting industries, as well as atmospheric deposition ^[8-9]. Especially around the mining area, due to the stacking and leaching of tailings, waste rocks, smelting residues and so on. The direct discharge of waste water produced by mining and beneficiation has

^aemail: 75391699@qq.com, ^bemail: 3155456304@qq.com, ^cemail: 2916244470@qq.com, ^demail: 1030762443@qq.com *Corresponding Author: zuyanqun@ynau.edu.cn caused a large amount of heavy metals accumulate in the soil. Yunnan Province is rich in lead-zinc mineral resources, which leads to the phenomenon of excessive Cd and Pb in the surrounding crops and farmland to endanger food safety ^[10]. The lead-zinc mine in Lanping County is the second largest in the world and the largest in Asia. According to the investigation, it was found that the historical pollution source in this area may be the main lead-zinc mine (Fenghuang lead-zinc mine) in the upstream of the Bi Jiang River, and dust generated during mining and mineral transportation. The farmland is polluted by dry and wet sedimentation, and the wastewater or percolation water from smelters and tailings ponds flows down the river or irrigation canals and enters the surrounding farmland. The maximum concentration of Cd in this area is 22.1 mg•kg-1, the minimum concentration is 1.92 mg•kg-1, the average value is 5.6 mg•kg-1, and the over-standard rate is 100%. The soil is moderately to severely polluted by Cd, and the Cd, Pb, and Zn in soil at some points are seriously exceeding the standard; the surrounding farmland is polluted by cadmium to different degrees.

Phytoremediation, as a new soil pollution control technology, has become a research focus because of its low cost, environmental friendly, economic benefits, and large-scale in-situ restoration. Dianthus caryophyllus as Caryophyllaceae Dianthus plants, because of their breeding fast, easy cultivation, adaptability, long flowering period, and high ornamental value and become one of the world's most popular application at present. According to previous tests, Dianthus caryophyllus is considered to have the characteristics of а low-accumulation plant. It can be used to remediate heavy metal cadmium pollution through plant extraction. It is also a non-used landscape plant and does not have ecological safety issues in the food chain. Cd as a non-essential element in plants which does not participate in the structure and metabolism of organisms, but it still will have a toxic effect on plants even at low concentrations. Research on the effects of cadmium on plants in China and abroad mostly focuses on the effects of cadmium on the above-ground parts of plants. For example, reduced plant photosynthesis, reduced nutrient and water absorption, nutrient element imbalance, interference with plant metabolism, leaf loss of green, reduced yield and quality, accelerated plant senescence; which can lead to death in severe cases [11-14]. The root system is an important interface between the plant and the soil, and it is more sensitive to the soil environment and easier to respond to the soil environment ^[15]. The damage of cadmium in the soil to plants is first manifested in the changes of roots. The morphological changes of roots can directly affect the physiological functions of roots and directly restrict the growth and yield of the above-ground parts ^[16]. In addition, a series of changes in root physiology can adapt to the stress environment. In particular, certain stress factors can induce a large amount of organic acids from plant roots, which is a relatively common active adaptive change ^[17]. Under heavy metal stress, plant roots can also secrete some organic acids, such as oxalic acid, citric acid, tartaric acid, succinic acid. These organic acids can affect

the availability of heavy metals in the soil and their absorption and accumulation in plants, which in turn affects the ability of plants to resist heavy metal stress ^[18]. For example, cadmium stimulates the secretion of organic acids, amino acids and other organic substances from soybean roots. The increase in the secretion of these organic substances promotes the complication of cadmium and enhances the ability of soybeans to actively resist cadmium poisoning^[19]; The root system of mangrove (Kandelia candel (L.) Druce) secretes a variety of organic acids, including formic acid, acetic acid, lactic acid, propionic acid, citric acid, tartaric acid. Among them, citric acid, lactic acid, and acetic acid accounted for 76.85%-97.87% of the total organic acid secretion ^[20]; The total amount of organic acids in the rhizosphere soil of the high-Cd durum wheat variety (Kyle) was significantly higher than that of the low-Cd-accumulation variety (Arcola), And the total accumulation of Pb and Cd in plant tissues is directly proportional to the organic acid content in rhizosphere [21]. Therefore, studying the response of roots to heavy metal stress is of great significance to reveal the mechanism of plant resistance to heavy metal stress and to further develop the phytotoxicological effects of heavy metal pollution. At present, although there are many researches on root morphology of plant, they focused on food crops and relationship between phosphorus deficiency, drought stress and root morphology. The research on the root system of heavy metal cadmium mostly focuses on the research of root biomass, root length and root tip cell microstructure, but there are few system researches on the physiological and ecological effects of cadmium on root system under the stress of cadmium. In order to explore the further toxic mechanism of cadmium stress on plant root growth, this experiment used two Dianthus caryophyllus cultivars with different tolerances as test materials, and used pot experiments to explore the effects of cadmium stress on Dianthus caryophyllus root morphology, root exudates organic acids and physiological and biochemical processes, and combine the study of Dianthus caryophyllus's absorption and accumulation of cadmium to explain the cadmium toxicity mechanism and the differences in response to cadmium stress among different cultivars. It provides a theoretical basis for further clarifying the mechanism of cadmium's toxicity to plants and the mechanism of biochemical detoxification.

2 Materials and methods

2.1 soil

Soil treatment group was collected from Lanping lead-zinc mining area in Nujiang, Yunnan Province. Soil control group was collected from the top soil (0-20cm) of Yunnan Agricultural University. It has been crushed, air-dried naturally, and the debris removed and passed through a 2mm sieve.

Soil background value: total nitrogen 2.07g/kg, total phosphorus 1.42g/kg, total potassium 89.50g/kg, available phosphorus 225mg/kg, available potassium

350.40mg/kg, organic matter 135.17g/kg, alkali hydrolyzed nitrogen 142.10mg/ kg and pH is 6.5. Soil background cadmium content: 1.42mg/kg.

2.2 Plant

Seedling of two different *Dianthus caryophyllus* cultivars purchased from Yunnan Binfen Flower Company, named "Master" and "Xiao Yan".

2.3 Method

Dianthus caryophyllus planted in soil with cadmium stress was the treatment group, and *Dianthus caryophyllus* planted in clean soil from Yunnan Agricultural University was the control group. Each treatment had 5 replicates and a total of 20 pots.

Pot experiment was set up in a greenhouse, Yunnan Agricultural University. Plastic pots (water storage pads placed under the pots) were used as cultivation containers, and each pot contained 8 kg of soil (calculated as dry soil). It is crushed, air-dried naturally, and the debris is removed and passed through a 2mm sieve. Put the seedlings in pots and plant one plant per pot in the soil without damaging the root system during the operation. Apply fertilizer per pot: Potassium fertilizer, when applying, the specific method is: fertilizer is water-soluble, dissolved in water at 1:800, fertilize uniformly, once every 14 days, after the plant survives and grows well.

2.3.1 Determination of plant morphological indicators

The morphological indicators of the plants shall be measured one month after the survival. Use a straightedge to measure the length of the longest leaf from the ground as its plant height. Number of leaves: the total number of all leaves above the root of the plant. Bud number: start counting when the plant has flower buds.

2.3.2 Measurement of rhizosphere space structure

Separate the *Dianthus caryophyllus* underground and the aboveground. The underground roots are scanned by EPSON PERFECTIONV 700 scanner one by one, and the pictures are scanned with the root analysis software Win RHIZOPro 2013 (Regent Instruments Inc.) analyzed the root system parameters ^[22].

2.2.3 Measurement of organic acids in root exudates

The detection and identification of the content of organic acids in root secretion usually use chemical instrumental analysis methods. The most commonly used detection and identification techniques are chromatographic separation, spectral identification and their combination. Take the concentrated root exudate collection and filter it with a 0.45 μ m membrane for testing. Root secretion organic acids were determined by external standard

method using high performance liquid chromatograph. The measurement conditions were ODS reversed-phase column, the injection volume was $20 \ \mu\text{L}$, and the mobile phase was $0.01 \ \text{mol} \cdot \text{L}^{-1} \ \text{KH}_2 PO_4$ prepared with deionized water. Adjust the pH to 2.65 with $20\% \ \text{H}_3 PO_4$, the flow rate is 1.0 mL•min⁻¹, and the UV detection wavelength is 214 nm. The standard acids measured are: oxalic acid, citric acid, acetic acid, malic acid, succinic acid and tartaric acid. The calculation of organic acid content adopts the peak area method, unit: mg•L⁻¹.

2.3.4 Determination of total soluble sugars and free amino acids in root exudates

The soluble sugar content is determined by the anthrone method, and the free amino acid composition and content are determined: take the prepared root exudates filtrated through a 0.45 μ m membrane, apply cation exchange, combine with the ninhydrin post-column derivatization method, and use the American Biochrom 30 amino acid Automatic analyzer for amino acid determination ^[23].

2.3.5 Determination of proline and glutathione in leaves

The determination method of proline refers to the method of Xu et $al^{[24]}$, and the method of determination of glutathione refers to the spectrophotometric method of Fan Chongdong et $al^{[25]}$.

2.3.6 Determination of cadmium content

Using pressure digestion tank digestion method, weigh 1g sample and place it in a polytetrafluoroethylene inner tank, add 6ml nitric acid and soak overnight, and then add 5ml hydrogen peroxide. Cover the inner cover, screw the stainless steel jacket tightly, put it in a constant temperature drying box, keep it at 120°C-140°C for 3h-4h, let it cool to room temperature naturally, use a dropper to wash or filter the digestive solution into a 25ml volumetric flask, wash the tank several times with a small amount of water, combine the lotion in a volumetric flask and make a constant volume, mix well for later use, and measure the value with a flame spectrophotometer.

Cadmium content (mg/kg)= $(\rho - \rho_0) \times V \times ts/m$ (1)

 ρ —The mass concentration of cadmium in the sample solution (µg/mL);

 ρ_0 —The mass concentration of cadmium in the blank solution (µg/mL);

V-determine the constant volume of the liquid;

ts-multiple of points;

m—the quality of the sample (g);

2.3.7 Calculation of Cd accumulation, transportation coefficient and aboveground enrichment coefficient

Cd accumulation = cadmium content ×biomass (2) Transport coefficient = plant Cd content / soil Cd content (3)

Enrichment factor = Cd content in the above-ground part

of the plant / Cd content in the underground part of the plant (4)

2.4 Data Statistics and Analysis

Software Excel and SPSS are used for data statistics and analysis, and Origin is used for drawing figures.

3 Results and analysis

3.1 Morphological indicators and biomass of two Dianthus caryllus cultivars under cadmium stress

Plant height, leaves, flower buds and biomass of the two plants were showed in Table 1 which were inhibited under cadmium stress, but the inhibition effect of "Master" is stronger. The plant height, leaves, flower buds and biomass of "Master" decreased separately by 39.7cm, 15.23, 6 flowers, 4.4g, and the plant height, leaves, flower buds and biomass of "Xiao Yan" decreased by 6cm, 15.6, 2 flowers, 1.7g, respectively. In the control soil, except for the biomass, the plant height, number of leaves and flower buds of "Xiao Yan" were higher than those of the "Master". In the cadmium-stressed soil, the morphological indicators and biomass of "Xiao Yan" were higher than those of the "Master". Compared with the control, there is a significant difference in the plant height of "Master", which indicates that the plant height of "Master" is significantly inhibited.

Table 1 Morphological indexes and biomass of two Dianthus caryophyllus cultivars under cadmium stress

Variety	Treatment	Plant height (cm)	Leaves	Flower	Biomass (g)
"Xiao Yan"	Cd	73.33±17.16a	61.33±38.07a	2.33±3.21a	5.83±3.06a
	Ck	79.33±11.02a	46.13±13a	4.33±0.58a	7.53±0.95a
"Master"	Cd	51.25±1.25a	38.5±0.5a	1.00±0a	3.44±0.61a
	Ck	76.17±10.3b	61.33±14.84a	6.67±1.53a	7.84±2.95a

Note: Data in the table are means \pm SD. Means in the column affixed with different lowercase letters are significantly different between treatment and control for the same plant (P<0.05). The same below.

3.2 Changes of root morphological parameters of two Dianthus caryophyllus cultivars under cadmium stress

For "Master", both root length and surface area under cadmium stress were promoted. Compared with the control group, the root length increased by 39.73cm, and the total root surface area increased by 2.09cm², both of which were significantly different from the control group (P<0.05). In the "Xiao Yan" variety, the total root length was inhibited under cadmium stress, and the total root surface area was promoted. Compared with the control group, the root length decreased by 23.84cm and the total root surface area increased by 0.548cm², which was not significantly different from the control group (P<0.05).

It can be seen from Table 2 that in the "Master" variety, the root volume is inhibited under cadmium stress, while the average root diameter and root projected area are promoted. Compared with the control group, the root volume is reduced by 0.091cm³, and the average root diameter is increased by 0.032mm, the root

projected area increased by 0.742 cm², the root volume inhibition reached a significant level (P < 0.05), and the average root diameter and projected area did not reach a significant level (P>0.05). In the "Xiao Yan" variety, root volume and projected area were inhibited under cadmium stress, and the average root diameter was promoted. In the "Xiao Yan" variety, root volume and projected area were inhibited under cadmium stress, and the average root diameter was promoted. Compared with the control group, the root volume decreased by 0.182cm³, the average root diameter increased by 0.081mm, and the root projected area increased by 1.026cm². The level of significant difference was not reached (P>0.05). In the control group, all root morphological parameters of Yan" were higher than "Master"; in "Xiao cadmium-stressed soil, the total length of roots, total root surface area, and total projected area of roots were higher than those of "Master", root volume and average diameter of roots in cadmium-stressed soil. Lower than "Master".

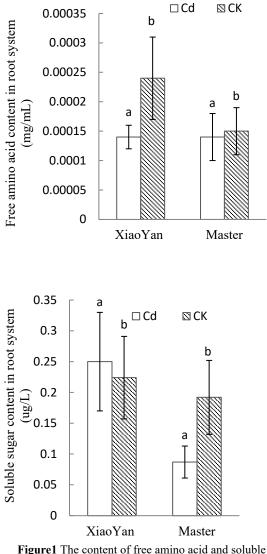
 Table 2 Changes of root parameters of the two cultivars in different soils

Variety	Treatment	Total root length (cm)	Total root surface area (cm ²)	Root volume (cm ³)	average diameter (mm)	Total projected area (cm ²)
Master	Cd	238.561±19.821a	17.536±1.173a	$0.303{\pm}0.048a$	0.427±0.045a	13.594±0.346a
	Ck	198.835±24.835b	15.443±1.395b	$0.394{\pm}0.070b$	0.395±0.069a	12.852±0.629a
Xiao Yan	Cd	221.859±40.881a	16.937±1.747a	0.580±0.074a	0.536±0.160a	13.015±1.221a
	Ck	245.698±23.199a	17.485±1.236a	0.762±0.170a	0.455±0.082a	14.041±0.630a

Note: Different letters in the same column indicate the significance of the difference between different cultivars of the same soil (P < 0.05). In the table, Cd indicates the soil in the Lanping lead-zinc mining area, and Ck indicates the clean soil, the same below.

3.3 Effects of cadmium stress on free amino acids and soluble sugars in root exudates of two Dianthus caryophyllus

It can be seen from Figure 1 that under cadmium stress, the levels of free amino acids in root exudates of the two cultivars were inhibited, and both reached significant levels with the control group (P < 0.05). In the "Master", cadmium stress showed an inhibitory effect on soluble sugar, and in the "Xiao Yan", it showed an promotion, and the soluble sugar content of the two cultivars reached a significantly different level from that of the control group (P < 0.05). In the control soil, the control group (P < 0.05). In the control soil, the content of free amino acids and soluble sugar in the root exudates of the "Xiao Yan" was higher than that of the "Master". In the cadmium-stressed soil, the free amino acid content in the root exudates of the "Xiao Yan" was consistent with that of the "Master", and the soluble sugar content was higher than that of the "Master".



sugar in root exudates of different cultivars

3.4 Effects of cadmium stress on five types of organic acids in root exudates of two Dianthus caryophyllus cultivars

The change trend of the content of organic acids secreted by the roots of the two cultivars under cadmium stress is basically the same, and both exhibited an inhibitory effect compared with the control group (Figure 2). In the "Xiao Yan", the content of five organic acids under cadmium stress is malic acid>oxalic acid>citric acid>acetic acid>tartaric acid. The inhibitory effect of Cd on oxalic acid, malic acid and acetic acid is weak and does not reach a significant level (P>0.05); The inhibitory effect on tartaric acid and citric acid is strong, reaching a significant level (P<0.05). In the "Master", the content of five organic acids under cadmium stress is citric acid>malic acid>oxalic acid>acetic acid>tartaric acid. The inhibitory effect of Cd on oxalic acid and tartaric acid is weak and does not reach a significant difference level (P>0.05); For malic acid, acetic acid has a strong inhibitory effect, reaching a significant difference level (P<0.05). For malic acid, acetic acid has a strong inhibitory effect, reaching a significant difference level (P<0.05).

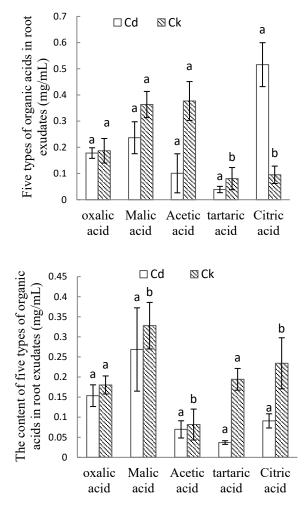


Figure 2 Organic acid content in root exudates of different cultivars

3.5 Effect of cadmium stress on glutathione and proline in leaves of two Dianthus caryophyllus cultivars

Under cadmium stress, the proline content of the "Master" was inhibited, and the proline content of the "Xiao Yan" was promoted and reached a significantly different level from the control group (P<0.05) (Figure 3). Under cadmium stress, the glutathione content of the two cultivars was inhibited. The glutathione content of the "Master" and the control group reached a significant level (P<0.05), while the "Xiao Yan" and the control group did not reach a significant level (P>0.05). In the control soil, the content of proline and glutathione in the leaves of the "Xiao Yan" was lower than that of the "Master"; in the soil under cadmium stress, the content of proline in the leaves of the "Xiao Yan" was higher than that of the "Master", the glutathione content is lower than the "Master".

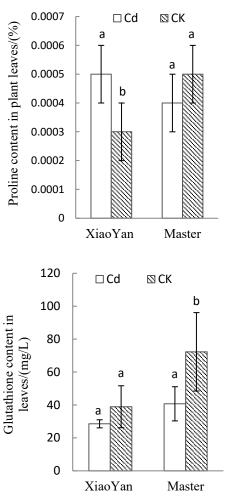
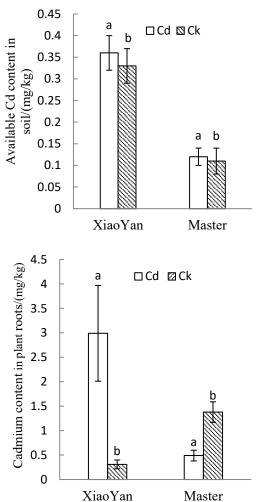


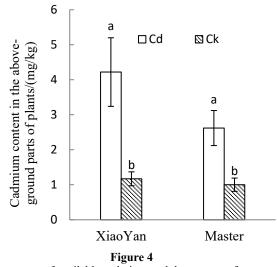
Figure3 The content of glutathione and proline in leaves of different cultivars

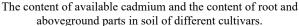
Note: Different lowercase letters in the figure represent significant differences between different treatments of the same cultivar (P < 0.05).

3.6 Changes in cadmium content in soil, cadmium content in plant roots and above ground

Figure 4 showed that the available cadmium content in the soil, the cadmium content in the roots, and the above-ground content of the "Xiao Yan" under cadmium 0.32~0.40mg/kg, 2.33~4.53mg/kg, stress are 3.23~4.75mg/kg, respectively. The Cd content of soil was 0.10~0.14mg/kg, 0.94~1.26mg/kg, 0.87~1.37mg/kg. In the "Master", the available cadmium content in the soil, the cadmium content in the root, and the above-ground content under cadmium stress were 0.29~0.37mg/kg, 2.33~4.53mg/kg, 3.23~4.75mg/kg. The effective cadmium content is 0.06~0.12mg/kg, 1.07~2.09mg/kg, 0.80~1.06mg/kg in the control soil. It can be seen that the cadmium content of the roots and aerial parts of the "Xiao Yan" under cadmium stress is higher than that of the "Master".







3.7 Cd accumulation, transportation coefficient and above-ground accumulation coefficient in plants under cadmium stress

The enrichment coefficient (aboveground) is used to evaluate the ability of plants to absorb heavy metals from the soil, and the transport coefficient characterizes the ability of plants to transport heavy metals in the body. It can be seen from Table 3 that under cadmium stress, the aboveground cadmium accumulation, aboveground enrichment coefficient and transport coefficient of each plant of "Xiao Yan" were 4.22, 11.72, and 1.65, respectively, while those in the control group were 1.17, 10.27, 4.18, respectively. The above-ground cadmium accumulation, above-ground enrichment coefficient, and transport coefficient of "Master" were 2.62, 7.94, and 5.69, respectively. In the control group, they were 1.00, 12.23, and 0.75, respectively. It can be seen that under cadmium stress, the enrichment coefficient (aboveground) and cadmium accumulation of aboveground cultivars are higher than "Master", and the transport coefficient is lower than "Master".

 Table 3 The accumulation, transfer coefficient and enrichment coefficient of cadmium in the aboveground part of the soil of the two plants

Treatment	Variety	Cd accumulation above ground (Per plant)	Aboveground enrichment factor (BCF)	Transshipment coefficient (TF)
Cd	"Xiao Yan"	4.22±0.98a	11.72±2.66a	$1.65{\pm}1.08a$
	"Master"	2.62±0.50b	7.94±1.20b	5.69±2.00b
Ck	"Xiao Yan"	1.17±0.20a	10.27±3.25a	4.18±1.56a
	"Master"	1.00±0.19b	12.23±6.20a	0.75±0.22b

4 Discussion

Cadmium is a non-essential element for plant growth. Plant growth is inhibited under cadmium stress, resulting in dwarf plants, slow growth, and reduced biomass. Plant height and biomass can be used as important indicators for evaluating plant tolerance to metal stress [26-27]. In this experiment, the plant height, leaves, flower buds and biomass of "Xiao Yan" and "Master" were all inhibited under cadmium stress. Compared with the control group, "Xiao Yan" decreased by 39.7cm, 15.23, 6 flowers, and 4.4, respectively. "Master" dropped 6cm, 15.67, 2 flowers, and 1.7 respectively. This may be due to a series of physiological changes in the plant under cadmium stress, causing the accumulation of free radicals and membrane lipid peroxidation, which damages and destroys the structure and function of the membrane system, increases permeability, and inhibits the growth of cells and the entire plant. Growth, resulting in plant dwarfing and slow growth ^[28]. The length of the root system mainly reflects the growth status of the plant and the ability to expand in the soil; the size of the root surface area affects the strength of the root system to absorb ions. The root volume is one of the important indicators reflecting the growth and development of plants, and the root projected area is an important indicator reflecting the combined area of the root system

and the soil. Studies have shown that low concentrations of cadmium promote the increase of total root length. root surface area, and root volume, while medium and high concentrations of cadmium inhibit root growth [29]. In this experiment, the root length, total root surface area, root volume, and root projected area of the "Xiao Yan" variety decreased under cadmium stress, and the average root diameter increased. This result is consistent with Li Ximing et al. ^[30] and Zhang Ling et al. ^[31] on the changes of wheat root morphological parameters under cadmium stress. It shows that plants under high cadmium concentration can optimize the resource acquisition ability (water, nutrients, etc.) by changing the root structure and distribution pattern, and then adapt to adversity stress. In this experiment, the root length, total root surface area, total root projected area, and average root diameter of the "Master" were promoted under cadmium stress, and the root volume was inhibited, which was different from the "Xiao Yan" .This may be due to the toxicity of different cultivars to cadmium reasons for different effects.

There are many ways in which root exudates affect the repair of heavy metals. It can secrete carbohydrates, amino acids, organic acids and other substances into the rhizosphere environment, by changing the physical and chemical properties of the rhizosphere environment such as pH and Eh; it affects the roots to metal absorption; through a series of chemical actions such as chelation,

complexation, acidification, reduction and activation, these heavy metal pollutants are discharged out of the roots, and the amount and effective concentration of metals in the rhizosphere environment are changed by changing the composition and activity of rhizosphere microorganisms. Therefore, root exudates have a great influence on the absorption of heavy metals by plants ^[32-33]. The main role of amino acids in root exudates is to acidify the rhizosphere or chelate Cd2+ in the soil, promote the migration of Cd²⁺ in the soil and root absorption; and soluble sugar is an important osmotic adjustment substance. In this experiment, the content of free amino acids in the root exudates of the two cultivars was significantly reduced under cadmium stress, while the content of soluble sugar in the "Xiao Yan" increased, and the content of soluble sugar in "Master" decreased. This result is consistent with the changes in wheat root exudates under the stress of medium and high concentrations of cadmium studied by Zhang Ling and others, and the decrease in "Master" 's soluble sugar content may be due to the different tolerance of different cultivars to cadmium toxicity [34]. In addition, in addition to the citric acid secreted by the roots of the "Master" variety, the five types of organic acids in the root exudates of the two cultivars are inhibited. Previous studies have shown that a certain concentration of cadmium stress can promote the secretion of organic acids in the roots of plants. This is due to the tolerance mechanism of plants, but when the concentration is too high, cadmium stress will destroy the root cell structure and inhibit the production of root exudates ^[35]. And Yang Renbin and others pointed out that organic acids and amino acids have a strong activation effect on heavy metals in the soil, among which oxalic acid, tartaric acid and citric acid have strong activation ability^[36].

Proline in leaves is an important osmotic adjustment substance. The increase in plant proline content is an adaptation to plant adversity stress. Proline will form a Cd-proline complex with heavy metal ions, reducing heavy metal oxidative damage to plants. Glutathione is a non-enzymatic antioxidant substance in the free radical scavenging system, which is beneficial to maintain the intracellular redox balance. In this experiment, the proline content of the "Xiao Yan" variety under cadmium stress was significantly higher than that of the control group, which was consistent with the results of Wang Yangyang et al. and Li Zifang's research on rice and wheat [37-38]. The increase in proline content has strong active oxygen free radical scavenging ability, reduces the degree of membrane lipidation, and can alleviate the toxicity of cadmium to plants. The reason for the decrease in the content of "Master" cultivars compared with the control group may be the same as mentioned before the tolerance of different cultivars to cadmium is related. In addition, the glutathione content in the leaves of the two cultivars decreased under cadmium stress. This is due to the outbreak of reactive oxygen species (ROS) in the plant under cadmium stress, resulting in the inactivation of glutathione reductase (GR), resulting in the glutathione content (GSH) is reduced.

The enrichment coefficient (aboveground) is used to evaluate the ability of plants to absorb heavy metals from

the soil, and the transport coefficient characteristics the ability of plants to transport heavy metals in the body. In this experiment, the cadmium accumulation in the roots and aerial parts of the "Xiao Yan" in the cadmium-stressed soil was higher than that of the "Master" In the control soil, except for the roots, the cadmium accumulation in the aboveground parts was also higher than that of the "Xiao Yan". "Master" breed. It can be seen that the tolerance of the smiling breed is higher than that of the "Master" breed. In addition, under cadmium stress, the cadmium accumulation in the aerial parts of the two cultivars is greater than the cadmium accumulation in the roots, and the enrichment coefficient and transport coefficient of the aerial parts are both greater than 1. The enrichment coefficient refers to the content of a certain heavy metal in the plant the ratio of the original content of the heavy metal in the soil is one of the important indicators for evaluating the plant's ability to accumulate heavy metals. It reflects the plant's ability to enrich a certain heavy metal element. The greater the enrichment coefficient, the stronger its enrichment ability. The transfer coefficient is the ratio of the heavy metal content of the above-ground part and the root of the plant, which can reflect the ability of the plant to transport heavy metals from the root to the above-ground part ^[39]. This proves that the two plants have strong cadmium accumulation and transportation capabilities.

5 Conclusion

Under the stress of cadmium, the growth of the two cultivars was affected. Both "Master" and "Xiao Yan" showed decline in plant height, leaves, flower buds and biomass, but "Master" was more inhibited. The root length, total root surface area, total projected area of roots, and average root diameter of the "Master" increased under cadmium stress, while the root volume decreased, but the root length, total root surface area, root volume, and root system of "Xiao Yan" cultivars under cadmium stress the projected area is reduced, and the average root diameter is increased.

Under the stress of cadmium, the glutathione in the leaves of the two cultivars of plants decreased, the proline content of the leaves of the "Xiao Yan" increased, and that of the "Master" decreased. In the root exudates, the free amino acid content of the two cultivars were reduced, and the secretion of organic acids was also inhibited (except for the citric acid secreted in the "Master"), while the soluble sugar content in the root exudates showed that the smile variety increased. Higher, lower "Master". According to the physiological response of the two cultivars under cadmium stress, the "Xiao Yan" is more suitable for soil restoration in the mining area of Lanping area.

Under the stress of cadmium, the plants of the two cultivars have certain tolerance, accumulation ability and transportation ability, but they did not reach the standard of hyperaccumulator plants, but this kind of plant has fast reproduction, easy cultivation, and adaptability. Strong, long flowering period, high ornamental value, can be used as a reference plant for soil restoration in the Lanping lead-zinc mining area.

Acknowledgments

This article is a key research and development project of Yunnan Province, "Research and Application Demonstration of Ecological Protection and Restoration Technologies in Small Watersheds in Typical Metal Mining Areas in Yunnan" (2019BC001-04), and Yunnan Agricultural University Innovation and Entrepreneurship Action Fund Project "Mechanisms of Different Carnations Alleviating Cadmium Stress Disasters Research" (2020ZKX033) one of the phased results.

References

- 1. Ministry of Environmental Protection and Ministry of Land and Resources. National Soil Pollution Survey Bulletin [Z]. April 2014
- State Council, Action Plan for Soil Pollution Prevention and Control, Guofa [2016] No. 31, May 2016
- Ying Huang, Lingyu Wang, Wenjia Wang, Tingqiang Li, Zhenli He, Xiaoe Yang (2019) Current status of agricultural soil pollution by heavy metals in China: A meta-analysis [J]. Science of the Total Environment, 651:,pp.3034-3042.
- 4. Qianqi Yang, Zhiyuan Li, Xiaoning Liu, Qiannan Duan, Lei Huang, Jun Bi(2018) A review of soil heavy metal pollution from industrial and agricultural regions in China: Pollution and risk assessment [J]. Science of the Total Environment, 642, pp.690-700.
- Libo Pan, Jin Ma, Xianliang Wang, Hong Hou (2016) Heavy metals in soils from a typical county in Shanxi province, China: Levels, sources and spatial distribution [J]. Chemosphere, 2016, 148, pp.248-254.
- Rubina Khanam, Anjani Kumar, A.K. Nayak, Md. Shahid, Rahul Tripathis, S.Vijayakumar, Debarati Bhaduri, Upendra Kumar, Sangita Mohanty, P.Panneerselvam, Dibyendu Chatterjee, B.S.Satapathy, H.Pathak. (2020) Metal (loid)s (As, Hg, Se, Pb and Cd) in paddy soil: Bioavailability and potential risk to human health [J]. Science of the Total Environment, 2020, 699, pp.134330.
- Sana Khalid, Muhammad Shahid, Nabeel Khan Niazi, Behzad Murtaza, Irshad Bibi, Camille Dumat (2017)A comparison of technologies for remediation of heavy metal contaminated soils [J]. Geochemical Exploration, 182, pp. 247-268.
- Javed Nawab, Sardar Khan, Muhammad Aamir, Isha Shamshad, Zahir Qamar, Islamud Din, Qing Huang (2016) Organic amendments impact the availability of heavy metal(loid)s in mine-impacted soil and their phytoremediation by Penisitum americanum and Sorghum bicolor [J]. Environ Science Pollution Research, 23(3), pp.2381-2390.

- Xuefeng Liang, Yi Xu, Yingming, Xu, Pengchao Wang, Lin Wang, Yuebing Sun, Qingqing Huang, Rong Huang (2016) Two-year stability of immobilization effect of sepiolite on Cd contaminants in paddy soil [J]. Environment Science Pollution Research, 23(13), pp.12922-12931.
- Cheng Xianfeng; Song Tingting; Chen Yu; Wei Yongming; Shen Jinxiang; Qi Wufu. Hyperspectral inversion analysis of soil heavy metal content in the Lanping lead-zinc mining area in western Yunnan[J]. Journal of Rock and Mineralogy, 2017, 36(165)
- 11. L. Sanita' di Toppi, R. Gabbrielli (1999) Response to cadmium in higher plants [J]. Environmental and Experimental Botany, 41,pp.105-130.
- Wu Feibo, Zhang Guoping. Variety differences in barley seedling growth and cadmium and nutrient uptake under different cadmium levels[J]. Chinese Journal of Applied Ecology, 2002, 13(12): 1595-1599.
- 13. Zhang Lihong, Li Peijun, Li Xuemei, et al. Effects of cadmium stress on the growth and physiological characteristics of wheat seedlings[J]Journal of Ecology, 2005, 24(4): 458-460.
- 14. Zhang Lei, Yu Yanling, Zhang Lei. Effects of exogenous cadmium stress on photosynthetic characteristics of maize seedlings[J]. North China Agricultural Journal, 2008, 23(1): 101-104.
- 15. He Junyu, Wang Yangyang, Ren Yanfang, Zhou Guoqiang, Yang Liangjing. Effects of cadmium stress on morphological and physiological characteristics of seedling roots of different rice varieties[J]. Acta Eco-Environmental Sciences, 2009, 18(5): 1863-1868.
- 16. Liu Ying, Gai Junyi, Lu Huineng. Research progress on the relationship between crop root morphology and abiotic stress tolerance[J]. Journal of Plant Genetic Resources, 2003, 4(3): 265-269.
- 17. Yuan Fanqi, Yuan Jinxi, Song Jinfeng, Zhang Hongguang. Study on the secretion of organic acids in the roots of Larix olgensis seedlings under Cd stress[J]. Soil Bulletin, 2019, 50(5):1218-1224.
- 18. Sun Ruilian, Zhou Qixing. The role and mechanism of organic acids in metal resistance in higher plants[J]. Chinese Journal of Ecology, 2006, 25 (10): 1275-1279.
- 19. Qiang Weiya, Chen Tuo, Tang Hongguan. The effects of Cd stress and enhanced UV-B radiation on soybean root exudates[J]. Acta Plant Ecology, 2003, 27 (3): 293-298.
- 20. Lu Haoliang, Yan Chonglin, Liu Jingchun (2007) Low-molecular-weight organic acids exuded by Mangrove (Kandelia candel (L.) Druce) roots and their effect on cadmium species change in the rhizosphere [J].Environmental and Experimental Botany, , 61(2), pp.159 – 166.
- G. Cie'sli'nski1, K.C.J. Van Rees, A.M. Szmigielska, G.S.R. Krishnamurti and P.M. Huang (1998) Low-molecular-weight organic acids in rhizosphere

soils of durum wheat and their effect on cadmium bioaccumulation [J]. Plant and Soil, 230,pp.109 -113.

- 22. Wang Jixiu, Zhan Fangdong, Li Yuan, Zu Yanqun, Qin Li, He Yongmei, Li Mingrui. The effect of intercropping floret Arabidopsis and maize on root exudates organic acids under lead stress[J]. Chinese Journal of Eco-Agriculture, 2016, 24(03): 365-372.
- 23. Fan Chongdong, Wang Miao, Wei Gongyuan, Huang Ling. Research progress in the determination of glutathione[J]. Biotechnology, 2004(01): 68-70.
- 24. Hao Wenya, Shen Qirong, Ran Wei, Xu Yangchun, Ren Lixuan. Effects of sugar and amino acids in root exudates of watermelon and rice on the growth of watermelon fusarium wilt pathogen[J]. Journal of Nanjing Agricultural University, 2011, 34(03) :77-82.
- Xu Tong, Chen Cuilian. Determination of Plant Resistance (Rapid Proline Determination) Method [J]. Journal of Huazhong Agricultural College, 1983(01): 94-95.
- 26. Song Jian; Jin Fengmei; Xue Jun; Liu Zhongqi. Research progress of cadmium stress on plant growth and physiological and ecological effects[J]. Tianjin Agricultural Sciences, 2014, 20(110): 23-26.
- 27. Zhao Huibo; Li Lili; Liang Tana; Zhang Yanxin; Huang Fenglan; Cao Qingguo. Research progress in plant response to heavy metals copper and cadmium stress[J]. Journal of Anhui Agricultural Sciences, 2019, 47(634): 22-24.
- Qin Li; Zu Yanqun; Li Yuan. Effect of Cd on the growth physiology of the super-accumulative plant Dipsacus chinensis[J]. Journal of Agricultural Environment Sciences, 2010, 29:56-60.
- 29. He Junyu; Wang Yangyang; Ren Yanfang; Zhou Guoqiang; Yang Liangjing. Effects of cadmium stress on morphological and physiological characteristics of seedling roots of different rice varieties[J]. Journal of Ecological Environment, 2009, 18:263-268.
- Li Ximing; Song Guilong. Effects of cadmium stress on cadmium absorption characteristics and root morphology of alfalfa[J]. Acta Prataculturae Sinica, 2016, 25(127): 180-188.
- Zhang Ling; Li Junmei; Wang Huanxiao. Physiological and ecological changes of wheat root system under cadmium stress[J]. Soil Bulletin, 2002, 63-67.
- 32. Kuang Yuanwen; Wen Dazhi; Zhong Chuanwen; Zhou Guoyi. Root exudates and their role in phytoremediation [J]. Chinese Journal of Plant Ecology, 2003, 137-145.
- Zhao Kuan; Zhou Baohua; Ma Wanzheng; Yang Limin. Research progress on the effects of different environmental stresses on root secretion of organic acids[J]. Soil, 2016, 48(282): 27-32.
- 34. Zhang Ling; Li Junmei; Wang Huanxiao. Physiological and ecological changes of wheat root

system under cadmium stress[J]. Soil Bulletin, 2002, 63-67

- 35. Wang Yuyun. The effect of Cd stress on the secretion of organic acids and amino acids and the Cd content of different rice roots[C]. Sichuan Agricultural University, 2011.
- 36. Yang Renbin; Zeng Qingru; Zhou Xihong; Tie Baiqing; Liu Shengyang. The activation effect of plant root exudates on heavy metals in soil contaminated by lead-zinc tailings[J]. Environmental Protection of Agriculture, 2000, 25-28.
- 37. Wang Yangyang; Ren Yanfang; Zhou Guoqiang; Zhang Xiaojian; He Junyu. Effects of cadmium stress on the growth and physiological characteristics of different resistant rice varieties[J]. Chinese Agricultural Science Bulletin, 2009, 25(198):460-464.
- Li Zifang; Liu Huifen; Xiong Xiaoxia; Liu Huisheng. Effects of cadmium stress on wheat seed germination, seedling growth and physiological and biochemical characteristics[J]. Journal of Agricultural Environment Sciences, 2005, 24-27.
- 39. Mao Haili; Yang Bo; Long Chengmei; Zou Hongtao; Zhong Caining. Research progress in the selection of heavy metal cadmium super-enrichment and enrichment plants[J]. Journal of Qiannan Teachers College for Nationalities, 2011, 31(156): 9-14.