RESISTANCE AND UPTAKE CHARACTERISTICS OF THREE GENOTYPES *DIANTHUS CARYOPHYLLUS* TO CHROMIUM CONTAMINATION OF TAILINGS SOILS

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Abstract. To investigate the physiological response and uptake characteristics of three genotypes of *Dianthus caryophyllus* to Cr stress, we used a heavy metal-contaminated soil sample from a lead-zinc mine in a pot trial to determine several parameters, including plant height, stem thickness, root architecture, biomass, heavy metal accumulation and transport capacity under different levels of Cr contamination. We discovered a dosage effect in the influence of the heavy metal Cr on the growth of *Dianthus caryophyllus*. Under low-dose conditions, it elicited a protective response favoring plant growth, whereas under high accumulation conditions, it inhibited plant growth. Additionally, chromium stress increased the growth of plant roots. In the range of chromium concentrations between 0-100 mg/kg, the enrichment and transit coefficient of the three species, namely Freedom, Master, and Dallas decrease as the concentration of chromium increases. The enrichment coefficient follows the order Freedom > Dallas > Master, whereas the transit coefficient follows the order Master > Freedom > Dallas. All three genotypes of *Dianthus caryophyllus* exhibited tolerance, accumulation, and transport capacities when subjected to chromium stress. As a result, they can serve as reference plants for on-site remediation of heavy metals in soils contaminated with tailings in mining regions.
Keywords: *metal tailings, ecological restoration, in situ repair, Dianthus caryophyllus, Cr stress*

Introduction

The rapid development of modern agricultural technology has led to the massive misuse of heavy metal-rich fertilizers and pesticides, leading to increasingly severe heavy metal pollution of soils globally (Qin et al., 2021; Wu et al., 2020). According to the China National Soil Pollution Survey Bulletin (2014), most of China’s farmland is involved in heavy metal pollution, with a total area of more than 840,000 hm^2. The cumulative, insidious, lagging, and difficult-to-biodegrade characteristics of heavy metal pollution facilitate accumulation in the soil or food chain, thus posing a threat to human health (Fei et al., 2018; Hu et al., 2020; Peng et al., 2022). Since the 1960s, environmental problems caused by heavy metal pollution have become an essential concern for governments, experts, scholars, and entrepreneurs worldwide (Long et al., 2021; Yuan et al., 2021; Yang et al., 2021). The role of heavy metals has been investigated by understanding the process and mechanism of heavy metal action in soil. The commonly used techniques for treating heavy metal-contaminated soil include physical, chemical, and combined physical and chemical methods. However, these methods often have the drawbacks of high cost, complex maintenance, and easily produce secondary contamination in the practical application (Wang et al., 2022). In comparison phytoremediation technology is environmentally friendly, has low

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investment and maintenance costs and is simple and easy to implement, so researchers in related fields have valued it in recent years, and it has become a hot spot of research in the academic community (Luo et al., 2023; Gavrilescu, 2020; He et al., 2020; Jin et al., 2019). The method mainly uses the plant root energy system, which not only reduces the concentration of heavy metal pollutants in the soil but also achieves the purpose of restoring the original soil geomorphology, improving the local climate and optimizing the microenvironment (Shikha and Singh, 2021).

Dianthus caryophyllus is a perennial herbaceous plant in the genus Dianthus, family Dianthus, and an ornamental crop whose growth habit is well adapted to environments such as barren mountains, wastelands, and rock crevices, and which is characterized by its vigorous vitality, rapid growth, easy propagation and easy maintenance (Mokarram et al., 2021). The crop is very well adapted to the environment of barren mountains and wastelands, such as rock crevices. In addition to its ornamental value, it is considered a high-potential plant for soil remediation due to its ability to absorb common heavy metal pollutants (Wu et al., 2021). In addition, many scholars have paid attention to the heavy metal remediation potential of this non-edible and economically valuable plant. However, the resistance and uptake characteristics of balsamroot to heavy metal contamination in tailings soil have been less studied, so the study of the harmless remediation of heavy metals in tailings soil is of great significance (Qiao et al., 2023; Liu et al., 2020).

Guangxi Zhuang Autonomous Region has the country’s fourth most significant amount of mineral resources. The mineral soils in this region have high concentrations of heavy metal pollution, poor physical structure, lack of adequate nutrients, drought, and extreme acidity or salinity, among other problems, which make the remediation of heavy metal pollution in this region more difficult. Therefore, this study took a lead-zinc mining area in the northeast of Guangxi Zhuang Autonomous Region as the study area. The area’s soil was taken to investigate the growth changes of three genotypes of *Dianthus caryophyllus*, Freedom, Master, and Dallas, under chromium stress and their resistance and uptake characteristics of heavy metal pollutants of tailings-contaminated soil using potting experiments. To provide reference plants for in situ remediation of heavy metals in tailings-contaminated soils in mining areas.

Materials and methods

Experimental materials

The tested soil was collected from the tailings-polluted soil of a mining area in the northeastern part of Guidong, Guangxi Zhuang Autonomous Region. A quadrant method was employed to collect soil samples from four directions within the mining area. Ten samples were collected at each location, totaling 40 samples for analysis. The basic physicochemical properties of the soil are as follows: pH value ranging from 7.36 to 8.36, organic matter content ranging from 23.45 to 35.77 g/kg, total nitrogen content ranging from 1.25 to 2.85 g/kg, and total potassium content ranging from 10.45 to 7.41 g/kg; the plants for the test were purchased from Kunming Colourful Flowers Co. Ltd.

Experimental methodology

The test soil (the average cadmium concentration is 110.45 mg/kg.) was evenly mixed and potted (pot height 18 cm, inner diameter 20 cm), each pot was filled with

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2 kg of soil, exogenous heavy metal pollutants were added to the soil, and two plants of the same genotypes were planted in each pot with similar sizes and morphology. Six experimental groups were set up for the test (exogenous heavy metal additions of 0, 10, 25, 50, 75, and 100 mg/kg, respectively), with three replications for each treatment. 90 days after transplanting, the height and stem thickness of the *Dianthus caryophyllus* were measured, and the roots were carefully washed with deionized water to remove soil particles from the root system, followed by scanning with a root morphology scanner to analyze the mean root diameter and total root volume. Subsequently, the plants were separated into roots, stems, leaves and flowers, and the samples were washed with deionized water and placed in a constant temperature oven (1000KW-1, Shanghai Farrer Instrument Technology Co., Ltd.) at 105°C for 60 min, and then dried in a constant temperature oven at 70°C until constant weight. After drying, the samples were crushed and sieved through a 100-mesh sieve and then subjected to microwave digestion (JUPITER, Shanghai Xinyi Microwave Chemistry Co., Ltd.) using a nitric acid-perchloric acid digestion system (8 mL, 3:1) for determination of sample biomass.

Physico-chemical analysis

The heavy metal content in both plants and soil was assessed using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) - Agilent 7850 (Agilent Technologies, Inc.). Soil samples underwent a series of preparatory steps including air-drying, grinding, sieving, and microwave digestion using the MARS 6 system (CEM Corporation) before the determination of heavy metal content via ICP-MS. Similarly, plant samples were subjected to drying, grinding, sieving, and digestion before the assessment of heavy metal content using ICP-MS. Furthermore, the root system data of the plants was measured using a root scanner (HTGX-A-I, Beijing Jingcheng Huatai Instrument Co., Ltd.). The bioconcentration factor (BCF) was calculated as shown in *Equation 1*.

$$BCF = \frac{m_0}{m_1} \qquad \text{(Eq.1)}$$

In *Equation 1*, m_0 is the heavy metal content in the plant; m_1 is the heavy metal content in the soil, and the heavy metal concentrations are in mg/kg. The transit coefficients (*TC*) was calculated as shown in *Equation 2*.

$$TF = \frac{m_2}{m_3} \qquad \text{(Eq.2)}$$

In *Equation 2*, m_2 is the heavy metal content in the above-ground part of the plant; m_3 is the heavy metal content in the plant’s roots; and the units of heavy metal concentration are mg/kg.

Data analysis

The data was processed and analyzed using software applications such as Excel, SPSS 24.0, and Origin 9.0. The results were represented using the mean values. Statistical analysis of the data was conducted through ANOVA followed by Duncan’s multiple comparisons test for further assessment.

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Results and discussion

Characteristics of heavy metal contamination of soil in lead and zinc tailing areas

Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the heavy metal content of tailings-contaminated soil in a mining area in northeast Gui, Guangxi Zhuang Autonomous Region, and the results of the analyses were compared with the limits of heavy metal content in the "*Soil Pollution Risk Control Standards for Soil Environmental Quality of Agricultural Land* (for Trial Implementation)" (GB15618-2018), and the results are shown in *Table 1*. The heavy metal content in the plant growth matrix in this region varied greatly, and their coefficients of variation all exceeded 30%, indicating that the heavy metal pollution in this region was characterized by significant spatial variability. Secondly, the average value of Cr content in the tailings-contaminated soil exceeded the background value of soil heavy metal content in the region. However, it did not exceed the national standard limit value. In contrast, Cd, Pb and Zn in the tailings-contaminated soil did not exceed the background value of soil heavy metal content in the region and the national standard limit value, which indicated that Cr contamination mainly existed in the region.

Table 1. *Heavy metal content of lead-zinc tailings contaminated soil*

Statistical value	Cd	Cr	Zn	Pb
Maximum (mg/kg)	0.28	190.43	60.78	70.68
Minimum (mg/kg)	0.07	42.36	23.46	15.46
Average (mg/kg)	0.12	110.45	43.25	30.19
Standard deviation (mg/kg)	0.07	33.45	32.19	10.07
Coefficient of variation (%)	58.33	46.35	74.42	33.35
Soil background (mg/kg)	0.13	101.89	65.77	34.16
National standard limit (mg/kg)	0.30	200	250	120

Growth of Dianthus in each treatment

Experiments were conducted to examine alterations in growth indices of three varieties of Dianthus, Freedom, Master, and Dallas, through employing varied exogenous heavy metal additions (control group CK, experimental groups T1-T5 received 0, 10, 25, 50, 75, and 100 mg/kg, respectively). *Figure 1a-d* indicates the experimental outcomes. With the addition of exogenous heavy metal Cr, the plant height and stem thickness of three Dianthus genotypes, namely Freedom, Master, and Dallas, exhibited a slight increase followed by a decrease. While low concentrations of Cr promote plant height in these genotypes, high concentrations of Cr inhibit it. The heights of the Freedom, Master and Dallas plants from the T5 treatment in the experimental group were the shortest, measuring 62.14, 53.12 and 58.77 cm, respectively. These were significantly lower than the heights of plants from the CK treatment group ($P < 0.05$). Furthermore, the stem thicknesses of the Freedom, Master and Dallas plants from the T5 treatment in the experimental group were the thinnest, measuring 3.98, 2.85 and 3.43 mm, respectively. These stem thicknesses were also significantly lower than those from the CK treatment group ($P < 0.05$). No improvements necessary as the text lacks context and already adheres to the principles. Secondly, chromium pollution had a stimulatory effect on the root dimensions and

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volume of three Dianthus genotypes, specifically Freedom, Master, and Dallas. As the concentration of Cr pollution increased, so did the root dimensions and volume of these three genotypes. The highest values for root diameter and volume were obtained from T5 of the experimental group, with values of 0.45, 0.49, and 0.41 cm, and 1.27, 1.41, and 1.27, 1.40, and 1.40 cm^3, respectively.

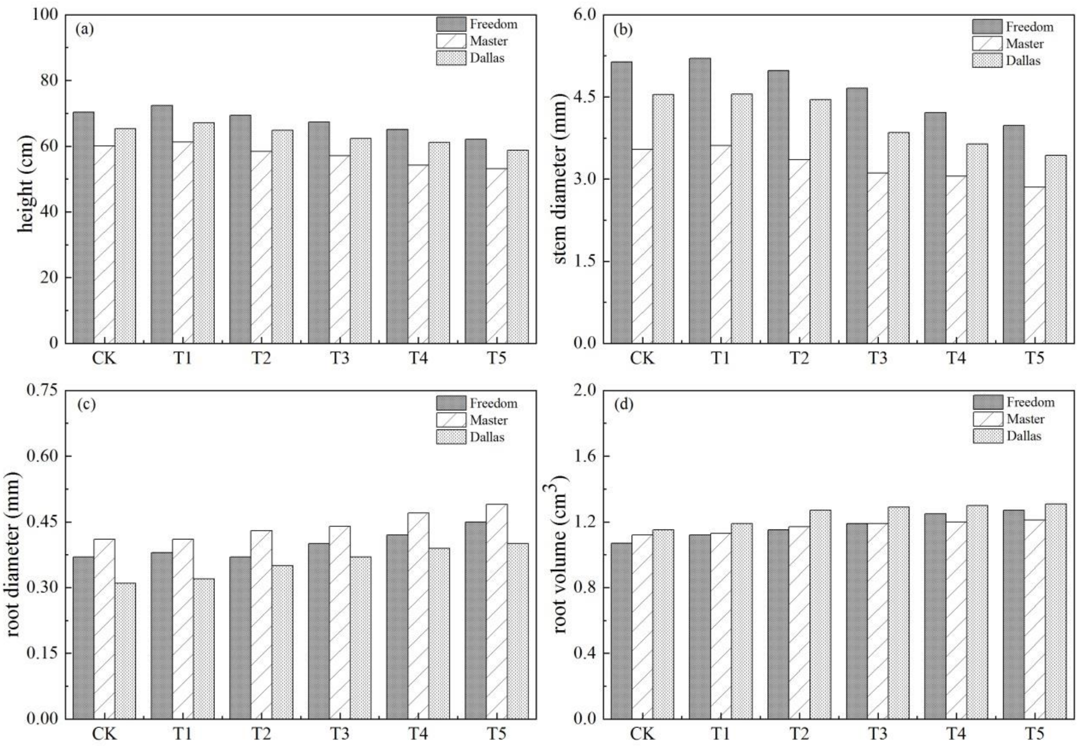

Figure 1. *Growth of three Dianthus genotypes under different treatment conditions*

Subsequently, an experiment was conducted to examine the dry weight of each section of the plants of the three Dianthus genotypes, specifically Freedom, Master, and Dallas, under various treatments (control group CK and experimental groups T1-T5, of 0, 10, 25, 50, 75, and 100 mg/kg correspondingly). The findings are depicted in *Figure 2*. With the rise in exogenous heavy metal exposure, the root, stem, leaf and total biomass of various *Dianthus caryophyllus* types displayed a minor increase and then decrease in trend. This suggests that the impact of Cr contamination on the biomass of all three *Dianthus caryophyllus* varieties was boosted by low concentration levels but reduced by higher concentrations.

In *Figure 2a*, the experimental group T5 treated with Freedom, Master, and Dallas exhibited significantly lower root biomass compared to the CK treatment group, with biomass of 4.23 g, 3.58 g, and 4.42 g respectively ($P < 0.05$). In *Figure 2b*, T5 treated with Freedom, Master, and Dallas also exhibited the lowest stem and leaf biomass at 7. 35, 6.84 and 8.25 g, respectively, were recorded for the Freedom, Master and Dallas treatment groups. These values were significantly lower than those of the CK treatment group ($P < 0.05$). *Figure 2c* shows that the lowest total biomass was obtained from the T5 treatment group consisting of Freedom, Master and Dallas. The values recorded for this group were 11.58 g, 10.42 g and 12.67 g, respectively, which were significantly lower than those of the CK treatment group ($P < 0.05$).

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According to certain studies (Lai and Han, 2022; Mzazumdar and Dax, 2015), low-level exposure to heavy metal pollutants can enhance plant growth. The results of this experimental study are in line with this finding, which is primarily due to the plant's protective reaction to heavy metal stress. As the plants grows, the relative concentration of heavy metals decreases. Furthermore, increased plant growth results in a lower relative concentration of heavy metals in the plant. As the concentration of heavy metals in the plant increased, the protective response decreased, resulting in a decrease in the growth rate of the plant.

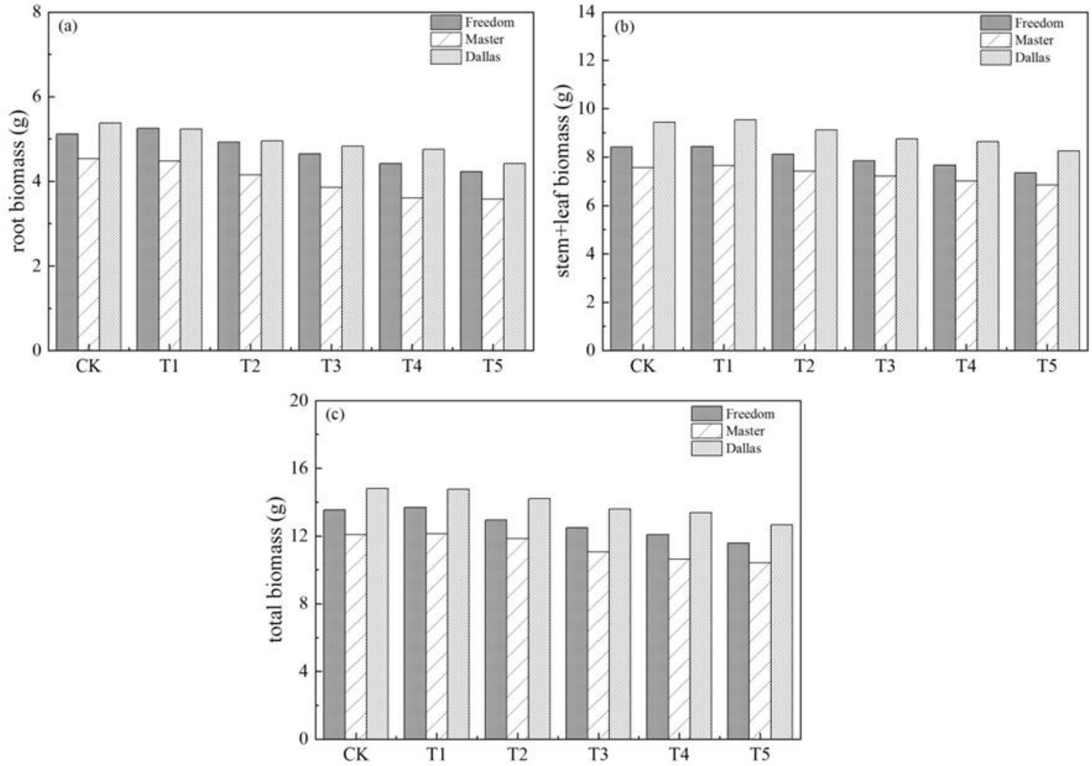

Figure 2. *Biomass of three carnation genotypes under different treatment conditions*

Characteristics of soil Cr uptake by the three genotypes of Dianthus caryophyllus under different treatments

The results of comparing the uptake and enrichment characteristics of heavy metal Cr in three genotypes of Dianthus caryophyllus under different treatment conditions (blank control group CK, experimental groups T1-T5 were 0, 10, 25, 50, 75 and 100 mg/kg, respectively) are shown in *Figure 3a-c*. With the increase of exogenous Cr addition concentration, the underground part, above-ground part, and soil Cr concentration of Freedom showed a rising trend. When the exogenous Cr addition concentration was up to 100 mg/kg, the concentration of its underground part, above-ground part and soil Cr reached the highest value of 223.4, 355.4 and 125.35 mg/kg.

With the increase of exogenous Cr addition concentration, the underground, above-ground, and soil Cr content of Master also showed an increasing trend. When the exogenous Cr addition concentration reached 100 mg/kg, the underground part, above-ground part and soil Cr, content reached the highest value, which were 300.9, 201.7 and 145.7 mg/kg.

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In addition, the above-ground Cr content of Freedom and Dallas was higher than that of the underground parts. In contrast, the Cr content of the above-ground parts of Master was lower than that of the underground parts, indicating that Master has a greater capacity to transport heavy metals than Freedom and Dallas.

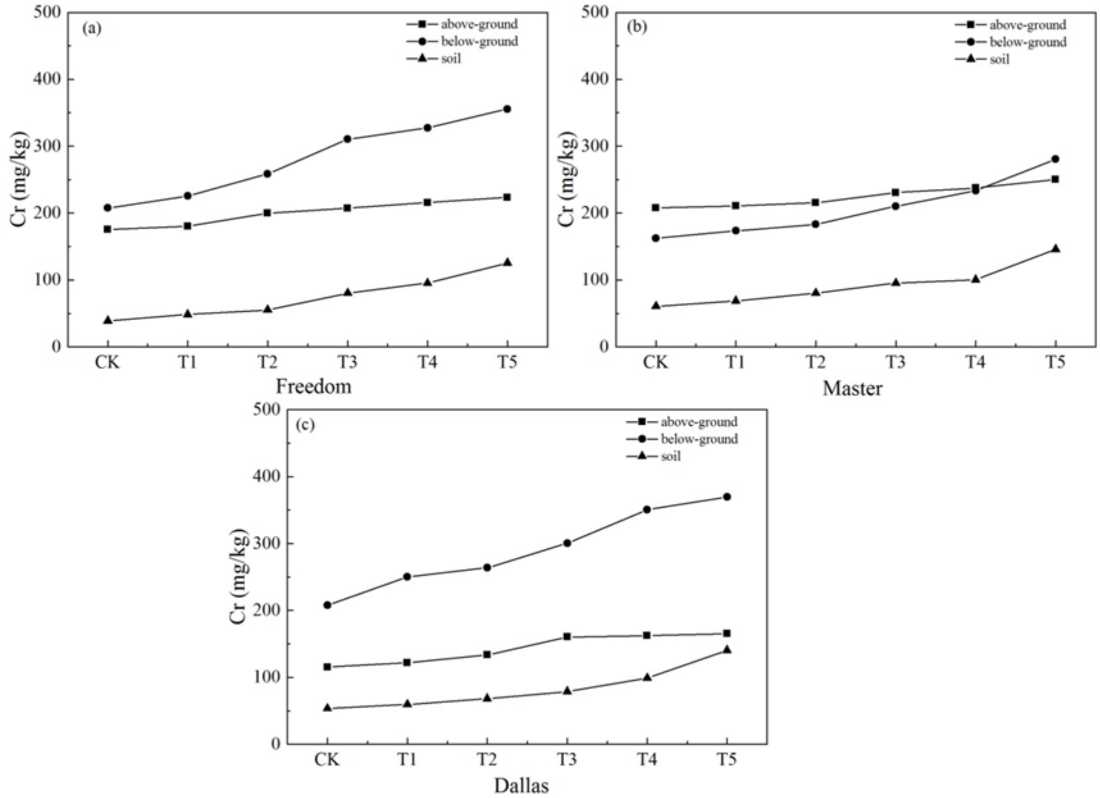

Figure 3. *Absorption and enrichment of Cr by Dianthus caryophyllus under different treatment conditions*

Enrichment and transport capacity of soil heavy metal Cr

The enrichment coefficient (BCF) is a commonly used index to assess the ability of a plant to absorb and accumulate heavy metals. The greater the ratio, the better the plant is at enriching heavy metals. As illustrated in *Figure 4a*, the BCF of Freedom, Master, and Dallas, exhibited a declining pattern as the concentration of external Cr was raised within the experimental bounds. The enrichment coefficient for Freedom, Master, and Dallas decreased from 9.88 to 4.62, 6.09 to 3.64, and 6.04 to 3.82, respectively, indicating a significant decrease. The declining trend of the three *Dianthus caryophyllus* varieties' enrichment coefficient demonstrated that increasing concentrations of exogenous Cr significantly impeded *Dianthus caryophyllus'* uptake of Cr. In addition, the enrichment coefficients for the three genotypes of *Dianthus caryophyllus* for soil heavy metal Cr were ranked in the order Freedom > Dalla > Master. This indicates that Freedom exhibits the most significant enrichment ability for soil heavy metal Cr.

The transit coefficient (TF) is commonly used to respond to the ability of the aerial part of the plant to transfer heavy metals absorbed by the roots, which is an essential indicator of phytoremediation of heavy metal pollution, and the higher the value, the better the heavy metal transfer effect of the plant. As illustrated by *Figure 4b*, the transit

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coefficients of the three types of *Dianthus caryophyllus* exhibit a declining trend with an increase in the concentration of exogenous Cr within the experimental range. Technical abbreviations, such as Cr, will be explained upon their first usage. The transit coefficients of Freedom declined from 0.85 to 0.63, Master from 1.28 to 0.89, and Dallas from 0.56 to 0.45. The transit coefficients of the three genotypes were less than 1 when the exogenous Cr addition was up to 100 mg/kg, which indicated that the high concentration of Cr stress significantly reduced the heavy metal transport capacity of the three varieties of *Dianthus caryophyllus*. Additionally, the transit coefficients of the three genotypes of *Dianthus caryophyllus* for soil heavy metal Cr were Master > Freedom > Dallas, indicating that Master has the most vital capacity for transport.

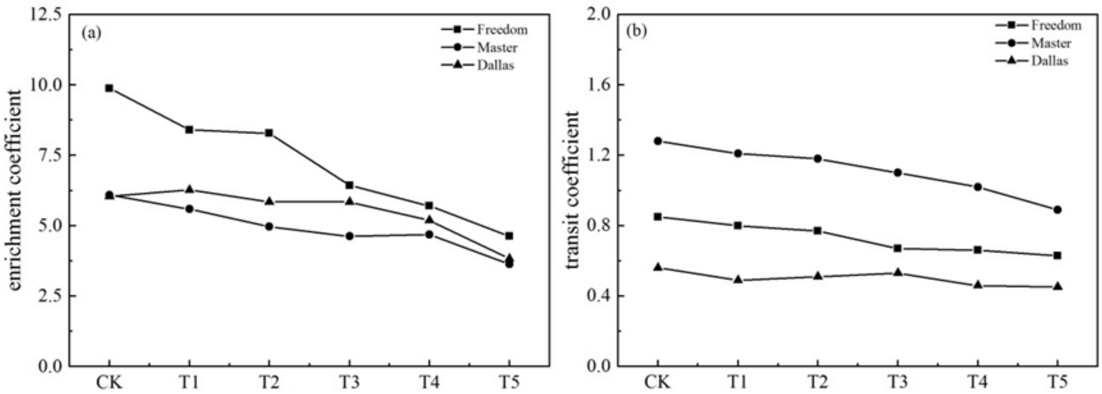

Figure 4. *Analysis of the enrichment and translocation capacity of heavy metal Cr*

Chromium (Cr) is a non-essential element for plant growth, and plant growth is efficiently inhibited under high concentrations of chromium stress, resulting in dwarfing, slow development and reduced total biomass. Plant height, stem thickness and biomass are important indicators for evaluating plant tolerance to metal stress. The plant height, stem thickness and total biomass of *Dianthus caryophyllus*, were all suppressed under Cr stress. Compared with the control group, the plant height, stem thickness and total biomass of “Freedom” were reduced by 8.21 cm, 1.16 mm and 1.96 g, that of “Master” by 7.01 cm, 0.69 mm and 1.67 g and that of “Dallas” by 1.67 g and 7.01 cm, 0.69 mm, respectively, 1.67 g, and “Dallas” decreased its plant height, stem thickness and total biomass by 6.58 cm, 1.11 mm and 2.146 g, respectively, which may be attributed to a series of physiological changes in plants under chromium stress, resulted in the accumulation of free radicals and peroxidation of membrane lipids, leading to the structural and functional damages to the membrane system and the increase of permeability, thus inhibiting the cellular permeability. The structure and function of the membrane system are damaged and destroyed, and the permeability is increased, thus inhibiting cell growth and development, ultimately leading to the slowing down of plant development (Shreya et al., 2020). Secondly, plant root development can reflect the plant’s ability to extract nutrients from the soil, and it has been shown that low chromium concentrations significantly promote plant root development. In contrast, medium and high chromium concentrations inhibit root growth (Ahmad et al., 2020). In this experiment, the root diameter and root volume of Freedom, Master and Dallas, were increased under Cr stress, and this result is in

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agreement with the findings of Hayat et al. (2012) on the changes in the morphology of the spider root system under chromium stress. That is, under high levels of chromium stress, plants adapt to chromium stress and optimize the ability to acquire resources necessary for growth by optimizing the distribution and structure of their root systems. In addition, the enrichment coefficient (above-ground) is used to evaluate the ability of plants to absorb heavy metals from the soil, and the translocation coefficient characterizes the ability of plants to translocate heavy metals in the body.

In this experiment, the enrichment coefficients of the three varieties of *Dianthus caryophyllus*, including Master and Dallas, showed a decreasing trend with the increase of exogenous Cr addition. The enrichment coefficients of Freedom were significantly higher than those of Dallas and Master, which indicated that the accessible varieties of *Dianthus caryophyllus* had a good capacity of heavy metal enrichment and that the above-ground Cr concentrations of the Freedom and Dallas plants were higher than those of the belowground parts of the soil under the high concentrations of Cr stress. In contrast, the above-ground Cr concentration was lower than that of the underground fraction, indicating that Master has a more vital heavy metal translocation capacity than Freedom and Dallas plants.

Conclusion

We used the soil in the area to investigate the growth changes of three genotypes of *Dianthus caryophyllus*, under Cr stress, as well as their resistance and uptake characteristics of heavy metal pollutants in tailing contaminated soil using potting experiments.

(1) Heavy metal Cr had a dose effect on the growth at low doses, it would induce a protective response in favor of plant growth. In contrast, at high accumulations, it would inhibit plant growth. Secondly, Cr stress will promote the growth of plant roots.

(2) In the range of Cr concentration 0~100 mg/kg, the enrichment coefficients and transit coefficients of Freedom, Master and Dallas, decreased with the increase of Cr concentration, in which the enrichment coefficients of the three varieties for the soil Cr were in the magnitude of Freedom > Dallas > Master and its transit coefficients for soil Cr was in the magnitude of Master > Freedom > Dallas, which showed that Freedom has better enrichment ability for Cr. Master has better transport ability for Cr.

(3) Under the stress of Cr, all three genotypes of *Dianthus caryophyllus* have certain tolerance, accumulation ability and transport ability, and these plants have the characteristics of fast propagation, easy cultivation, strong adaptability, long flowering period and high ornamental value, which can be used as reference plants for the in situ remediation of heavy metals in tailings contaminated soils of the mining area.

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