

## RESEARCH ARTICLE

## The influence mechanism of exogenous trehalose on the growth and development of maize under cadmium stress

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**Maize is an important agricultural product in China. Studying the effects of cadmium stress on maize growth and development is critical for its practical application. This study focused on the maize variety Guidan162 and conducted multiple experiments to investigate the effects of trehalose on the physicochemical and biochemical properties of maize seedlings under cadmium stress. The experimental outcomes showed that trehalose not only increased the root and stem length of maize seedlings under cadmium stress by 2.4 - 3.8 cm, but also increased the dry weight of seedlings by 40.8%. In addition, trehalose reduced the cadmium content in the plant and root parts of maize seedlings under cadmium stress by 37.0% and 32.4%, respectively. Consequently, exogenous trehalose had the potential to mitigate the growth mechanism stress caused by cadmium in maize.**

**Keywords:** exogenous trehalose; cadmium stress; maize growth; physicochemical properties; biochemical properties.

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### Introduction

In today's global environment, heavy metal pollution, especially cadmium (Cd) pollution, has become a serious problem. The accumulation of Cd in soil not only has a negative impact on plant growth, but also poses a threat to the physical health of humans and other animals through the food chain [1, 2]. There are two main ways for the formation of Cd in farmland. One is through natural phenomena such as volcanic activity, which results in excessive Cd in watershed soil. The other method involves human activity, which causes increased Cd levels in regional soil. For example, farmland near mining enterprises is prone to quantified Cd pollution caused by sewage irrigation. Ng *et al.* conducted a study on the growth of *Pinellia ternata* plants under Cd pollution and showed that the growth of *Pinellia*

*ternata* plants was severely hindered. When adding ground granulated blast furnace slag (GGBS) to alleviate Cd pollution, the leaf area index of the soil increased by about 30% [3]. Chen *et al.* studied the biological properties of sandy clay loam soil under Cd pollution. The results showed that plant growth was severely threatened at high Cd levels, disrupting the stability of soil microbial communities [4]. Han *et al.* investigated the pollution status of Cd on maize growing soil by randomly selected soil samples from areas surrounding the factory, and the results showed that the Cd pollution index in the maize-growing soil was 3.12, indicating severe tertiary pollution that seriously impacted the growth of maize [5]. Cd remains localized in soil but has high mobility in organisms. Due to Cd inhibiting cell growth, division, and photosynthesis, when Cd accumulates in crop

parts, plants may experience growth defects due to the lack of division process, resulting in reduced crop yields. Therefore, finding methods or substances that can alleviate Cd stress in plants has significant practical significance. Currently, many studies focus on enhancing soil cultivation and developing novel cultivars to alleviate the growth constraints of plants facing Cd stress [6, 7]. However, these studies have a long research cycle and high investment cost with high practical application difficulty [8, 9]. Trehalose is a natural disaccharide, mainly extracted from insect cocoons and composed of two glucose molecules and a non-reducing disaccharide, which plays an important protective role in organisms under various environmental pressures due to its unique structural characteristics of protecting and stabilizing cell membranes. In recent years, numerous studies have indicated that exogenous trehalose can regulate plant osmotic effect by maintaining balance between biofilms and proteins, ultimately enhancing the plants' resistance to external stress [10, 11]. As an important food product in China, many researchers have explored the growth mechanism of corn, among which heavy metal pollution is an important factor affecting the development of corn. However, for Cd pollution in heavy metal pollution, many scholars currently focus their research on its impact on seed pollution, neglecting the mechanism of its impact on the growth and development of maize seedlings. To explore the impact of trehalose on the growth mechanism of maize seedlings, this study innovatively explored the physicochemical and biochemical mechanisms of seedlings including investigating the morphology, rhizome height, and dry weight for physicochemical properties and examining reactive oxygen species (ROS) content, activity of antioxidant enzymes, and Cd content for the investigation of biochemical properties of maize seedlings. By exploring the growth status of corn seedlings in exogenous trehalose, this study aimed to further investigate the mechanism of the influence of exogenous trehalose on the growth and

development of maize under Cd stress and reveal the pathways of trehalose in alleviating Cd stress.

## Materials and Methods

### Culture and treatment of maize seedlings under Cd pollution by exogenous trehalose

The maize variety, Guidan162, from Guangxi Zhaohe Seeds Co. Ltd., Nanning, Guangxi, China was used in this study. 160 plump seeds were selected and soaked in a 0.5% sodium hypochlorite solution for 15 minutes before rinsed with distilled water. The seeds were then planted in a 32 well seedling tray with moistened base laid by a layer of damp newspaper. The seeds were arranged on the base newspaper at a spacing of 10 cm, and then were covered with vermiculite at the same height as the seedling tray. After the water was fully absorbed by vermiculite, the seedling tray was cultivated at 27 - 33°C, 65 - 75% humidity, and 620  $\mu\text{mol}/\text{m}^2 \cdot \text{s}$  light intensity for 48 hours. Most of the seeds were successfully germinated. The seedling nutrient solution including nitrogen, phosphorus, potassium, and necessary inorganic salts such as calcium nitrate for optimal plant growth and 20% oxygen at a flow rate of 160 mL/min were applied to the culture for 15 minutes and were replaced every three days. After the corn seedlings grow two leaves and one heart, the successfully germinated corn seedlings were divided into four groups with one experimental group in which 180 mg/L Cd (Sigma Aldrich (Shanghai) Trading Co., Ltd., Shanghai, China) and 22 mmol/L trehalose (Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China) were added to the seedling nutrient solution, and three control groups in which no additive, 22 mmol/L trehalose, and 180 mg/L of Cd were added, respectively. After 6 hours of cultivation, maize seedlings were removed for the nutrient solution [12]. After 6 hours treatments, photos of seedling growth morphology were taken and the differences in morphological characteristics between different groups of maize seedlings were recorded. Uproot seedlings from different planting groups and the roots of the seedlings below the soil were cut off

and measured separately. The length of the plant's main body was determined by the distance between the top and bottom of its leaves, while the length of the seedling's root was determined by the length of the underground roots. The rhizome parts from the four groups were placed in beakers and incubated in a constant temperature oven at 110°C for 30 minutes. The temperature of the oven was then adjusted to 75°C and the seedlings were dried to constant weight measured by using an electronic balance.

### Biochemical properties of maize seedlings under Cd pollution by exogenous trehalose

After seedling treatments, fresh and dried samples were collected. The third unfolded leaf was selected as the fresh maize seedling leaf sample, and the fresh root sample was the entire root system of the seedling. The Cd treated roots were soaked in 8 mmol/L EDTA-2Na solution for 15 minutes before rinsing with distilled water and absorbed surface moisture. The processed fresh samples were frozen by using liquid nitrogen and placed in the freezer for later use. All parts above ground and the entire root system of the maize were utilized to make the dry leaf samples and then stored in a dry place [13]. Three 0.2 g leaf samples were weighed and ground separately in a mortar with the addition of 1.5 g of calcium carbonate powder and 3 mL of 95% ethanol solution to make the samples into a uniform slurry. 7 mL of 95% ethanol were then added until the sample solution turned white. After standing for 5 minutes, the samples were filtered into a 25 mL volumetric flask by using filter paper and diluted to 25 mL by 95% ethanol solution [14]. The contents of chlorophyll a ( $C_a$ ) and chlorophyll b ( $C_b$ ) were detected at wavelengths of 665 nm and 649 nm, respectively by using a UV spectrophotometer and were calculated as:

$$\begin{cases} c_a = 13.95A_{665} - 6.88A_{649} \\ c_b = 24.96A_{649} - 7.32A_{665} \end{cases} \quad (1)$$

where 13.95 and 6.88 were the absorption coefficients of  $C_a$  at wavelengths 665 nm and 649

nm, respectively. 24.96 and 7.32 were the absorption coefficients of  $C_b$  at wavelengths 649 nm and 665 nm, respectively. The calculation of chlorophyll content (mg/g) was as follows.

$$c = \frac{(c_a + c_b) \times 25 \times k}{0.2} \quad (2)$$

where 25 was the volume of chlorophyll extraction solution.  $k$  was the dilution ratio of the extraction solution. 0.2 was the weight of the blade sample. The light efficiency was measured by using a plant efficiency meter to measure the photosynthetic parameters of plant leaves which underwent dark adaptation for 24 hours before the measurement. The relationship between the three photosynthetic parameters was shown in equation (3).

$$Fv = Fm - Fo \quad (3)$$

where  $Fv$  was the maximum value of variable fluorescence under dark adaptation.  $Fm$  was the maximum fluorescence of leaves after dark adaptation.  $Fo$  was the initial fluorescence of the leaves.  $Fv/Fm$  was the max photosynthetic efficiency, representing the maximum light energy conversion efficiency of the leaves.

### Determination of reactive oxygen species (ROS) content in maize seedlings

ROS in maize seedlings represent the biological activity of the plant, with higher levels of ROS indicating better growth and development of maize seedlings [15-17]. The ROS mainly includes the content of superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). Briefly,  $O_2^-$  was extracted by mixing 1.0 g of dark-treated maize seedlings with 3 mL of extraction buffer solution (Beijing Huakesheng Fine Chemical Products Trading Co., Ltd., Beijing, China) and grinding into a slurry in an ice bath. The homogenate was centrifuged at 8,000 rpm at 4°C for 15 minutes and the supernatant contained  $O_2^-$  was collected. 2.0 mL of the supernatant were mixed with 0.5 mL of 50 mmol/L potassium phosphate buffer and 0.1 mL of 10 mmol/L hydroxylamine

hydrochloride solution. After thoroughly mixing at room temperature, 1.5 mL of 56 mmol/L para aminobenzenesulfonic acid solution and 1.5 mL of 6 mmol/L *a*-naphthylamine solution were added and incubated at 30°C for 30 minutes. After equal volume chloroform extraction at 13,000 rpm for 5 minutes, the absorbance of the supernatant at 530 nm was measured by using a UV spectrophotometer and the amount of  $O_2^-$  was calculated.

$$O_2^- = \frac{2 \times V_t \times n}{F_w \times V_s} \quad (4)$$

where  $V_t$  was the total volume of the reaction reagent (mL).  $n$  was the  $NO_2^-$  content (the weight of the sample (g)) after hydroxylamine oxidation ( $\mu\text{mol}$ ).  $V_s$  was the volume of the extraction solution (mL). The  $H_2O_2$  content was extracted by mixing 3.0 g of leaves with 4°C acetone solution and grinding into a slurry with the final volume of 5 mL. The slurry was centrifuged at 6,000 rpm for 10 minutes. Titanium sulfate and concentrated ammonia solution were added into 1 mL of the supernatant, and then centrifuged again. 5 mL of 2 mol/L sulfuric acid solution was added to the centrifugal precipitation for colorimetric analysis by using a visible light spectrophotometer (Qingdao Lubo Jianye Environmental Protection Technology Co., Ltd., Qingdao, Shandong, China) at the wavelength of 415 nm. The concentration of  $H_2O_2$  was calculated as follows.

$$H_2O_2 = \frac{C \times V_q}{F_w \times V_c} \quad (5)$$

where  $C$  was the  $H_2O_2$  concentration obtained on the standard curve ( $\mu\text{mol}$ ).  $V_q$  was the total volume of the sample extraction solution (mL).  $V_c$  was the volume of sample extraction solution during colorimetric determination (mL).  $F_w$  was the weight of the sample (g).

#### Determination of antioxidant enzyme activity in maize seedlings

Antioxidant enzymes enhance plant antioxidant capacity and promote rapid plant growth, which consist of superoxide dismutase (SOD), catalase

(CAT), and peroxidase (POD) [18-20]. The SOD activity was determined by using the nitrogen blue tetrazole photoreduction method. Briefly, 0.5 g of corn seedlings in a pre-cooled mortar was mixed with 2 mL of extraction solution and ground to the pulp. The slurry was set to a constant volume of 10 mL with 5 mL of slurry being centrifuged at 4°C for 5 minutes at 13,000 rpm. The supernatant contained crude extract of SOD was mixed with 2 mL of biuret chromogenic reagent (Sigma Aldrich (Shanghai) Trading Co., Ltd., Shanghai, China) and then measured at 560 nm on the UV spectrophotometer. The SOD content was calculated by using equation (6).

$$SOD = \frac{(A_0 - A_s) \times V_t \times 60}{A_0 \times 0.5 \times F_w \times V_s \times t} \quad (6)$$

where  $A_0$  was the blank absorbance (distill water).  $A_s$  was the absorbance of the sample.  $V_t$  was the measured volume of the sample extraction solution (mL).  $V_s$  was the volume of the crude extract of the sample (mL). The CAT enzyme was measured by using ultraviolet absorption method. 0.5 g of fresh maize seedlings was mixed with 3 mL of 4°C phosphate buffer in a mortar and ground into a slurry. After making the total volume of 25 mL, the mixer was placed at 5°C for 10 minutes followed by centrifugation at 4,000 rpm for 15 minutes. 0.2 mL of supernatant that contained crude extract of peroxidase was then mixed with 0.3 mL of 0.1 mol/L  $H_2O_2$  solution before the absorbance at 240 nm was measured every 1 min for a total of 4 times. The CAT enzyme was calculated as below [21-23].

$$CAT = \frac{A_{240} \times V_T}{0.1 \times V_1 \times t \times F_w} \quad (7)$$

where  $A_{240}$  was the absorbance at 240 nm.  $V_T$  was the volume of the crude extract (mL).  $V_1$  was the measured sample volume (mL).  $t$  was the measurement time (minute).  $F_w$  was the weight of the sample (g). 0.1 was the enzyme activity unit of  $A_{240}$  decrease (u). The POD was determined by using the guaiacol method. 0.5 g

of fresh maize seedlings was mixed with calcium carbonate solution. The slurry was diluted to 50 mL before centrifugation at 4,000 rpm for 15 mins. 1 mL of resulted supernatant was then mixed with 1 mL of 0.1% guaiacol and 6.9 mL of distilled water and incubated at 25°C for 10 minutes. The POD was measured at 470 nm in a spectrophotometer with tetra methoxyphenol as the control. The POD was calculated as follows.

$$POD = \frac{(X - X_0) \times V_y}{F_w \times V_x \times t} \quad (8)$$

where  $X$  was the sample content (mg).  $X_0$  was the content of tetra methoxyphenol ( $\mu\text{g}$ ).  $V_y$  and  $V_x$  were the total volumes of the extraction solution and the enzyme solution measured, respectively (mL).  $t$  was the enzyme reaction time (minute).

#### Determination of Cd content in maize seedlings

The maize seedlings were crushed and passed through a 0.85 mm sieve. 0.5 g of sample powder was mixed with 3 mL of concentrated nitric acid, 2 mL of  $\text{H}_2\text{O}_2$ , and 3 mL of water. After digestion for 2 hours, the solution was mixed well and brought to a constant volume of 25 mL. 20  $\mu\text{L}$  of sample solution was mixed with 5  $\mu\text{L}$  of 99% purity Cd matrix modifier (Hubei Dongcao Chemical Technology Co., Ltd., Wuhan, Hubei, China) and injected into a pre-heated (100°C) graphite furnace (Guanhe Instrument Equipment (Shanghai) Co., Ltd., Shanghai, China) to measure the absorbance. The Cd content in the sample was decided according to the standard curve and calculated with equation (9) [24, 25].

$$Cd = \frac{(c_1 - c_0) \times V}{m \times 1000} \quad (9)$$

where  $c_1$  was the Cd content in the sample ( $\mu\text{g/L}$ ).  $c_0$  was the Cd content in the blank solution ( $\mu\text{g/L}$ ).  $V$  was the quantitative volume of the sample (mL).  $m$  was the sample mass (g).

#### Data processing

SPSS (version 20.0) (IBM, Armonk, NY, USA) was employed for data processing and linear model

construction. Furthermore, the data was analyzed by using t-test through Microsoft Excel (2010) (Microsoft, Redmond, WA, USA).

## Results

#### Growth characteristics of maize seedlings

The growth morphology of maize seedlings under normal, trehalose, trehalose plus Cd, and Cd treatments were observed. The maize seedlings under the normal condition exhibited excellent plant growth with superior plant height and leaf color in comparison to the other treated plants. Seedlings treated with Cd only had dry leaf tips, withered leaves, and thinner rhizomes, while seedlings treated with trehalose and Cd had relatively less leaf withering and less leaf tip withering, which confirmed that the application of trehalose had a mitigating effect on maize seedlings under Cd stress, ultimately contributing to the improvement of the seedlings' development.

#### Root and stem measurement of maize seedlings

The maize seedlings underground heights of different treatment groups were shown in Figure 1. During the 6-hour treatment, the seedlings in normal culture group showed a slow growth trend with a height from 24.3 cm to 26.1 cm. Conversely, the other three groups observed a decline trend in the seedlings' height to varying degrees with the Cd alone group demonstrating the significant decline trend from a height of 24.3 cm to 10.8 cm. When trehalose and Cd were added simultaneously, the decreasing trend of seedling height was alleviated with the decrease from 24.3 cm to 16.0 cm. Therefore, trehalose could increase the height of maize seedlings under Cd stress. The initial root length of the four groups of maize seedlings was 13.2 cm. Following various treatments, the length of the root system exhibited diverse changes with the normal treatment group demonstrating a slow upward trend reaching 14.6 cm at 6 h, while the other three groups showed a decreasing trend. The trehalose only group demonstrated a slight decrease reaching 12.2 cm after 6 hours, while

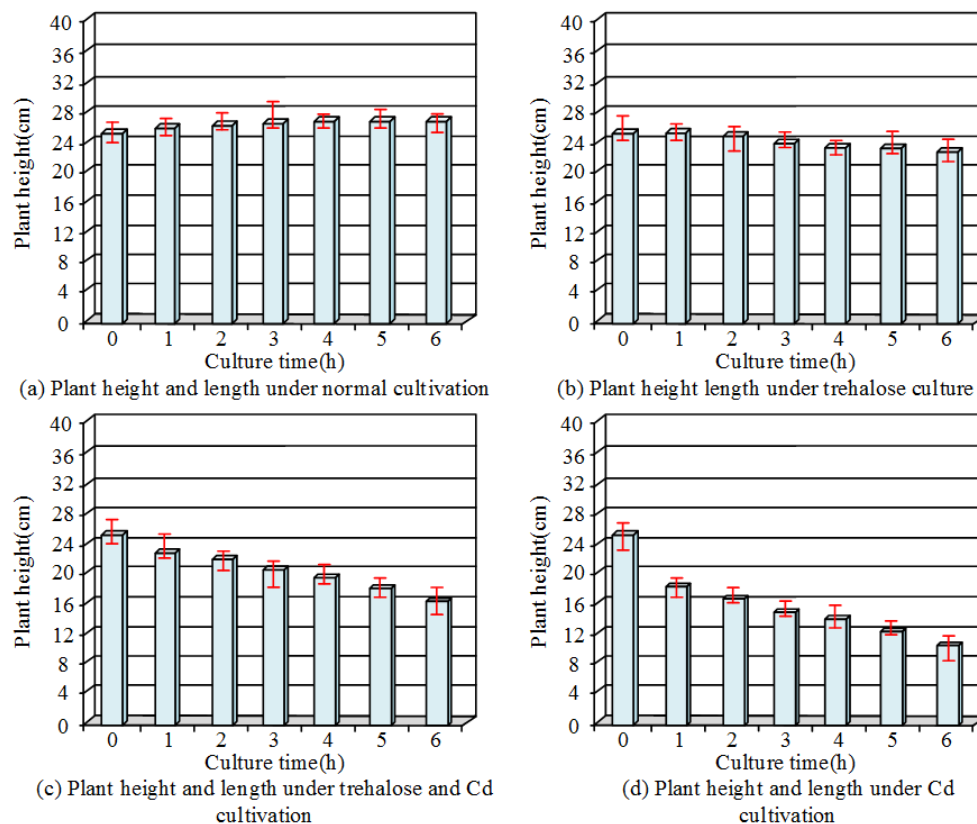


Figure 1. Plant height of maize seedlings under different treatment methods.

the Cd alone group showed the most significant shrinkage reaching 4.1 cm at 6 h, and the group of trehalose and Cd reached 8.3 cm, which slowed down the decreasing compared to the group treated with Cd only (Figure 2). The results indicated that trehalose might mitigate the damage caused by Cd to the roots of maize seedlings. Because trehalose could hinder the action of Cd by maintaining the stability of the root cell wall, the degree of Cd stress on the root system was reduced, and the speed of root growth was improved.

#### Determination of dry weight of maize seedlings

The dry weights of maize seedlings under different treatment methods were shown in Figure 3. Compared with the seedling dry weight of normal treatment, there was no significant difference in trehalose treatment groups, while the Cd only group demonstrated a significant reduction in seedling dry weight with a decrease

of 0.062 g in plant dry weight and 0.018 g in root dry weight. The seedlings treated with trehalose and Cd had a plant dry weight of 0.062 g and a root dry weight of 0.014 g, which was a 41.9% and 35.7% increases of plant and root dry weights, respectively and a 40.8% increase of total weight compared to that of Cd alone group. Therefore, trehalose had a promoting effect on the development of maize seedlings under Cd stress.

#### Measurement of photosynthetic parameters of maize seedlings

The changes in chlorophyll contents between normal and trehalose treatment groups exhibited a relatively small discrepancy. The chlorophyll content in Cd alone group was significantly reduced by 51.4% compared to the normal treated seedlings, while the decreasing trend of Cd and trehalose treatment group had been alleviated by 28.8% increase compared to

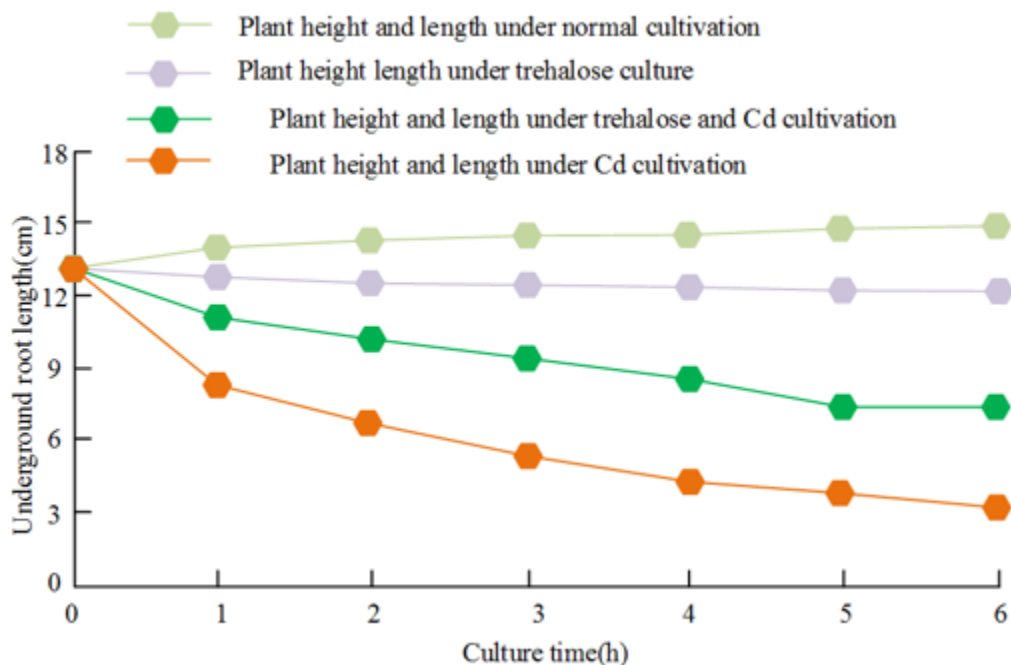


Figure 2. Root length of maize seedlings under different treatment methods.

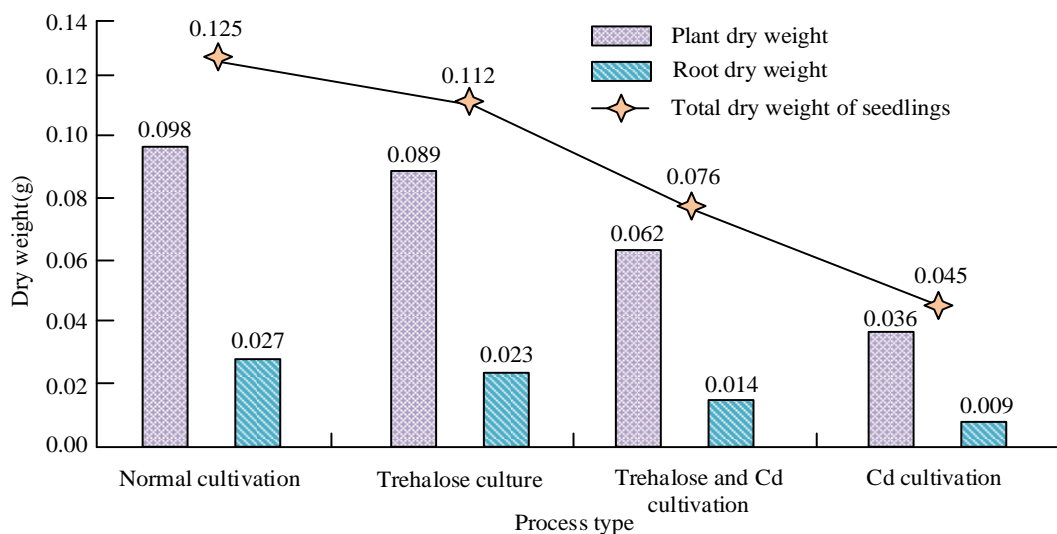


Figure 3. Dry weight of maize seedlings under different treatment methods.

Cd only group. However, the chlorophyll content was still lower than that of the normal treatment (Figure 4). The Fv/Fm values of maize seedlings under different treatment methods demonstrated varying degrees of improvement trend over the time. At the 6th hour of treatment, the Fv/Fm value of corn seedling

leaves under normal treatment reached 0.64, which was the same as the trehalose treatment. However, the value under Cd treatment was only 0.46 with significant difference to the normal group. The value of trehalose and Cd group reached 0.58, although still lower than normal leaves, it was higher than that of Cd only group

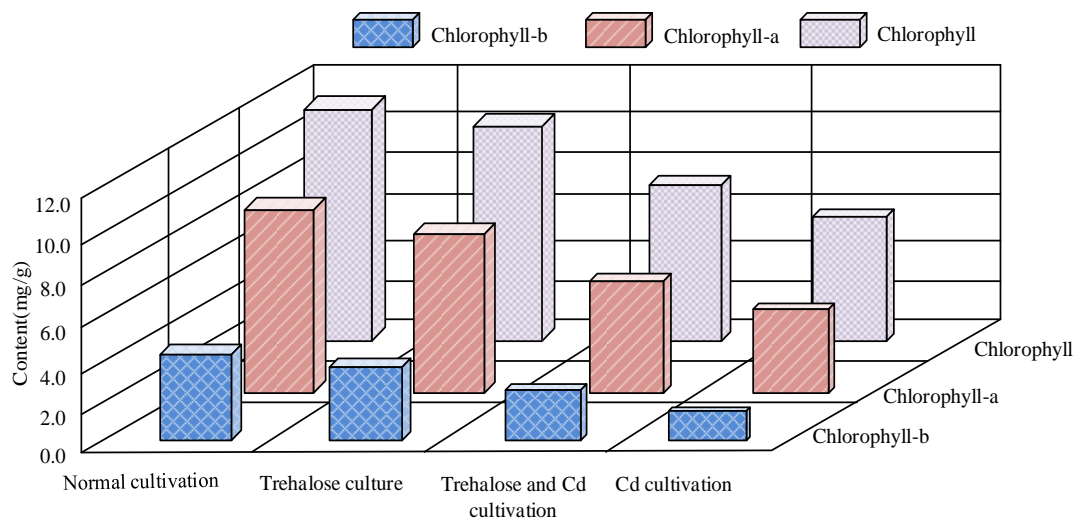


Figure 4. Changes in chlorophyll content under different treatment conditions.

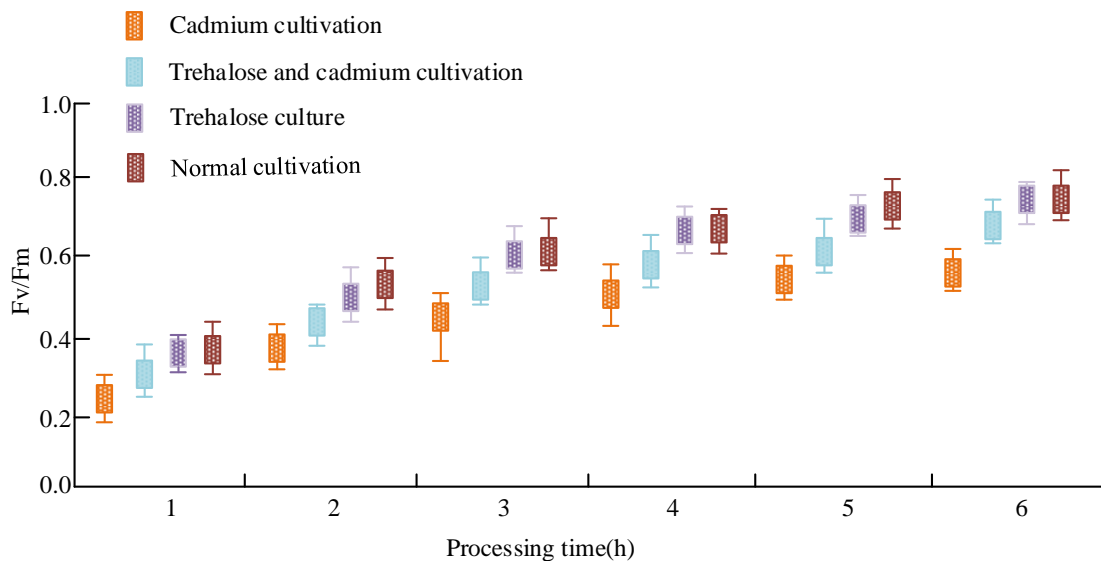


Figure 5. Fv/Fm values of maize seedlings under different treatments.

(Figure 5). The results confirmed that trehalose could increase the Fv/Fm value of leaves under Cd stress.

**Determination of ROS content in maize seedlings**

The O<sub>2</sub><sup>-</sup> contents of plant and root under different treatment methods were shown in Figure 6. The results showed that the higher the content of O<sub>2</sub><sup>-</sup>, the higher the degree of stress on the seedlings. The seedlings treated with Cd only had the

highest O<sub>2</sub><sup>-</sup> content in both plant and root parts, reaching 18.3 nmol/g and 0.36 nmol/g, respectively, indicating that the seedlings treated with Cd only had a higher degree of injury. The O<sub>2</sub><sup>-</sup> contents in seedlings of normal and trehalose treatment groups were 7.9 nmol/g and 0.14 nmol/g in the plant and root sections, respectively. The H<sub>2</sub>O<sub>2</sub> content of plant and root under different treatment methods were shown in Figure 7. The value of H<sub>2</sub>O<sub>2</sub> represents the degree of stress in the plant, and the higher the



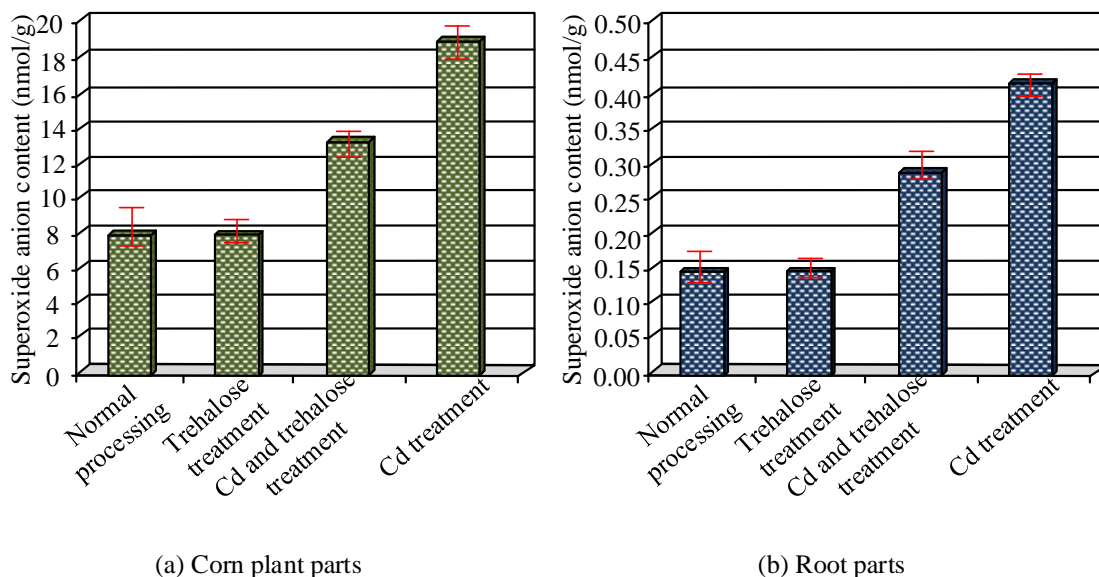


Figure 6. O<sub>2</sub><sup>-</sup> content under different treatments.

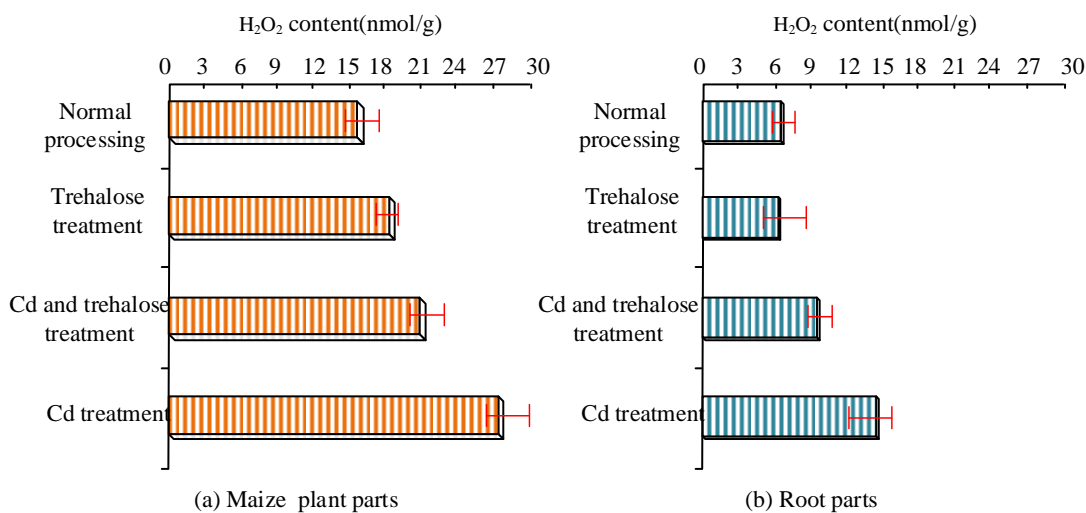


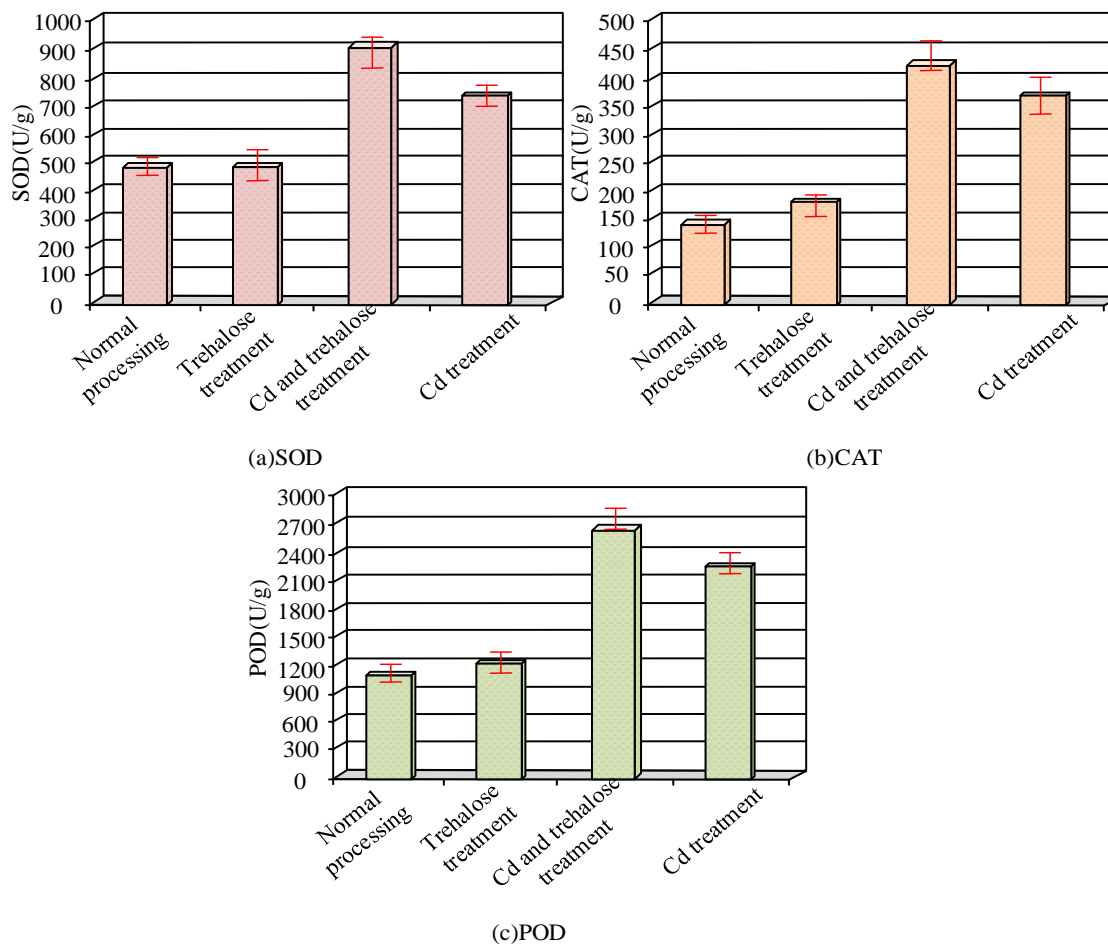
Figure 7. H<sub>2</sub>O<sub>2</sub> content of seedlings under different treatments.

value, the greater the degree of stress. The H<sub>2</sub>O<sub>2</sub> contents in the seedling plant and root parts of Cd treatment group were the highest one and reached 28.7 nmol/g and 16.1 nmol/g, respectively, while the H<sub>2</sub>O<sub>2</sub> content in the trehalose and Cd group reached 22.2 nmol/g and 10.3 nmol/g, respectively, which was a significant decrease in H<sub>2</sub>O<sub>2</sub> content. However, there was still a significant difference in H<sub>2</sub>O<sub>2</sub> content compared to that of the normal seedling plants

and roots which measured 15.93 nmol/g and 6.83 nmol/g, respectively.

**Determination of antioxidant enzyme activity in maize seedlings**

The antioxidant enzyme activity of seedlings under different treatment methods was determined by SOD, CAT, and POD contents. The seedlings treated with trehalose and Cd demonstrated the highest SOD, CAT, and POD



**Figure 8.** Changes in antioxidant enzyme activities of seedlings under different treatments.

contents, reaching 900 U/g, 423 U/g, and 2,516 U/g, respectively, while the SOD, CAT, and POD contents of normal group were 489 U/g, 141 U/g, and 1057 U/g, respectively (Figure 8). The combination of Cd and trehalose treatment could improve the antioxidant enzyme activity of maize seedlings, as trehalose could enhance the stress resistance of maize seedlings, thereby enhancing enzyme activity.

#### Determination of Cd content in maize seedlings

Figure 9 showed the Cd contents in maize seedling plants and roots subjected to varying treatment methods. The Cd contents of seedlings under normal and trehalose treatments were the same with the Cd contents in the plant and root parts of the seedlings reached 39.8 mg/g and 286.9 mg/g, respectively at the 6th hour of

treatment. The Cd content in the plant and root parts of Cd only group was as high as 235.6 mg/g and 1,259.3 mg/g, respectively. The difference of Cd contents in seedlings between the different treatment methods was significant. The combination of Cd and trehalose treatment resulted in 148.5 mg/g and 850.9 mg/g of seedling plants and root parts, respectively. Interestingly, in contrast to the seedlings treated with Cd alone, there was a significant decrease in Cd content in the seedlings treated with both Cd and trehalose. The Cd transport coefficients of maize seedlings under different treatment methods were listed in Table 1. The Cd transport coefficient of Cd alone group was the highest one reaching 0.19 at the 6th hour compared to 0.14, 0.14, and 0.17 in normal, trehalose, and trehalose and Cd combination groups,

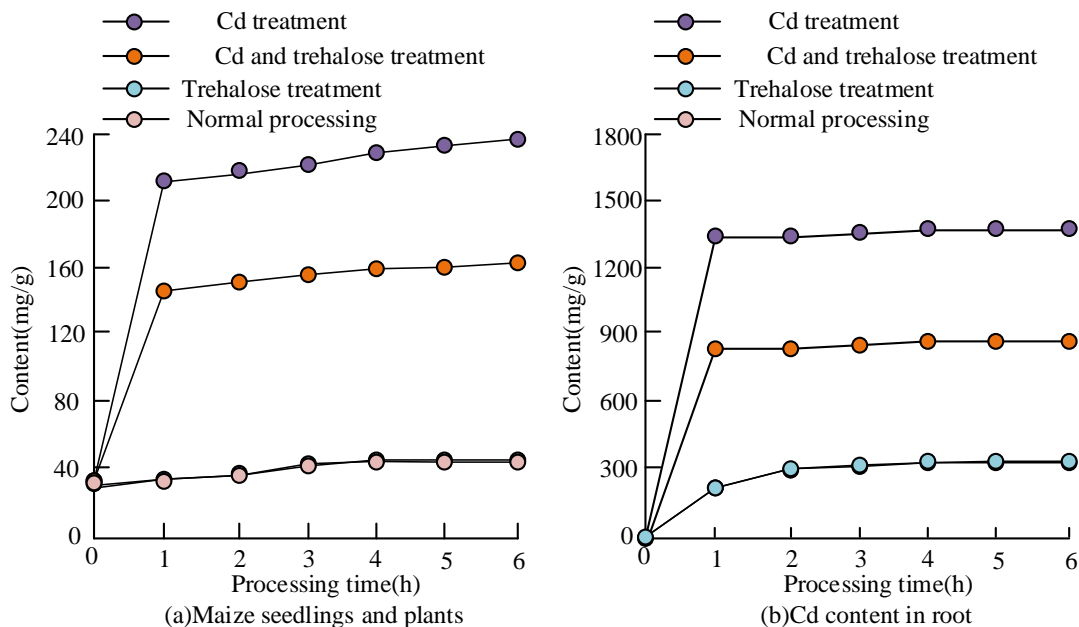


Figure 9. Cd contents of maize seedlings under different treatments.

Table 1. Cd transport coefficients of maize seedlings under different treatments.

Treatment	1 h	2 h	3 h	4 h	5 h	6 h
Cd alone	0.19	0.18	0.19	0.17	0.19	0.19
Cd and trehalose	0.16	0.17	0.16	0.17	0.17	0.17
Trehalose only	0.13	0.13	0.14	0.13	0.14	0.14
Normal processing	0.13	0.14	0.13	0.13	0.13	0.14

respectively. The increase of transport coefficient correlated with increased Cd content in the seedlings (Figure 9).

### Discussion

The plant appearance and physicochemical properties will be changed under the Cd stress. The results of this study indicated that the normal treatment and trehalose treatment of maize seedlings had excellent growth morphology and good growth momentum compared to Cd treatment plants that showed dry leaf tips, withered leaves, and thinner rhizomes with a plant height of only 10.8 cm and a root length of only 4.1 cm. However, the height of the maize seedlings cultivated by combining trehalose and Cd reached 16.0 cm with root length reached 8.3

cm, which suggested that the morphology and height of the plants had been improved when trehalose was added to Cd treatment group. Garcia *et al.* conducted a study on red trees and explored the effect of Cd on the root and stem morphology of plants. The study suggested that the xylem cells of plant vegetative organs were induced by Cd to precipitate, leading to hardening of the plant endoderm and hypoderm, and causing plant morphology to wither and shrink [18]. Additionally, Cd stress reduced the weight of maize seedlings to 0.045 g, which was much less than that of the normal seedling dry weight of 0.125 g. The dry weight of maize seedlings treated with trehalose and Cd was 0.076 g, indicating an increase in seedling dry weight, which also confirmed that trehalose could improve the growth morphology of maize seedlings.

In addition, the biochemical properties of maize seedlings were explored in this study. Compared to normal cultured seedlings, the chlorophyll contents of seedlings treated with Cd alone were decreased by 51.4%, and the Fv/Fm value of seedlings decreased from 0.64 to 0.46. Elisa *et al.* studied the mechanism of Cd's impact on young leaves of soybean plants and found that Cd bound to oxygen atoms in plant leaves, inhibiting nutrient absorption of plants. The toxicity of Cd to leaves was manifested in inhibiting the saturation of photosynthetic fluorescence parameters and net photosynthetic oxygen release rate [19], which led to the reduction of chlorophyll content and Fv/Fm value in plants under Cd stress. When trehalose and Cd acted together on maize seedlings, the chlorophyll contents of the seedlings were increased by 28.8%, and the Fv/Fm value was 0.58. Trehalose could reduce the degree of damage to the photosynthetic center of seedlings caused by Cd, uphold stable conversion efficiency of light energy, and therefore, enhance the photosynthetic capabilities of maize seedlings under Cd stress. The O<sub>2</sub><sup>-</sup> contents in the plant and root parts of trehalose and Cd treated maize seedlings reached 18.3 nmol/g and 0.36 nmol/g, respectively, while the H<sub>2</sub>O<sub>2</sub> contents reached 28.7 nmol/g and 16.1 nmol/g, respectively. Both results were higher than that in the normal treatment maize seedlings. The seedlings treated with trehalose and Cd showed the highest SOD, CAT, and POD activities as 900 U/g, 423 U/g, and 2,516 U/g, respectively, which might be caused by damaged antioxidant function and accumulated O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> contents in the plant that was affected by Cd [20]. In this study, trehalose induced antioxidant factors in plants, thereby reducing the oxidative performance. The Cd contents in the maize seedlings and roots treated with Cd alone reached 235.6 mg/g and 1,259.3 mg/g, respectively at 6 h, while the Cd contents reduced to 148.5 mg/g and 850.9 mg/g, respectively in the Cd group with the addition of trehalose. Trehalose reduced Cd content in seedlings through hindering the transport of Cd in plants by increasing the polysaccharide content in plant cell walls, thus increasing Cd

accumulation. In summary, trehalose could alleviate the tolerance of maize to Cd stress in agricultural production, and, as a carbohydrate molecule, could maintain the stability of plant cells and protect the effects of macromolecules such as proteins on plant growth activity, which ultimately reduced the accumulation of Cd in maize seedlings.

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