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Physiological Responses of Different Chinese Cabbage Varieties to Cd and Pb Stresses

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Abstract

Soil cadmium (Cd) and lead (Pb) pollution is becoming increasingly serious; heavy metals are accumulating in soil and agricultural products, and vegetables, as an indispensable part of people's daily diet, affect human health through the food chain. This study investigated the effects of different concentrations of Cd and Pb stress on growth root length, above-ground/below-ground biomass, chlorophyll, osmoregulatory substances (soluble proteins, soluble sugars, proline) content, and antioxidant enzymes (malondialdehyde (MDA), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT)) activities of four Cd and Pb high- and low-accumulating varieties of Chinese cabbage in hydroponic mode. The results showed that under Cd and Pb stress, Chinese cabbage could resist heavy metal stress by increasing the contents of osmoregulatory substances and the activities of antioxidant enzymes in the leaves. However, with the increase of heavy metal concentration, the physiological metabolic activities of Chinese cabbage leaf cells were affected, and the activities of POD, CAT, SOD, and osmoregulators decreased, while the osmoregulators of low Cd and Pb accumulating varieties were more stable, and the activities of POD, CAT, and SOD reached the peak value first compared with those of the high accumulating varieties. This study analyzed the physiological functions of different accumulating types of Chinese cabbage under Cd and Pb stress, in order to provide a theoretical basis for cultivating low Cd and Pb accumulating varieties.

Keywords

Heavy Metal Stress, Physiological Response, Osmoregulatory Substances, Antioxidant Enzymes

1. Introduction

With the expansion of cities and the rapid development of modern agriculture, heavy metals are increasingly being discharged into the soil system (Xue et al., 2019), destroying the basic functions of soil, affecting soil health and arable land quality, and leading to ecological and environmental risks (Ahado et al., 2021). Cd is characterized by high chemical activity, poor mobility, high enrichment, and persistent toxicity (Hu & Song, 2015; Guan, 2023) and has been classified as a primary soil pollutant (Li et al., 2016). Pb is a highly toxic pollutant, which is listed by the World Health Organization as one of the 10 most hazardous toxic substances to human health, with non-biodegradability and long half-life, and has received widespread attention (Xiao et al., 2021). Some studies have shown that leafy vegetables have the strongest enrichment ability for heavy metals (Zhou et al., 2016); Tu et al. (2020) and others investigated the heavy metal pollution of different kinds of vegetables in farmland soil around Nandan mining area, and found that Cd is the main polluting element of vegetables and leafy vegetables have the strongest enrichment ability for Cd. Low concentrations of Cd and Pb can promote plant seed germination and plant growth and development, but excessive Cd and Pb in the environment can easily produce toxic effects on plants and animals, causing a series of physiological and biochemical process disorders in plants (McLaughlin et al., 1994). After entering plants, heavy metals will not only accumulate and produce toxic effects in plants, but also enter the human body along with the food chain, causing harm to human health. Since the toxic effects of heavy metals on plants are multifaceted, the detoxification mechanism of heavy metals in plants is also a comprehensive effect of various physiological processes (Jiang & Zhao, 2001). Numerous studies have shown that plants detoxify heavy metals mainly through the antioxidant defense system, chelation and compartmentalization (Deng et al., 2018; Sharma & Dietz, 2009; Kolahi et al., 2020).

At present, most related studies focus on model plants, such as rice and Arabidopsis thaliana, or a few hyperaccumulators, such as Southeastern Sedum and Lobelia, etc., while there are fewer studies on vegetables, especially those with different levels of Cd and Pb accumulation. Therefore, in this study, four Cd- and Pb-high and low-accumulating varieties of cabbage (Cd low-accumulating variety Harmony Express (H), high-accumulating variety Violet F1 (F), Pb low-accumulating variety Green Crown (L), and high-accumulating variety Suzhou Green (S)), screened by the author's team in previous experiments, were used as the materials to study their response to the stress of different Cd and Pb concentrations, and to investigate the effects of different concentrations of Cd and Pb on high- and low-accumulating types of cabbage through a hydroponic mode. The effects of stress on root length, above-ground/below-ground biomass, chlorophyll, soluble protein, soluble sugar, proline content, and antioxidant enzyme activities (MDA, SOD, POD, CAT) of high- and low-accumulating cabbages were investigated by hydroponic culture mode to investigate the physiological functions of the different accumulating cabbages under heavy metal stress with a view to providing a theoretical basis for the cultivation of low-Cd and Pb-accumulating varieties for excavation.

2. Materials and Methods

2.1. Cultivation of Test Material

The indoor hydroponic test was conducted by selecting four Cd and Pb high and low accumulating varieties of cabbage screened out by the author's team in the preliminary test, namely, the Cd low accumulating variety H, the high accumulating variety F, the Pb low accumulating variety L, and the high accumulating variety S. Seeds with the same degree of fullness were selected and sterilized with 75% alcohol for 5 min, rinsed with distilled water, placed on a water cultivation seedling sponge, cultivated with distilled water, and then cultivated with 1/2 Hoagland nutrient solution. After the seedlings grew to about 5 cm, seedlings of the same size were selected, rinsed with distilled water, and then moved into the cultivation box for cultivation. In the pre-culture period, the bucket was filled with distilled water to acclimatize the plants for 24 h, and then changed to 1/2 Hoagland nutrient solution, followed by a change to full Hoagland nutrient solution after another 24 hours, and cultured for 28 days. Five Cd treatments (0, 20, 40, 60, 80 mg/L) and five Pb treatments (0, 300, 600, 900, 1200 mg/L) were set up, and each treatment was repeated three times. After the addition of Cd and Pb solutions, the nutrient solution was changed every 2 days and harvested after 10 days of incubation. Biomass, root length, chlorophyll, proline, soluble protein, soluble sugar, MDA, POD, SOD, and CAT were determined in cabbage after harvest.

2.2. Indicators and Measurement Methods

2.2.1. Determination of Chlorophyll Content

Select the same parts of the leaves from different groups of Chinese cabbage for sampling. Weigh 1.00 g of sample, grind in an ice bath with 80% acetone three times, extract by immersion, and dilute to 50 mL. Measure the absorbance values at 663 nm and 645 nm wavelengths, and use the following formula to determine the chlorophyll a, chlorophyll b, and total chlorophyll.

$$\text{Chlorophyll a} = 12.7 \times OD_{663} - 2.69 \times OD_{645} \quad (1)$$

$$\text{Chlorophyll b} = 22.9 \times OD_{645} - 4.68 \times OD_{663} \quad (2)$$

$$\text{Total chlorophyll concentration} = 8.02 \times OD_{663} + 20.21 \times OD_{645} \quad (3)$$

2.2.2. Determination of Soluble Protein Content

Determination of soluble protein content was carried out by the method of G-250 staining with Caulmers Brilliant Blue. The standard curve was drawn with bovine serum protein (BSA) as the standard protein, and the regression equation of the standard curve was Y = 0.0056X + 0.0129, $R^2 = 0.9931$ (Y is the absorbance, X is the mass concentration of bovine serum protein (mg/kg)).

2.2.3. Determination of Soluble Sugar Content

Referring to the anthrone sulfate colorimetric method of Li (2000). The standard curve was drawn with glucose as the standard, and the standard regression equation was Y = 0.0019X + 0.0025, $R^2 = 0.999$ (Y is the absorbance, X is the glucose concentration (mg/mL)).

2.2.4. Determination of Proline Content

Referring to the determination of proline content by Li (2000), the standard curve was plotted with proline as the standard, and the regression equation of the standard curve was Y = 0.0269X + 0.0007, R^2 = 0.9945 (Y is the absorbance, and X is the proline content (μg/2 ml)).

2.2.5. Determination of Antioxidant System Indices

The contents of MDA, CAT, POD, and SOD in plants were determined using kits, which were provided by Beijing Solebo Technology Co.

2.3. Data Statistics and Analysis

Excel 2019 was used for data statistics; SPSS Statistics 26 was used for data analysis; one-way analysis of variance (ANOVA) and Duncan's test for the level of significant difference ($P < 0.05$) were used; Origin 2021 was used to draw physiological index graphs.

3. Results

3.1. Effects of Cd and Pb Stress on the Growth Indices of Cabbage

As can be seen from Figure 1, different concentrations of Cd and Pb stress affected Chinese cabbage biomass and root length of different varieties to varying degrees. Under Cd stress, above-ground/below-ground biomass and root length of L and F varieties decreased with the increase in Cd concentration. At a Cd concentration of 40 mg/L, aboveground biomass of H and F varieties decreased by 70.05% and 61.45% compared with H0 and F0, respectively; at a Cd concentration of 80 mg/L, root length of H and F varieties decreased by 27.57% and 31.46% compared with H0 and F0, respectively, and belowground biomass decreased by 78.26% and 58.14%, respectively. Above-ground/below-ground biomass and root length under low-concentration conditions differed significantly among varieties for each index, and the difference between the two varieties was not significant at high concentration, indicating that high- and low-accumulating cabbages were differently sensitive to Cd stress at low Cd concentrations, and the low-accumulating variety (H) was more sensitive to Cd. Above-ground/below-ground biomass and root length of Chinese cabbage varied with increasing Pb concentration under different Pb treatments. All three growth indices of both varieties were lowest at a Pb concentration of 1200 mg/L, and the above-ground biomass of L and S varieties decreased by 48.92% and 26.11%, the belowground biomass by 50.00% and 86.27%, and the root length by 30.36% and 9.84%, respectively, when compared with the treatment of 0 mg/L.

3.2. Cd and Pb Accumulation in Different Varieties of Chinese Cabbage

There were significant differences in the Cd absorption and accumulation capacity

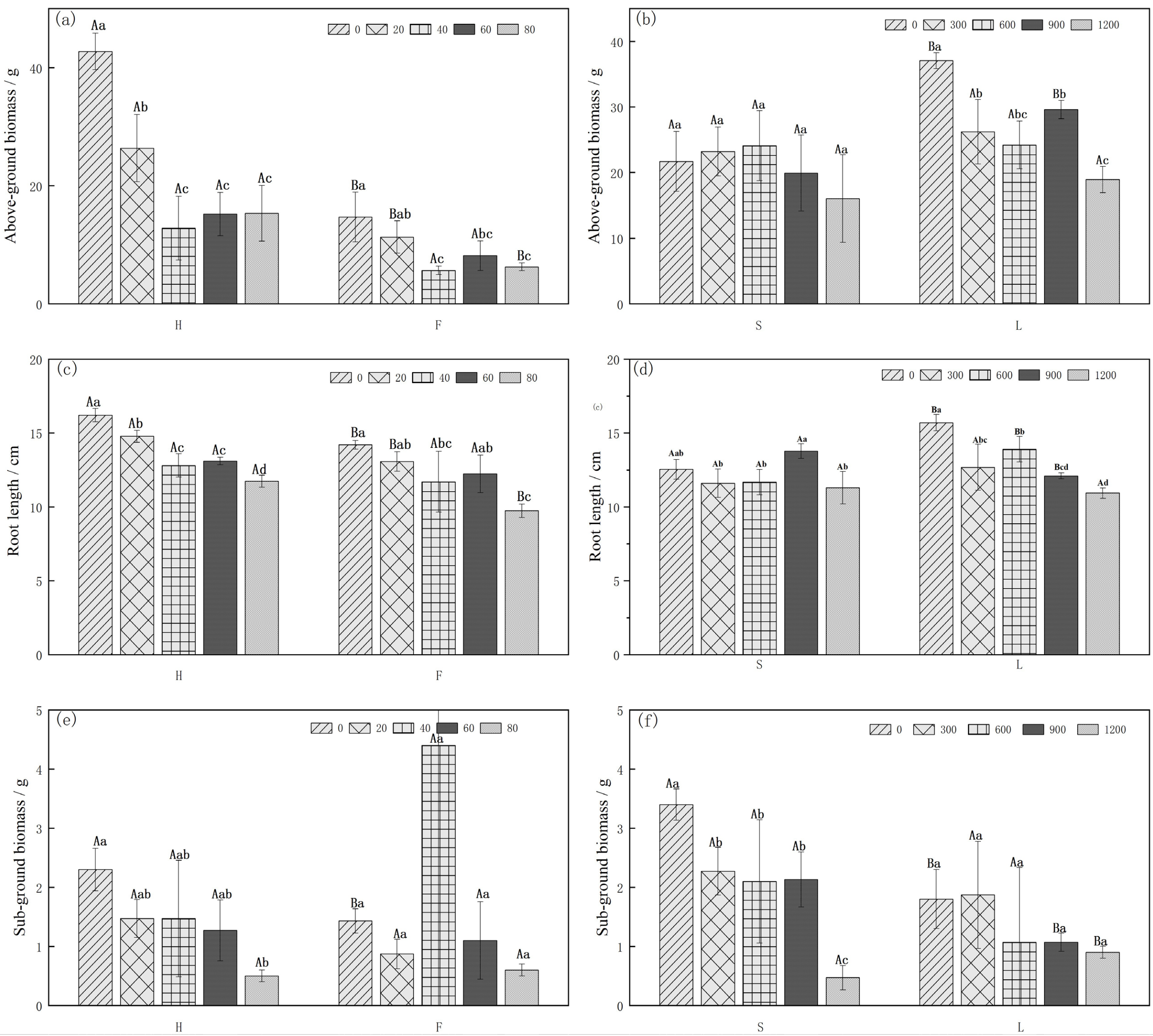

Figure 1. Effect of Cd and Pb stress on growth indexes of Chinese cabbage (a, c, and e are Cd treatments; b, d, and f are Pb treatments). Note: Different lowercase letters indicate that the difference between different treatments of the same variety reaches a significant level ($P < 0.05$); different uppercase letters indicate that the difference between different varieties under the same treatment reaches a significant level ($P < 0.05$), the same below.

of different varieties of cabbage (Table 1). In this study, for the aboveground part of cabbage, the variety with the highest Cd content was F (10.60 mg/kg), and the variety with the lowest Cd content was H (4.72 mg/kg), in which the Cd content of F was 1.25 times higher than that of H; the variety with the highest Pb content was S (18.00 mg/kg), the lowest variety was L (1.80 mg/kg), and the Pb content of S was 1.25 times higher than that of H. The variety with the highest Pb content was S (18.00 mg/kg), and the lowest variety was L (1.80 mg/kg), in which the Pb content of S was 9 times that of L. For the underground, the variety with the highest Cd content was F (13.20 mg/kg), and the variety with the highest Pb content was S (245.32 mg/kg).

Table 1. Above-ground and below-ground heavy metal contents of different Chinese cabbage.

Variety	Above-ground		Below-ground	
	Cd	Pb	Cd	Pb
Lvguan	9.87 ± 1.63	1.80 ± 1.71	15.96 ± 0.94	136.25 ± 1.70
Hexiekuaicai	4.72 ± 2.59	9.90 ± 0.85	9.56 ± 1.39	154.78 ± 0.51
Suzhouqing	10.00 ± 1.73	18.00 ± 1.00	13.21 ± 1.69	245.32 ± 0.71
ZiweiF1	10.60 ± 2.16	13.20 ± 2.71	17.69 ± 1.14	202.31 ± 1.49

3.3 Effects of Cd and Pb Stress on Chlorophyll Content

Chlorophyll is mainly composed of chlorophyll a, chlorophyll b, carotene, etc., and plays a role in the absorption and conversion of light energy in the photosynthesis of plants. Changes in chlorophyll content of high- and low-accumulating cabbages under different Cd concentration stresses are shown in **Table 2**. In H varieties, after the addition of Cd, their chlorophyll a content compared with the control group (H0) was reduced to a certain extent, among which the chlorophyll a content of the H40 treatment was inhibited the most, reduced by 0.96 mg/g compared with H0, with a reduction of 31.27%; the chlorophyll b content of the five treatments was H40 (1.38 mg/g) > H0 (1.16 mg/g) > H60 (1.03 mg/g) > H80 (0.91 mg/g) > H20 (0.87 mg/g), with an increase of 18.97% in H40 compared to H0, and a decrease of 25.00% in H20 compared to H0; chlorophyll ab content showed a trend of first decreasing, then increasing, and then decreasing with Cd concentration. The remaining four treatments were inhibited compared to H0, decreased by 28.84%, 17.49%, 15.51%, and 25.77%, respectively. The differences between chlorophyll a, chlorophyll b, and chlorophyll ab among treatments were not significant in H varieties.

The chlorophyll a content of F varieties was lower than that of the control (F0) in all treatments after the addition of Cd. F20, F40, F60, and F80 were reduced by 9.49%, 15.49%, 6.12%, and 28.29%, respectively, compared to F0. Chlorophyll b content decreased with the increase in Cd concentration, and compared to F0, it was reduced by 14.76%, 16.57%, 21.78%, and 32.94%. Chlorophyll ab content, with the increase of Cd concentration, showed a trend of decreasing, then increasing, and then decreasing, and the content of chlorophyll ab in the rest of the treatments was lower than that of the F0 treatment; the lowest inhibition was in F60, and the strongest was in F80, which was reduced by 6.98% and 29.78%, respectively, compared with F0. The chlorophyll a, chlorophyll b, and chlorophyll ab contents of the F0 treatments were significantly different from the rest of the treatments, while the contents of chlorophyll a, chlorophyll b, and chlorophyll ab of F80 were significantly different from the rest of the treatments. F80 showed significant differences, and the differences among the remaining treatments were not significant. Therefore, the chlorophyll content of F was more susceptible to different Cd concentration stresses compared to H. The chlorophyll content of F was significantly different from that of H. The chlorophyll content of F was significantly different from that of F80.

Table 2. Changes in chlorophyll content of high and low accumulating cabbage under different Cd concentration stresses.

Variety	Cd concentration (mg/kg)	chlorophyll a (mg/g)	chlorophyll b (mg/g)	chlorophyll ab (mg/g)
H	H_0	3.07 ± 0.90Aa	1.16 ± 0.23Aa	4.23 ± 1.13Aa
	H_{20}	2.14 ± 0.09Aa	0.87 ± 0.87Aa	3.01 ± 0.03Aa
	H_{40}	2.11 ± 0.92Aa	1.38 ± 1.38Aa	3.49 ± 0.46Aa
	H_{60}	2.56 ± 0.43Aa	1.03 ± 1.03Aa	3.59 ± 0.54Aa
	H_{80}	2.23 ± 0.47Aa	0.91 ± 0.91Aa	3.14 ± 0.68Aa
F	F_0	4.05 ± 0.69Aa	1.91 ± 0.34Ba	5.96 ± 1.02Aa
	F_{20}	3.66 ± 0.32Bab	1.63 ± 0.13Bab	5.29 ± 0.42Bab
	F_{40}	3.42 ± 0.29Aab	1.59 ± 0.15Aab	5.02 ± 0.39Bab
	F_{60}	3.80 ± 0.89Bab	1.49 ± 0.03Bab	5.54 ± 0.36Bab
	F_{80}	2.90 ± 0.40Ab	1.28 ± 0.06Bb	4.18 ± 0.36Ab

Changes in chlorophyll content of high and low cumulative cabbage under stress of different Pb concentrations are shown in Table 3. After the addition of Pb to S varieties, the chlorophyll a content of the rest of the treatments was higher than that of the control (S0), except for the S900 treatment, which was reduced by 3.95% compared to S0, and the rest of the treatments had higher chlorophyll a content than that of S0, with the values shown as S1200 (4.54 mg/g) > S600 (4.23 mg/g) > S300 (3.94 mg/g), which were 19.47%, 11.32%, and 3.68% higher than S0, respectively; the chlorophyll b content of the four Pb concentration treatments was higher than that of S0, with the values of S1200 (2.92 mg/g) > S600 (2.22 mg/g) > S900 (2.11 mg/g) > S300 (2.04 mg/g), which were elevated by 60.44%, 21.97%, 15.93%, and 12.09%, respectively, compared with S0; the chlorophyll ab content showed a trend of increasing, then decreasing, and then increasing with Pb concentration, with S1200 showing the largest increase of 32.74%, and S900 showing the smallest increase of 2.49%. Among treatments of S varieties, the differences between chlorophyll a, chlorophyll b, and chlorophyll ab were not significant.

After the addition of Pb, the chlorophyll a, chlorophyll b, and chlorophyll ab contents of L varieties showed a trend of decreasing, then increasing, and then decreasing with the increase of Pb concentration. The highest chlorophyll content was found in the L0 treatment, which was 4.86 mg/g, 2.01 mg/g, and 6.87 mg/g, respectively, and the sizes of the chlorophyll contents of the rest of the treatments were L300 > L900 > L1200 > L600. The most inhibited treatment was L600, with the contents of chlorophyll a, chlorophyll b, and chlorophyll ab reduced by 29.42%, 16.92%, and 25.91%, respectively, compared with L0; and the least inhibited treatment was L300, with the contents of chlorophyll a, chlorophyll b, and chlorophyll ab reduced by 17.49%, 7.46%, and 14.56%, respectively, compared with L0. The chlorophyll a content of the L300, L1200, and L1200 varieties was the most inhibited. The chlorophyll a content of the varieties was significantly different between the L0 treatment and the L600, L900, and L1200 treatments, and there was no significant correlation with the L300 treatment; there was no significant correlation between the chlorophyll b content of the treatments; and there was a significant correlation between the L0 and the L600, and no significant correlation with the rest of the treatments. Therefore, in comparison, L and S were more susceptible to chlorophyll content, and chlorophyll a was more susceptible to Pb concentration under different Pb stresses.

Table 3. Changes in chlorophyll content of high- and low-accumulating cabbages under different Pb concentration stresses.

Variety	Pb concentration (mg/kg)	chlorophyll a (mg/g)	chlorophyll b (mg/g)	chlorophyll ab (mg/g)
L	L_0	3.80 ± 0.83Aa	1.82 ± 0.26Aa	5.62 ± 1.08Aa
	L_{300}	3.94 ± 1.02Aa	2.04 ± 0.36Aa	5.99 ± 1.37Aa
	L_{600}	4.23 ± 1.62Aa	2.22 ± 0.48Aa	6.44 ± 2.11Aa
	L_{900}	3.65 ± 1.94Aa	2.11 ± 0.78Aa	5.76 ± 2.71Aa
	L_{1200}	3.54 ± 1.41Aa	1.92 ± 0.99Aa	5.46 ± 2.16Aa
S	S_0	4.86 ± 0.28Aa	2.01 ± 0.20Aa	6.87 ± 0.47Aa
	S_{300}	4.01 ± 0.81Aab	1.86 ± 0.21Aa	5.87 ± 1.02Aab
	S_{600}	3.43 ± 0.50Ab	1.67 ± 0.16Aa	5.09 ± 0.66Ab
	S_{900}	3.74 ± 0.61Ab	1.87 ± 0.28Aa	5.61 ± 0.88Aab
	S_{1200}	3.70 ± 0.38Ab	1.87 ± 0.06Aa	5.57 ± 0.41Aab

3.4 Effect of Cd and Pb Stress on Soluble Sugar Content

Figure 2 shows that the soluble sugar content of H increased with the increase in Cd addition, and the soluble sugar content of each treatment group was higher than that of the control group (H0). The soluble sugar content of H80, H60, H40, and H20 increased by 8.74%, 22.30%, 84.15%, and 187.32%, respectively, compared with that of H0. There were no significant differences between H0 and H20 and H40, and significant differences between H60 and H80. The soluble sugar content of F showed an increasing and then decreasing trend with the increase in Cd addition. F20 and F40 treatments increased by 35.82% and 0.37% compared with F0, and F60 and F80 treatments were lower than that of F0 treatment, with a decrease of 50.17% and 51.03% compared with F0 treatment, respectively. There was no significant difference between F0 and F40, and there was no significant difference between F60 and F80; there was no significant difference between F0 and F40, and there was no significant difference between F20, F40, F60, and F80. Compared with F varieties, the variation of soluble sugar content of F varieties was 177.34%, the variation of soluble sugar content of H varieties was 187.32%, and the variation of H varieties was 5.63% higher than that of F varieties. In the treatment of the same Cd concentration, the difference between the two varieties was significant, which showed that Cd had a promoting effect, but medium and high concentrations may inhibit the soluble sugar synthesis of F varieties and affect their normal growth.

The soluble sugar contents of both S and L showed wavy changes with the increase of Pb addition. The variations of soluble sugars in S varieties at different Pb concentrations were smaller than those in L varieties, and the differences between treatments of S varieties were not significant, and the differences between L0 and

L300 and L1200 were significant, indicating that the sensitivity of S varieties to changes in Pb concentration was smaller than that of L varieties.

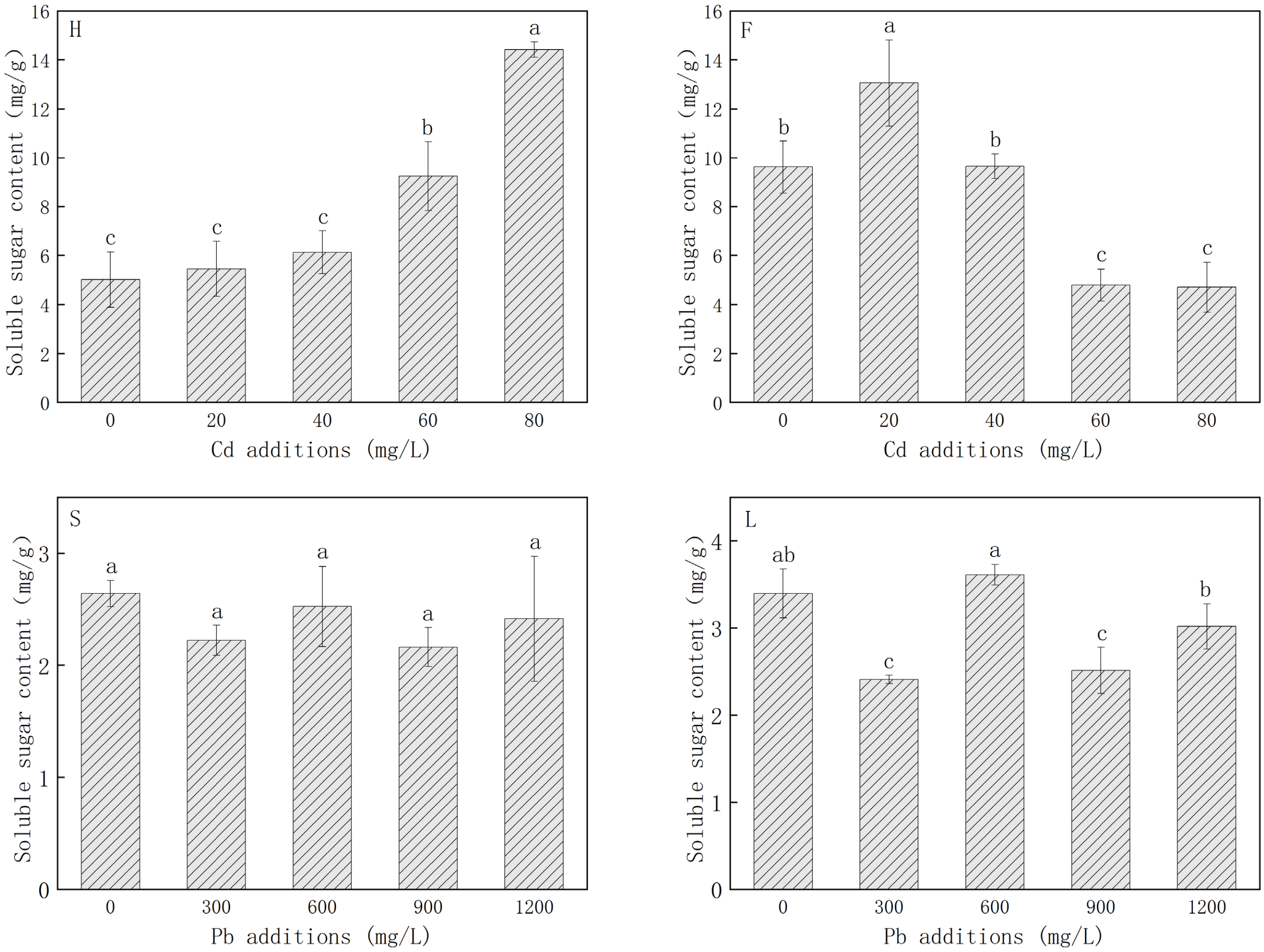

Figure 2. Effect of different concentration treatments on the soluble sugar content of cabbage.

3.5. Effect of Cd and Pb Stress on Soluble Protein Content

Figure 3 shows the effect of Cd and Pb stress on soluble protein in Chinese cabbage. It can be seen that under Cd stress, the soluble protein content of H varieties, in addition to the treatment under 20 mg/L, rose by 3.01% compared with the control (H0); in the rest of the treatments, compared with H0, its soluble protein content decreased, among which H80 showed the largest decrease in soluble protein content, which was significantly different from the rest of the treatments. The soluble protein content of F varieties showed a tendency to increase and then decrease with the increase of Cd concentration, and when the Cd concentration was 60 mg/L, the soluble protein content was the highest at 2.00 mg/kg, which was an increase of 37.56% compared with F0; the rest of the treatments showed a tendency to decrease with the increase of Cd concentration. There was no significant difference between F0, F20, and F40, and there was a significant difference between F60 and F80. Significant differences were observed between H and F varieties at Cd concentrations of 20 mg/L and 40 mg/L, and non-significant differences between varieties at Cd concentrations of 20 mg/L and 40 mg/L.

The soluble protein content of L varieties was most inhibited when Pb was added at 300 mg/L, which decreased by 58.58% compared with L0, and then showed a tendency to increase and then decrease with the increasing amount added. The soluble protein content reached the maximum value at 900 mg/L, which increased by 3.60% compared with L0, and the difference between the treatments was not significant. The content of soluble protein of S varieties showed a tendency to decrease first with the increase of Pb concentration, and then decreased with the increase of Pb concentration, and then decreased with the increase of Cd concentration, which was not significant. Pb concentration showed a trend of decreasing, then increasing and then decreasing; its maximum value of 1.43 mg/kg occurred at 900 mg/L of Pb concentration, which increased by 27.59% compared to S0. The rest of the treatments showed a decrease in soluble sugar content compared to S0, and there was a significant difference between S0 and S1200, but no significant difference between S0 and the other treatments. S varieties were significantly different from L varieties at a Pb concentration of 300 mg/L and 1200 mg/L compared to S0, and no significant difference was observed among other treatments.

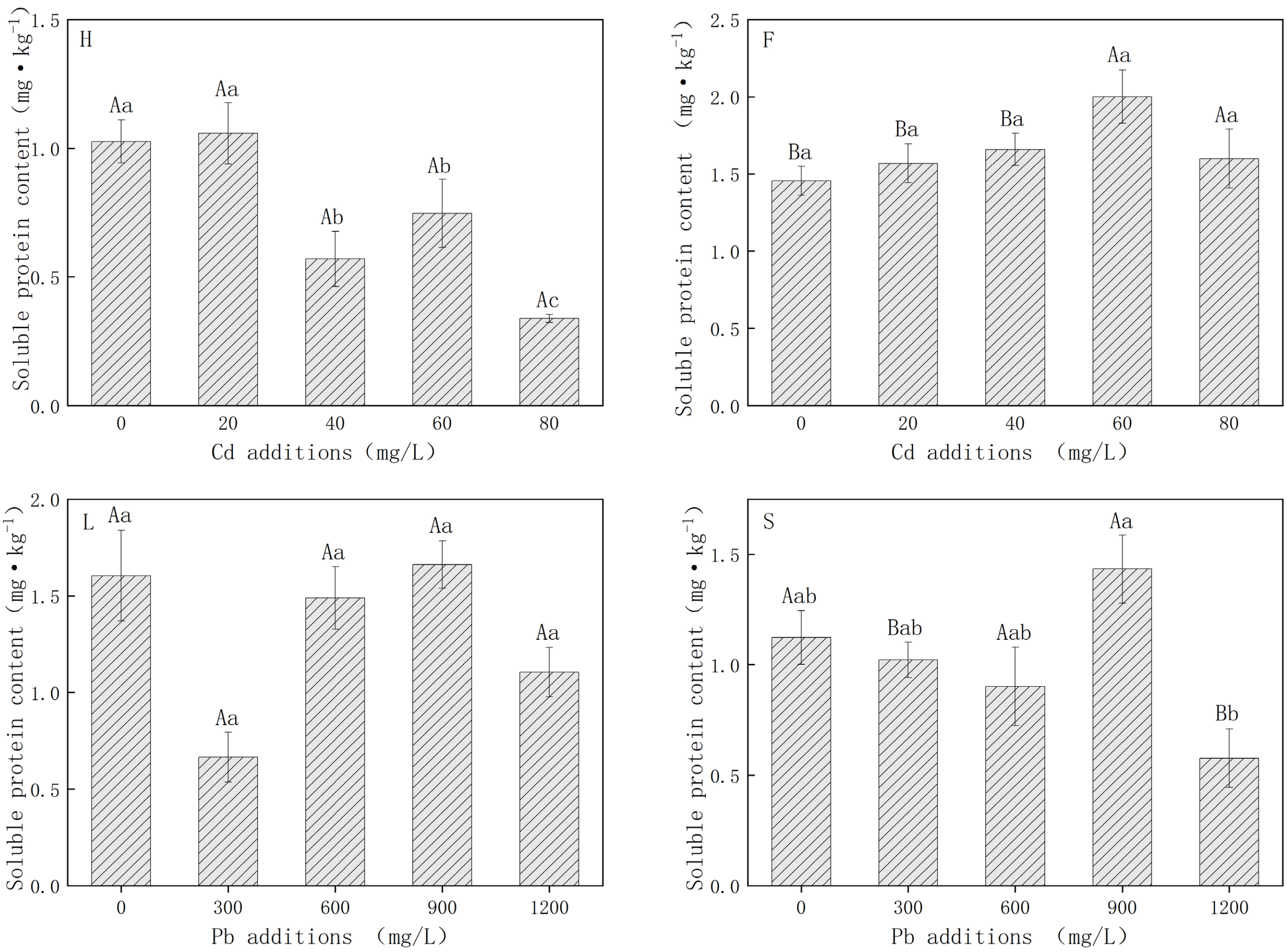

Figure 3. Effect of different concentrations of Cd and Pb treatments on the soluble protein content of cabbage.

3.6. Effect of Cd and Pb Stress on Proline Content

As can be seen in Figure 4, proline content of both varieties under Cd stress was higher than that of the control. In H varieties, H20, H40, H60, and H80 increased by 15.63%, 9.26%, 64.85%, and 41.88%, respectively, compared with H0, with the largest increase in H60 and the smallest in H40; there was no significant difference between H0 and H20 and H40, and a significant difference with H60 and H80. Among the F varieties, F20, F40, F60, and F80 increased by 53.87%, 55.01%, 54.55%, and 46.34%, respectively, compared to F0, with F60 having the largest and F80 the smallest increase; the difference between F0 and F60 was significant, and that with the rest of the treatments was not significant. Between the two varieties, the proline content of the H variety was higher than that of the F variety in the rest of the treatments, except for the Cd concentrations of 20 mg/L and 40 mg/L. The proline content of the H variety was higher than that of the F variety by 18.26, 26.14, and 14.66%, respectively, and there was no significant difference between the two varieties.

The proline content of L varieties showed a trend of increasing and then decreasing with the increase of Pb concentration, and L900 had the highest proline content of 13.97 mg/L, which increased by 183.27% compared with L0; there was no significant difference among L0, L600, and L900, and no significant difference between L300

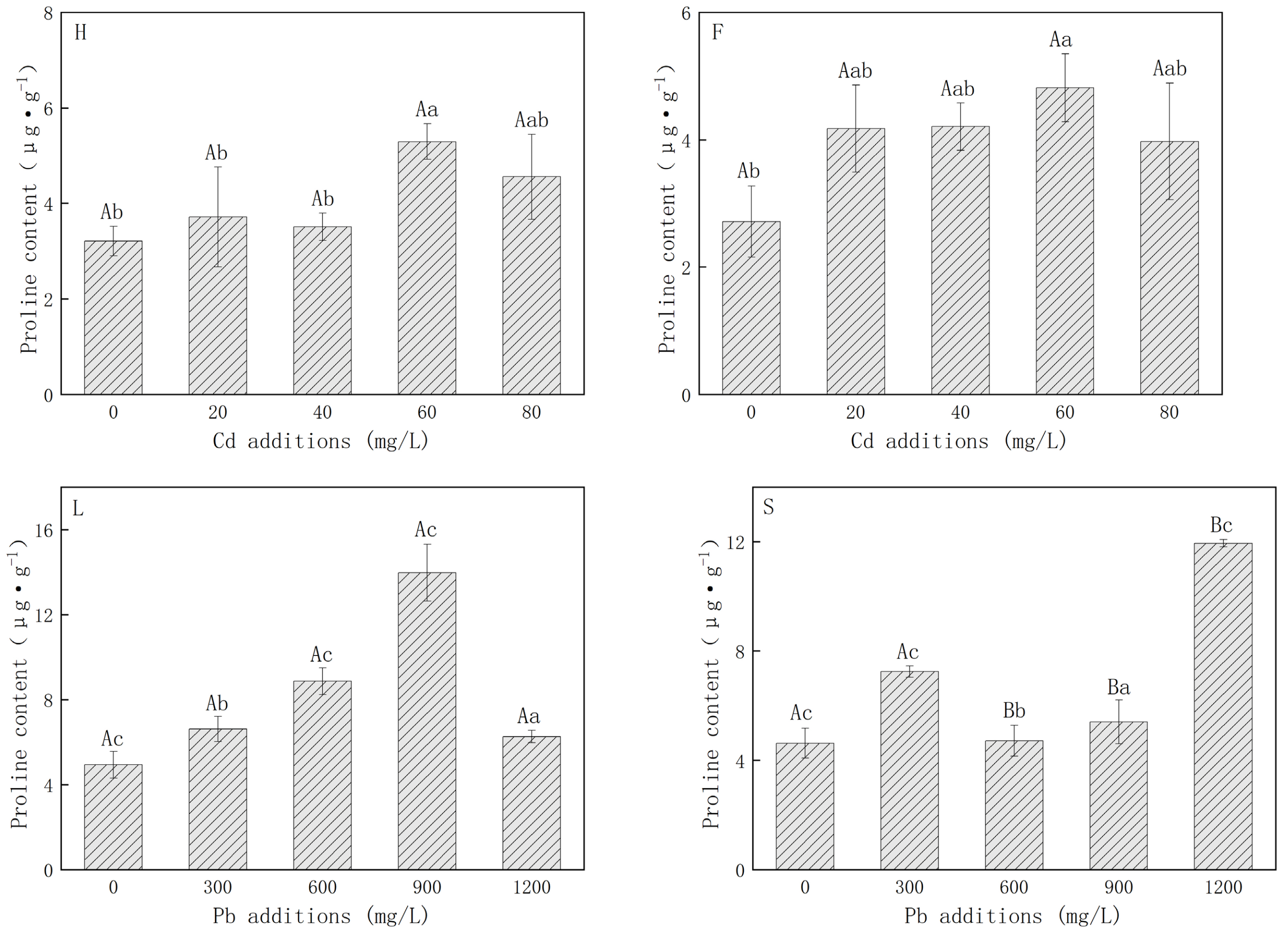

Figure 4. Effect of different concentrations of Cd and Pb treatments on the proline content of cabbage.

and L900. The proline content of S varieties showed a trend of first increasing, then decreasing, and then increasing with the increase of Pb content, and the proline content of the four treatments was higher than that of S0, with the lowest increase in S600 and the largest increase in S1200; there was no significant difference between S0 and S900 and S1200, and a significant difference with S600 and S900. There were no significant differences between the two varieties at Pb concentrations of 0 and 300 mg/L, and significant differences between the two varieties at 600, 900, and 1200 mg/L.

3.7. Effect of Cd and Pb Stress on Malondialdehyde (MDA) Content

MDA is the end product of lipid peroxidation, which can cause cross-linking polymerization of proteins, nucleic acids, and other life macromolecules and is cytotoxic (Yu, 2016). As shown in **Figure 5**, the MDA content of both H and F varieties increased with the increase of Cd addition. H20 and H40 increased by 45.45% and 68.18% compared to H0, and there was no significant difference between treatments. H60 and H80 increased by 272.73% and 388.67% compared to H0, and the difference between H0 and H60 and H80 was significant. F20, F40, F60, and F80 increased by 10.64%, 30.85%, 95.74%, and 153.19%, respectively, compared with F0, and there was no significant difference between the treatments and F0. The MDA content of F80 increased by 29.35% compared with F60, and the difference between the two treatments was significant. At the same treatment concentration, the MDA content of F varieties was higher than that of H varieties, and there was no significant difference between them and the treatments except that there was a significant difference between the two varieties at 0 mg/L concentration.

Under different Pb concentrations of stress, the MDA content in both L and S varieties increased to different degrees compared to the control, where in L varieties, the MDA content in the five treatments L0-L1200 was 13.55 mg/kg, 15.32 mg/kg, 21.29 mg/kg, 21.77 mg/kg, and 27.42 mg/kg, respectively. In each of the treatments compared to L0, the increases were 13.10%, 57.14%, 60.71%, and 102.38%, respectively, compared with L0, and the differences between L0 and L1200 were significant. Among the S varieties, the MDA contents of the five treatments, S0-S1200, were 7.10 mg/kg, 15.16 mg/kg, 17.26 mg/kg, 21.61 mg/kg, and 20.97 mg/kg, respectively; compared with S0, the treatments increased by 113.64%, 143.18%, 204.55%, and 195.45%, respectively, compared to S0. The differences were significant between S0 and L1200 and not significantly different from the rest of the treatments.

3.8. Effect of Cd and Pb Stress on Catalase (CAT) Content

As shown in **Figure 6**, CAT activity in plants under low concentrations of Cd and Pb stress increased to some extent, and CAT content gradually decreased with the increase of heavy metal concentrations. In the H variety, CAT reached its maximum value at 20 mg/L, which increased by 15.62% compared with H0, and the rest of the treatments decreased by 23.22%, 49.49%, and 59.57% compared with H0, respectively, and the differences among treatments were significant; in the F variety, CAT activity reached its maximum value at 40 mg/L, which increased by

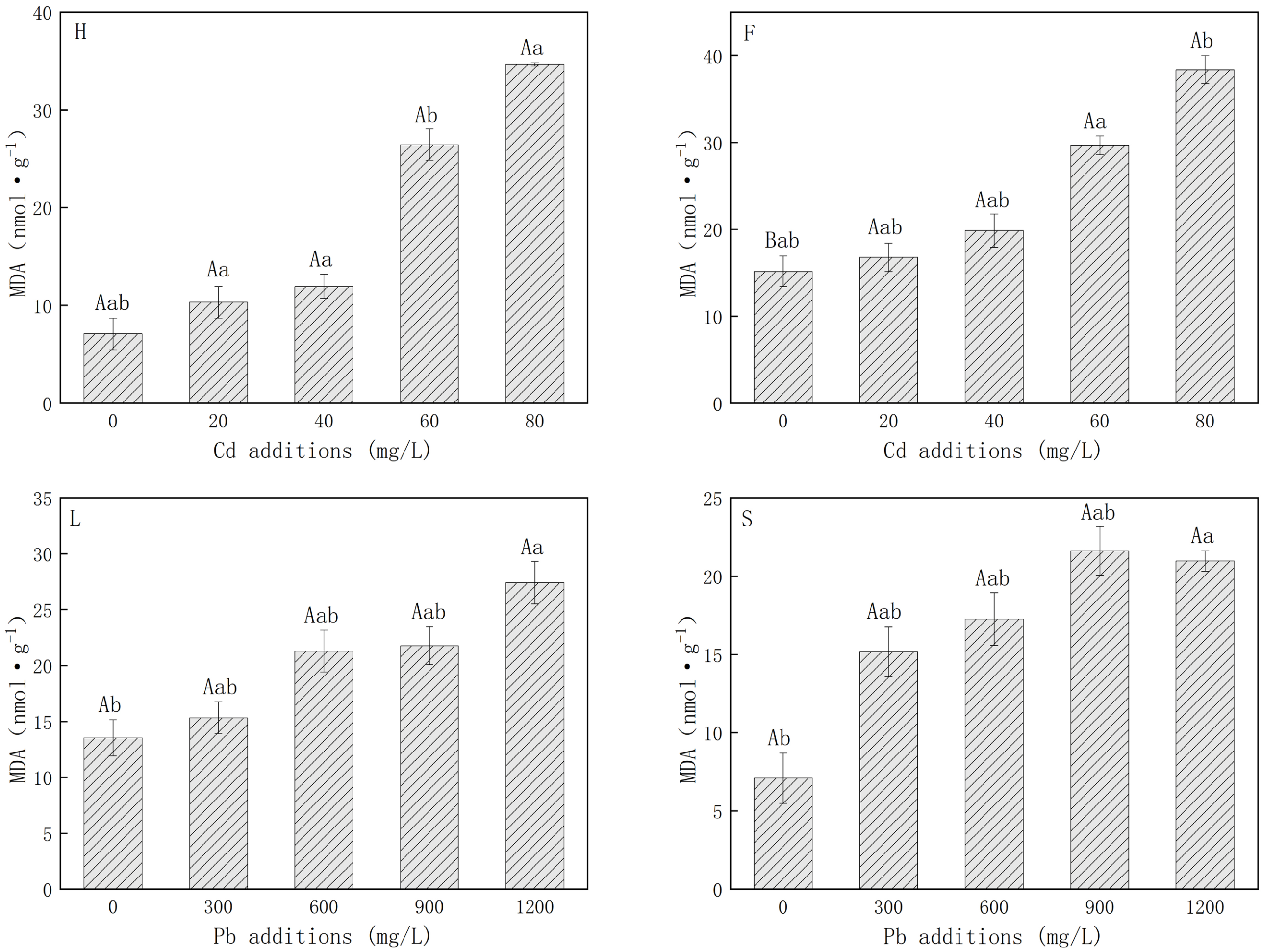

Figure 5. Effect of different concentrations of Cd and Pb treatments on the MDA content of cabbage.

9.85% compared with F0, and the F60 and F80 treatment CAT activity decreased by 26.43% and 33.00%, respectively, compared to H0, with no significant difference between F0 and F20 and F40, and significant differences between F0 and F60 and F80; the differences between the two varieties were significant at 0 and 20 mg/L concentrations, and not significant at the remaining concentrations. In the L variety, CAT activity reached its maximum value at 600 mg/L Pb concentration, and increased significantly compared to L0. Activity was the highest at a Pb concentration of 600 mg/L in the L variety, which increased by 14.55% compared to L0, and the differences between L900 and L1200 were significant compared to L0, which decreased by 36.88% and 46.97%, respectively; CAT activity was also the highest at 600 mg/L in the S variety, which increased by 51.18% compared to H0, and the differences between S900 and S1200 were significant compared to S0. The differences between the treatments were 17.82% and 17.82% lower than that of S0, and the differences were not significant at the remaining concentrations. Activity decreased by 17.82% and 26.10%, respectively; at a Pb concentration of 300 mg/L, there was a significant difference between the two varieties and no significant difference at the remaining concentrations.

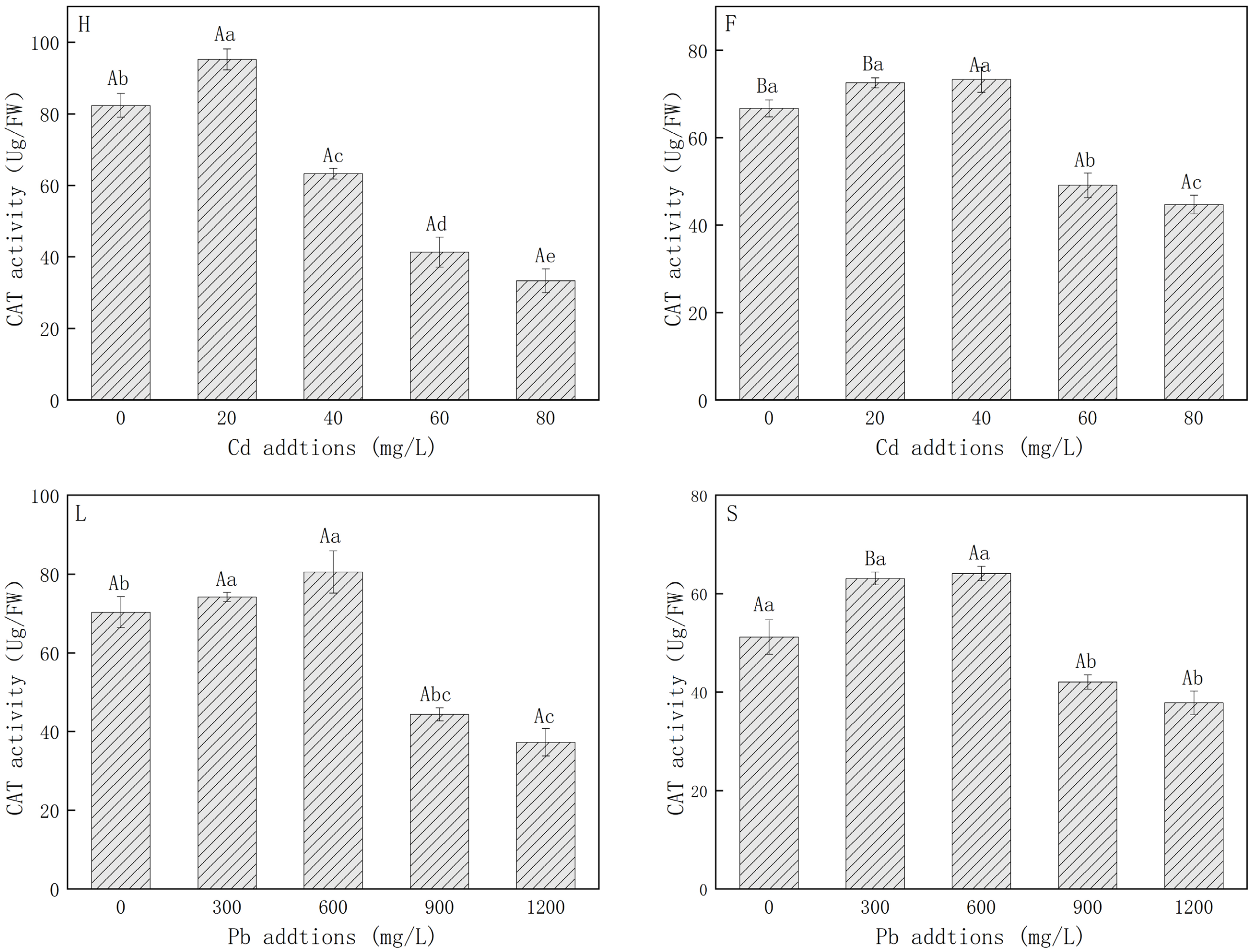

Figure 6. Effect of different concentrations of Cd and Pb treatments on CAT activity in cabbage.

3.9. Effect of Cd and Pb Stress on Peroxidase Activity (POD)

The changes in POD activity in four varieties of Chinese cabbage under different concentrations of Cd and Pb stress are shown in Figure 7, and the POD activity showed a tendency to increase and then decrease. The H variety showed the highest POD activity of 1645.96 U·(g·min)$^{-1}$ at a Cd concentration of 20 mg/L, and the POD activity at a Cd concentration of 0 mg/L was the lowest at 1016.74 U·(g·min)$^{-1}$. H40, H60, and H80 increased by 36.97%, 13.66%, and 4.41% respectively compared to H0, and there was no significant difference between H0 and H80, but significant differences with the other treatments. The F variety had the highest POD activity at a 40 mg/L Cd concentration of 1914.73 U·(g·min)$^{-1}$, which increased by 47.69% compared to F0, and the lowest POD activity was 1147.38 U·(g·min)$^{-1}$ at an 80 mg/L Cd concentration, which decreased by 11.32% compared to F0. Significant differences were observed among the treatments. Significant differences were observed between H and F varieties at 0, 40, and 60 mg/L of Cd concentration.

The highest POD activity of 1518.14 U·(g·min)$^{-1}$ was recorded in the L variety at 300 mg/L of Pb concentration, which increased by 48.02% as compared to L0. The lowest POD activity of 974.62 U·(g·min)$^{-1}$ was recorded at 1200 mg/L of Pb concentration, which decreased by 4.86% as compared to L0, and L600 and L900 showed an increase of 28.22% and 9.69% as compared to L0; there was no significant difference between L0 and L1200 and a significant difference with the rest of the treatments. The S variety had the highest POD activity of 1638.09 U·(g·min)$^{-1}$ at 600 mg/L Pb concentration, which was increased by 64.79% compared to S0; the S300, S900, and S1200 treatments increased by 26.49%, 37.84%, and 5.83%, respectively, compared to S0; there was no significant difference between S0 and S1200 and a significant difference with the other treatments. The two varieties differed significantly at 300, 600, and 900 mg/L Pb concentrations.

3.10. Effect of Cd and Pb Stress on Superoxide Dismutase Activity (SOD)

The changes in SOD activity of four varieties of cabbage under different concentrations of Cd and Pb stress are shown in Figure 8, and the SOD activity of all four varieties showed a trend of increasing and then decreasing. H varieties under different Cd concentrations of stress showed the following SOD activity: H40 (123.89 Ug/FW) > H60 (113.51 Ug/FW) > H20 (106.39 Ug/FW) > H0 (93.26 Ug/FW) > H80

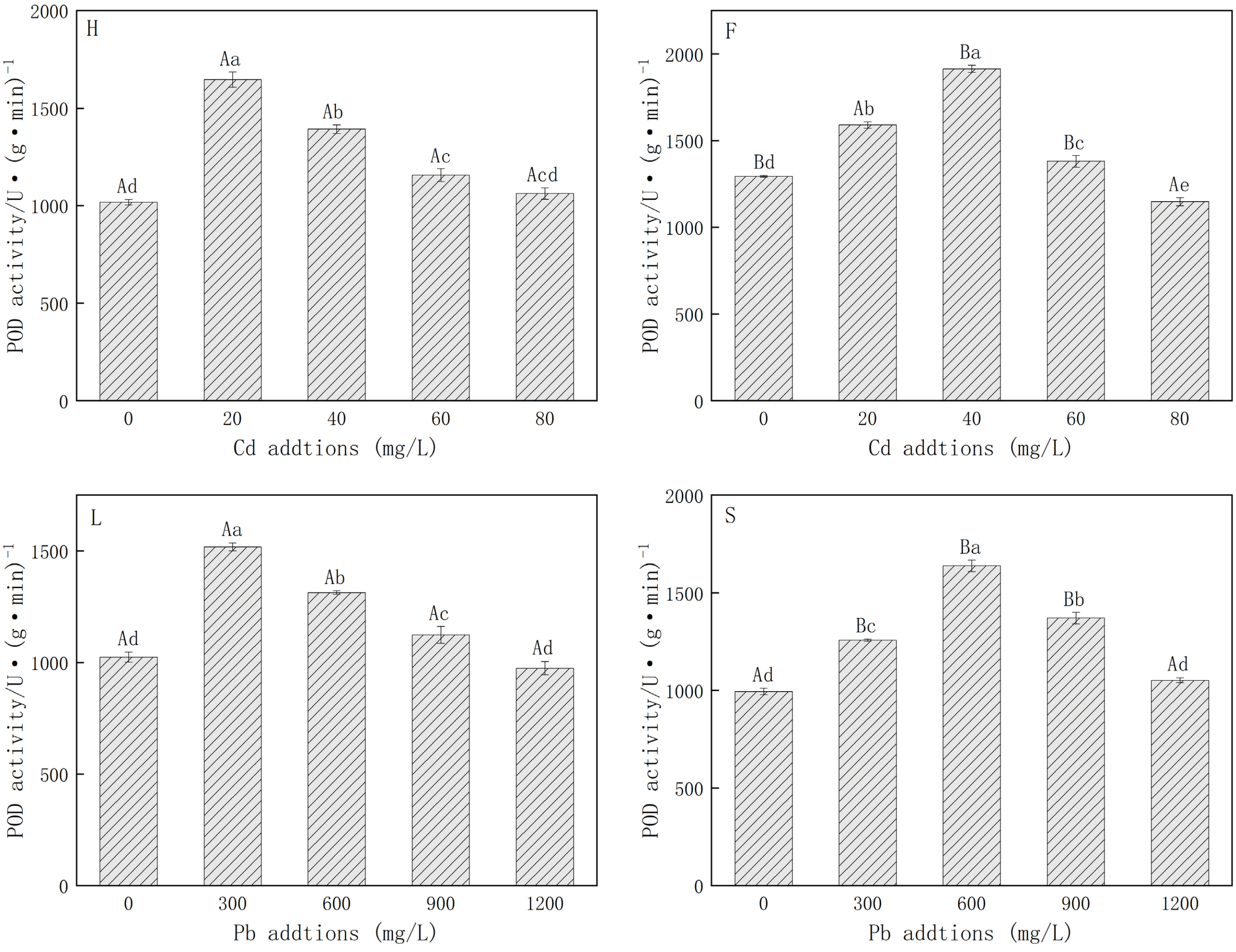

Figure 7. Effect of different concentrations of Cd and Pb treatments on POD activity in cabbage.

(88.49 Ug/FW). The differences were significant between the treatments of H0 and H40, and there was no significant difference with the other treatments. F varieties under different Cd concentration stress showed SOD activity of F20 (166.33 Ug/FW) > F40 (135.75 Ug/FW) > F60 (113.67 Ug/FW) > F80 (104.31 Ug/FW) > F0 (103.20 Ug/FW); the differences between F0 and F20 and F40 were significant. There was no significant difference in SOD activity between the two varieties at the same Cd concentration.

The L variety had the highest SOD activity of 110.10 Ug/FW at 600 mg/L Pb concentration and the lowest SOD activity of 87.06 mg/L at 1200 mg/L, while the S variety had the highest SOD activity of 106.60 Ug/FW at 300 mg/L Pb concentration and the lowest SOD activity of 78.16 mg/L at 1200 mg/L. SOD activity was the lowest at 78.16 mg/L; at 300 mg/L Pb concentration, SOD activity of L variety was 3.28% more than that of S variety; at 1200 mg/L Pb concentration, SOD activity of L variety was 11.39% more than that of S variety. There was no significant difference between treatments in L variety; in S variety, the difference between S300 and S1200 was significant; and there was no significant difference between the two varieties at the same Pb concentration.

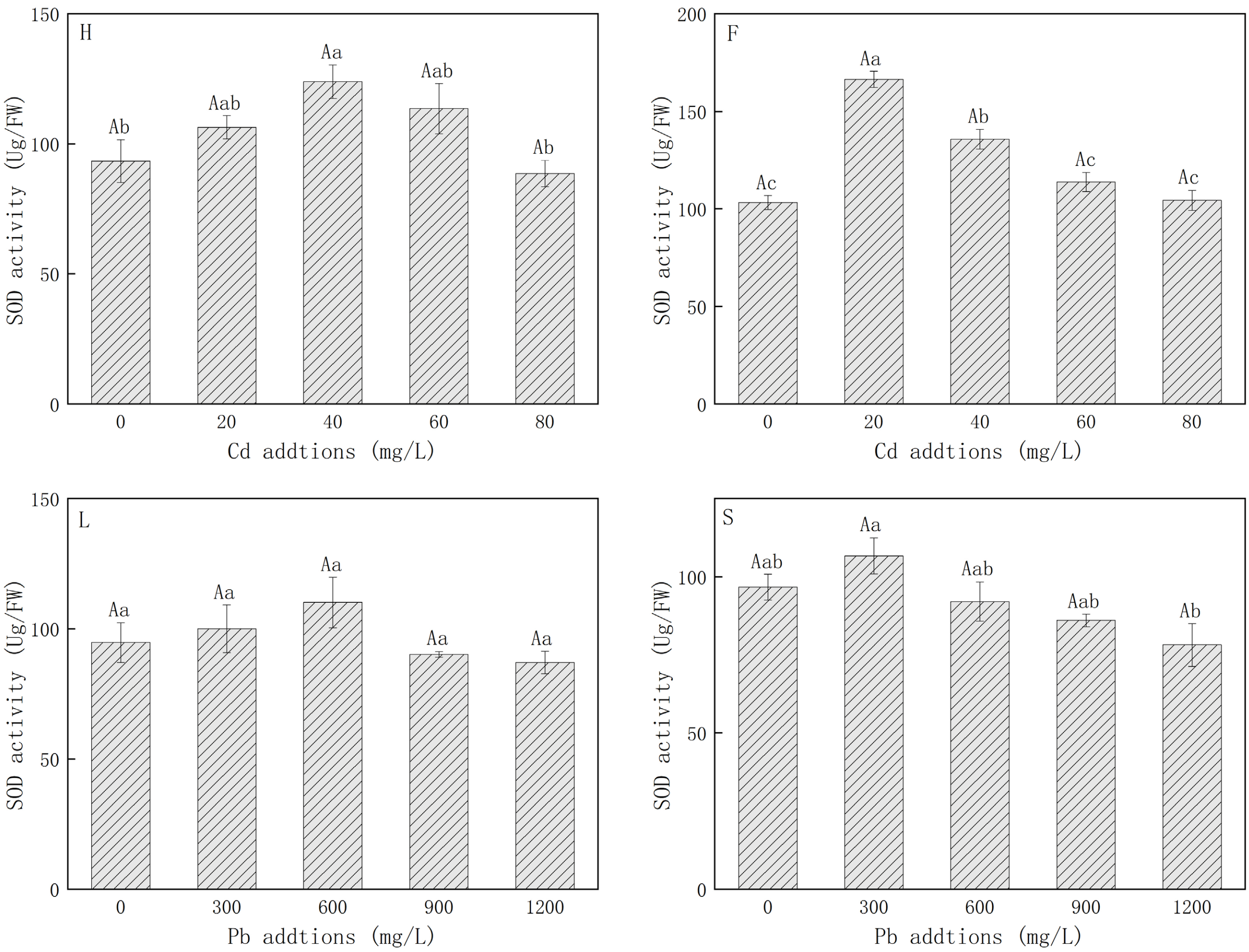

Figure 8. Effect of different concentrations of Cd and Pb treatments on SOD activity in cabbage.

4. Discussion

4.1. Effect of Heavy Metal Stress on Chlorophyll Content

It has been shown that Cd inhibits electron transfer and the regeneration phase of the Calvin cycle in the plant and photosynthetic system, causing the plant to exhibit moderate symptoms such as leaf yellowing and leaf wilting (Baker & Brooks, 1989). The heavy metal Cd interferes with chlorophyll synthesis by inhibiting photosynthetic pigment-producing enzyme activities or by inducing deficiencies in essential nutrients (Sabeen et al., 2013), and chlorophyll, as an important component of the Calvin cycle, can indicate whether or not the plant is exposed to the toxic effects of Cd (Zhang et al., 2014). In this study, the content of chlorophyll a and chlorophyll b was suppressed to a certain extent when the Cd concentration of H variety was less than 80 mg/L, but there was no significant difference compared with the control ($P > 0.05$), and the suppression of the chlorophyll content of this variety was not significant at the test concentration, and the content of chlorophyll a and chlorophyll b of F variety was suppressed when the Cd concentration was less than 80 mg/L, but there was no significant difference compared with the control ($P > 0.05$), and the content of chlorophyll b of F variety was suppressed when the test concentration was less than 80 mg/L. There was no significant difference in the content of chlorophyll a and chlorophyll b compared to the control at a Cd concentration of 80 mg/L, but when the Cd concentration was 80 mg/L, there was a significant difference compared to the control, and the inhibition of the chlorophyll content was the most obvious, with a significant decrease in the chlorophyll a and b content. There are two main reasons for the decrease in chlorophyll content. One is that after Cd is absorbed from the roots and reaches the leaves, oxygen radicals are formed in the leaf tissues, which destroy the structure and function of the chloroplasts, thus decreasing the activity of chlorophyll synthase (Zhang et al., 2022; Guo et al., 2016); the other is that Cd interacts with the chlorophyll synthase in the cells, which affects the synthesis of the chlorophyll precursors and accelerates the decomposition of chloroplasts, leading to the decrease in the number of chloroplasts. This leads to the blockage of mineral element uptake and inhibition of photosynthesis (Shi et al., 2022; Wang et al., 2016).

Some studies have shown that Pb^{2+} can enhance the activity of chlorophyllase and contribute to the degradation of chlorophyll (Yang, 1991; Gopal & Rizvi, 2008; Maria, 1994). Some studies have reported that plant chlorophyll content is affected under Pb stress. Cai Siqi's study showed that the chlorophyll content of two-podded cassia showed a tendency of increasing and then decreasing under Pb stress conditions (Cai, 2017); Long (2010) found that the chlorophyll content of southwestern pedicel was higher than that of control under Pb (300 - 4000 mg/L) treatment, and reached the maximum value at 4000 mg/L. However, it was also found that chlorophyll a, chlorophyll b, and total chlorophyll decreased with an increase in treatment (Yuan, 2011). In this study, we showed that chlorophyll a and chlorophyll b content of L variety under Pb treatment reached a maximum at 600 mg/L and decreased beyond 600 mg/L, while chlorophyll a and chlorophyll b content of S variety decreased with increasing Pb concentration. The reason for the difference was that L has higher tolerance to Pb, which not only did not destroy the structure of chloroplasts and inhibit the process of chlorophyll synthesis within the cabbage under low concentrations of Pb stress, but instead stimulated the activity of the key enzyme for synthesizing chlorophyll (Gupta et al., 2011; Thu Hoai et al., 2003).

4.2. Effects of Heavy Metal Stress on Osmoregulators

Plants subjected to heavy metal stress and other adversities will rapidly accumulate osmoregulatory substances such as proline, soluble proteins, and soluble sugars (Zhang et al., 2016), which are used for scavenging reactive oxygen radicals, maintaining cell membrane stability, storing energy, etc., in order to satisfy the normal growth and metabolism of the plant (Zhang et al., 2019), and also indirectly reflect the degree of cellular damage (Dai et al., 2017). Accumulation of proline is considered to be an indication of response to stress, which plays a protective role in the biofilm (Javed et al., 2017; Hui et al., 2018), and the higher the proline content, the stronger the resistance (Dai et al., 2006; Gupta et al., 2020). In this study, the proline content of both L and S was elevated compared with the control, and the proline content of S was higher than that of L. The changes in soluble sugar content of the two varieties varied considerably, with the soluble sugar content increasing with the increase of Cd concentration in H, and decreasing with the increase of Cd concentration in F. This suggests that there are some differences in the effects of Cd on the soluble sugar content of different plants. In addition, soluble proteins also play a crucial role in responding to adversity, and in this study, the soluble protein content in F was higher and differed significantly from that in H, which was similar to the study by Wenying Liu et al. (2014). The reason for the existence of the difference is because Cd^{2+} enters into different varieties of cabbage and reduces the toxicity of Cd by stimulating the protein to bind Cd, which is used to resist Cd toxicity (Kang et al., 2021), thus F is more resistant to Cd.

4.3. Effect of Heavy Metal Stress on the Antioxidant System

Under adversity, plants adapt to adversity by increasing the activity of antioxidant enzymes, thereby reducing the damage. This ability of plants to scavenge reactive oxygen species is limited, and the level of its capacity is the key to the strength of plant resistance (Xu et al., 2006; Shafi et al., 2015). Excess heavy metals produce ROS through the tonofen reaction and membrane peroxidation, resulting in the accumulation of MDA and oxidative damage term (Xiang et al., 2023). Under Cd and Pb stress, the MDA content of the leaves of the four cabbage species showed different degrees of increase, and the higher the concentration, the higher the content, indicating that the degree of cell membrane lipid peroxidation injury increased, and the plant's ability to scavenge reactive oxygen species has been reduced, which is the same as the conclusions of Guo et al. (2017) on the MDA of the five species of ornamental plants under short-term stress of heavy metals. It has been shown that superoxide radicals generated under stress conditions can be disproportionated by SOD to produce hydrogen peroxide, which can be scavenged by CAT and POD (Zhu et al., 2021). The changes in antioxidant enzyme activities of different plants for heavy metal stress are not consistent, Cui et al. (2022) found that Cd stress led to a decrease in CAT and POD activities and an increase in SOD activity, followed by a decrease in SOD activity in the leaves of meadow morning glory. Wang & Wang (2020) found that the SOD, POD, and CAT activities of the plants of Rendang bean seedlings under heavy metal stress increased at low concentrations of heavy metal stress, and the enzyme activities gradually decreased at high concentrations of heavy metal stress. In the present study, the stress of both heavy metals promoted the CAT, POD, and SOD contents of cabbage at the initial stage, but the activities of antioxidant enzymes gradually decreased with the increase in heavy metal concentration. Among them, CAT, SOD, and POD activities of H varieties under Cd stress reached their maximum values at 20 mg/L, while those of F varieties reached their maximum values at 40 mg/L. In the L variety under Pb stress, CAT and SOD activities reached maximum at 600 mg/L and POD at 300 mg/L, while in the S variety, SOD reached maximum at 300 mg/L and CAT and POD at 600 mg/L.

The earlier peak of antioxidant enzymes in low-accumulation varieties is a key physiological strategy for adapting to heavy metal stress, reflecting both rapid defense capabilities and potentially indicating the risk of premature aging (Zhou et al., 2013). In variety selection, enzyme activity dynamics (peak intensity and time), TF, and biomass indicators should be combined to comprehensively evaluate the repair potential and agronomic suitability of varieties (Ali et al., 2020). Future research should delve into the regulatory networks governing antioxidant enzyme gene expression to identify targets for molecular-assisted breeding.

5. Conclusion

This study investigated the effects of chlorophyll content, osmoregulatory substances, antioxidant enzyme activities, and other physiological and biochemical indexes of Cd- and Pb-sensitive and Pb-tolerant cabbage varieties under different concentrations of Cd (0, 20, 40, 60, and 80 mg/L) and Pb (0, 300, 600, 900, and 1200 mg/L) treatments to reveal the physiological and biochemical mechanisms of cabbage genotypes in response to Cd and Pb stress, revealing the physiological response mechanisms of different genotypes of cabbage in response to Cd and Pb stress. The main conclusions are as follows:

1. Under Cd and Pb stress, cabbage was able to resist heavy metal stress by increasing the contents of osmoregulatory substances (soluble sugars, soluble glycoproteins, and proline) and the activities of antioxidant enzymes (MDA, POD, CAT, SOD) in leaves.

2. However, as the concentration of heavy metals increases, the physiological and metabolic activities of cabbage leaf cells are affected and their functions are

impaired, and the activities of POD, CAT, and SOD, as well as the content of osmoregulatory substances, will subsequently decrease.

3. The osmoregulatory substances in low Cd- and Pb-accumulating varieties were more stable, and the activities of POD, CAT, and SOD peaked first in most of the varieties with high accumulation.

These findings provide new perspectives for understanding the response mechanism of cabbage to heavy metal stress, and provide ideas for breeding cabbage varieties with high tolerance to heavy metal stress.

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CRediT Authorship Contribution Statement

Shiqi Peng: Conceptualization**,** methodology, validation, writing—review and editing. **Hao Zhang:** validation, investigation, writing—original draft preparation, visualization. **Liyuan Mu:** Formal analysis, Validation. **Junlei Wang:** Investigation, Software. **Sijing Sun:** Investigation, Validation. **Ao Li:** Validation. **Naiming Zhang:** Resources, Project administration, Funding acquisition. **Li Bao:** Conceptualization, Data curation, Writing—review and editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that support the findings of this study are available upon reasonable request to the authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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