Asian Agricultural Research 2026, 18 (6): 13 - 21, 25 DOI: 10.19601/j.cnki.issn1943 - 9903.2026.06.002

Effects of Arsenic and Cadmium Stress on the Quality and Safety of Using Forage Mulberry Leaves

Lanxin WEI, Yuting LIANG, Yunqi ZHONG, Lijuan HUANG, Yuelian TAN, Yongrong QIN*, Xuhui LIU*

School of Chemistry and Bio-engineering, Hechi University, Hechi 546300, China

Abstract [**Objectives**] To investigate the effects of single and combined stress of As^{5+} and Cd^{2+} on the quality and safety of using forage mulberry leaves. [**Methods**] Using the mulberry cultivar 'Yuesang 51' as the experimental material, a hydroponic experiment was conducted with single and combined As^{5+} and Cd^{2+} stress at concentration gradients ranging from 0 to 100 mg/L. The contents of total chlorophyll, soluble sugar, soluble protein, and malondialdehyde (MDA) in the leaves of mulberry seedlings were measured. Arsenic contents and cadmium contents in the roots, stems, leaves, and branches of the seedlings were determined by atomic fluorescence spectrometry (AFS) and atomic absorption spectrometry (AAS), respectively. [**Results**] The severity of effects on mulberry seedlings under different heavy metal stress treatments followed the order: combined $As^{5+}-Cd^{2+}$ stress > single Cd^{2+} stress > single As^{5+} stress. Single stress of low-concentration As^{5+} or Cd^{2+} promoted physiological metabolism in mulberry leaves and increased the contents of soluble sugar and soluble protein. In contrast, combined $As^{5+}-Cd^{2+}$ stress inhibited physiological metabolism in mulberry leaves and markedly decreased the soluble protein content. Mulberry seedlings showed a relatively strong capacity for Cd^{2+} uptake. For both As and Cd, the contents in different plant parts were consistently ranked as root > stem > leaf > branch. Under combined stress, Cd content decreased while As content increased. The arsenic content in mulberry leaves was relatively low, but the cadmium content exceeded the national limit for plant-derived feed ingredients. [**Conclusions**] As^{5+} and Cd^{2+} stress exerts a considerable effect on the quality of forage mulberry leaves. Under both single and combined stress, when the concentrations of As^{5+} and Cd^{2+} in the solution exceed a certain range, the cadmium content in the leaves significantly exceeds the national limit, potentially posing a safety risk for their use as feed.

Key words As^{5+} and Cd^{2+} stress, Mulberry seedlings, Hydroponic experiment, Forage mulberry leaves, Safe utilization

0 Introduction

Mulberry (*Morus alba* L.) is an important cash crop and an excellent tree for landscaping. The nutrient-rich leaves represent a valuable feed resource[1-2], while the fruits are both edible and medicinal, providing notable health benefits[3-4]. The wood is suitable for furniture making, and the extensive root system makes mulberry an outstanding plant for soil and water conservation and the control of rocky desertification, offering considerable ecological and economic returns[5].

Northwest Guangxi is a major sericulture-producing region in China. Historically, this region has suffered from relatively serious heavy metal pollution due to various factors[6], which has significantly affected both the quality of forage mulberry leaves and human health. Mulberry possesses strong heavy metal tolerance and a certain uptake capacity, and is therefore regarded as an excellent plant resource for remediating heavy metal-contaminated soil. Studies have found that low concentrations of Cd^{2+} and Pb^{2+} can promote mulberry seed germination, whereas high concentrations inhibit growth[7]; even in soil contaminated with Pb^{2+} and Cd^{2+}, mulberry is able to maintain a relatively high biomass[8-9]. Under single-ion stress, mulberry can withstand a certain level of Sb^{3+} or Sb^{5+}, but high antimony stress suppresses chlorophyll synthesis[10]. Moreover, combined Mn^{2+} and Cd^{2+} stress can intensify chlorophyll degradation in mulberry leaves[11]. In addition, mulberry leaf extracts are capable of adsorbing heavy metals from industrial wastewater, which can be applied in water pollution control[12].

Although considerable progress has been made in mulberry research[13], studies on the safe utilization of forage mulberry leaves under combined heavy metal pollution remain scarce in the literature[14-15]. This study aimed to investigate, through an artificially simulated hydroponic experiment, the tolerance of mulberry seedlings to single and combined stress of As^{5+} and Cd^{2+}, as well as the distribution of arsenic and cadmium within the seedlings, so as to provide a scientific basis for the remediation of heavy metal-contaminated soil and the sustainable development of the sericulture industry in Northwest Guangxi.

1 Materials and methods

1.1 Screening and treatment of mulberry seedlings Based on literature review and field investigation, seedlings of the locally widely cultivated mulberry cultivar Yuesang 51 were selected as the experimental material. Newly purchased leafless mulberry seedlings were subjected to a certain pretreatment. First, the seedlings were planted in heavy metal-free soil, cultivated for 10 - 15 d, and periodically sprayed with deionized water. After

---

Received: April 2, 2026 Accepted: May 13, 2026

Supported by Innovation Training Program for College Students of Guangxi Zhuang Autonomous Region (202510605028); Project of Guangxi Modern Sericulture and Silk Collaborative Innovation Center (2022GXCSSC18); and the Northwest Guangxi Local Resources Conservation and Utilization Engineering Center (Gui Jiao Ke Yan [2012] 9).

Lanxin WEI, bachelor's degree, research fields: environmental chemical analysis and research on quality and safety of characteristic agricultural products in Northwest Guangxi. * Corresponding author. Yongrong QIN, master's degree, professor, research fields: soil heavy metal pollution control and ecological restoration; Xuhui LIU, master's degree, professor, research fields: the analysis and inspection of agricultural and sideline products and food safety research.

the seedlings sprouted and developed tender leaves, those showing good growth and uniform size were selected, carefully uprooted, washed with tap water to remove surface soil, then rinsed multiple times with purified water, and finally rinsed three times with deionized water and set aside for use.

1.2 Hydroponic experiment The pretreated mulberry seedlings were acclimated in a modified Hoagland nutrient solution[16] for 7 d. They were then subjected to single and combined stress treatments with As^{5+} and Cd^{2+} at concentration gradients of 0, 25, 50, 75, and 100 mg/L for 21 d, with the nutrient solution renewed every 7 d. The concentrations of As^{5+} and Cd^{2+} in the solution were calculated based on the arsenate ion and cadmium ion contents in $Na_3AsO_4 \cdot 12H_2O$ and $Cd(NO_3)_2$, respectively.

1.3 Collection and treatment of experimental samples After the hydroponic experiment, mulberry seedlings treated with different concentrations of As^{5+} and Cd^{2+} were collected. An appropriate amount of mature leaves was taken from the seedlings in each treatment group, washed clean, and stored in a refrigerator at 3 ℃ for the determination of chlorophyll, soluble sugar, soluble protein, and Malondialdehyde (MDA) contents. The remaining plant material was washed, dried, ground, sieved, and stored in labeled self-sealing plastic bags in a cool, dark place for the determination of arsenic and cadmium contents.

1.4 Determination of physiological indicators of nutrient metabolism in mulberry leaves MDA content was determined by the thiobarbituric acid (TBA) method[17]; chlorophyll content was measured by extraction using a mixture of acetone, absolute ethanol, and water[18]; soluble sugar content was determined by the anthrone colorimetric method[19]; and soluble protein content was determined by the Coomassie Brilliant Blue G-250 staining method[20].

1.5 Determination of arsenic and cadmium contents The cadmium content in mulberry samples was determined by mixed acid digestion-atomic absorption spectrometry (AAS)[21]; the arsenic content was determined by aqua regia digestion-atomic fluorescence spectrometry (AFS)[22]. To ensure data accuracy, the stability of the instruments was checked using quality control samples after every 15 sample measurements.

1.6 Scanning electron microscopy observation and X-ray energy dispersive spectroscopy analysis of different parts of mulberry seedlings The preparation of mulberry seedling samples for scanning electron microscopy and X-ray energy dispersive spectroscopy (SEM-EDS) analysis, as well as the analysis of arsenic and cadmium contents in different micro-areas, was performed according to references [23] and [24].

1.7 Data processing Three replicates were performed for each sample determination. The results are expressed as mean ± standard deviation (SD). Data were analyzed and processed using SPSS 27.0 and Excel 2010, and figures were plotted with Origin 2024. The least significant difference (*LSD*) method was used to analyze the significance of differences among experimental data. The bioconcentration factor (BCF) and translocation factor (TF) were calculated using the following formulas:

BCF = Heavy metal content in plant tissue/Heavy metal content in the growth medium (1)

TF = Heavy metal content in the aboveground part of the plant/Heavy metal content in the plant root (2)

2 Results and analysis

2.1 Physiological indicators of seedlings The measurement results of physiological indicators of nutrient metabolism in leaves of mulberry seedlings subjected to different concentrations of As^{5+} and Cd^{2+} stress are shown in Fig. 1 and Fig. 2. It can be seen that, compared with single As^{5+} or Cd^{2+} stress, the combined stress had a more pronounced effect on the physiological metabolism of mulberry seedlings. With increasing stress concentration, the contents of total chlorophyll, soluble sugar, soluble protein, and MDA in mulberry seedling leaves all showed obvious changes, and the effects of different stress modes on the measured physiological indicators differed significantly. Under high-concentration As^{5+} and Cd^{2+} stress, some of the measured physiological indicators may exhibit substantial changes, indicating that they were strongly affected.

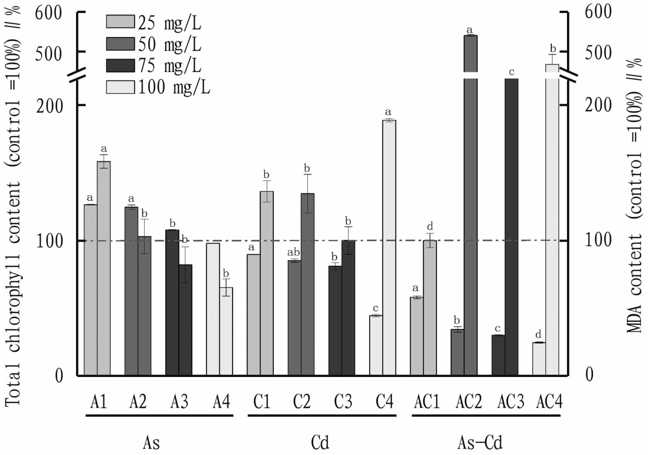


Fig. 1 Total chlorophyll and MDA relative content of mulberry seedlings under single or combined As^{5+} and Cd^{2+} stress

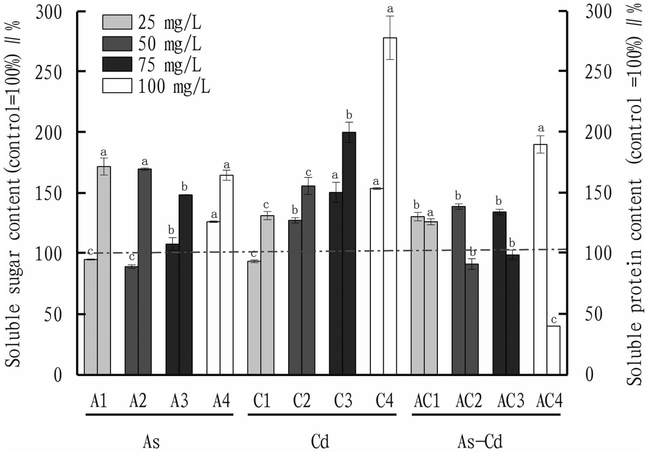


Fig. 2 Soluble sugar and soluble protein relative content of mulberry seedlings under single or combined As^{5+} and Cd^{2+} stress

2.2 Arsenic and cadmium contents in different parts of seedlings The results of arsenic and cadmium content determination in different parts of mulberry seedlings treated with different concentrations of As^{5+} and Cd^{2+} under single or combined stress are shown in Fig. 3 and Fig. 4. It can be seen that, under both single and combined stress, with increasing concentrations of As^{5+} and Cd^{2+}, their accumulation in different parts of the seedlings increased to varying degrees. The increases in roots and stems were more pronounced than those in branches and leaves, and the contents of arsenic and cadmium in different parts of the seedlings consistently followed the order: root > stem > leaf > branch. Compared with single stress, combined As^{5+} and Cd^{2+} stress resulted in a certain increase in arsenic content in different parts of the seedlings; conversely, combined stress led to a certain decrease in cadmium content. The former exhibited a synergistic effect, while the latter exhibited an antagonistic effect.

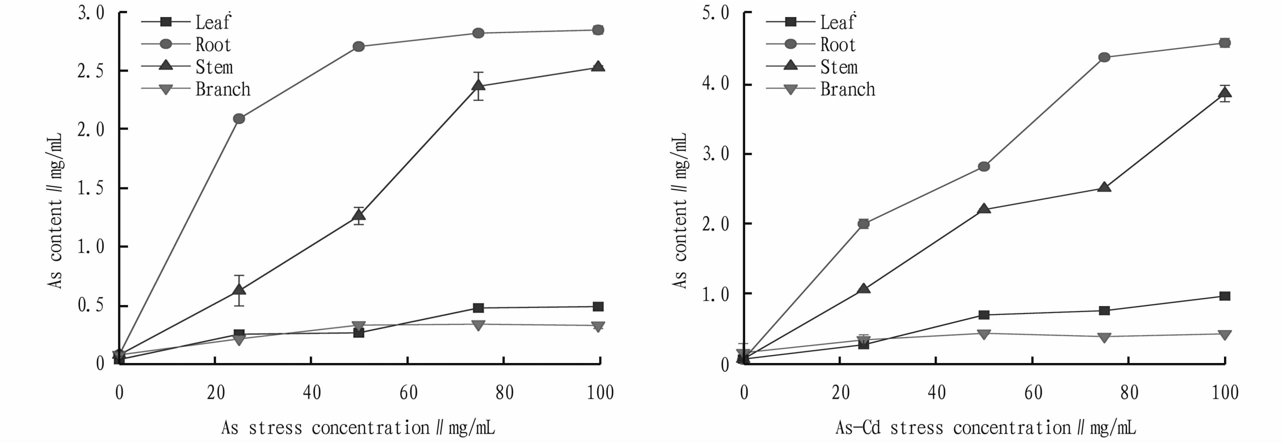


Fig. 3 Arsenic content in different parts of mulberry seedlings under single As stress or combined As and Cd stress

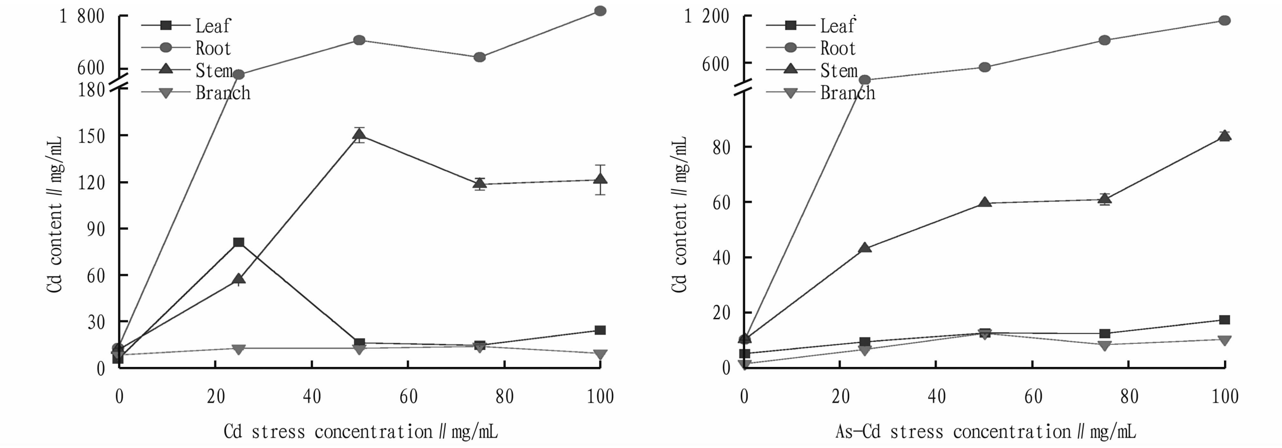


Fig. 4 Cadmium content in different parts of mulberry seedlings under single Cd stress or combined As and Cd stress

2.3 Scanning electron microscopy and X-ray energy dispersive spectroscopy analysis of different parts of seedlings Mulberry seedlings treated at a stress concentration of 100 mg/L were used for scanning electron microscopy and X-ray energy dispersive spectroscopy (SEM-EDS) analysis. Under combined arsenic and cadmium stress, the analysis results of arsenic and cadmium contents in micro-areas of roots, stems, leaves, and branches are shown in Fig. 5 – 7. Based on the analysis results of arsenic and cadmium contents (Wt%) in different micro-areas, the average arsenic and cadmium contents in the upper epidermis of mulberry leaves were 0.188% and 0.197%, respectively, and those in the lower epidermis were 0.147% and 0.413%, respectively; the average arsenic and cadmium contents in the roots of mulberry seedlings were 0.270% and 0.874%, respectively, those in the stems were 0.111% and 0.580%, respectively, and those in the branches were 0.078% and 0.744%, respectively. Under single arsenic stress, based on the analysis results of arsenic content (Wt%) in different micro-areas, the average arsenic content in the upper epidermis of mulberry leaves was 0.354%, and that in the lower epidermis was 0.146%; the average arsenic content in the roots of mulberry seedlings was 0.534%, that in the stems was 0.282%, and that in the branches was 0.456%. Under single cadmium stress, based on the analysis results of cadmium content (Wt%) in different micro-areas, the average cadmium content in the upper epidermis of mulberry leaves was 0.315%, and that in the lower epidermis was 0.278%; the average cadmium content in the roots of mulberry seedlings was 1.024%, that in the stems was 0.919%, and that in the branches was 0.855%. The analysis results indicate that although arsenic contents varied considerably among different parts of the seedlings, they were generally higher in the underground parts than in the aboveground parts. Compared with single arsenic or cadmium stress, the arsenic and cadmium contents under combined stress were lower, suggesting an antagonistic effect between the two metals. This finding differs slightly from the average arsenic and cadmium contents obtained by conventional detection methods, which may be attributed to the variation in content among different micro-areas.

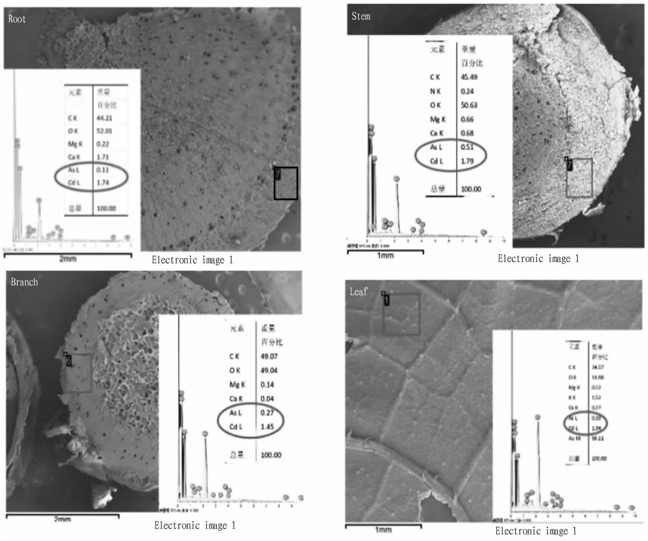


Fig. 5 Scanning electron microscopy and X-ray energy dispersive spectroscopy analysis of some micro-areas in different parts of mulberry seedlings under combined As^{5+} and Cd^{2+} stress

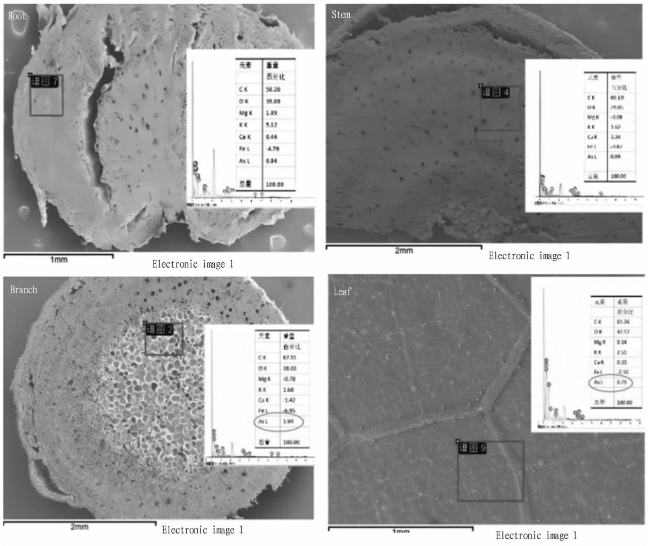


Fig. 6 Scanning electron microscopy and X-ray energy dispersive spectroscopy analysis of selected micro-areas in different parts of mulberry seedlings under single As^{5+} stress

2.4 Accumulation and translocation capacity of As in different parts of seedlings

The calculated BCF and TF of As in different parts of mulberry seedlings under different stress treatments are shown in Table 1. It can be seen that under single As^{5+} stress, the TF of As in the aboveground parts increased with increasing stress concentration. The roots were the main site of As accumulation, followed by the stems, while the leaves and branches exhibited relatively weak accumulation capacity. Under different concentrations of As^{5+} stress, no significant difference was observed in the BCF of As in the stems; however, the BCF in the roots and branches decreased significantly with increasing As^{5+} concentration. Under combined As^{5+} and Cd^{2+} stress, although the BCF of As in different parts of the seedlings showed a decreasing trend with increasing stress concentration, the BCF values in all parts were higher than those under single As^{5+} stress.

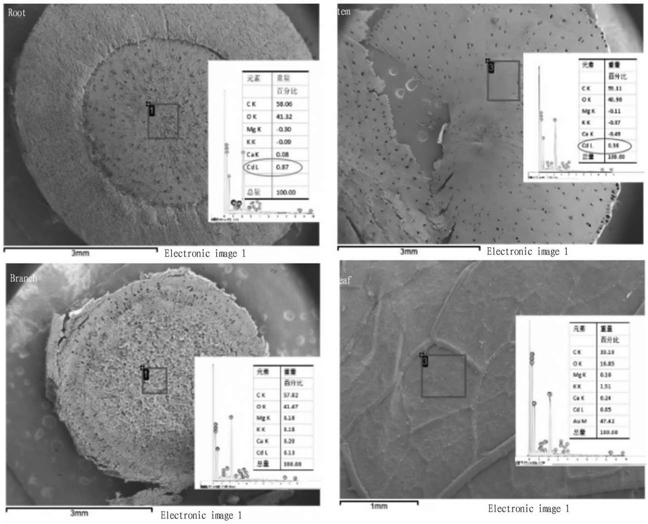


Fig. 7 Scanning electron microscopy and X-ray energy dispersive spectroscopy analysis of selected micro-areas in different parts of mulberry seedlings under single Cd^{2+} stress

2.5 Accumulation and translocation capacity of Cd in different parts of seedlings

The calculated BCF and TF of Cd in different parts of mulberry seedlings under different stress treatments are shown in Table 2. As can be seen, the accumulation and translocation characteristics of Cd in mulberry seedlings differed markedly under different stress conditions. Under both single Cd^{2+} stress and combined As^{5+} and Cd^{2+} stress, the translocation factor of Cd in the aboveground parts decreased with increasing stress concentration, and the Cd accumulation capacity in different parts of the seedlings consistently followed the order: root > stem > leaf > branch, with the root being the main organ of Cd accumulation. Under single Cd^{2+} stress, the BCF of Cd in leaves, stems, and branches all tended to decrease with increasing Cd^{2+} stress concentration; under the C2 stress treatment (likely referring to the Cd^{2+} single stress treatment at the second concentration level), the BCF of Cd in the root reached the highest value (24.926). Under combined As^{5+} and Cd^{2+} stress, the Cd accumulation in different organs of the seedlings was generally lower than that under single Cd^{2+} stress.

Table 1 Comparison of BCF and TF of As in mulberry seedlings under different stress concentrations

Treatment group		Stress concentration // mg/L		Aboveground part TF	BCF			
		As	Cd		Leaf	Root	Stem	Branch
Control	CK	0	0	0	0	0	0	0
As single stress	A1	25	0	0.529^{C}	0.010^{A}	0.084^{A}	0.025^{A}	0.009^{A}
	A2	50	0	0.691^{B}	0.005^{B}	0.054^{B}	0.025^{A}	0.007^{B}
	A3	75	0	1.133^{A}	0.006^{B}	0.038^{C}	0.032^{A}	0.005^{C}
	A4	100	0	1.184^{A}	0.005^{B}	0.028^{D}	0.025^{A}	0.003^{D}
As – Cd combined stress	A1C1	25	25	0.821^{b}	0.011^{b}	0.081^{a}	0.042^{a}	0.012^{a}
	A2C2	50	50	1.183^{a}	0.014^{a}	0.056^{b}	0.044^{a}	0.009^{b}
	A3C3	75	75	0.839^{b}	0.010^{c}	0.058^{b}	0.033^{c}	0.005^{c}
	A4C4	100	100	1.145^{a}	0.010^{c}	0.046^{c}	0.038^{b}	0.004^{d}

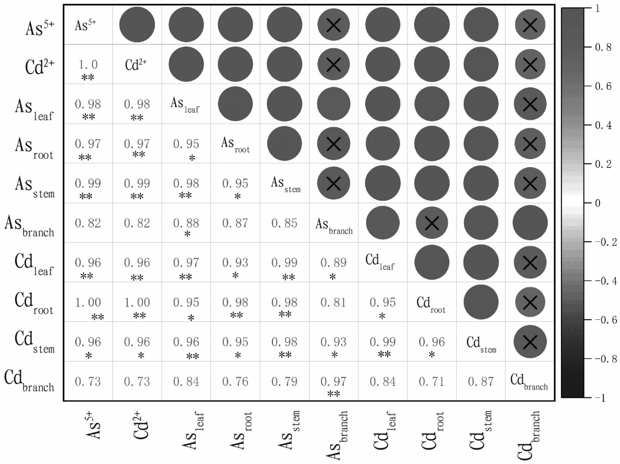
NOTE Different uppercase letters in the same column indicate significant differences among different treatments within the single stress group ($P<0.05$); different lowercase letters in the same column indicate significant differences among different treatments within the $As^{5+}-Cd^{2+}$ combined stress group ($P<0.05$). The same below.

Table 2 Comparison of BCF and TF of Cd in mulberry seedlings under different stress concentrations

Treatment group		Stress concentration // mg/L		Aboveground TF	BCF			
		As	Cd		Leaf	Root	Stem	Branch
Control	CK	0	0	0	0	0	0	0
Cd single stress	C1	0	25	0.322^{A}	3.246^{A}	18.721^{B}	2.275^{B}	0.505^{A}
	C2	0	50	0.144^{C}	0.322^{B}	24.926^{A}	3.006^{A}	0.252^{B}
	C3	0	75	0.171^{B}	0.195^{D}	11.496^{C}	1.581^{C}	0.185^{C}
	C4	0	100	0.081^{D}	0.243^{C}	19.101^{B}	1.214^{D}	0.094^{D}
As – Cd combined stress	A1C1	25	25	0.157^{a}	0.381^{a}	15.122^{a}	1.722^{a}	0.272^{a}
	A2C2	50	50	0.156^{a}	0.255^{b}	10.843^{b}	1.189^{b}	0.252^{a}
	A3C3	75	75	0.092^{c}	0.167^{c}	11.888^{d}	0.811^{c}	0.113^{b}
	A4C4	100	100	0.097^{b}	0.175^{c}	11.455^{c}	0.836^{c}	0.104^{b}

2.6 Correlation analysis

2.6.1 Correlation of arsenic and cadmium contents in different parts of seedlings. The results of the correlation analysis of arsenic and cadmium contents in different parts (roots, stems, branches, leaves) of mulberry seedlings under different stress conditions are shown in Fig. 8, Table 3 and Table 4. As can be seen, under combined arsenic and cadmium stress, the arsenic content and cadmium content in the roots, stems, leaves, and branches of mulberry seedlings showed a highly significant positive correlation. Additionally, the arsenic content in the roots was significantly positively correlated with the arsenic and cadmium contents in the stems, and highly significantly positively correlated with the cadmium content in the roots. The arsenic and cadmium contents in the branches showed some correlation with those in the roots and stems, but the correlations were not significant. Under single arsenic stress, the arsenic content in the leaves was highly significantly positively correlated with that in the stems, and significantly positively correlated with that in the roots and branches. The arsenic content in the branches was highly significantly positively correlated with that in the roots. The arsenic content in the stems was positively correlated with that in the roots and branches to a certain extent, but not significantly. Under single cadmium stress, the cadmium content in the leaves was negatively correlated with that in the roots and stems, but not significantly. The cadmium content in the branches was positively correlated with that in the roots, stems, and leaves, but the correlations were not significant.



NOTE ① * indicates $P<0.05$, significant correlation; ** indicates $P<0.01$, highly significant correlation. ② "As_{leaf}" denotes the arsenic content in the leaves of mulberry seedlings, and the same naming convention applies to the other parts. ③ Cd_{leaf} denotes the cadmium content in the leaves of mulberry seedlings, and the same naming convention applies to the other parts. The same below.

Fig. 8 Correlation analysis of arsenic and cadmium contents in different parts of mulberry seedlings under combined As^{5+} and Cd^{2+} stress

2.6.2 Correlation between arsenic and cadmium contents in seedling leaves and the measured physiological and metabolic indicators. The correlation analysis results between the arsenic and cadmium contents in the roots, stems, branches, and leaves of mulberry seedlings under different stress treatments and the measured physiological and metabolic indicators of the leaves are shown in Fig. 9, Table 5, and Table 6. Under combined As^{5+} and Cd^{2+} stress, the total chlorophyll content in the leaves of mulberry seedlings was highly significantly or significantly negatively correlated with the arsenic and cadmium contents in all parts of the seedlings. The soluble sugar content in the leaves was significantly positively correlated with the arsenic content in the stems and the cadmium content in the leaves, roots, and stems, and showed a certain but non-significant positive correlation with the arsenic and cadmium contents in other parts. The soluble protein content in the leaves was negatively but not significantly correlated with the arsenic and cadmium contents in different parts. The MDA content exhibited a strong or significant positive correlation with the arsenic and cadmium contents in different parts of the seedlings; among them, the arsenic content in the leaves and the cadmium content in the branches were significantly positively correlated with the MDA content. Under single As^{5+} stress, the total chlorophyll content in the leaves was significantly or highly significantly negatively correlated with the arsenic content in all parts. The soluble sugar, soluble protein, and MDA contents in the leaves showed certain positive or negative correlations with the arsenic content in different parts, but none were significant. Under single Cd^{2+} stress, the measured physiological indicators in the leaves all showed some correlation with the cadmium content in different parts of the seedlings. Specifically, the total chlorophyll content and MDA content in the leaves were significantly negatively correlated and significantly positively correlated, respectively, with the cadmium content in the stems, while the soluble sugar content in the leaves was significantly positively correlated with the cadmium content in the roots.

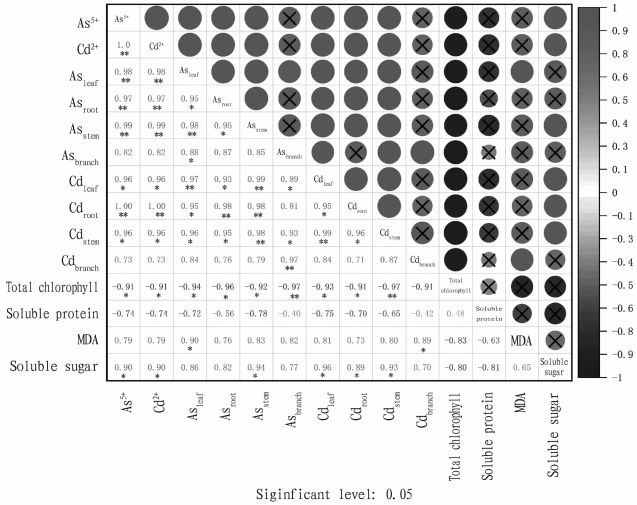


Fig. 9 Correlation between arsenic and cadmium contents in different parts of mulberry seedlings and measured physiological indicators of mulberry leaves under combined As^{5+} and Cd^{2+} stress

Table 3 Correlation of arsenic contents in different parts of mulberry seedlings under single As^{5+} stress

	As_{leaf}	As_{root}	As_{stem}	As_{branch}
As_{leaf}	1			
As_{root}	0.887 *	1		
As_{stem}	0.969 * *	0.823	1	
As_{branch}	0.882 *	0.977 * *	0.868	1

Table 4 Correlation of cadmium contents in different parts of mulberry seedlings under single Cd^{2+} stress

	Cd_{leaf}	Cd_{root}	Cd_{stem}	Cd_{branch}
Cd_{leaf}	1			
Cd_{root}	−0.119	1		
Cd_{stem}	−0.180	0.827	1	
Cd_{branch}	0.324	0.070	0.525	1

Table 5 Correlation between arsenic contents in roots, stems, branches, and leaves of mulberry seedlings and measured physiological indicators of mulberry leaves under single As^{5+} stress

	Total chlorophyll	Soluble sugar	Soluble protein	MDA
As_{leaf}	−0.919 *	0.802	−0.524	0.660
As_{root}	−0.986 * *	0.741	−0.346	0.763
As_{stem}	−0.891 *	0.801	−0.663	0.758
As_{branch}	−0.990 * *	0.718	−0.429	0.871

Table 6 Correlation between cadmium contents in roots, stems, branches, and leaves of mulberry seedlings and measured physiological indicators of mulberry leaves under single Cd^{2+} stress

	Total chlorophyll	Soluble sugar	Soluble protein	MDA
Cd_{leaf}	−0.038	0.074	0.456	−0.436
Cd_{root}	−0.866	0.951 *	−0.818	0.848
Cd_{stem}	−0.930 *	0.654	−0.458	0.954 *
Cd_{branch}	−0.545	−0.021	0.457	0.287

3 Discussion

3.1 Effects of As^{5+} and Cd^{2+} stress on the growth, development, and physiological metabolism of mulberry seedlings

Heavy metal elements have a considerable impact on plant growth and development. They can interfere with normal physiological activities through various pathways, thereby inhibiting plant growth, development, and physiological metabolism. Results from many related experimental studies both domestically and internationally indicate that low concentrations of heavy metals may promote plant growth to a certain extent[25], whereas high concentrations of heavy metals exhibit obvious inhibitory effects. Gao Hongpeng found that high concentrations of Cd^{2+} and Pb^{2+} stress inhibited the germination of mulberry seeds[26]. In other words, the effect of heavy metal stress on plant growth and development may exhibit a "low-concentration promotion and high-concentration inhibition" effect[27].

Chlorophyll is the photosynthetic pigment of plants. Related studies have found that with increasing Pb^{2+} content in soil, the total chlorophyll content of Chinese fir seedlings first increased and then decreased[28]. Zhang Jiatong *et al.* reported that combined stress of Pb^{2+} and Cd^{2+} inhibited the growth and photosynthetic efficiency of mulberry seedlings[29]. The present study found that the total chlorophyll content in mulberry seedling leaves was significantly or highly significantly negatively correlated with the arsenic and cadmium contents in the roots, stems, branches, and leaves of the seedlings, which is similar to the findings reported by Yu Xueze *et al.*[30].

Soluble sugar and soluble protein are the main osmoregulatory substances in plants. Under both single and combined stress of As^{5+} and Cd^{2+}, the soluble sugar content in mulberry seedling leaves showed an increasing trend with rising stress concentration. This stress-induced sugar accumulation may represent a self-protection mechanism of mulberry seedlings in response to heavy metal stress, helping to maintain cellular homeostasis in the short term[31]; however, a prolonged stress-induced high-sugar state could affect the nutritional balance of the leaves. Under single As^{5+} stress, the soluble protein content in the leaves changed to some extent with increasing stress concentration, but the difference was not significant. In contrast, under single Cd^{2+} stress, the soluble protein content increased markedly as the stress concentration rose, possibly due to the enhanced protein synthesis induced by Cd^{2+} stress[32], suggesting that within a certain concentration range, mulberry can cope with heavy metal stress through protein accumulation. Under combined As^{5+} and Cd^{2+} stress, the soluble protein content in the leaves showed a clear downward trend with increasing stress concentration, indicating that the combined stress may have an additive effect, impairing the protein synthesis function of mulberry seedlings and accelerating the degradation of existing proteins, thereby reducing the protein content[33].

In the present study, both single Cd^{2+} stress and combined As^{5+} and Cd^{2+} stress significantly increased the MDA content in mulberry leaves, which is consistent with the findings of Jiang N *et al.*[34]. When the concentration of the combined As^{5+} and Cd^{2+} stress reached 50 mg/L, the MDA content in mulberry seedling leaves was 469.01% of that of the control group. In contrast, under single As^{5+} stress, the MDA content in the leaves decreased gradually with increasing As^{5+} concentration, and the reason for this requires further investigation. The experimental results indicate that, compared with As^{5+} stress, Cd^{2+} exerted a greater toxic effect on the growth of mulberry, and the mulberry cultivar used in this study may possess relatively strong tolerance to As^{5+}.

3.2 Accumulation of arsenic and cadmium in different tissues and organs of mulberry seedlings In this study, the contents of arsenic and cadmium in different parts of mulberry seedlings under As^{5+} and Cd^{2+} stress consistently followed the order: root > stem > leaf > branch, indicating that mulberry seedlings can alleviate heavy metal toxicity by reducing their translocation to the aboveground parts and through leaf abscission[35]. However, under combined stress, the accumulation of As and Cd in the stems was significantly higher than that under single stress, suggesting that the two heavy metals may alter each other's translocation efficiency through competitive or synergistic interactions. The correlation analysis results showed that, under combined stress, the total chlorophyll content in mulberry leaves was significantly negatively correlated with the heavy metal content in various tissues and organs of the seedlings, indicating that heavy metal accumulation can directly inhibit the synthesis of photosynthetic pigments in plants[29], thereby affecting the nutrient metabolism of mulberry and the quality of mulberry leaves.

The SEM-EDS analysis results of arsenic and cadmium contents in different parts (roots, stems, branches, and leaves) of mulberry seedlings showed that the arsenic and cadmium contents differed significantly among the micro-areas, and within the same micro-area, the cadmium content was generally higher than the arsenic content. Overall, the arsenic and cadmium contents in the underground parts were markedly higher than those in the aboveground parts, which is consistent with the results obtained from conventional analysis and other related studies[36]. These findings suggest that the mulberry cultivar used in this study may be an arsenic excluder and a cadmium-tolerant plant[37-39]; however, specific issues require further investigation.

3.3 Translocation of arsenic and cadmium in mulberry and its implications for the feed safety of mulberry leaves The plant TF[40] and BCF[41] reflect the efficiency of heavy metal translocation from roots to aboveground parts and the plant's ability to accumulate heavy metals. In the present experiment, the translocation characteristics of As and Cd showed certain differences: As was more readily translocated from roots to aboveground parts, with TF values generally above 0.5, whereas the TF values of Cd in aboveground parts were generally below 0.5 and decreased with increasing Cd^{2+} concentration, indicating a pronounced "retention effect" of Cd^{2+} in the roots. This finding is similar to the results reported by Chen Chaoming[42]. Under both single and combined stress of As^{5+} and Cd^{2+}, the stem served as the main translocation pathway for heavy metal ions in mulberry seedlings, and its TF values were consistently higher than those of other organs.

Although the TF of As was generally higher than that of Cd, the absorption and accumulation capacity of mulberry seedlings for As^{5+} was far lower than that for Cd^{2+}. Under single Cd^{2+} stress, the BCF of the roots reached as high as 24.926. Although the As accumulation capacity of the roots was significantly greater than that of other parts, the highest BCF for As was only 0.084, and the As absorption capacity of the other parts was also far lower than that for Cd. This may be related to the uptake and translocation of different arsenic species in plants and the efflux of arsenic[43]. Kumar *et al.*[44] showed that mulberry absorbs arsenic mainly through the roots, but the absorption efficiency for As(III) is significantly higher than that for As(V). In the present experiment, As(V) was used as the stress source. Although many studies have demonstrated that plants possess a certain ability to reduce arsenic[45], the uptake of As(V) and its reduction capacity within mulberry seedlings under external As(V) supply require further verification.

According to *China's Hygienical Standard for Feeds* (GB 13078-2017)[46], the cadmium content in plant-derived feed ingredients must not exceed 1 mg/kg, and the arsenic content must not exceed 2 mg/kg. In the present experiment, under both single and combined stress treatments, the Cd content in mulberry leaves far exceeded the national feed standard limit. Under combined stress, although the As content in mulberry leaves was significantly higher than that under single As stress, even when both the As^{5+} and Cd^{2+} stress concentrations reached 100 mg/L, the maximum arsenic content in the leaves was only 0.964 2 mg/kg, which remained within the safe limit for feed use. In summary, despite the cadmium content exceeding the standard, the arsenic content in mulberry leaves under heavy metal stress still met the requirements of the Hygienical Standard for Feeds; however, greater attention should be paid to the risk of cadmium contamination. This study used 'Yuesang 51' as the experimental material, and the results may only reflect the characteristics of this specific cultivar. The general applicability of the findings needs to be further verified through multi-cultivar comparisons.

3.4 Remediation of heavy metal-contaminated soils using mulberry and safe utilization of forage mulberry leaves Mulberry has a strong absorption capacity for Cd^{2+}, with a translocation factor as high as 8.90, making it an excellent tree species for the remediation of Cd^{2+}-contaminated soils[47]. Huang Renzhi *et al.* found that in heavy metal-contaminated soils suitable for mulberry growth, the maximum contents of cadmium and lead could reach 39.25 and 601.69 mg/kg, respectively. When the leaf yield was reduced by 25%, the average tolerance thresholds of mulberry for cadmium and lead in soil were 40.88 and 527.00 mg/kg, respectively. Under high-concentration Cd^{2+} stress (>50 mg/kg), the cadmium contents in the leaves of different tested mulberry cultivars all exceeded the national limit for plant-derived feed ingredients[48], and the results obtained in this study are consistent with this finding. Therefore, the safety of using mulberry leaves as feed or food warrants careful attention when the trees are grown in cadmium-contaminated soil environments.

Eco-environmental protection is a hot topic of great public concern in contemporary society. In the process of using mulberry for phytoremediation of heavy metal-contaminated soils, ensuring the quality and safety of sericultural products while achieving desirable eco-economic benefits is a critically important issue. It has been reported that in some abandoned lead-zinc mining areas, *Miscanthus floridulus* and mulberry trees yield paper of good quality, with the heavy metal contents in the manufactured products meeting national standards[49]. Forage mulberry leaves serve as high-quality feed for silkworms, livestock, and poultry[1]. Tan Yongbi *et al.* investigated the feasibility of developing mulberry cultivation in heavy metal-contaminated farmland surrounding a mining area and found that the heavy metal-contaminated mulberry leaves exhibited relatively low biotoxicity to silkworms, that the sericultural products and their by-products were ecologically safe, and that the heavy metal contents complied with the relevant standards[14]. Therefore, planting mulberry and rearing silkworms on mining wastelands around mining areas can yield favorable ecological and economic benefits.

4 Conclusions

(i) As^{5+} and Cd^{2+} stress exerted a considerable impact on the growth, development, and physiological metabolism of mulberry. Owing to the synergistic effect between As^{5+} and Cd^{2+}, the impact of combined stress was significantly greater than that of single stress, manifesting as an additive effect. The severity of effects under different stress treatments followed the order: combined $As^{5+}-Cd^{2+}$ stress > single Cd^{2+} stress > single As^{5+} stress. Single stress of low-concentration As^{5+} or Cd^{2+} could promote, to a certain extent, the physiological metabolism of mulberry leaves and increase the contents of soluble sugar and soluble protein in the leaves. In contrast, combined $As^{5+}-Cd^{2+}$ stress inhibited nutrient metabolism in mulberry leaves and markedly decreased the soluble protein content.

(ii) Stress treatments with different concentrations of As^{5+} and Cd^{2+} influenced the arsenic and cadmium contents in mulberry seedlings. The seedlings showed a relatively strong absorption capacity for cadmium, with Cd contents far exceeding As contents. In all parts, both arsenic and cadmium contents consistently followed the order: root > stem > leaf > branch. Compared with single As^{5+} or Cd^{2+} stress, combined $As^{5+}-Cd^{2+}$ stress decreased the Cd content in different parts of the seedlings to a certain degree, while increasing the As content to some extent. That is, the combined stress exerted a synergistic promoting effect on As accumulation, but exhibited a certain inhibitory effect on Cd accumulation.

(iii) The arsenic content in the leaves of mulberry seedlings was relatively low, but the cadmium content had already exceeded the limit specified in the national feed standard, which warrants attention.

It can be concluded that As^{5+} and Cd^{2+} stress exerts a considerable impact on the quality of forage mulberry leaves. Under both single and combined stress, when the concentrations of As^{5+} and Cd^{2+} in the solution exceed a certain range, the cadmium content significantly surpasses the standard limit, potentially posing a safety risk for the use of the leaves as forage. Therefore, necessary measures should be taken to ensure the safe utilization of mulberry leaf products.

References

[1] YAN CH, CHEN FH, YANG YL, *et al.* Biochemical and protein nutritional potential of mulberry (*Morus alba* L.) leaf: Partial substitution improves the nutrition of conventional protein[J]. Journal of the Science of Food and Agriculture, 2024, 104(4): 2204-2214.

[2] GUO XQ, TANG CH, CHEN ZY, *et al.* The feeding value of mulberry leaves and their application in livestock and poultry production[J]. China Animal Husbandry & Veterinary Medicine, 2024, 51(10): 4301-4312. (in Chinese).

[3] ZOU YX, LIAO ST, XIAO GS, *et al.* Screening of superior mulberry va-

rieties based on edible value development[J]. Science of Sericulture, 2012, 38(5): 785 – 790. (in Chinese).

[4] CHAN CWE, WONG KS, TANGAH J, *et al*. Phenolic constituents and anticancer properties of *Morus alba* (white mulberry) leaves[J]. Journal of Integrative Medicine, 2020, 18(3): 189 – 195.

[5] LI HM, SUN ZM, LI Y. Suggestions for developing mulberry industry in Kashgar region[J]. Xinjiang Forestry, 2024(3): 23 – 24. (in Chinese).

[6] LI CZ. Study on heavy metal pollution and spatial distribution characteristics of paddy soil in Jinchengjiang District, Guangxi[J]. Journal of Dezhou University, 2021, 37(2): 51 – 58. (in Chinese).

[7] TANG LQ, XU ZL, CHEN RP, *et al*. Comprehensive evaluation of cadmium and lead tolerance of mulberry seed germination and seedling growth [J]. Journal of Jinggangshan University (Natural Science), 2024, 45 (6): 49 – 58. (in Chinese).

[8] H AM, IBRAHIM A, AF SE, *et al*. Removal of heavy metals from polluted aqueous media using berry leaf[J]. International journal of environmental analytical chemistry, 2023, 103(16): 4450 – 4466.

[9] WANG RK. Tolerance of cultivated plants to cadmium and their utilization in polluted farmland soils[J]. Engineering in life sciences, 2002, 22(1): 189 – 198.

[10] GENG LS, YANG ZF, XU ZN, *et al*. Effects of antimony stress on physiological indicators and accumulation and translocation characteristics of mulberry[J]. Journal of Agro-Environment Science, 2020, 39 (8): 1667 – 1674. (in Chinese).

[11] HAN SM, GAO RH, WANG AJ, *et al*. Effects of single and combined stress of manganese and cadmium on physiological and biochemical characteristics of mulberry[J]. Journal of Central South University of Forestry & Technology, 2023, 43(9): 164 – 170,198. (in Chinese).

[12] WANG L, JI G. Glutathione and calcium biomineralization of mulberry (*Morus alba* L.) involved in the heavy metal detoxification of lead-contaminated soil[J]. Journal of Soil Science and Plant Nutrition, 2021, 21(2): 1182 – 1190.

[13] TAGHIZADEH M, KAZEMI A. Potential health risk of heavy metals accumulation in cultivated mulberry in urban landscapes of arak, Iran: A case study[J]. Archives of Hygiene Sciences, 2019(4): 274 – 285.

[14] TAN YB. Feasibility study on developing mulberry planting industry in heavy metal-contaminated farmland around mining area[D]. Nanning: Guangxi University, 2008. (in Chinese).

[15] YU T. Transcriptome analysis of mulberry under zinc stress, cloning of related genes and expression analysis of differential genes[D]. Zhenjiang: Jiangsu University of Science and Technology, 2020. (in Chinese).

[16] LIU Y, PENG XD, LI Y. Effects of several hydroponic solutions on the growth of vetiver grass[J]. Agricultural Technology Service, 2016, 33 (13): 23 – 25. (in Chinese).

[17] TANG ZC. Experimental guide for modern plant physiology[M]. Beijing: Science Press, 1999. (in Chinese).

[18] CHI XX, GAO XY, ZHENG HX, *et al*. Effects of soda saline-alkali stress on MDA and chlorophyll contents in rice leaves[J]. Agriculture and Technology, 2023, 43(19): 38 – 40. (in Chinese).

[19] ZHANG XZ. Methods for crop physiology research[M]. Beijing: Agriculture Press, 1992: 139 – 145. (in Chinese).

[20] WANG XK. Principles and techniques of plant physiology and biochemistry experiments[M]. Beijing: Higher Education Press, 2006: 190 – 191. (in Chinese).

[21] LI YQ. Progress in the application of atomic absorption spectrometry in the analysis of heavy metals lead and cadmium[J]. Metallurgical Analysis, 2008, 28(6): 33 – 41. (in Chinese).

[22] ZHANG JR, YU XD, TU YF, *et al*. Instrumental analysis experiment (2^{nd} Edition)[M]. Beijing: Science Press, 2022. (in Chinese).

[23] LIU HK, MA R, ZHANG C. Study on dust-retention capacity of four street tree species in Lanzhou[J]. Tropical Agricultural Engineering, 2022, 46(6): 8 – 14. (in Chinese).

[24] LIU L, FANG YM, WANG SC, *et al*. Leaf micromorphology and characteristics of airborne particulate matter adsorption and heavy metal accumulation of seven tree species[J]. Environmental Science, 2013, 34 (6): 2361 – 2367. (in Chinese).

[25] LIU ZL, CHEN W, HE XY, *et al*. Effects of low-concentration cadmium on the growth and photosynthetic physiology of *Lonicera japonica* [J]. Environmental Chemistry, 2018, 37(2): 223 – 228. (in Chinese).

[26] GAO HP, ZHENG Z, LIU C, *et al*. Effects of cadmium and lead stress on seed germination, seedling growth and heavy metal accumulation of mulberry[J]. Journal of Anhui Agricultural Sciences, 2020, 48(11): 131 – 136. (in Chinese).

[27] SU S, HUANG JH, HE J, *et al*. Effects of cadmium stress on the growth of *Illicium verum* seedlings[J]. Chinese Journal of Tropical Agriculture, 2025, 45(10): 26 – 30. (in Chinese).

[28] GUAN P, HU HL, DAI DC, *et al*. Changes in growth and nutrient content and lead accumulation characteristics of Chinese fir seedlings under lead stress[J]. Journal of Northwest A&F University (Natural Science Edition), 2023, 51(2): 64 – 73. (in Chinese).

[29] ZHANG JT, GUAN YH, SI LQ, *et al*. Effects of combined Pb^{2+} and Cd^{2+} stress on photosynthesis of mulberry[J]. Journal of Beijing Forestry University, 2018, 40(4): 16 – 23. (in Chinese).

[30] YU XZ, GUO WZJ, SONG H. Tolerance, accumulation and translocation of *Iris ensata* under Zn stress[J]. Pratacultural Science, 2024, 41 (1): 67 – 76. (in Chinese).

[31] ZHOU Y. Effects of copper stress on chlorophyll and soluble sugar contents in *Conyza canadensis* in Daye Tonglushan[J]. Urban Geography, 2015(16): 225 – 226. (in Chinese).

[32] YIN K, ZHAO R, LIU Z, *et al*. Populus euphratica CPK21 interacts with heavy metal stress-associated proteins to mediate Cd tolerance in *Arabidopsis*[J]. Plant Stress, 2024: 11100328.

[33] SHANG YK, LIU SK, CHEN YH, *et al*. Effects of cadmium stress on the antioxidant system and soluble protein of Dongying wild soybean seedlings[J]. Journal of Sichuan Agricultural University, 2019, 37 (1): 15 – 21. (in Chinese).

[34] JIANG N, LI ZR, YANG JM, *et al*. Responses of antioxidant enzymes and key resistant substances in perennial ryegrass (*Lolium perenne* L.) to cadmium and arsenic stresses[J]. BMC plant biology, 2022, 22 (1): 145 – 145.

[35] WANG YX, ZHENG WY, HOU L, *et al*. Effects of combined stress of cadmium, lead and zinc on accumulation and translocation of cadmium in *Populus yunnanensis* seedlings[J]. Journal of Agro-Environment Science, 2023, 42(2): 310 – 318. (in Chinese).

[36] GOSWAMI, DAS S. Screening of cadmium and copper phytoremediation ability of *Tagetes erecta*, using biochemical parameters and scanning electron microscopy-energy-dispersive X-ray microanalysis[J]. Environmental Toxicology Chemistry, 2017, 36(9): 2533 – 2542.

[37] GHORI NH, GHORI T, HAYAT MQ, *et al*. Heavy metal stress and responses in plants[J]. International Journal of Environmental Science and Technology, 2019, 16: 1807 – 1828.

[38] CHI CN, DING GH. Research progress on molecular biology of plant tolerance to heavy metals[J]. Biotechnology Bulletin, 2017, 33(3): 6 – 11. (in Chinese).

(To page 25)

4 Conclusions

If the ripe fruit of sweet persimmon ‘Yangfeng’ is delayed for 15 – 20 d until early November, its total sugar content will significantly increase, and the frequency of main chromaticity will increase by 300%. The color will change from light yellow red to golden yellow, appearing more auspicious, bright and tempting, and the taste will become pleasant and sweet.

The fruits of delayed harvest have higher contents of calcium, magnesium, potassium, as well as vitamin C and protein, and they are all higher than the fruits during the normal harvest period. The nutritional quality of the fruits is significantly improved, which is more beneficial for human consumption and health care.

Production enterprises with the necessary conditions can appropriately delay the fruit harvesting period to around early November, which can achieve higher economic benefits while harvesting high-quality fruits[7–9].

This paper is reposted from the *Forest Science and Technology* in 2025, Issue 9, pages 107 – 109. Authors are Wu Haiyun, Wang Sanxing, Wang Chunmei, Hao Lilan, Zhang Shaobo, Zhang Xiaofei, Zhang Yanjun.

References

[1] ZHANG JW, ZHOU W, CAO QJ. Cultivation and management techniques for ‘Yangfeng’ sweet persimmons in Pingdingshan City[J]. Bulletin of Agricultural Science and Technology, 2020(9): 312 – 314. (in Chinese).

[2] LIU GG. Analysis on the development prospects of sweet persimmons in Guangxi[J]. Modern Agricultural Science and Technology, 2022 (2): 86 – 87, 91. (in Chinese).

[3] CHEN LY, SUN QH, SUN XH, *et al.* Comparative study on characteristics of Yangfeng and its bud variety Huangjin No. 1 sweet persimmon[J]. Journal of Shandong Forestry Science and Technology, 2021(1): 42 – 45, 48. (in Chinese).

[4] WENG HY. Cultivation techniques for the sweet persimmon variety ‘Yangfeng’: Adaptability research and application in Zhengzhou region [J]. Journal of Henan Forestry Science and Technology, 2020(2): 54 – 56. (in Chinese).

[5] ZHENG ZY. Analysis on the impact of fruit tree cultivation techniques and measures on fruits and quality[J]. Agricultural Development & Equipments, 2020(5): 101, 104. (in Chinese).

[6] LUO HJ, XIAO K, LIN YY, *et al.* Cultivation and management techniques for ‘Yangfeng’ sweet persimmon[J]. Fruit Growers’ Friend, 2024(2): 29 – 31. (in Chinese).

[7] DU GQ. Cultivation and management techniques for ‘Yangfeng’ sweet persimmons in Jingyang County[J]. Rural Science and Technology, 2023, 14(9): 96 – 98. (in Chinese).

[8] GUO DP. Cultivation techniques of ‘Yangfeng’ sweet persimmon in Yuncheng City[J]. Forestry of Shanxi, 2018(S1): 42 – 43. (in Chinese).

[9] YU QF. Research on the current status of soil nutrients and scientific fertilization in major persimmon orchards in Shaanxi Province[D]. Xianyang: Northwest A&F University, 2018. (in Chinese).

◆

(From page 21)

[39] LI Y, YU LJ, JIN XX. Tolerance mechanisms of plants to heavy metal stress[J]. China Biotechnology, 2015, 35(9): 94 – 104. (in Chinese).

[40] ZHOU Y. Tolerance and accumulation characteristics of *Leucaena leucocephala* under cadmium and copper stress[D]. Ya’an: Sichuan Agricultural University, 2014. (in Chinese).

[41] LI JH, WENG GY, WU HF, *et al.* Accumulation and translocation of lead and zinc in *Rumex acetosella* under different concentrations[J]. Jiangsu Agricultural Sciences, 2016, 44(7): 526 – 528. (in Chinese).

[42] CHEN CM, GONG HQ, WANG KR, *et al.* Uptake, accumulation and translocation of cadmium in mulberry-silkworm system[J]. Acta Ecologica Sinica, 1999, 19(5): 664 – 669. (in Chinese).

[43] YANG HC, FU HL, LIN YF, *et al.* Pathways of arsenic uptake and efflux[J]. Current Topics in Membranes, 2012, 69: 325 – 358.

[44] KUMAR A, SINGH RP, SINGH PK, *et al.* Arsenic uptake, toxicity and detoxification in mulberry (*Morus alba* L.) plants[J]. Environmental Science and Pollution Research, 2014, 21(16): 9940 – 9948.

[45] WANG JC, LI NN, XIE DT, *et al.* Research progress on absorption and metabolism mechanisms of arsenic in plants[J]. Chinese Bulletin of Botany, 2015, 50(4): 516 – 526. (in Chinese).

[46] National Feed Industry Standardization Technical Committee (SAC/TC 76). Hygienic Standard for Feeds: GB 13078-2017[S]. Beijing: China Standards Press, 2017. (in Chinese).

[47] LI GF, CHENG SQ. Absorption of soil heavy metals by trees around gold mines[J]. Journal of Northeast Forestry University, 2013, 41(1): 55 – 58. (in Chinese).

[48] HUANG RZ, LI YP, JIANG YB, *et al.* Effects of combined cadmium and lead stress on growth of mulberry seedlings and heavy metal content in mulberry leaves[J]. Science of Sericulture, 2018, 44(5): 665 – 671. (in Chinese).

[49] LIU XY, FANG DJ, WU LP, *et al.* Phytoremediation plants in heavy metal-contaminated soil of lead-zinc mining areas and their pulping and papermaking properties[J]. Transactions of China Pulp and Paper, 2018, 33(3): 14 – 19. (in Chinese).