

Effects of heavy metals Cu, Zn and its compound stress on physiological characteristics of *Cinnamomum camphora*

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Abstract: In order to investigate the effects of Cu, Zn and their complex stress on the physiological characteristics of *Cinnamomum camphora*, the seedlings of *C. camphora* were treated with different concentrations of Cu, Zn (0, 300, 600, 900, 1200, 1500 mg/kg) and their mixture (concentration of 300 mg/kg Cu + 300 mg/kg Zn, 300 mg/kg Cu + 600 mg/kg Zn, 600 mg/kg Cu + 300 mg/kg Zn, 600 mg/kg Cu + 600 mg/kg Zn). The results indicated that the transpiration rate, net photosynthetic rate and intercellular CO₂ concentration of *C. camphora* were significantly affected by Cu, Zn and its compound stress within one hour. With the increase of Cu or Zn concentration, the transpiration rate increased first and then decreased. At 1200 mg/kg, the transpiration rate reached the maximum. At the same concentration, the effect of Zn on net photosynthetic rate was more significant than that on Cu and Cu/Zn compound stress, and the effect of complex stress on intercellular CO₂ concentration was more significant than that of Cu and Zn. The CO₂ concentration reached the maximum at 900 mg/kg of Cu and the net photosynthetic rate reached the maximum at 1200 mg/kg of Cu. Under single Zn stress, the net photosynthetic rate reached the maximum at 900mg/kg. Under Cu/Zn stress, The effects of Cu on transpiration rate, intercellular CO₂ concentration and net photosynthetic rate of *C. camphora* were significantly higher than that of Zn concentration.

Introduction

Many heavy metals act as micronutrients and are essential for proper growth and development of plants. But these micronutrients also exhibit cytotoxic effects when accumulated in larger quantities [1]. Heavy metal toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons [2]. Zinc and copper are micronutrient elements required for the biological activity of proteins and enzymes as cofactors[3] and contributes to vital growth processes such as photosynthesis [4]. They can still be toxic to plants if present at extreme concentrations, and they are not biodegradable [5]. The effects on plants of heavy metals are a topic of many research projects in recent years. Most of the research related to model plant species such as *Arabidopsis* [6], *Oryza sativa* L.[7], *Gossypium hirsutum* L.[8], and *Zea mays* L. [9]. However, little is known about the effects of Zinc and copper on the physiological characteristics of *Cinnamomum camphora*.

In this paper, we aimed to investigate the effects of different concentrations of Cu, Zn and their complex stress on the physiological characteristics of *C. camphora*.

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Method

Plant material and growth conditions.

The experiment was carried out in a plastic flowerpot with the diameter of 20 cm and the height of 15 cm. In the experiment, *C. camphora* seedlings were cultivated for the same batch of seeds. Physical and chemical properties of cultivated soil such as Table 2.1. The experimental plantlets were cultured in a greenhouse with proper humidity and temperature of about 25 °C.

Tab. 2.1 Physical and chemical properties of cultivated soil

pH value	organic matter (g/kg)	Total nutrient (g/kg)			available nutrient (mg/kg)		
		nitrogen	phosphorus	potassium	nitrogen	phosphorus	
		potassium					
7.90	10.8	0.85	0.83	25.64	64.6	3.6	118.2

The experiments of copper and zinc stress were divided into four groups: (1) The control group was treated with distilled water with the concentration of copper and zinc as 0. (2) The group was sprayed with a single Cu^{2+} with a concentration of 300mg/kg, 600mg/kg, 900mg/kg, 1200mg/kg, 1500mg/kg respectively. (3) The group was sprayed with a single Zn^{2+} stress with a concentration of 300mg/kg, 600mg/kg, 900mg/kg, 1200mg/kg, 1500mg/kg respectively. (4) The compound stress of copper and zinc was used with ion concentration of 300mg / kg Cu^{2+} +300mg / kg Zn^{2+} , 300mg/kg Cu^{2+} +600mg/kg Zn^{2+} , 600mg/kg Cu^{2+} +300mg/kg Zn^{2+} , 600mg / kg Cu^{2+} +600mg / kg Zn^{2+} respectively spraying leaves of experimental seedlings.

The transpiration rate (E), net photosynthetic rate (A) and intercellular CO_2 concentration (ci) of seedling were measured with GFS - 3000 photosynthetic apparatus after spraying 20、40、60minutes respectively.

Statistical analysis

Statistical analyses were performed using ANOVA (IBM SPSS Statistics21), Mapping analysis using GraphPad Prism6.

Results

Effects of Cu^{2+} stress on transpiration rate of *C. camphora* seedlings

After 20 minutes of Cu^{2+} stress, the transpiration rate of *C. camphora* seedlings was lower than that of control group ($1.010278 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), indicating that the transpiration rate of *C. camphora* seedlings was sensitive to Cu^{2+} stress. When the concentration of Cu^{2+} changes from 900mg/kg to 1200mg / kg, the leaf transpiration rate increased from $0.01425 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.66423 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. While the concentration of Cu^{2+} was 1500mg/kg, the transpiration rate decreased to $0.40245 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

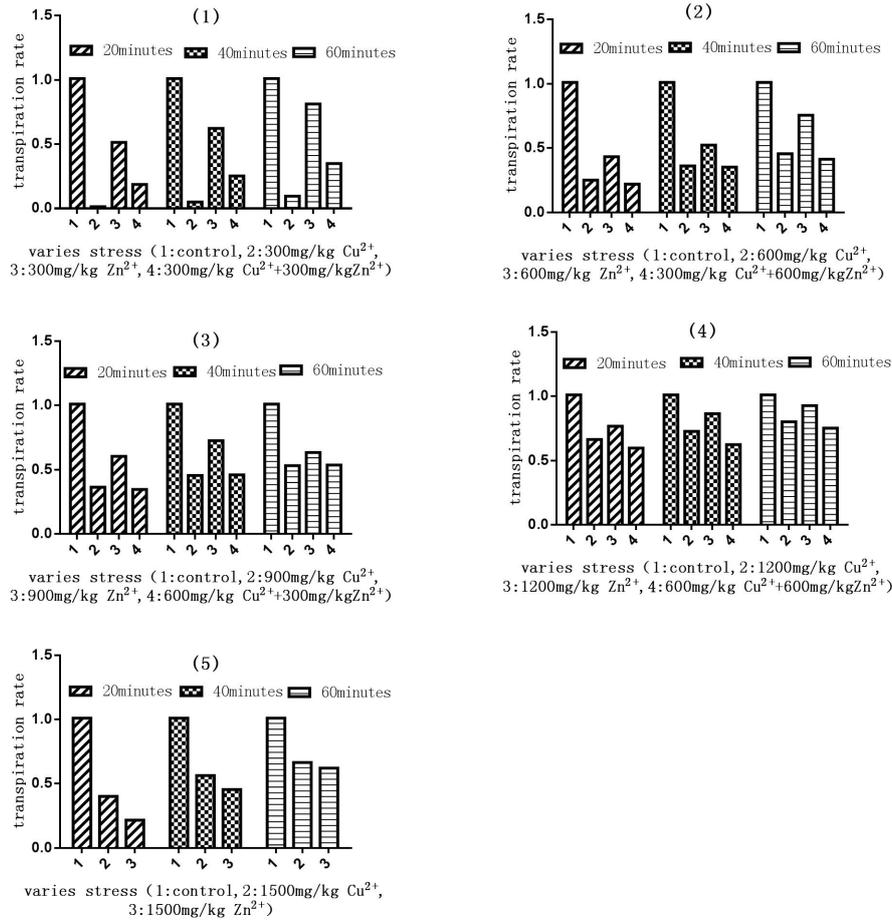


Fig. 1 The effect of different concentrations of Cu²⁺, Zn²⁺ and Cu²⁺ / Zn²⁺ on the transpiration rate of *C. camphora* seedlings

40 minutes later. The transpiration rate of leaves under stress increased from 0.0504 mmol·m⁻²·s⁻¹ to 0.72662 mmol·m⁻²·s⁻¹, and finally decreased to 0.56451 mmol·m⁻²·s⁻¹. After 60 minutes, the transpiration rate of the corresponding leaves was first increased from 0.09546 mmol·m⁻²·s⁻¹ to 0.801543 mmol·m⁻²·s⁻¹, and then down to 0.66551 mmol·m⁻²·s⁻¹. It shows that transpiration rate is less affected by stress. When the concentration of Cu²⁺ was 300mg / kg, the transpiration rate of *C. camphora* seedlings was 0.01425 mmol·m⁻²·s⁻¹. The transpiration rate was 0.01425 mmol·m⁻²·s⁻¹ in 20 minutes. It increased to a maximum of 0.09546 mmol·m⁻²·s⁻¹ in 40 minutes.

Effect of Zn²⁺ stress on transpiration rate of *C. camphora*

When single Zn²⁺ stresses *C. camphora* seedlings, its transpiration rate is less than that of the control group (1.010278 mmol·m⁻²·s⁻¹). The lowest transpiration rate is decreased to 0.76724 mmol·m⁻²·s⁻¹ (Zn²⁺200mg/kg) after 20 minutes. Maximum decrease is to 0.21689 mmol·m⁻²·s⁻¹ (Zn²⁺1500 mg / kg). After 40 minutes, the lowest transpiration rate decreased to 0.86461 mmol·m⁻²·s⁻¹ (Zn²⁺1200mg/kg), and the maximum decreased to 0.456149 mmol·m⁻²·s⁻¹ (Zn²⁺1500mg/kg). After 60 minutes, it decreased to 0.926425 mmol·m⁻²·s⁻¹ (Zn²⁺1200mg/kg) and maximum decrease to 0.622131 mmol·m⁻²·s⁻¹ (Zn²⁺15200mg/kg). When the stress concentration was 300 mg/kg, the Transpiration rate increased from 0.51399 mmol·m⁻²·s⁻¹ to 0.81342 mmol·m⁻²·s⁻¹. The effect of Zn²⁺ on transpiration rate was gradually weakened with time prolonging.

Effect of Cu/Zn compound stress on transpiration rate of *C. camphora*

After 20 minutes of 600mg/kg Cu²⁺+300mg/kg compound stress, the transpiration rate of *C. camphora* seedlings decreased the most that from 0.34653 mmol·m⁻²·s⁻¹ of control to 0.18741 mmol·m⁻²·s⁻¹. The secondly decreased transpiration rate to 0.219215 mmol·m⁻²·s⁻¹ by compound stress of 600mg/kg Cu²⁺+600mg/kg Zn²⁺. After 40 minutes, the minimum transpiration rate was 0.25346 mmol·m⁻²·s⁻¹ under the treatment of 600mg/kg Cu²⁺+300mg/kg Zn²⁺. After 60 minutes, the transpiration rate of *C. camphora* seedlings dealt with 300mg/kg Cu²⁺+600mg/kg Zn²⁺ was the highest (0.75162 mmol·m⁻²·s⁻¹). The minimum transpiration rate (0.34915 mmol·m⁻²·s⁻¹) caused by 600mg/kg Cu²⁺+300mg/kg Zn²⁺, which was also slightly higher than that of the control.

Effect of single Cu²⁺ stress on intercellular CO₂ concentration and net photosynthetic rate in *C. camphora*

After 20 minutes of stress with different concentrations of Cu²⁺, the concentration of intercellular CO₂ was always lower than that of the control (506.4156ppm). The concentration of intercellular CO₂ reached a minimum value of 101.4601ppm under the treatment of 900mg/kg Cu²⁺. The net photosynthetic rate of 1500mg/kg treatment decreased to a minimum of -1.0383μmol·m⁻²·s⁻¹. Under 300mg/kg stress, CO₂ concentration decreased by 63.8859ppm from 20 minutes to 40 minutes more than 40 minutes to 60minutes decline of 17.27422ppm. Between 20 and 40 minutes, the increase rate of net photosynthetic rate was greater than that of 40 minutes to 60 minutes. After 1500mg/kg stress for 20 minutes, the concentration of intercellular CO₂ reached a minimum of 233.59ppm. The net photosynthetic rate decreased to a minimum of -2.51245μmol·m⁻²·s⁻¹ after 60 minutes.

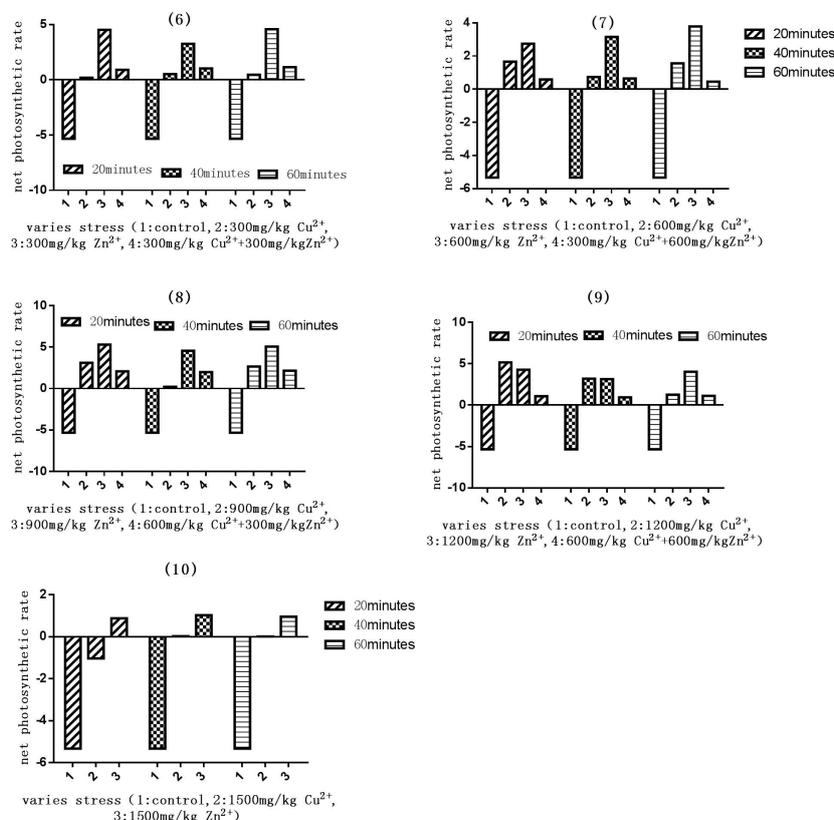


Fig. 2 The effect of different concentrations of Cu²⁺, Zn²⁺ and Cu²⁺ / Zn²⁺ on the net photosynthetic rate of *C. camphora* seedlings

The intercellular CO₂ concentration of *C. camphora* seedlings decreased and the net photosynthetic rate increased with the prolongation of stress time after 300mg/kg treatment.

However, with the increase of time of stress after other concentrations of Cu^{2+} treatment, the intercellular CO_2 concentration increased and the net photosynthetic rate decreased.

Effect of single Zn^{2+} stress on intercellular CO_2 concentration and net photosynthetic rate in *C. camphora*

After 20 minutes of single Zn^{2+} stress, the concentration of intercellular CO_2 was much lower than that of the control group (506.4156ppm), and with the increase of the Zn^{2+} concentration, the concentration of intercellular CO_2 increased from 92.80209 ppm to 245.8073 ppm. After treatment of 300mg/kg for 20 minutes, the concentration of intercellular CO_2 increased gradually, and it increased 17.45291ppm in 20-40 minutes more than 40-60 minutes that of increased by 5.06832ppm.

The net photosynthetic rate of *C. camphora* seedlings under different concentrations of Zn^{2+} for 20 minutes was lower than that of the control group $5.32672\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. It was at least $4.909172\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ treated with a concentration of 900 mg/kg. It decreased the maximum to $1.87550\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ treated by the concentration of 1500mg/kg. After the treatment of 300 mg/kg for 20 minutes, the net photosynthetic rate decreased gradually with the extension of time.

Effect of Cu^{2+} / Zn^{2+} stress on intercellular CO_2 concentration and net photosynthetic rate in *C. camphora*

After 20 minutes of Cu^{2+} / Zn^{2+} stress, the intercellular CO_2 concentration decreased from 223.5611ppm. Among them, 600mg/kg Cu^{2+} +300mg/kg Zn^{2+} decreased the most to 187.6236ppm. Then that was the treatment of 300mg/kg Cu^{2+} +300mg/kg Zn^{2+} . After 20 minutes of Cu^{2+} / Zn^{2+} compound stress, the net photosynthetic rate was less than the control value ($5.32672\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and 300mg/kg Cu^{2+} +600mg/kg Zn^{2+} treatment decreased the largest. The effect of Cu^{2+} / Zn^{2+} compound stress on intercellular CO_2 concentration of *C. camphora* seedlings decreased with time. Such as 300mg/kg Cu^{2+} +300mg/kg Zn^{2+} treatment for 60 minutes, the concentration of intercellular CO_2 increased to 235.9544 ppm. Cu^{2+} / Zn^{2+} compound stress decreased the net photosynthetic rate of *C. camphora* seedlings, and generally decreased rapidly within 20-40 minutes. Such as under the stress of 300mg/kg Cu^{2+} +300mg/kg Zn^{2+} , the net photosynthetic rate decreased by 0.06034 in 40-60 minutes less than 0.1072 in 20-40 minutes. The higher concentration of Zn^{2+} did the greater influence of the intercellular CO_2 concentration on the *C. camphora* seedlings. The higher concentration of Cu^{2+} did the greater effect on the net photosynthetic rate of *C. camphora* seedlings.

Discussion

Copper and zinc, as trace elements for plant growth, have the ability to promote plant growth [3] when the content of copper and zinc is low in the environment. Therefore, the physiological status of plants, such as transpiration, net photosynthesis rate and so on, showed a normal increase. In this experiment, the physiological state of camphor seedling was gradually recovered from stress for about 1 hour, which was related to the trace element of Cu and Zn in plants [3]. the relative utilization of the cover plant makes the excessive content of heavy metal Cu and Zn as the pollution source decrease, so the physiological index value measured is close to that of the control group step by step. When plants grow vigorously, their physiological activities are exuberant, and they need more copper and zinc, which are used as trace elements to supply plants. Therefore, the resistance of plants to heavy metal stress will be enhanced at this time. Perennial habit of camphor tree makes it resistant to heavy metal pollution such as copper and zinc and annual *O. sativa* [7], *Arabidopsis* [6], *Z. mays* [9]. There are obvious differences in the growth of camphor seedlings caused by copper and zinc pollution, and the time required to return to normal growth is short after the growth of camphor seedlings with heavy metal copper and zinc pollution. At the same time, the difference of

pollutant content in different pollutants combination will also lead to the difference of pollution stress degree and return to normal growth. For different plants, what are the individual concentrations of pollutants that have the greatest effect on plants? How long does the effect decrease, that is, how long after self-recovery? Which combination of pollutants has a greater effect on stress under complex pollution? These issues require further study.

Conclusions

Effect of Cu^{2+} , Zn^{2+} and $\text{Cu}^{2+}/\text{Zn}^{2+}$ compound stress on the transpiration rate of *C. camphora* seedlings

The transpiration rate of *C. camphora* seedlings under single Cu^{2+} or Zn^{2+} stress was lower than that of control. The transpiration rate decreased greater under higher concentration of stress heavy metal ions, especially after 20 minutes of treatment. After 40-60 minutes, the transpiration rate was gradually restored to the state of no stress. When Cu^{2+} concentration was higher than that of Zn^{2+} , transpiration rate decreased more obviously under compound stress.

Effects of Cu^{2+} , Zn^{2+} and $\text{Cu}^{2+}/\text{Zn}^{2+}$ compound stress on Intercellular CO_2 concentration and net photosynthetic rate of *C. camphora* seedlings

At the same concentration, the effect of Zn^{2+} on net photosynthetic rate was more significant than that of Cu^{2+} and $\text{Cu}^{2+}/\text{Zn}^{2+}$. The effect of $\text{Cu}^{2+}/\text{Zn}^{2+}$ stress on intercellular CO_2 concentration was more significant than that of single heavy metal stress. The effect of Cu^{2+} stress on the concentration of CO_2 and net photosynthetic rate is more significant than that of Zn^{2+} under $\text{Cu}^{2+}/\text{Zn}^{2+}$ stress.

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