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THE SUITABLE COPPER CONCENTRATION FOR CONTROLLING ROOT ENTANGLEMENT OF CONTAINER-GROWN SEEDLINGS

ZEJING WU[1], YING ZHANG[1], MING YANG[1,2], CAIFENG YAN[3], YUXIN LUO[1], JIAZHAO LIU[4], MEICHEN LIU[4] AND YUMEI ZHOU[1*]

[1]*School of Ecological Technology and Engineering, Shanghai Institute of Technology, Shanghai, 201418, China*
[2]*Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, 130102, China*
[3]*School of Pharmacy, Changzhi Medical College, Changzhi, Shanxi Provance, Changzhi 046000, China*
[4]*The Eighth Group of Geological and Mineral Exploration Development Bureau, Shandong Province, Shandong 276826, China*
**Corresponding author's ymzhou@sit.edu.cn*

Abstract

Coating high concentrations of copper (Cu) on the inner wall of containers can effectively control root spiraling, but it is not clear whether excessive Cu decreases root activity and absorption function during Cu root pruning. To test it, one-year-old *Camphora officinarum* seedlings were grown in containers coated with 0, 40, 80, 100, 120, 140, and 160 g L^{-1} Cu $(OH)_2$ with latex as the carrier. Over 100 g L^{-1} Cu $(OH)_2$ completely inhibited root entanglement. Contents of lignin and Cu in the roots under 100 g L^{-1} Cu $(OH)_2$ was the highest and the Cu transfer coefficient from roots to shoots ($TC_{roots/shoots}$) was the lowest. Compared with 100 g L^{-1} Cu $(OH)_2$, 120 g L^{-1} Cu $(OH)_2$ also maintained higher root activity while having lower lignin and Cu content. Although root circling was best controlled under 160 g L^{-1} Cu $(OH)_2$, higher $TC_{roots/shoots}$ and less new roots occurrence indicated that root function has been damaged. Notably, 40 and 80 g L^{-1} Cu $(OH)_2$ not only failed to control root entanglement but also caused Cu toxicity. Our results suggest that lignin deposition effectively immobilizes Cu in roots, thereby mitigating its toxicity to shoots. Suitable Cu concentrations not only effectively control root entanglement but also maintain higher root activity and lower Cu translocation from roots to shoots.

Key words: Root activity, Cu content, Cu translocation, Lignin.

Introduction

Root pruning with high levels of copper (Cu) has been proved to be an effective method to solve root entanglement of container-grown seedlings by suppressing root tip elongation and stimulating lateral root branching (Pardos *et al*., 2001; Dumroese *et al*., 2013; Yang *et al*., 2024). Chang & Lin (2006) reported that 100 g L^{-1} Cu $(OH)_2$ effectively controlled root circling of containerized *Alstonia scholaris* seedlings and increased plant height. The diameter, biomass, and volume of *Populus nigra* roots grown in containers increased after 6 weeks of 100 g L^{-1} Cu treatment (Montagnoli *et al*., 2022). However, negative effects like decreased growth and physiological function were also observed although root entanglement was obviously inhibited. Similarly, the content of nitrogen (N), phosphorus (P), and potassium (K) of *Physalis peruviana* seedlings decreased after 87 days of exposure to over 24 mg L^{-1} Cu (Marchioretto *et al*., 2020). The plant height and root weight of *Malus* × *micromalus* also declined under 120 g L^{-1} Cu $(OH)_2$ treatment (Zhou *et al*., 2023). Therefore, high concentrations of Cu may produce dual effects during root pruning process. So far, it is unclear if high Cu treatment leads to excessive Cu accumulation in vegetative organs and disturbs root activity.

Cu is one of the essential microelements for plant growth and development. It serves as a catalytic and structural cofactor for many enzymes that function in ion homeostasis, signaling transduction, protein transport, self-defense, cellular integrity, etc., (Kardos *et al*., 2018; Nazir *et al*., 2019; Zhang *et al*., 2019; Kumar *et al*., 2021). Generally, 5-30 mg kg^{-1} is considered a safe and normal concentration range for non-Cu resistant plants (Wuana & Okieimen, 2011). Excess of Cu interferes with composition of chloroplast, reduces content of photosynthetic pigments, inhibits nutrient absorption, and causes leaf wilting and cell necrosis (Printz *et al*., 2016; Zhang *et al*., 2019; Shabbir *et al*., 2020). For example, the chlorophyll content of *Vitis labrusca* L. significantly decreased under 50, 100, and 150 mg kg^{-1} Cu (Ambrosini *et al*., 2018). The contents of N, P, K, magnesium (Mg), and calcium (Ca) in the leaves and roots of *Lactuca sativa* L. and *Lens culinaris* Medik decreased under excessive Cu (Shams *et al*., 2019; Fardus *et al*., 2023).

Root is the main organ for Cu uptake and accumulation, so root structure and function might be firstly affected under an excess of Cu (Yang *et al*., 2015). For *Triticum aestivum*, 106 to 419 mg kg^{-1} Cu caused greater damage to roots than shoots and Cu accumulation ranked roots (57-410 mg kg^{-1}) > leaves (9-27 mg kg^{-1}) > stems (4-14 mg kg^{-1}) (Cook *et al*., 1997). Cu content in the roots of *Arabidopsis thaliana* growing under 2.5 μM Cu solution was 15.7 times higher than that in the shoots (344.4 mg kg^{-1} vs 21.9 mg kg^{-1}) (Lequeux *et al*., 2010). Cu enrichment in roots is a protection and tolerance mechanism to excessive Cu in order to preserve physiological functions of leaves (Marques *et al*., 2018). Cu transfer from roots to stems and leaves is limited under appropriate or normal Cu concentrations. However, under excessive Cu, more Cu might be transferred to the aboveground part (Shahid *et al*., 2017; Zlobin *et al*., 2017). In comparison with non-stressed control, 0.3 mM and 3.0 mM Cu significantly increased Cu content in the roots of *Lens culinaris*, while Cu content in the shoots increased only under 3.0 mM Cu treatment (Hossain *et al*., 2020), indicating that roots act as protective barrier.

Plants have evolved a variety of strategies against Cu stress by inhibiting absorption, immobilizing Cu in roots, converting active Cu ions into stable Cu complexes, stimulating secondary defense, etc. (Chen *et al*., 2020; Huang *et al*., 2021; Kumar *et al*., 2021). Increasing lignin biosynthesis and deposition in the cell walls of roots is an effective detoxification way (Huang *et al*., 2021; Wu *et al*., 2021). Lignin can chelate with Cu ions to form non-phytotoxic compounds and enhance the immobilization of Cu in root cells (Ren *et al*., 2022). However, lignin accretion also leads to thickening and hardening cell walls, thereby inhibiting cell elongation and growth.

In summary, there is a trade-off between controlling root entanglement and resisting Cu toxicity under excessive Cu. It is important to explore how plants balance positive and negative effects caused by high Cu during root pruning process. Here, one-year-old Camphor tree (*Camphora officinarum* Nees) seedlings were grown in the containers treated with a series of concentrations of copper hydroxide ($Cu(OH)_2$) (0, 40, 80,100, 120, 140, and 160 g L^{-1}) with latex as the carrier. The purposes of the present study are to determine an appropriate Cu concentration and then explore the balance measure of roots based on growth, physiology, and Cu distribution pattern within vegetative organs.

Material and Methods

Experimental design: This work was carried out at the botanical garden of Shanghai Institute of Technology, Shanghai, China (121°30′42″E, 30°50′42″N). The annual mean air temperature is 16°C, with the highest temperature occurring in July and August and the lowest temperature occurring in January. Average annual precipitation is 1000-1200 mm.

Uniform and well-grown one-year-old *C. officinarum* seedlings (47.3 cm in height and 4.2 mm in ground diameter) were planted in black plastic containers (15.0 cm in upper diameter, 11.0 cm in lower diameter, and 13.0 cm in height) on May 24, 2021. Before planting, the interior wall and bottom of containers were evenly coated with 40, 80, 100, 120, 140, and 160 g L^{-1} $Cu(OH)_2$ with latex (30 mg cm^{-2}) as the carrier. The unpainted containers (UC) and the containers only painted with latex (LC) were used as controls. The substrate in containers consisted of 2 peats: 1 perlite:1 vermiculite (by volume). There were 24 replications for each $Cu(OH)_2$ treatment and control, 192 containers and seedlings in total. All plants were watered two or three times a week depending on temperature. Samplings for all measurements were taken in September 2022 when control plants exhibited obvious root entanglement.

Root activity: Root activity was determined by the Triphenyl Tetrazolium Chloride (TTC) reduction method (Yamauchi *et al*., 2014). Fresh root tips were gently rinsed with deionized water and then dried with paper tissue. Roots were incubated in 10 mL of 4 g L^{-1} TTC and 67 mol L^{-1} phosphate buffer solution (pH 7.0) with 1:1 volume ratio at 37°C for three hours, and then 1 mL of 1 mol L^{-1} H_2SO_4 was added to the mixture to cease the reaction. After that, the roots were ground in 3 mL ethyl acetate and centrifuged at 4000 g. The absorbance of the supernatant was measured at 485 nm wavelength by a UV spectrophotometer (UV4800, Unocal, China). Root activity was calculated according to the following equation:

$$\text{Root activity (mg (g h}^{-1})^{-1}) = \frac{\text{Reduction amount of TTC (mg)}}{\text{Weight of root sample (g) x Time (h)}}$$

Root activity in new roots and mixed roots (new roots and old roots) was separately measured. The classification of new roots and old roots was mainly based on color and length, with new roots being white and shorter and old roots being brown and longer.

Biomass, volume, and tissue density of roots: Whole plants were gently removed from container and intact root systems were placed in water to avoid root damage, especially to root tips. Substrate adhering to roots was washed away and then the roots were dried with a tissue. Plants were divided into aboveground parts (stems + leaves) and belowground parts (roots), and then oven-dried at 70°C for at least 48 h to constant weight. Root/shoot ratio was calculated by the ratio of belowground dry weight to aboveground dry weight. Root volume was estimated by the volume of water discharged from completely immersed roots. Root tissue density (RTD) was calculated by the ratio of root dry mass to root volume (Birouste *et al*., 2014).

Lignin measurement: Lignin content was measured according to the procedure described by Bian (2019). Roots were dried at 80°C in the oven to constant weight and then ground into powder. One gram of root powder was soaked in 10 mL 1% acetic acid for 30 minutes to remove non-cell-wall fractions. After washing three times with a 5 mL mixture of 50% ethanol and 50% ether (v:v), the sample was digested in 72% H_2SO_4 for 16 h. The extraction was titrated with 0.1 mol L^{-1} sodium thiosulfate until the solution turned bright green.

Cu content and Cu transfer coefficient: Cu content in roots, stems, and leaves was measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, 7400, Thermo Electron Corporation, USA). Root powder was digested with strong acid (HCl: HNO_3 = 3:1) to a wet salt state and then pretreated with 25 mL ultra-pure water (Lafuente *et al*., 2023). Translocation coefficients of Cu from roots to aboveground part ($TC_{roots/shoots}$) and from stems to leaves ($TC_{stems/leaves}$) were calculated according to the following equations (Liu *et al*., 2023):

$$TC_{roots/shoots} = \frac{C_{stems} + C_{leaves}}{C_{roots}}$$

$$TC_{stems/leaves} = \frac{C_{leaves}}{C_{stems}}$$

where C_{roots}, C_{stems}, and C_{leaves} referred to the Cu content in roots, stems, and leaves.

Nutrient measurement: Eight plants were randomly selected from each treatment and control. Leaves from the top, middle, and bottom of each plant were picked and then mixed into one sample. All leaves were dried at 80°C in the oven for 48 hours to constant weight and then ground to powder. The leaf powder was digested with concentrated H_2SO_4 with an infrared digestion block (SKD-20S2, Shanghai Peiou Co., Ltd., China) to clear solution. N was determined by Kjeldahl method using Kjeltec Auto Analyzer (PEIOU SKD-800, China), P was determined by vanadium molybdate blue colorimetric method with UV spectrophotometer (UV4800, Unocal, China), and K was determined by flame photometer method (FP640, China).

Pigment measurement: Fresh leaves on the top of four randomly selected plants from each treatment and control were drilled along the midrib by a 5 mm diameter puncher. Four round discs on every leaf were ground and extracted by 95% ethanol (Brito *et al*., 2011). The absorbance of extract was measured at 646 nm, 663 nm, and 470 nm using UV4800 spectrophotometer (Unocal, China). The concentration of chlorophyll a (C_a), chlorophyll b (C_b), and carotenoids (C_{car}) were calculated by the following equations:

$$C_a = 12.21A_{663} - 2.81A_{646}$$

$$C_b = 20.13A_{646} - 5.03A_{663}$$

$$C_T = C_a + C_b$$

$$C_{Car} = \frac{(1000A_{470} - 3.27C_a - 104C_b)}{229}$$

Plant growth measurement: Plant height and ground diameter of all plants were measured by a tape and digital caliper, respectively.

Statistical analysis

All statistical analyses were conducted using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with an LSD test ($p<0.05$) was performed to test the differences in all parameters between treatments and controls. Two-way ANOVA was used to assess the effects of Cu $(OH)_2$ treatment on Cu content in the organs of leaves, stems, and roots.

Result

Root analysis

Root morphology: There were many dark and aged roots spiraling along the inner wall of containers in the UC (Fig. 1a). The roots in the LC were not completely aged, but they grew densely and entangled around the container wall (Fig. 1b). No root entanglement was observed when Cu $(OH)_2$ concentration was over 100 g L^{-1}, and the outermost roots gradually decreased with increasing Cu $(OH)_2$ concentration (Fig. 1c-1h). Although root entanglement under 40 and 80 g L^{-1} Cu $(OH)_2$ was still obvious, it was less than the UC and LC (Fig. 1c and 1d). There were more abundant, finer in diameter, and evenly distributed roots under 100, 120, and 140 g L^{-1} Cu $(OH)_2$ (Fig. 1e, 1f, and 1g). No roots were exposed on the substrate surface adjacent to container wall under 160 g L^{-1} Cu $(OH)_2$ (Fig. 1h).

Root activity and root tissue density: The activities of mixed roots and new roots were significantly affected by Cu $(OH)_2$ treatment and the activity of new roots was significantly higher than that of mixed roots for both Cu $(OH)_2$ treatments and controls (Fig. 2a; $p<0.05$). The activities of mixed roots and new roots in the LC and 120 g L^{-1} Cu $(OH)_2$ were the highest, 62.5% and 38.9% higher than that in the UC, 63.1% and 39.3% higher than the other Cu $(OH)_2$ treatments. No significant difference in root activity was observed between 80, 100, and 120 g L^{-1} Cu $(OH)_2$.

There was no significant difference in RTD between the UC and 40 and 80 g L^{-1} Cu $(OH)_2$, while 40.6% lower than the others (Fig. 2b) ($p<0.05$).

Fig. 1. Roots of *Camphora officinarum* seedlings growing in the unpainted control containers (UC, without Cu $(OH)_2$ and latex) (a), latex painted containers (LU) (b), and containers treated by 40 (c), 80 (d), 100 (e), 120 (f), 140 (g), and 160 (h) g L^{-1} Cu $(OH)_2$ with latex as the carrier.

Lignin and Cu content: Cu $(OH)_2$ treatment significantly increased lignin content in roots with the highest value under 100 g L^{-1} Cu $(OH)_2$ (Fig. 3a). Lignin content under 100 g L^{-1} Cu $(OH)_2$ was 137.5%, 241.0%, and 44.9% higher than that in the UC, LC, and other Cu $(OH)_2$ treatments ($p<0.05$). Among Cu $(OH)_2$ treatments, lignin content under 40 g L^{-1} Cu $(OH)_2$ was relatively lower.

Cu $(OH)_2$ treatment significantly increased Cu content and changed Cu distribution among organs, with the highest Cu content in the roots (Fig. 3b). On average, Cu content in the roots under Cu $(OH)_2$ treatments was 1.4 and 3.6 times higher than that in the UC and LU except 160 g L^{-1} Cu $(OH)_2$. Root Cu content under 40, 80, 120 and 140 g L^{-1} Cu $(OH)_2$ was 33.2% lower than that under 100 g L^{-1} Cu $(OH)_2$ while 88.1% higher than 160 g L^{-1} Cu $(OH)_2$ ($p<0.05$). Stem Cu content under Cu $(OH)_2$ treatments showed an almost opposite trend to root Cu content. Cu content in the stems under 40, 80, 120 g L^{-1} Cu $(OH)_2$ was 24.5% lower than that under 140 and 160 g L^{-1} Cu $(OH)_2$ while 34.1% higher than that under 100 g L^{-1} Cu $(OH)_2$ ($p<0.05$). There was no significant difference in leaf Cu content between UC and 100 g L^{-1} Cu $(OH)_2$, significantly lower than that under 120, 140, and 160 g L^{-1} Cu $(OH)_2$. Notably, there was no significant difference in root Cu content between 160 g L^{-1} Cu $(OH)_2$ and UC, but Cu content in the stems and leaves under 160 g L^{-1} Cu $(OH)_2$ was significantly higher than that in the UC. The plants treated with 40 g L^{-1} Cu $(OH)_2$ had the second highest total Cu content, accompanied by higher leaf Cu content.

The $TC_{roots/shoots}$ under 100 g L^{-1} Cu $(OH)_2$ were the lowest, 67.9% and 85.1% lower than in the UC and LU (Fig. 3c). There was no significant difference in $TC_{roots/shoots}$ between UC and 160 g L^{-1} Cu $(OH)_2$, 108.0% higher than the other Cu $(OH)_2$ treatments ($p<0.05$). Compared with UC, 140 and 160 g L^{-1} Cu $(OH)_2$ did not significantly change $TC_{stems/leaves}$. Higher $TC_{stems/leaves}$ under 40 to 120 g L^{-1} Cu $(OH)_2$ were mainly caused by lower stem Cu content.

Leaf physiology

Leaf nutrient: No significant differences in leaf N and leaf P were observed between Cu $(OH)_2$ treatments and controls, on average 0.2 g kg^{-1} N and 8.7 mg kg^{-1} P (Fig. 4a and 4b). Leaf K content showed a gradually decreased trend with increasing Cu $(OH)_2$ concentration ($p<0.05$) (Fig. 4c). The K content under 80 to 160 g L^{-1} Cu $(OH)_2$ was 31.6% lower than the controls.

Photosynthetic pigments: No significant differences in the contents of C_a, C_b, and C_{car} were observed between Cu $(OH)_2$ treatments and controls (Fig. 5). The average contents of C_a, C_b, and C_{car} were 11.5, 17.5, and 5.8 μg cm^{-2}.

Growth: The plant height under 40, 140, 160 g L^{-1} Cu $(OH)_2$ was 16.5% higher than that in the UC and LC, while 22.1% lower than that under 80, 100, 120 g L^{-1} Cu $(OH)_2$ ($p<0.05$) (Fig. 6a). There was no significant difference in ground diameter between 80, 100, and 120 g L^{-1} Cu $(OH)_2$, 11.1% higher than the controls and other Cu $(OH)_2$ treatments (Fig. 6b).

Over 100 g L^{-1} Cu $(OH)_2$ decreased root biomass by 19.3% compared with UC ($p<0.05$) (Fig. 6c). Root biomass in the LC was the lowest, 36.4% lower than that in the UC. The aboveground part biomass under Cu $(OH)_2$ treatments was relatively higher than the controls with the highest value under 80 g L^{-1} Cu $(OH)_2$.

Root/shoot ratio in the UC was the highest, on average 44.7% higher than that under Cu $(OH)_2$ treatments (Fig. 6d). The higher root/shoot ratio under 40 g L^{-1} Cu $(OH)_2$ was mainly attributed to the higher proportion of root biomass, opposite to 80 g L^{-1} Cu $(OH)_2$.

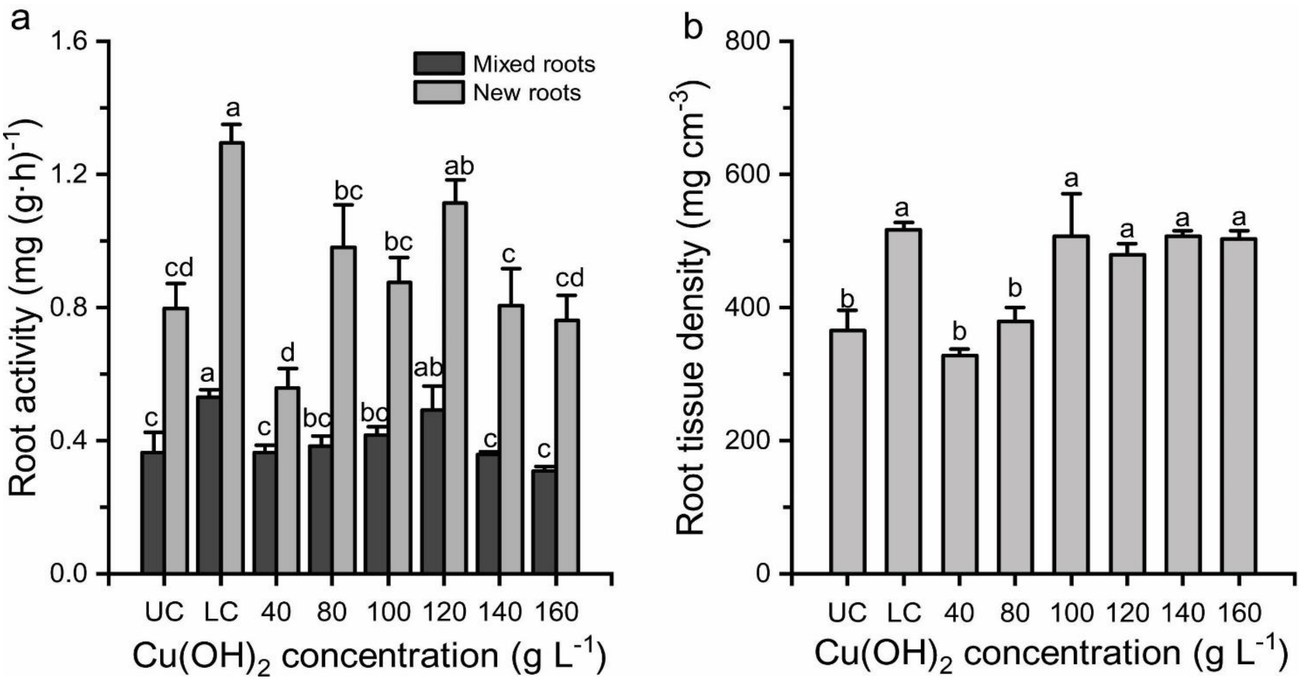

Fig. 2. Root activity (a) and root tissue density (b) of *Camphora officinarum* seedlings growing in the unpainted control containers (UC, without Cu $(OH)_2$ and latex), latex painted containers (LC), and containers treated by different concentrations of Cu $(OH)_2$ with latex as the carrier (Mean ± SE, n=4). Different letters indicated significant differences between treatments and controls at the level of 0.05.

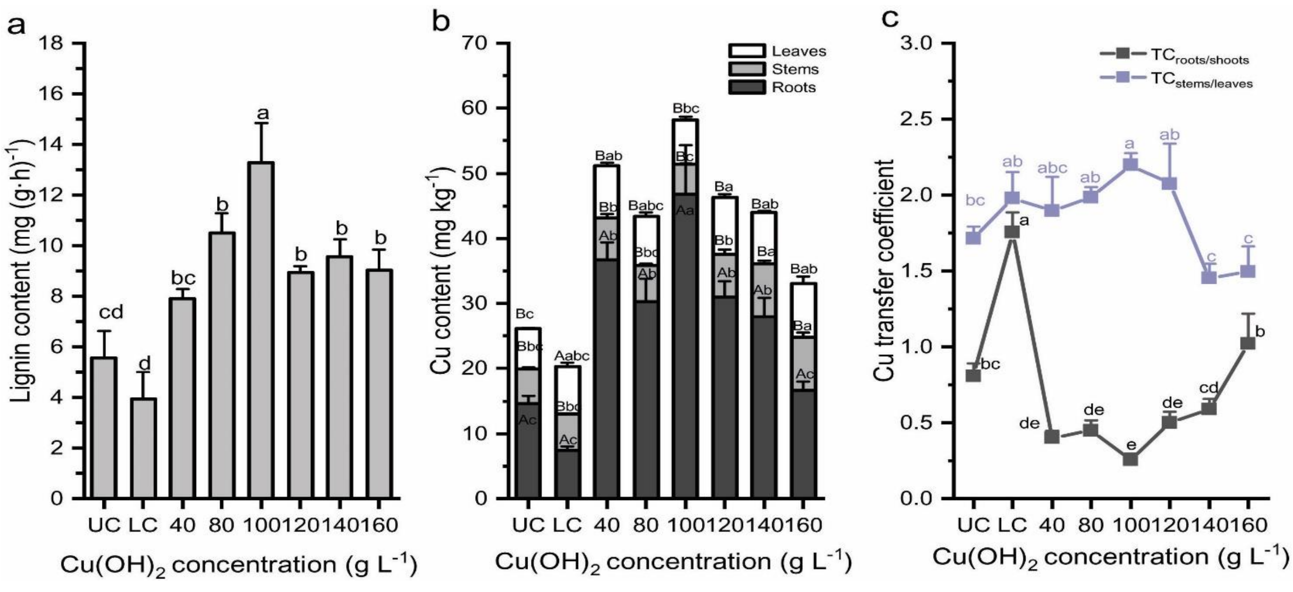

Fig. 3. Lignin content (a), Cu content in the roots, stems, leaves (b), and Cu translocation coefficients (c) of *Camphora officinarum* seedlings growing in the unpainted control containers (UC, without Cu $(OH)_2$ and latex), latex painted containers (LC), and containers treated by different concentrations of Cu $(OH)_2$ with latex as the carrier (Mean ± SE, n=8). Different lowercase letters indicated significant differences between treatments and controls, and different uppercase letters indicated significant differences in Cu content among organs at the level of 0.05.

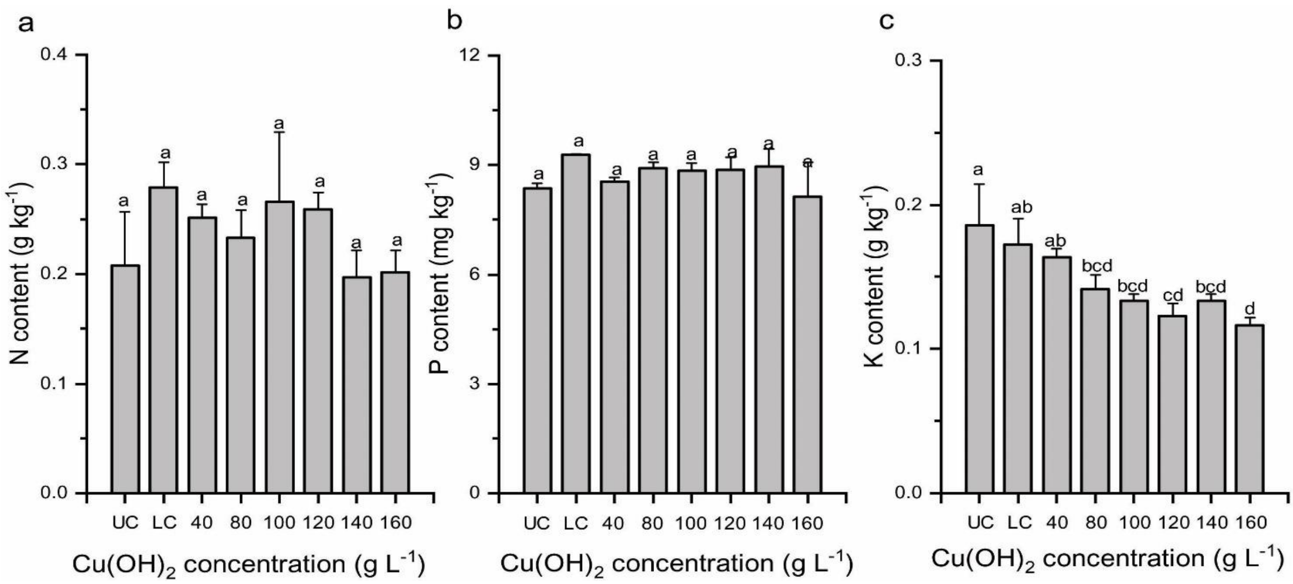

Fig. 4. Contents of N (a), P (b), and K (c) in the leaves of *Camphora officinarum* seedlings growing in the unpainted control containers (UC, without Cu $(OH)_2$ and latex), latex painted containers (LC), and containers treated by different concentrations of Cu $(OH)_2$ with latex as the carrier (Mean ± SE, n=8). Different letters indicated significant differences between treatments and controls at the level of 0.05.

Discussion

The number of peripheral roots close to the container wall decreased with increasing Cu $(OH)_2$ concentration. Over 100 g L^{-1} Cu $(OH)_2$ completely inhibited root circling of container-grown *C. officinarum* seedlings, especially under 160 g L^{-1} Cu $(OH)_2$ where no long roots were observed. However, 160 g L^{-1} was not the suitable concentration due to significant higher $TC_{roots/shoots}$, lower root activity and K content, and less root branching (Fig. 1h, 2a, 3c, 4c). Successful root pruning requires simultaneously improved root function and plant growth potential except inhibiting root entanglement.

Previous studies showed that the application of 100 g L^{-1} $CuCO_3$ increased the size of *Populus nigra* root system with a lower tissue density (Montagnoli *et al*., 2022). Coating containers with 8.3 and 33.0 mg L^{-1} Cu controlled root circling of *Pinus halepensis* and significantly increased the height, diameter, shoot and root biomass, and quality index (Tsakaldimi & Ganatsas, 2006). However, the phytotoxicity of Cu on asparagus, chicory, celery, fennel, lettuce and parsley seedlings was also observed at 2.5% dose although root spiraling was controlled. No detrimental effects were observed when less than this concentration (Cattivello & Danielis, 2008). In the present study, there was no entanglement under 160 g L^{-1} Cu $(OH)_2$. Root tips were unable to contact the container wall due to the high Cu concentration and no more lateral roots branching occurred. Root entanglement under 40 and 80 g L^{-1} Cu $(OH)_2$ was not completely controlled (better than the controls) but there were more live lateral roots than 160 g L^{-1} Cu $(OH)_2$. Therefore, the concentration of root pruning chemicals should be controlled within a reasonable range, and either too high or too low concentrations are detrimental. Reasonable excess of Cu not only inhibits root elongation but also stimulates secondary root branching without damaging root function (Lequeux *et al*., 2010; Mehra, 2022; Mir *et al*., 2022).

The inhibition of high Cu on root tip elongation was generally accompanied by lignin deposition in cell walls and Cu enrichment in roots. Many genes encoding the enzymes involved in each step of lignin biosynthesis were significantly upregulated after exposure to high Cu (Liu *et al*., 2015). Cu content in the roots of *C. officinarum* treated by Cu $(OH)_2$ was almost positively correlated with lignin content and they were significantly higher than the controls (Fig. 3). The efflux of Cu from vascular cylinder of roots into shoots was affected by lignin deposition. The similar relationship between lignin and Cu content was also found in *Arabidopsis thaliana* treated by 50 μM Cu (Lequeux *et al*., 2010). Furthermore, Lequeux *et al*. (2010) also found that high Cu decreased K concentration, consistent with our results. Lignin content was increased by three times in soybean roots treated with 10 μM $CuSO_4$ (Lin *et al*., 2005). Lignin is one of the main components of cell wall, playing an important role against Cu stress. Plant cells bind more Cu by increasing lignin content in cell wall, forming protective barriers to prevent Cu from entering cytoplasm (Derikvand *et al*., 2008; Sun *et al*., 2013; Ren *et al*., 2022). However, lignin content did not increase with increasing Cu concentration with the highest value under 100 g L^{-1} Cu $(OH)_2$ where Cu content in the roots was the highest and the lowest in the leaves, indicating that the blocked Cu in the roots prevents its transportation towards shoots (the lowest $TC_{roots/shoots}$) (Fig. 3c). No negative effects of 100 g L^{-1} Cu $(OH)_2$ on the contents of pigments, N, and P, growth, root activity, and root tissue density were observed.

Root activity is an important indicator for evaluating the ability of roots to absorb and transport water and mineral nutrients (Fageria & Moreira, 2011; Yusuf *et al*., 2011). The root activity and aboveground biomass of *C. officinarum* under 120 g L^{-1} Cu $(OH)_2$ were significantly increased (Fig. 2; Fig. 6). Although there was no significant difference in root activity between other Cu $(OH)_2$ treatments and UC, the response mechanism was different. The lower root activity under 40 and 80 g L^{-1} might be caused by partially root circling while under 140 and 160 g L^{-1} it might be due to excessive Cu toxicity. The highest root activity in the LC was mainly related to a warmer and wetter environment around roots created by latex (Yang *et al*., 2024). There was no high Cu stress in the LC and Cu contents in the three organs were within the safe range of 5-30 mg kg^{-1} (Wuana & Okieimen, 2011). Therefore, latex might be beneficial to root growth, but it cannot control root entanglement. The entanglement was not well controlled under 40 and 80 g L^{-1} Cu $(OH)_2$, which resulted in more thick roots growing along the container wall. Therefore, the root biomass under 40 and 80 g L^{-1} Cu $(OH)_2$ was the highest. For roots, concentrations of 40 and 80 g L^{-1} reached toxic levels but the lignin content did not increase as much as under 100 g L^{-1} Cu $(OH)_2$. The highest Cu $(OH)_2$ concentrations of 140 and 160 g L^{-1} obviously impaired root function, especially 160 g L^{-1}, where roots partially lost the ability to sequester Cu and block Cu upward translocation. Guimarães *et al*., (2016) reported that root activity reduced when Cu content in the environment was higher than the acceptable threshold of plants. Epidermal rupture, root crown reduction, root hair loss, cell structure fragmentation, and damage to membrane permeability were also observed when exogenous Cu concentration exceeded tolerance limit of plants (Nair & Chung, 2015; Guimarães *et al*., 2016; Kumar *et al*., 2021).

In commercial applications and previous studies, 100 g L^{-1} was the commonly used Cu concentration in root pruning for container-grown seedlings (Arnold, 1992; Pomper *et al*., 2002; Chang & Lin, 2006). Our results indicated that 100 g L^{-1} was also a suitable concentration, but the control effects under 120 g L^{-1} would last longer based on higher root activity, aboveground biomass, and future growth potential.

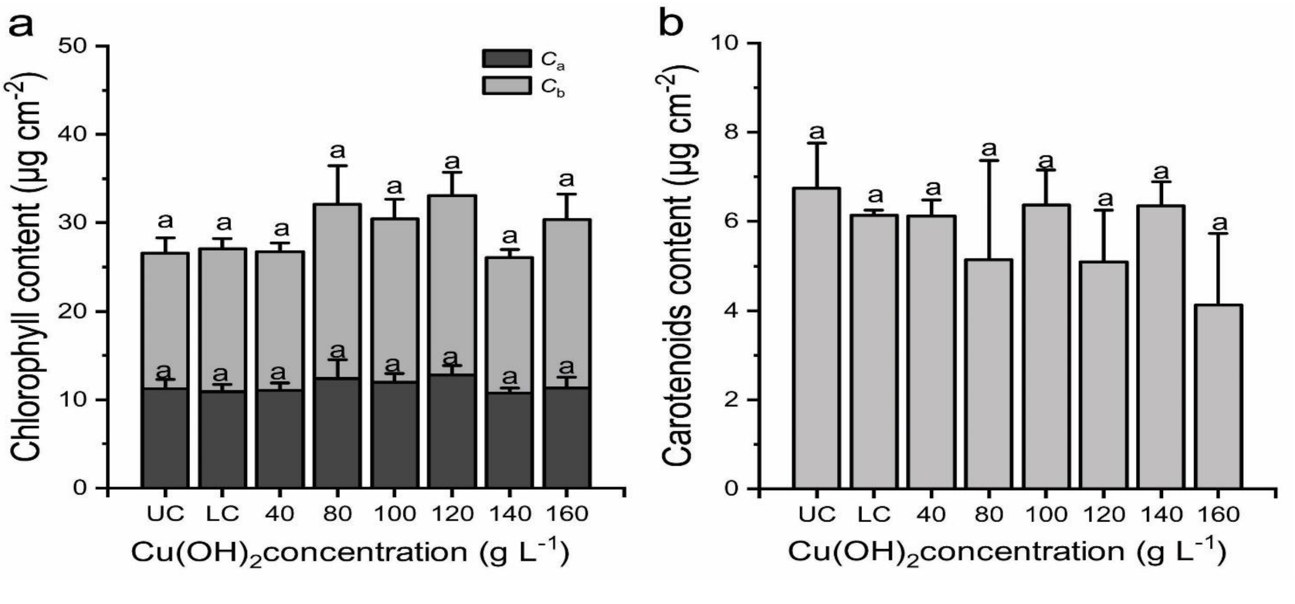

Fig. 5. Contents of chlorophyll a (C_a), chlorophyll b (C_b) (a), and carotenoids (C_{car}) (b) in the leaves of *Camphora officinarum* seedlings growing in the unpainted control containers (UC, without Cu $(OH)_2$ and latex), latex painted containers (LC), and containers treated by different concentrations of Cu $(OH)_2$ with latex as the carrier (Mean ± SE, n=4). Different letters indicated significant differences between treatments and controls at the level of 0.05.

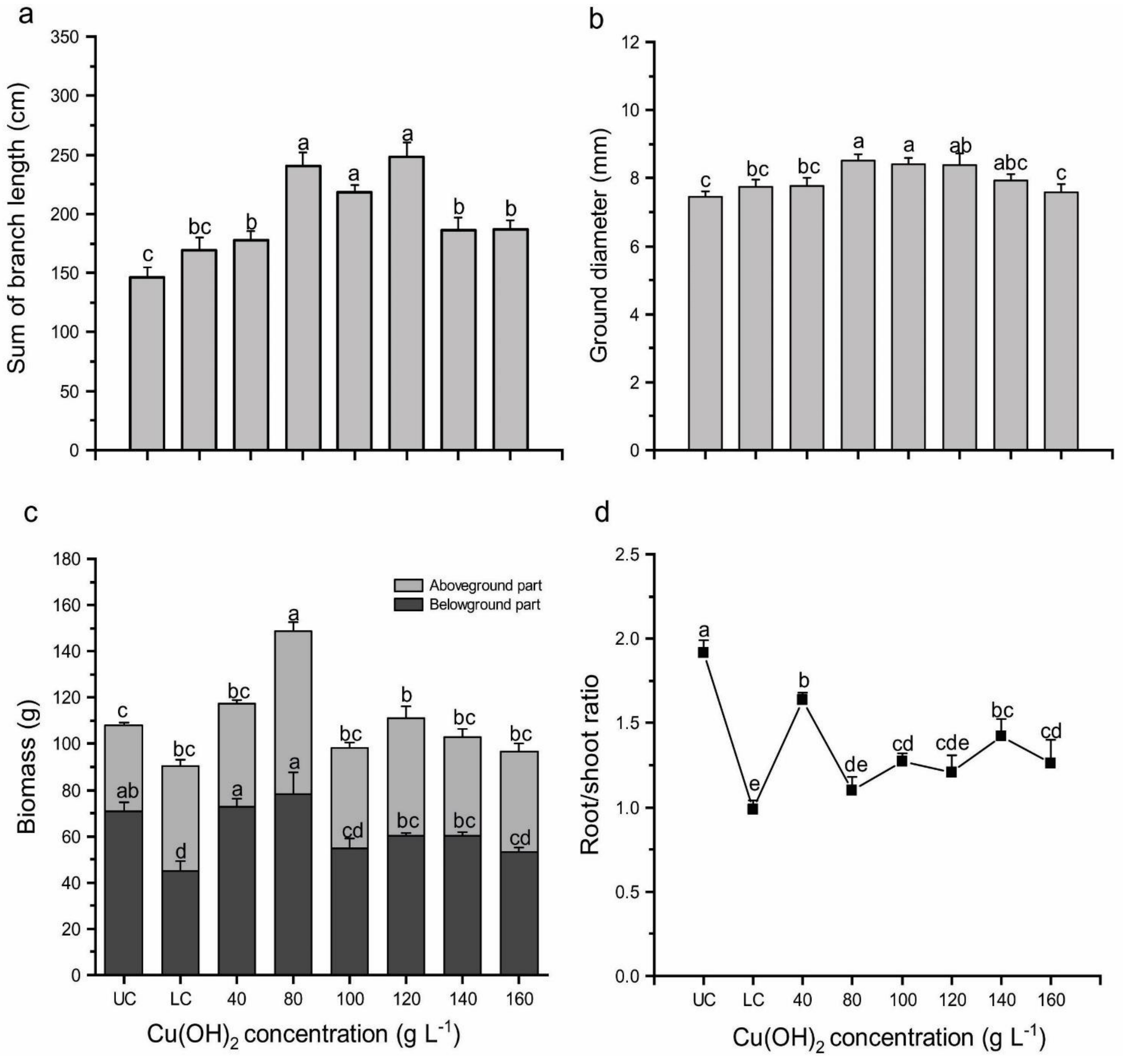

Fig. 6. Plant height (a), ground diameter (b), biomass (c), and root/shoot ratio (d) of *Camphora officinarum* seedlings growing in the unpainted control containers (UC, without $Cu(OH)_2$ and latex), latex painted containers (LC), and containers treated by different concentrations of $Cu(OH)_2$ with latex as the carrier (Mean ± SE, n=20 for plant height and ground diameter and n=8 for biomass allocation). Different letters indicated significant differences between treatments and controls at the level of 0.05.

Conclusion

Root entanglement was effectively inhibited when Cu $(OH)_2$ concentration was over 100 g L^{-1}, while 40 and 80 g L^{-1} Cu $(OH)_2$ only alleviated root circling. The less new roots occurrence and branching, lower root activity, higher $TC_{roots/shoots}$ and Cu distribution proportion in the leaves under 160 g L^{-1} Cu $(OH)_2$ indicated that root function has been damaged. Overall, 100 and 120 g L^{-1} Cu $(OH)_2$ optimally balanced root entanglement control and root function maintenance. Compared to 100 g L^{-1} Cu $(OH)_2$, relatively lower Cu accumulation in the roots and shoots under 120 g L^{-1} Cu $(OH)_2$ would be more beneficial for growth after transplantation. Higher lignin and Cu content in the roots under 100 g L^{-1} Cu $(OH)_2$ might limit growth potential although plants grew well during nursery phase.

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