Responses and tolerance mechanisms of K. paniculate seedlings under lead stress: physiological indicators, morphological distribution, enrichment effects and microstructure

Yonghua Chen (chenyonghua3333@163.com)
Central South University of Forestry & Technology
Tianzhi Xie
Central South University of Forestry & Technology
Rongkui Su
Central South University of Forestry & Technology
Haisong Yao
Central South University of Forestry & Technology
Lu Du
Central South University of Forestry & Technology
Jun Liu
Central South University of Forestry & Technology

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Abstract

Phytoremediation could be an alternative strategy for lead (Pb) contamination, The physiological mechanisms of plant resistance to Pd contamination are of less concern. In this study, Different concentrations of Pb solutions were added to explore the growth of *Koelreuteria paniculate* (Sapindaceae). physiology and microstructure of *K. paniculate* were determined. The results showed that plant growth increased up to 123.8% and 112.7% relative to the control group when the lead concentration was 200 and 400 mg/L. In all treatments, most of the Pb accumulation reached 81.31%-86.69% in the roots and slightly lower concentrations in the above-ground leaves than in the stems. Transmission electron microscopy (TEM) showed that Pb accumulates in the inactive metabolic parts (cell walls and vesicles) in roots and stems, which may be the main mechanism for plants to reduce Pb biotoxicity. In addition, by Fourier transform infrared spectroscopy (FTIR), Pb stress increased the content of intracellular -OH and -COOH functional groups, including compounds such as organic acids, polysaccharides and proteins that bind to Pb, attenuating the toxicity to plants and enhancing their adaptation and tolerance to heavy metals.

1. Introduction

Heavy metal pollution is one of many environmental problems worldwide (A et al. 2017). In 2014, the total production of lead mines in the world was 5.517 million tons, among which China owned the biggest share by 2.8533 million tons, accounting for 51.71% of the world distribution (Gu et al. 2017), and which has been growing at a consistent rate of 2.5% per year(Wang et al. 2016). Currently, the exposure of Pb slag has posed an incalculable potential threat to the nearby ecosystem (Mudd et al. 2017, Myung et al. 1996). The damage of excessive accumulation of lead, which brings about the reduction of biodiversity and the contamination of farmland and groundwater, should not be underestimated (Ruley et al. 2006). It also affects the normal function of the human nervous, reproductive and other systems and harms the gastrointestinal tract, liver, kidneys and brain (Vandame &Palacio 2010). The remediation of contaminated soil in tailings areas by phytoecology has attracted much attention in the past decades (Masarovicova et al. 2010).

Phytoremediation techniques offer various merits, such as eco-friendly, cost-effective and long-term benefits (Han et al. 2021, Laghlimi et al. 2015). It could be one of the ideal solutions for heavy metal contaminated soil (Rascio &Navari-Izzo 2011). So far, the high enrichment properties of hyperaccumulators have been extensively studied in phytoremediation (Yang et al. 2014). However, most plant species studied are annual herbaceous plants. Herbaceous hyper enriched plants do have outstanding enrichment advantages, but they hold slow growth and limited biomass (Epeldegui et al. 2007, Tandy et al. 2006)., which fails to provide an overall effective restoration of the herbaceous plants (e.g. *Thlaspi sp.*). Therefore, an urgent exploration of other plant species with advantages in rapid growth and biomass growth is needed.

In contrast, phyto-stabilized remediation mainly focuses on woody plants with heavy metal tolerance, whose enrichment concentration is less than that of hyperaccumulators. However, their huge biomass and biological root systems formed by soil micro-organisms reduce the effective form of heavy metals (Li et al. 2020) and they can reduce the migration and spread of heavy metals especially in some large contaminated paddies. As an inexpensive, high-efficiency and green technology (Ali et al. 2013, Wang et al. 2019), phyto-stabilized remediation has been widely concerned for its research and practical repair applications. Yet, additional research is still in need to short-list plants that are resistant to heavy metals for ecological restoration of contaminated areas (Heckenroth et al. 2016).

Black Locust (Shi et al. 2016), *Platanus acerifolia(Ait.) ,Willd* (Kang et al. 2018), *Poplar* (He et al. 2013), have been proven effective in tailings remediation. As a widely distributed fast-growing tree species in most provinces of southern China(Liu et al. 2018). *K. paniculate* is used for landscaping, energy, timber and industrial materials. It gives nutrients to the soil and encourages the growth of other plants (Kiboi et al. 2021). The vegetation could take an active role in restoring bare ground and reducing the spread of heavy metal pollutants in contaminated areas. In an earlier experimental selection of Pb-Zn slag plants for tolerance, it was found that *K. paniculate* could survive in Pb-Zn slag and showed good phytoremediation compared to 18 other tolerant plants(Chunfang et al. 2019). However, there have been no complete studies to analyze the physiological tolerance effects of *K. paniculate* to Pb, and its mechanism of enrichment tolerance remains unclear.

This study is intended to 1) explore the changes in physiological and physicochemical characteristics of *K. paniculate* under lead stress, including biomass, plant height, root structure, chlorophyll content and antioxidant enzyme activity; 2) analyze the enrichment mechanism of tolerance in K. paniculate by the content of Pb in each tissue part and each subcellular content ratio;3) clarify the tolerance and adaptation mechanism of *K. paniculate* to heavy metals lead through TEM and FTIR analysis. This investigation will provide a theoretical basis and technical support for the phytoremediation of heavy metal Pb by the woody plant.

2. Materials And Methods

2.1 Experimental materials and design

Experimental seedlings were obtained from the nursery of Central South University of Forestry & Technology. Seedlings were picked in good growth condition and maintained at the height of about 12 cm. Experimental cultivation sand (river sand) was purchased from Changsha Red-star Flower Market and Hoagland nutrient was purchased from the solution from Beijing Xiqing Agricultural Technology Company in Henan Province. The experiment started in May 2020 in the Nursery base of Central South University of Forestry Science and Technology, Changsha, Hunan Province, which is located in the subtropical monsoon climate zone with obvious continental climate characteristics and an average temperature of 25°C during the experimental period. River sand (2 mm) was used as a substrate for the experiment, soaked with 2% Hydrochloric acid, and rinsed with deionized water. The plants were moved

into the sand and incubated for 7 days until the growth state was stable. Here is the stress concentration (Pb stress solution mg/L) for each group: CK (0), A (200 mg/L), B (400 mg/L), C (600 mg/L), D (800 mg/L), E (1000 mg/L), F (1200 mg/L). Four replicates were set up for each gradient, with two seedlings containing 5 kg of sand in each pot. The Pb stress solutions were added twice a week (100 ml each time), and the whole stress process last ten weeks. Complete plants were harvested after 70 days of stress.

2.2 Experimental methods

2.2.1 Plant height, biomass and root length

The height of the plant was measured from the soil surface to the top of the plant, and root length was measured by a root analysis system (WinRHIZO PRO, 2007; Regent Instrument, Canada). After harvesting, plant samples were heated to 105°C for 30 minutes, dried to a constant weight at 75°C, and then Pb concentrations were determined with a flame atomic absorption 128 spectrophotometer (FAAS, AA-7002, Thermo Fisher Scientific, USA) (Zhang et al. 2019).

2.2.2. Pb changes in subcellular fractions in each tissue of the plant

Referring to the method (Han et al. 2020), with some modifications, the harvested plants were washed with water, soaked in EDTA-2Na solution to remove surface heavy metals, and later rinsed with distilled water. The homogenate was stirred with cooled extraction buffer [250 mM sucrose, 1.0 mM DTT ($C_4H_{10}O_2S_2$) and 50 mM Tris-HCl pH = 7.5]. The homogenate was centrifuged for 30 seconds (3000 rpm) and precipitated as the cell wall fraction (F1); the supernatant was continued to be centrifuged for 30 minutes (10,000 rpm) and precipitated as the organelle fraction (F2); the supernatant was the soluble fraction (F3). All operations were carried out at 3°C.

2.2.3. Determination of chlorophyll and antioxidant enzyme in plant leaves

Determination of chlorophyll content in leaves of *K. paniculate* by spectrophotometry; Determination of malondialdehyde (MDA) by thiobarbituric acid method Content; The decision of soluble protein content by Coomassie brilliant blue g250 (Nie et al. 2016); Superoxide dismutase (SOD) activity was determined by the *azotetrazolium* (NBT) method, guaiacol oxidation and UV absorption, while peroxidase (POD) and catalase (CAT) activities were determined respectively.

2.2.4 The microstructure and functional groups analysis of the plants

Roots and stems of fresh plants were washed with ultrapure water, cut into pellets (2mm*2mm*2mm) and stored in glutaraldehyde solution (2.5%) at 4°C to be measured. They were sent to CSU for measurement by TEM (FEI Tecnai Spirit, USA). Functional group composition was analyzed by Fourier transform infrared spectroscopy (FTIR). Dried tissue samples were crushed into powder with a pulverizer,

passed through a 200 mm sieve and analyzed by FTIR (Thermo Scientific Nicolet-iS10, USA) in the range of 400–4000 cm^{-1}. Measurements were operated by Shanghai Yeake detection Equipment Co.Ltd.

2.3 Statistics analysis

Data were statistically analyzed by using Microsoft Office Excel 2016.. The least significant difference (LSD) tests were performed for multiple comparisons. If the difference was significant, it was marked as ($P < 0.05$). And then, Duncan's test with 5% probability was performed to test for treatment differences. Finally, all data were expressed as mean ± standard deviation (SD) of three replicate experiments (n = 3).

3. Results And Analysis

3.1 Plant height and biomass under lead stress

In this experiment, *K. paniculate* survived to 100% under each concentration of Pb stress. Under the Pb concentration of 200 mg/L, it attained the highest height and biomass (Table 1), with its average height and total biomass increasing by 123.81% and 113.80% compared to CK. When the Pb concentrations further increased and was higher than that of 600 mg/L, physiological indicators such as plant height and biomass decreased inversely with the increase of Pb concentrations. Under 1200 mg/L treatment group, the leaf biomass and the root biomass decreased by 59.1% and 31.9% compared to CK, respectively. Besides, the average plant height after exposure to 1200 mg/L was similarly lower than that of the control.

Table 1
Effect of Pb Stress on growth parameters of *K. paniculate* seedling

Treatment group	Average height (cm)	Total biomass (g)	Root biomass (g)	Stem biomass (g)	Leaf biomass (g)
CK	42.54 ± 0.67c	9.71 ± 0.71b	4.98 ± 0.33a	2.64 ± 0.12c	2.08 ± 0.1a
200mg/L	52.67 ± 0.85a	11.05 ± 1.045a	5.28 ± 0.21a	3.60 ± 0.1a	2.17 ± 0.06a
400mg/L	47.95 ± 1.95b	10.14 ± 0.45ab	5.15 ± 0.38a	3.33 ± 0.16b	1.65 ± 0.11a
600mg/L	38.40 ± 1.01d	8.50 ± 0.54c	4.49 ± 0.16b	2.66 ± 0.15c	1.34 ± 0.17a
800mg/L	37.60 ± 2.0d	7.04 ± 0.59d	3.93 ± 0.14c	2.03 ± 0.15d	1.08 ± 5.26a
1000mg/L	37.20 ± 0.85d	6.60 ± 0.37de	4.07 ± 0.3c	1.61 ± 0.04e	0.92 ± 0.4a
1200mg/L	31.80 ± 1.39e	5.70 ± 0.3e	3.39 ± 0.28d	1.45 ± 0.78e	0.85 ± 0.18a

Note: Different lowercase letters represent significant differences in growth data at different processing concentrations ($P < 0.05$)

3.2 Lead Stress on root morphology

The changes in plant root morphology differed significantly($P < 0.05$) among all Pb treatment concentrations (Table 2). The promotion effect was pronounced in group A. Compared with CK, group A achieved 26.1%, 11.6% and 11.2% increases in total root length, root surface area and the number of fine roots, respectively. The total root length, surface area, and the number of fine roots were inhibited with the increase of Pb concentration, but the average root diameter increased proportionally with the increase of Pb stress concentration. Compared with group F, the total root length increased by 755.4%, the total surface area rose by 309.9%, and the average root diameter decreased by 45.5% in group A.

Table 2
Changes of root structure in seedlings of *K. paniculate*

Treatment group	Total root length (cm)	Total root surface area (cm^2)	Average root diameter (mm)	Number of fine roots
CK	3079.01 ± 169.49b	576.04 ± 115.39a	1.32 ± 0.80e	1621 ± 117.20ab
200mg/L	3883.7 ± 429.61a	665.97 ± 98.48a	1.21 ± 1.10e	1803 ± 187.19a
400mg/L	2705.99 ± 122.29b	558.89 ± 46.63a	1.46 ± 1.50de	1413 ± 188.56b
600mg/L	1621.44 ± 179.88c	396.78 ± 20.36b	1.73 ± 0.29cd	995 ± 169.14c
800mg/L	1040.36 ± 247.13d	330.42 ± 54.04bc	2.02 ± 0.08bc	641 ± 107.53d
1000mg/L	872.65 ± 123.18de	308.16 ± 51.49bc	2.25 ± 0.37b	582 ± 27.78de
1200mg/L	514.46 ± 83.001e	215.84 ± 24.76c	2.67 ± 0.27a	365 ± 50.10e

Note: the data are the average of three groups of parallel experiments (n = 3), and different lowercase letters represent significant differences at processing concentrations ($P < 0.05$)

3.3 Enrichment capacity of *K. paniculate* for Pb

The accumulation of Pb in all sections of K. paniculate increased proportionally with Pb stress concentration (Table 3), and there was an overall trend of root > stem > leaf(2.5%-8.2%) in the plant tissues. The root storage of lead was high, up to 80.3–87.3%. The enrichment factor of *K. paniculate* was inversely proportional to the concentration of Pb stress and showed a significant difference ($P < 0.05$). This indicates that the enrichment capacity of *K. paniculate* falls with the rise of Pb concentration, whilst the highest enrichment factor reached 0.65 at 400 mg/L. However, the trend of the transfer factor was stable between 0.07 and 0.1, and the highest reached 0.133 at 400 mg/L Pb stress.

Table 3
Heavy metal content of *K. paniculate* under Pb stress

Treatment group	Pb content (mg/kg)			BCF	TF
	Root	Stem	Leaf		
200mg/L	540.67 ± 13.05e	59.88 ± 5.38e	20.85 ± 4.49f	0.5 ± 0.014b	0.07 ± 0.003c
400mg/L	743.54 ± 55.48e	106.76 ± 1.75d	64.157 ± 5.49e	0.65 ± 0.013a	0.134 ± 0.01a
600mg/L	1143.25 ± 186.31d	119.24 ± 19.86d	93.21 ± 3.29d	0.46 ± 0.05b	0.09 ± 0.01b
800mg/L	1738.04 ± 192.28c	165.84 ± 7.81c	143.88 ± 17.16c	0.4 ± 0.02c	0.08 ± 0.005bc
1000mg/L	2338.69 ± 151.27b	280.78 ± 16.7b	188.22 ± 24.12b	0.36 ± 0.05c	0.08 ± 0.012bc
1200mg/L	3187.87 ± 251.77a	389.46 ± 21.7a	253.11 ± 7.81a	0.36 ± 0.12c	0.07 ± 0.003c

Note: Different letters in the same column indicate significant differences in Pb content in *K. paniculate* under different treatments ($P < 0.05$)

3.4 Physiological indexes of lead stress in *K. paniculate* leaves

The chlorophyll content showed a rise and then fell with increasing Pb stress concentration (Fig. 1), whilst chlorophyll content level reached the top at a stress concentration of 400 mg/L then followed by a significant decrease, and the changes of chlorophyll a/b in *K. paniculate* were consistent with the trend of total chlorophyll. The effect of chlorophyll content decreased with the increase of Pb^{2+} concentration.

Soluble protein content progressively rises with the increasing Pb levels (Fig. 1). The maximum amount in group E increased by about 423.0% compared to the minimum level CK group. However, the trend of MDA content was decreasing and then increasing, with a slight decrease in group A compared with group CK. Group F accumulated the highest amount of 36.06 *u*mol/g, which was 288.87% higher than group CK. Membrane lipid peroxidation was impaired, but soluble protein in K. paniculate leaves showed a coherent increase in response to MDA content, which counteracted some of the poor permeability caused by membrane lipid peroxidation.

The CAT activity showed a positive trend followed by a negative decline (Fig .2), with the highest point occurring in the B treatment group, reaching 2084.25 *U/g* (*FW*). The F group was equivalent to the content of the CK. The activity of SOD significantly increased ($P < 0.05$) with increasing levels of lead exposure stress. The maximum group F increased by about 206.6% relative to the CK. The strongest POD activity

appeared in group C, which showed 142.9% increase in activity compared to CK, and the bottom point of group E was 74.5% lower than that of CK.

3.5 The effects of Lead Stress on Plant Root and Stem Microstructure

Under various concentrations of Pb stress, the overall trend showed F1 > F3 > F2 (Fig. 3), with a large enrichment of Pb in F1 (68.2% – 87.2%). *K. paniculate* minimized the toxicity of Pb^{2+} mostly via storage of Pb in the weakly active site cell wall fraction. As the concentration of Pb^{2+} stress increased, the proportion of Pb in the cell wall (F1) gradually declined, and the subcellular distribution shifted to the soluble fraction (F3) as a whole.

3.6 The effects of Lead Stress on Plant Root and Stem Microstructure

Transmission electron microscopy (TEM) was used to photograph the microstructure of each tissue of *K. paniculate*. Under the treatment of CK, there are low concentration treatment group B (400 mg/L), medium concentration treatment group D (800 mg/L), and high concentration treatment group F (1200 mg/L) (Fig. 4). The comparative analysis showed that: in group CK of roots, stems, and leaves, the intercellular arrangement was regular, overall cell structure and cell membrane were intact, and no impurity materials filled the intercellular space. Group B root, stems, and leaves were less affected by heavy metal hazards. There was no obvious accumulation of impurities in the tissue cells. In the root, stems, and leaves of treatment group D, the suspected metal Pb accumulated inside the root cells and adhered to the cell walls, the membrane tissue was distorted and deformed, and the cell walls showed some damage but were able to can maintain normal cell morphology. Pb granules may have precipitated in the cell wall and cytoplasm, as black granules were different from starch granules (SG) (Dou et al. 2010). Conditions in leaf cells were slightly better than in stem cells, and no significant accumulation of Pb^{2+} was found. In Treatment group F, water loss of the vesicles in *K. paniculate* root cells resulted in the separation of the protoplasm layer from the cell wall, while a large number of fine black particles accumulated in the cell wall and intercellular spaces inside the cells. The stem cells were damaged locally and structurally, and a small amount of fine black particles were found in the intercellular spaces and cell wall, which were significantly less than those in the roots. Lead accumulation in *K. paniculate* leaves was fair, with swollen chloroplasts and uneven lamellar structure, but cell morphology was somewhat affected

3.7 FTIR Analysis functional group composition of K. paniculate tissue

Compared with group CK, the FTIR spectral peak shape of K. paniculate tissue cells in other groups remained similar, but their transmittances showed significant differences at 3340, 2920, 1630, and 1030 cm^{-1}(Fig. 5). At the vicinity of 3340 cm^{-1}, there is a strong absorption with a rounded and blunt peak band associated with the superposition of the stretching vibration of O-H and the stretching vibration peaks of

amino acids and protein amino groups (N-H), the change in absorbance at this point being mainly from changes in carbohydrates, such as cellulose, hemicellulose, and polysaccharides. The small and sharp absorption peak near 2920 cm^{-1} which represents -CH is mainly from protein, cellulose, and pectin in the cell wall. The absorption peak at 1610 cm^{-1} is mainly from -COO and C = O stretching vibrations in amino acids, peptides and proteins. The absorption peak near 1040 cm^{-1} is related to the stretching vibrations of S = O, mainly from polysaccharide carbohydrates (Ghori et al. 2019). The transmission of absorption peaks at 3420, 2920, 1630 and 1040 cm^{-1} decreased or shifted with increasing concentrations. These changes were evident in root cells, followed by leaves and stems, with relevant functional groups in the cell wall and soluble fraction, indicating that the cell wall and soluble fraction are important storage sites for Pb (James &Drenovsky 2007).

4. Discussions

4.1 Lead effects on the growth of K. paniculate

Growth morphology and biomass changes are the ultimate external form of plant growth adapted to the environment. Heavy metal stress inhibits plant growth, resulting in smaller leaf area, dwarfism and reduced plant biomass. In general, plants have a toxic response at a low level of heavy metals, and the biotoxicity of heavy metals is 10–100 times higher in hydroponic tests than in soil tests (Jinwei et al. 2008). In this test, Pb concentrations below 400 mg/L accelerated the growth of *K. paniculate* compared to the CK. When Pb concentrations were above 600 mg/L, growth was significantly inhibited. However, the plants did not show mortality, but adopted a set of physiological and biochemical tolerance changes to tackle the harm from Pb.

The roots serve as the site of direct exposure to heavy metals. In this experiment, the number of fine roots decreased with increasing stress concentration, the average root diameter decreased, and the specific surface area decreased in response to the lead stress. However, under growth-promoting stresses of 200 and 400 mg/L, the number of fine roots increased, and the specific surface area did not change significantly. It could be that *K. paniculate* actively adapts to lead stress by increasing biomass and root share in adversity, and improving nutrient and water extraction capacity. The increase in mean root diameter may provide protection of the root cells from heavy metals toxicity by increasing the thickness of the epidermis and non-protoplast barrier (Cabot et al. 2014, Lux et al. 2010, Ryser &Emerson 2007). At the same time, root endothelium strengthening and the Kjeldahl band barrier are also essential mechanisms of root resistance to heavy metal ions (Vaculik et al. 2012). Usage of physical and physiological avoidance strategies to intercept most of the heavy metal contaminants in the roots showed that *K. paniculate* can adapt to Pb contamination to some extent.

The root zone in *K. paniculate* accumulated more than 80% of the lead. Studies have also shown that plants have high metal tolerance and can usually accumulate and fix large amounts of heavy metals in

their roots, weakening the toxic effects on the plant body by preventing them from transferring from the subsurface to the ground (Cai-Ying et al. 2005). Overall, it seems that the transport of all parts of *K. paniculate* is relatively small, which is no more than 10%, and its above-ground parts have a weaker ability to accumulate Pb than hyperaccumulator plants, but with its huge biomass, the amount of enrichment should not be underestimated. Meanwhile, the *K. paniculate* roots stressed by the high Pb concentration (1200 mg/L) accumulated 3200 mg/kg of Pb, and no mortality occurred, which is sufficient to verify the high tolerance property of *K. paniculate* to Pb.

4.2 Defense mechanisms of K. paniculate leaves under lead stress

High concentrations of Pb stress inhibited plant leaf growth, and this decreasing trend may be related to the production of reactive oxygen species (ROS) (Bhaduri &Fulekar 2012). SOD, POD and CAT are essential components of the plant antioxidant system, scavenging excess O_2^- and H_2O_2 and their damage, inhibiting enzyme activity, poisoning membrane lipid peroxidation, and impairing the normal osmoregulatory capacity of cells (Bankaji et al. 2015). In this study, *K. paniculate* leaves did not show a single decline in enzyme activity. The CAT, POD activity of the leaves showed a trend of increasing and then decreasing, and they respectively reached the turning points of stress amounts of 400 and 600 mg/L. The results might attribute to the stimulation of antioxidant enzyme activity of *K. paniculate* within the threshold of lead stress, but as the stress concentration increased, the enzyme activity system of *K. paniculate* was disrupted, and the enzyme activity decreased. Similar to what previously had been reported (Zhengzheng et al. 2007), SOD activity maintained an upward trend to remove the harmful effects of reactive groups in the plant. In conclusion, *K. paniculate* reduces the toxicity of peroxides mainly by increasing leaf SOD activity and maintaining CAT, POD activity. The MDA content of *K. paniculate* leaves were at a stable levels under Pb 200–400 mg/L treatment, and significantly increased after the toxic effects of Pb stress became apparent. This might cause changes in the membrane structure of the leaf cells (Mahdavian et al. 2016, Vallee &Ulmer 1972). While Pb stress causes membrane lipid peroxidation damage in leaves, it also induces a response of antioxidant enzymes, antioxidants, and other resistance mechanisms in *K. paniculate*, leaving the total antioxidant capacity of *K. paniculate* leaves at a high level and enhances the overall antagonistic capacity of K. paniculate. This phenomenon, similar to the elevated soluble protein content, balances the osmotic potential between the cytoplasm and the vesicles, promotes the removal of reactive oxygen species in *K. paniculate*. It enables the normal proceeding of physiological activities such as cellular metabolism in *K. paniculate*, maintains the osmotic balance of cells and protects them from the toxicity of heavy metals (Peralta et al. 2001). It may be one of the main resistance mechanisms in *K. paniculate*.

Chloroplast chlorophyll content reflects the plants' photosynthetic capacity and directly affects their growth and development (Jain et al. 2009). Leaves in the treatment group above 1000 mg/L showed localized yellowing and black spots, similar to the response of many heavy metal-tolerant plants after suffering from excessive stress, including *Robinia pseudoacacia*(Yakun et al. 2016) and *Koelreuterie paniculate* (Zhang et al. 2019) ,etc. The toxicity of lead may affect the synthesis of prochlorophyll

reductase and amino-γ-ketovaleric acid, hindering the chlorophyll synthesis process and leading to a decrease in chlorophyll content (Yu et al. 2010). However, high chlorophyll a/b ratios suggest that the greater the stacking of cystoid bodies, the lower the photoinhibition and the more efficient plants are in using sunlight energy (Feixiang et al. 2012). In this study, the a/b ratio content of stressed plants still fluctuated between 78.14% and 104.86% compared to the CK group. Despite causing some damage, *K. paniculate* was still able to maintain its growth by stabilizing chlorophyll a/b values to improve light energy use efficiency under high levels of lead stress (Yan Ao.lei et al. 2010).

4.3. Microcosmic structure response of K. paniculate under lead heavy metals stress

Cell wall fixation and vacuole-dominated distribution of soluble components are two main ways to detoxify heavy metals in plants(Hall 2002). The same conclusion was obtained in this experiment, and the distribution pattern of lead in the subcellular fraction of *K. paniculate* was seen in the subcellular fraction of *K. paniculate* tissues and in the relationship of cell wall > soluble fraction > organelle content fraction.

In all tissues of *K. paniculate*, the concentration of Pb^{2+} distributed in the cell wall fraction always occupied the largest proportion, and the increase of lead ions in the cell wall was even more obvious. The cell wall contains polysaccharides such as pectin, cellulose, hemicellulose, and protein, and many pro-metal ion coordination groups can complex with positive-valent metal ions in an inactive state. The cell wall plays an important role in the cumulative fixation of heavy metals and the reduction of their toxicity. After the concentration of lead ions increases and the active sites in the cell wall are saturated and occupied, soluble fractions such as vesicles will absorb metal ions and thus reduce the toxic effect of lead.

Employing transmission electron microscopy (TEM), the root cells of *K. paniculate* under lead stress were observed: cell walls in the plants of group CK were smooth and well-organized; with the increasing concentration of Pb, the distribution of substances in each cell tissue became well-defined, and apparent aggregation effects were observed in the soluble components of the cell wall and vesicles. In addition, the cell interstitial space also occupied a certain proportion of the content in the high concentration group F. The cell wall is the first barrier for extracellular substances to enter the cell. It contains a variety of polysaccharides and is rich in carboxyl, aldehyde, amino, and other metal-friendly coordination groups, which can be easily complexed with and immobilized heavy metals(Ghori et al. 2019, Pan et al. 2019). When the heavy metal ions bound to the cell wall reach saturation, the thick wall of Pb ions interwoven by the cell wall dextran can block most harmful heavy metals outside the cell and stay in the interstitial cell space. Depending on the loading level, excess heavy metal ions loaded on the cell wall leach into the cell and are transferred to the vesicles, where they are complex with organic acids and inorganic salts(Cosio et al. 2004).

Fourier transform infrared spectroscopy (FTIR) is a technique for structural analysis based on the vibrations of functional groups and polar bonds in compounds(Bosch et al. 2006). In this experiment,

2920 cm^{-1} of -CH, -COO and 1630 cm^{-1} of C = O may be more associated with the breakage of polysaccharide-rich peptide chains in the cell wall, combined with the widespread carboxylic acid and ketone groups in their secreted polysaccharides to reduce the toxicity of lead to K. paniculate (Xue et al. 2019). Compared to CK, especially for treatment B, the root promotion effect may attribute to the increased secretion of amino acids and carboxylic acids in the cell wall and vesicles of *K. paniculate*, which increased the binding of polyester polysaccharides and heavy metals, and incidentally promoted the plant growth. This is consistent with the promotion of plant growth at low concentrations (Clemens 2001).

When stress levels increased, the peak absorbance of the above wavelength showed a downward trend. It could be that the hydroxyl group of rhizome and stem cell wall was complexed with Pb and the saturation of hydrogen bond decreases. At the same time, high Pb stress inhibited the root secretion of *K. paniculate*, organic acids, amino acids, polypeptide substances, and protein and root transport channels were affected. The absorbance of rhizome relative to leaf was significantly reduced in each peak spectrum change trend of 1030 cm^{-1} value in the infrared spectrum of leaf tissue with the increase of Pb treatment concentration, and finally tended to stabilize. As the Pb concentration increased, the level of cell membrane peroxidation was deepened, and the peroxide products of aliphatic ketones accumulated in the leaves to enhance the resistance of plates to Pb, which caused the increase in 1065 cm^{-1} values. The comparative analysis of the variation trend is 1030 cm^{-1}. When roots, stems, and leaves are under high concentration stress, leaf tissue can still show specific Pb adaptability. It is speculated that some of the toxicity matter might be left in the root in exchange for reducing the toxicity in other parts to ensure the relative balance of the whole plant's physiological metabolism, which is consistent with the physiological indexes of a plant root system.

5. Conclusions

Pb toxicity symptoms can affect physiological responses and inhibit plant growth and development. In this study, *K. paniculate* was found to be a highly Pb-tolerant woody plant. Under heavy metal Pb stress, the protective stress response of various antioxidant enzymes was obvious, and the threshold of stress damage production was high. Pb concentrations above 600 mg/L caused a decrease in various physiological indicators and inhibited plant growth, but the survival rate was not affected. Pb is mainly stored in the roots, and the local avoidance mechanism blocks the overall plant damage by optimizing the physiological and biochemical properties of the roots. In contrast, the fixation and sequestration of Pb by the cell wall and vesicles of root cells are the main pathways of Pb resistance in *K. paniculate*. As the concentration of Pb stress rose, the increase in soluble fractions acted as a fixation of Pb and the proportion of Pb content in the organelles. Microscopic TEM images showed slight damage to low and medium concentrations of Pb. However, at high concentrations of stress, a large amount of Pb was observed to accumulate in the cell wall, vesicles, and cell voids, breaking the structure of the cell wall and leading to the entry of Pb into the interior of the cell, which then disrupted the function of subcellular structures. At the same time, Pb stress increased the number of groups such as -COOH and $-CH_3$ in *K.*

paniculate, which can form stable compounds with Pb, thus reducing the toxic effect of Pb on *K. paniculate*. Thus, this study provides a theoretical basis for the phytoremediation of lead-contaminated soil by *K. paniculate.*

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

All data generated or analysed during this study are included in this published article. The datasets generated and/or analysed during the current study are not publicly available due The raw data are presented in the article but are available from the first author on reasonable request.

Competing interests:

I declare that the authors have no competing interests as defined by BMC, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Authors' contribution:

Tianzhi Xie: Methodology, Formal analysis, Writing - review & editing. Yonghua Chen: Supervision, Validation, Writing - review & editing. Rongkui Su: Supervision, Validation, Writing - review & editing. Haisong Yao: Writing - review & editing. Lu Du: Writing - review & editing. Jun Liu: Writing - review & editing

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References

1. A NS, B MI, C MRS, A WI, A MAK, D AM, B AR, F SHE (2017): Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. Chemosphere 171, 710-721
2. Ali H, Khan E, Sajad MA (2013): Phytoremediation of heavy metals: Concepts and applications. Chemosphere 91, 869-881
3. Bankaji I, CAdor I, Sleimi N (2015): Physiological and biochemical responses of Suaeda fruticosato cadmium and copper stresses: growth, nutrient uptake, antioxidant enzymes, phytochelatin, and glutathione levels. Environmental Science & Pollution Research 22, 13058-13069
4. Bhaduri AM, Fulekar MH (2012): Antioxidant enzyme responses of plants to heavy metal stress. Reviews in Environmental Science & Bio/technology 11, 55-69
5. Bosch A, Serra D, Prieto C, Schmitt J, Naumann D, Yantorno O (2006): Characterization of Bordetella pertussis growing as biofilm by chemical analysis and FTIR spectroscopy. Applied Microbiology and Biotechnology 71, 736-747
6. Cabot C, Sibole JV, Barceló J, Poschenrieder C (2014): Lessons from crop plants struggling with salinity. Plant Science 226, 2-13
7. Cai-Ying N, Ying-Xv C, Qi L, Guang-Ming T (2005): Subcellular localization of copper in tolerant and non-tolerant plant. Journal of environmental sciences (China) 17, 452-456, 2005
8. Chunfang T, Yonghua C, Qianni Z, Jianbin L (2019): Effects of peat on plant growth and lead and zinc phytostabilization from lead-zinc mine tailing in southern China: Screening plant species resisting and accumulating metals. Ecotoxicology & Environmental Safety 176, 42-49
9. Clemens S (2001): Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212, 475-486

10. Cosio C, Martinoia E, Keller C (2004): Hyperaccumulation of cadmium and zinc in thlaspi caerulescens and arabidopsis halleri at the leaf cellular level. Plant Physiology 134, 716-725
11. Dou CM, Fu XP, Chen XC, Shi JY, Chen YX (2010): Accumulation and detoxification of manganese in hyperaccumulator Phytolacca americana. Plant Biology 11, 664-670
12. Epeldegui M, Breen EC, Hung YP, Boscardin WJ, Detels R, Martínez-Maza O (2007): Elevated expression of activation induced cytidine deaminase in peripheral blood mononuclear cells precedes AIDS-NHL diagnosis. Aids 21, 2265-2270
13. Feixiang W, Miaoyuan W, Da L, Xiaoying Y (2012): Effects of soil Pb and Cd stress on photosynthetic properties and pigment content of leaf blades of Rhizophora rosea. Hunan Agricultural Sciences 15, 103-105
14. Ghori NH, Ghori T, Hayat MQ, Imadi SR, Gul A, Altay V, Ozturk M (2019): Heavy metal stress and responses in plants. International Journal of Environmental Science and Technology 17, 1807-1828
15. Gu J, Zhang X, Han J, Zhao Z (2017): Global lead resources situation and development of lead resources in China. China Mining 26, 16-20, 44
16. Hall JL (2002): Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53, 1-11
17. Han L, Chen Y, Chen M, Wu Y, Su R, Du L, Liu Z (2020): Mushroom residue modification enhances phytoremediation potential of Paulownia fortunei to lead-zinc slag. Chemosphere 253, 126774
18. Han M, Yang H, Ding N, You S, Yu G (2021): The role of plant-associated bacteria in the phytoremediation of heavy metal contaminated soils. E3S Web of Conferences 261, 04006
19. He J, Ma C, Ma Y, Hong L, Kang J, Liu T, Polle A, Peng C, Luo ZB (2013): Cadmium tolerance in six poplar species. Environmental Science & Pollution Research 20, 163-174
20. Heckenroth A, Rabier J, Dutoit T, Torre F, Prudent P, Laffont-Schwob I (2016): Selection of native plants with phytoremediation potential for highly contaminated Mediterranean soil restoration: Tools for a non-destructive and integrative approach. Journal of Environmental Management 183, 850-863
21. Jain P, Kachhwaha S, Kothari SL (2009): Improved micropropagation protocol and enhancement in biomass and chlorophyll content in Stevia rebaudiana (Bert.) Bertoni by using high copper levels in the culture medium. Scientia Horticulturae 119, 315-319
22. James JJ, Drenovsky RE (2007): A basis for relative growth rate differences between native and invasive forb seedlings. Rangeland Ecology & Management 60, 395-400
23. Jinwei S, Yizong H, Mengchun S, Yanshan C, Xiaofang LI, Lijun Z, Xin DU, Weiguo G (2008): The review of heavy metals biotoxicity in soil. Journal of ecology 28, 2861-2869
24. Kang W, Bao J, Zheng J, Xu F, Wang L (2018): Phytoremediation of heavy metal contaminated soil potential by woody plants on Tonglushan ancient copper spoil heap in China. International Journal of Phytoremediation 20, 1-7
25. Kiboi MN, Ngetich FK, Mucheru-Muna MW, Diels J, Mugendi DN (2021): Soil nutrients and crop yield response to conservation-effective management practices in the sub-humid highlands agro-

ecologies of Kenya. Heliyon 7, 7156

26. Laghlimi M, Baghdad B, Hadi HE, Boua Bd Li A (2015): Phytoremediation mechanisms of heavy metal contaminated soils: a review. Open Journal of Ecology 5, 375-388
27. Li C, Tian Q, Rahman M, Wu F (2020): Effect of anti-fungal compound phytosphingosine in wheat root exudates on the rhizosphere soil microbial community of watermelon. 456, 223-240
28. Liu T, Zhu F, Yan W, Chen X, Huang X, Wang R, Wang X, Kang H, Yi X (2018): Assessment of koelreuteria paniculata seedling for phytroremediation of pyrene-contaminated soils. Water, Air, and Soil Pollution 229, 1-396
29. Lux A, Sottníková A, Opatrná J, Greger M (2010): Differences in structure of adventitious roots in Salix clones with contrasting characteristics of cadmium accumulation and sensitivity. Physiol Plant 120, 537-545, 2004
30. Mahdavian K, Ghaderian SM, Schat H (2016): Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous Peganum harmala L. under Pb exposure. Environmental & Experimental Botany 126, 21-31
31. Masarovicova E, Kralova K, Kummerova M (2010): Principles of classification of medicinal plants as hyperaccumulators or excluders. Acta Physiologiae Plantarum 32, 823-829
32. Mudd GM, Jowitt SM, Werner TT (2017): The world's lead-zinc mineral resources: Scarcity, data, issues and opportunities. Ore Geology Reviews 80, 1160-1190
33. Myung, Chae, Jung, and, Iain, Thornton (1996): Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. Applied Geochemistry 11, 53-59
34. Nie J, Liu Y, Zeng G, Zheng B, Tan X, Liu H, Xie J, Gan C, Liu W (2016): Cadmium accumulation and tolerance of Macleaya cordata: a newly potential plant for sustainable phytoremediation in Cd-contaminated soil. Environmental Science & Pollution Research 23, 10189-10199
35. Pan G, Yan W, Zhang H, Xiao Z, Zheng L (2019): Subcellular distribution and chemical forms involved in manganese accumulation and detoxification for Xanthium strumarium L. Chemosphere 237, 124531
36. Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E, Parsons JG (2001): Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.). Bulletin of Environmental Contamination & Toxicology 66, 727-34
37. Rascio N, Navari-Izzo F (2011): Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Science 180, 169-181
38. Ruley AT, Sharma NC, Sahi SV, Singh SR, Sajwan KS (2006): Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in Sesbania drummondii grown in soil. Environmental Pollution 144, 11-18
39. Ryser P, Emerson P (2007): Growth, root and leaf structure, and biomass allocation in Leucanthemum vulgare Lam. (Asteraceae) as influenced by heavy-metal-containing slag. Plant & Soil 301, 315-324

40. Shi Y, Mu X, Li K, Shao H (2016): Soil characterization and differential patterns of heavy metal accumulation in woody plants grown in coal gangue wastelands in Shaanxi, China. Environmental Science and Pollution Research 23, 13489-13497
41. Tandy S, Schulin R, Nowack B (2006): The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere 62, 1454-1463
42. Vaculik M, Konlechner C, Langer I, Adlassnig W, Puschenreiter M, Lux A, Hauser MT (2012): Root anatomy and element distribution vary between two Salix caprea isolates with different Cd accumulation capacities. Environmental Pollution 163, 117-126
43. Vallee B, Ulmer D (1972): Biochemical effects of mercury, cadmium, and lead. Annual Review of Biochemistry 41, 91
44. Vandame R, Palacio MA (2010): Preserved honey bee health in Latin America: a fragile equilibrium due to low-intensity agriculture and beekeeping? Apidologie 41, 243-255
45. Wang B, Xie HL, Ren HY, Li X, Chen L, Wu BC (2019): Application of AHP, TOPSIS, and TFNs to plant selection for phytoremediation of petroleum-contaminated soils in shale gas and oil fields. Journal of Cleaner Production 233, 13-22
46. Wang Y, Xin N, Chang Q, Liang H, Zhou JE (2016): Characterization and iron removal treatment of ion-adsorption rare earth tailings in southern China. Journal of Minerals & Materials Characterization & Engineering 4, 127-134
47. Xue C, He CQ, Shi Z, Chen X, Muo Q (2019): Effect of spent mushroom substrate on strengthening the phytoremediation potential of Ricinus communis to Cd- and Zn-polluted soil. International Journal of Phytoremediation 20, 1-11
48. Yakun S, Xingmin M, Kairong L, Hongbo S (2016): Soil characterization and differential patterns of heavy metal accumulation in woody plants grown in coal gangue wastelands in Shaanxi, China. Environmental Science and Pollution Research 23, 13489-13497
49. Yan Ao.lei, Wu TT, Wang Youbao, Zhang X. q (2010): Tolerance and accumulation characteristics of three ornamental plants to heavy metal cadmium. Acta Ecologics Sinica 30, 2491-2498
50. Yang W, Zhang T, Li S, Ni W (2014): Metal removal from and microbial property improvement of a multiple heavy metals contaminated soil by phytoextraction with a cadmium hyperaccumulator Sedum alfredii H. Journal of Soils & Sediments 14, 1385-1396
51. Yu K, Meng Q-M, Zou JH (2010): Effect of cadmium on growth, chlorophyll content and cellular ultrastructure of maize seedlings. North China Journal of Agriculture 25, 118-123
52. Zhang Q, Chen Y, Du L, Zhang M, Han L (2019): Accumulation and subcellular distribution of heavy metal in Paulownia fortunei cultivated in lead-zinc slag amended with peat. International Journal of Phytoremediation 21, 1-8
53. Zhengzheng L, Lun W, Ya T, Guang Y (2007): Effect of pb, zn and their interactions on the chlorophyll content and antioxidant enzyme systems of houttuynia cordata thunb. Acta Ecologica Sinica 27, 5441-5446

Figures

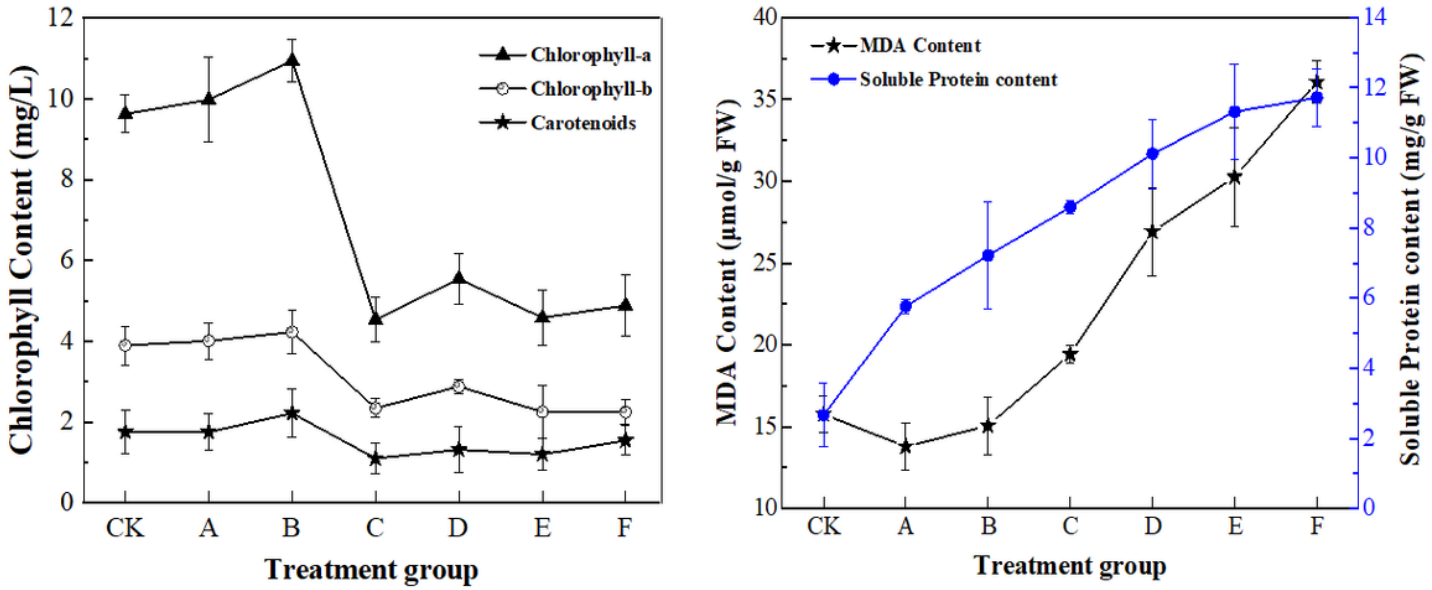

Figure 1

Pb stress on chlorophyll (left) and MDA content soluble protein content (right) in the K. paniculate leaf under different treatment group

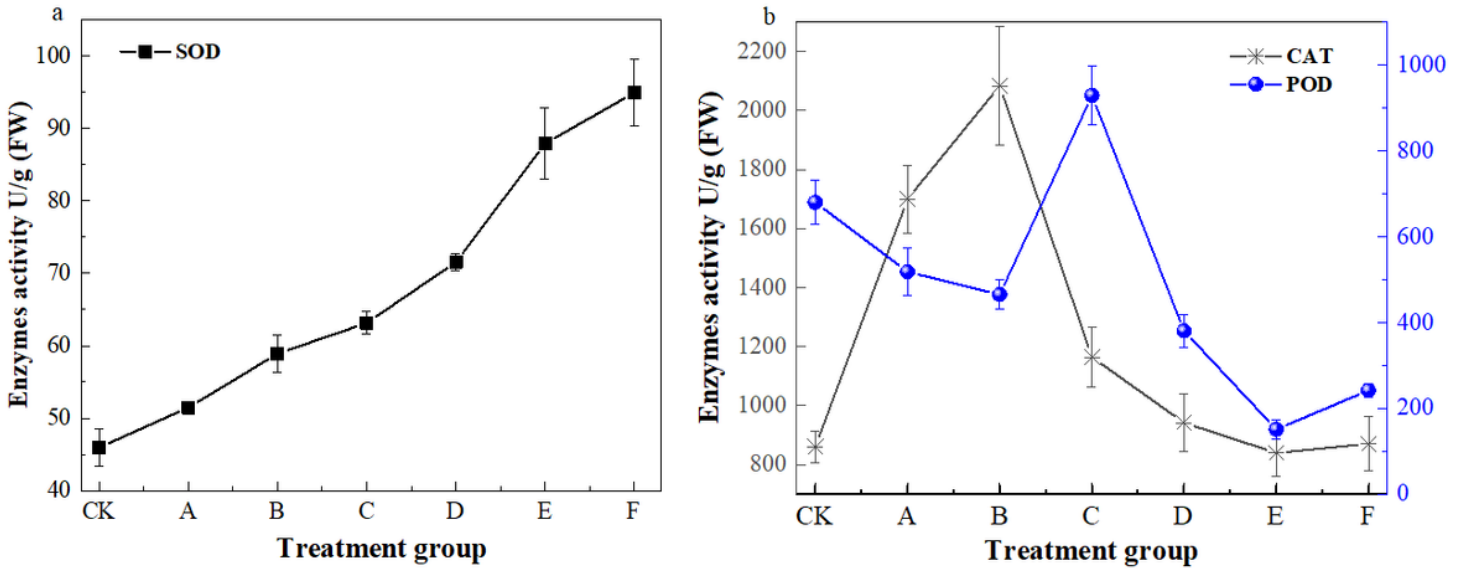

Figure 2

Effects of Pb stress on antioxidant enzymes activity in leaf blades

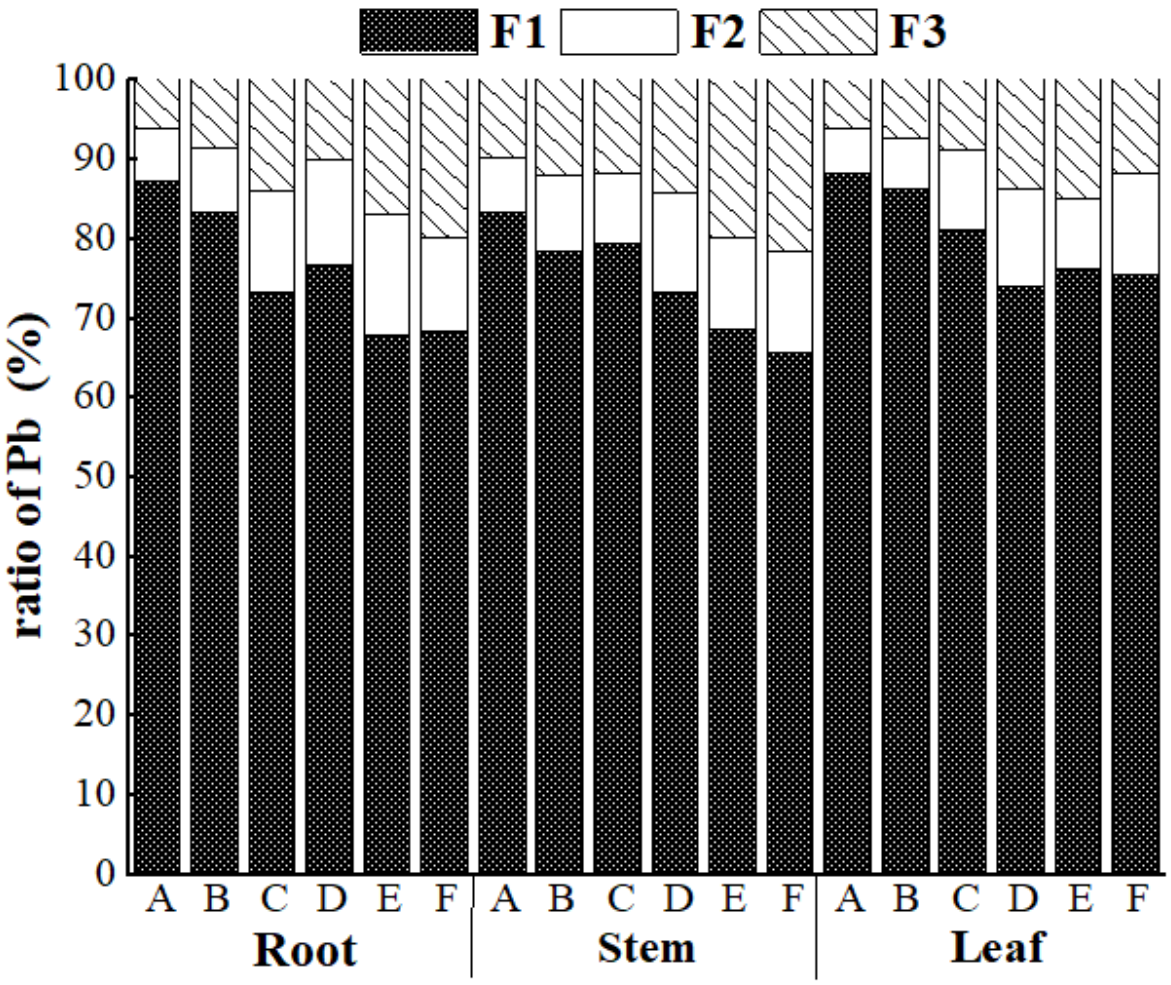

Figure 3

Distribution of Pb in different subcellular organs of *K. paniculate*

Note: F1 represents cell wall content fraction, F2 organelle content fraction and F3 soluble component content fraction.

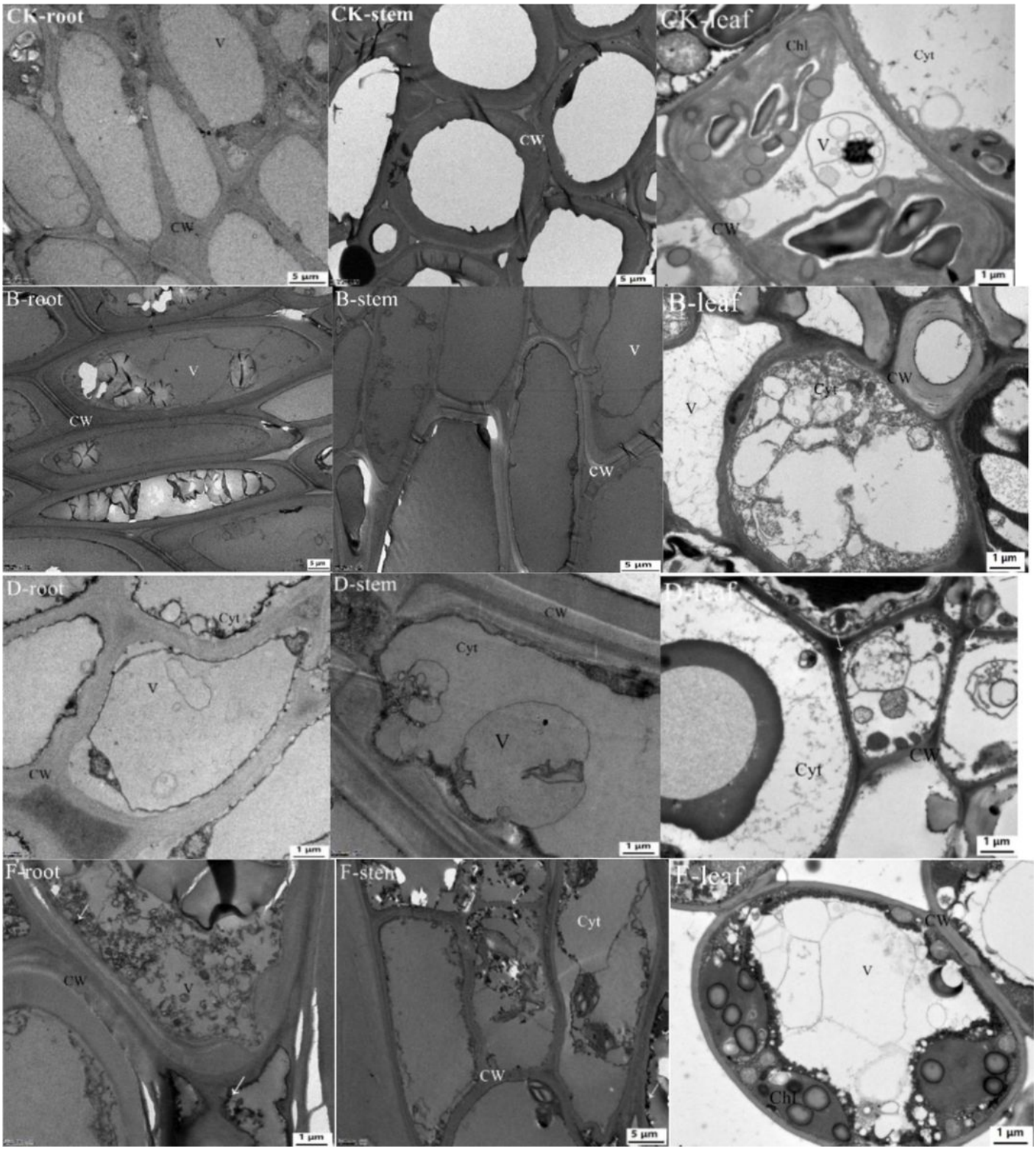

Figure 4

The effects of Pb stress on plant microstructure

Note: pictures of root, stem and leaf cells of K. paniculate seedlings in four treatments taken by TEM, CK treatment group, B treatment group (400 mg·L^{-1}), D treatment group (800 mg·L^{-1}), F treatment group (1200 mg·L^{-1}).; CW: cell wall; SG: starch granules; Cyt: cytoplasm; V: vacuoles; Chl: chloroplast

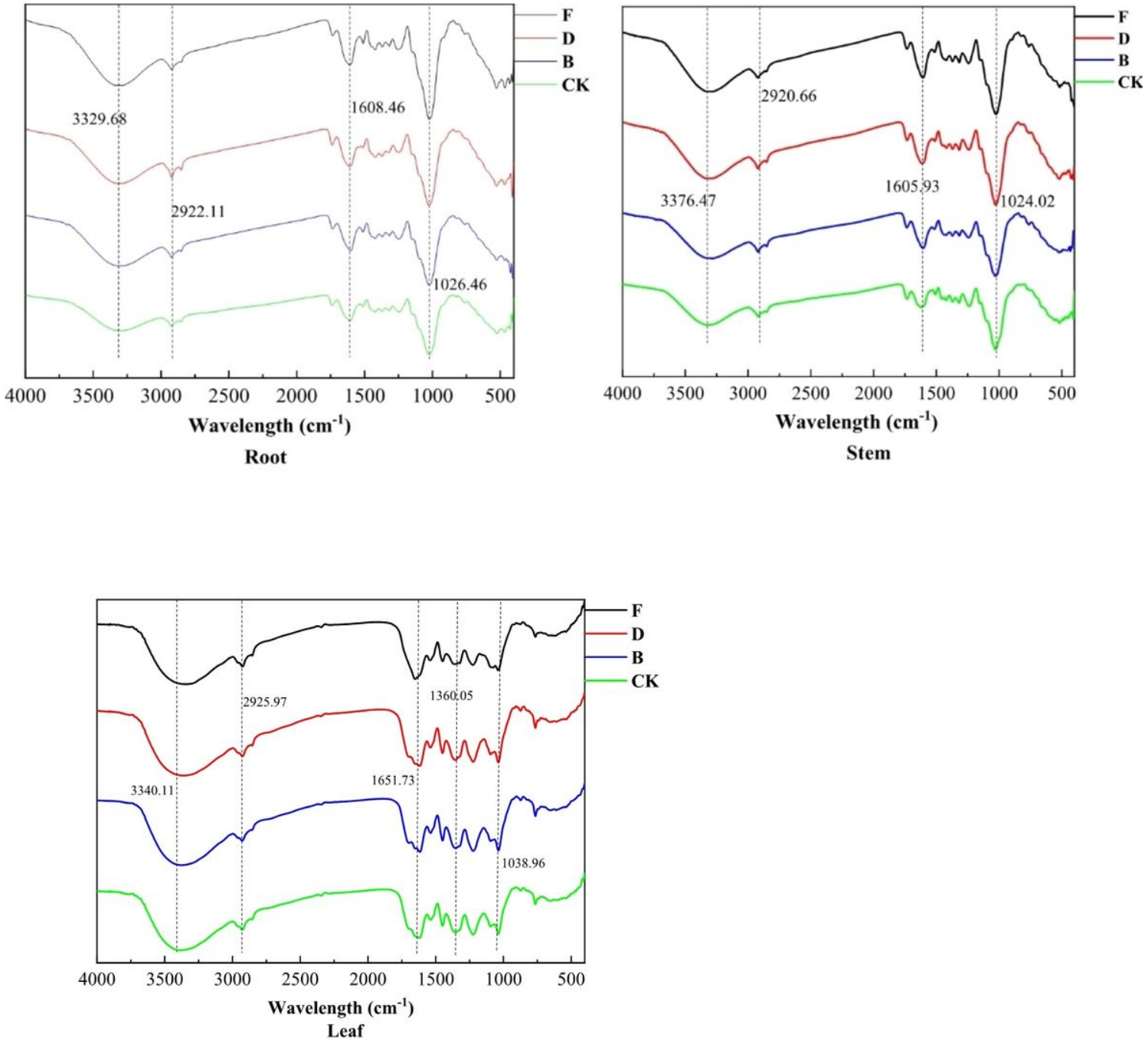

Figure 5

The functional groups of K. paniculate tissues on lead stress at different concentrations

Note: CK treatment group, B treatment group (400 mg·L^{-1}), D treatment group (800 mg·L^{-1}), F treatment group (1200 mg·L^{-1}).