

Mechanism of zinc alleviating cadmium toxicity in mangrove plant (*Kandelia obovata*)

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Research Article

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Abstract

Cadmium pollution is very common in mangrove ecosystem in China, and the pollution situation is serious. Zinc has been used to reduce cadmium accumulation in soil and plants, and phenolic acid metabolism plays an important role in plant response to stress. Therefore, the aim of this study was to clarify whether zinc alleviates cadmium toxicity in mangrove plants through phenolic acid metabolism. The cadmium contaminated *Kandelia obovata* was treated with different concentrations of (80,300,400mg/kg) ZnSO_4 in pot experiment. The results showed that the cadmium content and zinc content of *Kandelia obovata* in its leaves were in the range of $0.077 \sim 0.197 \text{ mg kg}^{-1} \text{ dw}$ and $90.260 \sim 114.447 \text{ mg kg}^{-1} \text{ dw}$ under different zinc treatment concentrations. The synthesis of phenolic acid metabolites such as 4-hydroxy benzoic acid, chlorogenic acid and cinnamic acid increased. Low dose zinc sulfate treatment had significant positive effects on the biomass, phenolic acid metabolization-related enzyme activities, antioxidant capacity, chlorophyll and soluble sugar contents of cadmium-contaminated mangrove plants. The results showed that low dose of Zn could enhance the metabolism of phenolic acid and synthesize more phenolic compounds such as hydroxycinnamic acid and hydroxybenzoic acid to increase the antioxidant capacity of mangrove plants.

Novelty Statement

This study found that zinc could alleviate cadmium toxicity of mangrove plants by enhancing phenolic acid metabolism. Zinc treatment can promote the synthesis of benzoic acid and cinnamic acid derivatives in the plants, and enhance the antioxidant capacity of plants so as to alleviate the oxidative damage caused by cadmium pollution. Low concentrations of ZnSO_4 complex repair agent would be an effective strategy in mangrove areas affected by cadmium stress. As it not only promotes the growth of mangrove plants, but also supplements the animal diet with phenolic compounds and trace element zinc.

Introduction

Mangroves are located in the interlaced zone of coastal areas in tropical regions and constitute a high-yield ecosystem that supports a variety of plants and animals through the food chain (Bharathkumar et al. 2007). Some common and widely distributed mangrove species like *Aegiceras corniculatum*, *Sonneratia caseolaris*, *Kandelia obovata* have a great medicinal value of their useful metabolites extracts (Chen et al. 2012). The economic value of mangroves brings a great wealth to human beings, however, due to various interference of human activities like mining wastes, metal smelting waste residues, untreated domestic sewage cause mangroves suffering from serious heavy metal pollution and their areas are decreasing (Das et al. 2016). Analuddin's research showed that mangroves were suffering from serious cadmium and zinc pollution, and mangroves could tolerant to various metals pollution in their environment (Analuddin et al. 2017, Sundaramanickam et al. 2016). Cadmium (Cd) is a widely existed nonessential element, which is classified as a harmful heavy metal to human health (Chao et al. 2009, Shang et al. 2020). Cadmium can be absorbed and accumulated by some special plants, and flows into higher nutrient level organisms through the intricate food chain (Adamczyk-Szabela et al. 2020), threatening human health (Bodin et al. 2013, Chen et al. 2021). Zinc (Zn) is a familiar essential element of plants and animals, plays a fundamental role in stabilizing and protecting biofilms from oxidative and peroxidation damage (Zhang et al. 2022). However, high levels of Zn can also cause heavy metal pollution in mangrove ecosystems, lead to restricted plant germination, reduced root development and induced plant aging (Chen et al. 2019, Lefevre et al. 2014). Cd and Zn are in the same group with similar physical and chemical properties, and always exist together in nature (H. G. Zha 2004, Mongkhonsin et al. 2016). Mongkhonsin had proved that Zn can reduce Cd toxicity under the dual treatments in *Gynura pseudochina* (Mongkhonsin et al. 2016). Our previous studies also found that 100 mg kg^{-1} Zn treatment can ease the cadmium toxicity in *Kandelia obovata* (Chen et al. 2019). Many researchers speculated that this phenomenon is caused by phenolic acid metabolism, but no one has specified what the mechanism is and tested the hypothesis.

Phenolic compounds are secondary metabolites consisting of hydroxylated aromatic compounds which contain carbon-based found only in plants and microorganisms (Tato et al. 2013), which can protect plant tissues from wounding, oxidative damage and insects, pathogen infections (Ali et al. 2005). For example, *Kandelia obovata* contains various common phenols such as cinnamic acids, flavonoid and phenylpropanoid derivatives, and their ecological function have been tested in vitro antioxidant and heavy metal bioavailability assays (Haoliang et al. 2007, Jiang et al. 2017, Li et al. 2016). Phenolic compounds play a variety of important chemical and biological functions in plants adapt to various changing environment. These physiological processes are metabolic plasticity, because plants can respond to external pressures by rapidly inducing phenolic compounds synthesis in a reversible way (Tanveer et al. 2022). For instance, the addition of cadmium significantly increased the total content of phenol in mangrove species such as *K. obovata* (Kováčik et al. 2009). Numerous studies have also reported that increased phenolic compounds in plant tissues and root secretions is a special response to different biological and abiotic stresses (Tato et al. 2013). Chen et al. observed that ZnSO_4 -treated *K. obovata*, as compared to only CdCl_2 -treated *K. obovata*, showed higher biomass and had stronger antioxidative capacity due to the enhancement of its phenolic biosynthesis (Chen et al. 2019, Zhao Hu & Wenjiao 2015). Phenolic acids are mainly synthesized by shikimic acid and phenylpropanoid pathways (Ali et al. 2005, Barros et al. 2019). The precursors of shikimic acid-mediated phenolic acid synthesis are mainly aromatic amino acids, phenylpropyl amino acids, and tryptophan produced by simple carbohydrate glycolysis and pentose phosphate pathway (Abdulrazzak et al. 2006). The shikimic acid pathway is a common pathway to provide precursors for subsequent secondary metabolites. It also shows that how primary and secondary aromatic metabolism related. It has been estimated that 60% of the total plant

biomass consists of molecules passing through the shikimic acid pathway (Tato et al. 2013). According to the above analysis, soluble sugar content in plant leaf is much directly influenced the amount of phenolic compounds synthesis, and the synthesis of soluble sugar is formed by the photosynthesis of plants, while plant photosynthesis ability is closely related to leaf chlorophyll content. So this experiment measured soluble sugar content and chlorophyll content of plant to assess its phenolic acids metabolism. Phenolic compounds metabolism related enzymes: L-phenylalanine ammonia-lyase (PAL), which can catalyze phenylalanine to cinnamate; shikimic acid dehydrogenase (SKDH), which can provide substrate for PAL; cinnamyl alcohol dehydrogenase (CAD), which can provide precursors for the synthesis of lignin; polyphenol oxidase (PPO), which can catalytic oxidation of catechol to catechol diquinone and act on the substrate of monophenol monooxygenase (Kováčik et al. 2009). These phenolic acids are considered to be effective substances protecting the plants against oxidative damage caused by heavy metal stress. Because the structure of phenolic acids makes them have a strong ability to scavenge free radicals and chelate heavy metals, which prevent Fenton reactions. In particular, phenolic acids like caffeic acid, chlorogenic acid, ferulic acid, and *p*-coumaric acid have been shown to have greater antioxidant capacity than hydroxyl derivatives of benzoic acid such as *p*-hydroxybenzoic acid, vanillic acid, and siringic acid (Cristina Sgherri 2003). Phenolic acid not only have the function of free radical scavenging but also inhibit lipid peroxidation and electron donors (Maqsood et al. 2014, Shi et al. 2010). So they can be used as excellent reaction substrates for some antioxidant enzymes (peroxidases) to reduce oxidative stress (Oh et al. 2009, Posmyk et al. 2009). In addition, phenolic acid can protect photosynthetic organs from light damage under heavy metal stress (P. Burchard 2000). Recently, Jiang et al. reported that phenolic acid content is related to heavy metal tolerance process of mangroves, particularly which can prevent mangrove plants against oxidative damage caused by heavy metals stress (Das et al. 2016, Jiang et al. 2017, Michalak 2006, Rui et al. 2016). DPPH is a free radical that can remain stable at room temperature and produces a violet solution in ethanol. However, it can be reduced in the presence of an antioxidant molecule, giving rise to uncolored ethanol solutions. The use of DPPH provides a simple and rapid method for evaluating antioxidants (Adjimani & Asare 2015, Mensor et al. 2001). Ferric reducing antioxidant power (FRAP) is another method to estimate the antioxidant capacity of phenolic acids (Afroz et al. 2016).

So far, nutrient supply has been one of the effective methods to induce tolerance responses to different heavy metals in plants, such as silicon (Se), phosphorus (P) and Silicon (Si) (Cui et al. 2017, Xie et al. 2014), or organic acid supply such as SA (salicylic acid) and JA (jasmonic acid). They act against stress by enhancing antioxidant activity or chelating with heavy metals that stimulates plant growth (Irtelli & Navari-Izzo 2006, Khan et al. 2016, Liu et al. 2016). However, there are rarely researches about the mechanism of plant tolerance to heavy metals by heavy metal interaction stimulate phenolic acid metabolism. Therefore, the purpose of this study was to solve the following problems: whether the addition of ZnSO_4 could alleviate the toxicity of cadmium on plant, and whether its resistance could be attributed to heavy metal interaction stimulate phenolic acid metabolism in *K. obovata*.

Materials And Methods

Plant Material Collection and Treatments

In this study, mature *Kandelia obovata* hypocotyls were obtained from Jiulongjiang Estuary, Fujian province, China. The hypocotyls of *Kandelia obovata* were soaked in 10‰ KMnO_4 solution for 24 h, then rinsing with distilled water. Hypocotyls with strong vitality were selected to sand culture until four-leaf seedlings, and then the same size seedlings were transplanted to 2 L plastic buckets for soil culture.

The soil was collected from cadmium-contaminated mangrove wetland, Jiulongjiang estuary. Sampling points are in 24°28' N, 117°24' E. Background values of Cd and Zn in sediments were 3.99 mg kg^{-1} and $367.54 \text{ mg kg}^{-1}$, respectively. The collected soil was treated with heavy metals after debris removal. Our experiment consisted of three treatments of 80, 300, and $400 \text{ mg} \cdot \text{kg}^{-1} \text{ ZnSO}_4 (\text{ZnSO}_4 \cdot 7\text{H}_2\text{O})$, and another control treatment that was only added to the same amount of distilled water without Zn application, which were respectively applied to the sediment and then stirred well for 30 days. After that transplanting the seedlings into the stirred sediment cultivating for 60 days. Each treatment was repeated three times in this experiment.

Determination of Chlorophyll Content in Leaves

Cut the mature leaves with no middle veins into small pieces, weighed 0.2 g of the leaves into a mortar containing a small amount of calcium carbonate and quartz sand. Then added 5 mL 95% ethanol to the mortar and homogenized them until the tissues turn white. The mixture was filtered through a funnel and the filtrate was filled to 25 mL with distilled water. The absorbance of the filtrate was measured at the wavelength of 645 nm and 663 nm. The chlorophyll content was calculated by the equation of Sae-Lee et al. (2012) (Chen et al. 2019).

Determination of Soluble Sugar in Roots and Leaves

Soluble sugar was extracted from root and leaf samples (0.1 g) with 1.5 mL sodium phosphate buffer (pH 6.8). The samples were centrifuged for 20 min, $12,000 \times g$, 0.5 mL supernatant was measured and mixed with 0.5 mL phenol (5%) and 2.5 mL concentrated sulfuric acid. Shake the mixture well and leave it at room temperature for 30 minutes to color. Then, the absorbance was measured with distilled water as the blank at the wavelength of 485 nm. The standard curve was drawn with sucrose ($100 \mu\text{g/L}$) as the standard solution (Falahi et al. 2018).

Determination of Cadmium and Zinc Content

In order to detect the content of Cd^{2+} and Zn^{2+} in plant leaves, 0.05 g of dried *K. obovata*'s leaves underwent digestion with 5 mL nitric acid and 1 mL H_2O_2 for 4 hours and was diluted with 50 mL distilled water, subsequently. The total content of Cd^{2+} and Zn^{2+} in leaves were measured directly by ICP-MS (Agilent 7500 ICP-MS, USA) (Chen et al. 2019).

Detection of Phenolic Acids by HPLC-QQQ-MS

Qualitative and quantitative determination of phenolic acids, 0.1 g of grinding lyophilized plant leaves leaching with 3 mL of water by oscillating in 300 rpm, 4 °C for 4 h, followed by overnight maceration. The resulting extract was centrifuged in 7500 rpm, 4 °C for 10 min and filtered with 0.45 μm PVDF membrane. The Agilent 1290 LC 6490 QQQ was used for HPLC-MS analysis. Chromatographic column specification: 00D-4462-YD, Kinetex 2.6 $\mu\text{C}18$ 100A, New column 100 $\times$ 3.0 mm high performance chromatographic column. The mobile phase consisted of two solvents: mobile phase (A) 2% glacial acetic acid and (B) methanol, the gradient program started with 75% of mobile phase A in 1 min, which decreased to 73% in 8 min and then to 50% in 3 min and after that increased to 75% in 2 min. The velocity of mobile phase was 0.3 mL min^{-1} and the injection samples volume was 0.5 μL . Column temperature was 30 °C, pressure was 500 bar. The mass spectrometer was worked in a multiple reaction monitoring mode (MRM). Mass spectrometric detection was operated in a negative ion mode after electrospray ionization. The following parameters were about the mass spectrometer: capillary voltage, 3371 V; drying gas (nitrogen) temperature, 350 °C; flow, 11.0 L min^{-1} ; collision gas, nitrogen (99.999%). The m/z ratios for precursor and product ions of target analytes, as well as collision energies and retention times, are presented in Figure. 2, Figure. S1 (Supplementary materials) and Table. 4, respectively.

Phenolic Acid Metabolism-Related Enzyme Assay

To determination of L-phenylalanine ammonia-lyase (PAL) activities, fresh leaves (0.2 g) were homogenized in liquid nitrogen and then extracted with 4 mL buffer solution (50 mM Tris pH 8.5, 14.4 mM 2-mercaptoethanol, 5% w/v insoluble polyvinyl pyrrolidone). The homogenate was centrifuged at 12000 $\times$ g for 15 min, 4°C. Then adding 0.5 mM, pH 8.0 Tris-HCl buffer and 10 μM L-phenylalanine solution to the obtained supernatant and incubating the mixture at 35°C for 2 h. The reaction was terminated with 50 μL 5 M hydrochloric acid acidification. The content of reacted L-phenylalanine in the presence of PAL was determined by UV-Spectrophotometer at 290 nm. The total protein concentration in the extract was measured by the Bradford (1976) method. PAL activity was expressed as $\text{nmol min}^{-1} \text{mg}^{-1} \text{protein}$ (Ali et al. 2006).

To determination of shikimic acid dehydrogenase (SKDH), cinnamyl alcohol dehydrogenase (CAD) and polyphenol peroxidase (PPO) activities, all of fresh leaves were homogenized in mortar with 50 mM potassium phosphate buffer (pH 7.0) at 4 °C. The obtained homogenates were stored in an ice bath and centrifuged successively at a speed of 15000 g for 15 min, 4°C (Blasco et al. 2013, Kováčik et al. 2009). The obtained supernatant is crude enzyme extract. SKDH activity detection was performed in 0.1 M pH = 9 Tris-HCl buffer, 2 mM shikimic acid 1.45 mL, 0.5 mM NADP 1.45 mL was gradually added to 0.1 mL crude enzyme solution. The absorbance was read at 340 nm over 1 min and the molar absorbance of 6.22 $\text{mM}^{-1} \text{cm}^{-1}$ was used to calculate its activity. CAD activity was measured by using 0.1 M Tris-HCl buffer (pH 8.8). The reaction tube contained 1.45 mL of 1 mM coniferyl alcohol, 1.45 mL of 1 mM NADP and 0.1 mL of supernatant. Measurement and calculation of CAD activity were the same as SKDH. PPO detection experiment was carried out in reaction centrifuge tube containing potassium phosphate buffer 50 mM 2.85 ml (pH 7.0), catechol 60 mM 50 μL and crude enzyme extract 0.1 mL. The absorbance was read at 420 nm after 2 minutes later (Kováčik et al. 2009).

DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical-Scavenging Activity and Ferric-Reducing Antioxidant Power (FRAP) of Phenolic Acids in Leaves Assay

Adding 3 mL distilled water to 0.1 g of ground freeze-dried leaves. Then the homogenate was shaken in a shaker at 300 rpm, 4 °C for 4 h. After that, centrifuging at 7500 rpm, 4 °C for 10 min. The supernatant was filtered with 0.22 μm membrane to obtain phenolic acids crude extracts of leaves.

0.1 $\text{g} \cdot \text{L}^{-1}$ DPPH solution was made by dissolving in a small amount of toluene firstly and then diluting with 60% ethanol. Transferring 2.0 ml crude extract of phenolic acid to 2mL of 0.1 $\text{g} \cdot \text{L}^{-1}$ DPPH solution, which was rapidly mixed and stored at room temperature for 20 min without light. The absorbance was determined at 517 nm with 60% ethanol as blank. DPPH radical scavenging activity was calculated (Afroz et al., 2016). The preparation method of FRAP reagent is the same as Afroz's method (Afroz et al., 2016). 200 μL of the above phenolic acid crude extract was mixed with 1.5 mL FRAP reagent and reacted at 37 °C for 4 minutes. The absorbance of the mixture was measured at the wavelength of 593 nm using distilled water as the blank. Then FRAP of phenolic acids in leaves was detected.

Statistical Analysis

All data related to biomass, cadmium, zinc and phenolic acid content and enzyme activity of the *K. obovata* leaves were submitted to SPSS 25 software for variance analysis (ANOVA), and Duncan multi-range test was conducted at the significance level ($P \leq 0.05$) of 5% to compare the significance of differences among them.

Results

Effects of Zn²⁺ treatment on the biomass and heavy metal content of cadmium-contaminated *K. obovata*'s leaves

Under the treatment of 400 mg kg⁻¹ ZnSO₄, the biomass of *K. obovata* leaves reached the lowest value, which was 33% lower than that of the control group (Table. 1). The 300 mg kg⁻¹ ZnSO₄ treatment also reduced in leaves' biomass contrast to control, but less severely than in the 400 mg kg⁻¹ ZnSO₄ treatment (Table. 1).

For the cadmium and zinc concentrations, the Cd²⁺ contents of leaves under 300 or 400 mg kg⁻¹ ZnSO₄ treatment was about 1.75 times higher than that of the control (Table. 1). But the addition of 80 mg kg⁻¹ ZnSO₄ treatment reduced in leaves' cadmium contents contrast to control. The addition of ZnSO₄ treatment made the Zn²⁺ concentration in leaves increased comparing with the control treatment (Table. 1). The application of 400 mg kg⁻¹ ZnSO₄ treatment could increase the content of Cd²⁺ and Zn²⁺ in plant leaves largely (Table. 1).

Effects of Zn²⁺ treatment on the chlorophyll and soluble sugar content of cadmium-contaminated *K. obovata*'s leaves

Total chlorophyll content in leaves was shown in Figure. 1A. The 80 mg kg⁻¹ ZnSO₄ treatment group had the highest content of chlorophyll (1.825 mg g⁻¹ FW) in leaves among these four treatments. Adding 80 mg kg⁻¹ ZnSO₄ treatment could increase the chlorophyll content comparing to control treatment (1.687 mg g⁻¹ FW). But adding 300 or 400 mg kg⁻¹ ZnSO₄ treatment could decrease the content of chlorophyll in leaves comparing with the control group.

The content of soluble sugar in leaves was increased significantly by 300 or 400 mg kg⁻¹ ZnSO₄ treatment (Figure. 1B). The most reduction in soluble sugar content in leaves was found at 80 mg kg⁻¹ ZnSO₄ treatment (60.452 mg g⁻¹ DW) as compared to control treatment (64.804 mg g⁻¹ DW). With the treatment of 400 mg kg⁻¹ ZnSO₄ treatment, soluble sugar content of leaves (79.219 mg g⁻¹ DW) showed lower increase than that of in 300 mg kg⁻¹ ZnSO₄ treatment (73.569 mg g⁻¹ DW) Figure. 1B.

Effects of Zn²⁺ treatment on phenolic acids of cadmium-contaminated *K. obovata*'s leaves

The maximum concentrations of both hydroxybenzoic acid and hydroxycinnamic acid, not including the benzoic acid, were all found in the 300 or 400 mg kg⁻¹ ZnSO₄ treatment, respectively. The minimum concentrations of these phenolic acids were found almost in the control (Table. 2). It was worth noting that for all detected kinds of phenolic acids, the different treatment concentrations of ZnSO₄ increased the content of above phenolic acids in comparison to plants treated with only 80 mg kg⁻¹ ZnSO₄, reaching maximum increases under the treatment concentration of 300 or 400 mg kg⁻¹ ZnSO₄ (Table. 2); nevertheless, plants treated with 400 mg kg⁻¹ ZnSO₄ raised larger content of Hyd and Cin than that of in 300 mg kg⁻¹ ZnSO₄ treatment. With regard to 4-hydroxy benzoic acid, cinnamic acid and chlorogenic acid, the levels were larger than other phenolic acids in all of the treatments (Table. 2 and Figure. S2).

Effects of Zn²⁺ treatment on phenolic acids metabolism related enzymes of cadmium-contaminated *K. obovata*'s leaves

The ability of phenolic acid metabolism related enzymes was also impacted by different concentration of ZnSO₄ treatment (Table. 3). PAL activities in the leaves of *K. obovata* showed a significant increase under 300 or 400 mg kg⁻¹ ZnSO₄ treatment, however, under 80 mg kg⁻¹ ZnSO₄ treatment, the PAL activity in leaves was lower than control group (Table. 3). The activity of PAL, SKDH, CAD and PPO in leaves reached to maximum values under 400 mg kg⁻¹ ZnSO₄ treatment, with increase of 43%, 86% 39% and 98% respectively comparing with the control group. On the other hand, the minimum enzyme activity values were almost found in control group plants. The treatment of different doses of ZnSO₄ resulted in an obviously enhancement in SKDH, PPO and CAD activities comparing with the control treatment (Table. 3). The SKDH, CAD and PPO activity, exceeding 5.4%, 3.0% and 4.3%, respectively, with respect to control under 80 mg kg⁻¹ ZnSO₄ treatment (Table. 3). In summary, the enzyme SKDH, CAD and PPO, given a rise activity with the increasing concentrations of ZnSO₄ treatments. In addition, the change of these enzymatic activities in 300 or 400 mg kg⁻¹ ZnSO₄ treatment was more than in 80 mg kg⁻¹ ZnSO₄ treatment (Table. 3).

Effects of Zn²⁺ treatment on antioxidant activity of cadmium-contaminated *K. obovata* leaves

The results of the DPPH free radical scavenging activity increased significantly after the application of ZnSO₄ treatments with respect to the control. However, no significant differences were observed between the control and 80 mg kg⁻¹ ZnSO₄ treatment (Figure. 1C). FRAP values at different concentration of ZnSO₄ treatment increased with respect to the control. The application of ZnSO₄ increased the reducing capacity of the ethanolic extracts comparing with the control, especially in the 300 or 400 mg kg⁻¹ ZnSO₄ treatment. It was observed that the FRAP values at 80 mg kg⁻¹ ZnSO₄ treatment were the same with the control treatment (Figure. 1D).

Discussion

Biomass, Cd and Zn content of *K. obovata*'s leaves

Biomass is one of the important indicators reflecting whether the plant is restrained (Kováčik & Bačkor 2007, Wang et al. 2016). Dealing with only 300 or 400 mg kg⁻¹ ZnSO₄ inhibited the growth of *K. obovata*, but treating with 80 mg kg⁻¹ ZnSO₄ could alleviate the cadmium toxicity on *K. obovata* (Table. 1). High concentration of ZnSO₄ treatment inhibited the growth of plants more apparent than low concentration of ZnSO₄ treatment (Table. 1). These results suggested that treatment with this concentration of Zn²⁺ (≤ 80 mg kg⁻¹ ZnSO₄) may initiate a special heavy metal tolerance mechanism to alleviate the cadmium toxicity in *Kandelia obovata* plants to some extent.

The toxicity of heavy metal stress to plants not only causes plant growth to slow down, but also leads to the accumulation of heavy metal in plant leaves (Armas et al. 2014). The accumulation of Cd²⁺ and Zn²⁺ ions in plant leaves can trigger membrane lipid peroxidation (Jia et al. 2017), cause to produce reactive oxygen species (ROS) (Weng et al. 2012). In this study, control plants showed lower ion concentrations of Cd²⁺ and Zn²⁺ than in high concentration of ZnSO₄ treatment like 300, 400 mg kg⁻¹ ZnSO₄ (Table. 1). The content of Cd²⁺ and Zn²⁺ in *K. obovata* leaves disposed with high concentration of ZnSO₄ could account for the depressed biomass in leaves found in these treatments (Table. 1). In addition, 300 or 400 mg kg⁻¹ ZnSO₄ caused the increasement of Cd²⁺ and Zn²⁺ concentration in respect to the control treatment (Table. 1), probably because of the synergism between the Cd²⁺ and Zn²⁺. However, 80 mg kg⁻¹ ZnSO₄ treatment inhibited the Cd²⁺ absorption in leaves (Table. 1), roughly due to the antagonism between the Cd²⁺ and Zn²⁺. Therefore, it can be seen that Cd and Zn presented antagonistic effects under low concentration of Zn treatment, while presented synergistic effects under high concentration of Zn treatment. Wang's report also confirmed these findings (Wang et al. 2016). This phenomenon could be explained by the competition between zinc and cadmium because of their similar chemical property (Wang et al. 2016). Zinc is essential element for plant growth at low concentrations, whereas cadmium is a non-essential metal for plants with a low toxicity threshold (5–10 mg /kg dw) (Wang et al. 2016). To counteract the toxicity of cadmium, plants must increase the transport of zinc from roots to shoots. Another group of authors have suggested that the interaction between cadmium and zinc is synergistic. In heavy metal hyper accumulative plants, the accumulation of zinc in stem was positively connected with the enhancement of Cd status in plants, and the author believed that the stimulation of Cd accumulation in stem was related to the increased xylem transport of Cd from roots to shoots (Wang et al. 2016). Besides, Turner and Marshall reported that the most accumulated zinc could bind to polygalacturonic acids and carbohydrates in cell wall (Xing et al. 2008). It has been reported that another possible mechanism for *A. marina* treating excessive zinc accumulation is to secrete excess zinc through leaf glandular (G.R. MacFarlane 2000). Under the condition of cadmium and zinc pollution, the growth of *K. obovata* was only slightly affected by 300 mg kg⁻¹ ZnSO₄ treatment, but an obvious reduction of foliar biomass of *K. obovata* was observed in 400 mg kg⁻¹ ZnSO₄ treatment. These results imply that low concentration of Zn²⁺ treatment could alleviate the Cd toxicity, but high concentration of Zn²⁺ treatment could increase the toxicity of heavy metals to plants. In this sense, Noa Lavid pointed out that the main reason of Zn²⁺ relating to the cadmium stress of *K. obovata* is to improve the antioxidant capacity induced by this trace element. As confirmed below, this may be related to the metabolism of phenolic acids (Cristina Sgherri 2003, Kováčik et al. 2009, Lavid et al. 2001).

Chlorophyll content, soluble carbohydrate content, and their relationship with phenolic acid metabolism

The synthesis of phenolic acids is from the amino acids produced by glycolysis, so it is closely related to the photosynthesis of plants (Cocetta et al. 2015). Chlorophyll content are the key parts of photosynthesis (Li et al. 2022). The reduction of total chlorophyll content was often found in biological stressed plants. Much of the decline in chlorophyll content is due to the fact that plants were under nutrient imbalance, oxidative stress like heavy metal stress (Wang et al. 2011). In this study, we found that the total chlorophyll content of leaves was decreased under 300 and 400 mg kg⁻¹ ZnSO₄ stress. On this basis, adding 80 mg kg⁻¹ ZnSO₄ treatment increased the total chlorophyll content of leaves comparing to the control. However, adding 300 or 400 mg kg⁻¹ ZnSO₄ treatment reduced the total chlorophyll content comparing to only add 80 mg kg⁻¹ ZnSO₄ treatment (Figure. 1A). The above results showed that adding 80 mg kg⁻¹ ZnSO₄ under high concentration of cadmium pollution could increase chlorophyll content and then enhance the photosynthesis of plants, while adding 300 or 400 mg kg⁻¹ ZnSO₄ could reduce chlorophyll content and weaken the photosynthesis of plants. Soluble sugar is the product of photosynthesis and soluble sugar levels are directly related to the synthesis of phenolic acid compounds. It has been reported that soluble sugar levels may influence the accumulation of flavonoid compounds (Cocetta et al. 2015). In our study, the change trend of soluble sugar content in leaves of *K. obovata* plants under zinc stress was contrary to that of chlorophyll content (Figure. 1B). According to Pearson correlation coefficients in Table. 5, chlorophyll content in leaves was highly correlated with soluble sugar content. Therefore, the soluble sugar content can indirectly reflect the amount of phenolic acid synthesis. However, we only know that the synthesis of phenolic acid is based on sugar metabolism, but we don't know whether the high sugar content does necessarily lead to the high metabolism of phenolic acid or not. So it is necessary to further explore the products of phenolic acid metabolism.

Effects of heavy metal stress on phenolic acid metabolism

Phenolic acids were often found when animals and plants are subjected to abiotic and biotic stresses. In previous studies, J. Lojonen found that birch leaves from contaminated areas had higher levels of several low-molecular weight phenols than birch leaves from control areas (J. Lojonen 2001). Begona Blasco put forward that phenolic acids are powerful antioxidants that can scavenge free radicals and other oxidizing substances (Blasco et al. 2013). However, so far this kind of research about the response of phenolic acids to heavy metals in mangrove plants is still very few. Bodin Mongkhonsin's (Mongkhonsin et al. 2016) studies indicated that herbal plant would increase more phenolic acids synthetic under high concentration of Zn and/or Cd treatment; K.Manquian-Cerda suggested that blueberry plantlets produced more phenolic acids to response Cd tolerance (Manquian-Cerda et al. 2016), whereas no one has studied low concentration of Zn alleviating Cd toxicity is due to the content of phenolic compounds upregulated.

Through statistics and comparison of these data, our study found that long-term exposure to 300 or 400 mg kg⁻¹ ZnSO₄ obviously enhanced the content of some phenolic acid metabolites (Table. 2). The application of 300 mg kg⁻¹ ZnSO₄ further raised the content of phenolic acids, but the increase of phenolic acids in plants treated with 80 mg kg⁻¹ ZnSO₄ was not as significant as that treated with 300 or 400 mg kg⁻¹ ZnSO₄. This discrepancy in findings may be due to the type of plant is different in our experiment. Cristina Sgherri et al also had reported the similar results in the past (Cristina Sgherri 2003). *Matricaria chamomilla* plants exposure in long periods of 60 and 120 µM CdCl₂ treatment resulted in higher levels of phenolic compounds in both young and old leaves (Kováčik et al. 2009). Also, previous results have shown that inhibiting phenolic acid metabolism would result in early death of transgenic tobacco plants' leaves and changes in its cell morphology (Lodovico Tamagnone 1998). Therefore, many studies have shown that phenolic acid metabolism is closely related to the normal growth of plants. It can also be seen from the above analysis that the content of phenolic acids change trend is the same as that of soluble sugar content in leaves of plants under zinc stress, so it can be concluded that the higher the soluble sugar content, the more phenolic acid synthesis.

Mechanism of heavy metal tolerance involved in phenolic acid metabolism

In our work, the application of ZnSO₄ treatment in cadmium-contaminated sediment, especially exposed to 300 or 400 mg kg⁻¹ ZnSO₄ treatment, raised the content of phenolic acids in leaves of *K. obovata* (Table. 2). The enhancement of phenolic acids content caused by ZnSO₄ addition made plants can survive in high concentrations of Cd and Zn stress (Table. 1). These phenolic acids are considered to play an important role in protecting the *K. obovata* from oxidative damage caused by Cd and Zn stress. Because phenolic acids are strong antioxidants which can scavenge free radicals and other reactive oxygen species (ROS) (Kim et al. 2006). The properties of phenolic acids may vary due to their different structures, mainly depending on the number and position of the hydroxyl groups on the aromatic ring (Kim et al. 2006). The phenolic acids in *K. obovata* plants are usually divided into two groups: benzoic and cinnamic acid derivatives. The antioxidant activity of cinnamic acid derivatives such as ferulic acid, caffeic acid and p-coumaric acid was better than that of hydroxybenzoic acid derivatives such as p-hydroxybenzoic acid, vanilic acid and salicylic acid (Kim et al. 2006). That is because that the presence of the CH = CH - COOH group in the hydroxycinnamic acids is considered to be key for the significantly higher antioxidative efficiency than the COOH in the hydroxybenzoic acids (Kim et al. 2006). In addition, phenolic acids can mitigate the effects of oxidative stress due to its electron donors function and can be used as an excellent substrates for some antioxidant enzymes (Blasco et al. 2013). From table 2, it can be seen that the application of ZnSO₄ significantly induces hydroxycinnamic acids and derivatives (chlorogenic acid, cinnamic acid, coumaric acid and ferulic acid), which can scavenge free radicals efficiently and stimulate antioxidant activity under different types of adversity stress in plants (Blasco et al. 2013). 300 or 400 mg kg⁻¹ ZnSO₄ treatment can stimulate the synthesis of benzoic and cinnamic acid derivatives more effectively than 80 mg kg⁻¹ ZnSO₄ treatment. Therefore, the application of ZnSO₄ treatment with a low concentration at the same cadmium concentration can stimulate the plant resistance to cadmium, which is achieved by stimulating the metabolism of phenolic acid to enhance the heavy metal tolerance of *K. obovata*. Bodin Mongkhonsin's studies indicated that Zn²⁺ and Cd²⁺ dominate with O and S ligands, which could be supplied by cell walls, phenolic compounds, and sulphur protein (Mongkhonsin et al. 2016). N. Garg found that Zn supplementation could be inhibitory for Cd-induced oxidative stress (Garg & Kaur 2013). It also has been reported that some certain metals like zinc are essential for adequate functional metabolism activities of plants, and they can be added to soil-crop systems as a remediation agent for heavy metals, such as in *Chenopodium album*, through application of ZnSO₄ or organics for chemical amendments (Rai et al. 2019). The plant phytoavailability of Cd (≥ 54.13%) was significantly reduced in the soil mixed with single superphosphate, triple superphosphate, and calcium magnesium phosphate sepiolite in conjunction with ZnSO₄ (Guo et al. 2017). The results of these authors and our study all indicated that ZnSO₄ can alleviate cadmium stress and ZnSO₄ is an effective remediation agent for cadmium pollution soil.

In order to determine the different reactions of different treatments to phenolic acids in our study, we measured the activities of major enzymes related to phenolic acids metabolism. PAL mediates phenylalanine to produce cinnamic acid, which is a key branching point in primary and secondary metabolism and it is also the first and most important regulatory step in the formation of many phenolic acids (Cocetta et al. 2015). Shikimate dehydrogenase (SKDH) is a member of shikimate pathway which can convert simple carbohydrates into aromatic amino acids including phenylalanine (Kováčik et al. 2009). It is one of the enzymes that controls the reaction of carbohydrates toward to phenolic metabolism and provides a substrate for PAL (phenylalanine ammonia-lyase) (Blasco et al. 2013, Chen et al. 2019). Plants can generate gallic acid and 4-hydroxybenzoate through shikimate pathways (Zhou et al. 2019). The 4-hydroxybenzoate and 3,4-dihydroxybenzoic acid are produced by general

phenylpropanoid pathway with cinnamic acid precursor. Cinnamic acid is also a precursor for the synthesis of benzoic acid, salicylic acid, caffeic acid, ferulic acid, coumaric acid and vanillin (Barros et al. 2019, Renault et al. 2017). Cinnamic acid and *p*-coumaric acid are all the substrates of cinnamyl alcohol dehydrogenase (CAD) (Barros et al. 2019), which provide precursors for the biosynthesis of lignin (Chen et al. 2019). In fact, phenolic compounds are the substrates of enzymatic browning reactions, which can form colored quinones under the enzymatic oxidation of polyphenol oxidases (PPO) (Léchaudel et al. 2018). However, the high level of PPO activity reduces the free oxygen level available for ROS production, which may be one of the reasons for the reduced ROS level of cellular under stress conditions (Léchaudel et al. 2018). As can be seen from Table 3, the lowest activities of the SKDH, CAD, and PPO found in the control plants (Table. 3), corresponding to the minimum concentrations of phenolic acids in *K. obovata* plants. On the contrary, the application of 300 or 400 mg kg⁻¹ ZnSO₄ treatment, increased primarily the activity of PAL, SKDH, CAD and PPO comparing with the only 80 mg kg⁻¹ ZnSO₄ treatment (Table. 3). These findings could well explain why the increase concentration of phenolic acids (Table. 2), why phenolic acids could protect plants against heavy metal stress, and why the leaves' biomass of 80 mg kg⁻¹ ZnSO₄ treatment was larger than that of 300 or 400 mg kg⁻¹ ZnSO₄ treatment. It has been reported that stronger phenolic acid metabolism related enzymes activities and the content of phenolic acids are related with the resistance of plants to abiotic stress (Blasco et al. 2013). Supporting our results, Barbara Irtelli et al (Irtelli & Navari-Izzo 2006) and Jozef Kovacik et al (Kováčik et al. 2009) have associated proved that PAL, SKDH, CAD and PPO activity were enhanced to assist plants to better resist cadmium pollution. Zn²⁺ contributes to the increase of SKDH enzyme activity, which may be due to the influence of trace element zinc promoting the photosynthesis and carbohydrate synthesis. Because additional carbohydrates can be provided to meet the increased synthesis of phenolic acids under the cadmium stress. PPO activity is correlated to the production of quinine and reactive oxygen species, so the increase of PPO activity will aggravate oxidative stress (Blasco et al. 2013). The results showed that *K. obovata* with the lowest foliar biomass under 400 mg kg⁻¹ ZnSO₄ treatment (Table. 1), but its corresponding polyphenol oxidase activity was the highest (Table. 3), which is related to the greater oxidative stress and the formation of reactive oxygen species in these plants. On the contrary, the application of 80 mg kg⁻¹ ZnSO₄ inhibited polyphenol oxidases activity (Table. 3), hence, ROS might be formed in *K. obovata*. These results are similar with the finds of Thipyapong et al., (Thipyapong et al. 2004) who reported that PPO activity reduced would decrease the concentration of H₂O₂ in tomato plants, thus, the plant's ability to resisting adversity stress was enhanced.

Phenolic acid radical clear ability

DPPH and FRAP are important indexes to measure the antioxidant potential of phenolic acid effectively (Cocetta et al. 2015). The DPPH and FRAP values in plant leaves increased under the treatment of ZnSO₄, adding 300 mg kg⁻¹ ZnSO₄ to 400 mg kg⁻¹ ZnSO₄ can increase the antioxidant capacity of phenolic acid extract from leaves. In addition, the plant's phenolic acid content under high concentration of ZnSO₄ treatment was higher than that of the other two groups under low concentration of ZnSO₄ treatment (Figure. 1C and 1D). Therefore, it can be seen that the higher the phenolic acid content of leaves is, the stronger the antioxidant capacity is, and the stronger the ability to resist the oxidative damage of cadmium/zinc is. It can also be seen from the Pearson correlation table in table. 5 that the content of cadmium and zinc in leaves is highly positively correlated with the activity of enzymes related to phenolic acid metabolism, the content of chlorophyll is highly negatively correlated with the activity of those enzymes, but the soluble sugar content and the antioxidant capacity were highly positively correlated with the activity of those enzymes. These showed that under the pollution of cadmium and zinc, *K. obovata* can stimulate the metabolism of phenolic compounds, sugar metabolism and photosynthesis related to phenolic acid metabolism, so as to improve the antioxidant capacity of plants to resist the oxidative damage caused by heavy metal stress, while 80 mg kg⁻¹ ZnSO₄ can alleviate the cadmium toxicity by improving the ability of phenolic acid metabolism of plants.

Conclusion

Our findings showed that the application of ZnSO₄ to Cd contaminated sediments, induced the increasing phenolic acid synthesis and higher related enzymes oxidation activity. These also led to an increase of hydroxycinnamic acids and derivatives and hydroxybenzoic acids synthesis in plants leaves which have a strong ROS scavenging ability. These compounds can be used as protective compounds under cadmium stress to enhance mangrove plants antioxidant activity. In the end, this work revealed that when mangrove wetland contaminated by cadmium, the application of complex repair agent containing low concentration of ZnSO₄ may be an effective strategy to raise the mangrove plant resistance to cadmium stress, in view of this, in addition to improving the growth of mangrove plants, it would increase the nutritional value of the animal diet, including phenolic compounds and trace elements zinc intake.

Declarations

Ethical Approval

Not applicable

Consent to Participate

Yes

Consent to Publish

Yes

Authors Contributions

The experiments and thesis writing are all done by Chen Shan alone.

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Competing Interests

The author have no relevant financial or non-financial interests to disclose.

Availability of data and materials

The data and materials are detailed and reliable.

References

1. Abdulrazzak N, Pollet B, Ehltling J, Larsen K, Asnaghi C, Ronseau S, Proux C, Erhardt M, Seltzer V, Renou JP, Ullmann P, Pauly M, Lapierre C, Werck-Reichhart D (2006): A coumaroyl-ester-3-hydroxylase insertion mutant reveals the existence of nonredundant meta-hydroxylation pathways and essential roles for phenolic precursors in cell expansion and plant growth. *Plant Physiol* 140, 30-48
2. Adamczyk-Szabela D, Lisowska K, Romanowska-Duda Z, Wolf WM (2020): Combined cadmium-zinc interactions alter manganese, lead, copper uptake by *Melissa officinalis*. *Sci Rep* 10, 1675
3. Adjimani JP, Asare P (2015): Antioxidant and free radical scavenging activity of iron chelators. *Toxicol Rep* 2, 721-728
4. Afroz R, Tanvir EM, Paul S, Bhounik NC, Gan SH, Khalil MDI (2016): DNA Damage Inhibition Properties of Sundarban Honey and its Phenolic Composition. *Journal of Food Biochemistry* 40, 436-445
5. Ali MB, Hahn EJ, Paek KY (2005): CO₂-induced total phenolics in suspension cultures of *Panax ginseng* C. A. Mayer roots: role of antioxidants and enzymes. *Plant Physiol Biochem* 43, 449-57
6. Ali MB, Singh N, Shohael AM, Hahn EJ, Paek K-Y (2006): Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress. *Plant Science* 171, 147-154
7. Analuddin K, Sharma S, Jamili, Septiana A, Sahidin I, Rianse U, Nadaoka K (2017): Heavy metal bioaccumulation in mangrove ecosystem at the coral triangle ecoregion, Southeast Sulawesi, Indonesia. *Mar Pollut Bull* 125, 472-480
8. Armas T, Pinto AP, de Varennes A, Mourato MP, Martins LL, Gonçalves MLS, Mota AM (2014): Comparison of cadmium-induced oxidative stress in *Brassica juncea* in soil and hydroponic cultures. *Plant and Soil* 388, 297-305
9. Barros J, Escamilla-Trevino L, Song L, Rao X, Serrani-Yarce JC, Palacios MD, Engle N, Choudhury FK, Tschaplinski TJ, Venables BJ, Mittler R, Dixon RA (2019): 4-Coumarate 3-hydroxylase in the lignin biosynthesis pathway is a cytosolic ascorbate peroxidase. *Nat Commun* 10, 1994
10. Bharathkumar S, RameshKumar N, Paul D, Prabavathy VR, Nair S (2007): Characterization of the predominant bacterial population of different mangrove rhizosphere soils using 16S rRNA gene-based single-strand conformation polymorphism (SSCP). *World Journal of Microbiology and Biotechnology* 24, 387-394
11. Blasco B, Leyva R, Romero L, Ruiz JM (2013): Iodine effects on phenolic metabolism in lettuce plants under salt stress. *J Agric Food Chem* 61, 2591-6
12. Bodin N, N'Gom-Ka R, Ka S, Thiaw OT, Tito de Moraes L, Le Loc'h F, Rozuel-Chartier E, Auger D, Chiffolleau JF (2013): Assessment of trace metal contamination in mangrove ecosystems from Senegal, West Africa. *Chemosphere* 90, 150-7
13. Chao Y-Y, Chen C-Y, Huang W-D, Kao CH (2009): Salicylic acid-mediated hydrogen peroxide accumulation and protection against Cd toxicity in rice leaves. *Plant and Soil* 329, 327-337
14. Chen H, Yang R, Zhang X, Chen Y, Xia Y, Xu X (2021): Foliar application of gibberellin inhibits the cadmium uptake and xylem transport in lettuce (*Lactuca sativa* L.). *Scientia Horticulturae* 288
15. Chen J, Xing XK, Zhang LC, Xing YM, Guo SX (2012): Identification of *Hortaea werneckii* Isolated from mangrove plant *Aegiceras comiculatum* based on morphology and rDNA sequences. *Mycopathologia* 174, 457-66
16. Chen S, Wang Q, Lu H, Li J, Yang D, Liu J, Yan C (2019): Phenolic metabolism and related heavy metal tolerance mechanism in *Kandelia Obovata* under Cd and Zn stress. *Ecotoxicol Environ Saf* 169, 134-143

17. Cocetta G, Rossoni M, Gardana C, Mignani I, Ferrante A, Spinardi A (2015): Methyl jasmonate affects phenolic metabolism and gene expression in blueberry (*Vaccinium corymbosum*). *Physiol Plant* 153, 269-83
18. Cristina Sgherri ECaFN-I (2003): Phenols and antioxidative status of *Raphanus sativus* grown in copper excess. *Physiologia Plantarum* 118, 21-28
19. Cui J, Liu T, Li F, Yi J, Liu C, Yu H (2017): Silica nanoparticles alleviate cadmium toxicity in rice cells: Mechanisms and size effects. *Environ Pollut* 228, 363-369
20. Das SK, Patra JK, Thatoi H (2016): Antioxidative response to abiotic and biotic stresses in mangrove plants: A review. *International Review of Hydrobiology* 101, 3-19
21. Falahi H, Sharifi M, Maivan HZ, Chashmi NA (2018): Phenylethanoid glycosides accumulation in roots of *Scrophularia striata* as a response to water stress. *Environmental and Experimental Botany* 147, 13-21
22. G.R. MacFarlane MDB (2000): Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Aquatic botany* 68, 45-59
23. Garg N, Kaur H (2013): Response of Antioxidant Enzymes, Phytochelatin and Glutathione Production Towards Cd and Zn Stresses in *Cajanus cajan* (L.) Millsp. Genotypes Colonized by Arbuscular Mycorrhizal Fungi. *Journal of Agronomy and Crop Science* 199, 118-133
24. Guo G, Lei M, Chen T, Yang J (2017): Evaluation of different amendments and foliar fertilizer for immobilization of heavy metals in contaminated soils. *Journal of Soils and Sediments* 18, 239-247
25. H. G. Zha RFJ, F. J. Zhao, R. Vooijs, H. Schat, J. H. A. Barker, S. P. McGrath (2004): Co-segregation analysis of cadmium and zinc accumulation in *Thlaspi caerulescens* interecotypic crosses
26. *New Phytologist* 163, 299-312
27. Haoliang L, Chongling Y, Jingchun L (2007): Low-molecular-weight organic acids exuded by Mangrove (*Kandelia candel* (L.) Druce) roots and their effect on cadmium species change in the rhizosphere. *Environmental and Experimental Botany* 61, 159-166
28. Irtelli B, Navari-Izzo F (2006): Influence of sodium nitrilotriacetate (NTA) and citric acid on phenolic and organic acids in *Brassica juncea* grown in excess of cadmium. *Chemosphere* 65, 1348-54
29. J. Loponen KL, V. Ossipov, M.V. Kozlov, A. Girs, K. Hangasmaa, E. Haukioja, K. Pihlaja (2001): Patterns in content of phenolic compounds in leaves of mountain birches along a strong pollution gradient
30. *Chemosphere* 45, 291-301
31. Jia X, Zhao YH, Liu T, He YH (2017): Leaf defense system of *Robinia pseudoacacia* L. seedlings exposed to 3 years of elevated atmospheric CO₂ and Cd-contaminated soils. *Sci Total Environ* 605-606, 48-57
32. Jiang S, Weng B, Liu T, Su Y, Liu J, Lu H, Yan C (2017): Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) Blanco (Ac). *Ecotoxicol Environ Saf* 141, 290-297
33. Khan I, Iqbal M, Ashraf MY, Ashraf MA, Ali S (2016): Organic chelants-mediated enhanced lead (Pb) uptake and accumulation is associated with higher activity of enzymatic antioxidants in spinach (*Spinacea oleracea* L.). *J Hazard Mater* 317, 352-361
34. Kim K, Tsao R, Yang R, Cui S (2006): Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry* 95, 466-473
35. Kováčik J, Bačkor M (2007): Changes of phenolic metabolism and oxidative status in nitrogen-deficient *Matricaria chamomilla* plants. *Plant and Soil* 297, 255-265
36. Kováčik J, Klejdus B, Hedbavny J, Štork F, Bačkor M (2009): Comparison of cadmium and copper effect on phenolic metabolism, mineral nutrients and stress-related parameters in *Matricaria chamomilla* plants. *Plant and Soil* 320, 231-242
37. Lavid N, Schwartz A, Lewinsohn E, Tel-Or E (2001): Phenols and phenol oxidases are involved in cadmium accumulation in the water plants *Nymphoides peltata* (Menyanthaceae) and *Nymphaeae* (Nymphaeaceae). *Planta* 214, 189-195
38. Léchaudel M, Darnaudery M, Joët T, Fournier P, Joas J (2018): Genotypic and environmental effects on the level of ascorbic acid, phenolic compounds and related gene expression during pineapple fruit development and ripening. *Plant Physiology and Biochemistry* 130, 127-138
39. Lefevre I, Vogel-Mikus K, Jeromel L, Vavpetic P, Planchon S, Arcon I, Van Elteren JT, Lepoint G, Gobert S, Renaut J, Pelicon P, Lutts S (2014): Differential cadmium and zinc distribution in relation to their physiological impact in the leaves of the accumulating *Zygophyllum fabago* L. *Plant Cell Environ* 37, 1299-320
40. Li J, Liu J, Lu H, Jia H, Yu J, Hong H, Yan C (2016): Influence of the phenols on the biogeochemical behavior of cadmium in the mangrove sediment. *Chemosphere* 144, 2206-2213
41. Li Y, Xin J, Ge W, Tian R (2022): Tolerance mechanism and phytoremediation potential of *Pistia stratiotes* to zinc and cadmium co-contamination. *Int J Phytoremediation*, 1-8
42. Liu Z, Ding Y, Wang F, Ye Y, Zhu C (2016): Role of salicylic acid in resistance to cadmium stress in plants. *Plant Cell Rep* 35, 719-31

43. Lodovico Tamagnone AM, Nicola Stacey, Kitty Plaskitt, Adrian Parr, Chi Feng Chang, David Lynn, J. Maxwell Dow, Keith Roberts, Cathie Martin (1998): Inhibition of Phenolic Acid Metabolism Results in Precocious Cell Death and Altered Cell Morphology in Leaves of Transgenic Tobacco Plants. *The Plant Cell* 10, 1801-1816
44. Manquian-Cerda K, Escudey M, Zuniga G, Arancibia-Miranda N, Molina M, Cruces E (2016): Effect of cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum* L.) plantlets grown in vitro. *Ecotoxicol Environ Saf* 133, 316-26
45. Maqsood S, Benjakul S, Abushelaibi A, Alam A (2014): Phenolic Compounds and Plant Phenolic Extracts as Natural Antioxidants in Prevention of Lipid Oxidation in Seafood: A Detailed Review. *Comprehensive Reviews in Food Science and Food Safety* 13, 1125-1140
46. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TCd, Coube CS, Leitão SG (2001): Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research* 15, 127-130
47. Michalak A (2006): Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *Polish J. of Environ. Stud* 15, 523-530
48. Mongkhonsin B, Nakbanpote W, Hokura A, Nuengchamnong N, Maneechai S (2016): Phenolic compounds responding to zinc and/or cadmium treatments in *Gynura pseudochina* (L.) DC. extracts and biomass. *Plant Physiol Biochem* 109, 549-560
49. Oh MM, Trick HN, Rajashekar CB (2009): Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. *J Plant Physiol* 166, 180-91
50. P. Burchard WB, G. Weissenbock (2000): Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet- induced chlorophyll fluorescence measurements. *Plant Cell and Environment* 23, 1373-1380
51. Posmyk MM, Kontek R, Janas KM (2009): Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol Environ Saf* 72, 596-602
52. Rai PK, Lee SS, Zhang M, Tsang YF, Kim KH (2019): Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environ Int* 125, 365-385
53. Renault H, Alber A, Horst NA, Basilio Lopes A, Fich EA, Kriegshauser L, Wiedemann G, Ullmann P, Herrgott L, Erhardt M, Pineau E, Ehrling J, Schmitt M, Rose JK, Reski R, Werck-Reichhart D (2017): A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nat Commun* 8, 14713
54. Rui H, Chen C, Zhang X, Shen Z, Zhang F (2016): Cd-induced oxidative stress and lignification in the roots of two *Vicia sativa* L. varieties with different Cd tolerances. *J Hazard Mater* 301, 304-13
55. Shang X, Xue W, Jiang Y, Zou J (2020): Effects of Calcium on the Alleviation of Cadmium Toxicity in *Salix matsudana* and Its Effects on Other Minerals. *Polish Journal of Environmental Studies* 29, 2001-2010
56. Shi C, Xu MJ, Bayer M, Deng ZW, Kubbutat MH, Watjen W, Proksch P, Lin WH (2010): Phenolic compounds and their anti-oxidative properties and protein kinase inhibition from the Chinese mangrove plant *Laguncularia racemosa*. *Phytochemistry* 71, 435-42
57. Sundaramanickam A, Shanmugam N, Cholan S, Kumaresan S, Madeswaran P, Balasubramanian T (2016): Spatial variability of heavy metals in estuarine, mangrove and coastal ecosystems along Parangipettai, Southeast coast of India. *Environ Pollut* 218, 186-195
58. Tanveer Y, Yasmin H, Nosheen A, Ali S, Ahmad A (2022): Ameliorative effects of plant growth promoting bacteria, zinc oxide nanoparticles and oxalic acid on *Luffa acutangula* grown on arsenic enriched soil. *Environ Pollut* 300, 118889
59. Tato L, De Nisi P, Donnini S, Zocchi G (2013): Low iron availability and phenolic metabolism in a wild plant species (*Parietaria judaica* L.). *Plant Physiol Biochem* 72, 145-53
60. Thipyapong P, Melkonian J, Wolfe DW, Steffens JC (2004): Suppression of polyphenol oxidases increases stress tolerance in tomato. *Plant Science* 167, 693-703
61. Wang C, Zhang S, Wang P, Hou J, Qian J, Ao Y, Lu J, Li L (2011): Salicylic acid involved in the regulation of nutrient elements uptake and oxidative stress in *Vallisneria spiralis* (Lour.) Hara under Pb stress. *Chemosphere* 84, 136-42
62. Wang S, Zhao Y, Guo J, Zhou L (2016): Effects of Cd, Cu and Zn on *Ricinus communis* L. Growth in single element or co-contaminated soils: Pot experiments. *Ecological Engineering* 90, 347-351
63. Weng Z-X, Wang L-X, Tan F-I, Huang L, Xing J-H, Chen S-P, Cheng C-L, Chen W (2012): Proteomic and physiological analyses reveal detoxification and antioxidation induced by Cd stress in *Kandelia candel* roots. *Trees* 27, 583-595
64. Xie X, Weng B, Cai B, Dong Y, Yan C (2014): Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth and nutrient uptake of *Kandelia obovata* (Sheue, Liu & Yong) seedlings in autoclaved soil. *Applied Soil Ecology* 75, 162-171
65. Xing JP, Jiang RF, Ueno D, Ma JF, Schat H, McGrath SP, Zhao FJ (2008): Variation in root-to-shoot translocation of cadmium and zinc among different accessions of the hyperaccumulators *Thlaspi caerulescens* and *Thlaspi praecox*. *New Phytol* 178, 315-25

66. Zhang H, Zhang W, Huang S, Xu P, Cao Z, Chen M, Lin X (2022): The potential role of plasma membrane proteins in response to Zn stress in rice roots based on iTRAQ and PRM under low Cd condition. J Hazard Mater 429, 128324
67. Zhao Hu, Wenjiao Z (2015): Effects of zinc stress on growth and antioxidant enzyme responses of *Kandelia obovata* seedlings. Toxicological & Environmental Chemistry 97, 1190-1201
68. Zhou K, Hu L, Li Y, Chen X, Zhang Z, Liu B, Li P, Gong X, Ma F (2019): MdUGT88F1-Mediated Phloridzin Biosynthesis Regulates Apple Development and Valsa Canker Resistance. Plant Physiology 180, 2290-2305

Tables

Table 1

Effects of Zn^{2+} Treatment on Leaves Biomass and Concentrations of Cadmium and Zinc in Leaves of Cadmium-contaminated *K. obovata*

Treatment	Leaves Biomass (g dw)	Cadmium (mg kg ⁻¹ dw)	Zinc (mg kg ⁻¹ dw)
Control	1.820 ± 0.170 c	0.113 ± 0.015 b	90.260 ± 4.860 a
80 mg kg ⁻¹ ZnSO ₄	2.070 ± 0.163 c	0.077 ± 0.006 a	100.450 ± 2.234 ab
300 mg kg ⁻¹ ZnSO ₄	1.545 ± 0.105 b	0.197 ± 0.003 c	109.339 ± 3.638 bc
400 mg kg ⁻¹ ZnSO ₄	1.213 ± 0.094 a	0.195 ± 0.005 c	114.447 ± 10.229 c
<i>P</i> value	***	***	**
Values are the mean ± SD (n = 3). Means followed by the same letter do not differ significantly. Levels of significance: *, <i>P</i> < 0.05; **, <i>P</i> < 0.01; ***, <i>P</i> < 0.001; ns, not significant. dw, dry weight.			

Table. 2 Effects of Zn^{2+} Treatment on Phenolic Compounds in Cadmium-contaminated *K. obovata*

Treatment PC (μg g ⁻¹ fw)	Control	80 mg kg ⁻¹ ZnSO ₄	300 mg kg ⁻¹ ZnSO ₄	400 mg kg ⁻¹ ZnSO ₄	<i>P</i> value
Gal	0.015 ± 0.003 a	0.013 ± 0.002 a	0.151 ± 0.027 b	0.038 ± 0.010 a	***
Hyd	1.359 ± 0.130 a	0.988 ± 0.291 a	3.633 ± 1.392 b	4.180 ± 0.727 b	***
Pro	0.033 ± 0.006 a	0.030 ± 0.002 a	0.076 ± 0.008 b	0.035 ± 0.016 a	*
Sal	0.027 ± 0.010 a	0.022 ± 0.003 a	0.079 ± 0.030 b	0.054 ± 0.032 ab	ns
Ben	0.068 ± 0.005 a	0.073 ± 0.004 a	0.222 ± 0.037 c	0.118 ± 0.016 b	***
Fer	0.089 ± 0.017 a	0.108 ± 0.025 a	0.204 ± 0.025 b	0.134 ± 0.050 a	***
Cou	0.022 ± 0.009 a	0.016 ± 0.001 a	0.415 ± 0.008 c	0.158 ± 0.074 b	*
Chl	2.007 ± 0.072 a	1.847 ± 0.133 a	6.228 ± 1.854 b	1.696 ± 1.974 a	***
Cin	0.601 ± 0.045 a	0.668 ± 0.223 a	2.589 ± 0.455 b	2.785 ± 0.374 b	***

Table 3
Effects of Zn²⁺ Treatment on Phenolic Compounds Metabolism Related Enzymes in Cadmium-contaminated *K. obovata*

Treatment	PAL activity (nmol·min ⁻¹ ·mg ⁻¹)	SKDH activity (nmol·min ⁻¹ ·mg ⁻¹)	CAD activity (nmol·min ⁻¹ ·mg ⁻¹)	PPO activity (UA·mg ⁻¹)
Control	20.302 ± 3.161 b	215.243 ± 4.333 a	223.820 ± 6.141 a	1.435 ± 0.060 a
80 mg kg ⁻¹ ZnSO ₄	14.673 ± 0.468 a	226.793 ± 4.601 a	230.547 ± 16.386 a	1.497 ± 0.049 a
300 mg kg ⁻¹ ZnSO ₄	24.840 ± 0.706 c	325.244 ± 13.289 b	294.486 ± 13.819 b	2.439 ± 0.123 b
400 mg kg ⁻¹ ZnSO ₄	29.108 ± 1.330 d	400.792 ± 18.437 c	310.687 ± 9.285 b	2.844 ± 0.087 c
P value	***	***	***	***

Values are the mean ± SD (n = 3). Means followed by the same letter do not differ significantly. Levels of significance: *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.

Table 4
LC-ESI-QQQ-MS/MS Analysis of These Phenolic Acids

Compounds	Molecular formula	Retention time (min)	Collision energy		ESI-MS m/z	
			E1 (V)	E2 (V)	[M-H]	MS/MS fragment
Pyrogalllic acid	C ₆ H ₆ O ₃	1.954	20	10	124.9	79.1, 97
Coumaric acid	C ₆ H ₄ O ₄	2.264	0	0	139.09	139.1, 95
Protocatechuic acid	C ₇ H ₆ O ₄	2.519	10	5	153	109.1, 153
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	3.259	15	5	353	190.8, 352.9
4-hydroxy benzoic acid	C ₇ H ₆ O ₃	3.844	10	0	137.1	93.1, 137.1
Caffeic acid	C ₉ H ₈ O ₄	4.418	30	30	179	134, 88.9
Syringic acid	C ₉ H ₁₀ O ₅	4.858	15	30	197.17	123, 95
Vanillin	C ₈ H ₈ O ₃	5.246	5	5	150.9	136.1, 151
Ferulic acid	C ₁₀ H ₁₀ O ₄	8.283	5	5	192.9	177.8, 134
Benzoic acid	C ₇ H ₆ O ₂	10.374	0	10	121	121, 77.1
Salicylic acid	C ₇ H ₆ O ₃	11.308	15	5	137.1	93.1, 137.1
Cinnamic acid	C ₉ H ₈ O ₂	12.592	5	5	147.1	103, 147

Table. 5 Table of Pearson Correlation Coefficients for Biomass, Cadmium and Zinc Content, Soluble Sugar, Chlorophyll Content, DPPH, FRAP, PAL, SKDH, CAD and PPO in leaves

	Biomass	Cd	Zn	Sugar	Chlorophyll	DPPH	FRAP	PAL	SKDH	CAD	PPO
Biomass	1	-0.890**	-0.608*	-0.899**	0.736**	-0.864**	-0.756**	-0.881**	-0.881**	-0.828**	-0.871**
Cd		1	0.656*	0.942**	-0.736**	0.953**	0.861**	0.899**	0.871**	0.913**	0.915**
Zn			1	0.697*	-0.56	0.794**	0.635*	0.574	0.784**	0.800**	0.803**
Sugar				1	-0.721**	0.928**	0.866**	0.963**	0.947**	0.910**	0.950**
Chlorophyll					1	-0.707*	-0.553	-0.672*	-0.718**	-0.742**	-0.758**
DPPH						1	0.896**	0.844**	0.942**	0.959**	0.970**
FRAP							1	0.731**	0.839**	0.887**	0.874**
PAL								1	0.884**	0.824**	0.871**
SKDH									1	0.934**	0.982**
CAD										1	0.973**
PPO											1

Note: *Correlation is less than or equal to 0.05 level (2-tailed); **Correlation is less than or equal to 0.01 level (2-tailed);

Figures

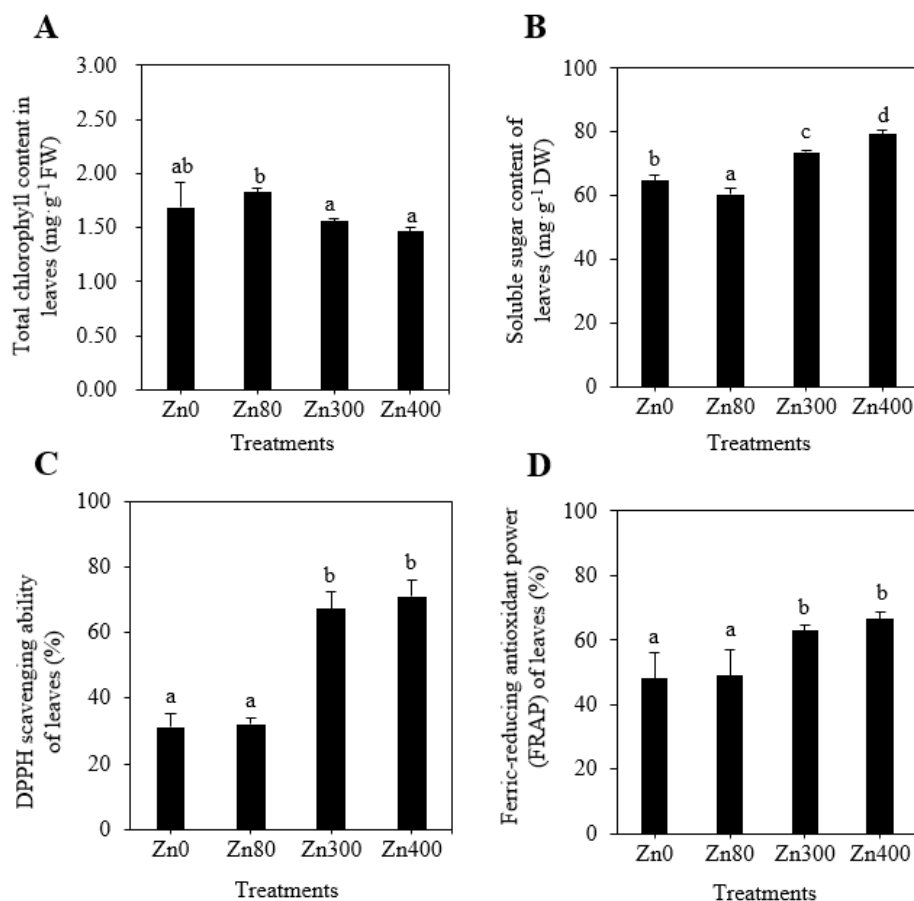


Figure 1

A- Chlorophyll content, B- Soluble sugar content, C- DPPH scavenging ability of phenolic acids, and D- Ferric reducing antioxidant power (FRAP) of phenolic acids in leaves of cadmium-contaminated *K. obovata*

under different concentration of Zn treatments. The data are shown as the means $\pm$ SD (n=4)

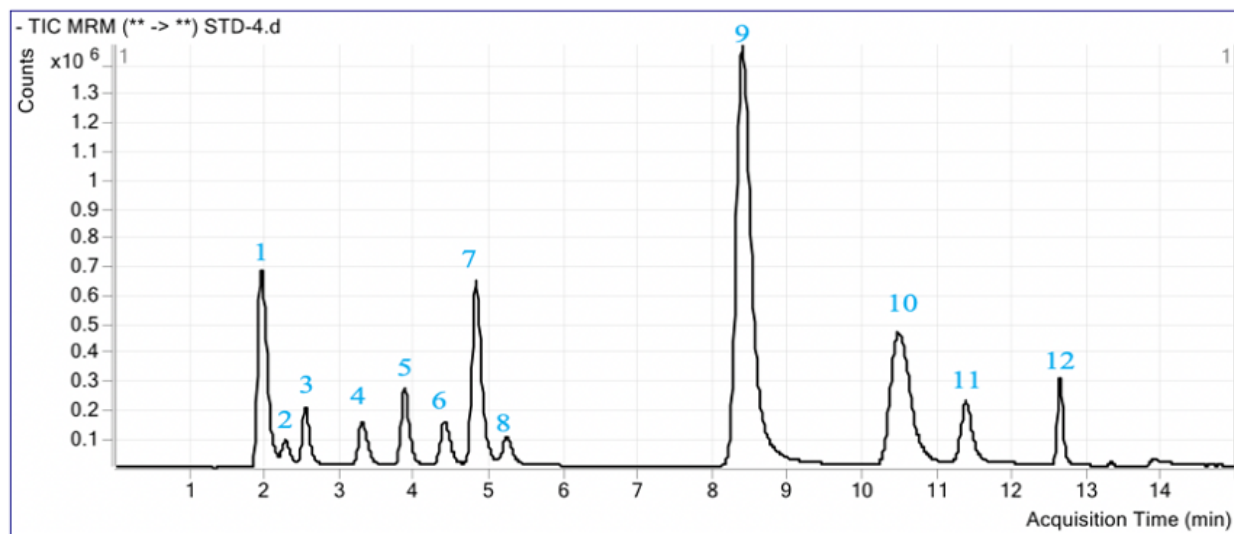


Figure 2

A total ion chromatogram (TIC) of phenolic acid mixture standards. The peak number represents the twelve phenolic acids as follows: 1– Pyrogalllic acid; 2– Coumaric acid; 3– Protocatechuic acid; 4– Chlorogenic acid; 5– 4-Hydroxy benzoic acid; 6– Caffeic acid; 7– Syringic acid; 8– Vanillin; 9– Ferulic acid; 10– Benzoic acid; 11– Salicylic acid; 12– Cinnamic acid

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