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Foliar application of Fe-fulvic acid: A strategy to reduce heavy metal accumulation and enhance nutritional quality

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ABSTRACT

Pepper is a key agricultural crop susceptible to accumulating heavy metals like cadmium (Cd) and barium (Ba), posing significant health risks. To address these issues, this study investigated the effects of foliar applications of fulvic acid (FA), Zn-fulvic acid (Zn-FA), and Fe-fulvic acid (Fe-FA) on Ba and Cd uptake in pepper tissues, as well as their impact on nutritional quality, biomass, and leaf enzyme activity. Results indicated that Fe-FA application significantly reduced Cd and Ba in pepper fruit by 25 % and 93 %, respectively. Additionally, Fe-FA enhanced pepper growth, increasing vitamin C and phenolic compounds by 136 % and 13 %, respectively. Metabolomics analysis revealed that Fe-FA application up-regulated 857 metabolites and down-regulated 1045 metabolites. Furthermore, Fe-FA primarily influenced amino acid, carbohydrate, and lipid metabolism, promoting pepper growth. These findings suggest that Fe-FA foliar application offers a promising strategy for reducing Ba and Cd accumulation in pepper fruits while enhancing its nutritional quality.

1. Introduction

Cadmium (Cd) and barium (Ba) are non-essential elements for human health (Lamb et al., 2013). Cd, recognized as a Group 1 carcinogen, can induce lung, kidney, prostate, and breast cancers with high levels of exposure (Nawrot et al., 2015). Even at low-level, Cd exposure is also associated with hypertension, metabolic syndrome, and atherosclerosis, leading to cerebrovascular and cardiovascular diseases (Bimonte et al., 2021). Similarly, exposure to Ba can cause severe health effects, including kidney disease, cardiovascular issues, pulmonary edema, cardiac failure, respiratory paralysis, as well as neurological, mental, and metabolic disorders (Peana et al., 2021). China is rich in Ba ore resources, especially in Guizhou Province, which hosts the world's largest barite mine (Li, 2004). The extraction and processing of Ba ores often involve the concomitant release of both Ba and Cd, leading to their co-occurrence as pollutants in the environment. Previous studies have shown that barite deposits are characterized by high levels of both Ba and Cd. For example, the concentrations of Cd and Ba in the soil of the Tianzhu barite mining area reach up to 91 mg/kg and 65,760 mg/kg, respectively, demonstrating a strong positive correlation between the two elements (Lu, Xu, Liang, et al., 2019; Lu, Xu, Xu, et al., 2019). This results in substantial accumulation of Ba and Cd in crops, posing significant risks to human and animal health through the food chain. Consequently, developing effective strategies to alleviate Ba and Cd uptake in crops is of paramount importance.

Pepper is a crucial vegetable (Souza et al., 2020). In Guizhou province, due to the widely cultivated area of more than 380,000 ha and large production of 7.87 million tons, pepper serves as a significant income source of local residents (Zou & Zou, 2021). It is rich in essential nutrients including vitamin C, flavonoids, carotenoids, and phenolic compounds. Additionally, pepper has been traditionally utilized in the treatment of various human ailments, such as ulcers, gastritis, and rheumatism (Cho et al., 2017). However, previous studies have documented the potential of pepper to accumulate heavy metals (Jidesh &

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Kurumthottical, 2000), thus prolonged consumption of peppers contaminated with these metals could pose substantial health risks. Therefore, it is imperative to develop strategies to mitigate heavy metal content in peppers to ensure food safety and protect public health.

Various strategies have been employed to mitigate the accumulation of metal(loid)s in agricultural products, including soil amendments, water management, and foliar spray (Hussan et al., 2021). Among these methods, foliar spray has garnered significant research interest due to its efficiency, operational simplicity, and cost-efficiency (Li, 2019). Fulvic acid (FA), an important natural organic matter, is characterized by its low molecular weight and is enriched with functional groups such as carboxyl, phenolic hydroxyl, and acylamine groups, making it highly suitable for foliar application. Its high solubility facilitates the modulation of heavy metals' migration toxicity and bioavailability (Li et al., 2018). Additionally, FA application has been documented to significantly enhance plant growth and nutrient uptake by improving nutrient availability, alleviating plant stress, activating enzymes, and regulating hormonal balance (Pettit, 2004). Moreover, chelation of FA with essential plant growth elements (e.g., Zn and Fe) has demonstrated a notable reduction in Cd accumulation in rice (Lu et al., 2024; Wang, Du, et al., 2022). For instance, Wang, Du, et al. (2022) reported that the foliar application of Fe-FA had a greater effect on reducing Cd content in rice grains compared to the control, and an even greater effect than the application of Fe alone. Nonetheless, the specific effects of FA and its chelation with iron and zinc on the heavy metal contents (Ba and Cd) in pepper fruits remain inadequately understood. Furthermore, it remains uncertain whether these substances can effectively enhance the quality of pepper fruits, including soluble sugar, protein, vitamin C, and total phenol content, or their influence on the associated metabolic pathways.

In the present study, soil naturally co-contaminated with Ba and Cd was collected for cultivating pepper plants in controlled pot experiments. The study aims to achieve the following objectives: 1) to investigate the effects of FA and its chelates with Fe or Zn on the accumulation of Ba and Cd in peppers; 2) to elucidate their influence on the growth parameters of pepper plants and the activity of antioxidant enzymes in the leaves; 3) to evaluate their effects on the nutritional quality of pepper fruits; 4) to explore the impact of the most effective treatment on the metabolite profile of pepper fruits. To the best of our knowledge, this study is the first to concurrently address the reduction of Ba and Cd while enhancing the quality of pepper fruits, offering a novel approach for the safe cultivation of chili peppers.

2. Materials and methods

2.1. Pot experiment

The Cd and Ba-contaminated soil was collected from Tianzhu County, Guizhou, southwest China. Pot experiments were conducted at the open-air experimental site of Guizhou Medical University. Cultivated pepper plants (*Capsicum annuum* L.) were divided into four treatment groups: a control group treated with ultra-pure water, a Fe-fulvic acid (Fe-FA) treatment group, a Zn-fulvic acid (Zn-FA) treatment group, and FA treatment group. The ratios of Fe to FA and Zn to FA are 1.3 % and 1.5 %, respectively. Each reagent solution, at a concentration of 3 g/L, was sprayed on both the front and back of the leaves at intervals of 10 days during the fruiting period. Pesticides, fertilizers and water management practices followed the local farming methods throughout the growing season. All experiments were performed in triplicate.

The plants were manually harvested at maturity, and their height was measured from the soil surface to the top using a ruler. The harvested plants were separated into roots, stems, leaves, and fruits, which were subsequently washed with tap water followed by ultrapure water. The fresh and dry biomass of each tissue was weighed. Subsequently, the fresh samples were stored in two portions. One portion was immediately stored in a − 80 °C refrigerator for non-target metabolomics analysis, while the other portion was freeze-dried and ground to pass through a 200-mesh sieve for Ba and Cd analysis.

2.2. Ba and Cd concentration analysis

The dry samples of roots, stems, leaves, and fruits were digested with HNO_3/H_2O_2 for the analysis of Ba and Cd concentrations. The detailed procedure is outlined in our previous reports (Lu, Xu, Liang, et al., 2019; Lu, Xu, Xu, et al., 2019). Briefly, 0.2 g of dried samples were digested at 150 °C for 36 h. Subsequently, the digestion solution was heated on a hot plate at 115 °C until the liquid phase completely evaporated. Finally, 3 mL of deionized water and 2 mL of ultra-pure HNO_3 were added to the residue. The Ba and Cd contents were subsequently determined using inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer, Waltham, MA, USA).

2.3. Determination of enzyme activities, soluble sugar, protein, vitamin C, and total phenol

For the analysis of enzyme activities, leaf samples were ground in ice-cold phosphate buffer at a weight to volume ratio of 1:10. The obtained homogenate was centrifuged for 10 mins at 8000 ×*g* and 4 °C. The supernatant was then stored at −20 °C for subsequent analysis. The activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were measured using standard kits from Nanjing Jiancheng Biotechnology Co., Ltd. (Jiangsu, China).

The soluble sugar content was measured using the anthrone-sulfuric acid assay method (Leng et al., 2016). The concentration of soluble proteins was determined by the Coomassie brilliant blue method (Cao et al., 2016). Vitamin C content was determined using the 2,6-dichloroindophenol titration method (GB/T 6195–86, 1986). The content of total phenols was determined using the Folin-Ciocalteu reagent (Ainsworth & Gillespie, 2007).

2.4. Pepper fruit non-target metabolomics detection

Approximately 25 mg of pepper fruit from both the Fe-FA treatment and control groups were weighed into an EP tube after liquid grinding for non-target metabolomics detection. 500 μL of the extraction solution (methanol: acetonitrile: water = 2:2:1, with the isotopically-labelled internal standard mixture) was added to the EP tube and homogenized at 35 Hz for 4 mins. This was followed by sonication of 5 mins in an ice-water bath. The homogenization and sonication processes were repeated three times. Subsequently, the extracted mixture was incubated for 1 h at −40 °C and centrifuged at 12000 rpm (RCF = 13,800 ×g, $R = 8.6$ cm) for 15 mins at 4 °C. The supernatant was then transferred to a glass vial for analysis.

LC-MS/MS analyses were performed using a UHPLC system (Vanquish, Thermo Fisher Scientific) with a UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm) coupled to an Orbitrap Exploris 120 mass spectrometer (Orbitrap Ms., Thermo). The raw data were converted to mzXML format using ProteoWizard and processed with an in-house program developed using R and based on XCMS, for peak detection, extraction, alignment, and integration. Subsequently, an in-house MS2 database (BiotreeDB) was utilized to metabolite annotation, with the cutoff for annotation set at 0.3.

2.5. Statistical analysis

Results are expressed as mean ± standard deviation. Statistical significance analysis was performed using SPSS 23. Figures were generated using OriginPro 2021 and Excel 2013. Data of metabolomics were analyzed using R software.

3. Results

3.1. Cadmium and barium concentrations

The effects of the application of FA, Zn-FA, and Fe-FA on Cd accumulation in the fruits, stems, and leaves of pepper is shown in Fig. 1a-c. Compared to the control group, the foliar application of Fe-FA resulted in a 25 % and 10 % reduction in Cd content in the fruits and leaves, respectively, while no significant difference was observed for FA and Zn-FA application. In the stems, foliar application of Zn-FA, Fe-FA, and FA reduced Cd content by 19 %, 23 %, and 20 %, respectively, compared to the control. The results indicated that the foliar application of Fe-FA significantly inhibit Cd uptake, as evidenced by the markedly lower Cd concentrations in pepper fruits, stems, and leaves compared to the control.

Similarly, Ba concentration in the fruit was reduced with Fe-FA and Zn-FA application (Fig. 1d), while an opposite trend was observed with FA application. Specifically, the Ba concentration in the fruit decreased from 9.2 mg/kg (control) to 0.64 mg/kg and 4.0 mg/kg, representing reductions of 93 % and 56 %, with Fe-FA and Zn-FA application, respectively. Conversely, Ba concentration increased from 9.2 mg/kg to 40 mg/kg with FA application. Furthermore, Ba concentration in the stems decreased with Zn-FA and FA applications, while no significant effects were observed with Fe-FA application (Fig. 1e). Specifically, Ba contents in the stems decreased from 72 mg/kg to 44 mg/kg and 45 mg/kg with the Zn-FA and FA application, respectively. In the leaves, Ba contents in the treatment groups were reduced from 67 mg/kg (control group) to 46 mg/kg and 39 mg/kg in the Zn-FA and Fe-FA treatment groups, respectively, while FA application increased Ba contents from 67 mg/kg to 103 mg/kg (Fig. 1f).

3.2. Growth parameters of pepper

The dry weights of pepper leaves, stems, and roots from each treatment are presented in Table 1. Leaf biomass increased by 32 % and 66 % with Zn-FA and Fe-FA applications, respectively, compared to the control group. Furthermore, all treatment groups exhibited an increase in stem and root biomass relative to the control group. Additionally, the single fruit weight and plant height of the pepper were determined (**Fig. S1**). Compared to the control group, Fe-FA application increased the single fruit weight by 14 % (from 28 g to 32 g). However, the application of Zn-FA had no significant effect on the single fruit weight, while FA application decreased it. Regarding height of the plant, both Zn-FA and Fe-FA applications increased the height of the pepper compared to the control group, while the FA application had no significant effect on the pepper height.

3.3. Activities of CAT, POD, and SOD

The enzyme activities of POD, CAT, and SOD in the leaves were determined to assess the plant health and stress tolerance (Fig. 2). With the application of Fe-FA, the activities of POD, CAT, and SOD were

Table 1
The dry biomass (g) of each pepper tissues with control, Zn-FA, Fe-FA, and FA application.

g/pot	Leaf	Stem	Root
Control	6.3 ± 0.66	16 ± 1.6	3.6 ± 0.36
Zn-FA	8.3 ± 2.5	19 ± 2.1	6.8 ± 1.1**
Fe-FA	10 ± 2.5	22 ± 0.15**	6.3 ± 0.70*
FA	5.8 ± 1.3	18 ± 0.53	5.4 ± 0.95

Note: one asterisk (*) indicates that p is less than 0.05. And two asterisks (**) indicate that p is less than 0.01.

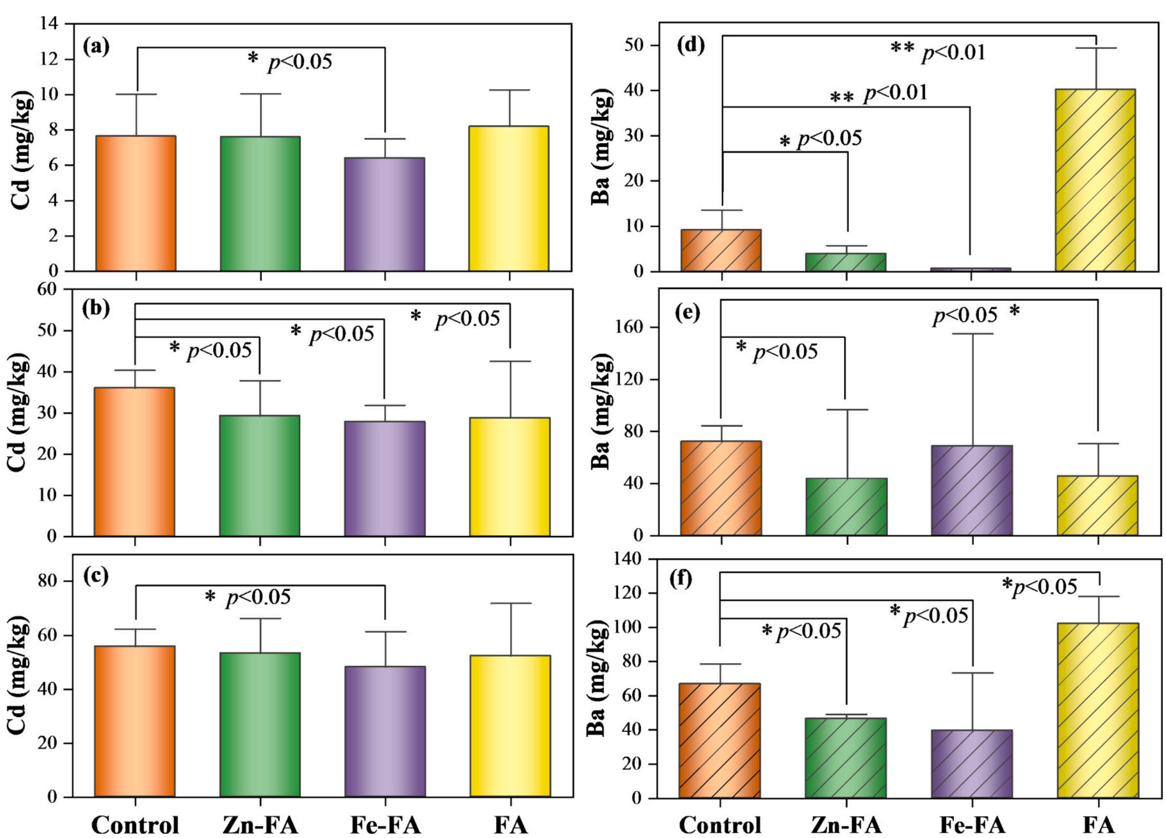

Fig. 1. The effects of Zn-FA, Fe-FA, and FA application on concentration of Cd (a-c) and Ba (d-f) in fruits (a, d), stem (b, e), and leaves (c, f) samples.

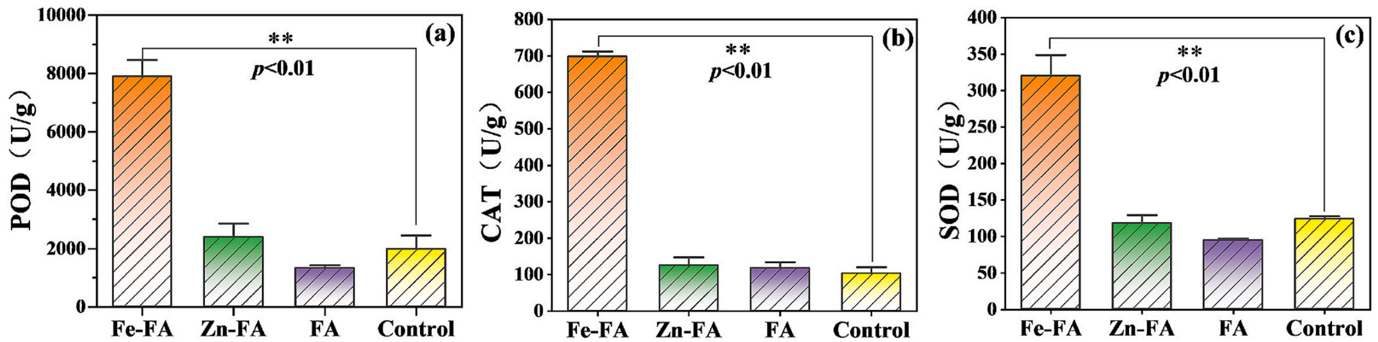

Fig. 2. Antioxidant enzymey activities of pepper leaves with Fe-FA, Zn-FA, FA and control application. (a) POD, (b) CAT, and (c) SOD.

significantly increased by 297 %, 578 % and 158 %, respectively, compared to the control group. However, there was no significant difference in leaves sprayed with Zn-FA and FA, relative to control group. With the highest levels of POD, CAT and SOD found in the Fe-FA group among all treatments, the study revealed that Fe-FA application resulted in the most significant improvement in antioxidant enzyme activity, effectively counteracting the toxic effects of Cd by activating mechanisms against oxidative stress.

3.4. Nutrients in pepper fruits

Overall, the application of Zn-FA, Fe-FA, and FA increased the levels of soluble sugar, protein, vitamin C, and total phenol in pepper fruits (Fig. 3), except for a decrease in soluble sugar content observed with FA application. Specifically, compared to the control group, Zn-FA and Fe-FA applications resulted in a 4.5 % and 12 % increase in soluble sugar content, while a 30 % decrease was observed with FA application. Similarly, the content of total protein in fruits was slightly increased by 8 % and 2 % for Zn-FA and Fe-FA applications, respectively. And there was no change observed with the foliar application of FA. Additionally, compared to the control group, the application of FA, Zn-FA, and Fe-FA significantly increased vitamin C content by 127 %, 102 %, and 136 %, respectively, with the highest vitamin C content up to 236 mg/100 g observed with Fe-FA. Furthermore, Zn-FA and Fe-FA application increased the total phenol level by 8 % and 13 % compared to the control, respectively. Overall, the application of Zn-FA, Fe-FA, and FA impacted the nutritional quality of pepper, with Fe-FA exhibiting the most pronounced effects on nutrient composition.

3.5. Pepper fruits non-target metabolomics

Given that Fe-FA application demonstrated the most promising results in reducing Ba and Cd content in pepper fruits and improving fruit quality, peppers treated with Fe-FA and the control were selected for the non-target metabolism analysis. In both groups, 40,285 metabolites were identified. The orthogonal partial least squares discriminant analysis (OPLS-DA) revealed a clear separation between the Fe-FA treatment and control groups, with the first and the second primary components accounting for 20.8 % and 26.4 % of variance, respectively (Fig. 4a). This indicates significant differences in the metabolites of

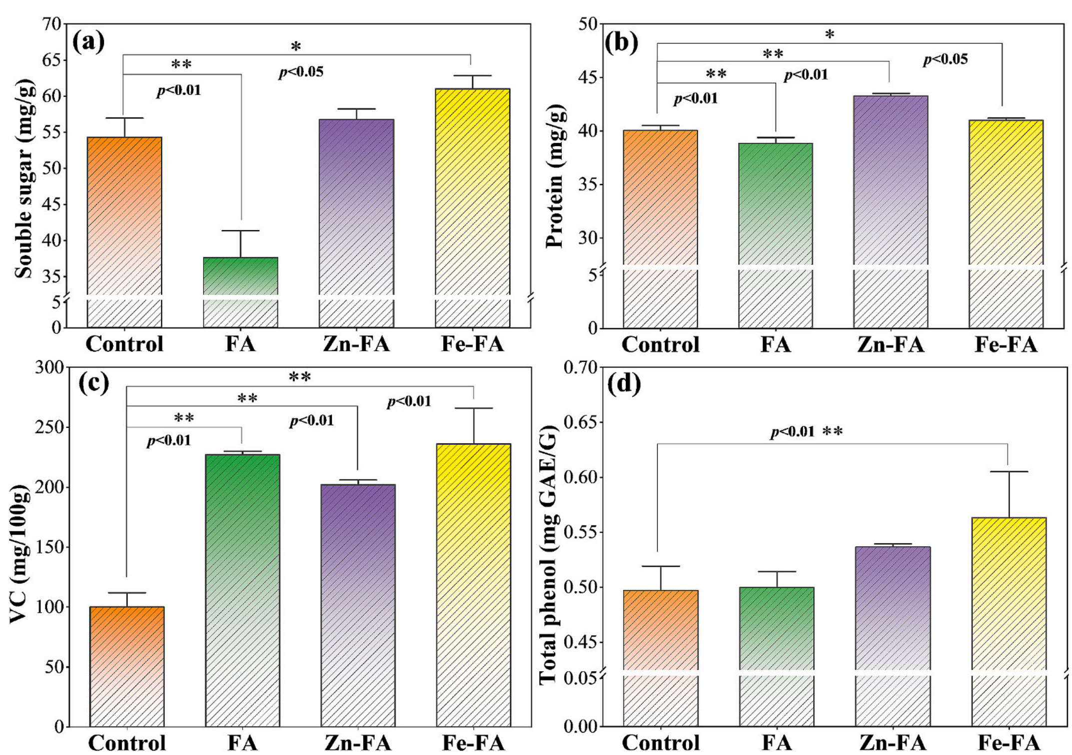

Fig. 3. Nutritional quality of pepper fruits with Fe-FA, Zn-FA, FA, and control application. (a) soluble sugar content, (b) protein content, (c) VC content and (d) total phenol content.

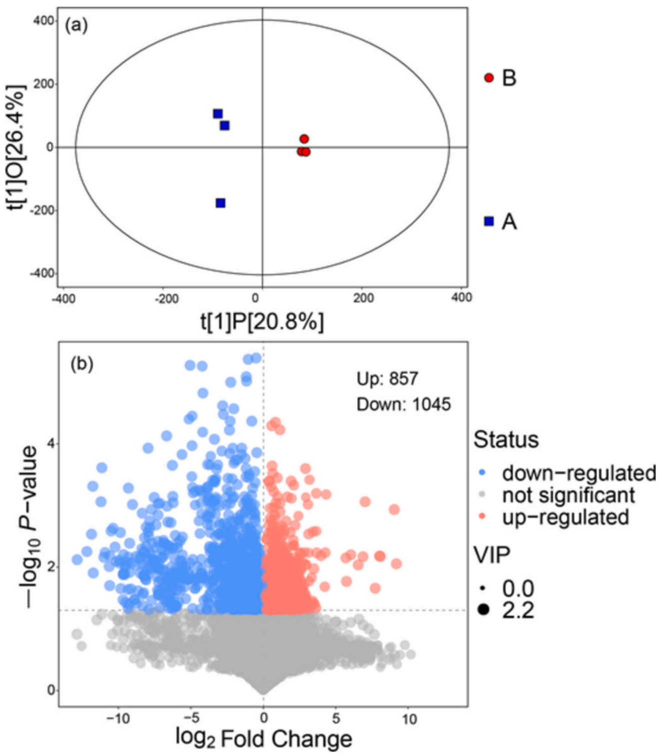

Fig. 4. Effect of Fe-FA foliar application on the differentially metabolites in the pepper fruits grown in Ba and Cd contaminated soil. (a) OPLS-DA score between Fe-FA application (B) and control (A), (b) volcanic map between Fe-FA application and control.

pepper between the two groups. Furthermore, a total of 1902 differential metabolites were identified between Fe-FA treatment and control groups (VIP > 1; $P < 0.05$), with 857 up-regulated and 1045 down-regulated (Fig. 4b). Classification analysis of differential metabolites revealed that the majority (30.8 %) were categorized as lipids and lipid-like molecules (**Fig. S2**), followed by organic oxygen compounds (15.8 %).

Differential metabolites between the control and Fe-FA applications were clustered and presented in a heatmap (Fig. 5a). A total of 26 distinct metabolites exhibiting significant variance in concentration were identified. The relative concentrations of Capsiamide (841 %), (2R,6×)-7-Methyl-3-methylene-1,2,6,7-octanetetrol 2-glucoside (100 %), Butyl-3-hydroxybutyrate glucoside (834 %), Italipyrone (141 %), Parakmerin A (73.7 %), Caryoptosidic acid (11,761 %), (7*R**,8*R**)-3-Methoxy-3′,4,7,9,9′-pentahydroxy-8,4′-oxyneolignan (366 %), Taurocholic acid (5922 %), and PS (20:5(5Z,8Z,11Z,14Z,17Z)/15:0) (12,187 %) were significantly up-regulated by foliar application of Fe-FA (Figs. 5a **and S3**). Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) was applied to examine the substances found in both the control and Fe-FA treatment groups (Fig. 5b). These differential metabolites are mainly involved in various pathways, such as valine, leucine, and isoleucine biosynthesis, as well as taurine and hypotaurine metabolism, and glycine, serine and threonine metabolism.

To further explore the interconnections among various metabolic pathways, a network-based enrichment analysis was performed using the identified differential metabolites. The results, as depicted in Fig. 6, revealed a strong correlation among valine, leucine, and isoleucine biosynthesis, Taurine and hypotaurine metabolism, C5-Branched dibasic acid metabolism, and betalain biosynthesis.

4. Discussion

4.1. Effect of FA, Fe-FA, and Zn-FA application on Cd and Ba uptake

High levels of Cd and Ba in crops pose a potential health threat to humans through dietary intake. Therefore, mitigating the uptake of Cd and Ba by plants is essential to ensure the safety of food production. In this study, the application of Zn-FA reduced the Ba content in pepper fruit, while Fe-FA applied not only decreased the Cd content but also simultaneously reduced the Ba content in pepper fruits (Fig. 1). Previous study indicated that Zn-FA application decreased the Cd content in rice grains by increasing the ratios of pectates and protein integration in the stems, alongside undissolved Cd phosphate in the leaves (Lu et al., 2024). Similarly, Wang, Du, et al. (2022) explored the impact of Fe-OM complexes on the accumulation of Cd in rice grains and found that Fe-FA exhibited the highest efficiency in reducing the Cd content in rice. They also found that the reduction achieved by Fe-FA application (from 0.48 ± 0.04 mg/kg to 0.19 ± 0.01 mg/kg) was significantly greater compared to Fe application alone (from 0.48 ± 0.04 mg/kg to 0.34 ± 0.01 mg/kg). Additionally, Fe-FA downregulated the expression of *OsNramp1* and *OsNramp5* in roots and stems, and upregulated the expression of *OsLCT1* genes in leaves. However, the application of FA did not reduce Cd and Ba content in pepper fruits, which partially contrasts with findings with previous studies. For instance, Wang et al. (2019) demonstrated that FA application reduced the Cd content in lettuce. This discrepancy is likely attributed to the variations in crop varieties, Cd concentration ranges, and the growth conditions.

4.2. Effects of FA, Fe-FA, and Zn-FA application on the growth and nutritional quality of pepper

Due to geological and anthropogenic activities, farmland soil contaminated with heavy metals is widespread in many countries, including China (Sun et al., 2019). Previous studies have indicated that Cd exposure can reduce plant growth, trigger reactive oxygen species production, and affect plant metabolism (Ahmad et al., 2016; Li et al., 2022). Similarly, Ba, a nonessential element in plants, can also affect plant growth and enzyme activity (Sleimi et al., 2021).

In this study, the effects of foliar applications of FA, Fe-FA, and Zn-FA on the accumulation of Ba and Cd in peppers, as well as their impact on biomass, enzyme activity, and beneficial nutrient components, were comprehensively studied. Results demonstrated that foliar application of these reagents increased the biomass of the stem and root of peppers. Particularly, Fe-FA exhibited a significant increase in the weight of individual pepper fruits. The comparatively lower biomass observed in the control can be attributed to the toxic effects of Cd and Ba. Cadmium inhibits normal cell division, decreases photosynthesis, and disrupts nutrient balance, leading to a lower biomass production (Rizwan et al., 2018). Similarly, Ba is also toxic for plants and affects growth, disturbing homeostasis and inhibiting photosynthesis (Sleimi et al., 2021; Suwa et al., 2008).

The results of this study illustrate that FA has the potential to enhance plant growth under Cd and Ba stress by alleviating Cd and Ba toxicity (**Fig. S1** and Table 1). This result aligns with previous studies. For instance, Wang et al. (2019) found that FA application increased chlorophyll content and facilitated the nutrient element translocation from root to shoot. FA, as a key constituent of organic matter characterized by its low molecular weight, high solubility, and strong migration activity, plays an important role in enhancing plant responses to environmental stress (Chiasson-Gould et al., 2014). This contributes to the higher root and stem biomass of pepper with FA application compared to the control group (Table 1). Furthermore, this study showed that Fe-FA was the most effective in promoting the growth of peppers. This is mainly attributed to the fact that Fe is crucial for plant growth and development, playing a vital role in multiple physiological processes, such as photosynthesis, respiration, and protein synthesis

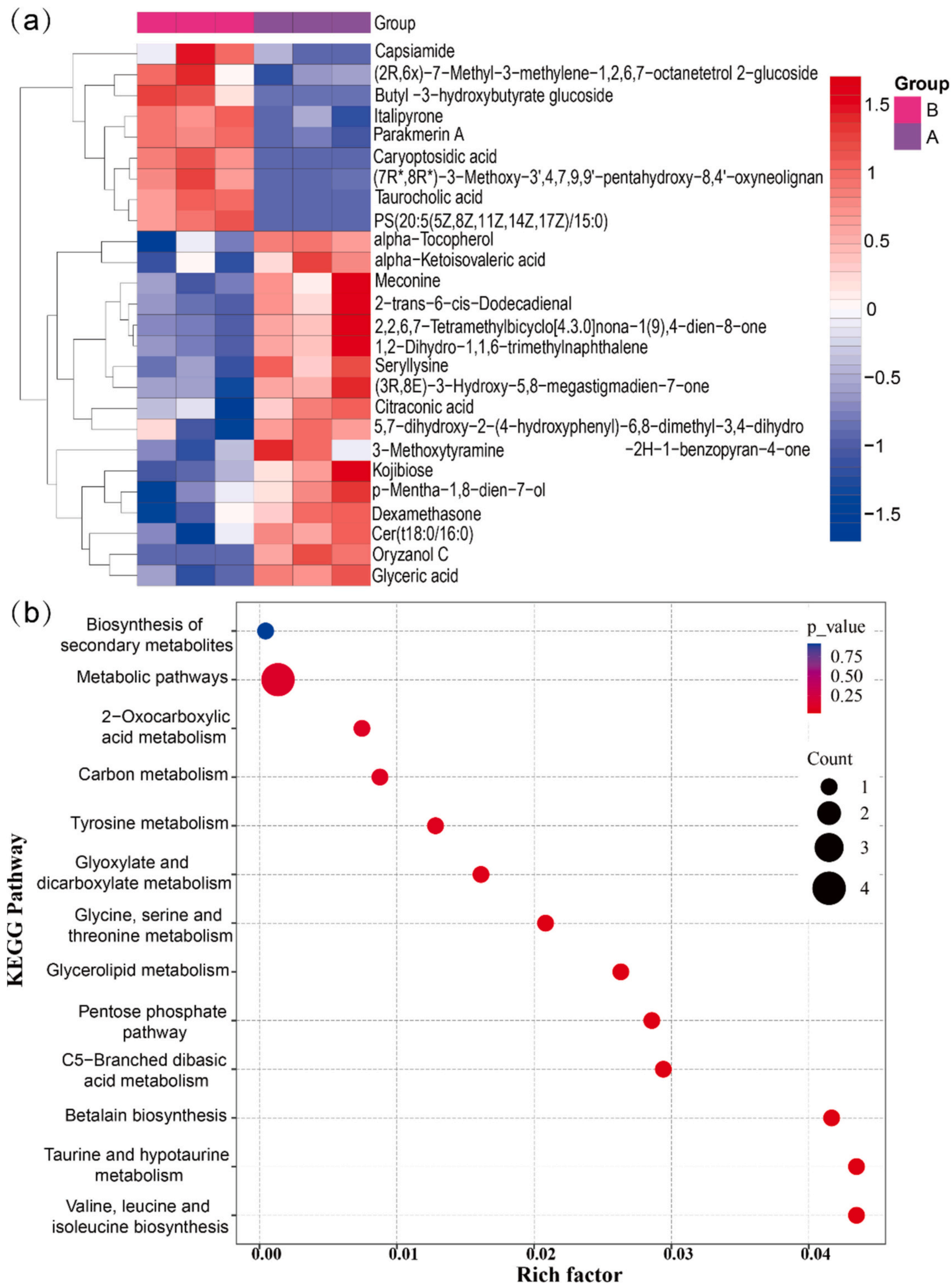

Fig. 5. Heatmap (a) and KEGG pathways (b) of differential metabolites in peppers with foliar application of Fe-FA (B) and control (A).

(Said-Al Ahl & Omer, 2009). This study also suggested that the concurrent application of FA and Fe had a synergistic effect on promoting the growth of peppers by improving the antioxidant system of peppers and modifying their metabolites (Figs. 2, 4, and 5).

Antioxidant enzymes such as POD, CAT, and SOD efficiently eliminate free radicals and peroxides, which are typically generated in plants under stress, thereby protecting crops from oxidative damage. Previous studies have indicated that Cd exposure to Cd leads to oxidative damage in plant cells, which hinders plant growth and photosynthesis (Molina et al., 2020). In this study, the application of Fe-FA notably enhanced the activity of POD, SOD, and CAT in pepper leaves, facilitating the mitigation of heavy metal (including Cd and Ba) induced oxidative stress, and consequently enhancing plant growth (Fig. 2). Similarly, Zhao et al. (2019) found that the application of auxin and brassinosteroid increased the gene expression of antioxidant enzymes in *Tetraselmis cordifolmis*. The application of H_2O_2 reduced oxidative stress caused by heavy metals by increasing the activity of antioxidant enzymes and promoting the expression of SOD proteins (Asgher et al., 2021). Wang, Jia, et al. (2022) demonstrated that arbuscular mycorrhizal fungi can alleviate heavy metal stress in plants by stimulating antioxidant enzymes like CAT, SOD,

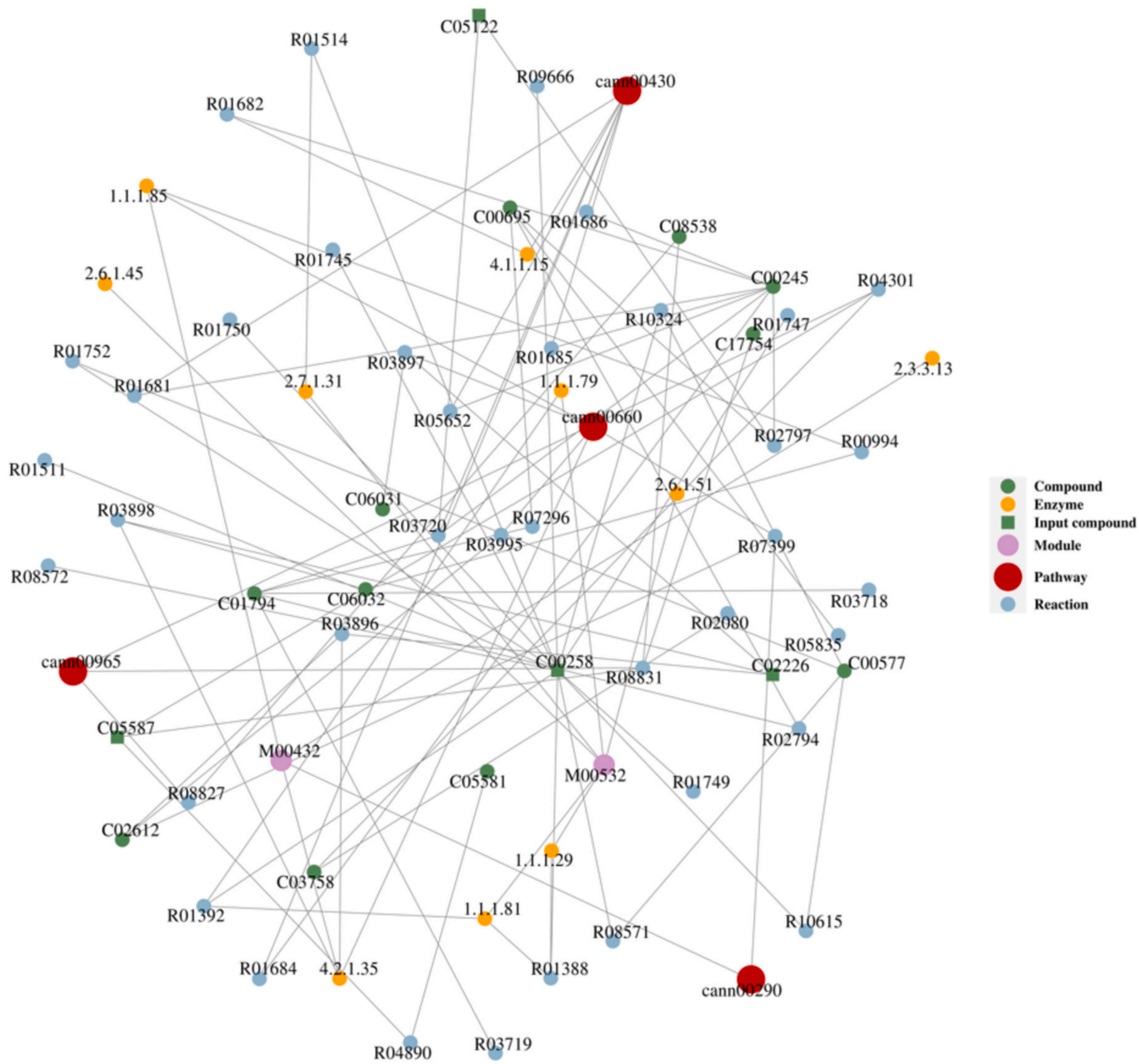

Fig. 6. Network diagram of group Fe-FA application vs group control. (Note: Red circle represent a metabolic pathway, yellow circles represent regulatory enzyme information related to a substance, green circles represent background substances of a metabolic pathway, purple circles represent information about a class of molecular modules, blue circles represent a chemical interaction reaction of a substance, and green squares represent differential metabolites obtained from the comparison.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and POD. Additionally, for pepper seedlings, melatonin relieved heavy metal-induced oxidative damage by enhancing the activity of antioxidative enzymes (Altaf et al., 2023). Collectively, the findings of this study suggest that Fe-FA application exerts a protective effect on pepper plants under Cd and Ba stress by stimulating antioxidative enzymes.

Foliar sprays of FA, Fe-FA, and Zn-FA positively affect the nutritional quality of pepper fruits, including soluble sugar, protein, vitamin C, and total phenol, particularly for vitamin C with a notable improvement. Vitamin C is an essential nutrient for the human body (Olatunji & Afolayan, 2018), and its deficiency can lead to scurvy, characterized by symptoms like purple spots on the skin, swollen gums, and tooth decay (Larralde et al., 2007). In this study, the vitamin C content of pepper fruits increased by 127 % (FA), 102 % (Zn-FA), and 136 % (Fe-FA) compared to the control group. Similarly, Li et al. (2022) also reported that FA application significantly increased the vitamin C content in tomatoes. The enhancement of vitamin C content is likely attributed to the promotion of gene expression related to vitamin C biosynthesis, such as PMI (phosphomannose isomerase), PMM (phosphomannose mutase), and VTC1 (GDP-mannose pyrophosphorylase), as observed in *Brassica chinensis* L. (Wolucka & Van Montagu, 2007). Additionally, a previous study demonstrated that up-regulated expression of L-GLDH in tobacco resulted in an increase in vitamin C content (Tokunaga et al., 2005).

4.3. The mechanism of Fe-FA application on Cd and Ba accumulation through non-target metabolomics analysis

In total, 26 distinct metabolites were identified, which significantly differed in their contents. The relative concentrations of capsiamide, (2R,6×)-7-Methyl-3-methylene-1,2,6,7-octanetetrol 2-glucoside, butyl-3-hydroxybutyrate glucoside, italipyrone, parakmerin A, caryoptosidic acid, (7*R**,8*R**)-3-methoxy-3′,4,7,9,9′-pentahydroxy-8,4′-oxyneolignan, taurocholic acid, and PS (20:5(5Z,8Z,11Z,14Z,17Z)/15:0) were found to be higher in peppers treated with Fe-FA foliar application compared to the control (Fig. 5a). Among these, capsiamide is an analog of capsaicin, the pungent component in chili peppers (Govindarajan & Sathyanarayana, 1991). Capsaicin has been extensively studied and is known for its therapeutic properties. It plays important biological roles, such as stimulating alkali and mucus secretion, inhibiting acid secretion, and particularly enhancing gastric mucosal blood flow, which contributes to the prevention and healing of gastric ulcers (Castro-Muñoz et al., 2022).

According to the KEGG database, differential metabolites were analyzed to identify significant metabolic pathways through enrichment analysis. This study demonstrated that these different metabolites primarily participated in 13 metabolic pathways (Fig. 5b). The results showed that glycine, serine and threonine metabolism; valine, leucine and isoleucine biosynthesis; C5-Branched dibasic acid metabolism; glyoxylate and dicarboxylate metabolism; pentose phosphate pathway, taurine and hypotaurine metabolism; betalain biosynthesis and glycerolipid metabolism were the most influenced biological pathways in the peppers treated with Fe-FA application, with a *P*-value less than 0.05 (Table S1). These pathways were primarily related to amino acid, carbohydrate, and lipid metabolism.

Amino acids play essential and central functions in various plant physiological processes, including the regulation of ion transport, involvement in heavy metal detoxification, and influence on the synthesis and interaction of numerous vital cellular enzymes (Sharma & Dietz, 2006). Zhao et al. (2023) showed that plants can enhance Cd tolerance by increasing amino acid metabolism, alleviating the inhibitory effect of Cd on growth. The biosynthesis of valine, leucine, and isoleucine plays a crucial role in counteracting osmotic stress caused by environmental pollutants (Xie et al., 2019). Additionally, plants can obtain more energy by increasing C5-Branched dibasic acid metabolism (Dong et al., 2023). The application of Fe-FA resulted in the up-regulation of amino acid and carbohydrate metabolism, which are crucial for protein synthesis, various physiological processes, energy production, metabolites, and aiding plants in adapting to changing environmental conditions. Previous studies have also indicated a close relationship between carbohydrate metabolism and plant responses to environmental stresses. Plants may modify their carbohydrate metabolism to maintain cellular homeostasis and increase their tolerance to stress when they are under stress conditions such as drought, high salinity, or extreme temperatures (Hasanuzzaman et al., 2018). Furthermore, earlier studies reported that glyceric acid and amino acids facilitate the detoxification of excessive heavy metals (e.g., Cu) in organisms (Feng et al., 2021). Therefore, the upregulation of glyceric acid through Fe-FA application may partially contribute to the mechanisms involved in the detoxification of Cd and Ba. Additionally, glycerolipid metabolism is a major tertiary metabolism of lipid metabolism (Wu et al., 2016). Lipids play an important role in regulating the stress resistance of plant cells. The impact of lipids on mitigating metal stress has been demonstrated (Zhang, Slaski, Archambault, & Taylor, 1997). Sun et al. (2020) reported that plants exposed to Cd stress experience a significant reduction in lipid concentration. Therefore, Fe-FA application might reduce Cd toxicity by increasing the glycerolipid metabolism. In the current study, the application of Fe-FA improved the amino acid pathway, carbohydrate metabolism pathway, and lipid metabolism, supporting the growth of pepper plants by enhancement in nutrient and energy availability.

5. Conclusions

Foliar application of FA, Fe-FA, and Zn-FA increased the biomass of chili pepper stems and roots. Notably, Fe-FA application led to a significant increase in the weight of pepper fruits and a marked reduction in Ba and Cd concentrations. Additionally, Fe-FA application enhanced the activity of POD, SOD, and CAT enzymes in pepper leaves, which positively impacted the nutritious quality of pepper fruits, including soluble sugar, protein, vitamin C, and total phenol content, particularly with a notable increase of vitamin C content. Metabolomics analysis revealed that Fe-FA application mainly affects amino acid, carbohydrate and lipid metabolism of plants, consequently providing more energy and nutrients for pepper plants under Cd and Ba stress. These findings offer valuable insights into the reduction of Ba and Cd accumulation and the improvement of quality in pepper fruits, providing effective measures for future research and practical application.

CRediT authorship contribution statement

Qinhui Lu: Writing – review & editing, Writing – original draft, Funding acquisition. **Zhidong Xu:** Writing – review & editing, Writing – original draft, Funding acquisition. **Qinghai Zhang:** Writing – review & editing. **Zhi Zhang:** Methodology, Investigation. **Yuxin Zhang:** Methodology. **Ting Zhang:** Investigation. **Jun Li:** Writing – review & editing, Supervision. **Xiaolin Wang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101904.

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