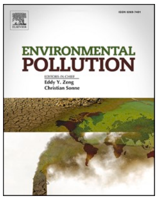
Environmental Pollution 337 (2023) 122578

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Physiological and metabolomic analyses reveal that Fe_3O_4 nanoparticles ameliorate cadmium and arsenic toxicity in *Panax notoginseng*☆

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ARTICLE INFO

Keywords:
Panax notoginseng
Fe_3O_4 NPs
Cadmium
Arsenic
Metabolomics

ABSTRACT

Heavy metal(loid)-contaminated available arable land seriously affects crop development and growth. Engineered nanomaterials have great potential in mitigating toxic metal(loid) stress in plants. However, there are few details of nanoparticles (NPs) involved in *Panax notoginseng* response to cadmium (Cd) and arsenic (As). Herein, integrating physiological and metabolomic analyses, we investigated the effects of Fe_3O_4 NPs on plant growth and Cd/As responses in *P. notoginseng*. Cd/As treatment caused severe growth inhibition. However, foliar application of Fe_3O_4 NPs increased beneficial elements in the roots and/or leaves, decreased Cd/As content by 10.38% and 20.41% in the roots, reduced membrane damage and regulated antioxidant enzyme activity, thereby alleviating Cd/As-induced growth inhibition, as indicated by increased shoot fresh weight (FW), the rootlet length and root FW by 40.14%, 15.74%, and 46.70% under Cd stress and promoted the shoot FW by 27.00% under As toxicity. Metabolomic analysis showed that 227 and 295 differentially accumulated metabolites (DAMs) were identified, and their accumulation patterns were classified into 8 and 6 clusters in the roots and leaves, respectively. Fe_3O_4 NPs altered metabolites significantly involved in key pathways, including amino sugar and nucleotide sugar metabolism, flavonoid biosynthesis and phenylalanine metabolism, thus mediating the trade-off between plant growth and defense under stress. Interestingly, Fe_3O_4 NPs recovered more Cd/As-induced DAMs to normal levels, further supporting that Fe_3O_4 NPs positively affected seedling growth under metal(loid)s stress. In addition, Fe_3O_4 NPs altered terpenoids when the seedlings were subjected to Cd/As stress, thus affecting their potential medicinal value. This study provides insights into using nanoparticles to improve potential active ingredients of medicinal plants in metal(loid)-contaminated areas.

1. Introduction

Excessive exploitation of ore resources can easily result in a great loss of accessory mineral materials worldwide, which has led to severe environmental consequences (Karak, 2022; Meharg and Whitaker-Hartley, 2002). *P. notoginseng* (Burk.) F.H. Chen, a traditional medicinal plant, is mainly distributed in southwest China (Guo et al., 2010). The content of cadmium (Cd) in *P. notoginseng* planted background soils ranges from 0.19 to 7.56 mg kg^{-1} (Liao et al., 2018), and arsenic (As) levels can reach 60.80 mg kg^{-1} (Liu et al., 2014), accompanied by high risks of metal(loid)s.

Toxic metal(loid) severely inhibits plant growth and quality. Heavy

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☆ This paper has been recommended for acceptance by Dr Parvaiz Ahmad.

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https://doi.org/10.1016/j.envpol.2023.122578
Received 30 July 2022; Received in revised form 4 August 2023; Accepted 16 September 2023

metal(loid)s can be absorbed and accumulated in plants and enter food chains, which has attracted great attention (Zhao et al., 2021). Plants have evolved different mechanisms for the uptake of essential and nonessential elements. Cd and As can be sequestered in vacuoles (Mendoza-Cózatl et al., 2011). Some ornamental plants play positive roles in heavy metal uptake and show higher tolerance to Cd contamination (Liu et al., 2008), implying that they can be used as hyperaccumulators for phytoremediation. Accompanying oxidative stress and disruption of ion homeostasis, elevated concentrations of Cd/As usually reduce plant growth and photosynthetic ability and can even lead to plant death (Ahsan et al., 2008; Küpper et al., 2007). Thus, it is still crucial to seek favourable measures to improve plant survival under toxic metal-(loid)s conditions.

Nanobiotechnology, widely used in the environment, biomedicine, and food agriculture, is one of the most promising technologies in the 21st century, and releasing nanoparticles (NPs) into the environment has become inevitable (Kopittke et al., 2019; Lv et al., 2019). Studies have shown that various NPs are translocated into plants and play positive or negative roles in growth and development (Lahiani et al., 2015; Lin and Xing, 2008). NPs have been widely used to combat biotic and abiotic stresses in plants (Kah et al., 2018; Zhao et al., 2016). Interestingly, NPs have great potential application in sustainable agriculture (Kah et al., 2019; Kolenčík et al., 2019; Rizwan et al., 2019). Recently, NPs serving as nanopesticides, nanoregulators, or nanofertilizers have attracted extensive attention (Wu et al., 2019; Zhao et al., 2020). The NPs interact with plant cells to affect gene expression or associated biochemical pathways, subsequently affecting plant growth and development (Faizan et al., 2022; Rahman et al., 2022; Rai et al., 2022; Wan et al., 2019).

Nano-based iron oxide (FeOx) NPs, one of the most promising NPs, have shown superior performance as regulators in mediating plant growth and response to stress (Basit et al., 2022; Gao et al., 2007; Rai et al., 2022). Ghafariyan et al. (2013) demonstrate that iron oxide NPs positively enhance the chlorophyll content. α-Fe_2O_3 NP priming facilitates legume root growth at low concentrations, whereas high concentrations cause growth inhibition (Palchoudhury et al., 2018). Nano-Fe_3O_4 exposure in *Arabidopsis thaliana* induces root growth inhibition (Lee et al., 2010). In contrast, Fe_3O_4 NPs facilitate nutrient uptake and plant development (De Souza et al., 2019), reducing heavy metal uptake and alleviating their toxicity to seedlings (Konate et al., 2017; Zhang et al., 2021).

Long-term exposure to increasing heavy metal(loid)s causes high health risks when using *P. notoginseng* and its products (Ou et al., 2016; Zhu et al., 2016). Bioactive components, such as saponins and flavonoids, are inevitably disrupted when exposed to an unfriendly environment (Liao et al., 2018; Liu et al., 2008). Previous studies have identified the complex mechanisms of *P. notoginseng* responses to biotic and abiotic stress (Kan et al., 2016; Tang et al., 2019; Zhu et al., 2016). Seedlings planted in 517 mg kg^{-1} As soil show increased enzyme activities associated with arsenic tolerance (Yan et al., 2011). Cd and As are two major metal(loid) stresses in the production area of *P. notoginseng*, which severely affects its growth and quality (Liao et al., 2018; Yan et al., 2011). However, the effects of NPs, especially oxide iron NPs involved in the *P. notoginseng* response to Cd/As stresses, remain to be further explained.

Herein, we examined the effects of Fe_3O_4 NPs on *P. notoginseng* under Cd and As stress. Combined with physiological and metabolomic analyses, we intended to examine the mechanisms of the *P. notoginseng* seedling response to Cd/As stress with or without foliar exposure to Fe_3O_4 NPs. The results will provide new insight into mitigating metal (loid) toxicity to *P. notoginseng* by spraying with Fe_3O_4 NPs. This study suggests that metal nanoparticles can serve as nanoregulators to combat metal(loid) stresses in the genuine production area of medicinal plants.

2. Materials and methods

2.1. Materials

Fe_3O_4 NPs were characterized in our previous study (Zou et al., 2022), and stock solution preparation was performed as described previously (Wang et al., 2011). The hydrodynamic size and zeta potential of 100 mg L^{-1} Fe_3O_4 NPs prepared with ddH_2O (pH 6.5) were 241.40 ± 11.13 nm and 12.03 ± 0.34 mV, respectively, which were determined by XFNANO (Jiangsu, China) using a dynamic light scattering apparatus (JEM-2100, Malvern). $Na_2HAsO_4{\cdot}7H_2O$ (Sigma-Aldrich, USA) and $CdCl_2$ (Macklin, China) were dissolved in ddH_2O to prepare stock solutions.

2.2. Seed germination and plant cultivation

The mature *P. notoginseng* fruits were thoroughly removed and rinsed clean with running water, and then the seeds were soaked in 5% $CuSO_4$ solutions for 30 min, followed by a rinse with water. Subsequently, the seeds were stratified in fine sands at a humidity of 20% for two months for after-ripening before germination. The soils used for seed germination were analyzed according to the methods described by Bao (2007), and the details of the soil properties were listed in Table S1.

The seeds were germinated and fostered in the soils for 3 months. Fifteen identical-looking seedlings were kept in a pot supported by a floating board covered with a plastic film which could prevent nanoparticles from entering the solutions when spraying with nanoparticles (Sun et al., 2020; Zou et al., 2022). Each pot containing 1 L of Hoagland solutions (pH 6.5; Table S2) and 0.5% Fe supplied as Fe-EDTA (Hoagland and Arnon, 1950; Li et al., 2013). The seedlings were fostered for additional growth for 30 days, and the solutions were renewed every 5 days.

2.3. Determination of stomatal aperture

Four-month-old seedlings with consistent growth were used for analysis. The stomatal assay of *P. notoginseng* leaves was performed as previously described (Yang et al., 2021; Zhang et al., 2020). At least 50 stomata were measured, and the stomatal aperture was calculated by the width/length ratio. The width and length of the stomata were 5.26 ± 0.12 μm and 11.32 ± 0.22 μm, respectively, and the stomatal aperture was 0.47 ± 0.08.

2.4. Foliar application of Fe_3O_4 nanoparticles

The foliar application was performed using spray bottles according to our previous study (Zou et al., 2022), and 100 ml of freshly prepared 100 mg L^{-1} Fe_3O_4 NPs with ddH_2O were applied every 3 days for five cycles. For the second spray, the Hoagland solutions were added with or without 5 μM $CdCl_2$ or 30 μM Na_2HAsO_4, which were specified as follows: Control; Cd, 5 μM Cd; FeNP, 100 mg L^{-1} Fe_3O_4 NPs; FeNP_Cd, 100 mg L^{-1} Fe_3O_4 NPs+5 μM Cd; As, 30 μM As; and FeNP_As, 100 mg L^{-1} Fe_3O_4 NPs+30 μM As. The treated seedlings were grown for 21 days and harvested for further experiments. Three pots with 45 seedlings for each treatment were used, and each seedling was exposed to approximately 1.11 mg of Fe_3O_4 NPs.

2.5. Determination of plant growth parameters

After the seedlings were treated for the indicated time, the plant height and root length were measured, and the fresh weight (FW) of shoots and roots was determined promptly after harvest.

2.6. Determination of plasma membrane integrity

The plasma membrane integrity of the root was detected using trypan blue staining according to a previously described method (De Vos et al., 1989), and the integrity was negatively correlated with the degrees of staining.

2.7. Determination of proline and antioxidant content

The malondialdehyde (MDA) content, proline content, and activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), were determined. The crude protein was extracted following procedures described by Chen and Zhang (2016). Next, the supernatants were collected to determine crude protein concentration using a Nanodrop 2000 (Thermo, USA). According to detailed procedures by Chen and Zhang (2016), MDA, proline, SOD, POD, and CAT levels were determined by a 6715 UV/Visible spectrophotometer (Jenway, UK). The ascorbate peroxidase (APX) activity was determined as previously described (Nakano and Asada, 1981).

2.8. Determination of mineral elements

The mineral elements were measured as previously described (Zou et al., 2022). The mineral elements, including arsenic (As), cadmium (Cd), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), were quantified by using an ICP−AES (iCAP6300, Thermo Fisher, MA, USA).

2.9. Metabolomics assays

The samples exposed to 100 mg L^{-1} Fe_3O_4 NPs with or without 5 µM $CdCl_2$ or 30 µM Na_2HAsO_4 were harvested at the indicated time and then quickly frozen in liquid nitrogen followed by storage at −80 °C until analysis. Five replicates of roots and leaves were examined for each treatment, and 60 arrays were performed for untargeted metabolomics analysis on a platform (UHPLC-Q Exactive HF-X, Thermo Fisher, MA, USA) as previously described (Chong et al., 2018; Xu et al., 2020). The details of the metabolomics analysis are presented in the Supplemental Information.

2.10. Statistical analysis

Each experiment was performed in three independent biological duplications. The data are presented as the means ± standard errors (SEs). Student's *t*-test (IBM SPSS Statistics 20.0) was conducted to determine the differences between the two treatments, and * indicates a significant difference at $p < 0.05$. One-way ANOVA followed Tukey's post-hoc test to determine significant differences for multiple comparisons at $p < 0.05$, as indicated by lowercase letters.

3. Results

3.1. Fe_3O_4 NPs affected the growth of *P. notoginseng* seedlings

Cd and As severely repressed plant development and growth. We first investigated the effect of Cd and As on *P. notoginseng* seedling growth. As shown in Fig. 1, Cd treatment markedly reduced shoot FW by 31.58%, root length by 17.90% and root FW by 34.15%. In addition, shoot and

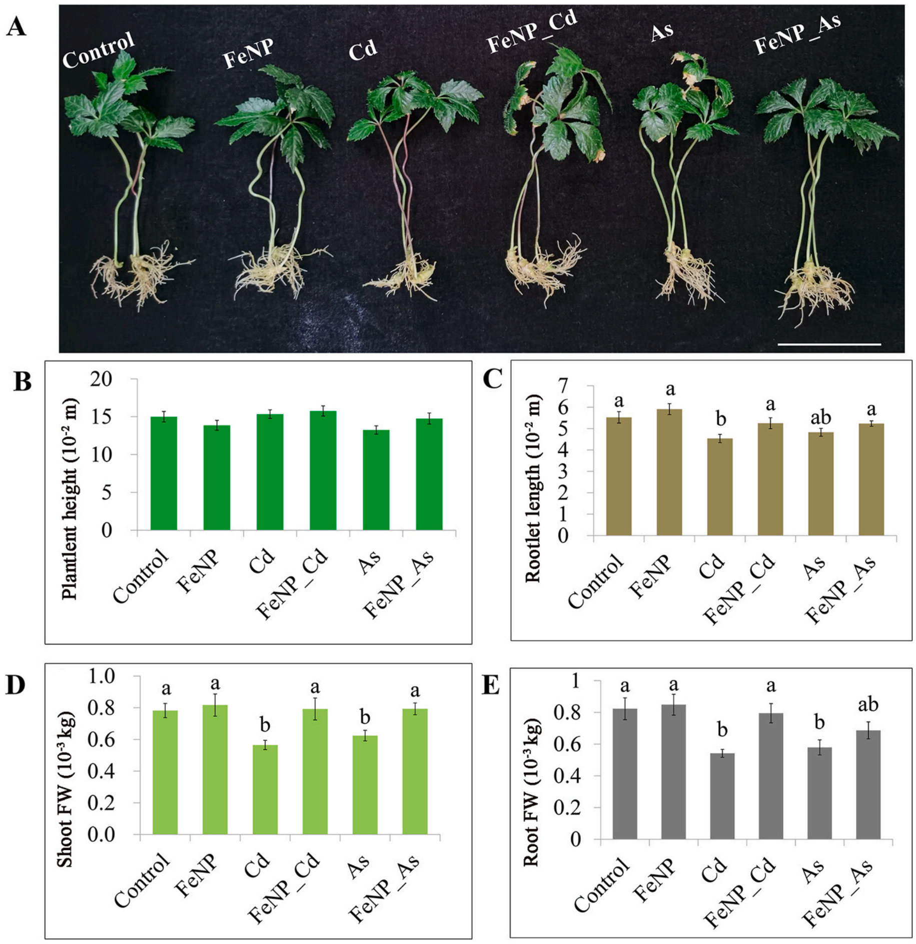


Fig. 1. Effects of Fe_3O_4 NPs on the growth of *P. notoginseng* seedlings. Representative phenotypes of Fe_3O_4 NPs supplemented with or without 5 µM Cd or 30 µM As for an additional 21 days (A). The plant height (B) and shoot FW (D). The root length (C) and FW (E). FW, fresh weight. Bars = 10 cm. The error bars represent ±SEs ($n = 3$; 15 plants/treatment/repeat), and different letters indicate significantly different values ($p < 0.05$ according to Tukey's test).

root FW were significantly reduced by 20.18% and 29.63% under As stress. To explore whether Fe_3O_4 NPs mediated the *P. notoginseng* response to Cd or As stress, we further performed foliar application of 100 mg L^{-1} Fe_3O_4 NPs to *P. notoginseng* seedlings. The results showed that NPs did not affect plant growth under normal conditions but markedly enhanced seedling growth under Cd/As stress (Fig. 1). Fe_3O_4 NPs dramatically increased shoot FW, the rootlet length and root FW by 40.14%, 15.74%, and 46.70% under Cd stress. Furthermore, Fe_3O_4 NPs markedly promoted the shoot FW by 27.00% under As stress. These results showed that Fe_3O_4 NPs played positive roles in *P. notoginseng* seedlings subjected to Cd/As stress.

3.2. Fe_3O_4 NPs affected the physiological and biochemical response in *P. notoginseng* seedlings

Cd or As toxicity disturbed nutrient uptake and metabolism, thus impairing ion balance and plant growth. We detected the element contents in the leaves and roots. Compared with the control conditions, Cd treatment increased the Cu, Fe, and Ca, but it decreased the Mn in the roots, and the Zn in the leaves (Fig. 2A). Arsenic treatment increased the Cu and Mg in the roots while it increased the Mn in the leaves but decreased it in the roots (Fig. 2A). Fe_3O_4 NPs exhibited a notable increase in Fe content in the leaves. Moreover, Fe_3O_4 NPs significantly increased the Fe content in the roots under Cd/As stress, indicating that NPs might be taken up by the leaves and translocated to the plant vasculature. Furthermore, Fe_3O_4 NPs increased the Cu in the roots but decreased it in the leaves under Cd/As stress (Fig. 2A). Compared with Cd or As stress alone, Fe_3O_4 NPs significantly decreased the Cd or As content by 10.38% and 20.41% in the roots but significantly increased the Cd content by 29.24% in the leaves (Fig. 2B and C). Fe_3O_4 NPs increased the Mn content in the roots and the Fe, Mg and Ca content in the leaves under Cd stress (FeNP_Cd/Cd), while they increased the Fe content in the roots and leaves under As stress (FeNP_As/As) (Fig. 2A). These results suggested that Fe_3O_4 NPs altered the element balance in *P. notoginseng* seedlings.

In addition to disrupting ion homeostasis, elevated concentrations of Cd/As usually accompany osmotic adjustment and oxidative stress. We then performed trypan blue staining to study the integrity of the root plasma membrane. Cd or As stress negatively affected the root tip cell membrane integrity (Fig. S1). The proline content was significantly increased in the Cd/As-treated seedling roots and leaves; however, Fe_3O_4 NPs decreased the proline content in Cd-treated seedling leaves and As-treated seedling roots and leaves (Fig. 3A and G).

We further examined the lipid peroxidation and the antioxidant system to clarify whether Fe_3O_4 NPs mediated antioxidant response in *P. notoginseng* seedlings. Cd/As treatment markedly increased the MDA content in the roots and leaves. Interestingly, Fe_3O_4 NPs decreased the MDA content in the As-treated seedling roots and Cd-treated seedling leaves compared with Cd/As stress alone (Fig. 3B and H). Cd treatment increased the activity of APX, POD and CAT in the roots and the activity of APX, CAT, and SOD in the leaves. Compared with Cd stress alone, Fe_3O_4 NPs decreased the activity of APX and CAT in the roots and APX and SOD in the leaves of Cd-treated seedlings (Fig. 3C–F and I-L). Similar effects were observed in the As-treated seedlings. Arsenic treatment increased the activity of SOD in the roots and the activity of APX, POD, CAT, and SOD in the leaves. Compared with As stress alone, Fe_3O_4 NPs

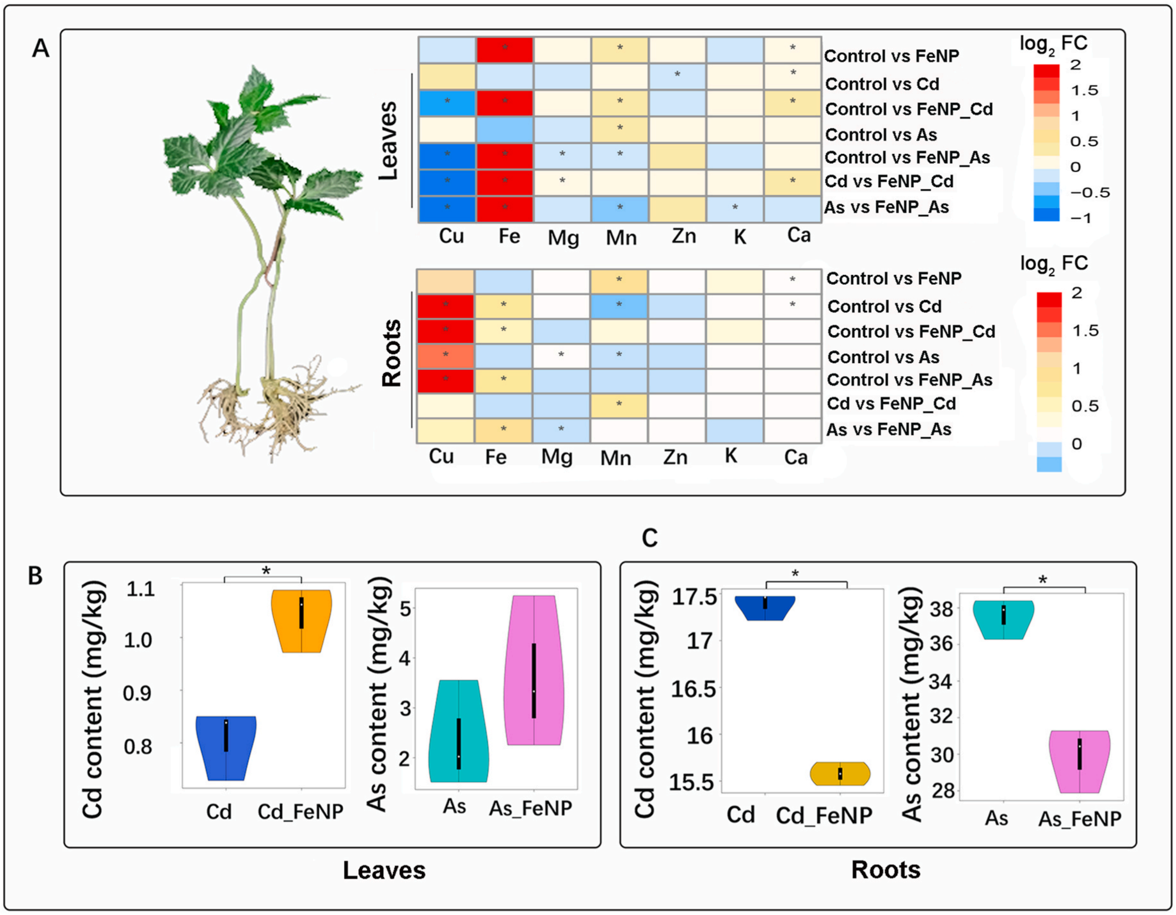


Fig. 2. Effects of Fe_3O_4 NPs on nutrient element accumulation in *P. notoginseng* seedlings. Heatmaps show mineral contents (log_2 fold change) compared between the two treatments (A). The violin plots show the Cd and As contents in the leaves (B) and roots (C). The * indicates significantly different values ($p < 0.05$) according to Student's *t*-test.

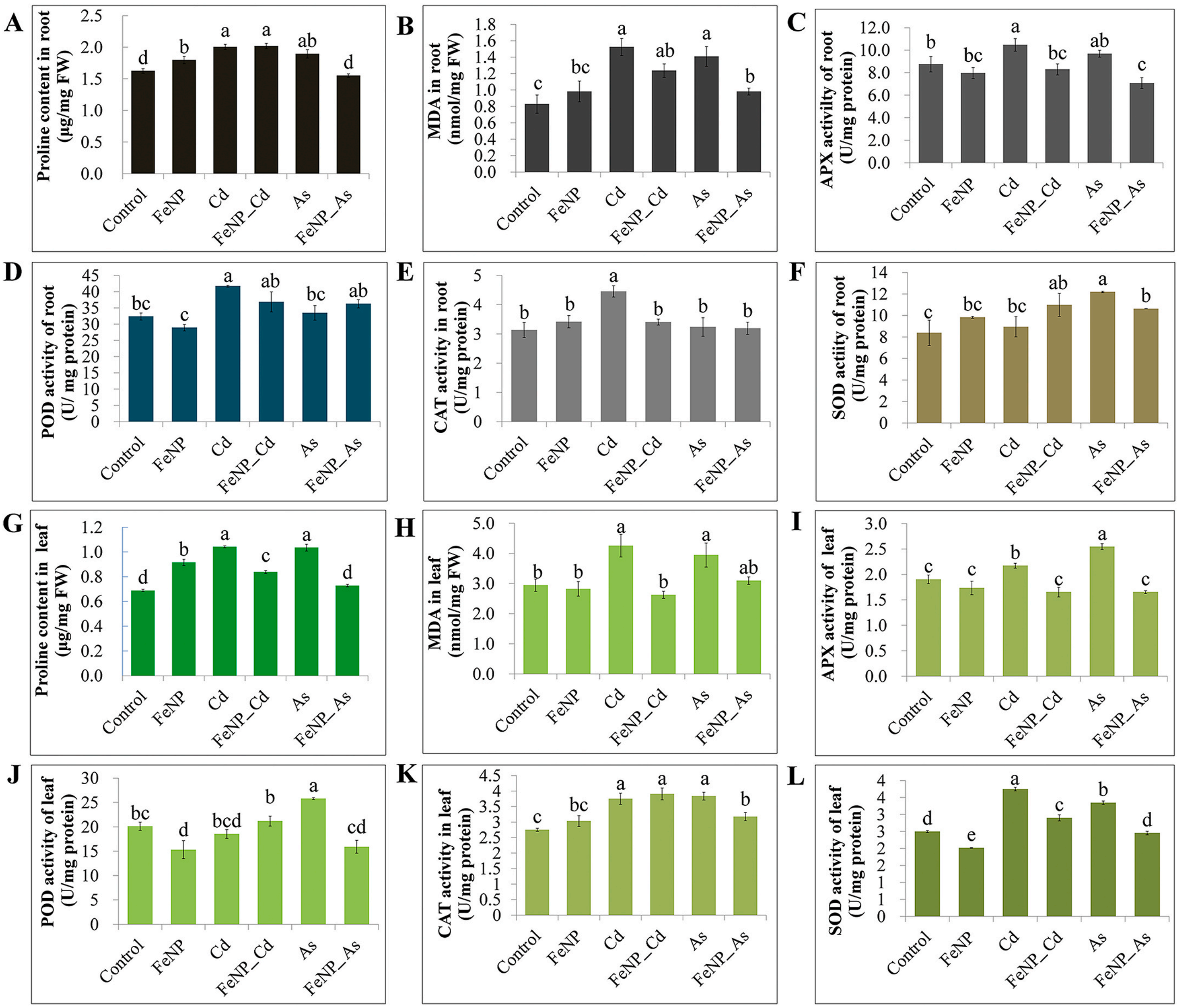


Fig. 3. Effects of Fe_3O_4 NPs on proline and MDA content and antioxidant enzyme activity in *P. notoginseng* seedlings. The proline and MDA content and the activities of antioxidant enzymes in roots (A–F) and leaves (G–L) of seedlings foliar sprayed with Fe_3O_4 NPs under 5 μM Cd or 30 μM As for an additional 21 days. The error bars represent ±SEs ($n = 3$), and different letters show significantly different values ($p < 0.05$ according to Tukey's test). MDA, APX, POD, CAT, and SOD represent malondialdehyde, ascorbate peroxidase, peroxidase, catalase, and superoxide dismutase, respectively.

decreased the activity of APX and SOD in the roots and APX, POD, CAT and SOD in the leaves of As-treated seedlings (Fig. 3C–F and I-L). These results indicated that Fe_3O_4 NPs altered the physiological response to Cd/As treatment in *P. notoginseng* seedlings.

3.3. Metabolomic profiling analysis

To explore the metabolomic profiling in the roots and leaves, we performed untargeted metabolomic analyses to identify the DAMs in the seedlings when exposed to Fe_3O_4 NPs, Cd/As stress, or their combinations. OPLS-DA analyses presented a clear separation among different treatments Figs. S2 and S3, indicating that Cd/As toxicity changed the metabolic profiles, and Fe_3O_4 NPs reprogrammed pronounced metabolites in the roots and leaves subjected to Cd/As stress.

We identified 227 and 295 DAMs (VIP ≥1 and fold change ≥2 or ≤0.5) in *P. notoginseng* roots and leaves, respectively (Fig. 4, Tables S3 and S4). In addition, 35 metabolites, including 7 amino acids, peptides and analogues (APAs), 4 terpenoids, and 3 flavonoids, were common in the roots and leaves (Fig. 4A). High levels of DAMs were observed in the roots under Cd stress with or without exposure to Fe_3O_4 NPs, and also in the leaves of Fe_3O_4 NP-treated and As-treated with Fe_3O_4 NPs (Fig. 4C and D). Furthermore, several top 20 significantly enriched pathways, including flavanoid biosynthesis, ascorbate and aldarate metabolism, and phenylalanine metabolism, were significantly enriched in roots and leaves (Fig. 4E and F).

3.4. Analysis of metabolite accumulation patterns

We identified different accumulation levels of metabolites among the treatments, and some metabolites showed similar changes in response to stress. Thus, we applied a *k*-means clustering algorithm to investigate the accumulation patterns of DAMs corresponding to different treatments, and 8 and 6 clusters were classified into the roots and leaves, respectively (Fig. 5A and B, Tables S5 and S6).

In the roots, 18 APAs were distributed in 7 clusters. Most of them were in Cluster IV (5) and Cluster VI (5), and their contents were

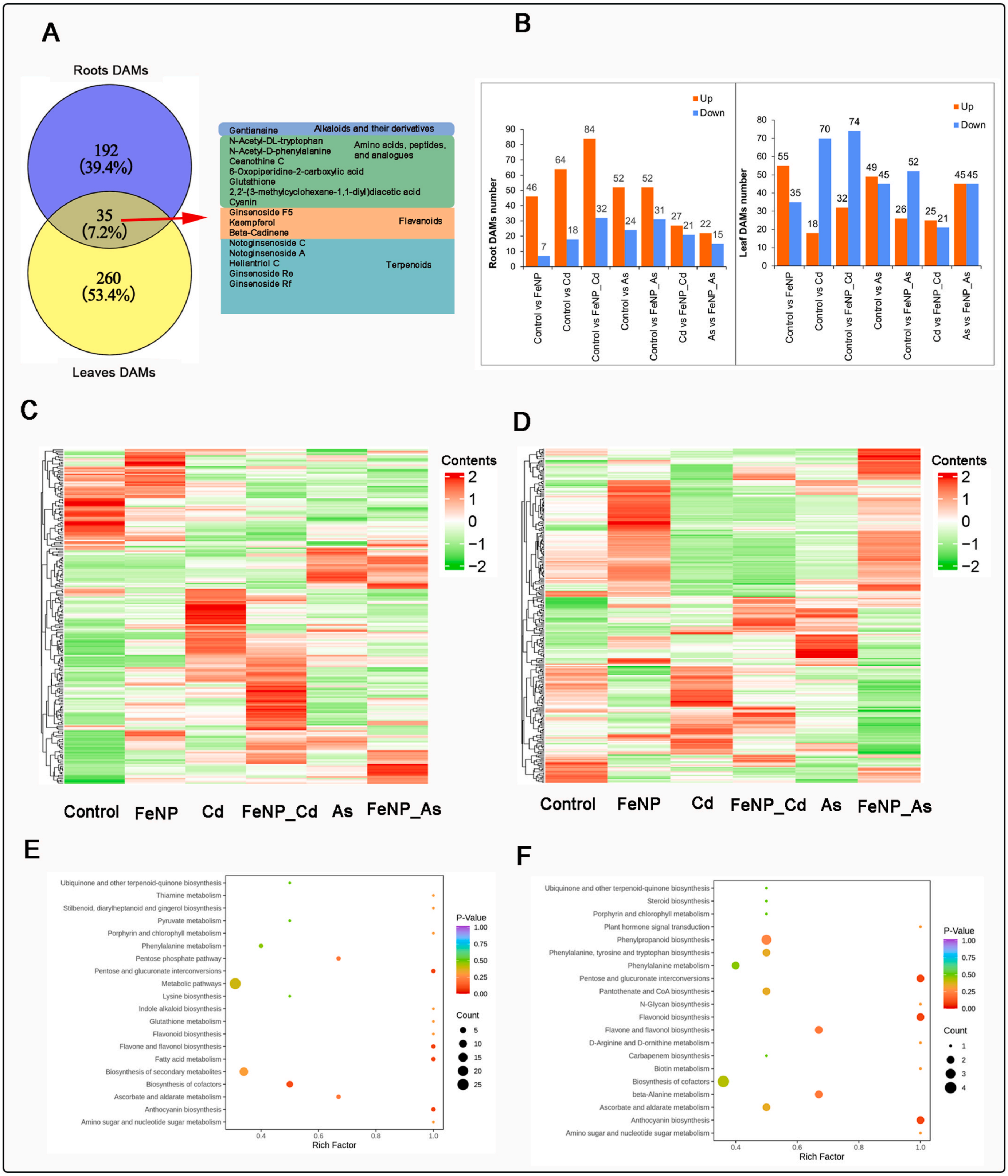
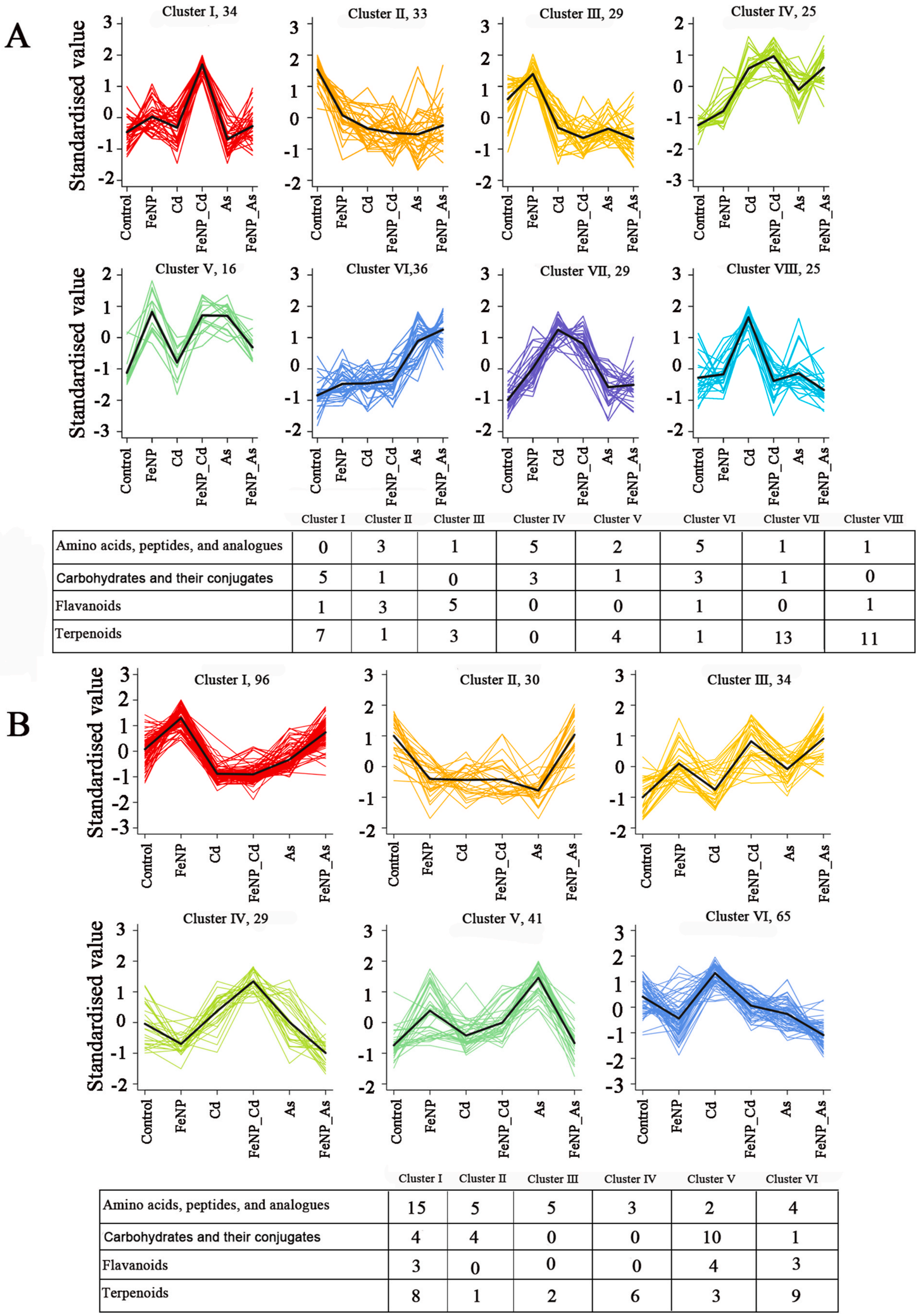


Fig. 4. DAMs analysis among various treatments. Venn diagram of root and leaf DAMs (A). Histogram statistics of DAM counts in roots and leaves among various treatments (B). Heatmaps of DAM contents in the roots (C) and leaves (D) among different treatments. Top 20 enriched KEGG pathways in the roots (E) and leaves (F).



	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Amino acids, peptides, and analogues	0	3	1	5	2	5	1	1
Carbohydrates and their conjugates	5	1	0	3	1	3	1	0
Flavanoids	1	3	5	0	0	1	0	1
Terpenoids	7	1	3	0	4	1	13	11

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Amino acids, peptides, and analogues	15	5	5	3	2	4
Carbohydrates and their conjugates	4	4	0	0	10	1
Flavanoids	3	0	0	0	4	3
Terpenoids	8	1	2	6	3	9

Fig. 5. Metabolite accumulation patterns analysis. The *k*-means clustering analysis of the DAMs in the roots (A) and leaves (B) among different treatments. The subclass represents that metabolites have the same change pattern in different treatments.

upregulated by different treatments compared with the control (Fig. 5A, Table S5). Flavonoids and carbohydrates and their conjugates were most abundant in Cluster III and Cluster I, respectively (Fig. 5A, Table S5). Terpenoids were the most abundant metabolites, and 40 terpenoids were abundant in 7 clusters, including 13 in Cluster VII and 11 in Cluster VIII (Fig. 5A, Table S5). We also found that terpenoids were presented in higher levels under Cd/As stress, whereas Fe_3O_4 NPs decreased the levels of these metabolites under Cd/As stress in Cluster VIII (Fig. 5A, Table S5). In addition, we found that coumarins and their derivatives were specifically abundant in Cluster II, and phenylpropanoic acids were abundant in Cluster VI (Fig. 5A, Table S5).

In the leaves, 295 DAMs were classified into 6 clusters. Ninety-six DAMs, including 3 alkaloids and their derivatives, 15 APAs, 4 carbohydrates and their conjugates and 3 flavonoids, were abundant in Cluster I. In addition, 65 DAMs, including 4 APAs, 12 glycerophospholipids and 3 flavonoids, were abundant in Cluster VI (Fig. 5B, Table S6). Furthermore, 29 terpenoids were abundant in all 6 clusters, including 8 in Cluster I and 9 in Cluster VI (Fig. 5B, Table S6). We also found that lineolic acids and their derivatives were more abundant in Cluster III and Cluster VI. Indoles and their derivatives were specifically abundant in Cluster II, and phenylpropanoic acids were abundant in Cluster IV (Fig. 5B, Table S6).

3.5. Fe_3O_4 NPs modulated the root and leaf metabolome

In Fe_3O_4 NP-treated seedlings, 46 and 55 metabolites were increased in the roots and leaves, respectively, whereas 7 and 35 metabolites were decreased (Fig. 4B, Tables S3 and S4). Venn diagram analysis showed that most DAMs were unique in Fe_3O_4 NP-treated roots and leaves. Fe_3O_4 NPs increased the levels of 4 metabolites, including 1 APA, 1 fatty acid ester and 1 terpene glycoside, in Fe_3O_4 NP-treated roots and leaves (Fig. S4, Tables S3 and S4).

Fe_3O_4 NPs increased the levels of 6 APAs, 2 carbohydrates and their conjugates, and 2 glycerophospholipids in the roots (Table S3). In addition, Fe_3O_4 NPs increased indoleacrylic acid and inosine in the roots (Table S3). However, 4-formyl indole showed decreased accumulation levels in the roots. In addition, Fe_3O_4 NPs increased the levels of 9 APAs and 5 carbohydrates and their conjugates but decreased the levels of 4 APAs and 6 glycerophospholipids in the leaves (Table S4).

3.6. Root and leaf metabolome subjected to Cd stress

Cd treatment upregulated 78% of the DAMs in the roots but downregulated 79.5% of the DAMs in the leaves (Fig. 4B, Tables S3 and S4). In the roots, Cd stress enhanced the levels of 64 metabolites, including 7 APAs, 2 glycerophospholipids and 19 triterpenoids (Table S3). In contrast, it decreased the levels of 18 metabolites, including 1 APA, 1 glycerophospholipid and 2 lineolic acids and their derivatives in the roots (Table S3). In addition, Cd increased the accumulation of indoleacrylic acid and inosine in the roots (Table S3). However, it decreased the levels of 4-formyl indole and thymine in the roots (Table S3).

Cd stress increased the levels of 18 metabolites in the leaves, including 2 APAs, 1 fatty alcohol and 2 triterpenoids (Table S4). In contrast, it decreased the levels of 70 metabolites in the leaves, including 11 APAs, 5 carbohydrates and their conjugates, 4 glycerophospholipids and 2 flavonoids (Table S4). Furthermore, kanzonol O showed an increased accumulation level, whereas quercetin and quercetin 3-beta-laminaribioside showed decreased accumulation levels in Cd-treated leaves (Table S4).

Venn diagram analysis revealed that most DAMs were unique in Cd-treated roots and leaves. The levels of three metabolites increased in the roots but decreased in the leaves of Cd-treated seedlings compared to the untreated control (Fig. S5, Tables S3 and S4). These results indicated that Cd toxicity reprogrammed different metabolic profiles in the roots and leaves.

3.7. Root and leaf metabolome subjected to As stress

Arsenic treatment upregulated 68.4% and 52.1% of the DAMs in the roots and leaves, respectively (Fig. 4B, Tables S3 and S4). In the roots, arsenic treatment increased the levels of 52 metabolites, including 9 APAs, 3 carbohydrates and their conjugates, 3 glycerophospholipids and 4 triterpenoids (Table S3). In contrast, it decreased 24 metabolites in the roots, including 2 APAs, 2 carbohydrates and their conjugates and 2 phenols (Table S3). In addition, arsenic treatment altered indoleacrylic acid level increased, and 4-formyl indole decreased in the roots (Table S3).

Arsenic treatment increased the levels of 49 metabolites in the leaves, including 7 APAs, 7 carbohydrates and their conjugates and 2 flavonoids. In contrast, it decreased the levels of 45 metabolites in the leaves, including 5 APAs, 4 glycerophospholipids and 3 lineolic acids and their derivatives (Table S4). Furthermore, the levels of coumarin increased, but the levels of indole-3-methyl acetate decreased in As-treated leaves (Table S4).

Venn diagram analysis indicated that 3 metabolites, including heliangin, N-acetyl-DL-tryptophan) and notoginsenoside A, showed increased accumulation levels in As-treated roots and leaves, whereas glutathione and inosine showed increased accumulation levels in the roots but decreased levels in the leaves of As-treated seedlings(Fig. S6, Tables S3 and S4).

3.8. Fe_3O_4 NPs reprogrammed Cd/As-induced DAMs

To further explore whether Fe_3O_4 NPs altered the metabolic profiling response to stress, we analyzed the Cd/As-induced common metabolites under Cd/As stress after foliar application of Fe_3O_4 NPs (Figs. 6 and 7, Tables S3, S4 and S7-S10). Cd/As toxicity reprogrammed primary metabolites, including APAs and glycerophospholipids, and secondary metabolites, including flavonoids, alkaloids and terpenoids in the roots and leaves (Figs. 6 and 7, Tables S7-S10).

We found that many Cd-induced metabolites recovered to normal levels in the Cd-treated seedlings when subjected to Fe_3O_4 NPs. Specifically, Fe_3O_4 NPs recovered 22 Cd-upregulated metabolites, including 2 APAs, 3 prenol lipids and 2 terpene glycosides, and 9 Cd-downregulated metabolites, including a glycerophospholipid, a lineolic acid and its derivative, in the Cd-treated roots to normal levels compared to the control (Fig. 6A and B, Table S7). In addition, Fe_3O_4 NPs recovered 7 Cd-upregulated metabolites, including 1 APA, 1 fatty acid and its conjugate and 1 flavonoid, and 17 Cd-downregulated metabolites, including 3 APAs, 1 carbohydrate and conjugate and 2 flavonoids, in the Cd-treated leaves to normal levels (FeNP_Cd/Control) (Fig. 6C and D, Table S8).

We also found that As-altered metabolites were recovered when subjected to Fe_3O_4 NPs. Fe_3O_4 NPs recovered 22 As-upregulated metabolites (including 5 APAs, 1 terpene glycoside and 3 triterpenoids) in the As-treated roots and 42 As-upregulated metabolites (including 6 APAs and 7 carbohydrates and their conjugates) in the As-treated leaves to normal levels compared to the control (Fig. 7, Tables S9 and S10). Furthermore, Fe_3O_4 NPs recovered 13 As-downregulated metabolites (including 2 phenols and 1 flavonoid) in the As-treated roots and 33 As-downregulated metabolites (including 4 APAs and 2 glycerophospholipids) in the As-treated leaves to normal levels (FeNP_As/Control) (Fig. 7, Tables S9 and S10).

3.9. Fe_3O_4 NPs reprogrammed the root and leaf metabolome under Cd and As stress

Compared with Cd-treated seedlings alone, Fe_3O_4 NPs increased the levels of 27 metabolites but decreased the levels of 21 metabolites in the roots, while they increased the levels of 25 metabolites but decreased the levels of 21 metabolites in the leaves of Cd-treated seedlings (Fig. 4B, Tables S3 and S4). Only ceanothine C showed an increased accumulation level in the leaves but decreased in the roots of Cd-treated seedlings

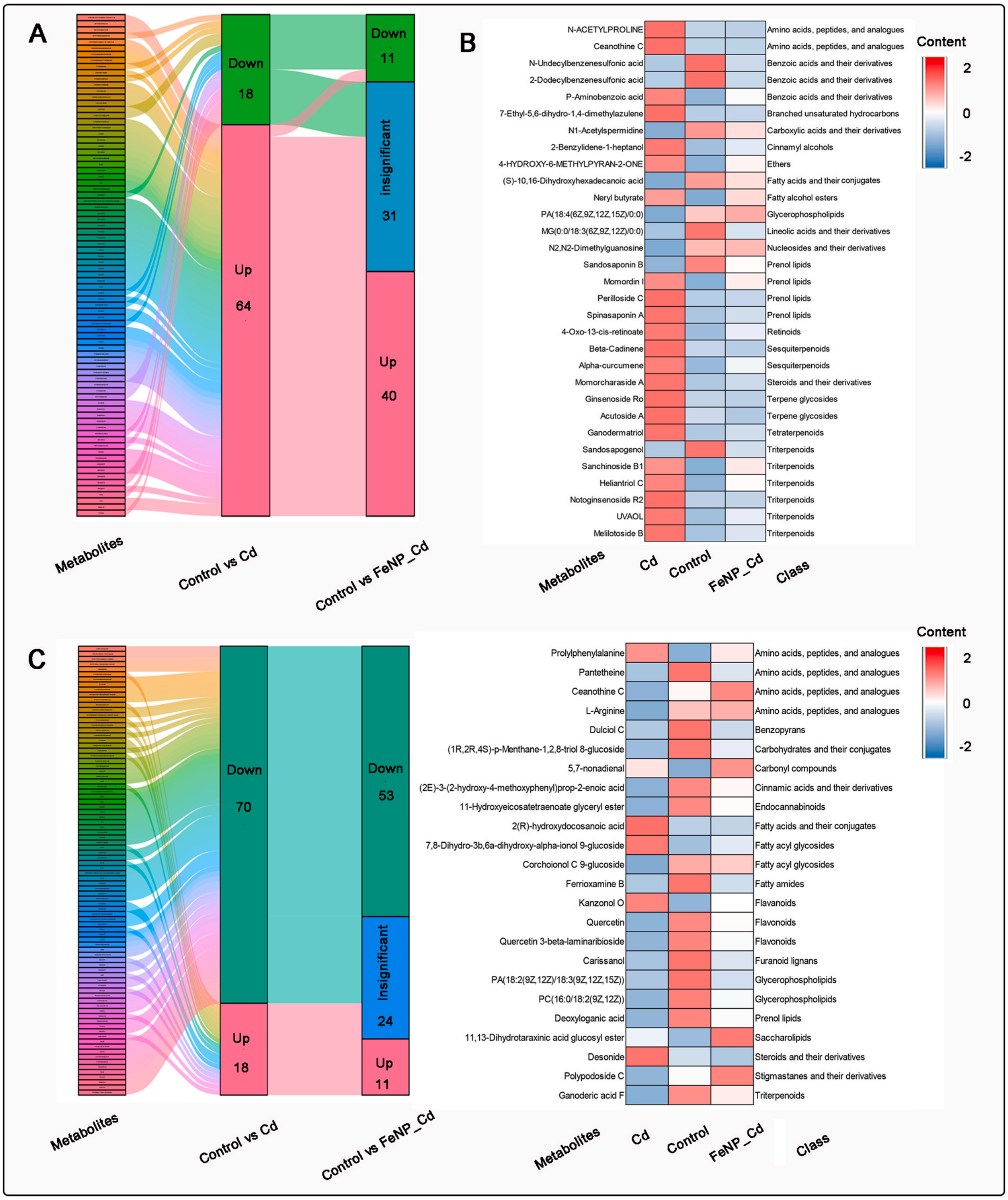


Fig. 6. Cd-induced DAMs in the roots and leaves of *P. notoginseng* seedlings. Sankey diagrams show DAMs in the roots (A) and leaves (C) of Cd-treated seedlings with or without Fe_3O_4 NPs compared to the control. Heatmaps of Cd-induced DAM contents recovered to the control level by spraying with Fe_3O_4 NPs under Cd stress in roots (B) and leaves(D).

subjected to Fe_3O_4 NPs (Fig. S7).

Compared with As-treated seedlings alone, Fe_3O_4 NPs increased the levels of 22 and 45 metabolites but decreased the levels of 15 and 45 in the roots and leaves of As-treated seedlings, respectively (Fig. 4B, Tables S3 and S4). One metabolite (ceanothine C) showed an increased level in the roots and leaves of As-treated seedlings subjected to Fe_3O_4 NPs. In addition, momordicoside D showed an increased level in the roots but decreased in the leaves of As-treated seedlings subjected to Fe_3O_4 NPs (Fig. S8).

After foliar application of Fe_3O_4 NPs, more terpenoids and glycerophospholipids were significantly changed under Cd stress than under As stress, and 4 flavonoids were significantly downregulated under Cd stress in the roots (Fig. 8A and C). In the leaves, the number of changed lineolic acids and their derivatives under Cd stress was 3 times more than those under As stress. In addition, flavonoids, carbohydrates and their conjugates, and alkaloids and their derivatives were significantly changed under As stress (Fig. 8B and D).

3.10. Comparative metabolic analyses of Cd and As stress with or without Fe_3O_4 NPs

To elucidate whether there were differences in *P. notoginseng*

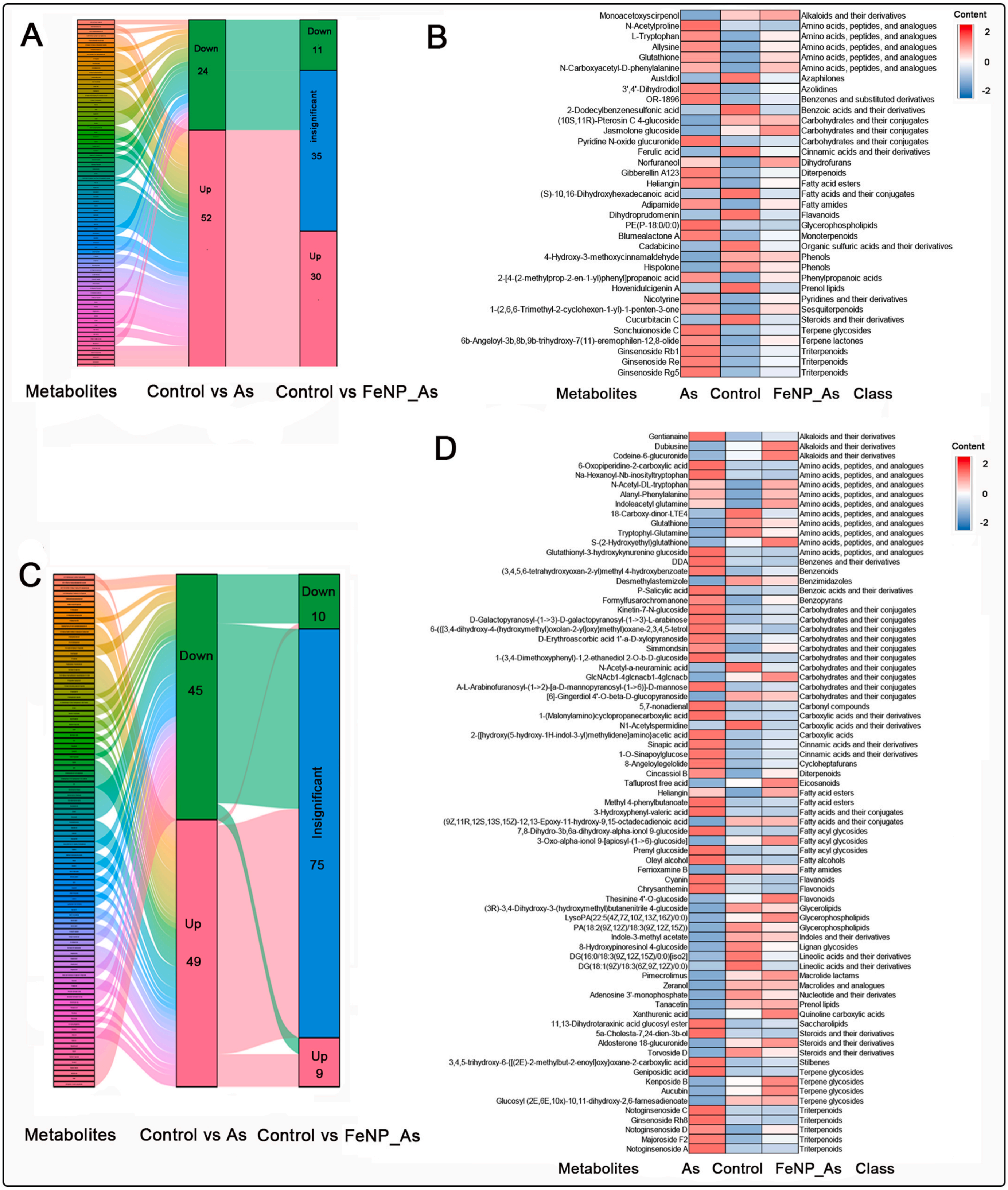


Fig. 7. Arsenic-induced DAMs in the roots and leaves of *P. notoginseng* seedlings. Sankey diagrams show DAMs in the roots (A) and leaves (C) of As-treated seedlings with or without Fe_3O_4 NPs compared to the control. Heatmap of As-induced DAM contents recovered to the control level by spraying with Fe_3O_4 NPs under As stress in roots (B) and leaves(D).

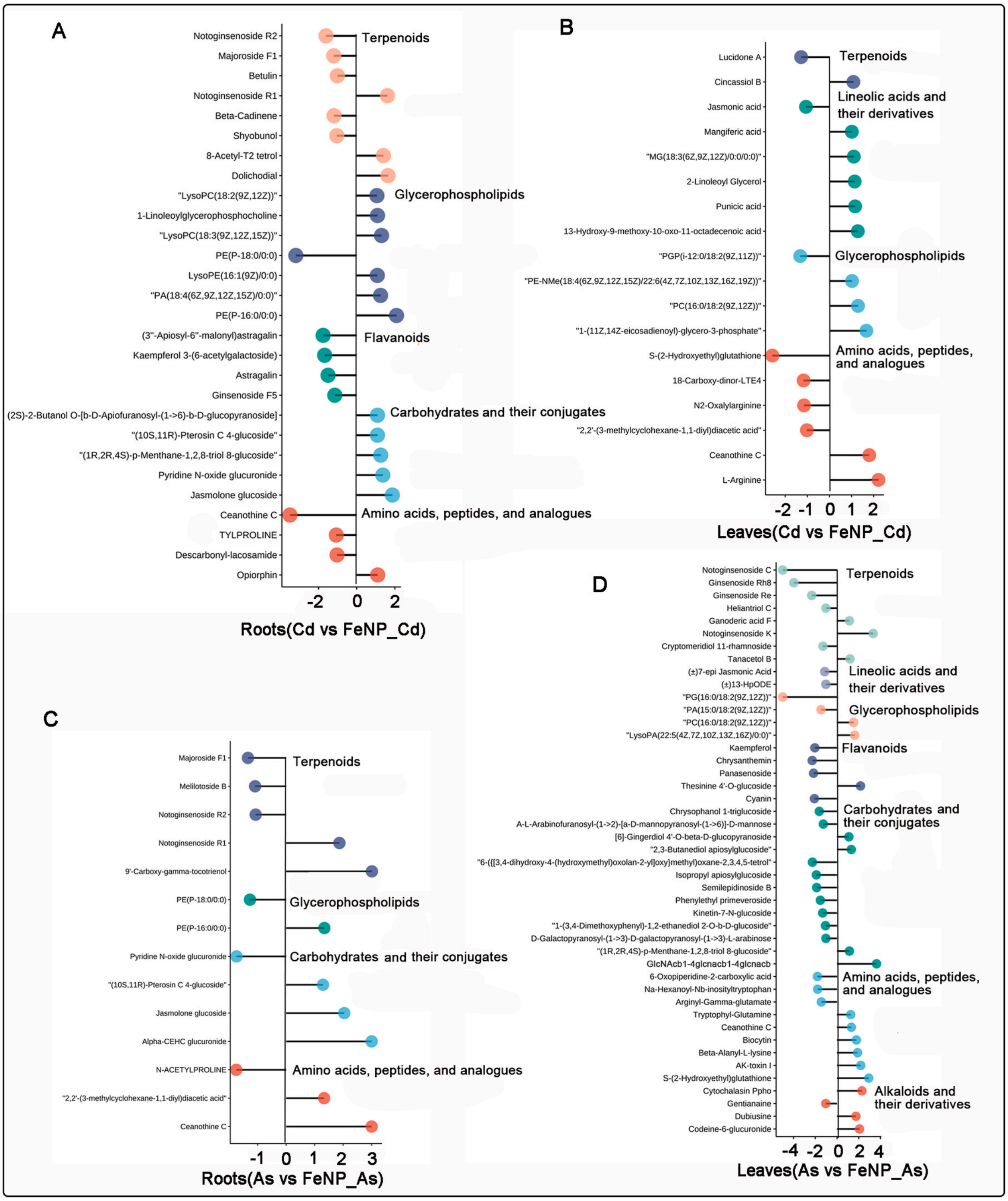


Fig. 8. Comparative analysis of DAMs response to Cd/As stress with or without foliar application of Fe_3O_4 NPs. The lollipop charts show DAMs ($\log_2$ fold change) between Cd and FeNP_Cd in the roots (A) and leaves (B) and between As and FeNP_As in the roots (C) and leaves (D).

responses to Cd/As toxicity, we further compared metabolic profiles in the roots and leaves under Cd stress and As stress. Arsenic increased the levels of 31 and 44 metabolites but decreased the levels of 40 and 24 in the roots and leaves, respectively (Tables S3 and S4). Two metabolites (ginsenoside Rf and ginsenoside F5) showed decreased accumulation levels in the roots and leaves (Fig. S9). The levels of terpenoids (13) and APAs (8) were the most changed in the roots and leaves under Cd/As stress, respectively (As/Cd) (Tables S3 and S4).

After foliar application of Fe_3O_4 NPs, arsenic increased 29 and 86 metabolite levels but decreased 38 and 53 in the roots and leaves, respectively (Tables S3 and S4). Venn diagram analysis indicated that ginsenoside Re showed a decreased accumulation in the roots and leaves (FeNP_As/FeNP_Cd) (Fig. S10). The levels of terpenoids (13) were the most changed in the roots, and the levels of 6 terpenoids (including notoginsenoside Fe and notoginsenoside R2) were also different when compared between seedlings under Cd stress and under As stress (Table S3). Terpenoids (9 showed increased accumulation levels, but 8 showed decreased accumulation levels) were the metabolites most commonly altered in the leaves, and musabalbisiane C was similar when compared between seedlings under Cd stress and As stress. Furthermore, the levels of 15 APAs (10 showed increased accumulation levels, but 5 showed decreased accumulation levels) were changed in the leaves, and 5 APAs (including N-lactoyl-tryptophan and 6-hydroxysandoricin) were similar when compared between seedlings under Cd stress and As stress

(Table S4).

3.11. *Comparative analyses of critical secondary metabolites in P. notoginseng seedlings*

Terpenoids, active pharmaceutical ingredients with potential medicinal value, are key secondary metabolites in *P. notoginseng*. We conducted comparative analyses of terpenoids among different treatments compared with the control. We found that terpenoids, including 3 and 1 monoterpenoids, 6 and 9 sesquiterpenoids, 3 and 4 diterpenoids, and 18 and 11 triterpenoids, were reprogrammed in the roots and leaves, respectively, especially under Cd and As stress with or without foliar application of Fe_3O_4 NPs (Fig. 9, Tables S3 and S4).

Specifically, Fe_3O_4 NPs increased the levels of 12 terpenoids in the roots compared to control, including 2 monoterpenoids (blumealactone A and 9′-carboxy-gamma-tocotrienol), 1 sesquiterpenoid (1-methyl-a-ionone), 2 diterpenoids (gibberellin A123 and cavipetin E isomer 1) and 7 triterpenoids (such as ginsenoside Re and ginsenoside Rg5) (Fig. 9A, Table S3). In addition, Fe_3O_4 NPs increased 3 terpenoids in the leaves, including beta-cadinene, cincassiol B and notoginsenoside D, whereas they decreased 4 terpenoids, including 1 sesquiterpenoid (lucidone A) and 3 triterpenoids (notoginsenoside K and majoroside F2) (Fig. 9B, Table S4).

Cd stress increased 19 but decreased 1 terpenoid in the roots, while it increased 2 but decreased 3 terpenoids in the leaves (Fig. 9, Tables S3 and S4). Fe_3O_4 NPs increased 17 terpenoids in the roots, while they increased 4 and decreased 6 terpenoids in the leaves of Cd-treated seedlings (Fig. 9, Tables S3 and S4). We found that 11 terpenoids, including 1 diterpenoid, 2 sesquiterpenoids and 8 triterpenoids, showed increased accumulation levels in the roots (Fig. 9A), while 2 triterpenoids (ginsenoside Rh8 and majoroside F2) showed increased accumulation levels and 2 diterpenoids (yucalexin P15 and musabalbisiane C) showed decreased accumulation levels in leaves exposed to Cd with or without Fe_3O_4 NPs (Fig. 9B).

Arsenic stress increased the accumulation levels of 8 terpenoids in the roots, while it increased the accumulation levels of 6 and decreased 3 terpenoids in the leaves (Fig. 9, Tables S3 and S4). Fe_3O_4 NPs increased 4 and 2 terpenoids and decreased 5 and 10 terpenoids in the As-treated roots and leaves, respectively (Fig. 9). Further analysis revealed that cavipetin E isomer 1 and notoginsenoside A showed increased accumulation levels in the roots (Fig. 9A), whereas heliantriol C and lucidone A showed decreased accumulation levels in leaves exposed to As with or without Fe_3O_4 NPs (Fig. 9B). In addition, two terpenoids showed increased accumulation in the roots under Cd/As stress with or without exposure to Fe_3O_4 NPs (Fig. 9, Tables S3 and S4). The above results showed that Cd/As treatment changed terpenoids in the roots and leaves, and Fe_3O_4 NPs under Cd/As treatment altered some common or distinct metabolites similar to Cd/As stress.

4. Discussion

Metal(loid) toxicity seriously inhibits plant growth and

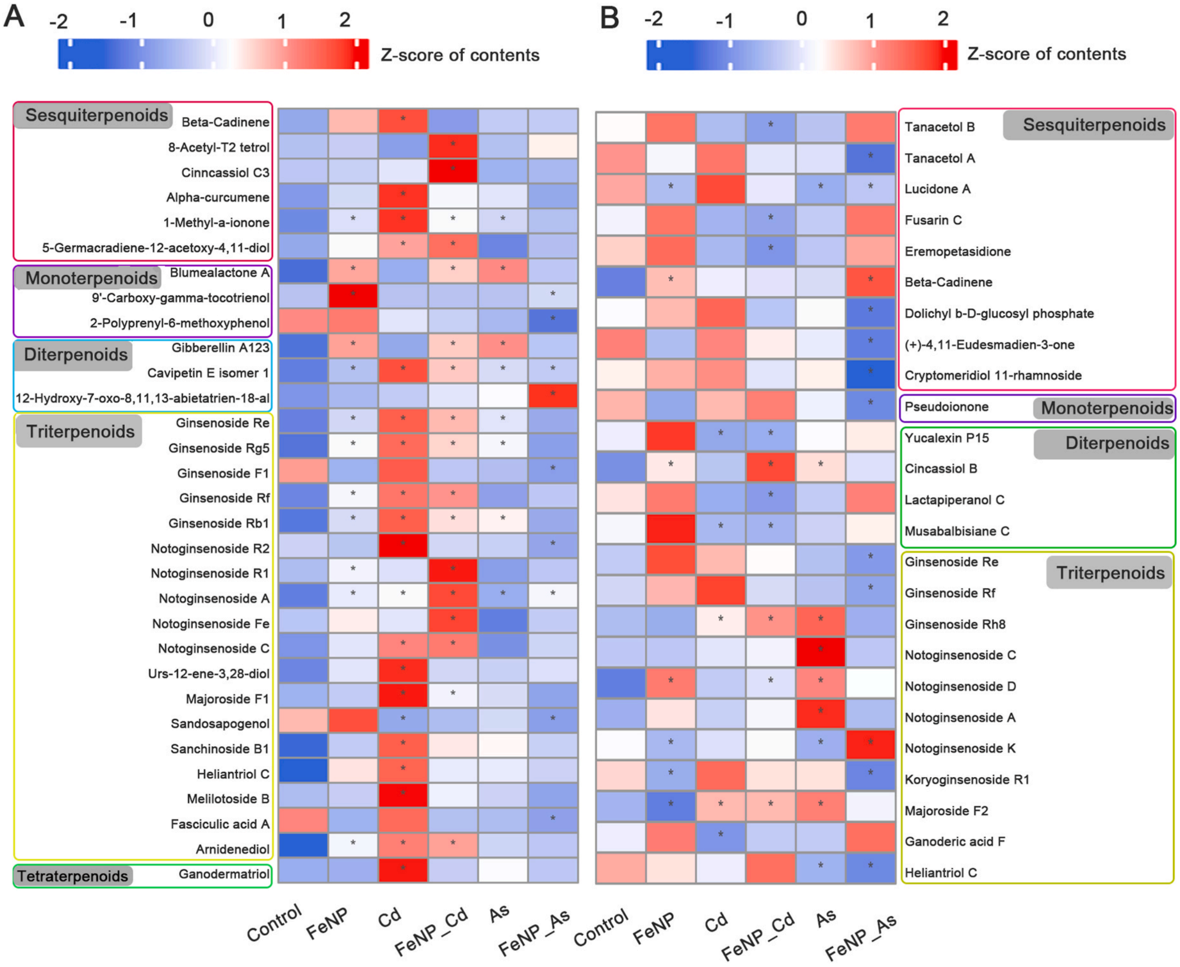


Fig. 9. Effects of Fe_3O_4 NPs on terpenoids in the roots and leaves of *P. notoginseng* seedlings. Heatmaps show terpenoids in the roots (A) and leaves (B). Each coloured cell represents the content of metabolite for different treatments. The * indicates that the metabolite content is significantly different compared with control conditions (VIP ≥1, fold change ≥2 or ≤ 0.5).

development, thus influencing yield and quality. This study found that Cd/As stress caused severe growth inhibition in *P. notoginseng* seedlings. However, foliar application of Fe_3O_4 NPs conferred tolerance to Cd/As stress by modulating ion balance and antioxidant enzyme systems and reprogramming metabolic profiles. Cd/As reduces plant biomass, root growth, and photosynthesis and even causes plant death (Ahsan et al., 2008; Küpper et al., 2007). In this study, we found that Cd/As treatment repressed seedling growth, as indicated by decreasing shoot FW, rootlet length and root FW (Fig. 1). Compelling evidence demonstrates that nano-Fe_3O_4 improves the growth of plants under abiotic stress (Konate et al., 2017). Our results revealed that Fe_3O_4 NPs markedly increased seedling growth subjected to Cd/As stress (Fig. 1). Similar alleviating effects of Fe_3O_4 NPs are also reported by Konate et al. (2017).

Ions uptake, and utilization is essential for plant growth (Fan et al., 2021), and their balances are vulnerable to disruption, especially under stress conditions (Mendoza-Cózatl et al., 2011; Zhao et al., 2021). We found that Cd and As toxicity disrupted the ion balance in the seedlings. Specifically, Cd treatment increased Cu, Fe, and Ca levels but decreased Mn levels in the roots and Zn levels in the leaves, while arsenic treatment increased Cu and Mg levels in the roots and Mn levels in the leaves (Fig. 2A), thereby affecting plant physio-biochemical processes. Fe_3O_4 NPs increased Fe and Cu levels in the roots and/or leaves under Cd or As stress. Importantly, Fe_3O_4 NPs decreased Cd or As levels in the roots, thus reducing their toxic effects on plant growth.

Furthermore, the relative accumulation ability of metal(loid) allows plants to survive under stress, as indicated by an increased antioxidant enzyme system under severe oxidative damage caused by metal-(loid)s stress (Basit et al., 2022; Liao et al., 2018). These changed elements, such as Fe, Mn, Cu and Zn, are key cofactors of FeSOD, MnSOD, and Cu/ZnSOD, which are essential components of the antioxidant enzyme system for regulating reactive oxygen species (ROS) homeostasis in plants (Miller, 2012). Cd and As toxicity increased APX, POD, CAT, and SOD activity in the roots and/or leaves compared with the control (Fig. 3), indicating that Cd/As induced oxidative damage to *P. notoginseng* seedlings and plants persistently improved antioxidant capacity for maintaining plant growth under continuous stress. FeOx NPs have the potential to scavenge ROS due to their antioxidant-enzyme-mimicking activities (Gao et al., 2007; Rai et al., 2022; Zhao et al., 2020). Interestingly, Fe_3O_4 NPs decreased the activity of APX, POD, CAT and SOD to a certain extent in the roots and/or leaves, suggesting that Cd/As-treated seedlings suffered less oxidation damage when subjected to Fe_3O_4 NPs. The reduction in MDA content and the trypan blue detection results further supported that Fe_3O_4 NPs played key roles in modulating the antioxidant system to repress oxidative stress.

Cd and As toxicity alter plant physiological and metabolic responses, thus severely repressing plant growth and development (Jiang et al., 2018; Meharg and Whitaker-Hartley, 2002). The above results showed that Cd and As induced different mineral element accumulation and physiological responses, thus influencing plant metabolism. Plants can optimize their carbon/nitrogen and energy metabolism to maintain growth under stress conditions (Wan et al., 2020; Zou et al., 2022). In addition, secondary metabolites play roles in coping with stressful conditions (Neilson et al., 2013; Yeshi et al., 2022). In this study, we found that Cd toxicity increased 78% of the DAMs in the roots but decreased 79.5% of the DAMs in the leaves, and arsenic treatment increased 68.4% and 52.1% of the DAMs in the roots and leaves, respectively (Fig. 4, Tables S3 and S4), indicating that numbers of DAMs were involved in stress responses.

Cd/As stress reprogrammed the accumulation of primary metabolites, such as APAs, carbohydrates and their conjugates and glycerophospholipids, and secondary metabolites, including alkaloids and their derivatives, flavonoids and terpenoids, indicating that *P. notoginseng* seedlings altered these key metabolites, which were favourable for maintaining survival under stress. We found that a key component for antioxidant resistance, glutathione, was upregulated in Cd-treated roots. A previous study demonstrated that improved glutathione metabolism could enhance Cd tolerance (Guo et al., 2016). In addition, Cd increased inosine but decreased thymine in the roots (Table S3), thus disrupting nucleic acid metabolism in plants. Quercetin is a key flavonoid involved in auxin transport and signal transduction (Lewis et al., 2011; Wan et al., 2018), thus affecting plant growth and development. We found that the level of one flavonoid (kanzonol O) increased, whereas the level of quercetin and quercetin 3-beta-laminaribioside decreased in Cd-treated leaves (Table S4). In addition, most DAMs were unique in Cd-treated roots and leaves, and 3 metabolites showed opposite accumulation levels in Cd-treated roots and leaves compared to the control (Fig. S5, Tables S3 and S4). For arsenic stress, L-tryptophan and glutathione in the roots and N-acetyl-DL-tryptophan and indoleacetyl glutamine in the leaves were upregulated in As-treated seedlings. However, the levels of glutathione and indole-3-methyl acetate decreased in the As-treated leaves (Tables S3 and S4). These key components of tryptophan metabolism are altered under As stress, suggesting that amino acid metabolism plays an essential role in modulating the stress response. In addition, arsenic treatment altered indoleacrylic acid and 4-formyl indole in the roots (Table S3). Most DAMs were unique in As-treated roots and leaves, similar to the Cd-treated seedlings. Three metabolites, heliangin, N-acetyl-DL--tryptophan and notoginsenoside A, increased in As-treated roots and leaves. However, glutathione and inosine showed opposite accumulation levels in the roots and leaves of As-treated seedlings (Fig. S6, Tables S3 and S4). These results collectively indicate that Cd/As toxicity reprograms different metabolic profiles in the roots and leaves, suggesting a distinct mechanistic response to Cd/As stress in *P. notoginseng* roots and leaves.

Fe_3O_4 NPs show great potential in modulating plant growth and development under toxic metal(loid) stress (Zhang et al., 2021; Zhao et al., 2020). Fe_3O_4 NPs increased the levels of 46 metabolites but decreased the levels of 7 in the roots, while they increased the levels of 55 metabolites but decreased the levels of 35 in the leaves (Fig. 4, Tables S3 and S4). Two amino acids and their derivatives, N2-malonyl-D-tryptophan and N-acetyl-DL-tryptophan, are key to tryptophan metabolism. Tryptophan, an aromatic amino acid, is fundamental for maintaining cellular functions (Mondanelli et al., 2022). The increased levels of these metabolites will help plants ameliorate cell functions. Furthermore, Fe_3O_4 NPs increased indoleacrylic acid and inosine levels but decreased 4-formyl indole levels in the roots (Table S3). These changes in purine nucleosides and indoles and their derivatives levels affect plant growth and development. Phenylalanine is a key amino acid for phenylpropanoid metabolism, which is responsible for maintaining plant growth under stressful conditions (Dong and Lin, 2020). We found that Fe_3O_4 NPs increased prolylphenylalanine and alanyl-phenylalanine in the leaves (Table S4), which might enhance and change the phenylalanine metabolic process. These results suggested that Fe_3O_4 NPs altered distinct metabolic responses in th e roots and leaves, and some increased metabolites participating in key metabolic pathways enhanced plant growth and development under stressful conditions.

Our previous study showed that Fe_3O_4 NPs modulated the metabolic response in tobacco under Cd toxicity and several critical metabolites, including amino acids, flavonoids, alkaloids, lipids and carbohydrates responsible for plant growth (Zou et al., 2022). A recent study found that nanoparticles reduced lipid peroxidation and ROS accumulation, thus enhancing antioxidant capacity to improve alfalfa tolerance to Cd stress (Kareem et al., 2023). In this study, Cd stress altered 16 lipids in the roots compared with control conditions, and the contents of PA (18:4 (6Z,9Z,12Z,15Z)/0:0) and MG (0:0/18:3 (6Z,9Z,12Z)/0:0) were down-regulation but restored to normal level after spraying with Fe_3O_4 NPs (Table S7). In addition, Cd toxicity altered 22 lipids in the leaves compared with control conditions, and the contents of PA (18:2 (9Z,12Z)/18:3 (9Z,12Z,15Z)) and PC (16:0/18:2 (9Z,12Z)) were down-regulation but recovered to normal levels after spraying with

Fe_3O_4 NPs (Table S8). Primary metabolites are indispensable for plant growth, while secondary metabolites are nonessential but key participants in the stress response (Ahanger et al., 2020; Pichersky and Gang, 2000). This study found that Cd/As toxicity induced primary and secondary metabolism in the roots and leaves (Figs. 6 and 7, Tables S7-S10). Fe_3O_4 NPs recovered many Cd-induced metabolites, including 22 Cd-upregulated and 9 Cd-downregulated metabolites, in the Cd-treated roots to normal levels (Fig. 6A and B, Table S7). Furthermore, Fe_3O_4 NPs recovered 7 Cd-upregulated metabolites and 17 Cd-downregulated metabolites in the Cd-treated leaves to normal levels (Fig. 6C and D, Table S8). In particular, this study found that Cd altered 2 APAs (N-acetylproline and ceanothine C) and 9 terpenoids (including beta-Cadinene and ganodermatriol) in the roots compared with control conditions but recovered to normal levels after spraying with Fe_3O_4 NPs (Table S7). In addition, Cd altered 3 flavonoids (kanzonol O, quercetin, and quercetin 3-beta-laminaribioside) and a terpenoid (ganoderic acid F) in the leaves compared with control conditions but recovered to normal levels after spraying with Fe_3O_4 NPs (Table S8).

Similar effects of Fe_3O_4 NPs on As toxicity were observed. Fe_3O_4 NPs recovered 22 and 42 As-upregulated metabolites in the As-treated roots and leaves, respectively, to normal levels compared to the control (Fig. 7, Tables S9 and S10). Furthermore, Fe_3O_4 NPs recovered 13 and 33 As-downregulated metabolites in the As-treated roots and leaves, respectively, to normal levels compared to control (Fig. 7, Tables S9 and S10). Plants usually promote higher levels of secondary metabolites to enable their survival at the expanse of retarding plant growth when subjected to stress (Neilson et al., 2013; Siemens et al., 2002). These recovered primary metabolites, including APAs, carbohydrates and their conjugates, glycerophospholipids, and secondary metabolites, including flavonoids, alkaloids and terpenoids, will be favourable for seedling growth, which was consistent with our previous study (Zou et al., 2022). These results, together with reduced MDA content, proline content and antioxidant enzyme activity, strongly suggested that Fe_3O_4 NPs enabled *P. notoginseng* to suffer less toxicity induced by Cd/As stress, thus improving plant growth under stress conditions.

Cd and As are taken up and translocated by transporters competing with essential elements that share similar physicochemical properties (Cao et al., 2022; Zhao et al., 2021). In this study, Cd and As stress caused similar growth inhibition, as indicated by decreasing shoot and root FW (Fig. 1). We found that arsenic toxicity increased 31 and 44 metabolites but decreased 40 and 24 metabolites in the roots and leaves, respectively, compared with Cd stress (Tables S3 and S4). Most of the metabolites were unique in the roots and leaves. The levels of two metabolites (ginsenoside Rf and ginsenoside F5) decreased in the roots and leaves compared to the Cd treatment (Fig. S9, Tables S3 and S4).

The above results indicated that Fe_3O_4 NPs improved plant growth under Cd/As toxicity by modulating primary and secondary metabolites. This study found that arsenic increased 29 and 86 metabolites but decreased 38 and 53 in the roots and leaves after spraying with Fe_3O_4 NPs, compared to the Cd stress (Tables S3 and S4). APAs, carbohydrates and their conjugates were the most changed metabolites, indicating that Fe_3O_4 NPs acutely altered primary metabolites under Cd/As stress, thus affecting carbon/nitrogen metabolism. Lipids are pivotal components of cell membranes and their recognition and signal transduction of proteins (Hirano and Sato, 2019; Hou et al., 2015). We found that As toxicity increased the levels of 1 and 3 glycerophospholipids but decreased 4 and 7 glycerophospholipids in the roots and leaves, respectively, compared with Cd-treated seedlings subjected to Fe_3O_4 NPs (Tables S3 and S4). In addition to 4 flavonoids, the arsenic treatment also altered the levels of 4 alkaloids and their derivatives in the roots and/or leaves (Tables S3 and S4). Most metabolites were unique in the Cd/As-treated roots and leaves after foliar application of Fe_3O_4 NPs. Only ginsenoside Re decreased in both As-treated roots and leaves exposed to Fe_3O_4 NPs compared with Cd-treated seedlings subjected to Fe_3O_4 NPs (Fig. S10, Ta bles S3 and S4). These results showed that Fe_3O_4 NPs induced different accumulation levels of metabolites when subjected to Cd/As stress, indicating a distinct mechanism of the *P. notoginseng* response to Cd and As toxicity.

Terpenoids are active pharmaceutical ingredients with potential medicinal value in *P. notoginseng* (Guo et al., 2010; Qiao et al., 2018; Zhang et al., 2018). In this study, we found that Fe_3O_4 NPs increased 12 terpenoids in the roots, including 1 sesquiterpenoid, 2 diterpenoids and 7 triterpenoids (Fig. 9A, Table S3), while Fe_3O_4 NPs increased 3 terpenoids but decreased 4 in the leaves (Fig. 9B, Table S4). Terpenoid contents are also profoundly affected by heavymetal (loid)s (Liao et al., 2018). Cd stress increased 19 and 2 terpenoids but decreased 1 and 3 terpenoids in the roots and leaves, respectively. Furthermore, arsenic stress increased 8 and 6 terpenoids in the roots and leaves, whereas it decreased 3 terpenoids in the leaves (Fig. 9, Tables S3 and S4). Interestingly, our previous study showed that Fe_3O_4 NPs played pivotal roles in reprogramming secondary metabolites (Zou et al., 2022). We found that 3 monoterpenoids, 6 sesquiterpenoids, 3 diterpenoids, 18 triterpenoids and 1 tetraterpenoid were altered in the roots of seedlings subjected to Cd/As stress with or without Fe_3O_4 NPs (Fig. 9A, Table S3); while 1 monoterpenoid, 9 sesquiterpenoids, 4 diterpenoids and 11 triterpenoids were reprogrammed in the leaves of seedlings under Cd/As stress with or without exposure to Fe_3O_4 NPs (Fig. 9B, Table S2). Surprisingly, Fe_3O_4 NPs altered more terpenoids in the leaves of Cd-treated seedlings than Cd stress, which might be related to increased Cd accumulation in the leaves (Fig. 2B and 9B, Table S4). These results suggested that applying Fe_3O_4 NPs under Cd/As stress reprogrammed some common or distinct metabolites similar to stress conditions, and Fe_3O_4 NPs played roles in modulating active medicinal ingredients.

5. Conclusion

Combined with physiological and metabolomic analyses, we elucidated the mechanism of Fe_3O_4 NP-mediated plant growth and Cd/As responses in *P. notoginseng*. Cd/As treatment severely inhibited seedling growth, and Fe_3O_4 NPs ameliorated Cd/As-induced growth inhibition through regulating ion balance, antioxidant contents and metabolic profiling. Fe_3O_4 NPs altered essential metabolites significantly enriched in amino sugar and nucleotide sugar metabolism, flavonoid biosynthesis and phenylalanine metabolism, thus modulating the trade-off between plant growth and defense against metal toxicity. More significantly, Fe_3O_4 NPs recovered many Cd/As-induced DAMs to normal levels, further supporting that Fe_3O_4 NPs were beneficial for seedling growth under stress. In addition, terpenoids were notably induced when subjected to Cd/As stress, and Fe_3O_4 NPs altered these key metabolites, thus affecting potential medicinal value. This study provides an alternative strategy using nanoparticles to maintain medicinal plants' growth and improve their quality in toxic metal(loid)-contaminated areas. The molecular mechanism of NPs involved in the response of *P. notoginseng* and other medicinal plants to Cd/As stress or combined toxic metal(loid) mixtures is worthy of further exploration.

CRediT authorship contribution statement

Tianquan Lu: Data curation, Visualization. **Xiaoning Wang:** Methodology, Writing – review & editing. **Xianliang Cui:** Formal analysis. **Jifang Li:** Investigation. **Jin Xu:** Conceptualization. **Peng Xu:** Supervision. **Jinpeng Wan:** Conceptualization, Supervision, Writing – original draft, 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.

Acknowledgements

This work was supported by the China National Natural Sciences Foundation (31902110), the Youth Innovation Promotion Association CAS (2020390), the CAS “Light of West China” Program, and the Science and Technology Projects of Yunnan Province, China (202003AD150007). We thank Institutional Center for Shared Technologies and Facilities of Xishuangbanna Tropical Botanical Garden, CAS, for providing research facilities. We also thank Majorbio and Biotree for metabolome detection and analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2023.122578.

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