

Differential physiological responses and tolerance to potentially toxic elements in *Primula forbesii* Franch.

Yin Jia (✉ jiayin_cn@163.com)

Sichuan Agricultural University <https://orcid.org/0000-0003-0044-2696>

Xiancai Yin

Sichuan Agricultural University

Jian Zhao

Sichuan Agricultural University

Yuanzhi Pan

Sichuan Agricultural University

Beibei Jiang

Sichuan Agricultural University

Qinglin Liu

Sichuan Agricultural University

Yifeng Li

Sichuan Agricultural University

Research Article

Keywords: *Primula forbesii*, aromatic plants, abiotic stress, heavy metal, hydroponic systems, physiological responses and tolerance, antioxidant system, ecological remediation

Posted Date: May 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1580150/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on April 27th, 2023. See the published version at <https://doi.org/10.1007/s11356-023-27259-9>.

Abstract

Soil pollution caused by the accumulation of potentially toxic elements (PTEs) has become a serious problem due to industrial and urban pollution as well as pesticide use. *Primula forbesii* Franch. is a biennial flower native to China with excellent stress resistance and ornamental value. In this study, we examined the phenotypic traits, growth indexes, and physiological properties of *P. forbesii* in response to five representative PTEs (Cd, Ni, Cr, Cu, and Zn) under hydroponic culture conditions. High concentrations of Zn and Cr had little effect on the growth and physiological properties of *P. forbesii*, indicating that the species has strong tolerance to Zn and Cr stress. Alternatively, high concentrations of Cd, Ni, and Cu seriously affected plant growth and development, resulting in leaf chlorosis and even death, and therefore may have a serious negative impact on the growth of *P. forbesii*. However, activity levels of some antioxidant enzymes and osmotic regulatory substances remained high, indicating that *P. forbesii* resisted heavy metal stress by regulating physiological and biochemical metabolism to a certain extent. Furthermore, principal component analysis and membership function were used to comprehensively evaluate *P. forbesii* resistance to PTEs. These analyses revealed that *P. forbesii* exhibits distinct sensitivities and physiological responses to different PTEs and suggested that the resistance to five PTEs in decreasing order is $\text{Zn} > \text{Cr} > \text{Cd} > \text{Cu} > \text{Ni}$. These results provide a theoretical basis for the future application of *P. forbesii* in environments with heavy metal pollution and may expand its practical utilization.

Introduction

Pollution by potentially toxic elements (PTEs) refers to the phenomenon in which the contents of metal elements in the soil exceed background values as a result of human activities, such as mining, industrial discharge, and overuse of chemical agents in agricultural production. This pollution is widespread and detrimental, seriously threatening sustainable ecological development as well as human health through natural biogeochemical cycles. Cadmium (Cd), zinc (Zn), copper (Cu), chromium (Cr), and nickel (Ni) are common, representative PTEs presenting ecological risks in cities. Although some of these PTEs, such as Cu, Zn, Cr, and Ni, are necessary for normal plant growth and development, excessive accumulation has toxic effects (Ghori et al. 2019). Other PTEs, such as Cd, are not regarded as nutrients and pose a threat to plant growth after safe values are exceeded (Shanying et al. 2017). Plants have evolved complex mechanisms for detoxification and protection in response to PTE stress. Plant resistance to PTEs mainly includes two strategies: avoidance and tolerance mechanisms (Toppi and Gabbrielli, 1999). The first strategy is to avoid PTE accumulation in sensitive tissues, reduce PTE absorption through the excretion of organic acids and carbohydrates, and accumulate excessive PTEs in roots, exoplasm, and vacuoles (Meharg, 1993). To protect against oxidative damage caused by PTEs, the second strategy is to promote the chelation of plant chelates (Pcs), metallothioneins, organic acids, and proteins with PTEs in plants and activate ion transporters to remove excess PTE ions from the protoplasm, in addition to activating and enhancing the activity of antioxidant systems to remove metal-induced reactive oxygen species (ROS) (Hall et al. 2002; Jozefczak et al. 2014; Gillet et al. 2010). Recent research on PTEs has mainly focused on crops and hyperaccumulators. In the practical application of metal-resistant plants, ornamental plants have lower biosecurity risks than those of crops and better landscape effects than those of most hyperaccumulators, with benefits related to

beautification, protection, and restoration of the environment. However, studies of the resistance and repair of ornamental plants under heavy metal stress are scarce.

Primula is a well-known ornamental flower worldwide. There are more than 500 species in the world, mostly distributed in the moist and cool regions of the northern hemisphere (Richards, 2003). China is a major center of *Primula* biodiversity with approximately 300 species (Hu, 1990). *Primula forbesii* Franch. is a biennial flower abundant in many locales across Sichuan and Yunnan Provinces in China; it was introduced to Europe by the seed company Vilmorin in 1891 (Richards, 2003). The species exhibits desirable horticultural traits, such as a long flowering season, plentiful flowers, and a pleasant fragrance (Jia, 2014). In addition, it has excellent resistance to environmental stresses. Studies have shown that *P. forbesii* has a high tolerance to salt and cadmium stress in the soil, although it has not been regarded as hyperaccumulator of PTEs (Jia, 2020a; Jia 2020b). Hydroponic culture enables direct measurements of plant physiological parameters and metabolism in a time-dependent manner, and this method is widely used to determine the phytotoxic effects of PTEs (Kopittke, 2010).

In this study, taking *P. forbesii* as the research object, the effects of various concentrations of Cd, Ni, Cr, Cu, and Zn on seedlings under hydroponic conditions were evaluated. Additionally, we measured growth and physiological indexes as well as heavy metal contents in the above-ground and underground parts of the plants. A principal component analysis (PCA) and membership function were used to analyze the resistance of *P. forbesii* to Cd, Ni, Cr, Cu, and Zn stress. This study provides a theoretical basis for the future application of *P. forbesii* in areas with heavy metal pollution and has the potential to expand the practical applications of the species.

Materials And Methods

Plant material and cultivation conditions

P. forbesii seeds were collected from plants grown in the Sichuan Agricultural University greenhouse. The seeds were sown in June 2019, grown in pots in a controlled-environment growth chamber programmed for 16/8 h at 20°C/15°C day/night. After 60 days, seedlings with 5–6 true leaves were transferred to a hydroponic container of 95 mm in diameter and 140 mm in height, which was filled with 600 mL of 1/8 modified Hoagland nutrient solution (147.594 mg/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 63.194 mg/L KNO_3 , 17.011 mg/L KH_2PO_4 , 6.161 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.040 mg/L $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.012 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.029 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.009 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.695 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 9.356 mg/L EDTA-Na_2 , 3.478 mg/L H_3BO_3). The pH of the solution was adjusted to 5.8 ± 0.1 with NaOH as required. Each container was used for one plant and the outer surface was wrapped with tinfoil to shade the roots. Hydroponic culture was carried out in a culture room set at 20°C, 16/8 h light/dark cycle, and a light intensity of 4000 Lux. The hydroponic solution was changed every 7 days. After 2 weeks of growth, healthy and uniform seedlings were selected and transplanted to the hydroponic solution, which was replaced with new 1/8 modified Hoagland nutrient solution containing either $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 2\text{H}_2\text{O}$, or $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at concentrations of 10, 50, 100, or 200 $\mu\text{mol/L}$ for each PTE. The PTE-containing hydroponic solutions were

changed at 7-day intervals up to day 21. There were 12 seedlings per treatment and three biological replicates. Plants grown in hydroponic solution without the addition of PTEs were used as controls.

Morphological observation and measurements

Morphological changes of plants were observed under different PTEs every day. After cultivation for 21 days, images were obtained to record the plant morphology. Plant height, crown width, and root length were measured using Vernier calipers. The fresh weights (FW) of the above-ground parts and roots were determined using an electronic balance. Then, the dry weight (DW) of the above-ground parts and roots was determined after samples were dried in a vacuum oven at 105°C for 30 min and at 80°C to a constant weight.

Chlorophyll content

Chlorophyll (Chl) contents were extracted from 0.2 g of fresh leaf samples in the dark for 24 h, following the method of Wellburn and Lichtenthaler (1984). Pigment was extracted in a mixed solution of acetone: ethanol = 2:1 (V:V) and centrifuged at 4000 rpm for 10 min. The absorbance of the extracts was measured at wavelengths of 663 and 645 nm for Chlorophyll a (Chl a) and Chlorophyll b (Chl b), respectively, by using a UV/visible spectrophotometer (BioMate 3S; Thermo, Waltham, MA, USA). According to the equation $\text{Chl (mg/L)} = (20.2 \times OD_{645} + 8.02 \times OD_{663}) \times (V/W \times 1000)$, the total chlorophyll (Chl T) contents were calculated. The ratios of Chl a to Chl b were calculated.

Detection of soluble sugar and soluble protein

Anthrone colorimetry was adopted to determine the content of soluble sugar (SS) (Li, 2002). The soluble protein (SP) content was determined according to the method described by Bradford (1976), using bovine serum albumin (BSA) as the standard protein and Coomassie brilliant blue G-520 as the staining dye.

Detection of malondialdehyde and hydrogen peroxide contents

The methods of Heath and Packer (1968) were followed to estimate malondialdehyde (MDA) contents. The hydrogen peroxide (H₂O₂) contents were evaluated by previously established procedures (Wang et al. 2013).

Assays of antioxidant enzyme activity

Fresh leaf samples (0.2 g) were ground in a precooled mortar and pestle. Then, the samples were homogenized by using phosphate buffer (0.05 mol/L, pH 7.8). The homogenate was centrifuged at 10000 rpm for 20 min at 4°C and the supernatant was collected. Levels of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbic acid (ASA), and glutathione (GSH), were evaluated by the methods of Hess and Foster (1976), Shah (2001), Aebi (1984), Law (1983), and Yang et al. (2018).

Measurements of mineral nutrient accumulation and PTEs

Plants were carefully separated from containers and rinsed with deionized water 5–6 times before element content analyses. The fresh leaves and roots were dried in a vacuum oven at 110°C for 30 min and at 80°C to a constant weight, ground into a fine powder, and sieved through a 2 mm nylon mesh screen. The samples

(0.2 g) were kept overnight in a mixture of concentrated nitric acid (HNO₃) and perchloric acid (HClO₄) (V:V = 4:1). Afterwards, the digestion process was completed on hot plate until a clear solution was obtained. The solution was allowed to cool to room temperature and then increased to 50 mL with ultrapure water. Contents of mineral nutrients (Kalium, K; Calcium, Ca; Magnesium, Mg; Manganese, Mn) and PTEs (Zn, Cr, Cd, Cu, and Ni) in the decomposed samples were measured by inductively coupled plasma atomic emission spectroscopy ICP-MS (Agilent 7700; Agilent Technologies, Santa Clara, CA, USA).

Statistical analysis

All statistical analyses were performed using SPSS 19.0 for Windows. Data are expressed as the mean $\pm$ standard deviation (S.D.). Least Significant Difference (LSD) tests were used to determine whether differences between treatments were significant ($P < 0.05$). Finally, a combination of a principal component analysis (PCA) and membership function (MF) method was used for a comprehensive evaluation of the effects of five PTEs on *P. forbesii*.

Results

Plant growth performance

During the first 7 days of PTE hydroponic stress, the phenotypes of *P. forbesii* plants were essentially similar to those of the control plants (Fig. 1). After 11 days of treatment, groups treated with high concentrations of Cu, Cd, and Ni (200 $\mu\text{mol/L}$) began to show symptoms of toxicity, e.g., the leaves turned yellow and wilted slightly; however, the symptoms in the Cu treatment group were mild. It took 16 days for groups treated with high concentrations of Cr and Zn (200 $\mu\text{mol/L}$) to show mild symptoms of toxicity. After 21 days of stress, the toxicity symptoms were severe under the 100–200 $\mu\text{mol/L}$ Ni treatment; a large number of leaves were withered and the whole plants became soft and died. In general, the lowest concentrations (10 $\mu\text{mol/L}$) of PTEs had no significant effect on the growth of seedlings of *P. forbesii*, while at the highest metal concentration of 200 $\mu\text{mol/L}$, plant growth was strongly inhibited, especially in plants treated with Ni, followed by Cd and Cu, with weaker effects of Cr and Zn stress.

After 21 days of exposure to a medium dose of PTEs, various plant properties were measured, i.e., height, crown width, root length, fresh and dry biomass production of shoots and roots (Table 1). At a PTE concentration of 10 $\mu\text{mol/L}$, the plant height and crown width after treatment with each of the five metals were not significantly different from those in the control, consistent with the observed phenotypic characteristics. Most of the PTEs began to reduce the plant height and crown width significantly when the concentration was 50–100 $\mu\text{mol/L}$, except for Cr at 50 $\mu\text{mol/L}$, which increased the plant height and crown width. Similar results were obtained for root length. Lower concentrations of Cr (10 $\mu\text{mol/L}$) and Zn (50 $\mu\text{mol/L}$) promoted root elongation. Except 10 $\mu\text{mol/L}$ Zn, most other concentrations of the PTEs led to remarkable decreases in dry and fresh weights of both aboveground and underground parts. In particular, the dry weights of plants treated with 200 $\mu\text{mol/L}$ PTEs decreased significantly by 55–70% as compared with those of the control.

Table 1

Growth parameters of *P. forbesii* after treatment with different concentrations of heavy metal. Values are means $\pm$ S.D. Lowercase letters (a, b, c, d, e) indicate significant differences at the $P < 0.05$ level between the same heavy metals under different concentrations according to LSD test.

Treatment	Crown width (cm)	Plant height (cm)	Fresh weight aboveground (g)	Dried weight Aboveground (g)	Root length (cm)	Fresh weight of underground (g)	Dried weight of Underground (g)
CK	8.53 $\pm$ 0.47 ^a	4.50 $\pm$ 0.26 ^a	2.58 $\pm$ 0.05 ^a	0.27 $\pm$ 0.03 ^a	6.80 $\pm$ 0.36 ^a	0.45 $\pm$ 0.02 ^a	0.0479 $\pm$ 0.0064 ^a
Ni 10	8.50 $\pm$ 0.17 ^a	4.53 $\pm$ 0.06 ^a	2.03 $\pm$ 0.19 ^b	0.22 $\pm$ 0.01 ^b	6.70 $\pm$ 0.53 ^a	0.27 $\pm$ 0.02 ^b	0.0264 $\pm$ 0.0015 ^b
Ni 50	7.00 $\pm$ 0.46 ^b	3.57 $\pm$ 0.29 ^b	1.50 $\pm$ 0.17 ^c	0.18 $\pm$ 0.02 ^{bc}	5.57 $\pm$ 0.35 ^b	0.21 $\pm$ 0.04 ^c	0.0197 $\pm$ 0.0033 ^{bc}
Ni 100	6.97 $\pm$ 0.59 ^b	2.60 $\pm$ 0.44 ^c	0.90 $\pm$ 0.06 ^d	0.20 $\pm$ 0.03 ^{bc}	5.30 $\pm$ 0.30 ^b	0.19 $\pm$ 0.02 ^{cd}	0.0203 $\pm$ 0.0026 ^{bc}
Ni 200	5.83 $\pm$ 0.32 ^c	2.40 $\pm$ 0.36 ^c	0.45 $\pm$ 0.14 ^e	0.16 $\pm$ 0.05 ^c	5.20 $\pm$ 0.10 ^c	0.14 $\pm$ 0.02 ^d	0.0146 $\pm$ 0.0012 ^c
CK	8.53 $\pm$ 0.47 ^a	4.50 $\pm$ 0.26 ^a	2.58 $\pm$ 0.05 ^a	0.27 $\pm$ 0.03 ^a	6.80 $\pm$ 0.36 ^a	0.45 $\pm$ 0.02 ^a	0.0479 $\pm$ 0.0064 ^a
Cd 10	8.73 $\pm$ 0.25 ^a	4.07 $\pm$ 0.74 ^{ab}	2.15 $\pm$ 0.17 ^b	0.21 $\pm$ 0.02 ^b	6.87 $\pm$ 0.21 ^a	0.22 $\pm$ 0.01 ^b	0.0253 $\pm$ 0.003 ^b
Cd 50	8.10 $\pm$ 0.53 ^a	3.57 $\pm$ 0.50 ^{bc}	1.32 $\pm$ 0.06 ^c	0.18 $\pm$ 0.03 ^{bc}	5.83 $\pm$ 0.42 ^b	0.19 $\pm$ 0.01 ^c	0.0191 $\pm$ 0.0004 ^c
Cd 100	6.80 $\pm$ 0.30 ^b	3.27 $\pm$ 0.21 ^{bc}	0.98 $\pm$ 0.09 ^d	0.13 $\pm$ 0.00 ^{cd}	6.20 $\pm$ 0.10 ^{bc}	0.16 $\pm$ 0.01 ^d	0.017 $\pm$ 0.0009 ^c
Cd 200	5.83 $\pm$ 0.46 ^c	2.67 $\pm$ 0.51 ^c	0.87 $\pm$ 0.06 ^d	0.13 $\pm$ 0.04 ^d	4.70 $\pm$ 0.53 ^c	0.14 $\pm$ 0.00 ^d	0.016 $\pm$ 0.0007 ^c
CK	8.53 $\pm$ 0.47 ^a	4.50 $\pm$ 0.26 ^a	2.58 $\pm$ 0.05 ^a	0.27 $\pm$ 0.03 ^a	6.80 $\pm$ 0.36 ^a	0.45 $\pm$ 0.02 ^a	0.0479 $\pm$ 0.0064 ^a

Treatment	Crown width (cm)	Plant height (cm)	Fresh weight aboveground (g)	Dried weight Aboveground (g)	Root length (cm)	Fresh weight of underground (g)	Dried weight of Underground (g)
Cu 10	7.63 ± 0.61 ^{ab}	4.53 ± 0.25 ^a	2.06 ± 0.23 ^b	0.22 ± 0.00 ^b	6.83 ± 0.25 ^a	0.30 ± 0.02 ^b	0.0279 ± 0.0009 ^b
Cu 50	6.73 ± 0.90 ^{bc}	3.43 ± 0.25 ^b	1.63 ± 0.15 ^c	0.16 ± 0.03 ^c	5.20 ± 0.87 ^b	0.21 ± 0.01 ^c	0.0209 ± 0.0017 ^c
Cu 100	6.70 ± 0.35 ^{bc}	3.27 ± 0.21 ^{bc}	1.03 ± 0.06 ^d	0.14 ± 0.01 ^{cd}	5.17 ± 0.12 ^b	0.18 ± 0.01 ^d	0.0162 ± 0.0015 ^c
Cu 200	5.83 ± 0.58 ^c	2.83 ± 0.35 ^c	0.93 ± 0.06 ^d	0.11 ± 0.03 ^d	4.27 ± 0.80 ^b	0.12 ± 0.01 ^e	0.0149 ± 0.0025 ^c
CK	8.53 ± 0.47 ^{bc}	4.50 ± 0.26 ^b	2.58 ± 0.05 ^a	0.27 ± 0.03 ^a	6.80 ± 0.36 ^{bc}	0.45 ± 0.02 ^a	0.0479 ± 0.0064 ^a
Cr 10	9.33 ± 0.76 ^{ab}	4.77 ± 0.60 ^b	2.29 ± 0.16 ^b	0.23 ± 0.01 ^b	7.83 ± 0.50 ^a	0.42 ± 0.04 ^{ab}	0.0401 ± 0.0017 ^b
Cr 50	10.43 ± 0.75 ^a	5.77 ± 0.31 ^a	1.88 ± 0.07 ^c	0.17 ± 0.00 ^c	6.97 ± 0.21 ^b	0.38 ± 0.01 ^b	0.0359 ± 0.0024 ^b
Cr 100	8.17 ± 0.90 ^{bc}	4.23 ± 0.21 ^b	1.67 ± 0.09 ^d	0.16 ± 0.00 ^c	6.60 ± 0.26 ^{bc}	0.28 ± 0.01 ^c	0.0217 ± 0.0003 ^c
Cr 200	7.8 ± 0.66 ^c	3.60 ± 0.17 ^c	1.54 ± 0.01 ^d	0.16 ± .03 ^c	6.20 ± 0.26 ^c	0.21 ± 0.01 ^d	0.0207 ± 0.0037 ^c
CK	8.53 ± 0.47 ^a	4.50 ± 0.26 ^{ab}	2.58 ± 0.05 ^a	0.27 ± 0.03 ^a	6.80 ± 0.36 ^b	0.45 ± 0.02 ^a	0.0479 ± 0.0064 ^a
Zn 10	8.40 ± 0.26 ^{ab}	4.40 ± 0.26 ^b	2.51 ± 0.24 ^a	0.27 ± 0.02 ^a	7.47 ± 0.93 ^b	0.40 ± 0.08 ^a	0.042 ± 0.0067 ^a
Zn 50	9.00 ± 0.87 ^a	5.20 ± 0.61 ^a	2.06 ± 0.19 ^b	0.20 ± 0.02 ^b	9.53 ± 0.84 ^a	0.28 ± 0.01 ^b	0.0308 ± 0.0019 ^b

Treatment	Crown width (cm)	Plant height (cm)	Fresh weight aboveground (g)	Dried weight Aboveground (g)	Root length (cm)	Fresh weight of underground (g)	Dried weight of Underground (g)
Zn 100	7.53 ± 0.38 ^{bc}	4.03 ± 0.21 ^b	1.68 ± 0.02 ^c	0.18 ± 0.00 ^{bc}	7.57 ± 0.32 ^b	0.24 ± 0.02 ^{bc}	0.0265 ± 0.0031 ^{be}
Zn 200	7.30 ± 0.26 ^c	4.03 ± 0.51 ^b	1.60 ± 0.07 ^c	0.14 ± 0.02 ^c	6.63 ± 0.23 ^b	0.17 ± 0.01 ^c	0.0196 ± 0.0016 ^c

Chlorophyll content

As shown in Fig. 2, as the concentrations of Cd, Ni, and Cu increased, Chl a and ChlT contents showed similar patterns characterized by an initial increase and subsequent decrease. Under Cd exposure, the content of Chl a reached the maximum value of 1.8410 mg/g at a concentration of 50 µmol/L. In Ni solutions, the contents of Chl a and ChlT were highest when the concentration was 10 µmol/L and were 12.51% and 5.59% higher than those of the control, respectively. Alternatively, under treatment with Cr and Zn solutions, the contents of Chl a and ChlT decreased gradually as the concentration increased. When the concentrations of Cr and Zn reached 200 µmol/L, the contents of Chl a and ChlT were the lowest. Compared with those in the control, the contents of Chl a and ChlT under Cr treatment were 38.81% and 33.70% lower, respectively, while those under Zn treatment were 38.96% and 33.21% lower, respectively.

Chl b showed a gradual increase as the Cd concentration increased. Conversely, under treatment with Ni, Cr, and Zn solutions, the content of Chl b decreased gradually as the solution concentration increased. With the increase in the Cu solution concentration, the content of Chl b increased initially and then decreased. As the concentrations of Cd, Cu, and Zn increased, the Chl a/b ratio in *P. forbesii* decreased gradually. When the stress concentration reached the maximum value of 200 µmol/L, Chl a/b decreased to the lowest value (i.e., it decreased by 32.62%, 23.79%, and 20.80% compared with that of the control, respectively). Under treatment with Ni and Cr solutions, Chl a/b increased initially and then decreased as the concentration increased. Based on the observed changes in the Chl content, responses to different heavy metals under the same concentration differed significantly.

Changes in plant osmotic regulation

As shown in Fig. 3, the SS content gradually increased as the Cd concentration increased and reached the maximum value when the concentration of Cd reached 200 µmol/L, which increased by 98.45% compared with that of the control group. In addition, with increases in the Ni, Cr, and Zn concentrations, the SS content of *P. forbesii* increased initially and then decreased. Under treatment with the Cu solution, the SS content of *P. forbesii* decreased first and then increased as the Cu concentration increased, and the differences were significant. When the Cu concentration was between 10 and 50 µmol/L, the SS content was significantly lower than that of the control. When the Cu concentration was 200 µmol/L, the SS content was 73.26% higher than that of the control group.

Under treatments with various concentrations of Cd, Cu, and Zn, the SP content tended to increase initially and then decrease as the metal concentrations increased. The increase in the SP content under Cd was significantly higher than those under Cu and Zn, and all SP contents reached the maximum value when the stress concentration was 10 $\mu\text{mol/L}$. Compared with those in the control group, SP contents increased by 30.16%, 13.27%, and 14.65%, respectively. Under Ni treatment, the SP content of *P. forbesii* decreased initially and then increased as the Ni concentration increased; the SP content of *P. forbesii* increased as the Cr concentration increased. When the Cr concentration was 200 $\mu\text{mol/L}$, the SP content was highest, i.e., 38.41% higher than that of the control.

Determination of membrane lipid peroxidation

As shown in Fig. 4, under treatment with Cd, Ni, Cr, and Zn solutions, the MDA content in *P. forbesii* leaves decreased initially and then increased as the concentrations increased. The MDA content reached a maximum value at 200 $\mu\text{mol/L}$. Of note, under treatment with Cd and Ni solutions, the final MDA content was significantly higher than that of the control (i.e., 51.42% and 64.47% higher, respectively). Under treatment with Cr and Zn solutions, the MDA content under the highest concentration in *P. forbesii* did not differ significantly from that of the control. In addition, as the Cu concentration increased, the MDA content showed a continuous upward trend and was significantly higher than that of the control at all concentrations.

With the gradual increase of the concentration of the five PTEs, the H_2O_2 content generally showed a continuous upward trend. However, under treatment with Cd, Cr, Cu, and Zn solutions, when the concentration was lowest (10 $\mu\text{mol/L}$), the H_2O_2 content was significantly higher than that of the control, whereas there was no significant difference among metals. When the stress concentration was higher than 10 $\mu\text{mol/L}$, the increase in the H_2O_2 content slowed. However, under Ni treatment, when the stress concentration was between 0 and 50 $\mu\text{mol/L}$, the H_2O_2 content increased slightly. When the stress concentrations were ≥ 100 $\mu\text{mol/L}$, the H_2O_2 content increased significantly and reached the highest value when the Ni concentration was 200 $\mu\text{mol/L}$ (i.e., 98.70% higher than that of the control). The H_2O_2 contents under Cd and Ni treatments (200 $\mu\text{mol/L}$) were significantly higher than those under Cr, Cu, and Zn treatments.

Determination of antioxidant system indicators

As summarized in Fig. 5, as the concentrations of Cd, Ni, Cr, and Cu increased, the SOD activity in *P. forbesii* showed an initial increase, followed by a decrease. The peak value was reached at concentrations of 100 $\mu\text{mol/L}$, 100 $\mu\text{mol/L}$, 50 $\mu\text{mol/L}$, and 10 $\mu\text{mol/L}$, respectively, and these values were significantly higher than those of the control.

Furthermore, the POD activity increased with as the concentrations of Cd and Cu increased and reached the peak values at 200 $\mu\text{mol/L}$. The peak value under Cu treatment was significantly higher than that under Cd treatment. In addition, similar POD activities were observed with the increases in Ni, Cr, and Zn concentrations, with an initial increase and subsequent decrease, and the increases in POD activity under Ni treatment were significantly higher than those for Cr and Zn.

As the Cd, Cu, and Zn concentrations increased, the CAT activity in leaves decreased initially and then increased. When the Cd concentration was 50 $\mu\text{mol/L}$, CAT activity was the highest and was significantly

higher than that of the control. While when Cu and Zn concentrations reached 200 $\mu\text{mol/L}$, CAT activities reached peak levels; however, only 200 $\mu\text{mol/L}$ Cu resulted in significantly higher CAT activity than that of the control.

With increases in Cd and Cu concentrations, the ASA content of the plant showed a gradual upward trend. Under the same concentration, there was no significant difference in ASA contents between Cd and Cu treatments. When the concentration was 200 $\mu\text{mol/L}$, the ASA content reached a peak, which increased by 47.96% and 42.91% compared with that of the control, respectively. Furthermore, the ASA contents of the leaves increased initially and then decreased as the Ni, Cr, and Zn concentrations increased. ASA contents reached the maximum value at 50 $\mu\text{mol/L}$ under both Ni and Cr treatments and at 100 $\mu\text{mol/L}$ for Zn; these maximum values were significantly different from those in the control.

With the continuous increases in Cd, Ni, and Cr concentrations, the GSH contents gradually increased and reached peak values at the highest concentration (200 $\mu\text{mol/L}$), which were significantly higher than those in the control. In addition, the GSH contents in leaves first increased and then decreased with increases in Cu and Zn concentrations. When the Cu concentration was 50 $\mu\text{mol/L}$, the GSH content reached peak values, which were 2.5 times higher than that in the control. When the Zn concentration was 100 $\mu\text{mol/L}$, the GSH content was the highest, (a 1-fold increase over that in the control).

Accumulation of heavy metals and metabolism of essential minerals

The PTE concentrations in shoots and roots of *P. forbesii* were measured (Fig. 6). PTE uptake in both parts increased due to PTE stress. The maximum uptake of Cd, Ni, and Cu in roots was observed at 200 $\mu\text{mol/L}$, whereas the peak uptake of Cr and Zn in roots was observed at 100 $\mu\text{mol/L}$, which then decreased significantly at higher concentrations. The accumulation of PTEs in the shoots under Cd, Ni, Cr, and Zn showed similar trends, characterized by a gradual increase with peak values at 200 $\mu\text{mol/L}$ (100 times, 50 times, 2 times, and 2.4 times higher than those of the control, respectively).

The influence of PTE exposure on the shoot and root contents of representative essential mineral nutrients, i.e., K, Ca, Mg, and Mn, were analyzed at a PTE concentration of 10 $\mu\text{mol/L}$ in hydroponic solution. Although the concentrations of these essential mineral nutrients did not change significantly under many conditions, a 13.21% decrease in the shoot Mg content and a 25.27% increase in the root Ca content were observed in Ni-treated plants. Both decreases and increases in nutrients in shoots and roots were observed in some cases. A decrease in the shoot K content was observed in Ni-, Cr-, and Cu-treated plants. Cd and Cu in roots significantly reduced the plant uptake of Mg, K, and Mn. Ca and Mn contents in the shoots increased in Ni- and Zn-treated plants.

Comprehensive evaluation of resistance to five PTEs in plants

Principal component analysis

As shown in Table 2, there were strong correlations between indicators. The role of each indicator in the protection of *P. forbesii* against heavy metal stress differed, and evaluations of a single indicator are

insufficient. Therefore, a PCA was used to comprehensively evaluate resistance to five PTEs in *P. forbesii*.

Table 2
Correlation coefficient matrix of various physiological indexes of *P. forbesii* under different heavy metal concentrations

Index	SS	SP	MDA	H ₂ O ₂	SOD	POD	CAT	ASA	GSH	ChIT
SS	1									
SP	-0.885	1								
MDA	0.349	-0.464	1							
H ₂ O ₂	0.172	-0.3	0.103	1						
SOD	-0.663	0.563	-0.288	-0.549	1					
POD	0.643	-0.692	0.868	-0.124	-0.308	1				
CAT	0.157	-0.389	0.68	-0.326	0.227	0.741	1			
ASA	0.944	-0.801	0.46	0.111	-0.679	0.682	0.213	1		
GSH	-0.075	0.151	-0.225	0.786	-0.456	-0.497	-0.755	-0.085	1	
ChIT	0.312	-0.217	0.256	0.794	-0.719	0.028	-0.437	0.355	0.839	1

The PCA of various resistance indexes under heavy metal stress, summarized in Table 3, revealed that the cumulative contribution of the first three principal components to the total variance was 91.765%, indicating that the three components represent most of variance in the resistance of *P. forbesii* to PTE stress. The eigenvalue of the first principal component was 4.536 and the proportion of variance explained was 45.364%. The main factors contributing to the first principal component were SS, SP, SOD, POD, and ASA. The eigenvalue of the second principal component was 3.553, and the proportion of variance explained was 35.525%. The main contributing factors were H₂O₂, CAT, GSH, and ChIT. The eigenvalue of the third principal component was 1.088, and the proportion of variance explained was 10.876%. The main contributing factor was MDA. According to Table 4, the corresponding feature vector expressions were as follows (X_1-X_{10} represent SS, SP, MDA, H₂O₂, SOD, POD, CAT, ASA, GSH, and ChIT, respectively):

Table 3
Principal component loading matrix and variance explained

Index	1	2	3
SS	0.891	0.042	-0.425
SP	-0.894	0.06	0.192
MDA	0.704	-0.291	0.614
H ₂ O ₂	0.296	0.79	0.329
SOD	-0.713	-0.559	0.121
POD	0.83	-0.487	0.194
CAT	0.389	-0.806	0.357
ASA	0.907	0.013	-0.328
GSH	-0.064	0.976	0.16
ChIT	0.43	0.829	0.269
Characteristic value	4.536	3.553	1.088
Contribution rate	45.364	35.525	10.876
Cumulative contribution rate	45.364	80.889	91.765

Table 4
Eigenvectors of each comprehensive index

Index	AF1	AF2	AF3
SS	0.418	0.022	-0.407
SP	-0.42	0.032	0.184
MDA	0.331	-0.154	0.589
H ₂ O ₂	0.139	0.419	0.315
SOD	-0.335	-0.297	0.116
POD	0.39	-0.258	0.186
CAT	0.183	-0.428	0.342
ASA	0.426	0.007	-0.314
GSH	-0.03	0.518	0.153
ChIT	0.202	0.440	0.258

$$F1 = 0.418X_1 - 0.420X_2 + 0.331X_3 + 0.139X_4 - 0.335X_5 + 0.390X_6 + 0.183X_7 + 0.426X_8 - 0.030X_9 + 0.202X_{10};$$

$$F2 = 0.022X_1 + 0.032X_2 - 0.154X_3 + 0.419X_4 - 0.297X_5 - 0.258X_6 - 0.428X_7 + 0.007X_8 + 0.519X_9 + 0.440X_{10};$$

$$F3 = -0.407X_1 + 0.184X_2 + 0.589X_3 + 0.315X_4 + 0.116X_5 + 0.186X_6 + 0.342X_7 - 0.314X_8 + 0.153X_9 + 0.258X_{10};$$

$F = 0.494F1 + 0.387F2 + 0.119F3$ (0.494, 0.387, and 0.119 represent the ratio of the contribution of each comprehensive indicator to the total variance).

Therefore, each physiological index under heavy metal stress was combined with the loading value of the principal component for each physiological index, and the linear equation was used to obtain the score values of the three principal components. The higher the score value, the better the resistance of *P. forbesii* to the heavy metal in this comprehensive index. As shown in Table 5, the resistance of *P. forbesii* to Cr and Zn was explained by the first principal component; the resistance of *P. forbesii* to Cd and Zn was explained by the second principal component. The resistance of *P. forbesii* to Cr and Cd was explained by the third principal component. According to the comprehensive scores, the resistance of *P. forbesii* to five PTEs, from strongest to weakest, was Zn, Cr, Cd, Cu, and Ni.

Table 5
Results of a principal component analysis of indexes of *P. forbesii* under different heavy metal stress

PTE	F1	F2	F3	F
Cr	7.767	-7.306	1.829	1.227
Cd	-5.045	3.404	4.89	-0.596
Ni	-6.837	-0.942	-2.225	-4.008
Cu	-2.67	-3.478	-2.681	-2.984
Zn	6.786	8.321	-1.813	6.361

Subordinate function evaluation

Based on the indexes obtained by PCA, the heavy metal resistance of *P. forbesii* was evaluated by the membership function method (Table 6). The membership function value (U) was obtained by numerical transformation using the membership function algorithm based on the principal component value, and its comprehensive evaluation value (D value) was calculated. A higher D value indicated stronger resistance of *P. forbesii* to the metal. The D values for each PTE in order from high to low were Zn, Cr, Cd, Cu, and Ni, consistent with the results of the PCA.

Table 6
Membership function U(X) values for *P. forbesii*
under PTE stress

PTE	U1	U2	U3	D	Order
Cr	1	0	0.596	0.565	2
Cd	0.123	0.685	1	0.445	3
Ni	0	0.407	0.060	0.165	5
Cu	0.285	0.245	0	0.236	4
Zn	0.933	1	0.115	0.948	1

Discussion

Our results suggested that exposure to different PTEs led to different degrees of growth retardation and physiological response in *P. forbesii*. Integrating various physiological indicators revealed that the toxicity of the five PTEs in *P. forbesii* decreased in the order of Ni > Cu > Cd > Cr > Zn, consistent with results obtained in barley (Hg > Pb > Cu > Cd > Cr > Ni > Zn) and *Chlorella vulgaris* (Hg > Cu > Cd > Fe > Cr > Zn > Ni > Co > Mn) (Nieboer and Richardson, 1980). However, it is noteworthy that Ni treatment resulted in the most severe growth defects in *P. forbesii* in this study, whereas this metal was reported to have relatively low toxicity in barley and *Chlorella*. However, this observation is consistent with results for physiological responses to PTEs in *Jatropha curcas* (Yamada et al. 2018). Ni is an essential trace element in plants and contributes to plant growth and development, morphology, physiology, and biochemistry (Li et al. 2003). However, excessive Ni will disrupt the normal balance of plant hormones, inhibit plant growth and development, cause plant phenotypic deformities, and destroy the roots (Ali et al. 2015). Since the effects of Ni toxicity on plant physiology depend on many factors, such as growth state and cultivation conditions, more detailed studies of the physiological processes of *P. forbesii* after Ni treatment are necessary.

Under exposure to low concentrations (10–50 µmol/L) of five PTEs, hydroponically grown *P. forbesii* showed normal growth and even showed increases in root length (10 µmol/L), crown width, and plant height (50 µmol/L) under low concentrations of Cr. Inversely, under high concentrations of Cd, Ni, and Cu (100–200 µmol/L), plant growth and biomass were seriously affected; the leaves began to wilt, curl, yellow, and even die. Although high concentration concentrations (100–200 µmol/L) of Zn and Cr also caused some damage to plants, the degree of damage was minor, and the plants exhibited normal growth.

Photosynthesis is the most basic and vital physiological activity of plants. The photosynthetic capacity of plants is closely related to plant health. The chlorophyll content is the most intuitive indicator of the strength of the photosynthetic capacity of plants. In this study, low concentrations of Cd, Ni, and Cu (10 µmol/L) increased the chlorophyll content of plants significantly, thereby enhancing photosynthesis and improving plant resistance to PTEs. In contrast, the chlorophyll contents under Cr and Zn stress decreased gradually, showing a negative correlation with the Cr and Zn concentrations. This may be due to the inhibition of ROS under stress, leading to a significant decrease in the photosynthetic capacity and downregulation of PSII via non-photochemical quenching (Huang et al. 2018).

SS plays significant roles in plant defense responses to environmental stress (Gill et al. 2001). The SS content of plants increased significantly under Cd stress, indicating that the plants responded positively to Cd stress and showed increased sugar accumulation to regulate the water potential balance and prevent the leakage of plant cell contents. Similar phenomena have been observed in wheat subjected to Zn stress, and this response is considered a stress adaptation in plants (Su et al. 2017; Li et al. 2013). Under 10–50 $\mu\text{mol/L}$ Cu, the SS content in *P. forbesii* plants was lower than that of the control, consistent with the response of *Cinnamomum camphora* under the same concentration of Cu (Zhou et al. 2019). This may be explained by the ability of plants under Cu stress to break down SS to regulate the osmotic balance and maintain normal growth under low concentrations of metals. However, under Ni, Cr, and Zn stress, the SS contents of plants increased initially and then decreased. This may be because low levels of heavy metal stress hindered SS metabolic pathways or the signal transmission pathways, resulting in elevated SS contents. However, osmotic regulation in plants was highly damaged under high concentrations of metals. To maintain the osmotic balance, plants consume large quantities of SS. The source of SS synthesis was hindered by stress, leading to a significant decrease in the SS content. The SP content in organisms is an important indicator of reversible and irreversible changes in metabolism and can respond to exogenous stimuli, such as heavy metal stress (Singh et al. 2003). Three general trends have been observed in SP variation under in response to five PTEs, characterized by an initial increase followed by a decrease, a continual increase, and an initial decrease followed by an increase. Therefore, the increase in the SP content under low concentrations of heavy metals might be an adaptation to heavy metal stress. Plants could reduce stress-induced damage by synthesizing specific proteins that bind to heavy metals (Yang et al. 2018). Under high concentrations of heavy metals, the decrease in SP content may be caused by the combination of heavy metal enrichment in the plants, causing damage to the SP, or may be explained by the heavy metal-induced inhibition of protein synthesis, resulting in a significant decrease in the SP content (Singh et al. 2003).

Plant cells produce high levels of ROS and free radicals under metal stress, which can destroy intracellular macromolecules and lead to membrane lipid peroxidation (Srivastava et al. 2007). With the production of excessive free radicals, MDA, a cytotoxic product of lipid peroxidation, increased significantly. The contents of MDA in *P. forbesii* varied substantially in response to the five PTEs, indicating that stress could influence MDA accumulation. High concentrations of Cd, Ni (100–200 $\mu\text{mol/L}$), and Cu (10–200 $\mu\text{mol/L}$) led to high levels of MDA accumulation, and the plants showed a high level of antioxidant stress, resulting in serious oxidative damage and a lack of self-repair. This is consistent with previous results for the response of *Koeleria paniculata* to Cd stress (Yang et al. 2018). However, Cr stress (10–100 $\mu\text{mol/L}$) reduced the MDA content significantly, and the antioxidant system functioned efficiently and repaired the stress-induced oxidative damage. There was no significant difference in the MDA content between Zn treatments and the control, indicating that the oxidative damage in *P. forbesii* was minor and the membrane lipid peroxidation was in a controllable state. These findings differ from those of a study of pigeon pea (Madhava et al. 2000). *P. forbesii* had strong adaptability under Zn and Cr stress and maintained normal metabolic processes via its repair mechanism.

In this study, the H_2O_2 content of *P. forbesii* tended to increase under PTE stress, indicating that the species showed an oxidative stress response to improve survival, resulting in the production and accumulation of H_2O_2 , consistent with the results of *Coronopus didymus* under Cd stress (Sidhu et al. 2017). The

accumulation of H_2O_2 in plant tissues can cause oxidative damage to plant cells and even plant death (Wrzaczek et al. 2013), which may be due to the effects of elevated ROS induced by heavy metals on membrane lipid peroxidation, thereby interfering with the oxidative repair function of cells and causing irreparable damage and H_2O_2 accumulation.

To remove the large amount of ROS produced by lipid peroxidation, several antioxidant defense systems are activated. SOD, POD, and CAT play crucial roles (Radoti et al. 2000; Matés et al. 2000). In this study, SOD activity continued to increase under Zn stress, indicating that the antioxidant enzyme system of plants promoted the removal of ROS (Sidhu et al. 2017). Under Cd, Ni, and Cu stress, the SOD activity in *P. forbesii* increased initially and then decreased rapidly, indicating that when the metal concentration exceeded a certain threshold, the antioxidant enzyme system began to fail and accumulated ROS continued to produce toxic effects, consistent with previous results for *Koeleria paniculata* (Yang et al. 2018). In addition, the trends in POD activity in *P. forbesii* under Cd and Cu stresses were consistent with previous results for *Mentha piperita* and *Pistia stratiotes* (Li et al. 2013; Khair et al. 2020). However, the changes of POD activity under Ni, Cr, and Zn stress showed an initial increase and subsequent decrease, consistent with results for *Zostera japonica* (Lin et al. 2016). CAT activity in *P. forbesii* under Cr and Ni stress showed a similar trend (i.e., an early increase followed by a decrease), indicating that under low concentration of Cr and Ni stress, leaves of *P. forbesii* could activate CAT activity, thereby enhancing lipid peroxidation in leaves to remove ROS, while high concentrations of heavy metals would harm the plant cell defense system, consistent with previous results (Hou et al. 2007; Singh et al. 2020). It is worth mentioning that although a high concentration of Cr led to a decrease in CAT activity, the rate of decrease was relatively slow and CAT activity was still higher than that of the control, indicating that the catalase system of *P. forbesii* could still effectively control the level of ROS; however, slight inhibition in CAT activity was detected.

ASA has an important role in ROS quenching and ascorbate-glutathione pathways of antioxidant metabolism (Noctor and Foyer, 1998). The ASA content increased significantly as the Cd and Cu concentrations increased, which promoted the removal of ROS and maintained the normal growth of *P. forbesii*. The production of a large amount of ASA not only contributes to heavy metal stress resistance but also affects the energy supply for plant growth and development. Therefore, the role of ASA to plant resistance needs to be studied further. Under Ni, Cr, and Zn stress, the ASA content increased and then decreased and was consistently higher than that of the control, indicating that ASA can effectively promote the removal of ROS in plants and maintain the balance of redox levels in cells under low concentrations of heavy metals. Nevertheless, as the concentration gradually increased, the level of ROS exceeded the capacity of ASA. With the consumption of a large amount of ASA, its concentration decreased significantly, indicating that the ROS scavenging pathway began to use ASA as a substrate (Hojati et al. 2017); however, the final content was slightly higher than that of the control, indicating that the synthesis of ASA was not inhibited, supporting the high tolerance of *P. forbesii* to Ni, Cr, and Zn stress. In addition, the effectiveness of plant oxidative defense systems can be measured by the levels of non-enzymatic antioxidants, such as GSH (Ahmad, 2012). Under Cd, Ni, and Cr stress, the GSH content in *P. forbesii* continued to increase, which improved the ROS and free radical scavenging ability of *P. forbesii*. Plants could use GSH to alleviate stress-induced damage. These findings indicated that *P. forbesii* had good tolerance to Cd and Cu stress, consistent with previous findings for tobacco seedlings (Ye et al. 2016).

Ion homeostasis refers to the balance of nutrient elements in plant tissues, which is an essential condition for normal growth and development (Rouached, 2011; Rouached et al. 2010). Once the heavy metal content exceeds a threshold value, the absorption and transport of essential mineral nutrients are altered, thus leading to an imbalance in nutrient elements in plants and eventually produce toxic effects (Matraszek et al. 2016a). In this study, notable abnormalities in the abundance of essential mineral nutrients were found after Cd exposure, including an increase in the Ca content in the shoots. Cd can gradually accumulate in plants through cation channels or other element transporters and interfere with normal photosynthesis (Matraszek et al. 2016b). The absorption of some nutrients is strengthened by self-protection mechanisms. For example, the absorption of Ca can prevent the replacement of Ca by Cd in proteins, thus alleviating the inhibition of photosynthesis induced by Cd (Zhang et al. 2014). The Mg and K contents in the Ni treatment were significantly lower than those in the control, and the Mg and K contents in the Cu treatment also showed a downward trend. In addition, the Mg and K contents in Zn and Cd treatments showed no significant change, suggesting that *P. forbesii* had stronger resistance to Zn and Cd than to Ni and Cu. This is consistent with the results obtained by a PCA. From the perspective of heavy metal accumulation, the accumulation of Ni in different parts of *P. forbesii* was greater in the roots than in the shoots, and the Ni contents in both parts of the plant increased as the Ni concentration increased, consistent with results for peppermint (Khair et al. 2020). Heavy metal accumulation in the underground part of *P. forbesii* increased significantly as the Cd and Cu concentrations increased, consistent with the results of studies of *Celosia argentea* under Cd stress (Liu et al. 2020) and *Cinnamomum camphora* under Cu stress (Zhou et al. 2019). Our quantitative results for *P. forbesii* indicated that the absorption of Cr and Zn in the roots of the plant was limited. The decrease in absorption under high heavy metal concentrations may be due to the loss and death of some roots of *P. forbesii*, resulting in a decrease in roots available for absorption.

Conclusion

Phenotypic and physiological indexes of *P. forbesii* varied substantially in response to five PTEs. By qualitative observations and quantitative analyses, including a PCA and membership function evaluation, the mechanisms underlying the response of *P. forbesii* to heavy metal stress were clarified from a physiological point of view. Our preliminary analysis showed that *P. forbesii* resistance to five PTEs decreased in the following order: Zn > Cr > Cd > Cu > Ni. Heavy metal pollution in soil is a serious issue, and various plants have been selected to repair the landscape and improve the soil ecology. As a fragrant plant in the genus *Primula*, *P. forbesii* not only has certain adaptability to heavy metal soil but also has landscape value; accordingly, it can be used as a component of phytoremediation strategies for soil contaminated by heavy metals. This study provides a reference for the application of *P. forbesii* to environments polluted by heavy metals. Nevertheless, this experiment adopted the hydroponic method, and it is necessary to verify the heavy metal resistance of *P. forbesii* in soil by culture experiments.

Declarations

Author contribution

All authors contributed to the study conception and design. Material preparation and implementation of experiments were performed by Jian Zhao and Yifeng Li, the data collection and analysis were performed by Yuanzhi Pan and Beibei Jiang. The manuscript was written by Yin Jia and Xiancai Yin, and Qinglin Liu made some modifications to the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the National Natural Science Foundation of China (32001356).

Ethics approval This study follows all ethical practices during writing.

Competing interests The authors declare no competing interests.

Consent to Participate Not applicable

Consent to Publish Not applicable

Availability of data and materials The authors confirm that the manuscript is an honest, accurate, and transparent account of the study reported; and that no vital features of the study have been omitted.

References

1. Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105(105):121–126
2. Ali MA, Asghar HN, Khan MY, Saleem M, Naveed M, Niazi NK (2015) Alleviation of nickel-induced stress in mungbean through application of gibberellic acid. *Int J Agric Biol* 17(05):990–994
3. Ahmad P (2012) Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *Afr J Biotechnol* 11(11):2694–2703
4. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1–2):248–254
5. Ghorri NH, Ghorri T, Hayat MQ, Imadiet SR, Gul A, Altay V, Ozturk M (2019) Heavy metal stress and responses in plants. *Int J Environ Sci Technol* 16(3):1807–1828
6. Gill SS, Tuteja N (2010) ROS and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48(12):909–930
7. Gill PK, Sharma AD, Singh P, Bhullaret SS (2001) Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulg J Plant Physiol* 27:72–84
8. Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J Exp Bot* 53(366):1–11
9. Hu CM (1990) Primulaceae. In: Chen FH, Hu CM (eds) *Flora Reipublicae Popularis Sinicae*. Science Press, Beijing, pp 1–321
10. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125(1):189–198
11. Hess JL, Foster JG (1976) Superoxide-dismutase in higher-plants. *Fed Proc* 7:1553

12. Huang MY, Ai HL, Xu XX, Chen K, Niu H, Zhu HH, Sun J, Du DY, Chen L (2018) Nitric oxide alleviates toxicity of hexavalent chromium on tall fescue and improves performance of photosystem II. *Ecotoxicol Environ Saf* 164:32–40
13. Hou WH, Chen X, Song GL, Wang QH, Chang CC (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). *Plant Physiol Biochem* 45(1):62–69
14. Hojati M, Modarres-Sanavy SAM, Enferadi ST, Majdi M, Ghanati F, Farzadfar S, Pazoki A (2017) Cadmium and copper induced changes in growth, oxidative metabolism and terpenoids of *Tanacetum parthenium*. *Environ Sci Pollut Res* 24(13):12261–12272
15. Jozefczak M, Keunen E, Schat H, Blik M, Hernandez LE, Carleer R, Remans T, Bohler S, Vangronsveld J, Cuypers A (2014) Differential response of *Arabidopsis* leaves and roots to cadmium: Glutathione-related chelating capacity vs antioxidant capacity. *Plant Physiol Biochem* 83:1–9
16. Jia Y, Zhao JL, Pan YZ, Xu Y, Sun LX, Liu QL (2014) Collection and evaluation of *Primula* species of western Sichuan in China. *Genet Resour Crop Evol* 61:1245–1262
17. Jia Y, Liu CL, Lan XY, Zhao J, Xiang YF, Pan YZ (2020a) Effect of cadmium stress on the growth and physiological characteristics of *Primula forbesii* seedlings. *Acta Bot Boreal -Occident Sin* 40(3):0454–0462
18. Jia Y, Xiang YF, Wang LL, Zhao J, Liu CL, Pan YZ (2020b) Effects of salt stress on the growth and physiological characteristics of *Primula forbesii*. *Acta Prataculturae Sinica* 29(10):119–128
19. Kopittke PM, Blamey FPC, Asher CJ, Menzies NW (2010) Trace metal phytotoxicity in solution culture: A review. *J Exp Bot* 61:945–954
20. Khair KU, Farid M, Ashraf U, Zubair M, Ali S (2020) Citric acid enhanced phytoextraction of nickel (Ni) and alleviate *Mentha piperita* (L.) from Ni-induced physiological and biochemical damages. *Environ Sci Pollut Res* 27(21):27010–27022
21. Li HS (2002) Experimental principle and techniques of plant physiology and biochemistry. Higher Education Press, Beijing, pp 194–197
22. Law MY, Charles SA, Halliwell B (1983) Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of paraquat. *Biochem J* 210(3):899–903
23. Li M, Wang GX, Lin JS (2003) Application of external calcium in improving the PEG-induced water stress tolerance in liquorice cells. *Bot Bull Acad Sinica* 44(4):275–284
24. Li Y, Zhang SS, Jiang WS, Liu DH (2013) Cadmium accumulation, activities of antioxidant enzymes, and malondialdehyde (MDA) content in *Pistia stratiotes* L. *Environ. Sci Pollut Res* 20(2):1117–1123
25. Lin HY, Sun T, Zhou Y, Zhang XM (2016) Anti-oxidative feedback and biomarkers in the intertidal seagrass *Zostera japonica* induced by exposure to copper, lead and cadmium. *Mar Pollut Bull* 109(1):325–333
26. Liu J, Yu G, Jiang P, Zhang X, Oiu R (2020) Interaction of Mn and Cd during their uptake in *Celosia argentea* differs between hydroponic and soil systems. *Plant Soil* 450(1–2):323–336
27. Meharg AA (1993) The role of the plasmalemma in metal tolerance in angiosperms. *Physiol Plant* 88(1):191–198

28. Madhava Rao KV, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci* 157(1):113–128
29. Matés JM (2000) Effects of antioxidant enzymes in the molecular control of ROS toxicology. *Toxicology* 153(1):83–104
30. Matraszek R, Hawrylak-Nowak B, Chwil S, Chwil M (2016a) Macronutrient composition of nickel-treated wheat under different sulfur concentrations in the nutrient solution. *Environ Sci Pollut Res* 23(6):5902–5914
31. Matraszek R, Hawrylak-Nowak B, Chwil S, Chwil M (2016b) Interaction between cadmium stress and sulphur nutrition level on macronutrient status of *Sinapis alba* L. *Water Air Soil Pollut* 227(9):355
32. Nieboer E, Richardson DHS (1980) The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environ Pollut Ser B* 1(1):3–26
33. Noctor G, Foyer CH (1998) ASCORBATE AND GLUTATHIONE: Keeping Active Oxygen Under Control. *Annu Rev Plant Physiol Plant Mol Biol* 49(1):249–249
34. Richards AJ (2003) *Primula*, 2nd edn. Timber Press, Oregon
35. Radoti K, Dui T, Mutavdi D (2000) Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. *Environ Exp Bot* 44(2):105–113
36. Rouached H (2011) Multilevel coordination of phosphate and sulfate homeostasis in plants. *Plant Signal Behav* 6(7):952–955
37. Rouached H, Secco D, Arpat BA (2010) Regulation of ion homeostasis in plants: Current approaches and future challenges. *Plant Signal Behav* 5(5):501–502
38. Shanying HE, Yang X, Zhenli HE, Baligar VC (2017) Morphological and physiological responses of plants to cadmium toxicity. *Rev Pedosphere* 27(3):421–438
39. Shah K, Kumar RG, Verma S, Dubey RS (2001) Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Sci* 161:1135–1141. [https://doi.org/10.1016/S01689452\(01\)00517-9](https://doi.org/10.1016/S01689452(01)00517-9)
40. Su C, Jiang Y, Li F, Yang Y, Lu Q, Zhang T, Hu D, Xu Q (2017) Investigation of subcellular distribution, physiological, and biochemical changes in *Spirodela polyrhiza* as a function of cadmium exposure. *Environ Exp Bot* 142:24–33
41. Singh PK, Tewari RK (2003) Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. *J Environ Biol* 24(1):107–112
42. Srivastava S, Mishra S, Tripathi RD, Dwivedi S, Trivedi PK, Tandon PK (2007) Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. *Environ Sci Technol* 41(8):2930–2936
43. Sidhu GPS, Singh HP, Batish DR, Kohli RK (2017) Tolerance and hyperaccumulation of cadmium by a wild, unpalatable herb *Coronopus didymus* (L.) Sm. (Brassicaceae). *Ecotoxicol Environ Saf* 135:209–215
44. Singh H, Kumar D, Soni V (2020) Copper and mercury induced oxidative stresses and antioxidant responses of *Spirodela polyrhiza* (L.) Schleid. *Biochem Biophys Rep* 23:100781
45. Toppi LSD, Gabbrielli R (1999) Response to cadmium in higher plants. *Environ Exp Bot* 41(2):105–130

46. Wellburn AR, Lichtenthaler HK (1984) Formulae and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Springer Netherlands, Dordrecht, pp 9–12
47. Wang J, Zeng Q, Zhu J, Liu G, Tang H (2013) Dissimilarity of ascorbate–glutathione (AsA–GSH) cycle mechanism in two rice (*Oryza sativa* L.) cultivars under experimental free-air ozone exposure. *Agr Ecosyst Environ* 165:39–49. <https://doi.org/10.1016/j.agee.2012.12.006>
48. Wrzaczek M, Brosche M, Kangasjarvi J (2013) ROS signaling loops—production, perception, regulation. *Curr Opin Plant Biol* 16(5):575–582
49. Yang LP, Zhu J, Wang P, Zeng J, Tan R, Yang YZ, Liu ZM (2018) Effect of Cd on growth, physiological response, Cd subcellular distribution and chemical forms of *Koeleria paniculata*. *Ecotoxicol Environ Saf* 160:10–18
50. Yamada M, Malambane G, Yamada S, Suharsono S, Tsujimoto H, Moseki B, Akashi K (2018) Differential physiological responses and tolerance to potentially toxic elements in biodiesel tree *Jatropha curcas*. *Sci Rep* 8(1):1635
51. Ye X, Ling T, Xue Y, Xu C, Zhou W, Hu L, Jian C, Shi Z (2016) Thymol mitigates cadmium stress by regulating glutathione levels and ROS homeostasis in tobacco seedlings. *Molecules* 21(10):1339
52. Zhou JH, Cheng K, Zheng JY, Liu ZQ, Shen WB, Fan HB, Jin ZN (2019) Physiological and biochemical characteristics of *Cinnamomum camphora* in response to Cu- and Cd-contaminated soil. *Water Air Soil Pollut* 230(1):15
53. Zhang XF, Zhang XH, Gao B, Li ZA, Xia HP, Li HF, Li J (2014) Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of an energy crop, king grass (*Pennisetum americanum* × *P. purpureum*). *Biomass Bioenergy* 67:179–187

Figures

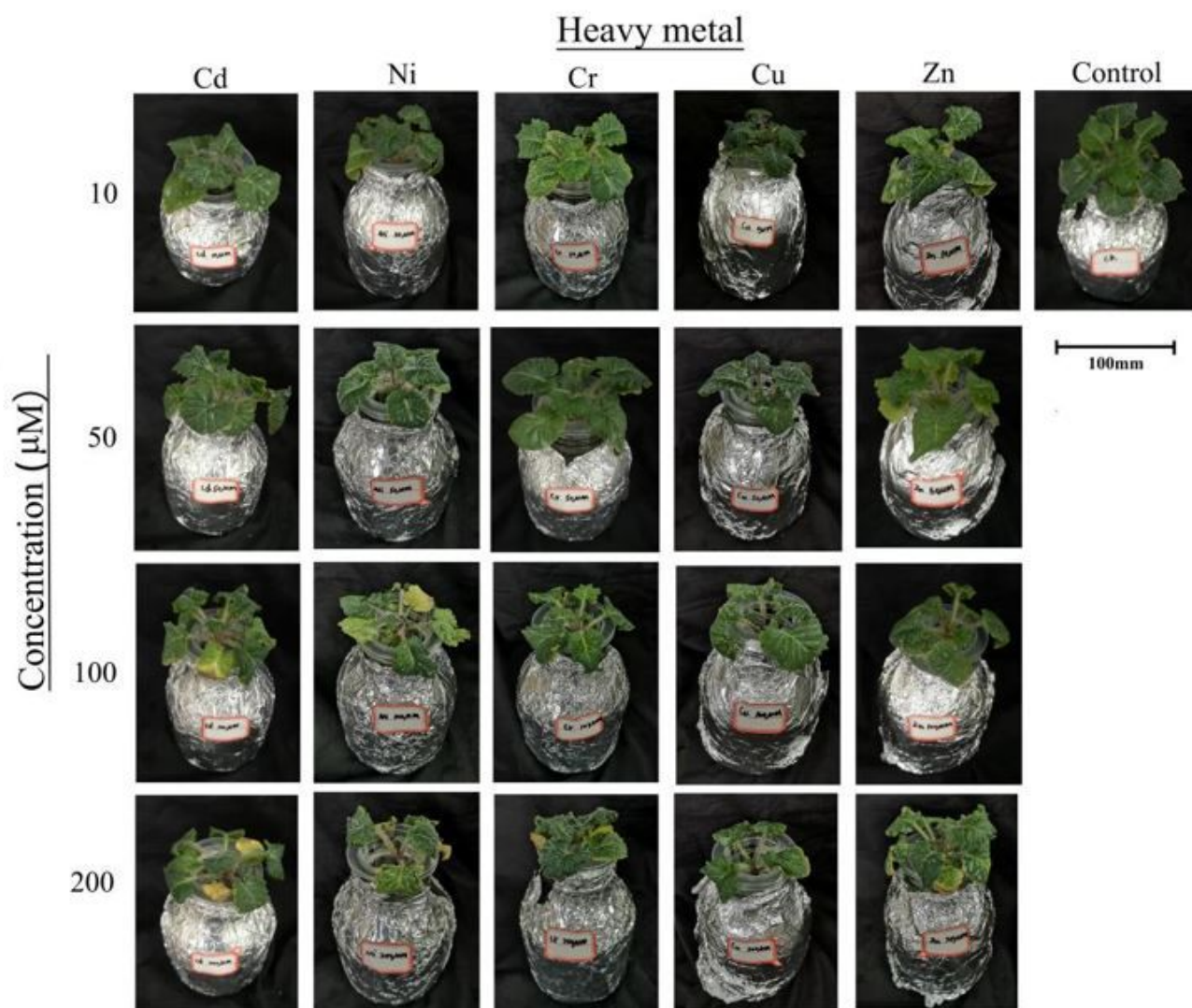


Figure 1

Phenotypic changes of *P. forbesii* after treatment with different concentrations of heavy metals

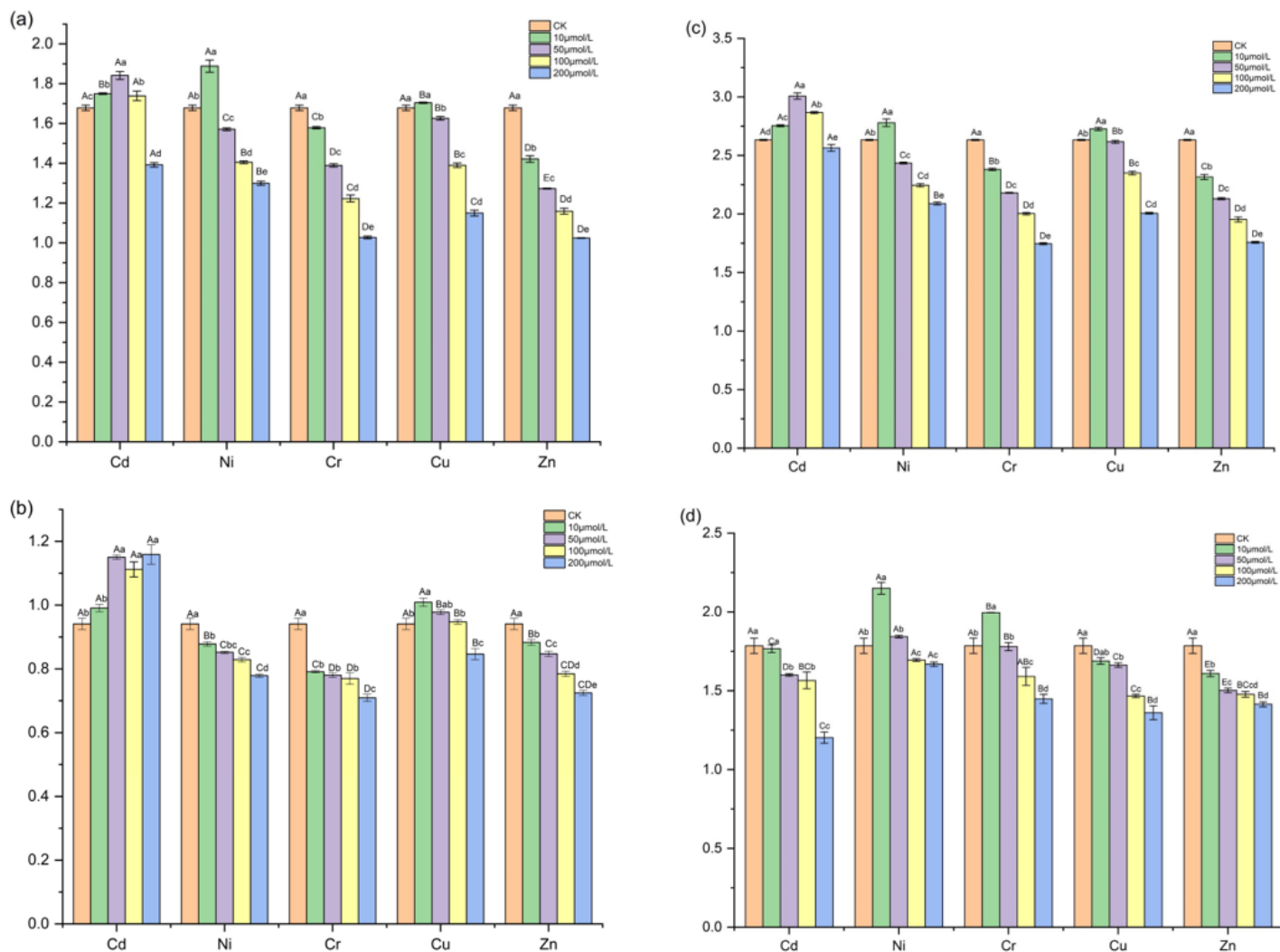


Figure 2

(a) Chlorophyll a content (mg/g). (b) chlorophyll b content (mg/g). (c) Total chlorophyll content (mg/g). (d) The ratio of chlorophyll a to chlorophyll b content (mg/g). Values are means $\pm$ S.D. Lowercase letters (a, b, c, d, e) indicate significant differences at the $P < 0.05$ level between the same heavy metals under different concentrations, and capital letters (A, B, C, D, E) indicate significant differences at $P < 0.05$ between treatments with different kinds of heavy metals at the same concentration according to LSD test. (The same definitions apply below.)

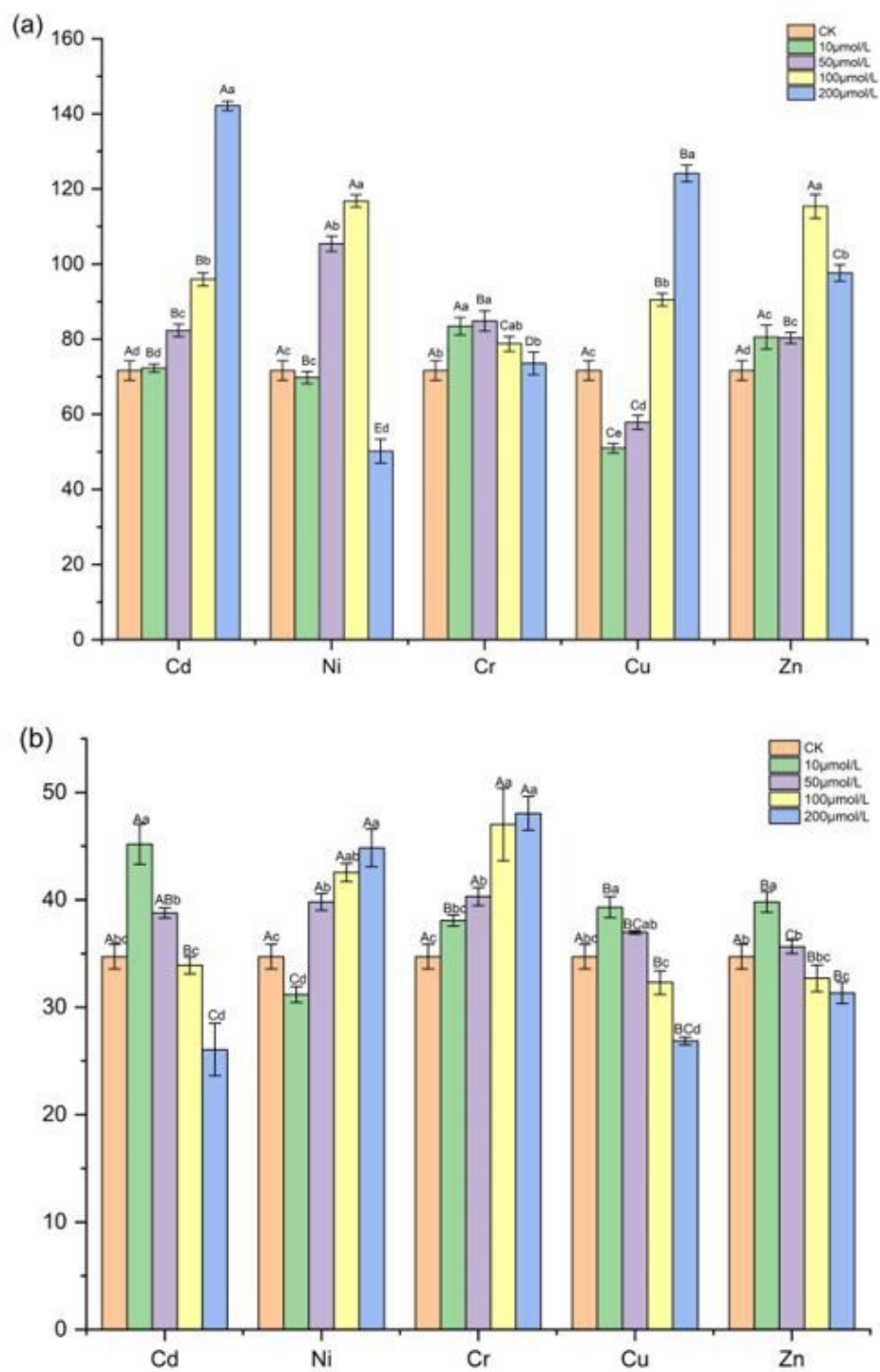


Figure 3

(a) Content of soluble sugar (mmol/L). (b) Content of soluble protein (mg/g).

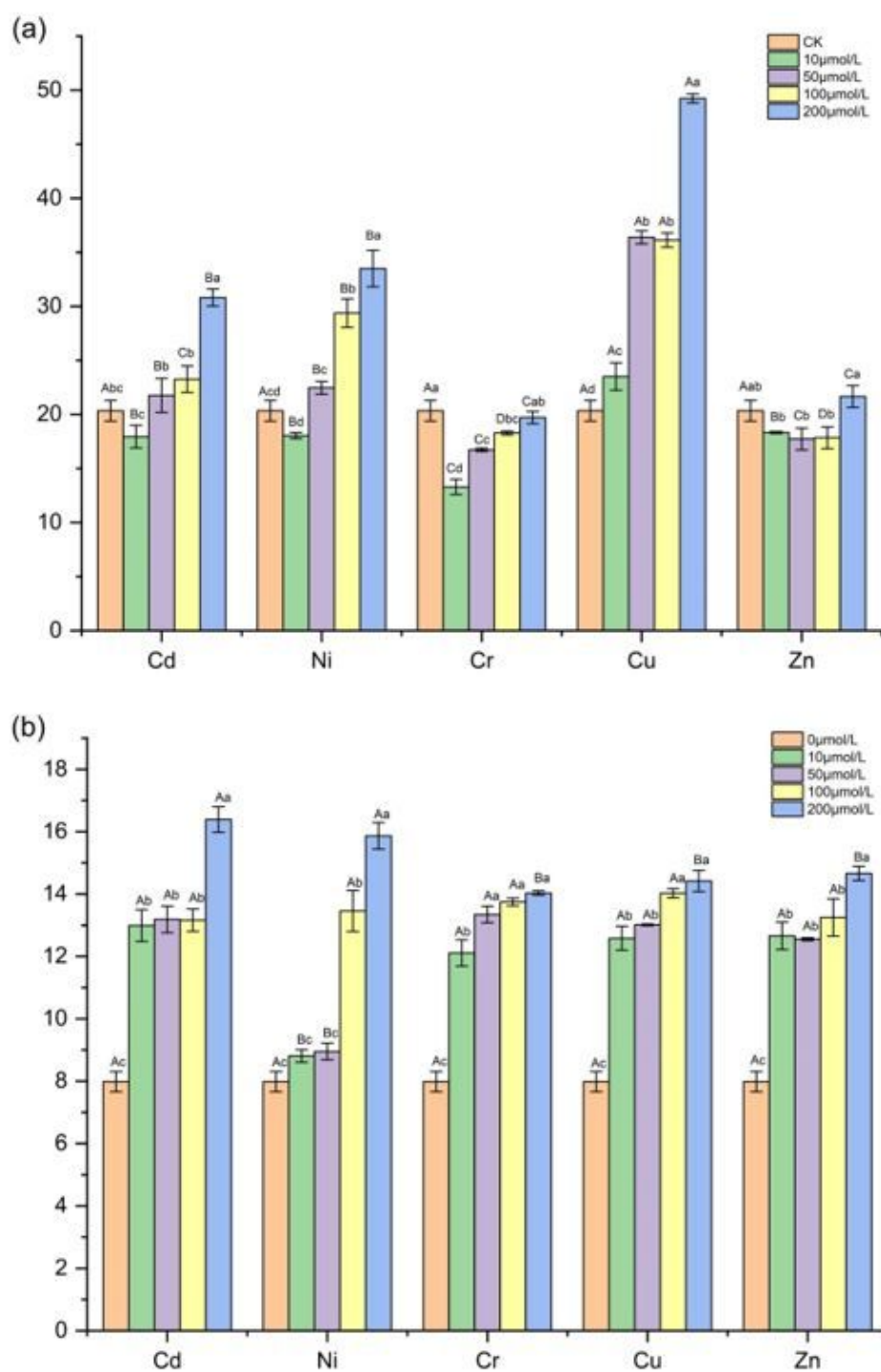


Figure 4

(a) Level of MDA (μmol/gFW). (b) Level of H₂O₂ (μmol/gFW).

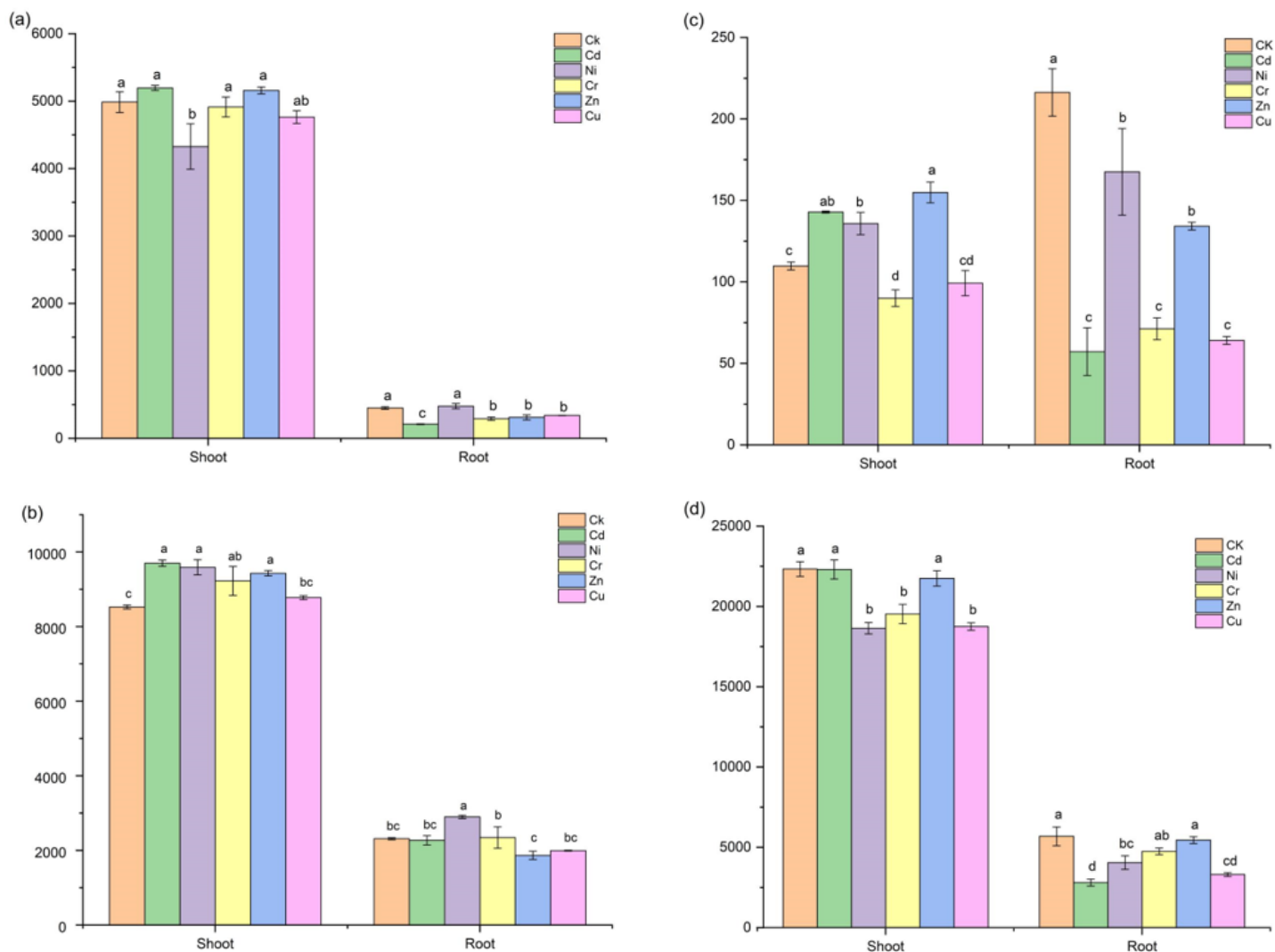


Figure 5

(a) SOD activity (U/gFW/min). (b) POD activity (U/gFW/min). (c) CAT activity (U/gFW/min). (d) ASA content ($\mu\text{g/gFW}$). (e) GSH content ($\mu\text{mol/gFW}$).

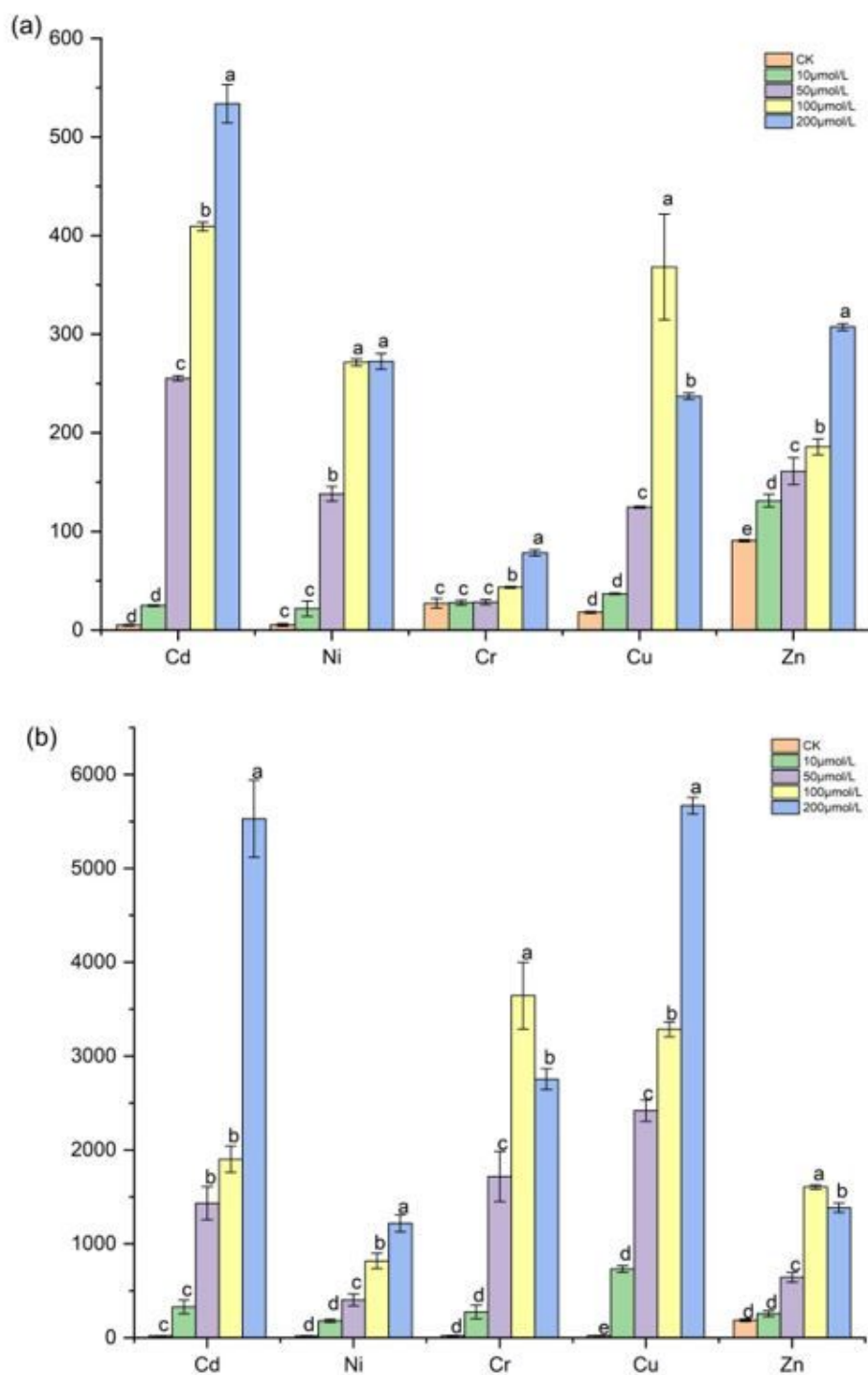


Figure 6

(a) Metal contents in roots ($\mu\text{g/gDW}$). (b) Metal contents in shoots ($\mu\text{g/gDW}$).

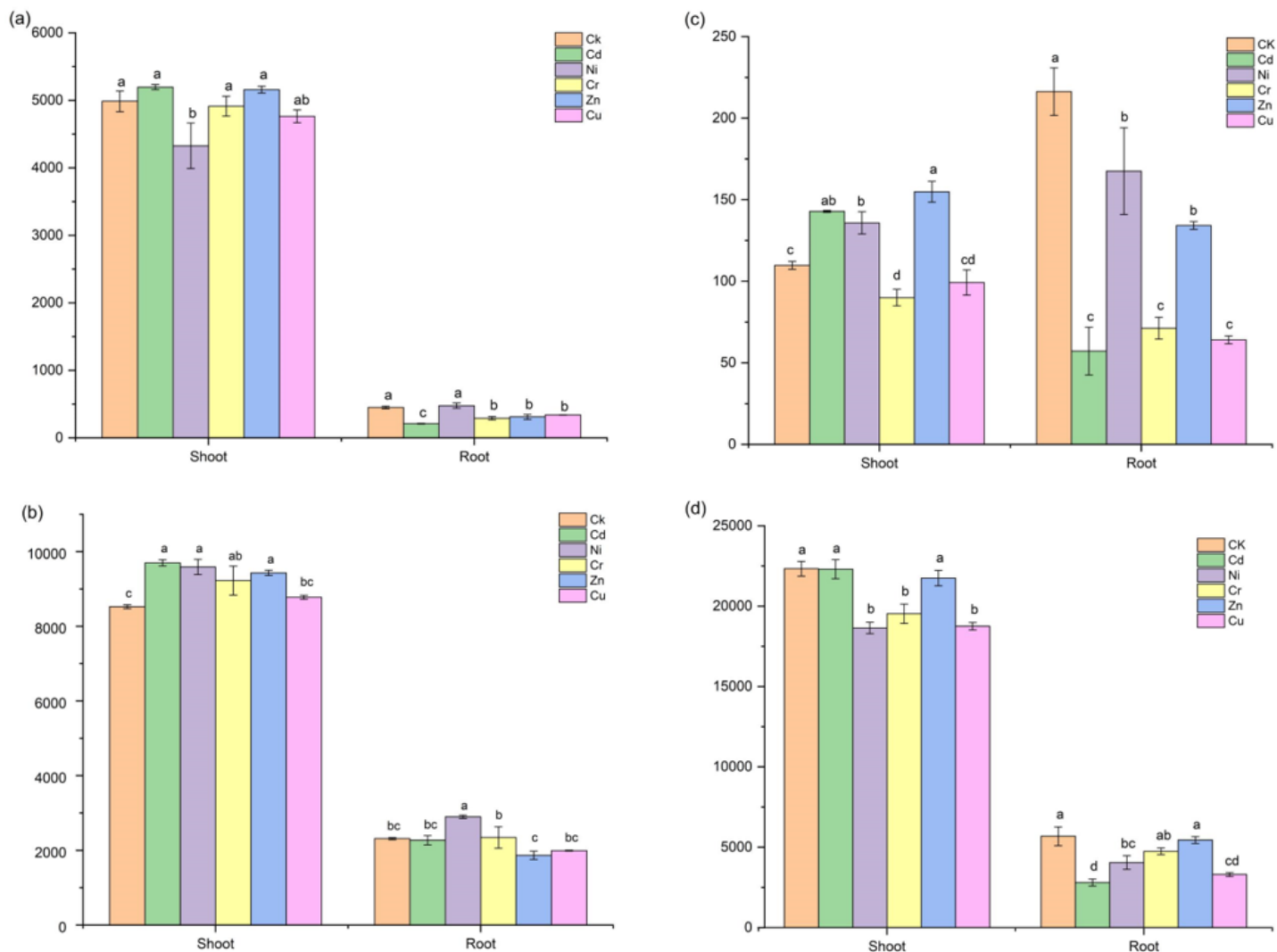


Figure 7

(a) Mg content ($\mu\text{g/gDW}$). (b) Ca content ($\mu\text{g/gDW}$). (c) Mn content ($\mu\text{g/gDW}$). (d) K content ($\mu\text{g/gDW}$). Values are means $\pm$ S.D. Lowercase letters (a, b, c, d, e) indicate significant differences at the $P < 0.05$ level between the same heavy metals under different concentrations, and capital letters (A, B, C, D, E) indicate significant differences at $P < 0.05$ between treatments with different kinds of heavy metals at the same concentration (10 $\mu\text{mol/L}$) according to LSD test.