

Original Research

Single and Combined Effects of Cadmium and Lead on Seed Germination and Early Seedling Growth in *Rhus typhina*

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Abstract

Cadmium (Cd) and lead (Pb) generally occur simultaneously with low concentration in soil. However, anthropogenic activities have significantly raised these non-biodegradable heavy metals and caused long-term deleterious effects on ecosystem health. To study single or combined effects of Cd and Pb on seed germination, early seedling growth and physiological response in *Rhus typhina*, a seed germination and sand culture experiment was established completely randomized with 0, 100, 300, and 500 mg·L⁻¹ Pb(NO₃)₂ or 0, 25, 75, 125 mg·L⁻¹ CdCl₂ individually or in combination. The present results showed seed germination and seedling growth of *Rhus typhina* decreased with increasing Cd and Pb, and the joint effect was more serious than single heavy metal stress. The lowest of seed germination rate (GR), germination index (GI), root length (RL) and shoot length (SL) in *Rhus typhina* decreased 65.85%, 73.46%, 84.33% and 61.95% compared to control in soil supplemented with combined Cd and Pb, respectively. The activity of superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA) and soluble protein (SP) changed significantly with increasing concentration of Cd and Pb, and MDA and POD played important roles in resisting Cd and Pb stress because of their significant correlation with seed germination and early seedling growth.

Keywords: *Rhus typhina*, Cd and Pb stress, MDA, Soluble Protein Content, SOD, POD

Introduction

During the last few decades, heavy metal contamination become one of the most noticeable environmental problems in developed as well as developing countries with the rapid development of industrial and modern agricultural practices [1]. Although soil heavy metals can naturally occur from the

erosion of certain rocks and minerals, they are released in excess into the environment by anthropogenic activities such as the use of pesticides, agricultural fertilizers, industrial production, sewage, metalliferous mines, and transportation [2, 3]. These non-biodegradable heavy metals in soil can cause long-term deleterious effects on ecosystem health as they can get absorbed by the root system and accumulate in different parts of plants, which disturbance plant physiological and metabolic processes, and further adversely affects human health and environment ecosystem by entering

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into the food chain in amount increases even at very low concentration [4-6]. Heavy metal toxicity in plants varies with specific metal and their content [1]. As the most hazardous pollutants presenting in agrochemicals and rich in industrially contaminated soils, Cd and Pb are the most toxic heavy metals and often occur simultaneously in soil, but single metals are commonly focused on toxic metal stress [7-9]. It was complex and important to understand the mechanisms of interaction between heavy metals due to design agronomic or genetic strategies to limit contamination of plants [10].

As the most significant stages in a seed plant's life, seed germination and seedling growth are the most sensitive to soil chemical and physical conditions, thus these parameters become the most prime important toxicity assays for detecting the inhibition effects caused by heavy metal [2, 11-13]. Many recent studies have confirmed that single heavy metals Cd or Pb and their interaction cause negatively influence on seed germination and seedling development by affected soluble protein (SP), malondialdehyde (MDA) and the degradation of antioxidant enzymes activities such as superoxide dismutase (SOD) and peroxidase (POD) [14-16]. However, some other experiments showed that seed germination was stimulated at low concentrations and inhibited under high concentrations, some other experiments documented gradual reduction in germination with concentration increase under single heavy metal stress [17-19].

More studies concentrated to the effects of combined heavy metal stress on herbaceous plants [1, 15]. However, woody plants are more widely used in urban landscaping and play an immeasurable role in improving the ecological environment in the urban ecosystem. *Rhus typhina* L. (Anacardiaceae) is a perennial shrub or a small tree and it may clone through rhizomes and production by seeds [20, 21]. As an introduced species from eastern United States, *Rhus typhina* has been distributed in most areas of China with anthropogenic assistance due to its expansive, admittedly decorative and attractive tree, and it may pose a threat to the environment because ripening inflorescences during pollination may pose an allergic hazard to humans [22, 23]. The fast-growing species with a brightly colored foliage tree during fall, has gradually become an ideal species for soil conservation and urban forestation because it can grow better under environmental stresses and highly effectively retain water and soil [24]. Compared to agricultural soils, heavy metal pollution soil in urban is becoming more and more serious with the development of urbanization, so it is essential to better understand how the seed germination and seedling growth of *R. typhina* will be affected by the environmental factors such as heavy metal stress in its non-native habitats where it is widely planted [25, 26].

The aim was to detect the phytotoxic effects of Cd and Pb in *Rhus typhina* by investigating the

morphological and physiological characteristics of seed germination and seedling growth of *Rhus typhina* under single or combine stress. With this experiment, we also evaluated how the contemporaneous presence of the Cd and Pb can affect each other. By examining, we will be able to assess its growth plasticity at different heavy metal pollution and to improve our mechanistic understanding of the survival and growth of *R. typhina* individuals in its introduced habitats. We hypothesized that the resistant capacity seed germination and seedling growth in *R. typhina* would be adversely affected by Cd and Pb stress, thus, to reveal whether it would be a potential species to resist heavy metal pollution.

Materials and Methods

Materials

Seeds of *R. typhina* were collected from campus of Jilin Agricultural University, Changchun (43°52' N, 125°21' E), China in Oct 2017, Then sun-dried, stored at 4°C. Heavy metal ions were made up in aqueous solutions at Pb(NO₃)₂ (100, 300, and 500 mg·L⁻¹) and CdCl₂ (25, 75, 125 mg·L⁻¹) for germination tests.

Seed Treatment and Seed Germination Experiments

Seeds of *R. typhina* were firstly surface-sterilized (1 % NaClO for approximately 10 min) and thoroughly washed with distilled water 3 times, then soaked into 90°C boiled water and kept for 24 hours after the water cooled down naturally, finally soaked in distilled water for 2 hours and dried with sterile filter paper. 30 seeds were distributed in 9 cm diameter Petri dishes, containing two sheets of filter paper, and 3 mL different concentrations of Pb(NO₃)₂ or/and CdCl₂, and distilled water was used as a negative control. The samples were incubated in a climate-controlled incubator at 85 % humidity with diurnal lighting (3500 lx light intensity, 25±1°C, 14 h light, and 20±1°C, 10 h dark) for 14 days. During the incubation, solution was supplied daily according to the amount of precipitation.

Seed Germination and Seedling Growth Measurements

The numbers of germinated seeds were counted daily at incubation time. Ten seedlings per Petri dish were selected at random for measurement. Germination rate (GR), germination index (GI), root length (RL) and shoot length (SL) were determined. Germination, defined as visible radicle emergence (>2 mm). GR was calculated by using the ratio of the final numbers of seed germination to the total numbers of seeds when no new germination occurred after 7 days of incubation. RL and SL were measured by using a digital caliper.

Physiological Measurements

For heavy metal quantification, MDA, SP content, SOD and POD activities, were plotted under the same conditions. All these characteristics were evaluated at the end of the experiment with 14-day seedlings.

The content of MDA was determined by thiobarbituric acid (TBA) reaction, as described by Heath and Packer [27]. Firstly, 0.5 g of fully expanded leaves were grinding in solution containing 6 mL 5% (v/v) trichloroacetic acid by mortar, then the solution was centrifuged at 4,000 rpm for 15 min. Next, 2 mL of enzyme extract was taken and then added 2 mL 0.6% (v/v) thiobarbituric acid. The mixture solution was heated at 100°C for 15 min using water bath, and then quickly cooled in an ice-water bath. Eventually, the solution was centrifuged at 4,000 rpm for 15 min. The absorbance of the supernatant at 450, 532 and 600 nm was recorded. The content of MDA was calculated as follow follows: $C_{(\mu\text{M})} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$; $\text{MDA}_{(\mu\text{mol}\cdot\text{g}^{-1})} = (C \times V_T) / (1\ 000 \times W_F)$. C: MDA concentration (μM); V_T : total volume of sample extract (mL); W_F : quality of fresh sample (g).

0.5 g fully expanded leaves of *R. typhina* were homogenized in a pre-chilled mortar and pestle with 6 mL of 50 mM ice-cold Na_2HPO_4 - NaH_2PO_4 buffer (pH 7.8) containing 0.1 mM Na_2 -EDTA and 1% (W/V) polyvinyl-polypyrrolidone (PVP). The extract was centrifuged at 10000 rpm for 15 min at 4°C, and the supernatant was prepared for measurement of SP content, SOD and POD activity.

The SP content in the supernatant was determined using the dye-binding method by Bradford [28], the absorbance of the reaction solution was measured at 595 nm after 2 min and before 1 h using a spectrophotometer (ultraviolet-2006). The activity SOD was determined by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT). The absorbance of the irradiated solution was recorded at 560 nm with a spectrophotometer (ultraviolet-2006), and one unit SOD activity was defined as the amount of extract required to cause 50% inhibition of the rate of NBT reduction at 560 nm [29]. The POD activity was determined by the change in absorbance of OD_{470} due to guaiacol oxidation according to the method described by Polle [30].

Statistical Analysis

All treatment values are expressed as mean of five replicates \pm standard deviation (SD). Data were analyzed by analysis of variance (ANOVA) using SPSS (SPSS Statistics, Shanghai, China, version 17.0) to evaluate the effect of Pb, Cd and their interaction on the growth indexes and physiological measurements. Statistically significant differences were set at $P < 0.05$. Duncan's new multiple range tests were calculated when treatments were significantly different at 0.05 level.

Results

Pb and Cd Inhibited Seed Germination of *R. typhina*

The GR and GI of *R. typhina* seed greatly decreased with the concentration of Pb^{2+} and Cd^{2+} increased ($P < 0.05$). GR decreased by 6.09-25.61% and 6.09-34.15%, and GI decreased by 10.2-22.35% and 3.91-41.62% under stress of single Pb^{2+} and Cd^{2+} , respectively (Fig. 1, Table 1). Interactive effects of two heavy metal pollutants Cd and Pb in seed GR and GI were examined on their stress, GR and GI significantly decreased by 21.95-65.85% and 35.89-73.46% ($P < 0.05$) (Fig. 1, Table 1). Compared with single heavy metal stress, combine stress significantly inhibited seed germination of *R. typhina*. When the concentration of Pb^{2+} , Cd^{2+} and combine heavy metal stress was at 500 $\text{mg}\cdot\text{L}^{-1}$, 125 $\text{mg}\cdot\text{L}^{-1}$ and Pb^{2+} 500 $\text{mg}\cdot\text{L}^{-1}$ + Cd^{2+} 125 $\text{mg}\cdot\text{L}^{-1}$, there were the lowest GR (67.78%, 60% and 31.11%) and GI (5.56, 4.18 and 1.9), respectively ($P < 0.05$) (Fig. 1, Table 1).

Pb and Cd inhibited Seedling Growth

Single Pb stress under 100 $\text{mg}\cdot\text{L}^{-1}$ slightly promoted the root growth, while high concentration Pb

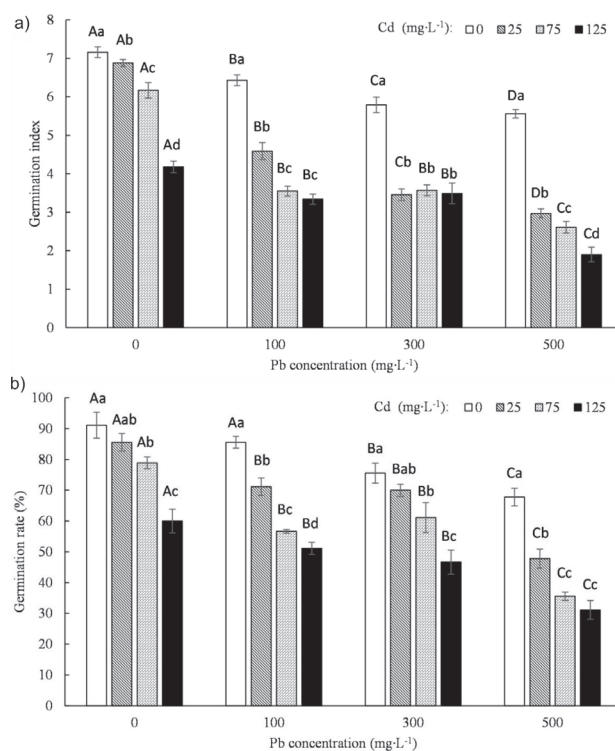


Fig. 1 Effect of Cd and Pb on Germination rate and Germination index of *R. typhina*. Each value represents mean \pm SE. Significant differences between different Cd concentration levels within a Pb concentration are indicated by lowercase letters (a-d); significant differences between different Pb concentration within a Cd concentration are indicated by capital letters (A-D; $P < 0.05$).

Table 1. ANOVA analysis of Pb²⁺ and Cd²⁺ on seed germination rate (GR), germination index (GI), root length (RL) and shoot length (SL).

Factors	DF	GR		GI		RL		SL	
		F	P	F	P	F	P	F	P
Pb ²⁺	3	121.991	<0.001	70.337	<0.001	273.043	<0.001	83.045	<0.001
Cd ²⁺	3	116.353	<0.001	59.678	<0.001	361.484	<0.001	82.858	<0.001
Pb ²⁺ × Cd ²⁺	9	3.084	0.009	3.354	0.005	27.105	<0.001	2.209	0.048

significantly inhibited the growth of roots and shoots of *R. typhina*. Compared with control, single Pb stress at 500 mg·L⁻¹ caused 60.54% and 25.73% decrease in root and shoot length, respectively (Fig. 2, Table 1). RL and SL significantly decreased with the increase of externally supplied Cd. Single Cd stress caused a 8.15-55.95% and 10.07-35.02% decrease in root and shoot length, respectively (Fig. 2, Table 1). The lowest RL (13.52 mm) and SL (19.95 mm) were observed at Cd 125, respectively.

Compared with control and single heavy metal stress, combined Pb and Cd stress significantly inhibited seedling growth of *R. typhina*, and RL and SL significantly decreased with the increase of Pb and Cd. Compared with control, combine heavy metal stress caused a 1.50-84.33% RL and 16.52-61.95% SL decrease, respectively (Fig. 2, Table 1). The lowest RL

and SL were 4.84 mm and 11.68 mm at Pb 500 mg·L⁻¹ and Cd 125 mg·L⁻¹, decreased by 84.33% and 61.99%, respectively.

Pb and Cd Impacted MDA and Soluble Protein Content

MDA content significantly increased with the increase of single externally heavy metal Pb and Cd stress (Fig. 3). Compared to control, the highest MDA content at 500 mg·L⁻¹ Pb was 21.4 nmol·g⁻¹ FW, or 125 mg·L⁻¹ Cd stress was 27.15 nmol·g⁻¹ FW, which significantly increased by 35.87% and 72.38%, respectively (Fig. 3, Table 1). The percent of increase in MDA content was relatively more in the single heavy stress of Cd than Pb. At combined heavy metal stress treatment, MDA content significantly increased with the increase of Cd concentration at Pb 500 mg·L⁻¹, however, there was no significant in MDA content between different Pb concentration at Cd 125 mg·L⁻¹. Although there was a significant interaction effect in Pb and Cd, MDA content was affected by Pb concentration when Cd reaches the maximum concentration 125 mg·L⁻¹ (Fig. 3, Table 2).

Single heavy metal (Pb or Cd) stress significantly increased soluble protein (SP) content (Fig. 3b). The highest SP content was observed 10.64 mg·g⁻¹ at single Pb 500 mg·L⁻¹ or 6.06 mg·g⁻¹ at single Cd 75 mg·L⁻¹, as compared with control, SP content significantly increased by 3.124 times and 1.359 times, respectively (Fig. 3b). Combined Pb and Cd stress also significantly increased SP content compared with control, but it was significant different between different Pb stress at same Cd stress. SP content increased and then decreased as the concentration of Pb increases at Cd 25 mg·L⁻¹, while decreased with the increase of Pb concentration at Cd 75 mg·L⁻¹ and 125 mg·L⁻¹ (Fig. 3b). The highest and the lowest SP content were 11.15 mg·g⁻¹ and 3.51 mg·g⁻¹ at combined stress, which increased by 3.322 times and 0.361 times compared with control, respectively (Fig. 3b).

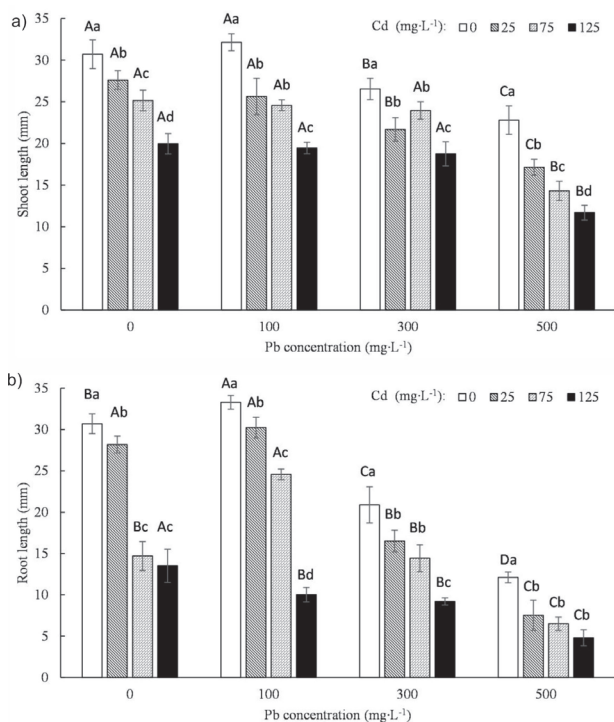


Fig. 2 Effect of Cd and Pb on Root length and shoot length of *R. typhina*. Each value represents mean±SE. Significant differences between different Cd concentration levels within a Pb concentration are indicated by lowercase letters (a-d); significant differences between different Pb concentration within a Cd concentration are indicated by capital letters (A-D); $P < 0.05$.

Pb and Cd Impacted Antioxidant Enzymes SOD and POD Activities

SOD activities fluctuated in different concentration of single Pb stress compared to the control, the dynamic tendency of SOD ascended, and then declined below

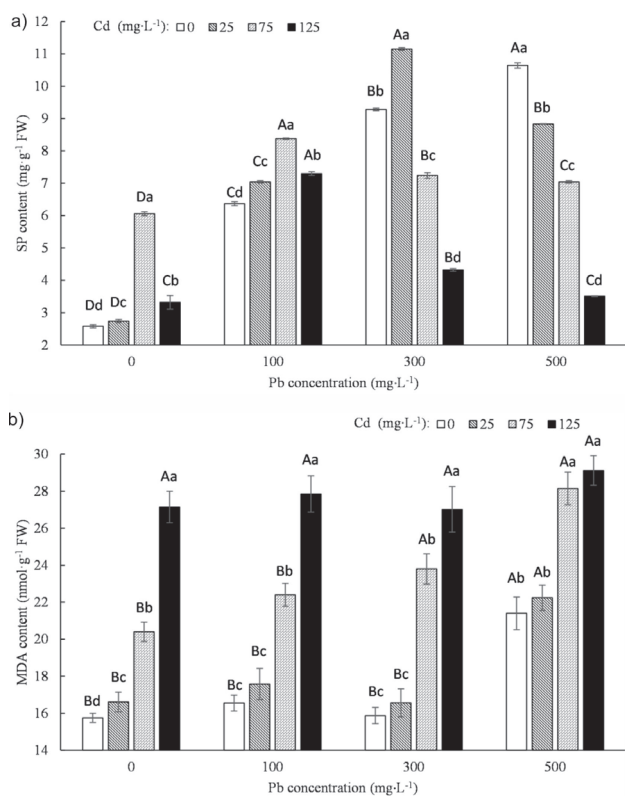


Fig. 3 Effect of Cd and Pb on malondialdehyde (MDA) and soluble protein (SP) of *R. typhina*. Each data point represents mean±SE. Significant differences between different Cd concentration levels within a Pb concentration are indicated by lowercase letters (a-d); significant differences between different Pb concentration within a Cd concentration are indicated by capital letters (A-D; $P<0.05$).

control levels at 500 mg·L⁻¹ Pb (Fig. 4a). The highest SOD was 214.86 U·g⁻¹ at 100 mg·L⁻¹ and the lowest was 104.79 U·g⁻¹ at 500 mg·L⁻¹, which increased by 53.82% and decreased by 24.98% in single Pb stress compared with control, respectively (Fig. 4a). While SOD activity decreased with the increase of single Cd stress concentration, the lowest SOD was 14.292 U·g⁻¹ at 125 mg·L⁻¹ ($P<0.05$). In combine heavy metal stress, SOD activity all significantly declined with ascend of one heavy metal stress at another heavy metal stress except 300 mg·L⁻¹ Pb and 75 Cd mg·L⁻¹ (Fig. 4a). The lowest SOD was 26.10 U·g⁻¹ at 500 mg·L⁻¹ Pb and 125 Cd mg·L⁻¹ (Fig. 4a), which decreased by 81.31%.

In single heavy metal stress, POD activity significantly increased with the increase of Pb or Cd

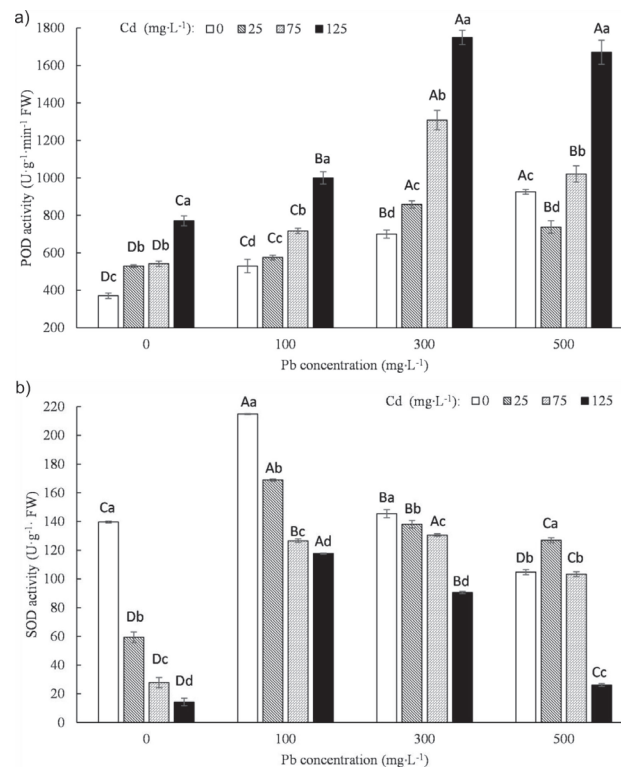


Fig. 4 Effect of Cd and Pb on superoxide dismutase (SOD) and peroxidase (POD) activity of *R. typhina*. Each value represents mean±SE. Significant differences between different Cd concentration levels within a Pb concentration are indicated by lowercase letters (a-d); significant differences between different Pb concentration within a Cd concentration are indicated by capital letters (A-D; $P<0.05$).

stress except no significant different between 25 mg·L⁻¹ and 75 mg·L⁻¹ single Cd stress (Fig. 4b). Compared to control, the highest POD at 500 mg·L⁻¹ Pb and 125 mg·L⁻¹ Cd stress were 925 U·g⁻¹·min⁻¹ and 770 U·g⁻¹·min⁻¹, which significantly increased by 149.44% and 107.87%, respectively (Fig. 4b, Table 2). In combined heavy metal stress, POD activity all significantly increased with the increase of Cd stress at same Pb level, while POD firstly significantly increased, and then declined with the increase of Pb concentration at same Cd level except no statistic significant changes between Pb 300 mg·L⁻¹ and 500 mg·L⁻¹ at Cd 125 mg·L⁻¹ (Fig. 4b, Table 2). The highest POD activity was 1750 U·g⁻¹·min⁻¹ at Pb 300 mg·L⁻¹ and Cd 125 mg·L⁻¹, which ascended by 371.91% compared with control ($P<0.05$).

Table 2. ANOVA analysis of Pb²⁺ and Cd²⁺ on MDA, soluble protein (SP), SOD and POD.

Factors	DF	MDA		SP		SOD		POD	
		F	P	F	P	F	P	F	P
Pb ²⁺	3	373.718<0.001		2627.049<0.001		5503.596<0.001		674.270<0.001	
Cd ²⁺	3	67.074<0.001		6295.351<0.001		6754.191<0.001		619.157<0.001	
Pb ²⁺ × Cd ²⁺	9	7.307<0.001		2241.736<0.001		622.261<0.001		53.921<0.001	

Table 3. Pearson correlation matrix for Pb²⁺, Cd²⁺ concentrations and seed germination rate (GR), germination index (GI), root length (RL) and shoot length (SL), MDA, soluble protein (SP), SOD and POD of *Rhus typhina*

Items	Pb ²⁺	Cd ²⁺	GR	GI	RL	SL	MDA	SP	SOD	POD
Pb ²⁺	1									
Cd ²⁺	0	1								
GR	-.642**	-.679**	1							
GI	-.578**	-.631**	.893**	1						
RL	-.665**	-.612**	.806**	.713**	1					
SL	-.645**	-.637**	.904**	.814**	.871**	1				
MDA	.308*	.872**	-.820**	-.692**	-.734**	-.753**	1			
SP	.486**	-.335*	-.084	-.189	-.217	-.066	-.162	1		
SOD	.027	-.619**	.324*	.185	.531**	.489**	-.533**	.404**	1	
POD	.580**	.662**	-.745**	-.708**	-.699**	-.685**	-.692**	-.031	-.307*	1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Correlation of Pb and Cd Stress on Seed Germination and Seedling Growth

Pearson correlation analysis showed that morphological and physiological parameters of seed germination and seedling growth in *Rhus typhina* were strongly affected by Pb and Cd concentrations except SOD under Pb stress (Table 3). Pb and Cd stress had significant negative correlations with all morphological indicators and significant positive with MDA and POD. Pb had significant positive correlations with SP, nevertheless, Cd had significant negative correlations with SP and SOD. MDA and POD had significant negative with all morphological indicators of seed germination and seedling growth in *Rhus typhina*, while SOD had significant positive with GR, RL and SL. The highest correlation of heavy metal stress was observed in Cd and GR of morphological parameters with 0.679 values, and MDA of physiological parameters with 0.872 values ($P < 0.01$).

Discussion

The present study examined the single and combined effects of the two key metal pollutants Cd and Pb in growth medium on their uptake and the contribution of oxidative stress in expression of their toxicities during establishment of *Rhus typhina* seedlings, in order to understand the combined effects of Cd and Pb on seed germination and early seedling growth in *Rhus typhina* and possible relationship of individual and combined excess levels as well as the response of antioxidative defence system of *Rhus typhina* seedlings.

Heavy metal contamination of soil causing toxicity has become one of the most damaging abiotic stress to restrict the survival and development of plants [31, 32]. Seed germination is the starting point in the

life cycle of a plant and is also the earliest stage of a plant's exposure to heavy metal stress, so detection of seed germination and seedling growth index is a good method to examine the capacity of higher plants to heavy metal toxicity pollutant [25, 33, 34]. Some previous studies and our results clearly indicate that seed germination and seedling growth index of higher plant significantly affected by Cd and/or Pb stress and greatly decreased with the concentration increased, regardless of whether individual effects of each metal or combined treatment [12, 35] (Figs 1-2, Table 3). Unlike other heavy metals (Cu, Zn, Mn, etc.) are considered as micronutrients by promoting seed germination and seedling growth in low concentrations and inhibiting in high concentrations, both Pb and Cd are known phytotoxicant to disturb metabolic and growth processes even at very low concentrations being nonessential for plant metabolic activities and without physiological function in plants [1, 36, 37]. Furthermore, compared with single heavy metal treatment, the toxicity on seed germination and seedling growth of *Rhus typhina* was strengthened remarkably under combined heavy metal stress (Figs 1-2). This phenomenon can be attributed to the synergistic effects of Cd and Pb [38].

MDA is generally used to indicate the degree of plant cell membrane damage as one final decomposition product of lipid peroxidation, and reflected the ROS level under heavy metal stress [39]. Our results showed an appreciable rise in MDA and SP compared to no heavy metal stress under single or combined Pb and Cd stress due to the free radical-induced membrane damage (Fig. 3). MDA significantly increased and correlated with the increase of single and combined Pb and Cd stress (Fig. 3a and Table 3). Nevertheless, different responses were observed in SP from MDA when exposed to Pb and various Cd levels (Fig. 3). Cd and Pb could induce plants to synthesize Phytochelatin (PC), and Pb could be complexed with metallothionein (MT)

to form metallothionein complex (Pb-MT) at their low concentrations to alleviate the toxicity of heavy metal stress to plant cells, which could be reflected by soluble proteins [40]. However, plant cells were damaged by excess heavy metal ions due to the inhibit expression of Pb-MT and Cd-PC with the increase of heavy metals, so SP content decreased. Pearson correlation analysis in present studies showed that although heavy metal stress caused the increase of SP, it was not related to the indicators of seed germination and seedling growth in *Rhus typhina* (Table 3).

Heavy metals stress can induce plants to produce reactive oxygen species (ROS) which can interact with DNA, pigments, proteins, lipids, and other essential cellular macromolecules ultimately leading to a chain of destructive processes [41, 42]. Being sessile organisms, plants have to develop a specific mechanism known as the plant antioxidant defense system that regulates ROS levels to keep ROS at physiological limit, preventing them from exceeding toxic threshold levels in the cellular system at a particular time [43]. As two primary antioxidative enzyme, previous studies revealed that SOD and POD play a relevant role in treatment of heavy metal enhances ROS formation [44, 45]. SOD can eliminate $O_2^{\cdot-}$, decrease peroxidation of membrane lipids and maintain cell membrane stability, and POD participate in the decomposition of H_2O_2 [25, 46]. In the present study and other studies showed that the activities of POD significantly increased and correlated with heavy metal stress, suggesting that plants adjusted their POD activity to protect membrane stability from heavy metal-induced oxidative damage [37, 47] (Fig. 4 and Table 3). Interestingly, our experiment showed that SOD activity significantly decreased with the increase of Cd stress whether Pb is presence or not, except in $500\text{ mg}\cdot\text{L}^{-1}$ Pb, but first increased and then decreased under Pb stress in the presence of Cd (Fig. 4). Although many experiments showed that the activity of SOD increased with the concentration of Cd and Pb under single and combined stress, some previous studies also showed that Pb or Cd inhibited the SOD activity, who concluded that it may be the dynamic balance of ROS is broken by the excessive accumulation of ROS under elevated concentrations of heavy metal stress in plants [48, 49]. Heavy metals could produce severe toxicity symptoms which destroyed the antioxidant enzyme system in plants. These results suggested that plant antioxidant enzymes have different sensitivity to Pb and Cd stress which results in different antioxidant enzyme response ability in different plant species [50, 51, 52]. These results also indicated that there were significant ion interactions and antagonistic effects between Pb and Cd (Fig. 4 and Table 3).

Conclusions

In summary, Cd and Pb stress significantly influenced early plant growth in *Rhus typhina*, and

seed germination and seedling growth of *Rhus typhina* decreased with increasing Cd and Pb, and the joint effect was more serious than single stress. MDA and POD can be used as critical indicators of the resistances to Cd and Pb stress and their interaction at seed germination and an early stage of seedling growth in *Rhus typhina*. Our results also confirmed that *Rhus typhina* was never completely inhibited in its early growth and could be used for phytoremediation on Pb and Cd contaminated soil, although seed germination and seedling growth of *Rhus typhina* decreased with increasing Cd and Pb whether single or combine stress.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. SENEVIRATNE M., RAJAKARUNA N., RIZWAN M., MADAWALA H.M.S.P., OK Y.S., VITHANAGE M. Heavy metal-induced oxidative stress on seed germination and seedling development: a critical review. *Environmental Geochemistry and Health*, **41** (4), 1813, **2019**.
2. HASSAN M., SIMEEN M. Oxidative stress and antioxidant defense mechanism in mung bean seedlings after lead and cadmium treatments. *Turkish Journal of Agriculture and Forestry*, **55**, **2014**.
3. SHETEIWY M. S., DONG Q., AN J., SONG W., GUAN Y., HE F., HUANG Y., HU J. Regulation of ZnO nanoparticles-induced physiological and molecular changes by seed priming with humic acid in *Oryza sativa* seedlings. *Plant Growth Regulation*, **83** (1), 27, **2017**.
4. SCHUTZENDUBEL A., POLLE A. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of experimental botany*, **53** (372), 1351, **2002**.
5. DEY S.K., DEY J., PATRA S., POTHAL D. Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. *Brazilian Journal of Plant Physiology*, **19**, 53, **2007**.
6. LESMANA S.O., FEBRIANA N., SOETAREDJO F.E., SUNARSO J., ISMADJI S. Studies on potential applications of biomass for the separation of heavy metals from water and waste water. *Biochemical Engineering Journal*, **44**, 19, **2009**.
7. CLEMENS S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie*, **88** (11), 1707, **2006**.
8. DAGO A., GONZALEZ I., ARINˆO C., DI ˆAZ-CRUZ J M., ESTEBAN M. Chemometrics applied to the analysis of induced phytochelatin in *Hordeum vulgare* plants stressed

- with various toxic non-essential metals and metalloids. *Talanta*, **118**, 201, **2014**.
9. APRILE A., SABELLA E., FRANZIA E., MILC J., RONGA D., PECCHIONI N., FERRARI E., LUVISI A., VERGINE M., DE BELLIS L. Combined effect of cadmium and lead on durum wheat. *International Journal of Molecular Sciences*, **20**, 5891, **2019**.
 10. ABIOYE O.P., AINA P.F., IJAH J.U., ARANSIOLA A.S. Effects of cadmium and lead on the biodegradation of diesel-contaminated soil. *Journal of Taibah University for Science*, **13** (1), 628, **2019**.
 11. BEWLEY J.D. Seed germination and dormancy. *The Plant Cell*, **9** (7), 1055, **1997**.
 12. HSU F.H., CHOU C.H. Inhibitory effects of heavy metals on seed germination and seedling growth of *Miscanthus* species. *Botanical Bulletin of Academia Sinica*, **33** (4), 335, **1992**.
 13. RODRÍGUEZ-ALONSO J., SIERRA M.J., LOMINCHAR M.Á., MILLÁN R. Effects of mercury on the germination and growth of *Quercus ilex* L. seedlings. *Environmental Science and Pollution Research*, **26** (30), 30930, **2019**.
 14. BARCELO J., POSCHENRIEDER C. Plant water relations as affected by heavy metal stress: A review. *Journal of Plant Nutrition*, **13** (1), 1, **1990**.
 15. SEREGIN I.V., IVANOV V.B. Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian journal of plant physiology*, **48** (4), 523, **2001**.
 16. GONZALES DE SOUZA G., MENDES PINHEIRO A.L., SILVA J.A. VERONEZE-JÚNIOR V., CARVALHO M., BERTOLI A.C., BARBOSA S., DE SOUZA T.C. Morphophysiological Tolerance Mechanisms of *Talinum patens* to Lead. *Water, Air, Soil Pollution*, **229**, 4, **2018**.
 17. MAHDIEH S., GHADERIAN S.M., KARIMI N. Effect of arsenic on germination, photosynthesis and growth parameters of two winter wheat varieties in Iran. *Journal of Plant Nutrition*, **36** (4), 651, **2013**.
 18. VA'ZQUEZ DE ALDANA B., GUNDEL P., GARCIA CRIADO B., GARCIA CIUDAD A., GARCIA SA'NCHEZ A., ZABALGOGEAZCOA I. Germination response of endophytic *Festuca rubra* seeds in the presence of arsenic. *Grass & Forage Science*, **69** (3), 462-469, **2014**.
 19. COKKIZGIN A., COKKIZGIN H. Effects of lead (PbCl₂) stress on germination of lentil (*Lens culinaris* Medic.) lines. *African Journal of Biotechnology*, **9** (50), 8608, **2015**.
 20. LOVETT-DOUST J., LOVETT-DOUST L. Modules of production and reproduction in a dioecious clonal shrub, *Rhus typhina*. *Ecology*, **69** (3), 741, **1988**.
 21. GANDHI K.N. Staghorn sumac: *Rhus typhina* or *R. hirta* (anacardiaceae). *Rhodora*, **118** (974), 232, **2016**.
 22. WANG C., LIU J., XIAO H., ZHOU J. Differences in leaf functional traits between *Rhus typhina* and native species. *Clean Soil Air Water*, **44** (11), 1591, **2016**.
 23. WANG G., JIANG G., YU S., LI Y., LIU H. Invasion possibility and potential effects of *Rhus typhina* on Beijing municipality. *Journal of Integrative Plant Biology*, **50** (5), 522, **2008**.
 24. KUDISH M. Adirondack Upland Flora: an Ecological Perspective. Saranac, NY, Chauncy Press, 320, **1992**.
 25. XU Z., ZHOU Q., LIU W. Joint effects of cadmium and lead on seedlings of four Chinese cabbage cultivars in northeastern China. *Journal of environmental sciences*, **21**, 1598, **2009**.
 26. ZITER C., TURNER M.G. Current and historical land use influence soil-based ecosystem services in an urban landscape. *Ecological Applications*, **28** (3), 643, **2018**.
 27. HEATH R.L., PACKER L. Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125**, 189, **1968**.
 28. BRADFORD M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248, **1976**.
 29. GIANNOPOLITIS C.N., RIES S.K. Superoxide dismutase: I. occurrence in higher plants. *Plant Physiology*, **59**, 309, **1997**.
 30. POLLE A., OTTER T., SEIFERT F. Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). *Plant Physiology*, **106** (1), 53, **1994**.
 31. SINGH S., PARIHAR P., SINGH R., SINGH V.P., PRASAD S.M. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Frontiers in Plant Science*, **6**, 1143, **2016**.
 32. JALMI S.K., BHAGAT P.K., VERMA D., NORAYANG S., TAYYEB A.S., SINGH K., SHARMA D., SINHA A.K. Traversing the links between heavy metal stress and plant signaling. *Frontiers in plant science*, **9**, 12, **2018**.
 33. BAE J., BENOIT D.L., WATSON A.K. Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes. *Environmental Pollution*, **213**, 112, **2016**.
 34. TAGHIZADEH M., SOLGI E. Impact of Heavy Metal Stress on In Vitro Seed Germination and Seedling Growth Indices of Two Turfgrass species. *Journal of Rangeland Science*, **7** (3), 220, **2017**.
 35. WANG J., LI W., ZHANG C., KE S. Physiological responses and detoxification mechanisms to Pb, Zn, Cu and Cd in young seedlings of *Paulownia fortunei*. *Journal of Environmental Sciences*, **22**, 1916, **2010**.
 36. BARUAH N., MONDAL S.C., FAROOQ M., GOGOI N. Influence of heavy metals on seed germination and seedling growth of wheat, pea, and tomato. *Water, Air, & Soil pollution*, **230** (12), 273, **2019**.
 37. NAGAJYOTI P.C., LEE K.D., SREEKANTH T.V.M. Heavy metals, occurrence and toxicity for plants: A review. *Environmental Chemistry Letters*, **8** (3), 199, **2010**.
 38. LOU Y., ZHAO P., WANG D., AMOMBO E., SUN X., WANG H., ZHUGE Y. Germination, physiological responses and gene expression of tall fescue (*Festuca arundinacea* Schreb.) growing under Pb and Cd. *PLoS ONE*, **12** (1), e0169495, **2017**.
 39. TAUQEER H.M., ALI S., RIZWAN M., ALI Q., SAEED R., IFTIKHAR U., AHMAD R., FARID M., ABBASI G.H. Phytoremediation of heavy metals by *Alternanthera bettzickiana*: growth and physiological response. *Ecotoxicology and Environmental Safety*, **126**, 138, **2016**.
 40. COBBETT C. Phytochelatin and metallothionein: roles in heavy metal detoxification and homeostasis. *Annual Review of Plant Biology*, **53**, 129, **2002**.
 41. CORTÉS-ESLAVA J., GÓMEZ-ARROYO S., RISUEÑO M.C., TESTILLANO P.S. The effects of organophosphorus insecticides and heavy metals on DNA damage and programmed cell death in two plant models. *Environmental Pollution*, **240**, 77, **2018**.
 42. SÜMER ERCAN, F., ERCAN, N., DEMIREZEN YILMAZ, D. Effect of heavy metal stress on antioxidant enzymes and DNA damage in *Nasturtium officinale* R. Br. (watercress). *Toxin Reviews*, **38** (4), 328, **2019**.
 43. APEL K., HIRT H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, **55**, 373, **2004**.

44. FAROOQ M.A., ALI S., HAMEED A., ISHAQUE W., MAHMOOD K., IQBAL Z. Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicology and Environmental Safety*, **96**, 242, **2013**.
45. BASHRI G., PRASAD S.M. Indole acetic acid modulates changes in growth, chlorophyll a fluorescence and antioxidant potential of *Trigonella foenum-graecum* L. grown under cadmium stress. *Acta Physiologiae Plant*, **37**, 1745, **2015**.
46. LI S., LEI N., CHEN J. Differential Effects of Natural Palygorskite and its Modified Form on Alleviation of Heavy Metals Toxicity to Tomato Grown in Soil Contaminated with Cd, Pb and Zn. *Polish Journal of Environmental Studies*, **28** (6), **2019**.
47. GAO Y., MIAO C.Y., XIA J., LUO C.Y., MAO L., ZHOU P., SHI W. Effect of citric acid on phytoextraction and antioxidative defense in *Solanum nigrum* L. as a hyperaccumulator under Cd and Pb combined pollution. *Environmental Earth Science*, **65**, 1923, **2012**.
48. MAO X., XB-CHEN L.L. Effects of heavy metal Pb and Cd stress on physiological characteristics of Japanese honeysuckle. *Applied ecology and environmental research*, **17** (3), 6415, **2019**.
49. CHEN Y.P., LIU Q., YUE X.Z., MENG Z.W., LIANG J. Ultrasonic vibration seeds showed improved resistance to cadmium and lead in wheat seedling. *Environmental Science and Pollution Research*, **20** (7), 4807, **2013**.
50. SUN R.L., ZHOU Q.X., SUN F.H., JIN C.X. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. *Environmental and Experimental Botany*, **60** (3), 468, **2007**.
51. DAI W., KE X., LI Z., GAO M., WU L., CHRISTIE P., LUO Y. Antioxidant enzyme activities of *Forsydia candida* and avoidance of soil metal contamination. *Environmental Science and Pollution Research*, **25** (3), 2889, **2018**.
52. SRIVASTAVA R.K., PANDEY P., RAJPOOT R., RANI A., DUBEY R. Cadmium and lead interactive effects on oxidative stress and antioxidative responses in rice seedlings. *Protoplasma*, **251** (5), 1047, **2014**.

