Effects of Arabidopsis thaliana-Maize Intercropping on Root Exudate Organic Acids under Lead Stress: Postprint

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Abstract

To reveal the effects of Pb stress on low-molecular-weight organic acids secreted by roots of hyperaccumulator plants and crops under intercropping and monoculture conditions, this study established a Pb stress condition of 400 mg · L ^{1} using a hydroponic aeration method. With monocultured maize and Arabidopsis thaliana as control treatments, the effects of maize and Arabidopsis thaliana intercropping under Pb stress on plant root morphology, root-secreted organic acids, and Pb uptake were investigated. The results showed that: compared with monoculture, when intercropped with Arabidopsis thaliana, lactic acid was detected in maize root exudates; the number of lateral roots, root surface area, and root density of maize increased by 60%, 15%, and 42%, respectively, compared with monoculture, and the dry weight biomass of roots and shoots increased by 108% and 75%, respectively, while Pb content in maize roots decreased by 44%; compared with monoculture, when intercropped with maize, acetic acid and lactic acid were detected in Arabidopsis thaliana root exudates, the amount of root exudates from Arabidopsis thaliana increased by 103%-1,700% compared with monoculture, the Pb accumulation in roots and shoots of Arabidopsis thaliana increased by 49% and 75%, respectively, compared with monoculture, and the translocation factor increased by 22%. Correlation analysis results showed that for monocultured Arabidopsis thaliana, only shoot Pb accumulation was significantly correlated with oxalic acid, whereas for intercropped Arabidopsis thaliana, both root and shoot Pb accumulation were significantly correlated with oxalic acid, citric acid, and malic acid. The study demonstrates that in the hyperaccumulator plant Arabidopsis thaliana and maize intercropping system, root-secreted organic acids alter the Pb accumulation characteristics in both Arabidopsis thaliana and maize, promoting Pb accumulation in the hyperaccumulator plant Arabidopsis thaliana while reducing Pb content in the crop maize. Intercropping hyperaccumulator plant

Arabidopsis thaliana with maize under Pb stress represents a feasible remediation model.

Full Text

Effects of *Arabis alpina* L. var. *parviflora* Franch and *Zea mays* L. Intercropping on Root-Exudated Organic Acids Under Lead Stress

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Abstract

To investigate the effects of Pb stress on low-molecular-weight organic acid exudation by hyperaccumulator and crop roots under intercropping versus monocropping systems, a hydroponic aeration experiment was conducted with 400 mg · L ^{1} Pb stress. Using monocropped maize and *Arabis alpina* as controls, we examined the impacts of intercropping maize with *A. alpina* on plant root morphology, root exudate composition, and Pb uptake.

The results demonstrated that compared with monocropped maize, lactic acid was detected in root exudates of intercropped maize. Intercropped maize showed increases of 60%, 15%, and 42% in root branching number, root surface area, and root density, respectively, while root and shoot biomass increased by 108% and 75%, respectively, and root Pb content decreased by 44%. Compared with monocropped *A. alpina*, acetic acid and lactic acid were detected in root exudates of intercropped *A. alpina*, with total root exudate amounts increasing by 103%-1,700%. Pb accumulation in underground and aboveground parts of intercropped *A. alpina* increased by 49% and 75%, respectively, with a 22% increase in Pb translocation coefficient.

Correlation analysis revealed that for monocropped *A. alpina*, only shoot Pb accumulation was significantly correlated with oxalic acid, whereas for intercropped *A. alpina*, both root and shoot Pb accumulation were significantly correlated with oxalic, citric, and malic acids. These findings indicate that in the *A. alpina*-maize intercropping system, root-exuded organic acids altered Pb accumulation patterns, promoting Pb accumulation in the hyperaccumulator *A. alpina* while reducing Pb content in the crop maize. Intercropping hyperaccumulator *A. alpina* with maize under Pb stress represents a feasible remediation strategy.

Keywords: Lead stress; *Arabis alpina* L. var. *parviflora* Franch; *Zea mays* L.; Intercropping; Root exudate; Organic acid

Introduction

Roots are the primary organs for plants to absorb nutrients and water from the environment, and they are influenced by both beneficial and toxic elements in their surroundings [1]. Simultaneously, roots exude protons and ions while releasing substantial amounts of organic substances into the environment. The interaction between plant roots and the soil interface is reciprocal and dynamic, with plant roots evolving various morphological adaptations to cope with pollution stress during growth [2,3]. Consequently, the rhizosphere microecological environment has attracted increasing attention, particularly regarding phytoremediation of heavy metal-contaminated soils [4]. Under heavy metal stress, low-molecular-weight organic acids secreted by roots can influence heavy metal speciation, mobility, and accumulation characteristics by altering physicochemical properties such as pH and redox potential in the rhizosphere. These root exudates can either promote or inhibit the growth of intercropped plants [5]. Currently, the relationship between root exudates and heavy metals has become a focal research topic.

Previous studies have documented relationships between low-molecular-weight organic acids in root exudates and heavy metals. In hydroponic and sand culture experiments with wheat (*Triticum aestivum* L.) exposed to 0.5 $\mathrm{mg \cdot L}^{\ 1}$ and 5 $\mathrm{mg \cdot L}^{\ 1}$ Cd, citric acid and oxalic acid were detected in root exudates [3]. In ryegrass (*Lolium perenne* L.), the types of secreted organic acids increased with Pb treatment concentration: at 0–400 $\mathrm{mg \cdot L}^{\ 1}$ Pb, oxalic, tartaric, and malic acids were secreted, while at 600–1,000 $\mathrm{mg \cdot L}^{\ 1}$ Pb, acetic and citric acids were additionally detected [4,5]. Aluminum stress induced citric and malic acid secretion in rye roots [6,7]. Exogenous addition of organic acids to wheat cultures at different pH levels revealed that at pH 4.0, tartaric acid increased shoot Cd accumulation while malic acid decreased it [3,8]. Malonic and tartaric acids at 1–3 $\mathrm{mmol \cdot L}^{\ 1}$ increased Pb concentration in ryegrass shoots, whereas acetic acid increased root Pb concentration [4]. In different Cd-accumulating amaranth cultivars (*Amaranthus mangostanus* L.), increased secretion of low-molecular-weight organic acids correlated positively with Cd uptake [9].

Studies on root exudates in hyperaccumulator plants have yielded varying results regarding heavy metal resistance and tolerance. Research on *Elsholtzia haichouensis* Sun and *Commelina communis* L. demonstrated that root exudates could mobilize Cu in contaminated soils, with *C. communis* showing greater Cu-mobilizing capacity than *E. haichouensis* [10]. However, studies on two ecotypes of *Sedum alfredii* H. found no significant differences in Zn accumulation related to root exudate composition or quantity [11]. Previous research indicates that different plant species in intercropping systems exhibit distinct root exudate components and amounts [12], with greater phylogenetic distance between plants corresponding to larger differences in root exudate composition and quantity. Furthermore, the types and amounts of root exudates vary with environmental stress factors such as heavy metal type and concentration.

Current research has documented effects of heavy metal stress and monocropping or crop-crop intercropping patterns on plant growth, heavy metal accumulation, and low-molecular-weight organic acid secretion. For example, intercropping *Solanum nigrum* L. with *Aloe vera* L. under Cd stress alleviated Cd toxicity in *A. vera* [13]. Under As stress, citric acid and total organic acids in *Panax notoginseng* (Burk.) F. H. Chen root exudates increased with As concentration [14]. Wheat and faba bean intercropping increased both the types and amounts of organic acids secreted by both species compared with monocropping [12]. However, previous studies have analyzed heavy metal stress effects in isolation, with few reports on the relationship between root exudate organic acids and heavy metal content under hyperaccumulator-crop intercropping systems. The role of root exudates in heavy metal ion uptake by hyperaccumulator roots in intercropping systems warrants further investigation. This study established monoculture controls of hyperaccumulator *Arabis alpina* L. var. *parviflora* Franch and maize (*Zea mays* L.), and an intercropping treatment, to investigate the effects of Pb stress (400 mg · L^{1}) on root morphology, biomass, and root exudate organic acids. Our objective was to explore the relationship between root exudate organic acids and Pb content in the *A. alpina*-maize intercropping system.

Materials and Methods

1.1 Experimental Materials

The test plant *Arabis alpina* L. var. *parviflora* Franch (Brassicaceae) is an annual herbaceous hyperaccumulator. In mining areas, shoot Pb content can reach 1,711.8 mg · kg^{1} and root Pb content 1,963.2 mg · kg^{1}, classifying it as a Pb hyperaccumulator [15].

A. alpina seeds were collected from abandoned Pb-Zn mining areas in Huize County, Yunnan Province, and stored in the laboratory for future use. The Huize Pb-Zn mine is located in the Qujing region of northeastern Yunnan, within the Wumeng Mountain range (elevation: 2,463–2,516 m; 103°03 –103°55 E, 25°48 –28°38 N), covering an area of 5 km^2. The mine has reserves of 15.28 million tons, ranking second in Yunnan, with ore grades of 28 g · kg^{1} Pb and 77 g · kg^{1} Zn.

For seed cultivation, uniformly sized, plump seeds of *A. alpina* were selected and sterilized with 10% Antiformin (20× dilution) for 20 min, then rinsed with distilled water. Seeds were transferred to floating trays (1–2 seeds per cell) using tweezers. During seedling cultivation, conventional watering and light management were performed under controlled conditions of 16 h light: 8 h dark. Seedlings were transplanted after developing five true leaves.

The maize cultivar 'Huidan 4' was used as the test crop. Uniformly sized, plump seeds were surface-sterilized with 10% H O for 10 min, rinsed with distilled water, and sown in a seedbed. Seedlings were transplanted after developing two true leaves.

1.2 Experimental Methods

The pot experiment was conducted at the Soil and Fertilizer Laboratory of the Agricultural Science Basic Experimental Teaching Center, Yunnan Agricultural University. Hydroponic containers were white plastic boxes (30 cm × 44 cm × 24.3 cm). The nutrient solution was modified Hoagland's formula (30 L volume), replaced every 3 days, with pH adjusted to 6.0 using 0.1 mol · L ^{1} NaOH and HCl. Three planting patterns were established: maize monocropping, *A. alpina* monocropping, and maize-*A. alpina* intercropping. Seedlings were transplanted with 10 cm spacing between plants and rows. Intercropping followed a 1:1 ratio of *A. alpina* to maize. Monocropped pots contained 12 plants each, while intercropped pots contained 6 plants of each species. Continuous aeration was provided 24 h daily. Each treatment had 4 replicates arranged in a completely randomized design (12 pots total). Natural light was provided for 6 h daily by moving pots outdoors. After 20 days of growth, Pb stress was applied at 400 mg(Pb) · L ^{1} using analytical grade Pb(NO) , following Qiao et al. [5]. Following Pb addition, the nutrient solution was not replaced; instead, evaporative losses were replenished daily. After 10 days of Pb stress treatment, root exudates were collected.

1.3 Root Morphology Measurement

After 10 days of Pb stress, plants were removed from the nutrient solution and rinsed with tap water. Underground and aboveground parts of maize and *A. alpina* were separated. Root systems were scanned individually using an EPSON PERFECTION V700 scanner. Root parameters were analyzed using WinRHIZO-Pro 2013 software (Regent Instruments Inc.). Following Costa et al. [16], root length, surface area, density, and lateral root number were determined for *A. alpina*.

1.4 Collection and Analysis of Root Exudates

The Pb-containing nutrient solution was removed from hydroponic containers, and plants were rinsed with deionized water and aerated for 12 h. Roots were then washed three times with deionized water. Plants were placed in beakers containing 500 mL deionized water, and exudates were collected under natural light with aeration for 12 h. All collected solutions were filtered slowly through 0.45 m microporous membranes [13,14]. The filtrate was concentrated to approximately 10 mL using a rotary evaporator at 60°C and stored below -20°C for low-molecular-weight organic acid analysis. Plant stems, leaves, and roots were collected separately, oven-dried at 105°C for 30 min, then at 70°C for over 24 h until constant weight for biomass determination. Dried plant samples were ground and passed through a 0.149 mm nylon sieve for subsequent Pb content analysis.

Low-molecular-weight organic acids were analyzed using high-performance liquid chromatography (HPLC) under the following conditions: Agilent 1100

HPLC system with Ionpac AS11-HC separation and guard columns, ASRS-11 anion micro-membrane suppressor, conductivity detector, CQ250 degassing unit, and Agilent Zorbax SB-C18 column (4.6 mm × 250 mm, 5 m). The eluent consisted of 2% methanol and 98% water (containing 0.4% phosphoric acid), with a flow rate of 0.6 mL · min ^{1} at 30°C column temperature, 15 L injection volume, 210 nm wavelength, and 1 s conductivity detection sensitivity. Organic acids measured included oxalic, tartaric, citric, malic, lactic, and acetic acids.

Standard solution preparation: Standards were purchased from Aladdin Chemistry Co. Ltd (purity: 100%). Accurately weighed amounts of oxalic acid (7.04 mg), tartaric acid (7.07 mg), citric acid (2.06 mg), malic acid (2.73 mg), lactic acid (8.47 mg), and acetic acid (15.10 mg) were dissolved in 10 mL volumetric flasks with methanol to prepare stock solutions. Stock solutions were serially diluted with methanol at 10×, 20×, 50×, 100×, and 200×. Five microliters of each diluted standard were injected into the HPLC system to establish calibration curves using external standard methods.

Heavy metal Pb content determination: Plant samples were digested with HNO -HClO , cooled, filtered, and analyzed for Pb using atomic absorption spectrophotometry (Beijing Purkinje General TAS-990 AAS).

1.5 Statistical Analysis

All data represent means of four replicates. Statistical analysis was performed using Microsoft Excel 2000. Significant differences were determined using Duncan' s new multiple range test (DPS software) at $P < 0.05$ significance level.

Results

2.1 Effects of Intercropping on Root Morphology of *A. alpina* and Maize Under Pb Stress

Intercropping significantly altered root morphology of both *A. alpina* and maize compared with monocropping. Intercropped maize exhibited increases of 59.83%, 14.85%, and 42.11% in root branching number, root surface area, and root density, respectively. Intercropped *A. alpina* showed increases of 25.68%, 103.89%, 46.85%, and 78.26% in root length, branching number, surface area, and density, respectively. The intercropping system significantly modified root morphological structures of both species under Pb stress (Table 1).

Table 1 Effect of Pb stress on root morphology of intercropped and monocropped *Arabis alpina* and maize

Plant	Planting pattern	Root length (cm)	Root number per plant	Root surface area (cm^{2})	Root density (cm · cm ^{3})
Maize (*Zea mays*)	Monocropping	40.3±1.9a	6.7±1.52b	125.6±6.8b	0.38±0.06b
	Intercropping	58.3±12.1b	13.6±4.0a	144.3±8.6a	0.54±0.07a
A. alpina	Monocropping	38.7±3.6a	14.8±2.1b	52.2±8.5b	0.23±0.09b
	Intercropping	93.2±7.0a	18.6±1.12a	76.6±7.5a	0.41±0.10a

Values are means ± SD. Different lowercase letters within the same column for the same plant indicate significant differences between monocropping and intercropping ($P < 0.05$). The same below.

2.2 Effects of Intercropping on Plant Biomass Under Pb Stress

Plant biomass was separated into underground and aboveground components. Intercropped maize showed a significant 108% increase in aboveground dry weight compared with monocropped maize (Figure 1 [Figure 1: see original paper]A), while aboveground biomass of intercropped *A. alpina* did not differ significantly from its monocropped counterpart. However, underground dry weight of intercropped *A. alpina* increased significantly by 75% compared with monocropped plants (Figure 1B).

Root-to-shoot ratio analysis revealed that intercropped maize biomass was 0.79 g · plant ^{1} compared with 0.66 g · plant ^{1} in monoculture (20% increase). For *A. alpina*, the root-to-shoot ratio was 0.78 under intercropping versus 0.50 under monoculture (56% increase). Intercropping significantly affected root biomass of *A. alpina*.

Figure 1 Effect of Pb stress on biomass of intercropped and monocropped maize (A) and *Arabis alpina* (B). Different lowercase letters indicate significant differences between monocropping and intercropping for the same plant ($P < 0.05$). The same below.

2.3 Effects of Intercropping on Root Exudate Organic Acids Under Pb Stress

Monocropped maize root exudates contained five organic acids: oxalic, citric, tartaric, malic, and acetic acids. Intercropped maize exudates included these five plus lactic acid. Contents of oxalic, citric, malic, and acetic acids differed significantly between monocropped and intercropped maize ($P < 0.05$), increasing by 167%, 103%, 127%, and 1,700%, respectively. Lactic acid was not detected in monocropped maize but was present in intercropped maize at 0.06 mg · plant ^{1}.

Tartaric acid was detected in both systems without significant differences (Table 2).

A. alpina showed significant differences in both types and quantities of root exudates between monocropping and intercropping ($P < 0.05$). Monocropped *A. alpina* exudates contained three organic acids: oxalic, citric, and malic acids. Intercropped *A. alpina* exudates increased to five types, adding acetic and lactic acids to the three found in monoculture. Oxalic, citric, and malic acid contents differed significantly ($P < 0.05$), increasing by 1,558%, 264%, and 367%, respectively. Acetic and lactic acids were not detected in monocropped *A. alpina* but were present in intercropped plants at 0.03 mg · plant ¹ and 0.09 mg · plant ¹, respectively. Tartaric acid was not detected in either monocropped or intercropped *A. alpina*.

Table 2 Effect of Pb stress on low-molecular-weight organic acid contents secreted by intercropped and monocropped *Arabis alpina* and maize

Plant	Planting pattern	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Acetic acid	Lactic acid
Maize	Monocropping	1.21±0.48b	3.50±1.10b	0.09±0.04a	0.78±0.22b	0.02±0.005b	–
	Intercropping	3.23±0.86a	7.09±0.63a	0.31±0.24a	1.77±0.47a	0.36±0.13a	0.06±0.02
A. alpina	Monocropping	0.12±0.04b	0.91±0.06b	–	0.06±0.02b	–	–
	Intercropping	1.99±0.11a	3.31±0.85a	–	0.28±0.07a	0.03±0.007	0.09±0.01

"–" indicates not detected.

2.4 Effects of Intercropping on Pb Content in Plants Under Pb Stress

Monocropping and intercropping treatments showed significant differences in Pb accumulation in aboveground and underground plant parts ($P < 0.05$). Underground Pb accumulation in intercropped maize was 144.81 mg · kg ¹ compared with 256.73 mg · kg ¹ in monoculture, representing a 44% reduction. Aboveground Pb accumulation did not differ significantly between monocropped and intercropped maize (79.55 mg · kg ¹ vs. 68.93 mg · kg ¹). Translocation coefficients were 0.41 for monocropped and 0.32 for intercropped maize, with no significant difference (Figure 2 [Figure 2: see original paper]A).

For *A. alpina*, intercropping significantly affected underground and aboveground Pb accumulation and translocation coefficient ($P < 0.05$). Monocropped *A. alpina* accumulated 373.61 mg · kg ¹ and 394.62 mg · kg ¹ Pb in underground and aboveground parts, respectively, while intercropped plants accumulated 555.31 mg · kg ¹ (49% increase) and 690.22 mg · kg ¹ (75% increase). The translocation coefficient increased from 1.05 to 1.28 (22% increase) (Figure 2B).

These results demonstrate that the maize-*A. alpina* intercropping system significantly reduced Pb accumulation in maize roots while increasing Pb accumulation in both root and shoot tissues of *A. alpina* and enhancing its translocation coefficient.

Figure 2 Effects of Pb stress on Pb contents of intercropped and monocropped maize (A) and *Arabis alpina* (B).

2.5 Correlation Analysis Between Root Exudate Organic Acids and Pb Accumulation

Table 3 presents correlations between root-exuded organic acids and Pb accumulation in plant tissues. For monocropped maize, root Pb content was significantly correlated with malic acid secretion. For intercropped maize, root Pb content correlated significantly with oxalic, citric, malic, and tartaric acids, while shoot Pb content correlated with oxalic and tartaric acids. For monocropped *A. alpina*, shoot Pb accumulation correlated significantly only with oxalic acid. In contrast, for intercropped *A. alpina*, both root and shoot Pb accumulation correlated significantly with four organic acids. These findings suggest that the altered composition and quantity of root-exuded organic acids in the intercropping system may enhance Pb accumulation and translocation in the hyperaccumulator *A. alpina*.

Table 3 Correlation analysis between organic acid contents and Pb content in intercropped and monocropped *Arabis alpina* and maize

Plant	Planting pattern	Organic acid	Root Pb	Shoot Pb
Maize	Monocropping	Malic acid	0.984*	0.944*
	Intercropping	Oxalic acid	0.948*	0.996*
		Citric acid	0.994*	0.976*
		Malic acid	0.952*	0.988*
		Tartaric acid	0.971*	0.965*
A. alpina	Monocropping	Oxalic acid	0.904*	0.950*
	Intercropping	Oxalic acid	0.984*	0.901*
		Citric acid	0.937*	0.916*

- indicates significant correlation at P < 0.05.

Discussion

3.1 Effects of Intercropping on Root Morphology and Pb Uptake

Roots are vital organs for water and nutrient absorption and participate in substance synthesis and transformation. When sensing different environmental conditions, roots may alter the quantity and concentration of ion uptake and transport [16,17]. Previous studies found that in intercropping systems with ryegrass (*Lolium perenne* L.) and *Astragalus sinicus* L. with *Brassica juncea* L. and *B. napus* L., Cd and Pb contents in shoots and roots of *B. juncea* and *B. napus* decreased significantly, while Cd and Pb contents in roots of ryegrass and *A. sinicus* increased significantly [18]. In maize-pea (*Pisum sativum* L.) intercropping, Cu content in maize shoots was significantly lower than in monoculture at the same Cu concentration, while root Cu content was significantly higher. The bioconcentration factor of intercropped maize was lower than that of monocropped maize, and translocation coefficients were significantly lower in all intercropping patterns, indicating reduced Cu translocation from roots to shoots [19].

In this study, the maize-*A. alpina* intercropping system significantly reduced Pb content in maize roots while increasing Pb content in both roots and shoots of *A. alpina*. The intercropping system altered metal accumulation sites in plants, possibly through changes in root architecture that modified uptake and transport mechanisms. For instance, under low P stress, ryegrass developed shorter, denser primary and lateral roots with increased root hair length and density [20]. Under Al stress, low Al concentration (10 mol·L^{1}) promoted root elongation in soybean (*Glycine max* L.) but not in rice, while high Al concentration (50 mol·L^{1}) inhibited root growth in both species [21]. In this study, intercropped maize showed significant differences in root branching number, surface area, and density compared with monoculture, while all root morphological parameters of intercropped *A. alpina* increased significantly. Underground biomass of both maize and *A. alpina* increased by approximately 2-fold. While root morphological parameters represent surface manifestations of altered heavy metal content in plants, the underlying mechanisms regarding whether root architectural changes modify metal uptake and transport patterns require further molecular-level investigation.

3.2 Effects of Intercropping on Low-Molecular-Weight Organic Acid Secretion

Changes in root morphological structure in intercropping systems may result from synergistic interactions among soil-plant-microorganism systems in the rhizosphere microecological environment, where root-secreted substances influence the transformation of mineral nutrients and pollutants, which in turn affects root morphology through low-molecular-weight organic acids. Zuo et al. [23] found that direct root contact between intercropped species was not the critical factor; rather, intercropped plants influenced each other through root exudates released into the soil. In maize/peanut (*Arachis hypogaea* L.) intercropping, maize root exudates could enter the peanut rhizosphere and affect Fe nutrition whether roots were in direct contact or separated by nylon mesh. Intercropped plants influence each other' s nutritional status through root exudates, which is transmitted to shoots, regulating physiological metabolism and consequently promoting or inhibiting plant growth. Exogenous organic acid treatments revealed that acetic acid reduced root active absorption area, while citric acid enhanced root activity, inhibited Pb uptake, and facilitated conversion of Pb to less active forms in shoots. Organic acid addition generally alleviated plant responses to Pb stress [24,26]. Different planting patterns showed that soybean root exudates significantly affected maize growth and yield, while sweet potato inhibited maize growth and reduced yield [27]. Wheat-maize intercropping increased root quantity and biomass in both species while markedly increasing the types of root-exuded organic acids [28]. Peanut root exudates significantly promoted growth of rice, maize, and ryegrass [29].

In this experiment, intercropping significantly increased both the quantity and diversity of root-exuded organic acids in maize and *A. alpina*. Maize exudates included additional lactic acid, while *A. alpina* exudates added malic, acetic, and lactic acids. Based on the increased underground biomass of *A. alpina* and both underground and aboveground biomass of maize under intercropping, we infer that low-molecular-weight organic acids may have promoted plant growth.

3.3 Relationship Between Low-Molecular-Weight Organic Acids and Pb in Plants

Arabis alpina is a Pb hyperaccumulator, while maize growth is inhibited under 400 mg $\cdot$ L ^{1} Pb^{2} stress. During the experiment, neither species exhibited toxicity symptoms. Previous studies demonstrated that exogenous citric acid forms stable cyclic citrate-Pb complexes [30], and Al treatment increased citric acid secretion in soybean roots [21]. We hypothesize that the 103% increase in detected citric acid in this study may have formed citrate-Pb complexes, alleviating Pb stress on maize growth. Other research showed that citric acid increased exchangeable Pb content in soil [31], and increased exchangeable Pb concentration facilitated plant uptake. In this study, under intercropping conditions with the Pb-hyperaccumulating *A. alpina*, maize grew normally while *A. alpina* Pb accumulation increased, possibly related to ion uptake and transport channels in the two species. Sasaki et al. [32] cloned the aluminum resistance gene ALMT1, which encodes a malate transporter that enhances Al resistance and can be expressed in yeast (*Saccharomyces* spp.), rice, and tobacco (*Nicotiana tabacum* L.) to secrete large amounts of malic acid. Al stress increased citric acid content and secretion in soybean roots but only increased secretion (not content) in rice roots [21]. Kitagawa et al. [33] reported positive correlations between Al resistance and malic acid secretion in different wheat varieties. Under Pb stress, citric acid detoxification primarily inhibited root uptake while promoting Pb translocation to shoots, where it was converted to less active forms. Tartaric acid did not alter the leaf/root ratio and showed relatively weak detoxification effects [24]. Different plants, organic acids, and heavy metal stresses produce varied effects, suggesting that organic acid secretion may be related to anion channels.

Under Pb stress, intercropping significantly altered root morphology and increased root biomass in both maize and *A. alpina* compared with monoculture. Intercropping also significantly changed the types and quantities of root-exuded organic acids, with lactic acid detected in maize and malic, acetic, and lactic acids detected in *A. alpina*. Compared with monoculture, acetic acid secretion in intercropped *A. alpina* correlated with root Pb content, while lactic acid correlated with shoot Pb content. Maize-*A. alpina* intercropping significantly reduced Pb content in maize roots, increased Pb content in both roots and shoots of *A. alpina*, and enhanced the translocation coefficient of *A. alpina*. In summary, intercropping altered the composition and quantity of root-exuded organic acids, changed Pb content in the hyperaccumulator *A. alpina* and crop maize, promoted Pb accumulation in *A. alpina*, and reduced Pb content in maize. Intercropping hyperaccumulator *A. alpina* with maize under Pb stress represents a feasible remediation strategy.

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Note: Figure translations are in progress. See original paper for figures.

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