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Relationship between Lead Uptake by Lettuce and Water-Soluble Low-Molecular-Weight Organic Acids in Rhizosphere As Influenced by Transpiration

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The relationship between Pb uptake by leaf lettuce (*Lactuca sativa* L.) and water-soluble low-molecular-weight organic acids (LMWOAs) in rhizosphere, as influenced by transpiration (high and low), has been studied. Studies were carried out by culturing lettuce plants grown for 2 weeks in pots filled with quartz sand mixed with anion-exchange resin and then for 30 days in a greenhouse. The potted lettuce plants were subjected to stress by the addition of $Pb(NO_3)_2$ solutions (100, 200, and 300 mg of Pb L^{-1}) and by high and low transpiration treatments for another 10-day period. Blank experiments (without addition of $Pb(NO_3)_2$ solutions to the pots) were also run. There were no significant differences in the growth of the plants with the addition of $Pb(NO_3)_2$ solutions in either of the transpirations studies. Uptake of Pb by the shoots and roots of the plants was found to be proportional to the concentration of Pb solutions added, and more accumulation was observed in the roots than in the shoots at the end of days 3 and 10. High transpiration caused more Pb uptake than did low transpiration. One volatile acid (propionic acid) and nine nonvolatile acids (lactic, glycolic, oxalic, succinic, fumaric, oxalacetic, D-tartaric, *trans*-aconitic, and citric acids) in rhizosphere quartz sand or anion-exchange resin were identified and quantified by gas chromatography analysis with a flame ionization detector. The amount of LMWOAs in rhizosphere quartz sand or anion-exchange resin increased with higher amounts of Pb in quartz sand solution and also with longer duration of the study. The total quantities of the LMWOAs in the rhizosphere quartz sand or anion-exchange resin were significantly higher under high and low transpiration with a 300 mg of Pb L^{-1} solution addition at the end of day 10. Compared with our previous related studies (published work), the present study shows that the presence of LMWOAs in rhizosphere does not significantly affect Pb uptake by lettuce plants under high and low transpiration. A physiological mechanism of the roots of lettuce plants governing the relationship between Pb contamination level and quantity of water-soluble LMWOAs in rhizosphere quartz sand and resin, as influenced by transpiration, was proposed.

KEYWORDS: Low-molecular-weight organic acids; rhizosphere quartz sand; transpiration; Pb; anion-exchange resin; lettuce plants

INTRODUCTION

In a soil environment, organic acids originate from decomposition of soil organic matter (SOM) in the upper soil horizons, plant root exudates, and microbial metabolites (*1, 2*). Therefore, the kinds and concentrations of organic acids in a soil environment as well as rhizosphere do vary with microbial population, pathway of biodegradation, kinds of organic matter, plant species, and soil properties.

Plants' mechanisms of heavy metal tolerance include exclusion and detoxification (*3*). The exclusion mechanism involves increasing rhizosphere pH or excreting root exudates, which prevents heavy metals from transporting to or accumulating in the above-ground parts of plants (*4*). It was reported that the organic ligands excreted from the tips of rootlets form complexes with heavy metals in rhizosphere to decrease the bioavailability of heavy metals (*5*), which in turn reduces the absorption of heavy metals by plant roots. The specific mechanism of exclusion, such as the excretion of exudates in rhizosphere,

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inhibits the absorption of heavy metals by plant roots. However, when the concentration of heavy metals in soils exceeds the limits, the function of this exclusion mechanism diminishes, and then the absorption and subsequent accumulation of heavy metals by plants increases (*5*)

Brune et al. (*6*) stated that there are five pathways for the detoxification mechanism for Zn present in the cytoplasm of plants: (1) The plasma membrane may have low permeability for Zn, thereby restricting Zn uptake into the cell. (2) After uptake into the cell, Zn could be transported rapidly into subcellular compartments such as the large central vacuole. (3) Alternatively, Zn could be precipitated as insoluble salts. (4) Zn may be detoxified by complex formation or binding, for example, to phytochelatins. (5) Cells may also reduce their heavy metal load by rapid and active extrusion across the plasma membrane into the apoplast. Plants have the capability to render heavy metals bonding with low-molecular-weight organic compounds ($<$10 kDa) to reduce heavy metal toxicity (*7, 8*). When the concentration of heavy metals in plant tissues increases, it provokes the syntheses of phytochelatins (PCs) (*9*). The presence of Cd, Cu, Ag, Hg, or Pb in plant tissues—especially Cd and Cu—provokes the syntheses of PCs (*10*). The bonding of heavy metals with PCs can reduce the toxicity of heavy metals to plants (*11*). PCs are one kind of protein in cytoplasm, also called cadystin, phytometallothionein, or metallothiopeptide (*12*), usually containing cysteine (S-containing amino acid), with a structural formula of $(\gamma\text{-GluCys})_n\text{-Gly}$ ($n = 2$–12). Many kinds of low-molecular-weight organic acids (LMWOAs) have been found in rhizosphere soils of agricultural and forest lands, including acetic acid, citric acid, formic acid, fumaric acid, maleic acid, malic acid, malonic acid, lactic acid, oxalic acid, and succinic acid (*13*). However, depending on the type of plant species, the kinds and contents of LMWOAs differ significantly (*1, 14, 15*). Though the kinds and contents of LMWOAs are different under different vegetation conditions, the kinds of LMWOAs associated with a particular crop species are the same (*16*).

Crop roots excrete organic acids into rhizosphere environment and then use these acids to extract low-bioavailablilty forms of heavy metals, or to increase the bioavailability of heavy metals by decreasing rhizosphere soil pH (*7, 17–19*), which in turn increases heavy metal absorption and accumulation by crops. Klassen et al. (*17*) reported that some crops can accumulate heavy metals by means of the excretion of organic compounds from roots or by decreasing soil pH. Moreover, Hammer and Keller (*19*) pointed out that planting the super-accumulator plant, *Thlaspi caerulescens*, can change the form of existing heavy metals in soil. LMWOAs in the rhizosphere environment acidify rhizosphere soil, decrease soil pH by up to 2 units, and transform the distribution of forms of heavy metals (*20, 21*). It was reported that LMWOAs including fumaric acid, maleic acid, malonic acid, oxalic acid, succinic acid, and tartaric acid amended to soil decrease the soil pH and promote the extraction of Cd and Cu. Among these acids, maleic acid was reported to be the best extractant for Cd and Cu (*22*). Wang et al. (*23*) and Lin et al. (*24*) investigated the distribution of solid-phase forms of heavy metals in rhizosphere soil of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) and pointed out that the strong-bonding forms of Cd, Cu, Pb, and Zn (bound to Fe–Mn oxides or organics) were converted to highly mobile forms (water-soluble or carbonate) in the presence of LMWOAs.

Qin et al. (*25*) investigated the difference in the extractability for heavy metals between acetic acid and malic acid and pointed out that, in the presence of LMWOAs, pH decrease is not the main factor influencing the solubility of heavy metals in soils. Yong et al. (*26*) and Krishnamurti and Naidu (*27*) pointed out that different characteristics as well as functional groups of soil organic components may lead to different binding capabilities with heavy metals, which in turn affects protonation and subsequent dissolution of heavy metals by LMWOAs.

Different dissociation constants of LMWOAs, representing their different degrees of dissociation, thus affect their complexation with heavy metals (*25, 28, 29*). Krishnamurti et al. (*30*) reported that, among LMWOAs, fumaric acid has a higher effect in promoting Cd desorption from soil particles than other acids. They concluded that the pK_a of fumaric acid is much lower than those of acetic acid, citric acid, oxalic acid, and succinic acid, resulting in easy dissociation of fumaric acid to form a complex with Cd and its subsequent extraction. Shan et al. (*31*) pointed out that the amount of heavy metal extracted from soil was in good correlation with the concentration of heavy metal in plant tissue. The correlation of Cd was the highest ($r^2 > 0.90$), while that of Pb was the lowest ($r^2 > 0.50$).

Liao et al. (*32*) found that Pb uptake by lettuce plants (*Lactuca sativa* L.) increased with increasing Pb concentration in quartz sand solutions up to 300 mg L^{-1}. However, the results showed that, under high and low transpiration, the total amount of water-soluble LMWOAs in rhizosphere increased with increasing Pb concentration in quartz sand solution. This study was thus aimed to investigate consecutively the relationship between Pb uptake by leaf lettuce and water-soluble LMWOAs in rhizosphere, as influenced by high and low transpiration, in the presence of anion-exchange resin (which fixes the LMWOAs released by the lettuce plant) thoroughly mixed with rhizosphere quartz sand.

MATERIALS AND METHODS

The experimental conditions (pot experiment, transpiration conditions, and levels of lead treatments and nutrients) were maintained similar to those described in our previous experiment (*32*), except that anion-exchange resin was amended and thoroughly mixed with rhizosphere quartz sand in the pots.

Pot Experiment. Leaf lettuce, a very common winter and spring dietary vegetable in Taiwan, was selected as the pot crop in this study. Leaf lettuce plants were transplanted to 32 rectangular pots in a greenhouse (air temperature range of 26.0–30.5 °C) after 2 weeks of seedling culture in a basin. Each pot (30 cm length, 23.5 cm width, and 10 cm depth) was lined with a clean, unpunched plastic bag and filled with 8 kg of quartz sand (particle size 0.1–0.5 mm, washed with 0.1 M HCl solution and then with deionized water). Before the lettuce plants were planted in the pots, 5.00 g of anion-exchange resin (Amberlite IRA402, Rohm and Haas, Philadelphia, PA; strongly basic anion-exchange resin, previous saturated with Cl^- and then washed with deionized water) per lettuce plant (total 60.0 g of anion-exchange resin was mixed with rhizosphere quartz sand for 12 lettuce plants in each pot) was thoroughly mixed with rhizosphere quartz sand, and then the lettuce plants were planted. Characteristics of the resin: total anion-exchange capacity, $\geq 6.5 \times 10^4$ equiv L^{-1}; water holding capacity, 50–60%; specific gravity, 1.063–1.093; bulk density, 610–680 g L^{-1}; and particle size range, 0.62–0.77 mm. The lettuce plants were grown in the greenhouse for 30 days. During the growth of lettuce plants in the greenhouse, liquid nutrient solution was added to the pots, containing the following: NH_4^+-N, 21 mg L^{-1}; NO_3^--N, 49 mg L^{-1}; P, 10 mg L^{-1}; K, 78 mg L^{-1}; Ca, 80 mg L^{-1}; Mg, 36 mg L^{-1}; Fe, 2 mg L^{-1}; Mn, 1 mg L^{-1}; B, 0.5 mg L^{-1}; Zn, 0.2 mg L^{-1}; Cu, 0.05 mg L^{-1}; and Mo, 0.005 mg L^{-1}. Based on the proposed suitable water content of 20% (w/w) of general sandy soil for plant growth (*33*), 1 L of nutrient solution complemented with 600 mL of deionized water was irrigated to each pot to make 20% water content at day 1 of the greenhouse growth stage. The lettuce plants were then irrigated with deionized water daily to bring the water content to 20% for each pot, but 100 mL of nutrient solution was present in the irrigated water at days 7, 14, 21, and 28 of the growth stage. After 4 weeks of growth of lettuce plants in the greenhouse, one set of 16 pots of lettuce plants plus four additional pots, which contained only quartz sand, anion-exchange resin, and 1600 mL of deionized water, was subjected to high transpiration treatment. The four additional quartz sand and anion-exchange resin pots were used for calibrating evaporation of water from the pots. In the same manner, the other set of 16 pots of lettuce plants plus the other four additional quartz sand and anion-exchange resin pots was subjected to low transpiration treatment. The low transpiration was maintained at greenhouse temperature under normal flow of air, while high transpiration was made effected blowing air above the lettuce plants with a big electric fan to promote the convection of the air-stream and subsequently increase the humidity gradient at the surface of the leaves of the lettuce plants. The high and low transpiration treatments were carried out for 2 days for modulation between lettuce plants and transpiration treatments. During the 2-day period, the water lost by transpiration of lettuce plants and evaporation of quartz sand of each pot was complemented with deionized water to 20% water content by weight daily. After 2 days of modulation by the high and low transpiration treatments, lettuce plants from both treatments were subjected to the additional stress of heavy metal treatment. At the end of the 2-day modulation (defined as day 0 of the heavy metal stress stage), $Pb(NO_3)_2$ solutions of different concentrations (0, 100, 200, and 300 mg L^{-1}) of Pb, prepared by using lead(II) nitrate (Merck KGaA, Darmstadt, Germany; ACS analytical grade, minimum 99.5%), were added to the two sets of pots of lettuce plants. For each set representing high or low transpiration treatment, four replicates of pots were designated for 0 (check), 100, 200, and 300 mg L^{-1} of Pb treatments. An attempt was made initially to also add $Pb(NO_3)_2$ solutions of Pb concentrations 400 and 500 mg L^{-1} to the quartz sand mixed with anion-exchange resin, but these solutions were found to be toxic to the lettuce plants.

During the 10-day growth with the Pb stress, the rates of evaporation of quartz sand pots and of evapotranspiration of lettuce plant pots were measured by weighing every pot at the end of each day, and the four levels of Pb-content solutions described above were added to their respective treatments to bring the pots back to 20% water content. The width and length of leaves of any four randomly chosen lettuce plants among the 12 plants in each pot were measured at the end of the days 0, 3, and 10. The roots of the four measured lettuce plants were removed carefully from each pot, and any quartz sand and anion-exchange resin loosely adhering to the roots was gently shaken off, back into the respective pot. It was found that most of the anion-exchange resin was released back into pot by shaking the plant, and the trace amount of resin adhering to the roots was picked off with tweezers (previously washed with tap water and rinsed with deionized water) and put back into pot. The whole anion-exchange resin (about 5.0 g) mixed with quartz sand in a pot for each lettuce plant was collected and then sieved, with a sieve of opening 0.50 mm, to separate resin from quartz sand. Because of the distinct particle size range difference between quartz sand and resin, most of the quartz sand was passed through the sieve opening, and resin was retained on the screen of the sieve. The whole retained resin was then poured onto an adequate size of filter paper (previously washed with deionized water), and then tweezers were used to separate resin from any trace amount of quartz sand left. The collected quartz sand was transferred back to the bulk quartz sand of the respective pot. Ten grams of bulk quartz sand was taken from each pot, and then 10 mL of deionized water was added to make a suspension for measuring and elucidating the pH regime of pot quartz sand on the three days (*34*). Quartz sand attached to the roots of the four lettuce plants was then washed off with 500 mL of deionized water, and this quartz sand was considered as the rhizosphere quartz sand (*15, 35*). The shoots and roots of each lettuce plant were then cut apart and separately collected on these three days, washed with tap water, and rinsed with deionized water. The plant materials were dried in an oven at 70 °C for 24 h, and the dry matter was weighed.

Lead Concentrations in Plant Tissues. The samples of plant materials, including shoots and roots, were prepared according to the method described by Jones and Case (*36*). Concentrated HNO_3 (nitric acid, Merck KGaA; 2.5 mL, minimum 65.0%) was added to 200 mg of oven-dried plant tissue, and the mixture was kept overnight. The plant tissue was subsequently decomposed by heating at 80 °C for 1 h, followed by addition of concentrated $HClO_4$ (perchloric acid, Merck KGaA; 2.5 mL, 70.0–72.0%) and further heating at 180–200 °C for 2–3 h. The Pb content in the digested solutions was determined using inductivity coupled plasma atomic emission spectroscopy (ICP-AES, JY 138 UL-TRACE, Jobin Yvon, S.A.S., Longjumeau Cedex, France). To determine Pb concentration in digested solutions of the roots and shoots of the lettuce plants, the emission line of 220.353 nm was selected. For each batch of samples, three replicates of each standard and each sample were subjected to the determination of Pb concentration. It was found that, for each determination of Pb concentration, the reproducibility was very high, with standard deviation <3% of the mean. The method detection limits (MDLs) for the determination of Pb in lettuce plant tissue by ICP-AES was 0.86 mg kg^{-1}. Throughout the experiment, the average of four replicates for each Pb concentration stress under high or low transpiration treatment was considered for each sample.

Extraction of Water-Soluble LMWOAs in Rhizosphere Quartz Sand Mixed with Anion-Exchange Resin. Each portion of rhizosphere quartz sand washed from each pot as described above was extracted with deionized water (500 mL) using an end-over-end shaker (amplitude of 2.5 cm and 200 rpm) for 8 h at 25 ± 1 °C. The extract was centrifuged (18600*g*) for 15 min at 25 °C to separate the supernatant from the sediment. The anion-exchange resin (about 5.0 g) collected as described above for each lettuce plant from each pot was extracted with 10 mL of 0.5 M HCl (minimum 37.0%, Merck KGaA) using an end-over-end shaker, and then the extract was centrifuged to separate the supernatant from the sediment. The four portions of supernatants for the four lettuce plants from a pot were combined for each of the three days. Each of the two kinds of supernatants was then filtered through a 0.45 μm pore size cellulose nitrate membrane filter (Whatman International Ltd., Maidstone, England). The filtrate was transferred to a plastic vial containing an anion-exchange membrane (1 cm × 7 cm) in hydroxyl form (IONAC MA-3475, Sybron Chemicals, Birmingham, NJ) and shaken in an end-over-end shaker (amplitude of 2.5 cm and 200 rpm) for 8 h at 25 ± 1 °C. In a preliminary test, the efficiency of the anion-exchange membrane in hydroxyl form was verified, and it showed better adsorption of LMWOAs than that of the membrane in bicarbonate form. The membrane was then transferred to another vial containing HCl solution (5 mL of 0.1 M) and again shaken in an end-over-end shaker (amplitude of 2.5 cm and 200 rpm) for 8 h at 25 ± 1 °C (*14, 15, 35*). All the acidified aqueous extracts were used directly for gas chromatography (GC) analysis of volatile LMWOAs (Shimadzu GC-14B, Shimadzu Corp., Kyoto, Japan). For the samples of nonvolatile LMWOAs, the acidified aqueous extract was re-extracted with ethyl acetate (3 × 5 mL, minimum 99.0%; Sigma Chemical Co., St. Louis, MO), and the organic portion was used for GC analysis.

Determination of Water-Soluble Volatile and Nonvolatile LMWOAs. All the standard samples of volatile and nonvolatile LMWOAs were purchased from Supelco Inc. (Bellefonte, PA). For nonvolatile LMWOAs analysis, glutaric acid was used as an internal standard. Each 1 mL aliquot of HCl-soluble solution, with 0.1 mL of 100 μg mL^{-1} glutaric acid added, was extracted with 2 mL of ethyl acetate (Sigma). The ethyl acetate layer was transferred to a V-shape vial, heated to 50 °C, and then blown dry with nitrogen gas. This extraction procedure was repeated three times. A 200 μL portion of trimethylsilyl (TMS) derivatizing agent (bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS), 99:1; Supelco Inc.) was then added to the dried extract, covered with lid, placed into a 70 °C water bath, and heated for 30 min (*35*). This transforms nonvolatile LMWOAs into highly volatile, low-polarity, and thermostable products which are suitable for separation on a capillary column. Quantitative estimation of water-soluble LMWOAs was carried out by GC analysis (*14, 15, 35*). For analysis of volatile water-soluble LMWOAs, a separate column (glass column, 2.1 m in length and 4 mm of inner diameter) filled with SP-1000 (Supelco Inc.) was used as the stationary phase, and a flame ionization detector (FID) was used. For analysis of water-soluble nonvolatile LMWOAs, a Supelco SPB-1 capillary column (30 m length, 0.53 mm inner diameter, and 1.5 μm film thickness) and a FID were used. The glass column and capillary column were used accordingly. The injector, column, and detector were kept at 200, 150, and 200 °C,

Table 1. Comparison of Rates of High and Low Transpiration (g pot^{-1}) Based on the Measured Mass of Amended Irrigated Water Treated with Different Pb Concentrations for 10 Days

	day										
Pb treatment	1	2	3	4	5	6	7	8	9	10	total[a]
					High Transpiration						
blank	9.8	9.5	10.4	11.0	11.5	12.4	15.6	18.2	19.3	21.5	139.2 b
100 mg L^{-1}	9.9	10.2	10.1	10.7	11.6	12.8	15.7	17.9	19.6	22.1	140.6 b
200 mg L^{-1}	10.3	10.9	11.1	11.3	12.8	13.1	15.2	18.2	19.4	22.4	144.7 a
300 mg L^{-1}	9.9	10.1	11.3	11.5	12.6	13.2	14.8	18.3	18.9	20.3	140.9 b
					Low Transpiration						
blank	9.4	9.3	8.7	9.2	10.7	11.5	13.3	15.8	17.4	20.1	125.4 d
100 mg L^{-1}	9.8	9.6	9.1	10.2	10.9	11.5	13.8	16.3	17.4	20.7	129.3 c
200 mg L^{-1}	9.6	9.6	9.3	10.2	10.7	11.3	12.8	15.5	17.9	19.8	126.7 cd
300 mg L^{-1}	9.2	9.9	10.0	10.4	11.1	11.8	13.4	14.7	17.3	20.8	128.6 c

[a] Means followed by different letters are significantly different at the 5% probability level by the analysis of variance and least significant difference values.

Table 2. Effect of Transpiration on the Growth and Yield of Lettuce at Days 0, 3, and 10 of Growth after Treatments with Irrigated Water with Different Pb Concentrations

	leaf[a]		shoot[a]		root[a]	
Pb treatment	width (cm)	length (cm)	fresh weight (g plant^{-1})	dry weight (g plant^{-1})	fresh weight (g plant^{-1})	dry weight (g plant^{-1})
			High Transpiration			
blank	6.01	19.21	10.87	0.94	2.42	0.28
	6.28	19.63	11.08	1.01	2.51	0.31
	6.54	20.01	11.23	1.08	2.74	0.33
100 mg L^{-1}	6.02	19.18	10.93	0.93	2.40	0.28
	6.23	19.74	11.12	1.02	2.49	0.31
	6.42	20.33	11.21	1.09	2.75	0.33
200 mg L^{-1}	6.08	19.12	10.64	0.95	2.38	0.30
	6.34	19.58	10.68	1.00	2.52	0.29
	6.48	20.05	11.17	1.07	2.68	0.32
300 mg L^{-1}	6.21	19.23	11.03	0.98	2.44	0.29
	6.62	19.55	11.05	0.98	2.53	0.30
	6.89	20.14	11.09	1.09	2.80	0.34
			Low Transpiration			
blank	6.08	19.28	10.43	0.96	2.23	0.26
	6.32	19.81	10.87	0.98	2.44	0.30
	6.61	20.56	11.33	1.02	2.53	0.32
100 mg L^{-1}	6.11	19.34	10.58	0.95	2.28	0.25
	6.41	19.92	11.02	0.97	2.31	0.30
	6.72	20.81	11.24	1.04	2.62	0.32
200 mg L^{-1}	6.07	19.23	10.42	0.97	2.31	0.37
	6.43	19.88	10.46	0.97	2.52	0.31
	6.81	20.44	11.05	1.03	2.68	0.30
300 mg L^{-1}	6.23	19.42	10.81	0.94	2.33	0.27
	6.58	19.96	11.15	0.98	2.51	0.32
	6.78	20.39	11.22	1.05	2.53	0.31

[a] Within the same column, the first, second, and third rows of each data set are the means of days 0, 3, and 10, respectively. The growths of leaf, shoot, and root are not significantly different at the 5% probability level by the analysis of variance and least significant difference values at days 0, 3, and 10 for the treatments with four different Pb concentrations.

respectively, for the detection of volatile LMWOAs and at 250, 225, and 300 °C, respectively, for that of nonvolatile LMWOAs. Nitrogen gas (purity 99.9995%) was used as a carrier gas at a flow rate of 60 mL min^{-1} for volatile LMWOAs analysis, while the nitrogen gas pressure was kept at 1 kg cm^{-2}, with the same nitrogen gas as makeup gas at a pressure of 0.5 kg cm^{-2} for nonvolatile LMWOAs analysis. Fixed 5 μL needle syringes (No. 75, Hamilton Co., Reno, NV) were used to inject the samples (1 μL for each splitless injection). The chromatograms were recorded, and peaks were integrated using a HP 3395 integrator (Hewlett-Packard Development Co., Wilmington, DE). For each batch of samples, three replicates of 1 μL volume of each standard and each sample were manually injected with a splitless technique for the analysis of LMWOAs using the fixed 5 μL needle syringe. It was found that the reproducibility for each determination of LMWOAs was very high, and the corresponding standard deviation was <3% of the mean. Further, the required QA/QC procedure that involves analysis of samples spiked with standard and QC sample was also carried out for each batch of determinations. All the data obtained were within the upper and lower warning as well as control limits. This clearly indicates that, though a fixed 5 μL needle syringe was used in this study, the values obtained for the determination of LMWOAs were very accurate and precise.

RESULTS AND DISCUSSION

Rate of Transpiration and Lettuce Growth. .The rate of transpiration of the volatiles through the lettuce plants was studied for 10 consecutive days during the growth stage of lettuce plants under high and low transpiration treatments and stress of different Pb concentrations. The lettuce plants were grown in quartz sand mixed with anion-exchange resin with three different Pb concentrations and also in quartz sand mixed with anion-exchange resin without lead contamination (blank experiment). Additionally, the lettuce plants were subjected to low as well as high transpiration. The rate of transpiration of

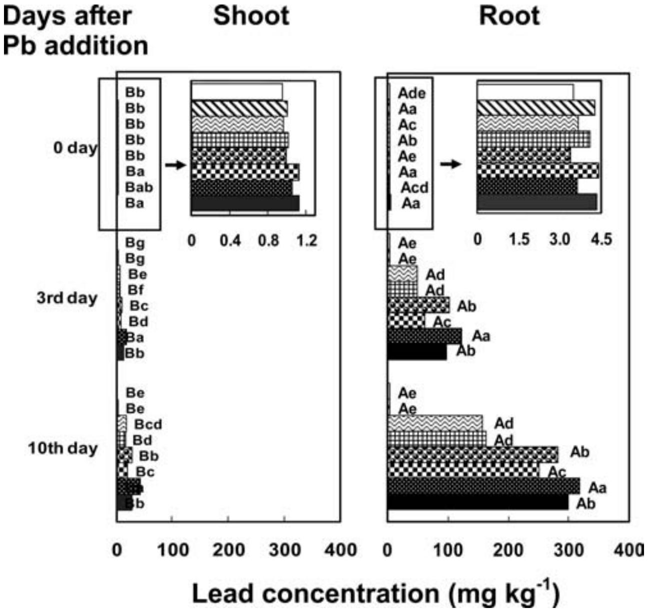

Figure 1. Lead concentrations (expressed as mg kg^{-1}) in the shoots and roots of the lettuce plants at days 0, 3, and 10 under high and low transpiration treatments and stress of four levels of Pb concentrations in the irrigated water. The eight bars in columns within each day from the top were divided into four sets of Pb concentration stress: blank and 100, 200, and 300 mg L^{-1}, respectively. The first bar of each set is for high transpiration, and the second bar of each set is for low transpiration. Different lowercase letters following the bars in columns within each day indicate significant difference at the 5% probability level by the analysis of variance and least significant difference (LSD) values. Different uppercase letters following the bars in rows within each level of Pb concentration in irrigated water indicate significant difference at the 5% probability level by the analysis of variance and LSD values. The alphabetical order for both lower- and uppercase letters indicates the sequence for concentration.

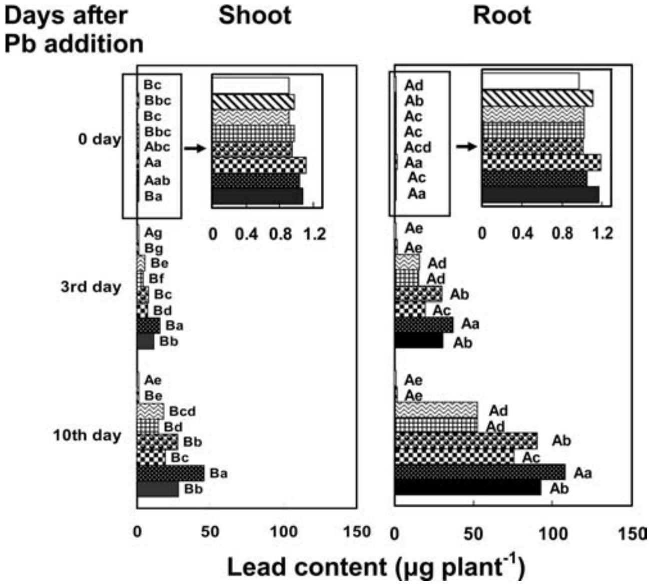

Figure 2. Lead contents (expressed as μg plant^{-1}) in the shoots and roots of the lettuce plants at days 0, 3, and 10 under high and low transpiration treatments and stress of four levels of Pb concentration in the irrigated water. The meanings of the eight bars in columns within each day and the lower- and uppercase letters following the bars in the figure are explained in the caption of Figure 1.

the volatiles was found to be almost similar for each of the initial four days and then increased gradually under the effect of both transpirations (**Table 1**). Kastori et al. (*37*) reported that crop growth and transpiration rate were pronouncedly affected when heavy metal concentration attained a certain level. However, in our study, the amount of the volatiles transpirated per day did not depend on the concentration of Pb in quartz sand mixed with anion-exchange resin, but with high transpiration the rate of transpiration was somewhat higher than with low transpiration. As a result, total transpiration of volatiles during the 10 days of the growth stage of lettuce plants was higher in the case of high transpiration than in low transpiration, and their differences were statistically significant but almost independent of the availability of Pb in quartz sand mixed with anion-exchange resin (**Table 1**).

Leaf lettuce plants were grown in quartz sand mixed with anion-exchange resin containing three different concentrations of $Pb(NO_3)_2$ solution (100, 200, and 300 mg of Pb L^{-1}). Blank experiments (plants grown in quartz sand mixed with anion-exchange resin without addition of $Pb(NO_3)_2$ solution) were run in parallel. The lettuce growth was studied under the effect of high and low transpirations at the end of days 0, 3, and 10, and the lettuce plants grown on all three quartz sand samples mixed with anion-exchange resin with three different concentrations of Pb appeared healthy. The width and length of the plant leaves and fresh and dry weights of shoots and roots were examined (**Table 2**). Minimal variations in the growth of the lettuce plants with the addition of three concentrations of $Pb(NO_3)_2$ solutions were observed with both low and high transpirations. Mostly, at the end of days 0, 3, and 10, the leaves grew a little more in width and length under low transpiration than under high transpiration. However, the fresh and dry weights of both shoots and roots were slightly higher in the case of high transpiration. Because the differences in width and length of leaves and in fresh and dry weights of shoots and roots of the lettuce plants were not statistically significant (**Table 2**), it is clear that both high and low transpiration treatments and stress of different Pb concentrations did not affect the growth of lettuce plants in this experiment. Hopkins (*38*) pointed out that movement of mineral nutrient elements in the sap of xylem depends on transpiration rate, but the limitation of nutrient supply rate seems to play a key role in affecting nutrient absorption by roots and subsequent translocation in xylem of plants. Although the high and low transpiration rates set in this experiment were significantly different (**Table 1**), the high transpiration rate may not be great enough to promote the absorption of nutrient elements by roots and their subsequent translocation in xylem and the growth of lettuce plants. Comparison of the present study (**Tables 1** and **2**) to our earlier related study (*32*), in which only quartz sand with the same three concentrations of Pb in the pots, without anion-exchange resin, were used for growing lettuce plants, shows no significant differences in the rate of transpiration and lettuce growth.

Concentration of Pb in Lettuce Plants. The accumulation of Pb in lettuce plants in all three quartz sand samples mixed with anion-exchange resin with three different concentrations of Pb was investigated under high and low transpirations at the end of days 0, 3, and 10. Blank experiments were run in parallel. The concentration of Pb per kilogram of lettuce plant as well as per plant were determined (**Figures 1** and **2**). In the blank experiment, these concentrations were very low and their differences were not significant during days 0, 3, and 10 (significance labels not shown in **Figures 1** and **2**). More accumulation of Pb was observed in the roots than in shoots in all the cases of high and low transpiration treatments and different Pb concentration stress (**Figures 1** and **2**). This clearly indicates that the translocation of Pb from roots to shoots of the lettuce plants is limited, and thus Pb accumulated in the roots. Similar results of heavy metals translocation from roots to shoots were also observed for other plants, according to reports by Murillo et al. (*39*) and Liu et al. (*40*). Except for the blank experiment, the ratios in terms of milligrams of Pb per kilogram in shoots to their respective roots were in the range of 0.08–0.13 (**Figure 1**), while the ratios in terms of micrograms of Pb per plant in shoots to their respective roots were in the range of 0.26–0.43 (**Figure 2**). This shows higher ratios of the latter than of the former, which implies that there is risk to consumers because the shoots of lettuce plants are usually consumed by people as a dietary vegetable. Lead uptake by lettuce plants was found to be dependent on the concentration of Pb present in quartz sand mixed with anion-exchange resin and also on the rate of transpiration (**Figures 1** and **2**). As the concentration of Pb increased in quartz sand mixed with anion-exchange resin, the accumulation of this metal also increased in both shoots and roots at the end of days 3 and 10. Lead uptake by lettuce plants was also significantly higher under high transpiration than under low transpiration at the end of days 3 and 10, but this is not the case with the 100 mg L^{-1} Pb treatment at day 3 and/or 10 (**Figures 1** and **2**). Klassen et al. (*17*) reported that the absorption of Pb by crop roots is via passive uptake. This indicates that transpiration drives water-soluble Pb in quartz sand mixed with anion-exchange resin to move into the roots and upward to the shoots of the lettuce plants via the transpiration stream of water. Hence, a high transpiration rate produced a significantly larger quantity of transpiration stream of water to drive water-soluble Pb uptake across the plasma membranes in the roots and shoots of the lettuce plants than did a low transpiration rate (**Table 1**). Moreover, because higher Pb concentration treatment gave higher water-soluble Pb concentration in rhizosphere quartz sand mixed with anion-exchange resin, a significantly higher quantity of water-soluble Pb was absorbed by the roots and then transported to the shoots of the lettuce plants under high and low transpiration treatments at the end of days 3 and 10 of the growth stage (**Figures 1** and **2**). Grifferty and Barrington (*33*) studied Zn uptake by young wheat plants under two transpiration regimes and reported that the low transpiration rate produced Zn uptake half that produced by the high transpiration rate. However, in the present study, the transpiration rate did not show such prominent effects. This may be attributed to the characteristics of the crop species as well as the chemical behavior of heavy metal contaminants. As the duration of the present studies increased (from 0 to 10 days), total accumulation of Pb in both shoots and roots also increased (**Figures 1** and **2**). This indicates that the edible part of mature vegetables contains a higher quantity of harmful heavy metals and causes more risk to consumers than that of young vegetables. There were no significant differences in the absorption of Pb by the roots and subsequent accumulation in the shoots of lettuce plants between our previous related studies (*32*), in which only quartz sand with the same three concentrations of Pb in the pots, without anion-exchange resin, were used, and the present work (**Figures 1** and **2**).

Water-Soluble LMWOAs in Rhizosphere Quartz Sand Mixed with Anion-Exchange Resin. The lettuce plants were subjected to high and low transpirations in quartz sand mixed with anion-exchange resin with three different concentrations of Pb, as well as quartz sand mixed with anion-exchange resin without $Pb(NO_3)_2$ solution (blank experiment). The composition of the water-soluble LMWOAs, including nonvolatile and volatile acids, released by lettuce plants and subsequently adsorbed by anion-exchange resin or rhizosphere quartz sand was mostly found to be the same at the end of days 0, 3, and 10, though their amounts were variable (**Tables 3** and **4**). This finding was close to those reported by Cieśliński et al. (*16*). However, the amounts of nonvolatile and volatile acids adsorbed by rhizosphere quartz sand were much less than those adsorbed by anion-exchange resin (**Tables 3** and **4**). The nonvolatile and volatile acids adsorbed by rhizosphere quartz sand were found at the end of day 3 and/or 10 (**Table 4**). In comparison to our earlier related study (*32*) (**Tables 3** and **4**), in which only quartz sand in the pots without anion-exchange resin was used, there were no significant differences in the total amounts of nonvolatile and volatile LMWOAs adsorbed by rhizosphere quartz sand and by quartz sand mixed with anion-exchange resin, respectively. LMWOAs originate from decomposition of SOM in the upper soil horizons, plant root exudates, and microbial metabolites (*1*, *2*). In our study, no organic matter was amended to the quartz sand mixed with anion-exchange resin in each pot. The LMWOAs determined were thus mostly derived from root exudates and their microbial metabolites in rhizosphere of the lettuce plants in quartz sand mixed with anion-exchange resin. The composition and quantities of these LMWOAs were determined by GC analysis with a FID. One volatile acid (propionic acid) and nine nonvolatile acids (lactic, glycolic, oxalic, succinic, fumaric, oxalacetic, D-tartaric, *trans*-aconitic, and citric acids) were identified in the rhizosphere quartz sand mixed with anion-exchange resin (**Tables 3** and **4**). D-Tartaric and citric acids were found to be major among the nonvolatile acids. However, glycolic, fumaric, and *trans*-aconitic acids were not detected in the anion-exchange resin and rhizosphere quartz sand of blanks (without $Pb(NO_3)_2$ solution) under high and low transpiration on the three days. Moreover, these three acids were not detected in the anion-exchange resin and rhizosphere quartz sand treated with 100 mg L^{-1} $Pb(NO_3)_2$ solution under high and low transpiration on day 0, 3, and/or 10 (**Tables 3** and **4**). Under high and low transpiration, the quantities of the total acids in the rhizosphere quartz sand mixed with anion-exchange resin were higher at the end of day 10, and the amount of volatile propionic acid was always higher than those of other volatile acids on all three days (**Tables 3** and **4**). Moreover, under high and low transpiration, the quantities of the total nonvolatile acids were significantly greater than that of volatile acid (significant labels not shown in **Tables 3** and **4**).

Correlation of Pb Uptake with LMWOAs. The amount of the water-soluble LMWOAs present in the resin and rhizosphere quartz sand increased with increasing amount of Pb uptake, which was again dependent on the concentration of Pb present in the resin and rhizosphere quartz sand (**Figures 1** and **2**, **Tables 3** and **4**). The equilibrated Pb concentration in quartz sand amended with anion-exchange resin (the amount of Pb absorbed by lettuce plant roots was subtracted from the amount of Pb added and then expressed as milligrams of Pb per kilogram of quartz sand) and Pb content of lettuce plant root (expressed as microgram of lead per plant) were correlated with the quantity of total nonvolatile and volatile LMWOAs on the days 0, 3, and 10 under high and low transpiration. Each correlation tendency was divided into two linear correlations with different slopes (**Figure 3a,b**). Ross (*5*) reported that the organic ligands excreted from the tips of rootlets form complexes with heavy metals in rhizosphere to reduce the absorption of heavy metals by plant roots. The exclusion mechanism of plants is to excrete root exudates, which prevents heavy metals from transporting to or accumulating in above-ground part of the plants (*4*). The

Table 3. Effect of Transpiration on the Amount of Low-Molecular-Weight Organic Acids (μg) in 10 g of Anion-Exchange Resin in Rhizosphere at Days 0, 3, and 10 of Growth of Lettuce after Treatments with Irrigated Water with Different Pb Concentrations

Pb	nonvolatile^a										volatile^a	total^a (μg/10 g
treatment	lactic	glycolic	oxalic	succinic	fumaric	oxalacetic	D-tartaric	*trans*-aconitic	citric	total	propionic	of resin)
High Transpiration												
blank	0.82 Ebc	ND	2.31 Cc	1.41 Dd	ND	1.41 Dab	5.73 Ae	ND	3.11 Ba	14.8 d	3.63 bc	18.4 e
	1.28 Ee	ND	3.48 Ce	2.08 De	ND	1.58 Ed	7.81 Ae	ND	5.46 Be	21.7 f	10.2 d	31.9 f
	2.12 Ef	ND	5.67 Ce	3.27 Df	ND	3.42 De	12.1 Ad	ND	7.69 Bg	34.3 e	11.6 d	45.9 e
100 mg L^{-1}	0.98 Dabc	ND	2.71 Bb	1.58 Ccd	ND	1.38 Cab	6.81 Ac	ND	2.96 Ba	16.4 c	3.72 abc	20.1 d
	3.74 DEc	ND	4.89 CDd	4.44 CDbc	ND	5.67 Ca	24.5 Abc	2.11 Ec	10.8 Bd	56.2 d	27.5 c	83.7 d
	5.34 Ee	0.95 Fd	11.2 Ccd	6.92 Dd	ND	11.8 Cc	54.7 Ab	4.87 Eb	28.1 Be	124 c	44.5 c	168 c
200 mg L^{-1}	1.07 Eab	ND	2.02 Ccd	1.76 Cbc	ND	1.45 Dab	7.83 Aa	ND	3.04 Ba	1.71 b	3.87 ab	21.0 c
	4.76 CDb	4.32 CDc	5.33 Cd	5.07 Ca	1.56 Ec	5.12 Cb	29.8 Aa	3.08 EDb	17.6 Bc	76.2 c	36.8 ab	113 c
	5.55 Ee	6.53 Ec	11.8 Cc	8.73 Dc	6.33 Ec	12.1 Cbc	58.2 Aa	5.12 Eb	35.6 Bc	150 b	56.7 b	207 b
300 mg L^{-1}	1.21 Ea	0.97 EFa	2.23 Ccd	1.69 Dc	0.77 FGa	1.53 Da	7.36 Ab	0.56 Ga	3.12 Ba	19.5 a	4.03 a	23.5 a
	5.28 DEb	9.73 Ca	7.14 Dc	4.83 EFab	2.28 Gb	5.89 DEa	27.5 Aab	2.87 FGb	24.5 Bb	89.8 b	39.2 a	129 b
	6.30 Gd	16.3 Da	18.7 Cb	10.7 EFb	9.05 Fa	12.5 Eab	61.5 Ab	6.73 Ga	50.8 Ba	193 a	69.5 a	262 a
Low Transpiration												
blank	0.72 Ec	ND	2.23 Bcd	2.03 BCa	ND	1.22 Dbc	5.12 Af	ND	1.87 Cc	13.2 e	3.02 de	16.2 f
	1.15 Ee	ND	4.22 Cde	2.23 De	ND	1.76 Dd	8.73 Ae	ND	5.12 Be	23.2 f	8.53 d	31.7 f
	2.64 Ef	ND	5.89 Ce	3.77 Df	ND	3.33 De	12.8 Ad	ND	6.73 Bg	35.2 e	10.8 d	46.0 e
100 mg L^{-1}	0.83 Dbc	ND	1.97 Bd	1.72 BCc	ND	1.53 Ca	5.23 Af	ND	1.93 Bc	13.2 e	3.18 de	16.4 f
	2.69 Cd	ND	10.7 Bb	3.12 Cd	ND	3.43 Cc	20.5 Ad	ND	10.2 Bd	50.6 e	26.7 c	77.3 e
	8.73 CDc	ND	9.76 Cd	5.34 EFe	ND	6.68 DEd	43.6 Ac	4.37 Fc	23.9 Bf	102 d	47.1 c	149 d
200 mg L^{-1}	0.97 Dabc	0.96 Da	2.87 Bb	1.97 Cab	0.53 Eb	1.08 Dc	5.76 Ae	0.59 Ea	2.16 Cbc	16.9 bc	2.96 e	19.9 d
	4.12 Ec	3.72 Ed	11.8 Cb	2.99 EFd	2.27 Fb	5.76 Da	22.6 Acd	2.81 EFb	19.1 Bc	75.5 c	34.5 b	110 c
	11.8 Da	7.77 Eb	17.8 Cb	6.85 Ed	5.01 Fd	12.6 Dab	52.7 Ab	4.83 Fbc	32.6 Bd	152 b	54.9 b	207 b
300 mg L^{-1}	1.01 Fabc	0.95 FGa	3.76 Ba	2.01 Da	0.62 Gab	1.42 Eab	6.23 Ad	0.62 Ga	2.48 Cb	19.2 a	3.34 cd	22.5 b
	7.33 DEa	8.69 Db	14.1 Ca	4.32 FGc	3.85 Ga	5.83 EFa	27.5 Bab	4.13 FGa	32.1 Aa	108 a	37.6 ab	146 a
	11.3 Fb	15.6 Da	25.6 Ca	13.6 Ea	7.29 Gb	13.1 EFa	51.9 Ab	5.14 Hb	47.3 Bb	191 a	67.3 a	258 a

[a] Within the same column, the first, second, and third rows of each data set are the means of days 0, 3, and 10, respectively. Different lowercase letters following the data at day 0, 3, or 10 in a column indicate significant difference at the 5% probability level by the analysis of variance and least significant difference (LSD) values. Different uppercase letters following the data in a row indicate significant difference at the 5% probability level by the analysis of variance and LSD values. ND indicates the means are smaller than the method detection limits of glycolic acid (0.93 μg/10 g ofresin), fumaric acid (0.42 μg/10 g of resin), and *trans*-aconitic acid (0.36 μg/10 g of resin).

Table 4. Effect of Transpiration on the Amount of Low-Molecular-Weight Organic Acids (μg) in 10 g of Dry Quartz Sand in Rhizosphere at Days 0, 3, and 10 of Growth of Lettuce after Treatments with Irrigated Water with Different Pb Concentrations

Pb	nonvolatile^a										volatile^a	total^a (μg/10 g
treatment	lactic	glycolic	oxalic	succinic	fumaric	oxalacetic	D-tartaric	*trans*-aconitic	citric	total	propionic	of sand)
High Transpiration												
blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	ND	ND	ND	0.98 Ab	ND	ND	1.53 Aa	ND	1.23 Aab	3.74 c	1.36 b	5.10 c
	0.84 Cd	ND	1.24 ABCa	1.02 BCb	ND	1.02 BCa	1.62 Aa	ND	1.34 ABa	7.08 bc	1.58 a	8.66 b
100 mg L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	0.71 Cb	ND	1.32 ABa	1.01 BCb	ND	1.12 BCa	1.63 Aa	0.62 Cb	1.32 ABab	7.11 b	1.47 ab	8.58 b
	1.02 Bcd	ND	1.56 ABa	1.25 ABab	ND	1.26 ABa	1.58 Aa	0.93 Ba	1.28 ABa	8.88 b	1.52 a	10.4 b
200 mg L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	0.75 CDEb	1.23 ABCa	1.48 ABa	1.11 BCDb	0.58 Ea	1.17 ABCDa	163 Aa	0.71 DEab	1.39 ABab	10.0 a	1.69 ab	11.7 a
	1.12 BCbcd	1.44 ABCa	1.62 ABa	1.25 ABCab	0.96 Ca	1.23 ABCa	1.72 Aa	0.89 Ca	1.42 ABCa	11.7 a	1.72 a	13.4 a
300 mg L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	0.71 Db	1.46 ABCa	1.53 ABa	0.84 Db	0.98 BCDa	1.16 BCDa	1.81 Aa	0.93 CDab	1.53 ABa	11.0 a	1.81 a	12.8 a
	1.18 BCabcd	1.58 ABCa	1.69 ABa	1.15 Cab	1.15 Ca	1.18 BCa	1.98 Aa	1.09 Ca	1.48 ABCa	12.4 a	1.86 a	14.3 a
Low Transpiration												
blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	ND	ND	1.18 ABa	0.83 Bb	ND	ND	1.44 Aa	ND	1.12 ABab	4.57 c	1.41 ab	5.98 c
	0.84 Cd	ND	1.36 ABa	0.96 BCb	ND	0.93 BCa	1.56 Aa	ND	1.21 ABCa	6.86 c	1.53 a	8.39 b
100 mg L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.02 Bab	ND	1.23 ABa	1.12 ABb	ND	0.97 Ba	1.63 Aa	ND	1.18 ABab	7.15 b	1.48 ab	8.63 b
	1.36 ABabc	ND	1.36 ABa	1.18 ABab	ND	1.02 Ba	1.72 Aa	0.88 Ba	1.29 ABa	8.81 b	1.49 a	10.3 b
200 mg L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.11 ABab	1.02 Ba	1.32 ABa	1.15 ABb	1.12 ABa	1.18 ABa	1.58 Aa	0.91 Bab	1.21 ABab	10.6 a	1.62 ab	12.2 a
	1.48 ABab	1.33 ABa	1.43 ABa	1.36 ABab	1.35 ABa	1.21 Ba	1.74 Aa	1.09 Ba	1.28 ABa	12.3 a	1.59 a	13.9 a
300 mg L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.32 ABa	1.11 Ba	1.28 ABa	1.75 Aa	1.04 Ba	1.06 Ba	1.73 Aa	1.12 Ba	1.03 Bb	11.5 a	1.63 ab	13.1 a
	1.58 ABa	1.46 ABCa	1.47 ABCa	1.63 ABa	1.18 BCa	1.25 BCa	1.89 Aa	1.09 Ca	1.21 BCa	12.8 a	1.74 a	14.5 a

[a] Within the same column, the first, second, and third rows of each data set are the means of days 0, 3, and 10, respectively. Different lowercase letters following the data at day 0, 3, or 10 in a column indicate significant difference at the 5% probability level by the analysis of variance and least significant difference (LSD) values. Different uppercase letters following the data in a row indicate significant difference by the analysis of variance and LSD values. ND indicates the means are smaller than the method detection limits of acids: lactic (0.69 μg/10 g of dry quartz sand), glycolic (0.93 μg/10 g of dry quartz sand), oxalic (1.12 μg/10 g of dry quartz sand), succinic (0.73 μg/10 g of dry quartz sand), fumaric (0.42 μg/10 g of dry quartz sand), oxalacetic (0.81 μg/10 g of dry quartz sand), D-tartaric (1.32 μg/10 g of dry quartz sand), *trans*-aconitic (0.36 μg/10 g of dry quartz sand), citric (1.02 μg/10 g of dry quartz sand), and propionic (1.11 μg/10 g of dry quartz sand).

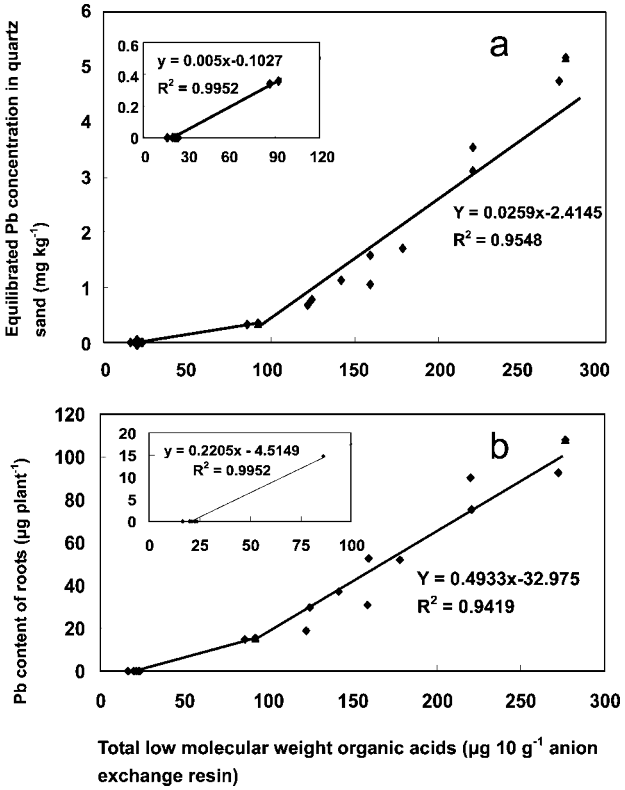

Figure 3. Correlations of (a) equilibrated Pb concentration in quartz sand (expressed as mg kg^{-1}) and (b) lead contents (expressed as μg plant^{-1}) in the roots of lettuce plants with the quantity of total low-molecular-weight organic acids in 10 g of anion-exchange resin in rhizosphere.

results of our study suggest that concentrations of LMWOAs in rhizosphere quartz sand mixed with anion-exchange resin of lettuce plants in each pot were lower than the critical concentrations and are within the range of linear correlation, as revealed by the lower slope of **Figure 3a** or **Figure 3b**. Since the concentration of LMWOAs in rhizosphere quartz sand mixed with anion-exchange resin was lower than the critical concentration, the concentration of Pb in lettuce roots did not increase with that in rhizosphere quartz sand mixed with anion-exchange resin. However, when the concentration of Pb in rhizosphere quartz sand mixed with anion-exchange resin exceeded the exclusion limit of LMWOAs, the function of the exclusion mechanism of LMWOAs diminished, and then absorption and subsequent accumulation of Pb by the roots of lettuce plants increased. Accordingly, the physiologically induced exudation of LMWOAs by the roots of lettuce plants into rhizosphere quartz sand mixed with anion-exchange resin increased with increasing Pb concentration in rhizosphere as well as in roots of lettuce plants (**Tables 3** and **4**, **Figure 3a,b**).

Comparing the effect of transpiration to that of Pb concentration stress in quartz sand mixed with anion-exchange resin on the quantity of water-soluble LMWOAs in rhizosphere of lettuce plants, the high and low transpiration treatments were not as prominent as Pb concentration stress (**Tables 3** and **4**). This indicates that stress from Pb concentration in quartz sand mixed with anion-exchange resin strongly promoted the increase in the quantities of water-soluble LMWOAs in rhizosphere of lettuce plants, even under a normal transpiration rate. Crops excrete organic acids to the rhizosphere environment to complex the forms of heavy metals with lower bioavailability, or to decrease the rhizosphere soil pH, which in turn increases the bioavailability of heavy metals (*17–19*). This results in the absorption and accumulation of heavy metals by crops. Petersen and Bottger (*41*) reported that LMWOAs are the main source of acidity in the rhizosphere environment. Higher quantities of water-soluble LMWOAs in rhizosphere soil may thus decrease the soil pH, which subsequently reduces the adsorption of heavy metals by soil colloids and increases the concentrations and bioavailability of heavy metals in soil solution (*42*). Salt et al. (*43*) proposed that the formation of cadmium citrate in rhizosphere soil promoted the movement of Cd via transpiration in plants. Therefore, whether the absorption of Pb by the roots of the lettuce plants in this study is due to the decrease in the rhizosphere pH of quartz sand mixed with anion-exchange resin by the excretion of LMWOAs and/or the complexation of LMWOAs with Pb needs to be further investigated. In this study, 5.0 g of anion-exchange resin was mixed thoroughly with rhizosphere quartz sand for each lettuce plant, for a total of 60.0 g of resin for 12 lettuce plants in a pot of 8 kg quartz sand. In our previous study (*32*), the experimental conditions were similar to those in the present experiment, except that anion-exchange resin was not mixed with rhizosphere quartz sand in the pots. These two studies revealed that there were no significant differences in the quantities of volatiles under high and low transpiration treatments, the effect of transpiration on the growth and yield of lettuce plants, the effect of Pb concentration (expressed as mg kg^{-1}) as well as Pb content (expressed as μg plant^{-1}) in the shoots and roots of lettuce plants, and the effect of transpiration on kinds and yield of LMWOAs in rhizosphere of lettuce plants (**Tables 1–4**, **Figures 1** and **2**). Moreover, a very important key point in this study is that the LMWOAs exuded by lettuce plants and from other sources in the rhizosphere environment were mostly exchanged and adsorbed by the anion-exchange resin that was mixed with the rhizosphere quartz sand. Accordingly, the LMWOAs do not exert their ability to complex with Pb present in rhizosphere of lettuce plants. This clearly indicates that the kinds and quantities of LMWOAs in rhizosphere, excreted by the roots and from other sources in the rhizosphere environment of lettuce plants, do not affect the absorption and subsequent accumulation of Pb by the roots and transport to the above-ground part of lettuce plants. Except for the complexation reaction of LMWOAs with heavy metals in rhizosphere, the acidification effect resulting from their dissociations, depending on the various pK_a values, may also play a role in converting heavy metals from a lower bioavailability form to a higher bioavailability form for plants. Therefore, the role of LMWOAs excreted from the roots of lettuce plants and from other sources in the rhizosphere environment in promoting the absorption of Pb by the roots of lettuce plants is mainly through the acidification effect on the rhizosphere, as demonstrated by our previous and present studies. This effect was verified by measuring the pH of the bulk quartz sand in the present study. As can be seen from **Table 5**, high and low transpiration treatments did not significantly affect the pH of the bulk quartz sand (significant labels were not shown) containing four concentrations of $Pb(NO_3)_2$ solutions (including blank) on days 0, 3, and 10 of growth of the lettuce plants. However, the pH of the bulk quartz sand significantly decreased with increasing concentration of $Pb(NO_3)_2$ solution in quartz sand mixed with anion-exchange resin on days 3 and 10 of growth of the lettuce plants under high and low transpiration treatments. Further, except for the blank (without treatment with $Pb(NO_3)_2$ solution), addition of each concentration of $Pb(NO_3)_2$ solution in quartz sand mixed with anion-exchange resin significantly decreased the pH of the bulk quartz sand with increasing growth of lettuce plants on days 0, 3, and

Table 5. Effect of Transpiration on the pH of Bulk Quartz Sand at Days 0, 3, and 10 of Growth after Treatments with Irrigated Water with Different Pb Concentrations

	pH[a]		
Pb treatment	day 0	day 3	day 10
	High Transpiration		
blank	5.82 Aa	5.73 Aa	5.61 Aa
100 mg L^{-1}	5.84 Aa	4.97 Bb	3.54 Cbc
200 mg L^{-1}	5.77 Aa	4.73 Bbc	3.23 Ccd
300 mg L^{-1}	5.82 Aa	4.53 Bc	2.93 Cde
	Low Transpiration		
blank	5.79 Aa	5.67 Aa	5.63 Aa
100 mg L^{-1}	5.80 Aa	5.02 Bb	3.64 Cb
200 mg L^{-1}	5.75 Aa	4.66 Bbc	3.11 Cde
300 mg L^{-1}	5.84 Aa	4.47 Bc	2.87 Ce

[a] Different lowercase letters following the data in a column indicate significantly different at 5% probability level by the analysis of variance and least significant difference (LSD) values. Different uppercase letters following the data in a row indicate significantly different at 5% probability level by the analysis of variance and LSD values.

10 (**Table 5**) for both high and low transpiration treatments. Because the quartz sand (particle size 0.1–0.5 mm, washed with 0.1 M HCl solution and then with deionized water) used in this study is regarded as a nonactive exchanger and the anion-exchange resin (Amberlite IRA402, previously saturated with Cl$^-$ and then washed with deionized water) used is inert to exchange of cations, including HO_3^+, in aqueous solutions of quartz sand mixed with anion-exchange resin, the pH of rhizosphere quartz sand mixed with anion-exchange resin can be regarded as the pH of the bulk quartz sand.

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