Article

Low Molecular Weight Organic Acids Increase Cd Accumulation in Sunflowers through Increasing Cd Bioavailability and Reducing Cd Toxicity to Plants

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Abstract: The use of low molecular weight organic acids (LMWOAs) for the phytoremediation of heavy metals has become a promising technique. We chose five kinds of organic acids (oxalic acid (OA), acetic acid (AA), tartaric acid (TA), malic acid (MA), and citric acid (CA)) with six application rates (1, 2, 3, 4, 5, and 6 mmol/kg) and planted sunflowers (*Helianthus annuus* L.) in Cd-polluted soil to study the efficiency of the phytoremediation of Cd and the degree of Cd toxicity to plants. Treatment with no acid application served as the control (CK). We analyzed the plant height dry matter and the Cd and nonprotein sulfhydryl (NPT) contents in the soil and plant tissues. OA, AA, TA, MA, and CA increased plant heights by 17.6–47.40%, 21.25–39.17%, 12.5–35.52, 5.10–30.50%, and 16.15–49.17%, respectively; shoot biomass of the sunflowers was increased except with MA. NPT decreased under LMWOA application, which, in the roots, increased with the increase in root Cd under LMWOA treatment; however, there was no obvious relationship in the stems and leaves. The composition of Cd in the soil changed significantly under the LMWOA treatments compared to the CK, and the changes in carbonate Cd and Fe-Mn oxide Cd were the most prominent. The plant Cd accumulation of OA, AA, TA, MA, and CA increased by 43.31%, 55.25%, 48.69%, 0.52%, and 32.94%, respectively, and the increase in root Cd content and shoot dry matter quality promoted the increase in Cd accumulation. The LMWOAs were more likely to affect the phytoremediation of Cd by changing total P (TP) rather than total N (TN).

Keywords: sunflowers; Cd; low molecular weight organic acids; nonprotein sulfhydryl; biomass

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1. Introduction

With the rapid development of industry, the number of heavy metal-contaminated lands in different regions of the world has increased significantly [1–3]. In particular, mining, industrial water, and battery factories are responsible for polluting a lot of farmland with Cd, Pb, Cu, etc. [4,5], which leads to heavy metals penetrating the food chain and thereby entering the human body, threatening people's lives and health [6–9]. Heavy metal dissipation with mineral fertilizers also adds considerable amounts of heavy metals to agricultural soil [10]. At present, the research on phytoremediation of heavy metal pollution has attracted wide attention [11–14], mainly focusing on two aspects: the enrichment ability and the biomass of heavy metals in plants.

Important progress has been made in the screening of heavy metal hyper accumulators, such as *Celosia argentea* L. [15], *Spinacia oleracea* L. [16], *Populus* and *Salix* [17], and Jack Bean [18]. The bioavailability of Cd in soil is an important factor affecting the uptake of Cd by plants, and exchangeable and carbonate forms are the main forms of Cd [19,20]. The

efficiency of phytoextraction is related to the ability of plants to accumulate the toxicant, and there are significant differences in the remediation of heavy metals among different crops [21,22].

Low molecular weight organic acids (LMWOAs) can increase the bioavailability of heavy metals in soils by forming water-soluble complexes with said metals [23], as well as enhancing their phytoextraction efficiencies [15,24,25]. LMWOAs may inhibit or facilitate Cd^{2+} transport under different environmental conditions [26]. The desorption of heavy metals by different organic acids is different [27–29], which is due to the differences in the complexation strength between organic acids and heavy metals [30]. Therefore, for different crops, the effect of different organic acids on these two aspects needs to be quantitatively evaluated, and the reasons for the change need to be analyzed.

Nonprotein sulfhydryl (NPT) compounds mainly include glutathione (GSH), phytochelatins (PC), and cysteine (Cys), and the complexation of heavy metals with sulfhydryl compounds forms the response of plants to heavy metal stress [31]. NPT is induced by various metals and metalloids in cells [32], and GSH can scavenge several potentially toxic ROS (such as O_2^- or $\text{OH}^\cdot$) and can help cells cope with the oxidative stress caused by oxidation; meanwhile, PC shows hypersensitivity to Cd stress [33]. Studying the change of NPT is helpful for determining the main factors for plants to improve the accumulation of heavy metals.

Cd is a very toxic and ubiquitous heavy metal in soil that seriously threatens food safety in China [15], and higher Cd in soil could produce toxic effects on crops, thereby inhibiting crop growth and reducing biomass [34]. LMWOAs could enhance nutrient uptake and translocation into the aboveground parts of plants [35]. Sunflowers have broad application potential in the remediation of soil Cd pollution because of their large biomass, strong Cd tolerance, and low requirement of water and fertilizer, and they can be used as biomass energy [36–39]. The Cd absorption of sunflower increased linearly with the increase in Cd levels [40], although Cd inhibited the growth of oil sunflower, the application of plant growth regulators could improve the Cd removal efficiency [41]. In the present study, we investigated the phytoextraction efficiency of sunflowers using Cd-polluted soil with a Cd content of 19.8 mg/kg. In addition, we assessed the effects of oxalic acid (OA), acetic acid (AA), tartaric acid (TA), malic acid (MA), and citric acid (CA) on Cd mobilization and total Cd accumulation. The objectives were (i) to investigate the effectiveness of these five LMWOAs in phytoremediation and (ii) to clarify the role of NPT in the phytoremediation of Cd.

2. Methods and Materials

2.1. Soil Preparation

This experiment was conducted from October 2018 to January 2019 in a greenhouse ($20 \times 50 \text{ m}^2$), located at the Hongmen Experimental Station of Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, in Henan province (latitude $51^\circ 5' 45'' \text{ N}$ and longitude $113^\circ 55' 5'' \text{ W}$). The soil was collected from a Cd-contaminated area (soil Cd concentrations varied from 3.38 to 44.94 mg/kg) [42] near a battery factory (35.35696 N , 113.839256 E) in 2018, of which the texture is silt clay loam, and the percentages of particle size for 0.02–2 mm, 0.002–0.02 mm and $< 0.002 \text{ mm}$ are 27.88%, 54.77% and 17.35%, respectively. The total amount of Cd in the soil was 19.5 mg/kg (measured by the atomic absorption spectrometry method). The total amounts of soil nitrogen, phosphorus, and potassium were 1.14, 0.63, and 0.086 g/kg, respectively. The soil organic matter content was 2.70% and the pH was 8.20. The soil was first air-dried for three months prior to passing through a 2 mm sieve to remove debris. It was then fertilized with base fertilizers (analytical grade, Tianjin Bodi Chemical Co., Ltd., Tianjin, China): 18.44 g/kg of urea, 12.2 g/kg of KH_2PO_4 , and 50.2 g/kg of KNO_3 (fertilizer mass/soil mass) with Cd content of 0 mg/kg [43].

2.2. Experimental Design and Sampling

Xianrun 2 (*Helianthus annuus* Linn., Xianrui Seed Technology Co., Ltd., Beijing, China) was used as the test material. Two seeds were sown in the middle of each pot on 2 October 2018. After all seedlings had emerged four days later, on October 6, the seedlings in each pot were thinned to one. Water was sprayed on the soil surface in each pot during the experiment to maintain its soil moisture at 70–100% of the soil field capacity. All pots were weighed daily to estimate the water loss from evapotranspiration, and water was added until its content was 100% of the field capacity whenever the soil moisture was less than 70% of the field capacity. We sprayed solutions of OA, AA, TA, MA, and CA of 1, 2, 3, 4, 5, and 6 mmol/kg (mass of acid/mass of soil) onto the soil surface in different pots at 20 days after seedling emergence, based on the application rates used in the literature [43,44]. Table 1 shows the chemical properties of the five used organic acids. Pots without added acids were used as controls (CK; 0 mmol/kg). Overall, there were 31 treatments, each with three replicates. The sunflower plants were harvested on 9 January 2019, and the test period lasted 100 days. The plants were divided into four parts: roots, stems, leaves, and buds, which were all packed into paper bags. Rhizosphere soil taken from each pot was thoroughly mixed and then sealed in a plastic bag before being frozen at -80°C until chemical analysis.

Table 1. Abbreviations and concentration of the five low molecular weight organic acids (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China).

Acid	Abbreviation	Concentration (%)
Glacial acetic acid	AA	99.8
Oxalic acid	OA	99.99
Citric acid monohydrate	CA	99.99
DL-malic acid	MA	99.99
DL-tartaric acid	TA	99.99

2.3. Measurement

2.3.1. Plant Height, Biomass, and Nonprotein Sulfhydryl (NPT)

The plant height from the soil surface to the highest point was measured using a meter stick (100 cm) on 26 November 2018. The roots and shoots of the sunflower plants were washed thoroughly with both tap and deionized water. Next, they were sterilized at 105°C for 30 min, and then dried at 75°C until the mass was constant. The dry weights of the sunflower roots and shoots (stems, leaves, and buds) were measured.

The NPT of the stems, leaves, and buds at harvest was determined by the method of Rama and Prasad [45]. The reaction system was as follows: 1 mL of supernatant, 1.85 mL of 0.2 mol/L Tris HCl (pH = 8.2), and 0.15 mL of 10 mmol/L 2-nitrobenzoic acid (DTNB). After reaction for 20 min, the absorbance was measured at 412 nm with TECAN infinite M200, with no DTNB as the blank.

2.3.2. Soil Index

Soil total N (TN) and total P (TP) were measured using an AA3 flow analyzer (Brown Rupee Pte Ltd.) [46]. Available phosphorus (AK) was determined using flame photometry [47].

2.3.3. Cadmium in the Plant Tissues and Soil

The Cd content in the roots, stems, leaves, and buds was measured. Ground plant samples, each weighing 0.2 g, from each plant component were mixed with 10 mL of pure concentrated nitric acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) in a digestion tube and soaked for 7–8 h at room temperature ($24 \pm 1.0^{\circ}\text{C}$). The total Cd in the soil was measured using the same method as that used for plants using a graphite furnace atomic absorption spectrophotometer (AA-6300FG, Japan) [43]. We also measured

exchangeable Cd, carbonate Cd, Fe-Mn oxide Cd, organic Cd, and residual Cd using the synchronous extraction method following the protocol presented in the literature [48].

The transport factor (*TF*), bioconcentration factor (*BF*), and bioavailability coefficient (*BC*) were calculated as follows:

$$TF = \text{Cd in shoot} / \text{Cd in root} \quad (1)$$

$$BF = \text{Cd in shoot} / \text{Cd in soil} \quad (2)$$

$$BC = (\text{Exchangeable Cd} + \text{Carbonate Cd}) / \text{soil Cd} \quad (3)$$

2.4. Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) with SPSS 19.0 (SPSS Inc, Chicago, IL, USA), and each treatment contains three replicates. Multiple comparisons were made using Duncan's new multiple range test (MRT). In all analyses, differences were considered significant at $p < 0.05$. Correlation analysis based on Pearson's method and on principal co-ordinates analysis (PCoA) was carried out using R 3.6.0.

3. Results

3.1. Plant Height and Biomass

Figure 1 shows plant heights on 26 November 2018 and biomass at harvest. The LM-WOAs increased the plant height of the sunflowers (Figure 1a), with OA, AA, TA, MA, and CA increasing by 17.6–47.40%, 21.25–39.17%, 12.5–35.52%, 5.10–30.50%, and 16.15–49.17%, respectively, compared to CK. In addition, the plant heights under 1 mmol/kg of OA and TA, 2 mmol/kg of AA and MA, and 4 mmol/kg of CA had significant differences with CK ($p < 0.05$).

The application of microorganisms did not significantly reduce the biomass of the roots and shoots, and 3 mmol/kg of OA, 5 mmol/kg of AA, 4 mmol/kg of TA and CA, and 2 mmol/kg of MA significantly increased the biomass of the roots and shoots (Figure 1b,c). The mean biomass of shoots for OA, AA, TA, MA, and CA treatments increased by 59.18%, 66.36%, 66.92%, 11.23%, and 63.34%, respectively.

3.2. Cd Form in Soil

The order of Cd content in rhizosphere soil was carbonate Cd > exchangeable Cd > Fe-Mn oxide Cd > residue Cd > organic Cd, indicating that the change of soil Cd content was mainly determined by the first three forms of Cd. The carbonate Cd content was increased under OA treatment (Figure 2a), which increased by 51.46%, 41.65%, and 43.73% ($p < 0.05$) under 4, 5, and 6 mmol/kg, respectively; however, OA treatment decreased the content of Fe-Mn oxide Cd, with 2, 3, 4, and 6 mmol/kg resulting in significant decreases of 50.44%, 49.40%, 45.87%, and 48.35%, respectively ($p < 0.05$).

Figure 1. Cont.

Figure 1. Plant height and biomass under low molecular weight organic acid (LMWOA) treatments and the control (CK). (a) Plant height; (b) biomass of the roots; (c) biomass of the shoots. Different lowercase letters show significant differences for $p < 0.05$.

The application of AA increased the content of carbonate Cd (Figure 2b), with the 4 mmol/kg treatment resulting in significant differences compared to the CK (54.65%, $p < 0.05$); meanwhile, the content of Fe-Mn oxide Cd under 1–6 mmol/kg decreased by 36.72–52.01% ($p < 0.05$). Additionally, exchangeable Cd under the 2–5 mmol/kg treatments also decreased, with the contents following the 5 mmol/kg treatment being the most significantly different.

As Figure 2c shows, the application of TA increased the content of exchangeable Cd and carbonate Cd with no significant difference to the CK, but the 3 mmol/kg treatment decreased Fe-Mn oxide Cd by 57.86% ($p < 0.05$) compared to the CK.

The application of MA increased carbonate Cd in the soil by 45.27–72.95% ($p < 0.05$) and decreased Fe-Mn oxide Cd by 4.03–30.69% (Figure 2d), while the results of the 2–6 mmol/kg treatment were significantly difference compared to the CK. In addition, the exchangeable Cd after the 5 mmol/kg treatment and the residue Cd after the 6 mmol/kg treatment were significantly lower than the contents in the CK ($p < 0.05$).

The application of CA reduced exchangeable Cd and Fe-Mn oxide Cd by 16.02–43.95% and 29.46–50.48%, but increased carbonate Cd by 19.73–76.34%, when compared to the CK (Figure 2e).

3.3. Nonprotein Sulphydryl (NPT) in Plant Tissues

As Figure 3 shows, the NPT in the plant tissues showed as root > stem > leaf. Compared to the CK (Figure 3a), the application of OA significantly reduced the content of NPT in the roots by 14.11–43.79% ($p < 0.05$). Additionally, the content of NPT in the stems under the 1, 3, 4, and 6 mmol/kg treatments significantly decreased by 40.83%, 13.44%, 25.32%,

and 23.51%, respectively ($p < 0.05$), while in the leaves under the 2, 3, 4, and 6 mmol/kg treatments, it significantly decreased by 51.09%, 41.12%, 39.56%, and 29.60%, respectively ($p < 0.05$).

Figure 2. Cont.

Figure 2. Cd content of different forms in the soil under LMWOA treatments and the CK; (a) oxalic acid (OA), (b) acetic acid (AA), (c) tartaric acid (TA), (d) malic acid (MA), and (e) citric acid (CA). Different lowercase letters show significant differences for $p < 0.05$.

Figure 3. Cont.

Figure 3. Nonprotein sulfhydryl (NPT) in the plant tissues under LMWOA treatments and the CK; (a) oxalic acid (OA), (b) acetic acid (AA), (c) tartaric acid (TA), (d) malic acid (MA), and (e) citric acid (CA). Different lowercase letters show significant differences for $p < 0.05$.

For AA (Figure 3b), the NPT content in the roots significantly decreased by 27.37–50.32% ($p < 0.05$), and the NPT content in the stems under the 1–5 mmol/kg treatments was close to that of the CK, but the 6 mmol/kg treatment significantly decreased it by 45.99% ($p < 0.05$). Except for the 4 mmol/kg treatment, the other treatments significantly reduced the NPT content in the leaves ($p < 0.05$).

The application of TA (Figure 3c) significantly reduced the NPT content in the roots by 10.11–52.21% ($p < 0.05$), while the content of NPT in the stems under the 2–5 mmol/kg treatments significantly decreased by 22.22–34.37% ($p < 0.05$). Additionally, the 1, 4, and 6 mmol/kg treatments significantly reduced NPT in the leaves by 37.07%, 51.71%, and 29.60%, respectively ($p < 0.05$).

The application of MA (Figure 3d) reduced the content of NPT in the roots (22.95–45.68%) and the stems (5.17–25.06%); except for the 2 mmol/kg treatment, the other treatments resulted in significant differences from the CK. NPT in the leaves increased by 38.32% and 38.32% ($p < 0.05$) under the 1 and 5 mmol/kg treatments but decreased by 57.01% under the 2 mmol/kg treatment ($p < 0.05$).

When CA was applied (Figure 3e), NPT in the roots decreased by 26.74% and 19.79% ($p < 0.05$) under the 2 and 5 mmol/kg treatments, while NPT in the roots increased by 14.11% in the 4 mmol/kg treatment ($p < 0.05$). Compared to the CK, NPT in the stems decreased significantly under the 4 and 6 mmol/kg treatments ($p < 0.05$), while NPT in the leaves decreased by 38.94%, 33.64%, and 57.32% under the 4, 5, and 6 mmol/kg treatments ($p < 0.05$).

3.4. The Relationship between Soil Cd, Soil NPK, and Plant Cd

Correlation analysis was performed on soil Cd, soil NPK, and plant Cd ($n = 93$; Table 2). Root Cd had a significant negative correlation with stem and bud Cd ($r = -0.340$ and -0.219 , respectively), and had a significant positive correlation with TP ($r = 0.345$). Stem Cd had a significant positive correlation with bud Cd, Fe-Mn oxide Cd, and TN ($r = 0.648$, 0.306 , and 0.208 , respectively), and had a significant negative correlation with TP ($r = -0.208$). Leaf Cd had a significant positive correlation with bud Cd and exchangeable Cd ($r = 0.291$ and 0.302 , respectively). Bud Cd had a significant positive correlation with Fe-Mn oxide Cd and Organic Cd ($r = 0.319$ and 0.239). Soil total Cd had a significant positive correlation with exchangeable Cd, carbonate Cd, and Fe-Mn oxide Cd ($r = 0.373$, 0.397 , and 0.282). TP had a significant positive correlation with exchangeable Cd ($r = 0.255$) and a significant negative correlation with carbonate Cd and Fe-Mn oxide Cd ($r = -0.219$ and -0.215). AK had no significant correlation with soil Cd or plant Cd.

Table 2. The correlation coefficients between soil Cd, soil NPK, and plant Cd.

Indexes	Root Cd	Stem Cd	Leaf Cd	Bud Cd	Exchangeable Cd	Carbonate Cd	Fe-Mn Oxide Cd	Organic Cd	Residue Cd	Soil Total cd	TN	TP	AK
Root Cd	1.0000												
Stem Cd	−0.340 **	1.0000											
Leaf Cd	0.0530	0.0490	1.0000										
Bud Cd	−0.219 *	0.648 **	0.291 **	1.0000									
Exchangeable Cd	0.0200	0.0210	0.302 **	0.0250	1.0000								
Carbonate Cd	−0.0540	−0.1030	−0.0510	−0.0800	−0.414 **	1.0000							
Fe-Mn oxide Cd	−0.1830	0.306 **	−0.1560	0.319 **	−0.1520	0.0040	1.0000						
Organic Cd	0.0560	0.1150	−0.0230	0.239 *	−0.0730	0.0960	0.628 **	1.0000					
Residue Cd	0.0280	0.0140	0.1360	0.0210	0.0880	−0.403 **	−0.348 **	−0.370 **	1.0000				
Soil total Cd	−0.1360	0.1210	0.1960	0.1620	0.373 **	0.397 **	0.282 **	0.1940	0.1410	1.0000			
TN	0.0210	0.208 *	0.1540	0.1550	0.0240	0.0000	−0.0540	0.0740	−0.0310	−0.0330	1.0000		
TP	0.345 **	−0.208 *	0.0140	−0.1910	0.255 *	−0.219 *	−0.215 *	−0.0770	−0.0100	−0.1570	0.1840	1.0000	
AK	−0.0520	0.1110	−0.1540	0.0040	−0.0220	0.0080	0.0480	−0.0510	0.0580	0.0600	−0.0360	0.1100	1.0000

Note: * and ** represent that there was a significant correlation at $p < 0.05$ and $p < 0.01$, respectively. $n = 93$. AK—available phosphorus; TN—total N; TP—total P.

4. Discussion

4.1. The Effect of Organic Acid on Plant Growth and Soil TN and TP

Organic acids can promote the growth of sunflowers (*Helianthus annuus* Linn.), mainly in terms of increasing the plant height and the shoot biomass, which is consistent with the results of maize using citric and oxalic acids [49] and rapeseed using OA, CA, TA, MA, and AA [50]. OA, AA, and CA are beneficial for increasing the dry weight of the root system (Figure 1), which is mainly due to the increase in soil nitrogen and phosphorus content (Figure 4), especially the significant increase in TP. Under the action of plant root and soil water change, soil nutrients moved from nonrhizosphere to rhizosphere, increasing the TN and TP in rhizosphere soil [51–55]. Additionally, LMWOAs can be seen as a carbon source [56], which could promote the reproduction of soil microorganisms [57,58], resulting in an increased supply of soil N and P [59,60]. In addition, the activities of urease, catalase, and alkaline phosphatase are increased by applying organic acids [57]. In addition, MA or AA application more or less reduces Cd phytotoxicity by increasing the growth parameters and photosynthetic pigment concentrations, by decreasing the accumulation of H_2O_2 , and by improving the root activity [61], which can also promote crop growth.

Figure 4. TN and TP in rhizosphere soil under organic treatments and CK; oxalic acid (OA), acetic acid (AA), tartaric acid (TA), malic acid (MA), and citric acid (CA). Different lowercase letters show significant differences for $p < 0.05$.

Despite the fact that phosphorus and nitrogen were increased as the LMWOAs were applied [62], the effect of TP on dry matter accumulation was greater than that of TN. TP was closely related to exchangeable Cd, carbon Cd, Fe-Mn oxide Cd, root Cd, and stem Cd (Table 2). As Table 3 shows, TN was positively correlated with plant biomass ($p > 0.05$), while TP was positively correlated with the biomass of the stems, leaves, shoots, and plants ($p < 0.05$). The addition of LMWOAs mainly increased the content of available phosphorus, and the LMWOAs had no significant effect on the content of hydrolytic nitrogen [63]. The amount of nitrogen applied in the experiment was higher than that of TP (see Section 2.1), resulting in the content of TN being higher than that of TP; therefore, nitrogen is sufficient or even excessive for plants, and at this point, the supply of phosphorus is critical [64,65]. Some low molecular organic acids (e.g., citric acid, tartaric acid, and acetic acid) can further increase the content of phosphate in soil through reducing the binding of phosphate ions to aluminum and iron [66].

Table 3. The correlation coefficients between TN, TP, and AK in the rhizosphere and plant biomass.

Indexes	Root	Stem	Leaf	Bud	Shoot	Total Biomass
TN	0.135	0.082	0.069	0.007	0.073	0.081
TP	0.185	0.236 *	0.235 *	0.113	0.250 *	0.250 *
AK	0.002	−0.044	−0.014	−0.06	−0.045	−0.042

* represents a significant correlation at $p < 0.05$.

4.2. The Effects of Organic Acids on Soil Cd

Compared to the CK, the application of organic acids increased the content of carbonate Cd and decreased the content of Fe-Mn oxide Cd (Figure 2), but the average exchangeable Cd of the other treatments also decreased, except for TA. The change of total Cd in the soil was mainly determined by these three forms of Cd (Table 2), which was similar to our previous research results on oil vegetables [43]. There were significant differences in the composition of the soil Cd forms between the LMWOA treatments and the CK-treated soil (the blue ellipse formed by A1, A2, and A3 is away from the other ellipses in Figure 5), and significant differences were also found among the different application rates of AA, MA, and CA (Figure 5). The transformation of Fe-Mn oxide Cd to other forms is the reason for the improvement in the bioavailability of Cd. The driven dissolution of Fe and Mn by LMWOAs had a major control on the mobilization of Cd [67,68]. It can also be seen that the organic acids were able to change the form of Cd and improve the bioavailability of Cd (Figure 6a); it is beneficial for plants to absorb more Cd. As Figure 5b shows, the plant Cd accumulations of OA, AA, TA, MA, and CA increased by 43.31%, 55.25%, 48.69%, 0.52%, and 32.94%, respectively. The important reason for this is that organic acids can effectively activate the solid heavy metals adsorbed on the surface of soil particles and can transform said solid heavy metals into effective forms easily absorbed by plant roots [69].

The addition of acid did not significantly reduce Cd in rhizosphere soil; the mean reductions in Cd in soil for OA, AA, and CA increased by 1.72%, 3.54% and 8.38% compared to the CK, which for TA and MA decreased by 11.68% and 5.97%, and even the 5 and 6 mmol/kg TA treatments significantly increased Cd (Figure 6c), indicating that the sunflowers mainly increased the root surface area to engage with more Cd [70] or increased Cd migration from nonrhizosphere soil to rhizosphere soil. In addition, it is not that a higher application rate of organic acids results in better remediation of Cd in soil, because the toxicity of Cd increases with an increase in the concentration of low molecular weight organic acids [71]. For example, the 6 mmol/kg OA, TA, CA, and AA treatments reduced NPT in the roots, stems, and leaves (Figure 3), which is not conducive to maintaining a high Cd content in plant tissues.

4.3. Organic Acids Are Beneficial to the Cd Detoxification of Plants

The application of organic acids significantly reduced the NPT content in the roots, but the effect on the NPT content in the stems and leaves changed with the application rates (Figure 3). In full, the NPT content in the roots was higher than that in the stems and leaves, which is consistent with the Cd content in the roots, stems, and leaves (the average Cd contents in the roots, stems, and leaves were 36.6, 7.22, and 12.95 mg/kg). NPT plays an important role in the tolerance and detoxification of Cd in plants, which can be complexed with excessive Cd [72–74].

We found that there was a high correlation between Cd and NPT in the roots; with the increase in root Cd content, NPT also showed an increasing trend. Except for CA, the R^2 of the fitting linear equation of other acids was greater than 0.39 (Figure S1). There was no significant increase or decrease in the NPT content of the stems and leaves as the Cd in the stems and leaves changed, which is different from the results of *Posidonia oceanica* L. [75]. NPT contains glutathione (GSH) and other NPT compounds (plant chelating peptide (PC), cysteine (Cys), etc.). The contributed NPT compounds react differently to the detoxification of Cd in different plants, such as maize [76], *Arabis paniculata* Franch [77], *Eichhornia crassipes* [78], and different plant tissues [79,80]. Not all of the PCs induced by Cd were involved in the detoxification of Cd [32] and the change trend of NPT and GSH in the leaves may be opposite under Cd stress [81] or in different growth stages [82]. Therefore, further analysis of GSH, PC, and Cys is needed.

Figure 5. Principal co-ordinates analysis (PCoA) on the soil Cd forms of the organic treatments and the CK (0 mmol/kg); (a) oxalic acid (OA), (b) acetic acid (AA), (c) tartaric acid (TA), (d) malic acid (MA), and (e) citric acid (CA). A1, A2, and A3 are the three samples of CK (0 mmol/kg); B1, B2, and B3 are the three samples of 1 mmol/kg; C1, C2, and C3 are the three samples of 2 mmol/kg; D1, D2, and D3 are the three samples of 3 mmol/kg; E1, E2, and E3 are the three samples of 4 mmol/kg; F1, F2, and F3 are the three samples of 5 mmol/kg; G1, G2, and G3 are the three samples of 6 mmol/kg.

Figure 6. Bioavailability coefficient (a), plant Cd accumulation (b), and reduction in Cd in the soil (c) with the organic acids and their application rates, along with the CK (0 mmol/kg)—oxalic acid (OA), acetic acid (AA), tartaric acid (TA), malic acid (MA), and citric acid (CA).

NPT is helpful for Cd migration from roots to shoots [83,84], reducing the toxicity of Cd to roots [79]. Increasing the toxicity of Cd to shoots is not conducive to improving the efficiency of the phytoremediation of heavy metals. In our experiment, NPT in the stems and leaves showed a negative correlation with the biomass of said stems and leaves (Figure S2), indicating that a decrease in NPT was beneficial to biomass accumulation. The reason for this is that OA, AA, TA, and CA significantly reduced the migration of Cd from the roots to shoots (Table S1), but NPT in the shoots did not change with the content of Cd and weakened the toxic effect of Cd on the shoots, which was conducive to maintaining the strong photosynthetic capacity of the plants and accumulating more dry matter [85]. Although the content of Cd in the shoots did not increase significantly, the dry matter in the

shoots increased dramatically (Figure 1), which led to an increase in the Cd accumulation in the shoots (Figure 6).

The purpose of phytoremediation is to increase the quality of soil and serve human beings [86], which is not only reflected in the removal of soil pollution, but also can bring income in the process of phytoremediation, such as selling the thallium from phytoremediation [87]. Although there is a two-way relationship between tourism and Cd pollution [88], for polluted land, combining agriculture with tourism is a benefit to save the cost of soil remediation, as sunflowers are very beautiful (Figure S3), which were introduced as new crops for enhancing rural landscape [89]. We could reduce the persecution of heavy metals on sunflower by applying LMWOAs and Cd accumulation. The order of maximum Cd accumulation was $AA > OA > TA > CA > MA$, but the corresponding application rate was different. Additionally, the Cd accumulation changed in a wave shape with different application rates. The effects of organic acids on heavy metals in soil are twofold, mainly depending on the concentration and types of organic acids, the forms of heavy metals, the soil quality, and action the environment [90], through influencing the complexing ability of Cd with organic acids [91]. There is evidence that LMTOAs have different effects on the transfer of heavy metals from soil to vegetables [16]: MA is more effective in the alleviation of Cd stress in sunflowers than AA, because MA diminishes Cd toxicity by enhancing root activity and reducing H_2O_2 levels [92]. Meanwhile, OA was the only acid effective in increasing the metal micronutrients at the lowest Pb level, and citric acid was not at the highest Pb level [49]. Based on the above results, for specific soil and different crops, it is necessary to select suitable organic acids to promote phytoremediation.

5. Conclusions

The five investigated LMWOAs (i.e., OA, AA, TA, MA, and CA) had positive effects on the bioavailability coefficient of Cd in the soil. After the application of the LMWOAs, the main toxic effect of Cd on the plants was on the roots. NPT in the roots increased with the increase in root Cd content, but the OA, AA, TA, and CA reduced the transfer coefficient of Cd from the roots to the stems and leaves; however, they also weakened the toxic effect on the stems and leaves, manifested as a lack of a correlation with the Cd and NPT content of the stems and leaves. This effect helped to maintain normal development aboveground, while the increased supply of N and P in the soil further promoted the growth of the sunflowers, especially the shoot biomass, which was increased by 59.18%, 66.36%, 66.92%, 11.23% and 63.34% for OA, AA, TA, MA, and CA treatments, respectively. TP played a more important role in the change of Cd in the soil and plant tissues than TN. Although application of the LMWOAs did not significantly reduce Cd in rhizosphere soil (the reduction in Cd in soil for OA, AA, and CA increased by 1.72%, 3.54% and 8.38 compared to the CK, which decreased for TA and MA by 11.68% and 5.97%), it significantly increased the aboveground dry matter quality of the oil sunflowers and increased the Cd accumulation of plants.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2075-163X/11/3/243/s1>: Figure S1: Linear fitting results of NPT and Cd in roots under 1–6 mmol/kg organic acid treatments; Figure S2: Linear fitting results of the NPT content and biomass of roots (a), stems (b), and leaves (c); Figure S3: Sunflower during experiment period; Table S1: The transport factor (TF) and bioconcentration factor (BF) of different plant tissues.

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