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Effects of chlorsulfuron and cadmium on metabolites of maize seedlings

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Abstract

The impact of persisting herbicide residues on succeeding crops is of great concern to farmers because even the presence of very low concentrations can inhibit growth of crop and cause crop reduction. Furthermore, wastewater irrigation can lead to cadmium accumulation in soils. Thus, the co-occurrence of low amounts of herbicide residues and cadmium within agricultural fields are difficult to avoid. How the combination of these two pollutants affect plant metabolites remains to be elucidated and thus warrants investigation. Maize seeds were planted in soil that had been sprayed with chlorsulfuron and Cd, then we studied the effects of exposure to the herbicide chlorsulfuron (0.001, 0.003, 0.005, 0.008, and 0.010 mg kg^{-1}) and cadmium (as 5.0 mg kg^{-1} $CdCl_2$) on maize seedlings by utilizing nuclear magnetic resonance (NMR) after 21 d. Principle component analysis of ^{1}H NMR spectra clearly discriminated between control and treatment groups. Compared with chlorsulfuron-only treatments, treatments using both contaminants showed higher content of phenolic acids, aspartic acid, choline, β-galactose, and α-glucose in the seedlings. Contrary to previous reports, we found larger pools of branched-chain amino acids in seedlings exposed to chlorsulfuron and $CdCl_2$. These findings indicate that $CdCl_2$ did not aggravate the effects of chlorsulfuron on maize seedlings metabolites. $CdCl_2$ elicited significant changes in plant metabolism at a concentration that did not impair plant growth. Moreover, chlorsulfuron did not inhibit branched chain amino acid synthesis.

Keywords: branched-chain amino acid, joint effect, metabolomics, nuclear magnetic resonance, *Zea mays*.

Introduction

Chlorsulfuron (2-chloro-N-[(4-methoxy-6-methyl-1,3, 5-triazine-2-yl) aminocarbonyl]-benzene-sulfonamide) is a selective, systemic post-emergence herbicide. It was the first sulfonylurea herbicide approved in the United States (Zhou *et al.* 2020), and globally, it was one of the most commonly used herbicides of its type for removing broadleaf weeds and other weedy annuals in wheat and barley fields. According to existing reports, the primary target of chlorsulfuron and other sulfonylurea herbicides is acetolacetate synthase (ALS), the enzyme specific to branched-chained amino acid biosynthesis (Fan 2003). In addition, chlorsulfuron inhibits spermidine accumulation in mitotic tissues of root tips more effectively than other specific inhibitors of spermidine synthesis. Because of its high efficacy as a weed killer, chlorsulfuron is applied to wheat fields at very low concentrations. However, several field studies indicate a long residual time, with a half-life of 4 to 10 weeks, depending on soil properties (Shan 1998). Moreover, the previous study reported that chlorsulfuron persisted for 3 - 5 years in alkaline soils (Hollaway *et al.* 2006). The other study also reported that it would theoretically take 271.8 d for chlorsulfuron to degrade to 0.2 μg kg^{-1} (below this amount is considered safe for rice roots) (Zhang *et al.* 2015). Chlorsulfuron has been used

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Abbreviations: ALS - acetolacetate synthase; CAT - catalase; GST - glutathione S - transferase; MDA - malondialdehyde; NMR - nuclear magnetic resonance; SOD - superoxide dismutase; POD - peroxidase; TSP - sodium-3-trimethylsilylpropionate.
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for several years in China and the soil in northern China is alkaline, so chlorsulfuron degrades slowly there. Thus, it is likely that application of chlorsulfuron produces residual effects on terrestrial plant reproduction.

The heavy metal cadmium has long been known for its detrimental physicochemical properties that can result in environmental damage. Consequently, the environmental discharge limit of Cd is far lower than for most heavy metals. Cadmium is non-biodegradable and its accumulation in crops poses a threat to human health. In plants, Cd binds to apoplastic and symplastic target sites and disrupts the active components affecting fundamental processes such as cell division, mineral nutrition, and saccharide metabolism (Ahmad *et al.* 2009, Moussa and El-Gamal 2010, Deng *et al.* 2020, Li *et al.* 2020).

Soil pollution is rarely caused by a single contaminant and toxic effects on an individual organism are not easily attributable to a single chemical (Olsvik and Søfteland 2020). Such is the case in agricultural fields in which both pesticide and heavy metal residues may affect non-target plants. Because heavy metals are likely to co-occur with sulfonylurea herbicides in contaminated soils, their combined effects on non-target plants are a matter of interest to researchers. Cadmium, the most toxic heavy metal, and chlorsulfuron, an effective herbicide commonly applied to wheat fields, were investigated in this study. We tested their effects on content of metabolites in maize seedlings.

According to our previous study (Zhao *et al.* 2018), chlorsulfuron inhibited shoot growth more severely after 21 d, with inhibitory rate 28 % after exposure to 0.001 mg kg^{-1} chlorsulfuron in test soil. Chlorsulfuron also significantly ($P < 0.05$) impaired root growth. Even at the very low concentration of 0.001 mg kg^{-1}, chlorsulfuron reduced root length by 50 %. Cadmium at 5.0 mg kg^{-1} had no significant effect on shoot elongation, but it did enhance root growth ($P < 0.05$). We also found that chlorsulfuron negatively affected the chlorophyll content, photochemical efficiency of photosystem II in the dark-adapted state, the maximum efficiency of photosystem II, photochemical quenching coefficient, and steady-state fluorescence decline ratio in the leaves of maize seedlings. However, cadmium did not produce noticeable changes on these parameters.

Nuclear magnetic resonance (NMR) spectroscopy and multivariate data analysis have been used to detect differences between samples and identify biomarkers in diverse fields (Gall *et al.* 2001, Charlton *et al.* 2002, Azam *et al.* 2020, Mascellani *et al.* 2020). We used NMR in order to clarify the changes of metabolites in maize seedlings when chlorsulfuron and cadmium are added in alkaline soil.

Materials and methods

Plants and treatments: Maize (*Zea mays* L.) seeds were obtained from *Shanxi Qiangsheng Seed Company* (Taiyuan, Shanxi, China). Soils were collected from the 0 - 20 cm surface layer of an uncontaminated field at the Shanxi Experimental Station. Soil properties were as follows: pH = 8.08 (in 1 mol dm^{-3} KCl); organic matter = 1.21 %; cation exchange capacity = 25.4 cmol kg^{-1}; and texture (% by volume) = 24.7 % clay, 32.9 % silt, and 42.4 % sand. Samples of soil were dried at room temperature, gently crumbled, and then passed through a 2-mm mesh sieve; the samples were stored for subsequent analysis. The soil used in this paper meets the GB 15618-2018 soil environmental quality standard, all elements meet the environmental background value, and do not contain chlorsulfuron. The herbicide chlorsulfuron was a commercial formulation of 25 % wettable powder (*Jiangsu Institute of Ecomines Company*, Jiangsu, China). $CdCl_2$ was from *Chengdu XiYa Chemical Technology Company* (Chengdu, China) and methanol-d4 for NMR, 99.8 atom % D from *Sigma-Aldrich* (St. Louis, USA).

All experiments were performed in controlled laboratory conditions, at a constant temperature of 25 ± 2 °C, a relative humidity between 40 and 60 %, a 12-h photoperiod, and an irradiance of 87.5 µmol m^{-2} s^{-1} on soil surfaces. The moisture of soil was 60 % of the water holding capacity during the experiment. Depending on the treatment conditions, a single application of herbicide and/or $CdCl_2$ were added to soils before maize seeds were sown. According to previous studies (Zeng and Zhu 2005, Chen *et al.* 2013), cadmium accumulates up to 5.0 mg kg^{-1} in sewage irrigation areas in China and content of cadmium 5.0 mg kg^{-1} was selected in experiments. According to Shan *et al.* (2001), residue of chlorsulfuron found in field was 0.005 - 0.013 mg kg^{-1}(soil) after spraying at 15 and 30 g ha^{-1}. The five concentrations of chlorsulfuron (0.001, 0.003, 0.005, 0.008, or 0.01 mg kg^{-1}) were used in this study. Six single-treatments received $CdCl_2$ at 5 mg kg^{-1} or chlorsulfuron at 0.001, 0.003, 0.005, 0.008, or 0.01 mg kg^{-1}. Six combined-treatment conditions received both $CdCl_2$ and chlorsulfuron at the same concentrations. The control was grown at the same type of soil but without added chlorsulfuron or Cd.

Seed germination and seedling growth tests were performed following the procedures in *ISO11269-2* (2013), with the following modifications. Plastic vessels were filled with soil of 155 g (dry mass equivalent). The diameters of each test vessel were 9 cm (top) and 6.5 cm (bottom) and the height was 6.5 cm; the bottom of each vessel had a drainage hole. Soil moisture was checked by weighing several randomly selected pots daily. Ten uniform and undressed seeds were planted in each pot filled with approximately 40 g of soil. After the emergence of 50 % of the seedlings in the control (uncontaminated soil) condition, emergence rates were determined for all conditions. After two weeks, the remaining plants were harvested for analyses of growth, lipid peroxides, enzyme activity and metabolites.

Chlorophyll content, lipid peroxidation, and enzyme activities: The content of chlorophyll and malondialdehyde was detected according to the method described in our previous study (Zhao *et al.* 2018). The activities of superoxide dismutase (SOD), peroxidase (POD), catalase

Table 1. Metabolites from extracts of maize seedlings identified in the 600 MHz ^{1}H spectrum. ^{1}H chemical shift is referenced to a 1 mM sodium-3-trimethylsilylpropionate (TSP) internal standard. Abbreviations are reported in parentheses. Chemical shifts are referenced to TSP signal (δ=0.000). s - singlet, d - doublet, dd - double doublets, t - triplet, q - quartet, m - multiplet, - unknown.

Classification	No.	Metabolite	Assignment	^{1}H δ	Multiplicity	Coupling constant J [Hz]
Amino acids	1	isoleucine (Ile)	δ-CH_3	0.950	t	7.5
			γ-CH_3	1.030	d	7.2
			γ-CH	1.250	m	
			Γ′-CH	1.450	m	
	2	leucine (Leu)	δ-CH_3	0.980	d	6.0
			Δ′-CH_3	0.970	d	6.0
	3	valine (Val)	Γ′-CH_3	1.000	d	7.2
			γ-CH_3	1.060	d	6.6
	4	threonine (Thr)	γ-CH_3	1.340	d	6.6
			β-CH	4.250	m	
	5	alanine (Ala)	R-CH	3.770	q	
			β-CH_3	1.480	d	7.2
	6	arginine (Arg)	β-CH_2	1.650 1.720	m	
			γ-CH_2	1.920	m	
	7	proline (Pro)	γ-CH_2	2.010	m	
			β-CH	2.360	m	
			B′-CH	2.080	m	
	8	glutamine (Gln)	β-CH_2	2.150	m	
			γ-CH_2	2.450	m	
	9	γ-aminobutyrate (GABA)	R-CH_2	2.320	t	7.2
			γ-CH_2	3.010	t	7.8
	10	aspartate (Asp)	R-CH	3.950	dd	8.1, 4.2
			β-CH	2.680	dd	17.7, 9.6
			B′-CH	2.790	dd	12.0, 4.2
	11	asparagine (Asn)	R-CH	3.950	dd	8.1, 4.2
			β-CH	2.830	dd	17.4, 8.4
			B′-CH	2.950	dd	17.4, 3.9
	12	tyrosine (Tyr)	C2,6H ring	7.170	d	9.0
			C3,5H 510 ring	6.890	d	9.0
	13	glycine (Gly)	1-CH_2	3.570	s	
	14	trimethylamine	CH_3	2.900	s	
Saccharides	15	sucrose (Suc)	CH-1 (Glc)	5.420	d	3.6
			CH_2-1′ (Fru)	3.650	s	
			CH-3′	4.170	d	9.0
	16	β-glucose (β-Glc)	CH-1	4.590	d	7.8
	17	α-glucose (α-Glc)	CH-1	5.190	d	3.6
	18	α-galactose (α-Gal)	C1H	5.270	d	3.6
	19	β-galactose (β-Gal)	C1H	4.600	d	3.6
Organic acids	20	succinic acid (SA)	αβ-CH_2	2.481	s	
	21	acetic acid (AA)	α-CH_3	1.910	s	
	22	lactic acid (LA)	β-CH_3	1.390	d	6.6
	23	malic acid (MA)	α-CH	4.300	dd	8.4, 3.9
			β-CH	2.730	dd	15.9, 3.9
	24	citric acid (CA)	α γ-CH	2.540	d	17.4
			α′γ′-CH	2.700	d	16.8
	25	pyruvic acid (PA)	β-CH_3	2.350	s	
	26	fumaric acid (FA)	α,β-CH=CH	6.530	s	
	27	formic acid	HCOOH	8.460	s	
	28	*p*-hydroxybenzoic acid	-	6.930	d	9.0
			-	7.930	d	8.4

Miscellaneous	29	choline	$N(CH_3)_3^+$	3.210	s	
compounds	30	choline chloride O-(dihydrogen phosphate)	$N\text{-}CH_3$	3.280	s	
	31	adenosine	H-7	8.213	s	
			H-2	8.350	s	
	32	phenolic acids	H-7′	7.610	d	16.2
			H-8′	6.430	d	16.2
			$-OCH_3$	3.800	s	
Unassigned	33		-	7.330	d	9.0
resonances	34	-	-	7.266	s	
	35	-	-	7.178	d	8.4
	36	-	-	7.140	d	9.0
	37	-	-	7.104	d	8.4
	38	-	-	7.551	d	12.0
	39	-	-	6.720	d	5.4

(CAT), and glutathione-S-transferase (GST) were also described in detail in the previous published article (Zhao *et al.* 2018).

NMR spectroscopy: The whole plants were ground to a powder in liquid nitrogen, and 200 mg of the powder was extracted using 6 cm^3 of CH_3OH and water (1:1) in a 10-cm^3 centrifuge tube. The extracts were vortexed, sonicated for 60 min, and centrifuged at 2 012 *g* for 20 min. Supernatant (4 cm^3) was transferred to a 25 cm^3 round flask and evaporated to dryness at 45 °C using a rotary evaporator. The dried extract was dissolved in 0.4 cm^3 deuterated methanol-d4 and 0.4 cm^3 buffer (pH 6.0) containing 0.05 % (m/v) 2,2,3,3-D4-3-(trimethylsilyl) propionic acid sodium (TMSP-2,2,3,3-D4). Then the solution was centrifuged at 6 540 *g* for 15 min. Supernatant (0.6 cm^3) was transferred to a NMR tube and analyzed by NMR.

The ^{1}H NMR spectra of metabolites in each seedling sample were recorded at 25 °C on a 600 MHz AV 600 spectrometer (*Bruker*, Rheinstetten, Germany) equipped with a cryo-probe operating at a proton NMR frequency of 600.13 MHz and 64 scans requiring a 3 min 15 s acquisition time. Other parameters were as follows: pulse width (PW) = 30° (12.7 μs) and relaxation delay (RD) = 5.0 s. Free induction decay (FID) was Fourier transformed with line broadening (LB) = 0.3 Hz and residual H_2O signals were inhibited by a pre-saturation sequence.

The ^{1}H NMR spectra were automatically reduced to ASCII files using *MestReNova* (*v. 6.1.1*, *Mestrelab Research*, Santiago de Compostela, Spain). Spectral intensities were sorted into bins of equal width (δ0.04) corresponding to the region of δH9.28 - 0.64. The regions of δH4.96 - 4.80 (water) and δH3.34 - 3.28 (methanol) were excluded from the analysis. Although the differences between spectra could be clearly visualized, we further examined the data using multivariate analysis for a more objective comparison. Principal component analysis (PCA) is one of the most widely used techniques in multivariate analysis. The purpose of PCA is to describe the variance in a set of multivariate data in terms of underlying orthogonal variables (principal components). The original variables (metabolite concentrations) can be expressed as linear combinations of the principal components (Sumner *et al.* 2003, Brereton 2018). The PCA and partial least squares discriminant analysis (PLS-DA) were performed with *SIMCA-P* software (*v. 11.0*, *Umetrics*, Umea, Sweden), using the Pareto scaling method for PCA and the unit variance method for PLS-DA.

Results

After exposure to the chlorsulfuron, the inhibition on shoot and root length was 28 - 87 % (Fig. 1*A*). However, the inhibitions on shoot and root length were 47 - 90 % after exposure to chlorsulfuron and cadmium.

Chlorsulfuron reduced fresh mass by 55.8 - 93 % (Fig. 1*A*); the combined pollutants also led to a significant decrease in fresh mass (63 - 93 %, Fig. 1*A*). However, Cd alone did not have a significant effect on the fresh mass ($P > 0.05$).

Both chlorsulfuron alone and in combination with Cd caused significant decrease in the amount of chlorophyll (26.78 - 83.93 %, Fig. 1*B*). The content of MDA was the highest (approximately 1.5-times in comparison with control) after exposure to 0.005 mg kg^{-1} chlorsulfuron. As the concentration of chlorsulfuron continued to increase, the content of MDA in seedling showed a downward trend. Only at the concentrations of 0.003 and 0.005 mg kg^{-1}, the content of MDA was significantly different from the control ($P < 0.05$), and the other treatments had no significant effects. The content of MDA showed no significant change after exposure to chlorsulfuron and cadmium ($P > 0.05$).

Chlorsulfuron caused a significant increase in SOD, POD, CAT, and GST activities. Cd alone had no significant effect on SOD, POD, CAT, and GST (Fig.1*C*,*D*). Meanwhile, there was no significant difference between the effect of chlorsulfuron and Cd combination and chlorsulfuron single pollution on SOD and POD. But, there were some significant differences between chlorsulfuron and Cd pollution and chlorsulfuron single pollution on GST and CAT.

Table 1 lists 39 identified metabolites, including 14 amino acids, 9 organic acids, 5 sugars, 4 miscellaneous

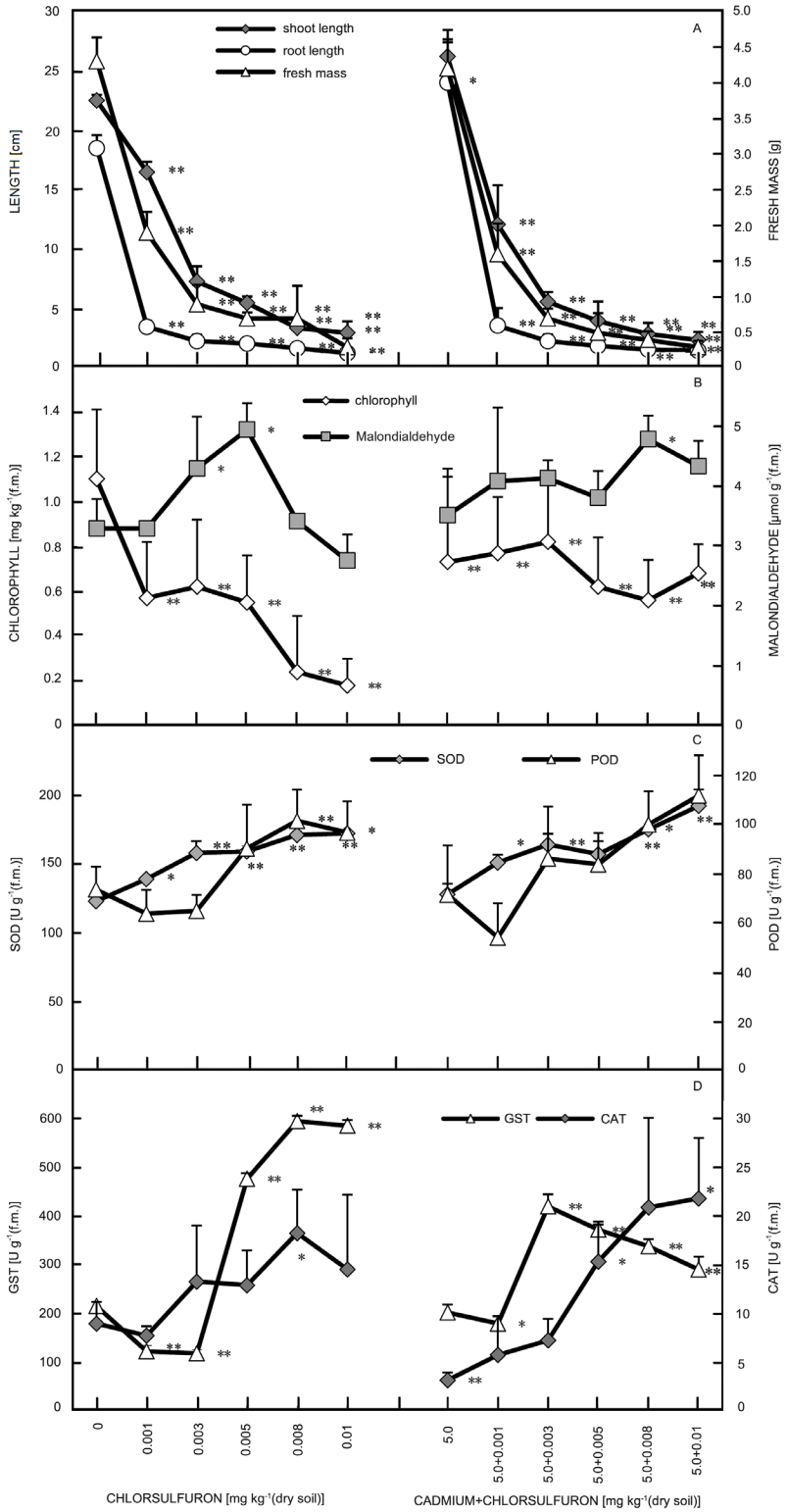

Fig. 1. Effects of chlorsulfuron, Cd, and the mixture of chlorsulfuron and Cd on fresh mass, shoot length and root length (*A*), on chlorophyll *a*+*b* and malondialdehyde (MDA) content (*B*), superoxide dismutase (SOD) and peroxidase (POD) activities (*C*), and glutathione S - transferase (GST) and catalase (CAT) activities (*D*) of maize seedlings grown for 14 d in soil. Means ± SEs, $n = 4$, **and * indicate significant difference at $P < 0.01$ and $P < 0.05$ between the treated and control plants, respectively. One unit of SOD is defined as amount of enzyme necessary for 50% inhibition of photochemical reduction of nitroblue tetrazolium; one unit of POD is defined as 0.01 increase of absorbance per min; one unit of GST is defined as amount of enzyme that conjugated 1nmol dm^{-3} of dinitrobenzene with reduced glutathione per min; one unit of CAT is defined as 0.01 decrease of absorbance per min.

compounds, and 7 unknown compounds with their characteristic chemical shifts and coupling constants.

As shown in Fig. 2*A*, the 2-D score plot produced by PCA showed distinct separation between NMR data from control and chlorsulfuron-only treatment groups. Principle component 1 (PC1) and principle component 2 (PC2) accounted for 30 and 19 % of the variation, respectively. Similarly, Fig. 2*B* shows the distinct separation between NMR data from the treatment group exposed to $CdCl_2$ alone and the groups exposed to $CdCl_2$ and chlorsulfuron. Principle component 1 (PC1) and principle component 2 (PC2) accounted for 31 and 15 % of the variation, respectively.

To better understand the variables (*i.e.*, metabolites) contributing to the classification components, the spectral data were further subjected to PLS-DA (partial least squares discriminant analysis) and OPLS-DA (orthogonal partial least squares discriminant analysis). Figs. 3 - 4 and 1 - 3 Suppl. show PCA score plots, validation models, and corresponding coefficient plots for the control treatment and 0.001, 0.003, 0.005, 0.008, and 0.01 mg kg^{-1} chlorsulfuron treatments. The random left values of R^2 and Q^2 in Figs. 3*B*, 4*B*, 1*B* Suppl., 2*B* Suppl., and 3*B* Suppl. that were lower than the right values indicated that these models were of reasonable quality. The loading plots of the NMR data (Figs. 3*C*, 4*C*, 1*C* Suppl., 2*C* Suppl., and 3*C* Suppl.), which were obtained from comparisons of control seedlings and those exposed to chlorsulfuron, indicate that the herbicide had marked effects on plant metabolites. Chlorsulfuron treatments increased the content of glutamine, the primary product of ammonia (re) assimilation. This increase in glutamine was accompanied by markedly increased pools of two other major amino acids, such as alanine and tyrosine.

The content of arginine, asparagine, proline, and glutamine increased with the increasing dose of chlorsulfuron. The content of proline, another messenger involved in plant response to stress, also increased in maize seedlings exposed to chlorsulfuron. Chlorsulfuron decreased content of malic and citric acids, which are important in the tricarboxylic acid cycle.

As shown in Fig. 4 Suppl., OPLS-DA analysis of the NMR data revealed a pronounced separation between data from maize seedlings grown in control and $CdCl_2$ treatment. The values of R^2 and Q^2 indicated that these models were of reasonable quality (Fig. 4*B* Suppl.). The NMR data in the loading plot obtained by comparing control and 5.0 mg kg^{-1} $CdCl_2$ (Fig. 4*C* Suppl.) show that maize seedlings grown under the $CdCl_2$ treatments had lower content of sucrose, malic acid, asparagine, alanine, isoleucine, and valine; however, they had higher content of phenolic acids, glycine, β-galactose, and α-glucose. The results show that addition of 5.0 mg kg^{-1} $CdCl_2$ to soil markedly inhibited biosynthesis of branched-chained amino acids, such as isoleucine and valine. The decrease in malic acid observed from this analysis for $CdCl_2$-exposed seedlings also indicates that Cd affected the tricarboxylic acid cycle.

There were significant differences ($P < 0.05$) between the only chlorsulfuron exposed groups and chlorsulfuron (0.001 and 0.003 mg kg^{-1}) and Cd exposed groups (Fig. 5 Suppl. and 6 Suppl.). The values of R^2 and Q^2 indicated that these models were of reasonable quality (Fig. 5*B* Suppl., 6*B* Suppl.). At 0.005, 0.008, and 0.010 mg kg^{-1} chlorsulfuron, there were no significant differences between chlorsulfuron-exposed groups and chlorsulfuron and Cd exposed groups. For these, PLS-DA validation models were not used.

Loading plots (Fig. 5*C* Suppl., 6*C* Suppl.) show that phenolic acids, which are synthesized through shikimate-independent pathways, increased in maize seedlings exposed to both $CdCl_2$ and chlorsulfuron, as did sucrose, aspartate, choline, β-galactose, and α-glucose. In contrast, the branched-chain amino acids, which include valine and isoleucine, decreased in maize seedlings exposed to $CdCl_2$ and chlorsulfuron. These results indicate differences in content of metabolites between maize seedlings exposed to $CdCl_2$ and chlorsulfuron, and those

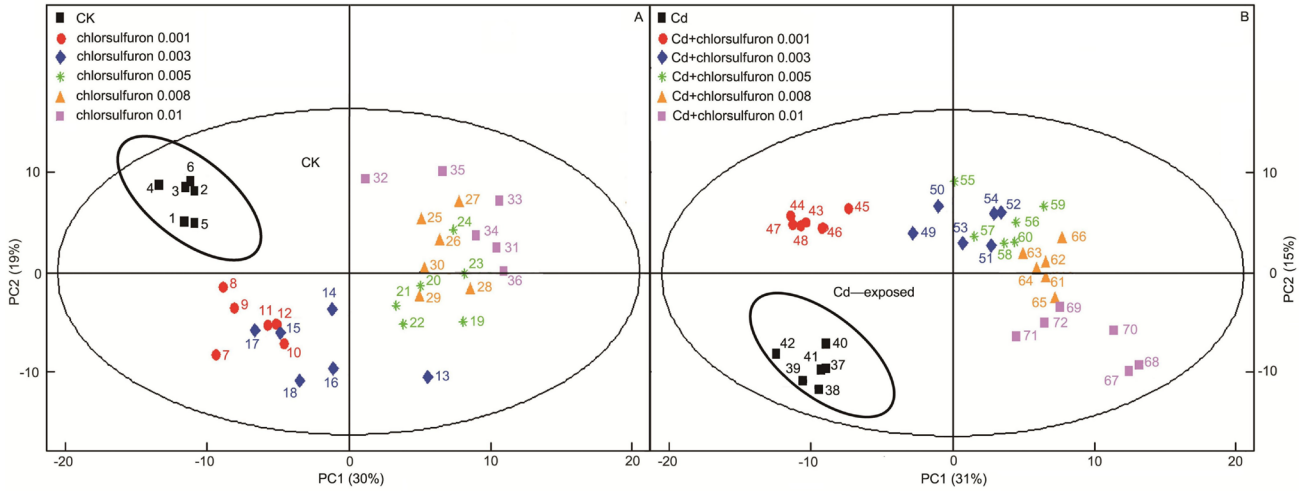

Fig. 2. Results of principle component analysis of nuclear magnetic resonance data. *A* - Comparison of data from maize seedlings grown in control (CK) and chlorsulfuron-exposed conditions. *B* - Comparison of data from maize seedlings grown in soil treated with cadmium chloride (Cd) alone or in addition to chlorsulfuron. PC1 - Principle component 1, PC2 - Principle component 2.

exposed to chlorsulfuron alone, at low concentrations of chlorsulfuron.

Discussion

In our present study, we investigated shoot length, root

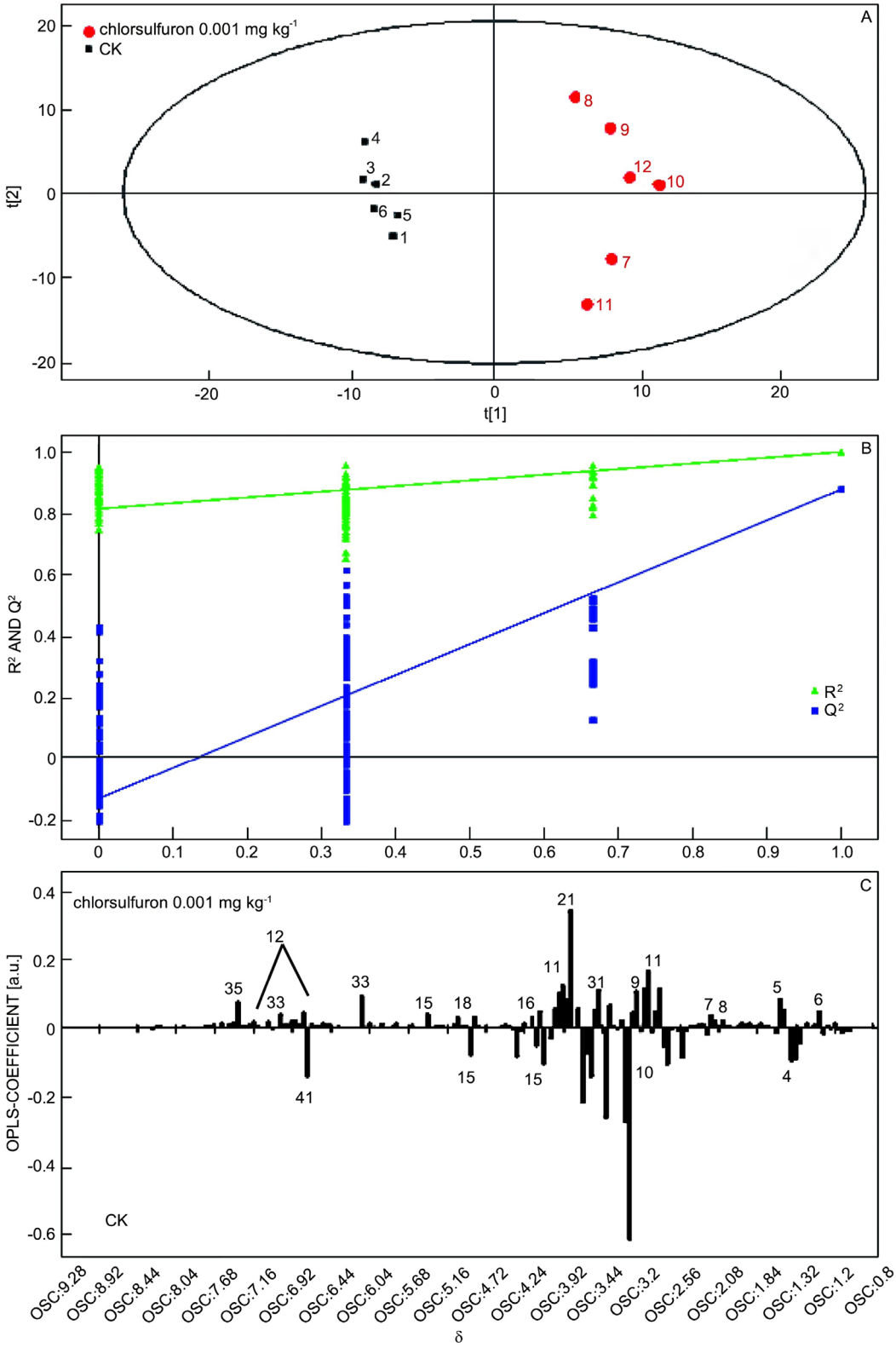

Fig. 3. Comparison of control and chlorsulfuron-exposed (0.001 mg kg^{-1}) maize seedlings. *A* - PCA score plot. *B* - Validate mode, $R^2 = 0.998$, $Q^2 = 0.887$. R^2 - the predictable variables of the model, Q^2 - the predictive degree of the model. *C* - OPLS-DA loading plot. OSC is orthogonal signal correction. The numbers at peaks represent the corresponding metabolites, which can be found in Table 1.

length, and fresh mass of maize seedlings after exposure to chlorsulfuron and Cd. Chlorsulfuron was the main factor inhibiting the growth of maize seedlings, especially the growth of their roots. This finding suggested that small amounts of chlorsulfuron residue in the soil affected maize seedlings by inhibiting root growth, leading to plant growth retardation at later developmental stages. Moreover, we also found that low-concentration of chlorsulfuron and Cd did not increase MDA. These results indicated that chlorsulfuron was the main factor causing the change of

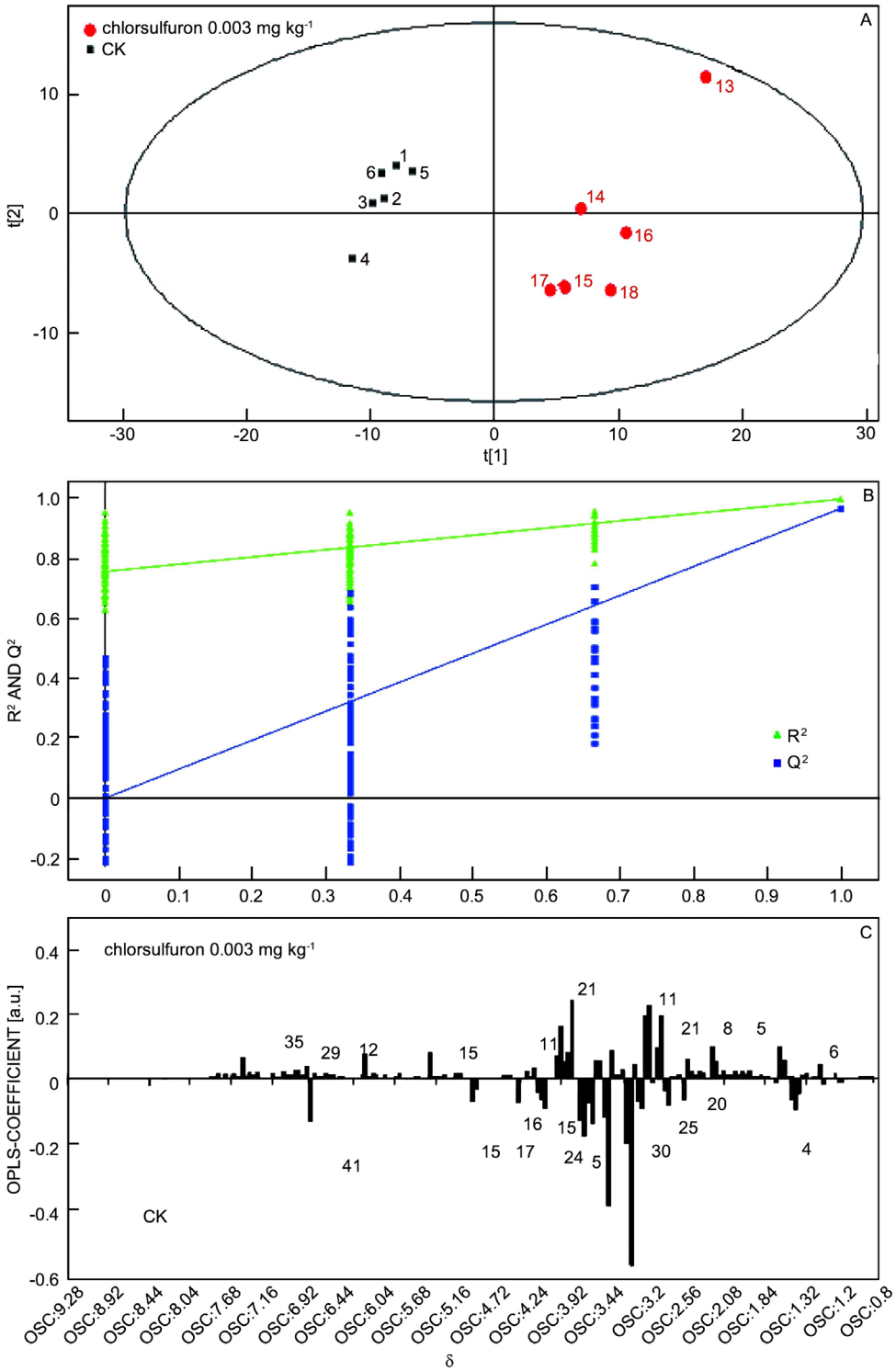

Fig. 4. Comparison of control and chlorsulfuron-exposed (0.003 mg kg^{-1}) maize seedlings. *A* - PCA score plot. *B* - Validate mode, $R^2 = 0.991$, $Q^2 = 0.961$. R^2 - the predictable variables of the model, Q^2 - the predictive degree of the model. *C* - OPLS-DA loading plot. OSC is orthogonal signal correction. The numbers at peaks represent the corresponding metabolites, which can be found in Table 1.

growth, fresh mass, and activity of antioxidant enzymes in maize seedlings.

In agreement to information from previous reports (Piccioni *et al.* 2009, Shuib *et al.* 2011, López-Gresa *et al.* 2012, Wang *et al.* 2012a,b, Zhi *et al.* 2012, D'Abrosca *et al.* 2013, Mi *et al.* 2013, Pereira *et al.* 2014) 39 metabolities were identified from maize seedlings under chlorsulfuron or/and Cd stress.

Comparing the results of the metabolites in the control and chlorsulfuron-treated groups, we found that different concentrations of chlorsulfuron induced an increase in asparagine, arginine, and proline. The content of asparagine is significantly increased in wheat grains under severe disease (Navrotskyi *et al.* 2018). Moreover, the upregulation of glutamine, asparagine and malonic acid was found in recovered-tolerant genotype of rice, suggesting a role in the regulation of panicle branching and spikelet formation for survival (Ma *et al.* 2021). Arginine, an important amino acid in plants, not only serves as a nitrogen reserve and in nitrogen recycling, but also as a precursor of polyamines and nitric oxide, important messengers in almost all physiological and biochemical processes. Therefore, asparagine and arginine accumulation in the treatment group can be used as one of the signs of stress in maize seedlings. $CdCl_2$ induced the decrease of sucrose, malic acid, asparagine, alanine, isoleucine, and valine.

The previous studies reported that the primary target of chlorsulfuron and other sulphonylurea herbicides is acetolactate synthase, the enzyme specifically involved in branched-chain amino acids biosynthesis (Zhou *et al.* 2007, Orcaray *et al.* 2010, 2011). But, there was no significant decrease of branched-chain amino acids under chlorsulfuron stress in maize. However, $CdCl_2$ inhibited the biosynthesis of branched-chain amino acids.

Moreover, the content of sucrose increased in maize seedlings exposed to chlorsulfuron and $CdCl_2$, it is maybe associated with the increase in energy metabolism. The increase in sucrose content in roots also suggests that it was transported from the leaves to the roots at a higher rate than it was utilized. Under stress, the sugar gradient required for long-distance transport is abolished; thus, phloem transport is inhibited, suggesting that the saccharide accumulation in the leaves of treated plants reflected a reduction in sink strength (Zabalza *et al.* 2013).

Conclusions

This study revealed that chlorsulfuron is a main limiting factor in growth of maize seedlings, and the addition of Cd aggravated or ameliorated the inhibitory effect of chlorsulfuron. Moreover, there were synergism between chlorsulfuron and cadmium on activity of some antioxidant enzymes. Variation of metabolites in maize seedlings under chlorsulfuron or/and $CdCl_2$ suggested that chlorsulfuron was the main factor, and $CdCl_2$ did not aggravate the effects of chlorsulfuron on maize seedlings metabolites. The present work also found that $CdCl_2$ elicited significant changes in plant metabolism at a concentration that did not impair plant growth. Furthermore, the results also suggest that the changes of metabolites in maize seedlings could be a good indicator for the early toxic effects from environmental pollutants.

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