



Separation and analysis of chlorine isotopes in higher plants



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ABSTRACT

A novel chemical mass spectrographic method was used in the determination of chlorine (Cl) isotopes in plant tissues. The procedure includes dry ashing, three-step ion chromatographic separation of Cl isotopes, and isotope ratio determination based on Cs_2Cl^+ ion in positive thermal ionization mass spectrometry. The recovery of the method and the fractionation of Cl isotopes were validated using certified reference standard materials. The pre-treatment strongly eliminated the effects of organic impurities and other anionic interferences, especially soluble nitrates and sulfates. The results show that there was severe fractionation of Cl isotopic composition in the tissues of plant samples, which might be caused by different molecular mechanisms of uptake and translocation of Cl within plants. The observed Cl isotopic variation is considered to be a useful isotope signature of living systems, which may be used to understand better the Cl cycling process in the environment.

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1. Introduction

Chlorine (Cl) is a critical micronutrient in plants (Broyer et al., 1954). Through its physicochemical behavior, it plays an important role in growth, photosynthesis, and enzyme activation in plants (Churchill and Sze, 1984; White and Broadley, 2001; Kusunoki, 2007; Hänsch and Mendel, 2009). At high concentrations, Cl is toxic to plants but a deficiency of it results in death of leaves and shoots. Cl is also applied in a chemical fertilizer to accelerate the growth and enhance the yields of crops (Whitehead, 1985). However, widespread salinization and fertilization in urban areas would be a severe ecological and environmental issue that affects the growth of plants; it increases the amount of Cl in the food chain.

The mechanism of mobilization of Cl from soil to plants, known as the geochemical pump, retards the release of Cl to aqueous systems. Although the requirements for Cl uptake by plants vary in magnitude, the effect of its application to global Cl cycling is immense. Cycling of Cl by uptake from soil and processing in plant tissues by a series of chemical reactions can lead to Cl isotopic fractionation. Because the reaction does not need the transfer of all of the Cl from one plant to another, the fractionation of Cl isotopes occurs within the plants. Lastly, although in the process of global cycling chlorine can be easily washed

by rainwater from the surface of dry plants and is integrated in the migration processes without decomposition of dead plants (Kashparov et al., 2007b), plants return Cl to the soil through leaf litter with a Cl isotopic composition different from that of the growth medium. The transfer, translocation, and mechanism of processing of Cl within plants are another powerful contribution to the global cycling of Cl (Kashparov et al., 2007a; Henner et al., 2013; Hurtevent et al., 2013).

In recent years, the acquisition and availability of Cl in biological systems have been investigated (Johnson et al., 1957; Hänsch and Mendel, 2009; Sharp et al., 2013). However, no evidence of discrimination of biological processes between stable isotopes of Cl has been found, except in the case of the radioisotope ^{36}Cl (Kashparov et al., 2007a,b), which has been identified as an environmental contaminant (Sheppard et al., 1996). Unlike in the case of the other well-established stable isotopes (C, H, O, N, S) (Grusak, 1997; Kelley et al., 2005), no attempt has been made to determine the use of Cl isotopes in biological matrices such as higher plants. There are many methods developed for separation of Cl for its isotope determination in environmental samples, such as ion exchange (Xiao et al., 2002a; Sun et al., 2004; Wei et al., 2012), AgCl sediment (Magenheim et al., 1994), and CH_3Cl transformation of chlorinated organic compounds (Tanaka and Rye, 1991; Long et al., 1993). However, large amounts of organic matter present in plants can interfere with the emission of Cs_2Cl^+ ion currents in thermal ionization mass spectrometry (TIMS). Therefore, it is critical to develop an efficient separation method and to eliminate the interferences due to impurities during the enrichment of Cl and isotopic determination of Cl in a complex matrix. In our study, we performed a series of

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chromatographic experiments on Cl extraction in plants, and determined its isotopic composition in the various tissues by positive thermal ionization mass spectrometry (PTIMS). Fractionation of Cl isotopes during separation was estimated using a reference standard. Fractionation variations in the Cl isotopic composition in the various tissues of plants are discussed.

2. Materials and methods

2.1. Plants and soil site

To examine the fractionation in chlorine isotope among different plant species, samples were collected from five different sites at three areas. Plant samples investigated in this study included various tissues of *Swertia mussotii* Franch. and *Halenia elliptica* D. Don collected in the Qinghai–Tibet Plateau area, *Weigela florida* cv. Red Prince and *Echinacoa angustifolia* in Shandong area, and *Cynomorium songaricum* Rupr. in Inner Mongolia area, China. *S. mussotii*, generally called as “Zang Yin Chen” by Tibet in China, as well as *H. elliptica*, is a biennial famous medicinal plant in the family Gentianaceae, and grows mainly in the alpine and subalpine meadow in the Qinghai–Tibet Plateau (Yang, 1991). These two samples were collected during the months of September and October (the flowering and fruiting period), 2012, from Yushu county and Banma county of Qinghai province in China, respectively. The root holoparasite *C. songaricum* (Cynomoriaceae genus, Saxifragales family), known in Chinese herbal medicine as “suoyang”, is a classic Mongolian pharmaceutical plant. It usually parasitizes the roots of *Nitraria* spp. *E. angustifolia*, a herbaceous plant species in the daisy family Asteraceae, which blooms from late spring to mid summer. It is also found growing in dry prairies and barrens with rocky to sandy-clay soils. *W. florida*, belonging to the family Caprifoliaceae, is a deciduous shrubs and collected in June, 2012. The information about sample site, plant species and soil-climate conditions in the regions are summarized in Table 1.

2.2. Instruments, reagents, and samples

Nitric acid, silver nitrate, barium sulfate, and cesium nitrate were of guaranteed reagent grade. Graphite slurry of 13 mg/g was prepared by adding high-purity graphite to an aqueous ethanol solution (80%). The isotopic reference standard for Cl used in this study was ISL 354 NaCl (Xiao et al., 2002b). Ag^+ , Ba^{2+} , and Cs^+ resins were prepared using solutions of AgNO_3 , BaSO_4 , and CsNO_3 , respectively. High-purity water, which was used to prepare stock and working solutions, was redistilled by sub-boiling distillation and then passed through a resin column filled with Ag^+ resin.

2.3. Separation of Cl isotopes in plants

Cl matrix separation was performed on a series of strong cation exchange resin, DOWEX 50 W \times 8, purchased from Sigma-Aldrich

(Shanghai, China). To eliminate potential exogenous contaminants, only quartz glasses and Teflon containers were used for Cl pretreatments. The process included two steps: the first was dry ashing, and the second was three-step ion-exchange chromatography using three different types of anion resins connected in series.

2.3.1. Dry ashing

Traditionally, plant tissue is decomposed through the wet chemical digestion method using $\text{HNO}_3/\text{H}_2\text{O}_2$, which can lead to the loss of Cl by formation of HCl. Dry ashing was therefore used to decompose plant tissue. Through this method, almost all organic impurities could be removed (Rosner et al., 2011) and loss of Cl could be avoided, as reported by Kashparov et al. (2005). Approximately 0.5 g of dried plant material was weighed into a quartz crucible. The crucible samples were placed in a closed muffle furnace. To avoid the effect of bubbles in samples due to rapid heating, the temperature was raised to 200 °C for 1 h during the carbonization of organic matter. The temperature was then raised to 550 °C for 4 h until the ash changed color from whitish to black. A 0.5 mol/L HNO_3 solution (1 mL) was used to dissolve the ash. The resulting sample solution was then transferred to a polypropylene tube after filtration.

2.3.2. Ion-exchange chromatography

Because of the presence of SO_4^{2-} , NO_3^- , and other contaminants that could interfere with determination of Cl isotopes in mass spectrometry (Xiao et al., 2002a), three different types of cation resins were used to extract and purify Cl in the aqueous sample solution. Ag^+ resin was used to enrich the Cl in the first step. The columns were twice cleaned with 3 mL HNO_3 followed by three 1 mL portions of ultrapure water. The sample solution was loaded into the conditioned Ag^+ resin column. Cl in the form of AgCl , together with other ions such as SO_4^{2-} , Br^- , and so forth, remained at the top of the resin bed, which prevented the lease of AgCl particles. This step removes nitrate compounds completely and prevents the loss of Cl. The eluate was checked for the loss of Cl by using AgNO_3 solution. After storing the sample solution for about 6 h in darkness, it was passed through the resin. The resin was subsequently washed twice with 2 mL of ultrapure water, and then 0.1 mol/mL ammonia hydroxide solution was used to dissolve the deposited AgCl . The collected eluates were then completely transferred to 1 mL of the Ba^{2+} resin column to eliminate the sulfate compounds. The resin was subsequently rinsed three times with 0.5 mL ultrapure water. Finally, the eluates were pooled, transferred to a 20-mL conical beaker, and then evaporated to dryness at 80 °C under a stream of air. Three batches of 50 μL of ultrapure water were then added to the container. The solution was passed through the Cs^+ resin, and the resin was washed with 0.5 mL of ultrapure water three times. The solutions were collected together and evaporated to about 2–4 mg Cl/mL at 80 °C with an air stream. The solution was then stored at 4 °C for isotopic analysis of Cl.

An outline of the general workflow of Cl separation in plant samples mentioned above is shown in Fig. 1.

Table 1
Sampling sites and plant species.

Species	Sampling location	Altitude (m)	Longitude	Latitude	T. range ^a /Av. T ^b (°C)	Precipitation ^c (mm)	Habit type	Soil type
<i>Weigela Florida</i>	Linyi, Shandong	71	118°17'13.21"E	35°6'21.24"N	–2–26 13	830	Sand	Shantung soil
<i>Echinacoa angustifolia</i>	Pingyi, Shandong	242	117°40'23.52"E	35°15'56.88"N	–3–27 14	800	Sand	Cinnamon soil
<i>Cynomorium songaricum</i>	Jilantai, Inner Mongolia	1060	105°37'13.83"E	39°34'42.61"N	–10–27 8	110	Sand	Sandy soil
<i>Swertia mussotii</i>	Yushu, Qinghai	3585	97°53'23.28"E	33°20'12.12"N	–18–16 2.9	460	Shrub grassland	Alpine steppe soil
<i>Halenia Elliptica</i>	Banma, Qinghai	3514	100°47'3.48"E	32°46'27.12"N	–11–12 3	640	Bottomland meadow	Meadow soil

^a Temperature range per year.

^b Average temperature per year.

^c Average precipitations per year.

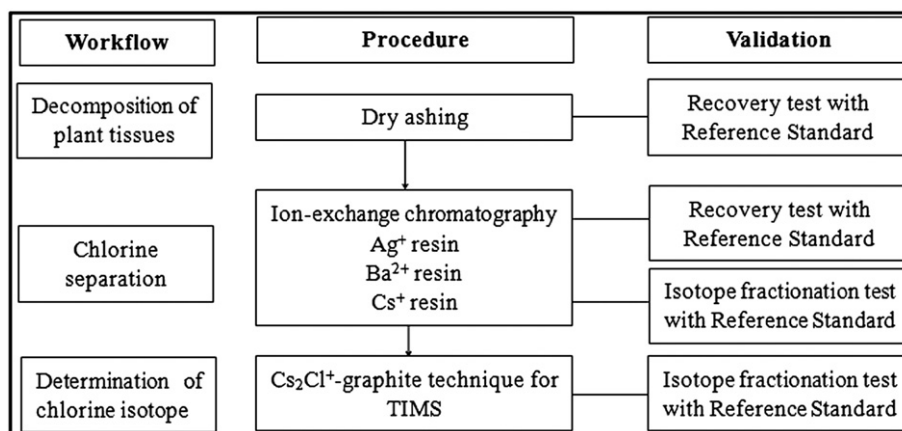


Fig. 1. Workflow of the determination of Cl isotope in plant tissues.

2.4. Analysis of Cl isotopes by TIMS

Measurement of the Cl isotopic composition was performed using a Triton thermal ionization mass spectrometer (Thermo Fisher Scientific Inc., USA) equipped with a special double cup system allowing static multi-collection of Cs₂³⁷Cl⁺/Cs₂³⁵Cl (303/301) ions (Xiao and Zhang, 1992). Tantalum filaments were degassed at a current of 3 A for 1 h and then coated with 1 μL of graphite slurry. When the slurry was almost dry, the sample solution was loaded on top of the graphite. The determination of Cl isotopes followed the procedure reported by Xiao and Zhang (1992).

The Cl isotopic compositions of the samples are expressed as δ³⁷Cl value according to the following formula.

$$\delta^{37}\text{Cl}(\text{‰}) = \left[\left(\frac{\text{Cl}^{37}}{\text{Cl}^{35}} \right)_{\text{Sam}} / \left(\frac{\text{Cl}^{37}}{\text{Cl}^{35}} \right)_{\text{Std}} - 1 \right] \times 1000 \quad (1)$$

where (³⁷Cl/³⁵Cl)_{Sam} and (³⁷Cl/³⁵Cl)_{Std} are the Cl isotopic ratios of the sample and isotope reference standard material NaCl named as ISL 354 NaCl by Xiao et al. (2002b), respectively. The average of the measured ³⁷Cl/³⁵Cl ratios of ISL 354 NaCl was 0.31909 ± 0.00006 (2σ = 0.02%, n = 5).

3. Results and discussion

3.1. Effect of anion interferences

Soluble nitrate (NO₃⁻) and sulfate (SO₄²⁻) compounds are the main interferences for the emission of Cs₂Cl⁺ current in the ion chamber of TIMS. Reports by Xiao et al. (2002a) on the variation of chlorine isotope composition determined by PTIMS showed that when the molecular ratio of NO₃⁻ to Cl⁻ is <1.0 and that of SO₄²⁻ to Cl⁻ is <3.0 in the loading solution, NO₃⁻ and SO₄²⁻ did not influence the measurements of Cl isotopes. Because of the large amounts of nitric acid used to dissolve the sample ashes, the elimination of NO₃⁻ and SO₄²⁻ is a key problem in the determination of Cl isotope composition. The ratios of NO₃⁻/Cl⁻ and SO₄²⁻/Cl⁻ in the reference standard and samples measured through the procedure ranged from 2.2 × 10⁻³ to 6.3 × 10⁻², lower than those reported by Xiao et al. (2002a). These results indicate that NO₃⁻ or SO₄²⁻ did not affect the emission of Cs₂Cl⁺ ion current and that the pretreatment strongly removed NO₃⁻ and SO₄²⁻ in plant samples.

3.2. Effect of pH of the loading solution

According to the reports by Xiao and Zhang (1992) and Xiao et al. (2002a), the optimum pH of loading solution for chlorine isotope determination ranges from 2.5 to 6.0. In this range, the ³⁷Cl/³⁵Cl ratios are

obtained at higher precision and accuracy. However, Sun et al. (2004) found that the measured Cl isotope ratios of neutral loading solution with low concentrations of Cl (e.g., rainwater and snow water samples) were similar to those reported by Xiao et al. (2002a). In this experiment, pH at 7 was the optimum for the loading solution and was therefore selected.

3.3. Recovery of dry ashing and process blank

In our method, the most critical concern is Cl loss and the fractionation of Cl isotopic composition in dry ashing and ion-exchange chromatography of biological samples. Recoveries of Cl after dry ashing and ion-exchange procedures in the reference standard NaCl and plant samples were determined by a standard addition calibration method of ion chromatography.

The mass concentrations of Cl in the plant tissues, and in soils and reference standard NaCl determined after dry ashing are listed in Table 2. The results reveal a weighted mean recovery of 100.9% with an uncertainty of 9.5%.

The amount of Cl in the blank in the ashing procedure was less than 0.6% in the entire procedure. This result was considered as a negligible effect of the recovery of Cl. The findings show that there was no Cl loss during dry ashing at ≤600 °C for the conservative behavior of Cl, which was in accordance with the results reports by Kashparov et al. (2005) that ³⁶Cl activity values are located on the plateau with no release of chlorine during the ashing process up to 500 °C. The quantitative recovery of Cl in the dry ashing is demonstrated by the agreement in the weighted comparison of the values for the reference standard NaCl before and after dry ashing in five replicates (mean recovery of 100.1%). The Cl recovery yields were determined for the complete procedure as well as for three-step ion chromatographic separation of Cl in the matrix (results are shown in Fig. 2).

3.4. Variation of the fractionation of Cl isotopic composition

Accurate determination of isotopic composition requires quantitative recovery of Cl during the procedure to avoid isotope fractionation (Magenheim et al., 1994). In the three-step ion-exchange chromatography for separation of Cl in the matrix, the change in Cl isotope composition may occur because of the introduction of the Cl blank or because of isotopic fractionation effects during ion-exchange chromatography. These analytical problems were addressed by running a blank, reference standard, and isotopic reference standard in parallel with the sample. The variation of Cl isotope composition during introduction of chemical separation was impossible because the Cl isotopic composition of the spiked ISL 354 NaCl was determined by isotope dilution mass spectrometry in the entire procedure. This sample showed a very low mean level

Table 2
Chlorine contents of plant tissues (number of measurements, $n = 3$).

Tissues	Plants	Mass concentration (g/kg)	Added amount (μg)	RSD (%)	Recovery (%)
Flower	<i>Weigela florida</i>	2.59	0.5	5.5	99.6
	<i>Echinacoa angustifolia</i>	1.56	0.5	3.4	105.3
	<i>Cynomorium songaricum</i>	0.68	0.5	6.8	98.1
	<i>Swertia mussotii</i>	0.12	0.5	4.5	101.9
	<i>Halenia elliptica</i>	0.70	0.5	4.3	104.7
Leaf	<i>Weigela florida</i>	0.60	0.5	2.7	99.0
	<i>Echinacoa angustifolia</i>	0.73	0.5	3.1	97.7
	<i>Swertia mussotii</i>	0.15	0.5	4.5	102.3
	<i>Halenia elliptica</i>	0.14	0.5	5.5	98.7
Stem	<i>Weigela florida</i>	0.99	0.5	6.2	96.0
	<i>Echinacoa angustifolia</i>	2.44	0.5	3.8	103.4
	<i>Cynomorium songaricum</i>	0.23	0.5	5.7	102.4
	<i>Swertia mussotii</i>	0.42	0.5	3.9	105.9
	<i>Halenia elliptica</i>	0.28	0.5	6.0	99.6
Root	<i>Weigela florida</i>	1.28	0.5	5.6	98.9
	<i>Swertia mussotii</i>	0.72	0.5	5.3	101.1
	<i>Halenia elliptica</i>	0.41	0.5	5.3	100.8

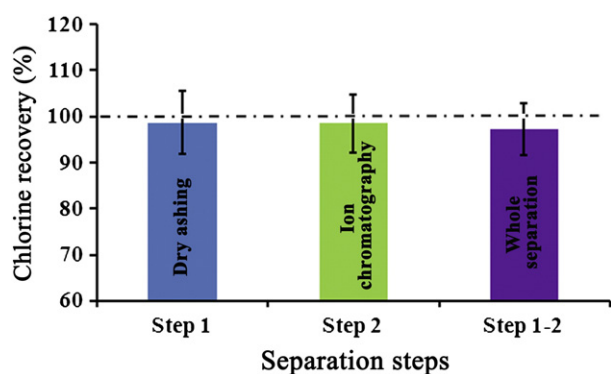


Fig. 2. Mean recovery yields of Cl for the individual step and the entire procedure.

of Cl (16 ng). This was confirmed further by the $\delta^{37}\text{Cl}$ value of +0.42‰ and +0.41‰ before and after the determination of the reference standard, respectively, as well as the value for ISL 354 NaCl (0.01‰). The value for ISL 354 NaCl is indistinguishable from the validated $\delta^{37}\text{Cl}$ value of +0.00‰ \pm 0.28‰ (2SD).

3.5. $\delta^{37}\text{Cl}$ values in higher plants

Cl separation in tissues of higher plant samples, including leaf, root, stem, and flower, was performed through the proposed method. The measured chlorine isotopic ratios related to that of ISL 354 NaCl

(0.319644) reported by IAEA in 2004 and $\delta^{37}\text{Cl}$ values in the tissues are shown in Table 3. The $\delta^{37}\text{Cl}$ values range from -1.79‰ to $+4.77\text{‰}$. The mean value of 1.15‰ and SD of 1.97‰ are within the range of $\delta^{37}\text{Cl}$ values (-7.7‰ to $+7.5\text{‰}$) in environmental samples such as rock, mineral, seawater, and aerosols (Coplen et al., 2002; Sharp et al., 2013). These also indicate that transference and transformation but not transport could influence the fractionation of Cl isotopes in water, rock minerals, and atmospheric samples. The uptake and translocation of Cl in plant samples may be considered as another critical reason for the fractionation of Cl isotope composition in the nature.

The fractionation between two components i and j was determined as follows (Moynier et al., 2009):

$$\Delta\delta^{37}\text{Cl}_{i-j} = (\delta^{37}\text{Cl})_i - (\delta^{37}\text{Cl})_j \quad (2)$$

The variations of $\Delta\delta^{37}\text{Cl}$ among the tissues (stem, leaf, flower, and root) in the plant samples, *W. florida*, *S. mussotii*, and *H. elliptica* are described in Fig. 3. There is severe Cl isotopic fractionation among the plant species. The maximum of $\Delta\delta^{37}\text{Cl}$ ($>+0\text{‰}$) was found in the root–stem, root–leaf, and root–flower of *H. elliptica*. The minimum of those values ($<-0\text{‰}$), which was observed in *S. mussotii*, may be caused by the different manner of uptake and transference or by the biophysiological effect of Cl on plants. This presence would be another important channel for the variation of Cl isotope composition in the global cycle of Cl by further uptake and translocation of Cl in the tissues of plants.

Table 3
Chlorine weights and its isotopic composition of plant samples.

Tissues	Plants	Total chlorine weight (mg)	Measured $^{37}\text{Cl}/^{35}\text{Cl}$	$\delta^{37}\text{Cl}$ (‰) \pm 2SD
Flower	<i>Weigela florida</i>	0.83	0.31971	+0.21 \pm 0.15
	<i>Echinacoa angustifolia</i>	0.53	0.31919	-1.42 \pm 0.06
	<i>Cynomorium songaricum</i>	0.30	0.32012	+1.48 \pm 0.42
	<i>Swertia mussotii</i>	0.02	0.32054	+2.81 \pm 0.95
	<i>Halenia elliptica</i>	0.10	0.32073	+3.39 \pm 0.35
Leaf	<i>Weigela florida</i>	0.21	0.31910	-1.71 \pm 0.05
	<i>Echinacoa angustifolia</i>	0.23	0.31945	-0.60 \pm 0.10
	<i>Swertia mussotii</i>	0.03	0.31982	+0.55 \pm 0.46
	<i>Halenia elliptica</i>	0.02	0.32077	+3.53 \pm 0.36
Stem	<i>Weigela florida</i>	0.33	0.32028	+1.99 \pm 0.37
	<i>Echinacoa angustifolia</i>	0.77	0.31908	-1.79 \pm 0.16
	<i>Cynomorium songaricum</i>	0.07	0.32021	+1.76 \pm 0.37
	<i>Swertia mussotii</i>	0.10	0.32007	+1.34 \pm 0.39
	<i>Halenia elliptica</i>	0.07	0.32117	+4.77 \pm 0.62
Root	<i>Weigela florida</i>	0.58	0.31937	-0.88 \pm 0.22
	<i>Swertia mussotii</i>	0.17	0.32054	+2.80 \pm 0.59
	<i>Halenia elliptica</i>	0.07	0.32007	+1.31 \pm 0.25

SD: Standard deviation.

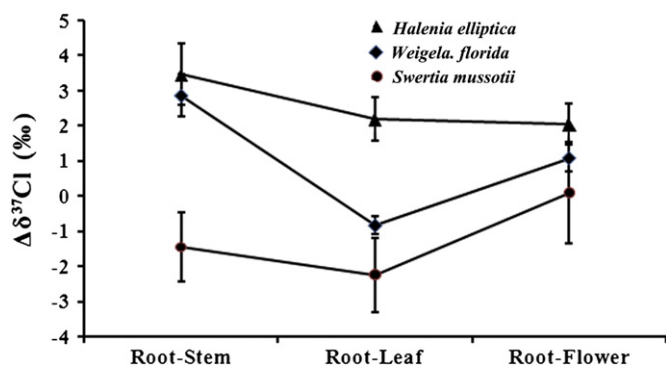


Fig. 3. Cl isotope fractionation in tissues (stem, leaf, and flower) vs. root.

4. Conclusion

The newly proposed chemical and mass spectrometric method for the determination of Cl isotopic composition in plant samples enables applications of Cl isotopes in biogeochemistry, plant nutrition, and plant metabolism. Dry ashing and ion-exchange chromatographic Cl matrix technique allow the separation of Cl in plant samples with Cl concentrations as low as 1 mg/kg. The variation of Cl isotopic composition in plants may be used in the investigation of Cl isotopic fractionation in intraplant and interplant samples. The observed $\delta^{37}\text{Cl}$ data of higher plant samples reveal a large variability of Cl isotope values in the tissues, which covers more than 40% of the natural overall variation of $\delta^{37}\text{Cl}$. Cl isotope fractionation in transport, translocation, and compartmentalization observed in the present study can be used to investigate the function and transport mechanisms of Cl in biological systems. Moreover, knowledge of cycling, accumulation, and isotopic fractionation of Cl by the uptake of plants will contribute to better understanding of the global biogeochemical cycle of Cl.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.chemgeo.2014.04.006>.

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