Isotopic fractionation of Cu in plants

Charlotte Weinstein [a], Frédéric Moynier [a,*], Kun Wang [a], Randal Paniello [a], Julien Foriel [a], Jeff Catalano [a], Sylvain Pichat [b]

[a] Department of Earth and Planetary Sciences and McDonnell Center for the Space Sciences, Washington University in St Louis, One Brookings Drive, St Louis, MO 63130, USA
[b] Laboratoire de Sciences de la Terre, Ecole nomale superieure de Lyon, 46 allée d'Italie, 69007 Lyon, France

ABSTRACT

Knowledge of the copper cycle in the plant–soil–water system is needed in order to better constrain proper plant micronutrient nutrition, control pollution, and determine sustainable soil management practices. Here, we report the Cu isotopic compositions of different components (seeds, germinated seeds, leaves, and stems) of the dicot, lentil (*Lens culinaris*), and of two monocots, Virginia wild rye (*Elymus virginicus*) and hairy-leaved sedge (*Carex hirsutella*). According to our data, the isotopic compositions of these plants are systematically enriched in the lighter isotope of Cu (^{63}Cu) in comparison to the soil in which they grow. Furthermore, different components within the plants themselves are isotopically fractionated. The shoots (stems, leaves and seeds) are systematically lighter than the germinated seeds of the plants and the Cu isotopic compositions of individual leaves correlate with their heights on the plant. These results are similar to what has been observed for Zn isotopes, which are assumed to be transported through plants by means of diffusion and kinetic fractionation across cell membranes. Because of this similarity, we suggest that the same transport mechanisms are also responsible for the observed isotopic fractionation of Cu. As a side-note, the Cu isotopic variations measured in plants are similar in magnitude to the differences previously measured in various soils, and therefore should not be neglected while interpreting the isotopic composition of soils.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Copper is a vital micronutrient in organisms, particularly plants, most notably because Cu participates in protein synthesis, membrane activities, and photosynthesis (Koch et al., 1997; Frausto da Silva and Williams, 2001). However, Cu may also be toxic at high concentrations (Shioi et al., 1978; Maksymiec, 1997; Manceau et al., 2008). Cu is often applied to soil and crops as fertilizer to ameliorate micronutrient deficiencies caused by prolonged, intensive agriculture (Karamanos et al., 2005). It is also often used as a fungicide, especially in viniculture (Epstein and Bassein, 1998). Given this widespread use and the expansion of urban areas, pollution by Cu is an important issue that could affect plant growth and increase the concentration of heavy metals within the food chain.

The theory behind mass-dependent stable isotopic fractionation was developed in two seminal papers (Bigeleisen and Mayer, 1947; Urey, 1947). The theory specifies that the fractionation between two isotopes is proportional to their relative difference in mass. Until recently, it was very difficult to measure the small natural variations in the stable isotopes of transition metals, and so the technique was applied almost exclusively to light elements (e.g. H, C, N, O). With the

advent of high precision multi collection-inductively coupled plasma-mass spectrometry (MC-ICP-MS), precise measurement of "non-traditional" stable isotopes of alkali earth elements and transition metals became possible (for reviews, see Johnson, et al., 2004). Analyses of the 'new' stable isotope systems, notably those of Mg (Young and Galy, 2004), Cr (Ellis et al., 2002; Moynier et al., 2011a), Fe (Dauphas and Rouxel, 2006; Johnson et al., 2008), Ni (Moynier et al., 2007; Cameron et al., 2009), Cu (Maréchal et al., 1999; Albarede, 2004), and Zn (Albarede, 2004; Cloquet et al., 2008; Moynier et al., 2009a,b,2011b) are starting to provide novel insights into geochemical cycles and biological processes. A major preliminary conclusion of these early studies is that biological processes play an important role as one of the few types of processes that can fractionate transition metal stable isotopes (Albarede, 2004; Johnson et al., 2008).

Recently, use of the Cu isotopic ratio has been shown to be a good method to trace long-term processes in soil that are difficult to quantify by other means (e.g. analysis of element transport and variations in redox conditions) (Bigalke et al., 2010a, b). However, the influence of plant-induced isotopic fractionation on soil composition has not yet been quantified, significantly limiting the practicality of this technique (Bigalke et al., 2010a,b). The main known processes that can induce Cu isotopic fractionation in soils are 1) adsorption onto mineral surfaces and organic matter (Pokrovsky et al., 2008; Navarette et al. 2011), 2) inorganic and organic complexation to some

* Corresponding author.
E-mail address: moynier@levee.wustl.edu (F. Moynier).

ligands (Balistrieri et al., 2008; Pokrovsky et al., 2008), 3) variation in redox conditions (Cu(II) vs. Cu(I)) (Zhu et al., 2002), and 4) biological fractionation by plants or micro-organisms (Jouvin et al., 2008; Zhu et al., 2010) . The first 3 mechanisms have already been studied in detail (see references above). However, biological fractionation caused by plants has not yet been evaluated, except for two preliminary studies (Jouvin et al., 2008; Zhu et al., 2010). Jouvin et al. (2008) suggest that Cu is isotopically fractionated in wheat, such that the shoots of the plant become enriched in light isotopes compared to the roots, while Zhu et al. (2010) found that *Elsholtzia splendens* plants are enriched in ^{63}Cu when compared to the growth medium. Further understanding of biological fractionation is critical to interpreting other Cu isotopic data in soils and in the environment.

In addition, knowledge about the fractionation of stable isotopes within the plant body can also be used to understand the transport mechanisms of some metals (e.g. Fe, Zn, Ca, Mg) (Weiss et al., 2005; Guelke and Von Blanckenburg, 2007; Viers et al., 2007; Black et al., 2008; Cenki-Tok et al., 2009; von Blanckenburg et al., 2009). The pioneer study of Weiss et al. (2005) showed that tomato (*Lycopersicon esculentum*), rice (*Oryza sativa*) and lettuce (*Lactuca sativa*) plants were enriched by ~0.2‰ per atomic mass unit (amu) in light isotopes of Zn compared to their growth solution and that the shoots of the plants were isotopically lighter than their roots by ~0.2‰/amu. This general trend was subsequently confirmed, and a correlation between plant height and the magnitude of Zn isotopic fractionation suggested that Zn is transported by the xylem and is progressively enriched in light isotopes either by diffusion or by the complexation of heavier Zn isotopes as they travel upward (Viers et al., 2007; Moynier et al., 2009a,b).

The goals of the present study are to determine whether Cu stable isotopes are fractionated during 1) uptake by plants, or 2) transport from germinated seeds or roots to shoots/leaves/seeds in three angiosperm plants with different growing patterns: a dicot (lentil: *Lens culinaris*), a graminaceous monocot [Virginia wild rye (*Elymus virginicus*)] and an another monocot [hairy-leaved sedge (*Carex hirsutella*)]. We also aim to test the 3) influence of the plant's source of nutrients on the isotopic fractionation between the germinated seeds and the different components (stems, leaves, seeds) of the plant and 4) whether there is a correlation between the heights of the leaves and the degree of isotopic fractionation, as observed for Zn isotopes (Viers et al., 2007; Moynier et al., 2009a,b).

2. Material and methods

2.1. Samples

The lentils were cultivated in a clean chemistry laboratory at Washington University in St Louis (WUSTL) following the same protocol described in Moynier et al. (2009b). Around 200 seeds were grown in large Petri dishes lined with high-purity kimwipes. No nutrient solution was added, and the lentils were grown on their own nutrient reserves with the addition of 18.2 MΩ water. In order to test reproducibility, two identical experiments were conducted together (exp. A1 and A2). After 15 days of growth, the germinated seeds (including roots that were either not present or not visible to the naked eye), and shoots (stems and leaves) were separated (see Fig. 1A), dissolved and prepared for Cu isotopic measurements. The dissolved masses of the different parts of the lentils are reported in Table 1.

A dozen Virginia wild rye and hairy-leaved sedge plants were harvested in a field in St. Louis, MO in Spring, 2010 by FM. The plants were simply cut with scissors, placed in ziploc bags and brought back to the clean lab in WUSTL. In order to avoid any possible post-harvest redistribution of the Cu isotopes, the different parts of the plants were separated later that same day. All plants were taken from the same field over a ~1 m² area. A soil sample was also collected from a depth of 10 cm near the roots of the Virginia wild rye at the time of harvest. The soil was an alfisol of silt loam texture developed in loess and had a pH as measured in 0.01 M CaCl$_2$ of 6.42. For the Virginia wild rye, the different parts (leaves, stems, and seeds) of 4–5 plants were separated
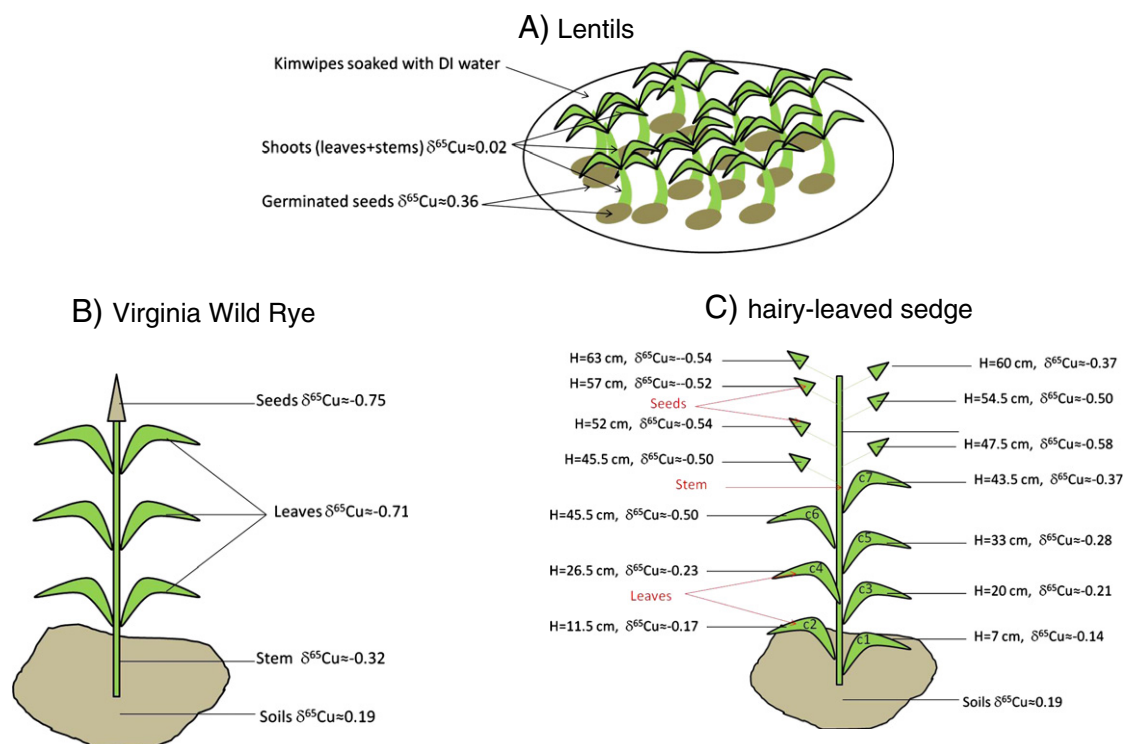

Fig. 1. Sampling strategy for the different experiments: A (lentil), panel A; B (Virginia wild rye), panel B; C (hairy-leaved sedge), panel C. The Cu isotopic composition is reported in permil δ notation with NIST 976 as the reference material.

Table 1
Cu isotopic composition and elemental abundance (ppm) from experiments A (lentils), B (Virginia wild rye) and C (hairy-leaved sedge), as well as a terrestrial granite. The external reproducibility is 0.10 (2sd) for the δ^{65}Cu and ~20% for the concentrations.

Sample name	δ^{65}Cu (‰)	[Cu] ppm wet matter	Height (cm)
GA (granite)	0.06		
Ungerminated lentil seed	0.24	8.35	
Lentil experiment A1			
Shoots-A1	0.02	4.69	
Germinated seeds A1	0.36	0.42	
Lentil experiment A2			
Shoots-A2	0.03	6.28	
Germinated seeds A2	0.36	1.74	
Soil (growth media for exp. B&C)	0.19	30.4	
Virginia Wild Rye B1			
Leaves B1	−0.71	0.77	
Stem B1	−0.32	0.65	
Seeds B1	−0.75	5.60	
Virginia Wild Rye B2			
Leaves B2	−0.30	5.01	
Stem B2	−0.17	2.86	
Seeds B2	−0.36	1.01	
Hairy-leaved sedge C			
Leaf C1	−0.14	5.37	7
Leaf C2	−0.17	2.80	11.5
Leaf C3	−0.21	4.68	20
Leaf C4	−0.23	4.16	26.5
Leaf C5	−0.28	3.60	33
Leaf C6	−0.48	6.28	38.5
Leaf C7	−0.37	5.66	43.5
Seeds C1	−0.50	4.87	45.5
Seeds C2	−0.58	4.03	47.5
Seeds C3	−0.54	5.85	52
Seeds C4	−0.50	6.22	54.5
Seeds C5	−0.52	9.92	57
Seeds C6	−0.48	10.19	60
Seeds C7	−0.54	8.11	63
Stem C	−0.60	3.07	

(exp. B1, Fig. 1B). The exact same protocol was applied to another group of 4–5 Virginia wild rye plants to test the reproducibility of the experiment (exp. B2). For the hairy-leaved sedge, only one plant was used. This plant was used to test a possible correlation between the heights of the leaves and seeds and the degree of Cu isotopic fractionation (exp. C, Fig. 1C). We sampled single leaves and clusters of seeds at different heights (7 to 43.5 cm from the bottom of the plant for the leaves and 45.5 to 63 cm for the seeds). We also dissolved the remaining stem for analysis (after removal of all additional leaves and seeds). The dissolved masses of all these samples are reported in Table 1.

CRPG GA, a granite from Andlau, France, was also analyzed as a geological reference material.

2.2. Chemical purification and mass-spectrometry

Plant and soil samples were first cleaned twice using 18.2 MΩ water and then dissolved using a mixture of HNO_3 (4 times sub-boiled) and Seastar© optima grade H_2O_2 in closed Teflon beakers. They were subsequently treated with a mixture of HF/HNO_3 to ensure dissolution of siliceous plant matter. A final treatment using aqua regia ensured that all the samples were completely dissolved. The geostandard was dissolved in a mixture of HF/HNO_3, followed by a treatment of 6 N HCl. The samples' Cu content was then purified following the method described in Maréchal et al. (1999) and Moynier et al. (2006). The column chemistry was performed twice in order to achieve a complete separation of the Cu from the matrix. The total yield of Cu was checked before starting the first chemical purifications and was found to be >95%, as previously observed in Maréchal et al. (1999). The blank of the full procedure is <10 ng.

The ^{65}Cu/^{63}Cu ratio was measured by MC-ICP-MS on a Thermo-Fisher Neptune Plus at WUSTL. The samples were introduced by free aspiration in 0.1 HNO_3 using a Teflon microcentric nebulizer (uptake rate of 100 μl/min) and a glass cyclonic spray chamber. The masses 63 and 65 were positioned on the L2 and axial collectors, respectively. Zn isotopes (64, 66, 67 and 68) were also measured on the collectors L1, H1, H2, H3, respectively. The intensity of ^{62}Ni was measured on the collector L3 in order to monitor and correct for the interference of ^{64}Ni. Instrumental mass fractionation was corrected by Zn doping and standard-sample bracketing following suggestions found in Maréchal et al. (1999) and Albarède (2004). All of the samples' intensities (after chemical purifications) were first checked with the MC-ICP-MS using a small aliquot and were then compared to the intensity of a standard of known concentration. The raw concentrations deduced are reported in Table 1. Then the samples were diluted to match the concentration of the standard mixture (200 ppb of Zn and Cu). Replicate analyses of the same samples carried out during different analytical sessions define an external reproducibility of ±0.10‰ (2sd) for δ^{65}Cu (Maréchal et al., 1999; Herzog et al. 2009; Moynier et al., 2010). The excellent reproducibility of the two different sets of lentil experiments (A1 and A2) for two pools of shoots (δ^{65}Cu = 0.02 v. 0.03) and germinated seeds (δ^{65}Cu = 0.36 v. 0.36) further confirmed the external reproducibility of the full procedure (dissolution, chemical purification and mass-spec measurements). We calculated the elemental concentrations of Cu in our samples from the measured intensities of ^{63}Cu through a comparison with standard solutions, as described in the subsequent Results section.

3. Results

Isotope ratios are expressed as parts per 1000 deviations relative to a standard:

$$\delta^{65}Cu = \left(\frac{\left(\frac{^{65}Cu}{^{63}Cu}\right)_{sample}}{\left(\frac{^{65}Cu}{^{63}Cu}\right)_{NIST976}} - 1 \right) \times 1000 \qquad (1)$$

NIST 976 was used as the reference material (Maréchal et al., 1999).

The isotopic fractionation between two components i and j is defined in the same fashion as in Moynier et al. (2009b):

$$\Delta\delta^{65}Cu_{i-j} = \left(\delta^{65}Cu\right)_i - \left(\delta^{65}Cu\right)_j \qquad (2)$$

The Cu isotopic compositions of the geostandard and plant samples are reported in Table 1. The geostandard GA (δ^{65}Cu = +0.06 ± 0.05) shows almost no fractionation and has a typical terrestrial composition (Moynier et al., 2010). This result, together with our previous geostandard measurements for NIST SRM 278 (δ^{65}Cu = 0.06), BHVO-2 (δ^{65}Cu = 0.10), and AGV-2 (δ^{65}Cu = 0.10), as well as Archer and Vance's (2002) results for BCR-1 (δ^{65}Cu = 0.07 ± 0.08‰), suggests a homogeneous distribution of Cu within the silicate crust of the Earth.

The lentil experiments A1 and A2 give similar results (Table 1): shoots (+0.02<δ^{65}Cu<+0.03) are lighter than the germinated seeds (δ^{65}Cu = +0.36 ± 0.05) and $\Delta\delta^{65}$Cu$_{germinated\ seeds-shoots}$ = 0.34 and 0.33. The lentil seeds before germination (δ^{65}Cu = +0.24 ± 0.05) have Cu isotopic compositions that lie in between those of the shoots and germinated seeds, suggesting that the masses of the different Cu isotopes present within the plant were conserved. However, since some Cu might have been leached from the plant by the culture medium, this mass-balance should only be taken as a first-order calculation.

The Virginia wild rye and the hairy-leaved sedge samples (stem, leaves and seeds) show systematic enrichment in light isotopes compared to the soil sample with $+0.33<\Delta\delta^{65}Cu_{soil\text{-}shoot}<+0.94$ (see Fig. 1). The Virginia wild rye experiments B1 and B2 both indicate the same trend (although on a slightly different magnitude), with $\delta^{65}Cu_{stem}>\delta^{65}Cu_{leaves}>\delta^{65}Cu_{seeds}$ (see Figs. 1B and 2). The seeds from the hairy-leaved sedge (exp. C) are also isotopically lighter than the leaves, and the stem sample is lightest of all (see Table 1, Figs. 1C and 2).

There is a correlation between $\delta^{65}Cu$ and the heights (L) of 7 leaves taken from a single hairy-leaved sedge plant. The highest leaves become more enriched in light isotopes (Fig. 3), this trend is described by a regression line with the equation $\delta^{65}Cu = -0.01L - 0.07$, where L is measured in cm. On the other hand, seeds collected at different heights all have the same Cu isotopic composition.

Fig. 3. Cu isotopic fractionation of the leaves from experiment C (hairy-leaved sedge) vs the height of the leaves. The highest leaves are isotopically lighter than the lower ones. This observation suggests that the isotopic fractionation is enhanced during the course of transport of Cu within the plant.

4. Discussion

4.1. Influence of plants on the Cu isotopic budget of soils

Our measurements of the granite standard GA ($\delta^{65}Cu = 0.06‰$) support the results from Li et al. (2009) that indicate that terrestrial granites have a nearly homogeneous Cu isotopic composition scattered around zero ($0.03 \pm 0.15‰$ for I-type granites and $-0.03 \pm 0.42‰$ for S-type granites). This confirms that terrestrial igneous rocks all have about the same Cu isotopic composition ($\delta^{65}Cu \sim 0$) (Marechal, 1998; Ben Othman et al., 2006; Moynier et al., 2010). Sedimentary rocks, on the other hand, seem to be slightly more fractionated in Cu isotopes. Maréchal et al. (2000) demonstrated that Mn-nodules have a $\delta^{65}Cu = 0.31 \pm 0.23‰$, although the availability of other data is still very limited. The largest isotopic fractionations (from -4 to $+9‰$) seem to be limited either to ores and hydrothermal systems where redox reactions or supergene recycling are taking place, or to low temperature environments (Zhu et al., 2000; Larson et al., 2003; Rouxel et al., 2004; Mathur et al., 2005; Markl et al., 2006; Asael et al., 2007; Mathur et al., 2009). The general rule is that the phases containing Cu(II) are isotopically heavier than Cu(I)-phases. For example, Ehrlich et al. (2004) showed that during the exchange of Cu in a water/CuS system, the heavy isotope of Cu is preferentially enriched in the oxidized phase (aqueous Cu(II)) compared to CuS. Recently, Asael et al. (2009) showed that Cu isotopes could be used as a strong tracer of redox conditions in rock. Complexation of Cu with oxy-hydroxides enriched the oxides in the heavy isotope compared to the solution (Balistrieri et al., 2008; Pokrovsky et al., 2008).

Bigalke et al. (2010b) studied the isotopic composition of Cu in four soil profiles and found isotopic variation in $\delta^{65}Cu$ up to 0.60‰. The layers of soil enriched in organic materials generally have a lighter $\delta^{65}Cu$ than layers made of inorganic materials. These results suggest that isotopic fractionation of Cu occurs during soil-plant-soil transfer and that plants should therefore be enriched in lighter isotopes of Cu. Our new results not only support this, but also show that the Cu isotopic fractionation induced by plants is on the same order of magnitude as the fractionation observed in soils. Therefore, this plant-induced source of fractionation must be taken into account in order to accurately interpret the Cu isotopic record of soils.

The lentils (exp. A) were grown from their own reserve of nutrients in our clean laboratory, with no external addition of Cu. Since no external materials or nutrients were added, the isotopic fractionation between shoots and germinated seeds could have only
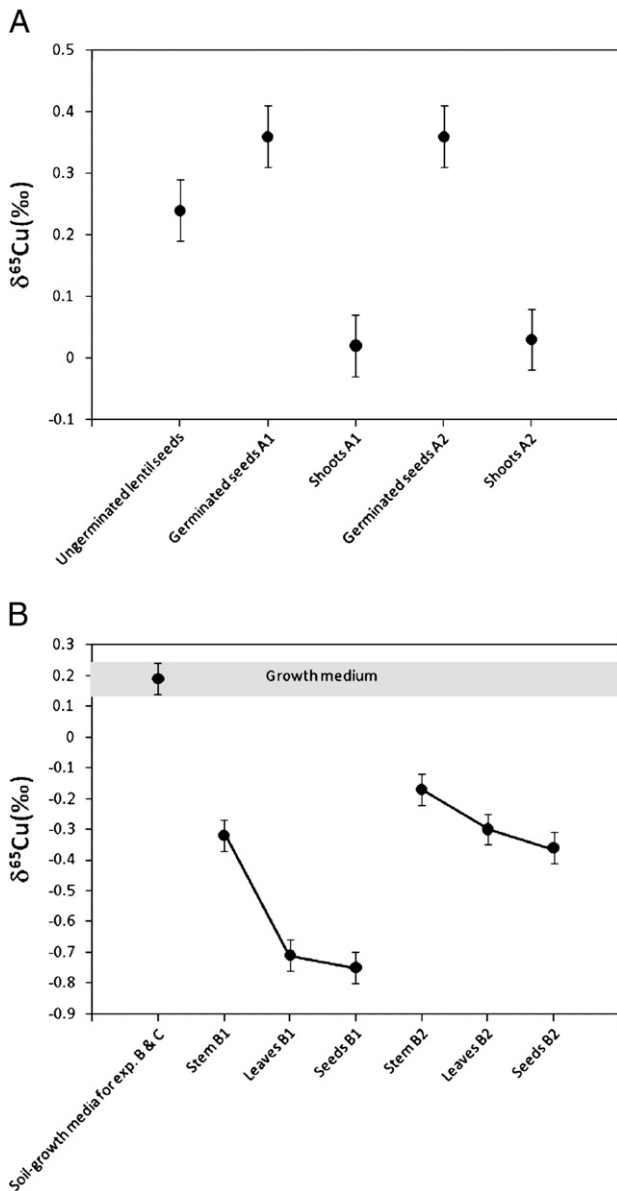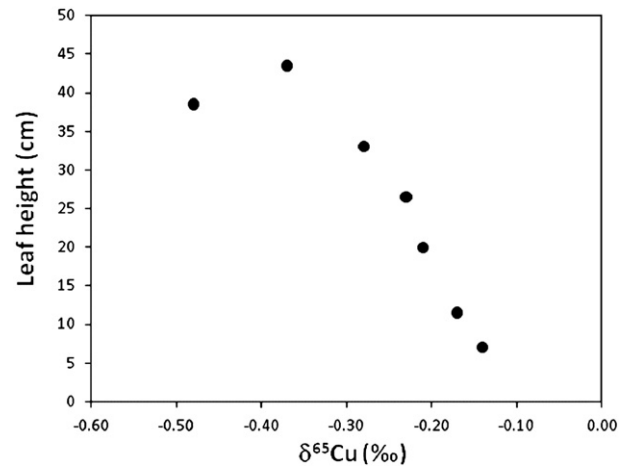

Fig. 2. Cu isotopic composition for experiment A (lentil, panel A) and experiment B (Virginia Wild Rye, panel B). The isotopic composition is reported in permil δ notation with NIST 976 as the reference material. The error bars are 2sd. In exp. A, germinated seeds and shoots are isotopically fractionated compared to the original seeds. In exp. B, all the plant components are enriched in light isotopes of Cu when compared to the growth medium (soil). The different parts of the plant are distinct in isotopic composition and become isotopically lighter in the order stem-leaves-seeds.

happened during the mobilization of the seed reserve. This indicates that the transport of the Cu is the key mechanism responsible for the observed fractionation.

4.2. Transport of Cu within the plant

4.2.1. Similarity between Zn and Cu isotopic behavior

Moynier et al. (2009b) used the same experimental design (lentils cultivated in a clean lab, with no addition of Zn) as for our experiment A (lentils) and found a $\Delta\delta^{66}Zn_{germinated\ seeds-shoots} = 0.34 \pm 0.18$, which is the same magnitude as the Cu isotopic fractionation from our experiment A ($\Delta\delta^{65}Cu_{germinated\ seeds-shoots} = 0.34$ and 0.33). This correlation between the isotopic compositions of Zn and Cu in lentils suggests that both elements are fractionated by the same mechanism. Moynier et al. (2009b) suggested a combination of diffusion and movement through transporters in the cell membrane to explain their observations. The coefficient of diffusion for Cu in an aqueous solution is similar to the one for Zn ($\sim 5 \times 10^{-10}$ m²/s), and since we used the same length scale (~ 10 cm) and timescale (15 days) in our experiment A as in Moynier et al. (2009b), the difference between the coefficient of diffusion of ^{63}Cu and ^{65}Cu (^{63}D and ^{65}D) calculates to $^{65}D/^{63}D-1 = -2 \times 10^{-5}$ (see Moynier et al. (2009b) for details of the equations). The transport of Cu through cell membranes is known to favor light isotopes: living organisms are usually enriched in the light isotope of Cu, compared to non-biological materials (Marechal, 1998; Zhu et al., 2002). Recently, Navarrete and Borrock (2010) found that two species of bacteria (*Bacillus Subtilis* and *Escherichia Coli*) preferentially absorbed ^{63}Cu during Cu uptake from the culture media. Therefore, the enrichment in light isotopes observed in plants could be the result of transport through cell membranes.

Experiment B ($+0.33 < \Delta\delta^{65}Cu_{soil-shoot} < +0.94$) shows that the plants are enriched in the light isotope of Cu, compared to the growing environment (the soil). This indicates that the plant preferentially absorbs the light isotope of Cu, most likely due to kinetic isotope fractionation during transport from the medium to the plant. These observations confirm preliminary measurements by Zhu et al. (2010) who found that *E. splendens* are enriched in light isotopes when compared to the growing medium. They also imply that, on the short term, the plants would deplete the soil in light isotopes and would therefore noticeably impact the Cu isotopic composition of the soil. The Cu isotopic fractionation is further enhanced during the translocation of the Cu within the plant, with $\delta^{65}Cu_{stem} > \delta^{65}Cu_{leaves} > \delta^{65}Cu_{seeds}$. The two plants B1 and B2 show the same pattern, but the magnitude of the fractionation is different. Plant B1 is clearly isotopically lighter than plant B2. This can be explained by a difference in the ages of the two plants: We collected them at the same time, but they might have been growing for a slightly different number of days.

The correlation between $\delta^{65}Cu$ and the height of the leaves in experiment C probably has the same origin as the isotopic fractionation pattern observed in experiments A and B. When you go higher in the plant, lighter isotopes are more enriched by a combination of diffusion and transport through cell membranes. The absence of varying fractionations in the seeds collected at different heights is puzzling. This could be because the range of height is much smaller for the seeds (45.5 to 63 cm) than for the leaves (7 to 43.5 cm), so any fractionation that may have occurred would have been too small for us to measure. The absence of further isotopic variations between the seeds could also suggest that the mechanism of entrance of Cu into the seeds is different than that for the leaves and does not further fractionate Cu isotopes. The assimilation of the Cu by the seeds could also be quantitative. More experiments focusing on the seeds (comparisons between seeds collected from different plants, comparison to the isotopic composition of the stem at the same height as the seeds, etc.) would help to resolve these questions.

The slope and origin of the correlation lines between the degree of Cu isotopic fractionation and the height of the leaves for the hairy-leaved sedge (exp. C) (slope = −0.01 and origin = −0.07) and for the Zn in a bamboo plant (slope = −0.01 and origin = −0.06 (Moynier et al., 2009a,b)) were almost identical. This surprisingly good correlation between Zn and Cu isotopes is another argument in favor of similar transport mechanisms for these two elements within a plant. Both Zn and Cu have been proposed to be complexed to a ligand during transport within the xylem (Liao et al., 2000; Colangelo and Guerino, 2006). The chelator is probably Nicotianamine, a non-proteino amino acid (Budesinsky, 1980) that is involved in the translocation of many metals in xylem sap (Pich et al., 1994; Welch, 1995; Pich and Scholz, 1996; Trampczynska et al., 2010). Our results suggest that both Zn and Cu share this transporter.

5. Conclusion

Cu isotopes are fractionated in both monocots and dicots 1) during the uptake of Cu by the plant and 2) within the plant, with an enrichment in the lighter isotope for any growth environments. In lentils, shoots (leaves and stems) are isotopically lighter than the germinated seeds. In monocot plants, seeds growing on the plant are isotopically lighter than leaves, and leaves are generally lighter than the stem, which is lighter than the soil from which the plants have grown. Cu strongly correlates with Zn, and so we suggest that both elements are fractionated by the same mechanisms: diffusion and transport through cell membranes.

Furthermore, because the amount of Cu isotopic fractionation induced by plants is of the same order of magnitude as the fractionation observed in soils, this factor should be taken into account in order to accurately interpret the Cu isotopic record of soils.

References

Albarede, F., 2004. The stable isotope geochemistry of copper and zinc. In: Johnson, C.M., Beard, B.L., Albarede, F. (Eds.), Rev Mineral Geochem. Reviews In Mineralogy & Geochemistry. Mineralogical Society of America, pp. 409–427.

Archer, C., Vance, D., 2002. Mass discrimination correction in multiple collector plasma source mass-spectrometry: an example using Cu and Zn isotopes. J. Anal. At. Spectrom. 64, 356–365.

Asael, D., Matthews, A., Bar-Matthews, M., Halicz, L., 2007. Copper isotope fractionation in sedimentary copper mineralization (Timna Valley, israel). Chem. Geol. 243, 238–254.

Asael, D., Matthews, A., Oszczepalski, S., Bar-Matthews, M., Halicz, L., 2009. Fluid speciation controls of low temperature copper isotope fractionation applied to the Kupferschiefer and Timna ore deposits. Chem. Geol 262, 147–158.

Balistrieri, L.S., Borrok, D.M., Wanty, R.B., Ridley, W.I., 2008. Fractionation of Cu and Zn isotopes during adsorption onto amorphous Fe(III) oxyhydroxide: experimental mixing of acid rock drainage and ambient river water. Geochim. Cosmochim. Acta 72, 311–328.

Ben Othman, D., Luck, J.M., Bodinier, J.L., Arndt, N.T., Albarede, F., 2006. Cu–Zn isotopic variations in the Earth's mantle. Geochim. Cosmochim. Acta 70, 46.

Bigalke, M., Weyer, S., Kobza, J., Wilcke, W., 2010a. Stable Cu and Zn isotope ratios as tracers of sources and transport of Cu and Zn in contaminated soil. Geochim. Cosmochim. Acta 74, 6801–6813.

Bigalke, M., Weyer, S., Wilcke, W., 2010b. Stable copper isotopes: a novel tool to trace copper behavior in hydromorphic soils. Soil Sci. Soc. Am. J. 74, 60–73.

Bigeleisen, J., Mayer, M.G., 1947. Calculation of equilibrium constants for isotopic exchange reactions. J. Chem. Phys. 15, 261–267.

Black, J.R., Epstein, E., Rains, W.D., Yin, Q.Z., Casey, W.H., 2008. Magnesium-isotope fractionation during plant growth. Environmental Science & Technology 42, 7831–7836.

Budesinsky, M., 1980. The normalizing factor for the tomato mutant chlorerva. 10. Nicotinamine, a possible phytosiderophore of general occurence. Phytochemistry 19, 2295–2297.

Cameron, V., Vance, D., Archer, C., House, C., 2009. A biomarker based on the stable isotopes of nickel. Proc. Natl. Acsd. Sci. 27, 10944–10948.

Cenki-Tok, B., Chabaux, F., Lemarchand, D., Schmitt, A.D., Pierret, M.C., Viville, D., Bagard, M.L., Stille, P., 2009. The impact of water-rock interaction and vegetation on calcium isotope fractionation in soil and stream waters of a small, forested catchment (the Strengbach case). Geochim. Cosmochim. Acta 73, 2215–2228.

Cloquet, C., Carignan, J., Lehmann, M.F., Vanhaecke, F., 2008. Variation in the isotopic composition of zinc in the natural environment and the use of zinc isotopes in biogeosciences: a review. Anal. Bio. Chem. 390, 451–463.

Colangelo, E., Guerino, M.L., 2006. Put the metal to the petal: metal uptake and transport throughout plants. Current opinion in plant biology 9, 322–330.

Dauphas, N., Rouxel, O., 2006. Mass spectrometry and natural variations of iron isotopes. Mass Spectrom Rev 25, 515–550.

Ehrlich, S., Butler, I., Halicz, L., Rickard, D., Oldroyd, A., Matthews, A., 2004. Experimental study of copper isotope fractionation between aqueous Cu(II) and covellite, CuS. Chem Geol 209, 259–269.

Ellis, A.S., Johnson, T.M., Bullen, T.D., 2002. Chromium Isotopes and the Fate of Hexavalent Chromium in the Environment. Science 295, 2060–2062.

Epstein, L., Bassein, S., 1998. Pesticide applications of copper on perennial crops in California, 1993 to 1998. Journal of environmental quality. 30, 1844–1847.

Frausto da Silva, J.J.R., Williams, R.J.P., 2001. The Biological Chemistry of the Elements. The Inorganic Chemistry of Life. Univ. Press, Oxford. 584 pp.

Guelke, M., Von Blanckenburg, F., 2007. Fractionation of stable iron isotopes in higher plants. Environ. Sci. Tech. 41, 1896–1901.

Herzog, G.F., Moynier, F., Albarede, F., Berezhnoy, A.A., 2009. Isotopic and elemental abundances of copper and zinc in lunar samples, Zagami, Pele's hairs, and a terrestrial basalt. Geochim. Cosmochim. Acta 73, 5884–5904.

Johnson, C.M., Beard, B.L., Albarede, F. (Eds.), 2004. Non traditional stable isotopes. Mineralogical society of America. Rev. Mineral. Geochem., 454 pp.

Johnson, C.M., Beard, B.L., Roden, E.E., 2008. The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient earth. Annual Review of Earth and Planetary Sciences 36, 457–493.

Jouvin, D., Louvat, P., Marechal, C., Benedetti, M.F., 2008. Fractionation of copper isotopes in plants. Geochim. Cosmochim. Acta 72, A441–A.

Karamanos, R.E., Walley, F.L., Flatten, P.L., 2005. Effectiveness of seedrow placement of granular copper products for wheat. Canadian journal of soil science 85, 285–306.

Koch, K.A., Pena, M.M.O., Thiele, D.J., 1997. Copper-binding motifs in catalysis, transport, detoxification and signaling. Chem Biol 4, 549–560.

Larson, P.B., Maher, K., Ramos, F.C., Chang, Z., Gaspar, M., Meinert, L.D., 2003. Copper isotope ratios in magmatic and hydrothermal ore forming environments. Chem. Geol. 201, 337–350.

Li, W., Jackson, S., Pearson, N.J., Alard, O., Chappell, B.W., 2009. The Cunext term isotopic signature of granites from the Lachlan Fold Belt, SE Australia. Chem Geol. 258, 38–49.

Liao, M.T., Hedley, M.J., Woolley, D.J., Brooks, R.R., Nichols, M.A., 2000. Copper uptake and translocation in chicory (Cichorium intybus L. cv. Grasslands Punia) and tomato (Lycopersicon esculentum Mill. cv. Rondy) plants grown in NFT system. I. Copper uptake and distribution in plants. Plant and Soil 131, 135–142.

Maksymiec, W., 1997. Effect of copper on cellular processes in higher plants. Photosynthetica 34, 321–342.

Manceau, A., Nagy, K.L., Marcus, M.A., Lanson, M., Geoffroy, N., Jacquet, T., Kirpichtikova, T., 2008. Formation of metallic copper nanoparticles at the soil-root interface. Environmental Science & Technology 42, 1766–1772.

Marechal, C., 1998. Géochimie des isotopes du Cuivre et du Zinc. Méthode, variabilités naturelles, et application océanographique. PhD Thesis, Ecole Normale Supérieure de Lyon, 253 pp.

Maréchal, C., Télouk, P., Albarède, F., 1999. Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. Chem. Geol. 156, 251–273.

Maréchal, C.N., Douchet, C., Nicolas, E., Albarède, F., 2000. The abundance of zinc isotopes as a marine biogeochemical tracer. Geochem., Geophys. Geosyst. 1, 1999GC–000029.

Markl, G., Lahaye, Y., Schwinn, G., 2006. Copper isotopes as monitors of redox processes in hydrothermal mineralization. Geochim. Cosmochim. Acta 70, 4215–4228.

Mathur, R., Ruiz, J., Titley, S., Liermann, L., Buss, H., Brantley, S., 2005. Cu isotopic fractionation in the supergene environment with and without bacteria. Geochim. Cosmochim. Acta 69, 5233–5246.

Mathur, R., Titley, S., Barra, F., Brantley, S., Wilson, M., Phillips, A., Munizaga, F., Maksaev, V., Vervoort, J., Hart, G., 2009. Exploration potential of Cu isotope fractionation in porphyry copper deposits. Journal of Geochemical exploration 102, 1–6.

Moynier, F., Albarede, F., Herzog, G., 2006. Isotopic composition of zinc, copper, and iron in lunar samples. Geochim. Cosmochim. Acta 70, 6103–6117.

Moynier, F., Blichert-Toft, J., Telouk, P., Luck, J.M., Albarede, F., 2007. Comparative stable isotope geochemistry of Ni, Cu, Zn, and Fe in chondrites and iron meteorites. Geochim. Cosmochim. Acta 71, 4365–4379.

Moynier, F., Beck, P., Jourdan, F., Yin, Q.Z., Reimold, U., Koeberl, C., 2009a. Isotopic fractionation of Zn in tektites. Earth. Planet. Sci. Lett. 277, 482–489.

Moynier, F., Pichat, S., Pons, M.L., Fike, D., Balter, V., Albarede, F., 2009b. Isotopic fractionation and transport mechanisms of Zn in plants. Chem. Geol. 267, 125–130.

Moynier, F., Koeberl, C., Beck, P., Jourdan, F., Telouk, P., 2010. Isotopic fractionation of Cu in tektites. Geochim. Cosmochim. Acta 74, 799–807.

Moynier, F., Paniello, R., Gounelle, M., Albarede, F., Beck, P., Podosek, F., Zanda, B., 2011a. Nature of volatile depletion and genetic relationships in enstatite chondrites and aubrites inferred from Zn isotopes. Geochim. Cosmochim. Acta. 75, 297–307.

Moynier, F., Yin, Q.Z., Schauble, E., 2011b. Isotopic evidence of Cr partitioning into the Earth's core. Science 331, 1417–1420.

Navarrete, J.U., Borrock, D.M., 2010. Fractionation of Cu isotopes during adsorption and metabolic uptake by bacteria. Geochim. Cosmochim. Acta 74, A749.

Navarette, J.U., Borrok, D.M., Viveros, M., Ellzey, J.T., 2011. Copper isotope fractionation during surface adsorption and intracellular incorporation by bacteria. Geochim. Cosmochim. Acta 75, 784–799.

Pich, A., Scholz, G., 1996. Translocation of copper and other micronutrients in tomato plants (Lycopersicon esculentum Mill.): nicotiamamine-stimulated copper transport in the xylem. Jounral of Experimental Botany 47, 41–47.

Pich, A., Scholz, G., Stephan, U.W., 1994. Iron-dependant changes of heavy metals, nicotianamin, and citrate in different plant organs and the xyleme exudate of 2 tomato genotypes -nicotianamine as possible copper translocator. Plant and Soil 165, 189–196.

Pokrovsky, O.S., Viers, J., Emnova, E.E., Kompantseva, E.I., Freydier, R., 2008. Copper isotope fractionation during its interaction with soil and aquatic microorganisms and metal oxy(hyd)oxides: Possible structural control. Geochim. Cosmochim. Acta 72, 1742–1757.

Rouxel, O.D., Fouquet, Y., Ludden, J.N., 2004. Copper isotope systematics of the Lucky Strike, Rainbow, and Logatchev hydrothermal fields on the Mid-Atlantic Ridge. Econ. Geol. 99, 585–600.

Shioi, Y., Tamai, H., Sasa, T., 1978. Effects of copper on photosynthetic electron transport systems in spinach chloroplasts. Plant & Cell Physiol. 19, 203–209.

Trampczynska, A., Kupper, H., Meyer-Klaucke, W., Schmidt, H., Clemens, S., 2010. Nicotianamine forms complexes with Zn(II) in vivo. Metallomics 2, 57–66.

Urey, H.C., 1947. The thermodynamic properties of isotopic substances. J. Chem. Soc. (London) 562–581.

Viers, J., Oliva, P., Nonelle, A., Gelabert, A., Sonke, J., Freydier, R., Gainville, R., Dupre, B., 2007. Evidence of Zn isotopic fratination in a soil-plant system of a pristine tropical watershed (Nsimi, Cameroon). Chem. Geol. 239, 124–137.

von Blanckenburg, F., von Wiren, N., Guelke, M., Weiss, D.J., Bullen, T.D., 2009. Fractionation of metal stable isotopes by higher plants. Elements 5, 375–380.

Weiss, D.J., Mason, T.F.D., Zhao, F.J., Kirk, G.J.D., Coles, B.J., Horstwood, M.S.A., 2005. Isotopic discrimination of zinc in higher plants. New Phytologist 165, 703–710.

Welch, R., 1995. Micronutrient nutrition of plants. Critical review in plant science 14, 49–82.

Young, E.D., Galy, A., 2004. The isotope geochemistry and cosmochemistry of magnesium. In: Johnson, C.M., Beard, B.L., Albarede, F. (Eds.), Rev Mineral Geochem. Mineralogical Society of America, pp. 197–230.

Zhu, X.K., O'Nions, R.K., Guo, Y., Belshaw, N.S., Rickard, D., 2000. Determination of Cu-isotope variation by plasma souce mass spectrometry: implications for use as geochemical tracers. Chem. Geol. 163, 139–149.

Zhu, X.K., Guo, Y., Williams, R.J.P., O'Nions, R.K., Matthews, A., Belshaw, N.S., Canters, G.W., de Waal, E.C., Weser, U., Burgess, B.K., Salvato, B., 2002. Mass fractionation processes of transition metal isotopes. Earth Planet. Sci. Lett. 200, 47–62.

Zhu, X.K., Li, S.Z., Luo, Y.M., Wu, L.H., 2010. Copper isotopic fractionation by higher plants. Geochim. Cosmochim. Acta Supp. 74, A1234.