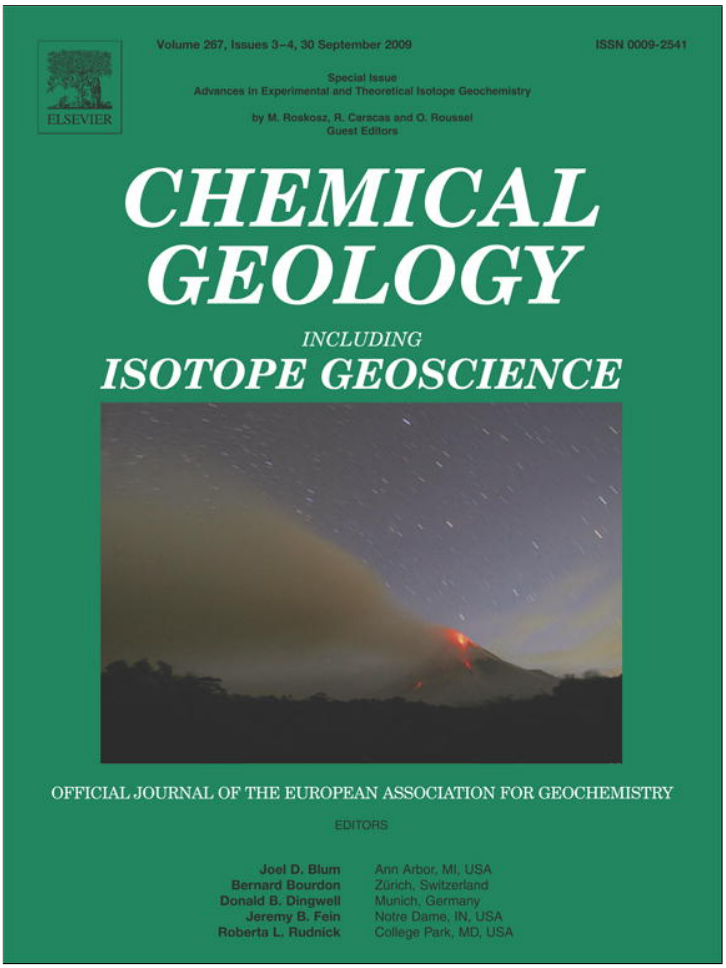

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Isotopic fractionation and transport mechanisms of Zn in plants

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ABSTRACT

We have analyzed by MC-ICP-MS the Zn isotopic composition of different components (seeds, leaves, and rhizome, stem and leaves) of lentils (*Lens culinaris*) and bamboos (*Phyllostachys aurea*), respectively. Zn isotopes are systematically fractionated between seeds and leaves of lentil and between stem and leaves of bamboos. Leaves are enriched in light Zn isotopes compared to the other parts of plants. The range of the fractionation is up to 0.52‰ per amu and is clearly mass dependent. The observed Zn isotopic fractionation is consistent with that occurring during both diffusive processes and cross-cell membrane transport. Our study also shows a clear interspecies variability for Zn isotopic fractionation. We conclude that the Zn could be used as a tracer for biological activities.

1. Introduction

Zn is a vital micronutrient in living organisms and particularly in plants, notably because Zn participates in protein synthesis and membrane activities (Welch et al., 1982). Zn concentration in soil is a key parameter for the growth of plants of economical importance (e.g. cereals, rice). Soils with low concentrations of Zn are frequent in the world (Sillanpaa, 1982), and Zn deficiency appears as a major agronomical issue (Cakmak et al., 1999). In addition, plants growing in Zn-depleted environments tend to accumulate heavy metals, like Cd (Wolnik et al., 1993) in concentrations that are potentially toxic either for the plant itself or for the associated trophic chain. With the expansion of industrial production and city size, the pollution by heavy metals is becoming an issue that could severely affect plant growth and increase the concentration of heavy metals within the food chain.

The transport mechanisms of Zn in plants remain poorly understood. One way to improve our knowledge about these mechanisms is to study the natural stable isotopic fractionation of Zn during translocation processes (transport of Zn within the plant body). In the late-90s, the development of multicollector inductively-coupled plasma mass-spectrometry (MC-ICP-MS) (Maréchal et al., 1999; Zhu et al., 2001) opened up a new field in stable isotope geochemistry. In particular, numerous applications of the mass fractionations of transition metals have been found in planetary, earth, ocean, biological and environmental sciences (Johnson et al., 2004; Cloquet et al., 2008 for reviews). In the field of biological and environmental sciences, MC-ICP-MS has led to the investigation of natural isotopic fractionations in metabolically essential transition metals such as Fe, Cu and Zn.

Recently, Weiss et al. (2005) measured the isotopic fractionation of Zn between roots and shoots in tomato (*Lycopersicon esculentum*), rice (*Oryza sativa*) and lettuce (*Lactuca sativa*) during their growth on two different nutrient solutions: EDTA (ethylenediaminetetraacetic acid) and HEDTA + NTA (nitrilotriacetic acid). The growth media were concentrated in Zn to avoid reservoir depletion effects. The experiments conducted by Weiss et al. (2005) show a systematic depletion in the heavy isotopes of Zn from roots to shoots from −0.13‰ to −0.26‰ per atomic mass unit (amu). Viers et al. (2007) followed up with Zn isotopic data in a soil–plant system of a tropical watershed in Cameroon. While the emphasis of the study was on the soil horizon, they also measured the Zn isotopic composition of roots, shoots and leaves for three herbaceous species and three tree species. Their main conclusions are somehow different from the ones drawn by Weiss et al. (2005): (1) only 1 out 4 of root-shoot pairs has a significant enrichment in light Zn isotopes in shoot, (2) Zn isotopic compositions of the leaves, shoots and roots of the 3 analyzed herbaceous species do not significantly differ and (3) tree leaves have $\delta^{66}Zn < 0‰$ and on the two species were data are available, tree leaves are depleted in heavy

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Table 1
δ^{66}Zn and δ^{68}Zn results for experiments A (lentils).

sa. name	δ^{66}Zn	δ^{68}Zn	sa. name	δ^{66}Zn	δ^{68}Zn	sa. name	δ^{66}Zn	δ^{68}Zn
Experiment A1			Experiment A2			Experiment A3		
Leaf-1a	0.91	1.74	Leaf-1a	0.90	1.98	Leaf-1a	1.18	2.33
Leaf-1b	0.85	2.00	Leaf-1b	0.98	1.89	Leaf-1b	1.03	2.04
Leaf-2	1.00	2.00	Leaf-2	0.89	1.88	Leaf-2	1.07	2.08
Leaf-ave ± 2σ	**0.92 ± 0.15**	**1.91 ± 0.30**	**Leaf-ave ± 2sσ**	**0.93 ± 0.01**	**1.91 ± 0.11**	**Leaf-ave ± 2sσ**	**1.09 ± 0.16**	**2.25 ± 0.31**
Seed-1a	1.24	2.46	Seed-1a	1.31	2.65	Seed-1a	1.21	2.34
Seed-1b	1.22	2.38	Seed-1b	1.19	2.28	Seed-1b	1.32	2.51
Seed-2	1.32	2.76	Seed-2	1.30	2.68	Seed-2	1.37	2.90
Seed-ave ± 2σ	**1.26 ± 0.11**	**2.53 ± 0.40**	**Seed-ave ± 2sσ**	**1.27 ± 0.13**	**2.54 ± 0.45**	**Seed-ave ± 2sσ**	**1.30 ± 0.16**	**2.59 ± 0.57**

In all experiments the leaves are depleted in heavy isotopes of Zn compared to the seeds − 1 and − 2 are two different samples. Samples noted -a and -b are replicate measurements of the same sample digestion. No nutrient solution was added and lentils were grown on the seed reserves with addition of either 18.2 MΩ water (Exp. A1 and A3) or 18.2 MΩ water with 1 ppm Zn (Exp.A2). Experiment A1 and A2 have been performed inside a class 10,000 clean room to avoid any contamination whereas Exp. A3 was conducted in a standard laboratory.

Zn isotopes compared to either the shoot (*Musanga cecropioies*) or both the root and the shoot (*Raphia vinifera*). They proposed that the xylem is progressively enriched in light isotopes by diffusion and/or by preferential complexation of heavy Zn isotopes by proteins along the transport pathway. They also hypothesized a correlation between plant length (tree>herbs) and the intensity of Zn isotopic fractionation in leaves.

The existence of isotopic fractionation in plants and, in particular the existence of isotopically depleted Zn of biological origin, may help achieve isotopic mass balance in the terrestrial Zn cycle. Most terrestrial materials are enriched in heavy isotopes of Zn (compared to the JMC 3-0749 L standard), with a maximum of 1.4‰ in the carbonate fraction of a deep-sea sediment core (Pichat et al., 2003). So far, depletions in heavy Zn isotopes in geological material have only been observed in sulphide ore deposits (Mason et al., 2005; John et al., 2008), where they are attributed to the preferential precipitation of light Zn in zinc sulfides (John et al., 2008). Therefore, isotopically light biological components are of interest for their potential to balance the Zn isotope cycle. However, the isotopic fractionation mechanisms that account for the enrichment in light Zn isotope are still unclear and need to be clarified before any unambiguous interpretation of the natural variations in Zn isotopic compositions and accurate tracing of heavy metal pollution in plants.

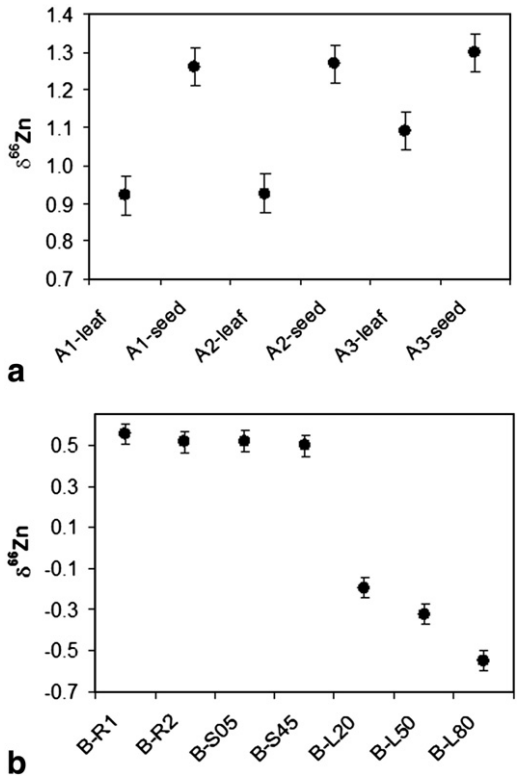

Fig. 1. a. Results for experiment A (lentils). b. Results for experiment B (bamboo). The average values for different experiments are plotted. For lentils, Exp. A1 and A2 were conducted inside a class 10,000 clean room whereas Exp. A3 was performed in a standard laboratory. Exp. A1 and Exp. A3 were grown with only the addition of 18 MΩ water, whereas Exp A2 additionally contained 1 ppm of Zn. For lentils, the heavy isotopes of Zn are systematically depleted in leaves compared to the germinated seeds in every experiment. For bamboo (Exp. B), R1 and R2 are rhizome samples, S5 and S45 are samples from the stem collected at two different height (5 and 45 cm) and L20, L50 and L80 are leaves samples taken at the height of 20, 50 and 80 cm (L20, L50 and L80). For the bamboo, no isotopic fractionation of Zn is observed between rhizome and stem, whereas the leaves are systematically depleted in heavy isotopes compared to rhizome/stem.

The objectives of this paper are 1) to test whether stable isotopic fractionation of Zn occurs during its transportation from seeds or roots to shoots/leaves in two plants that have different growing patterns, a dicotyledon (lentil: *Lens culinaris*) and a monocotyledon (golden bamboo: *Phyllostachys aurea*); 2a) to check the hypothesis proposed by Viers et al. (2007) that there is a correlation between plant length and the intensity of Zn isotopic fractionation in leaves by comparing bamboo and lentils leaves data and 2b) to test whether there is a correlation between the height of the leaves in a given plant and the Zn isotopic fractionation by measuring the isotopic composition in leaves collected at different height on a bamboo; 3) to constrain the mechanisms of Zn mobilization from the seed to the various physiological compartments using controlled growing medium; and 4) to explore the natural Zn isotopic fractionation in plants growing in a natural environment. We will interpret the results with respect to physiological mechanisms.

2. Analytical

2.1. Samples

Lentils were cultivated in a clean chemistry room to minimize external (airborne) contamination and to control growth conditions.

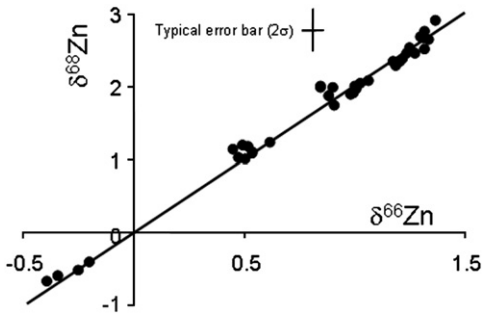

Fig. 2. δ^{68}Zn vs δ^{66}Zn. All the samples fall on the mass-dependent fractionation line of slope 1.94.

Table 2
$\Delta\delta^{66}Zn_{i-j}$ for the experiments A and B.

	Exp. A1	Exp. A2	Exp. A3	Exp. BL20	Exp. BL50	Exp. BL80
$\Delta\delta^{66}Zn_{i-j} \pm 2\sigma$	0.34 ± 0.18	0.34 ± 0.17	0.21 ± 0.23	0.71 ± 0.15	0.85 ± 0.15	1.07 ± 0.15

For Exp. A $\Delta\delta^{66}Zn_{i-j} = (\delta^{66}Zn)_{seeds} - (\delta^{66}Zn)_{leaves}$ and for Exp. B $\Delta\delta^{66}Zn_{i-j} = (\delta^{66}Zn)_{rhizome/stem} - (\delta^{66}Zn)_{leaves}$.

This is important because of the high solubility of Zn in water and low abundance of zinc in lentils ~20 ppm (μg/g). Lentils were grown for 5 days under controlled conditions (12 h light per day, temperature 21.5 °C) in a clean room. No nutrient solution was added and lentils were grown on their seed reserves with addition of either 18.2 MΩ water (Exp. A1) or 18.2 MΩ water with 1 ppm Zn (Exp. A2). The Zn solution used for the experiment was extracted from manganese nodule DR8607 (Maréchal et al., 1999), which has a known $\delta^{66}Zn = 1.12 \pm 0.04$‰ compared to Lyon Zn JMC 3-0749 L ($\delta^{x}Zn = [(^{x}Zn/^{64}Zn)_{sample}/(^{x}Zn/^{64}Zn)_{standard} - 1] \times 10^3$ and $x = 66$, 67 or 68). All $\delta^{66}Zn$ values in the text are with respect to the Lyon JMC 3-0749 L standard and all precisions are reported as 2σ standard deviations or 2se standard error. In order to test the effect of atmospheric pollution during growth, one experiment was conducted outside of a clean room laboratory under summer daylight, ca. 12 h of light/day with the addition of 18.2 MΩ water (Exp.A3). After 5 days of growing up, the germinated seeds and leaves of each experiment were separated, dissolved and prepared for Zn isotopic measurements.

A 80 cm high bamboo was harvested in a field near the town of Lyon (France) after 14 days of growth in spring (ca. 11 h light/day); its natural culture media is unknown (Exp. B). We analyzed 3 types of samples: the main root, or rhizome (secondary roots were cut off), the main stem, and leaves. The two samples from the rhizome (R1 and R2) were cut at the base of the main stem using a diamond microsaw. These two samples were taken from the same plant 2 mm apart. To check the influence of the height on the Zn concentration and isotopic composition, we analyzed two samples from the main stem at 5 and 45 cm (S5 and S45). Finally, 2–3 leaves where taken at the height of 20, 50 and 80 cm (L20, L50 and L80). Leaves grow on secondary stems that have not been analyzed. The pedicel from the leaves was removed prior to cleaning.

2.2. Chemical purification

Bamboo leaves (150–200 mg), stems (250–350 mg) and rhizome (300–400 mg) were cleaned with 18.2 MΩ water prior to dissolution. The procedure was repeated 4 times to ensure the removal of surface-coated Zn coming from atmospheric sources. After this step, the rhizome samples were immersed in 18.2 MΩ water and ultrasonicated for 15 min. The procedure was repeated until no soil could be seen under binocular at the surface of the rhizome. Lentils leaves (100–200 mg) and seeds (100–200 mg) were directly digested with no additional H_2O cleaning.

The digestion solution consists of a 2.5:1 mixture of concentrated sub-boiled distilled HNO_3 and 30% H_2O_2 (analytical grade). The solution was subsequently converted to bromide form in 1.5 N HBr (Seastar™). Zn was purified by anion-exchange chromatography using a procedure adapted from Moynier et al. (2006). Briefly, samples were loaded in 1.5 N HBr on a 0.5 ml AG-1x8 (200–400 mesh) chromatographic columns and Zn was extracted in 0.5 N HNO_3. The process was repeated on a 100 μl column to further purify Zn. Anion-exchange chromatography is known to fractionate Zn isotopes when yields are incomplete (Maréchal and Albarède, 2002). Using Zn standard JMC 3-0749 L, we checked that the yields were better than 99%. The test was made with each batch of experiments. We further checked that the Zn-standards were not fractionated by the chromatographic process.

2.3. Mass spectrometry

Zn isotopic ratios were measured by MC-ICP-MS at ENS-Lyon. For lentils, the samples were analyzed on a VG Plasma 54 as described by Maréchal et al. (1999). For the bamboo samples, the analyses were made on a Nu plasma 500 HR. The samples were introduced by free aspiration in 0.05 N sub-boiled distilled HNO_3 using a glass microconcentric nebulizer (uptake rate: 80 μl/min) and a glass cyclonic spray chamber. Zn isotopes ($M = 64$, 66, 67 and 68) were measured in collectors H4, H2, Axial and L3. Cu isotopes ($M = 63$ and 65) were measured in collectors L2 and L4. ^{62}Ni was measured in collector L5 in order to correct for the Ni contribution at mass 64 using the Ni natural abundance. Peak intensities were measured in Faraday detectors in static mode with a spectral resolution of $M/\Delta M = 1000$. Instrumental mass fractionation was corrected using Cu-doping and standard-sample bracketing following the recommendations provided in Albarede et al. (2004). We used Cu NIST-SRM 976 for doping and an exponential law to correct the instrumental mass bias. Samples were randomized and measurements were usually duplicated to avoid systematic errors. Sample measurement solutions were diluted to match the concentration of the standard mixture (Zn 0.5 ppm–Cu 0.5 ppm). The total procedural blank, (dissolution, chemical purification and mass spectrometry) of Zn, was <15 ng and represents less than 0.5% of the total signal. The external reproducibility based on the repeated measurement of a Zn standard is 0.05‰ (Maréchal et al., 1999).

3. Results

Results for both plants are summarized in the Table 1 and Fig. 1. We checked that isotopic fractionation is mass-dependent for all the samples, i.e. $\delta^{68}Zn \approx 1.94 \times \delta^{66}Zn$ (Fig. 2).

Table 3
$\delta^{66}Zn$ and $\delta^{68}Zn$ results for experiments B (bamboo) -a, -b, -c: replicate measurement of the same sample, -ave: average value.

sa. name	$\delta^{66}Zn$	$\delta^{68}Zn$	sa. Name	$\delta^{66}Zn$	$\delta^{68}Zn$
Experiment B (bamboo)					
Rhizome-R1-a	0.48	0.98	Leave-L20	−0.19	−0.41
Rhizome-R1-b	0.62	1.24	**Leave-L20-ave**	**−0.19**	**−0.41**
Rhizome-R1-ave ± 2σ	**0.55 ± 0.20**	**1.11 ± 0.37**	Leave-L50-a	−0.34	−0.68
Rhizome-R2-a	0.52	1.17	Leave-L50-b	−0.39	−0.70
Rhizome-R2-b	0.51	0.99	Leave-L50-c	−0.25	−0.54
Rhizome-R2-ave ± 2σ	**0.51 ± 0.01**	**1.08 ± 0.25**	**Leave-L50-ave ± 2σ**	**−0.32 ± 0.14**	**−0.64 ± 0.17**
Rhizome R1–R2 average ± 2σ	**0.53 ± 0.06**	**1.10 ± 0.04**	Leave-L80	−0.55	−1.07
Stem-S05-a	0.50	1.20	**Leave-L80-ave**	**−0.55**	**−1.07**
Stem-S05-b	0.54	1.10			
Stem-S05-ave ± 2σ	**0.52 ± 0.06**	**1.15 ± 0.14**			
Stem-S45-a	0.45	0.92			
Stem-S45-b	0.54	1.08			
Stem-S45-ave ± 2σ	**0.50 ± 0.13**	**1.00 ± 0.23**			

Leaves are systematically depleted in heavy isotopes of Zn compared to the stem and rhizome.

3.1. Lentils

Lentils show enrichment in heavy Zn isotopes compared to the standard both in germinated seed and leaves. The germinated seeds and the leaves were taken at the same growth stage (after 5 days). Leaves are systematically lighter than the germinated seeds. In the following discussion the isotopic fractionation between two compartments i and j or the same compartment between two experiments *i* and *j* will be expressed as:

$$\Delta\delta^{66}Zn_{i-j} = \left(\delta^{66}Zn\right)_i - \left(\delta^{66}Zn\right)_j$$

e.g., for the isotopic difference between leaves and seeds for a given experiment: $\Delta\delta^{66}Zn_{seeds-leaves} = (\delta^{66}Zn)_{seeds} - (\delta^{66}Zn)_{leaves}$. The precision on the $\Delta\delta^{66}Zn_{seeds-leaves}$ is estimated by propagating the standard errors measured on the $(\delta^{66}Zn)_{seeds}$ and $(\delta^{66}Zn)_{leaves}$. The different $\Delta\delta^{66}Zn$ are reported in the Table 2. In all experiments, $\Delta\delta^{66}Zn_{seeds-leaves}$ is positive which implies that the germinated seeds are isotopically heavier than the leaves. The $\Delta\delta^{66}Zn_{seeds-leaves}$ (0.34‰) is the same for the 2 experiments conducted into the clean chemistry room while the experiment conducted outside the clean room has a $\Delta\delta^{66}Zn_{seeds-leaves}$ of 0.21‰ (which is only slightly outside the analytical error). The experiment conducted out of the clean laboratory (Exp. A.3) shows a slightly heavier Zn isotopic composition for the leaves ($\Delta\delta^{66}Zn_{A1-A3} = 0.16$‰) while the $\delta^{66}Zn$ are identical for the seeds. Finally, there is no difference in Zn isotopic composition between the lentils grown with water (Exp. A.1) and the ones grown with water + 1 ppm Zn (Exp.A.2).

3.2. Bamboos

Samples from the rhizome and the stem show a systematic enrichment in heavier Zn isotopes compared to the standard. The two rhizome samples (R1 and R2), which were taken 2 mm apart from each other, show similar $\delta^{66}Zn$ values (0.55‰ and 0.51‰, respectively). Moreover, these values are almost identical to the values measured in the main stem at two different heights, 5 and 45 cm (S05 and S45: $\delta^{66}Zn = 0.52$‰ and 0.50‰, respectively). Thus, there is no apparent fractionation between the rhizome and the main stem. Leaves show a systematic enrichment in light isotopes compared to the Zn standard ($\delta^{66}Zn = -0.19$‰ to -0.55‰, Table 3). Therefore, a significant fractionation occurs between the main stem and the leaves. There is a correlation ($R^2 = 0.98$) between the height of the leaves and the amplitude of the Zn fractionation (Fig. 3). However, there is no variation of the Zn isotopic composition associated with height in the stem (S05 and S45). The correlation between height and Zn isotopic composition is only based on three samples and may be fortuitous.

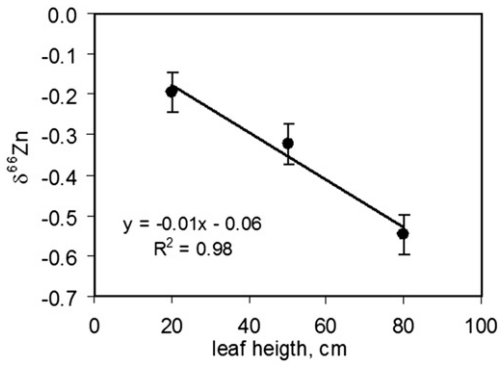

Fig. 3. $\delta^{66}Zn$ vs leaf height. The Zn isotope compositions of leaves from a bamboo are correlated with the height of the leaves.

The $\Delta\delta^{66}Zn$ between the leaves and the closest sampled stem are: $\Delta\delta^{66}Zn_{L20\text{-}S05} = -0.71$‰ and $\Delta\delta^{66}Zn_{L50\text{-}S45} = -0.82$‰.

4. Discussion

4.1. Range of biological Zn isotopic variations

The $\delta^{66}Zn$ values for bamboo leaves (-0.19 to -0.55‰) are among the lightest Zn isotope composition reported in terrestrial material. They are within the same range as the values reported for 3 tree leaves: -0.03 to -0.91‰ (Viers et al., 2007) and much lower than the values reported for 3 herbaceous leaves (0.26 to 0.63‰) by the same authors. Lighter values are found in lunar materials (Moynier et al., 2006) and some meteorites (Luck et al., 2005; Moynier et al., 2007). The light $\delta^{66}Zn$ values measured in bamboo leaves are very unlikely resulting from external pollution because most of the terrestrial values recorded to date have positive $\delta^{66}Zn$ (Maréchal et al., 1999; Weiss et al., 2007; John et al., 2007b; Cloquet et al., 2008). Our results on bamboo show that plants could represent a reservoir for light Zn isotopes in nature.

4.2. Possible mechanisms of isotopic fractionation in lentils and bamboos

The $\Delta\delta^{66}Zn_{seeds-leaves}$ of 0.34‰ in lentils, i.e. an isotopic fractionation of 0.17‰/amu, is consistent with the results of Weiss et al. (2005). In their experiments, plants were cultivated for more than 40 days, and the addition of nutrient solutions was needed to allow a normal growth. In this study, we worked on lentil, a quick growing plant, which has been cultivated on its own reserves for 5 days, only with the addition of 18.2 MΩ water, which is virtually Zn free. Our results lead to the conclusion that the isotopic fractionation between seeds and leaves took place during the mobilisation of the seed reserves. This result is confirmed by the fact that the same enrichment in light isotopes in leaves is observed for the lentils that have been supplied with water (Exp.A.1) and for the ones that have been supplied with a 1 ppm Zn solution where $\delta^{66}Zn$ was 1.12‰ (Exp.A.2).

For the bamboo, rhizome and stem have the same Zn isotopic composition. Therefore, the transport of Zn between these two compartments of the plant does not involve fractionation. On the other hand, Zn isotopic fractionation does occur between the main stem and the leaves, i.e. over 2 to 10 cm. Zn transport in bamboo therefore requires at least two different types of mechanisms: from rhizome to stems, mechanisms that imply no fractionation and from stems to leaves, mechanisms that require significant fractionation over short scales (<10 cm). At least, two mechanisms can account for the latter aspect: diffusion or transport (active or passive) through a transporter across the cell's membrane. The short scale transport of Zn from seed to leaves for the lentils and the transport from stems to leave for the bamboo could be modelled by Zn diffusion. Diffusion of Zn stable isotopes in an infinite, one dimension medium can be treated with the following equation:

$$\frac{\partial {}^{i}Zn}{\partial t} = {}^{i}D\frac{\partial^{2i}Zn}{\partial x^2} \quad (1)$$

where the superscript *i* is the mass of the isotope of interest, *t* is the time of diffusion in seconds, ${}^{i}D$ is the diffusion coefficient of the isotope of mass *i* and *x* is the space dimension. Eq. (1) can be solved for *x* and *t* using the error function complement (erfc) by:

$${}^{i}Zn(x,t) = \frac{{}^{i}Zn_0}{2}\,\mathrm{erfc}\frac{x}{\sqrt{4{}^{i}Dt}} \quad (2)$$

where ${}^{i}Zn(x,t)$ is the concentration of ${}^{i}Zn$ in position *x* at the time *t*, and ${}^{i}Zn_0$ is the Zn uniform initial concentration, i.e. Zn in seed for the lentils and Zn in the stalk for the bamboos.

Considering the $^{66}Zn/^{64}Zn$ stable isotope ratio, Eq. (2) can be written with the δ notation as follow:

$$\delta^{66}Zn = \left[\frac{\mathrm{erfc}\left(x/\sqrt{4^{66}Dt}\right)}{\mathrm{erfc}\left(x/\sqrt{4^{64}Dt}\right)} - 1\right] \times 10^3 \quad (3)$$

The value of ^{i}D (5.10^{-10} m^2s^{-1}) is estimated from previous works on Zn diffusion in aqueous solutions (Zhang and Davison, 1999; Rodushkin et al., 2004). Given that lentils and bamboos have grown up for 5 days and 14 days, respectively, and that the seed-to-shoot and stem-to-leaf distances for lentils and bamboos are about 10 cm, a simple calculation of optimisation of $\delta^{66}Zn$ with respect to $^{66}D/^{64}D$ leads to $^{64}D = 5.0001\times10^{-10}$ m^2s^{-1} for the lentils and between 5.0004×10^{-10} m^2s^{-1} and 5.0009×10^{-10} m^2s^{-1} for the bamboos and $^{66}D/^{64}D-1 = -2\times10^{-5}$ for the lentils and between -7.6×10^{-5} and -1.6×10^{-4} for the bamboos. These values are of the same order of magnitude as the results from Rodushkin et al. (2004) ($^{66}D/^{64}D-1 = -6\times10^{-5}$) for diffusion in aqueous solutions. Although simplistic, this model could explain a part of the Zn isotopic fractionation observed for both lentils and bamboos on a short scale (<10 cm). A chromatographic interpretation of the differential rate of transport of the different isotopes would clearly give similar results.

Isotopic fractionation of Zn during the transport through transporters across cells membrane is a second possible mechanism. Recently, John et al. (2007a) demonstrated that Zn isotopic fractionation occurred during the uptake of Zn though a diatom's cell membrane with light isotopes preferentially taken up by the cell. The magnitude of the isotopic fractionation is different in the case low-affinity transporters ($\Delta\delta^{66}Zn_{diatom\text{-}media} = -0.8‰$) and high affinity transporters ($\Delta\delta^{66}Zn_{diatom\text{-}media} = -0.2‰$). Therefore, the enrichments in Zn light isotope observed in leaves could represent the fractionation for trans-membrane low-affinity transport. We note that the isotopic fractionation observed in bamboo's leaves (−0.7 to −0.8‰) is in a surprisingly good agreement with the values reported by John et al. (2007a) for low-affinity transporters in diatom cells (−0.8‰).

Therefore either diffusion processes or cross-membrane transport can account for the observed isotopic fractionation of Zn during its transport in plant.

4.3. Influence of the growth medium

There is no difference between the Zn isotopic compositions of the lentils that have grown with a 18.2 MΩ water supply (Exp.A.1) and those which have grown with a supply of water with 1 ppm Zn enriched in heavy isotopes ($\delta^{66}Zn = 1.12‰$, Exp.A.2). While the isotopic composition of the Zn solution added to seeds in Exp A.3 was very close to that of the germinated seeds (1.2–1.3‰), the offsets are slightly larger than our error bars and we infer that growth medium has no effect on plant $\delta^{66}Zn$ during germination. Further studies conducted by adding an isotopically distinct Zn solution to the media are necessary to resolve the effect of the growth medium and to determine whether there is an uptake of Zn by the seed during the germination. We have not been able to measure the Zn isotopic composition of the soil associated with the bamboo at the time of its growth. Zn isotopic composition variations for plants growing in natural environment (Exp. B.) appear to be higher with a $\Delta\delta^{66}Zn_{stem\text{–}leaves}$ up to 1.04‰. An exhaustive study should be conducted to determine whether this phenomenon is due to the species, to the growth media, or to a contribution from airborne particles.

4.4. Effect of height

Viers et al. (2007) hypothesized a correlation between the $\delta^{66}Zn$ in leaves and the length of the plants with tree leaves having lower $\delta^{66}Zn$ than herbaceous leaves. Our results seem to confirm this hypothesis with bamboo leaves having $\delta^{66}Zn$ values lower that the ones measured in lentils. However, there is also an apparent effect of the height of leaves on the amplitude of isotopic fractionation, the highest leaves showing the highest enrichment in light Zn isotopes (Fig. 3). A study using marked ^{65}Zn on wheat plants showed that the concentration in ^{65}Zn increases during the expansion of leaves then decreased at its late stage of development to reach low values in the oldest leaves (Page and Feller, 2005). Translocation of Zn from root to shoots apparently occurs via the xylem (e.g. Salt et al., 1995). However, the redistribution of Zn from old to young leaves occurs via the phloem (Haslett et al., 2001; Page and Feller, 2005). For the bamboo, the oldest leaves are located in the lower part of the plant. If redistribution of Zn from old to young leaves occurs, a fractionation of Zn is expected with younger leaves showing the highest enrichment in light Zn isotopes. A similar enrichment with height should also be observed along the stem. Two interpretations could be given to our results. (1) The redistribution of Zn from old to young leaves does not occur in bamboos since we could not observe a fractionation between stem samples taken at two different heights. (2) There is a redistribution of Zn from old to young leaves, but the fractionation of Zn could not be observed within the stem because the fraction of Zn that is transported between leaves is transported via the phloem and the concentration of Zn in the phloem is much lower than the one in the xylem. A simple mass balance calculation shows that the fraction of Zn transported in the phloem should be lower than 5%. This calculation however does not take isotope fractionation of Zn upon transfer from the leaves to the main stem into account.

4.5. Implications for the Zn isotopic cycle

If the fractionation of Zn stable isotopes between seed and leaves for the lentils and between stem and leaves for the bamboos is an ubiquitous mechanism in higher plants, this could have important implications for the biological cycle of Zn. Zn isotopes have a potential to trace the origin of pollutants in the environment (Cloquet et al., 2006; Dolgopolova et al., 2006; Weiss et al., 2007; John et al., 2007b; Cloquet et al., 2008). While many mechanisms can affect the elementary abundance of Zn, igneous processes are inefficient at fractionating Zn isotopes on Earth (Ben Othman et al., 2006). Our results and the ones obtained by Weiss et al. (2005) and Viers et al. (2007) show that plants fractionate Zn isotopes and that these fractionations vary from one species to the other. Therefore, biological fractionation(s) needs to be considered in studies that attempt to trace zinc pollution by isotopic methods.

5. Conclusions

We have shown that the Zn isotopic composition is fractionated between germinated seeds and leaves of lentil and between stem and leaves of bamboos. Leaves are enriched in light Zn isotopes compared to the other parts of plants. The range of the fractionation is up to 0.52‰ per amu and is clearly mass dependant. Our results confirm the previous work of Weiss et al. (2005) and of Viers et al. (2007). We have shown that either diffusion processes or cross-membrane transport can account for the observed Zn isotopic fractionation. We have also demonstrated that Zn uptake from the growth media is unlikely for plants growing on reserves (lentil seeds). In addition, there is no fractionation from the rhizome to the stem in bamboos. Our study also shows a clear interspecies variability for Zn isotopic fractionation.

Finally, this study indicates that Zn isotopes could be used as a tracer for biological activities. Further research has to be conducted to elucidate controls on biological fractionation. In particular, plants are the primary producer in terrestrial vertebrate trophic chains. It would be very interesting to understand how the enrichment in light Zn isotopes is transmitted to higher trophic levels. Our results show that

there could be a change in the Zn biological cycle in relation to plant evolution, in particular with the appearance of deciduous plants at the Cretaceous. There is a need to explore other plant systems such as ferns (which do not possess true leaves) or conifers (which are not deciduous) to check whether similar isotopic fractionations was present in primitive plants such as ferns and cycads, which were widespread during the Mesozoic.

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References

Albarede, F., 2004. The stable isotope geochemistry of copper and zinc. Geochemistry Of Non-Traditional Stable Isotopes 55, 409–427.

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

Cakmak, I., et al., 1999. Zn defiancy stress responses in stimulating heavy-metal transport in plants. Plant Physiology 116, 175–188.

Cloquet, C., Carignan, J., Libourel, G., 2006. Isotopic composition of Zn and Pb atmospheric depositions in an urban/periurban area of northeastern France. Environmental Science & Technology 40, 6594–6600.

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. Analytical and Bioanalytical Chemistry 390, 451–463.

Dolgopolova, A., Weiss, D.J., Seltmann, R., Kober, B., Mason, T.F.D., Coles, B.J., Stanley, C., 2006. Use of isotope ratios to assess sources of Pb and Zn dispersed in the environment during mining and ore processing within the Orlovka–Spokoinoe mining site (Russia). Applied Geochemistry 21, 563–579.

Haslett, B.S., Reid, R.J., Rengel, Z., 2001. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. Annals of Botany 87, 379–386.

John, S.G., Geis, R., Saito, M., Boyle, E.A., 2007a. Zn isotope fractionation during high-affinity zinc transport by the marine diatom *Thalassiosira oceanica*. Limnology and Oceanography 52, 2710–2714.

John, S.G., Park, J.G., Zhan, Z.T., Boyle, E.A., 2007b. The isotopic composition of some common forms of anthropogenic zinc. Chemical Geology 245, 61–69.

John, S.G., Rouxel, O.J., Craddock, P.R., Engwall, A.M., Boyle, E.A., 2008. Zinc stable isotopes in seafloor hydrothermal vent fluids and chimneys. Earth and Planetary Science Letters 269, 17–28.

Johnson, C.M., Beard, B.L., Albarede, F., 2004. Geochemistry of non-traditional stable isotopes 55 Min.Soc.Am.

Luck, J.M., Othman, D.B., Albarede, F., 2005. Zn and Cu isotopic variations in chondrites and iron meteorites: early solar nebula reservoirs and parent-body processes. Geochimica et Cosmochimica Acta 69, 5351–5363.

Maréchal, C., Albarède, F., 2002. Ion-exchange fractionation of copper and zinc isotopes. Geochimica et Cosmochimica Acta 66, 1499–1509.

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

Mason, T.F.D., Weiss, D.J., Chapman, J.B., Wilkinson, J.J., Tessalina, S.G., Spiro, B., Horstwood, M.S.A., Spratt, J., Coles, B.J., 2005. Zn and Cu isotopic variability in the Alexandrinka volcanic-hosted massive sulphide (VHMS) ore deposit, Urals, Russia. Chemical Geology 221, 170–187.

Moynier, F., Albarede, F., Herzog, G., 2006. Isotopic composition of zinc, copper, and iron in lunar samples. Geochimica et Cosmochimica 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. Geochimica et Cosmochimica Acta 71, 4365–4379.

Page, V., Feller, U., 2005. Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves in young wheat plants. Annals of Botany 96, 425–434.

Pichat, S., Douchet, C., Albarede, F., 2003. Zinc isotope variations in deep-sea carbonates from the eastern equatorial Pacific over the last 175 ka. Earth and Planetary Science Letters 210, 167–178.

Rodushkin, I., Stenberg, A., Andren, H., Malinovsky, D., Baxter, D.C., 2004. Isotopic fractionation during diffusion of transition metal ions in solution. Analytical Chemistry 76, 2148–2151.

Salt, D.E., Prince, R.C., Pickering, I.J., Raskin, I., 1995. Mechanism of cadmium mobility and accumulation in Indian mustard. Plant Physiology 109, 1427–1433.

Sillanpaa, M., 1982. Micronutrients and the nutrient status of soils: a global study. FAO Soils Bulletin 48, 75–82.

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

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.

Weiss, D.J., Rausch, N., Mason, T.F.D., Coles, B.J., Wilkinson, J.J., Ukonmaanaho, L., Arnold, T., Nieminen, T.M., 2007. Atmospheric deposition and isotope biogeochemistry of zinc in ombrotrophic peat. Geochimica et Cosmochimica Acta 71, 3498–3517.

Welch, R., Webb, M., Loneragan, J., 1982. Zinc in membrane function and its role in P toxicity, Ninth Plant Nutrition Colloquium Commonwealth Agricultural Bureau. Warwick, pp. 710–715.

Wolnik, F., et al., 1993. Elements in major raw agricultural crops in the United States. 1. Cadmium and lead in lettuce, peanuts, potatoes, soybeans, sweet corn and wheat. Journal of Agricultural and Food Chemistry 31, 1240–1244.

Zhang, H., Davison, W., 1999. Diffusional characteristics of hydrogels used in DGT and DET techniques. Analytica Chimica Acta 398, 329–340.

Zhu, X.K., Guo, Y., O'Nions, R.K., Young, E.D., Ash, R.D., 2001. Iron isotope homogeneity in the early Solar nebula. Nature 412, 311–313.