Atomic Spectroscopy

Article

Zinc Isotopic Compositions of 23 Certified Biological Reference Materials (Plants, Animal Tissues, Foods) Measured by MC-ICP-MS

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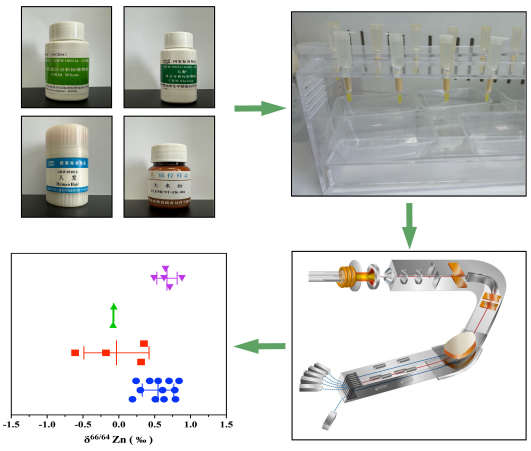
Received: *September 09, 2025;* ***Revised:*** *November 30, 2025;* ***Accepted:*** *December 01, 2025;* ***Available online:*** *December 01, 2025.*

DOI: 10.46770/AS.2025.151

ABSTRACT: Zinc (Zn) isotopes are increasingly applied in biogeochemistry, nutrition, and environmental sciences, but systematic data for biological reference materials remain scarce. In this study, we present high-precision Zn isotope measurements of a wide range of certified biological reference materials, including plants, animal tissues, human tissues, and processed food, using multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) combined with a one-column ion-exchange purification protocol. The analytical procedure yielded excellent repeatability, with long-term precision better than ± 0.07 ‰ (2SD) for $\delta^{66/64}$Zn. Isotopic analyses were performed on 23 certified biological reference materials, covering plants (*e.g.*, wheat, spinach leaves, ginseng), animal/human tissues (*e.g.*, scallop, pork liver, human hair), and processed foods (*e.g.*, milk powder, infant formula). The measured $\delta^{66/64}$Zn values span distinct ranges: plants (0.19‰ to 0.84 ‰), animal tissues (-0.61 ‰ to 0.35 ‰), human hair (-0.07 ‰ to -0.08 ‰), and food products (0.49 ‰ to 0.88 ‰). These results were consistent with previously reported data for selected standards (*e.g.*, BCR-2, BHVO-2, GBW10051), confirming method accuracy. Importantly, this study provides the first systematic dataset of $\delta^{66/64}$Zn values for 18 previously unreported biological reference materials, thereby establishing robust baselines for future research in Zn biogeochemical cycling, food authenticity, nutritional assessment, paleodiet and biomedical applications.



INTRODUCTION

Zinc (Zn), an essential trace element for humans as well as for plants and animals, is ubiquitous in living organisms and participates in numerous physiological processes[1]. It is the second most abundant transition metal in human body after iron and serves as a cofactor for hundreds of enzymes, playing key roles in immune function, protein and nucleic acid synthesis[2]. For example, Zn is involved in the activation of carbonic anhydrases, carboxypeptidases, and superoxide dismutase, contributes to membrane stability, and promotes the synthesis of proteins, carbohydrates, lipids, and nucleic acids[3]. Zn also supports normal growth and development (*e.g.*, in fetuses, infants, and adolescents) and sensory functions such as taste perception. Similarly, in plants, Zn plays essential structural and catalytic roles in growth, reproduction, and signal transduction, acting as a necessary cofactor for a wide range of enzymes such as carbonic anhydrases and ribosomal proteins[4].

Zn has five stable isotopes (^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, and ^{70}Zn) and

their fractionation occurs during biological and geochemical processes such as redox reactions and ligand coordination[5]. Biogeochemical studies have shown that heavier Zn isotopes are preferentially absorbed, while lighter isotopes tend to precipitate with phytic acid in plant tissues[6]. In plant, Zn isotopes have been applied to elucidate uptake pathways and internal allocation mechanisms. There are differences in isotope signatures between roots, stems, and leaves ($\delta^{66}Zn_{root} > \delta^{66}Zn_{stem} > \delta^{66}Zn_{leaf}$) [7, 8], enable the distinction between passive xylem transport, which generally enriches lighter isotopes, and active protein-mediated uptake, which preferentially incorporates heavier isotopes[9]. In animals, Zn isotopes serve as valuable tracers of dietary sources, tissue-specific metabolism, and ecological interactions. Systematic isotope differences among tissues, heavier isotopes in bone and serum versus lighter isotopes in hair and urine, reflecting variations in protein binding affinity and turnover rates[10]. At the ecosystem level, trophic-level trends in $\delta^{66}Zn$ values, with herbivores enriched in heavier isotopes relative to omnivores and carnivores, provide a robust basis for dietary reconstruction and paleoecological studies[11]. The dietary effects on Zn isotope composition in consumers were investigated using a combined controlled feeding experiment, which implies that diet is the primary factor controlling consumer tissue $\delta^{66/64}Zn$ values, independent of its specific composition[12]. The isotope systematics have highlighted the potential of Zn isotopes as tracers in food authentication, nutritional metabolism, and ecological reconstructions[13].

High-precision Zn isotope analyses are essential for resolving the subtle fractionations (0.1 ‰ ~ 3 ‰) that occur during migration, absorption, and metabolism within organisms[14-16]. Such precise measurements provide critical insights into Zn dynamics during digestion, circulation, and tissue allocation. Multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has become the preferred technique for Zn isotope ratio determination due to its high ionization efficiency, high sample throughput and high mass resolution[17,18]. Zn isotopic measurements using MC-ICP-MS are subject to instrumental isotopic fractionation, interferences, as well as contamination, which introduces a bias between the measured and true values. To obtain accurate Zn isotope ratios, effective chemical purification and rigorous correction of instrumental mass bias are required. Double-spike (DS) technique, elemental doping and sample-standard bracketing (SSB) were used to correct mass discrimination effect during the Zn isotopic measurements[19-21].

Reference materials (RMs) are indispensible to enable a reliable method validation. A sustainable RM ensures data quality, reproducibility, and comparability between studies[22, 23], these RMs cover much of the variability of $\delta^{66/64}Zn$, including geological, environmental and biological matrix. Previous studies reported some biochemical reference materials[24-33], however, these materials require further confirmation and some of which are no longer available, leaving a gap in baseline datasets for food- and tissue-based materials. It is of great importance to obtain readily available and stable Zn isotope values for biological reference materials, which are necessary to provide robust benchmarks for Zn tracing research. In this study, high-precision Zn isotope analyses of a suite of certified biological reference materials, including plants, animal tissues, and food products, using a one-column ion-exchange purification method coupled with MC-ICP-MS. The results establish robust baseline values for $\delta^{66/64}Zn$, providing critical reference data for future applications in food safety, nutritional studies, environmental monitoring, and biomedical research.

EXPERIMENTAL

Reagents and reference materials. AG MP-1M resins (100-200 mesh, Bio-Rad) were employed for Zn purification. Prior to sample loading, the resin underwent precleaning with ultrapure H_2O (18.2 MΩ·cm), followed by 6 mol L^{-1} HNO_3, in three alternating cycles. High-purity chemical reagents, such as HNO_3, HCl, and HF, were purchased at the BVIII grade from the Beijing Institute of Chemical Reagents (BICR). Subsequently, they were sub-boiled individually using the Savillex DST-1000 system. The Bio-Rad Poly-Prep columns, pipette tips and tubes were sequentially immersed in 10% HNO_3 (v/v) and ultrapure H_2O at a temperature of 90 °C for three times. All the PFA beakers were cleaned by purifying them in 6 mol L^{-1} HCl, 6 mol L^{-1} HNO_3 and then ultrapure water for three times.

Different types of biological reference materials were employed, including plant reference materials (GBW10011a, GBW10014, GBW10015, GBW10020, GBW10023, GBW10026, GBW10027, GBW10028, GBW07602, GBW07604 , and GBW07605), spirulina (GBW10025), animal tissue reference materials (GBW10018, GBW10024, GBW10050, and GBW10051), human tissue reference materials (GBW09101, GBW09101b), and processed food (GBW10017, CDIF-ELEMENT-ZK-004, GB2025-5050, GB2025-5051, and GBW10055). Two geological rock reference materials, BHVO-2 and BCR-2 were used to ensure the accuracy and precision of the measurements. The geological and biological reference materials used in this study were procured from Institute of Geophysical and Geochemical Research (IGGE), Shanghai Institute of Applied Physics (SINAP), the Academy of State Administration of Grain (ASAG), Beijing Weibiao Standard Materials Technology, and the United States Geological Survey (USGS). The investigation focuses on the intrinsic relationship of Zn isotope composition across these diverse sample types, most of which are being reported for the first time in this study. The foundational data of these reference materials is presented in Table 1.

Table 1. The information of biochemical reference materials

Sample name	Description	Zn (mg kg^{-1})
GBW10011a	Wheat	12.3
GBW10014	Cabbage	26.0
GBW10015	Spinach leaves	35.3
GBW10020	Citrus leaves	18.0
GBW10023	Laver	28.0
GBW10025	Spirulina	42.0
GBW10026	Pollen	32.0
GBW10027	Ginseng	11.1
GBW10028	Astragalus membranaceus	22.3
GBW07602	Shrub branches and leaves	20.6
GBW07604	Poplar leaves	37.0
GBW07605	Tea leaves	26.3
GBW10018	Chicken	26.0
GBW10024	Scallop	75.0
GBW10050	Prawns	76.0
GBW10051	Pork liver	211.0
GBW09101	Human Hair	191.0
GBW09101b	Human Hair	191.0
GBW10017	Milk powder	34.0
CDIF-ELEMENT-ZK-004	Rice meal	13.6
GB2025-5050	Infant formula milk powder	48.5
GB2025-5051	Rice meal	11.1
GBW10055	Whole soybean	36.3

Sample digestion and chromatographic purification. All reference materials were analyzed at the Institute of Geographic Sciences and Natural Resources Research, CAS. The reference materials utilized in this study encompass plant samples, animal tissues (including human hair), and other biological specimens. Comparing to geological samples, biological samples exhibit complex matrices. A mixture of HNO_3 and high-purity H_2O_2 was used to dismantle the residual organic matter structure, and its decomposition products are straightforward, exerting minimal impact on the reaction matrix. Besides, HF has been used in the treatment of plant[24, 29]. The experimental process is as follows. Different amounts of reference powder (30 ~ 500 mg) containing 5 μg Zn were weighed into vessels (50 mL), to which 3 mL of sub-boiled concentrated HNO_3, 1 mL of sub-boiled concentrated HCl, and 2 mL high-purity H_2O_2 were added. The samples were equilibrated for 12 hours at room temperature and then evaporated to dryness at 100 °C to remove organic matter. Before being placed in a microwave digestion instrument (Multiwave PRO, Anton Paar, AT), 6 mL of sub-boiled concentrated HNO_3, 2 mL of sub-boiled concentrated HCl and 2 mL of sub-boiled concentrated HF was then added to vessels. The instrument operated by incrementally increasing the temperature to 190°C and maintaining for 40 minutes. This process was repeated twice to ensure complete digestion. Afterward, the digestion solutions were evaporated at 160 °C until dry. The remaining dried residues underwent reflux with HCl twice to ensure solution homogeneity. Ultimately, the digested samples were redissolved in 7 mol L^{-1} HCl + 0.001% H_2O_2 prior to purification.

The separation protocol for Zn is adapted from Maréchal [16], described in Table S1. The process employed a 10 mL Bio-Rad Poly-Prep column packed with 1.6 mL of AG MP-1M resin, the resin was subsequently flushed with 7 mol L^{-1} HCl + 0.001% H_2O_2 (10mL) and ultrapure H_2O (10mL), then conditioned with 7 mol L^{-1} HCl + 0.001% H_2O_2 (7 mL). The sample, containing 5μg of Zn, was introduced to the column in 1 mL of 7 mol L^{-1} HCl + 0.001% H_2O_2. Following the elution of impurities such as Na, Mg, Ti, Al, Ni, K, Ca, Cu, and Fe using 7 mol L^{-1} HCl + 0.001% H_2O_2 (37 mL) and 2 mol L^{-1} HCl + 0.001% H_2O_2 (20 mL), Zn was collected using 0.5 mol L^{-1} HNO_3 (11 mL). Samples were evaporated to dryness and dissolved twice in concentrated HNO_3, then diluted to 500 μg L^{-1} Zn solutions in 2% HNO_3 (v/v) for isotopic analysis. Before instrumental analysis, a split sample aliquot was assessed to confirm Zn yield, which determined by comparing expected and observed Zn ion beam intensities.

Zn isotope measurements. The isotope ratios of Zn solutions were measured using a multiple-collector plasma source mass spectrometer (MC-ICP-MS, Nu Plasma II, Nu Instruments, UK). The introduction of sample solutions was facilitated via a pneumatic nebulizer, operating at an uptake rate of 0.2 mL min^{-1}. Wet plasma mode was used for the measurement of Zn isotopes, employing standard wet cones and a quartz nebulizer, the instrument parameters shown in Table S2. Four Zn isotopes ^{64}Zn, ^{66}Zn, ^{67}Zn, and ^{68}Zn were collected through L1, H3, H5, and H7, respectively. To ensure the reliability of the data, the introduction system underwent a thorough cleaning with 2% HNO_3 (v/v) for 70 seconds to remove potential cross-contamination, continuing until the ^{64}Zn signal fell below 3 mV. Generally, samples and measurement standards were diluted to 500 μg L^{-1} using 2% HNO_3 (v/v) before being introduced into the instrument. The overall procedural blanks varied between 10 to 12 ng, which is considered negligible in comparison to the approximately 5 μg of Zn in the samples loaded onto the columns. Background signals arising from potential memory effects and gas-related interferences in the sample introduction system were subtracted using on-peak-zero (OPZ) measurements of the 2% HNO_3.

The sample-standard bracketing (SSB) method were employed to address instrumental mass bias. To ensure repeatability, a minimum of three analyses of the same sample solution was performed. Zn isotope data are measured in standard delta-notation (‰) relative to the IRMM 3702 reference material [Eq. (1)]. ^{6x}Zn standards for ^{66}Zn and ^{68}Zn. The Zn isotope data are reported relative to an isotope standard using the δ notation relative to JMC-Lyon[34].

$$\delta^{x/64}\mathrm{Zn}(‰) = \left[\frac{(^{6x}\mathrm{Zn}/^{64}\mathrm{Zn})_{\mathrm{sample}}}{(^{6x}\mathrm{Zn}/^{64}\mathrm{Zn})_{\mathrm{standard}}} - 1\right] \times 1000 \qquad (1)$$

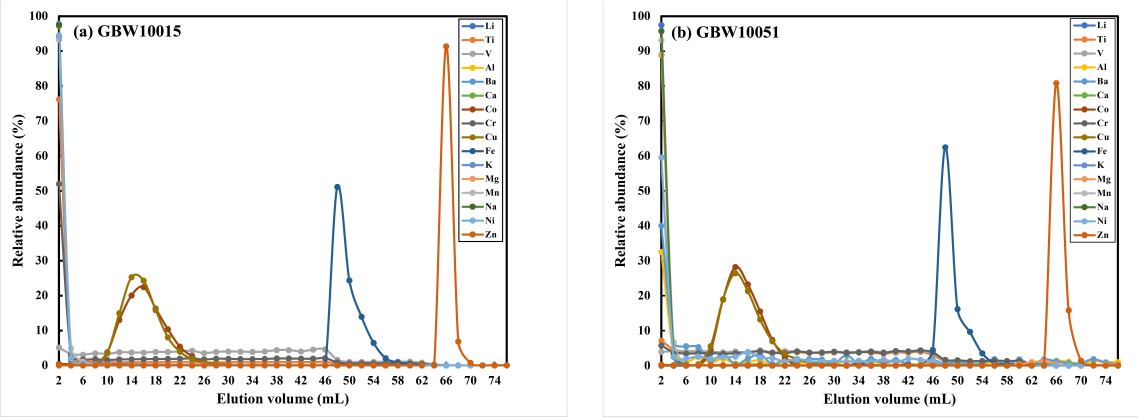


Fig. 1 Elution curves for (a) spinach leaves (GBW10015), (b) pork liver (GBW10051).

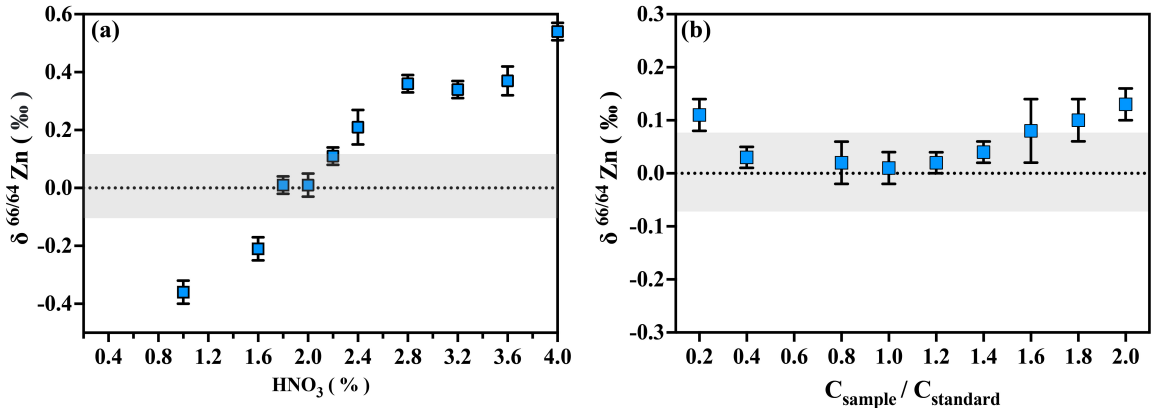


Fig. 2 The results of mismatching conditions of Zn isotopic measurement. The grey horizontal area represents the 2SD range of ± 0.07 ‰.

RESULTS AND DISCUSSION

Precision and accuracy of Zn isotope measurement. The reliability of the Zn purification procedure was evaluated using certified reference materials representing plant and animal matrices, specifically spinach leaves (GBW10015) and pork liver (GBW10051), as illustrated in Fig. 1. Matrix ions including Na, Mg, Ti, Al, K, Ca and Ni were eluted at the beginning of the elution curve, Cu and Fe eluted follow matrix ions. Zn was collected at 11 mL 0.5 mol L^{-1} HNO_3. The measurement of element mass fractions was conducted via ICP-OES and ICP-MS. The Zn recovery after separation was better than 95 %, indicating effective purification for both plant and animal tissues.

To assess potential biases in Zn isotopic measurements, the effects of acid molarity and sample concentration relative to the bracketing standard were examined. A high-purity Zn solution (Alfa -Zn), which is an in-house reference was diluted to 500 μg L^{-1} in 2% HNO_3 (v/v) for use as the bracketing measurement standard. Meanwhile, aliquots of the same tested material were diluted to 500 μg L^{-1} in nitric acid solutions ranging from 1.0% to 4.0% for use as unknown samples to evaluate the effect of acid molarity. The concentration of the same tested material from 100 to 1000 μg L^{-1} in 2% HNO_3 (v/v) also used as samples to evaluate the effect of the element mass fractions. As shown in Fig. 2(a), $\delta^{66/64}Zn$ is not caused by an acid concentration difference of 10% between samples and standard. When the acid concentration ratio between the sample and the standard exceeds 1.1 or falls below 0.9, the offset in $\delta^{66/64}Zn$ markedly deviates from the true value. Fig. 2(b) shows that Zn isotope measurements exhibit strong tolerance to mass fraction mismatch when the sample mass fraction exceeds that of the standard. No isotopic offset in $\delta^{66/64}Zn$ could be observed when the C_{sample} / $C_{standard}$ ratio were between 0.4 and 1.4, while ratios outside this range introduce measurable isotopic offsets. To ensure the precision and accuracy of Zn isotope determination, the Zn concentration mismatch between sample and standard solutions was kept at less than ± 10%, samples and standard were diluted in 2% v/v HNO_3 for the whole isotope measurement process.

Table 2. Zn isotope compositions of reported reference materials

Reference materials	Description	Zn (mg kg^{-1})	$\delta^{66/64}$Zn [a]	n	References
GBW10050 (N=2)	Prawns	76.0	0.31 ± 0.03	6	This Study
			0.28 ± 0.03		Guo *et al.* (2023)[24]
GBW10051 (N=2)	Pork liver	211.0	-0.61 ± 0.02	6	This Study
			-0.65 ± 0.02		Guo *et al.* (2023)[24]
GBW09101 (N=2)	Human hair	191.0	-0.07 ± 0.04	6	This Study
			-0.05 ± 0.06		Retzmann *et al.* (2024)[26]
GBW09101b (N=2)	Human hair	191.0	-0.08 ± 0.03	6	This Study
			-0.14 ± 0.02		Guo *et al.* (2023)[24]
GBW10055 (N=2)	Whole soybean	36.3	0.88 ± 0.01	6	This Study
			0.87 ± 0.03		Guo *et al.* (2023)[24]

a: $\delta^{66/64}$Zn values were converted to $\delta^{66/64}Zn_{JMC}$ values in accordance with standard protocol. For the conversion, a $\delta^{66/64}Zn_{IRMM\ 3702}$ values of -0.27 ‰ for JMC was used[34]. N is the number of independent digestions of the same sample. n is the repeated measurements of the same sample. The error represents the two times standard deviation of n repeated measurements of the reference materials.

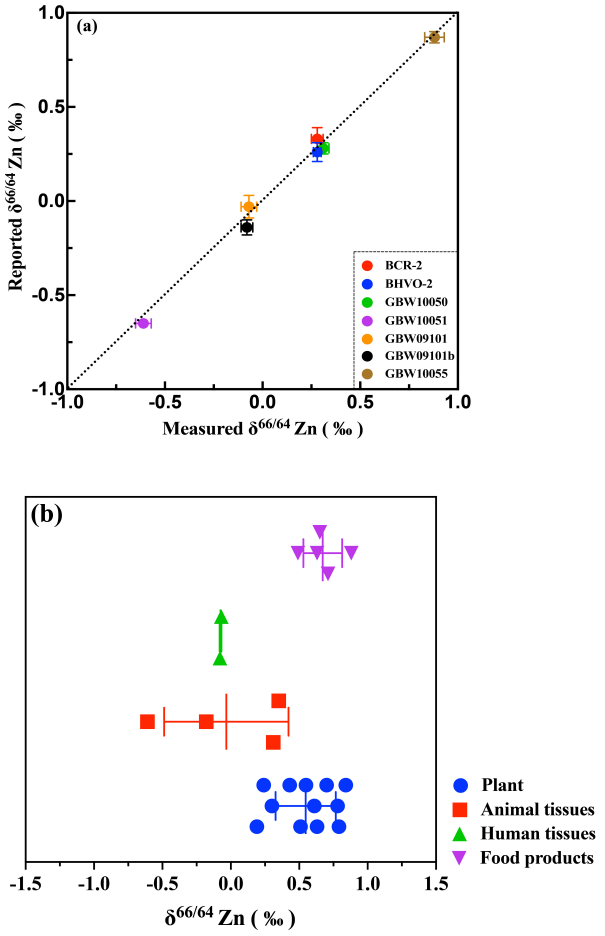


Fig. 3 (a) The mean $\delta^{66/64}$Zn values of well-documented reference materials measured in this study and reported values. (b) Zn three-isotope plot ($\delta^{68/64}$Zn vs $\delta^{66/64}$Zn) of certified biological reference materials analyzed in this study.

The presence of matrix elements can significantly affect the precision and accuracy of Zn isotopic ratio determinations. In this study, the effects of Ni, Ti, Mg, Al, Cu, and Fe on Zn isotope analysis were systematically evaluated. Notably, $^{64}Ni^{+}$ can generate strong isobaric interferences on $^{64}Zn^{+}$. Ti, Mg, and Al were specifically considered due to the formation of molecular ions that are isobaric with Zn isotopes, such as $^{48}Ti^{16}O+$ and $^{24}Mg^{40}Ar^{+}$ on $^{64}Zn^{+}$, and $^{50}Ti^{16}O^{+}$ on ^{66}Zn, $^{27}Al^{40}Ar^{+}$on $^{67}Zn^{+}$. In addition, the co-elution of Cu and Fe with Zn can also impact isotopic measurements. Significant deviations in $\delta^{66/64}$Zn were observed when the molar ratios exceeded the following thresholds: Ti/Zn > 0.01, Mg/Zn >0.1, Ni/Zn> 0.001, Cu/Zn >0.5, Fe/Zn > 1.0 and Al/Zn>0.5, meeting the limit requirements for interfering ions as stipulated in the literature[24], shown in Fig. S1.

Zn isotopic compositions in biological reference materials. The reliability of this study, particularly its precision and accuracy, has been rigorously evaluated through the replicated measurements of IRMM 3702, the average value of IRMM 3702 against itself was determined to be 0.00 ± 0.05 ‰ (2SD, n = 33) (Fig. S2). To ensure the accuracy and reliability of the data, multiple samples with known isotope ratios were subjected to the same procedures and tested simultaneously. Geochemical standard samples (BCR-2 and BHVO-2) and biochemical reference materials (GBW10050, GBW10051, GBW09101, GBW09101b, and GBW10055) were used as reference materials. Prior to conducting sample testing, measurements of BCR-2 and BHVO-2 were taken to verify the measurement accuracy, followed by the testing of additional samples, with results summarized in Fig. S3. The mean $\delta^{66/64}$Zn values of basalts BCR-2 and BHVO-2 were 0.28 ± 0.03 ‰ (2SD, n = 15) and 0.27 ± 0.04 ‰ (2SD, n = 18), respectively, which were consistent with the previously reported data[34, 35].

The $\delta^{66/64}$Zn values of biochemical materials GBW10050, GBW10051, GBW09101, GBW109101b and GBW10055 were 0.31 ± 0.03 ‰ (2SD, n = 6), -0.61 ± 0.02 ‰ (2SD, n = 6), -0.07 ± 0.04 ‰ (2SD, n = 6), -0.08 ± 0.03 ‰ (2SD, n = 6), and 0.88 ± 0.01 ‰ (2SD, n = 6), respectively. These values closely match the corresponding published data, confirming the reliability and consistency of the present measurements. The result is presented in Table 2 and Fig. 3(a), demonstrate the robustness of the analytical

Table 3. Zn isotope compositions in biological reference materials

Reference name	Description	Zn (mg kg^{-1})	$\delta^{66/64}$Zn	n
GBW10011a (N=2)	Wheat	12.3	0.51 ± 0.03	6
GBW10014 (N=2)	Cabbage	26	0.24 ± 0.02	6
GBW10015 (N=2)	Spinach leaves	35.3	0.78 ± 0.03	6
GBW10020 (N=2)	Citrus leaves	18	0.43 ± 0.06	6
GBW10023 (N=2)	Laver	28	0.63 ± 0.03	6
GBW10025 (N=2)	Spirulina	42	0.30 ± 0.02	6
GBW10026 (N=2)	Pollen	32	0.55 ± 0.04	6
GBW10027 (N=2)	Ginseng	11.1	0.84 ± 0.02	6
GBW10028 (N=2)	Astragalus membranaceus	22.3	0.61 ± 0.02	6
GBW07602 (N=2)	Shrub branches and leaves	20.6	0.70 ± 0.04	6
GBW07604 (N=2)	Poplar leaves	37	0.79 ± 0.06	6
GBW07605 (N=2)	Tea leaves	26.3	0.19 ± 0.02	6
GBW10018 (N=2)	Chicken	26	-0.18 ± 0.03	6
GBW10024 (N=2)	Scallop	75	0.35 ± 0.02	6
GBW10017 (N=2)	Milk powder	34	0.71 ± 0.04	6
CDIF-ELEMENT-ZK-004 (N=2)	Rice meal	13.6	0.65 ± 0.02	6
GB2025-5050 (N=2)	Infant formula milk powder	48.5	0.49 ± 0.03	6
GB2025-5051 (N=2)	Rice meal	11.1	0.63 ± 0.07	6

Note: N represents the number of independent digestions of the same sample; n represents the repeated measurements of the same sample; the error represents the two times standard deviation of n repeated measurements of the reference materials.

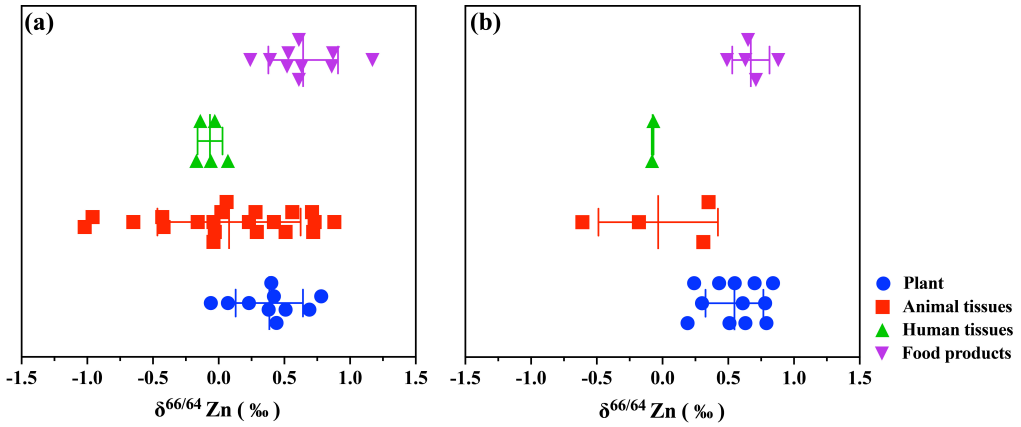


Fig. 4 (a) The $\delta^{66/64}$Zn values for biological reference materials reported in previous studies[9,10,24-33,37] (b) The $\delta^{66/64}$Zn values for biological reference materials reported in this study. The vertical lines in the figure represent the average values and relative deviations of samples of different categories.

method in generating precise and accurate isotopic data. The three-isotope plot of Zn for all biochemical reference materials is depicted in Fig. 3(b). The fractionation line for $\delta^{66/64}$Zn and $\delta^{68/64}$Zn (defined as y = 1.91x + 0.07, with an R^2 value of 0.995) displays a slope that aligns with the theoretical equilibrium mass-dependent fractionation[36, 37].

Zn isotope compositions of 18 biological reference materials, including 11 plant materials (GBW10011a, GBW10014, GBW10015, GBW10020, GBW10023, GBW10026, GBW10027, GBW10028, GBW07602, GBW07604, and GBW07605), Spirulina (GBW10025), 2 animal tissues materials (GBW10018 and GBW10024), and 4 processed food materials (GBW10017, CDIF-ELEMENT-ZK-004, GB2025-5050, and GB2025-5051) were first determined in this study, the result is shown in Table 3 (details in Table S3). In this study, the results reveals that the $\delta^{66/64}$Zn values in plant tissues (*e.g.*, Spinach leaves (GBW10015)) ranged from 0.19 ‰ to 0.84 ‰. Animal tissues (*e.g.*, chicken (GBW10018)), ranged from -0.61 ‰ to 0.35 ‰. The $\delta^{66/64}$Zn values in human hair are ranged from -0.07 ‰ to -0.08 ‰. The $\delta^{66/64}$Zn values in food products is ranging from 0.49 ‰ to 0.88 ‰. The $\delta^{66/64}$Zn values of prawns (0.31 ± 0.03 ‰), scallop (0.35 ± 0.02 ‰), as well as spirulina (0.30 ± 0.02 ‰) are significantly different from the Zn isotope composition of chicken (-0.18 ± 0.03 ‰) and pork liver (-0.61 ± 0.02 ‰). This may indicate that aquatic animals exhibit higher zinc isotope compositions compared to terrestrial animals.

Application of Zn isotope in biological samples. Zn stable isotopes provide a powerful tool for tracing absorption, transport, and redistribution processes in biological systems, thereby offering unique insights into nutrient cycling and metabolism.

Isotopic fractionation of Zn is governed by multiple factors, including coordination chemistry, transport pathways, protein binding specificity, and environmental conditions[34]. Fig. 4(a) shows reported $\delta^{66/64}$Zn values of reference materials in previous studies, details in Table S4. The $\delta^{66/64}$Zn values vary systematically among different biological materials. Plant tissues (*e.g.*, spinach leaves) exhibit relatively heavy values (0.07‰ to 0.78‰), except for the peach leaves, which give a $\delta^{66/64}$Zn value of -0.06‰. Animal tissues (*e.g.*, bovine muscle) fall within a broad range, display lighter signatures (-1.02‰ to 0.88‰). Human tissues, such as hair, fall within a narrower range (-0.17‰ to 0.07‰). Food products range from 0.24‰ to 1.17‰. In this study, the $\delta^{66/64}$Zn values exhibit a decreasing trend with increasing trophic level. these values fall within the ranges of Zn isotope variation reported for different trophic levels, as shown in Fig. 4(b). The mean of $\delta^{66/64}$Zn value of human tissue (-0.07‰) overlaps with the range observed in animal materials. Collectively, these results demonstrate a trophic-level trend in which $\delta^{66/64}$Zn values decrease from plants (0.19‰ to 0.84‰) to animals (-0.61‰ to 0.35‰).

Beyond trophic discrimination, variations in $\delta^{66/64}$Zn have also been linked to pathological states, suggesting potential applications as isotopic biomarkers in medical diagnostics[38-40]. For example, Zn isotope ratios have been used as a marker and analyzed in different types of cancer[41]. Indicating Zn isotope analysis in cancer would be in the early detection of certain tumors. However, Zn isotope data for biological reference materials remain limited, and some certified reference materials (*e.g.*, BCR-281) are no longer available. This highlights the necessity of characterizing alternative reference materials to ensure robust inter-laboratory comparability. The present results, together with literature data, confirm the application of Zn isotope in tracing the nutrient transfer across trophic levels, as well as reconstructing dietary habits of extant and extinct organisms. This study provided the $\delta^{66/64}$Zn values of different kinds of biological reference materials, aiming to facilitate further research on isotope fractionation within organisms, biological utilization across species, and related fields such as biomedicine.

CONCLUSION

In this study, we established a reliable method for Zn isotope analysis of biological reference materials using MC-ICP-MS following a one-column ion-exchange purification protocol. Rigorous evaluation of matrix effects, acid molarity, and concentration mismatches ensured measurement accuracy and repeatability, achieving a long-term precision of ± 0.07 ‰ (2SD). The results revealed clear isotopic distinctions among different biological groups, with heavier Zn isotopes generally enriched in plant materials and lighter values in animal tissues, consistent with known biological fractionation mechanisms. These new data significantly expand the available baseline database for certified biological reference materials, providing indispensable benchmarks for future Zn isotope applications in environmental geochemistry, nutritional metabolism, food safety, and disease-related biomarker studies.

ASSOCIATED CONTENT

The supporting information (Figs. S1−S3, Tables S1−S4) is available at https://www.at-spectrosc.com.

AUTHOR INFORMATION

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Notes

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ACKNOWLEDGMENTS

This research was financially supported by the National Natural Science Foundation of China (42203011). The authors also sincerely thank Dr. Xiaoqiang Li from Shanxi University, for his help with sample analysis.

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