

EFFECTS OF LONG-TERM STORAGE ON THE ISOTOPIC COMPOSITIONS OF  
DIFFERENT TYPES OF ENVIRONMENTAL WATERS

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Water input components such as fog, dew, and rain, are crucial for sustaining ecosystem functions, especially in water-limited regions. However, these water resources are subject to isotopic changes induced by storage due to their small sample volumes and inherent sensitivity to ambient atmospheric particulates. A comprehensive understanding of the long-term storage effect on isotopic compositions of these water inputs is essential for maintaining isotopic integrity throughout both field collection and laboratory analysis. In this study, the extent of such changes in the isotopic compositions ( $\delta^{2}\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$ ) of fog, rain, and dew was investigated under different storage times (4.5–9 years) and different sample bottle fill levels (4.8% to 92.4%) using the Los Gatos Research Inc. GLA431 series analyzer. The long-term storage could lead to a large variation in oxygen isotopes ( $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$ ) for the fog samples with minor effects on oxygen isotope measurements of dew and rain samples. In addition, no significant difference in  $\delta^{2}\text{H}$  values was observed between present and past measurements for three types of water samples. The isotopic changes of  $\delta^{18}\text{O}$  for fog waters were negatively correlated with the sample bottle fill level ( $p < 0.01$ ) but positively related to the storage time ( $p < 0.01$ ). We argue that chemical reactions between high concentrations of solutes and water molecules within fog samples may induce oxygen fractionation, leading to fog oxygen isotopes showing higher sensitivity to long-term storage compared to the  $\delta^{2}\text{H}$  values of dew and rain samples. Our findings could help understand the long-

term isotopic accuracy and precision of fog, dew, and rain waters by providing information on isotopic changes after long-term storage.

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## LIST OF ABBREVIATIONS

$\delta^2\text{H}$ ,  $\delta^{17}\text{O}$ ,  $\delta^{18}\text{O}$ : Represent the isotopic compositions of hydrogen and oxygen.

VSMOW: Vienna Standard Mean Ocean Water.

SLAP: Standard Light Antarctic Precipitation.

PET: Polyethylene Terephthalate (a type of plastic often used in bottles).

DI: Deionized (water)

## 1. Introduction

The stable isotopes of hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$ ) in water have become one of the most useful tools for quantifying critical ecohydrological fluxes such as evapotranspiration partitioning<sup>1,2</sup>, spatiotemporal origins of plant water sources<sup>3-5</sup>, and identification of precipitation/fog/dew origins<sup>6-8</sup>. Establishing global and long-term stable isotope datasets of the different water input components (e.g., fog, dew, and rain) for water-limited ecosystems is an urgent need for understanding and interpreting the spatiotemporal patterns of hydrological fluxes and the linkages between plant ecology and hydrological fluxes<sup>4,9-11</sup>. The storage of water samples in glass bottles for long-distance transport and isotopic analysis is a common operation method in isotope-based ecohydrological studies<sup>6,12</sup>. It is well established that the accuracy of trace element measurements in water samples can be significantly compromised by various factors, including storage conditions, potential contamination from aqueous solutions, and evaporation during storage. Previous studies have emphasized the importance of understanding the effects of long-term storage on the isotopic compositions of water samples, including filtered bottled water<sup>13</sup>, deionized tap water<sup>14</sup>, rain, and groundwater<sup>15</sup>. Fog and dew may be particularly susceptible to such storage-induced isotopic changes given their small water input, small sample volumes, and inherent sensitivity to ambient atmospheric particulates<sup>16,17</sup>. However, the difference of such long-term storage effect on the isotopic signatures in different water input components (fog, dew, and rain) has, as yet, been poorly studied. Understanding the effects of long-term storage on the isotopic composition of different water input components can offer valuable insights for preserving isotopic integrity during both field collection and laboratory analysis.

Evaporation of water in the water-air interface has been regarded as the most important factor for the isotope variations of water samples during long-term storage<sup>13,18</sup>. The stable isotope ratios of hydrogen ( $\delta^1\text{H}/\delta^2\text{H}$ ) and oxygen ( $\delta^{16}\text{O}/\delta^{17}\text{O}/\delta^{18}\text{O}$ ) are generally fractionated during evaporation, because heavier isotopes ( $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$ ) preferentially retain in residual water and lighter isotopes ( $\delta^1\text{H}$  and  $\delta^{16}\text{O}$ ) are easier to release into the atmosphere<sup>19</sup>. A rapid molecular exchange between liquid and vapor has been shown to expedite the evaporation-induced enrichment of isotopes in residual water samples<sup>18,20</sup>. Hence, the fill level in sample bottles during water storage serves as a crucial indicator of the molecular exchange rate between water and air within the storage container, fundamentally influencing isotopic changes in stored water samples. For instance, Rozanski, et al.<sup>18</sup> concluded that the mass loss of water samples through evaporation within the high-density polyethylene containers in the order of 1% will result in an increase in isotopic enrichment of ca. 1.1‰ and 0.35‰ for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , respectively. In addition, the solute content (e.g., organic components, heavy metals, ions) likely increases with the increasing fill levels of water samples. This could induce an increase in chemical reaction-derived isotope fractionation of the water sample, consequently leading to its isotopic variations<sup>21-25</sup>. Nevertheless, Terzer-Wassmuth, Wassenaar<sup>14</sup> found that the water samples stored in stainless-steel beverage kegs maintained original isotopic signatures over the 2-year experimental period regardless of different initial fill levels (100%, 70%, and 50%). Thus, the mechanism related to the fill level effects on isotope changes during water storage remains an open question.

In addition to the fill level of water samples, duration of storage time could also affect long-term storage impacts on the isotopic changes of water samples<sup>18,26</sup>. Williams,

Lartey, Sanders <sup>27</sup> recently assessed the isotopic integrity of water samples stored in automatic bottles during short-term storage (< 30 days), and they found the isotopic integrity would decrease with increasing storage time. In the meantime, a change of about +4.0‰ for  $\delta^2\text{H}$  and +0.7‰ for  $\delta^{18}\text{O}$  was measured after 253 days of storage in the PET plastic bottle<sup>28</sup>, while a change of +5.0‰ for  $\delta^2\text{H}$  and +2.0‰ for  $\delta^{18}\text{O}$  was observed for the water samples after more than one year of storage in plastic bottle<sup>13</sup>. However, few studies have focused on the long-term storage (> one year) effect on isotopic values of water samples within the glass bottle. It is an urgent need to investigate the effects of fill level and storage time on the isotopic compositions of water samples within glass bottles over a long-term period in the laboratory.

Recently, hydrogen and oxygen isotopes have been used to interpret and predict the availability and variability of water sources in the hyper-arid Namib Desert ecosystem<sup>29</sup>.  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  are regarded as effective tools for tracing different hydrometeorological processes related to rainfall and non-rainfall (i.e., fog, dew, and water vapor) water sources in water-limited ecosystems<sup>6,7,29</sup>. At the same time,  $\delta^{17}\text{O}$ , a new hydrological tracer, can inform additional interpretation for differentiations between different drought types (e.g., synoptic drought and local drought) and different types of condensation (e.g., fog and dew) in drylands<sup>6,8,30</sup>. Understanding long-term changes in water resources using isotopes requires the knowledge of accuracy and precision changes for hydrogen and oxygen isotopes in storage water samples. Therefore, it is necessary to investigate the effect of long-term storage on isotopic changes ( $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$ ) in different water input components (i.e., fog, dew, and rain) for the water-limited ecosystem.

In this study, the three types of water input components (i.e., fog, dew, and rain) collected in Namibia from 2014 to 2018 were analyzed soon after collection and then measured again in 2022 to investigate the effect of storage time (with a range of 4.5-9 years) and fill level of sample bottle (with a range of 14.8%-92.4%) on the isotopic ratio of  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$ . The objectives of this study were to: 1) examine the long-term storage effects on isotopic variations among different water components; 2) understand how and to what extent the storage time can affect stable isotopic compositions ( $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$ ) in water samples; and 3) investigate how and to what extent the fill level can affect stable isotopic compositions in water samples.

## **2. Materials and Methods**

### **2.1 Sample selection and preparation**

There was a total of 129 water samples used in this study, comprising 46 fog samples, 13 dew samples, and 70 rain samples. The water samples were collected between 2014 and 2018 in Namibia ( $23.56^{\circ}$  S,  $15.04^{\circ}$  E). All the isotopic compositions of water samples were promptly analyzed upon collection, which has been documented in previous studies<sup>6-8,31</sup>. All water samples were collected, isotopically analyzed, and stored in liquid water form, additional details related to the sample collection during 2014 and 2018 can be found in our previous studies mentioned above. In 2022, these water samples were re-classified according to the sample collecting date, sample collecting location, water fill level in the sample bottle, and water type (fog, dew, and rain). Wassenaar, et al.<sup>15</sup> and Spangenberg<sup>13</sup> have demonstrated that isotopic compositions of water samples stored in plastic bottles can be more easily affected by evaporation enrichment compared to those stored in glass bottles. In this regard, all water samples stored in plastic bottles were excluded from this study.

All water samples used in this study were stored in the standard Qorpak 15 ml glass bottles with an actual water volume capacity of 21 ml. In addition, all water samples were measured for the remaining fraction of water within the glass bottle (i.e., fill level) before conducting the present measurements in 2022. The storage time was calculated for each water sample using the time difference between the previous isotopic analysis time and the present isotopic analysis time. All water samples were filtered using a centrifuge and all particles present in water samples were removed based on visual inspection. After samples were centrifuged, they were transferred to Fisher brand screw thread autosampler glass

vials with 9 mm screw threaded autosampler caps for isotopic analysis. The glass vials were filled with 1 ml of the water sample.

## 2.2 Isotope analysis

The previous isotopic measurements of different water samples during 2014 and 2018 were described in detail in our previous studies<sup>6-8,31</sup>. The Los Gatos Research Inc GLA431 series analyzer (LGR, Los Gatos Research Inc., Mountain View, CA, United States) was used to measure isotopic values of water samples in 2022. The precision of the present measurements was 0.4‰ for  $\delta^2\text{H}$ , 0.15‰ for  $\delta^{18}\text{O}$ , and 0.09‰ for  $\delta^{17}\text{O}$ . LGR working standards 1C-4C were used to ensure the best performance of LGR instrument. The standard isotope values of LGR 1C were  $-154.0\text{‰}$  for  $\delta^2\text{H}$ ,  $-19.49\text{‰}$  for  $\delta^{18}\text{O}$ , and  $-10.30\text{‰}$  for  $\delta^{17}\text{O}$ ; LGR 2C were  $-123.7\text{‰}$  for  $\delta^2\text{H}$ ,  $-16.24\text{‰}$  for  $\delta^{18}\text{O}$ , and  $-8.56\text{‰}$  for  $\delta^{17}\text{O}$ ; LGR 3C were  $-97.3\text{‰}$  for  $\delta^2\text{H}$ ,  $-13.39\text{‰}$  for  $\delta^{18}\text{O}$ , and  $-7.06\text{‰}$  for  $\delta^{17}\text{O}$ ; LGR 4C were  $-51.6\text{‰}$  for  $\delta^2\text{H}$ ,  $-7.94\text{‰}$  for  $\delta^{18}\text{O}$ , and  $-4.17\text{‰}$  for  $\delta^{17}\text{O}$ <sup>12</sup>. One of the four LGR working standards were measured after every fourth sample measurement. In particular, at the beginning and end of each run, all four LGR working standards were measured. To further eliminate possible contamination between samples, DI water was used to flush out the instrument syringe after every eight water samples. The isotopic values of  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$  are expressed in  $\delta$ -notation and calculated relative to Vienna standard mean ocean water (VSMOW)-Standard Light Antarctic Precipitation (SLAP) scale.

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{VSMOW}}} - 1 \right) \quad (1)$$

where  $R_{\text{sample}}$  is the ratio of heavy and light isotopes in the sample and  $R_{\text{VSMOW}}$  is the ratio of heavy and light isotopes for the standard. The difference in isotopic composition for the same water sample was calculated by subtraction of past isotopic measurements from the present isotopic measurements.

### **2.3 Statistical analysis**

We used the one-way analysis of variance (ANOVA) to examine differences in isotopic changes (i.e.,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$ ) of different water components (i.e., fog, dew, and rain) between present and past measurements. The analysis of covariance (ANCOVA) was applied to establish relationships of isotopic changes of different water samples with different storage times and different water fill levels in sample bottles. All statistical analysis was conducted using Microsoft Excel (v2022). The significance level was set at  $\alpha < 0.05$ .

### 3. Results and Discussion

#### 3.1 The variations of hydrogen and oxygen isotopes of different water components

The stable hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$  and  $\delta^{17}\text{O}$ ) isotopic differences of fog, dew, and rain between the present (measured at the end of 2022) and past (measured in 4.5-9 years ago) measurements were summarized in Table 1 and Figure 2. There was a significant isotope depletion ( $-3.0 \pm 3.2\text{‰}$ ) in  $\delta^{17}\text{O}$  values of fog samples after 4.5-9 years of storage ( $p < 0.01$ ) (Table 1). No significant  $\delta^{17}\text{O}$  change for dew and rain samples were observed after 4.5-9-year storage ( $p > 0.05$ ). Compared to our observed full natural range of  $\delta^{17}\text{O}$  in the three types of water samples, the percentage of variations in  $\delta^{17}\text{O}$  between present and past measurements were 26.0%, 8.9%, and 2.8% for fog, dew, and rain, respectively. This also indicated that the long-term storage could lead to a large variation in  $\delta^{17}\text{O}$  for the fog samples with minor effects in dew and rain samples. At the same time, no significant difference in  $\delta^{18}\text{O}$  values was observed for all three types of water samples between present and past measurements ( $p > 0.05$ ) (Table 1, Figure 1 and Figure 2). The percentage of  $\delta^{18}\text{O}$  variations relative to the corresponding full natural range was 11.1%, 6.1%, and 1.5% for fog, dew, and rain, respectively. In regard to the full natural range of  $\delta^{18}\text{O}$  measurements, the impact of long-term storage on  $\delta^{18}\text{O}$  measurements is relatively larger for fog samples, in comparison to rain and dew samples in which the effect is negligible. There was no significant difference in  $\delta^2\text{H}$  values between present and past measurements for three types of water samples ( $p > 0.05$ ). Compared to the stable oxygen isotopes ( $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$ ) in water samples,  $\delta^2\text{H}$  values of all three types of water samples showed greater variations in both present and past measurements (Table 1 and Figure 2). Nevertheless, the long-term storage has less impact on the  $\delta^2\text{H}$  values than oxygen isotopic

compositions in the three types of water samples because of the lower percentages of  $\delta^2\text{H}$  variations compared to the  $\delta^2\text{H}$  natural range (with 7.2%, 7.0%, and 0.9% for fog, dew, and rain samples, respectively) (Table 1).

Water samples may undergo evaporation at different rates contingent upon the water-air exchange rates during water storage<sup>20</sup>. The different degree of the isotope fractionation during evaporation of three water components could be one of the most possible reasons for the substantial oxygen isotope changes after 4.5-9 years of storage observed in fog samples and negligible changes of dew and rain samples<sup>18,20</sup>. However, our results showed no significant difference in fill levels and storage time between these three types of water samples ( $p > 0.01$ ) (Table 1). This indicates that there is no significant difference in the molecule exchange between water and air within the storage container among three types of water components during the 4.5-9 years of storage. The substantial depletion in  $\delta^{17}\text{O}$  values of fog samples observed after 4.5-9 years of storage, on the other hand, contradicts the theory of heavy-isotope enrichment induced by the evaporation of water samples. Thus, such differences in oxygen isotope change values after long-term storage between fog and two other types of environmental waters (dew and rain) cannot be attributed to the oxygen isotope fractionation during the evaporation process.

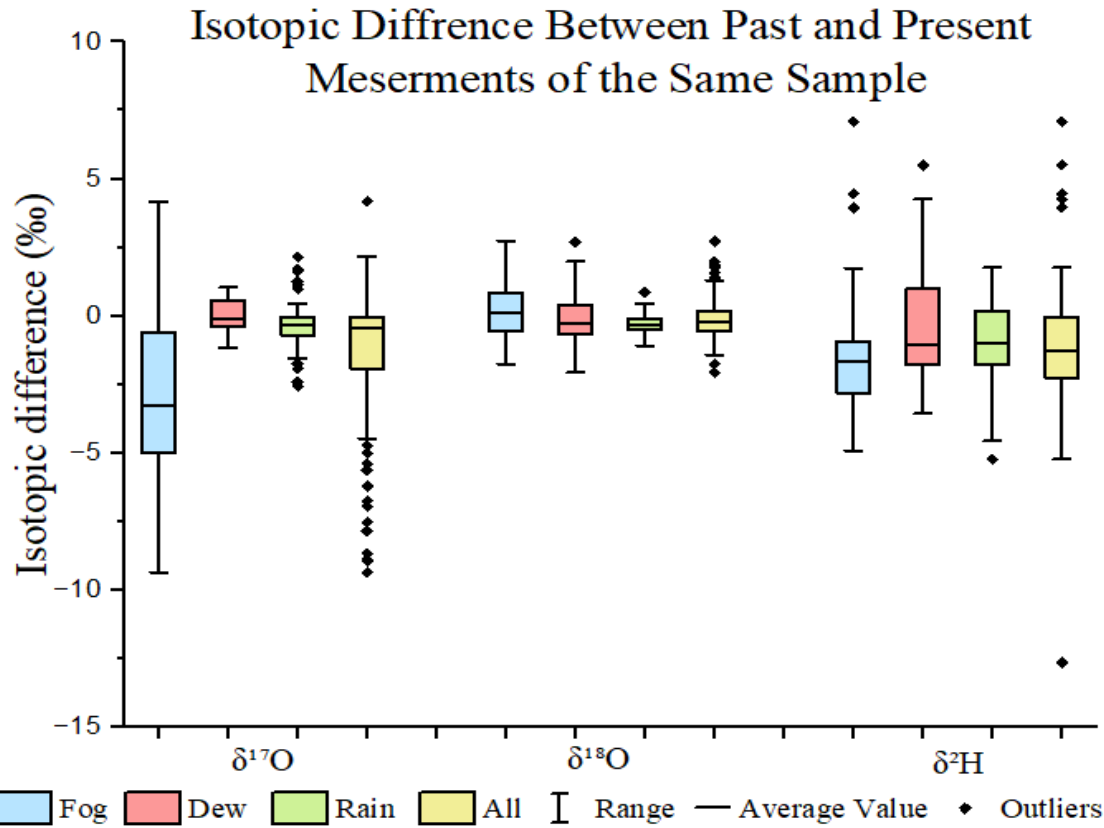
Recently, several studies showed that much higher concentrations of solutes (e.g., suspending mineral particles, dissolved ions) in fog samples were observed than those in dew and rain samples at identical sites<sup>16,17,32</sup>. The longer residence time in the atmosphere and much smaller droplet size (nearly 100 times smaller than dew drops) enable fog water a higher capacity for capturing particles<sup>16,17,32</sup>. In addition, fog could be subject to more intense exposure to near-surface aerosol particles (e.g., sea salt, mineral particles,

emissions) as a result of lower height of formation compared to rain samples. As shown in Photo 1, the precipitated mineral particles within fog water samples are much higher than those in dew and rain water samples in this study area. The higher solute concentrations in fog water are expected to induce the oxygen isotope fractionation by accelerating kinetic processes, facilitating isotopic exchange reactions, and promoting chemical diffusion between water and solutes<sup>12,30,33-35</sup>. Therefore, compared to the dew and rain samples, the larger oxygen isotope variations in fog samples after the long-term storage could be explained by the significant isotope fractionations during the isotope exchanges between chemical solutes and fog water<sup>21,36-38</sup>.

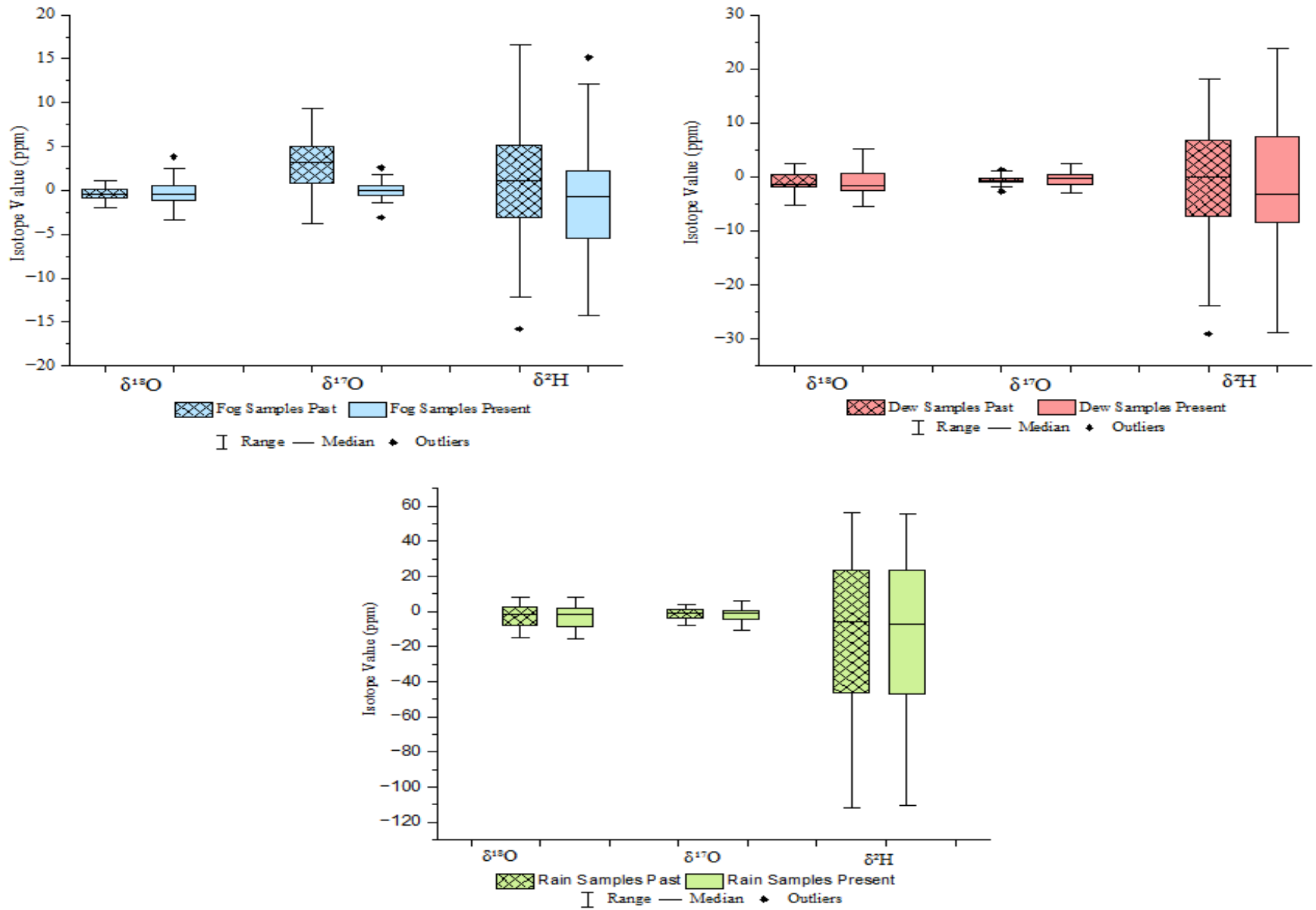
In addition to the difference in long-term storage effects on isotope changes between different environmental waters, long-term storage substantially affects oxygen isotope fractionation in fog with minor effects on hydrogen isotopes. This can be explained by two possible reasons with regard to the high concentration of chemical solutes within fog water. On the one hand, the aerosol particle samples collected in the Namib Desert have been documented to contain a high concentration of Na<sup>+</sup> (41.3 ppm), Ca<sup>2+</sup> (12.9 ppm), and Mg<sup>2+</sup> (4.9 ppm)<sup>39</sup>. Such cations within fog water samples could generate a more structured organization of water molecules and thus lead to the isotopic fractionation of oxygen isotopes<sup>40</sup>. While the isotope fractionation between cations and water molecules was not observed for hydrogen isotopes<sup>36</sup>. On the other hand, the kinetical oxygen isotope exchange between all mineral particles (e.g., carbonates, sulfides, silicas) and fog water during the dissolution and re-crystallization processes could lead to a substantial oxygen isotope fractionation of water samples<sup>21,23,41,42</sup>. In contrast, hydrogen isotopes are not involved in this isotope exchange process, either.

**Table 1.** Summary of Isotopic and Storage Characteristics of Fog, Dew, and Rain Samples  
The different types of water samples, number of water samples, average sample volume, average fill level, average storage time, full natural range of  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ , and  $\delta^{17}\text{O}$ , average isotopic difference between measurements, and magnitude of variations for  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ , and  $\delta^{17}\text{O}$ . The full natural range refers to the full range of the isotopic values observed for both the past and present measurements of each water type used in this study.

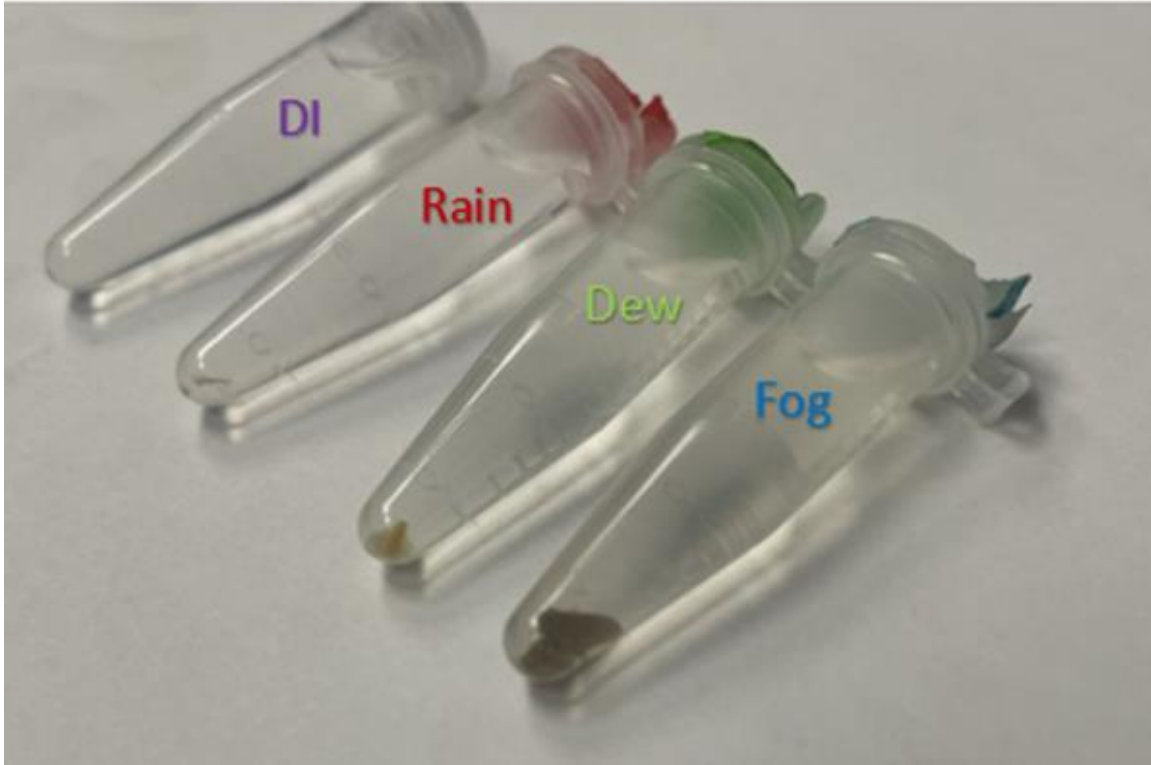
Different Types of Water Samples	Fog	Dew	Rain
Number of Water Samples	46	13	70
Sample Volume (mL)	$10.5 \pm 5.2$	$11.1 \pm 6.5$	$9.0 \pm 4.0$
Fill Level (%)	$50.1 \pm 24.5\%$	$53.0 \pm 30.7\%$	$42.9 \pm 18.8\%$
Storage Time (years)	$5.9 \pm 0.5$	$6.6 \pm 1.0$	$6.51 \pm 0.3$
Full Natural Range for $\delta^{18}\text{O}$ (‰)	$-3.4 - 3.9\text{‰}$	$-5.5 - 5.3\text{‰}$	$-15.5 - 8.0\text{‰}$
Full Natural Range for $\delta^{17}\text{O}$ (‰)	$-3.8 - 9.4\text{‰}$	$-3.1 - 2.4\text{‰}$	$-10.4 - 5.8\text{‰}$
Full Natural Range for $\delta^2\text{H}$ (‰)	$-15.8 - 16.6\text{‰}$	$-29.1 - 23.7\text{‰}$	$-111.7 - 56.0\text{‰}$
Average $\delta^{18}\text{O}$ difference between present and past measurements (‰)	$0.2 \pm 1.0\text{‰}$	$-0.3 \pm 0.8\text{‰}$	$-0.4 \pm 0.8\text{‰}$
Average $\delta^{17}\text{O}$ difference between present and past measurements (‰)	$-3.0 \pm 3.2\text{‰}$	$-0.1 \pm 0.6\text{‰}$	$-0.4 \pm 0.8\text{‰}$
Average $\delta^2\text{H}$ difference between present and past measurements (‰)	$-1.5 \pm 2.3\text{‰}$	$-0.6 \pm 3.0\text{‰}$	$-0.9 \pm 1.4\text{‰}$
Magnitude Percentage of $\delta^{18}\text{O}$ Variations (%)	11.1%	6.1%	1.5%
Magnitude Percentage of $\delta^{17}\text{O}$ Variations (%)	26.0%	8.9%	2.8%
Magnitude Percentage of $\delta^2\text{H}$ Variations (%)	7.2%	7.0%	0.9%



**Figure 1.** The isotopic differences in  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ,  $\delta^{17}\text{O}$  between present (measured at the end of 2022) and past (measured 4.5-9 years ago) measurements for fog, dew, and rain samples.



**Figure 2.** The present and past isotope measurements of  $\delta^{18}\text{O}$ ,  $\delta^{17}\text{O}$ ,  $\delta^2\text{H}$  for fog samples (a), dew samples (b), and rain samples (c)



**Picture 1.** Visual particles observed in deionized water (DI) as well as in water samples collected from rain, fog, and dew in this study. This photo was taken after the water samples were centrifuged.

### 3.2 Storage time effects on isotopic compositions of water samples

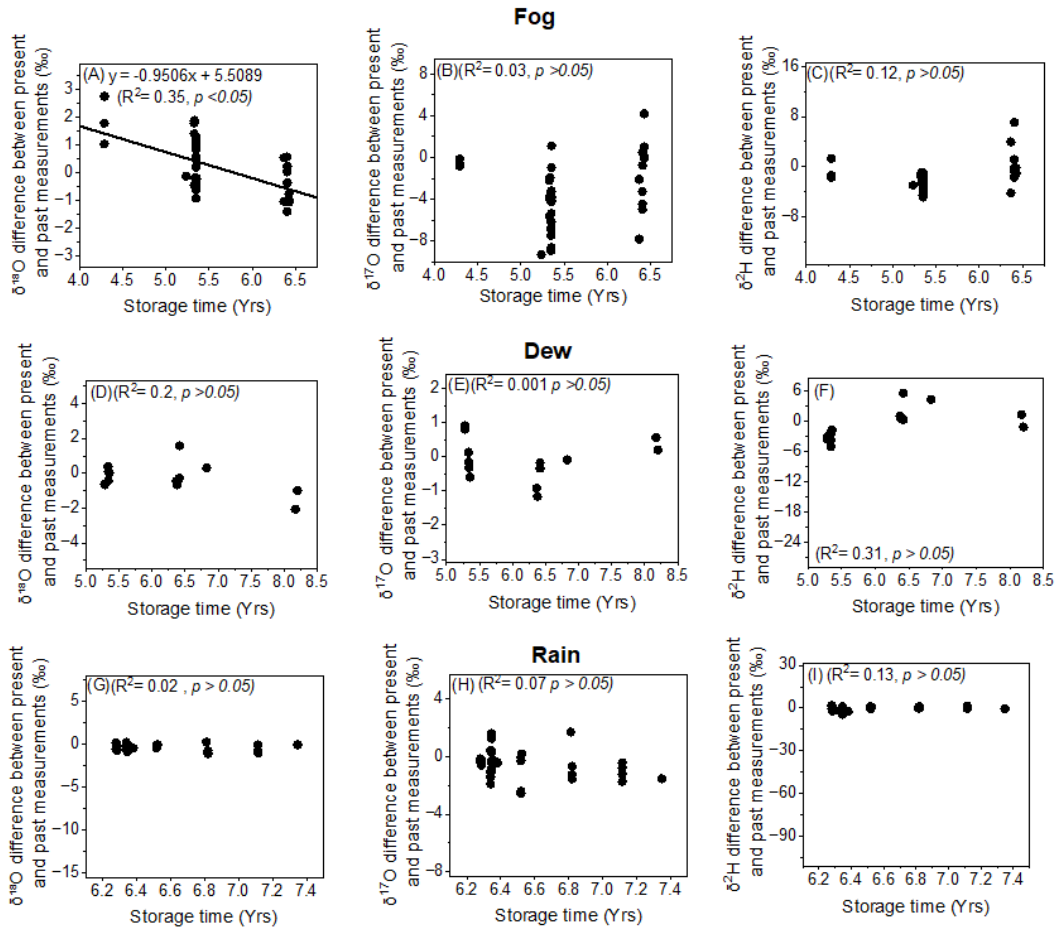
To investigate the effects of storage time on the isotopic composition of various water samples, we established relationships of isotope changes between present and past measurements with storage time for each water type. There was a statistically significant negative relationship between  $\delta^{18}\text{O}$  changes and storage time ( $R^2 = 0.36$ ,  $p < 0.01$ ) (Figure 3A). Moreover, the  $\delta^{18}\text{O}$  variations between present and past measurements for fog samples gradually shifted from positive values to negative values with the increase in storage time (Figure 3A). This shift resulted in no significant difference in fog  $\delta^{18}\text{O}$  values was observed between present and past measurements ( $p > 0.05$ ). In contrast, no significant relationship between the  $\delta^{18}\text{O}$  variations and storage time was observed for both dew or rain samples ( $p > 0.05$ ) (Figure 3D and 3G). In despite of substantial  $\delta^{17}\text{O}$  variations after long-term

storage reported in Table 1 and Figure 2, there was no significant relationship between storage time and  $\delta^{17}\text{O}$  changes for fog ( $p > 0.05$ ), dew ( $p > 0.05$ ), or rain samples ( $p > 0.05$ ) (Figure 3B, 3E, and 3H). No significant relationship was found between storage time and  $\delta^2\text{H}$  changes for fog ( $p > 0.05$ ), dew ( $p > 0.05$ ), and rain samples ( $p > 0.05$ ) (Figure 3C, 3F, and 3I).

Our results suggest that while the substantial  $\delta^{18}\text{O}$  enrichment was initially observed in fog water samples, such enrichment trend was counteracted by  $\delta^{18}\text{O}$  depletion effects as storage time increased (Figure 3A). Consequently, the overall  $\delta^{18}\text{O}$  changes in fog water samples storage for 4.5-9 years exhibited no statistically significant difference from zero (Table 1 and Figure 2). The isotope fractionation during the evaporation process is expected to be more apparent for hydrogen than oxygen, as the substitution of  $^2\text{H}$  for  $\delta^1\text{H}$  often leads to a more significant difference in vibrational energy between the isotope species compared to the substitution of  $^{18}\text{O}$  for  $\delta^{16}\text{O}$ <sup>43</sup>. Thus, in this study, the initial  $\delta^{18}\text{O}$  enrichment effects of fog samples cannot be attributed to the evaporation-induced isotope fractionation due to the absence of observed  $\delta^2\text{H}$  enrichment in the same fog samples (Figure 3C).

Given that such a shift from  $\delta^{18}\text{O}$  enrichment to  $\delta^{18}\text{O}$  depletion effects over time is exclusive to fog samples, we speculate that it could be linked to the different chemical reaction-induced isotope exchanges between solutes and fog water. The energy required to disassociate individual molecules from water aggregates is less for  $^{16}\text{O}$  bonds than for  $^{18}\text{O}$  bonds of fog water<sup>44</sup>. Following dehydration from fog samples, the lighter isotope of oxygen (i.e.,  $^{16}\text{O}$ ) tends to preferentially exchange with dissolved mineral species such as carbonates, sulfates, silicates, among others, ultimately leading to crystallization<sup>21,23,41,42</sup>.

This kinetics of oxygen exchange between dissolved mineral species and fog water prior to and during their crystallization can lead to heavier  $\delta^{18}\text{O}$  enrichment in the residual fog water. In addition, the isotope exchange between cations (i.e.,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ ) and fog water could also enrich  $\delta^{18}\text{O}$  in the fog water samples<sup>36</sup>. In the meantime, the lighter  $^{16}\text{O}$  of fine and tiny crystals suspending or precipitating in fog samples prefer to be dissolved into solutions. Such crystal dissolution process would result in the depletion of heavier  $^{18}\text{O}$  in fog water. Furthermore, the crystal dissolution-induced oxygen depletion becomes much stronger compared to oxygen enrichment effects as storage time increased, which can be derived from the shift from  $^{18}\text{O}$  enrichment to  $^{18}\text{O}$  depletion effects of fog samples over time (Figure 4A). However, the precise underlying mechanisms responsible for this phenomenon require further research.



**Figure 3.** Relationships between storage time and isotopic changes between present and past measurements for fog, dew, and rain samples. The Y-axis range in each panel is adjusted to accommodate the full natural range of isotopic values specific to each isotope for fog, dew, and rain samples.

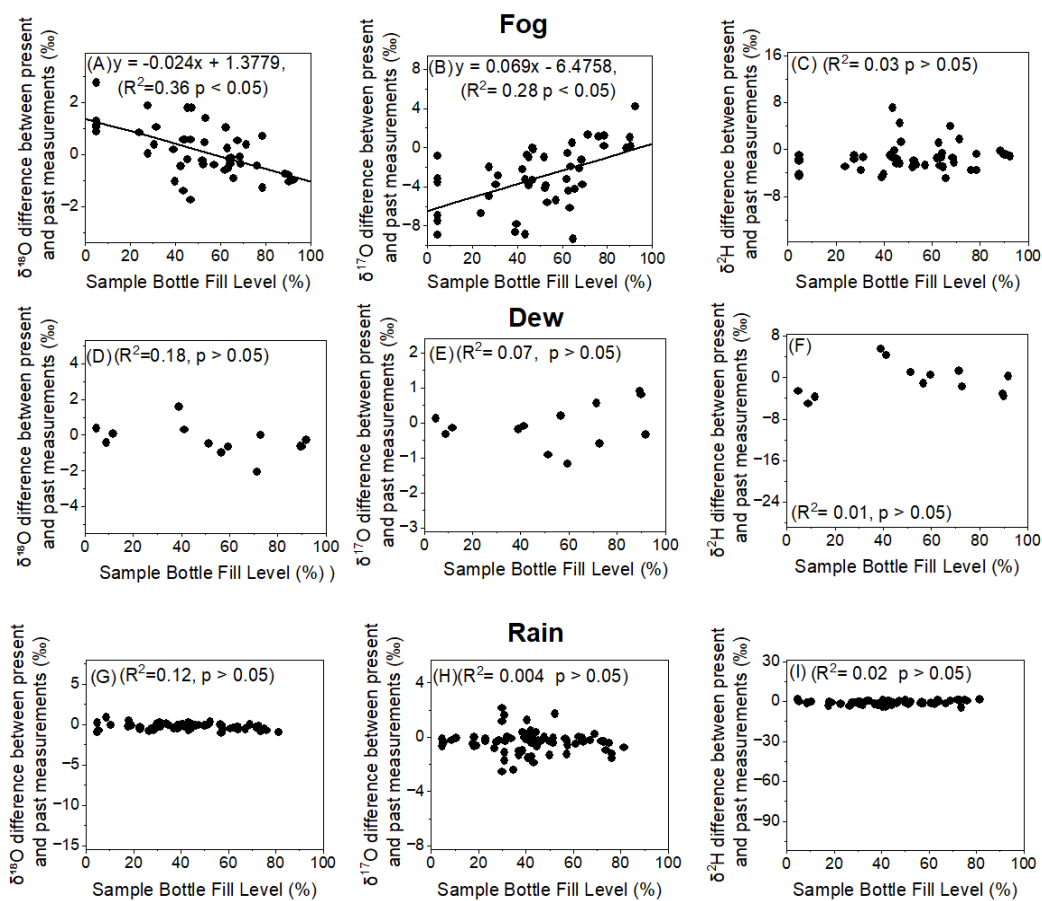
### 3.3 Storage fill level effects on isotopic compositions of water samples

We analyzed relationships between isotopic changes and sample bottle fill level after 4.5-9 years of storage. There were statistically significant negative relationships between the sample bottle fill level and  $\delta^{18}\text{O}$  changes for fog samples ( $R^2 = 0.36$ ,  $p < 0.01$ ) (Figure 4A). The  $\delta^{18}\text{O}$  variations between present and past measurements for fog samples gradually shifted from positive values to negative values with the increase in bottle fill level (Figure 4A). No significant relationship between the  $\delta^{18}\text{O}$  variations and storage bottle fill level was found for dew and rain samples ( $p > 0.05$ ) (Figure 4D and 4G). In

addition, the dew and rain samples with various fill levels located to the line of “ $y = 0$ ”, further indicating that when considering rain and dew samples, fill level has a negligible effect on  $\delta^{18}\text{O}$  changes between present and past measurements. A statistically significant positive relationship between the sample bottle fill level and the observed differences of  $\delta^{17}\text{O}$  after 4.5-9-year storage was found for fog samples ( $R^2 = 0.28$ ,  $p < 0.01$ ) (Figure 4B). As the fill level in the bottles increased, the  $\delta^{17}\text{O}$  variations of fog samples between present and past measurements decreased (Figure 4E). This implies that the increasing fill level in the water bottles could reduce  $\delta^{17}\text{O}$  changes. No significant difference between the sample bottle fill level and the observed difference in  $\delta^{17}\text{O}$  was found for dew or rain samples ( $p > 0.05$ ) (Figure 4E and 4H).

Our results show that  $\delta^{18}\text{O}$  changes of fog samples after long-term storage gradually shifted from positive values to negative values with the increasing fill level, while  $\delta^{17}\text{O}$  changes in fog samples exhibited a contrary increase and became closer to zero with the increasing storage fill level (Figure 4A and 4B). The positive linear relationship between  $\delta^{17}\text{O}$  changes and fill level can be attributed to the enhanced preservation of the original isotopic composition, which increases with higher sample fill levels<sup>20</sup>. This is because the increasing fill level could decrease the molar ratio of substances that can interact with the water sample, resulting in a decreasing variation of the isotopic composition of the water sample during long-term storage<sup>13,20</sup>. In contrast, regarding the negative correlation observed between  $\delta^{18}\text{O}$  changes after long-term storage and fill level, we speculate that the  $\delta^{18}\text{O}$  values of fog water samples show an increasing sensitivity to the crystal dissolution-induced oxygen fractionation but a decreasing sensitivity to the crystallization-derived oxygen fractionation as the volume of fog samples increases.

Further, there were no significant relationships between  $\delta^2\text{H}$  changes after long-term storage and sample bottle fill level for all three water types (Figure 4C, 4F, and 4I), indicating a minor effect of sample fill level storage on  $\delta^2\text{H}$  changes for all three water types. This result was consistent with our previous result demonstrating that in terms of natural isotopic variations, the changes in  $\delta^2\text{H}$  observed after long-term storage were relatively smaller than changes in oxygen. This could be explained that oxygen isotopes could be more sensitive to physicochemical factors<sup>45,46</sup>.



**Figure 4.** Relationships between the fill level of the sample bottle and isotopic differences between present and past measurements for fog, dew, and rain samples. The Y-axis range in each panel is adjusted to accommodate the full natural range of isotopic values specific to each isotope for fog, dew, and rain samples.

### 3.4 Implications and further directions

In this study, we demonstrated that chemical reactions between solutes or suspending particles and water significantly influenced isotopic changes in fog water samples stored in standard, well-sealed glass bottles, whereas evaporation had no detectable effect. In contrast, dew and rain water samples stored under similar standard conditions maintained their isotopic integrity over long-term storage, remaining unaffected by isotopic fractionation induced by either evaporation or chemical reactions. These

findings suggest that environmental waters in hyper-arid deserts, which are highly susceptible to isotopic fractionation due to their small sample volumes and sensitivity to ambient atmospheric particulates, can retain their isotopic integrity over long-term storage when properly filtered and preserved using standard methods. Thus, it is feasible to collect environmental water samples under standard storage conditions from other research groups and subsequently reanalyze their isotopic compositions to develop stable isotope datasets of environmental waters with high spatial and temporal resolution, particularly for water-limited ecosystems. This can help to understand and interpret the spatiotemporal patterns of hydrological fluxes and the linkages between plant ecology and hydrological fluxes in the context of global climate change.

Further investigations should also examine the long-term storage effects on isotopic changes in fog water samples collected from areas around megacities, as the study sites in this research are located in the central Namib Desert, an area largely unaffected by human activity. Ecological and environmental studies based on fog waters in urbanized areas can offer critical insights into the impacts of urban development on hydrological cycles and environmental quality. Additionally, recent observations revealed significant differences in  $\delta^2\text{H}$  values between plants and source waters, but not in  $\delta^{18}\text{O}$  values<sup>5,47,48</sup>. These changes have been attributed to either hydrogen isotope fractionation during root water uptake and subsequent water transport from roots to leaves or methodological artifacts during the cryogenic vacuum extraction water extraction process<sup>5,47,48</sup>. In contrast, the chemical reactions and evaporation over long-term storage showed less impacts on  $\delta^2\text{H}$  values in three types of environmental water, but a significant influence on  $\delta^{18}\text{O}$  values, particularly

for fog water. Further investigations should continue to focus on the distinct fractionation mechanisms of hydrogen and oxygen isotopes in isotope-based ecohydrological studies.

#### 4. Summary

In this study, we investigated the effect of the storage time (with a range of 4.5-9 years) and fill level in sample bottle (with a range of 4.8%-92.4%) on the isotopic ratio of  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{17}\text{O}$  in three types of environmental water samples (fog, dew, and rain). The long-term storage had less impact on the  $\delta^2\text{H}$  values in the three types of water samples, while it led to a large variation in oxygen isotopes for the fog samples with minor effects on oxygen isotope measurements of dew and rain samples. The notable isotopic changes observed after 4.5-9 years of storage cannot be attributed to evaporation-induced isotope fractionation. We expect that the higher concentrations of solutes in fog samples could result in higher isotopic exchanges and more active chemical diffusions within water samples, consequently causing oxygen isotope fractionation. In addition, such chemical reaction-induced isotope exchange between solutes and fog water could result in an increasing depletion in  $\delta^{18}\text{O}$  of fog water with the increasing storage time as well as fill level of the sample bottle. Therefore, it seems that long-term storage should not be recommended in oxygen isotopic analysis for fog samples, if no initial filtering or distillation was conducted. Because the higher concentration of solutes in fog water may result in substantial oxygen isotope fractionation with a large oxygen isotope variation during fog water storage. This study enhances our understanding of the long-term storage effect on isotopic variations in different types of water samples. It provides critical insights on how to establish comparable global and long-term stable isotope datasets of water samples, providing a foundation for reliable interpretations of isotope-based ecohydrological studies.

## References

- Bötcher ME. Stable Isotope Fractionation during Experimental Formation of Norsethite (BaMg[CO<sub>3</sub>]<sub>2</sub>): A Mineral Analogue of Dolomite. *Aquatic Geochemistry*. 2000;6:201-212.
- Cernusak LA, Barbeta A, Bush RT, et al. Do <sup>2</sup>H and <sup>18</sup>O in leaf water reflect environmental drivers differently? *New Phytologist*. 2022.
- Chacko T, Cole DR, Horita J. Equilibrium Oxygen, Hydrogen and Carbon Isotope Fractionation Factors Applicable to Geologic Systems *Reviews in Mineralogy and Geochemistry*. 2001;43(1).
- Craig H, Gordon LI, Horibe Y. Isotopic exchange effects in the evaporation of water: 1. Low-temperature experimental results. *Journal of Geophysical Research*.
- Dijk JV, Fernandez A, Müller IA, Lever M, Barnasconi SM. Oxygen isotope fractionation in the siderite-water system between 8.5 and 62 °C. *Geochim Cosmochim Acta*. 2018;220:535-551.
- Eckardt FD, Schemenauer RS. Fog water chemistry in the Namib desert, Namibia. *Atmospheric Environment*. 1998;32(14-15).
- Ellsworth P, Williams D. Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant and Soil*. 2007;291(1):93-107.
- Evaristo J, Jasechko S, McDonnell JJ. Global separation of plant transpiration from groundwater and streamflow. *Nature*. 2015;525(7567):91-107.
- Evaristo J, McDonnell JJ. Prevalence and magnitude of groundwater use by vegetation: a global stable isotope meta-analysis. *Scientific Reports*. 2017;7.
- Gat JR. Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences*. 1996;24(1):225-262.
- Geldern RV, Barth JAC. Optimization of instrument setup and post-run corrections for oxygen and hydrogen stable isotope measurements of water by isotope ratio infrared spectroscopy (IRIS). *Limnology and Oceanography: Methods*. 2012;10(12):1024-1036.
- Greule M, Rossmann A, Schmidt H, Amosandl A, Keppler F. A Stable Isotope Approach to Assessing Water Loss in Fruits and Vegetables during Storage. *Journal of Agricultural and Food Chemistry*. 2015.

- Hu G, Clayton RN. Oxygen isotope salt effects at high pressure and high temperature and the calibration of oxygen isotope geothermometers. *Geochimica et Cosmochimica Acta*. 2003;67(17):3227-3246.
- Jasechko S, Sharp ZD, Gibson JJ, Birks SJ, Yi Y, Fawcett PJ. Terrestrial water fluxes dominated by transpiration. *Nature*. 2013;496(7445):347-352.
- Kaseke KF, Tian C, Wang LX, et al. Fog spatial distributions over the central Namib Desert - An isotope approach. *Aerosol and Air Quality Research*. 2018;18(1):49-61.
- Kaseke KF, Wang L, Wanke H, Tian C, Lanning M, Jiao W. Precipitation origins and key drivers of precipitation isotope ( $^{18}\text{O}$ ,  $^2\text{H}$ , and  $^{17}\text{O}$ ) compositions over Windhoek. *Journal of Geophysical Research: Atmospheres*. 2018;123(14):7311-7330.
- Kaseke KF, Wang LX, Seely MK. Nonrainfall water origins and formation mechanisms. *Science Advances*. 2017;3(3).
- Kaseke KF, Wang LX. Reconciling the isotope-based fog classification with meteorological conditions of different fog types. *Journal of Hydrology*. 2022;605:10.
- Khoury D, Millet M, Jabali Y, Delhomme O. Fog Water: A General Review of Its Physical and Chemical Aspects. *Environments*. 2023;10(12).
- Kim H, Collier S, Ge X, et al. Chemical processing of water-soluble species and formation of secondary organic aerosol in fogs. *Atmospheric Environment*. 2019;200:158-166.
- Klemm O, Schemenauer RS, Lummerich A, et al. Fog as a fresh-water resource: overview and perspectives. *Ambio*. 2012;41(3):221-234.
- Landais A, Barkan E, Luz B. Record of  $\delta^{18}\text{O}$  and  $^{17}\text{O}$ -excess in ice from Vostok Antarctica during the last 150,000 years. *Geophysical Research Letters*. 2008;35(2).
- Li Y, Ma Y, Song X, Wang L, Han D. A  $\delta^2\text{H}$  offset correction method for quantifying root water uptake of riparian trees - R Discovery. *Journal of Hydrology* 2021.
- Li Y, Song X, Wang L, Sprenger M, Ma Y. Quantitative contribution of cryogenic vacuum extraction and radial water transport to xylem-source water deuterium offset. *Agricultural and Forest Meteorology*. 2024;345.
- Liebermann O. Synthesis of Dolomite. *Nature*. 1967.

- Matthews A, Amitai K. Oxygen isotope fractionation during the dolomitization of calcium carbonate. *Geochim Cosmochim Acta*. 1977;41(10):1431-1438.
- Miguez-Macho G, Fan Y. Spatiotemporal origin of soil water taken up by vegetation. *Nature*. 2021;598(624-628).
- Millar C, Janzen K, Nehemy MF, et al. On the urgent need for standardization in isotope-based ecohydrological investigations. *Hydrological Processes*. 2022;36(10).
- Morrow DW, Ricketts BD. Chemical controls on the precipitation of mineral analogues of dolomite: The sulfate enigma *Geology*. 1986;14(5).
- Nan Y, Tian F, Hu H, Wang L, Zhao S. Stable Isotope Composition of River Waters across the World. *Water*. 2019;11(9).
- Nyamgerel Y, Han Y, Kim M, Koh D, Lee J. Review on applications of  $^{17}\text{O}$  in hydrological cycle. *Molecules*. 2021;26(15):4468.
- Oi T, Sato K, Umcmoto K. Oxygen and Hydrogen Isotopic Preference in Hydration Spheres of Magnesium and Calcium Ions. *Zeitschrift für Naturforschung A* 2013;68(5).
- O'Neil JR, Clayton RN, Mayeda T. Oxygen Isotope Fractionation in Divalent Metal Carbonates. *The Journal of Chemical Physics*. 1969;51(12):5547-5558.
- Rozanski K, Chmura L. Isotope effects accompanying evaporation of water from leaky containers. *Isotopes in Environmental and Health Studies*. 2008;44(1):51-59.
- Spangenberg JE, Vennemann TW. The stable hydrogen and oxygen isotope variation of water stored in polyethylene terephthalate (PET) bottles. *Rapid Communications in Mass Spectrometry*. 2008;22(5):672-676.
- Spangenberg JE. Caution on the storage of waters and aqueous solutions in plastic containers for hydrogen and oxygen stable isotope analysis. 2012.
- Terzer-Wassmuth S, Wassenaar LI. Performance of low-cost stainless-steel beverage kegs for long-term storage integrity and easy dispensing of water isotope ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ) reference materials. *Rapid Communications in Mass Spectrometry*. 2021;35(19):e9164.
- Tian C, Wang L. Stable isotope variations of daily precipitation from 2014–2018 in the central United States. *Scientific Data*. 2019;6:190018.
- Tian C, Wang LX, Tian FQ, Zhao SH, Jiao WZ. Spatial and temporal variations of tap water  $^{17}\text{O}$  excess in China. *Geochimica et Cosmochimica Acta*. 2019;260:1-14.

- Vasconcelos C, McKenzie JA, Warthmann R, Bernasconi SM. Calibration of the  $\delta^{18}\text{O}$  paleothermometer for dolomite precipitated in microbial cultures and natural environments. *Geology*. 2005;33(4).
- Wang L, Caylor KK, Villegas JC, Barron-Gafford GA, Breshears DD, Huxman TE. Partitioning evapotranspiration across gradients of woody plant cover: assessment of a stable isotope technique. *Geophysical Research Letters*. 2010;37:L09401.
- Wang L, Good SP, Caylor KK, Cernusak LA. Direct quantification of leaf transpiration isotopic composition. *Agricultural and Forest Meteorology*. 2012;154-155:127-135.
- Wang L, Kaseke KF, Seely MK. Effects of non-rainfall water inputs on ecosystem functions. *Wiley Interdisciplinary Reviews-Water*. 2017;4(1).
- Wassenaar LI, Coplen TB, Aggarwal PK. Approaches for Achieving Long-Term Accuracy and Precision of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for Waters Analyzed using Laser Absorption Spectrometers. *Environmental science & technology*. 2013;48(2):1123-1131.
- Williams M, Lartey J, and, Sanders L. Isotopic ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) Integrity of Water Samples Collected and Stored by Automatic Samplers. *Agricultural & Environmental Letters*. 2018;3(1).
- Zhao L, Wang L, Cernusak LA, et al. Significant difference in hydrogen isotope composition between xylem and tissue water in *Populus euphratica*. *Plant, Cell & Environment*. 2016;39(8):1848–1857.
- Zheng Y-f, Böttcher M. Oxygen isotope fractionation in double carbonates. *Isotopes in Environmental and Health Studies*. 2014;52(1-2).
- Zheng Y-f. Oxygen isotope fractionation in carbonate and sulfate minerals. *Geochemical Journal*. 1999;33(2):109-126.

## Curriculum Vitae

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### Education

**M.S.** Indiana University Indianapolis, *May 2025*  
Geology  
Concentration: Geography  
Cumulative GPA: 4.0

**B.S.** Indiana University Purdue University Indianapolis, *December 2022*  
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### Honors, Awards, and Fellowships

**Science Scholar - IUPUI** *Fall 2021 & Spring 2022*  
IUPUI science undergraduate with a GPA of 3.7 or above

**Dean's List – IUPUI** *Spring 2020 & Spring 2021*  
IUPUI undergraduate with a GPA of 3.5 or above

**IUI Earth and Environmental Science Department's Best Presentation Award for MS students** *Spring 2024*  
Presentation of MS thesis project

**Travel Fellowship – Indiana University Indianapolis** *Fall 2024*  
Graduate school fellowship for travel to AGU24

### Research Experience

**Laboratory Manager, Ecohydrology, Dr. Lixin Wang**  
**Indiana University Purdue University Indianapolis** *8/2022 to 7/2023*

- Perform isotopic analysis of various water samples using a liquid water isotope analyzer
- General maintenance and upkeep of all laboratory equipment, including ordering all supplies and maintaining contact with company support

**Research Assistant, Sedimentology, Dr. Broxton Bird  
Indiana University Purdue University Indianapolis**

*1/2022 to 8/2022*

- Collected and sampled sediment cores using a manual corer.
- Processed sediment cores for magnetic susceptibility, grain size, and bulk density lost on ignition
- Used x-ray fluorescence (XRF) to analyze sediment samples

**Research Assistant, Ecohydrology, Dr. Lixin Wang  
Indiana University Purdue University Indianapolis**

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- Filtered and prepared various types of water samples for isotopic analysis
- Assisted graduate students with building meta-analysis
- Construct and maintain inventory logs of all samples and standards
- Designed laboratory website using Weebly

**PRESENTATIONS**

**Poster Presentation**, Pre-Columbian Population Dynamics, and the Role of Late Holocene Midcontinental Flooding, CRL Summer Research Symposium, 8/2022

**Poster Presentation**, Effects of long-term storage on the isotopic compositions of different types of environmental waters, International Conference on Fog, Dew and Fog Collection, 7/2023

**Poster Presentation**, Effects of long-term storage on the isotopic compositions of different types of environmental waters, American Geophysical Union, 12/2024

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Diersing et al. in review., Effects of long-term storage on the isotopic compositions of different types of environmental waters, Rapid Communications in Mass Spectrometry.

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- Educate the public on state flora and fauna through educational programs and hikes
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