

Methodological recommendations for measurements with deuterium-labelled drops in the context of soil erosion caused by rain studies**

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Abstract. Splash erosion represents the initial stage of rainfall-induced soil degradation. Among the many methods used to investigate this process, some employ labelled water as a tracer of splashed material however, studies utilizing deuterium-labelled water remain scarce. The application of deuterium-labelled water in splash measurements introduces methodological challenges that may significantly affect results and are often negligible in other soil science or hydrological investigations. The aim of this study was to identify the principal sources of potential error and to propose methodological recommendations for experiments using deuterium-labelled water. Laboratory splash experiments were conducted with drops of 4.2 mm enriched in deuterium ($\delta^2\text{H} \sim 1500\%$ vs. VSMOW), falling from various heights of 1-7 m under controlled conditions, including temperature (20°C) and relative humidity (value depending on the experimental variant). The key recommendations are: i) the temperature and relative humidity should be monitored; ii) the isotopic enrichment must be sufficiently high to enable detection of the studied process, yet low enough to limit the drive toward thermodynamic equilibrium; iii) to minimize evaporation effects, experiment duration should be kept as short as possible. However, since sampling time cannot be eliminated, it should remain constant within a given experiment to reduce systematic error in trend analysis.

Key words: stable isotopes, deuterium-labelled water, IRMS, splash, soil water erosion

1. INTRODUCTION

Erosion caused by water is an unfavourable and highly significant phenomenon of physical soil degradation due to its large scale (Quinton and Fiener, 2024). Therefore, it is not surprising that extensive research is devoted to this topic (Dziczek *et al.*, 2025; Juliev *et al.*, 2024; Scholten and Seitz, 2019; Smolska *et al.*, 2025). Erosion studies are carried out using different approaches. It seems that the two most popular techniques are the estimation of the mass of soil lost (Xiong *et al.*, 2024) and modelling (Akpa *et al.*, 2024). However, studies of different types of erosion use more specialized tools that facilitate a more detailed description of the phenomena.

In many studies of soil water erosion, splash is an important issue because it is recognized as the first stage of many types of soil erosion caused by water (Fernández-Raga *et al.*, 2017). For the quantification of splash erosion, most researchers use such methods as splash cups (Zumr *et al.*, 2020), high-speed cameras (Beczek *et al.*, 2020), and surface deformation analysis (Mazur *et al.*, 2025). Studies are often conducted by analysing single drops (Mazur *et al.*, 2023) or using rainfall simulators (Davidová *et al.*, 2015).



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Moreover, splash can be studied in the context of a drop hitting, for example, the surface of other solid bodies (Bauer and Gilet, 2024; Yonemoto *et al.*, 2022), the surface of plants (Papierowska *et al.*, 2023; Ryzak *et al.*, 2023) or liquids (Faghiri *et al.*, 2026; Sochan *et al.*, 2022).

All the above examples indicate studies in which important elements are the physicochemical properties of drops, the characteristics of the water drop, and the ability to monitor its behaviour during and after the splash. One way to enable this monitoring is to label the water in the drop.

Previous methods of water labelling in splash erosion studies have primarily relied on fluorescent or visible dyes, such as brilliant blue or rhodamine, for visualizing infiltration pathways and transport (Ersoy and Eslamian, 2019). These techniques enable tracking of water and soil particle movement in rainfall simulators; however, they are limited by dye adsorption onto soil and the lack of precision in distinguishing stable isotopes (Schneider *et al.*, 2018). Other approaches, like radioactive markers (*e.g.*, $^{239+240}\text{Pu}$) or salts, trace soil redistribution but not water directly in splash processes (Alewell *et al.*, 2017; Loba *et al.*, 2022). The methodological gap lies in the absence of non-invasive, persistent tracers that allow isotopic analysis without affecting drop or soil behaviour.

In contrast to the approaches described above, water labelling with deuterium (D_2O) involves adding the heavy hydrogen isotope (^2H), which enables tracking its movement in erosion processes without altering the physical properties of the drop. Deuterium is stable, non-toxic, and inexpensive, making it ideal for field and laboratory experiments in splash studies (Beyer *et al.*, 2015). Analysis using isotope ratio mass spectrometry (IRMS) allows precise measurement (precision $<1\%$) of the D/H ratio in water extracts from soil (Wu *et al.*, 2020). In the context of splash erosion, it facilitates the quantification of water and soil particle transport in the initial erosion phase and potentially enables the calibration of erosion models, studies of climate change effects on splash intensity, and evaluation of anti-erosion practices in sustainable agriculture (Mook, 2001). It may also be important in terms of the description of the transport of soil microorganisms through splash, as it allows tracing their origin in the splashed water.

The study considered two main processes that could affect the results of experiments using deuterium-enriched water in splash analyses. The first was evaporation, which reduces the mass of drops/droplets and may alter the isotopic ratio of the liquid from which they originate. The second was isotopic exchange, in which the water in the drops tends to equilibrate its isotopic ratio with the water vapour in the surrounding air.

Apart from the publication by Polakowski *et al.* (2022), to the best of the authors' knowledge, labelling water with stable water isotopes in splash erosion studies has not been used so far (Polakowski *et al.*, 2022). The study considered two main processes affecting deuterium-enriched water

experiments: evaporation, which reduces droplet mass and alters the isotopic ratio of the source liquid, and isotopic exchange, where droplet water equilibrates its ratio with the surrounding air vapour. Therefore, there are no developed methods and methodological guidelines, nor are there any indications of the sources of potential errors. We are convinced that the method of labelling with stable isotopes is safe, versatile, and has enormous potential for such research. Therefore, in order to fill the gap in the knowledge, the study was aimed to describe potential sources of errors in the isotopic monitoring of the splash phenomenon and to propose methodological recommendations for this type of research.

2. MATERIALS AND METHODS

For clarity description in the experiment and results, the regular water would be described as deionized water – H_2O ($\delta^2\text{H} \sim -61.6\%$) (Duliński *et al.*, 2019) and the deuterium-enriched water as D_2O ($\delta^2\text{H} \sim 1500\%$). The deuterium-enriched water was prepared using class I water (EN ISO 3696:1995) and 99.8% purity deuterium oxide (Thermo Scientific).

Measurements of the splash phenomenon were conducted in a laboratory with a mounted platform that allowed the release of water drops from a height 7 m. The falling water will be called drop, while the splashing water will be called droplets. The platform was a drop dosing system consisting of a peristaltic pump and a glass capillary through which the drop was released. In all experiments related to the splash phenomenon, the drop size was 4.2 ± 0.02 mm for both H_2O and D_2O (Ryzak *et al.*, 2023). The conditions in the laboratory were controlled. The temperature was $20 \pm 2^\circ\text{C}$, whilst the relative humidity was adjusted to the selected experimental variant, as detailed below.

2.1. Evaporation measurement

The issue of evaporation was considered 2 aspects: i) evaporation from a drop during its flight, ii) evaporation from droplets after the splash.

2.1.1. Evaporation from a drop during its flight

To determine the effect of inflight drop evaporation, 10 subsequent drops of H_2O and D_2O were separately dropped in each experiment from 0 and 7 m (0 m is the notation for the reference (control) height – in practice the drip test was conducted with the dispensing nozzle at its minimum distance, *i.e.*, 1 cm from the surface).

To prevent water loss due to splashing, a tall beaker was used to collect water. The beaker was placed on a scale. The measurements were performed in 10 repetitions. During the experiments, the humidity in the laboratory was maintained at $45 \pm 5\%$. This air humidity was measured at the level conventionally assumed to be 0 m (see above); however,

before the experiments, we measured the humidity distribution over the entire height of the falling drop, and the maximum difference was 0.5%.

Immediately after the last drop in the series fell, the mass was measured on the scale. The total time for these 10 drops to fall was ~ 30 s. The time interval between successive released drops was set to ~ 3 s based on preliminary measurements and to relate this to the high-frequency of drop dripping in *e.g.* the throughfall phenomenon (Nanko *et al.*, 2006). The purpose of these measurements was to assess the magnitude of changes in the mass of a water drop during flight. The choice of 10 drops in the series was a compromise between two opposing premises: i) measuring the mass of a single drop would be subject to high uncertainty; ii) the experiment time should be short enough so that the water from the drops that have already fallen does not have time to evaporate.

2.1.2. Evaporation from droplets after a splash

As in the previous measurements, H_2O and D_2O were separately dripped into a tall beaker placed on the scale. However, this time, 100 water drops were dropped (it took about ~ 5 min.) from the following heights: 0 (control), 1, 2, 4, and 7 m. The drop generation frequency was the same as before, *i.e.*, 10 drops per ~ 30 s. After dripping, the increase in the mass of water in the vessel was measured, which enabled comparison of evaporation from droplets splashed after the impacts of drops falling from different heights. The values obtained for each height were related to the control sample, *i.e.*, the mass of 100 drops falling into the vessel from a height of 0 m. The measurements were performed in 10 repetitions. During these experiments, the humidity in the laboratory was maintained at $45 \pm 5\%$.

2.2. Isotope changes

2.2.1. Isotope changes in drop flight

The experiment tested the extent to which the phenomenon of isotope changes, which occurs during the fall of drops, affects the water they form. In this case, 40 drops were dripped into a tall vessel for 2 min. After the dripping process was finished, the water was collected for analysis as quickly as possible into a 2 ml vial using a pipette and then tightly closed to prevent evaporation. The experimental conditions were consistent with those presented in subsection 2.1.2: drops of H_2O and D_2O were dropped separately; the humidity was $45 \pm 5\%$; the dropping heights were 0 (control), 1, 2, 4, and 7 m; 10 repetitions were performed separately for each type of water and each height.

2.2.2. Isotope changes in splash

To determine the isotopic exchange with water vapour in the air and splashed droplets, H_2O and D_2O were separately dispensed onto the water layer in a Petri dish. The amount of water in the dish was sufficient to cover the

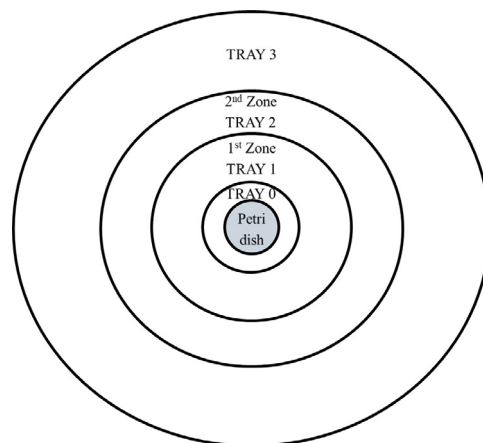


Fig. 1. Diagram of the location of trays and the coverage area of the zones where splashed liquid droplets were collected. The diameter of the Petri dish containing water was 10 cm.

entire surface, and all the water in the splashed droplets came from the surface of the water layer in the Petri dish – in practice about 3–4 mm (Polakowski *et al.*, 2022). H_2O dripped into H_2O and D_2O dripped into D_2O . The experimental conditions followed those described in subsection 2.1.2: drops of H_2O and D_2O were dropped separately; the humidity in the laboratory was $45 \pm 5\%$; the drops fell from the height of 7 m; 10 repetitions were performed.

The droplets ejected as a result of the splash were collected using a tray system (Fig. 1). Three trays were placed around the Petri dish. Two measurement zones were designated for analysis (Tray 1 and Tray 2), with the dimensions given in Fig. 1, from which the droplets were collected immediately after the droplet splash. Tray 3 was a “control” tray and was not included in the analysis. The selection of only two zones was a compromise between the best possible characterization of the phenomenon and technical limitations. In this case, the greater the number of narrow zones, the more complete the description of the phenomenon; however, to collect an appropriate amount of water for analysis from a higher number of smaller zones, a greater number of drops would be required. This, in turn, would extend the experiment time and cause evaporation from the trays, which might alter the hydrogen isotope ratio. Next, the collected water was transferred into a 2 ml vial using a pipette and tightly sealed to prevent evaporation.

2.2.3. Isotope changes during static evaporation

The study refers to situations where the amount of water after splashing is large enough to form a “puddle,” *i.e.*, water droplets merge into a larger body, or when a drop hits the water surface from the very beginning. Four variants were investigated in this regard:

- H_2O droplet contact with free surface at a relative humidity of: 1) $40 \pm 5\%$, 2) $80 \pm 5\%$;
- D_2O droplet contact with free surface at a relative humidity of: 3) $40 \pm 5\%$, 4) $80 \pm 5\%$.

In each of the above variants, 5 ml of H₂O or D₂O were placed in 10 cm diameter Petri dishes. This amount created “a puddle” at the bottom of the vessel with a minimum achievable water layer thickness of about 0.6 mm. The thickness of this layer was the balance between the force of gravity, surface tension, and internal cohesion forces. Of course, the size of this “puddle” was incomparably larger than the droplets, but this was necessary to ensure that, after the evaporation time, an appropriate amount of water could be collected for further isotopic research.

The dishes were placed in a climate chamber (Biogenet Fito 700) with controlled temperature and humidity (the water vapour in the chamber was generated from unenriched water – H₂O). The variation in the relative humidity allowed the verification of the significance of the analysed processes, *i.e.*, the variant with lower humidity involved increased evaporation but reduced isotope exchange with water vapour present in the air, whilst the variant with higher humidity involved reduced evaporation but enhanced isotope exchange with water vapour in the air. During the experiments, the chamber temperature was set at 20 ± 2°C. Once the temperature and humidity conditions in the climate chamber had stabilized, Petri dishes filled with fresh water were placed inside. Changes in the isotopic ratios were monitored by taking measurements repeated 10 times for each variant at intervals of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 60 min.

2.3. Isotopic analysis

Isotope measurements were conducted using a Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo Scientific, USA) coupled with a vario PYRO cube elemental analyzer (Elementar, Germany). The δ²H value of the sampled water was expressed relative to VSMOW (Vienna Standard Mean Ocean Water). The measurements were performed in 10 repetitions. The results were expressed in delta notation (δ²H vs. VSMOW) using equation:

$$\delta^2H = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) 1000,$$

where: *R* is the ²H/¹H ratio for δ²H (‰).

2.4. Statistical analysis

Statistical analyses were conducted using Statistica 13.1 software (TIBCO Software Inc, USA). Shapiro-Wilk normality tests Kruskal-Wallis (rank-based analogue of one-way ANOVA) and Dunn’s post-hoc test were performed with a significance level of *p* < 0.05.

3. RESULTS

3.1. Changes in the mass of drops/droplets during measurements

One of the mechanisms of the δ²H change may be the evaporation of water forming the drops/droplets or, more precisely, the preferential evaporation of water molecules

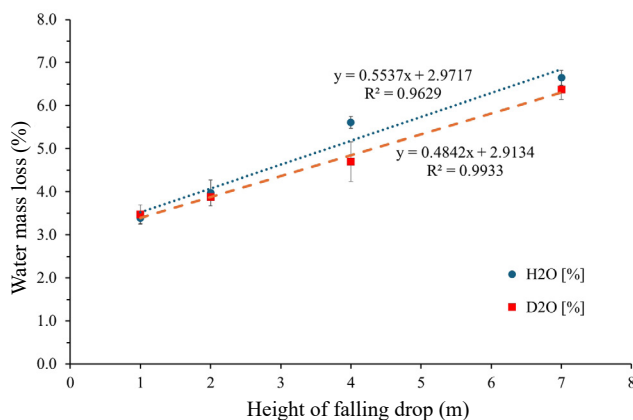


Fig. 2. Changes in droplets mass (relative to the initial droplet mass) after splash from different heights. Ambient humidity was 45 ± 5%. Whiskers indicate standard deviation.

composed of a lighter isotope of hydrogen. Therefore, first of all, we assessed how the mass of the drops changed during the fall from different heights and how the mass of the droplets formed as a result of splash changed over time.

The mass measurements based on a series of H₂O and D₂O drops falling from a height of 0 and 7 m indicated that there were no statistically significant differences. In other words, the time of the drop falling was so short that even at relatively low air humidity (45 ± 5%) did not change its mass significantly.

The situation was different when the measurement concerned splashed droplets. The results of this experiment are shown in Fig. 2. The observed trend indicated that an increase in the height of the drop release results in greater evaporation of the splashed water. The water loss due to evaporation after the splash is practically the same in both cases (*i.e.* H₂O and D₂O). the statistical analysis did not show any statistically significant differences in water mass change. It ranged from ~3.5% for droplets released from 1 m to ~6.5% for droplets falling from 7 m. Noteworthy is the fact that the measurement of evaporation of splashed droplets took 5 min and was longer than the measurement of the evaporation during drop falling.

3.2. Isotope changes during drop fall

The changes in hydrogen isotope ratios in H₂O and D₂O during the drop fall from different heights are presented in Fig. 3.

The analysis of Fig. 3a may suggest that there is no clear trend in the changes in δ²H for H₂O (based on the R² value). The points seem to be highly scattered, but it is worth noting that the absolute values of the scatter are small. The situation is different in the case of D₂O – here, there is a clear trend towards a reduction in the δ²H value, reflecting a decrease in deuterium content in water due to the contact with water vapour during drop fall (Fig. 3b).

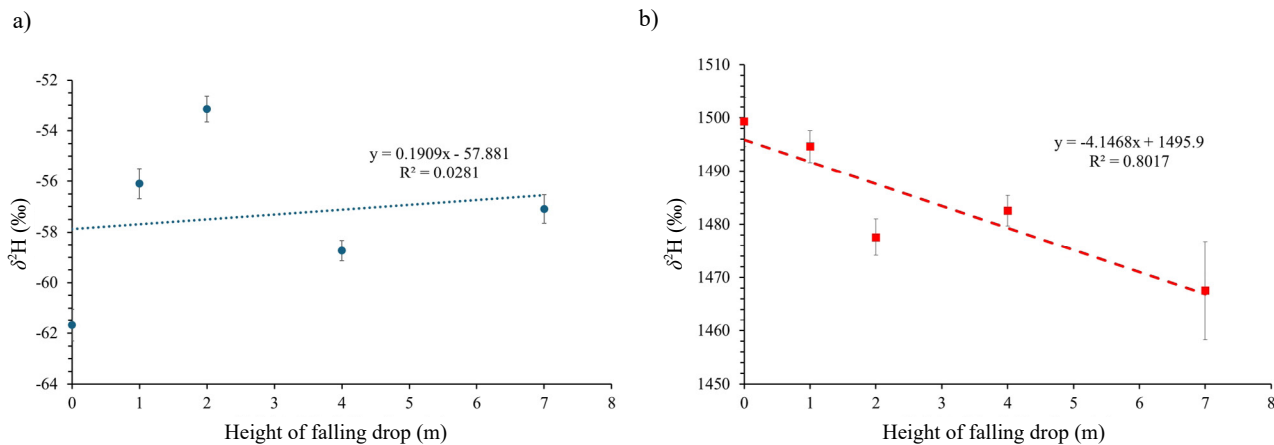


Fig. 3. Changes in hydrogen isotope ratios in H₂O (a) and D₂O (b) during the drop fall from different heights. Whiskers indicate standard deviation.

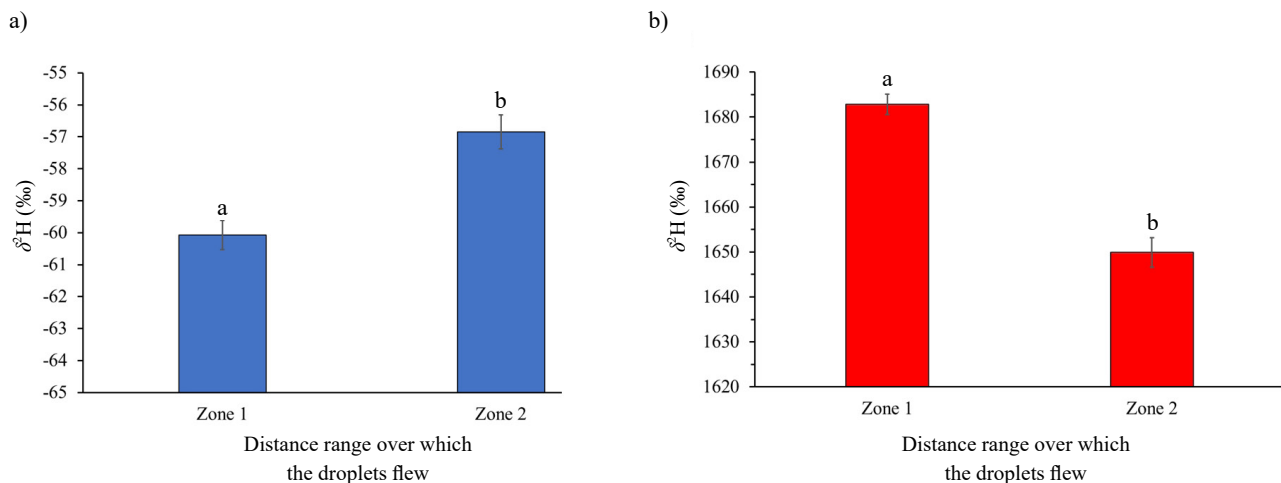


Fig. 4. Changes in hydrogen isotope ratios in H₂O (a) and D₂O (b) during the droplets fly over various distances after splashing. Whiskers indicate standard deviation. The results of Dunn's post-hoc test are shown by the letters above the bars.

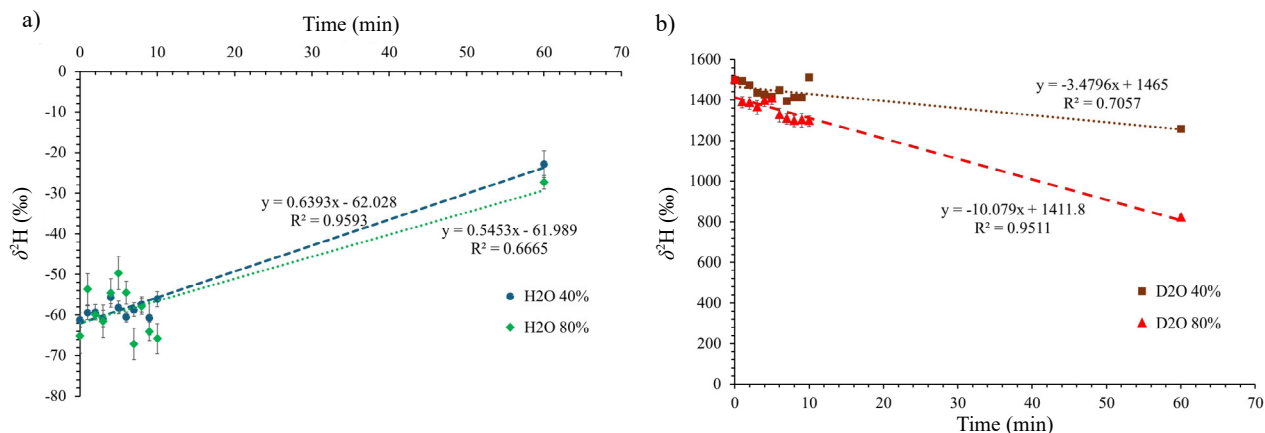


Fig. 5. Changes in hydrogen isotope ratios in H₂O (a) and D₂O (b) during contact of water free surface with air at a relative humidity 40 and 80%. Whiskers indicate standard deviation.

3.3. Isotope exchange from splash to collection

Isotope exchanges in both investigated media (*i.e.* H₂O and D₂O), depending on the droplet ejection distance, are presented in Fig. 4.

As shown in Fig. 4, opposite trends were observed in both water variants. In the case of H₂O, the $\delta^2\text{H}$ values decreased, whereas the $\delta^2\text{H}$ values increased in the case of D₂O. In both cases the differences were statistically significant.

3.4. Isotope exchange during static evaporation

The changes in hydrogen isotope ratios in a shallow H₂O and D₂O layer during static contact with air at the specified relative humidity values are presented in Fig. 5.

The analysis of Fig. 5 revealed a fundamental difference in the changes in the ratios between hydrogen isotopes in non-enriched water (H₂O) and deuterium-enriched water (D₂O). The $\delta^2\text{H}$ values increased in the case of H₂O but decreased in the D₂O. The H₂O increasing trends were smaller than the decreasing trends for D₂O.

It is also worth noting the differences in the results recorded for H₂O and D₂O due to the effect of air humidity. In the case of H₂O, the changes in the isotope ratios did not depend on the ambient humidity ($\delta^2\text{H}$ values are very similar in experiments conducted at relative humidity of 40 and 80%). In the case of D₂O, this dependence was visible, and a greater change was observed at higher humidity values

4. DISCUSSION

Studies on the effect of hydrogen isotope fractionation during water evaporation have been conducted repeatedly by many researchers under various experimental (Cappa *et al.*, 2005) and natural conditions (Gallart *et al.*, 2024), covering both equilibrium (Hochscheid *et al.*, 2025) and kinetic approaches (Xie *et al.*, 2021) and taking into account the influence of atmospheric conditions (Galewsky *et al.*, 2016), turbulence (Benetti *et al.*, 2014), and isotopic composition of the liquid (Chen *et al.*, 2025).

Referring to the purpose of this work, *i.e.*, the assessment of the extent to which natural phenomena occurring between water (in drops and droplets) and air can influence the results of subsequent splash tests, two most important processes should be mentioned: i) fractionation as a result of preferential evaporation of H₂O molecules in relation to D₂O and DHO (as it is well known that DHO molecules are formed when D₂O is added to H₂O), and ii) the establishment of thermodynamic equilibrium between water molecules in drops and droplets and water in the form of water vapour in the air. The question is which of these processes plays a greater role and whether the $\delta^2\text{H}$ change is large enough to be taken into account in splash studies using deuterium-labelled water.

The preferential evaporation of water molecules containing lighter hydrogen over the evaporation of water molecules containing heavier hydrogen (deuterium) has long been known and repeatedly described (Cappa *et al.*, 2005). To assess the magnitude of this effect when a water drop falls, it was first necessary to determine the amount of evaporated water, including unlabelled (H₂O) and deuterium-labelled water (D₂O). As already mentioned in subsection 3.1, no statistically significant differences were recorded for either H₂O or D₂O for the drop falling from a height of 0 and 7 m. This observation seems to be valid for laboratory studies. With the drop size used in our experiment (4.2 mm), a height of 7 m (the maximum height from which the drops fell) seems to be sufficient for most splash erosion studies (Epema and Riezebos, 1987).

As can be seen in Fig. 2, a longer duration of the experiment (~5 min) resulted in measurable decreases in the mass of splashed water. This change depended on the height of the fall - the greater the height from which the drop fell, the greater the loss of mass (for both H₂O and D₂O). Therefore, if there was no significant evaporation of droplets during the flight and the duration of the measurement was the same at each altitude (100 drops in 5 min), a question arises as to the cause of the trend observed in Fig. 2. An explanation for this fact may be that the greater height from which the drop falls yields its higher energy, which results in fragmentation into a larger number of smaller droplets. Additionally, with the greater energy of falling, the droplets settled higher on the wall of the beaker, increasing the extent of beaker wall wetting. In turn, a larger number of smaller droplets resulted in a larger evaporation surface, which directly translates into greater evaporation dynamics, as observed in Fig. 2.

It is difficult to directly relate the evaporation rate obtained in our experiment (Fig. 2) to literature reports, as the evaporation rate depends on the conditions - primarily on temperature, humidity, and possible air turbulence. Depending on weather conditions, this range may vary from a few to 100% (Wang *et al.*, 2024). Taking into account the conditions prevailing in the laboratory and the high repeatability of the results, the data presented in Fig. 2 should be considered reasonable. However, the fact that evaporation occurs in the splashed droplets does not, in itself, answer the question of the significance of this process for changes in the isotope ratio in deuterium-labelled water. Resolving this issue requires a comparison of the results of evaporation with the isotopic exchange process that occurs when the water surface comes into contact with water vapour in the surrounding air.

Changes in stable isotope ratios observed during our methodological experiments should be considered separately. Let us first consider the case where H₂O was used for dripping. The analysis of the graphs presented in Fig. 3a may lead to the conclusion that the change in $\delta^2\text{H}$ in the case of H₂O may be neglected. The scatter of results appears to be random, and its range is of the order of the uncertainty

of the method. This approach may be supported by the fact that the changes observed in the H₂O isotope ratio occur much slower (Fig. 3a) than in enriched water (Fig. 3b). Furthermore, the observed trend (an increase in the isotope ratio in H₂O) suggests that the change is due to the preferential evaporation of the lighter isotope. However, it should be noted that, even in the case of water not enriched with deuterium, the duration of the experiments may be significant for the recorded results, as demonstrated by the exposure of free water surfaces to air of varying relative humidity, where significant changes in the isotope ratio were observed for 60 min.

The second case involved deuterium-enriched water. As already mentioned, during contact of static water surface with air at a relative humidity level (Fig. 5b), evaporation prefers the separation of water molecules containing lighter hydrogen. This should be reflected in an increasing $\delta^2\text{H}$ value, undergoing a slower change under higher relative humidity (slower evaporation) and a faster change under lower relative humidity (faster evaporation). It should also be noted that, based on the results presented in Fig. 2, it can be assumed that evaporation of H₂O and D₂O under the same humidity conditions was the same. As opposed to evaporation, the exchange of water isotopes from the drops/droplets with water vapour should cause the delta value to decrease. The $\delta^2\text{H}$ of water molecules in air is similar to that of surface waters in Poland, *i.e.*, on the order of several tens of ‰ (Palige *et al.*, 2008). It is therefore much smaller than the $\delta^2\text{H}$ D₂O used in our experiment (~ 1500‰). The results presented in Figs 3b, 4b, and 5b indicate that the process of establishing thermodynamic equilibrium between water molecules in drops/droplets and water in the form of water vapour in the air is responsible mainly for the decrease in the $\delta^2\text{H}$ value.

Moreover, it is clearly visible there that the decrease in the $\delta^2\text{H}$ value is much greater in the case of higher humidity (80%), when evaporation is certainly lower than at 40% humidity (Fig. 5). Higher humidity means more water molecules in the air. An indirect support for this statement comes from atmospheric studies showing that, at very high air humidity, variations in hydrogen isotope ratios are mainly controlled by equilibration processes toward thermodynamic equilibrium (Graf *et al.*, 2019).

5. CONCLUSIONS

The use of deuterium-labelled water, commonly applied in natural sciences, has great potential in splash erosion research, *e.g.*, to determine the origin of water formed in splash (whether the water comes from a falling water drop or from water stored in the soil). However, due to the specific nature of the splash phenomenon (mainly due to the fact that the mass of splashed water is very small), one may encounter methodological problems that are not relevant in other soil or hydrological studies. To limit the impact of these limitations on the results obtained, it is worth following the recommendations:

1. The temperature and relative humidity should be monitored. Reporting the experimental conditions and sample collection time in the methodological section of the publication will allow other researchers to estimate the potential error, and thus the results obtained in different laboratories will be more comparable.

2. Given a very large difference in the deuterium content in the labelled water compared to the water vapour present in the air, the main mechanism of change in $\delta^2\text{H}$ is the system's striving to achieve thermodynamic equilibrium, and the rate of this process increases with the magnitude of this difference. Therefore, $\delta^2\text{H}$ in the labelled water should be large enough to monitor the process being tested, but small enough to limit the dynamics of these changes resulting from the system's striving to achieve the aforementioned thermodynamic equilibrium.

3. To minimize the impact of evaporation, the experiment time should be as short as possible. However, since the sampling time (*i.e.* from the moment of ending the active experiment to the time of closing the collected water in a sealed container) cannot be reduced to zero, this error will always be present. Therefore, one should strive to ensure that this time is always the same in a given experiment. In this way, random error will be converted into systematic error and thus the error made during trend analysis will be minimized.

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Conflict of interest. The authors do not declare any conflict of interest.

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