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Water uptake of Haloxylon ammodendron in desert regions of Iran

Pouyan Dehghan Rahimabadi
University of Tehran
Vahideh Faghihi
Nuclear Science and Technology Research Institute
Hossein Azarnivand (hazar@ut.ac.ir)
University of Tehran
Javad Fasihi
Nuclear Science and Technology Research Institute
Arash Malekian
University of Tehran
Behzad Yadollahzadeh
Expert, Nuclear Science and Technology Research Institute

Research Article

Keywords: Groundwater, IsoSource, Stable Isotopes, Semnan, Soil Water, CVD

Posted Date: January 17th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2471811/v1

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Abstract

The complex relationships in the functioning of desert ecosystems and their environmental conditions can be expressed by the patterns of plant water use. In these areas, the water use sources of plants are a useful tool for resource management. Therefore, it is necessary to identify and track the available water resources and water use patterns. In this study, we investigated water use pattern of *Haloxylon ammodendron* growing in Semnan province, a semi-arid area of IRAN, using the stable isotope method. We used a simple, home-made Cryogenic Vacuum Distillation (CVD) system to direct extract the water in soil samples and different plant components before isotope analysis by mass spectrometer. The contribution of each possible water source of the plant xylem water was estimated using the IsoSource mixing model. The patterns in $\delta^{18}O$ values in plant xylem water indicates the ability of *H. ammodendron* to use groundwater as the most important water resources in wet season to maintain its life. The results showed that there is a significant correlation between the isotope values with sand particles in the soil layers ($P < 0.05$). The results of this research can be of great help in strategic planting of *H. ammodendron* to prevent damages to groundwater resources and maintaining the long-term sustainability of groundwater.

1. Introduction

Many countries are faced with land degradation and desertification as a serious problem, which causes the loss of available resources. Hence, the preservation and development of resources will be of particular importance for the proper use and exploitation of the land, especially in arid and semi-arid areas. Knowledge of energy, water and biogeochemical cycles in the ecosystem of these areas is considered a tool for proper management. The main part of the arid and semi-arid ecosystems is vegetation cover, which can express certain aspects of the ecosystem's performance. Vegetation cover also plays an important role in ecological cycles, although its survival and growth are basically limited by water resources (Zhao and Wang 2018), and due to characteristics such as immobility, relatively high growth rate, tolerance to acute conditions and relatively simplicity, the sampling is an important environmental criterion in ecological assessments and studies (Raab and Bayley 2012).

Plants prioritize the use of stable water resources, although they can change the water absorption strategy under certain conditions (Zhao and Wang 2018). Thus, it is necessary to identify the water sources available to plants to track the source of plant growth. (Barbeta et al. 2020). Traditional methods cannot distinguish different water sources in regions with multiple water sources (Meinzer et al. 2001). In the past decades, the use of water stable isotopes as natural tracers has become a common tool in plant ecological research (Orlowski et al. 2013) and understanding plants' water resources, as well as identifying ecological and hydrological processes. (Chen et al. 2020; Tiemuerbieke et al. 2018). Water uptake by plants (Hill et al. 2021; Lanning et al. 2020; Penna et al. 2020; Wu et al. 2019a), physiology of plants (Wilkinson et al. 2017), infiltration processes and runoff production (Gou et al. 2018; Orlowski et al. 2019; Tetzlaff et al. 2015) and study of evaporation processes (Gonfiantini et al. 2020; Martín-Gómez et

al. 2017; Yong et al. 2020) are among the fields in natural sciences, where water stable isotopes are used as tracers.

The use of water stable isotopes relies on high-precision measurements (Wassenaar et al. 2012). However, application of this method in investigation of water absorption by plants has advantages, including: A) this method is very simple and only requires the isotopic matching of the sampled water with the composition of different water sources. B) In plant studies, it is possible to determine the contribution of different plant available water sources. C) A small amount of sampling is required and therefore it is a non-destructive method for the environment.

Water use patterns employed by plants are closely related to the rooting depth and distribution of their roots (Phillips et al. 2016; Warren et al. 2015). In plants with shallow roots, water absorption takes place from the upper layers of the soil, on the other hand, plants with deep roots can absorb water from deep soil layers (Wu et al. 2019a). By using the method of stable isotopes, it is possible to estimate the quantity of water used in different soil depths.

Various studies have investigated water resources in different species of plants in a wide range of environmental conditions and ecosystems. Schwinning et al. (2005) examined the pattern of water use in three species inclluding *Oryzopsis hymenoides*, *Gutierrezia sarothrae*, and *Ceratoides lanata* in the cold desert ecosystem of the Colorado Plateau and found that all these three species absorb water from deeper soil layers under drought conditions, while using water in shallower soils after heavy summer rainfall. Rossatto et al. (2012) by investigating the response of plants to the level of underground water, found that plants at higher altitudes extract water from both shallow and deep layers of the soil, while plants that are located only at lower altitudes extract more water from the shallow soil. Zhao and Wang (2018) assessed the strategy and pattern of water use in plants by studying and reviewing published information about stable isotopes in plant tissues of 368 species in China. The results showed that there is a strong correlation between δ^{2}H and δ^{18}O in stem of plants and water resources. Also, the main sources of water for plants are largely influenced by rainfall patterns. So that plants in normal conditions use stable and continuous water sources, but in drought conditions they have a more variable water absorption strategy. Zhang et al. (2018) studied the water resources for *Tamarix laxa* in the Great Badain Jaran desert hills in China using δ^{18}O stable isotope analysis. The results indicated that the pattern of water consumption in this specie is closely related to the events of precipitation and soil water content. In this way, *T. laxa* depends on water from the middle soil layer (60–120 cm) and deep soil layer (120–200 cm) at the beginning of its growing season. Then, in the wet season, it mainly absorbs the water from the shallow soil layer (0–60 cm), and in September, it uses the water from the deep soil layer (120–200 cm).

Considering that there are long periods of drought and limited water resources in dry and semi-arid regions of Iran, plants show different behaviors to provide the water they need. Therefore, the aim of this study is to determine and track water sources in *H. ammodendron* using water stable isotopes in spring for first time in Iran. For this purpose, xylem water and soil water content in different soil layers and groundwater in the hand-planted forests of *H. ammodendron* in Semnan and Damghan plain, Iran, were

collected. The water in soil samples and plant components were extracted using home-made CVD system before isotope analysis by mass spectrometer. Finally, IsoSource model was used to estimate the contributions of different sources to the *H. ammodendron* xylem water.

2. Materials And Methods

2.1. Study area

Semnan province is located on the central plateau of Iran and the southern slopes of the Alborz Mountain range, which has arid and semi-arid climate. In general, the height decreases from north to south and often includes flat lands without slopes. Groundwater table in late winter and early spring is higher than summer and autumn. Due to the lack of surface water, groundwater plays an essential role in providing water needed for drinking purposes and agricultural activities. The average annual precipitation and evaporation are 180 and more than 2400 mm, respectively. This area does not have rich vegetation. In order to prevent the desertification and the movement of sands, *H. ammodendron* has been planted in the form of hand-planted forests. Figure 1 shows the location of the studied areas and the sampling points in Semnan and Damghan plains.

2.2. Sampling

Six hand-planted *H. ammodendron* forests (3 points in Semnan Plain and 3 points in Damghan Plain) were selected. Plants, groundwater and different layers of soil were sampled at each point on May 29 and 30, 2021. These were sampled in morning between 08:00 and 11:00 AM; after the dew disappeared and before warm-up. It should be noted that there was no rainfall during sampling and the last rainfall event had occurred 17 days before sampling day (< 5 mm, May 12, 2021).

At each sampling point, twig samples with a diameter of about 10 mm and a length of about 100 mm were selected and collected at a height of about 1.5 meters from the ground. As soon as sampling, the dry barks were scraped to avoid contamination of xylem water (Liu et al. 2016; Phillips and Gregg 2003). Then, they were immediately placed in glass vials, caped and sealed with parafilm and stored in well-sealed containers with ice for transport from the field to the laboratory. The characteristics of *H. ammodendron* at the sampling points are presented in Table 1.

At the same time the twigs were sampled, close to the trunk of each plant the soil samples were collected using a hand augar at 30 cm intervals up to the depth of 180 cm. We collected three replicated soils at each interval. Two replicates were mixed to analyze soil water isotope and soil water content and the other one was kept to determine the soil texture. The soil samples were immediately placed in glass vials sealed with caps and wrapped with parafilm to prevent evaporation and stored in insulated containers at freezing temperature.

Finally, groundwater samples were collected from adjacent wells around each plant. For this, first, the glass vials were washed with the same well water and then filled to the top. The filled vials were sealed

with caps and wrapped with Parafilm, then stored in the insulated containers. In the laboratory, the samples were stored in the refrigerator (at 4°C) for later processing.

For the purpose of soil texture determination, the soils were passed through a 2 mm sieve and the soil texture was determined by hydrometer method. Table 2shows the proportion of different soil particles six sampling points.

2.3. Plant and soil water extraction

There are various methods for extracting water from plant and soil samples (Fischer et al. 2019). Vacuum extraction is the most commonly used technique for extracting water from these types of samples (Koeniger et al. 2011). Using of a small sample size has made this method attractive and widely applicable (Orlowski et al. 2018). In this method, the plant or soil sample is heated in a tube under certain vacuum conditions. Then sample water is evaporated and the vapor is frozen in a cooling trap (liquid nitrogen) (Ingraham and Shadel 1992; Orlowski et al. 2013) and after defrosting, the isotopic composition of the sample water can be analyzed (Fig. 2).

2.4. Isotope analysis

In this study, vacuum extraction system includes a port that connects the sample tube to the collection tube and the vacuum system through a T-shaped tube. The vacuum system includes a vacuum pump (RVP Oil Rotary Vacuum Pump), a pressure gauge (Edwards WRG-S-NW25) and a valve that isolates the port from the vacuum system. Before water extraction process, the port is first checked for leaks. The port must be able to maintain a vacuum of less than 100 mTorr (Goebel and Lascano 2012), otherwise the connections must be checked to achieve this vacuum. In the process of water extraction, firstly, the sample whose water is to be extracted is placed inside the sample tube and thin tube is placed inside the liquid nitrogen (LN_2), so that the sample freezes to prevent the loss of water vapor during creating a vacuum and then placed on the port. Then vacuum (up to 100 millitorr) is applied to the system to make a vacuum and remove any possible water contamination, and then the valve of the vacuum system is closed and the pump is turned off to isolate the port from the vacuum system. The sample tube is submerged into the dewar filled with distilled water, which is heated on a hot plate to a temperature of nearly 90°C, and the sample water evaporates under heat conditions and collected in the collection tube, which is immersed in the dewar filled with LN_2, it accumulates and freezes. At the end of the extraction process, the remaining water in the joints of the glasses are directed to the collection tube using a heat gun. The resulting ice after melting (liquid water sample) is available for isotope analysis. The water samples were passed through 0.45 micrometer filters and then transferred in 2 ml vials caped sealed with parafilm and stored in a refrigerator at 4°C until isotopic analysis.

To prove the reliability of the water extraction system, the isotopic composition of water samples with known isotopic composition were measured by mass spectrometer after extraction from the above

system. The performance test of the system showed an acceptable agreement of the isotopic values of the extracted water with its actual values.

Isotopic analysis

$^{2}H/H$ and $^{18}O/^{16}O$ isotopic ratio measurements of water samples were determined by Dual Inlet-IRMS. In measurements of $^{2}H/H$ ratios, the water sample is equilibrated with hydrogen gas for 1.5 hours using a platinum catalyst. The H_2 gas absorbs the isotopic signatures of water and then enters the cryogenic water trap to remove the remaining water vapor. Then the dry H_2 gas enters the IRMS through the dual inlet. The 3/2 mass ratio was used to evaluate $^{2}H/H$ ratio.

For $^{18}O/^{16}O$ analyses, the water samples is equilibrated with CO_2 for 4.5 hours. Equilibrated CO_2 enters the IRMS through a dual inlet. The 46/44 mass ratio of the equilibrated CO_2 gas is used to evaluate the $^{18}O/^{16}O$ ratio.

The result of the measurements is usually expressed with the symbol δ (Leibundgut et al. 2011):

$$\delta\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

Where: R_{sample} denotes the isotope ratio of $^{2}H/H$ or $^{18}O/^{16}O$ of a sample and $R_{standard}$ those of the standard.

Finally, the contribution of each possible water source of xylem water of *H. ammodendron* was estimated using the IsoSource 1.3.1 model (Phillips and Gregg 2003; Phillips et al. 2005). In this study, the IsoSource model used in small increments (1%). It should be also noted that the oxygen isotopes were used to estimate the water sources of *H. ammodendron*, due to the high- fractionation of hydrogen isotopes in xerophytes tissue (Ellsworth and Williams 2007; Zhang et al. 2018; Wu et al. 2019b).

3. Results

3.1. Soil water content (SWC) and isotopic variations

The SWC was obtained in different soil layers. Weight analyzes before and after soil water extraction showed that water present in the soil was almost completely extracted. In all sampling points of Semnan and Damghan plains, SWC increased with increasing soil depth ($p < 0.01$). Figure 3 shows the results of water extracted from soil samples in both Semnan and Damghan plains at different depths.

SWC in both plains increased gradually with increasing soil depth. The moisture content in the shallow soil layers was much lower than the deep layers, which indicates the effect of evaporation. The high surface temperature due to the solar radiation in May is an important cause of decreasing the soil moisture content in these layers. As the soil depth increased, the influence of temperature on soil water

content decreased and evaporation decreased, as a result, the moisture content in the lower soil depths increased (as Cao et al. (2013) have also reported).

The values of δ^2H and $\delta^{18}O$ in soil water content in different depths also showed changes. Figure 4 shows the values of δ^2H and $\delta^{18}O$ in different soil depths. At points S1, S2, D2 and D3, the abundance of heavy isotopes was generally higher in the shallow soil layer than in deeper soil layers due to intense evaporation (Zhang et al. 2018; Gazis and Feng 2004). In general, the values of δ^2H and $\delta^{18}O$ almost gradually decreased and reached a relatively constant trend at lower depths with increasing soil depth (the last two layers)

In the middle soil layers (60–120), the changes in δ^2H and $\delta^{18}O$ values were more than in other layers, which can be attributed to changes in the soil particles. Therefore, depending on the SWC and the similarities in the values of δ^2H and $\delta^{18}O$ in different soil layers, the soil profile is divided into three main layers:

The shallow soil layer (0–60 cm): which is more affected by evaporation.

The middle soil layer (60–120 cm): where the phase change occurs and has dynamic changes.

Deep soil layer (120–180 cm): which is almost stable.

In points S3 and D1, the values of δ^2H and $\delta^{18}O$ decreased up to a depth of 120 cm and then increased with depth. This might be due to the capillary action, indicating that groundwater could charge the deep soil layer. However, the isotopic values of groundwater were depleted that those of soil water. This denotes that groundwater charges the deep soil layers in the form of gaseous water.

Table 3 shows the relationship between the soil particles and the isotope values. As it can be seen, there is a high correlation between heavy isotope values and sand particles. The correlation of δ^2H and $\delta^{18}O$ with sand particles are 0.507 and 0.573, respectively. This high positive correlation indicates that the more sand particles in the soil texture, the more water loss from soil moisture due to evaporation and the higher values of δ^2H and $\delta^{18}O$ (Zhao et al. 2021).

3.2. Variable stable isotope values of groundwater

$\delta^{18}O$ values of groundwater in Semnan plain varies from − 9.11 to -8.08 and in Damghan plain from − 10.76 to -9.54. The range of δ^2H values in Semnan and Damghan plains is from − 66.50 to -62.9 and − 79.60 to -74.20, respectively (Fig. 5). The small changes in the abundance of stable isotopes of groundwater show that the groundwater source is relatively stable and the water input from outside the groundwater table from the nearby plains is either non-existent or very little.

3.3. Variable stable isotope values of *H. ammodendron*

The δ^2H and $\delta^{18}O$ values in xylem are shown in Table 4. The isotope values in the green branch tissues are higher than those in the main stem. The abundance of heavy isotopes in the upper parts of the plant shows more transpiration in these parts, which causes the loss of lighter isotopes in this part of the plant. In Semnan plain, where the age of the plants is different (30, 20 and 10 years old), the δ^2H and $\delta^{18}O$ values of the old plant are higher than those of the younger ones. In Damghan Plain, where all plants are the same age, the δ^2H and $\delta^{18}O$ values have a smaller range of difference.

3.4. Analysis of variation of δ^2H and $\delta^{18}O$ values in *H. ammodendron* and water sources

The contribution of different water sources in the xylem of *H. ammodendron* was assessed by plotting polygons in dual isotope space in the IsoSource model. At most sites isotopes in xylem water fall outside the convex polygon. While xylem water composition could be better explained using polygons to enclose respective data sets when plotted in oxygen isotope space. This is due to the fact that the hydrogen isotope fractionation is high in the stem and root tissues of plants in arid areas. Also, Ellsworth and Williams (2007) have reported that the δ^2H values in xylem water do not accurately represent the δ^2H values of the soil water and groundwater, while the values of $\delta^{18}O$ in the xylem water accurately represent its values in the soil water and groundwater. Therefore, in this study, we used only the $\delta^{18}O$ values for tracing the feasible different water resources of *H. ammodendron*. Table 5 shows the proportions of feasible different water sources in *H. ammodendron* in wet season based on $\delta^{18}O$ (%) using the IsoSource model. At point S1, *H. ammodendron*obtained water mainly from the shallow and middle layers (81.9%), but it obtained only 6% from the groundwater. At point S2, the plant used water uniformly and equally from the shallow, middle and deep soil layers as well as the groundwater. At point S3, the primary water use source was groundwater with a contribution of 54.6% and the second water use source was soil water of the middle layer (25.4%). At point D1, the plant obtained a large proportion (49.3%) from the groundwater, and the rest almost uniformly from the shallow, middle and deep soil layers. At point D2, the plant used the highest proportion of groundwater (95.8%) and almost used no water from the surface and middle layers. At point D3, the plant mainly used groundwater (54%) and (26.9%) water from the shallow soil layer and almost a fifth (19.1%) from the the middle and deep soil layers.

4. Discussion

4.1. CVD system

CVD is one of the most common and accurate methods of direct water extraction in soil samples and different plant components before isotope analysis with mass spectrometer. In this study, a simple research sample of CVD system is basically similar to the system made by Goebel and Lascano (2012) was designed, built and set up. The performance of the system was validated by comparing isotope values of waters with known isotopic composition before and after extraction through the system. A vacuum threshold of 24 mTorr was usually established about 2–3 minutes after vacuum was applied to the system, and after the vacuum was cut off and the valve was closed, the vacuum port was maintained

at 100 mTorr for 2–3 hours, the time required to extract water from the plant and soil samples according to the studies of Goebel and Lascano (2012). The water extraction time for soil samples was at least 2 hours, and for soils with finer texture, more time for extraction (up to 3 hours) was considered.

4.2. SWC distribution and isotopic variations

In all 6 sampling points, the surface layers had a lower SWC than the deeper soil layers. In points S1, S2, D2 and D3, the δ^2H and $\delta^{18}O$ values in the shallow soil layers were higher and gradually decreased with increasing depth and SWC. The δ^2H and $\delta^{18}O$ values in shallow soil layer (0–30 and 30–60 cm) have different behavior which is due to high evaporation in this layer and isotopic fractionation as a result and previous days' rainfall input. The isotopic values decreased with increasing depth. But, in points S3 and D1, the water in deep soil layer was heavier than the water in upper soil layers which was caused by the capillary action. The soil texture can affect the isotopic composition of soil water. The results showed the direct relationship between abundance of heavy isotopes and sand particles; with increasing sand particles in the soil, the abundance of heavy isotopes in soil water increases which is consistent with the results of Meißner et al. (2014). The small changes in the δ^2H and $\delta^{18}O$ values of groundwater samples indicate that the groundwater source is completely stable and the presence of external water do not exist or are very small. The δ^2H and $\delta^{18}O$ values in the green branch tissues were higher than those in the woody stem. This is due to the fact that evaporation occurs farther from the leaf surface which is more exposed to sunlight than other parts of a plant.

4.3. Contributions of water sources in *H. ammodendron*

The studies generally show that in dry areas, *H. ammodendron* rely on soil water from different depths and the groundwater in the wet season. Water sources use differentiation by *H. ammodendron* can be related to the wide range of environmental conditions of this species. Previous studies have reported that *H. ammodendron* used mainly water from different soil layer and the groundwater (Hui et al. 2008; Liu et al. 2016). In recent years, some researchers have stated that *H.ammodendron* can absorb many available dense water sources, and more researches are needed to judge which is the main source. *H.ammodendron* maintains its growth under seasonal variation in water-sources-use. Findings of another research have indicated that the water in the soil around the root and the groundwater are the most important water sources for *H. ammodendron*. Liu et al. (2016) reported that *H. ammodendron* is dormant in winter, so they cannot effectively use winter rainfall and snow (if any) directly. Previous studies also showed that *H. ammodendron* does not use rainfall directly and surface roots absorb it indirectly when rain penetrates into the soil.

In this study, we employed the natural abundance of water isotopes to assess water uptake of *H. ammodendron* in desert region of Iran. While both the δ^2H and $\delta^{18}O$ values of extracted soil water were depleted with increasing soil depth and soil moisture, the hydrogen isotopic fractionation was observed when plotting polygons in dual isotope space in the IsoSource model. This was already mentioned by other studies (Ellsworth and Williams 2007) that the δ^2H values in xylem water do not accurately represent the δ^2H values of the soil water and the groundwater, while the values of $\delta^{18}O$ in the xylem

water accurately represent its values in the soil water and groundwater. The hydrogen isotopic composition may be affected by water exchange with soil particles; this could either be exchangeable hydrogen on the clay surface or exchangeable O-bonded hydrogen in the organic matter, which can be the result of mutual effects of water and soil and hydrogen exchange or hydrogen exchange with exchangeable Oxygen bonds in organic materials (Meißner et al. 2014). Therefore, we used only the ^{18}O isotopic values in our study. As the recorded amount of last rainfall received was < 5 mm, it was not sufficient enough to penetrate into the soil layers. Therefore, soil water content in soil layers did not charge by rainfall.

In our study, the $\delta^{18}O$ values for the xylem water of the *H. ammodendron* at six sampling sites imply that *H. ammodendron* mainly use the groundwater at wet season. In 4 points (S3, D1, D2 and D3) *H. ammodendron* used generally groundwater, in one point used from different layers of soil and groundwater uniformly (S2) and in just one sampling point it used from the surface and middle layers of the soil (S1). These findings are consistent with the results of Liu et al. (2016) that reported that groundwater is the main source of *H. ammodendron* following with the shallow soil layer water.

5. Conclusions

The stable isotope technique was used to find out the dependency of *H. ammodendron* on soil water and groundwater in two selected plains of Iran, Semnan and Damghan. The enriched isotope values of shallow soil layer could be explained by fractionation effect resulting from evaporation. The $\delta^{18}O$ values of *H. ammodendron* xylem water from five of six selected sites were similar to those of groundwater, suggesting that this species mainly used groundwater, but it was found at only one site, that both the shallow and the middle soil water were its main water source. Overall, the patterns in $\delta^{18}O$ values from five of six selected sites indicate the ability of *H. ammodendron* to absorb water from the groundwater as its main water source in wet season.

It should be noted that to the best of our knowledge, this is the first study on stable isotope analysis of water sources for *H. ammodendron* in the arid region of Iran. It is obvious that with data only from 6 sites, our understanding of water uptake of *H. ammodendron* is limited. We should extend our studies to seasonal variation in the water source dependency of *H. ammodendron* over a long period of years and including more sampling sites in order to obtain significant conclusion.

Considering that planting *H. ammodendron* is developing in different dry areas of Iran, it is crucial to create strategic plans to prevent damages to groundwater resources and maintaining the long-term sustainability of groundwater.

Declarations

Funding: No funding was received for conducting this study.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Ethical Permissions: Not declared by the authors.

Consent for publication: Not applicable

Authors Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Dr. Pouyan Dehghan Rahimabadi], [Dr. Vahideh Faghihi], [Prof. Dr. Hossein Azarnivand], [Dr. Javad Fasihi], [Dr. Arash Malekian] and [Eng. Behzad Yadollahzadeh]. The first draft of the manuscript was written by [Dr. Pouyan Dehghan Rahimabadi] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Tables are available in Supplementary Files section.

Figures

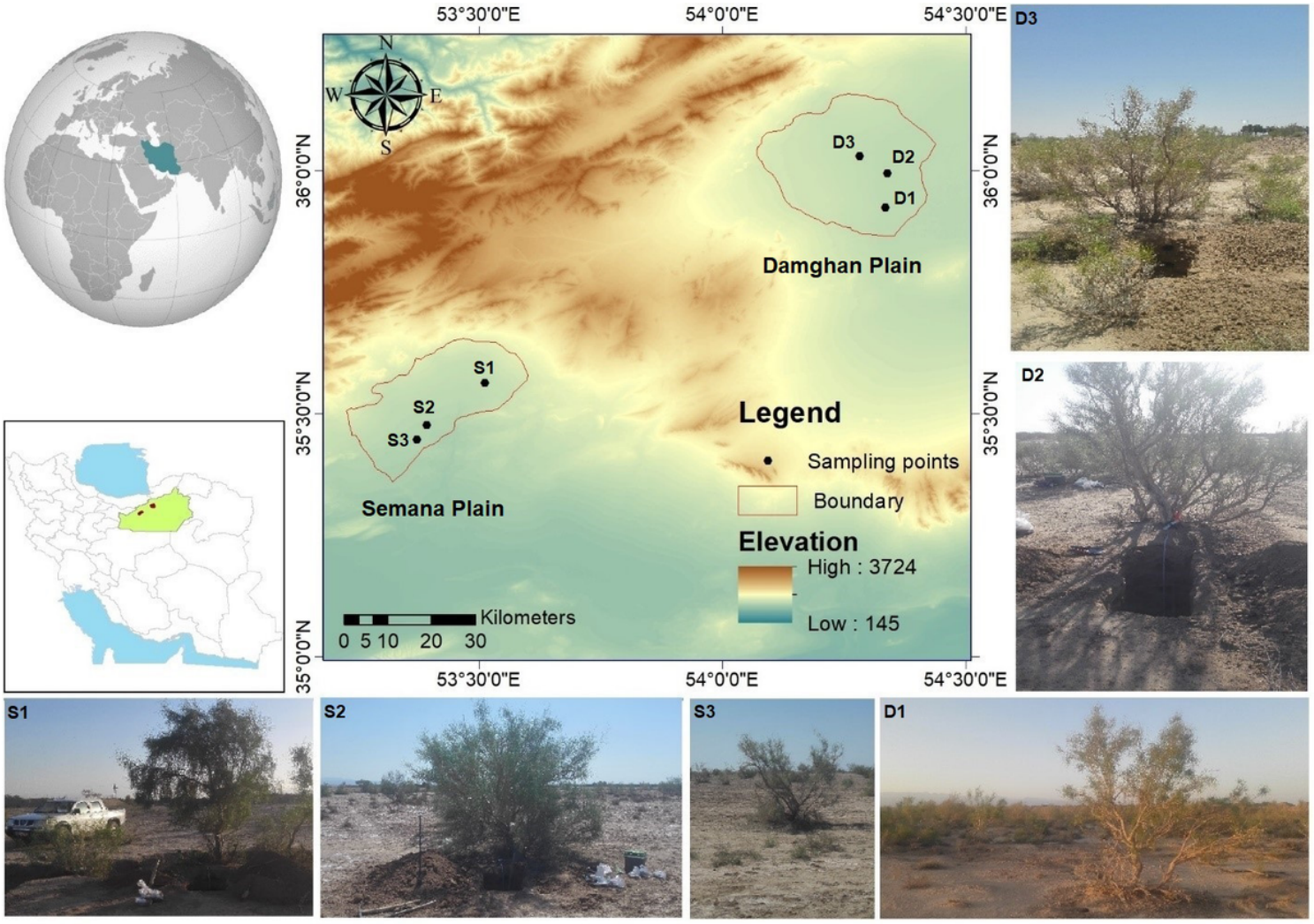


Figure 1

The location of sampling points

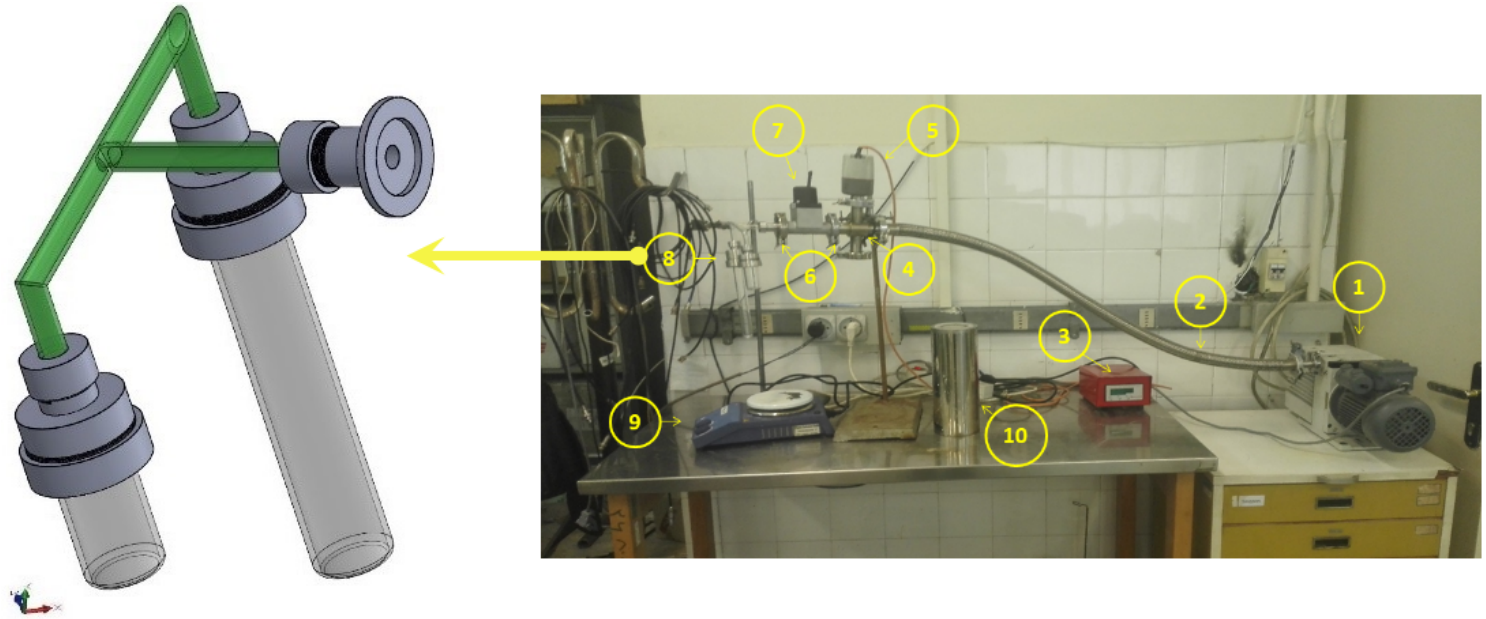


Figure 2

Cryogenic vacuum extraction system in the laboratory

Including 1. Vacuum Pump, 2. Connector Hose, 3. Vacuum Gauge Display, 4. Four-Way Vacuum Line Connector, 5. Vacuum Gauge, 6. Clamp as Connector, 7. Vacuum Valve, 8. Glass Setup, 9. Plate Heater, 10. Liquid Nitrogen Dewar

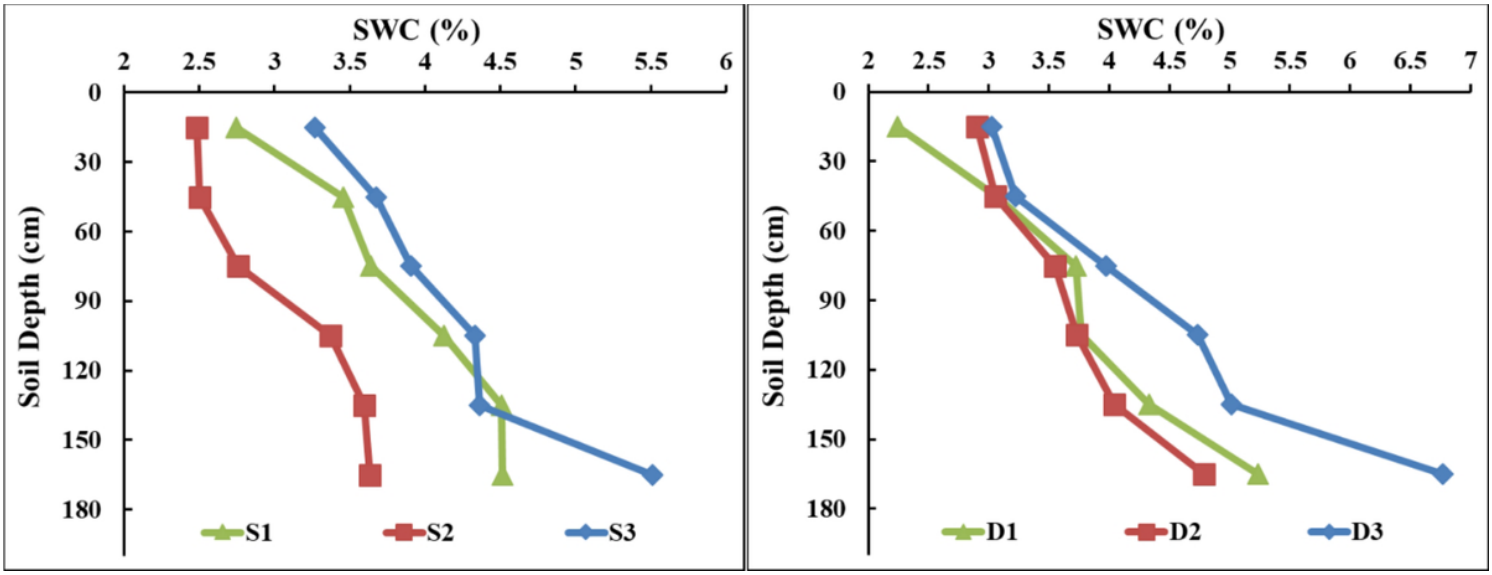


Figure 3

SWC at different depths

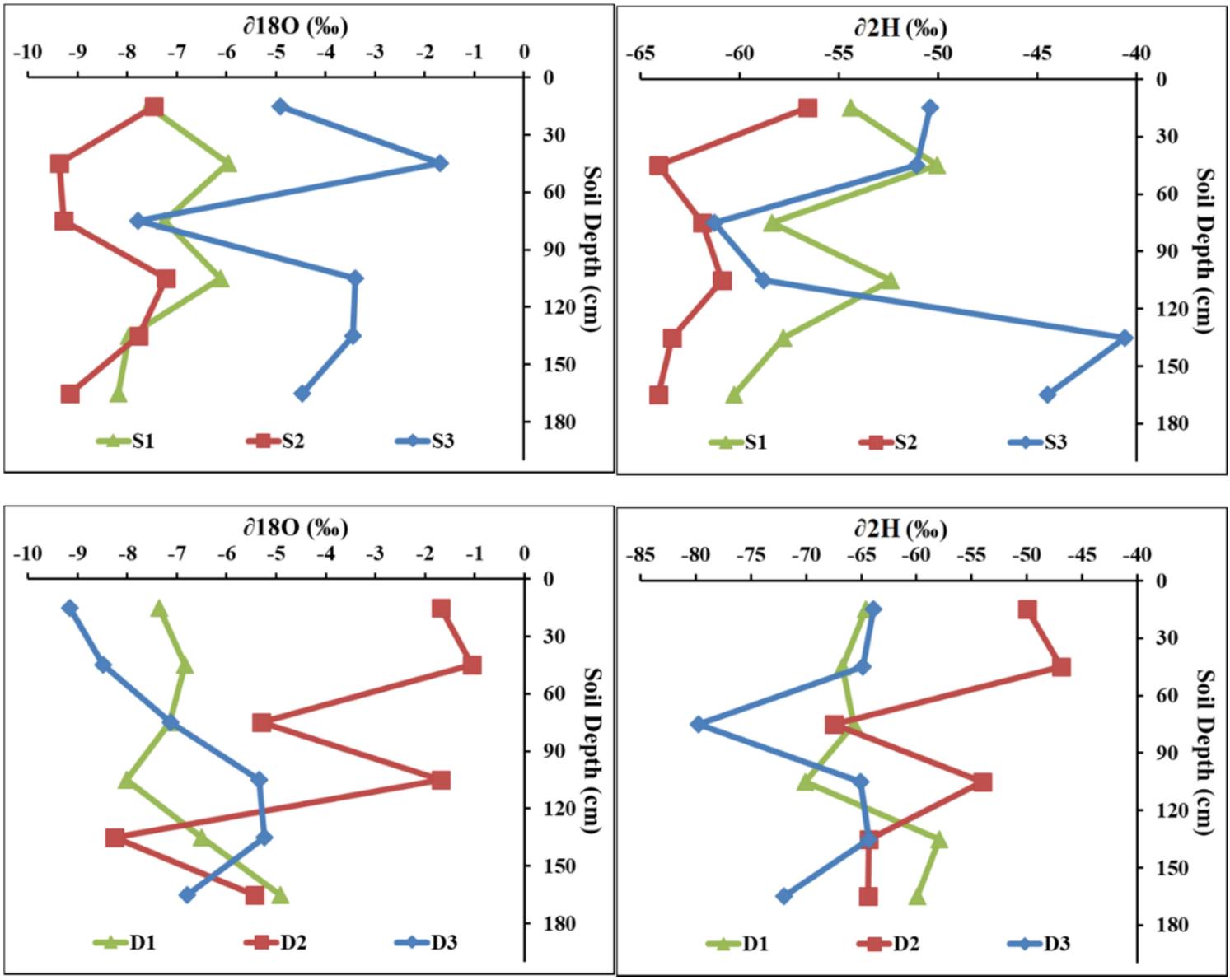


Figure 4

Vertical distributions of ^{18}O and ^{2}H at different soil depths

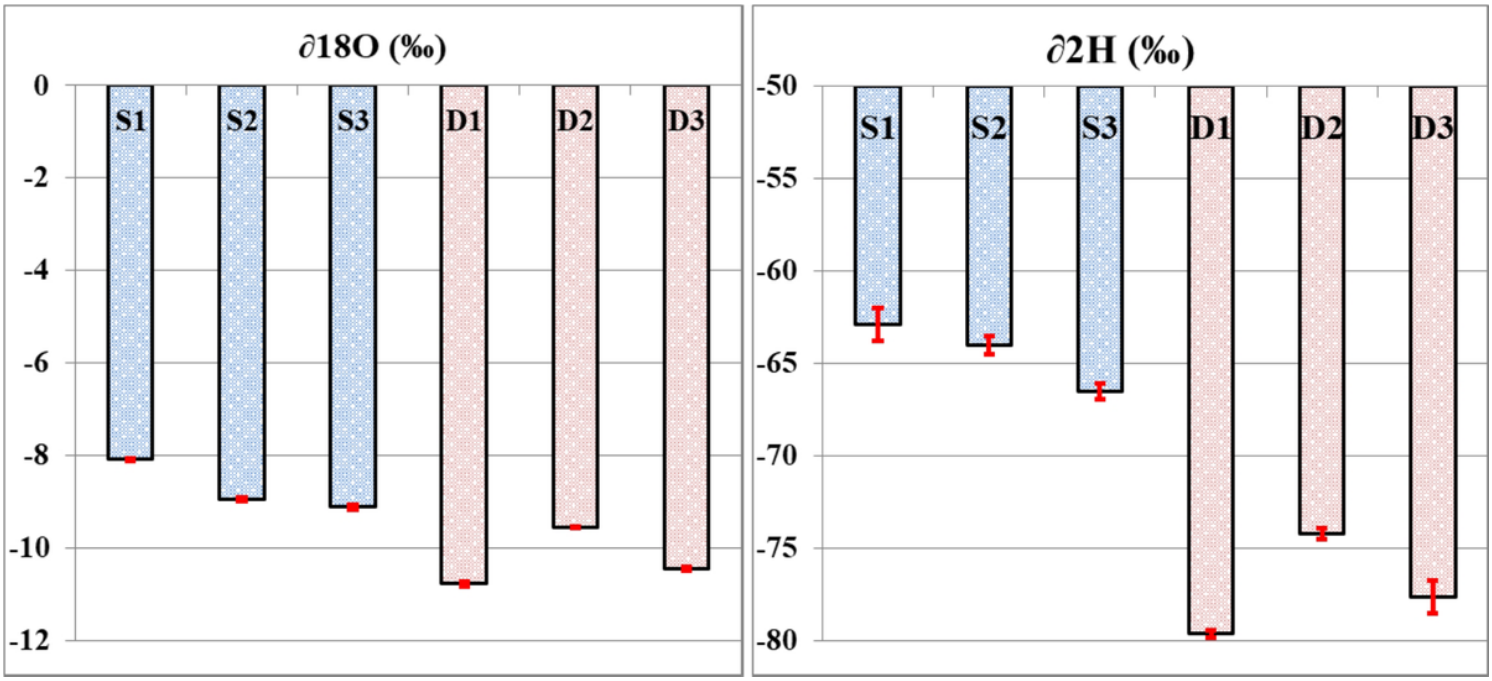


Figure 5

Distributions of ^{18}O and ^{2}H at groundwater samples

Supplementary Files

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