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Determination of the $\delta^{2}H$ and $\delta^{18}O$ of Soil Water and Water in Plant Matter; RSIL Lab Code 1700

Chapter 19 of
Section C, Stable Isotope-Ratio Methods
Book 10, Methods of the Reston Stable Isotope Laboratory

Techniques and Methods 10–C19

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KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

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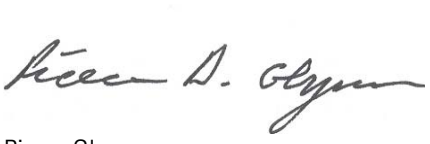
Foreword

The Reston Stable Isotope Laboratory (RSIL) provides stable isotope analyses on a routine basis for a large user community within the U.S. Geological Survey (USGS) and elsewhere. The RSIL also serves the USGS National Research Program (NRP) through its project on Stable Isotope Fractionation in Hydrologic Processes. The NRP conducts basic and problem-oriented hydrologic research in support of the mission of the USGS. The Stable Isotope Fractionation in Hydrologic Processes project conducts research on the use of isotope-ratio measurements in studies of water resources and environmental quality. One objective of this project is to develop new techniques for isotopic analysis of hydrogen, nitrogen, oxygen, carbon, and sulfur in environmental samples. New analytical techniques expand the range of tools available for studying the movement of those elements in hydrologic and biogeochemical systems. Another objective of the project is to test new applications of isotope measurements in specific field settings. Field studies of isotope behavior have contributed to understanding water-supply sustainability, groundwater/surface-water interactions, paleoclimate history, biologic cycling of nutrients, groundwater contamination, and natural remediation. This project also contributes to the improvement of measurement science and the development of isotope databases.

Book 10 of the Techniques and Methods series of the USGS contains detailed descriptions of methods of the RSIL, including routine analytical methods called standard operating procedures (SOPs), along with safety guidelines, maintenance procedures, and other information about the operation of the RSIL. Section C of book 10 contains the SOPs for a variety of methods to measure stable isotope ratios, each of which constitutes a chapter. Each chapter is limited to a narrow field of subject matter to permit flexibility in revision as the need arises.



Pierre Glynn
Chief, Branch of Regional Research, Eastern Region

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Conversion Factors

Multiply	By	To Obtain
	Length	
micrometer (μm)	0.00003937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
	Volume	
microliter (μL)	0.0610210^{-3}	cubic inch (in^3)
milliliter (mL)	0.06102	cubic inch (in^3)
cubic centimeter (cm^3)	0.06102	cubic inch (in^3)
liter (L)	61.02	cubic inch (in^3)
	Mass	
nanogram (ng) = 10^{-3} μg	3.527×10^{-11}	ounce (oz)
microgram (μg) = 10^{-3} mg	3.527×10^{-8}	ounce (oz)
milligram (mg) = 10^{-3} g	3.527×10^{-5}	ounce (oz)
gram (g) = 10^{-3} (kg)	3.527×10^{-2}	ounce (oz)
kilogram (kg) = 10^{3} g; 10^{6} mg; 10^{9} μg; 10^{12} ng	2.205	pound (lb)
	Temperature	
Celsius (°C)	°F = 9/5 (°C) + 32	Fahrenheit (°F)

Acronyms and Abbreviations

ANSI	American National Standards Institute
cm	centimeter
DI-IRMS	dual-inlet isotope-ratio mass spectrometer
DIW	deionized water
g	gram
HPLC	high-performance liquid chromatography
IAEA	International Atomic Energy Agency
LIMS-LSI	Laboratory Information Management System for Light Stable Isotopes
mm	millimeter
mL	milliliter
MSDS	Material Safety Data Sheet
NFPA	National Fire Protection Association
NIST	National Institute of Standards and Technology
per mil, ‰	one part in one thousand parts, with value 10^{-3}
QA	quality assurance
QC	quality control
RSIL	Reston Stable Isotope Laboratory
SLAP	Standard Light Antarctic Precipitation
SOP	standard operating procedure
std	international measurement standard
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
VSMOW	Vienna Standard Mean Ocean Water

Symbols

<	less than
>	greater than
≤	less than or equal to

Determination of the δ^2H and δ^{18}O of Soil Water and Water in Plant Matter; RSIL Lab Code 1700

By Kinga Révész, Bryan Buck, and Tyler B. Coplen

Summary of Procedure

The purpose of the Reston Stable Isotope Laboratory lab code 1700 is to determine the $\delta(^2H/^1H)$, abbreviated as δ^2H, and the $\delta(^{18}O/^{16}O)$, abbreviated as δ^{18}O, of soil water and water in plant matter. This method is based on the observation that water and toluene form an azeotropic mixture at 84.1 °C. This temperature is substantially lower than the boiling points of water (100 °C) and toluene (110 °C), but water and toluene are immiscible at ambient temperature. The water content of a soil or plant is determined by weighing, drying, and reweighing a small amount of sample. Sufficient sample to collect 3 to 5 milliliters (mL) of water after distillation is loaded into a distillation flask. Sufficient toluene is added so that the sample is immersed throughout the entire distillation to minimize evaporation of water, which would affect the δ^2H and δ^{18}O values. The mixture of sample and toluene is heated in a flask to its boiling point (84.1 °C) so that water from the sample and toluene can distill together into a specially designed collection funnel. The temperature of 84.1 °C is maintained until the water has been quantitatively transferred to the collection funnel, at which time the temperature is raised to the boiling point of the remaining component (toluene, 110 °C). The collection funnel is maintained at ambient temperature so that the sample water and toluene can be separated physically. After separation, the sample water is purified by addition of paraffin wax to the container with the sample water, capping the container, and heating to approximately 60 °C to melt the wax. Trace amounts of toluene will dissolve in the wax, purifying the sample water for isotopic analysis.

The isotopic composition of the purified water is then determined by equilibration with gaseous hydrogen or carbon dioxide, followed by dual-inlet isotope-ratio mass spectrometry. Because laser-absorption spectrometry is sensitive to organic compounds, such as trace toluene remaining in water samples, water samples should be analyzed for isotopic composition only by mass spectrometry and not by laser-absorption spectrometry.

Reporting Units and Operational Range

Variations in stable isotope ratios typically are small. Stable isotope ratios commonly are measured and expressed as the relative difference in the ratio of the number of the less abundant isotope (usually the heavy isotope) to the number of the more abundant isotope (usually the light isotope) of a sample with respect to the measurement standard, std (Coplen, 2011). This relative difference is designated δ^iE, which is a shortened form of $\delta(^iE/^jE)$ or $\delta(^iE)$, and is defined according to equation 1 (Coplen, 2011):

$$\delta^i\mathrm{E} = \delta\left(^i\mathrm{E}\right) = \delta\left(^i\mathrm{E}/^j\mathrm{E}\right) = \frac{N\left(^i\mathrm{E}\right)_\mathrm{P}/N\left(^j\mathrm{E}\right)_\mathrm{P} - N\left(^i\mathrm{E}\right)_\mathrm{std}/N\left(^j\mathrm{E}\right)_\mathrm{std}}{N\left(^i\mathrm{E}\right)_\mathrm{std}/N\left(^j\mathrm{E}\right)_\mathrm{std}} \quad \textbf{(1)}$$

where $N(^iE)_p$ and $N(^jE)_p$ are the numbers of the two isotopes ^iE and ^jE of element E in specimen P, and equivalent parameters follow for the international measurement standard, "std." A positive δ^iE value indicates that the specimen is enriched in the heavy isotope, ^iE, relative to the std. A negative δ^iE value

indicates that the specimen is depleted in the heavy isotope, ${}^{i}\mathrm{E}$, relative to the std. For stable hydrogen isotopes, $\delta^{2}\mathrm{H}$ (sometimes written in the literature as $\delta\mathrm{D}$) is defined as follows:

$$\delta^{2}\mathrm{H} = \delta\left({}^{2}\mathrm{H}\right) = \delta\left({}^{2}\mathrm{H}/{}^{1}\mathrm{H}\right) = \frac{N\left({}^{2}\mathrm{H}\right)_{\mathrm{p}}/N\left({}^{1}\mathrm{H}\right)_{\mathrm{p}} - N\left({}^{2}\mathrm{H}\right)_{\mathrm{std}}/N\left({}^{1}\mathrm{H}\right)_{\mathrm{std}}}{N\left({}^{2}\mathrm{H}\right)_{\mathrm{std}}/N\left({}^{1}\mathrm{H}\right)_{\mathrm{std}}} \quad \textbf{(2)}$$

For stable oxygen isotopes, $\delta^{18}\mathrm{O}$ is defined as follows:

$$\delta^{18}\mathrm{O} = \delta\left({}^{18}\mathrm{O}\right) = \delta\left({}^{18}\mathrm{O}/{}^{16}\mathrm{O}\right) = \frac{N\left({}^{18}\mathrm{O}\right)_{\mathrm{P}}/N\left({}^{16}\mathrm{O}\right)_{\mathrm{p}} - N\left({}^{18}\mathrm{O}\right)_{\mathrm{std}}/N\left({}^{16}\mathrm{O}\right)_{\mathrm{std}}}{N\left({}^{18}\mathrm{O}\right)_{\mathrm{std}}/N\left({}^{16}\mathrm{O}\right)_{\mathrm{std}}} \quad \textbf{(3)}$$

The international measurement standards for both $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ measurements are Vienna Standard Mean Ocean Water (VSMOW) and Standard Light Antarctic Precipitation (SLAP) (Gonfiantini, 1978; Coplen, 1994). By interspersing local laboratory isotopic reference waters that were calibrated using international reference waters (VSMOW and SLAP) among the unknowns, the $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ values of unknown waters can be determined relative to the international measurement standards (Coplen, 1988, 1996).

The distillation and separation system was tested by analyzing water samples with known $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ values. The $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ values of test samples ranged from approximately –65 to –400 ‰ for $\delta^{2}\mathrm{H}$ measurements and from approximately –6.5 to –51.3 ‰ for $\delta^{18}\mathrm{O}$ measurements, which approximately covers the ranges of $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ of natural water. In addition, the system was tested by distilling soil water with known isotopic compositions. The routine analysis requires soil with a mass fraction of water of 5 percent or higher; however, with a reduction in accuracy of isotopic measurements, the system is capable of analyzing samples with a mass fraction of water as low as 3 percent. The accuracies of analytical measurements are similar to those of pure water that does not go through the distillation process (2-σ standard deviations of $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ measurements are 2 and 0.2 ‰, respectively). Between-sample memory is below detection.

Reference Materials and Documentation

Reference Materials Used, Storage Requirements, and Shelf Life

The standard reference scale used for reporting results of both $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$ measurements is the VSMOW-SLAP scale (Gonfiantini, 1978; Coplen, 1994), anchored by VSMOW (distributed by the International Atomic Energy Agency (IAEA) and the National Institute of Standards and Technology (NIST) of the United States as RM 8535) and SLAP (distributed by the IAEA and by NIST as RM 8537). Results expressed relative to the VSMOW-SLAP scale can be identified by the subscript VSMOW-SLAP, such as $\delta^{2}\mathrm{H}_{\mathrm{VSMOW\text{-}SLAP}}$ and $\delta^{18}\mathrm{O}_{\mathrm{VSMOW\text{-}SLAP}}$. These international measurement standards are assigned exact values of $\delta^{2}\mathrm{H}_{\mathrm{VSMOW\text{-}SLAP}} = 0$ and –428 ‰, respectively; they are assigned exact values of $\delta^{18}\mathrm{O}_{\mathrm{VSMOW\text{-}SLAP}} = 0$ and –55.5 ‰, respectively. The Reston Stable Isotope Laboratory (RSIL) has calibrated local laboratory waters against VSMOW and SLAP reference waters to develop secondary reference materials. Some of the secondary reference materials include deionized water (DIW) assigned a $\delta^{18}\mathrm{O}_{\mathrm{VSMOW\text{-}SLAP}}$ value of –6.54 ‰, a second DIW reference material assigned a $\delta^{2}\mathrm{H}_{\mathrm{VSMOW\text{-}SLAP}}$ value of –62.3 ‰, and Antarctic water assigned $\delta^{2}\mathrm{H}_{\mathrm{VSMOW\text{-}SLAP}}$ and $\delta^{18}\mathrm{O}_{\mathrm{VSMOW\text{-}SLAP}}$ values of –399.5 and –51.23 ‰, respectively.

Documentation

All measurement data for the calibrations are stored in the Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI) (Coplen, 2000) under sample identifiers with a prefix of W; local laboratory reference waters analyzed daily include W-62000, W-50000, and W-63333, which are DIW, DIW, and Antarctic water, respectively.

Labware, Instrumentation, and Reagents

The apparatus for sample preparation of soil water and water in plant matter (fig. 1) consists of a heating element that can be adjusted by a variable transformer, a 250- or 500-mL flask, a specially designed separatory receiving funnel (fig. 2), and a straight condenser. The funnel has a sidearm at an angle of 105°. This angle allows toluene to overflow and drip back into the flask without obstructing the funnel. The distillation is continued until all water drops that condense on the interior glass surfaces fall into the collection funnel. In addition, the coalescence of water drops on the interior glass walls of the entire apparatus is facilitated by coating the condenser and the funnel with a solution of dimethyldichlorosilane compound to change the surface from hydrophilic to hydrophobic. The monolayer formed is permanent; that is, the apparatus needs only a single treatment.

The instrumentation used for performing δ^2H measurements of water is the VG hydrogen dual-inlet isotope-ratio mass spectrometer (DI-IRMS) equipped with a H_2-H_2O platinum equilibration peripheral (RSIL lab code 1574; Révész and Coplen, 2008a). The instrumentation used for performing δ^{18}O measurements is the DuPont carbon dioxide DI-IRMS equipped with a CO_2-H_2O equilibration peripheral (RSIL lab code 489; Révész and Coplen, 2008b).

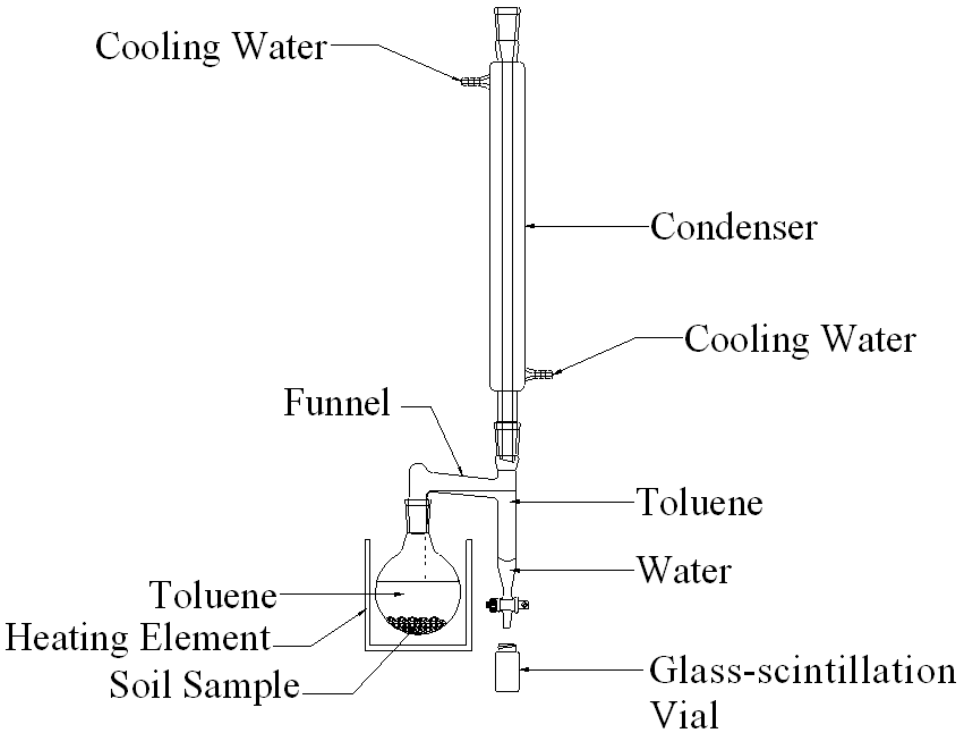

Figure 1. Diagram of distillation apparatus (Révész and Woods, 1990). Close up of funnel is shown in fig. 2.

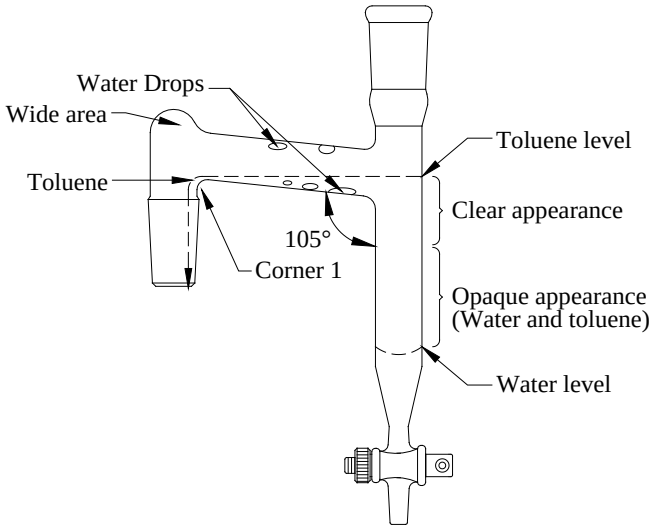

Figure 2. Specially designed receiving funnel (Révész and Woods, 1990).

Sample Collection, Preparation, Analysis, Retention Times, and Disposal

Sample Containers, Preservation, and Handling Requirements

Each soil or plant-matter sample is collected in a wide-mouth jar, which is tightly capped with a metal lid and sealed with electrical tape to prevent loosening and potential evaporation of sample water, which generally would increase the remaining sample in ^{2}H and ^{18}O. Twigs are cut approximately in 2.5-centimeter (cm) lengths, with a maximum thickness of 1.2 cm; bark may be removed if desired. The jars are labeled with isotopes to be determined. In the field, containers should be filled and capped as quickly as possible to minimize evaporation of the water sample. An amount of sample sufficient to yield a minimum of 3 mL of sample water is required for $\delta^{2}H$ and $\delta^{18}O$ analyses. Sufficient sample to prepare two aliquots of sample water is desired. Regardless of the amount of sample collected, the container size should be matched to the sample size. To minimize the effects of evaporation of water into the head space of the sample container, the containers should be full.

Treatment and preservation are not required; however, it is advisable to freeze plant samples and refrigerate soil samples to minimize microbial activity. If a sample is submitted from outside the continental United States (for example, from Hawaii and Puerto Rico), a copy of the RSIL U.S. Department of Agriculture soil permit must accompany the sample, which is available from the RSIL. An example is provided as appendix A.

Data Handling

Sample preparation involves logging samples into the LIMS-LSI (discussed below in the section titled “Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI)”). The login procedure involves logging samples in batches into LIMS-LSI, and batches can contain from 1 to 100 samples per batch (also called a project). Upon successful login, the LIMS-LSI program outputs printed labels for each sample container and a summary project report (Révész and Coplen, 2008a,b). Each sample label contains the Field ID (the identifier by which the sample submitter knows each sample) and

the unique RSIL Lab ID assigned by the LIMS-LSI. By using four parallel sample preparation systems, 15 to 20 samples can be processed each day. Each distillation requires about 20 minutes. The current distillation procedure, which includes the sections "Determining How Much Water is Contained in the Soil Sample," "Performing the Distillation," and "Removing the Toluene," is provided in appendix B. The systematic procedure is listed in the "Lab Procedures" binder. The isotopic measurements of the clean water obtained from the soil or plant-matter samples are made by DI-IRMS for $\delta^{2}H$ and $\delta^{18}O$ values (Révész and Coplen, 2008a,b). The $\delta^{2}H$ measurements are made with a DI-IRMS (Révész and Coplen, 2008a), which alternatively measures six cycles of the unknown H_2 gas and seven cycles of the "working reference" hydrogen H_2 gas. The $\delta^{18}O$ measurements are made with a DI-IRMS (Révész and Coplen, 2008b), which measures, alternatively, the unknown CO_2 gas and of the "working reference" CO_2 gas. Final daily correction factors are determined on the basis of the daily analyses of local laboratory reference waters using equations 4 and 5 or 6 and 7, which are represented with numerical values:

$$-6.54\ ‰ = m \times \delta^{18}O_{W\text{-}62000/VSMOW} + b \quad \textbf{(4)}$$

$$-51.23\ ‰ = m \times \delta^{18}O_{W\text{-}63333/VSMOW} + b \quad \textbf{(5)}$$

$$-62.3\ ‰ = m \times \delta^{2}H_{W\text{-}50000/VSMOW} + b \quad \textbf{(6)}$$

$$-399.5\ ‰ = m \times \delta^{2}H_{W\text{-}63333/VSMOW} + b \quad \textbf{(7)}$$

The values –6.54, –51.23, –62.3, and –399.5 ‰ are the values of secondary reference waters that have been calibrated using the international reference waters VSMOW and SLAP. The $\delta^{18}O$ and $\delta^{2}H$ values in equations 4 to 7 are the mean daily delta values of the respective local laboratory reference water relative to VSMOW, b is the additive correction factor, and m is the expansion coefficient. The procedure to apply correction factors is given in Révész and Coplen (2008a,b).

As a check on quality assurance, samples regularly are distilled and analyzed in replicate. If the replicates do not agree within acceptable tolerances, they are distilled and analyzed again until the desired statistically acceptable agreement among the results is achieved. The computerized LIMS-LSI will not release data until the tolerances are acceptable.

Problematic Samples

Problematic samples include those that have a mass fraction of water less than 3 percent. At such low mass fractions, water is strongly adsorbed to the soil particles or plant matter, and quantitative yield by distillation is difficult to achieve. If a quantitative yield is not achieved, the sample water may be isotopically fractionated. If soil contains minerals with water of crystallization (such as gypsum) and it loses this hydration water at a temperature less than 84.1 ºC, the isotopic composition of soil water of the sample must be determined by isotope balance of water of crystallization and the soil water by other methods; otherwise, the measured isotopic composition might be erroneous.

Soil samples have a high surface area; therefore, evaporation is enhanced. Evaporation alters the isotopic composition of the sample water. Any samples that were not properly capped are noted and returned to the sample submitter. Samples with cracked caps can be problematic because they enable evaporation. Caps with foam liners should be avoided and have been especially problematic because they allow water to evaporate due to the permeability of the foam.

Interferences

No interferences are known. One note of caution is that isotopic analysis should not be performed by laser-absorption spectrometry because small amounts of organic material can bias the measurement results (Brand and others, 2009; Singleton and others, 2009).

Troubleshooting and Bench Notes

If there are any leaks between the joints of the distillation apparatus, water can be lost during distillation, and the samples can be isotopically fractionated. If this occurs, the distillation should be terminated and the apparatus reassembled until a perfect seal is achieved. It is crucial for the distillation to continue an additional 10 to 15 minutes after the appearance of the first clear toluene drops in the funnel. The high-temperature toluene vapor will rinse water drops from the inner wall into the receiving funnel. The drops on the glass wall usually have different isotopic compositions than the bulk water accumulated in the funnel, resulting in an incorrect isotopic measurement result. If any water drops remain on the surface, a glass rod with an O-ring at the end should be used to push down the drops into the funnel so that they can be combined with the remainder of the sample water.

Maintenance and Maintenance Records

It is imperative to keep the apparatus clean. After each distillation, the funnel assembly must be thoroughly dried by wiping the residual toluene and water from the inside of the apparatus with a clean, dry, lint-free tissue. The inner part of the straight condenser must also be dried. This is done by blowing dry, oil-free, compressed air though the condenser until all traces of moisture are removed.

Sample Retention Time and Disposal

Samples are retained by the RSIL for at least 6 months after reporting data. Samples are then discarded unless the submitter has requested that the samples be returned.

The sample analysis records from the DI-IRMS computers are kept indefinitely on two different hard disks of the Data Back-Up computer. Paper reports are kept for a few weeks. Analytical results from each DI-IRMS are transmitted in real time to the LIMS-LSI and kept indefinitely. No data are entered by hand, and no manual calculations are performed on the data.

Data Acquisition, Processing, Evaluation, Quality Control, and Quality Assurance

Laboratory Information Management System for Light Stable Isotope (LIMS-LSI)

In the RSIL, the LIMS-LSI (Coplen, 2000) is used for data processing and evaluation. This system is a database program capable of (1) storing information about samples, (2) storing the results of mass spectrometric delta values of samples, (3) calculating analytical results using standardized algorithms stored in a database, (4) normalizing delta values using isotopic reference materials, and (5) generating templates to facilitate loading of samples. With this system, the following are ensured: (1) quality assurance (QA), (2) laboratory efficiency, (3) reduction of workload and errors owing to the elimination of retyping of data by laboratory personnel, and (4) a decrease in errors in data reported to sample submitters. This database provides a complete record of information on how laboratory reference materials have been analyzed and provides a record of what correction factors have been used as an audit trail for the RSIL.

Quality Control (QC) Samples

Approximately two local laboratory reference waters are distilled and analyzed daily with samples. One of these is Antarctic water that is highly depleted in ^{18}O and ^{2}H, and the other is laboratory DIW with typical $\delta^{18}O$ and $\delta^{2}H$ values. These are our QC samples.

An analyst examines the mass spectrometric analysis reports daily for problems. Additive correction factors and expansion factors are determined daily with the LIMS-LSI and applied to isotopic data. The analyst reviews the results and determines which samples need to be redistilled and reanalyzed to achieve acceptable statistics (for example, $\delta^{18}O$ standard deviation equal to or better than 0.2 ‰).

For each batch of samples, a graph is made of $\delta^{2}H$ plotted against $\delta^{18}O$. The $\delta^{2}H$ and $\delta^{18}O$ values of natural waters commonly have a high natural correlation; the loci of points are called the meteoric water line. Outliers are noted and redistilled, and they are reanalyzed for either or both $\delta^{2}H$ and $\delta^{18}O$ because these may be interesting research findings or laboratory errors, such as a mix up in samples.

Acceptance Criteria for All QC Samples

Acceptance criteria for QC samples are the same as acceptance criteria for the other samples. The rules are as follows:

For $\delta^{18}O$:

- If the standard deviation is <0.30 ‰, use the mean delta.
- If there are three or more analyses, delete the outlier and recalculate the mean.
- If the standard deviation of this recalculation is ≤0.30 ‰, use the mean from this recalculation.
- If none of the above is the case, the result is not acceptable, and corrective action is required.

For $\delta^{2}H$:

- If the standard deviation is <2 ‰, use the mean delta.
- If there are three or more analyses, delete the outlier and recalculate the mean.
- If the standard deviation of this recalculation is ≤2 ‰, use the mean of this recalculation.
- If the recalculation was performed on exactly three analyses and if recalculation of the mean results in a standard deviation value ≤2.5 ‰, use it.
- If the difference between the original standard deviation and the recalculated standard deviation is >1.1 and <3 ‰, use the mean of the recalculated standard deviation.
- If none of the above is the case, the result is not acceptable, and corrective action is required.

Uncertainty

The RSIL estimates expanded uncertainty ($U = 2\mu_c$) of $\delta^{2}H_{VSMOW\text{-}SLAP}$ measurement results. The expanded uncertainty provides an envelope that represents a 95 percent probability of encompassing the true value that has been determined from the aggregation of results over a period of years. The "long-term" expanded uncertainty is ±3 ‰ for the reporting of hydrogen isotopic compositions of soil water and plant water. The expanded uncertainty can be determined using the guide to the expression of uncertainty (Joint Committee for Guides in Metrology, Working Group 1 (JCGM/WG1), 2010). The application of expanded uncertainty to the reporting of stable isotope measurements is discussed by Coplen and others (2006). Our results suggest that if any given sample were resubmitted to the RSIL for hydrogen isotopic analysis, the measured value would fall within the uncertainty bounds of the previous result more than 95 percent of the time.

Similarly, the expanded uncertainty ($U = 2\mu_c$) estimated for reporting of $\delta^{18}O_{VSMOW\text{-}SLAP}$ measurement results has been determined from aggregating measurement results over time, and the long-term

uncertainty ($2\mu_c$) for soil water and plant water is ±0.3 ‰. Again, if any given sample were resubmitted to the RSIL for oxygen isotopic analysis, the measured value would fall within the uncertainty bounds of the previous result more than 95 percent of the time.

Corrective Action Requirements

If an analyst finds any problem with the daily reference-sample data, the analyst contacts the supervisor. The troubleshooting process will require an evaluation and reanalysis of certain samples to identify the origin of the problem.

If samples do not give satisfactory results after three or more separate distillations and analyses, the analyst averages all the data and reports the mean value. Such analytical results are indicated with a comment, and the customer will be advised of the problem by e-mail or other means.

Responsible Parties for All QA/QC Functions for Procedures Covered in RSIL SOPs

The analyst, with supervisory approval, is responsible for qualifying data and notifying customers.

Data Management and Records

In addition to evaluating daily sample analyses, an analyst also evaluates the data for each project every week, reports results to the customers, and files final project data reports in the laboratory "Correspondence" binder (Révész and Coplen, 2008a,b).

Health, Safety, and Waste-Disposal Information

Applicable Health and (or) Safety Issues

Personal Protection

Laboratory safety glasses and protective latex gloves are recommended whenever samples are handled, especially when the samples are of biological origin. When the submitter indicates hazardous chemical in the samples, the technician should consult the Material Safety Data Sheets (MSDS), which are on file in the laboratory and at the URL *http://www.ilpi.com/msds/#Manufacturers*. This URL provides links to the MSDSs of most chemical companies. Depending on the hazardous chemical, nitrile gloves may be needed.

Electrical Hazards

Electrical systems must conform to the National Electric Code, National Fire Protection Association Code (NFPA 70–1971), and the American National Standards Institute (ANSI) Code (C1–1971). For more information, consult the U.S. Geological Survey's *Safety and Environmental Health Handbook* (U.S. Geological Survey, 2002, sec. 4–4.1).

Shock hazards exist inside the instruments. Only an authorized service representative or an individual with training in electronic repair must remove panels or circuit boards where voltages are greater than 20 volts. The instruments require a third-wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.

Chemical Hazards

Toluene is flammable, and its vapor has a higher density than that of air. It is toxic via inhalation of vapors and skin contact. (See MSDS for personal protective equipment for working with toluene.) Each distillation should be carried out in a hood with flash-proof electrical heating equipment. Upon receipt, samples should be carefully inspected visually for obvious cracks. When the submitter submits a sample containing a hazardous chemical, the analyst should consult the appropriate MSDS about handling the sample.

The SO_2 reference gas is colorless and has a suffocating odor. Exposure to SO_2 can cause respiratory tract burns. Therefore, an appropriate ventilation unit must be installed in the Finnigan MAT ConFlo II interface. A leak test on the tank of gas and the line carrying SO_2 to the Finnigan MAT ConFlo II interface must be performed routinely.

Gas Cylinder Handling

Compressed gas cylinders must be handled and stored according to the U.S. Geological Survey's *Safety and Environmental Health Handbook* (U.S. Geological Survey, 2002, sec. 4–4.5.1). Each cylinder must be (1) carefully inspected when received; (2) securely fastened at all times with an approved chain assembly or belt; (3) capped at all times when not in use; (4) capped when transported; (5) transported only by a properly designed vehicle (hand truck); and (6) stored separately with other full, empty, flammable, or oxidizing tanks of gas, as appropriate.

Specific Waste-Disposal Requirements

The U.S. Department of Agriculture (USDA) requires that unanalyzed soil and plant samples from areas outside the continental United States be autoclaved or otherwise treated to kill biologically active organisms before disposal. The analyst should follow the rules in USDA Circular Q–330.300–1 at *http://www.geotech.org/survey/geotech/pdf/soil-circular.pdf.* At the RSIL samples are autoclaved with steam for 30 minutes at a pressure of 15 lb/in^2.

Waste toluene should be poured into a collection container and clearly labeled for disposal. Toluene-soaked wipes should be collected in plastic bags. Both used toluene and wipes should be given to the safety, health, and environmental officer for disposal.

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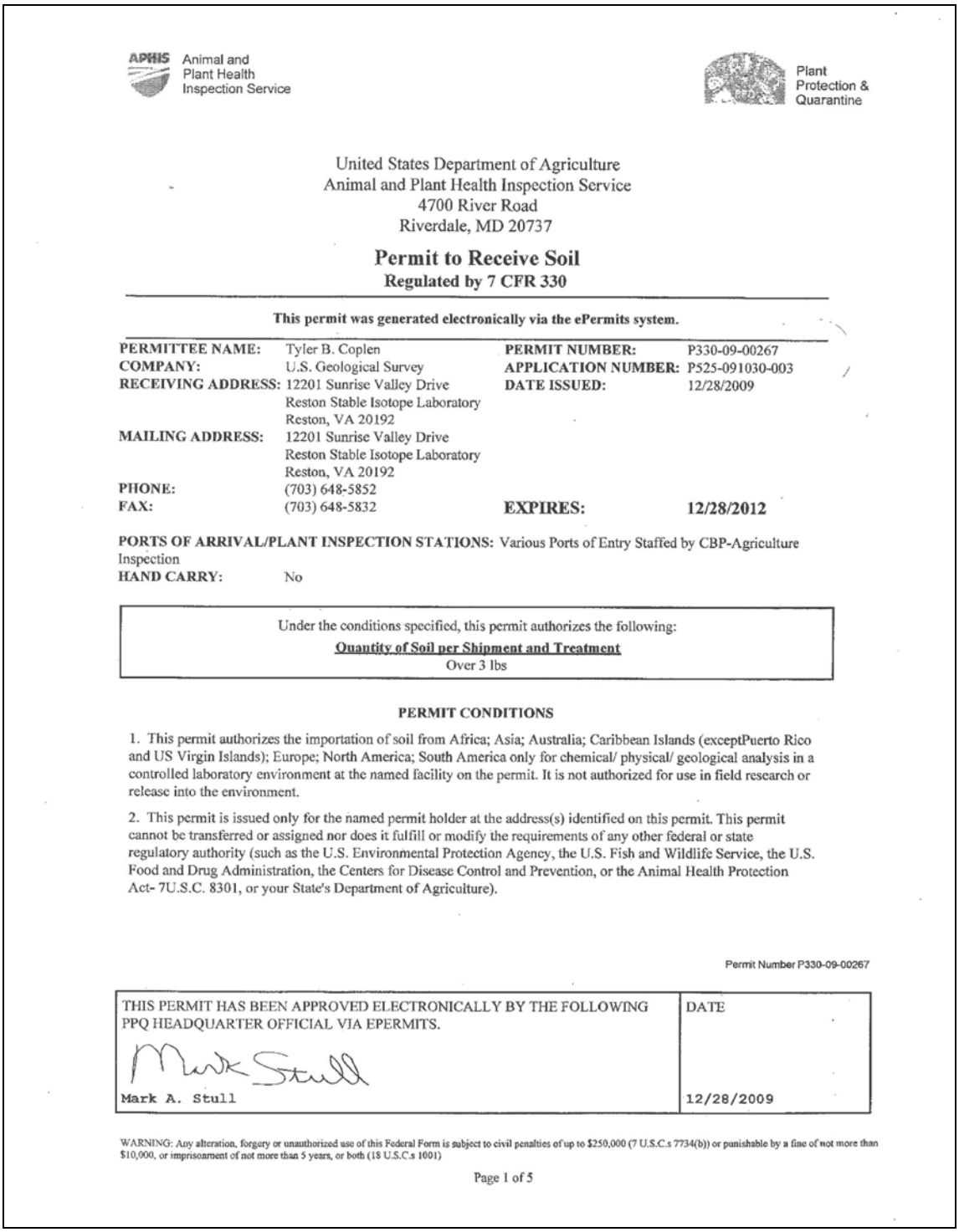
Appendix A. Example of a United States Department of Agriculture Permit to Receive Soil



APHIS Animal and Plant Health Inspection Service

Plant Protection & Quarantine

United States Department of Agriculture
Animal and Plant Health Inspection Service
4700 River Road
Riverdale, MD 20737

Permit to Receive Soil
Regulated by 7 CFR 330

This permit was generated electronically via the ePermits system.

PERMITTEE NAME:	Tyler B. Coplen	**PERMIT NUMBER:**	P330-09-00267
COMPANY:	U.S. Geological Survey	**APPLICATION NUMBER:**	P525-091030-003
RECEIVING ADDRESS:	12201 Sunrise Valley Drive Reston Stable Isotope Laboratory Reston, VA 20192	**DATE ISSUED:**	12/28/2009
MAILING ADDRESS:	12201 Sunrise Valley Drive Reston Stable Isotope Laboratory Reston, VA 20192		
PHONE:	(703) 648-5852		
FAX:	(703) 648-5832	**EXPIRES:**	**12/28/2012**

PORTS OF ARRIVAL/PLANT INSPECTION STATIONS: Various Ports of Entry Staffed by CBP-Agriculture Inspection
HAND CARRY: No

Under the conditions specified, this permit authorizes the following:
Quantity of Soil per Shipment and Treatment
Over 3 lbs

PERMIT CONDITIONS

1. This permit authorizes the importation of soil from Africa; Asia; Australia; Caribbean Islands (exceptPuerto Rico and US Virgin Islands); Europe; North America; South America only for chemical/ physical/ geological analysis in a controlled laboratory environment at the named facility on the permit. It is not authorized for use in field research or release into the environment.

2. This permit is issued only for the named permit holder at the address(s) identified on this permit. This permit cannot be transferred or assigned nor does it fulfill or modify the requirements of any other federal or state regulatory authority (such as the U.S. Environmental Protection Agency, the U.S. Fish and Wildlife Service, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, or the Animal Health Protection Act- 7U.S.C. 8301, or your State's Department of Agriculture).

Permit Number P330-09-00267

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Mark A. Stull	12/28/2009

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to $250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)

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Appendix B. Step-by-Step Procedure to Prepare and Analyze Samples for Distillation

Determining How Much Water is Contained in the Soil Sample

After the samples are logged into the LIMS-LSI database and sample numbers have been assigned, the first thing that must be done is to determine how much water is contained in the soil sample. This is done by weighing out 5 grams (g) of sample, drying it in either a convection or vacuum oven, and then reweighing the remainder of the sample. The weight of the dried sample can then be used to determine the amount of sample needed to yield a desired quantity of water that can be extracted per weighed sample. A minimum of 3 to 4 mL of water is needed for a satisfactory analysis, but quantities as low as 0.5 mL can be used. If sufficient sample has been provided or the sample is overly saturated, as much as 10 mL of water should be extracted.

Evaporating Water from the Sample

Choose a container to weigh out the soil sample. Any type of suitable container can be used, but it should be able to withstand at least 90 °C. This same container will be used to dry the sample in the oven. Figures B–1 and B–2 show the 100 × 15-millimeter (mm) disposable polystyrene Petri dishes used.

Weighing Out the Sample

1. Label each container with the corresponding number of the sample that was assigned in LIMS-LSI (fig. B–2). Label each dish as you prepare the sample for weighing to avoid any mix ups. A permanent sharp-tip-type marker works well.
2. Open the container of sample and weigh out at least 5 g of sample into the Petri dish.
3. Close the sample container and reseal. Do this as quickly as possible to minimize evaporation.
4. On a sheet of paper, write down the sample ID number plus the combined weight of the soil sample and the container. To keep things simple, try to weigh out as close to 5 g of sample as possible for each sample taken.
5. Repeat steps 2, 3, and 4 for the remaining samples.

Drying Samples

1. After all samples are weighed out into individual containers, the water must be evaporated. A convection oven or a vacuum oven can be used. To expedite the evaporation process, the latter method is preferred.
2. Place the samples in the oven. If using a convection oven, set the temperature to 90 °C. If using a vacuum oven, set the temperature to 45 °C.
3. Evaporation times will vary depending on the amount of water a sample contains.
4. Reweigh the samples after they are thoroughly dried.

Calculating Water Content

1. Reweigh the evaporated soil samples in their containers and write down the results on the same sheet of paper used previously. Write down the combined weight of the soil sample and container.

2. Determine how much sample will be required to yield 5.0 mL of water; multiply the mass of the wet soil by 5 and then divide that answer by the yield of water (for example, 6.2 g (mass of wet soil) × 5.0 mL (amount of water that needs to be extracted) ÷ 4.7 mL (yield of water) = 6.59 g).
3. The result, 6.59 g, is the mass of soil needed to yield 5.0 mL of water.

Water in Plant Samples

The entire plant sample is usually distilled because it contains little water. If there is water condensation on the inside of the glass vial, the entire vial can be dropped into the toluene through the wide opening of the flask holding the toluene.

Performing the Distillation

Equipment and Reagents Needed

1. Paraplast™ wax, pellet form (used to remove toluene from the extracted water).
2. Heating plate with water bath to melt the wax with the extracted water.
3. Toluene (high-performance liquid chromatography (HPLC) grade).
4. Thermometer (general-purpose laboratory type).
5. Support stands.
6. Adjustable angle clamps.
7. Cold-water connections for condenser.
8. Specially designed receiving funnel (fig. B–3).
9. Variac™ (fig. B–4) for each heater to control temperature.
10. One 500-mL flask with heavy wall, round bottom, and taper-ground joint (fig. B–5).
11. Large disposable wipes.
12. Disposable latex gloves.
13. Bench top scales (for water-content determination, use 0.00-g accuracy scale; for weighing samples, 0.0-g accuracy).
14. One 100-mL glass beaker.
15. Glass sample (scintillation) vials with cone-shaped cap (11 mL) (fig. B–6).
16. Glass rod with O-ring (fig. B–7).
17. Condenser (Liebig, 300 mm, fig. B–8).
18. Electrothermal hemispherical mantle-bottom heater (fig. B–9).
19. Boiling chips (used only when distilling standard waters for checking the system).

Distilling the Sample

1. Before starting to weigh out samples, all necessary equipment should be set up and be operational (fig. B–10). Do not turn on heaters.
2. Weigh out the amount of sample needed to yield the desired amount of water into a 250- to 500-mL flask (fig. B–11). Add sufficient toluene (200–250 mL) to each sample so that the soil will remain covered throughout the distillation process. Do this as quickly as possible to minimize evaporation.
3. Repeat step 2 for the remaining distillation set ups.
4. Put each flask in position on its heater and attach it to the receiving funnel, which is attached to the condenser.
5. Start the water flow through the condensers and turn on the heaters.
6. Using the Variac(s), adjust the heaters to approximately 70 °C so that the water and toluene in the flask is not being forcibly boiled into the receiving funnel.

7. During the distillation process, drops of water will form in the upper section of the receiving funnel and on the inside of the condenser. A glass rod with a piece of plastic tubing or an O-ring at its end can be used to push the drops down into the distilled water that accumulates in the receiving funnel (fig. B–12). Water drops can be pushed down in this way during the distillation process through the top of the condenser and afterward in both the condenser and the receiving funnel (fig. B–13).
8. After no water droplets are observed to be forming in the condenser or the receiving funnel, turn off all heaters. Allow the flask and receiving funnel to cool.
9. When the water and toluene contained in the receiving funnel has cooled sufficiently, a clear "line" can be seen separating the two components, as seen in fig. B–13. If there is an opaque, narrow segment in between, stir it in with the clear water using the glass rod. Let it separate again for a short time.
10. Take an appropriate-sized glass-sample (scintillation) vial and place the corresponding label on the vial to identify the sample (fig. B–6).
11. Position the vial under the stopcock of the receiving funnel and slowly open the stopcock to allow the water to drain into the scintillation vial.
12. As soon as all the water is emptied, quickly close the stopcock to minimize the amount of toluene in the sample.

Removing the Toluene

1. Before placing the cap on the scintillation vial, wax must be added to remove any toluene in the water.
    a. Add approximately 1.5 g of wax per 5 mL of sample.
    b. Replace the cap and place the vial in a glass dish of heated water (fig. B–14) to melt the wax.
    c. Turn on the heater under the set up used to melt the wax in the glass scintillation vials.
    d. Adjust the temperature to maintain 56 °C.
    e. Be careful to prevent the temperature of the water from rising above the melting point of the wax (56 ºC).
2. When all the wax has melted, remove the vial from the hot water and turn it upside down on the counter top.
3. After the wax has solidified, remove the cap and decant the distilled water into a second scintillation vial with the appropriate label.
4. Make sure the water sample is free of residual toluene. Note: If the solidified wax seems soft or if a toluene odor is detected, it is a good indication that there was a large amount of toluene present. The harder the wax is, the lower the amount of toluene in the distilled water.
5. If there is still a strong odor of toluene in the distilled water, the waxing must be repeated a second time. Repeat as necessary until the water is odor free. Sometimes the sample will smell of toluene until it is decanted away from the wax. Be sure to decant into clean vials each time. Normally, two treatments with wax are sufficient to remove the toluene from the distilled sample.

Preparing for the Next Run

1. After each distillation, approximately 25 to 30 mL of toluene will collect in the receiving funnel. This should be discarded into an appropriate waste-disposal container within the hood and 25 mL of fresh toluene is added back to each flask before the next distillation. It is not necessary to remove the prior sample from the flask, but it is advisable. Nevertheless, ensure that when the next sample is added, it remains completely immersed with toluene.
2. Wipe the funnel dry with a disposable wipe. Collect all toluene-soaked wipes in a plastic bag for discarding by the safety, health, and environmental officer.
3. Discard the toluene with the distilled soil samples into a clearly labeled, larger container for disposal by the safety, health, and environmental officer.

4. Assemble the apparatus for the next sample.

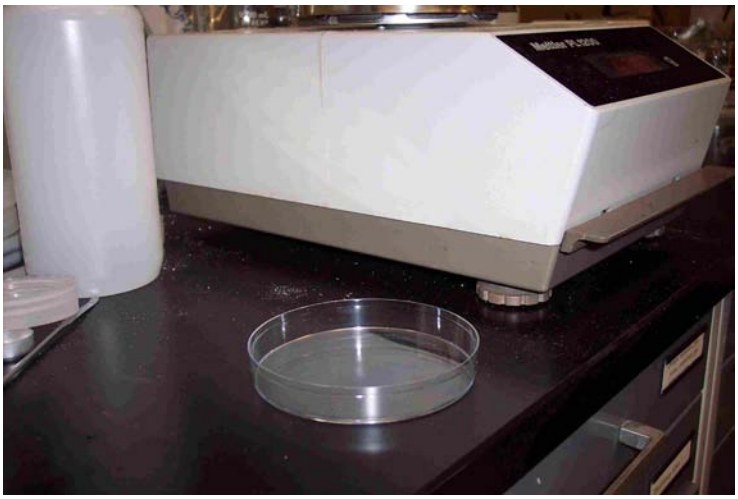

Figure B–1. Preparation for determining the water content of sample.

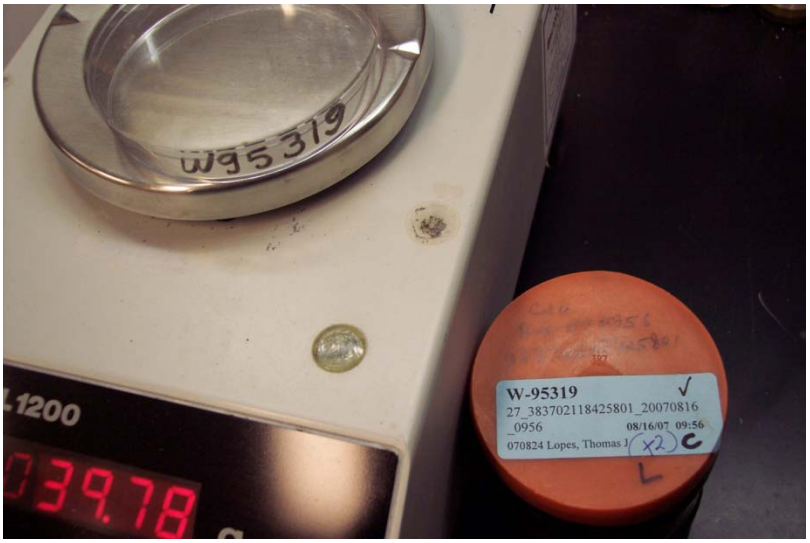

Figure B–2. Preparation for determining the water content of the sample with sample to right of balance and Petri dish labeled with sample ID.

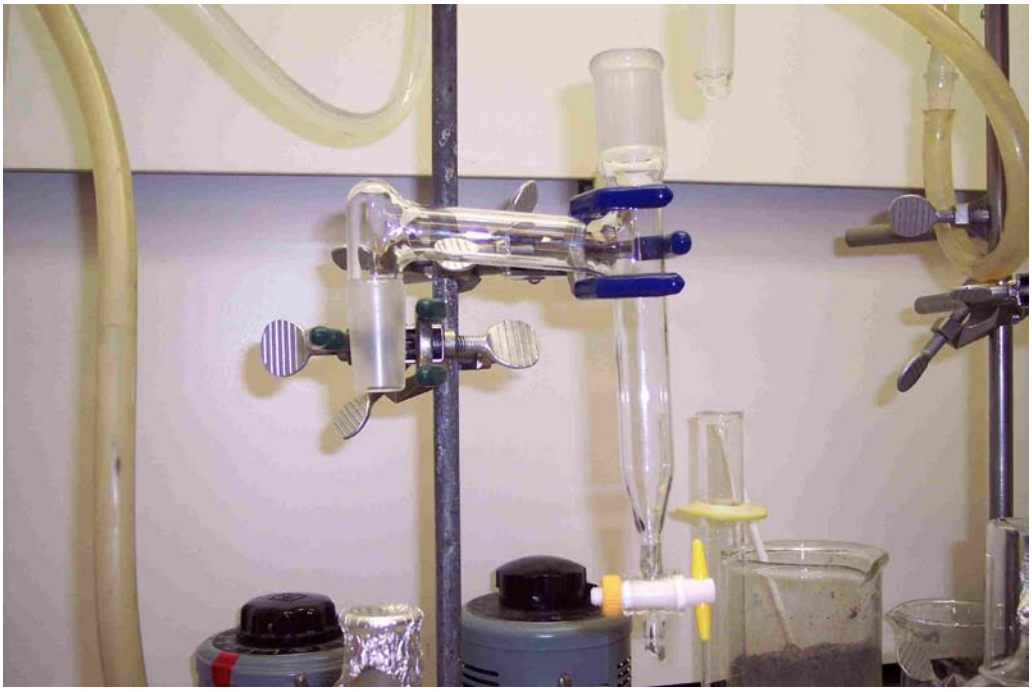

Figure B–3. Specially designed receiving funnel.

Figure B–4. Variac to control temperature of the heating element.

Figure B–5. Flask (500-mL) with heavy wall, round bottom, and taper-ground joint.

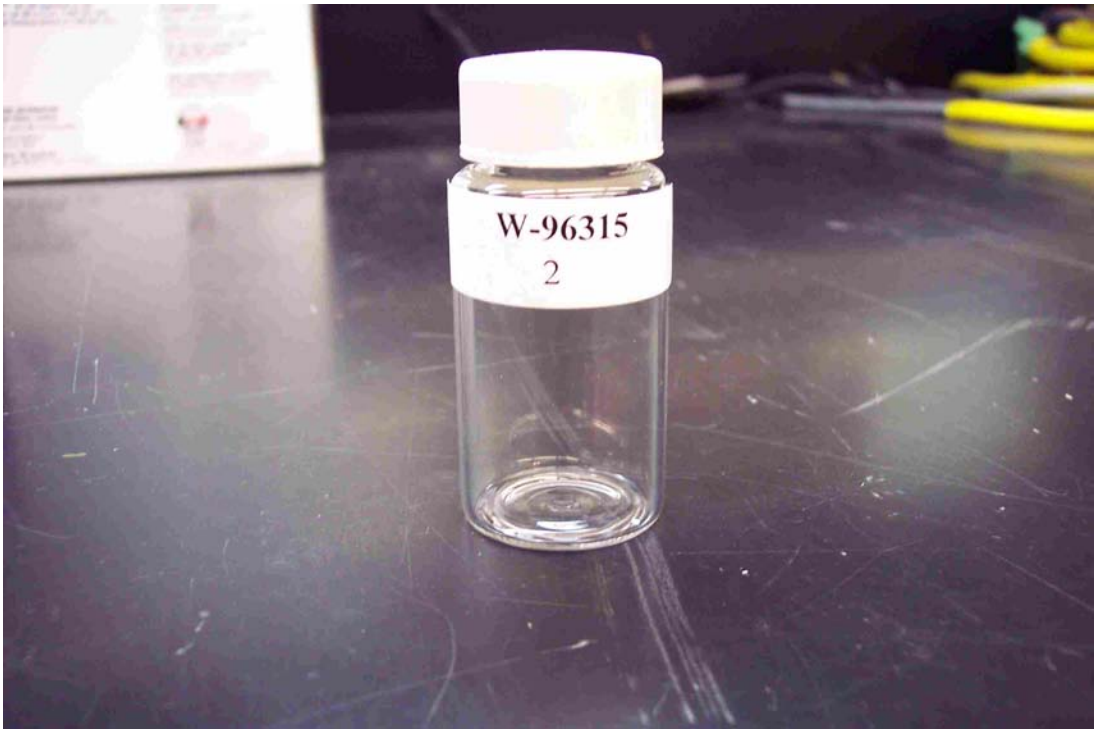

Figure B–6. Glass-scintillation vial with label.

Figure B–7. Glass rod with O-ring.

Figure B–8. Condenser (Liebig, 300 mm).

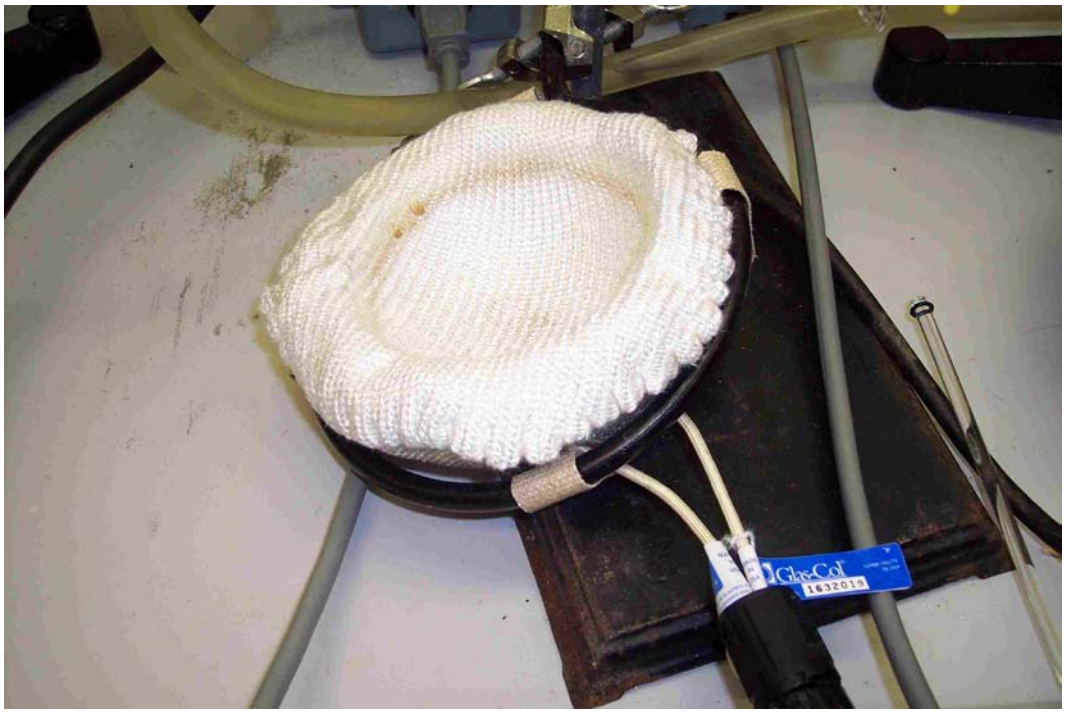

Figure B–9. Electrothermal hemispherical mantle-bottom heater.

Figure B–10. Four-station distillation set up.

Figure B–11. Weighing out samples.

Figure B–12. Distillation of soil samples.

Figure B–13. Glass rod with O-ring used to push water drops into distilled water.

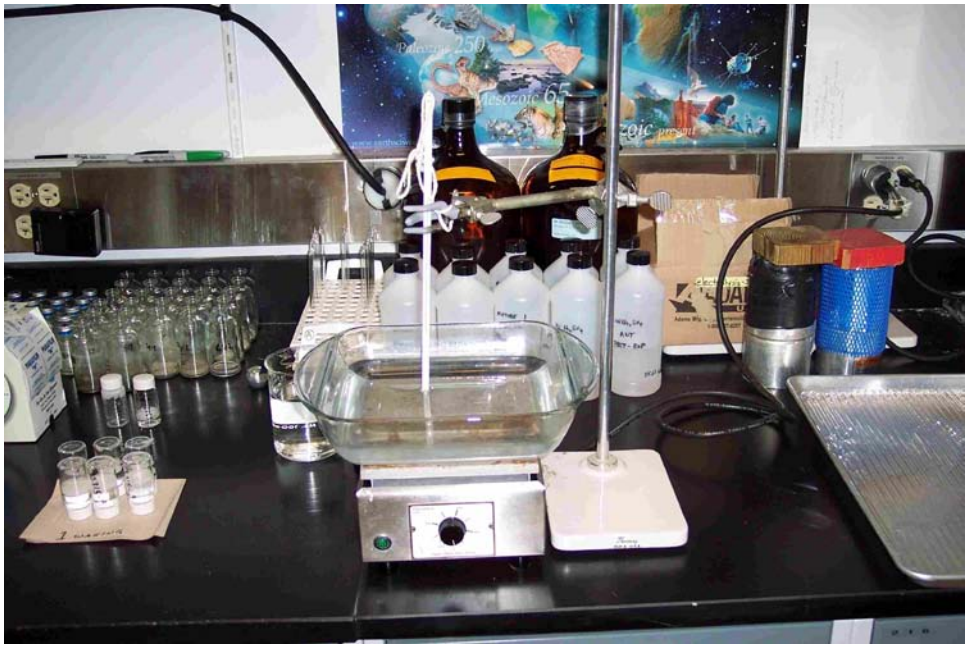

Figure B–14. Set up used to melt wax.