Project Harvest

DIY GUIDE

Thank you so much for participating in the University of Arizona's and SERI's Project Harvest. Thank you for your time and consideration.

PROJECT HARVEST OVERVIEW

What is Project Harvest?

Project Harvest is a co-created citizen science project, meaning: members of the public are involved in most or all steps of the scientific process to generate new knowledge. Project Harvest is designed to improve environmental health education and monitoring in underserved rural and urban communities. Together, we will monitor the quality of harvested water, soil, and plants while learning more about our environmental health. The goals of this project are to:

- Learn about potential pollutants in harvested water such as: microorganisms, inorganics/ metals, and organic compounds.
- Learn how these possible pollutants might impact soil, plant and human health
- Evaluate the learning and action-based outcomes of a citizen science and community-engaged research

Project Harvest aims to co-generate a robust environmental monitoring dataset, while informing the safe production of food sources in underserved communities. By participating in Project Harvest, you will: 1) Learn the scientific method and how to collect harvested water, soil, and/or vegetable samples from your garden for environmental analysis and 2) meet others in your community who are interested in environmental and food quality.

How does Project Harvest work?

Project Harvest is using a peer education model! Our researcher team has trained local community health workers (promotoras) in each of the targeted Arizona communities: Tucson (Southern metropolitan area), Dewey-Humboldt, Hayden-Winkelman, and Globe/Miami.
The trained community health educators are recruiting and training participants at their homes and providing all the necessary materials for sampling harvested water, soil, and/or vegetables.

As a participant, you will work with traditional laboratory (LAB) supplies and Do-It-Yourself (DIY) gear to monitor your environment. Sample collection will begin in Winter 2017 and run through Winter 2020 (see **Master Checklist**). Samples will be analyzed by University of Arizona researchers and you! The data will be interpreted and shared with all participants and communities.

What is my role in Project Harvest?

You are part of the team! The work you do, and the samples you collect will be used in a scientific study to determine the quality of your harvested water, soil, and plants. This manual provides step-by-step instructions on how to collect samples from your garden for:

- Analysis at University of Arizona laboratories with traditional laboratory (LAB) supplies
- Conducting experiments at home with Do-It-Yourself (DIY) gear

Thank you again for participating in Project Harvest! You are helping us better understand the quality of harvested rainwater, soil, and plants in underserved rural and urban communities. It is an honor to work with you all. If you have questions, please contact me anytime.

Warm regards,

Mónica Ramírez-Andreotta
Project Harvest Director
Assistant Professor of Soil, Water and Environmental Science (home) and Public Health's Division of Community, Environment & Policy (joint)
University of Arizona
1177 E Fourth Street, Rm. 429, Tucson, AZ 85721
Email: mdramire@email.arizona.edu
Phone: 520-621-0091

THE UNIVERSITY OF ARIZONA COLLEGE OF AGRICULTURE & LIFE SCIENCES Environmental Science
THE UNIVERSITY OF ARIZONA MEL AND ENID ZUCKERMAN COLLEGE OF PUBLIC HEALTH
seri
Ramirez-Andreotta Integrated Environmental Science & Health Risk Laboratory
NSF
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GENERAL INSTRUCTIONS

For all sampling activities, you will need to follow the directions below.

- **Always sample from the same cistern.** The rainwater harvest cistern that you have chosen to use in this project should be used for all of the rain water sample collections. Please do not switch between cisterns during the project.
- **Label your cistern clearly with kit number so that it can be easily read from 5 feet away.** Use a sharpie pen or paint. You can use a sticker or mark directly on the cistern. Over the course of the project make sure this label remains clearly visible, and repair if needed.
- **Find a nice and clean area to set-up.** Identify a clean, dry area outside (like a picnic table, patio, or a bare and flat surface) in the shade and out of the wind.
- **Gloves are a must!** Wear gloves at all times when sampling. You may keep the same pair of gloves on during all water sampling procedures. Change gloves in between water soil and plant sampling. Once you are done, remove gloves and place them in provided waste bag.
- **Run water for 10 seconds before sampling.** Before any water sample is collected from the harvesting system, open the faucet and let the water run at full speed for 10 seconds (you can water a plant or catch water in a bucket if you want).
- **Do not worry if your cistern is empty at sample collection time.** It's okay that you do not have any water to sample. You can sample next time. Just let us or your promotora know in advance.
- **Avoid sample contamination. Hold the cap!** Try your best to always hold the cap while taking your water sample. Do not allow sampling vial or bottle to touch the faucet at any time – this may contaminate the sample.
- **Label everything! Use the provided permanent marker for all labeling.** In DIY, you will label reactive strips for Inorganic Arsenic testing and the 5 vials used in the Microbial experiment.
- **Mark your soil sampling spots with the tent stakes and whiskers (you may need a hammer). Sample at these locations for both irrigated and not irrigated every time (LAB and DIY)!** You will collect soil samples only once a year during Project Harvest.
  - You have the following whisker colors to mark the locations:
    - Blue: Irrigated soil
    - Orange: Non-irrigated soil
    - White: MICROBIAL sampling site to also be used for INORGANIC in both the irrigated and not irrigated soil
- **Take Notes and Pictures!** Document your experience and observations by taking notes in either your Project Harvest Field Notebook or online at **https://projectharvest.arizona.edu/.**
- **Check it off your list!** After you finish collecting a set of samples, go to your "Master Checklist" and check the sampling off your list!
- **Waste Disposal – We got you covered!** Dispose of all waste in the provided waste bag or the Tupperware container if it is liquid waste. We are collecting all of the waste for proper disposal at the University of AZ. You can drop off waste at the end of the summer.

You will see each methodology labeled with colors and icons. They are as follows...

Orange=	Organic
Yellow=	Inorganic
Green=	Microbial

WASTE DROP-OFF PROCEDURES

You will need to:

- Deliver waste bags to your designated local drop-off location by the time set by your promotora.

  - **Tucson - University of Arizona Visitor's Center**
    Address: 811 N Euclid Ave, Tucson, AZ 85719
    Hours: 9AM–5PM Monday – Friday; closed the weekends
    Phone: 520-621-5130

  - **Globe/Miami: Globe Public Library**
    Address: 339 S Broad St, Globe, AZ 85501
    Hours: Monday – Tuesday 10:30 am - 5:30 pm
    Wednesday-Thursday - 10:30 am - 6:00 pm
    Friday - 10:30am - 5:30pm
    Saturday - 10:00 am - 2:00 pm
    Closed Sundays
    Phone: 928-425-6111

  - **Hayden-Winkelman:**
    Hayden High School Room 1320
    Address: 824 Thorne Ave, Winkelman, AZ 85192
    Hours: 7:00AM – 5:00PM, Monday – Thursday; closed the weekends

  - **Dewey-Humboldt: Town Library**
    Address: 2735 Corral St, Humboldt, AZ 86329
    Phone: (928) 632-5049
    Hours: Tuesday, Wednesday& Friday:
    10:00 am - noon &12:30pm - 5:00 pm
    Thursday: 12:30 pm - 7:00 pm
    Saturday: 10:00 am - 2:00 pm

If you are unable to access the drop-off location at the available times, please discuss other options with your promotora.

GLOSSARY OF TERMS

Arsenic - A naturally occurring toxic metalloid (an element that has properties in common with metals and some in common with non-metals) that can be high in regions associated with mining. In drinking water, the USEPA has set the maximum contaminant level for drinking water at 10 micrograms of arsenic per liter of water (equal to 10 parts per billion); above that level water utilities and private well owners must treat the water to remove arsenic.

Arsine Gas – A toxic gas (AsH_3) that at elevated levels, may be lethal.

Chain of Custody - Documentation of the control and transfer of samples. The chain of custody establishes the proof that the sample remains the same, and does not get mixed up with other samples, through all of the analyses.

Compositing - A technique where multiple samples are taken from different, separate, and distinct locations. These samples are then combined, thoroughly mixed, and treated as a single sample. Composite sampling can improve coverage of an area without increasing the number of samples that have to be taken (sample quantity).

Control Sample - The control sample provides a baseline that lets us see if the areas irrigated with harvested water are different and affected by the harvested water. Control samples help assure that results are reliable. Also called controls, known samples, and knowns. This is located in your Project Harvest notebook.

GLOSSARY OF TERMS

Escherichia coli (abbreviated as E. coli) - Bacteria found in the environment, foods, and intestines of people and animals. E. coli are a large and diverse group of bacteria. Although most strains of E. coli are harmless, others can make you sick. Some kinds of E. coli can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

Fecal Coliforms - A group of bacteria that generally originates in the intestines of warm-blooded animals. Their presence in food or water indicate water or food contamination.

Indicator Bacteria - Indicator bacteria are types of bacteria used to detect and estimate the level of fecal contamination of water. They are not dangerous to human health but are used to indicate the presence of a health risk.

Inorganic Compounds - Metals, minerals, or compounds that contain little or no carbon.

Microorganism/Microbes/Microbial - An organism that is microscopic, which means it is too small to be seen by the unaided human eye. Microbes refers to any of the microorganisms, including bacteria, fungi, protozoa, and viruses. In this study, we will be measuring bacteria that can indicate fecal contamination and pathogens.

Mercury – A toxic heavy metal. The mercury in the test kit is mercuric bromide, which is extremely toxic.

Most Probable Number (MPN) - A method used to estimate the concentration of viable microorganisms in a sample. For this project, the MPN will be used to estimate the number of sulfur reducing bacteria in the harvested rainwater sample.

Nanopure - Free of any elements, organic compounds and/or pathogens

Organic Chemicals/Compounds – A chemical (or compound) is a substance consisting of two or more elements (from the periodic table) associated by chemical bonds. An "organic" compound contains the element carbon. Organic compounds may be arranged in rings or chains of carbon atoms, along with atoms of other elements. Common elements besides carbon (C) that are found in organic compounds include hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), sulfur (S) and halogens like chlorine (Cl) and fluorine (F).

Pathogens – Microorganisms (Bacteria, Virus, etc.) that can cause illness (e.g. diarrhea).

Precipitate - A solid forming from a liquid solution

Sulfur Reducing Bacteria (SRB) - Sulfur-reducing bacteria get their energy by reducing elemental sulfur to hydrogen sulfide. For this project, SRB are going to be used as indicators of fecal contamination.

Scientific Method – a process of inquiry that begins with observations which lead to a question. From the question a hypothesis is developed to explain the observation(s). Data are then collected by reproducible experiment(s) to test the hypothesis. Data are then analyzed and interpreted to reach a conclusion. Detailed record keeping is essential to aid in recording and reporting experimental results, and to support the effectiveness and integrity of the procedure.

MASTER CHECKLIST 2017–2018

Kit # _____

Sample Collection happens once per year		2017 - 2018			
		Dec 1 – Jan 6	Feb 15 - Feb 28	June 15 - July 15	Sept 8 – Sept 30
		First Winter rain	Last Winter rain	First monsoon rain	Last monsoon rain
Inorganic	Water				
	Soil	Irrigated			
		Not Irrigated:			
Microbial	Water				

You will test water samples 4 times per year – 2 in winter and 2 in summer (during monsoon) after a significant amount of rainfall. You will also test soil (irrigated and not irrigated) samples once a year. Login to Project Harvest and create a new journal entry.

Significant rainfall = 1/10 of an inch of rainfall. You will use the provided rain gauge. Once you see 1/10 of an inch of rain in the gauge, you can sample. **Empty rain gauge after every rainfall event.**

WINTER
December 1 - February 28/29

You will collect a water sample **AFTER the first significant rainfall** in winter. **Create a new journal entry with observations and images to projectharvest.arizona.edu between December 1 – January 6.** If you do not have computer access, write observations in provided Project Harvest notebook.

Then, you will collect a water sample **AFTER one of the last significant rainfall in winter.**

2018–2020

		2018-2019				2019-2020			
		Dec 1 – Jan 6	Feb 15 - Feb 28	June 15 - July 15	Sept 8 – Sept 30	Dec 1 – Jan 6	Feb 15 - Feb 29	June 15 - July 15	Sept 8 – Sept 30
		First Winter rain	Last Winter rain	First monsoon rain	Last monsoon rain	First Winter rain	Last Winter rain	First monsoon rain	Last monsoon rain
		Irrigated				Irrigated:			
		Not Irrigated:				Not Irrigated:			

Create a new journal entry with observations and images to projectharvest.arizona.edu between February 15 - February 28/29.
If you do not have computer access, write observations in provided Project Harvest notebook.

MONSOON
June 15 - September 30

You will collect a water sample **AFTER the first significant rainfall** in the monsoon season. **Create a new journal entry with observations and images to projectharvest.arizona.edu between June 15 - July 15.** If you do not have computer access, write observations in provided Project Harvest notebook.

Then, you will collect a water sample **AFTER one of the last significant rainfall event in the monsoon season. If you have not collected a soil or plant samples for the three previous collection periods, this is the time to sample! Create a new journal entry with observations and images to projectharvest.arizona.edu between September 8 – September 30.** If you do not have computer access, write observations in the provided Project Harvest notebook.

Watch the sampling videos online on our Project Harvest website:

WATER
https://www.youtube.com/watch?v=1Ym4TcjlJNg
SOIL
https://www.youtube.com/watch?v=HkvwtLdX5D4

INORGANIC

Do-It-Yourself method for measuring arsenic in water and soil

This Do-It-Yourself method will measure the concentration of arsenic (As) in water and soil samples. This test is based on a chemical reaction and will provide the estimated concentration range of total arsenic in the water and soil sample.

KIT MATERIALS

Gloves

DIY reaction bottle
with flip top cap
and screw top cap

Coin envelope with
test strips

Empty coin envelopes
in ziploc bag

Scissors

DIY chemical
packets 1 and 2

Project Harvest
Field Notebook
(hard copy or online)

Waste bag

Camera (not included)

INSTRUCTIONS

Collecting Harvested Rainwater Samples for Arsenic DIY

For this experiment, you will be handling mercury and possibly generating arsine gas, **these are toxic and can harm you.** You must be outside and carefully follow the steps below. Keep your gloves on at all times. This experiment should be done only by an adult.

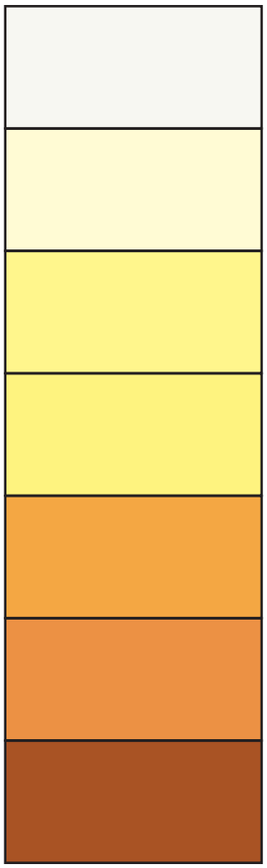
WARNING: Perform this test outside in the shade out of the wind. Be sure to wear gloves and dispose of the test strips in one of the provided blank coin envelopes. Don't touch the tip of the indicator strip contains mercury bromide, which is very toxic.

Set-up: Cover experiment surface with paper, newspaper, or a flat tray before conducting your experiment. That way, if some of the compound is spilled, it will be easy to dispose of that waste.

1. Put your gloves on.
2. Remove flip-top lid from DIY reaction bottle
3. Open flip-top and insert test strip into flip top with reactive side towards the center of the bottle. Secure strip by closing the flip in the cap.
4. Open the cistern faucet so your rain barrel water flows at full speed and let it flow out for 10 seconds (feel free to capture this water and use it for gardening purposes).
5. Fill reaction bottle with harvested water to the mark.
6. Use scissors to open packet #1 and #2. Add packet #1 and packet #2 to the reaction bottle. Packet 1 contains a dry acid, packet 2 is zinc. Handle carefully and do NOT spill. Once you dispense the chemical contents of packets place empty pouches in the provided waste container.

Continued on page 16

Arsenic Color Chart
(micrograms per liter, parts per billion)



Notice that the unreacted strip is off-white.

7. Immediately cap the bottle being careful to get the test strip in the bottle, and gently swirl for 60 seconds. **Do not tip upside down or let liquid contact test strip. It is very important that the test strip does not get wet.**
8. Every 5 minutes, gently swirl the bottle for 10 seconds until 40 minutes total have passed. After 40 minutes, the color will fully develop. Record observations.
9. At 40 minutes, in the shade, take off the lid, open the flip-top, and remove the test strip. **Immediately compare to the color chart on the left. Exposure to the sun will lighten the test strip, complete your comparison quickly.**
10. Record observations. What concentration range did your samples most closely resemble? Write down what you see. **Take a photograph of your test strip with the color chart; ensure you capture both the test strip and the color chart.**
11. Keep the strip for your records! Place used strip in one of the provided empty coin envelopes. **Label coin envelope with: Kit Number, Sample Type (Water, Soil-Irrigated, or Soil-Not-Irrigated), & Date.**
12. Save all of your labeled coin envelopes. In order to keep track of the strips, you can tape them to the back of your Project Harvest notebook.
13. Log in to the projectharvest.arizona.edu website and upload your images and type up your observations. If you do not have access to a computer or smart phone, please take notes in your Project Harvest notebook. **If you have any questions about your results, please contact the community health educator (promotora) in your area.**
14. Remove the flip top lid from the **used** reaction bottle and place it on an **unused** reaction bottle.
15. Put the screw cap on **used** reaction bottle. Seal tightly and place in waste bag.

KIT MATERIALS


Hand trowel


DIY reaction bottle
with flip top cap
and screw top cap


Whiskers (4 blue,
2 white)

Tent stakes (6)


Marker


Plastic spoon


Weigh boat

DIY Chemical packets
1 and 2


Tube filled with clean water
labeled “Nanopure”

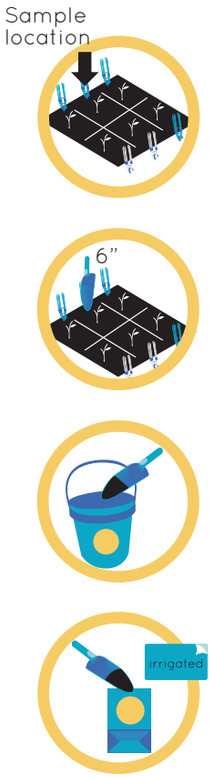

Scissors


Coin envelope
with test strips


Empty coin envelopes
in ziploc bag


Project Harvest Field Notebook
(hard copy or online)

Waste bag



Collecting a soil sample where you have irrigated with harvested rainwater

WARNING: Perform this test outside in the shade out of the wind. Be sure to wear gloves and dispose of test strip in the provided sealable bag. The tip of the indicator strip contains mercury bromide, which is very toxic.

Important Notes: You will collect soil samples only once a year during Project Harvest. Try to collect your soil samples after you have prepared your garden for the season. Make sure to wash the hand trowel before every use.
Mark your soil sampling spots with the tent stakes and whiskers. Sample at these locations for both irrigated and not irrigated every time!
You have the following whisker colors to mark the locations (see illustrations on page 17):
Blue: Irrigated soil
Orange: Not irrigated soil
White: Microbial sampling site to also be used for inorganic soil sampling.

Set-up: Cover experiment surface with paper, newspaper, or a flat tray before conducting your experiment. That way, if some of the compound is spilled, it will be easy to dispose of that waste.

1. Select 6 spots to sample in your garden roughly in a grid-like pattern. Mark these with the tent stakes and whiskers (4 will be blue, 2 will be white).
2. With your gloves on and using the hand trowel provided, loosen the top 6 inches (the approximate length of the hand trowel blade) of each of the 6 soil spots.
3. At each location, take one full scoop of soil and place it into the bucket labeled **“Irrigated.”**
4. Mix the six soil samples thoroughly inside the bucket. This process is called *compositing*.
5. Pick out rocks and gravel that are pea-sized or larger.

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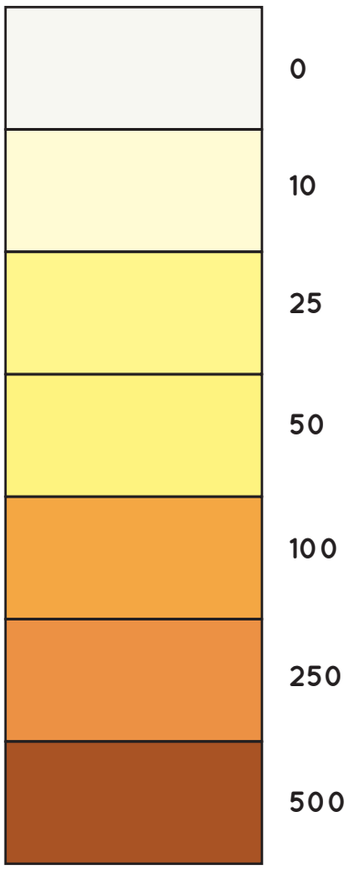
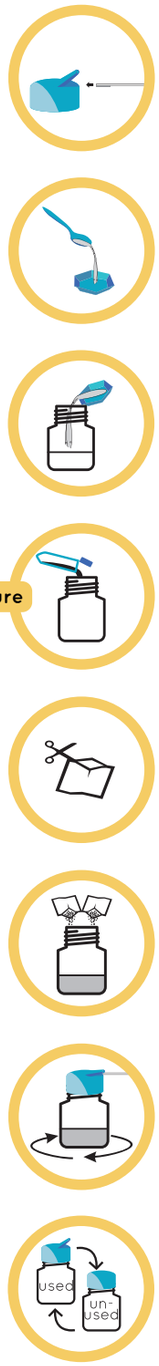
Arsenic Color Chart
(micrograms per liter, parts per billion)



Notice that the unreacted strip is off-white.



Now, with the irrigated soil sample from the irrigated bucket, you will:

6. Remove lid from DIY reaction bottle
7. Open flip-top and insert test strip into flip top with reactive side towards the center of the bottle. Secure strip by closing the flip in the cap.
8. From the composited soil, use the provided plastic spoon to scoop some soil.
9. Use hexagonal plastic boat to scrape the spoon so you have a level spoonful, this will be very close to 5 g.
10. Transfer the soils from the spoon to the empty weigh boat - this is for easier transfer to the reaction bottle.
11. Fold the weigh boat (like a taco) to pour the soil into the reaction bottle.
12. Fill reaction bottle with provided water labeled “Nanopure” to the mark.
13. Use scissors to open packet #1 and #2. Add packet #1 and packet #2 to the reaction bottle. Packet 1 contains a dry acid, packet 2 is zinc. Handle carefully and do NOT spill.
14. Once you dispense the chemical contents of packets place empty pouches in the provided waste container.

    Immediately cap the bottle, being careful to get the test strip in the bottle, and gently swirl for 60 seconds. **Do not tip upside down or let liquid contact test strip. It is very important that the test strip does not get wet.**
15. Every 5 minutes, gently swirl the bottle for 10 seconds until 40 minutes total have passed. After 40 minutes, the color will fully develop. Record observations.
16. At 40 minutes, in the shade, take off the lid, open flip-top and remove test strip. **Immediately compare to color chart. Exposure to the sun will lighten the test strip, complete your comparison quickly.**

Continued on page 22

KIT MATERIALS


Hand trowel


DIY reaction bottle
with flip top cap
and screw top cap


Whiskers (4 orange,
2 white)


Tent stakes (6)


Marker


Plastic spoon


Weigh boat

DIY Chemical packets
1 and 2


Tube filled with clean water
labeled “Nanopure”

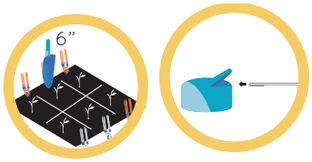

Scissors

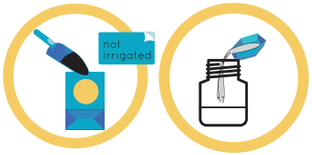

Coin envelope
with test strips


Empty coin envelopes
in ziploc bag


Project Harvest Field Notebook
(hard copy or online)

Waste bag





17. Record observations. What concentration range did your samples most closely resemble? Write down what you see. **Take a photograph of your test strip with the color chart; ensure you capture both the test strip and the color chart.**

18. Keep the strip for your records! Place used strip in one of the provided empty coin envelopes. **Label coin envelope with: Kit Number, Sample Type (Water, Soil-Irrigated, or Soil-Not-Irrigated), & Date.**

19. Log in to the projectharvest.arizona.edu website and upload your images and type up your observations. If you do not have access to a computer or smart phone, please take notes in you Project Harvest notebook and save all your test strips in the provided folder. **If you have any questions about your results, please contact the community health educator (promotora) in your area.**

20. Place empty reagent packets and tightly closed bottle (with water, soil and contents of packets) in provided waste bag.

Collecting Soil Samples where you have NOT irrigated with harvested rain water (control sample)

You will do the same soil sampling process as described above for your garden soil, but now for an area in the yard that has **not** been exposed to rainwater from the rain barrels AND has **not** been exposed to rain running off of the roof. Complete steps 1-20 above, note that you will now be using the 1-gallon bucket labeled “Not-Irrigated.”

Important Notes: Complete steps 1-20 on pages 18-22, with the following exceptions:

Step 1 - You will now use 4 orange and 2 white whiskers.

Step 3 - You will now use the 1-gallon bucket labeled “Not Irrigated”

You will be using the same hand trowel to collect the “Soil – Not Irrigated” sample. Please thoroughly rinse the hand trowel with water and completely dry before use. Always collect the soil samples from the exact same area each year.

Watch the sampling videos online on our Project Harvest website:

WATER
https://www.youtube.com/watch?v=BtIVMv77fEM

MICROBIAL

Do-It-Yourself method for testing fecal contamination in water

This method will involve testing for presence or absence of sulfur reducing bacteria as an indicator of fecal contamination in water. The test is based on measuring bacteria that produce hydrogen sulfide. If these bacteria are present, the hydrogen sulfide produced reacts with iron, which is present in the testing tube to form iron sulfide. Iron sulfide will appear as black precipitate in the testing tube.

KIT MATERIALS

250mL Plastic Sampling bottle

Pipet

Sharpie

Gloves

25mL sterile vials (5)

Chloraprep Alcohol swabs
in silver foil packets (2)

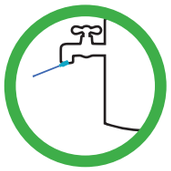
Thermometer

Labeling tape

Silver packets (5)

Scissors

Project Harvest Field Notebook
(hard copy or online)



INSTRUCTIONS

Harvested Rainwater sample collection

1. Using labeling tape, label five 25 mL sterile glass vials 1 through 5.
2. Find a tray or rack to hold all the five 25 mL vials. An egg carton can also be used as a tray to hold the bottles in place.
3. Put gloves on.
4. Loosen the lid from the 250 mL plastic sampling bottle without completely removing the lid.
5. Clean the rim of the cistern faucet using one of the alcohol swabs (ChloraPrep).
6. Open the cistern faucet so your rain barrel water flows at full speed and let it flow out for 10 seconds (feel free to capture this water and use it for gardening purposes).
7. Quickly open the lid from the 250 mL plastic sampling bottle and fill water to the top. Make sure the rim of the cistern faucet and the bottle do not touch to minimize contamination. Immediately cap the bottle to also minimize any contamination.
8. Remove the lid from one 25 mL sterile vial.
9. Very carefully add 20 mL of the water sample– the 20 mL level is clearly marked on the side of each vial. Do this carefully to avoid contamination and spillage. If needed, use the provided pipet to add or remove water.
10. Using an alcohol swab (ChloraPrep), clean the outside of the packets and the scissors.
11. Using the scissors, cut one packet at a time and pour the entire content into the vial and cap immediately.
12. Repeat steps 8-11, to fill each bottle with the water sample and packet contents for all the remaining four vials.

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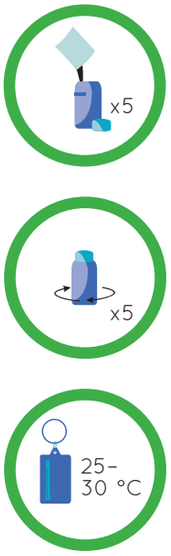

Bottle # 4 is negative for SRB.
Bottle # 5 is positive for SRB.

Table 1. Estimating Number of Sulfur Reducing Bacteria (SRB)

Number of Bottles Positive for SRB	Most Probable Number for each 100mL
0	<1.1
1	1.1
2	2.6
3	4.6
4	8.0
5	>8.0

What does Most Probable Number mean?
It is an estimate of the number of bacteria in the sample.

13. Gently swirl the vials to mix and dissolve the powdered content. The samples will turn yellow.
14. Put all waste in the provided waste bag.
15. With the thermometer, find a safe location with a constant temperature between 25–30 °C.
16. Incubate samples in this location for a total of five days. Each day, check your samples, write down:
    - The temperature
    - How many vials have changed color to black
    - Write daily observations in your Project Harvest Field Notebook. It is a good practice to conduct your observation around the same time every day. **It is important that the vials remain as still as possible during the five-day incubation. Do not shake or swirl the vials.**
17. After 5 days look at each of the five bottles:
    - If no black solids have formed, the water sample is negative for bacteria that produces hydrogen sulfide and thus, the water does not contain bacteria that indicate fecal contamination.
    - If black solids have formed, this indicates the presence of hydrogen sulfide reducing bacteria. The water may be contaminated with fecal matter. Note: When black solids or black precipitates form, the entire bottle changes color from yellow to black. See examples above Table 1.
    - Count how many vials changed color and have black solids. Compare your results with the **Table 1** to estimate the amount of the sulfur reducing bacteria in your water sample. Record how many bottles changes in your notebook and take a picture.
18. Login to the **projectharvest.arizona.edu** website and upload your images and type up your observations. If you do not have access to a computer or smart phone, please take notes in you Project Harvest notebook. **If you have any questions about your results, please contact the promotora in your area.**

KIT MATERIALS

Gloves

25mL sterile vials (5)

Plastic container with lid (for liquid waste)

Bottle of diluted bleach labeled “Bleach”

Small cleaning brush

Waste bag

Cleaning the 25 mL bottles

Once the test is completed, all of the 25 mL vials should be cleaned and returned into the kit for the next test. To clean the bottles:

1. Squirt diluted bleach into each filled vial. The amount of bleach added should fill the bottle to the top. Cap the bottles and let them sit for 10 minutes. The bleach will kill living bacteria remaining in the bottle.

   NOTE: For vials that are positive (have produced black precipitates), a rotten egg smell will be noticeable once the top is removed. Complete the bleach addition step outdoors, or in a very well-ventilated area.

2. After 10 minutes, pour all liquid waste content into the Tupperware and seal the top of the Tupperware tightly. The waste should be returned to the laboratory for safe disposal.

3. Using a brush and liquid dish detergent, clean the bottles and caps thoroughly

4. Rinse the bottles and caps in a bowl with hot boiled water three times or until all residual soap is removed. You want to completely remove any residual soap since liquid soap is highly toxic to microorganisms.

5. Dry the bottles in a safe isolated clean area. To dry, the vials and caps should be placed face-down on a paper towel to make sure the bottles are placed on a clean surface.

6. Let the bottles dry overnight.

7. Once dried, cap the vials and return the vials into the kit until next test.