SEDI-LAB
BACTERIAL TOXICITY EDUCATIONAL KIT

STREAM

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Introduction

EBPI's Sedi-Lab™ kit employs a direct sediment toxicity testing method to assess total toxic responses from all soluble, insoluble, organic, inorganic, and volatile molecules in a given sample. The kit is a rapid, bacterial-based colorimetric bioassay that determines acute toxicity in sediments, suspended sediments, soils and solid wastes. Perhaps most importantly, the Sedi-Lab™ assay will pick up additive, synergistic and antagonistic effects from toxicant mixtures.

Purpose

The Sedi-Lab™ kit is sensitive to a wide spectrum of toxic substances including heavy metals, organic and inorganic pollutants, antibiotics and priority contaminants. The assay detects toxicity directly without labour intensive solvent extraction procedures. The sensitivity of the assay combined with its ease of use make it an essential research tool that can be used qualitatively for field measurements to compare relative toxicity between samples.

WARRANTY

EBPI warrants that, at the time of shipment, the Sedi-Lab™ kit is free of defects in material and workmanship, and complies with the company specifications. Since actual experimental conditions prevailing at user's laboratory are beyond the control of EBPI or its representatives, EBPI makes no other warranty, express or implied, with respect to the product. Notification of any breach of warranty must be made within 120 days of delivery. The sole and exclusive remedy of the customer for any liability of EBPI of any kind, including liability based upon warranty (express or implied, whether contained herein or elsewhere) is limited to the replacement of the product or the refund of the invoice price of the product.

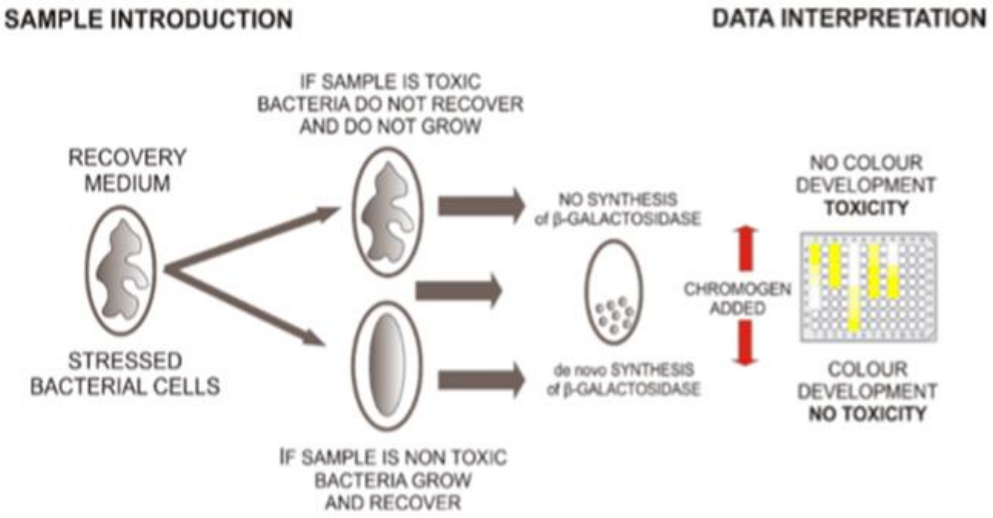
Concept: Testing Acute Toxicity in Soil and Sediments

Soils are an essential component for life on Earth. Soil provides the foundation for plant growth, habitats for species, it acts as filtration for surface water, carbon store and maintenance of atmospheric gases. Soil pollution occurs when materials such as chemicals are out of place or present in concentrations that are higher than normal which will affect humans. Soil pollution can also be caused by means other than the direct addition of substances such as agricultural runoff waters and industrial waste.

Soil has a natural ability to retain most pollutants released into the environment. Accidental spills and a history of various land disposal and storage practices can result in the release of hazardous substances into soil environments. Soil and sediment contamination is a significant toxicological problem that has been extensively studied for the last 35 years. Soils and sediments can become polluted from industrial spills, improper disposal of chemical waste or normal deposition from chronic release of environmental contaminants. Often analysis of pore water or solvent extracts are used to estimate the level of soluble or bioavailable toxicants in a soil sample. Our kit studies the toxicity of chemical, physical or biological substances to the soil. Factors such as absorption, degradation, bioaccumulation, chemical composition, topography, climate, biotic activity, and other variables can influence the risks toxic substances pose to the environment.

The Reaction

The Sedi-Lab™ kit's assay is based on the ability of a toxicant to inhibit the de novo synthesis of an inducible enzyme (β-galactosidase) in a highly permeable mutant of E. coli and the results manifest as an easily observable colour change after a short incubation period. Sensitivity and performance of the test is enhanced by exposing the bacteria to stressing conditions prior to lyophilization, after which they are rehydrated in a cocktail containing a specific inducer of β-galactosidase and essential factors required for recovery of the bacteria from their stressed condition. The activity of the induced enzyme is detected by hydrolysis of a chromogenic substrate to produce a clearly visible blue or yellow colour change. Toxic materials that interfere with the recovery process will decrease enzymatic synthesis and inhibit colour production once the chromogen is added. Comparing the amount of colour produced between a test sample and a reference soil provides a measure of toxic potency.



Preparation – Sedi-Lab™

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Expectations

The Sedi-Lab™ kit employs a direct sediment toxicity testing method to assess total toxic responses from all soluble, insoluble, organic, inorganic, and volatile molecules in a given sample. The Sedi-Lab™ kit should be performed no later than **6 weeks** after collecting the samples. Once your class completes the experiment, the results can be recorded to determine sediment toxicity levels and each group will complete a lab report for evaluation. The Sedi-Lab™ is designed for students to work in five groups of three to four students.

Contents of the Sedi-Lab™ Kit

Each kit contains sufficient components for a class-sized lab. The bottles and vials in the Sedi-Lab™ are labelled with clear, bold letters.

Note: Each Sedi-Lab kit contains bacteria, buffers, chemical solutions and all plastics required for running 5 samples with 6 dilution levels and all necessary controls. Unprepared sediment samples can be used but toxicity levels may not be as sensitive due to factors like decreased surface area and sample moisture content. Soil or sediment samples should be collected from a range of sites at different depths to fully investigate a contaminated site.

Each kit should contain the following:

A. Reaction mixture - a cocktail containing an enzymatic inducer and cofactors required for the recovery of test bacteria from their stressed condition. (3 vials) B. Sedi-Lab™ lyophilized bacteria - a highly permeable selected mutant of E. coli. (1 vial) C. Rehydration solution - a solution to rehydrate the lyophilized bacteria. (1 vial) D. Positive control HgCl2 1 mL (1 vial) --- Bleach F. Blue Chromogen (1 vial) G. Sedi-Lab™ diluent 10 mL (5 vials)	Plastics and additional components: ▪ Disposable plastic pipettes. (6 pipettes) ▪ Disposable test tubes. (32 tubes) ▪ Reference Soil (1 unit)

Collecting the Samples

You have the option of either having your students acquire samples from pre-determined or assigned sources that you and/or your class discussed or you can acquire the samples. Three samples are required from each source. Ensure your students understand and follow safety measures if they are acquiring the samples. When acquiring the samples, read the following below:

Samples need to be mixed manually using a nontoxic device (i.e. stainless steel spoon or spatula)

Samples should be stored in cool dry place, in a glass container, and under cool temperatures (4 °C).

Samples should be crushed, screened, and dried.

Measure the pH and organic content of the soil. Do NOT adjust the pH of the soil before doing the experiment.

The class should have a total of 5 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5
- Sample 1	- Sample 2	- Sample 3	- Sample 4	- Sample 5

Review the Experiment Controls with your Students

Negative Control: A negative control sample should be collected from an uncontaminated site in the same area that represents the local soil conditions while being free of toxicity. These results will be compared to the reference soils to strengthen toxicity results.

Positive Control: The positive control should be used to ensure that the bacteria are functioning properly. This sample contains HgCl2 (bleach) and give a dose-dependent positive response with increased dilutions. It should be run with the samples experiment functions properly.

Student Station Preparation

Below is the suggested distribution of supplies for the preparation of the lab stations for the experiment:

FRONT OF THE CLASSROOM

For use by all Student Groups:

- Negative and Positive Control test tubes (for analysis)

STUDENT WORKSTATION

For each Student Group:

- Sedi-Lab diluent (1 vial, 10 mL)
- Negative control soil sample
- 6 small test tubes
- Pipette
- Bacteria (Bottle B) and aqueous solution (Bottle C)
- Reaction Mixture
- Chromogens (ONPG & Diluent)
- STOP solution

Lab Safety Protocols – Review this with your class before beginning the experiment

Storage:

- The main materials should be stored under refrigeration (2°C to 8°C).
- The Sedi-Lab™ bacteria should be stored in -20°C until use.
- Under conditions of proper storage, the shelf life of the Sedi-Lab™ kit is up to a year.

Handling:

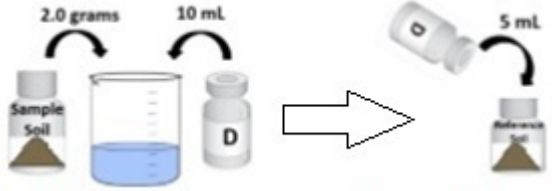
- Handle the kit and tested samples like any potentially hazardous material.
- Note that the bacterial strain is not a known pathogen.
- The E.coli bacterial strain included in the kit are considered a bio-safety level 1 handling strain.
- It is advisable and good laboratory practice to sterilize the remains of the kit before disposal (use biohazard bag).

Procedure – Sedi-Lab™

1. Sample & Control Suspension Preparation

Mix and stir for 10 minutes 2 g of your dried sample with a Teflon stir bar and 10 mL of Sedi-Lab™ diluent into a glass beaker.

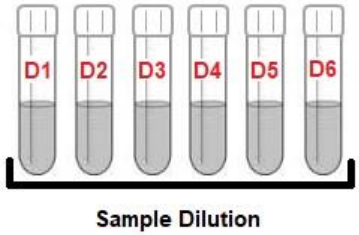
Thoroughly mix 5 mL of Sedi-Lab™ diluent with 1 g of negative control (soil reference). Cover this solution until use.



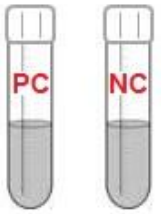
2. Bacterial Rehydration

Under cold temperatures, rehydrate the bacterial in Bottle B with the solution in Bottle C and mix gently. Leave at room temperature for 15 minutes.

3. Assay Preparation with Controls

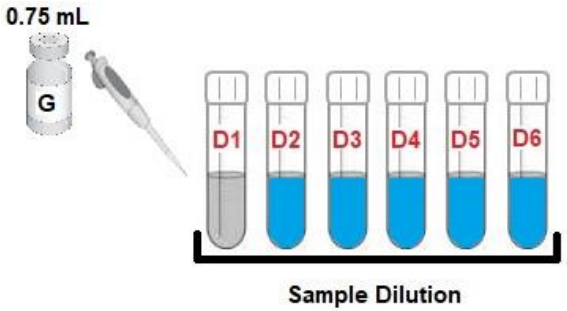


Set up your group of 6 test tubes to test the 6 dilutions of your sample.



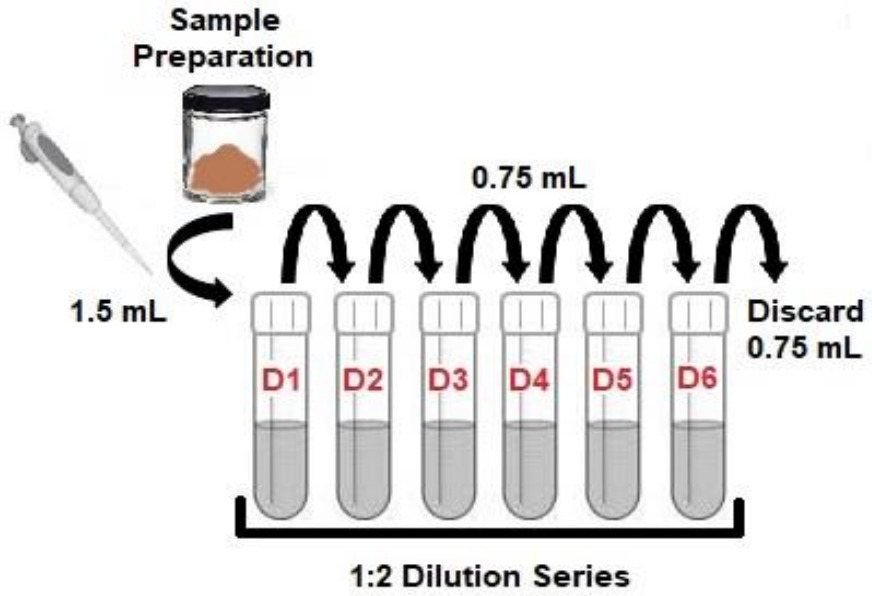
In front of the class, 1 test tube will be used for the negative control and 1 test tube for the positive control.

3. Assay Preparation with Controls (continued)



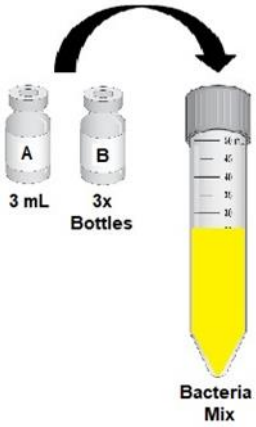
Pipette 0.75 mL of diluent to test tubes 2 to 6. The educator will pipette 0.75 mL of the diluent to the positive and negative control test tubes in the front of the class.

Note: Leave test tube 1 empty.

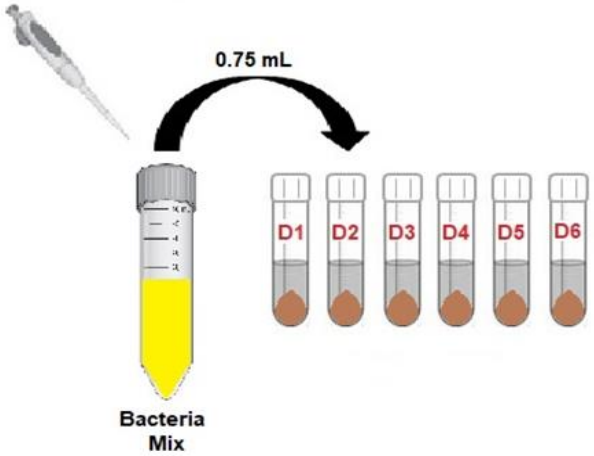


Pipette 1.5 mL of the sample to the D1 test tube. Perform a series of 1:2 dilutions by mixing the contents of the D1 test tube 3 times and quickly draw a volume of 0.75 mL to transfer to the D2 test tube. Repeat until all test tubes up to the D6 test tube are mixed. One pipette can be used for the sample and mix each sample well. Remove 0.75 mL from the D6 test tube.

4. Inoculation and Incubation



Transfer 3 mL of bacterial suspension (B) into the sterile Bacteria Mix centrifuge tube. Transfer the entire contents of 3 Reaction Mixture (A) bottles into the Bacteria Mix centrifuge and mix well. Keep it at room temperature for 10 minutes.

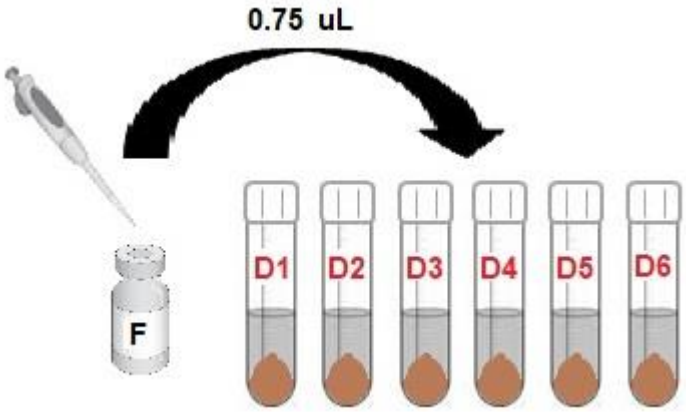


Dispense 0.75 mL of bacterial reaction mixture into all tubes containing samples or controls. Hand shake the sediment-bacteria mix vigorously.

Leave the test tubes at room temperature (20°C) overnight.

If you have an incubator, incubate the test tubes for 2 hours at 37°C with shaking or rotation if possible. After the two hour incubation period, remove the test tubes from the incubator.

5. Chromogen Addition and Colour Development



Remove the seal from the bottle of chromogen. Pipette 75 uL of the chromogen to each test tube that contains a sample. The test tubes can be left at room temperature for colour development.

You can speed up the reaction using heat by placing the plate in an incubator at 37°C or a 60°C oven. The time for colour development will vary, but you should check the test tubes every 15 minutes.

To Educators: Please review the information as a visual guideline of the experiment results, recording the results, further analysis, and the answer sheet for the questions.

Recording the Results

Criteria for a Valid Test: For the Sedi-Lab™ assay to be considered valid, the following conditions must be met:	a) A result must be observed with the **positive control** included with the assay. The positive control must also show a dose response. b) **Blank** test tubes should not contain any colour development. Colour development in the blank wells indicates bacterial contamination as no bacteria is added to these tubes. c) **Negative control** wells should show a red/brown development which are non-toxic.

1. Students will first record their group observations within the 'Sedi-Lab™ Results Table.' Compare the amount of colour development in each sample dilution with the negative control test tube in the front of the classroom.

2. Once this occurs, have each group will go to the other group results and record those results within the 'Sedi-Lab™ Results Table.' Have the students compare the amount of colour development in each sample dilution with the negative control test tube. Please note that the colour development between the samples should be very close and used to eliminate outliers.

When students analyze your results, please note below as a guideline:

Visual Guidelines:

Colour	This represents viable bacteria that are producing enzymes normally.
No Colour	This represents signs of toxicity levels.

The degree of toxicity will be classified into four categories based on a visual inspection of the plate wells:

(-)	No red/brown colour development, high toxicity
(+)	< 50% red/brown colour intensity compared to control, moderate response
(++)	< 100% but > 50% colour intensity compared to control, low response
(+++)	Red/brown colour development is equivalent to the negative control, non-toxic

Comparisons should be made to the average colour development from the negative control test tube and positive control test tube.

Please note that it may be useful to compare samples to each other by categorizing the concentration of sediment that causes an EC100 to the bacteria. This will help provide your group acquire some comparative toxicity data between many samples run in the field.

Sample Observation Table for the Sedi-Lab™ Results

Sample	Dilution	Response (-, +, ++, +++)	Toxicity Sedi-Lab™ EC_{100} (mg/ml)
Sample 1	1	+++	100
	2	+++	
	3	+++	
	4	++	
	5	+	
	6	+	
Sample 2	1	+++	50
	2	+++	
	3	+++	
	4	+++	
	5	+++	
	6	+++	
Sample 3	1	+++	50
	2	+++	
	3	+++	
	4	++	
	5	++	
	6	++	
Sample 4	1	+++	100
	2	+++	
	3	+++	
	4	++	
	5	+	
	6	+	
Sample 5	1	+++	25
	2	++	
	3	+	
	4	+	
	5	-	
	6	-	

Interpreting the Results: Once the students record their results, the students can answer the following questions:

1. Did you note any toxicity levels? Explain further using the concepts you learned. Answers will vary depending on the sites.
2. Describe three factors that can affect toxicity levels in soil? Answer: pH, Soil Moisture, Organic Content

Further Analysis: You can pick further questions based on your preference and the project stream's overall objectives.

Questions and Answers

1. Explain the reasons why negative and positive controls are needed for this experiment. Include details in relation to sampling.	Negative Control: A negative control sample is important because it provides a comparison to strengthen toxicity results from the samples collected. The sample also needs to be representative of the local soil conditions (TOC, fine vs. coarse grain, moisture content) while being free of toxicity. Positive Control: A positive control is included with the kit and used to ensure that the bacteria are functioning properly. Our reference soil sample contains HgCl2 and will give a dose-dependent positive response with increased dilutions.
2. Describe how sediment size can affect toxicity levels in soil.	The composition of the sediment (fine vs coarse granules) can have a significant impact on bacterial toxicity. Uncontaminated sediments and soils with high percentages of fine particulate can demonstrate higher levels of toxicity.

Additional Notes and Assay Observations for Teachers:

A) Make sure that your set up makes use of replicates. The Sedi-Lab kit can run up to 5 samples with 6 dilutions. If your experiment requires the testing of more samples, you can reduce dilution amounts and run more samples using the provided reagents.

B) The composition of the sediment (fine vs coarse granules) can have a significant impact on bacterial toxicity. Uncontaminated sediments and soils with high percentages of fine particulate can demonstrate higher levels of toxicity.

C) It is important to test an uncontaminated soil from the same location with roughly the same particle composition to directly compare. Rough guidelines based on ref 4 and 5 to judge toxicity are given below:

Guideline 1: A sediment is judged to fail the sediment toxicity test if the IC50 is less than 1000 mg/L regardless of grain size characteristics.

Guideline 2: Any test sediment which contains < 20% fine particulate (particles ≤0.063 mm in size or silt and clays) and has an IC50 ≥ 1000 mg/L, the IC50 of this sediment must be compared to a reference sediment of approximately the same particulate composition. Based on this comparison, the sediment fails the toxicity test if and only if:
a) The IC50 is greater than 50% lower than the IC50 of the reference sediment … or

b) The IC50 of the reference and sample sediments differ significantly. Follow ref 6 and used a p values of 0.05.

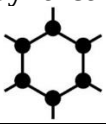
D) Colour development will continue to occur until the stop solution is added. Too much colour development time will blur the difference between toxic and non-toxic responses. If more time is needed, slow the reaction placing the plate into a refrigerator overnight.

E) To more accurately compare the data sets to one another between tests, EBPI recommends calculating toxicity equivalency factors (TEF) to the standard HgCl2 positive control. For more information on how to do this, please contact a representative at EBPI.

Student Lab Report

Group #	Group Student Names	Role

Checkmark the appropriate Stream below:

Water 	Chemical 	Soil 	Synthetic 

Note: At the beginning of class, your teacher will have the lab stations ready. Remember to check your lab station, ensure you have the appropriate supplies, and read over the safety protocols.

Pre-Lab Notes	

Instructions: Read over the preparation and the procedure for the specific biotechnology kit that you will be working on. Complete the lab and record the results.

Preparation – Sedi-Lab™

To Students: Please follow the instructions and information below to help prepare for the lab experiment.

Expectations

The Sedi-Lab™ kit employs a direct sediment toxicity testing method to assess total toxic responses from all soluble, insoluble, organic, inorganic, and volatile molecules in a given sample. The Sedi-Lab™ kit should be performed no later than **6 weeks** after collecting the samples. Once your group completes the experiment, your group will record the results from your sample and then record the results of the other groups. Each group will determine sediment toxicity levels and complete a lab report for assessment and evaluation.

Collecting and Preparing the Samples

Your educator will discuss whether your group will need to acquire samples from a pre-determined or assigned sites or if your educator will provide the samples. If you will be doing field work, ensure you read and understand the safety measures. You will be required to collect one sample. When acquiring the sample, read the following below:

Your sample needs to be mixed manually using a nontoxic device (i.e. stainless steel spoon or spatula)	The sample should be stored in cool dry place, in a glass container, and under cool temperatures (4°C).	The sample should be crushed, screened, and dried.	Measure the pH and organic content of the soil. Do NOT adjust the pH of the soil before doing the experiment.

The class will have a total of 5 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5
- Sample 1	- Sample 2	- Sample 3	- Sample 4	- Sample 5

If you are completing the site sample study: Once you acquired the sample, submit your sample to the Educator.

Lab Station Preparation

At the beginning of the lab, you should have the following available in the front of the classroom and lab station:

FRONT OF THE CLASSROOM

For use by all Student Groups:
- Negative and Positive Control test tubes (for analysis)
- Incubator

GROUP LAB STATION

For each Student Group:
- Sedi-Lab diluent (1 vial, 10 mL)
- Negative control soil sample
- 6 small test tubes
- Pipette
- Bacteria (Bottle B) and aqueous solution (Bottle C)
- Reaction Mixture
- Chromogens (ONPG & Diluent)
- STOP solution

Before starting an experiment it is important to ensure all of the materials, supplies and equipment needed are available and ready. Be sure to speak to your educator if there is anything missing.

Ensure that you review the Lab Safety Protocols before beginning the experiment.

Storage:
- The main materials should be stored under refrigeration (2°C to 8°C).
- The Sedi-Lab™ bacteria should be stored in -20°C until use.
- Under conditions of proper storage, the shelf life of the Sedi-Lab™ kit is up to a year.

Handling:
- Handle the kit and tested samples like any potentially hazardous material.
- Note that the bacterial strain is not a known pathogen.
- The E.coli bacterial strain included in the kit are considered a bio-safety level 1 handling strain.
- It is advisable and good laboratory practice to sterilize the remains of the kit before disposal (use biohazard bag).

Results & Analysis – Sedi-Lab™

Instructions: Once you obtained the results, complete the following steps below. Read over the guidelines before recording your results.

If you have any errors from the experiment, please describe them here and the reasons why this occurred:

Observing the Results:

Criteria for a Valid Test:	
For the Sedi-Lab™ qualitative assay to be considered valid, the following conditions must be met:	d) A result must be observed with the **positive control** included with the assay. The positive control must also show a dose response. e) **Blank** test tubes should not contain any colour development. Colour development in the blank wells indicates bacterial contamination as no bacteria is added to these tubes. f) **Negative control** wells should show a red/brown development which are non-toxic.

When you analyze your results, please note below as a guideline:

Visual Guidelines:

Colour	This represents viable bacteria that are producing enzymes normally.
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The degree of toxicity will be classified into four categories based on a visual inspection of the plate wells:

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(+++)	Red/brown colour development is equivalent to the negative control, non-toxic

Comparisons should be made to the average colour development from the negative control test tube and positive control test tube.

Please note that it may be useful to compare samples to each other by categorizing the concentration of sediment that causes an EC100 to the bacteria. This will help provide your group acquire some comparative toxicity data between many samples run in the field.

Recording the Results:

1. Have your group record the observations in the 'Sedi-Lab™ Results Table' in the next page. Compare the amount of colour development in each sample dilution with the negative control test tube in the front of the classroom.

2. Once you are done this, have your group go to the other group results and record those results within the 'Sedi-Lab™ Results Table.' Compare the amount of colour development in each sample dilution with the negative control test tube. Please note that the colour development between the samples should be very close and used to eliminate outliers.

Sedi-Lab™ Results Table

Sample	Dilution	Response (-, +, ++, +++)	Toxicity Sedi-Lab™ EC_{100} (mg/ml)
Sample 1	1		
	2		
	3		
	4		
	5		
	6		
Sample 2	1		
	2		
	3		
	4		
	5		
	6		
Sample 3	1		
	2		
	3		
	4		
	5		
	6		
Sample 4	1		
	2		
	3		
	4		
	5		
	6		
Sample 5	1		
	2		
	3		
	4		
	5		
	6		

Interpreting the Results: Complete the following questions.

Did you note any toxicity levels? Explain further using the concepts you learned.

Describe three factors that can affect toxicity levels in soil?

Further Analysis: Complete the following questions.

Explain the reasons why negative and positive controls are needed for this experiment. Include details in relation to sampling.

Describe how sediment size can affect toxicity levels in soil.