FOR 3457 – Forest Watershed & Water Quality Watershed Management Tools & Methods

Testing Stream Water for Bacteria

This lab is used to show the count of bacteria in stream water based on the growth of colonies on petri dishes. Using water sample from various sources will show the range in water quality from urban to rural sources. Each water sample will be placed in a petri dish with Agar growing medium which will encourage bacterial growth. After an incubation period (24-72 hours), bacteria colonies will be visible on the petri dish. This simple technique is a useful method of determining bacterial density of different samples.

Equipment and Supplies

- Water samples
- 50 ml flasks, sterilized
- Petri dish, sterile, 100 mm x 15 mm
- Pipets & pipet tips, sterile
- Incubator, maintained at 35±0.5°C
- Glass lens with magnification of 2-5x, or stereoscopic microscope
- Nutrient Agar, sterile and prepared

Procedure

- 1. Write the name of the site and sample number on the bottom rim of the petri dish.
- 2. Melt the ice/water samples. Shake samples 25 times to ensure good bacterial distribution. Measure 49 ml of distilled water into a glass flask. Prepare initial 1:50 dilution by pipetting 1 ml of sample into the distilled water using a sterile pipette.
- 3. Replace the parafilm on the flask and shake the sample vigorously. Then add 1 ml of the diluted sample to the petri dish followed by 12-15 ml of melted agar to the plate. Rotate the plate 5 times to the right, left, front, and back to thoroughly coat the plate. Tape lid shut and place on a level surface to harden (10 minutes). Invert the plates and incubate at 35°C. Plates can be stacked 4 high.
- 4. After 24-72 hours, remove the plate from the incubator, count and record the number of colonies with the aid of an illuminated lens or stereomicroscope. If there is time, get a second count done by different students to verify the count. Follow the general formula to determine the total bacteria/ml:

 $bacteria \ count \ / \ ml = \frac{Number \ of \ colonies}{Dilution \ factor \ (1:50 = 0.02)}$

Report Outline

The purpose of this lab is to compare the results of bacteria (#/ml) across the study sites. You can use your previous sites analyzed, or start a new analysis with any sites that interest you. In your analysis, try and showcase any trends that form. Use previous lab results and compare bacteria to pH levels, DO levels, water colour, etc... Also you could potentially look at previous year's data to show trends. In your introduction, introduce the water born bacteria. Elaborate and research the importance of looking at bacteria levels in terms of water quality, and the role different bacteria play in various watershed types. Methods should include a detailed overall explanation at what was done in the lab with the plates, as well as any explanations needed for any statistical work. Results should include a table of results (might be suitable for an appendix depending on size), as well as any statistical results. Focus on any relationships between different lab results from previous labs and the bacteria level. Use previous lab's knowledge about statistics and make sure to have an analysis. (I.e. some sort of regression?) (Please do not forget to talk about results in this section. Don't just plop tables and graphs here, you need to introduce and talk about them). Your discussion section is where you talk about your results, and elaborate on the trends/relationships found. This section is important and should include research and references to support your findings. You should also focus on the belief and trust regarding the outcomes of the results i.e. reliability, uncertainty, representativeness, appropriateness. Discuss the results but also the methods in which you found them and their reliability. Conclude your report with stated objectives and the overall lesson learnt. (Don't forget appendices and reference)

Report worth: Introduction - 20 Methods - 15 Results - 20 Discussion - 30 Conclusion - 5 Title, References, and Appendix - 10

Notes: You need a title page with a title that is descriptive and creative. Make sure to have appropriate references. We discourage the use of web based references. Utilize the library and the online archives to find actual scientific references and books. Make sure to have a properly formatted list of references. Appendices should each have a title. Number your pages, and remember that page 1 starts with your writing, not with the table of contents (if you chose to add one). This report is marked from a 3rd year university level, therefore be sure to write and format appropriately.

Marking Guide

Introduction (20)

- Did they introduce the topic and do some background research on the importance/impact of soil bacteria? Are there references in their research? (Proper references)

- Did they introduce quickly the sites they are analyzing?

- Did they state the objective of the report? (Where? Why? How?)

Methods (15)

- How where the bacteria plates prepared? As specific as possible.

- What materials were used? (Actual names of products)

- What did they use to count the plates, and what was the procedure? (i.e. split the plate in 4, all the categories, description). Did they show the equation that determines bacteria density?

- Did they keep the writing in the proper tense? No first person.

Results (20)

- Are the figures introduced before they are placed in text? (i.e. need to reference them before they show up)

- Are the figures formatted properly? (Proper axis, significant digits, axis labels, colour/pattern to differentiate, clean looking?)

Did they do any statistical analysis between bacteria and previously measured results (i.e. colour?
DO?). Something that shows critical thought in the analysis, not just showing straight lab results.
Quick mention of trends in the results? Elaborate on this in the discussion but a quick mention of

drastic differences. The "what" goes in results, the "why" in discussion.

- Don't just copy and paste the same paragraphs over and over again.

Discussion (30)

- Is there a discussion about the results? Do they go into detail about the "WHY"?

- Did they go over any trends found and explain them with research to support their explanations?

- Are there appropriate references backing up their discussion?

- Did they deal with the matter of belief and trust regarding the results? (Reliability, uncertainty, representativeness, context)

- Did they suggest ways to enhance reliability? How would they make the lab better? (Sampling? Procedure? Human errors?)

- Why is knowing about water bacteria important?

Conclusion (5)

- What was learned from looking into the bacteria levels at each site?

- No personal comments about what they feel, but just what was accomplished in the lab?

Title, Appendix & References (10)

- Is the title creative and informative? Does it specifically state what will be looked at in the report?

- Is the bibliography formatted properly? (Alphabetical order, APA, consistent format) Are all of the reference academic? (I.e. no URL/wiki references)

- Is the appendix formatted properly? (I.e. proper titles for each appendix) If necessary, data should be in appendix if it's too large or complex for the body of the report.

- Is the paper grammatically correct? Does it flow well? Proper paragraph and sentences? Spelling?