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Faculty of Science

Laboratory Manual

Toxiocology

Bachelor of Biotechnology (Hons.)

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Toxicology

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')

- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful
- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

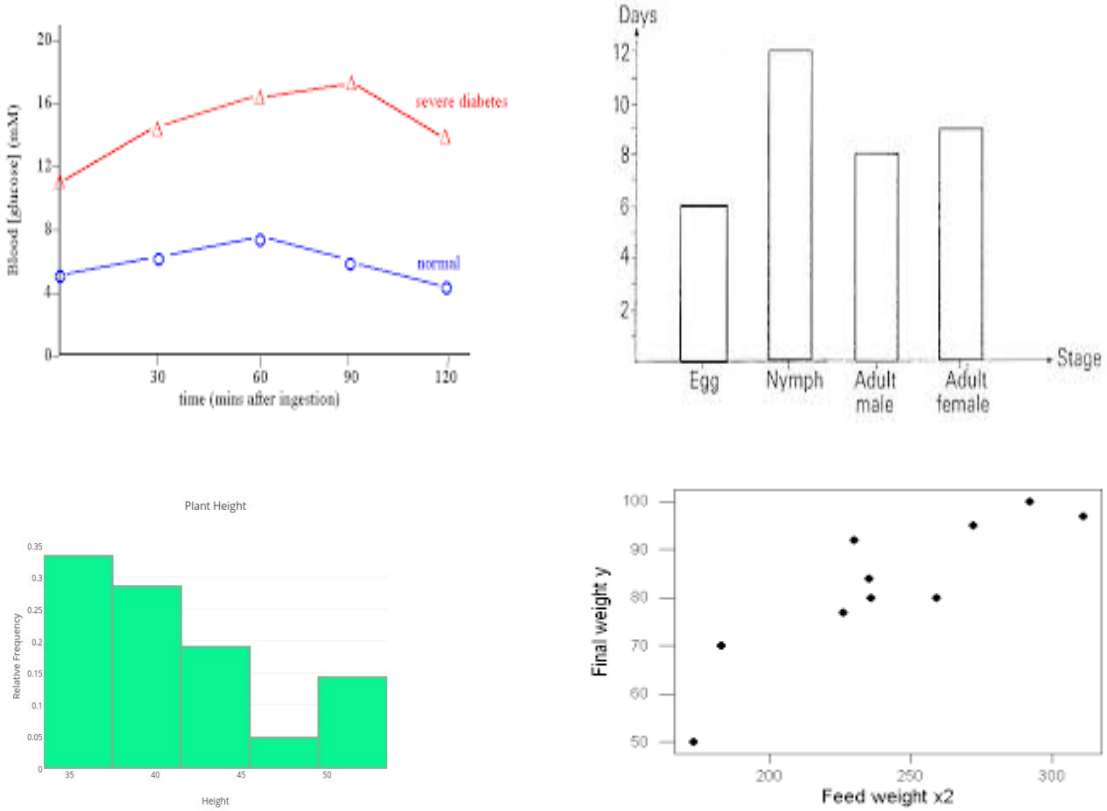

Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.

- Cite sources to substantiate background information.
- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1
Title: Determination of death percentage due to toxicity

Objective:
After completing the practical, you will be able:
1. To predict the potential death percentage in a risk assessment study

Introduction:

Toxicity is the degree to which a chemical substance or a particular mixture of substances can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity). By extension, the word may be metaphorically used to describe toxic effects on larger and more complex groups, such as the family unit or society at large. Sometimes the word is more or less synonymous with poisoning in everyday usage.

A central concept of toxicology is that the effects of a toxicant are dose-dependent; even water can lead to water intoxication when taken in too high a dose, whereas for even a very toxic substance such as snake venom there is a dose below which there is no detectable toxic effect. Toxicity is species-specific, making cross-species analysis problematic. Newer paradigms and metrics are evolving to bypass animal testing, while maintaining the concept of toxicity endpoints.

Problem statement:

A risk assessment study scenario involves 1200 people being exposed to chlorine vapour due to a train car rupture in a suburban area. Predict the potential death.

Group	People	Number	Exposure in time (Minute)	Concentration (ppm)
Group A	600	1	40	150
		2	30	120
		3	20	100
Group B	600	1	60	140
		2	40	110
		3	30	100

Procedure:

Formula:

$y = a + b \ln c^n t$

where, a, b and n are experimentally determined constant
c is concentration in ppm
t is time exposure in minutes
ln is function

Results:

Practical 2
Title: Determination of death percentage due to toxicity

Objective:

After completing the practical, you will be able:

1. To predict the potential death percentage in a risk assessment study

Introduction:

Toxicology is a discipline, overlapping with biology, chemistry, pharmacology, and medicine, that involves the study of the adverse effects of chemical substances on living organisms and the practice of diagnosing and treating exposures to toxins and toxicants. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Factors that influence chemical toxicity includes the dosage (and whether it is acute or chronic), route of exposure, species, age, sex, and environment. The science of toxicology is based on the principle that there is a relationship between a toxic reaction (the response) and the amount of poison received (the dose).

An important assumption in this relationship is that there is almost always a dose below which no response occurs or can be measured. A second assumption is that once a maximum response is reached any further increases in the dose will not result in any increased effect.

Materials:

- Four large sized glasses
- One small sized glass
- Colouring material
- Distilled water

Procedure:

1. Fill the large and small glasses with approximately ¾ of distilled water.
2. The small glass represents a child and the large glass represents a matured person.
3. Add same number of sample in each glass.
4. The small glass would be showing darker coloration and show high dose response compared to the other glass.

Observation/ Results:

Questions:

1. Explain dose- response relationship.
2. What are the implications of dose- response relationship in toxicological studies?

Practical 3

Title: Determination of degree of hardness (dkh) in surrounding water bodies

Objective:

After completing the practical, you will be able:

1. To learn how to assess the degree of hardness in water bodies.

Introduction:

Hard water is formed when water passes through or over limestone or chalk areas and calcium and magnesium ions dissolve into the water. The hardness is made up of two parts: temporary (carbonated) and permanent (non-carbonated) hardness.

Degrees of general hardness (dGH or °GH) is a unit of water hardness, specifically of general hardness. General hardness is a measure of the concentration of divalent metal ions such as calcium ($Ca^{2+)}$ and magnesium (Mg^{2+}) per volume of water. Specifically, 1 dGH is defined as 10 milligrams (mg) of calcium oxide (CaO) per litre of water. Since CaO has a molar mass of 56.08 g/mol, 1 dGH is equivalent to 0.17832 mmol per litre of elemental calcium and/or magnesium ions.

In water testing, paper strips often measure hardness in parts per million (ppm), where one part per million is defined as one milligram of calcium carbonate ($CaCO_3$) per litre of water. Consequently, 1 dGH corresponds to 10 ppm CaO but 17.848 ppm $CaCO_3$ which has a molar mass of 100.09 g/mol.

Materials:

- Water bottle
- Kit for detection of degree of hardness in water

Procedure:

1. Rinse the sample several times to make it free from contamination.
2. Fill the test tubes with sample water up to 5 ml mark.
3. Carefully add one drop of reagent at a time from the bottle and swirl gently to mix the solution after each drop.
4. Count the number of drops until the solution becomes light yellow to a red- purple colour.
5. Once the solution turns red-purple, leave it to stand for 30 seconds. If the colour disappears, add on more drops of reagent until the solution turns into purple colour.
6. Temporary water hardness is given German degrees (dkh) and corresponds to the number of drops reagent utilized to obtain the red-purple colour.

1 drop= 1 degree dkh

Observation/ Results:

Questions:

1. What is temporary and permanent hardness?
2. What do you mean by degree of hardness?

Practical 4

Title: Determination of nitrate (NO^{3-}) and nitrite (NO^{2-}) in water sample

Objective:

After completing the practical, you will be able:

1. To learn and assess how to estimate the nitrate and nitrite level in water sample.

Introduction:

Nitrate (NO^{3-}) as well as nitrite (NO^{2-}) level determines the degree of decomposition of organic matter at the bottom of the water bodies.

As we know, due to the constant excretion and other decomposition of inhabitants of water body (flora and fauna), there is a constant accumulation of organic substances at the bottom of the lake.

Therefore, to certain, the level of organic matter in the sample water taken from water body must be tested to identify the nitrate level in the water body.

Materials:

- Water bottle
- Kit for detection of nitrate and nitrite in water

Procedure:

1. Rinse the test tube provided in the nitrate testing kit and fill with 5ml of water sample.
2. The nitrate test kit contains 2 bottle of reagent which must be shaken well before adding into water sample.
3. Shake reagent in bottle 1 and add 10 drops of reagent from bottle 1 into the test tube provided containing 5ml of water sample and mix thoroughly.
4. Shake reagent in bottle 2 and add 10 drops of reagent from bottle 2 into the test tube provided containing 5ml of water sample and mix thoroughly.
5. Leave the test tube containing 5ml of water sample for 1 min which is an important step for a more accurate result.
6. Wait for 5 minutes for colour to develop and tally with the strip provided.

Questions:

1. What do you mean by organic deposition in water bodies?
2. Explain the interconversion of nitrate and nitrite?

Notes

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