Experiment 1

Theme: Water Quality

NUI, Galway
Monday 16th May, 2005

General instructions.

Wear a laboratory coat and safety spectacles at all times within the laboratory.

Eating and drinking are prohibited in the laboratory.

Disposable gloves are provided and must be worn when handling chemicals and microbiological samples.

All paper used, including rough work paper, must be handed in at the end of the experiment.

All results must be entered into your answer book.

Your graph must be handed up along with the answer book.

Only the final answer book, and attached graph, will be marked.

Where you are asked to get a laboratory supervisor to sign off on a result before proceeding to the next step, marks will only be awarded if the supervisor has signed off on the result at the correct time.

The tasks may be carried out in which ever order you wish.

Marks for each task.

Task 1 carries 9 marks.

Task 2 carries 25 marks.

Task 3 carries 32 marks.

Task 4 carries 11 marks.

Task 5 carries 11 marks.

Task 6 carries 12 marks.

The scenario.

Inhabitants have requested an investigation into both the source and the nature of pollution, which is being discharged into the river running through their town.

Several small residential settlements and local industries discharge waste water (sewage, dairy and chemical waste) after treatment into the local river that flows into the sea near a local seaside resort and beach. Some of the treatment plants at the various sites are old and may not be effective.

The inhabitants have requested that the nature and consequences of these discharges be determined for the local environment.

To carry out this study, samples of both river water and marine water were collected at seven different sites (Figure1; Sites 1 – 7) and tested to determine the nature of the samples and the organic and chemical pollution. All seven samples were tested by the State Analysis Laboratory and the results of the tests from each sample were reported to the Local Environmental Protection Authority.

Prior to analysis, all seven samples were taken in duplicate and a subset was sent to other approved testing laboratories for confirmation and validation of the results (using alternative methods where possible). Your team represents one of these other testing laboratories.

Your team has been given three of these water samples to test. The samples were re-labelled blindly as Samples A, B and C.

Using the samples which are provided from three of the study sites (Samples A, B and C) determine the following:

1. The nature of the water sample (freshwater or marine).
2. The nature and, where possible, the level of the organic pollutants.
3. The identity of the chemical pollutant, and the source of chemical pollution.
4. The location of the sampling sites.

In order to do this, you have been asked to carry out the following specific tasks (in which ever order you wish).

1. Determine the **Bacterial** levels present (in colony forming units per 100 millilitres).
2. Determine the **Chemical pollutant (R)** present in only **Sample A**.
3. Determine the **Density** of each of the samples.
4. Determine the type of bacteria present by way of a **Gram stain**, cell shape and arrangement.
5. Determine the **Organic loading** present by way of BOD tests (day 5 only). (See page 14 for a detailed explanation of BOD.)
6. Determine the **Sampling Sites** for Samples A, B and C.

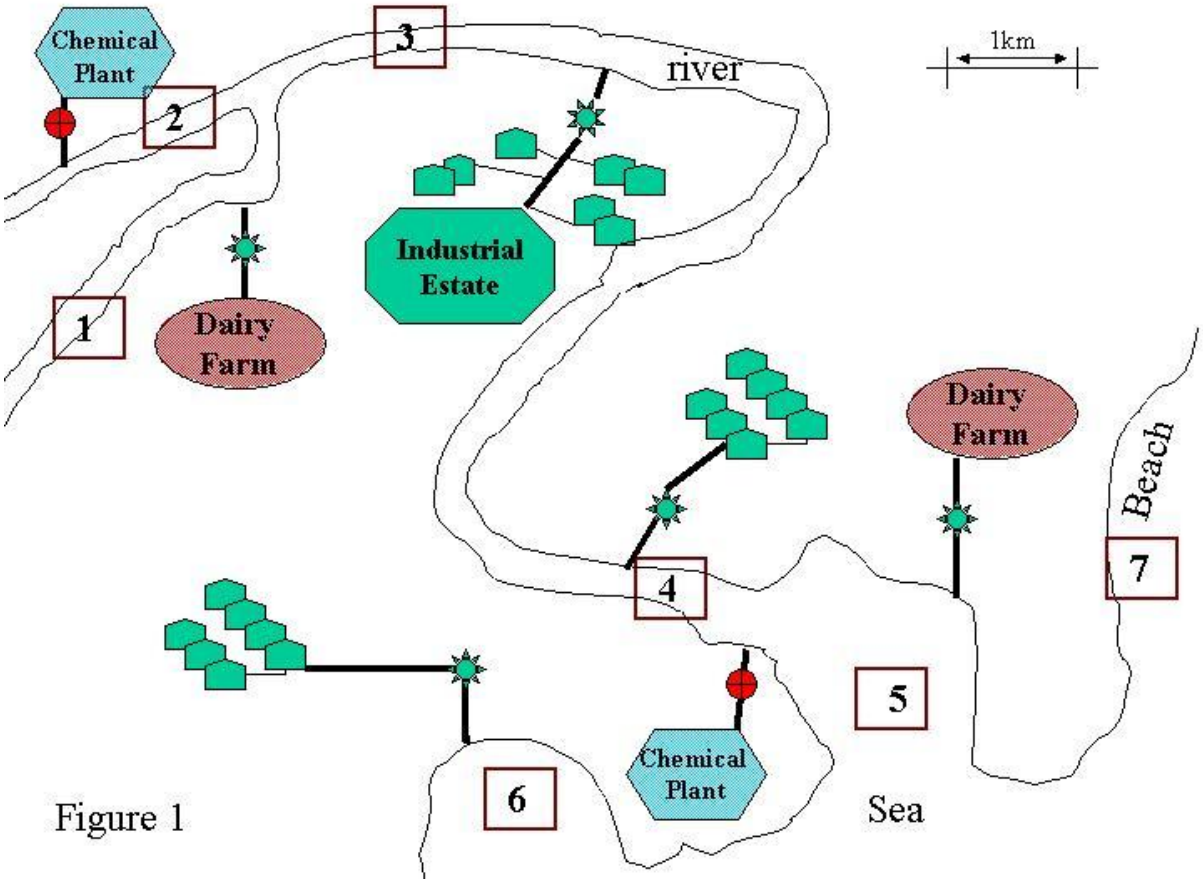


Figure 1

Key to symbols

- Domestic households
- Sewage treatment works
- Chemical waste treatment works
- Outflow pipe
- Sampling site

Task 1 - Determine Bacterial levels.

You are given three plates of serial ten-fold dilutions (one from each of the three water samples labelled A, B, and C) and asked to estimate the count per 100 ml of the original samples A, B, and C. The extent of the serial dilution is indicated on each plate.

Calculation of Colony* Forming Units (CFU's) per 100 ml of Sample.
In this instance, 0.1 ml of sample was used to prepare each of the plates. Divide the number of colonies on a plate by the dilution factor to get the number of CFU's in 0.1 ml of the sample. For example, if 125 colonies are counted on a 10^{-7} pour plate, there are

$$(125) / (10^{-7}) = 1.25 \times 10^{9} \text{ CFU's per 0.1 ml of sample}$$

You then have to scale your results to express them in CFU's per 100 ml.

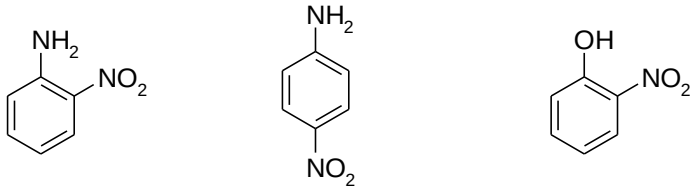
Results:
Record your results for Question 1 in the answer book.

* A colony is a macroscopic visible population growing on solid medium arising from a single cell.

THIS COMPLETES TASK 1.

Task 2 - Determine the Chemical pollutant present in Sample A only.

Problem:
One of the chemical plants shown on the map (Figure 1) is a dyes factory, which uses anilines and phenols to make azo dyes. One of the following three compounds; *ortho*-nitroaniline **(X)**, *para*-nitroaniline **(Y)** and *ortho*-nitrophenol **(Z)** has been accidentally released by the factory into the water. This chemical pollutant was extracted from only water **Sample A** into a suitable organic solvent. This compound is chemical pollutant **(R)**. You are requested to use thin-layer chromatography (TLC) to identify the pollutant compound **(R)**, from three authentic samples of **(X)**, **(Y)** and **(Z)**. You will make the azo dyes made by the factory by reacting the diazonium salt of **(R)** with phenol and 1-naphthol. You are required to know the colours of these dyes in order to monitor future emissions by the factory.



(X) **(Y)** **(Z)**

You can now answer Question 2 in the answer book.

Brief Description of Thin Layer Chromatography (TLC):
Chromatography separates compounds in a mixture according to interactions of the compounds between the stationary phase and mobile phase. In this case the stationary phase is silica gel adsorbed onto aluminium, which is commonly called a TLC plate. The mobile phase is a mixture of organic solvents. The most polar compound interacts with the silica gel the strongest, and requires the greatest polarity solvent to move up the plate. The least polar compound moves up the plate the quickest, and requires the least polar or non-polar solvent to move up the plate. Ethyl acetate is the polar solvent, and hexane is the non-polar solvent provided.

TLC Procedure:
1. Using a pencil, mark off four equidistant points along the pencil line (base-line) of the TLC plate provided, and label them underneath the line X, Y, Z and R. Do not place finger marks on the plate, as the organic compounds in your fingers will interfere with the analysis. Use tweezers to handle the TLC plates.
2. Using the glass capillary tubes (TLC Spotters), apply or spot the compounds onto the corresponding pencil marks X, Y, Z and R. Your spots should be no more than 3 mm in diameter in order to get maximum resolution. You should also make sure that your spots are clearly visible (yellow or orange) on the white silica background. This may require re-spotting of the samples.
3. The first TLC plate should be run using a mobile phase of 5-6 cm^3 of 20% (volume) ethyl acetate and 80% (volume) hexane. It is essential that the base-line is above the level of the solvent, when the plate is placed inside the Development Tank.

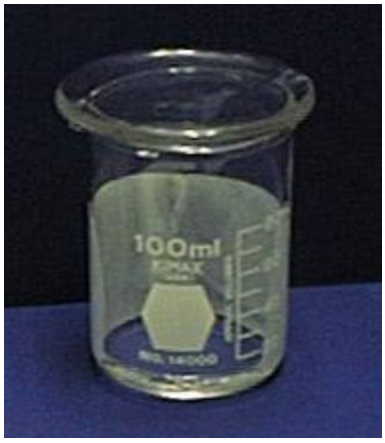


Figure 2: Example of Development Tank

Place a watch-glass on top of the beaker to prevent evaporation of the solvent. Along with the filter paper, it maintains a saturated atmosphere inside the tank.

4. Carefully place the TLC plate inside the Development Tank. Keep the tank covered with the watch glass during the chromatography. You should allow the mobile phase to rise to about 1cm from the top of the TLC plate, before taking the plate out of the tank using tweezers. The height to which the mobile phase rose is immediately marked. This is the solvent front.
5. Circle the spots with a pencil, and measure the distance from the baseline to the centre of each spot **(a)**, and the distance from the baseline to the solvent front **(b)**. You should now estimate the Retardation Factor (R_f) for each compound **(X)**, **(Y)** and **(Z)**, where $\mathbf{R_f = (a)/(b)}$.

You can now answer Questions 3-5 in the answer book.

Carry out one further TLC analysis using the mobile phase mixture of 30% ethyl acetate and 70% hexane.

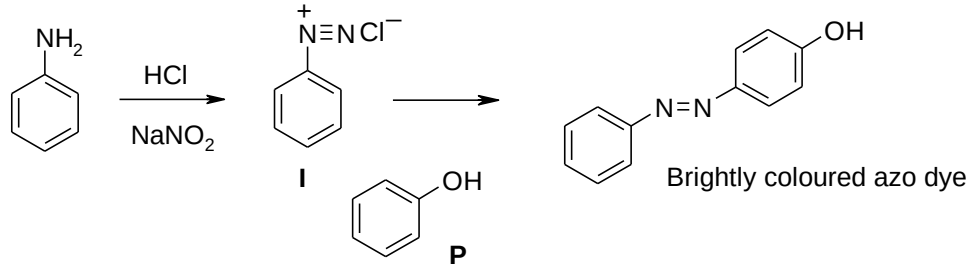
You can now answer Questions 6-8 in the answer book.

The R_f of unknown compound **(R)** will be the same as either **(X)**, **(Y)** or **(Z)** at the given mobile phases. This can be used to identify it.

You can now answer Questions 9 and 10 in the answer book.

Synthesis of Azo Dyes:
A dye can generally be described as a colorant that has an affinity to the substrate to which it is being applied. The following task involves preparing two of the azo dyes made by this factory. Different azo compounds possess different colours.

Brief Description of the Reactions
Azo-compounds have been used as dyes for over 150 years, and are made by coupling an aryl diazonium salt (*e.g.* compound **I** in the Scheme 1 diagram below) with an activated aromatic compound (*e.g.* phenol **P** in the Scheme 1 diagram). The diazonium salt, *e.g.* **I,** is isolated from the reaction of an aniline with nitrous acid. Nitrous acid (HNO_2) is generated *in situ* from the reaction of sodium nitrite ($NaNO_2$) with dilute hydrochloric acid (HCl). Diazonium salts are unstable, and should be kept at 0 ºC.



Scheme 1:
You can now answer Question 11 in the answer book.

Procedure (using the 12 test-tubes provided):
Note – You should ask for ice when required.

1. Add approximately the tip of a spatula full of the solid unknown **(R)** to 2-3 cm^3 of hydrochloric acid. Stir solution well using the glass rod provided, while cooling on ice.
2. Make up a separate similar concentration of sodium nitrite in 2-3 cm^3 of water. Stir solution well, while cooling on ice.
3. Add the cooled nitrite solution to the cooled acidic **(R)** solution to give the diazonium salt. Keep this solution cooled on ice.
4. In a third test-tube, stir the tip of a spatula full of phenol in 3 cm^3 of sodium hydroxide (alkali).
5. Mix a <u>small</u> portion of the diazonium salt solution with the alkali solution of phenol. The reaction occurs quickly and a coloured solid precipitates form rapidly. The precipitates are the crude azo dyes.
6. Prepare the azo dye from diazonium salt **(R)** and activated aromatic compound, 1-naphthol.

You can now answer Questions 12 and 13 in the answer book.

THIS COMPLETES TASK 2.

Task 3 – Determine the density of each of the samples.

Introduction.
Freshwater has a density of 1000 kg m^{-3} at 4 ^{o}C. The density of a seawater sample depends on its temperature and the salts and other material dissolved in it. Typical densities at the ocean surface range from 1025 kg m^{-3} to 1030 kg m^{-3}. Water in estuaries, bays, and near the mouths of rivers, where freshwater from rivers mixes with salty seawater, has a density between the density of freshwater and the density of seawater. Its density depends on the precise proportions of freshwater and seawater in the sample. This is influenced by the location of the sampling point relative to river outlets, by the amount of freshwater flowing from the rivers, and by tides.

A hydrometer is a device that allows you to measure the density of a liquid. For this task, you are asked to construct a hydrometer and measure the densities of samples of water.

Construction of a hydrometer.

Principle of operation:
Archimedes' Principle tells us that when an object is wholly or partly immersed in a fluid, it experiences an upward buoyancy force equal to the weight of the fluid that the object displaces.

Construction procedure:
You have been given the components required to build a working hydrometer, illustrated in Figure 3. Construct the hydrometer as follows:

(i) Thread the plastic pipette through the hole in the rubber bung, keeping the tapered end of the pipette outside the test tube.

(ii) Peel the paper backing off the self-adhesive scale. Attach the scale firmly to the pipette while the pipette is still dry. Position the "0" on the scale a few centimetres from the top of the pipette. In case the scale becomes detached later on, mark the point on the pipette corresponding to "0" on the scale

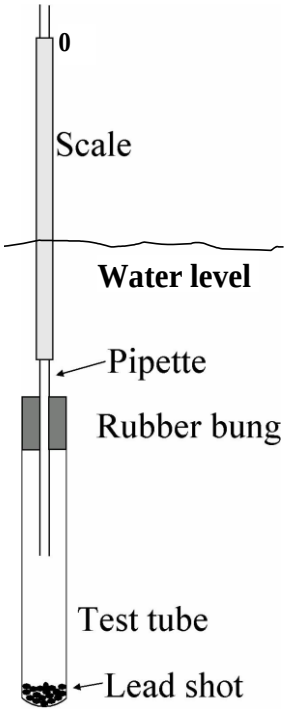


Figure 3: A completed hydrometer.

(iii) Estimate the mass of lead shot you will have to add so that your hydrometer floats in freshwater with most of its volume below the surface. The masses of the different components of your hydrometer (excluding the lead shot) are given in the table below.

Component	Mass (g)
Test tube	42
Rubber bung	16
Pipette	1.5
Scale	0.5

(Hint: You need to measure or estimate the volume of the hydrometer to answer this question.)

Enter your calculation and result in Question 14 in your answer book.

BEFORE YOU PROCEED TO THE NEXT STEP, ask your laboratory supervisor to sign off on this calculation.

(iv) Add sufficient lead shot to the test tube so that the hydrometer floats upright in freshwater (tap water), with the pipette breaking the surface of the liquid at a scale reading close to "0". In case the rubber bung becomes loose later on, mark with the felt-tipped pen where the rubber bung meets the glass of the test tube.

Record the hydrometer scale reading where the scale breaks through the surface of the freshwater in Question 15 in your answer book.

Calibration of the hydrometer:
The hydrometer scale must be calibrated before you can use the hydrometer to measure densities of water samples. To do this, you are asked to draw a calibration curve for your hydrometer. The calibration curve is a graph of hydrometer scale reading plotted against density. The data for this graph are obtained by recording scale readings obtained when you float your hydrometer in liquids of known densities.

You are not allowed to assume that hydrometer scale reading varies linearly with density. You have been provided with approximately 1000 cm^3 of seawater, which has a density of 1026 kg m^{-3}. You also have freshwater (tap water) available to you, which you may assume has a density of 1000 kg m^{-3}. These solutions provide two data points for your calibration curve. For additional data points, solutions of other known densities may be obtained by carefully diluting the seawater with appropriate amounts of freshwater. The calibration curve must be drawn with data obtained using diluted samples of seawater.

(i) Derive a formula giving the density of the solution you would obtain if you mix a volume V_1 of a liquid of density ρ_1 with a volume V_2 of a liquid of density ρ_2.

Enter the formula you obtain in Question 16 of your answer book.

(ii) Before carrying out any measurements, plan how you are going to carry out each of the dilutions you will use in turn to obtain solutions of different densities.

Begin with the pure seawater sample. Including those for densities 1026 kg m^{-3} and 1000 kg m^{-3}, your graph should have seven points. While you can use as much freshwater as you wish, you only have a 1000 cm^3 of seawater to work with. You may discard portions of solutions as part of your dilution scheme.

Draw up a step-by-step plan for how you will carry out dilutions, giving the density of the mixture after each dilution. This planning needs to be done very carefully and will take quite a bit of time.

Check your scheme carefully before recording it in the table provided in Question 17 in your answer book

BEFORE YOU PROCEED TO THE NEXT STEP, ask your laboratory supervisor to sign off on your dilution scheme. Any subsequent changes made to this dilution scheme must be initialled by your laboratory supervisor.

(iii) Pour the seawater sample into the graduated cylinder and float your hydrometer in it.

Record the hydrometer scale reading where the scale breaks through the surface of the seawater in the appropriate line of the table in Question 18 in your answer book.

(iv) Carry out dilutions as per your dilution scheme.

For each diluted solution, record the density of the solution and the hydrometer scale reading in the table in Question 18 in your answer book. Your laboratory supervisor must observe and initial certain readings indicated in the table in the answer book.

Ask your laboratory supervisor to sign off on your table of data BEFORE YOU PROCEED TO PLOT A CALIBRATION CURVE.

(v) *(Question 19) Plot a calibration curve for your hydrometer on the graph paper provided. If you feel it is appropriate, you may draw a line of best fit to your data. Do not forget to include your calibration curve with your answer book.*

Measurement of the densities of samples.
You have been provided with three samples labelled A, B, and C. Use your hydrometer to measure the densities of these samples. You need not determine errors.

Record the hydrometer scale readings and the corresponding densities, for each of the samples A, B, and C in Question 20 in your answer book.

THIS COMPLETES TASK 3.

Task 4 - Gram Stain, Cell shape and arrangement.

Carry out a Gram stain on each of the fixed slides of the bacterial cultures from Task 1 (Samples A, B, and C).

Background:
The Gram stain divides bacteria into two physiological classes: Gram positive, and Gram negative. If Gram' s method is carried out properly:

- Gram positive (+) organisms are stained *dark violet* in colour.
- Gram negative (–) organisms are stained *pink / light red* in colour.

Note: Your are provided with a duplicate Sample B for testing.

Gram Staining procedure:
Before starting, make sure that all reagents, as well as the squirt-bottle of water, are easily accessible because you won't have time to go get them during the staining procedure. Also, make sure you are doing this over the staining bowl because it can get really messy. Wear protective gloves.

STEP 1: Place your slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for 60 seconds. When the time has elapsed, rinse your slide for 5 seconds with water from the squirt bottle. The specimen should appear blue-violet when observed with the naked eye.

STEP 2: Now, flood your slide with the iodine solution. Let it stand for a minute as well. When time has expired, rinse the slide with water for 5 seconds and immediately proceed to Step 3. At this point, the specimen should still be blue-violet.

STEP 3: This step involves addition of the decolourising solution. Add the solution drop-wise until the blue-violet colour is no longer seen from your specimen. As in the previous steps, rinse with the water for 5 seconds.

STEP 4: The final step involves applying the counter-stain, safranin. Flood the slide with the dye as you did in Steps 1 and 2. Let this stand for a minute to allow the bacteria to incorporate the saffranin. Gram positive cells will incorporate little or no counter-stain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink colour and are easily distinguishable from the Gram positives. Again, rinse with water for 5 seconds to remove any excess of dye.

After you have completed Steps 1 through 4, you should blot the slide gently with filter paper or allow it to air dry before viewing it under the microscope. DO NOT RUB THE SMEAR!

Finally, examine the stained slide preparations under the microscope using the X40 lens first followed by the oil immersion (X100 magnification) lens

N.B. THIS TASK MUST BE VERIFIED BY YOUR LABORATORY SUPERVISOR SIGNING THE APPROPRIATE BOXES IN QUESTION 21 OF THE ANSWER BOOK BEFORE YOU RECORD YOUR RESULTS.

Results:
Answer the following questions by ticking the appropriate boxes in the tables for Question 21 in the answer book. See Appendix 1 for an explanation of the different terms used.

(i) Give a description of shape and cell arrangement of each sample.
(ii) Determine which sample contains *E.coli* and therefore is polluted from sewage.
(iii) Determine which sample contains lactic acid bacteria and therefore is polluted from dairy waste.

THIS COMPLETES TASK 4.

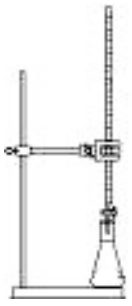
Task 5 - Organic loading by Biological Oxygen Demand (BOD).

Introduction.
Microorganisms such as bacteria are responsible for decomposing organic waste. When organic matter such as dead plants, manure, sewage, or food waste is present in a water supply, the bacteria begin the process of breaking down this waste. When this happens, aerobic bacteria consume much of the available dissolved oxygen, robbing other aquatic organisms of the oxygen they need to live. Biological Oxygen Demand (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. If there is a large amount of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. Therefore, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water, BOD levels will begin to fall.

The BOD test takes five days to complete. The BOD level is determined by comparing the Dissolved Oxygen (DO) level of a water sample immediately the sample is taken with the DO level of a water sample that has been incubated in a dark location for five days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level. Each team will be given the results of Day 1 DO and will have to determine the Day 5 DO level.

Each group is given three water samples (A, B, and C) in which the Dissolved Oxygen has been fixed using the Winkler method. Sample A is a 1 in 20 dilution, Sample B is a 1 in 100 dilution, and Sample C is a 1 in 200 dilution of the original sample.

NOTE: You are given the samples to titrate against sodium thiosulphate (M/80, i.e., 0.0125 M) to determine the oxygen present in the sample.



Treatment of Samples:
Pour, using a graduated cylinder into a conical flask, 100 ml of sample into a conical flask and titrate this solution with sodium thiosulphate (M/80) until the colour has faded to a pale yellow. (In order to observe the colour change more easily, place the conical flask on the white tile provided or on a white sheet of paper). Add a few drops of starch indicator. An intense blue colour will appear. Titrate (once for each sample) until this blue colour has disappeared (the end point is colourless). Repeat the procedure for each bottle.

Results:
For the purpose of the experiment it is sufficient to assume that the number of millilitres of sodium thiosulphate titrated is equivalent to the number of mg L^{-1} of dissolved oxygen present in the test sample. Please take note of any dilution factors when calculating results.

The results of day 1 DO levels at the three sites are as follows:

Day 1 DO levels for Sample A = 11.5 mg L^{-1}
Day 1 DO levels for Sample B = 11.8 mg L^{-1}
Day 1 DO levels for Sample C = 11.2 mg L^{-1}

Answer the following questions by ticking the appropriate boxes in the tables for Question 22 in the answer book.

- *Indicate approximate BOD results in mg L^{-1}.*
- *Determine which sample has most biological load.*
- *Determine which sample has least biological load.*

THIS COMPLETES TASK 5.

Task 6 - Determine the sampling sites for samples A, B, and C.

Using the results of the various tests you carried out on Samples A, B, and C in Tasks 1 to 6, you are asked to determine the sampling sites at which each of the samples were collected.

Indicate the site at which each sample was taken by ticking the appropriate boxes in Question 23 in the answer book.

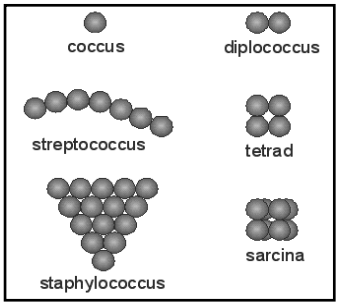
THIS COMPLETES TASK 6 AND ALSO COMPLETES THIS EXPERIMENT.

Appendix 1 - Bacterial cell morphology and Gram reaction.

Most bacteria come in one of three basic shapes: coccus, rod/bacillus, and spiral.

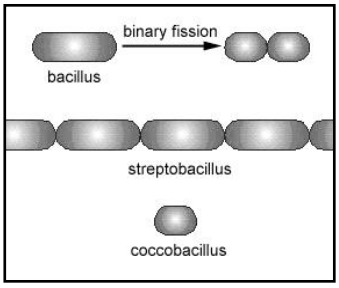
The coccus:
The cocci are spherical or oval bacteria having one of several distinct arrangements:

- Pair of cocci: Diplococcus:
- Chain of cocci: Streptococcus:
- Tetrad arrangement: Rectangular group of four cocci
- Cube of 8 cocci: Sarcina
- Random: Staphylococcus



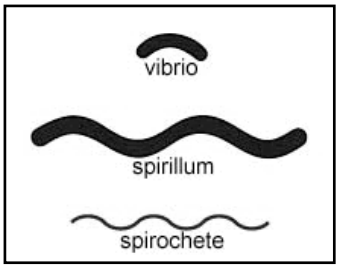
The rod/bacillus:
Bacilli are rod-shaped bacteria.
Bacilli produce the following arrangements:

- Bacillus: a single rod / bacillus
- Streptobacillus: a chain of bacilli
- Coccobacillus: oval and similar to a coccus



The spiral:
The spirals come in one of three forms:

- a vibrio
- a spirillum
- a spirochete



If Gram's method is carried out properly:
Gram positive (+) organisms are stained ***dark violet*** in colour
Gram negative (–) organisms are stained ***pink / light red*** in colour (by the counterstain).

The coliform group of bacteria is used as an indicator of pollution, and includes many environmental species of bacteria found in soil, on fruit, leaves, grains, and in runoff water. Coliform bacteria of faecal origin are referred to as faecal coliforms. *Escherichia coli* (*E.coli*) is a faecal coliform found in the faeces of humans, mammals and birds. Analyses for indicator organisms such as *E.coli* provide information on the microbiological quality of water. The detection of *E.coli* in water indicates contamination from a faecal source.

- ***E.coli* are Gram negative cells with a slender rod morphology**

Lactic acid bacteria refer to a large group of beneficial bacteria that have similar properties, all of which produce lactic acid as an end-product of the fermentation process. They are widespread in nature and are also found in our digestive systems. These microbes are used in the production of fermented food products, such as yogurt (*Streptococcus spp.* and *Lactobacillus spp*.), cheeses, butter, buttermilk and kefir.

- Lactic acid bacteria are Gram positive and vary in morphology from long, slender rods to short coccobacilli or cocci which frequently form short chains or clusters.

European Union Science Olympiad

Marking Scheme for Experiment 1

NUI, Galway
Monday 16th May, 2005

This experiment is marked out of 100 marks.

Task 1 carries 9 marks.

Task 2 carries 25 marks.

Task 3 carries 32 marks.

Task 4 carries 11 marks.

Task 5 carries 11 marks.

Task 6 carries 12 marks.

Task 1 - Determine Bacterial levels (9 marks in total)

1. Indicate the bacterial count in each sample in colony forming units (CFU) per 100 ml by ticking the appropriate boxes in the table below.
[3 x 3 marks per sample = 9 marks]

Task 2 - Determine the Chemical pollutant present in Sample A only (25 marks in total)

2. Draw the chemical structure of *para*-nitrophenol *[1 mark]*

3. Calculate the R_f value of compound (**X)** using the mobile phase, 20% ethyl acetate and 80% hexane. *[2 marks]*

4. Calculate the R_f value of compound (**Y)** using the mobile phase, 20% ethyl acetate and 80% hexane. *[2 marks]*

5. Calculate the R_f value of compound (**Z)** using the mobile phase, 20% ethyl acetate and 80% hexane. *[2 marks]*

6. Calculate the R_f value of compound (**X)** using the mobile phase, 30% ethyl acetate and 70% hexane. *[2 marks]*

7. Calculate the R_f value of compound (**Y)** using the mobile phase, 30% ethyl acetate and 70% hexane. *[2 marks]*

8. Calculate the R_f value of compound (**Z)** using the mobile phase, 30% ethyl acetate and 70% hexane. *[2 marks]*

9. Place compounds (**X)**, (**Y)** and (**Z)** in order of polarity with the most polar first. *[3 marks]*

10. According to your TLC investigations, what is the most likely identity of pollutant compound **(R**) in water **Sample A**, is it compound **(X)**, **(Y)** or **(Z)**?
[2 marks]

11. Assuming an analogous reaction takes place as in scheme 1. Draw the chemical structure of the azo dye made from the reaction of the diazonium salt of **(R)** with phenol. *[1 mark]*

12. What is the colour of the dye made by the reaction of the diazonium salt of **(R)** with phenol? Tick the correct box. *[3 marks]*

13. What is the colour of the dye made by the reaction of the diazonium salt of **(R)** with 1-naphthol? Tick the correct box. *[3 marks]*

Task 3 – Determine the density of each of the samples (total of 32 marks).

14. Calculate the approximate mass of lead shot required for your hydrometer and enter your calculation and result below.

[3 marks] ***For good estimate/measurement of volume (within 10%) 1 mark***

For answer: within 5 g to get 2 marks

outside 5 g but right method 1 mark

15. In the space below, record the hydrometer scale reading where the scale breaks through the surface of the freshwater.

[1 mark] ***Hydrometer scale must be between 0 and 2 cm on scale to get mark.***

16. In the space below, enter a formula giving the density of the solution you would obtain if you mix a volume $\mathbf{V_1}$ of a liquid of density ρ_1 with a volume $\mathbf{V_2}$ of a liquid of density ρ_2.
[2 marks] ***Marks for totally correct formula only (all or nothing).***

17. Enter your dilution scheme and solution densities in the table below.
[5 marks]

Number of points:
- *Seven or more calibration points to get* — *1 marks*
- *Six or less calibration points to get* — *0 marks*

Good spread of readings:
- *no gaps greater than 6 kg m^{-3} to get* — *2 marks*
- *one gap between 6 kg m^{-3} and 10 kg m^{-3} to get* — *1 mark*
- *gap > 10 kg m^{-3} or more than one gap > 6 kg m^{-3}* — *0 marks*

Systematic approach taken:
- *for all readings to get* — *2 marks*
- *for a subset (4 or more) of readings only* — *1 mark*
- *non-systematic approach* — *0 marks*

Max. penalty for not having scheme signed off before proceeding *3 marks*

18. Enter the density and the corresponding hydrometer scale reading for each calibration curve data point in the table below.
[8 marks]

Scheme executed with the 1000 cm^3 of seawater provided *4 marks*

Original scheme set out in Question 17 executed without changes *2 marks*

Seven or more data points to get *2 marks*
Five or six data points to get *1 mark*
Fewer than five data points to get *0 marks*

Max. penalty for not having readings witnessed (per reading) *1 mark*

19. Using the data in your table for Question 18, plot a calibration curve for your hydrometer on the graph paper provided. If you feel it is appropriate, you may draw a line of best fit to your data. Include your calibration curve with your answer book.
[7 marks]

Good use of page (must occupy at least half A4 page)	*1 mark*
Sensible scales (easy to work with)	*1 mark*
Scale reading plotted against density	*1 mark*
Axes labelled correctly (including density units)	*1 mark*
Points clearly marked	*1 mark*
Spread of points around properly drawn line of best fit	*2 marks*

(e.g. two thirds of points vertically within 1.5 mm (of scale reading) of line, with no points more than 3.0 mm from line would probably attract full marks)

20. Enter the hydrometer scale reading and corresponding density for each of the unknown samples in the table below.
[3 x 2 marks per sample = 6 marks]

Answers within 4 kg m^{-3} of correct values to get	*2 marks*
Answers within.4 kg m^{-3} to 10 kg m^{-3}	*1 mark*
Answers outside this range to get	*0 marks*

(The upper limits of the two ranges correspond to errors of about 1.5 mm and 4.0 mm respectively in the scale reading.)

Task 4 - Gram Stain, Cell shape and arrangement (total of 11 marks).

21(i). Give a description of shape and cell arrangement of each sample by ticking the appropriate boxes in the table.
[3 x 3 marks per sample = 9 marks] [No marks if not signed off]

(ii) Determine which sample contains *E.coli.* and therefore is polluted from sewage. (Tick the appropriate boxes.)
[1 mark]

(iii) Determine which sample contains Lactic acid bacteria and therefore is polluted from dairy waste. **(Tick the appropriate boxes.)**
[1 mark]

Task 5 - Organic loading by Biological Oxygen Demand (BOD) (total of 11 marks).

22(i). Record BOD in mg L^{-1} for each sample by ticking the appropriate boxes.
[3 x 3 marks per sample = 9 marks]

(ii) Determine which sample has the most biological load and which sample has the least biological load. Indicate your answers by ticking the appropriate boxes in the table.
[2 x 1 marks per sample = 2 marks]

Task 6 - Determine the sampling sites for samples A, B, and C (total of 12 marks)

23. Indicate the sites at which each sample was taken by ticking the appropriate boxes in the following table.
[3 x 4 marks per sample = 12 marks] (all or nothing in each case)