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Environmental health lab (Z222)

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Preface :

The topics related to the environmental issue are extremely important. This is related to that they are in close association with the human life and constitutes the lone way to human welfare. The aquatic environment constitutes the major bulk in the Globe, so considerable attention is projected to it. Of the aquatic environment, the marine habitat is its major. Therefore, in this practicum part concern is thrown to studying the water quality criteria either in the field or in the lab. The author of this chapter hopes to provide some information that builds background related to aquatic ecology. Also, try to present some recent methods related to aquatic ecology is one of the present targets.

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1. What is Ecology?

Ecology is the study of plants and animals in relation to each other and to the physical and chemical component in the environment in which they naturally occur.

2. Ecosystem

An Ecosystem is an assemblage of plant, animal and microbial species in a particular place which interacts with each other and with their physical and chemical environment in such a way as to constitute a self-maintaining and self-regulating system Abeech wood represents, at one level, a community, but at a deeper one, it functions as intricate ecosystem which can maintain itself without the help of any forester and stays the same for thousands of years. The ecosystem requires :

1) An external energy source, 2) Recycle inorganic substances (by decomposer).

3. MEASURING ENVIRONMENTAL FACTORS IN WATER

3.1. Water Habitat

The ocean provided the more stable environment because of the way the properties of a large mass of water differ from those of air. Freshwater habitats share some of this stability, but as much smaller volumes of water are involved, to a lesser extent.

Freshwater habitats provide plenty of variety- some have running water, while in others the water is stagnant. Running water represents streams and rivers. Stagnant water represents the pools, puddles, ponds, canals and ditches. Running water has higher oxygen content because of several factors that include its lower temperature, the absence of humus which reduces the amount of oxygen used for oxidation of the products of decomposition, and as water flows over stones or waterfalls bubbles of air are trapped and some of this gas dissolves. There are very few rooted plants in fast-moving waters, and

substratum is mainly rocks and stones because the soil is washed away by the current.

Stagnant water differs in several ways. The temperature of running water remains fairly constant because of the continual mixing; stagnant water undergoes a diurnal and annual variation. The stillness of the water means that dead plant and animal material sinks to the bottom and provides food for animals as well as minerals for plants and phytoplankton (microscopic and motionless plants). In the spring there may be so much phytoplankton presents that the water looks green. As the material decomposes, oxygen is used up and methane gas may accumulate as oxygen runs out, so perhaps not all bubbles that may be visible are bubbles of oxygen from photosynthesis.

Activity:

Please make a field visit for each of running and stagnant water bodies, write a comment for your visit attached with its date and make photos as possible.

Important safety:

Polluted water is particularly interesting to study, but remember it may contain pathogenic microbes and toxic chemicals which could harm you. Wash your hands as soon as possible after working in such places (have a bowl of water with added disinfectant with you in the field), and certainly before eating. Avoid altogether obviously extreme kinds of organic pollution, such as where a whole stream is highly turbid and evil-smelling. Treat any kind of pollution with respect: Wearing rubber gloves is recommended. Be especially careful not to fall into the polluted water.

Sampling :

Freshwater is surprisingly difficult to sample because they are rarely homogeneous and their quality varies during the day and during the year. In addition, the most representative sampling locations are often at a distance from the shore. Because of this, to get samples for ecological studies follow the following directions:

A -Take a sample at a specific distance from the shore.

B- Take samples monthly basis at a fixed day all over one year and sometimes two years.

C –In case of shallow water avoid making turbidity or any circulation.

3.2. Physical characteristics of water

Physical characteristics of water (temperature, colour, taste, odour and etc.) are determined by senses of touch, sight, smell and taste. For example temperature by touch, colour, floating debris, turbidity and suspended solids by sight, and taste and odour by smell.

3.3. Dissolve oxygen as an ecological factor

The level of oxygen is high in fast-moving waters, simply because of the sheer turbulence (in which water gets mixed with air), but slower –moving waters may have high levels because of an abundance of plant life. Shallow water that runs over a stony stream bed will have more oxygen than water that runs at the same speed but is half a meter deep with a muddy bed.

Oxygen levels often indicate the quality of freshwater. A common form of pollution is sewage, which increases the rate of fungal and bacterial growth which in turn leads to deoxygenating of water and the death of animal life. Well, oxygenated water, by contrast, has a high species diversity.

Note the following:

- Percentage saturation 90-100% dissolved oxygen----------Safely water, not polluted.
- Percentage saturation 50-90% dissolved oxygen----------Water is of fair quality.

- Saturation below 50% dissolved oxygen---------- indicate the presence of pollution.

Important notes should be taken for Oxygen measurements:

- Dissolved oxygen varies by the daytime, so try to fix the time of sampling.
- Oxygen saturation falls overnight when photosynthesis tops or when the temperature rises as the higher the temperature the less oxygen saturation.

The effects of organic pollution can be measured by working out the biological oxygen demand (BOD). The higher the BOD then the more polluted is the water. Polluted water characterized by high BOD and low oxygen level.

3.3.1.Measuring oxygen level using Winkler's method

-The firsts and most reliable is **the Azide-Winkler** titration method, against which the others are compared to test for accuracy. However, this method also requires the most training and the use of some strong chemicals. For these reasons, it is not often used in citizen monitoring programs.

-If you are hand dipping the BOD bottle, lower the bottle about halfway into the water and let it fill slowly. If you are sampling in a stream, allow the water to overflow for a least 2 minutes or until the water in the bottle has replaced itself two or three times. Check to be sure no air bubbles are present before you lift the bottle – look closely just below the neck of the bottle, where bubbles often get caught. If you see bubbles, gently tip the bottle to either side to allow the bubbles to escape. Carefully stopper the bottle so no air pockets form below the cap. Do this by tilting the BOD bottle slightly and slowly lowering the cap. You may want to turn the bottle upside down and watch for bubble movement. If you see bubbles, dump the sample and start over.

Reagents

A. Manganous sulphate solution for 1L

Dissolve one of the following in 1 L of distilled water:
480 g $MnSO_4 \cdot 4H_2O$ –or-
400 g $MnSO_4 \cdot 2H_2O$ –or-
364 g $MnSO_4 \cdot H_2O$

B. Alkali-iodide (add azide* if necessary) for 1L

Dissolve in 1 L of distilled water:
500 g NaOH –or- -AND- 135 g NaI –or-
700 g KOH 150 g KI
*Use azide for bacteria or nitrite laden samples:
Dissolve 10 g NaN_3 in 40 ml distilled water.
Add to 1 L of above solution.

C. Sulfuric acid (concentrated)

D. Starch for 0.1L

Dissolve 2 g soluble potato starch in 100 ml hot distilled water; stir.
(note: very important to add to hot water as starch may take some time to dissolve)
Salicylic acid (0.2 g) may be added as a preservative.(not necessary if starch is used on the same day it is dissolved)

E. Sodium thiosulfate for 1L

- Dissolve in 1 L distilled water 6.25 g $Na_2S_2O_3 \cdot 5H_2O$ (note: this solution is perishable and should be made up on the day it is used)

Procedure:

1. Fill a 300-mL glass stoppered BOD bottle with sample water. Remember – no bubbles!
2. Immediately add 2mL of manganese sulphate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner.

4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settled to the bottom, mix the sample by turning it upside down several times and let it settle again.
5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper and cap the bottle with aluminium foil and a rubber band during the storage period.
6. In a glass flask, titrate 201 mL of the sample with sodium thiosulfate to a pale straw colour. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continue stirring or swirling the sample water.
7. Add 2 mL of the starch solution till blue colour forms.
8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue colour. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue colour.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of millilitres of titrant used. Each millilitre of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolved oxygen.

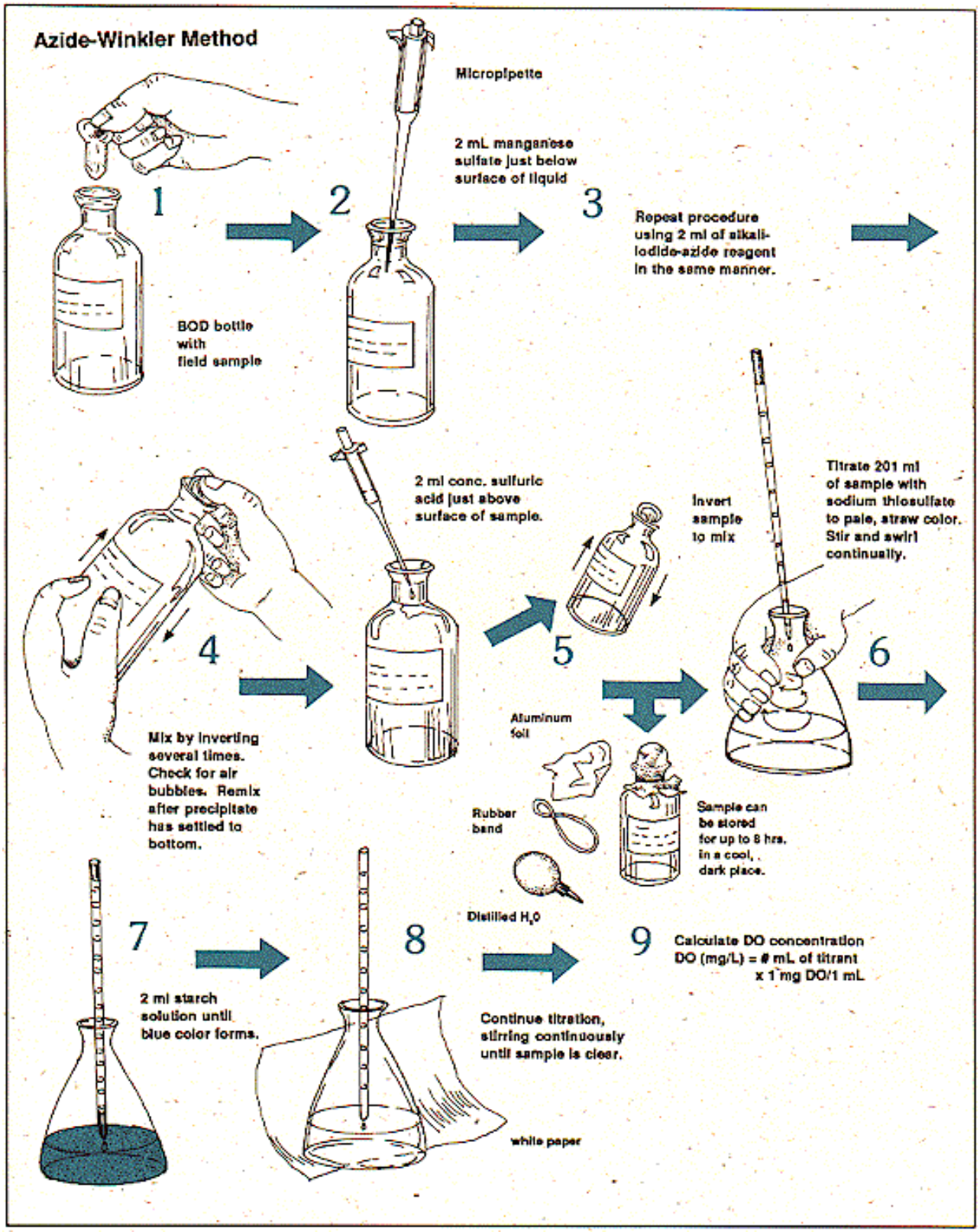


Fig.(1): Illustration of the DO procedure

NOTE: Be very careful when doing DO analyses. The reagents are corrosive, so keep them away from your skin and clothes. Wear safety goggles and wash your hands when you are done.

- Once the endpoint is reached record the amount of $Na_2S_2O3•5H_2O$ that was added. One mL is equal to 1 mg/L (or p.p.m.) dissolved oxygen in your sample. (Note: 1/12 mL or 83µL equals 1mg/L dissolved oxygen…thus 2/3 of an mL will be equal to 8 mg/L (water in equilibrium with atmospheric O_2) titrate carefully.

Reactions and principle:

$MnSO_4$ + NaOH $\longrightarrow$ Na_2SO_4+ $Mn(OH)_2$

$Mn(OH)_2 + O_2 + H_2O \longrightarrow Mn(OH)_3$ precipitate

$Mn(OH)_3 + H_2SO_4 + KI \longrightarrow I_2 + K_2SO_4 + MnSO_4 + H_2O$

$I_2 + Na_2S_2O_3 \longrightarrow NaI$

The added manganous sulphate is reacted with NaOH of Winkler reagent to form manganous hydroxide which is reacted with oxygen in the water and for a manganic hydroxide precipitate. So, the amount of the manganic hydroxide ppt is equal to the amount of dissolved oxygen. Sulphuric acid dissolves the ppt of Manganic hydroxide in the presence of KI to form K2so4 and MnSO4 and H2O with the release of Iodine as a free molecule. The Iodine is determined by titration with sodium thiosulphate using starch as indicator. Thus, I2 equal Manganic hydroxide ppt that equal to Dissolved oxygen.

References

Standard Methods for the Examination of Water and Wastewater, 1995, New York, American Public Health Association.

3.4. Dynamics of Dissolved Oxygen in Natural Systems

Objective

To apply your chemical knowledge of dissolved oxygen analyses in order to observe how various organisms affect the dissolved oxygen concentration of natural waters.

Experimental Method

Materials:

- 4 glass tanks of equal size/water content
- 2 plants
- 2 goldfish

Procedure:
Using the aquariums provided in class, create four experimental systems comprised of one fish, one plant, one fish and one plant, and a vacant tank (control) respectively.
For each system…

- Obtain three water samples according to the method you practised in class, and analyze each sample for its dissolved oxygen concentration. Record the average value of your three essays in the table below.
- Given your knowledge of dissolved oxygen from previous experiments, make a hypothesis on how the dissolved oxygen concentration will change over the course of one week (Increase/Decrease/Remains Constant). **Important:** Be sure to record your hypothesis for each system in the chart below.
- At the end of one week obtain three more water samples, and again, analyze each sample for dissolved oxygen. Record your average value of your results.

system	Initial DO	Hypotheses	Final DO
Fish			
plant			
Fish and plant			
Control			

Data Analysis
On the back of this sheet, summarize your results by concluding how the dissolved oxygen concentration of each system changed over the course of the week. Also, determine whether your results did or did not support your hypotheses, and give reasons for the observed behaviour of each system.

3.5. Measuring biochemical oxygen demand (BOD)

Measuring biochemical oxygen demand (BOD) is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. BOD value is most commonly expressed in mg of oxygen consumed per litre of the sample during 5 days of incubation at 20 C, so it is called BOD_5.

Procedure:

-Collect the sample from the field.Then determine DO at zero time (DOi).

-Incubate the sample at 20 °C for 5 days and then determine the DO (DO_f).

$BOD_5 = DO_i - DO_f$.

3.6.Measuring the gross and net primary productivity

Definitions:

1- Gross primary productivity is the total energy produced by the producer during the process of photosynthesis.
2- Net primary productivity is total energy stored in the body of producer after energy utilization in the process of respiration, i.e., producer produces energy and used some of it to perform vital activities.

Procedure:

1- Collect available water from the field at the morning time.
2- Determine the amount of oxygen, let it as Oa.
3- Bring two glass bottles (300 ml), Cover one of it with aluminium foil.
4- Fill the two glass bottles completely with the field water. As known, the field water contains phytoplankton as a producer. In addition to phytoplankton producer, add to both bottles some fresh green plant leaves.
5- Leave both bottles for 4 hrs to perform photosynthesis (not covered bottle) and respiration in case of covered one.
6- Determine the Oxygen content for water in both bottles.

Calculation:

-Let Oxygen in not covered bottle as O_1

-Let Oxygen in covered bottle as O_2

-Gross production= Oxygen content in not covered bottle (mg/l or ppm) - Oa

-Net primary productivity= $(O_1 - O_a) - (O_2 - O_a)$.

Note:
O_a is the measure of oxygen at the initial time.
Subtracting the Oa, initial oxygen, is necessary to indicate the photosynthetic or respiratory activities.

3.7.Measuring turbidity

Turbidity is the name given to the clarity of the water, which is affected by the amount of suspended solids in it. High turbidity often accompanies organic pollution. If the water contains a lot of suspended solids, these particles gather on the gill filaments, block them up and reduce the surface area for gas exchange (asphyxia). As this continues the animals will die as they are unable to obtain sufficient oxygen to supply their metabolism.

Turbidity reduces the depth to which light can penetrate, and hence reduces the growth of plants (primary productivity).
Turbidity can be measured by the following methods:
1- Using 1 meter test tube with 2.5 cm diameter.
2- Using Secchi disc: the procedure is

- ❖ Secchi disc is divided into four alternate black and white sections.
- ❖ It is lowered on a piece of string into the stream until the sections can no longer be seen.
- ❖ The disc is most easily used from a boat or a bridge.

Results:

- Good waters above 600 mm
- Satisfactory about 300 mm
- Poor waters less than 100 mm

3.8.Measuring the suspended solid content

-Take a sample of water of known volume (100 ml).
-Weight the filter paper that it will be used.
-Pour it through a filter funnel, letting the water drain into the beaker.
- Remove the filter paper and let it dry naturally.
-Weight the filter paper again and find out the weight of the sample.
Results:
Total suspended solids (g/ L) = [Weight of solid (weight of dried filter paper with solid- weight of filter paper)/ Volume of water sample] 1000

3.9. Conductivity as an ecological indicator

The level of conductivity in water gives a good indication of a qantity of ionizable substances dissolved in it, such as phosphates, nitrates and nitrites which are washed into streams and ponds after fertilizer is applied to surrounding fields or are present in the effluent from sewage-treatment installations. The sodium chloride and other salts in seawater give it a higher conductivity than freshwater.

In unpolluted waters the conductivity increases by approximately 3 percent with every 1°C rise in temperature, so the temperature must be noted and corrections made for comparative purposes.

The easiest way to measure conductivity is by conductivity meter and probe. The unit of its measurement is microsiemen (mS/cm).

Fig.(3). Electrical conductivity meter

3.10. Determining Concentration of salinity by Titration with Silver Nitrate (Boyle's Method)

BACKGROUND:

Salinity is the concentration of salts in water. Salinity is an important characteristic of natural waters, and in fact is the key factor in determining what species of plants and animals will be found in a certain area. It is usually expressed in ppt of total dissolved salts and ranges from 0 ppt in fresh water to 35 ppt in the open ocean. Because the composition of the major ions in seawater is constant, we will measure salinity as molarity of chloride ions. This corresponds to about 0.0M - 0.5M of chloride ions.

In this lab, you will employ a classic method to determine the salinity of water samples, one first devised by Robert Boyle for the British Navy in the early 1700's. This method is based on two precipitation reactions. The main reaction is a double replacement reaction between silver nitrate and sodium chloride, in which silver chloride precipitates. The idea is to add enough silver nitrate to the saltwater to precipitate out all the chloride ions as silver chloride. In order to know exactly when all the chloride ions have been precipitated, we will "spike" the saltwater samples with potassium chromate. Potassium chromate also reacts with silver nitrate, to produce a red-orange silver chromate precipitate. The key is that the silver ions prefer to combine with the chloride ions rather than the chromate ions. Thus, the silver chromate ppt. will not appear until all the chloride ions have reacted. By measuring exactly what volume of silver nitrate is needed to reach the endpoint, where the first hint of a red-orange ppt. appears, we can use a simple proportion to determine the amount of chloride in the sample, and hence its salinity. This method, of quantitatively adding a solution of known concentration to a solution of unknown concentration until an endpoint is reached, is called a TITRATION.

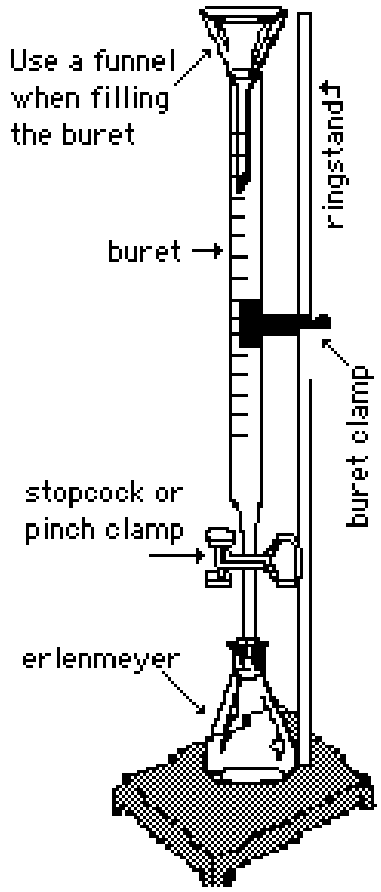
FOCUS QUESTIONS:

[1] What are your class's values for the salinity (in molarity of Cl-) of the stock 0.100M NaCl (aq) sample? Discuss your precision and accuracy. If a skewed error occurred, discuss it.

[2] What is the salinity of the class unknown estuarine water sample? What can you conclude about accuracy and precision?

PROCEDURE:

1. Titrate 10.0 mL of the "unknown estuarine water" provided by your instructor with the 0.016 M $AgNO_3$ solution. Use potassium chromate as your indicator.
2. Repeat until you have two trials within 0.10 mL.



Analysis:

- 1000 ml of 1 M AgNO3= 58.5 g NaCl
- 1 ml of 1 M AgNO3= 58.5 mg NaCl
- 1 ml 0.016 M AgNO3 =0.936 mg NaCl
- Cholorinity of sample (mg/L)= volume in ml of 0.016 M AgNO3 X 0.936 X 100
- Salinity of sample= [Chlorinity (mg/L) X 1.805 + 0.03]/1000
- Note: 1.805 is the molecular weight of NaCl
  0.03 is correction factor
  The formula is divided by 1000 to give salinity unit in g (as chlorinity unit is given in mg).

Salinity is also measured using salinometer:

A salinometer is a device designed to measure the salinity, or dissolved salt content, of a solution. Since the salinity affect both the electrical conductivity and the specific gravity of a solution, a salinometer often consists of an ec meter or hydrometer and some means of converting those readings to a salinity reading. A salinometer may be calibrated in either micromhos.

3.11. Calcium as an ecological factor in freshwater

It is important to determine hardness. A good indication that water is hard is an abundance of gastropods, gammarids and bivalves which are absent in soft water. As hardness decreases, pH increases (4.3) and mayfly and stonefly appear. Hard waters generally not harmful to man health. It can cause a serious problem in industrial settings where it breakdown boilers…etc.

There are two types of water hardness:

1-**Temporary hardness**

It is a type of water hardness caused by the presence of dissolved bicarbonate minerals (calcium bicarbonate and magnesium bicarbonate). When dissolved these minerals yield calcium and magnesium cations (Ca2+, Mg2+) and carbonate and bicarbonate anions (CO_3^{2-}, HCO_3^-). The presence of the metal cations makes the water hard. The "temporary" hardness can be reduced either by boiling the water or by the addition of lime (calcium hydroxide) through the softening process of lime softening.[4] Boiling promotes the formation of carbonate from the bicarbonate and precipitates calcium carbonate out of solution, leaving water that is softer upon cooling.

2-**Permanent hardness**

Permanent hardness is hardness (mineral content) that cannot be removed by boiling. When this is the case, it is usually caused by the presence of **calcium sulphate and/or magnesium sulphates** in the water, which does not precipitate out as the temperature increases. Ions causing permanent hardness of water can be removed using a water softener, or ion exchange column.

Total Permanent Hardness = Calcium Hardness + Magnesium Hardness

The calcium and magnesium hardness is the concentration of calcium and magnesium ions expressed as equivalent of calcium carbonate.

Total permanent water hardness expressed as equivalent of CaCO3 can be calculated with the following formula: Total Permanent Hardness (CaCO3) = 2.5(Ca2+) + 4.1(Mg2+).

❖ Measurements of hardness:

Hardness can be quantified by instrumental analysis. The total water hardness is the sum of the molar concentrations of Ca^{2+} and Mg^{2+}, in mol/L or mmol/L units. Although water hardness usually measures only the total concentrations of calcium and magnesium (the two most prevalent divalent metal ions), iron, aluminium, and manganese can also be present at elevated levels in some locations.

Water hardness is often not expressed as a molar concentration, but rather in various units, such as degrees of general hardness (dGH), German degrees (°dH), parts per million (ppm, mg/L, or American degrees), grains per gallon (gpg), English degrees (°e, e, or °Clark), or French degrees (°F). The table below shows conversion factors between the various units.

Hardness unit conversion.

	mmol/L	**ppm, mg/L**	**dGH, °dH**	**gpg**	**°e, °Clark**	**°F**
mmol/L	1	0.009991	0.1783	0.171	0.1424	0.09991
ppm, mg/L	100.1	1	17.85	17.12	14.25	10
dGH, °dH	5.608	0.05603	1	0.9591	0.7986	0.5603
gpg	5.847	0.05842	1.043	1	0.8327	0.5842
°e, °Clark	7.022	0.07016	1.252	1.201	1	0.7016
°F	10.01	0.1	1.785	1.712	1.425	1

For example: 1 mmol/L = 100.1 ppm and 1 ppm = 0.056 dGH.

Hard/ soft classification:

Because it is the precise mixture of minerals dissolved in the water, together with the water's pH and temperature, that determine the behaviour of the hardness, a single-number scale does not adequately describe hardness. However, the United States Geological Survey uses the following classification into hard and soft water,[18]

Classification	**hardness in mg/L**	**hardness in mmol/L**	**hardness in dGH/°dH**	**hardness in gpg**
Soft	0–60	0–0.60	0.3-3.00	0-3.50
Moderately hard	61–120	0.61–1.20	3.72-6.75	3.56-7.01
Hard	121–180	1.21–1.80	6.78–10.08	7.06-10.51
Very hard	≥ 181	≥ 1.81	≥ 10.14	≥ 10.57

❖ **Laboratory Procedure**

1. For water with relatively high hardness, you should put 25.0 mL of sample water in a beaker and dilute it to about 50 mL by adding distilled water. If the water is relatively soft, you can use a larger sample size of 100 to 1000 mL without dilution. Record the amount of sample used.
2. Add 1 to 2 mL of buffer solution to the beaker (or more if you use a larger water sample.) This will change the pH of the sample water to about 10.0 to 10.1. Stir.
3. Add 1 to 2 drops of indicator solution (calamigite) to the beaker (or more if you use a larger water sample.) Stir.
4. Titrate with EDTA titrant, stirring at intervals, until the indicator changes colour. The colour will change from reddish to blue, although incandescent lights tend to give the final solution a reddish tinge.
5. Record the amount of EDTA titrant which was required to reach the endpoint.
6. Calculate and record the amount of hardness (as ppm $CaCO_3$) in the water using the following formula:

$$\text{Hardness} = \frac{(\text{mL titrant}) \text{ X } (\text{molarity of titrant}) \text{ X } (100{,}090)}{(\text{mL sample})}$$

3.12. Alkalinity of water

Why is alkalinity important?

Aquatic organisms benefit from a stable pH value in their optimal range. To maintain a fairly constant pH in a water body, a higher alkalinity is preferable. High alkalinity means that the water body has the ability to neutralize acidic pollution from rainfall or basic inputs from wastewater. A well-buffered lake also means that daily fluctuations of CO2 concentrations (discussed above) result in only minor changes in pH throughout the course of a day.

Table 1: U.S. E.P.A. Classification[1] of lakes and ponds based on alkalinity as measured in a concentration of calcium carbonate ($CaCO_3$).

U.S. E.P.A. category	Concentration of $CaCO_3$ (mg/L)
Acidified	< 1 and pH < 5

Critical < 2
Endangered 2 – 5
Highly Sensitive 5 – 10
Sensitive 10 – 20
Not Sensitive > 20

How is alkalinity measured?

Alkalinity, reported as mg/L CaCO3, is measured as the amount of acid (e.g., sulfuric acid) needed to bring the water sample to a pH of 4.2. At this pH, all the alkaline compounds in the sample are "used up." Laboratory technicians use a burette (a graduated glass tube with a small opening at its base and stopcock for delivering measured quantities of liquid) to dispense the sulfuric acid drop by drop into the water sample while continuously monitoring the change in pH with a pH meter and electrode or pH "pocket pal." Field kits are also available, but they typically target a higher range of alkalinity than in RI waterways.

3.13. Determination of free carbon dioxide in water

Introduction

Carbon Dioxide is present in water in the form of a dissolved gas. Surface waters normally contain less than 10 ppm free carbon dioxide, while some ground waters may easily exceed that concentration. Carbon dioxide is readily soluble in water. Over the ordinary temperature range (0-30 C) the solubility is about 200 times that of oxygen. Calcium and magnesium combine with carbon dioxide to form carbonates and bicarbonates.

Environmental Significance

Aquatic plant life depends upon carbon dioxide and bicarbonates in water for growth. Microscopic plant life suspended in the water, phytoplankton, as well as large rooted plants, utilize carbon dioxide in the photosynthesis of plant materials; starches, sugars, oils, proteins. The carbon in all these materials comes from the carbon dioxide in water.

When the oxygen concentration in waters containing organic matter is reduced, the carbon dioxide concentration rises. The rise in carbon dioxide makes it more difficult for fish to use the limited amount of oxygen present. To take on fresh oxygen, fish must first discharge the carbon dioxide in their blood streams and this is a much slower process when there are high concentrations of carbon dioxide in the water itself.

Reagent

1. Standard N/44 Sodium Hydroxide,
2. Phenolphthalein Indicator

Procedure

(1) Take a 100 ml of sample in a beaker and add 10 drops of Phenolphthalein indicator. If a pink colour develops, no carbon dioxide is present in the water sample.

(2) Add N/44 sodium Hydroxide solution from a burette to the sample and stir gently until a slight permanent pink colour appears as compared with distilled water. Record ml of sodium hydroxide used.

Note:

Since excess CO2, if present easily escapes to the atmosphere, so tests should be performed immediately after collection of the water sample. If this is not possible sample bottle should be completely filled and stoppered and be kept at a temperature lower than that at which it was collected.

Calculation

Carbon dioxide present (mg/L) = (normality of NAOH * equivalent wt of CO2 * 1000 * milliliter of of N/44 NaOH added) / milliliter of sample taken X 10

3.14. Determination of Total Calcium and Magnesium Ion Concentration

Equipment Needed

Burette

20 mL pipette

250 mL conical flasks

100 mL volumetric cylinder

Solutions Needed

EDTA: (ethylene-diamine-tetra-acetic acid) 500 mL of a 0.05 molL-1 solution. Weigh 9.31 g of the EDTA salt and dissolve it in 500 mL of distilled water in a volumetric flask.

Buffer: Dissolve 7.0 g of ammonium chloride in 57 mL concentrated ammonia (see safety notes). Dilute to 100mL with distilled water in a volumetric flask. The pH should be 10.5.

MgCl2.6H2O: 0.025 molL-1 solution. Weigh 2.54 g of magnesium chloride hexahydrate and dilute to 500 mL with distilled water in a volumetric flask.

ErioT indicator: Dissolve 0.2 g of Eriochrome Black T indicator in 15 mL of concentrated ammonia solution (or 15 mL of triethanolamine) (see safety notes) and 5mL absolute ethanol. Do not store more than one to two days before use. You may be able to get the ErioT indicator from the University of Canterbury - see the contact details at the end.

Titration Method for Fresh or Tap Water Samples

1. Add a 100 mL of the sample solution into a 250 mL conical flask.
2. Prepare a 0.005 mol L−1 EDTA solution by diluting the 0.05 mol L−1 EDTA solution by a factor of 1/10. Add 20mL of this diluted EDTA to the sample solution.
3. Add 10 mL of the ammonia buffer and 1 mL of Eriochrome Black T indicator solution.
4. Prepare a 0.0025 mol L−1 magnesium chloride solution by diluting the 0.025 mol L−1 magnesium chloride solution by a

factor of 1/10.

5. Titrate the sample solution with this 0.0025 molL−1 magnesium chloride solution until a permanent pink colour appears. Repeat the titration with further samples until concordant results (titres agreeing within 0.1 mL) are obtained.

Figure 1 Colour changes for magnesium chloride back-titration in clear solution using Eriochrome Black T indicator. Left flask: blue colour well before endpoint (all Ca2+/Mg2+ ions complexed by excess EDTA, all indicator molecules uncomplexed). Centre flask: last trace of blue/purple colour just before endpoint (excess EDTA almost totally complexed by added Mg2+). Right flask: pink/red colour at endpoint (all EDTA complexed by added Mg2+, the indicator also complexed).

Result Calculations

1. Calculate the total moles of EDTA added to the sample solution.
2. Calculate the moles of the magnesium chloride solution used in the back titration from your concordant results. From the equation of the titration below, the moles of Mg2+ will be equivalent to the moles of excess EDTA.

$$EDTA^{4-} + Mg^{2+} \rightarrow [Mg\text{-}EDTA]_{2-}$$

3. Given the ratio of Ca^{2+} + Mg^{2+}: EDTA = 1: 1, calculate the moles

of Ca2+ and Mg2+ that must have been complexed with EDTA by subtracting the excess EDTA from the total moles of EDTA added to the sample.

This result is the moles of Ca^{2+} and Mg^{2+} in the sample solution.

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4.Standard method for the examination of Plankton in water and wastewater

Introduction

The term ‘‘plankton’’ refers to those microscopic aquatic forms having little or no resistance to currents and living free-floating and suspended in natural waters. Planktonic plants, ‘phytoplankton,’’ and planktonic animals, ‘‘zooplankton,’

1. Significance

Plankton, particularly phytoplankton, long have been used as indicators of water quality.[1-4]. Some species flourish in highly eutrophic waters while others are very sensitive to organic and/or chemical wastes. Some species develop noxious blooms, sometimes creating offensive tastes and odors[5] or anoxic or toxic conditions resulting in animal deaths or human illness.[6] The species assemblage of phytoplankton and zooplankton also may be useful in assessing water quality.[7,]

Because of their short life cycles, plankters respond quickly to environmental changes, and hence their standing crop and species composition are more likely to indicate the quality of the water mass in which they are found.

2.Sample collection

- Because water of rivers and streams usually is well mixed vertically, subsurface sampling, i.e., the upper meter or a composite of two or more strata, often is adequate for collection of a representative sample.
- In lakes, reservoirs, and estuaries where plankton populations can vary with depth, collect samples from all major depth zones or water masses. In shallow areas of 2 to 3 m depth, subsurface samples collected at 0.5 to 1 m may be adequate.

3.Sampling procedure

a. Phytoplankton: In oligotrophic waters or where phytoplankton densities are expected to be low collect a sample of up to 6 L. For richer, eutrophic waters collect a sample of 0.5 to 1 L. Because of their small size, nannoplankton and picoplankton can pass through collection nets, making nets unsuitable for most phytoplankton sampling.

For qualitative and quantitative evaluations collect whole (unfiltered and unstrained) water samples with a water collection bottle consisting of a cylindrical tube with stoppers at each end and a closing device. Different size categories of phytoplankton can be separated by subsequently filtering these whole water samples through the netting of the appropriate mesh size. The specimen can be examined fresh or preserved. Preservation can be done using 0.7 mL Lugol's solution per 100 mL sample after 1 h or few drops formalin immediately after collection.

Details of Lugol's solution:

Lugol's solution: To preserve samples with Lugol's solution add 0.3 mL Lugol's solution to 100 mL sample and store in the dark. For long-term storage add 0.7 mL Lugol's solution per 100 mL sample and buffered formaldehyde to a minimum of 2.5% final concentration after 1 h. Prepare Lugol's solution by dissolving 20 g potassium iodide (KI) and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid

b- Zooplankton

For collecting microzooplankton (20 to 200 μm) such as protozoa, rotifers, and immature microcrustacea, use the bottle samplers described for phytoplankton. The small zooplankters usually are sufficiently abundant to yield adequate samples in 5- to 10-L bottles.

5.TOXICITY TEST

Definition :

It is a test to indicate the toxicity of a specific :

- **Wastewater (treated or Untreated)**
- **Pesticides**
- **Chemicals commonly used for different purposes in the environment.**
- **Oil refinery products**
- **Any other environmental pollutants**

The following photo is for Daphnia, a most famous species used for toxicity evaluation. It belongs to order Cladocera, class crsutacea.
Why is Daphnia commonly used?
Because of:
-Dominant throughout the year
-Easy to rear and to handle
-Sensitive to environmental variables
-Distributed all over the world.
-Easy to assess its vital activity as heart rate, movement, feeding and egg production
- Easy to get similar age or size individuals that are needed for toxicity testing to minimize the error.

Fig. Indicating Daphnia photo with evident appendages, alimentary canal and eggs.

- **Procedure:**

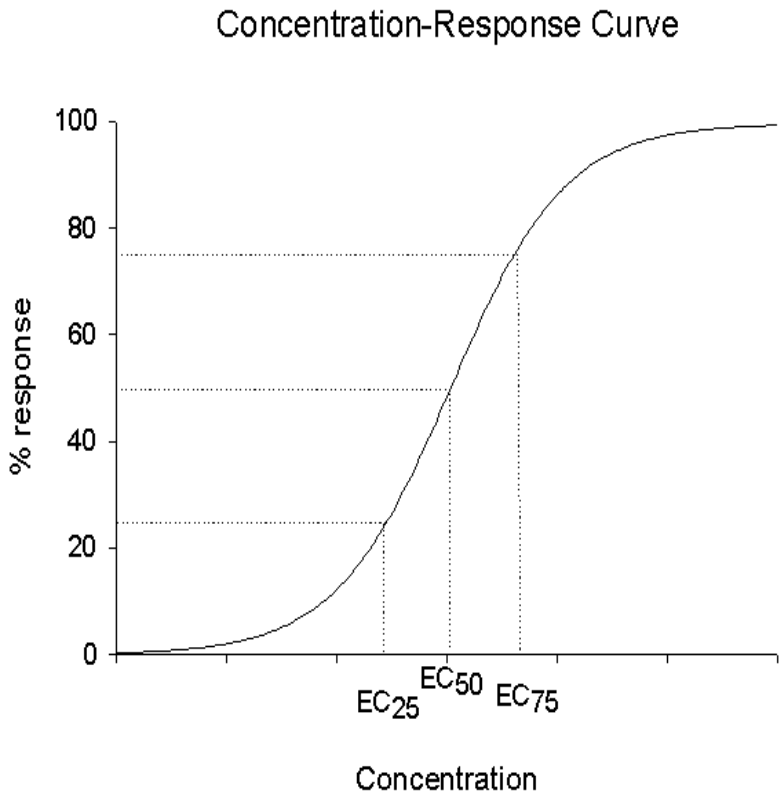
- In case of wastewaters, Make serial dilutions of the test substances (0, 5, 10, 20, 40, 60, 80, 100%) from the stock wastewater.
- In case of chemical substances make a stock solution (10%) and then make serial dilutions to give serial concentration depend upon

the toxicity of the substance.

- In triplicate order, expose the test organism to every serial dilution or concentration.
- In case of Daphnia, small Petri dishes are used and currently microplate is used to conduct the test. It is placed in an incubator for 24 and 48 h.
- In case of the fish model, aerated glass aquaria are used for 24 and 48 h.
- At the end of 24 and 48 h the dead animals are counted for each serial dilution or concentrations.
- Make the following table:

Dilutions (%) of concentration (mg or ug/L)	% Death		
	Replicate 1	Replicate 2	Replicate 3

- Depict a graph between % Mortality at Y axis versus dilution or concentration at X axis, this is termed Toxicity Curve. From this curve, LC50 can be estimated.



- **Find the LC50** by hand from the hand fitted curve or most recently using specific software.
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ACTIVITY:

-Pls bring wastewater (treated or Untreated) and make serial dilutions as indicated above estimate DO in each.

-Pls make a different concentration of Potassium dichromate as a standard pollutant.

Note:

Potassium permanganate is considered as a standard pollutant to compare the toxicity of the pollutants for the following:

-It a common and available chemical.

-It is also one of the common pollutants in an aquatic habitat.

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