INTERNSHIP REPORT

Birla Institute of Technology and Science, Pilani (BITS Pilani), Applied and Environmental Biotechnology Laboratory - Dept. of Biological Sciences

Duration: 01-12-2022 to 31-12-2022

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BITS Pilani

GLOBAL SANITATION GRADUATE SCHOOL

Certificate of Completion

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Himanshi Upadhyay

For having actively completed a 1 month Internship
at BITS Pilani, KK Birla Goa Campus from 01-12-2022 to 31-12-2022

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Acknowledgement

I am incredibly grateful to my internship guide, Dr. Srikanth Mutnuri, Professor, FSM Laboratory, Dept. of Biological Sciences, BITS, Goa, for his guidance and support throughout my internship. He provided me with clear direction and advice, and were always available to answer my questions and provide valuable feedback.

I would also like to express my gratitude to my mentors, Ph.D. scholar Mrs. Rajashree Yaragal and Associate Researcher Mr. Guruprasad Talekar for their warm welcome and invaluable guidance during my internship. It was a pleasure to work with such a talented and supportive team. From the moment I arrived, they were quick to introduce themselves and make me feel welcome. They were always willing to share their knowledge and expertise, and were patient in answering my questions and helping me to understand the laboratory. Their experience and expertise helped me to better understand the work, and allowed me to make the most of my internship.

With great pleasure, I would like to extend my gratitude and thanks to Department of Biotechnology, Ministry of Science and Technology, Government of India and School of Biological Sciences and Biotechnology, Department of Biotechnology, Goa University for giving me the opportunity to do an internship in Birla institute of technology (BITS), Applied and Environmental Biotechnology Laboratory - Dept. of Biological Sciences.

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Chemical Oxygen Demand

Introduction: Chemical Oxygen Demand (COD) is a test that measures the amount of oxygen required to chemically oxidize the organic material and inorganic nutrients, such as Ammonia or Nitrate, present in water. COD analysis is used as an indirect measure of pollutants in a water sample. It is an important parameter in water quality analysis, helping to reduce risk to humans and the environment. A higher COD in a sample indicates that it contains higher levels of oxidisable material. If this is the case, then the water will have reduced dissolved oxygen levels. Where this happens, the effects can be environmentally damaging to higher aquatic lifeforms. The aim of wastewater treatment, therefore, is to reduce levels of COD in water. The use of the dichromate procedure was pioneered and perfected for wastewater in 1949. COD is measured via a laboratory assay in which a sample is incubated with a strong chemical oxidant for a specified time interval and at constant temperature (usually 2 hours at 150°C). The most commonly used oxidant is **potassium dichromate**, which is used in combination with boiling **sulphuric acid**

Principle:

A known volume of sample is refluxed with a known excess of Potassium dichromate in sulphuric acid medium using silver sulphate as a catalyst. Potassium dichromate is a strong oxidizing agent under acidic conditions. Mercuric sulphate is added to the sample to eliminate the interference of halides. Silver sulphate acts as a catalyst.

Materials Required:

COD tubes, Test tube stand, Pipette(10mL), Pipette tips, Conical flasks etc. Instruments: Block digestion assembly, spectrophotometer

Fig 1. Sample from Sewage Treatment Plant

Fig 2. Block digestion assembly

Preparation of reagents:

1. Digestion reagent (Potassium dichromate solution):

Dissolve 33.3 gm of Mercuric sulphate and 167 ml of Sulphuric acid (96 %) and 10.216 gm of Potassium dichromate (previously dried at 105°C for 2 hours) and finally make up the volume to 1000 ml with distilled water.

2. Sulphuric acid reagent

Dissolve 2 gm of silver sulphate in 200 ml of sulphuric acid (96 %), (1gm/100ml).

Procedure:

1. Digestion:

Take 1.2 ml digestion reagent in the COD tubes and add 2.8 ml of conc. sulphuric acid reagent slowly and carefully and mix it thoroughly. Add 2 ml of sample. Load the tubes in digestion blocks which is set to 150°C and ensure perfect sealing of condensers for 2 hours. Keep the tubes at 150°C and after removing, cool down to room temperature. Take the absorbance at 600 nm.

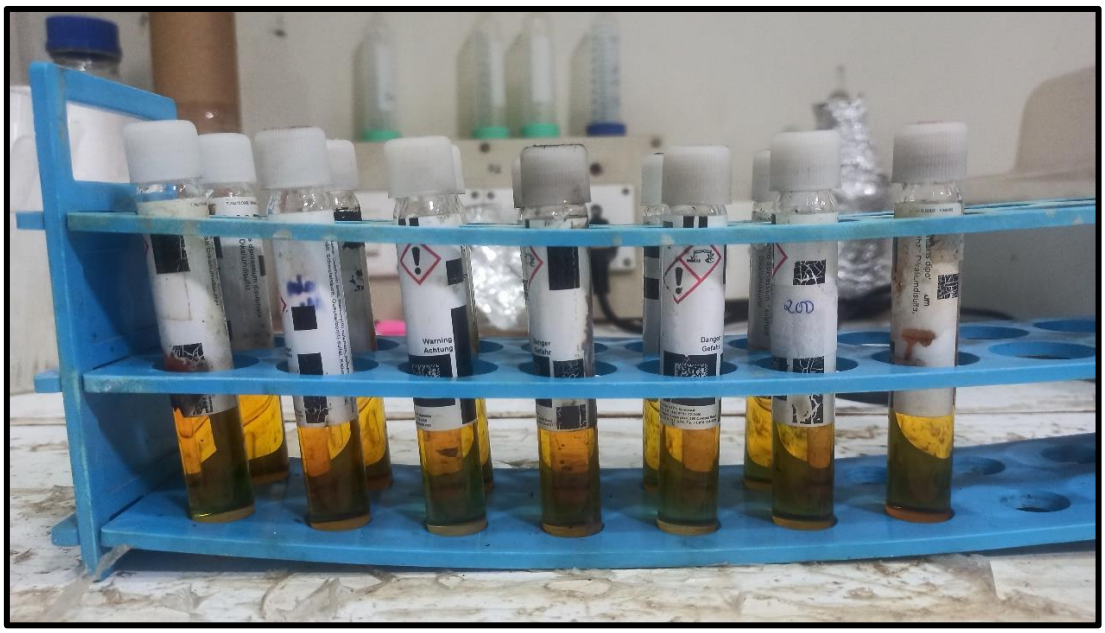

Fig 3. COD tubes with reaction mixture

Observation table:

Sample from sewage treatment plant	Absorbance at 600 nm
STP 2 a **STP 2 b**	0.007 0.003
STP 3 a **STP 3 a**	0.120 0.128
31 MLD STP a **31 MLD STP b**	0.023 0.021
10 MLD STP a **10 MLD STP b**	0.019 0.010
1.5 MLD STP a **1.5 MLD STP b**	0.117 0.126
1.5 MLD STP diluted (1:1) a **1.5 MLD STP diluted (1:1) b**	0.057 0.059
120 MLD a **120 MLD b**	0.081 0.100
120 MLD diluted (1:1) a **120 MLD diluted (1:1) b**	0.044 0.035

Calculations for COD

	Absorbance	**(At 600nm)**		
Sample	A	B	Average	Average/0.0004 = COD (mg/L)
STP 2	0.007	0.003	0.005	12.5
STP 3	0.120	0.128	0.124	310
31 MLD STP	0.023	0.021	0.022	55
10 MLD STP	0.019	0.010	0.0145	36.25
1.5 MLD STP	0.117	0.126	0.121	302.5
120 MLD	0.081	0.100	0.0905	226.25

Results and Interpretations: The chemical oxygen demand (COD) for the samples STP 3, 1.5 MLD STP and 120 MLD STP is more than samples STP 2, 31 MLD STP, 10 MLD STP which indicates they are highly polluted and needs further treatment before discharging into water bodies.

Determination of Biological oxygen demand (BOD)

Introduction:

Microorganisms such as bacteria are responsible for decomposing organic matter. When organic matter such as dead plants, leaves, grass clippings, manure, sewage, food waste is present in a wastewater, the aerobic bacteria will start the oxidation of these wastes. When this happens, much of the available Dissolved Oxygen (DO) is consumed by aerobic bacteria, robbing other aquatic organisms of the oxygen they need to live. The biochemical oxygen demand is measure of oxygen utilized by aerobic micro-organisms during biological oxidation of organic matter. Generally, when BOD levels are high, there will be low DO levels.

Drinking water must have a BOD of less than 01 mg/L and the water is considered fairly up to 03 mg/L of BOD, but when the BOD value ≥ 05 mg/L the water is doubtful in purity.

Ordinary domestic sewage may have a BOD of 200 mg/L. As per CPCB standards the treated or untreated sewage to be discharged into surface water bodies must have of BOD of less than 30 mg/L.

Requirements: BOD bottles, Water samples

Calculations

BOD = 80% of COD

Expected BOD values are calculated from the COD values from the previous experiment.

Expected BOD value (mg/L)	Amount of sample to be used (mL)
0-40	432
0-80	365
0-200	250
0-400	164
0-800	97
0-2000	43.5
0-4000	22.7

Sample	Expected BOD (mg/L)	Amount of sample used (ml)
STP 2	10	432
STP 3	248	164
31 MLD STP	44	365
10 MLD STP	29	432
1.5 MLD STP	242	164
120 MLD STP	181	250

Fig 4. BOD test bottles

Sample	Measured value of BOD after 5 days (mg/L)
STP 2	3.3
STP 3	83.5
31 MLD	42.4
10 MLD	17.1
1.5 MLD	171.6
120 MLD	122.0

Result and interpretation: The BOD for water sample STP 3, 1.5 MLD and 120 MLD is more than other samples used. These water samples are highly polluted and can not be discharged in the environment without proper treatment. As per CPCB standards the treated or untreated sewage to be discharged into surface water bodies must have of BOD of less than 30 mg/L.

Helminth microscopy method

Introduction

The prevalence of helminth infection in people living with rudimentary water and sanitation in low-income countries is generally high. Due to the extreme hardiness of the eggs of the roundworm, *Ascaris lumbricoides*, they are used in the waste and sanitation field as a marker or indicator for the safe end-use of resource recovery products from faecal sludge. Since *Ascaris* eggs are so difficult to inactivate, if treatment of faecal sludge is successful in killing *Ascaris* eggs, then it is likely that other pathogens are also inactivated (viruses, bacteria, protozoa and helminths). Other commonly found helminths are *Trichuris trichiura, Taenia spp. (Necator americans* and *Ancylostoma duodenale)* and *Strongyloides sterocoralis.* Various animal parasites are also commonly encountered. In countries where piped water is not chlorinated, the presence of free-living soil and water organisms are encountered and need to be differentiated from pathogens.

Principle:

Helminths eggs are thought to adhere to soil particles, possibly as a result of charge interations with, or adsorption of, eggs to the particles. Faecal sludge samples are often contaminated with silica particles, hence the use of ammonium bicarbonate as a wash solution. Liquid samples that have a high fat content need to be treated differently. Here, it is suggested that a surfactant such as Tween 20^{D}, TritonX-100^{D}, or $7X^{D}$ is used to break up the fats, rather than ammonium bicarbonate.

Laboratory testing for helminths is based on four main principles: washing, filtration, centrifugation and flotation of eggs to remove them from the various waste media.

1) Ammonium bicarbonate is used as both a wash solution and also to dissociate the eggs from the soil particles (surfactant should be used for fatty samples).

2) Filtration using 100µm and/or 20µm sieves is used to separate larger and smaller particles from the eggs both after washing and after flotation.

3) Centrifugation is used to sediment the deposit so water can be discarded after washing, to aid the separation process during flotation, and to sediment the washed eggs after flotation.

4) Flotation, using a solution of zinc sulphate at a specific gravity (SG) of 1.3 is used to separate eggs (with a relative density of < 1.3) out of the matter retained (retentate) with them on the 20µm sieve.

Safety precautions:

Appropriate personal equipment (PPE) should be used.

Required chemicals:

- Physiological saline (8.5g/L NaCl)

Dissolve 8.5g sodium chloride in 1,000 mL deionised water. If this amount will not be utilised in less than a week, it is preferable to decant it into smaller containers and autoclave for 15 min at 121°C. Cool to room temperature and store.

- Ammonium carbonate (AmBic)

Dissolve 119g of Ammonium carbonate in 1L de-ionised water (use a magnetic stirrer and bar magnet)- store in a glass jar.

- Tween 80, TritonX100, or 7X.
- Zinc sulphate ($ZnSO_4.7H_20$)

Dissolve 500g zinc sulphate in approximately 800mL deionised water (use the magnetic stirrer and bar magnet) and adjust SG using more of the chemical or water to raise or lower the SG to 1.3.

- 0.1 N sulphuric acid (H_2SO_4)

Add 500mL deionised water to a 1L plastic bottle, pour 3mL concentrated sulphuric acid into a 10mL graduated cylinder, then pour the H_2SO_4 into the plastic bottle containing the water, then recap and shake. Uncap, add 497mL of deionised water to the plastic bottle, recap and shake.

Required apparatus and instruments:

- Compound microscope with 10× and 40× objectives (and preferably, a camera)
- Bench-top centrifuge with a swing-out rotor that can spin a minimum of 8 × 15 mL plastic conical test tubes (e.g., Falcon tubes) and, if possible, buckets that can also spin a minimum of 4 × 50 mL plastic conical test tubes
- 15 mL plastic conical test tubes (Falcon tubes)
- 50 mL plastic conical test tubes (Falcon tubes)
- Sink with hose attached to tap for washing using strong water pressure
- Top-pan balance (for weighing up to 1.20 g and accurate to 2 decimal places)
- Magnetic stirrer and bar magnets
- Vortex mixer
- Hydrometer that can measure SG between 1.2 and 1.3
- 100 μm mesh stainless steel pan sieve, diameter 200 mm, height 50 mm
- 20 μm mesh stainless steel pan sieve, diameter 200 mm, height 50 mm
- 20 μm mesh stainless steel pan sieve, diameter 100 mm, height 45 mm
- Plastic conical test tubes (Falcon tubes), 15 mL or 50 mL
- Plastic test tube racks to hold the 15 mL Falcon tubes (and if using 50 mL tubes, one for these)
- Plastic 200 mL beakers

- Plastic ‘hockey-stick’ shaped spreaders
- Plastic 3 mL Pasteur pipettes (non-sterile)
- Non-sterile gloves
- Applicator sticks and wooden tongue depressors
- Microscope slides (76 × 26 × 1.2 mm)
- Cover glasses (22 × 40 mm). 8.8.1.4.5

Sample preservation

After collection, the samples should be stored at approximately 4-10 °C. Processing is always best carried out as soon after sampling as possible, but providing that there is sufficient moisture and the samples are fairly large (≥ 100 mL/100 g), the eggs should be unharmed and development will be arrested at these low temperatures.

Procedure for slurry, semi-solid and solid faecal sludge samples (TS > 5%):

1. Place a 200 mL plastic beaker (labelled with the sample number) on the top-pan balance, zero the balance, and weigh 10 g or 20 g of the sample into the beaker. Note: if waste material is very dry (e.g., pelletised or completely desiccated), then soak the weighed sample for 12 – 24 hr in ± 80 mL physiological saline to soften. Next, break up and mix the sample well in the saline. Allow to stand to sediment the solids for 4 hr. Remove as much supernatant as possible without disturbing the deposit, and continue with the next step below.
2. Add 50-80 mL AmBic and a magnetic stirring bar, and mix on the magnetic stirrer for 10 min.
3. Pour this mixture over the 100 µm mesh sieve placed on top of the 20 µm sieve (wet the sieves with tap water first).
4. Rinse the beaker with tap water and pour over the sieves.

5. Wash the magnet over the sieves and remove, wash the 100 μm sieve well (using a 'hockey stick'-shaped spreader, or preferably, a gloved hand) over the 20 μm filter, regularly checking the bottom sieve for fluid build-up. Use the same hockey stick spreader to stir the sample on the 20 μm sieve while holding the 100 μm sieve directly above so as not to lose any sample. When the 20 μm sieve has drained sufficiently, place the 100 μm sieve back on top and continue washing. Repeat this until the sample on the 100 μm sieve is sufficiently well washed.

6. Separate the sieves and then rinse the 20 μm sieve well. Use water pressure to wash the material to one side of the sieve to make collection easier.

7. Rinse all the material off the 20 μm sieve into the original rinsed-out, labelled beaker.

8. Pour the beaker contents into 4 × 15 mL conical test tubes labelled with a sample number or if the retentate is large, use 50 mL labelled tubes. Rinse the beaker with a small volume of water and add this to the test tubes until all the sample is collected. (The aim after the next step is to have ≤1 mL deposit in a 15 mL tube and ≤5 mL in a 50 mL tube.)

9. Centrifuge at 3,000 rpm (1,512 g) in the centrifuge with a swing-out rotor for 10 min.

10. Pour off the supernatant, leaving deposits in the test tubes.

11. Place the test tubes in the rack with the applicator stick in each (as a stirring rod) and pipette in $ZnSO_4$, 3 mL at a time, vortexing in between the addition of the chemical, until the tubes are filled to the 14 mL mark for the 15 mL tubes/45 mL mark for the 50 mL tubes.

12. Centrifuge at 2,000 rpm (672 g) for 10 min.

13. Pour the supernatant flotation fluid over the 100 mm diameter 20 μm sieve. Wash the remaining deposits out of the test tubes and keep one aside for re-use.

14. Wash the material on the sieve well with tap water and rinse it down to one side of the sieve for collection. Using a 3 mL plastic pipette, transfer the material back into the test tube kept aside.

15. Centrifuge at 3,000 rpm (1,512 g) for 10 min to obtain the final deposit. 16. Pour off the supernatant water and pipette up the deposit, place it on one or more microscope slides (but make one slide at a time so they do not stand for long periods and dry out), place a 22 × 40 mm coverslip on top, examine and count every Ascaris egg, classifying them as viable, potentially viable or dead. Trichuris, Taenia, and hookworm spp. eggs must also be counted and assessed simply as potentially viable or dead.

Observation:

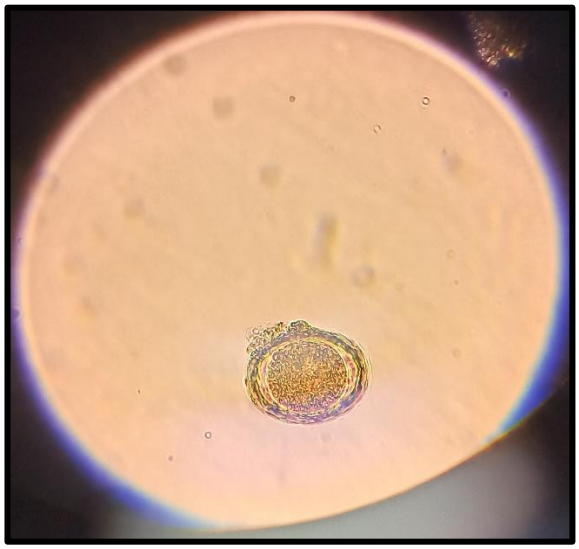

Fig 5. Helminth egg under microscope

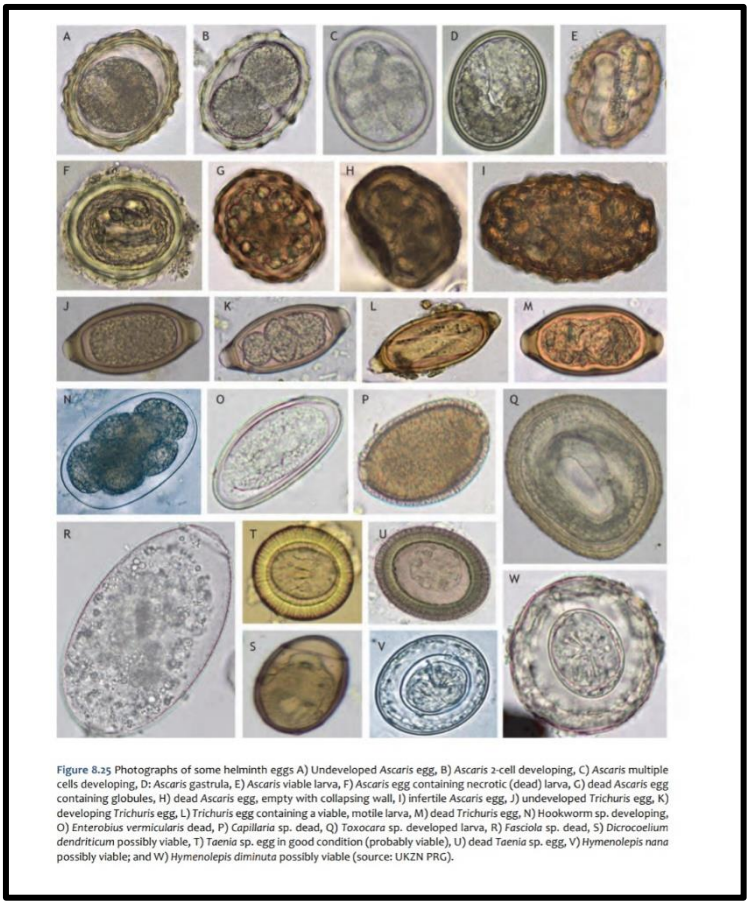

Figure 8.25 Photographs of some helminth eggs A) Undeveloped *Ascaris* egg, B) *Ascaris* 2-cell developing, C) *Ascaris* multiple cells developing, D: *Ascaris* gastrula, E) *Ascaris* viable larva, F) *Ascaris* egg containing necrotic (dead) larva, G) dead *Ascaris* egg containing globules, H) dead *Ascaris* egg, empty with collapsing wall, I) infertile *Ascaris* egg, J) undeveloped *Trichuris* egg, K) developing *Trichuris* egg, L) *Trichuris* egg containing a viable, motile larva, M) dead *Trichuris* egg, N) Hookworm sp. developing, O) *Enterobius vermicularis* dead, P) *Capillaria* sp. dead, Q) *Toxocara* sp. developed larva, R) *Fasciola* sp. dead, S) *Dicrocoelium dendriticum* possibly viable, T) *Taenia* sp. egg in good condition (probably viable), U) dead *Taenia* sp. egg, V) *Hymenolepis nana* possibly viable; and W) *Hymenolepis diminuta* possibly viable (source: UKZN PRG).

Fig 6. Reference picture of Helminth eggs

Calculation:

Once all the eggs have been counted, the results should be calculated to report the number of eggs per litre or per gram for each species of helminth and within each species, and those that are viable and non-viable.

Estimation of Total phosphorous

Introduction:

Controlled concentration of phosphorus in water is critical for a stable ecosystem. Aquatic plants are dependent on a certain amount of phosphorus in order to survive; however, excess phosphorus leads to eutrophication, the over enrichment of a water body with nutrients. This can lead to a surge in biomass growth manifesting as an algal bloom that blocks sunlight from other aquatic plants and starves the water of oxygen adversely affecting the environment. The EPA Water Quality Criteria recommends a maximum phosphorus concentration of 100 µg/L in rivers and streams and 25 µg/L in lakes in order to prevent eutrophication from occurring.

The method we will use to measure the concentration of pollutants in the water is called Spectrophotometry, which is a procedure that determines how much a chemical compound absorbs and transmits light. Having the light absorbance profile of a solution, we can compare it with a known sample and identify which compounds are present and also their quantities.

Materials required: COD tubes, test tube stand, Reagent A, Reagent B, Mixed reagent (freshly prepared)

Equipment: Spectrophotometer etc

Preparation of reagents:

1. Reagent A: Ammonium molybdate solution

Dissolve 25 gm of Ammonium molybdate in 400 ml distilled water.

2. Reagent B: Ammonium vandate

Dissolve 1.25 gm of Ammonium vandate in 300 ml distilled water.

3. Mixed reagent: should be prepared freshly.

To prepare 50 ml of mixed reagent, take 20 ml of reagent A, 15 ml of reagent B, 12.5 ml of concentrated HNO3 and 2.5 ml distilled water.

Procedure:

1) Prepare stock solution of Phosphorus: 0.16 mg/L
2) Dissolve 179 mg of KH_2PO4 in 250 ml distilled water to give final concentration of (PO_4-P) 0.16 mg/ml. Use this stock to prepare different concentrations of PO_4-P as given in following table.
3) Prepare one blank sample.

Sr. No.	Volume of stock (ml)	Volume of distilled water (ml)	Total Volume (ml)	Conc. (mg/L)
1	0	5	5	0
2	0.1	4.9	5	0.0032
3	0.2	4.8	5	0.0064
4	0.3	4.7	5	0.0096
5	0.4	4.6	5	0.0128
6	0.5	4.5	5	0.016
7	0.6	4.4	5	0.0192
8	0.7	4.3	5	0.0224

9	0.8	4.2	5	0.0256
10	0.9	4.1	5	0.0288
11	1	4	5	0.032

4) Now take 3.5 ml from each of above tubes, add 1 ml of mixed reagent and dilute to make final volume of 5.0 ml as given in following table and use this diluted stock samples for construction of standard (PO_4 – P) curve.

Sr. No.	Volume diluted stock sample (ml)	Volume of mixed reagent (ml)	Distilled water (ml)	Total Volume (ml)	Concentration (mg/L)	OD at 540 nm
1	3.5	1	0.5	5	0	0
2	3.5	1	0.5	5	0.00224	0.085
3	3.5	1	0.5	5	0.00448	0.177
4	3.5	1	0.5	5	0.00672	0.297
5	3.5	1	0.5	5	0.00896	0.372
6	3.5	1	0.5	5	0.0112	0.459

7	3.5	1	0.5	5	0.01344	0.572
8	3.5	1	0.5	5	0.01568	0.691
9	3.5	1	0.5	5	0.01792	0.751
10	3.5	1	0.5	5	0.02016	0.871
11	3.5	1	0.5	5	0.0224	0.943

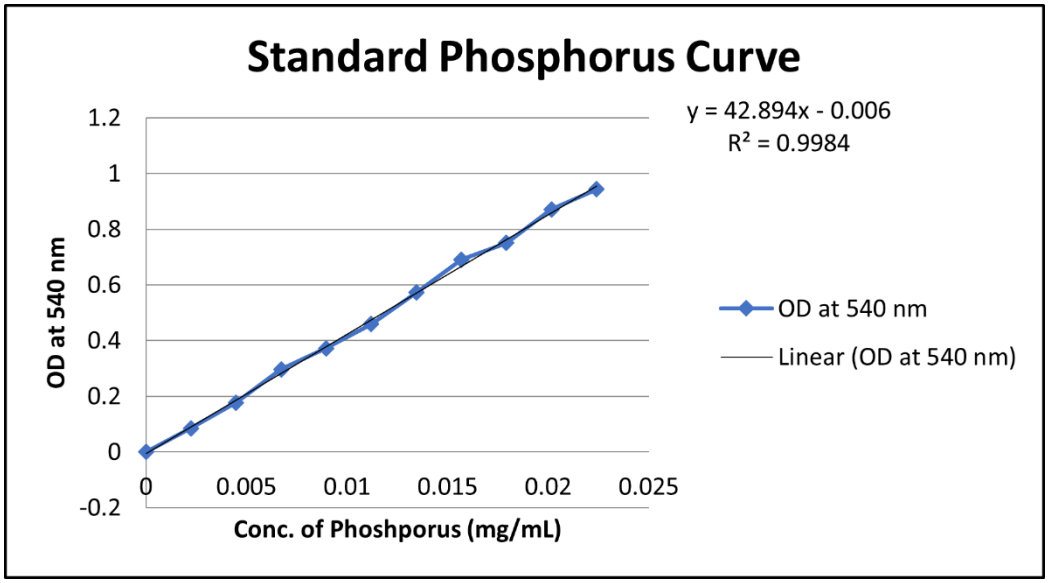

Fig 7. Standard Phosphorous curve

Observation table for the given sample.

Sample from sewage water treatment plants	Absorbance at 450 nm	Average	Phosphorous value calculated from the standard curve (mg/L)
1) STP 2 a STP 2 b	0.003 0.002	0.0025	0.198
2) STP 3 a STP 3 b	0.038 0.032	0.035	0.955
3) 31 MLD a 31 MLD b	0.105 0.095	0.1	**2.471**
4) 10 MLD a 10MLD b	0.055 0.033	0.044	1.165
5) 1.5 MLD a 1.5 MLD b	0.352 0.360	0.356	**8.439**
6) 120 MLD a 120 MLD b	0.181 0.192	0.1865	**4.487**

Result and interpretation: The sewage samples named 31 MLD, 1.5 MLD and 120 MLD have very high concentrations of phosphorous which indicates that they are highly polluted. The possible reasons might be the presence of fertilizers, run-off from urban areas, poor agricultural practices, etc. Although there is no actual phosphorus discharge limit for wastewater into general water bodies, it is generally now considered that for a water body to actually achieve 'good' status under the WFD, a limit as low as **0.1 mg/l** may be necessary.

Determination of Total Suspended Solids of sludge sample

Aim: To determine the total suspended solids

Principle:

Pre-weighed sample is evaporated in pre-weighed dish and dried until constant weight is observed in dry air oven at 105 ^{0} C. The increase in weight over that of empty dish represents the total suspended solids.

Materials and Methods:

- Evaporating dishes – Dishes of 100 ml capacity made of porcelain.
- Analytical balance
- Drying oven, for operation at 103 to 105 ^{0} C.

Procedure:

Centrifuge the suspension at 8000 rpm for 10 minutes and concentrate all the TSS in one pre-weighed centrifuge tube. Centrifuge again at 8000 rpm for 10 minutes and discard the supernatant. Wash the pellet with 1 % NaCl twice with centrifugation and discard the supernatant. Now remove all the pellet in pre-weighed porcelain evaporating dishes and determine the wet weight of solid. Now evaporate the dishes in dry air oven at 100 ^{0} C to constant weight.

Note: All the experiments were carried out in duplicates.

Calculation formulae:

Total solids (%) = (A-B) X 100 /Sample wet weight (gm)

A = weight of dried sludge sample including dish

B = weight of empty dish

Estimation of Ammonium using kit

Method:

Ammonium nitrogen (NH4-N) occurs partly in the form of ammonium ions and partly as ammonia. A pH-dependent equilibrium exists between the two forms. In strongly alkaline solution ammonium nitrogen is present almost entirely as ammonia, which reacts with hypochlorite ions to form monochloramine. This in turn reacts with a substituted phenol to form a blue indophenol derivative that is determined photometrically. Due to the intrinsic yellow coloration of the reagent blank, the measurement solution is yellow-green to green in color.

Applications: This test measures both ammonium ions and dissolved ammonia.

Preparation

• Rinse glassware ammonium-free with distilled water. Do not use detergent!

• Analyze immediately after sampling.

• Check the ammonium content with the MQuant® Ammonium Test.

Samples containing more than 150 mg/l NH4-N must be diluted with distilled water.

• The pH must be within the range 4 - 13. Adjust, if necessary, with sodium hydroxide solution or sulfuric acid.

• Filter turbid samples.

Procedure

Sample was diluted with distilled water (1:5)

Measuring range 2.0 - 75.0 mg/l NH4-N (2.6 - 96.6 mg/l NH4+):

1) Add 5mL Reagent NH4-1 (20-30°C), Pipette into a test tube.
2) Add 20mL Pre-treated sample (20-30°C). Add with pipette and mix.
3) Add Reagent NH4-2 (1 level blue micro spoon). Add and shake vigorously until the reagent is completely dissolved.
4) Leave to stand for 15 min (reaction time), then fill the sample into a 10-mm cell, and measure in the photometer.

Results:

Sample 1 NH4-N = 146mg/L x 5

Sample 2 NH4-N = 124mg/L x 5

Demonstration of TOC analyzer for measurement of Total organic carbon in Solid samples like sludge, Soils, Sediments etc., and TOC-L for measurement of TOC, TC (Total carbon), TIC (Total inorganic carbon) along with total nitrogen (TN) in liquid samples like water, wastewater, etc.,

Carbon measurement

TOC refers to a Total Organic Carbon analyzer, which utilizes a catalytic oxidation combustion technique at high temperature (the temperature raises up to 720 ºC), to convert organic carbon into CO_2. The CO_2 generated by oxidation is measured with a Non-dispersive Infra-Red (NDIR) sensor. By using special kits and (dilution) methods the device can be applied to determine the carbon concentration over an extremely broad range (theoretically from 4µg/L to 30, 000mg/L), from pure drinking water to sea water with sludge. In addition, it is possible to indirectly determine the fraction of IC (= "inorganic carbon") arising from dissolved CO_2 and acid salts containing carbon.

The Shimadzu TOC-L uses a high temperature combustion method to analyze aqueous samples for total carbon (TC), total organic carbon (TOC) and dissolved organic carbon (DOC), also known as non-purgeable organic carbon (NPOC). TOC and TC concentrations are derived from whole unfiltered water. NPOC concentrations are derived from water that has been filtered through a 0.7 um (nominal pore size) GF/F glass fiber filter, or equivalent.

Nitrogen measurement

By means of a chemi-luminescence reaction also the nitrogen content can be determined in above mentioned samples. Shimadzu's TNM-1, the Total Nitrogen Module, can accurately measure nitrogen over a broad range: Total Nitrogen (TN) from 100ppb to 4000ppm. A nitrogen-containing sample is combusted to NO and NO_2. The reaction products react with

ozone to an excited state of NO_2. When falling back to the ground level, energy is emitted as light. The nitrogen is measured with a chemi-luminescence detector.

Demonstration of Flame photometry.

Introduction

The principle of flame photometer is based on the measurement of the emitted light intensity when a metal is introduced into the flame. The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample.

Flame photometer can be used to determine the concentration of certain metal ions like sodium, potassium, lithium, calcium and cesium etc.

Principle:

The compounds of the alkali and alkaline earth metals (Group II) dissociate into atoms when introduced into the flame. Some of these atoms further get excited to even higher levels. But these atoms are not stable at higher levels.

Hence, these atoms emit radiations when returning back to the ground state. These radiations generally lie in the visible region of the spectrum. Each of the alkali and alkaline earth metals has a specific wavelength.

Element	Emitted wavelength	Flame color
Sodium	589 nm	Yellow
Potassium	766 nm	Violet

Element	Emitted wavelength	Flame color
Barium	554 nm	Lime green
Calcium	622 nm	Orange
Lithium	670 nm	Red

For certain concentration ranges:

The intensity of the emission is directly proportional to the number of atoms returning to the ground state. And the light emitted is in turn proportional to the concentration of the sample.

Parts of flame photometer

A simple flame photometer consists of the following basic components:

Source of flame: A Burner in the flame photometer is the source of flame. It can be maintained in at a constant temperature. The temperature of the flame is one of the critical factors in flame photometry

Nebuliser: Nebuliser is used to send homogeneous solution into the flame at a balanced rate.

Fig 8. Flame Photometer

Optical system: The optical system consists of convex mirror and convex lens. The convex mirror transmits the light emitted from the atoms. Convex mirror also helps to focus the emissions to the lens. The lens helps to focus the light on a point or slit.

Simple colour filters: The reflections from the mirror pass through the slit and reach the filters. Filters will isolate the wavelength to be measured from that of irrelevant emissions.

Photo-detector: The intensity of radiation emitted by the flame is measured by photo detector. Here the emitted radiation is converted to an electrical signal with the help of photo detector. These electrical signals are directly proportional to the intensity of light.

Working procedure

- Both the standard stock solution and sample solution are prepared in fresh distilled water.
- The flame of the photometer is calibrated by adjusting the air and gas. Then the flame is allowed to stabilize for about 5 min.
- Now the instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters.
- The readings of the galvanometer are adjusted to zero by spraying distilled water into the flame.

- The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame. Now the full-scale deflection of the galvanometer is recorded.
- Again, distilled water is sprayed into the flame to attain constant readings of galvanometer. Then the galvanometer is readjusted to zero.
- Now each of the standard working solutions is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Finally sample solution is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Calculate the mean of the galvanometer reading.
- Plot the graph of concentration against the galvanometer reading to find out the concentration of the element in the sample

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Applications of flame photometer

1. Flame photometer can be applied both for quantitative and qualitative analysis of elements. The radiations emitted by the flame photometer are characteristic to particular metal. Hence with the help of Flame photometer we can detect the presence of any specific element in the given sample.
2. The presence of some group II elements is critical for soil health. We can determine the presence of various alkali and alkaline earth metals in soil sample by conducting flame test and then the soil can be supplied with specific fertiliser.
3. The concentrations of Na+ and K+ ions are very important in the human body for conducting various metabolic functions. Their concentrations can be determined by diluting and aspirating blood serum sample into the flame.
4. Soft drinks, fruit juices and alcoholic beverages can also be analysed by using flame photometry to determine the concentrations of various metals and elements.