Technical Assistance
HYDROLOGY PROJECT

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Training module # WQ - 41

How to measure Total Phosphorus: Ascorbic Acid Method

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with
HALCROW, TAHAL, CES, ORG & JPS

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1. Module context

This module deals with the significance of phosphorus to water quality and methods for measuring phosphorus. Modules in which prior training is required to complete this module successfully and other available, related modules in this category are listed in the table below.

While designing a training course, the relationship between this module and the others would be maintained by keeping them close together in the syllabus and placing them in a logical sequence. The actual selection of the topics and the depth of training would, of course, depend on the training needs of the participants, i.e. their knowledge level and skills performance upon the start of the course.

No.	Module title	Code	Objectives
1.	Basic water quality concepts	**WQ - 01**	• Discuss the common water quality parameters • List important water quality issues
2	Basic chemistry concepts	**WQ - 02**	• Convert units from one to another • Discuss the basic concepts of quantitative chemistry • Report analytical results with the correct number of significant digits.
3	Oxygen balance in surface waters	**WQ- 25**	• Explain the importance of oxygen in water • Identify the main processes of oxygen addition and depletion in surface waters
4	Basic ecology concepts	**WQ - 26**	• Explain how energy flows through an aquatic ecosystem • Explain how nutrients are cycled in the environment • Explain the causes and problems of eutrophication
5	Absorption Spectroscopy	**WQ - 34**	• Understand the principle of absorption spectroscopy • Explain the use of absorption spectroscopy for chemical analysis
6	Measurement of Oxidised Nitrogen by Cd reduction method and UV Spectrophotometric methods	**WQ - 37**	• Measure oxidised nitrogen by Cd-reduction and UV spectrophotometric methods • Appreciate limitations of the UV method.

No.	Module title	Code	Objectives
7	How to measure Ammonia and Organic Nitrogen: Kjelhahl method	**WQ - 38**	• Understand the relevance of nitrogen to water quality. • Know how to make analysis of ammonia and organic nitrogen by Kjeldahl method.
8	How to measure Chlorophyll-a	**WQ - 40**	• Explain the process of extracting chlorophyll-a • Apply the spectrophotometric method of chlorophyll-a measurement

2. Module profile

Title	:	How to measure Total Phosphorus: Ascorbic Acid Method
Target group	:	HIS function(s): Q2, Q3, Q5, Q6
Duration	:	1 Theoretical session of 40 min, plus 1 Practical Laboratory session of 120 min, plus 1 Report writing session of 20 min
Objectives	:	After the training the participants will be able to*:* • Understand the relevance of phosphorus to water quality • Know how to make analysis for phosphorus
Key concepts	:	• Ascorbic acid method for phosphorus • Relation of Phosphorus concentration to different water types
Training methods	:	Lecture, laboratory analysis
Training tools required	:	Board, flipchart, OHS, Complete laboratory facilities for phosphorus analysis
Handouts	:	As provided in this module Including SAP for Analysis of Phosphorus
Further reading and references	:	• Chemistry for environmental engineers - C. N. Sawyer, P. L. McCarty & G. F. Parkin, McGraw - Hill, Inc., 1994 • Standard methods for the examination of water and wastewaters, AWWA, 19th edition, 1995

3. Session plan

No	Activities	Time	Tools
1	***Preparations*** • Collect samples as detailed in the main text • Prepare reagents required for the test as described in the Standard Analytical Procedure		
2	***Introduction***: • Introduce the session • Ask the question, 'Why do we need to measure phosphorus?' • Talk about the phosphorus cycle • Talk about the problems caused by phosphorus in the aquatic environment • Discuss the effect that phosphorus has on eutrophication	20 min	OHS
3	***Ascorbic Acid Method*** • Describe the method in terms of its chemistry and how it is measured.	20 min	OHS
4	***Practical Session*** • Allow participants to conduct analysis according to SAP • Stress the need to write-up material as the analysis is proceeding • Be available to guide participants and answer questions	120 min	Laboratory
5	***Report Writing*** • Allow participants to complete their report • Discuss results	20 min	

4. Overhead/flipchart master

OHS format guidelines

Type of text	Style	Setting
Headings:	OHS-Title	Arial 30-36, with bottom border line (not: underline)
Text:	OHS-lev1 OHS-lev2	Arial 24-26, maximum two levels
Case:		Sentence case. Avoid full text in UPPERCASE.
Italics:		Use occasionally and in a consistent way
Listings:	OHS-lev1 OHS-lev1-Numbered	Big bullets. Numbers for definite series of steps. Avoid roman numbers and letters.
Colours:		None, as these get lost in photocopying and some colours do not reproduce at all.
Formulas/Equations	OHS-Equation	Use of a table will ease horizontal alignment over more lines (columns) Use equation editor for advanced formatting only

Measurement of Phosphorus (1)

- Phosphorus in the environment switches between organic and inorganic forms due to the action of bacteria and plants
- Orthophosphate (H_3PO_4, $H_2PO_4^-$, $HPO4^{2-}$, PO_4^{3-}) is the only form of phosphorus that plants and micro-organisms can use
- Organic phosphorus is changed to inorganic by bacterial action
- Bacteria are also involved in converting insoluble inorganic phosphorus to soluble forms

Measurement of Phosphorus (2)

- Phosphate is often used as a synonym for orthophosphate
- Phosphate is lost from water through uptake by plants
- Phosphate promotes algal growth
- If nitrate is also present, explosive algal growth can occur (eutrophication)
- Eutrophication can lead to severe water quality problems

Measurement of Phosphorus (3)

- Eutrophication also induces:
  - *wide daily variations in dissolved oxygen concentration*
  - *wide variations in the pH of the water*
- The excessive algal growth associated with eutrophication can also give problems at water treatment works

Ascorbic Acid Method:

- Orthophosphate reacts, in acid medium, with ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid
- This is reduced by ascorbic acid to form highly coloured molybdenum blue
- Measured spectrophotometrically at 880 nm
- Arsenate, chromium (VI) and nitrite interfere

Aim:

- To determine the concentration of total phosphorus in a number of different samples

Sample	Source	Expected concentration mg/L
A	Polluted pond water	2 – 3
B	River water	0.5 – 1.0
C	Tap water	0 – 0.2

Method:

- Collect a sample from each of the buckets A, B and C
- Determine phosphorus in each sample using SAP

Observations

1. Fill in the following table as you proceed with the test:

Sample	Absorbance at 880 nm (R)	Absorbance of Blank (S)	Corrected Absorbance (R – S)
0.15 mg/l Standard Solution			
0.3 mg/l Standard Solution			
0.6 mg/l Standard Solution			
0.9 mg/l Standard Solution			
1.3 mg/l Standard Solution			
A			
B			
C			

2. Plot calibration curve and read conc. values for the samples

Report

- The aim of the investigation
- The results that you have produced
- The phosphorus concentration of the samples and what this means in terms of water quality

5. Evaluation sheets

6. Handout

Measurement of Phosphorus

- Phosphorus in the environment switches between organic and inorganic forms due to the action of bacteria and plants
- Orthophosphate (H_3PO_4, $H_2PO_4^-$, $HPO4^{2-}$, PO_4^{3-}) is the only form of phosphorus that plants and micro-organisms can use
- Organic phosphorus is changed to inorganic by bacterial action
- Bacteria are also involved in converting insoluble inorganic phosphorus to soluble forms
- Phosphate is often used as a synonym for orthophosphate
- Phosphate is lost from water through uptake by plants
- Phosphate promotes algal growth
- If nitrate is also present, explosive algal growth can occur (eutrophication)
- Eutrophication can lead to severe water quality problems
- Measurement of Phosphorus
- Eutrophication also induces:
  - *wide daily variations in dissolved oxygen concentration*
  - *wide variations in the pH of the water*
- The excessive algal growth associated with eutrophication can also give problems at water treatment works

Abscorbic Acid Method

- Orthophosphate reacts, in acid medium, with ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid
- This is reduced by ascorbic acid to form highly coloured molybdenum blue
- Measured spectrophotometrically at 880 nm
- Arsenate, chromium (VI) and nitrite interfere

Aim:

- To determine the concentration of total phosphorus in a number of different samples

Sample	Source	Expected concentration mg/L
A	Polluted pond water	2 – 3
B	River water	0.5 – 1.0
C	Tap water	0 – 0.2

Method:

- Collect a sample from each of the buckets A, B and C
- Determine phosphorus in each sample using SAP

Observation

1. Fill in the following table as you proceed with the test:

Sample	Absorbance at 880 nm (R)	Absorbance of Blank (S)	Corrected Absorbance (R – S)
0.15 mg/l Standard Solution			
0.3 mg/l Standard Solution			
0.6 mg/l Standard Solution			
0.9 mg/l Standard Solution			
1.3 mg/l Standard Solution			
A			
B			
C			

2. Plot calibration curve and read conc. values for the samles

Report

- The aim of the investigation
- The results that you have produced
- The phosphorus concentration of the samples and what this means in terms of water quality

Add copy of Main text in chapter 8, for all participants.

7. Additional handout

8. Main text

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How to measure Total Phosphorus: Ascorbic Acid Method

1. Introduction

Phosphorus in the environment switches between its organic and inorganic forms due to the action of bacteria and plants in much the same way as nitrogen does. Orthophosphate (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) the only form of phosphorus which can be used by most living things, is converted to organic phosphorus by plants and micro-organisms. This organic phosphorus is, in turn, changed to inorganic phosphorus by the action of bacteria on the waste products and dead bodies of plants and animals. Bacteria are also involved in the conversion of insoluble inorganic phosphorus species to soluble forms.

Because phosphate (often used as a synonym for orthophosphate) is an essential plant nutrient it promotes the growth of algae and other aquatic plants in surface waters. If sufficient concentrations of phosphate and nitrate are present, there is sometimes an unnatural, explosive growth of algae in water bodies; a process known as 'eutrophication'. Once this abnormal mass of algae die, their bodies are decomposed by bacteria which leads to reduction in the dissolved oxygen in the water (in severe cases to zero concentration).

The presence of both phosphate and nitrogen is necessary for eutrophication to occur. However, because the concentration of phosphate is almost always lower than that of nitrogen, the growth of algae and the onset of eutrophication is usually controlled by the amount of phosphorus present in the water. If this is the case the concentration of phosphorus is said to be 'limiting'.

A further consequence of eutrophication is that it induces wide daily variations in the dissolved oxygen concentration. This is due to the fact that algae are net producers of oxygen via photosynthesis during daylight hours but net consumers of oxygen through respiration during darkness. The oxygen concentration in the water is therefore very high during the day and low at night. This daily cycle can also be observed in the pH of the water body which also varies widely. This variation is due to the fact that algae consume acidic carbon dioxide gas during the day and release it at night. Such wide variations in the chemical parameters of a eutrophic water body can lead to significant implications for the health and use of the water. Fish for example can become stressed if ammoniacal nitrogen is present in the water because as pH increases more toxic free ammonia (NH_3 gas) is produced. Further, excessive algal growth can cause problems with treatment of the water if it is to be used as a drinking water source.

It can be seen from the above that phosphate affects water quality. Therefore its determination is important in evaluating the quality of surface waters.

2. Ascorbic Acid Method

The ascorbic acid method for determining phosphorus relies on the fact that orthophosphate reacts, in acid medium, with ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid. This is reduced by ascorbic acid to form highly coloured molybdenum blue which can be measured in a spectrophotometer at 880 nm wavelength. Arsenate, chromium (VI) and nitrite ions can interfere with the method.

In order to determine phosphorus, it is necessary to convert all forms of phosphorus to orthophosphate. This can be done by the 'Persulphate Digestion Method' in which the sample is heated with sulphuric acid and either ammonium or potassium persulphate.

3. Experiment

Aim

a. To determine the concentration of total phosphorus in a number of different samples by abscorbic acid method.

Method

a. Collect a sample from each of the buckets marked A, B and C.

Sample	Source	Expected concentration mg/L
A	Polluted pond water	2 – 3
B	River water	0.5 – 1.0
C	Tap water	0 – 0.2

b. Read the SAP for determination of total phosphorus
c. Determine the phosphorus in each sample according to the Standard Analytical Procedure for total phosphorus.

Observations & calculations

a. Fill in the following table as you proceed with the method:

Sample	Absorbance at 880 nm (R)	Absorbance of Blank (S)	Corrected Absorbance (R – S)
0.15 mg/l Standard Solution			
0.3 mg/l Standard Solution			
0.6 mg/l Standard Solution			
0.9 mg/l Standard Solution			
1.3 mg/l Standard Solution			
A			
B			
C			

b. Use the values of the standard solutions in the table to plot a graph of phosphorus concentration versus absorbance.
c. Read the phosphorus concentration of the three samples from the standard curve.

Report

When writing your report the following aspects should be addressed:

- the aim of the investigation
- the results that you have produced
- the phosphorus concentration of the samples and what this could mean in terms of water quality