Technical Assistance
HYDROLOGY PROJECT

World Bank & Government of The Netherlands funded

Training module # WQ - 40

How to measure Chlorophyll-a

New Delhi, March 2000

CSMRS Building, 4th Floor, Olof Palme Marg, Hauz Khas,
New Delhi – 11 00 16 India
Tel: 68 61 681 / 84 Fax: (+ 91 11) 68 61 685
E-Mail: dhvdelft@del2.vsnl.net.in

DHV Consultants BV & DELFT HYDRAULICS
with
HALCROW, TAHAL, CES, ORG & JPS

Table of contents

1. Module context

This module introduces the significance of chlorophyll a to water quality, and gives practical experience with the spectrophotometric method for measuring chlorophyll a. 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 place 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.	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
4.	Absorption spectroscopy	**WQ - 34**	• Understand the principle of absorption spectroscopy • Explain the use of absorption spectroscopy for chemical analyses .

2. Module profile

Title	:	How to measure Chlorophyll-a
Target group	:	HIS function(s): Q2, Q3, Q5, Q6
Duration	:	1 Lecture session of 30 min 1 Laboratory session of 120 min 1 Report Writing session of 30 min
Objectives	:	After the training the participants will be able to*:* • explain the process of extracting chlorophyll a • apply the spectrophotometric method of chlorophyll a measurement
Key concepts	:	• Significance of determining chlorophyll a in water • Spectrophotometric method of chlorophyll determination
Training methods	:	Lecture, Laboratory Analytical Exercise, Report preparation
Training tools required	:	Board, flipchart, OHS, Complete Laboratory Facilities for Chlorophyll a analysis
Handouts	:	As provided in this module, including SAP for Analysis of Chlorophyll a
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*** • Prepare reagents and glassware according to SAP • Collect samples A, B and C		
2	***Introduction:*** • Introduce the subject of chlorophyll • Talk about the link between chlorophyll and eutrophication • Discuss the significance of chlorophyll in water	5 min	OHS Flipchart/ Board
3	***Chlorophyll a Analysis – background*** • Describe the basis of the chlorophyll a method in terms of its chemistry and how it is applied. Refer to SAP for Analysis of Chlorophyll-a • Describe the acetone extraction spectrophotmetric method • Ask the participants to read the handouts	10 min	OHS, Handout
4	***Practical Session – Aim and Method*** • Explain the overall aim of this practical module • Explain how to calculate chlorophyll a concentrations • Describe the content of the report and how it should be written	15 min	OHS, Flipchart/ Board
5	***Practical Session*** • Allow participants to conduct analysis according to SAP: Refer to SAP for Chlorophyll a • Stress the need to write-up results as the analysis proceeds • Be available to guide participants and answer questions	120 min	Laboratory Facilities
6	***Report Writing and Wrap up*** • Allow participants to complete their reports • Discuss results	30 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 Chlorophyll

- Chlorophyll *a* is found in all green plants
- Gives an estimate of algal concentraion, 1 – 2% of dry weight of algae.
- Chlorophyll *a* is used to indicate the state of fertilisation of a water body.

Sample Treatment

- Concentrate sample by centrifugation or filtration
- Extract chlorophyll a into acetone

Methods of measurement

- Fluorometry which relies upon the fact that chlorophyll a fluoresces at 663 nm
- HPLC which separates chlorophylls before determination by fluorescence
- Spectrophotometry which measures the absorbance of light by chlorophyll a at 664 nm

Spectrophotometric Method (1)

- Based on absorbance at 664 nm
- Correction for interference by pheophytin, common degradation product
  - *acidify sample to convert chlorophyll a to pheophytin a*
  - *second measurement at 665 nm*
  - *substract from the first measurement at 664 nm*
  - *calculate concentration using correction factor and absorbance coefficient.*
- Correction for turbidity
  - *substract absorbance at 750 nm*

Spectrophotometric Method (2)

Sample	Source
A	Pond water
B	River water
C	Drain water

Spectrophotometric Method (3)

- Fill in the following table as you proceed with the method:

Sample	Absorbance at 750 nm before acidification (W)	Absorbance at 664 nm before acidification (X) (NOTE: should be 0.1 to 1.0)	664b (= W – X)	Absorbance at 750 nm after acidification (Y)	Absorbance at 665 nm after acidification (Z)	665a (= Y – Z)
A						
B						
C						

Spectrophotometric Method (4)

- Calculate the chlorophyll a concentration of each sample:

$$\text{Chlorophyll a, mg/m}^3 = \frac{26.7 \times \left(664_b - 665_a\right) \times V_1}{V_2 \times L}$$

V_1 = Volume of extract, L

V_2 = Volume of sample, m^3

L = light path of cuvette, cm

664_b & 665_a = absorbance before and after acidification

Report

- the aim of the investigation
- the results you have produced
- the algal content of the samples and what this could mean in terms of water quality

5. Evaluation sheets

6. Handout

Measurement of Chlorophyll

- Chlorophyll *a* is found in all green plants
- Gives an estimate of algal concentraion, 1 – 2% of dry weight of algae.
- Chlorophyll *a* is used to indicate the state of fertilisation of a water body.

Sample Treatment

- Concentrate sample by centrifugation or filtration
- Extract chlorophyll a into acetone

Methods of measurement

- Fluorometry which relies upon the fact that chlorophyll a fluoresces at 663 nm
- HPLC which separates chlorophylls before determination by fluorescence
- Spectrophotometry which measures the absorbance of light by chlorophyll a at 664 nm

Spectrophotometric Method (1)

- Based on absorbance at 664 nm
- Correction for interference by pheophytin, common degradation product
    - *acidify sample to convert chlorophyll a to pheophytin a*
    - *second measurement at 665 nm*
    - *substract from the first measurement at 664 nm*
    - *calculate concentration using correction factor and absorbance coefficient.*
- Correction for turbidity
    - *substract absorbance at 750 nm*

Spectrophotometric Method (2)

Sample	Source
A	Pond water
B	River water
C	Drain water

Spectrophotometric Method (3)

- Fill in the following table as you proceed with the method:

Sample	Absorbance at 750 nm before acidification (W)	Absorbance at 664 nm before acidification (X) (NOTE: should be 0.1 to 1.0)	664b (= W – X)	Absorbance at 750 nm after acidification (Y)	Absorbance at 665 nm after acidification (Z)	665a (= Y – Z)
A						
B						
C						

Spectrophotometric Method (4)

- Calculate the chlorophyll a concentration of each sample:

$$\text{Chlorophyll a, mg/m}^3 = \frac{26.7 \times (664_b - 665_a) \times V_1}{V_2 \times L}$$

V_1 = Volume of extract, L
V_2 = Volume of sample, m^3
L = light path of cuvette, cm
664_b & 665_a = absorbance before and after acidification

Report

- the aim of the investigation
- the results you have produced
- the algal content of the samples and what this could mean in terms of water quality

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

7. Additional handout

8. Main text

Contents

How to measure Chlorophyll-a

1. Introduction

Chlorophyll a is found in all green plants including algae. For this reason it can be used to estimate the quantity of algae present in a water body. Chlorophyll a constitutes approximately 1 to 2% of the dry weight of planktonic algae. Level of chlorophyll a indicates the quality of a water body with respect to its fertilization.

2. Extraction of chlorophyll a

Before the chlorophyll a can be determined it must be extracted from the bulk of the water sample as it would normally be too dilute to measure directly. This should be done by concentrating the sample by centrifugation or filtration and then extracting the chlorophyll pigment with acetone as given in the Standard Analytical Procedure (SAP) for chlorophyll a.

3. Methods of chlorophyll a determination

A number of methods are available for the determination of chlorophyll a as follows:

Fluorometry

This method relies upon the fact that chlorophyll a fluoresces at a wavelength of 663 nm when excited by radiation at a wavelength of 430 nm. Determination of chlorophyll in this way is therefore carried out in a fluorometer which has been previously calibrated with various known chlorophyll a solutions.

It is also possible, with a suitable meter, to carry out this determination in the field.

High-performance Liquid Chromatography (HPLC)

This method makes use of HPLC to separate various algae-derived chlorophylls and their degradation products. Following separation in the chromatography column, chlorophyll a and other species of interest can be determined using a fluorescence detector.

This method is capable of detecting chlorophylls a, b and c and many of their degradation products in a single instrument run.

Spectrophotometry

Chlorophyll a absorbs light of 664 nm wavelength and can be measured spectrophotometrically. Pheophytin a, a common degradation product of chlorophyll a, also absorbs light in the same region of the spectrum. Therefore correction for the presence of pheophytin a is made by taking another absorbance reading at 665 nm after converting chlorophyll a in the sample to pheophytin a by adding an acid and subtracting this value from the 664 nm value before addition of the acid. A correction factor based on ratios of absorbances at 664 nm and 665 nm for pure chlorophyll a and for pure pheophytin a and absorbance coefficient for chlorophyll a at 664 nm, is used in the final calculations. Correction is also made for turbidity in the sample by subtracting absorbance at 750 nm.

4. Experiment

Aim

To determine the chlorophyll a in a number of different samples by spectrophotometry

Method

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

Sample	Source
A	Pond water
B	River water
C	Drain water

b. Read SAP for measurement of chlorophyll

c. Determine the chlorophyll a in each sample according to the Standard Analytical Procedure for chlorophyll a.

Observations & calculations

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

Sample	Optical Density at 750 nm before acidification (W)	Optical Density at 664 nm before acidification (X) (NOTE: should be, 0.1 to 1.0)	664b, (= W – X)	Optical Density at 750 nm after acidification (Y)	Optical Density at 665 nm after acidification (Z)	665a, (= Y – Z)
A						
B						
C						

b. Use the values in the table to calculate the chlorophyll a concentration in each sample using the form.

Report

When writing your report the following aspects should be addressed:
- the aim of the investigation
- the results that you have produced
- the algal content of the samples and what this could mean in terms of water quality