Nile Basin Initiative
Nile Trans boundary Environmental Action Project

Selected Standard Methods for Water & Waste Water Quality Analysis for Transboundary Samples

July 2007

NILE BASIN INITIATI

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FOREWORD

The Basin wide Water Quality Monitoring Component of the NTEAP has developed four Water Quality Operational Manuals which will assist in the transboundary water quality monitoring of the Nile Basin.
The four Manuals that have been developed are:

- **Simple Procedures for Water & Waste Water Sampling for Nile Basin Countries for Transboundary Water Quality Monitoring.**
- **Selected Common Standard Analytical Methods for Nile Basin Countries for Transboundary Water Quality Monitoring.**
- **Guidelines for Data Reporting Forms for Nile Basin Countries for Transboundary Water Quality Monitoring.**
- **Manual for On-Site Tests by Local Communities & Schools for Nile Basin Countries for Transboundary Water Quality Monitoring.**

The Manuals will also:

- *Promote basin wide networking on Water Quality Management, to ensure transboundary water quality assessment;*
- *Promote continued exchange of information on key transboundary parameters;*
- *Enhance continued awareness on water quality issues;*
- *Assist and enhance capacities for Water Quality Monitoring, and improve the understanding of transboundary Water Quality Management issues.*

The Manuals will promote good comparability of the water quality data produced, and also ensure data reporting consistency on a regional and international level, so that the analytical results produced can be compared on a level platform.

The NBI through NTEAP is proud to produce and launch these simply designed and user-friendly series of Manuals which will compliment the already on-going national water quality monitoring initiatives.

On behalf of the NBI, the NTEAP wishes to acknowledge with gratitude the technical and administrative support by the Regional Water Quality Working Group Members, the Consultant, the PMU Staff, the National Project Coordinators and Water Quality Lead Specialist for contributing to the development of these Manuals.

It is our hope that the users of these Manuals will find them beneficial, as a first step towards harmonizing transboundary water quality monitoring practices in the Nile basin countries.

Gedion Asfaw,
Regional Project Manager,
Nile Transboundary Environmental Action Project.

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In the preparation of these Manuals, other sources of information such the Standard Methods for Water and Waste water Analysis by the American Water Works Association (AWWA) as well as other standard sources were referred to. All these sources are hereby acknowledged.
Our thanks go to all those persons and institutions that played a role in the compilation of this Manual.

Selected Standard Methods for Water & Waste Water Quality Analysis for Transboundary Samples

CONTENTS

Abbreviations

AQC……Analytical Quality Control
ATC…….Automatic Temperature Compensation
ATU ……Allylthiourea
BOD ……Biochemical Oxygen Demand
COD……Chemical Oxygen Demand),
DEHP……..Di(2-ethylhexyl)phthalate
DO ……..Dissolved Oxygen
EC……Electrical Conductivity
EDTA …….Ethylene Diamine Tetra acetic Acid (
FAO…….Food &Agriculture Organisation
GPS ……Geographical Positioning System)
N…….Nitrogen,
NBI ……Nile Basin Initiative
NTEAP ……Nile Trans boundary Environmental Action Project
NTU…….Nephelometric Turbidity Units
OS……On site (analysis)
P……...Phosphorous
PAH ……Poly Aromatic Hydrocarbons
PCB……..Polychlorinated Biphenyls
SCA……..Standard Committee of Analysts
TDS……..Total Dissolved Solids
TSS…….Total Suspended Solids
UNOPS……United Nations Office for Project Services
µs/cm……...micro seimans/cm

BACKGROUND

The Nile Trans boundary Environmental Action Project (NTEAP) is one of seven projects under the Nile Basin Initiative Shared Vision Programme and is of five years duration. The main objective of the project is to provide a strategic environmental framework for the management if transboundary waters in the Nile Basin.

The basin wide Water Quality Monitoring Components is one of the five components of the NTEAP. This component's objectives include:

iv. Initiate basin-wide dialogue on water quality.
v. Improve capacities for monitoring and management of water quality.
vi. Provide a platform for the exchange and dissemination on information on key parameters.

This manual is one a series of four manuals, which meets these objectives. This manual is a controlled document and amendments can only be authorised by The NBI Water Quality Manager.

The other manuals of are:

- Simple Procedures for Water & Waste Water Sampling for Nile Basin Countries for Transboundary Water Quality Monitoring.

- Guidelines for Data Reporting Forms for Nile Basin Countries for Transboundary Water Quality Monitoring

- Manual for On-Site Tests by Local Communities & Schools for Nile Basin Countries for Transboundary Water Quality Monitoring.

INTRODUCTION

This Laboratory Manual is a selection of analytical methods submitted for monitoring transbounday water along the Nile for the nine member countries. Though each country has their own sampling and analytical national schemes, to ensure consistency on an international transboundary level, it is proposed to submit these methods in a standard format, such that the results can be compared on level platform.

The methods have been divided into up to the nine following sections:

1) **Principle**: A simple explanation of the theory of the method.
2) **Performance**: Characteristics of the method such as the limit of detection, speed of analysis.
3) **Apparatus**: The main equipment required.
4) **Reagents**: The main chemicals and standards required- some methods have analytical quality control standards recommended.
5) **Calibration**: Step by step procedures to calibrate the analysis.
6) **Procedure:** Step by step operations to analyse the samples.
7) **Calculation**: Conversion of measured results to reporting results.
8) **References**: Further detailed information.
9) **Ordering Information**: Suppliers of the reagents.

In certain methods, it was expedient to combine sections 5 & 6. These methods were written to be as brief and simple as possible, and is not envisaged that these will replace the more comprehensive National Standard Methods. The methods are in a consistent format and should enable an analyst who is unfamiliar with the method to easily undertake the analysis. References are included in the methods to provide more in-depth information.

The format has been accepted by accrediting agencies and could be used as a template for internal laboratory manuals for full accreditation.

Application of the Manual

Where the member country's methods fall under the three categories below, then the following italicised actions should be undertaken:

1) No methods are currently being used: - *this manual should prove the most useful and the recommended methods applied.*

2) A different national method is in place but has been validated, with analytical quality controls and with performances similar to those detailed: - *this additional method could be applied, subject to the approval by the NBI Lead Water Quality Specialist, and included in the manual as an alternative method.*

3) The national method is the same, but with a slight variation, for good reason*;- this modified method could be applied, subject to the approval by the NBI Lead Water Quality Specialist, it would be documented and apportioned different reference, issue and file numbers.*

This Manual is an NBI controlled document and no changes can be made without the approval of the NBI Lead Water Quality Specialist, who is responsible for any updated

issues. However, Members may add constructive supplementary information to the methods within their own laboratories to assist their staff but these documents will be regarded as uncontrolled copies.

Laboratory Quality Assurance

To ensure the analytical results are acceptable, the following quality assurance procedures should be undertaken:

iv. The method should be validated at the laboratory to check the performance of the method i.e.: precision, accuracy, and limit of detection of the method. This entails a rigorous testing of the method using a number of different types of samples analysed on at least twelve different occasions.
The performance values included with the methods in this manual are values obtained from reference laboratories and are included for guidance only.

v. Shewhart control charts should be constructed for each chemical and physical parameter. Analytical Quality Control (AQC) samples should be included with the analyses and these results plotted on the control chart.

vi. In addition to the internal quality controls outlined above, the laboratory should participate in external quality control schemes with other laboratories to analyse the distributed replicate samples.

The NBI will submit a full training module on Laboratory Quality Assurance to all member states.

NILE BASIN INITIATIVE	Method No 1
DETERMINATION OF TOTAL DISSOLVED SOLIDS BY GRAVIMETRIC ASSAY	Number of Pages: 2
	Date:1/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: TDSMETH

1. PRINCIPLE

Suspended matter is removed from a measured volume of sample by filtration. The filtrate is evaporated to dryness at dried at 180˚C and then the total dissolved solids determined gravimetrically.

2. PERFORMANCE

Limit of detection……..10 mg/1
Analysis time:…….5 hrs
Interferences:……..bicarbonates & other hygroscopic salts
Results reported ……...……..mg/l to the nearest whole number

3. APPARATUS

Glass fibre filter papers, Whatman GF/C grade (934AH) without organic binder, 70 mm diameter.
Three component Hartley- type filter funnels, 70 mm perforated plate.
Filter flasks.
Suction pump.
Drying oven capable of reaching 180˚C.
250 ml Evaporating dishes.
Desiccator.
100ml glass pipette.
4 decimal place analytical balance.

4. PROCEDURE

4.2) Dry a clean evaporating dish at 180˚C for 1 hour, cool in a desiccator and weigh on the analytical balance to record the weight to 0. l mg 'A'.

4.3) Insert a glass fibre filter paper into the funnel assembly and clip together.

4.4) Using slight suction wash with 100 ml of distilled water.

4.5) Thoroughly mix the sample to be analysed, using a glass pipette filter 100ml (0. 1 litre) of the sample.

4.6) Transfer the filtrate to the evaporating dish and evaporate to dryness at approximately 100˚C in the drying oven. Then increase the temperature to 180˚C and dry to a constant weight.

4.7) Cool in a desiccator and weigh 'B'.

5. CALCULATION

Total Dissolved Solids = **(B – A) X 10 mg/l**

6. REFERENCES

6.1) Methods for the Examination of Waters and Associated Materials (UK).
6.2) American Standard Methods for the Examination of Water & Waste Water –Method 209B.

7 ORDERING INFORMATION

Whatman GF/C grade Filter (934AH) without organic binder, 70 mm diameter.

NILE BASIN INITIATIVE	Method No 2
DETERMINATION OF pH by pH METER	Number of Pages: 3
	Date:3/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: phMet

1 PRINCIPLE

The pH of a solution is defined by the equation pH = -log aH, where aH is the activity of hydrogen ions in the solution expressed in gram-moles. As the hydrogen ion activities cannot be determined experimentally, the pH of a solution is determined by measuring the electromotive force (emf) of a cell containing the test solution and comparing it with the emf of a similar cell containing standard buffer solution. The emf of the cell is measured with a pH meter, which is a high impedance volt meter calibrated directly in pH units.

2 PERFORMANCE

Range of application:……..0-14
Limit of detection:……..Record results to 2 decimal places
Analysis time:……..2 minutes per sample.
Interferences:……...Sodium at pH 10 interferes. Oil grease and suspended matter
……can interfere by blocking the electrode surface.

3 REAGENTS

The buffer solutions should be stored in the dark for up to 2 yrs.

Calibration
Buffer solutions: pH 4.0
(Ready prepared) pH 7.0
pH 9.0

Analytical Quality Control (A.Q.C)
Merck buffer solution: pH 8.0
(Ready prepared)

4 APPARATUS

4.1) pH meter
4.2) 100 ml plastic Beaker

5 CALIBRATION AND PROCEDURE

5.7) Pour out 40mls of pH 7 and pH 4 buffers into the respective beakers.

5.8) On the meter press CAL and then press the 2PT arrow.

5.9) Remove the electrode and ATC probe from the storage water, shake them and very gently wipe with a tissue. Check that the KCl level in the electrode is within l cm of the filling hole and that this hole is free of obstruction.

5.10) Immerse the electrode and probe in the pH 7 buffer and swirl the beaker gently for not more than 10 secs. Press READ.

5.11) When the meter indicates it is ready for the second buffer, follow the same procedure with the pH 4 or 9 buffer.

5.12) Remove from the buffer and rinse the electrode and probe in deionised water and shake the electrode.

5.13) Immerse the electrode and probe in the AQC buffer and swirl and then press READ.

5.14) When the reading stabilises, record the final meter reading.

5.15) Remove and shake the electrode rinsing with deionised water and immerse the electrode and probe in 40 mls sample and record the stabilised reading and repeat this for each sample.

5.16) Finally, store the electrode and probe in the tap water.

6 CLEANING and CONDITIONING

When the AQC result is out of range or the response is slow, the probe and electrode can be both cleaned and conditioned by leaving it in 0. 1M Hydrochloric acid overnight. It should then be washed thoroughly with distilled water and the calibration checked.

7 REFERENCES

7.1) SCA Methods for the Examination of waters and associated Materials: Book 13. The measurement of Electrical Conductivity and the Laboratory Determination of the pH Value of Natural, Treated and Waste Waters 1978; Method B; Page 9 The Laboratory determination of the pH of Natural waters, treated waters, aqueous solutions and effluents by use of an electrode system.
7.2) American Standard Methods for the Examination of Water & Waste Water 16th Edition – Method 209B.

8 ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 3
DETERMINATION OF ELECTRICAL CONDUCTIVITY	Number of Pages: 2
	Date:4/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: ConductivityMET

1 PRINCIPLE

Electrical conductivity is a measure of a solution's ability to conduct electricity. The SI units for conductivity are Siemens/metre (S/m), however it is usually reported in µS/cm.

2 PERFORMANCE

Range of application……..5 - 1500 µS/cm
Relative Standard Deviation…….10%
Limit of detection:…….5 µS/cm
Analysis time:……2 mins per sample
Interferences:…….Suspended or greasy matter may interfere by sealing the electrode system.

3 REAGENTS

Stock Calibration Standard (0.1M KCI)
7.4550g potassium chloride (KCl), dissolved in 1 litre deionised water.
Store in dark for up to 6 months.

Calibration Standard - (1278µS/cm**)**
Dilute 200ml stock calibration standard to 200ml in a volumetric flask.
Store in dark for up to 3 months

Stock Analytical Quality Control (AQC) Standard
Dissolved 11.67g potassium chloride (KCl) in 1 litre deionised water.
Store in dark for up to 6 months.

Analytical Quality Control 400 µS/cm
Dilute 10ml stock AQC standard in a 500ml volumetric flask with deionised water.
Store in dark for up to 3 months.

4 APPARATUS

4.11) Electrical conductivity meter.
4.12) 100 ml Beaker.

5. CALIBRATION

5.8) Calibration should be done daily before use.

5.9) Insert the electrode and Automatic Temperature Compensation (ATC)probe into the calibration standard solution and leave for about 10 minutes to attain a steady reading.

5.10) Adjust the reading to 1278 µS/cm using the control marked CAL.

5.11) Rinse the electrode and ATC probe with deionised water.

6 PROCEDURE

6.1) Pour the AQC/sample into a 100 ml glass beaker and insert the electrode and ATC probe, allow the reading to stabilize before recording the reading.

6.2) AQC standards should be measured at the beginning and end of a batch and at least after every 16 samples.

7 CALCULATION

If the reading on the meter is in S/m multiply by 1000 to give the results in µS/cm. Report to the nearest whole number.

8 REFERENCE

8.1) SCA Methods for the Examination of Waters and Associated Materials: Book 13 The Measurement of Electrical Conductivity and Laboratory Determination of the pH Value of Natural, Treated and Waste Waters 1978.
8.2) American Standard Methods for the Examination of Water & Waste Water 19th Edition pp 4-59 to 4-69.

9 ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 4
DETERMINATION OF TOTAL SUSPENDED SOLIDS BY GRAVIMETRIC ASSAY	Number of Pages: 2
	Date:6/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: TSSMETH

1. PRINCIPLE

Suspended solids are determined by filtering the sample through a weighed glass fibre filter paper and determining the increase in weight of the paper.

2. PERFORMANCE

Limit of detection……2 mg/1 (for 250 ml sample)
Standard deviation……....3 at 50 mg/l & 0.4 at 5mg/l
Speed of Analysis…….For 10 samples: Total analytical time 2.5 hours & total
……..operator time 1 hour
Interferences…….High concentrations of dissolved solids. Oil and grease
Results reported ……...mg/l to nearest whole number

3. REAGENTS

3.1) Petroleum Spirit (40-60ºC Boiling Range)
3.2) 95% Ethanol

4. APPARATUS

Glass fibre filter papers, Whatman GF/C grade, 70 mm diameter.
Three component Hartley- type filter funnels, 70 mm perforated plate.
Filter flasks.
Suction pump.
Oven (105ºC).
Desiccator.
250ml Measuring Cylinder.

5. PROCEDURE

5.1) Ensure the conductivity of the sample is less than 10,000 µS/cm.
5.2) Insert a glass fibre filter paper into the funnel assembly and clip together.
5.3) Using slight suction wash with 100 ml of distilled water. Remove the paper, place on a filter paper of larger diameter. Dry in an oven at 105ºC for one hour.
5.4) Allow the paper to cool in a desiccator for 5 minutes and weigh. Let the weight be B mg.
5.5) Replace the paper in the funnel and moisten with distilled water.

5.6) Shake the sample vigorously and immediately transfer, by means of a suitable measuring cylinder, the volume indicated in table 1 into the funnel. Let the volume used be V ml:

Table 1	
Type of sample	Volume used (V ml)
Clean River	250
Effluents	100
Sewage	25

5.8) Filter under suction, transferring any solids remaining in the measuring cylinder to the funnel with distilled water. Wash the residue with 10 ml of distilled water three times.

5.9) When oil is present in the sample, the filtered suspended solids should be washed first with 30 ml of 95% ethanol and then with 30 ml of petroleum ether.

5.10) Remove the paper, place in an oven on a filter paper of larger diameter and dry at 105ºC for one hour.

5.11) Allow the paper to cool in a desiccator for 5 minutes and weigh.

5.12) Let the weight of the paper plus the solids be A mg.

6. CALCULATION

$$\text{mg total suspended solids/l} = \frac{(A - B) \times 1000}{V}$$

7 REFERENCES

7.1) American Standard Methods for the Examination of Water & Waste Water 16th Edition–Method 209 C.

8. ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 5
DETERMINATION OF NITRATE BY DIRECT ULTRA-VIOLET SPECTROPHOTOMETRY SCREENING METHOD	Number of Pages: 3
	Date: 2/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: NitrateMet

1. PRINCIPLE OF METHOD

The nitrate ion has a strong absorption band in the far ultraviolet range with a peak at 203 nm, and in the absence of other substances which absorb in this region of the spectrum, nitrate can be determined rapidly by direct measurement of its ultra, violet absorbance. A limited correction for absorption due to organic material can be applied by measuring the absorption at 275 nm.

2 PERFORMANCE

Calibration linearity……0 to 2 mgN/1
Criterion of detection……..0.004 mgN/l
Sensitivity……F = 2.2 (1 cm cell)
Standard deviation (Within batch)……...up to 0.084 mg/l
Speed of analysis……..10 samples per hour.
Interferences : The following can be tolerated when using a 40 ml sample: Up to 2,000 mg/1Carbonate or hydroxyl ions (as CaCO3). Up to 6.4 mg/l.Nitrite (as N).

3 REAGENTS

Distilled water.
Sulphuric acid (5% v/v).
Sulphamic acid solution (l%).
Dissolve 5 gm sulphamic acid in 500 ml of 5%V/V sulphuric acid. stable two months.

3.4) Standard nitrate solution (A) (1 ml = 100 µg N).
Dry potassium nitrate by heating in an oven at 1 dissolve 0.7216gm potassium nitrate (Dried analytical grade) in 1 litre distilled water.

3.5) Dilute standard nitrate solution (B) (1 ml =2 µg N). *Pipette 10 ml of standard nitrate solution (A) into a 500 ml graduated flask, dilute to volume with distilled water.*

4 APPARATUS

4.1) UV spectrophotometer capable of measuring at wavelengths down to 200 nm.
4.2) 1 cm silica cells.
4.3) GF/C glass fibre filter papers.

5 CALIBRATION

5.1) Measure out the following volumes into a series of 50 ml graduated flasks, and then make up the volume to 50mls to make the series of standards in the Table 1.

Volume ml	Equivalent to µg Nitrate Nitrogen N
0	0
5	10
10	20
15	30
25	50
40	80

5.1) To each flask add 1. 0 ml, sulphamic acid solution. Dilute to 50 ml with distilled water and mix well.
5.2) Measure the absorbances at 210 nm in a 1 cm silica cell against distilled water in the reference cell.
5.3) Subtract the cell blank reading from these absorbance readings, and plot the corrected readings against the amounts of nitrate nitrogen in micrograms. Draw in the 'best-fit' straight line which pass through the origin.
5.4) Derive the equation µg N = (Absorbance-Blank) x F (Where F is the gradient of graph).
5.5) Once F is derived it can be checked on a regular basis, e.g. monthly.

6 PROCEDURE

6.1) If the sample is turbid or contains suspended matter, filter 80 ml through a GFC paper. Reject the first 20 ml of filtrate.
6.2) Pipette the volume of sample indicated below into a 50 ml graduated flask. [V ml].

Expected NO concentration (mg/1 N)	Volume of sample (ml) [V]
0-2	40
2-8	10
8-16	5
16-40	2

6.1) Add 1.0 ml of mixed reagent.

6.2) Dilute to 50 ml with distilled water, and mix well.

6.3) Prepare a blank solution by treating 40 ml of distilled water as described under 6.3 to 6.4.

6.4) Measure the absorbance of the sample and blank solutions on a spectrophotometer against distilled water in a 1 cm cell at wavelengths of 210 and 275 nm respectively. Let the absorbance of the sample and blank solutions be A_{s210} nm & $A_{b210.}$ respectively.

6.5) Similarly let the absorbances at 275 nm be A_{s275} and A_{b275} respectively.

7 CALCULATION

$$\text{Nitrate Concentration (mg/l N)} = \frac{[A_{s210} - A_{b210} - (C)A_{s275} + A_{b275}]}{V} \times F$$

(C) = Correction Factor = 3 for most waters but this can be confirmed or modified by comparing with another method e.g. Method 6.

Note: if the absorbance at 275 nm exceeds 10% of the absorbance at 210 nm, then the method is no longer applicable.

8. REFERENCES

8.1) American Standard Methods for the Examination of Water & Waste Water –Method 418A.
8.2) Yorkshire Water Authority (UK) Method 130-02.

9 ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 6
DETERMINATION OF NITRATE COLORIMETRICALLY	Number of Pages: 3
	Date:19/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: NITRATECOLMET

1 PRINCIPLE

Nitrate (NO_3^-) is to Nitrite (NO_2^-) in the presence of Cadmium. The nitrite produced is then determined by reacting it with sulphanilamide and N-(1-naphthylethylenediamine dihydrochloride) to form a highly coloured azo dye that is measured colorimetrically. A correction is made for any NO_2^- present in the sample by analysing without the reduction step.

2 PERFORMANCE

Interferences…….Suspended matter in the column will restrict sample flow.
……..Concentrations of Fe, Cu, or other metals above several
……..mg/l lowers the reduction efficiency. Add Ethylene
…….Diamine Tetra acetic Acid (EDTA) to samples to
……..eliminate this interference.
…… Residual chlorine can interfere by oxidising the Cd
…….column reducing its efficiency. Remove this using
…….Sodium thiosulphate($Na_2S_2O_3$).

3 REAGENTS

Copper-Cd granules.
Wash 25g of Cd granules with 6N HCl and rinse with distilled water. Swirl the Cd with 100ml 2% $CuSO_4$ for 5 minutes until the blue colour partially fades. Decant and repeat with fresh $CuSO_4$ solution until a brown colloidal precipitate begins to develop. Gently flush with distilled water to remove all the brown particles.

Colour reagent
To 800 ml of distilled water add 100 ml 85% phosphoric acid and 10g of sulphanilamide. After dissolving the sulphanilamide add 1.0 of N-(1-naphthylethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1litre with distilled water in a volumetric flask. Store in dark bottle in a refrigerator. Store up to 1 month.

Ammonium chloride-EDTA solution:
Dissolve 13g NH_4Cl and 1.7g EDTA-Na salt in 900ml water. Adjust to pH 8.5 with conc. NH_4OH and dilute to 1 litre. Store in refrigerator for up to 1 month.

Dilute ammonium chloride-EDTA solution;
Dilute 300ml NH_4Cl-EDTA solution to 500ml with distilled water.

Hydrochloric acid 6N
Gradually Add 250ml of Conc. Hydrochloric conc. to 250ml distilled water.

2% Copper Sulphate solution
Dissolve 20g $CuSO_4.5H_2O$ in 500ml distilled water and dilute to 1litre.

Calibration Solutions

Stock nitrate solution 100mg/l
Dry KNO_3 in an oven at 105^oC for 24hr.Dissolve 0.7218g in distilled water and dilute to 1000ml. (Store up to 6 months in dark in refrigerator)

Intermediate 10 mg/l nitrate solution
Dilute 100ml of stock to 1000ml. Preserve with $CHCl_3$. Store up to 1 month in dark in refrigerator.

Stock 100mg/l nitrite solution.
Dissolve 0.4922g $NaNO_2$ previously dried at 105^oC for at least 2 hours in distilled water and dilute to 1000ml. Store up to 1 month in dark in refrigerator.

Intermediate 10mg/l nitrite solution
Dilute 25ml of the stock nitrite solution to 250ml in a volumetric flask.

Working solution 1mg/l
Dilute 10ml of the intermediate nitrate to 1000ml.

AQC 0.54mg/l Nitrate
Dilute 5.4ml of the intermediate nitrate standard (10mg/l) to 100ml.

Working Calibration Standards
Using the intermediate nitrate standard solution 10mg/l prepare standards 0.05, 0.1, 0.2, 0.5, 1.0mg/l NO_3^- by diluting 0.5, 1, 2, 5 and 10ml to 100ml in a volumetric flask.

4 APPARATUS

4.1 Reduction Column.
Use a 100ml volumetric pipette from which the top has been removed or equilalent (10cm long bulb,3cm ID to 25cm long column with 3.5mm I.D.)

4.2 Visible Spectrophotometer

5 PROCEDURE

5.1 Preparation of Reduction Column

5.1.4) Insert a glass wool plug into the bottom of the reduction column and fill with water. Add Cd granules to produce a column 18.5cm long.
5.1.4) Maintain the water level above Cu-Cd granules to prevent entrapment of air.
5.1.4) Wash the column with 200ml dilute NH_4Cl-EDTA solution.
5.1.4) Activate the column by passing through it at 10ml/min of a solution composed of 25% 1.0 mg NO_3/l standard and 75% NH_4Cl-EDTA standard solution.

5.2 Sample Analysis

5.2.4) Adjust pH of the sample to between 7 and 9 using a pH meter and dilute HCl or NaOH.
5.2.4) To 25ml of sample or sample diluted to 25ml, add 75ml NH_4Cl-EDTA solution and mix.Pour the mixed sample into the column and collect at the rate of 7 to 10 ml/min. Discard the first 25ml. Collect the rest in original sample flask.
5.2.4) When all samples have been eluted pour dilute NH_4Cl-EDTA solution on top and let it pass through the system. Store Cu-Cd column in this solution.
5.2.4) As soon as possible, and not more than 15minutes after reduction, add 2.0ml colour reagent to 50ml of sample and mix.
5.2.4) Between 10 minutes and 2hrs afterwards measure the absorbance at 543nm.
5.2.4) Carry out reduction of standards as for samples.
5.2.4) Compare at least one NO_2^- standard to a reduced NO_3^- standard at the same concentration to verify reduction column efficiency.

6 CALCULATION

Obtain a standard curve by plotting absorbance of standards against NO_3^- concentration. Compute sample concentration directly from standard curve. Subtract NO_2^- separately determined.

7 REFERENCES

7.1) Cadmium reduction method for the Determination of N-NO3- in Standard method for the Examination of waters and waste waters 19th Edition 1995 p4-87.
7.2). Oxidised Nitrogen in Waters 1981 in HMSO Methods for the Examination of Waters and Associated Materials p.31.

ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 7
DETERMINATION OF ORTHO-PHOSPATE BY COLORIMETRIC METHOD	Number of Pages: 3
	Date:
	Author:
	Authorised by:
	Issue No: 1
	File Name: PHOSPHATEMETH

1 PRINCIPLE

Ortho-phosphate reacts with ammonium molybdate to form molybdo-phosphoric acid. This is reduced by ascorbic acid to the intensely coloured complex known as molybdenum blue.

4 PERFORMCE

Calibration linearity…….0 to 15 µg P
Limit of detection ……0.01 mg/l P
Sensitivity ……..F = 79.5 (1 cm cells)
Blank value……0.005 absorption units (4cm cell)
Standard deviation……. 0.005 mg,/1 P at the 1,00 mg/1 P
Speed of Analysis ……..For 10 samples, the operator time is 30 minutes and
…… total analytical time is 40 minutes.
Interferences……..……Arsenates react with the molybdate reagent to produce a blue
……...Colour similar to that formed with phosphate. Concentrations
……as low as 0.10 mg/1 arsenic interfere. Chromium and nitrite.give
……results about 3% lower at concentrations of 1.0 mg/1and 10-15%
……...lower at concentrations of 10 mg/l.

3. REAGENTS

3.1 Ammonium molybdate tetrahydrate solution.
Dissolve 40.0 g of ammonium molybdate tetrahyarate in about 800 ml of distilled water, warming slightly if necessary, then make up to 1 litre with distilled water.

3.2 Sulphuric acid, ~ 5N.
Add carefully with mixing 140 ml sulphuric acid, SG 1.84, to water, cool and make up to 1 litre with distilled water.

3.3 Potassium antimonyl tartrate solution.
Dissolve 2.7g potassium antimonyl tartrate in about 800 ml of distilled water. Then make up to 1 litre with distilled water.

3.4 Reducing agent.
Mix together 600 ml of distilled water, 1000 ml of 5N sulphuric acid, 300 ml of ammonium molybdate solution and 100 ml of potassium antimonyl tartrate solution and mix well. This solution is stable. Immediately before use add 1.3 g of ascorbic acid to 250 ml of this solution and shake to dissolve, this solution is stable one day only.

3.5 Modified reducing agent for colour and/or turbidity blank.
For the correction for colour and turbidity, omit the ascorbic acid and the 100 ml potassium antimonyl tartrate solution, adding instead 100 ml water to maintain the correct concentrations of sulphuric acid and ammonium molybdate.

3.6) Standard Phosphate (Stock) Solution (1 ml = 1000 µg P). *Dissolve 4.390 g of potassium dihydrogen phosphate, KH 2P0 49 (dried at 110 OC) in about 100 ml of distilled water. Transfer to a 1 litre graduated flask and dilute to volume with distilled water. Mix well.*
3.7) Standard Phosphate (Working) Solution (1.0 ml = 1.0 pg P*) Pipette 10.0 ml of standard phosphate (stock) solution into a 100 ml graduated flask and dilute to volume with distilled water. Mix well. Pipette 10.0 ml of this dilution into a 1 litre graduated flask and dilute to volume with distilled water. Mix well. This solution is not very stable and should be freshly prepared as required.*

4. APPARATUS

UV/Visible Spectrophotometer with 1 & 4 cm cells.
Volumetric Flasks that should not be cleaned with detergents containing phosphate.

5. CALIBRATION PROCEDURE

4.0 Into a series of 50 ml graduated flasks measure (by means of a burette) the volumes of standard phosphate (working) solution shown in the table below:

Volume of Standard (Working) ml	Wt of P µg	Measure in cell (cm)
0	Blank	1 & 4
2	2	4
4	4	4
6	6	1
8	8	1
10	10	1
12	12	1
14	14	1

5.2 Continue as under Procedure 6.2 to 6.6 omitting 6.5.

5.3 Subtract the absorption of the blank solution from the absorptions obtained for all the other standards. For each cell size plot the corrected absorptions against the concentration of phosphate. Draw in the 'best-fit' straight lines, which pass through the origin.

5.4 For each cell derive the equation:

µg P = (Absorption - Blank) x F

(Where F = Gradient of graph)

6. PROCEDURE

6.1) Pipette the volume of sample indicated below into a 50 ml graduated flask. Let the volume of sample taken be V ml. Add distilled water (if necessary) to give a total volume of 30 to 40 ml.

EXPECTED PO4 CONCENTRATION (mg/l)	VOLUME OF SAMPLE ml
<0.5	40
0.5-1.5	10
1.5-3.0	5
>3.0	1

6.4) Add 8.00 0.02 ml of reducing agent. Mix well.
6.4) Dilute to volume with distilled water. Mix well.
6.4) Allow to react for between 10 and 15 minutes.
6.4) Prepare a blank solution by treating 40 ml of distilled water as described under 6.3 to 6.4.
6.4) Measure the absorption of the sample and blank solutions on a spectrophotometer against Water in a suitable size cell, at a wavelength of 870 nm. Let the absorbance of the sample solution be As and that of the blank solution Ab.
6.4) If it is thought that a significant error may be introduced by colour or turbidity in the sample, a duplicate sample should be treated exactly as under 6.1 to 6.6 omitting 6.5 and using the modified reducing agent. Let the absorbance obtained be Ac.

7. CALCULATION

$$\text{Ortho.phosphate content} = \frac{(As-Ab) \times F}{V} \text{ mg/l P}$$

Or if colour/turbidity correction is used:

$$\text{Orthophosphate content} = \frac{(As-Ab-Ac) \times F}{V} \text{ mg/l P}$$

8. REFERENCES

8.1) Determination of phosphorous using the Ascorbic Acid method in American Standard Method for the Examination of Waters and Waste Waters 19th Edition 1995 p4-111 4500-PE.
8.2) Phosphorous in Waters, Effluents & Sewages 1980 in Methods for the Examination of Waters and Associated materials HMSO. P-5.

9. ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 8
DETERMINATION OF DISSOLVED OXYGEN IODIMETRIC METHOD	Number of Pages: 2
	Date:12/9/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: DOMETH2

1. PRINCIPLE OF METHOD

This is an iodimetric titrimetric procedure based on the original work of Winkler (9.1). Managanous hydroxide is precipitated in a closed bottle and the dissolved oxygen combines with this to form higher hydroxides. When this is acidified in the presence of iodide, iodine is liberated in an amount chemically equivalent to the original dissolved content in the sample. The iodine is determined by titration with a standard of sodium thiosulphate solution.

2. PERFORMANCE

Limit of detection……..0.08 to 0.46 mg/1
Standard deviation ……0.04 to 0.07 mg/l
Speed of analysis……...10 minutes operator time for one sample
Interferences……..Oxidising or reducing agents, e.g. ferric and ferrous salts,
……...residual chlorine, sulphites, sulphides, thiourea, nitrite & chromate.

3. REAGENTS

3.1 Manganous sulphate reagent
Dissolve 500g of manganous sulphate (4H20) in water and dilute to 1000ml.

3.2 Alsterber reagent
Dissolve 500ml sodium hydroxide in 500 ml of water and cool. Dissolve 135g sodium iodide (or 150 g potassium iodide) in 100 ml water. Dissolve 2.00g sodium azide in 40 ml water mix the three solutions together, when all are at room temperature, dilute to 1000 ml in a stoppered measuring cylinder.

3.3 Sulphuric acid, concentrated (S.G. 1.84)

3.4 Potassium Iodate solution (0.0042M)
Dissolve 0.8920 g of potassium iodate (dried for one hour at 110ºC) in distilled water and dilute to 1000 ml in a graduated flask.

3.5 Stock Sodium Thiosulphate Solution (~0.25M)
Dissolve 62.5g of Sodium thiosulphate (5H2O) in distilled water and dilute 1000ml in a graduated flask.

3.6 Working Standard Sodium Thiosulphate Solution
Dilute 50 ml of Stock Sodium thiosulphate Solution (~0.25M) with distilled to 1000ml. Add 1ml of chloroform as preservative. This solution should b standardised daily as detailed below.
3.7 Starch indicator

Dissolve 2g of laboratory grade soluble starch and 0.2g salicylic acid (preservative) in 100ml hot distilled water.

4. HAZARDS

Sodium azide solutions evolve hydrazoic acid when rendered acid in this method. Prolonged exposure to atmospheres containing more than 1 mg/l of hydrazoic acid is hazardous and good ventilation is therefore essential. Azide wastes may react with metal sink waste pipes to produce explosive compounds and hence plastic waste pipes are recommended.

5. APPARATUS

4.1 Glass 250 ml bottles with well fitting glass or plastic stoppers.

6. CALIBRATION

It is necessary to standardise the 0.0125 M sodium thiosulphate solution as follows:

6.4) Dissolve 2.0 g potassium iodide (iodine free) in 100 ml of water in a conical flask.
6.4) Add 2.0 ml of conc.sulphuric acid, swirl, and then add 20.00 ml of potassium iodate solution.
6.4) Dilute with water to 200 ml and titrate with the sodium thiosuphate solution Add 2ml starch indicator towards the end of the titration.
6.4) The sodium thiosulphate should be adjusted to exactly 0.0125 M.
Then 1.00 ml ≡ 0.100 mg dissolved oxygen.

7. PROCEDURE

7.4) Carefully remove the stopper from the bottle containing the sample. Add 2.0 ml of manganous sulphate solution followed by 2.0 ml of Alsterbergs reagent, making both additions below the surface. Replace the stopper and mix thoroughly by inversion.
7.4) Allow the precipitate to settle to the lower third of the bottle and repeat the mixing. Then allow the precipitate to settle completely.
7.4) Remove stopper and add 2.0 ml of conc. sulphuric acid. Replace stopper and mix thoroughly, the precipitate should then dissolve.
7.4) Using a measuring cylinder, transfer 100ml of the solution into a conical flask. Titrate with 0.0125 M sodium thiosulphate solution to a pale straw colour. Add 2ml starch indicator towards the end of the titration. Continue to titrate until a pale straw yellow colour is reached. Let T ml be the volume of titrant used.

8. CALCULATION

Dissolved oxygen (DO) = T mg/l

9. REFERENCES

9.1) Winkler LW., Ber Deutsch. Chem. Ges 21 2843, 1888.
9.2) American Standard Methods for the Examination of Water & Waste Water 19th Edition pp 4-59-4-69
9.3) Dissolved Oxygen in Natural and Waste Waters 1979. Methods for the Examination of Waters and Associated Materials. HMSO series.

10. ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 9
DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND	Number of Pages: 3
	Date:25.7.06
	Author:
	Authorised by:
	Issue No: 1
	File Name: BODMET

DETERMINATION OF BIOCEMCAL OXYGEN (ATU)

1. PRINCIPLE

BOD is an empirical test in which standardised laboratory procedures are used to determine the relative oxygen requirements of a sample. The test measures the oxygen required for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidise inorganic material such as sulphides and ferrous iron. Allyl-thiourea is added to prevent oxygen being consumed in the oxidation of reduced forms of nitrogen (nitrogenous demand).

The method consists of placing a sample in a full, air-tight bottle and incubating the bottle under specified conditions for five days. Dissolved oxygen (D.O.) is measured initially and after incubation. The difference in DO is the oxygen used and from it the BOD can be computed.

2. PERFORMANCE

Range……1 to 7 mg/l
Sensitivity……..not applicable
Blank value……less than 0.5 mg/l
Speed of analysisfor 10 samples……Total analytical time= 5 days
……Total operator time = 2 hours
Interferences…… Heavy metals, bacteriocides, polythionates, & herbicides

3. REAGENTS

3.1) Phosphate Buffer Solution
Dissolve 42.5 g of potassium dihydrogen phosphate in 700 ml of water. Add 8.8 g of sodium hydroxide and dissolve. Dilute to 1000 ml in a stoppered measuring cylinder and mix well. The pH of the solution should be 7.20 adjust if necessary, and then add 2.0 g of ammonium sulphate, dissolve and mix well.

3.2) Magnesium sulphate solution.
Dissolve 25.0g of magnesium sulphate 1000 ml of distilled water.

3.3) Ferric chloride solution.
Dissolve 0.125g of ferric chloride ($6H_2O$) in 1000 ml of distilled water.

3.4) Calcium chloride solution.
Dissolve 27.5g of calcium chloride (2H 0) in 1000 ml of distilled water.

3.5) Dilution water.
Add 1.0 ml of reagents 3.3, 3.49 3.2 & 3.1,in that order,to each litre of distilled water.The water should be adjusted to 20.0º C. Containers must be kept clean by periodic cleaning with chromic acid. The water should be 'seeded' before use by addition of 5.0 ml per litre of sewage effluent obtained from a settling tank following aerobic biological purification, after settlement in the laboratory.

Allylthiourea solution (ATU).
Dissolve 0.50 g of allylthiourea to 1000 ml in a graduated flask.

4. APPARATUS

4.1) Incubation bottles: 250 ml glass bottles with ground glass stoppers.
4.2) Air incubator or water bath, thermostatically controlled at 20ºC. Exclude all light to prevent formation of DO by algae in the sample.

6. PROCEDURE

6.1 For samples which require no dilution, warm to 20ºC before proceeding. Clean rivers may be supersaturated and therefore excess oxygen must be allowed to escape before BOD analysis.

6.2 Samples which require dilution should be diluted so that dilutions result in a residual DO of at least 2 mg/1 and a DO depletion of at least 2 mg/1 after 5 days, in order to produce the most reliable results.

6.3 If Nitrification inhibition is required, then add 1.0ml of allylthiourea solution to 1000m1 of sample, or diluted sample, in a 1000 ml stoppered measuring cylinder, Mix the contents of the cylinder thoroughly, but without violent agitation and carrry on with the next step, but record the results as BOD(ATU).

6.4 Dispense the water sample into two BOD incubation bottles, filling them until they overflow. Allow the bottles to stand a few minutes, and then gently tap them a few times to remove air bubbles. Carefully introduce a stopper to each bottle without trapping any air bubbles.

6.5 Determine the D.O. of the contents of one bottle by method no. 8. Let this be X mg/l.

6.6 Place the other bottle in the incubator for 5 days at 20ºC. On removal determine the D.O. of the contents (as in 6.5).Let this be Y mg/l.

6.7 A blank determination should be carried out with each batch using seeded dilution water, For this determination the value of X-Y should not exceed 0.5mg/l.

4. CALCULATION

The BOD of a sample is calculated as follows:

When no Dilution Water is required: BOD mg/l = X- Y

When Dilution Water is added …….BOD mg/l = (X-Y) x D
(Where D= Dilution Factor)

When Dilution Water is seeded……..BOD mg/l = [(X-Y) – (S1- S2)F] D
(Where S1= DO mg./l before incubation
S2= DO mg/l after incubation)
……F= ratio of seed in sample to seed in control = (% seed in X/ % seed in S1)

4 REFERENCES

8.0) American Standard Methods for the Examination of Water & Waste Water 19th Edition pp 5-2.
8.0) Yorkshire Water Authority (UK) Method 0502
8.0) 5 - day Biochemical Oxygen Demand (BOD) in HMSO series of Methods for the Examination of Waters and Associated Materials, Second Edition, 1988.

9 ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 10
DETERMINATION OF CHEMICAL OXYGEN DEMAND SCA METHOD	Number of Pages: 2
	Date:14/9/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: CODMETH

1 PRINCIPLE

The Chemical Oxygen Demand (COD) is used as a measure of the oxygen equivalent to the organic matter content of a sample that is susceptible to oxidation by strong chemical oxidant.
The dichromate reflux method applies the oxidation of organic matter by a boiling mixture of chromic and sulphuric acids. The sample is refluxed in a strongly acid solution with potassium dichromate in the presence of silver sulphate as a catalyst. Mercuric sulphate is added to complex halides and eliminate their interference. The oxygen equivalent of the oxidisable organic matter is determined titrimetrically with ferrous ammonium sulphate.

2. PERFORMANCE

2.1 Range…….0 to 400 mg/l 0
2.2 Limit of detection……..3 to 8 mg/l
2.3 Sensitivity……..1 ml of titrant = 20 mg/l
2.4 Standard deviation ….4 to 7.0 mg/l
2.6 Speed of analysis……For 10 samples the operator
2.7 Interferences……Pyridine related compounds resist oxidation,Volatile straight ……...chain aliphatic compounds are oxidised only to the extent that ……they remainin contact with the oxidant. Halides interfere but they can ……be overcome using mercuric sulphate.

3. REAGENTS

3.1 Standard potassium dichromate solution (M/48)
Dissolve 6.129g of potassium dichromate (dried 140 - 150 ºC) in about 200 ml of distilled waters dilute to 1000ml in graduated flask.

3.2 Ferrous ammonium sulphate (0.025M)
Dissolve 9.8 g of ferrous ammonium sulphate hexahydrate in about 100 ml of distilled water, cautiously add 20.0 ml of sulphuric acid (S.,g. 1.84). Cool and dilute to volume in a one litre graduated flask.

3.3 Ferroin indicator solution
Dissolve 3.5g of ferrous sulphate heptahydrate in 50 ml of distilled water. Add 7.4 g of 1:10 phenanthroline monohydrate and shake until dissolved.

3.4 Silver sulphate solution (l% m/v)
Dissolve 10.0 g of silver sulphate in 1000 of sulphuric acid (s.g. 1.84).
3.5 Mercuric sulphate solution (20% m/v)

Prepare by adding cautiously, with swirling, 50ml sulphuric acid (s.g. 1.84) to 500 ml of water. Dissolve 100g of laboratory grade mercuric sulphate in the diluted sulphuric acid.

4. APPARATUS

4.1 150 ml boiling flasks fitted with water cooled condensers.
4.2 Pre-digested anti-bumping granules.
4.3 Means of heating to 160-170ºC. Either an electrical heating mantle or a heated sand tray. Point sources of heating are considered unsatisfactory.

5. CALIBRATION PROCEDURE

5.1 Pipette 5.00 ml of the potassium dichromate solution into 250 ml conical flask.
5.2 Add 55 ml of distilled water.
5.3 Add 15 ml of sulphuric acid (s.g.1.84) and cool.
5.4 Add two drops of ferroin indicator solution and titrate with ferrous ammonium sulphate solution until the faint blue colour changes to red. Let the titre obtained be S ml.
5.5 The molarity of the ferrous ammonium sulphate is given by: $\frac{0.625}{S}$

6. PROCEDURE

Safety Points: The method involves the handling of boiling and strong solutions of sulphuric acid and dichromate. Protective clothing, gloves and full face protection are essential.

6.1 Insert a few anti-bumping granules into the 150ml flask.
6.2 Pipette 10 ml of sample into the flask.
6.3 Add 1.0 ml of the 20% m/v mercuric sulphate solution and swirl to mix.
6.4 Add 5.00 ml of the potassium dichromate solution.
6.5 Add 15.0 ml of the 1% m/v silver sulphate solution.
6.6 Fit the condenser and swirl the flask and its contents. Then boil gently under reflux for 120 minutes.
6.7 Remove the flask from the source of heat and allow to cool for approximately 10 minutes. Add 25 ml of water via the condenser. Disconnect the flask from the condenser and cool the flask to room temperature in running water.
6.8 Add 2 drops of ferroin indicator and mix well. Titrate with ferrous ammonium sulphate until the blue colour changes to red. Let the titre be T ml.
6.9 Carry out a blank determination by proceeding as under 6.1 to 6.8 but using 10.00 ml of distilled water instead of sample in 6.2.
Let the blank titre be B ml.

7. CALCULATION

Chemical oxygen demand = 800 M (B-T)

8. RFERENCES

8.1 Methods for the Examination of Waters & Associated Materials HMSO 1977.

9. ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 11
FLAME ATOMIC ABSORPTION SPECTROMETRY INSTRUMENT SET UP	Number of Pages: 2
	Date:31/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: AASFlameMET

1. Insert Burner Head

2. Switch on compressor and extraction fan

3. Switch on AA spectrophotometer

4. Turn on acetylene supply at cylinder. If the acetylene pressure in the cylinder is less than 80 psi, the cylinder should be replaced.

5. Turn on air supply and acetylene supply in laboratory

6. Plug in lamp, turn SIGNAL switch to "LAMP" and set lamp current.

7. Leave the lamp to warm up for 1/2hour

8. Set slit the width

9. Turn SIGNAL switch to "set up"

10. Set wavelength using the Coarse & Fine adjustment to produce maximum energy.

13. Adjust the two alignment knobs on the lamp holder to maximise energy.

14. Re-check the wavelength is finely adjusted

15. Set the SIGNAL control to "Abs"

16. Lower the burner assembly using the burner vertical adjustment knob (right) Press AZ

17. Raise the burner until the display starts indicating absorbence (the burner head is starting to interfere with the beam). Then, lower the burner again slightly so that no absorbence is indicated.

18. Turn on air supply at instrument and set appropriate flow rate.

19. Turn on fuel supply at instrument.

20. Ignite flame.

23. Press Autozero (AZ).

RUNNING SAMPLES

1. Aspirate the top calibration standard solution. Adjust the horizontal and rotational burner adjustment knobs until the reading in the READOUT display is maximum. Note the reading and record it as the sensitivity result.

2. Aspirate blank Turn SIGNAL switch to "Conc" and MODE switch to "Hold"

3. Using the numerical keyboard:
Enter standard 1 concentration.
Enter standard 2 concentration.
Enter standard 3 concentration if required.
Enter reslope standard concentration.
Enter number of readings to be averaged.
Enter the measurement interval in seconds.

5 . Aspirate a blank solution and zero the display by pressing the AZ key

6. Aspirate a standards to calibrate.

7. Aspirate samples recording the stable results adding the AQC standards after every 10 samples.

8. When analysis is complete Aspirate the blank solution to rinse out nebuliser and burner assembly Then aspirate air, turn off acetylene, then compressed air at the instrument

ROUTINE MAINTENANCE

WEEKLY

1. Clean burner head by removing and placing it in a 5 % Decon 90 solution in the ultrasonic bath for approximately 10 mins, then rinsing thoroughly with deionised water and baking for 1 hour at 180 ºC to remove all moisture.

2. Clean windows and lamps with methylated spirits.

3. Do a visual inspection of the water trap for algae. If some algae is found, the water trap should be cleaned with a mild bleach solution, the rinsed thoroughly.

MONTHLY

1. Clean the nebulizer by placing it in a 50% alcohol solution in the ultrasonic bath for approximately 10 mins, then rinsing thoroughly with deionised water.When replacing the nebulizer, it will be necessary to re-optimize the impact bead.

2. The blank beaker should be decontaminated by soaking overnight in a 5 % solution of Decon 90.

NILE BASIN INITIATIVE	Method No 12
DETERMINATION OF COPPER BY FLAME ATOMIC ABSORPTION SPECTROMETRY	Number of Pages: 2
	Date:1/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: CuMet2

1) PRINCIPLE

Copper in raw and potable waters is detertnined directly in acidified samples by atomic absorption spectrophotometry by aspirating directly into an air/acetylene flame.

2) SAMPLE PREPARATION

On receipt the samples are acidified with 10ml of 50% v/v Nitric acid to 1000ml of sample.

3) PERFORMANCE

Range of application:	5.0 - 1000 µg/1
Limit of detection:	5.0 µg/1
Sensitivity:	1000 µg/1 ≈ 0. 10 absorbance
WHO GV:	3000 µg/1
Analysis time:	36 in 30 mins
Interferences:	none
Results	µg Cu/1 (whole numbers)

4) STANDARDS

4.1) 50% v/v Nitric acid.

4.2) STOCK CALIBRATION STANDARD 1000 mg/1

Copper standard solution 1000 mg/1. Store in dark for up to 2 years.

4.3) CALIBRATION STANDARD – 1: 200 µg/1

Dilute 0. lml stock calibration standard and 5ml of 50% nitric acid to 500ml in deionised water,
Store for up to 1 week.

4.4) CALIBRATION STANDARD -2 (1000 µg/1)

Dilute 0.5ml stock calibration standard and 5ml of 50% nitric acid to 500ml in deionised water. Store for up to 1 week.

4.5) BLANK

To 500ml deionised water add 5ml of 50% nitric acid.

4.6) STOCK ANALYTICAL QUALITY CONTROL STANDARD (1000 mg/l)

Copper standard solution 1000 mg/1. Store in dark for up to 2 years.

4.7) LOW STOCK ANALYTICAL QUALITY CONTROL STANDARD 50 µg/1

Dilute 0.025ml stock control standard plus 5ml of 50% nitric acid to 500ml in a volumetric flask with deionised water. Store for up to 1 week.

4.8) HIGH ANALYTICAL QUALITY CONTROL CONTROL STANDARD 200 µg/1. *Dilute 0.05ml stock control standard plus 2.5ml of 50% nitric acid to 250ml in a volumetric flask with deionised water.Store for up to 1 week.*

5) APPARATUS

5.1 Atomic Absorption Spectrophotometer (Flame)(AAS)
5.2 Volumetric Flasks

6) PROCEDURE

Follow Instructions for Instrument Setup Flame absorption method No. 10.

The parameters required for copper are as follows:

Gases:	Air/Acetylene
Air flow rate:	40 on scale
Acetylene flow rate:	10 on scale
Burner size:	long
Slit Width:	0.7 mm
Lamp:	Cu
Lamp current:	9 mA
Wavelength:	324.8 nm
Calibration standard 1:	200 µg/1
Calibration standard 2:	1000 µg/1
Reslope standard:	1000 µg/1
Low AQC standard:	50 µg/1
High AQC standard:	200 µg/1
Number of reading to be averaged:	3
Measurement intervals:	4 seconds

7 REFERENCES

7.1) Methods for the Examination of Waters and Associated Materials: Copper in potable waters by atomic absorption spectrophotometry 1980.
7.2) Perkin Elmer Manual 2380 AAS: Recommended conditions.

8 ORDERING INFORMATION

Copper standard solution 14139 2N (Spectrosol)	1000 mg/1	100ml	Merck
Copper standard solution 20,707-1	1000 mg/1	100ml	Aldrich
Nitric Acid (Analar)	2500mI	Merck	

NILE BASIN INITIATIVE	Method No 13
DETERMINATION OF CADMIUM BY FURNACE ATOMIC ABSORPTION SPECTROMETRY	Number of Pages: 2
	Date:8/8/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: Cadmiumfurnace.met

1) PRINCIPLE

Cadmium in raw and potable water is determined directly in acidified samples by electrothermal atomization atomic absorption spectrophotometry.
A lanthanum salt is added to the samples in order to minimize the negative bias due to suppressive interference effects.

2) SAMPLE PREPARATION

On receipt the samples are acidified with 10ml of 50% v/v Nitric acid to 1000ml of sample.

3) PERFORMANCE

Range of application:	0.5 - 6µg/1
Limit of detection:	0.5µg/1
WHO GV:	5 µg/1
Analysis time:	10/hour
Interferences:	None
Results	Reported in µgCd/1 to one decimal place

4) REAGENTS/STANDARDS

4.1) 50% v/v NITRIC ACID (Analar)

4.2) MATRIX MODIFIER - 0. 6 % LaNO3 / 0.5% HNO3
To a 100ml Volumetric flask add approximately 50ml deionised water.
Dissolve 0.8g La(N03)3.6H20. Add lml 50% v/v HNO3.
Make up to the mark with deionised water. Store in dark for up to 6 months.

4.3) STOCK CALIBRATION STANDARD
Merck Standard solution - 1000mg/1 Cadmium
Store in dark for up to 2 years.

4.4) WORKING STOCK CALIBRATION STANDARD (1mg/l)
To a 500ml Volumetric flask add approximately 400mi deionised water.
Add 0.5ml of stock calibration standard. Make up to the mark with deionised water.
Then add 5ml 50% nitric acid. Store in dark for up to 1 week.

4.5) CALIBRATION STANDARD (6µg/1)
To a 200mI Volumetric flask add approximately 150mI deionised water.
Add 1.2ml of stock standard. Make up to the mark with deionised water.Add 2ml 50% nitric acid. Store in dark for up to 1 week.

4.6) ANALYTICAL QUALITY CONTROL (AQC) STOCK STANDARD
Aldrich Standard solution - 1000mg/1 Cadmium. Store in dark for up to 2 years.

4.7) WORKING (AQC) STOCK CONTROL STANDARD (lmg/l)
To a 500 ml Volumetric flask add approximately 400ml deionised water.
Add 0.5ml of stock control standard. Make up to the mark with deionised water.
Then add 5ml 50% nitric acid. Store in dark for up to 1 week.

4.8) LOW AQC (1µg/l)
To a 200ml Volumetric flask add approximately 150ml deionised water.
Add 0.2ml of stock standard. Make up to the mark with deionised water.
Then add 2ml 50% nitric acid. Store in dark for up to 1 week.

4.9) HIGH AQC
To a 200ml Volumetric flask add approximately 150ml deionised water.
Add 1.0ml of stock standard. Make up to the mark with deionised water.
Then add 2ml 50% nitric acid. Store in dark for up to 1 week.

5) APPARATUS

Atomic Absorption Spectrophotometer (Furnace)(AAS)
Volumetric Flasks

6) PROCEDURE

1 . Follow the Manufacturer's Instrument Start up' Instructions for Furnace.

The parameters required for Cadmium are as follows:-

Matrix modifier -2 µl 0.6%LaNO3/0.5%HNO3 per 30 µl sample.

Pretemperature-	400ºC
Atomization temperature-	1800ºC
Slit width -	0.7 nm
Lamp current -	6mA
Wavelength -	228.8
Standard 1 -	6 µg/1
Standard 2 -	4 µg/1
Standard 3 -	2 µg/1
Low AQC -	1 µg/1
Standard AQC -	5 µg/1
Read delay on A/S-	0.0 sec
Read time on A/S-	3.0 sec
Background correction-	yes
Tube with platform-	yes

7) REFERENCES

7.1) Methods for the Examination of Waters and Associated Materials: Lead and Cadmium in Fresh Waters by Atomic Absorption Spectrophotometry (2nd Edition). A General Introduction to Electrothermal Atomization Atomic Absorption Spectrophotometry 1986 P7 - P17.

7.2) Perkin Elmer Manual for HGA-700, Graphite Furnace: Recommended conditions for standard graphite furnace AAS.

8) ORDERING INFORMATION

Cadmium standard solution 1000 g/1 100m1 Merck(Spectrosol)	14135 2F
Cadmium standard solution 1000 g/1 100m1Aldrich	20,701-2
Lanthanum Nitrate 100g Merck (Analar)	10412 3J
Nitric Acid 2.5 litre Merck (Analar)	

NILE BASIN INITIATIVE	Method No 14
DETERMINATION OF TOTAL OIL & GREASE GRAVIMETRICALLY	Number of Pages: 2
	Date: 9/9/06
	Author:
	Authorised by:
	Issue No: 1

1. PRINCIPLE

The sample is acidified to a pH of <2 with hydrochloric acid then serially extracted with dichloromethane in a separating funnel. The solvent is evaporated from the extract and the residue weighed.

2. PERFORMANCE

Detection Limit……1 mg/l
Interferences ……None known
Recoveries 90 – 110%
Speed of analysis……..6 samples per day for the whole procedure

The method is not applicable for measurement of light hydrocarbons that volatilise at temperatures below 70ºC. Petroleum fuels from gasoline up to number 2 fuel oils are completely of partially lost in the solvent removal.

3. REAGENTS

3.1 Hydrochloric acid, concentrated, reagent grade
3.2 Dichloromethane, HPLC grade or better.
3.3 Sodium Sulphate, anhydrous, crystal

4. APPARATUS

2000ml Separating funnel.
Boiling flask, 125 ml, one per sample.
Laboratory stand with support rings.
Filter paper, Whatman No. 541.
Evaporation system such as Rotovap apparatus, water temperature set to 70ºC.
Centrifuge set at >2000 rpm's.
Graduate cylinder, 1000 ml.

6. PROCEDURE

6.1 Sampling and Preservation

Collect the sample in a 1 litre glass bottle and capped with a teflon lined cap.
The sample is preserved with 5ml Hydrochloric acid per litre and stored in a refrigerator set at 4ºC.

6.2 Analysis

6.2.4) Mark the bottle at the water meniscus level with a permanent pen marker.
6.2.4) Rinse out the boiling flask with dichloromethane and blow out remaining solvent with an airline.
6.2.4) Place the boiling flask in a desiccator for at least one hour, then weigh the flask on an analytical balance recording the weight (A) gm.
6.2.4) Pour the acidified sample into the separating funnel.
6.2.4) Add 30ml of dichloromethane to the sample bottle, rotate bottle to rinse sides and pour the solvent into the separating funnel.
6.2.4) Extract by shaking the separating funnel for at least two minutes. Allow the layers to separate.
6.2.4) If an emulsion forms, drain the emulsion into a centrifuge tube and centrifuge the sample for 10-15 minutes.Place a filter paper in a funnel and add ~1 g of sodium sulphate. Rinse the salt and filter paper with 10 ml of dichloromethane and discard the rinse.
6.2.4) Filter the solvent layer through the funnel and into a boiling flask and rinse the filter paper with 5 ml of dichloromethane into the boiling flask.
6.2.4) Repeat steps 6.2.5 to 6.2.8 two more times combining the solvent layers into the boiling flask.
6.2.4) Insert the boiling flask in to the rotovap and evaporate the solvent.
6.2.4) Place boiling flasks in a desiccator for at least one hour then weigh the flasks on an analytical balance recording the weight (B) gm.
6.2.4) Fill the sample bottle with water up to the marked meniscus level then pour the water into a graduate cylinder to measure the volume of the sample in L.

7 CALCULATION

$$\text{Oil \& Grease mg/l} = \frac{[B - A]gm \times 1000}{\text{Volume of Sample (L)}}$$

8 REFERENCES

8.1) US EPA, 1983. Methods for Chemical Analysis of water and wastes, EPA-600/4-79-020, Method 413.1, Oil and Grease Total Recoverable.

9. ORDERING INFORMATION

NILE BASIN INITIATIVE	Method No 15
DETERMINATION OF MINERAL & TOTAL OIL BY INFRARED SPECTROSCOPY	Number of Pages: 3
	Date: 10/9/06
	Author:
	Authorised by:
	Issue No: 1
	File Name: OILMET2

1. PRINCIPLE

The water sample is extracted with carbon tetrachloride. Total oil (i.e. all material soluble in carbon tetrachloride) is determined by measuring the aliphatic CH2 absorption in the infrared at 2962 cm^{-1}, 2926 cm^{-1} and 2853 cm^{-1}. The extract is then chromatographed through florisil to remove any polar material. The mineral oil is then similarly determined in the eluate.

2. PERFORMANCE

2.1 Calibration linearity…….0-400 mg/l mineral oil
2.2 Criterion of detection……..0.03 mg/1
2.3 Sensitivity……..F = 175(1 cm cell)
2.5 Relative Standard Deviation ……..4-6% at 0.3 mg/l level on extracts (within batch).
2.6 Recoveries ……90 -110%
2.7 Speed of Analysis…….5 samples per day for the whole procedure

3.0 REAGENTS

3.1 Carbon tetrachloride (Spectroscopic or A.R. grade).
3.2 Stock solution of mineral oil.
Dissolve 0.2gm oil in carbon tetrachloride and make up to 100 ml, in a graduated flask.
3.3 Stock solution of vegetable oil
Corn oil or other polar material in carbon tetrachloride, 2000 mg/1. As for 3.2 using the selected oil.
3.4 Florisil, 100-200 mesh. *Activate at 180ºC for two hours and keep in a sealed bottle*.
3.5 Quartz Wool.

4. APPARATUS

4.1 Infrared spectrophotometer with 1 cm quartz cells.
4.2 Glass chromatography columns. Columns 40 cms long by 0.7 cms i.d. have been found to be suitable. Fit the columns with a quartz wool plug and add 2.0 gm of activated florisil. Wash with 10.0 ml of carbon tetrachloride before use. After determination of the blank, one column can be used for the determination of about ten water samples depending on the water quality.
4.3 Kuderna-Danish evaporator fitted with a micro-Snyder column (Fig.1).
4.4 10 ml graduated centrifuge tubes with ground glass stoppers.

Wash all glassware with carbon tetrachloride. Scan the washings from 3200 cm^{-1} to 2700 cm^{-1} at slow speed and normal energy level using carbon tetrachloride as blank in the transmission mode. If any absorbance is noted, repeat the washing until it is eliminated.

5.0 CALIBRATION PROCEDURE

5.1 Preparation of Calibration Curve

5.1.1 Prepare six solutions of the mineral oil containing: 20 mg/l, 50 mg/1, 100 mg/1, 200 mg/1, 300 mg/l and 400 mg/1. Pipette into 100 ml graduated flasks the following volumes of stock solution and make up to the mark.

Volume of Stock Solution ml	Final Concentration of Solution mg/l
1	20
2.5	50
5	100
10	200
15	300
20	400

5.1.2 Continue as under 6.9 to 6.11.

5.1.3 Construct a calibration of absorbance versus mineral oil concentration.

5.2 Efficiency of Chromatography Column

Newly activated florisil should be checked before use in order to determine how efficiently it will separate polar and non-polar materials.

5.2.1 Pipette 1.5 ml of each stock solution into a 100 ml graduated flask and make up to the mark with carbon tetrachloride.

5.2.2 Proceed as under 6.6 to 6.9.

5.2.4 Pipette 5.0 ml. of mixed standard onto the column.

5.2.5 Continue as under 6.14 to 6.17.

5.2.6 Calculate the amount of mineral oil recovered. This should be in the range 90-110%.

6.0 PROCEDURE

Determination of Total Oil

6.4) Shake the bottle of sample and pour off enough water to leave 1 litre in the bottle.

6.4) Add 10.0 ml of carbon tetrachloride to the sample and stopper the bottle.

6.4) Shake for 1 minute.

6.4) Allow to separate. If the separation is poor, centrifuge at 2000 rpm for 15 minutes.

6.4) Separate off the solvent layer and run into a 1 cm quartz cell.

6.4) Scan from 3200 cm $^{-1}$to 2700 cm $^{-1}$' at slow speed and normal energy level using carbon tetrachloride as blank.

6.4) Construct a baseline from 3200- 2700 cm $^{-1}$. Read off the transmission due to the peak and baseline at 2962 cm $^{-1}$., 2926 cm $^{-1}$and 2853 cm $^{-1}$

6.4) Calculate the absorbance due to each peak by calculating the value log (baseline transmission) - log (peak transmission).

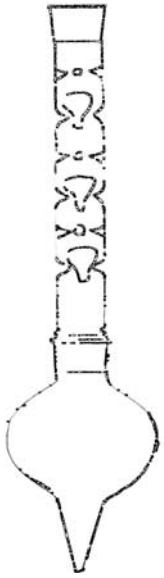
6.4) Add the three absorbances together for each sample. Let this value be A1.

6.4) Read off the concentration of carbon tetrachloride solubles in the extract from the calibration curve.

Determination of Mineral Oil

6.4) Prepare a chromatography column as in section 4.2.
6.4) Pipette 15.0 ml of carbon tetrachloride onto the column and proceed as under 6.15 and 6.16. Let the value obtained be A2.
6.4) Add 5.0ml of extract to the column.
6.4) Elute with 10.0 ml.. of carbon tetrachloride.
6.4) Collect the eluate and reduce the volume to about 3 ml in Kudurna -Danish evaporator fitted with a micro-Snyder column.

Figure 1 Kudurna -Danish evaporator fitted with a micro-Snyder column.



6.4) Transfer to a graduated centrifuge tube, make up to 5.0 ml., and continue as under 6.9 to 6.10. Let the absorbance value obtained be A3.

7.0 CALCULATION

7.1 The absorbances for the three types of oil are:

Total oil absorbance..=……..A1
Mineral oil absorbance……...= A4 = A3-A2
Absorbance due to polar materials.= A5 = A1-A4

7.2 The calibration graph, is used to convert the absorbances into concentrations in mg/l. i.e. Total Oil Concentration, Mineral Oil Concentration & Polar Oil Concentraion respectively.
7.3 Correcting for the liquid /liquid extraction to convert the concentrations to the actual concentration in the initial water sample, divide each result by 100 (10ml/1000ml).
7.4 If there is a substantial amount of oil present, the sample to solvent ratio may be varied and the concentration altered similarly.

8.0 REFERENCES

8.4) Jeltes, R. and den Towkelaar, W.A.M., Water Research 1972, 6, 271 - 278
8.4) Mallevialle, J., Water Research 1974, 8, 1071 - 1075
8.4) Jones, B.A., and Firth, M.G., Determination of Mineral Oil in Water by InfraredtSpectroscopy YWA Report 54/77

9.0 ORDERING INFORMATION

Florisil, 100-200 mesh (Merck).
Quartz Wool (Gallenkamp).

NILE BASIN INITIATIVE	Method No 16
DETERMINATION OF PRESUMPTIVE TOTAL COLIFORMS & E-COLI BY MEMBRANE FILTRATION	Number of Pages: 2
	Date:
	Author:
	Authorised by:
	Issue No: 1
	File Name: coliformsMet

1) PRINCIPLE

A measured volume of the sample is filtered through a membrane of cellulose nitrate. The pore size (0.45µm) is such that the coliform bacteria are retained on the surface of the membrane. This is then placed face-upwards on a pad soaked in a differential selective medium containing lactose, and phenol red as an indicator of acidity. On incubation for a stated time at a specified temperature, the coliforms retained by the membrane will form colonies of characteristic morphology and colour, which are counted.

2) PEFORMANCE

Speed of Analysis…….For 10 samples (Presumptive Coliforms) Operator time 2 Hours
…….& analytical time 18hours.
Interferences……..The method is not suitable for waters for waters containing small numbers of coliform organisms in the presence of large numbers of other organisms capable of growth on the membrane and the medium used.

3. REAGENTS

Membrane Lauryl Sulphate Broth (MLSB) Hydrated media
Dissolve 37.1gm of Hydrated MLSB (Oxoid) in 500ml and sterilise in autoclave.

4) APPARATUS

Membrane Filter units with suction pump
Sterile 47mm 0.45µm membranes
Hand lens, X4 magnification
Membrane Forceps
Large pan or boiler to disinfect funnels
Tongs to handle hot funnels
Autoclave or large pressure cooker
Test tube racks
Pipette canisters
Sterile Absorbent Pads 47mm
Waterproof marker pens
5mm Sterile Petri-dishes

5. PROCEDURE

Filtration

5.4) Dispense sterile absorbent pads aseptically into sterile petri- dishes.
5.4) Dispense 2.7 ml of sterile medium aseptically onto each sterile pad.
5.4) Remove the funnel and place a sterile membrane, grid-side upwards, on the porous disc of the filter base. Grasp only the outer part of the membrane filter with flat-ended sterile forceps
5.4) Replace the sterile funnel securely on the filter base.
5.4) With the stopcock turned off, pour the required volume of water sample into the funnel.
5.4) Open the stopcock to apply the vacuum and filter the water slowly though the membrane.
5.4) Pour off any excess medium in the petri-dish containing the saturated pad.
5.4) Close the stopcock, remove the funnel and transfer the membrane carefully to the pad saturated with medium. Ensure that no air-bubbles are trapped between the membrane and the pad.

Incubation

5.8) Invert the petri-dishes containing the membranes and place them inside a container with a tight fitting lid to prevent drying out. Alternatively, a polythene bag may be used and carefully folded over, tied or sealed. Incubate the membranes for 4 hours at 30ºC, and then for 14 hours at 37ºC but incubate at 44ºC. A total incubation period of 18 -24 hours is recommended.

Examination of Membranes

5.4) Examine the membranes under good light, if necessary with a hand lens. Count all yellow colonies irrespective of size; do this within a few minutes of removal from the incubator, as colours are liable to change on cooling and standing.

5.4) For confirmation and differentiation of coliforms, a representative number of yellow colonies can be sub-cultured. This is not normally required for river samples or effluent samples but these methods are fully explained in the references.

6 REFERENCES

6.1) Methods for the Examination of Waters & Associated Materials Environmental Agency UK The Microbiology of recreational & Environmental Waters (2000)
6.2) Yorkshire Water Authority (UK) Method 0502

7 ORDERING INFORMATION