Nile Basin Initiative
Nile Trans boundary Environmental Action Project

Report Name

TRAINING MODULES & QUALITY ASSURANCE PROGRAM – RWANDA

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Acronyms

A Absorbance, in spectrophotometric analysis
A_o Educational grade assigned to a graduate on completion of four years at university
A_1 Educational grade assigned to a graduate on completion of two years at university
A_2 Educational grade of a six-year secondary school graduate
A_3 Graduate on completion of only three years of secondary education
AAS Atomic Absorption Spectroscopy
AOAC Association of Official Analytical Chemists
BOD Biochemical Oxygen Demand
COD Chemical Oxygen Demand
DO Dissolved Oxygen
EDTA Ethylene Diamine Tetraacetic Acid, usually a disodium salt of this acid
ELGZ ELECTROGAZ, a body in charge of production and distribution of water and gas in Rwanda
ET Enriched Teepol, a bacterial growth medium in bacteriological analysis
G Glass, container for sampling water
GLC Gas-Liquid Chromatography
HPLC High Performance (or Pressure) Liquid Chromatography
KHI Kigali Health Institute
MINITERE Ministry of Lands, Water, Mines and Environment
MOH Ministry Of Health
NBI Nile Basin Initiative
nm nanometer
NUR National University of Rwanda
P Plastic, container for sampling water
pH A measure of hydrogen ion concentration in solution, given as $pH = -\log_{10}[H^+]$
ppm parts per million, a measure of concentration in mg/L
PTFE PolyTetraFluoroEthene, Teflon, used for making containers
QA Quality Assurance
RBS Rwanda Bureau of Standards
WQAP Water Quality Assurance Program

Executive Summary

In this report, capacities of institutions in Rwanda that are involved in analysis of water for quality control or monitoring are reviewed with a view to developing appropriate training modules for analysts and technicians that will assist the country and NBI in water quality management. It is pointed out that there are about six institutions in Rwanda that are for one reason or another involved in water quality assessment, namely: Ministries of Health and Environment, ELECTROGAZ, National University of Rwanda, Rwanda Bureau of Standards and Kigali Health Institute. Out of all these, it is only the laboratory now shared by the Ministry of Environment and the National University of Rwanda and some ELECTROGAZ laboratories that regularly test water for quality assessment. These laboratories train their own workers in water analysis. There is no institution in Rwanda that is responsible for training water analysts.

Since different laboratories may be employing different methods for testing similar parameters, the training modules developed in this report are a collection of standard procedures proposed to be used by the national laboratory that will assist NBI in water quality monitoring. There is no single laboratory in the country that uses all the methodologies presented in the training modules. Moreover there is no single laboratory that tests water for all the essential parameters. So the quality assurance program presented in this report contained additional parameters that should be tested for by the assembled National Laboratory. Additional training, particularly in analysis for the additional parameters utilizing new and more sophisticated instruments is suggested in this report.

Water Quality Program, which is currently non-existent in Rwanda, is designed in accordance with rain patterns and drainage systems in Rwanda. Sampling points and frequency of sampling and therefore of analysis are suggested, but can be changed to suit the national budget and that of other stakeholders and sponsors.

It is recommended that in future the NBI should have their own standard methods of analysis, results-report forms, storage and retrieval of information regulations for ease of comparison of characteristics of different waters in the Nile Basin.

PART I : TRAINING MODULES

1.0 Introduction

In Rwanda, there are seventeen laboratories that are regularly or occasionally involved in analysis of surface water. Nine of them are owned and run by **ELECTROGAZ (ELGZ)**, a national establishment charged with the provision of piped drinking water and electricity in Rwanda, although two of them in Gisenyi and Cyagugu in Western Rwanda do not deal with water of the Nile basin. However, these two can assist in quality-monitoring of Nyabarongo water in the upper region of western Rwanda, at least by taking samples on behalf of other laboratories. Three of these ELGZ laboratories are situated in Kigali in central Rwanda, while the rest are scattered in the western, eastern and southern provinces of the country. The distribution of these laboratories throughout the country would allow a wider coverage of water-quality monitoring in the basin**.**
The **National University of Rwanda(NUR)** has in its possession four laboratories at the university in Butare in Southern Province, and another one in Kigali, all of which carry out analysis of water, including microbiological analysis for research purposes on behalf of the university, and are very well equipped.
The **Ministry of Health (MOH)** occasionally uses some of its medical laboratories for screening for microbial agents of water-borne diseases in times of epidemics outbreaks.
There are two other laboratories that once in a while get involved in analysis of water…one is owned by **Rwanda Bureau of Standards (RBS)** and the other by **Kigali Health Institute**
(KHI). Both of these laboratories are based in Kigali, and have most of the basic equipments necessary for analysis of water.
The performance of each of these laboratories would be described as fair, endowed with modest equipments and relevant analytical reagents and materials, and manned by qualified analysts and technicians, who can assist in water-quality management countrywide.
Of all these laboratories, it is only the ELGZ laboratory branch at the Training Centre in Kigali that used to train, in conjunction with the main laboratory at Kimisagara, also in Kigali, their members of staff throughout the country in water-quality assessment through chemical analysis. For now, each laboratory trains its own staff on recruitment.
In developing the required technical training modules, the listed laboratories will first be assessed in terms of their physical infrastructures, analytical equipments, materials and manpower qualifications and competence. Each sectoral assessment will reveal the available and the shortfall of equipments, from which recommendations to be made will be derived.
It should be mentioned here and now that what they lack in common is the means to travel to far places to collect samples from Akagera River and its not-so-few tributaries for laboratory analysis. There would be need for at least one vehicle in the class of four-wheel drive double cabin pickup for a Kigali-based laboratory in particular.

2.0 Assessment of Technical Training Facilities

2.1 Laboratories

As already noted above, ELGZ alone has nine laboratories, and these are scattered all over the country, which would conveniently allow wider assessment of the water-quality in this part of the Nile basin.

NUR has five laboratories, four of which are based around the university at Butare , while the fifth is situated in Kigali. This NUR laboratory in Kigali is relatively free to engage in fulltime water-quality monitoring. The four NUR laboratories at the university are mainly engaged in research on behalf of the university, and can hardly participate in fulltime analysis of water for quality monitoring. But they do carry out analysis of raw surface-water, including microbiological screening for pathogenic organisms.

Overall, the laboratories that will be assessed here are: three ELGZ laboratories and the NUR one based in Kigali. The evaluation of the NUR, RBS and KHI laboratories are deliberately left out simply because they have nearly all the necessary standard equipments for analysis of water, and the required manpower. The MOH laboratory is a medical laboratory that occasionally analyses water for pathogenic micro-organisms, and as such cannot be compared with the ordinary water-analysis laboratory. It is also left out for the same reasons. It is neither necessary to include in the evaluation every ELGZ laboratory because nearly all of them have similar equipments, and use similar methods of analysis. Only one- Kadahokwa in Butare- will be considered for evaluation as a representative sample of the remaining six ELGZ laboratories based upcountry. Two other ELGZ laboratories based in Kigali are here included in evaluation.

These two ELGZ laboratories are reasonably well-equipped and manned by qualified manpower. One of these laboratories based at Kimisagara is involved in day-to-day activities of production and distribution of drinking water in Kigali. But they do carry out analysis of raw water before treating it; and they do train their new staff in the area of water-quality

assessment. The second ELGZ laboratory at the Training Centre in Kigali is largely free from water production activities. It was originally set aside for training of staff in the business of water treatment, quality control and research in these fields.

All these laboratories enumerated above are housed in buildings with enough space and large enough desks equipped with gas and water outlets and enough sinks. They are generally all well designed and ventilated except some do not have hoods, but this may not be a serous hindrance to carrying out standard analyses in which volatile products are formed. The laboratory infrastructures are spacious enough and capable of handling a sizeable manpower for training in water analysis.

The NUR laboratory is the only laboratory that is directly involved in water-pollution monitoring in the country on fulltime basis. It used to belong to a government ministry in charge of Environment and Natural Resources, and was involved in water-quality assessment, mainly for rural communities. Recently, it was acquired by the National University of Rwanda, but still carries out some analytical work on behalf of the ministry. It has enough space, even for expansion if need be, with basic infrastructure like desks etc necessary for chemical analytical work. It should be supported in every way possible to achieve this objective. It was proposed that the training of analysts and technicians in the country by the selected expert should take place at this laboratory.

It is then clear that there is no shortage of laboratories in the country which can assist in water-quality monitoring for quality assurance, and they are well distributed, which

would at least make sampling easier. However, a national laboratory should be established to take charge of water-quality monitoring in the Nile basin using standardised analytical methods to harmonise analysis of water all over the country. The national laboratory should have at least three sections responsible for:

- Analysis and training
- Quality assurance and records keeping, and
- Finance, administration and public relations

2.2 Equipments

Here below is given the Table of Equipments and Reagents for different laboratories:

Table I. 1 . Standard Equipments available in four selected laboratories

Laboratory / Equipment	ELGZ 1	ELGZ 2	ELGZ 3	NUR
Spectrophotometer	Y	Y	Y	Y
Colorimeter	X	X	X	X
pH meter	Y	Y	Y	Y
Conductivity meter	Y	Y	Y	Y
DO Analyser	Y	Y	Y	Y
COD Set	X	X	X	Y
BOD Set	X	X	X	Y
Autoclave	Y	Y	X	Y
Incubator	Y	Y	Y	Y
Membrane Filtration set	Y	X	X	Y
Microscope	X	X	X	Y
Analytical Balance	Y	Y	Y	Y
Top-pan Balance	Y	Y	Y	Y
Water still	Y	Y	Y	Y
Water bath	Y	Y	Y	Y
Hot plate	Y	Y	Y	Y
Refrigerator	Y	Y	Y	Y
Flame photometer	X	X	X	X
Turbidimeter	Y	Y	Y	Y
Dessicators	Y	X	Y	Y
Computer-Desktop	Y	Y	Y	Y
Printer	Y	Y	Y	Y
Fume cupboard	Y	Y	Y	X
Titration Equipment	Y	Y	Y	Y
Oven	Y	Y	Y	Y
Centrifuge	X	Y	X	Y
AAS	X	X	X	X
GLC	X	X	X	X
Water sampling equipment	Y	Y	Y	Y
Comparator . Field photometer	X	X	X	X

Portable Bact. Equipment	X	X	X	Y

Key: ELGZ 1= Kimisagara (Kigali), 2 = Training Centre (Kigali), 3 =Kadahokwa (Butare, Southern Province, NUR = NUR Laboratory in Kigali

As can be seen, almost all the laboratories under review have basic equipments for elementary analysis of water for quality-monitoring. Some of the heavy metals in water are analysed for spectrophotometrically at **Kimisagara** using **HACH DR 2400 SPECTROPHOTOMETER**. Alternatively, heavy metals can be analysed for at one of the four NUR laboratories at Butare where AAS equipment is available. This leaves COD, BOD, pesticides and a few other analyses, among the advanced analyses, not catered for. The NUR laboratory once in a while carries out COD and BOD analyses. If competent manpower were available, pesticides could be tested for at the university since they have a GLC machine. Training in these areas i.e. AAS and GLC may also be undertaken here. Assessment for bacteriological quality of water can be done at any of the two laboratories of the university, and at one of the ELGZ at Kimisagara in Kigali. It is recommended that modern equipments, mainly AAS, GLC and or HPLC be requisitioned for the selected national laboratory, and analysts trained in their use. It is also important that some field instruments as Oxygen meter, comparators or field photometers, membrane filtration sets and also flame photometer for analysing for potassium and sodium in water be available for field work.

2.3 Manpower

The following Table indicates the number of analysts and technicians available in different institutions in Rwanda, together with their levels of education:

Table I.2. Manpower in the most prominent institutions involved in water-quality testing

	Chemists		**Biologists / Microbiologists**	
Personnel / Level / Laboratory	**Analysts ($A_O + A_1$)**	**Technicians ($A_2 + A_3$)**	**Analysts ($A_O + A_1$)**	**Technicians ($A_2 + A_3$)**
ELGZ	8	9	1	8
RBS	1	1	4	3
KHI	4	-	7	-
N.U.R.	3	4	5	1
TOTAL NUMBER	**16**	**14**	**17**	**12**

Note: Ao is a University Graduate (4years) while A_1 is also a University Graduate(2 years).

A_2 is an equivalent of a secondary school leaver (6years). A_3 is an 'O' Level graduate.

From the point of view of qualified manpower, each laboratory has at least a chemical analyst and a biologist/ microbiologist and some technicians who have specialised in chemical analysis of water through training. The analysts (16 chemists and 17 biologists / microbiologists) are university graduates while technicians are high school leavers with training of some king.
The training they may need is in analysis for parameters that are new to them, for example COD and BOD, and using advanced instruments such as AAS or GLC.

2.4 Nature, Type and Level of Training Offered

It has already been remarked that there is no laboratory set aside specifically for provision of training in water analysis. The ELGZ laboratory at the Training Centre (TC) used to train their own manpower in the area of water analysis, but they have not done so for a number of years now. But this does not mean individual laboratories do not train their own manpower. They do to the extent that some have now become specialists in physical and chemical analysis of water; and others still in microbiological screening for pathogenic micro-organisms in water, treated or not. They are capable of performing basic or elementary and some advanced analyses of water; and also bacteriological screening of water for total coliforms, faecal coliforms, and sometimes faecal streptococci and clostidium perfringens..
As already noted, analysts that are recruited in water-testing laboratories in Rwanda are university graduates, while technicians are generally school leavers . University graduates who join the Water Department of ELGZ are trained at Kimisagara and Training Centre laboratories in elementary as well as advanced analyses of water, while technicians (A_2 and below) are trained to carry out mainly elementary, and some in bacteriological analysis of water.
Parameters analysed for in these laboratories are given are given in Section 2.5 below.

2.5 Training Courses Offered

When the need of training their own staff arises, they train them in the following areas:

- Water treatment
- Analysis of treated water
- Analysis of raw water
- Microbiological analysis of raw and treated water

The following parameters are normally covered, especially on raw water:

- pH
- Turbidity
- Colour
- Electrical Conductivity
- Total Dissolved Solids
- Suspended Solids
- Nitrates
- Nitrites
- Ammonia
- Phosphates

- Sulphate
- Fluoride
- Heavy metals in general, and
- Microbiological analysis for total coliforms, faecal coliforms, streptococci and clostidium at Kimisagara.
- Other parameters analysed for on raw water not listed in the treated water analysis include chloride, hardness, alkalinity and silica.

This occasional training is offered by ELGZ laboratories that employ a sizeable number of workers. The training, when it occurs, is offered to their workers only, and is therefore national in character. Otherwise every laboratory trains its own manpower in analysis for most of the above parameters whenever required. It should be noted here that Heavy Metals are analysed for at Kimisagara using a spectrophotometer. The preferred AAS method can only be employed after the analysts have been trained in this field. Moreover, additional training is required for BOD; COD; pesticides; oils and fats; and determining organic characteristics of raw surface water. Methods employed in analysis of raw water for various parameters are given in Section 2.8 below.

2.6 Water-Quality Monitoring: Sampling, Testing, Data Storage and Results Interpretation

In this section, we shall consider only the ELGZ laboratory at Kimisagara in Kigali, not only because it is the only laboratory that carries out water-quality testing reasonably regularly, but also that even when the others do, they use similar methods of sampling and analysis using similar instruments. It will therefore represent all nine similar laboratories that analyse surface water, owned by the same institution.

The waterworks at Kimisagara draws water from Yanze River nearby. The raw water is sampled from a standpipe just before the water collects into the dosing well. The water is collected in 20-L plastic containers and taken to the laboratory for analysis. Some of the water is tested for pH, colour, suspended solids, turbidity and other parameters, including microbiological screening for micro-organisms like total coliforms and faecal coliforms. Some of the raw water is used to ascertain treatment conditions by carrying out jar tests. The clear water so obtained after treatment is tested for such parameters as turbidity, colour, pH, residual aluminium and chlorine, heavy metals, bacteriological load etc. From the jar-test results, they can work out the quantities of various water treatment chemicals required for that batch of water. The quality of treated water is monitored hourly to detect any unexpected changes in the water characteristics. The data generated in all these analyses are daily entered during testing onto ready-designed forms which are filed. The head of the laboratory is the supervisor for this work, and is responsible for the interpretation of the results. The comments, particularly on discordant results, are made against the entered results. Any deviations from the acceptable standards are corrected and recorded.

Overall, the comments made and the results obtained were found to be pertinent and accurately reflect the true physical, chemical and bacteriological characteristics of the Yanze River water as are known over a long period of time. The files are accessible to fellow laboratory workers, but can also be obtained by any inquisitive person through the

official channels, especially easily if that person is a representative of a government institution or organisation concerned with the quality of drinking water.

2.7 Review of Water-Testing Results in Rwanda

We have already noted that there is a good number of laboratories in Rwanda, seventeen in number, that are involved in water analysis, though not necessarily in water-quality monitoring on fulltime basis. The only institution that has been participating in water-quality assessment in the country is the laboratory formerly owned by the Ministry of Environment and Natural Resources, now MINITERE. The laboratory now belongs to NUR. This institution still serves the same ministry and the country at large, at the same time remaining at the university's service. They analyse for most of physical, chemical and bacteriological parameters of raw water. . It is the only laboratory in Rwanda that has analysed for COD and BOD. However, no properly compiled results were available for review in spite of the fact that they at times carry out water analysis. This can be attributed to lack of direction as the institution does not at the moment seem to know where it belongs: either to the ministry or university.

The university has four other analytical laboratories at Butare in the southern region of the country. These laboratories are well equipped. They even have AAS and GC instruments. The university laboratories have the capacity to act as a regional centre for water-quality monitoring or assurance. One of them carries out research on fish farming and need to analyse water for its nutrients contents. They particularly analyse for nitrates, phosphates, dissolved oxygen and organics such as chlorophylls and planktons.

There is the Environmental Chemistry laboratory that occasionally carries out water-quality assessment in some rivers nearby, including the Akanyaru River some distance away. Most of the water parameters are analysed for.

There is a Microbiology laboratory that occasionally performs bacteriological analyses on raw water in the surrounding areas.

ELGZ laboratories are mainly involved in testing of treated water. They only carry out very few parameters like pH, colour and turbidity on raw water. This is the same for RBS whose main activity is analysis of water for the purpose of testing for conformity to set standards.

The MOH laboratory undertakes microbiological analysis of raw water only when epidemics of water-borne diseases have broken out.

It is to be noted that in Rwanda, there exists no water-quality assurance program. Even the NUR laboratory that was charged with the duty of water-quality monitoring does not have any established testing program, and so the activity is irregular. This has been exacerbated by the current unclear sense of belonging, either to the university or ministry. The quality-assurance program that is to be developed in this report should be adopted nationally if acceptable, for regular water-quality assurance in the country.

Parameters analysed for, methods employed in analysis and the kinds of results obtained by some laboratories are summarised in the sections that follow.

2.7.1 Parameters and Results of Environmental Chemistry Laboratory at NUR

A summary of parameters analysed for, methods employed in analyses and results obtained on 17 samples collected in 2000 from different rivers in western Rwanda is presented in the Table below:

Table I.3 . Parameters and Results of Analysis of 17 samples of different rivers in the country:

Parameter	T °C	pH	D.O. mg/l	Salinity mg/l	Cond µs/cm	Acid mg/l	Alkalin.mg/l TA	Alkalin.mg/l TAC	Colour Hazen	SM mg/l	Turbidity FTU
AverageVal.	21.81	7.14	7.16	0.04	112.00	17.80	0.60	38.43	415.53	70.40	118.87

Parameter	Total Hardness	Calcium Hrdness	CO_2	Ca^{++}	Mg^{++}	Cl^-	F^-	NO_2^-	I_2	N-NH_3	SO_4^{2-}	PO_4^{3-}
Aver.Val. (mgl^{-1})	33.40	17.30	5.87	6.99	4.70	10.43	0.37	0.09	<020	0.38	18.70	0.58

Parameter	Cu^{2+}	Mn^{2+}	Cr^{3+}	Fe^{2+}	Na^+	K^+	
Average Val. (mgl^{-1})	0.17	0.22	0.28	1.35	10.87	4.86	

Samples were taken from Rivers: Mwogo, Rukara, Mbirurume, Nyabarongo, Mukungwa, Nyabugogo, Base, Agatobwe, Migina, Akanyaru, Kabogobogo, Ruvubu, Akagera, Ngoma, Muvumba I, Muvumba II. All these rivers directly or indirectly drain into Akagera River

Comments

These rivers are found in very different environments and therefore have waters with very different characteristics. For example, turbidity of water from Mukungwa River (7 units) cannot be compared with that of water from River Nyabarongo (273 units). So the average values do not make sense. Similarly, it is not fair to take the average value of heavy metal contents like chromium and copper of waters of rivers Mbirurume and Nyabugogo. The latter flows through Kigali city where there is a lot of metal scrap, while Mbirurume flows through the rural countryside of the western highlands of Rwanda, where there is very little probability of contamination by such heavy metals. Nyabugogo river water is indeed a hot spot for such pollutants as organic matter and heavy metals. So it has the highest content of ammonia and copper.

It has also been pointed out that additional training be provided in analysis for.

- COD
- BOD
- Heavy metals using AAS
- Pesticides using GLC and / or HPLC
- Some elements in sediments
- Soaps and detergents
- Oils and fats, and
- Organic matter in water

2.7.2 Parameters, Methods and Results of Analysis by the Agriculture Laboratory at NUR.

The laboratory carried out analysis of water sampled from five points on Lake Ihema, one of the several lakes in the flat Akagera River valley in eastern Rwanda, in order to determine the quantity of nutrients available for fish in that lake. D1 and D4 refer to the lake-water sampling depths of 1m and 4m respectively. The parameters, methods used and results of analyses are given below:

Table I.4 Parameters, methods and results of analysis of Lake Ihema water

Parameter / Site	T^oC	Conduct. µS/ cm	pH	D.Oxygen mg/l	Total Alk.mg/l	PO_4^{3-} mg/l
KANYINYA D1	24.60	121.00	8.29	4.28	34.03	0.08
D4	23.00	126.00	8.30	3.01	38.04	0.08
CYOGO 1 D1	23.60	116.00	8.49	5.07	38.04	0.07
D4	23.40	117.00	8.69	4.59	37.04	0.39
CYOGO 2 D1	25.20	121.00	8.75	5.07	39.04	0.13
D4	24.60	122.00	8.73	4.12	39.04	0.11
KABUGA D1	25.20	120.00	8.39	6.18	38.04	0.09
D4	25.40	122.00	7.40	5.39	37.04	0.11
RUZIZI D1	24.80	121.00	8.33	4.75	22.02	0.09
D4	24.60	121.00	7.34	3.17	20.02	0.11
Method of Analysis	Thermo meter	Cond. meter	pH meter	Winkler method	Acid-base titration	Colorimetric

Parameter / SITE	Total N mg/l	NH_3 mg/l	NO_3^- mg/l	NO_2^- mg/l	SILICA mg/l
KANYINYA D1	1.96	0.28	0.02	0.15	10.72
D4	2.80	0.28	0.05	0.17	29.82
CYOGO 1 D1	1.68	0.28	0.01	0.13	10.78
D4	2.38	0.28	0.01	0.17	12.02
CYOGO 2 D1	1.26	0.21	0.01	0.16	25.00
D4	1.68	0.28	0.01	0.16	10.39
KABUGA D1	2.80	0.21	0.01	0.18	12.08
D4	2.38	0.21	0.02	0.18	11.99
RUZIZI D1	1.68	0.28	0.01	0.17	11.96
D4	2.66	0.28	0.02	0.20	12.62
Method of Analysis	Kjeldhal	Spectro.	Specro.	colorimetric	molybdate

It is impossible to comment on their accuracy because there are no such results anywhere else. ELGZ laboratories are mainly concerned with testing treated water, and the results for water analysis are rarely compiled. The NUR in Kigali did not seem to be in possession of any compiled results, ostensibly because nobody has ever shown the interest in using them for any purpose.

3.0. MODULES

Introduction

The modules developed have been divided into three categories:

- Elementary Analysis of Water
- Advanced Analysis of Water, and
- Microbiological Analysis of Water.

It is necessary to highlight the need for sampling, preservation, storage and sample pre-treatment in water-quality monitoring. The table below summarises the most important elements of the activities (sampling, preservation etc…..) for various parameters.

Table I.5. Sampling containers, Preservation Techniques and Holding Times:

Parameter No. / Name	**Container 2**	**Preservation 3,4**	**Maximum Holding Time 5**
Coliforms, faecal and total Faecal streptococci	P,G P,G	Cool, 4 ^{o}C, 0.008 %$Na_2S_2O_3$ Cool, 4 ^{o}C, 0.008% $Na_2S_2O_3$	6 hours 6hours
Table 1B- Chemical Tests			
. Acidity	P,G	Cool, 4 ^{o}C	14 days
Alkalinity	P,G	Cool 4 ^{o}C	14 days
Ammonia	P,G	Cool, 4 ^{o}C, H_2SO_4 to pH< 2	28days
BOD	P,G	Cool, 4 ^{o}C	48 days
COD	P,G	Cool, 4 ^{o}C, H_2SO_4 to pH < 2	28 days
Chloride	P,G	Cool, 4 ^{o}C, H_2SO_4 to pH< 2	28 days
Colour	P,G	Cool, 4 ^{o}C	48 days
Fluoride	P	None required	28 days
Hardness	P,G	HNO_3 to pH < 2, H_2SO_4 to	6 months

		pH< 2	
pH .Kjeldahl and organic nitrogen	P,G P,G	None required Cool 4 ^{o}C, H_2SO_4 to pH < 2	Analyse immediately 28 days
Metals			
Chromium VI	P,G	Cool 4 ^{o}C	24 hours
Other metals except B, Cr VI and Hg	P,G	HNO_3 to pH < 2	6 months
Nitrate	P,G	Cool, 4 ^{o}C	48 hours
Nitrate-nitrite	P,G	Cool 4 ^{o}C, H_2SO_4 to pH < 2	28 days
Nitrite	P,G	Cool, 4 ^{o}C	48 hours
Oil and Grease	G	Cool, 4 ^{o}C, HCl or H_2SO_4 to pH< 2	28 days
Orthophosphate	P,G	Filter immediately;Cool, 4 ^{o}C	48hours
Oxygen,dissolved probe	G bottle and top	None required	Analyse immediately
Winkler	G bottle and top	Fix on site and store in dark	8 hours
Phosphorus, elemental	G	Cool,4 ^{o}C	48 hours
P , Total	P,G	Cool, 4 ^{o}C, H_2SO_4 to pH< 2	28 days
P Residue, Total	P,G	Cool, 4 ^{o} C	7days
P residue, fillterable	P,G	,,	,,
P residue,Nonfilterable(TSS)	,,	,,	,,
P residue, Settleable	,,	,,	48 days
P Residue, Volatile	,,	,,	7 days
Silica	P, PTFE or quartz	,,	28 days
Specific conductance	P,G	,,	,,
Sulphate	,,	,,	,,
Surfactants	,,	,,	48 hours
Temperature	,,	None required	Analyse immediately
Turbidity	,,	Cool, 4 oC	48 hours

P = Plastic
G = Glass
PTFE = PolyTetraFluoroEthene (or ethylene)

Sample Pre-treatment
Sample pre-treatment is necessary to remove substances that would otherwise interfere in the analysis for the determination of the analyte in the sample and give unacceptably high or low value. There are several methods for sample pre-treatment. Some of these are given below:

- pH adjustment

- Masking
- Wet digestion
- Ashing
- Distillation

Different samples are pre-treated according to the nature of the interfering impurities that have to be removed, and during the analysis, the described analytical procedure may indicate the pre-treatment method for the parameter to be analysed for.

MODULE 1

3.1 ELEMENTARY ANALYSIS OF WATER

This module is made up 17sections, each section dealing with a specific type of analysis of water. The sections are arranged as follows:
Section 3.1.1: pH
Section 3.1.2: Colour, True and Apparent
Section 3.1.3: Suspended Solids
Section 3.1.4: Acidity
Section 3.1.5: Alkalinity
Section 3.1.6: Solids: Dissolved and Undissolved
Section 3.1.7: Total Nitrogen
Section 3.1.8: Ammonia Nitrogen
Section 3.1.9: Nitrate Nitrogen
Section 3.1.10: Nitrite Nitrogen
Section 3.1.11: Chloride
Section 3.1.12: Fluoride
Section 3.1.13: Carbonate and Bicarbonate
Section 3.1.14: Total Hardness
Section 3.1.15: Phosphorus
Section 3.1.16: Sulphate
Section 3.1.17: Silica

3.1.1 pH of Water

A. Principle
pH, which is an accepted measure of acidity or alkalinity, is determined by change in potential of glass-saturated calomel electrodes, as measured by commercial apparatus standardized against standard buffer solutions whose pH values are assigned by NIST. pH of most natural H_2O falls within 4-9. Majority of waters are slightly basic from presence of CO_3-HCO_3 system. Method is applicable to drinking, surface, and saline waters, and domestic and industrial wastes. Oils and greases, by coating electrodes, may cause sluggish response.

B. Apparatus and Reagent
(a) *pH meter* – Commercial instrument with flow-type electrodes (preferred for relatively unbuffered specimens such as condensates) or immersion electrodes. Operate in accordance with manufacturer's instructions.

(b) Standard buffer solutions

C. Determination
Thoroughly wet electrodes in accordance with manufacturer's instructions. Standardize instrument with standard buffer solutions with pH near that of specimen and then with 2 others to check linearity of electrode response.
Analyze specimen as soon as possible, preferably within few hours. Do not open specimen bottle before analysis. With immersion electrodes, wash 6-8 times with portions of specimen, particularly when unbuffered solution follows buffered solution. Equilibrium, as shown by absence of drift, must be established before readings are accepted.

3.1.2 Colour, True and Apparent

Examination of water for true and apparent colour using platinum-cobalt standards is given in the HACH manual for 2400 portable spectrophotometer.

3.1.3 Suspended Solids

A photometric method of estimating the total amount of solids suspended in water is given in the HACH manual of the 2400 portable specrtrophotometer. The absorbance is measured at 810 nm.

3.1.4 Acidity of Water

A. Principle

Specimen is titrated to pH 8.3, using phenolphthalein as indicator, and results are reported as mg $CaCO_3$/L. Method is applicable to drinking and surface waters, domestic and industrial wastes, and saline waters.

B. Apparatus

(a) *Illumination* – Daytime fluorescent lamps provide uniform lighting conditions.

(b) *Potentiometric equipment*-Automatic titrators and pH meters, suitably calibrated, may be substituted for visual titration and end point.

C. Reagents

(a) *Carbon dioxide –free water* – If pH is<6.0. Deionized H_2O may be substituted if conductance is <2 $\mu mhos/cm$ and pH >6.0.

(b) *Sodium hydroxide standard solution*- 0.02M.

D. Determination

Use specimen volume requiring <25 mL titrant. If indicator is used, remove free Cl_2 with one drop 0.1M $Na_2S_2O_3$.

To 50 or 100 mL specimen in white porcelain casserole or in Erlenmeyer over white surface, add 0.15mL phenolphthalein. Titrate with standardized 0.02M NaOH to faint pink (pH 8.3)

mg $CaCO_3$/L= mL NaOH x molarity NaOH x 50 000/mL specimen

3.1.5 Alkalinity of Water

A. Principle

Unaltered (undiluted, unconcentrated, unfiltered) specimen is titrated potentiometrically to pH 4.5. Applicable to drinking and surface waters, domestic and industrial wastes, and saline waters. Suitable for all concentration ranges.

B. Reagents

(a) *Carbon dioxide-free water*

(b) *Acid standard solutions*- 0.1M HCl or 0.05MH_2SO_4

1 mL 0.02M acid=1.00 mg $CaCO_3$/L.

C. Determination

Use specimen volume requiring <25mL titrant. Titrate potentiometrically to pH 4.5.

mg $CaCO_3$/L= mL HCl x molarity x 50 000/mL specimen

or

2 x mL H_2SO_4 x molarity x 50 000/ mL specimen

3.1.6 Solids in Water

A. Total Solids

Thoroughly shake specimen, and pipet 100 mL unfiltered specimen into weighed Pt dish. If specimen contains much suspended matter, shake, pour rapidly into 100 mL graduate, and immediately transfer to weighed Pt dish. Evaporate to dryness and heat to constant weight at 100^oC.

B. Solids in Solution

Let specimen stand until all sediment settles and filter if necessary to secure perfectly clear liquid. (Occasionally, clear filtrate can be obtained only by use of alumina cream; avoid if possible.) Evaporate 100-250 mL to dryness in weighed Pt dish. Heat to constant weight at 100°C.

Alumina cream-Prepare cold saturated solution of alum in H_2O. Add NH_4OH with constant stirring until solution is alkaline to litmus, let precipitate settle, and wash by decantation with H_2O until wash H_2O gives only slight test for sulfates with $BaCl_2$ solution. Pour off excess H_2O and store residual cream in glass-stoppered bottle. (Alumina cream is suitable for clarifying light-colored sugar products or as adjunct to other agents when sugars are determined by polariscopic or reducing sugar methods.)

C. Ignited Residue

Ignite residue from A at 525-550°C in furnace or over burner until dish shows dull red glow and ash is white or nearly so. Note any odor or change in color produced during ignition. Determine weight ignited residue and calculate loss on ignition.

3.1.7 Nitrogen (Total) In Water

A. Principle

Specimen is digested with H_2SO_4. to convert organic N to NH_3, which is distilled after alkalinization and determined by nesslerization or titrimetry. Preserve specimens by addition of 40 mg $HgCl_2$/L and store at 4°C. Analyse as soon as possible, as conversion of organic N to NH_3 may occur even with preservation. Method is applicable to surface and saline waters and domestic and industrial waster. Some industrial wastes containing materials such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines may not be converted to NH_3.

B. Apparatus

(a) *Digestion apparatus*.

(b) *Distillation apparatus*- Use all-glass apparatus with 800 or 1000 mL distilling flask and 500 mL glass- stoppered Erlenmeyers, marked at 350 and 500 mL, as receivers. Prepare for use by distilling mixture of NaOH-$Na_2S_2O_3$ solution and H_2O (1+1) until distillate is NH_3-free by Nessler reagent, (j). Repeat each time apparatus is out of service $\geq 4h$.

(c) *Nessler tubes*-Matched, ca 300 mm long, 17 mm id, and marked at 225 ± 1.5 mm inside measurement from bottom.

(d) *Spectrophotometer or filter photometer*-For use at 425mm

C. Reagents

(a) Water-Distilled, NH_3-free.

(b) *Mercuric sulfate solution*

(c) *Digestion solution*

(d) *Sodium hydroxide-sodium thiosulfate solution*
(e) *Phenolphthalein indicator solution*
(f) *Sulfuric acid standard solution*-0.01M.
(g) *Ammonia standard solutions*-(1) Stocksolution-100 mg N/mL.. (2) Working solution-0.01 mg N/mL.
(h) *Boric acid solution*
(i) Mixed indicator
(j) *Nessler reagent*

D. Digestion and Distillation

Determine specimen size as follows:

mg N/L	mL Specimen
0-5	500
5-10	250
10-20	100
20-50	50.0
50-100	25.0

Place specimen, or residue from NH_3 determination (for organic Kjeldah N only), into 800 mL Kjeldah flask. Dilute, if necessary, to 500 mL and add 100 mL digestion solution, (c). Boil until SO_3 fumes are evolved and test solution becomes colorless or pale yellow. Cool, and dilute with 300 mL H_2O. Add NaOH-$Na_2S_2O_3$ solution slowly down neck of tilted flask to underlay acid solution in amount sufficient to make final solution strongly alkaline as shown by phenolphthalein (60 mL NaOH-$Na_2S_2O_3$ solution will neutralize 20 mL H_2SO_4.) Connect flask to condenser, with tip of condenser dipping into 50 mL 2% H_3BO_3 solution in 500 mL glass-stoppered Erlenmeyer. If test solution is to be titrated, 100 or 200 mL H_3BO_3 may be used. Mix solutions and distil 300 mL at 6-10 mL/min. If NH_3 concentration is $\geq 1 mg/L$, determine titrimetrically, E; if less, determine colorimetrically, F.

E. Titrimetric Determination

Add 3 drops mixed indicator, (i), to distillate and titrate with 0.01M H_2SO_4 (f), matching end point against blank containing same volume of NH_3-free H_2O,H_3BO_3 solution, and indicator.

mg Total N/L=[(mL standard H_2SO_4 for specimen
-mL standard H_2SO_4 for reagent blank)
x 2 x molarity standard H_2SO_4 x 14.01 x 1000]
/mL specimen digested

F.Colorimetric Determinatin

Prepare series of standards containing 0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mL NH_3 working standard solution, (g) (2), diluted to 50 mL with NH_3-free H_2O (contains 0.0, 0.04, 0.10, 0.20, 0.30, 0.40, 0.60, and 0.80 mg NH_3 N/L). Add 1 mL Nessler reagent, (j), and mix. After 20 min, read A at 425 mm against 0.0 (blank) standard, and plot A against

concentration to obtain standard curve. Distil one or more high and low standard solutions daily to ensure adequate recoveries.
As estimated by preliminary determination, determine NH_3 in 50 mL aliquot, or aliquot diluted to 50 mL, as above, and read NH_3 concentration from standard curve.

mgTotal N / L =[(mg NH_3-N from curve × 1000) / ml specimen taken for distillation] × (ml final distillate, including H_3BO_3 solution / (ml distillate taken for nesslerisation).

3.1.8 Nitrogen (Ammonia) in Water

A. Principle
Specimen buffered at pH 9.5 is distilled into H_3BO_3 solution. Depending upon concentration, NH_3 is determined colorimetrically (0.05-1.0 mg N/L) by nesslerization or titrimetry (1.0-25 mg N/L). Hg, if present as preservative, and residual Cl must be removed by addition of $Na_2S_2O_3$ before distillation.

B. Apparatus
See **3.1.8 (b) to(d)**

C. Reagents
See **3.1.8. (f) to (j),** and following
(a) *Borate buffer*-pH 9.5

(b) *Sodium hydroxide solution*-1M.
(c) *Dechlorinating reagent*

D. Distillation
Add 500 mL NH_3-free H_2O and few boiling chips previously treated with NaOH solution of Kjeldahl distilling flask. Adjust 400 mL specimen to ph 9.5 with 1N NaOH, using pH meter or short range test paper. If specimen contains residual Cl_2, remove by adding equivalent amount dechlorinatin reagent, (c). Transfer to distilling flask and add 25 mL buffer, (a). Distil 300 mL at 6-10 mL/min into 50 mL H_3BO_3 solution, 3.17.C(h). Dilute distillate to 500 mL in receiving flask. Determine NH_3 in 50 mL aliquot as in colorimetric determination. If NH_3 concentration is $\geq 1 mg/L$, determine titrimetrically, E; if less, determine colorimetrically, F.

E. Titrimetric Determination
Proceed as in 3.1.7 E using remaining 450 mL distillate.

$$\text{mg } NH_3\text{-N/L} = \frac{\text{mL } 0.01M\ H_2SO_4 \times 1000}{\text{Equivalent mL specimen in aliquot titrated}}$$

F. Colorimetric Determination
Proceed as in 3.1.7 F

$$\text{mg } NH_3\text{-N/L} = \frac{NH_3 \text{ concentration from standard curve} \times 1000}{0.8 \times \text{mL distillate taken for determination}}$$

3.1.9 Nitrogen (Nitrate) in Water

A. Principle

Nitrate ion reacts with brucine in H_2SO_4 at 100°C to form colored compound whose A is measured at 410 mm. Temperature control of reaction is critical. Applicable to 0.1-2mg NO_3-N/L in surface and saline waters and domestic and industrial wastes.
Organic matter developing color with H_2SO_4 and natural color are compensated for by blank; effect of salinity is compensated for by addition of NaCl. Strong oxidizing and reducing agents interfere. Determine presence of free Cl_2 with o-tolidine reagent. Eliminate residual Cl_2 by addition of $NaAsO_3$ solution. Effect of Fe^{2+}, Fe^{3+} and Mn^{4+} is negligible at <l mg/L.

B. Apparatus

(a) *Spectrophotometer or filter photometer*-Capable of accommodating 25 mm diameter tubes and measuring A at 410 mm.
(b) *Tubes* – Matched tubes for conducting reaction and measuring A.
(c) *Racks* – Neoprene, wire coated, evenly spaced, to permit uniform flow of bath H_2O between tubes.
(d) *Water baths* – (1) 100°C-Boiling H_2O bath of sufficient size so that when tubes are inserted, temperature drop is $\leq 1-2^{o}C$. Should have tight-fit cover, preferably of gable construction, with circulator or stirrer to maintain uniform temperature. Uniform temperature control of this bath is critical. (Caution:Check H_2O bath to assure it is electrically grounded.) (2) 10-15°C.-For cooling tubes.

C. Reagents

(a) *Water*-Use distilled or deionized H_2O for preparation of all reagents and standard.
(b) *Salt solution*
(c) *Sulfuric acid* -6.5M
(d) *Brucine-sulfanilic acid reagent*
(e) *Nitrate standard solutions*-(1) Stock solution-100 mg N/L. (2) Working solution-1 mg/L.

D. Determination

Preserve specimens with 40 mg $HgCl_2$/L and store at 4°C. Adjust pH to ca 7 with CH_3COOH (1+3) and, if necessary, filter through $0.45\eta m$ filter.
Prepare set of matched tubes for blanks, standards, and specimens. If necessary to correct for color or for organic matter which will cause color on heating, add extra set of tubes to which all reagents except brucine will be added.
Pipet 10 mL specimen, or aliquot diluted to 10 mL, into specimen tubes. For saline specimen, add 2.0 mL 30% NaCl (w/v) solution to specimens, standards, and blank tubes. Swirl tubes and place in 0-10°C bath. Pipet 10 mL 6.5M H_2SO_4 into each tube and swirl. Let all tubes come to thermal equilibrium. Pipet 0.5 mL brucine reagent to all tubes except color control tubes and swirl. Then place entire rack containing all tubes in boiling

H_2O bath for exactly 25 min. remove rack and transfer to cold H_2O bath and let cool to 20-25°C.
Dry tubes and read A against reagent blank at 410 mm.
Prepare set of standard containing 0.1-2 mg N/L and conduct standard along with specimens. Color may not follow Beer's law. If necessary, subtract A of color controls from A of specimens.

3.1.10 Nitrite-Nitrogen

A diazotisation method for the determination of nitrite in water is described in the HACH manual for the DR 2400 spectrophotometer. This diazotisation involves the nitrogen of nitrite and sulphanilic acid.

3.1.11 Chloride in Water

A. Principle
Chloride titrated with mercuric ions forms soluble, slightly dissociated $HgCl_2$. In pH range 2.3-2.8 diphenylcarbazone indicates end point by forming purple complex with excess Hg^{2+}. Xylene cyanol FF serves as pH indicator and background color to facilitate end point detection. $NaHCO_3$ added to both blank and specimen followed by constant amount of HNO_3 added with indicators provides pH of 2.5±0.1.Increasing strength of titrant and modifying indicator mixture permits determination of high Cl concentrations common in waste water.
Br and I titrate as chloride. Chromate, Fe^{3+}, and SO_3^{2-} interfere when present at >10 mg/L. Sulfites may be removed with 0.5-1 mL H_2O_2/50 mL specimen. Methods are applicable to drinking, surface, and saline waters, and domestic and industrial wastes at all Cl concentrations. However, to avoid large titration volumes, use specimen containing >20 mg Cl/50 mL.

B. Reagents
(a) *Sodium chloride standard solution*-0.014M.
1 mL=0.500 mg Cl.
(b) *Chlorine-free water*-Redistilled or deionized.

For Low Chloride Concentration

(c) *Indicator-acidifier reagent*
(d) *Mercuric nitrate standard*
Store in dark bottle away from light. 1 mL=0.500 mg Cl.

For High Chloride Concentration

(e) *Mixed indicator*
(f) *Mercuric nitrate standard solution*-0.0705M.

C. Determination
(a) *For low chloride (drinking water)*-To≤100 mL specimen containing ≤10 mg Cl, add 1.0 mL indicator-acidifier, (c). Color should be green-blue. If not, adjust pH of specimen

to 8 before addition of reagent. Titrate with 0.00705M $Hg(NO_3)_2$ to definite purple end point. (Solution becomes blue few drops before end point.) Determine blank by titration of equal volume H_2O containing 10 mg $NaHCO_3$.
(b) *For high chloride*-To 50.0 mL specimen (5.00 mL if ≥5 mL titrant needed) in 150 mL beaker, add 0.5 mL mixed indicator, (e) and mix well. Color should be purple. Add 0.1M HNO_3 dropwise until just yellow. Titrate with 0.0705M $Hg(NO_3)_2$ to first permanent dark purple. Determine blank by titration of equal volume H_2O.
(c) **Calculation**

mg Cl/L = [(mL specimen titration-mL blank titration)
2x molarity $Hg(NO_3)_2$ x 35340]/mL specimen
mg NaCl/L=(mg Cl/L) x 1.65

3.1.12 Fluoride in Water

A. Reagents
(a) *Fluoride standard solution*-0.01 mg F/mL.
(b) *Thorium nitrate solution*
(c) *Alizarin red indicator*-0.01% aqueous solution sodium alizarin sulfonate (alizarin red S).
(d) *Hydrochloric acid*-Exactly 0.05M.
(e) *Sodium hydroxide solution*-Exactly 0.05M
(f) *Hydroxylamine hydrochloride solution*-1.0 g/100 mL.

B. Apparatus

(a) *Claisen flask*-250 mL.
(b) *Nessler tubes*-6 long-form 50 mL tubes with double optically plane disks fused to tubes. Match tubes for length and test for optical similarity as follows: Add ca 40 mL H_2O,

1 mL indicator, 2 mL 0.05M HCl, and H_2O to mark on tube. To one tube add amount of $Th(NO_3)_4$ solution such that, after diluting to mark and mixing, color is barely changed to faint pink. Note amount of $Th(NO_3)_4$ solution used. Add same amount of $Th(NO_3)_4$ solution to each of remaining 5 tubes. Reject tubes showing detectable differences in shade or intensity.

C. Preparation of Specimen
If specimen has odor of H_2S, oxidize with 0.1 mL30% H_2O_2 solution before evaporation. Place 100 mL specimen in porcelain or Pt dish, make alkaline to phenolphthalein with 10% NaOH solution (w/v) (Avoid excess), and evaporate to 20 mL over burner at temperature just below bp. During evaporation, keep sample alkaline by adding small amounts of 0.05M NaOH from time to time. Transfer the 20 mL evaporated specimen to Claisen flask containing glass beads or boiling tube previously rinsed with boiling 10% NaOH solution (w/v) to eliminate all traces of gelatinous SiO_2 accumulating in flask. Place flask containing specimen on insulating board (15 x 15 x 0.6 cm with 2.5 cm center hole) over burner adjusted for medium flame. Close straight neck of flask with 20-hole rubber stopper through which pass thermometer and stem of small separator with outlet constricted to 2 mm diameter. (Adjust thermometer and outlet tube of separator to extend

almost to bottom of flask.) Close other neck of flask with solid rubber stopper. (Alternatively, all-glass distillation assembly may be used.)
Connect flask with H_2O condenser; add 20 mL 60% $HClO_4$ to flask, rinsing evaporating dish and separator; then add amount of saturated $AgClO_4$ solution that will precipitate chlorides (determined previously by titration with standard $AgClO_3$ solution), and distil at $132^o \pm 3^oC$, adding H_2O dropwise through separator to maintain temperature during distillation. Collect nearly 200 mL distillate. Dilute to volume (200 mL) and mix well. To determine acidity, use 40 mL distillate, add 1 mL indicator, mix thoroughly, and note mL 0.05N NaOH required for neutralization.
Repeat preparation and distillation, using 100 mL H_2O in place of specimen, to determine blank.

D. Determination

Prepare one standard, one color comparison tube, and one or more specimen tubes as follows:
(a) *Color comparison tube*-To 40 mL H_2O add 2 mL 0.05M HCl, 1 mL alizarin red indicator, 1 mL $NH_2OH.HCl$ solution, and enough $Th(NO_3)_4$ solution to give faint but definite pink end point. Compare all end point colors with this color.
(b) *Specimen tube*-To specimen tube containing 40 mL distillate add 1 mL indicator, 1 mL $NH_2OH.HCl$ solution, and volume 0.05M HCl such that total volume acid in tube (acidity previously determined plus volume 0.05M HCl added) equals 2 mL 0.05M HCl. Dilute to volume and mix. If in preliminary acidity determination it is found that the 40 mL distillate required >2 mL 0.05M NaOH solution for neutralization, do not add the HCl solution to specimen tube, but add to standard tube same amount of acid as was found present in sample tube. If 40 mL distillate requires >5 mL 0.05M NaOH, repeat distillation under conditions favorable to low acidity. From 10 mL buret, graduated to 0.05 mL, add $Th(NO_3)_4$ solution with frequent mixing until faint pink appears, comparable to comparison tube, (a) Note volume $Th(NO_3)_4$ solution used.
(c) *Standard tube*-To standard tube containing 40 mL H_2O, add 1 mL indicator. 1 mL $H_2NOH.HCl$ solution, and ≥2 mL 0.05M HCl, as was required in specimen tube in (b). If aliquot chosen for determine already contains 2-5 mL 0.05M acid, add exactly same amount to standard tube. Add exactly same amount of $Th(NO_3)_4$ solution as was added to specimen tube. To standard tube (now more highly colored than specimen tube), add standard F solution from 10 mL buret with mixing until color matches that of specimen tube. Dilute contents of both standard and specimen tubes to same volume. Mix solution in each tube and let all air bubbles escape before making color comparisons. Check end point by adding 1-2 drops standard F solution to standard tube. Distinct color change should develop.

E. Calculation

Subtract mL solution required by blank from mL F solution required by specimen.

$$\frac{\text{mL F solution} \times \text{mL total distillate} \times 10}{\text{mL aliquot titrated} \times \text{weight specimen taken}} = F(\mu g/mL)$$

3.1.13 Carbonate and Bicarbonate in Water: Total Alkalinity

To 100 mL specimen add few drops phenolphthalein, and if pink is produced, titrate with 0.05M HCl or 0.025M H_2SO_4, adding drop every 2-3s until color disappears. Multiply buret reading by factor 3 to obtain mg CO_3^{2-} ion in 100 mL. To colorless solution from this titration, or to original solution if no color is produced with phenolphthalein, add 1-2 drops methyl orange, continue titration without refilling buret, and note total reading. If CO_3^{2-} is absent, multiply total buret reading by factor 3.05 to obtain value of HCO_3^- ion in mg/100 mL. If $CO_3^{2-}$is present, multiply reading with phenolphthalein by 2 and subtract from total reading of buret. Multiply difference by 3.05 to obtain HCO_3^- ion in mg/100 mL. Express results as mg/L.

3.1.14 Hardness of Water

A. Calculation Method

Calculate hardness as sum of $CaCO_3$ equivalent (mg/L) obtained by multiplying concentration (mg/L) found of following cations by factor shown:

Table I.6 Factors for calculating hardness of water due to different cations

Cation	Factor	Cation	Factor
Ca^{2+}	2.497	Al^{3+}	5.564
Mg^{2+}	4.116	Zn^{2+}	1.531
Sr^{2+}	1.142	Mn^{2+}	1.822
Fe^{2+}	1.792		

Factors to be used for calculating hardness using given cations.

EDTA Titrimetric Method

B. Principle

Ca and Mg at pH 10 in presence of dye eriochrome blank T are wine red. When completely complexed with EDTA, solution becomes blue. Mg must be present for satisfactory end point and is added as MgEDTA. End point sharpness increases with pH, but high pH may cause precipitation of $Ca(OH)_2$ or $Mg(OH)_2$ and cause color changes of dye. pH of 10.0±0.1 is satisfactory compromise. Limit of 5 min for titration minimizes precipitation. Heavy metal interference is minimized by complexing with cyanide. Method is applicable to drinking and surface waters and domestic and industrial wastes. To avoid large titration volumes, use aliquot containing <25 mg $CaCO_3$.

C. Reagents

(a) *Buffer solution*
(b) *Indicator*
(c) *EDTA standard solution*-0.01M.
(d) *Calcium standard solution*-1.000 mg $CaCO_3$/mL.

D. Determination

Dilute 25 mL specimen (or such volume as to require <15 mL titrant) to ca 50 mL with H_2O
in porcelain casserole, add 1-2 mL buffer solution, 250 mg NaCN (pH of solution should be

10±0.1), and ca 200 mg indicator powder, and titrate with EDTA standard solution slowly,
with continuous stirring, until last reddish tinge disappears, adding last few drops at 3-5 s intervals. Color at end point is blue in daylight and under daylight fluorescent lamp. Complete titration within 5 min from time of buffer addition.
For waters of low hardness (<5 mg/L), use 100-1000 mL specimen, proportionately larger amounts of reagents, microburet, and blank of distilled H_2O equal to specimen volume.

Hardness (EDTA) as mg $CaCO_3$/L= T x B x 1000/mL standard specimen

Where T=mL EDTA standard solution and B=mg $CaCO_3$ equivalent to 1.00 mL EDTA standard solution.

3.1.15 Phosphorus in Water

A. Definitions
(a) *Phosphorus-(P).* All P present in specimen, regardless of form, measured by persulfate digestion method. (1) Orthophosphate-(P, ortho). Inorganic P, (PO_4^{3-}), in specimen as measured by direct colorimetric analysis. (2) Hydrolyzable phosphorus.-(P, hydro). P as measured by H_2SO_4 hydrolysis method minus orthophosphate; includes polyphosphates, $(P_2O_7)^{4-}$, $(P_3O_{10})^{5-}$ and some organic P. (3) Organic phosphorus.-(P, organic)=
P-[(P, ortho)+(P,hydro)].
(b*) Dissolved phosphorus-(P-D*). P present in filtrate of specimen filtered through 0.45µm pore filter, measured by persulfate digestion method. (1) Dissolved orthophosphate-(P-D, ortho). Inorganic P as measured by direct colorimetric method. (2) Dissolved hydrolysable phosphorus-(P-D, hydro). P as measured by H_2SO_4 hydrolysis-(P-D, ortho). (3) Dissolved organic phosphorus-(P-D, organic) =(P-D)-[(P-D, ortho)+(P-D, hydro)].
(c) *Insoluble phosphorus-(P-I).* When sufficient amount is present, calculate following: (1) P-I=(P)-(P-D). (2) Insoluble orthophosphate-(P-I, ortho)=[(P, ortho)-(P-D, ortho)]. (3) Insoluble hydrolysable phosphorus-(P-I, hydro)=[(P, hydro)-(P-D, hydro)]. (4) Insoluble organic phosphorus.-(P-I, organic)={(P, organic)-(P-D, organic)}.

B. Principle
Ammonium molybdate and potassium antimonyl tartrate react in acid solution with dilute solutions of PO_4^{3-} to form antimonyl phosphomolybdate complex which is reduced to intensely blue complexes by ascorbic acid. Method is specific for orthophosphate and for compounds that can be converted to orthophosphate. Various forms of P are determined, depending on pretreatment, in range 0.01-0.5 mg P/L.
Method is applicable to surface and saline waters and domestic and industrial wastes. Most commonly measured forms are total P, dissolved P, orthophosphate, and dissolved orthophosphate. Hydrolysable P is normally found only in sewage-type specimens. Concentrations of Cu, Fe, silicate, and arsenate many times greater than those in sea

water do not interfere. Interference of $HgCl_2$, used as preservative, is overcome by adding minimum of 50 mg NaCl/L to specimens.

C. Apparatus

(a) *Glassware*-Wash all glassware with hot HCl (1+1) and rinse with H_2O. Remove last traces of P by filling with H_2O containing all color-developing reagents. Use treated glassware only for P determinations and after use, rinse with H_2O and keep covered until used again. Under such conditions, hot HCl and reagent treatment need be applied only occasionally. Never use commercial detergents on glassware.

(b) *Photometer-Spectrophotometer or filter photometer* -Measuring at 880 mm, using ≥2.5 cm light path.

D. Reagents

(a) *Dilute sulfuric acid*-2.5M.

(b) *Potassium antimony tartrate solution*

(c) *Ammonium molybdate solution –*

(d) *Ascorbic acid solution*-0.1M.

(e) *Combined reagent*-Warm reagents (b)-(d) to room temperature, and add with mixing in following order : 50 mL 2.5M H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. If turbidity forms, shake, and let stand few min before proceeding. Stable 1 week at 4°C.

(f) *Hydrolyzing acid solution*-Slowly add 310 mL H_2SO_4 to 600 mL H_2O, cool, and dilute to 1 L.

(g) *Phosphorus standard solutions*-(1) *Stock solution*-50 mg P/L.. (2) *Intermediate solution*-0.5 mg/L. Dilute 10.0 mL stock solution to 1 L. (3) *Working solutions*-Dilute 0.0, 1.0, 3.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mL intermediate solution to 50 mL to prepare standard solution containing 0.0, 0.01, 0.03, 0.05, .0.10, 0.20, 0.30, 0.40 and 0.50 mg P/L.

E. Determination

Store specimen in plastic or Pyrex containers. If analysis cannot be performed on day of collection, preserve with 40 mg $HgCl_2$/L and refrigerate at 4°C. In such case, add 50 mg NaCl/L before analysis.

(a) *Phosphorus*-Add 1 mL hydrolyzing acid solution to 50 mL specimen in 125 mL Erlenmeyer. Add 0.4 g ammonium persulfate, and boil gently on preheated hot plate 30-40 min or until volume is 10 mL. Do not let specimen evaporate to dryness. Alternatively, autoclave 30 min at 121°C. Cool, add few drops phenolphthalein, adjust to pink with 1M NaOH, and then to colorless with 1 drop hydrolyzing acid solution. Cool, and dilute to 50.0 mL. If turbid, filter. Proceed as in (c), beginning "Add 8.0 mL combined reagent,….."

(b) *Hydrolyzable phosphorus*-Proceed as in (a), except omit addition of ammonium persulfate.

(c) *Orthophosphate*-Add 1 drop phenolphthalein to 50.0 mL specimen; if red develops, add hydrolyzing acid solution dropwise until color is discharged. Add 8.0 mL combined

reagent, and mix thoroughly. After specific time within 10-30 min, measure A at 880 mm against reagent blank as reference.
(d) *Standard curve and calculation*-Process standards and blank as in (c) and plot A against mg P/L. Include blank and ≥2 standard solutions with each series of test solutions. If standards do not agree with standard curve within ±2%, prepare new standard curve. Obtain mg P/L specimen directly from standard curve.

3.1.16 Sulfate in Water

Turbidimetric Method

A. Principle

Sulfate is precipited in dilute HCl with $BaCl_2$ under controlled conditions to form $BaSO_4$ crystals of uniform size. A of suspension measured and sulfate concentration is obtained from standard curve.
Method is applicable to drinking and surface waters and domestic and industrial wastes. Dilute specimens to concentration of ≤40 mg SO_4/L. Color and suspended matter interfere. Some suspended matter is removed by filtration. Remaining interference is corrected by blank which omits $BaCl_2$.

B. Apparatus

(a) *Magnetic stirrer*-Adjustable, but once set must operate at constant speed. Stirring bars must be of identical shape and size. Exact speed is not critical, but it should be constant for each series of samples and standards and should be at maximum at which no splashing occurs. Timing device to permit operation for exactly 1 min is desirable.
(b) *Photometer*-Nephelometer, spectrophotometer set at 420 mm with 4-5 cm cell, or filter photometer with filter having maximum T near 420 mm with 4-5 cm cell.

C. Reagents

(a) *Conditioning reagent*
(b) *Barium chloride-Crystals, 20-30 mesh*. Dispense from 0.2-0.3 mL measuring spoon.
(c) *Sulfate standard solution*-100µg SO_4/mL.

D. Determination

Pipet 5 mL conditiong reagent into 100 mL specimen or aliquot diluted to 100 mL in 250 mL Erlenmeyer, and mix on magnetic stirrer. While stirring, add spoonful of $BaCl_2$ crystals and begin timing. Stir exactly 1 min at constant speed. Immediately transfer some solution into cell and measure turbidity at 30s intervals for 4 min. Record maximum reading. Conduct blank determination without $BaCl_2$ and subtract reading. Prepare standard curve by carrying 0-40 mg SO_4/L, in 5 mg increments, through entire determination. Introduce standard solution with every 3-4 specimens.

mg SO_4 / L = mgSO_4/L from curve x 1000/mL specimen.

3.1.17 Silica in Water

The HACH 2400 portable spectrophotometer manual describes a method for analysis of silica in water.
The silica and phosphate in the sample react with molybdate ions under acidic conditions to form yellow complexes. Addition of citric acid destroys the phosphate complex. Silica is then determined by measuring the remaining yellow colour. The results are measured 452 nm.

MODULE 2

3.2 ADVANCED ANALYSIS OF WATER

This Module contains sections on analysis of,

- Biochemical Oxygen Demand (BOD)
- Dissolved Oxygen (DO)
- Chemical Oxygen Demand (COD)
- Manganese
- Iron
- Zinc
- Copper
- Barium
- Chromium, and
- Cadmium

Where appropriate, detailed methods of analysis are described. References of methods used are given. References picked from the HACH Manual are not detailed because instrument operations in the manual are given in form of diagrams, which are not necessary to reproduce.

Analysis for DO, COD and BOD

3.2.1. Oxygen (Dissolved) in Water

A. Applications

Azide (Alsterberg) method is ordinarly used; it is not affected by most common interference, nitrite, but most other oxidizing or reducing agents should be absent. Effect of Fe^{3+} is eliminated with F^- Permanganate (Rideal-Stewart) method is used in presence of Fe^{2+} but not organic matter.Pomeroy-Kirshman-Alsterberg method is used for waters supersaturated with O_2 or containing high organic matter content.

Method I: Azide Method

B. Reagents

(a) *Alkaline iodide-sodium azide solution*

(b*) Manganese sulfate solution* .

(c)*Potassium biiodate standard solution*-0.00208M.

(d) *Potassium fluoride solution*

(e) *Sodium thiosulfate standard solutions*-(1) 0.1M (2) 0.025M

1mL=0.2 mg O.

(f) *Starch indicator solution*

C. Determination

(Add all reagents, except H_2SO_4, well below surface of sample from 10 mL pipets graduated in 0.1 mL, with tips elongated ca 50mm). Add 2.0mL $MnSO_4$ solution and 2.0 mL alkaline I-NaN_3 solution to specimen in 250 or 300 mL BOD bottle, replace stopper, excluding air bubbles, and invert several times to mix. Let floc settle and repeat mixing. (Water with high chloride concentration requires 10 min contact with precipitate.) After floc has settled, leaving $\geq 100mL$ clear supernate, remove stopper and add 2.0 mL H_2SO_4 down neck of bottle. (If>100 ppm Fe^{3+} is present, add 1.0 mL KF solution before acidifying.) Restopper and mix by inversion until I_2 is uniformly distributed. Immediately titrate 203 mL (3 mL is allowance for added reagents) with 0.025M $Na_2S_2O_3$ to pale straw yellow. Add
1-2 mL starch indicator and titrate to disappearance of blue. Disregard reappearance of blue.

mg/L (ppm) Dissolved O_2= (mL 0.025M $Na_2S_2O_3$ x 0.2/200) x 1000

3.2.2 Chemical Oxygen Demand (COD) of Water

A. Principle

Organic substances are oxidized by $K_2Cr_2O_7$ in H_2SO_4 (1+1) at reflux temperature with Ag_2SO_4 as catalyst and $HgSO_4$ to remove Cl interference. Excess dichromate is titrated with Fe^{2+}, using orthophenanthroline as indicator. Method is independent determination of organic matter in specimen and has no definable relationship to biological oxygen demand (BOD).

Method is applicable to surface and saline waters and industrial wastes. Apply Method I, using 0.25M reagents, to specimens containing >50 mg COD/L; apply low level modification, Method II using 0.025M reagents, to specimens in range 5-50 mg/L; apply special modification, Method III, to saline waters containing > 1000 mg Cl/L and >250 mg COD/L. Organic matter from glassware, atmosphere, and distilled H_2O must be excluded. Condition glassware by using it for blank determination to eliminate organic matter.

B. Preparation of Specimen

Collect specimens in glass bottles if possible; plastic may be used if it contributes no organic material to specimen. Test biologically active specimens as soon as possible. Mix or homogenize specimens containing settleable materials. Specimens may be preserved with H_2SO_4, 2 mL/L.

C. Apparatus and Reagents

(a) *Reflux apparatus*-500 mL Erlenmeyer or 300 mL round-bottom flask with standard taper joint connected to 30 cm (12") Allihn condenser.

(b) *Distilled water*-Low in organic matter. Ordinary distilled H_2O is satisfactory; do not use deionized H_2O.

(c) *Potassium dichromate standard solution*-(1) 0.04M (2) 0.004M

(d) *Sulfuric acid reagent*

(e) *Ferrous ammonium sulfate standard solution*.-(1) 0.125M (2) 0.0125M

(f) *Phenanthroline ferrous sulfate (ferroin) indicator solution*

D. Standardization of Ferrous Solutions

(a) Concentrated solution-Dilute 25.0 mL 0.04M $K_2Cr_2O_7$, (c) (1), to ca 250 mL with H_2O. Add 75 mL H_2SO_4 and cool. Titrate with 0.125M $Fe(NH_4)_2(SO_4)_2$, using 10 drops ferroin indicator. Molarity = (mL $K_2Cr_2O_7$ x molarity/mL $Fe(NH_4)_2(SO_4)_2$.

(b) *Dilute solution*-To 15 mL H_2O add 10.0 mL 0.004M $K_2Cr_2O_7$, (c)(2). Add 20 mL H_2SO_4 and cool. Titrate with 0.0125M $Fe(NH_4)_2$ $(SO_4)_2$, using 1 drop ferroin indicator. Blue-green to reddish brown color change is sharp. Calculate molarity as in (a).

Method I-High Level

Place several boiling chips and 1 g $HgSO_4$ in reflux flask. Add 5.0 mLH_2SO_4 and swirl until $HgSO_4$ dissolves. Place in ice bath and slowly add, with swirling, 25.0 mL 0.04M $K_2Cr_2O_7$. Slowly, and with swirling, add 70.0 mL H_2SO_4- Ag_2SO_4 reagent. While still in bath, pipet in 50 mL specimen (or aliquot diluted to 50 mL) with continuous mixing. Attach solution condenser and reflux 2 h. (Shorter period may be used on waste H_2O of constant or known composition where time of maximum oxidation has been determined previously.)

Cool, and wash down condenser with ca 25 mL H_2O. If round-bottom flask has been used, quantitatively transfer solution to 500 mL Erlenmeyer. Dilute to ca 300 mL with H_2O, and let cool to ca room temperature. Add 8-10 drops ferroin indicator, and titrate excess $K_2Cr_2O_7$ with 0.125M $Fe(NH_4)_2$ $(SO_4)_2$ to sharp, reddish end point (S mL). Perform blank determination with all reagents, including refluxing, on distilled H_2O in place of sample and determine mL 0.125M $Fe(NH_4)_2$ $(SO_4)_2$ required (B mL).

$$\text{mg COD/L} = (B-S) \times M \times 8000/V$$

where M=molarity $Fe(NH_4)_2$ $(SO_4)_2$ and V=volume specimen used.

Method II Low Level

Proceed as in high level determination, E, except use 0.004M $K_2Cr_2O_7$ and 0.0125M $Fe(NH_4)_2$ $(SO_4)_2$.

Method III-Saline Waters

Pipet 50 mL specimen of 250-800 mg COD/L and Cl^->1000 mg/L (or aliquot diluted to 50 mL with distilled H_2O having Cl^- concentration equal to that of specimen) into 500 mL Erlenmeyer and add 25.0mL 0.004M $K_2Cr_2O_7$ and 5.0 mL H_2SO_4. Add 10 mg $HgSO_4$/mg Cl in 4 specimen and swirl until dissolved. Carefully add 70.0 mL H_2SO_4.- Ag_2SO_4 reagent with swirling. Add several boiling chips. Attach condenser, and reflux 2 h. (If volatile organic compounds are present in specimen, attach condenser prior to addition of H_2SO_4.- Ag_2SO_4 reagent and add reagent through condenser while cooling flask in ice bath.)

Cool and proceed as in low level determine, F, including blank. Disregard reappearance of blue-green after end point is reached. For saline waters, prepare standard curve of COD against mg Cl^- /L, using NaCl solutions with intervals of ≤ 4000 up to 20000 mg Cl^- /L, carried through entire determination.

COD, mg /L =[B-S) x M x 8000-50D] x 1.20/V
Where D= Cl^- correction from standard curve, and 1.20 is compensation factor to account for extent of Cl^- oxidation which is dissimilar in organic and inorganic systems. Other symbols are defined in E.

3.2.3 Biochemical Oxygen Demand (BOD) of Water

A. Principle

Specimen is incubated 5 days at 20°C in presence of acclimated biological system. Comparison of O_2 content of specimen at beginning and end of incubation is measure of BOD. Method is applicable to raw or treated domestic wastes, industrial water, and industrial waste water. Following classes of materials exert O_2 demand: (1) organic material usable as food by aerobic organisms (source of BOD of many waste waters); (2) oxidizable N from nitrites, NH_3, and organic N compounds which serve as food for specific bacteria (e.g., Nitrosomonas and Nitrobacter) (a source of some of O_2 demand of biologically treated effluents); (3) chemically oxidizable materials e.g., Fe^{2+}, S^{2+} SO_3^{2-}. (When present, test must be based upon calculated initial dissolved O_2 content). Many synthetic organic components of industrial wastes are not degraded by common organisms. Without special seeding material, effect is manifested as retardation of aerobic metabolism because of toxic effect or deficiency or absence of appropriate microorganism. Toxic compounds in distilled H_2O, frequently Cu, may result in low BOD.

B. Apparatus

(a) *Incubation bottles*-250 or 300 mL with glass stoppers.
(b) *Incubator*-Air or H_2O bath maintained at 20 ± 1°C and which excludes light.

C. Reagents

(a) *Water*-Containing ≤ 0.01 mg cu/L, obtained by double demineralization of distilled H_2O or distillation from all-glass or Sn-lined system.
(b) *Calcium chloride solution*
(c) *Ferric chloride solution*
(d) *Magnesium sulfate solution*
(e) *Phosphate buffer solution*-pH 7.2
(f) *Seeding material*
(g) *Sodium hydroxide solution*-50g NaOH/L.
(h) *Sodium sulfite solution*-

D. Preparation of Dilution Water

Store H_2O, C(a), in cotton-plugged bottles long enough to saturate with atmospheric O_2 at 20°C or aerate with air filtered to remove any oil from compressor ($\leq 1h$ may be required for 19L [5 gal.]). Add desired volume of O_2-saturated H_2O to suitable bottle and add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions/L. Seed this dilution H_2O with seeding material and with volume found by experience to be most satisfactory for particular waste being examined. Use seeded dilution H_2O, within 24 h of preparation.

Periodically check quality of dilution H_2O, effectiveness of seed, and technique with particular organic compound if known to be present in waste or, for general work, with mixture of glucose and glutamic acid (150 mg each/L) which should show BOD ca $220 \pm 30 mg/L$ in 95% of determinations. Appreciable divergence requires examination of quality of H_2O, variability of seeding material, or technique.

E. Preparation of Specimens

Keep time between collection specimen and start of analysis to absolute minimum. Protect specimens from atmospheric O_2. If necessary, pretreat specimens as follows:

(a) *Acidity or caustic alkalinity*-Neutralize to ca pH 7 with dilute H_2SO_4 or 5%NaOH (w/v), using pH meter or bromothymol blue as external indicator. PH of seeded dilution H_2O should not be changed by dilution of specimen.

(b) *Residual chlorine* – Let stand 1-2 h to dissipate Cl. If not effective, use $Na_2S_2O_3$ treatment. Determine volume to be used by adding 10mL CH_3COOH (1+1) or H_2SO_4 (1+49) and 10 mL 10% Kl to 1L specimen. Titrate to starch-I_2 end point with $Na_2S_2O_3$ solution. Add indicated volume to specimen, and test small portion with starch-I_2 solution to check that treatment is complete.

(c) *Toxic substances* –Remove or neutralize. Test for toxicity as follows: add same amount seed to duplicate set of BOD bottles. Add dilution H_2O to each bottle, leaving room for amount of specimen to give final concentrations of 0.06, 0.12. 0.25, 0.50, 1.0, 2.5, 5, 10, 20, and 40%. Neutralize specimen, add required volume specimen to duplicate bottles, and fill with dilution H_2O. Determine dissolved O_2. in second series after 3 days. Plot consumption of dissolved O_2 against concentration. Magnitude of O_2 concentration change will depend on amount of food available and toxicity of specimen. If toxicity is factor, O_2 consumption will decrease at higher concentrations.

(d) *Supersaturation with oxygen* – specimens containing >9.2 mg O_2 /L at 20°C may be encountered during winter or where algae are actively growing. To prevent loss of O_2 during incubation, reduce O_2 content to saturation by transferring specimen at ca 20°C to partially fill bottle and shake vigorously.

F. Determination

Specimen must be diluted with seeded dilution H_2O so that at least 1 dilution will achieve dissolved O_2 depletion of 1 mg/L (ppm) during 5 day test period but will not reduce residual dissolved O_2 to <1 mg/L. [Preliminary chemical O_2 demand (COD) determination, 3.2.2, may serve as guide to estimate range of BOD].

Carefully siphon seeded dilution H_2O into 1 or 2 L graduate, filling it ½ full. Add volume of carefully mixed sample to desired dilution and fill to mark with dilution H_2O. Mix well with plunger-type mixing rod, avoiding entrainment of air. If possible BOD range is large, prepare geometric series of dilutions to cover possible range. Siphon, with continued mixing, diluted specimen to completely fill 3 BOD bottles – one for incubation, one for determination of dissolved O_2 content, and one for determination of immediate dissolved O_2 demand (IDOD). Insert stoppers without entrainment of any bubbles. Determine dissolved O_2 by method indicated in 3.2.1. **Alternatively**, prepare diluted specimens directly by pipetting specimen with wide-tip pipet into BOD bottles of known capacity and filling bottles with seeded dilution

H_2O. If dilution >1:100 is required, prepare in graduate before adding to BOD bottles. Prepare blank of seeded dilution H_2O containing volume used for dilution of specimens for determination of initial dissolved O_2 content. Prepare control of 2 BOD bottles with unseeded dilution H_2O. Stopper and H_2O –seal 1 bottle for incubation. (If special H_2O –sealed bottles are not used, H_2O –seal by immersion in tray of H_2O.). Determine dissolved O_2 in other bottle before incubation. Quality of unseeded dilution H_2O is satisfactory if depletion obtained is ≤ 0.1. Do not use this value as blank correction. If dilution H_2O is seeded, determine O_2 depletion of seed used in such dilution that will result in 40-70% depletion in 5 days. Use this depletion, not seeded blank, to calculate correction due to small amount of seed in dilution H_2O. Incubate prepared mixtures, H_2O-sealed, 5 days at 20 ± 1^oC and determine final dissolved O_2 content.

G. Calculation

Calculate in mg/L (ppm) as follows:
Immediate dissolved O_2 demand (IDOD) = $(D_c-D_1) / P$
When seeding is not required,
BOD = $(D_1-D_2) / P$
When using seeded dilution H_2O,
BOD = $[(D_1-D_2)-(B_1-B_2)\ f\ ] / P$
Including IDOD, if small or not determined,
BOD = $(D_c-D_2) / P$
Where D_o=dissolved O_2 (DO) of original dilution H_2O; D_1 =DO in diluted specimen 15 min after preparation; D_2 =DO of diluted specimen after incubation; S=DO of original undiluted specimen; D_c=DO available in dilution at zero time =(D_{oP}) + SP; p=decimal fraction of dilutionH2O used,P= decimal fraction of specimen used, B1= DO of the dilution of seed control before incubation; B_2=DO of the dilution of seed control after incubation; f=ratio of seed in specimen to seed in control = (percent seed in D_1)/(percent seed in B_1).

H. Interpretation

Arbitrary standard 5 day incubation period is satisfactory measurement of the O_2 load on receiving water for raw or treated domestic sewage. It may be misleading for wastes containing organic compounds not easily amenable to biological oxidation. Studies with 3 incubation periods on series of dilutions of the waste will provide information on lag periods, suitability of inocula rate of biochemical oxidation, ultimate O_2 demand, and amenability to biochemical self-purification. Particularly important is ratio of 5 day BOD to ultimate O_2 demand.

Analysis for Heavy Metals

The methods presented for analysis for heavy metals in water were obtained from Hach DR 2400 Spectrophotometer manual.

3.2.4 Manganese in Water

The method for manganese analysis is described in the Hach manual. This method is based on oxidation of Mn2+ in water sample to Mn7+ (manganate VII, or permanganate) ions whose solution in water is purple. The oxidizing agent in this case is sodium periodate, but ammonium persulphate could be used. The purple colour developed is proportional to the manganese II ions present in the water sample. Absorbance is measured at 525 nm.

3.2.5 Determination of Iron in Water

This method is available in a manual by HACH which describes a spectrophotometric analysis for iron in various forms using DR/ 2400 portable spectrophotometer. One of the methods describes a procedure in which a certain reagent converts all the iron in the sample to the ferrous form. The ferrous iron is reacted with 1,10-phenanthroline to give an orange complex whose colour is proportional to iron concentration. Absorbance is read at the wavelength of 510 nm.

3.2.6. Determination of Zinc in Water

This spectrophotometric analytical method is also available in the manual by HACH which describes the procedure for zinc analysis.The pH of sample is adjusted to 3-5 using 0.5M sulphuric acid, after sample digestion if necessary.
The zinc ions in water react with 4- 2-pyridylazo –resorcinol PAR at pH 5. An orange-red complex is formed and the absorbance is measured at 490nm.
The calibration curve is constructed using standard zinc solution of 1000mg/ L as zinc.

3.2.7. Determination of Copper in Water

The determination is also described in the HACH manual. The method is based on the formation of an orange complex when copper I ions react with disodium bathocuproine disulphonic acid. Any copper II ions that may be present are reduced to copper I ions using ascorbic acid before the complex is formed. Absorbance of the complex is taken at 478nm. The calibration curve is constructed using standard copper standard solution of 1000mg/ L as copper.
There is another method for determining copper using porphyrin which forms an intense yellow complex with copper. This method is said to be more sensitive than the former method as it is less liable to interferences and does not require sample extraction or concentration.

3.2.8. Determination of Barium in Water

The procedure for determining barium in water is also described in HACH manual. It is a turbidimetric method involving precipitation of barium sulphate which is held in suspension by an emulsifying agent like ethylene glycol. The amount of turbidity caused

by the suspension is proportional to the concentration of barium present. The absorbance is measured at 450 nm.

3.2.9 Determination of Chromium in Water

The HACH manual describes the procedure for determining total and hexavalent chromium. One method uses 1,5-diphenylcarbohydrazide which forms a red complex with chromium VI ions. Chromium VI is reduced to chromium III ions. The analysis is performed at 543 nm.
The other method uses alkaline hypobromite which oxidises chromium III in the sample to chromium VI. The total chromium content is determined by the 1,5-diphenylcarbohydrazide method. Trivalent chromium is determined by subtracting the results of a separate hexavalent chromium test from the results of the total chromium test. The absorbance is measured at 540 nm.

3.2.10 Determination of Cadmium in Water

According to the HACH manual, cadmium ions react with a substance named cadion to form a red complex. This complex is then broken down, thus reducing the colour intensity. The resulting reduction in colour is measured at 552 nm.

Reference HACH DR / 2400 Spectrophotometer Manual

Before the instrument for AAS analysis becomes available, heavy metals and many other parameters are analysed for in water at the ELGZ and NUR laboratories using the procedures described in the **Hach Manual for the 2400 spectrophotometer**.

MODULE 3

3.3 MICROBIOLOGICAL ANALYSIS OF WATER

3.3.1 Overview of Bacteriological Analysis

Surface water is liable to contamination by sewage or any other excreted matter. Such water, if consumed untreated, may cause outbreaks of intestinal infections such as typhoid fever.
Bacteriological tests become necessary to indicate the presence or absence of such micro-organisms. The principal bacteria indicative of faecal contamination water are:

- *Coliforms*
- *Faecaj coliforms*
- *Faecal streptococci*
- *Clostidium perfringens*

Pathogenic bacteria found in water include:

- *Salmonella*
- *Shigella*
- *Esherichia coli*
- *Vibro cholerae*
- *Clostidium perfringens*

The demonstration of pathogenic bacteria, e.g *Typhoid bacillus* would indicate the presence of dangerous impurity. But their test is not practical because such pathogens are scanty, and therefore difficult to detect. Instead tests that will reveal the presence of bacteria of intestinal origin, such as those of coliform group: *Streptococcus faecaIis* and *clostidium welchii* are relied on. Water grossly polluted with human excretal matter, e.g. sewage, contains these bacteria in large numbers. Faecal coliforms, e.g. *E. coli,* are almost exclusively of intestinal origin and their presence is an indication of excretal contamination. Atypical coliforms such as
Kl. Aerogenes may grow in soil and on vegetation.
The routine tests generally used in bacteriological examination of water are:

1. A quantitative test for all coliform bacilli known as the **presumptive coliform count.**
2. A differential test for typical coliform bacilli (*Esch. Coli*) known as the **differential coliform test.**
3. An enumeration of viable bacteria known as the **plate count**; this is done in duplicate , cultivating at 37 ^{o}C and 22 ^{o}C

Because these methods are lengthy, their details cannot be included here. They can be found in any standard microbiology textbook. Only **Membrane Filtration Technique** will be briefly presented here.

Collection of Specimens

Specimens are taken in bottles of about 250 ml, with ground-glass stoppers having an overhanging rim; they are sterilized by autoclaving, the stopper and neck of the bottle being covered over by two layers of kraft paper. Care must be taken to avoid any bacterial contermination from an outside source.

When water is drawn from a tap, the mouth of the tap should be flamed with a blow-lamp or spirit lamp, and the water allowed to run for 5 min before filling the bottle. In the case of streams, rivers and lakes, the stopper should be removed carefully with one hand, and with the other the bottle held at its base should be inserted, mouth downward, a few centimeters below the surface of the water; the bottle is then turned so that the mouth is directed to the current and the water flows into the bottle without coming into contact with the hand. If there is no current, the bottle should be moved horizontally, the mouth foremost, so that water flows into it. The bottle is then brought to the surface and the stopper replaced. Do not contaminate the stopper.

When a sample is to be taken from a depth, a bottle weighted with lead is used, having two cords attached- one to the neck, the other to the stopper; the bottle is lowered to the required depth, and is filled by jerking out the stopper by means of the attached cord; the bottle is then quickly raised to the surface and re-stoppered.

When three hours or more must elapse before the laboratory examination can be carried out , the bottles should be kept on ice. Special insulated boxes for the purpose are available for transporting the sample for a long distance.

3.3.2 The Membrane Filter Technique for the Bacteriological Examination of Water.

This method is based on the use of a highly porous cellulose membrane, the pore structure of which enables fairly large volumes of water or aqueous solutions to pass through rapidly under pressure, but prevents the passage of any bacteria present in the sample. These are retained on the surface of the membrane which is then brought into contact with suitable liquid nutrients. These diffuse upwards through the pores thereby inducing the organisms to grow as surface colonies which can be counted. Membrane filters may also be used for the isolation of pathogens from water and sewage and for the demonstration of tubercle bacilli in cerebrospinal fluid and other fluid specimens including sputa from cases of tuberculosis.

A. Apparatus

One of the most suitable consists of a cylindrical aluminum filter funnel graduated at 50 and 100 ml attached by means of a bayonetlocking device to the base of the apparatus which contains a disk of sintered glass on which the membrane is supported. The outlet is provided with a tap and fits into the rubber stopper of a suction jar. Membranes that have been used for the coliform count may be washed in running water, dried between blotting-paper and sterilized for further use. This may be done up to twelve times, but damaged membranes should always be discarded.

B. Sterilization

The filtering apparatus is assembled without the membrane, wrapped in kraft paper and sterilized by autoclaving at 212^{o}C for 15 min. thereafter, between each test, it is sufficient to apply a jet of live steam, or to immerse the apparatus in boiling sterile distilled water for 1 minute. Both the inner and outer surfaces of the funnel as well as its base and the sintered glass disk require to be sterilized in this way. The routine examination of large numbers of water samples is facilitated by the use of several funnels for each piece of apparatus. While one sample is being filtered, the spare funnels for subsequent samples can be sterilized and cooled. The membranes may be sterilized by one of two methods.

1. Gentle boiling in prefiltered distilled water on two occasions each of 20 min duration. Vigorous boiling tends to make the membranes buckle. This method not only sterilizes but washes out residual solvent and air present in the pores.
2. Autoclaving at 115^{o}C for 10 min. for this purpose bundles of 10 membrane filters are interleaved with disks of good quality absorbent paper (subsequently to be used for holding the liquid medium). The bundles are secured between two pieces of thin card held in position by adhesive tape and the whole is wrapped in kraft paper and sterilized.

C. Media for Culture on Membrane Filters

1.M-Yeast extract broth (for the enumeration of viable bacteria)made of:

Yeast extract	6 g	
Peptone	40 g	
Distilled water	to	1000 ml
PH		7.4

2. Membrane enriched Teepol broth (0.4 ET) (for estimations of Esch. coli)

D. Filtration

The following amounts are recommended:

Purified tap water	250-500 ml
Well water	10 and 100 ml
River water	1 and 10 ml

When the water has been filtered and a small amount of sterile distilled water allowed to pass through as a final rinse the funnel is removed and the vacuum released. The membrane is then transferred with sterile flat-bladed forcepts to a 2-in Petri dish containing a sterile absorbent pad, saturated with about 2 ml of the appropriate liquid

medium. The membrane should be placed on the moist pad in such a way as to exclude any air bubble. The Petri dish is then inverted with the pad and membrane adhering to the base and incubated in a moist atmosphere.

E. Incubation.
For total colony counts at 37^{o}C incubation for 18 h on yeast extract broth gives results that are comparable with the agar plate counts at that temperature. For colony counts at 22^{o}C incubation for 3 days is necessary. All the colonies that develop on the membranes are counted and the number of bacteria per ml of undiluted water may then be calculated. For membrane coliform counts, the cultures on 0.4 per cent enriched Teepol broth are incubated for 4 h at 30^{o}C followed by 14 h at 35^{o}C. The number of yellow colonies is then counted.

3.3.3 Summary of Membrane Filtration Method

- **Sterilise the filtration apparatus using burning alcohol**
- **Aseptically place the membrane (0.45 μm) onto the filter support using sterile pair of tongs**
- **Fit the filter setup into the filter flask**
- **Pour about 100ml of test sample into the filter system and filter using a pump. Rinse funnel with about 25ml of sterile water**
- **When filtration is complete, remove the funnel together with membrane filter.**
- **Remove membrane using sterile tongs.**
- **Place the membrane into the Petri dish containing the appropriate culture medium**
- **Incubate the Petri dish and contents at specified temperature eg at 37^{o}C for specified period of time eg 18-24 hours for toyal coliforms, but at 44^{o}C for for faecal coliforms.**
- **Depending on the culture medium used, count yellow, orange or grey colonies surrounded by halos.**

It mat be necessary to subculture and confirm the doubtful colonies on a confirmative medium.

4.0 PUBLIC-WATER-QUALITY AWARENESS

4.1 Water Quality Appreciation

A normal adult human being will recognise water as good or dirty. This water consumer relies on his or her senses to pass judgement on the quality of the water. The senses are appearance or colour, odour and taste. Water that is turbid, coloured or has an objectionable taste or odour may, or in fact should be regarded as unsafe and therefore can be rejected for drinking purposes. Tastes, odour and colour therefore may be the first indication of potential health hazard. However, everybody should know that the absence of any of these adverse sensory effects does not guarantee the safety of the water. The unseen dissolved components may be injurious to the human body. Some of these could be organic, for example pathogenic bacteria, or inorganics like cyanide and heavy metals. For such water containing toxic substances to be safe for human consumption, it has to be treated.

4.2 Demonstration of Water-Quality Improvement

Colour and turbidity in water are both caused by the presence of dissolved or suspended impurities. This can be demonstrated in schools, health centers and to the general public gatherings in social halls through the following simple experiments:

- **Sedimentation**. Brown water like that taken from river Nyabarongo, if left standing in a beaker for some time, will tend to clear as soil particles settle to the bottom of the beaker. Less heavy particles may remain floating on the surface. These solid particles are impurities in the water.
- **Filtration.** If the clear portion of the water in the above experiment is filtered using a filter paper, or a piece of fine cloth using a funnel, most of the insoluble dirt will be trapped on the filter paper or cloth. For large-scale production of

filtered water, sand, instead of a filter paper, is used. There are some homesteads that have some sand-filtration apparatus.

- **Evaporation and Distillation.** Pure water can be obtained from sea-water by evaporation or distillation using heat. The more volatile water is changed into steam by the application of heat. The evaporated water may be condensed back into water during distillation. The residue is a mixture of many soluble salts that were dissolved in water that makes the sea. This method cannot be used for the production of drinking water because it is expensive.
- **Desalination.** Another method of removing most of the dissolved minerals from sea water is desalination. Here the undesirable salts ions (cations and anions) are removed by ion exchangers: cation and anion exchangers. The exchangers can at the end of the day be regenerated using acids or alkalis. Cation exchanger will retain positively charged ions (cations) like Na^{+} and Ca^{2+}, while anion exchanger will trap and remove anions like SO_4^{2-} and Cl^{-}.
- **Field instruments** may include pH meter, turbidity meter, conductivity meter, comparator and portable spectrophotometer. The pH meter shows how acidic or alkaline the water is. Good drinking water should have pH between 6.5 and 8.3. The acidity of water cannot be detected visually. In place of a pH meter, litmus paper can be utilised for the purpose. Turbidity meter shows how heavily loaded the water is with suspended matter that cannot be filtered. It is then a measure of such impurities in water. Conductivity meter may indicate qualitatively and even quantitatively the amount of dissolved solids in the water. Comparators are used to measure colour and other parameters like nitrates, nitrites, ammonia etc that produce coloured substances if correct reagents are available. With right reagents, the spectrophotometer can be utilised to determine more accurately the quantities of many impurities in water that cannot be seen or estimated using simple instruments like the conductivity meter. These impurities include heavy metals like lead and mercury that have cumulative adverse health effects in man and animals.
- **Disinfection**. We have already noted that the clarity of water does not necessarily mean the water is safe for drinking. The microscopic pathogenic organisms may be present in the water. But they ca be introduced into the water by man if hygiene rules are not observed. The quality of the water can be improved by boiling for example. In Rwanda, many people are aware of 'sur eau' that is said to treat normal tap-water that is suspected not to be clean enough for drinking. This 'sur eau' or 'sure water' is just chlorine solution in water. Large water-treatment works the world over utilise disinfectants like chlorine or ozone that kill the dangerous micro-organisms. In nature, germs present in water may be destroyed by ultraviolet rays naturally present in sunlight. Portable bacteriological analysis apparatus can be used to demonstrate how the micro-organisms in water can be detected.

Communities should be reminded that the dirt (contaminants) we find in water comes from the environment through which the water flows. This environment includes man and his habitat. Man should protect the environment and water sources so that rainwater does not sweep the top soil with all the dirt into water bodies like rivers and lakes. We

must devise ways of disposing of our own wastes, domestic or otherwise, so that they do not find their way into rivers and lakes which are our sources of water.

PART II: QUALITY ASSURANCE PROGRAM

5.0. Introduction: Quality Assurance Program

In the development of this water-quality assurance program, the following issues will be discussed:

- Justification for the Quality-Assurance Program,
- Water Resources in Rwanda: Nile-basin river systems in Rwanda,
- Sources of Pollution,
- Review of Quality Assurance Capacities in Rwanda,
- Development of Water-Quality Assurance Program,
- Sampling Points and Frequency of Sampling and Testing,
- Analysis: Methods Used and Parameters to be Tested for,
- Proposed Additional Parameters to be Analysed for, and
- Public Sensitisation for Awareness

5.1. Justification of Quality Assurance Program

It appears the two terms quality assurance and quality assessment are generally erroneously used interchangeably as if the mean or imply the same thing. In essence, water-quality assurance would entail elimination of all possible sources of pollution of the water sources and courses, natural and human. Domestic, agricultural, industrial and

commercial wastes in this case would deliberately be prevented from finding their way into the water sources and courses. Soil erosion would totally be prevented from taking place through adequate afforestation and appropriate land use; and water would flow through, or be found in, an environment without materials that are harmful to plant, animal or human life.
Under the present circumstances, quality assurance and quality assessment and monitoring will be conveniently used interchangeably. The latter idea would simply involve
monitoring of the quality of the water through continuous testing for chemical and physical characteristics of the water.
In Rwanda, like in other countries in the region, waters are polluted by industrial, commercial, agricultural and domestic wastes. Industrial heavy metals like copper, lead, chromium, zinc, iron and manganese are found in water. The country is highly dependent on agriculture; and fertilizers and pesticides are extensively applied. Eventually, these find their way into the water-bodies. Mining activities for metals like tin, tantalum, niobium, tungsten and others also take place in many places in Rwanda; and wastes from these mines easily become pollutants of surface water especially.
Domestic human and animal wastes from rural or urban homesteads in Rwanda as a whole are not treated; and so pathogenic bacteria like faecal coliforms have been found in raw waters especially during rainy seasons. It is then no surprise that such epidemics as cholera have occurred from time to time in the recent history of the country.
For all the above reasons and more, it is imperative that water bodies in the country and other countries that are served by the same water system be regularly monitored for their physical, chemical and microbiological levels of contamination.

5.2. Water Resources in Rwanda

In order to appreciate the need to have in place a water-quality assurance program, we have to understand the type of water resources in our possession, and therefore the kind of environment of the resources. The environment will affect the water resources in terms of quality, the assessment of which is the sole objective of quality assurance program.
In Rwanda, Nile River basin constitutes 67% of the total landmass, and is home to 90% of the total national surface waters found in lakes, rivers and wetlands. All these waters collect into the Akagera River that drains into Lake Victoria, officially the source of River Nile.
The major tributaries of the Akagera River are rivers Nyabarongo, Akanyaru and Muvumba. The Akagera River is in the east, Muvumba in the north and Nyabarongo runs trough the middle of the country before it (Nyabarongo) changes its name to Akagera in the south-east. The main tributaries of Nyabarongo River are Mbirurume and Mwogo rivers in the western highlands of the country, and Mukungwa River in the north-easten highlands. The importance of this geographical description will be apparent when it comes to the idea of establishing sampling points, and perhaps of selecting water-quality monitoring laboratories. This suggests that there should be at least three sampling points on Nyabarongo, Akanyaru and Muvumba rivers.

There are two rainy seasons in a year in Rwanda: from March to May and then from September to November. These are the periods during which maximum pollution of the river systems by all sorts of pollutants in the surrounding areas. The seasons will dictate the times and frequency of sampling if this process is to be undertaken cost-effectively.

5.3. Sources of Water-Pollutants n in Rwanda

Rwanda's economy is based mainly on agriculture, that is, arable and stock farming. Pesticides and fertilisers are thus extensively used. The agrochemical residues and their biotransformation products find their way into water bodies, especially during the rainy seasons. High concentrations of pesticides, nitrates, nitrites, ammonia and phosphates are therefore expected in river and lake waters in the country. Rwanda is a hilly country. As such, soil erosion is prevalent especially in hilly deforested areas. The water is then expected to be highly turbid, coloured and loaded with exceptionally high quantities of iron and manganese, among the heavy metals, particularly during the wet seasons. The water bodies in low-lying areas in the eastern and north-eastern regions of the country become overly silted.

Domestic wastes of all kinds in municipal centres are not treated and sanitation in rural areas is poor. Micro-organisms from human and animal wastes, etc, are expected in surface waters during the rainy seasons.

There are many industries and commercial centres scattered all over the country. Industrial wastes include organic matter from coffee and tea factories, abattoirs, hotels, large-scale farms, food processing industries etc. As already mentioned, these wastes are discharged into rivers and wetlands untreated.

Heavy metals, oils and greases are mainly discharged from garages, workshops, printing works and mining sites that are found scattered all over the country.

This topic is raised here because the types of pollutants in any particular location will dictate the parameters to analyse for in a laboratory.

5.4. Review of Quality Assurance Capacities in Rwanda

A review of water-quality monitoring capacity in Rwanda in terms of infrastructure, equipments, materials and manpower necessary for was presented in previous chapter. It was pointed out that ELGZ alone has about ten laboratories scattered all over the country and the NUR has five. One can even include the Rwanda Bureau of Standards laboratory that is involved in setting up national standards, including those of drinking water. All these laboratories have reasonably competent analysts, most of who have been involved in water-quality assessment, though not necessarily on fulltime basis. The only institution that has been participating in water-quality assessment in the country is the laboratory formerly owned by the Ministry of Environment and Natural Resources, now MINITERE. The laboratory now belongs to the National University of Rwanda (NUR). It is believed that this institution will still serve the same ministry and the country at large, at the same time remaining at the university's service. They analyse for most of physical, chemical and bacteriological parameters of raw water, but mainly underground water for rural communities. However,no records for Nile Basin surface water-quality monitoring results.

The university has four other analytical laboratories at Butare in the southern region of the country. These laboratories are well equipped. They even have AAS and GC instruments. The university laboratories have the capacity to act as a regional centre for water-quality monitoring or assurance. The problem is that their analytical activities are research-oriented.
One of them carries out research on fish farming and need to analyse water for its nutrients contents. They particularly analyse for nitrates, phosphates, dissolved oxygen and organics such as chlorophylls and planktons.
There is the Environmental Chemistry laboratory that occasionally carries out water-quality assessment in some rivers nearby, including the Akanyaru River some distance away. Most of the water parameters are analysed for.
There is a Microbiology laboratory that occasionally performs bacteriological analyses on raw water in the surrounding areas.
Overall, manpower and laboratories are available. It would only be necessary to train the available manpower in analysing for parameters they normally do not analyse for, e,g, COD, BOD and pesticides, using new equipments they do not possess at the moment, such as AAS and GLC. If mobilised and their objectives to analyse water redirected towards water-quality monitoring on regular basis, the analysts can assist the Government of Rwanda and NBI in the business of water-quality assurance in the Nile basin. They have all it takes to produce analytical results that would be useful for coordinated quality assurance of the diverse transboundary waters of the basin. They would also adopt the habit of keeping records of the results obtained.
For modernisation of equipments for quality and reliable analytical results, however, it would not be too much to suggest the acquisition of new equipments like AAS, GLC, HPLC or both for analysis of heavy metals and such organics like pesticides. One can also add Oxygen-meter and modern COD and BOD equipments whose operations are less tedious for analysis for such parameters.
It is to be noted that there exists no water-quality assurance program in the country. Even the NUR laboratory in Kigali that was charged with the duty of water-quality monitoring does not have any established testing program, and so the activity is irregular. This has been exacerbated by the current unclear sense of belonging, either to the university or ministry.
Parameters analysed for, methods employed in analysis and the kinds of results obtained by each laboratory are summarised in the sections that follow.

5.5. Development of Water-Quality Assurance Program

As already mentioned, water-quality cannot be assured through water-quality testing alone. The quality of water can be assured if the water is totally protected from pollution, assuming that that water flows through clean environment that is itself free of pollutants. Good quality of water can therefore be ensured by preservation of the environment and protecting the water against pollutants which are in most cases man-made. In the process of water-quality assurance program development therefore, a brief section on protection of water from pollution has been added.

5.5.1 Sensitisation for Awareness-Creation

The government of Rwanda appreciates the value of having good-quality water for sustaining good human health in particular; but its efforts in assuring this quality may be hampered by limited resources. The government should therefore be encouraged and assisted in its efforts in sensitisation of the general public to conserve the environment through:

- Proper land-use through terracing hilly countryside for cultivation; not cultivating near river-banks or catchment areas; avoiding overgrazing etc.
- Afforestation, particularly of hilly areas, all in order to avoid soil erosion
- Proper disposal of sewage and other wastes: domestic, commercial, agricultural and industrial.

Everyone is a waste-producer. So every homestead should have a waste pit or compost in which to dispose of the litter. Once in a while the organic portion can be burnt off. The same homestead should in addition have a pit latrine.

Large institutions, like hospitals, colleges and universities, far removed from municipal centres need to have their own sewage treatment works on a small scale in form of oxidation ponds that will achieve at least partial waste treatment. The oxidation ponds should discharge the effluent into a distant water- body such as a wetland. Cities that cannot afford sewage treatment works can also use large oxidation ponds in a similar way.

Industrialists should be encouraged and advised on how to treat their wastes before they are released into the environment. For example, coffee and tea factories should have small oxidation ponds into which the effluent flows over cascades downstream of the factory before settling into the ponds. This allows the waste to dissolve enough oxygen that will be used in the oxidation pond for the waste biodegradation. A channel constructed downstream of the pond will release the wastewater with less oxidisable organic matter into the environment. The released effluent should be made to flow for a good distance before discharging into a water system.

Printing works, workshops and garages should also have waste-disposal pits where oils, greases and heavy metal wastes collect. The oils and greases may be burnt off. Metal products, after their natural chemical transformation, will percolate through the soil, their origin. Metal scrap should be collected together in one place and sold off to the abundant dealers for recycling. The collected and selected paper and other combustible organic materials are collected in a pit and are later burnt.

Pollution by fertilizers and pesticides is difficult to control. It was suggested in the 'Baseline Report Draft 25/05' that 'to reduce run-off of used pesticides, farmers should be encouraged to localize run-offs by use of ditches or channels ……which could then be used as sampling points ….'. These conduits, if made wide enough, would in addition act as oxidation ponds since most organic pesticides are biodegradable into less toxic products. Inorganic fertilizers would naturally disappear into their natural soil home, but would spare the surface water for a while when man devises more sophisticated methods for dealing with them.

Industrialists involved in mining must arrest their wastes in concentration pits for a while so that if possible they can reuse them, for example for refilling mined areas. Maybe the concentrates can find other uses somewhere else.

Sensitisation of the public for the above activities may be done through rallies involving the general public including community and government leaders at all levels, through newspapers, radio and television, at least during the months of June-August to December and then March of the following year just before the onset of rainy seasons. Every one of us must be made to be responsible for wastes that one produces and for the environment one lives in. The government should be assisted to promulgate environmental legislations and implement them so that the polluter of the environment is penalized according to the established law.

5.5.2 Q.A. Program: Sampling Points, Sampling and Frequency of Sampling and Testing

Under this activity, it will be assumed that the water sample collected will be transported immediately to the laboratory where it will be analysed. Probably the analysis will not be performed immediately on delivery. In this case then, there must be preservation of the sample. It has already been pointed out that the commonest method of water sample preservation is refrigeration, perhaps after pH adjustment if that is necessary. Ice boxes are available for transporting cooled samples if it is necessary, especially if such parameters like dissolved oxygen have not been determined at sampling point. Parameters that should be tested for at the sampling point include temperature, pH and dissolved gases like oxygen and carbon dioxide.

Sampling from a river should be done some distance from the river-bank to avoid as much as possible the water that has settled out of the main stream. Such water loses some properties characteristic of the whole river water. The sample should be taken from below the surface of the water, into plastic or glass container that has been rinsed several times with the water to be sampled.

Siltation is not a serous problem in Rwanda, a country that is generally hilly. It appears not a single test has been done on a silt sample of any river in the Nile Basin. But some siltation does occur in the Nyabarongo river valley near Kigali city, and also in the Akagera National Park along the Akagera river valley where a few lakes were formed long time ago, probably as a result of siltation of the Akagera river. Silt samples can be taken from each side of the river during dry seasons.

It would be ideal to take samples for analysis from at least four tributaries of River Nyabarongo, i.e. Mwogo, Mbirurume, Mukungwa and Nyabugogo because these rivers have waters with widely differing characteristics, at least from their physical appearance. Again, it would be better to also sample and analyse waters from the Akagera river and its other tributaries Akanyaru and Muvumba rivers in order to understand the totality of River Akagera water characteristics before it empties itself into Lake Victoria. For economic reasons, we propose four sampling points on rivers Akanyaru in Butare at the bridge connecting Rwanda and Burundi; Akagera at Rusumo at the border with Tanzania; Nyabarongo at Kanzenze and Muvumba at Kagitumba at the border with Uganda. Hydrological stations are found only at Kanzenze and Rusumo on rivers Nyabarongo and Akagera respectively. If found unnecessary, the Muvumba sampling point can be omitted because the confluence of Akagera and Muvumba rivers is almost in Uganda, and so water-quality assessment can be taken care of in Uganda.

Intuitively, the sampling frequency should be dictated by, among other things, rainy seasons. It is during the rainy seasons that water characteristics change appreciably due to soil erosion and large quantities of pollutants swept into rivers and other water bodies. Consequently, it is suggested that sampling takes place at least twice each month of April and May and then October and November. During the other months of dry season, sampling can take place once every month. Specifically, sampling of sediments can be done during the dry months of January-February and July-August. If prevailing circumstances allow, the frequency of sampling can be doubled.

Table II.1: A summary of minimum sampling frequencies at sampling points on the rivers indicated:

Period / **Sampling Point**	**April**		**May**		**October**		**November**	
Nyabarongo at Kanzenze	Week 1	Week 3	Week 2	Week 4	Week 1	Week 3	Week 2	Week 4
Akanyaru at Butare	Week 1	Week 3	Week 2	Week 4	Week 1	Week 3	Week 2	Week 4
Akagera at Rusumo	Week 2	Week 4	Week 1	Week 3	Week 2	Week 4	Week 1	Week 3
Muvumba at Kagitumba	Week 2	Week 4	Week 1	Week 3	Week 2	Week 4	Week 1	Week 3

Table showing Sampling Points and Sampling Periods. The Table given in the Introduction in Part I should be consulted on storage and preservation of samples before analysis.

5.5.3 Analysis: Parameters and Methods of Analysis.

Sampling, storage and preservation of collected samples have already been outlined. The next stage is analysis of the laboratory sample. Detailed methods of analysis recommended for use nationally are described in Modules 1 and 2 and 3.

TableII.2 Parameters analysed for and methods that have been used in most of the laboratories in Rwanda

Parameter	**Method of Analysis**	**Reference**
pH	pH Meter	Official Methods of AOAC
Turbidity	Turbidity Meter	HACH Manual
Colour, True and Apparent	Platinum-Cobalt standards	HACH Manual
Total Dissolved Solids	Filtration and Evaporation	Official Methods
Suspended Solids	Colorimetric	HACH Manual
Acidity	Titration with a base	Officia Methods of AOAC

Alkalinity	Titration with acid	,,
Hardness	EDTA Titration	,,
Total Nitrogen	Kjeldahl digestion	,,
Ammonia Nitrogen	Nesslerisation / Titrimetry	,,
Nitrate Nitrogen	Colorimetric with brucine	,,
Nitrite Nitrogen	Diazotisation, Spectrophotometric	HACH 2400 manual
Fluoride	Colorimetric	Official Methods
Carbonate and Bicarbonate	Titration with acid	,,
Phosphorus	Photometric	,,
Sulphate	Turbidimetric	,,
Chloride	Titration with mercury nitrate	,,
Silica	Spectrophotometric	HACH Manual
Dissolved Oxygen	Azide Method	Official Methods
Chemical Oxygen Demand	Titration with Dichromate	,,
Biochemical Oxygen Demand	Comparision of O_2 content of sample at beginning and end of incubation for 5 days	,,
Manganese	Oxidation by Na periodate, colorimetric	HACH Manual
Iron	1,10-phenanthroline, spectrophotometric	,,
Zinc	PAR, specrtophotometric	,,
Copper	Porphyrin, spectrophotometric	,,
Barium	Sulphate precipitate turbidimetry	,,
Chromium	1,5-diphenylcarbohydrazide, spectrophotometric	,,
Cadmium	Cadion, spectrophotometric	,,

5.5.4 Reporting and Storage of Results, and Role of NBI in W.Q.A.

Whenever possible, at least three tests should be performed on a given sample, and the average of the three taken. The results should be recorded as soon as they are obtained, and on well-designed forms. Different institutions involved in analysis of water have their own forms on which they record their results. When NBI gets directly involved in supervision of water-quality monitoring, it is suggested that an appropriate standard reporting form be designed and its use by the institutions that will be participating in analysis of water for the purpose of water-quality assurance enforced. The forms can be saved in hardcover files and the files stored in safe shelves, the custodian of which is the head of the laboratory. The same results should also be saved on a computer and flash-discs. In whichever form the results are stored, the access to them should be protected from unauthorised users as much as possible and only limited to fellow participants in water-quality assurance program.
The NBI should in future design its own quality-assurance program document containing standards, policies and procedures for activities related to the collection, processing, storage, analysis and publication of surface-water data and review of reports, safety and training of manpower. This program would serve as a guide to all national, regional and

basin-wide personnel involved in surface-water activities. Regular updates of these activities should be part and parcel of the quality assurance process.
NBI should produce a list of potential chemical contaminants of known origins that are used habitually in the region, in one way or other, that are likely to contaminate surface waters of the Nile basin. In the program, mention should be made of water-quality objectives that match specific parameters that are more stringent than national or regional objectives. NBI should be in position to judge when to or not to release the publication of test results to individual governments and the general public.

6.3.4 Recommended Additional Parameters

These parameters include:

- COD
- BOD
- Other heavy metals
- Analysis for or screening for pesticides in general
- Microbiological analysis for persistent micro-organisms
- Soaps and Detergents
- Oils and Greases
- Biological materials like green algae, planktons and chlorophylls.

COD and BOD parameters are only occasionally analysed for by only the NUR laboratory. This particular laboratory has instruments that are simple to use, perhaps the simplest ever seen, for the determination of the parameters. However, it is not yet clear if their accuracy and reproducibility are yet established or are reliable. They do not have results of analysis for these parameters of their own anywhere on record. Other laboratories do not carry out these important analyses. Any laboratory carrying out surface-water-quality monitoring on behalf of the NBI should be able to analyse for these extra parameters.
Other **heavy metallic elements** like lead, mercury and arsenic are abundantly present in the environment especially in surface waters and may have unimaginably adverse consequences on human and animal health for direct consumers of the water. These three elements should also be analysed for. The methods of analysis are available in the **HACH 2400 Spectrophotometer** manual. Probably the AAS method would be better.
Additional **microbiological analysis** for human pathogens that are agents of high health significance should be carried out on water. In particular, tests for the more environmentally persistent micro-organisms like *salmonella,Yersinia enterocolitic;, entamoeba histolytica, giardia intestinalis, cryptosporidium parvum; enteroviruses and hepatitis A* should be screened for in water, at least by regional laboratories.
Screening surface-water for **pesticides** in general can and should be carried out by the national laboratory on behalf of NBI. Many pesticides can be extracted from water using cheap reagents like pentane, hexane or dichloromethane. Thin-Layer Chromatography can then be utilised to qualitatively detect the presence or absence of organophosphate, organochloride or carbamate pesticides in water. More sophisticated instruments such as GC, GLC or HPLC would be required for quantitative determination of the pesticides. NUR laboratory should be able to do the analysis for these dangerous impurities.

Detergents or surfactants such as straight-chain or branched-chain alkyl benzene sulphonates can be analysed for using the method available in the HACH 2400 Spectrophotometer Manual at national or regional level. The ELGZ laboratory can carry out this analysis.
Oils and greases in water can be determined at a national laboratory as '**Total Petroleum Hydrocarbons'** employing the analytical methodology described in the HACH 2400 Spectrophotometer Manual. The ELGZ laboratory should be able to do this analysis.
One of the laboratories of the National University of Rwanda has done some research on Lake Ihema, partly concerning **biological parameters** like phytoplanktons and chlorophyll. It is possible they can also carry out studies on algae in general. There is a particular kind of algae that is sometimes called blue-green alga that is suspected to produce dangerous toxins known as *hepatotoxins*. These blue-green algae are just a collection of 'cyanobacteria blooms' that are found in fresh-water lakes, the types that are found in eastern Rwanda along the Akagera River valley. The river- and lake-waters do sometimes mix during flood times; and the presence of such bacteria should be confirmed as the river leaves the last lake downstream.

6.0 CONCLUSION AND RECOMMENDATIONS

Water-quality monitoring and training for water-quality assurance capacities in Rwanda have been reviewed, technical training modules together with water-quality assurance program developed, and additional requirements in terms of equipments and manpower training exposed. The remedial action to be taken to eliminate the shortfall in these requirements is proposed in form of recommendations. Recommendations will be made on the following topics:

- Training
- Water-Quality Assurance Program (W.Q.A.P.)
- Acquisition of Equipments and Materials
- Design Awareness Campaign Program

1. Overall Training.
Two local experts, one biological / bacteriological analyst and the other a chemist should be identified and appointed for training all analysts and technicians in analysis for water-quality assurance. NBI should reach a memorandum of understanding with ELGZ, NUR and the other institutions so that each contributes at least two people for training for at least three months. All trainees should assemble in the suggested central training place of NUR laboratory in Kigali. They should be trained in analysis for all parameters, the emphasis being placed on the parameters they are not used to, such as COD, BOD, heavy metals using AAS, pesticides using GLC or HPLC, bacteriological analysis for more persistent microbial agents, soaps and detergents, fats and oils, and biological quality of water. The second objective for training is using harmonised and standard methods acceptable nationally and approved by NBI, probably as developed and presented in Modules 1 to3 in this report, in order to have comparable results of analysis and realistic characteristics of the basin waters. After training, some of them can form the backbone of the manpower for the national laboratory in charge of water-quality monitoring and

assurance. They will all later train others in their respective laboratories. The training should include sampling, storage, preservation and analysis of surface water and results-report making, interpretation of results and record-keeping. Statistical treatment of results of analysis should also be taught to the trainees for them to appreciate the accuracy and precision of their findings.

2. Quality Assurance Program.
The WQAP presented in thus report should be implemented wholesome or revamped as appropriate to match financial standing of the organisation. For example the sampling points and frequencies may be increased to improve on quality monitoring. A system of keeping records of results obtained should be established by the national or regional laboratory selected for water-quality management for ease of reference, and the exercise made a culture by the analysts involved.

3. Equipments and Materials.
The HACH 2400 SPECROPHOTOMETER and its manual are very useful for a water analytical laboratory. In fact it can be used for analysis of all parameters in Module 1 and 2, but not pesticides, COD or BOD. While AAS and GC are being awaited, this instrument and the accompanying reagents is a must-have, although admittedly it probably was originally designed for use by robots since the worker does not have to know what reagents are being employed in the analysis. In addition, DO meter, COD and BOD sets like those used at the NUR laboratory should be acquired because their operations are les tedious than those traditional methodologies described in Module 2, although the in-charge at the NUR laboratory seems to prefer the said traditional methods.

4. Awareness Campaign Program.
Last but certainly not least of all, it would be good to design a sensitisation campaign program and implement it in collaboration with the Rwanda government to develop awareness by the general public about the need, and promulgate environmental legislations, to conserve the environment in order to protect our water from avoidable pollution. This is the primary obligation every individual should have if the quality of water is to be assured.
Community institutions like schools, health centres and development associations should be reached for sensitisation on the need for sanitation in order to keep our water sources safe from pollution. Simple intervention methods of improving the quality of water, such as those described in section 4.2 above should be internalised by all community members who should adopt them as a culture for obtaining safe drinking water.