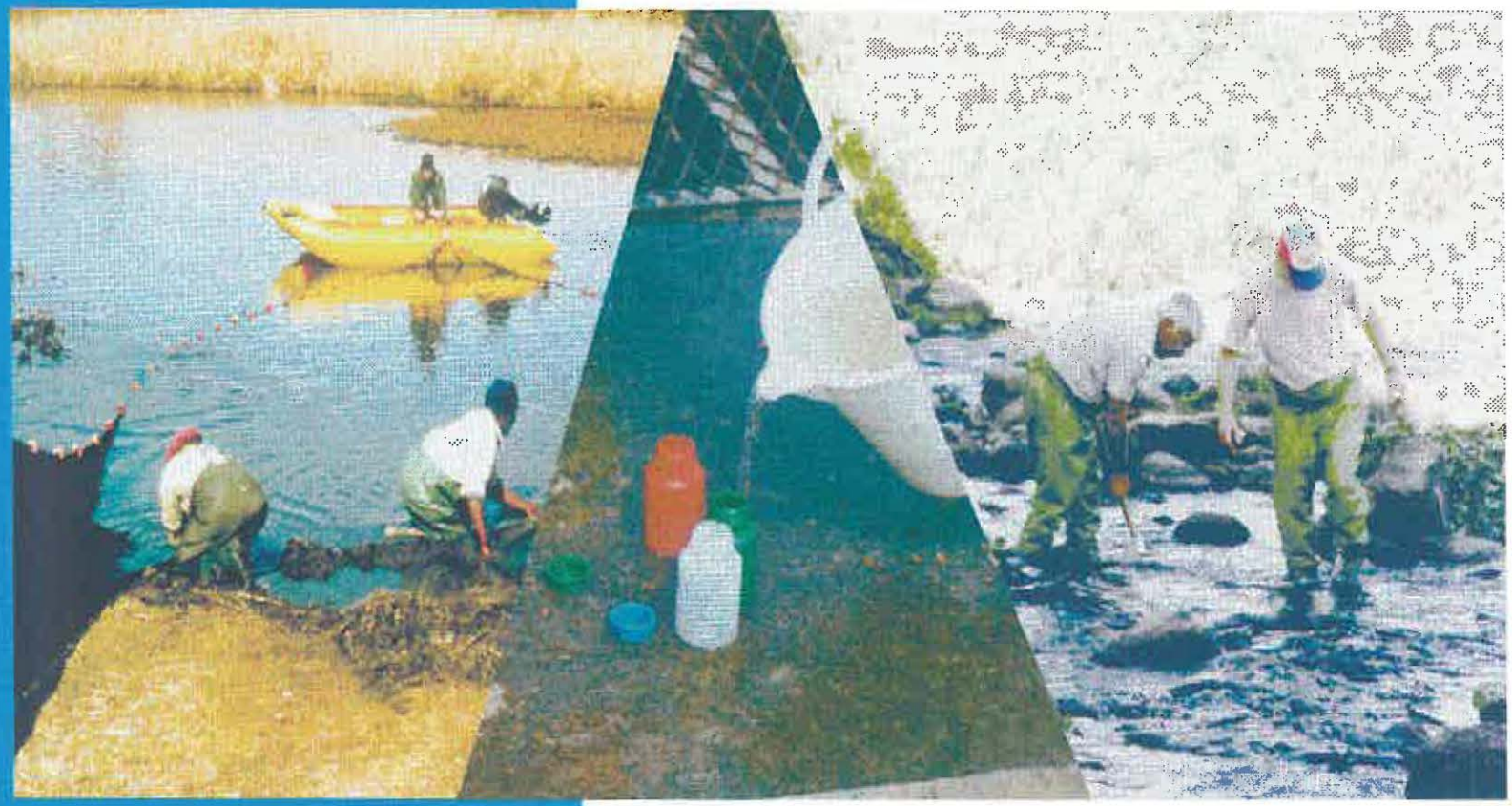
WATER QUALITY SAMPLING MANUAL FOR THE AQUATIC ENVIRONMENT



INSTITUTE FOR WATER QUALITY STUDIES
DEPARTMENT OF WATER AFFAIRS AND FORESTRY

IWQS

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1. PURPOSE OF WATER QUALITY MONITORING

Water quality management may be defined as an effort to control the physical, chemical, and biological characteristics of water. The efforts are directed at controlling the impact of society upon the quality of water. Water quality monitoring is therefore an effort to obtain quantitative information on the physical, chemical, and biological characteristics of water via statistical sampling. The type of information sought depends upon the objectives of the monitoring network. Objectives range from detecting stream standard violations (compliance monitoring) to determining temporal water quality trends for strategic planning.

The main reason for the assessment of the quality of the aquatic environment has been, traditionally, the need to verify whether the observed water quality is suitable for its intended uses. The use of monitoring has also evolved to determine trends in the quality of the aquatic environment and how it is affected by the release of contaminants, other anthropogenic activities, and/or by waste treatment operations. It is also used simply to check whether any unexpected change is occurring in otherwise pristine conditions (Chapman and Hall, 1992).

General definition for *monitoring* is that the water quality assessment operation is a long-term, standardised measurement, observation, evaluation and reporting of the aquatic environment in order to define status and trends (Chapman and Hall, 1992).

Data are principally collected at given geographical locations in the water body, often described by the longitude and latitude of the sampling site (x and y coordinates) and further characterised by the depth at which the sample is taken. Monitoring data must also be characterised and recorded with regard to the time at which the sample is taken or the *in situ* measurement made. Thus any physical, chemical or biological variable will be measured as a concentration, or a number.

No assessment programme should be started without critically scrutinising the real needs for water quality information. Since water resources are usually put to several competing beneficial uses, the monitoring should reflect the data needs of the various users involved.

Consequently, there are two different types of monitoring programs (Chapman and Hall, 1992):

(i) *Single-objective monitoring* which may be set up to address one problem area only. This involves a simple set of variables such as total dissolved solids (TDS), cations and anions for salinisation, nutrients and chlorophyll pigments for eutrophication, various nitrogenous compounds for nitrate pollution, or sodium, calcium, chloride and a few other elements for irrigation, etc.

(ii) *Multi-objective monitoring* which may cover various water uses such as drinking water supply, industrial manufacturing, fisheries or aquatic life, thereby involving a large set of variables.

Maintenance of the fitness for use of South Africa's water resources on a sustained basis is the Department of Water Affairs and Forestry's major overall water quality management goal. The fitness for use concept implies the evaluation of water quality in terms of the requirements of a particular user or categories of users. It is usually measured against water quality criteria, norms or guidelines that have been established as representing the ideal water quality for a particular use.

This implies that most water quality monitoring is performed as a result of government attempting to control (or manage in an equitable manner) the economic externalities associated with water quality degradation.

The South African Water Quality Guidelines serve as the primary source of information for determining the water quality requirements of different water uses and for the protection and maintenance of the health of aquatic ecosystems.

These guidelines form an integral part of the water quality management strategy to maintain South Africa's water resources fit for use. The guidelines are presently divided into two sets:

1. Water Quality Guidelines of Fresh Water
   - Volume 1: Domestic Water Use
   - Volume 2: Recreational Water Use
   - Volume 3: Industrial Use
   - Volume 4: Agricultural Use: Irrigation
   - Volume 5: Agricultural Use: Livestock Watering
   - Volume 6: Agricultural Use: Aquaculture
   - Volume 7: Aquatic Ecosystems
   - Volume 8: Field Guide

2. Water Quality for Coastal Marine Waters
   - Volume 1: The Natural Environment
   - Volume 2: Recreational Use
   - Volume 3: Industrial Use
   - Volume 4: Mariculture (the effects and target values related to *human health* also apply to the *collection of seafood* along the coast)

This *Water Quality Sampling Manual* is intended to be used by the IWQS personnel as a practical field guide to the sampling of the aquatic environment. It accommodates routine as well as special sampling techniques which are currently practiced at the IWQS. Each technique includes short background information, equipment needed, and the necessary procedure. The sampling and handling requirements for each variable are summarised in Appendix A with page references.

2. REGISTRATION OF MONITORING PROGRAMS

2.1 BACKGROUND

The objective of registering monitoring programs is to ensure the incorporation of all monitoring activities and the generated monitoring results into the current electronic data handling and storage facilities. This will ensure access to all data generated within the Department of Water Affairs and Forestry and related monitoring information.

The current electronic data handling and storage facilities include the following:

1. WQIMS - Water Quality Information Management System. The purpose of this system is to incorporate and consolidate all monitoring programs and activities and to facilitate the operation of a number of monitoring activities simultaneously.

2. HIS-QDB (Hydrological Information System - Quality Databank). The central databank for all departmental and related monitoring results.

2.2 INFORMATION REQUIRED WHEN REGISTERING A MONITORING PROGRAMME

The following information is required with the registration of a monitoring programme:

1. Name of the programme

2. Description of the monitoring programme
   - purpose of the monitoring programme
   - the information expectations

3. Custodian
   - person responsible for the design and implementation of the programme

4. Other interested parties
   - data users or client

5. Operational responsibilities
   - the persons responsible for the sample and data collection at the various stations identified in the programme
   - laboratories responsible for the analysis of the samples address, telephone numbers of the contact persons
   - The responsible laboratories should supply the IWQS with the analytical methods that are used

6. Identification and incorporation of sampling stations
   Each sampling station should be accompanied with the following information:
   - a *station number* if it is already part of the national flow gauging or water quality monitoring networks

- a copy of a *1:50 000 map* indicating the location of the sampling station
- the *latitude and longitude* of the site
- a *description of the site*,
- with the *name of the water body*

7. Variables to be included
   - Identify all the variables to be analyzed at the identified monitoring stations. (Consult the appointed laboratories for the variables and the methods used)

8. Frequency of sampling for the identified variables

9. Monitoring actions
   - Type of samples and method of sample collection to be used

10. Duration of the monitoring programme
    - Start date
    - End date

The IWQS will then register the requested monitoring programme and the relevant parties will receive the following information:

1. A project name and number
2. Station numbers for all the requested sites
3. The programme will be incorporated and consolidated with existing monitoring activities
4. Sampling schedules will be sent to the identified personnel responsible for sample collection
5. The generated data will be available from the HIS (QDB) if the analysis was conducted at the IWQS. The data generated at other laboratories will be transferred to the HIS (QDB).

2.3 REQUEST FOR DATA FROM THE HIS (QDB)

Requests for data from the HIS can be sent directly to J Wentzel, either by fax (012) 326 1488 or by letter (Patterson K536, Private Bag X313, Pretoria, 0001). This office can also provide an inventory of all the monitoring stations in a specific drainage region (primary, secondary, or tertiary, etc.), from which specific stations can be selected for that area.

The following information can be provided:

- water quality sampling results
- summary statistics of the sampling results
- monitoring frequencies
- station location and site description

The data can be downloaded for a specific period:

- for a single station
- for a number of stations
- per drainage region (primary, secondary or tertiary)
- for a list of stations (batch file)
- according to latitude and longitude
- according to the project identification code on the WQIMS system as registered at the IWQS

Data can be provided in the following formats:

- A file in an ASCII format, which can be imported into most computer software packages. This includes Quattro Pro, Excel, MSWord, Word Perfect, Statgraphics and Statistica. This file can be obtained by e-mail (if not too big) or on disc.

3. SAMPLING REQUIREMENTS

3.1 INTRODUCTION

The four basic factors which affect the quality of environmental data are sample collection, preservation, analysis and data recording. Improper action in any one of these areas will result in poor data from which poor judgements are certain to follow.

The collection of samples is the first step towards providing water quality information. Obtaining representative samples and maintaining their integrity are critical parts of any monitoring programme. Analytical methods have been standardised, but the results of analyses are only as good as the sampling and the sampling preservation methods. The objective of sampling is to remove a small portion of an environment that is representative of the entire body. The portion of material must be small enough in volume to be transported conveniently and handled in the laboratory. Once the sample is taken, the constituents of the sample must not deteriorate or become contaminated before the sample reaches the laboratory. The length of time that these constituents will remain stable is related to their individual character and the preservation method used (Badenhorst, 1994).

The sampling technique is determined by the type of matrix to be sampled. This manual primarily addresses the following sample types:

- Surface water,
- sediments,
- drinking water,
- industrial effluents,
- and biota.

3.2 GENERAL CONSIDERATIONS

Most definitions of water quality are user related. Most users produce waste waters containing pollutants which impact the environment in different ways. The broad spectrum of ground water, surface waters, lakes, estuaries, coastal waters, municipal wastes, industrial waste waters and surface run-offs make monitoring of water quality a formidable task.

The sampling network forms the basis of a water quality monitoring program. It must be performed properly to assure valid data.

(i) When selecting the location of sampling sites, the following must be considered (Badenhorst, 1994):

- Homogeneity of water
- general characteristics of water
- pronounced water quality degradation
- flow measurement
- location representative of site
- convenience, accessibility and practicability.

(ii) To obtain representative water samples, the following should be recognised (Gerber and van Deventer, 1996):

- A clear distinction should be made between sampling techniques performed in flowing waters (rivers, canal etc.) and stationary waters (reservoirs, lakes, etc.).
- When sampling rivers and canals it is essential to take representative samples of the component of the water column, stationary water should be avoided. The exception may be drought related monitoring programmes where the river puddles could be sampled to evaluate the impact of drought conditions on the aquatic fauna and flora.
- In a river with an existing measuring weir, the ideal place to take a sample is directly downstream of the weir (Figure 1). It is important to take turbidity samples where the water is most turbulent (water flowing fast and is well mixed).
- The same principles apply in a river where there is no measuring structure. Ensure that a representative water sample is taken from the main stream of the moving water column, or as close as possible to where the water is still thoroughly mixed and flows fast (Figure 2).
- Reservoir samples differ from river samples, because monitoring in a dam continues regardless of whether there is an inflow into the water body or not. Samples should never be taken next to the dam wall or the shore. Point Q01 is always opposite the middle of the dam wall and about 50 meters away from the wall (Figure 3). Other monitoring sites are chosen according to the designing needs of the specific programme and could differ from reservoir to reservoir.
- Samples should preferably always be taken at the same place and, if possible, at the same time of the day.
- Sample with bottle mouth facing upstream to avoid contamination.

Once collected, water samples must be analysed immediately or stored in a container with a preservative (where applicable) to maintain the integrity of the sample. Complete preservation is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that take place in a sample after the sample is removed from the parent source.

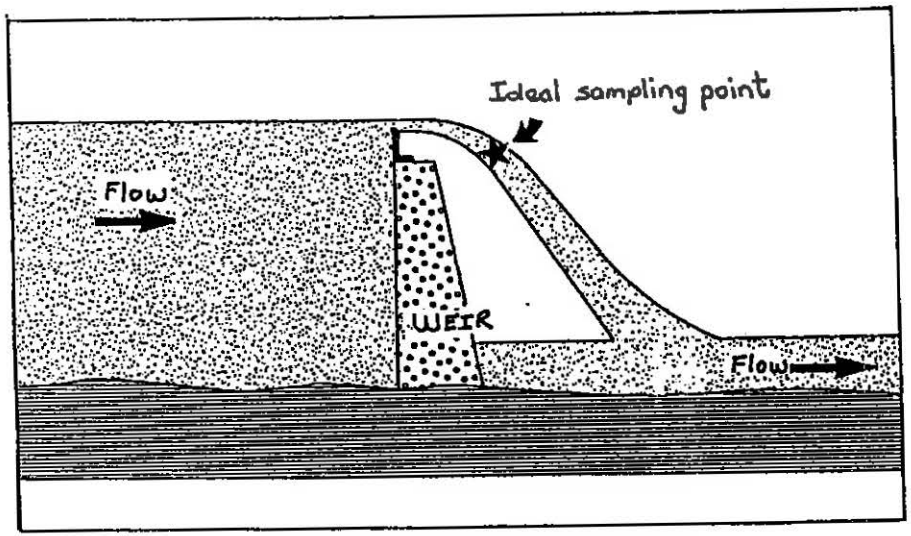

Figure 1: Ideal sampling point at a river flow measuring weir (Du Plessis and van Deventer, 1993).

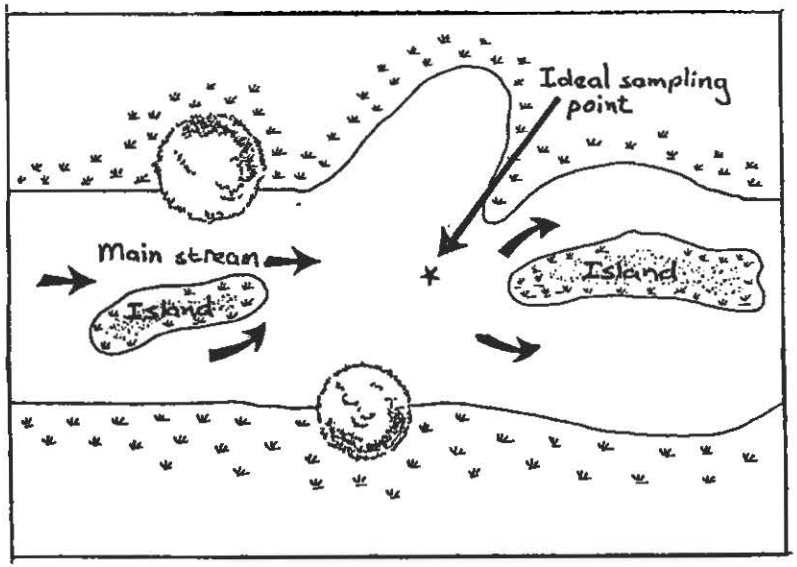

Figure 2: Ideal sampling point in a river, where there is no measuring structure (Du Plessis and van Deventer, 1993).

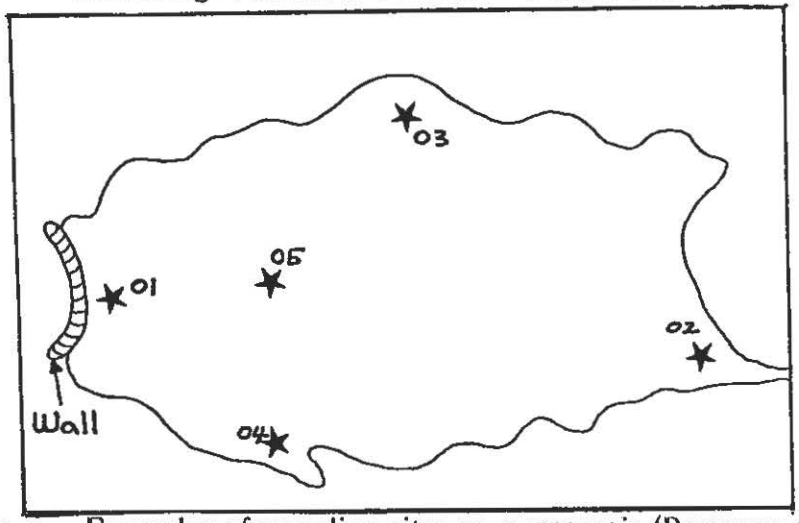

Figure 3: Examples of sampling sites on a reservoir (Rossouw and Badenhorst, 1987).

(iii) Methods of preservation are relatively limited and are intended generally to:

- retard biological action,
- retard hydrolysis of chemical compounds and complexes, and
- reduce volatility of constituents.

(iv) Preservation methods are generally limited to:

- chemical addition,
- pH control,
- refrigeration, and
- freezing.

Combinations of these methods are often used for the preservation of the water sample.

3.3 SAMPLE CONTAINERS AND SAMPLE LABELS

The type of sample container used is of utmost importance. Containers for water samples are made typically of plastic or glass, but one type of material may be preferred over the other. For example, silica and sodium may be leached from glass but not plastic, and trace levels of metals may adsorb onto the walls of glass containers. Glass containers are preferred with samples containing organic compounds.

The identification of samples is essential and therefore a label is used on which basic information is filled in. Labels are printed on special water resistant paper. This label accompanies the water sample to the laboratory. The label is filled in with a waterproof pen immediately after the sample is taken and tied to the neck of the bottle with a piece of string. These labels can be obtained at the sample reception laboratory of the IWQS.

(i) The following information must be filled in by the sampler (Figure 4):

- station number, always use the departmental station numbers; example C2H018Q01 (river station number) or C2R001Q01 (reservoir station number),
- name of the dam or river sampled,
- name of location,
- date and time of collection,
- state whether or not the sample has been preserved,
- sampling depth,
- gauge plate reading when available, and
- name of sampler.

(ii) Collection of consecutive samples at the same monitoring site:

- If more than one sample is collected at the same monitoring site, at least a minute's difference should be filled in on the label.
- Water samples collected at the same site but at different depths or different types of samples at the same site.
- For example 10:05 and 10:06 to give each sample an unique identity.

3.4 SUBMISSION OF SAMPLES

The samples should be submitted at the sample reception. An *analysis request form* should be completed for each type of analysis (macro, trace, biological) with the following information:

- number of samples
- project name
- project code
- date of collection
- client name
- contact person, telephone number and address
- specify if samples are filtered or not, preserved (type of preservative) or not, data listed, data on floppy
- laboratory (macro, trace, turbidity or biology)

If the sampling was an once off event or if the project is not registered at the IWQS, then an *analysis request form for ad hoc* samples should be completed, with the above information.

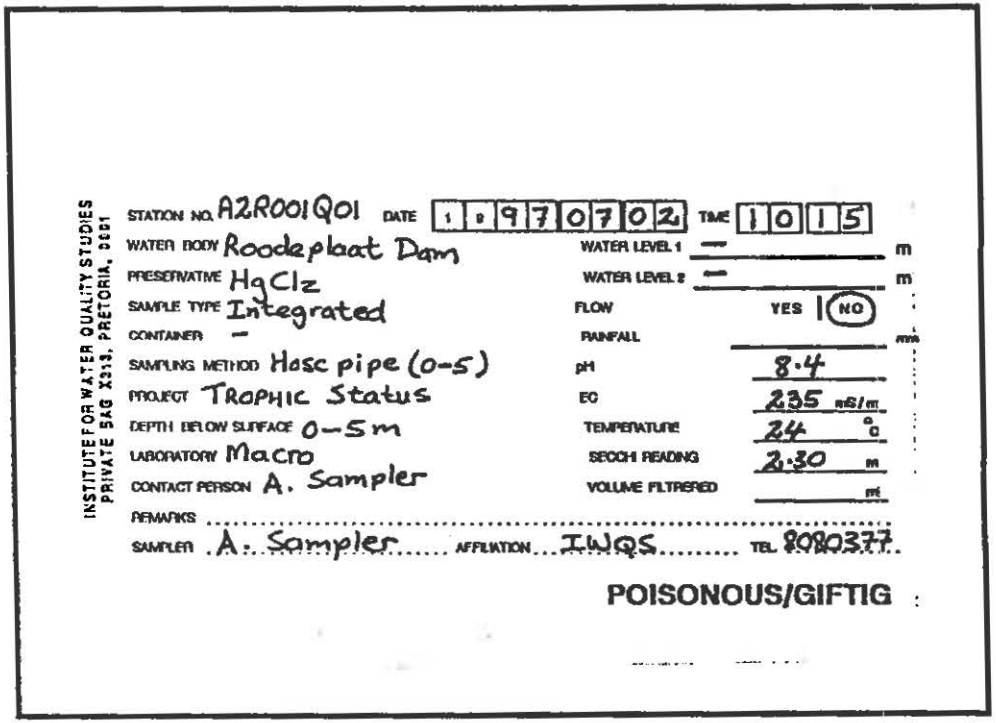

Figure 4: Label for the identification of a water sample.

4. SAFETY AND HEALTH

4.1 INTRODUCTION

It is important to remember that accidents occur easily and unexpectedly. While safety is often not considered an integral part of routine sampling, the sampler must be aware of unsafe working conditions, hazards connected with the operation of sampling gear, and other risks.

4.2 GENERAL PRECAUTIONS

Basic good practice should be followed both in the field and in the laboratory. These practices should be aimed at protecting the staff from physical injury, preventing or reducing exposure to hazardous or toxic substances, avoiding interference's with laboratory operations, and producing valid data.

Field personnel should be able to swim. Waders should always be worn with a belt to prevent them from filling with water in case of a fall. A life jacket at dangerous wading stations is advisable if one is not a strong swimmer, because of the possibility of sliding into deep holes. Always wear shoes, preferably boots with non-slip soles when not working in the water. In the summer season make sure to have enough drinking water and wear a hat and sunscreen.

Many hazards lie out of sight in the bottom of lakes, rivers and streams. Broken glass or sharp pieces of metal embedded into the substrate can cause serious injury if care is not exercised when walking or working with the hands in such environments. Infectious agents and toxic substances that may be absorbed through the skin or inhaled may also be present in the water or sediment. Make sure to wear hand gloves and to wash hands when samples were taken from sewage effluent.

Personnel must consider and prepare for hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment.

Prior to the sampling trip, personnel should determine that all necessary equipment is in safe working condition and that the operators are properly trained to use the equipment.

Safety equipment and a first aid emergency kit should be available in the field at all times. When sampling from a boat, nobody is allowed to go out on a boat alone. Both personnel on the boat should know how to handle a boat and to swim. Life jackets should be worn and fire extinguishers should be on board at all times.

4.3 SAFETY EQUIPMENT AND OPERATIONS

Necessary and appropriate safety equipment such as waders, gloves, hats and sunscreen should be available. First aid emergency kits, fire extinguishers and blankets should be readily available.

Supplies should be available for cleaning of exposed body parts that may have been contaminated by pollutants in the water. Soap and an adequate supply of clean water or ethyl alcohol may be suitable for this purpose.

At least two persons should be involved in all field collecting trips and no one should be left alone while in the field.

All surface waters should be considered potential health hazard due to toxic substances of pathogens and exposure to them should be minimised as much as possible. Exposed body parts should be cleaned immediately after contact with these waters.

Persons working in areas where poisonous snakes may be encountered should carry a snake bite kit and be familiar with its use. Any person allergic to bee stings or other insect bites should take proper precautions and have any needed medications handy.

It is also useful to have a cellular phone available when visiting remote areas.

4.4 DISEASE PREVENTION

- Wear masks when sampling at sewage works where the waterspray can get into your face.
- It is a safe practice to wear surgical gloves when sampling effluent from sewage works.
- Take malaria tablets when entering high risk areas.
- Personnel, who may be exposed to water known or suspected to contain human wastes, should be immunized against tetanus, hepatitis, typhoid fever, and polio.
- Have yourself tested for bilharzia every six months.

5. MEASUREMENT OF PHYSICAL PROPERTIES OF WATER

5.1 INTRODUCTION

This section deals with the measurement of the physical properties of a sample or water body. The determinations included are:

- Oxygen
- temperature
- pH
- electrical conductivity
- turbidity
- light penetration

5.2 OXYGEN

5.2.1 Background

Dissolved oxygen (DO) is a fundamental constituent of natural waters and is "probably the most important measure of water quality" (Vesilind & Peirce, 1983), at least for aquatic life. Aquatic life is so dependent on DO that low concentrations can indicate the unsuitability of the aquatic habitat in the absence of any other information. The physiological efficiency of aquatic species such as fish is reduced when dissolved oxygen levels decrease in the water column. The depletion of dissolved oxygen in the water body may cause fish kills. The oxygen requirements of fish are influenced by a variety of factors, which include species, size, age activity, physiological condition, nutritional status, temperature and solutes in the water (Vesilind & Peirce, 1983).

An adequate supply of dissolved oxygen is essential for the maintenance of self-purification processes in natural water systems and water treatment plants (Vesilind & Peirce, 1983). Dissolved oxygen levels in natural and waste waters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

Dissolved oxygen can be reported in two ways; either as a concentration, or as a percentage of the saturation concentration. Atmospheric conditions, temperature, and salinity affect the solubility of oxygen in water. As the atmospheric pressure on the Highveld is only 80 % of that at the coast, the saturation concentration for identical waters will differ by the same margin. In order to be able to compare different waters in different parts of the country, the dissolved oxygen should be reported as a percentage of the saturation concentration (Harris *et. al.*, 1992).

The DO content of the water has the following influences on its environment (Badenhorst, 1994):

- Distribution of living organisms
- solubility of inorganic nutrients and heavy metals such as iron and manganese
- distribution of nutrients and metals
- overall productivity of a water body
- conductivity of water.

5.2.2 Equipment

- DO field instrument (polarographic oxygen electrodes).

5.2.3 Procedure

DO can be measured in the field using a field instrument (portable probe) or in the laboratory. The simplest procedure is to use a polarographic oxygen electrodes and measure the concentration *in situ*. This method provides the fastest results. DO measurements can be recorded at the surface or at given depths (1m intervals) throughout the water column. Calibrate the instrument according to the manufacturer's instructions. Lower the probe into the water and record the DO at 1 m or more or less frequent intervals. Allow sufficient time for the probe to stabilize. DO is recorded in mg/*l* or percentage (%) saturation.

Special preservation techniques are necessary if the DO is to be determined in the laboratory. It requires the fixing of the oxygen when the sample is collected, then measuring the concentration in the laboratory. The Winkler titration method is often used to measure DO (see Standard methods).

5.3 TEMPERATURE

5.3.1 Background

Water absorbs solar energy as heat which is retained in the water. The distribution of heat in a water body is influenced by currents, wind energy, morphometry and depth of the reservoir and water losses.

Temperature measurement is influenced by the depth, site and time of day at which it is recorded. The elevation of water temperature affects oxygen solubility and may also increase the biotic toxicity of certain chemicals, both which result in increased stress on the associated organisms. Many life cycle characteristics of aquatic organisms are cued into temperature, i.e. temperature is the cue for migration, breeding, emergence, etc. Temperature change influences metabolic processes and life cycle patterns by altering reproductive cycles, rates of development of eggs and other life stages, physiology, growth, behavior and feeding of aquatic organisms (Dallas & Day, 1993).

Some effects of temperature on a water body are manifested in:

- Temperature stratification of a reservoir,
- water movement,
- distribution of organisms with different temperature tolerances.

5.3.2 Equipment

- Mercury and electronic field thermometer

5.3.3 Procedure

Temperature can be measured in the field using a field instrument (portable electrode and meter) or in the laboratory. The simplest procedure is to use a mercury or electronic thermometer and measure the temperature *in situ*. Temperature measurements can be recorded at the surface or at given depths (1m intervals) throughout the water column. No calibrate of the instrument is necessary. Lower the probe into the water and measure the temperature (°C) at the surface and at approximately 1 m intervals (when applicable). Allow sufficient time for the probe to stabilize.

5.4 pH

5.4.1 Background

Measurement of pH is one of the most important and frequently used tests in water chemistry. The pH is largely determined by the concentration of the hydrogen ion (H^+) in moles/litre:

$$pH = -\log_{10} [H^+]$$

where $[H^+]$ is the hydrogen ion concentration
pH < 7 water is acidic, while pH > 7 water is alkaline

The pH scale provides a useful and convenient method of expressing the intensity of the alkalinity and acidity of a water, although it gives no indication of the quantity of alkali or acid present. Since pH is a log scale, a change of one unit means a ten-fold change in $[H^+]$. Most fresh waters are usually relatively well buffered and more or less neutral, with a pH range between 6.5 and 8.5, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals (Bath, 1989).

Human-induced acidification of rivers is normally the result of industrial effluents, mine drainage and acid precipitation. Alkaline pollution is less common but may result from certain industrial effluents and anthropogenic eutrophication (Dallas & Day, 1993). Where photosynthesis by aquatic organisms take up dissolved carbon dioxide during the daylight hours, a diurnal pH fluctuation may occur with maximum pH reaching as high as 9.0 (USGS, 1970).

Practically every phase of water supply and wastewater treatment, for example, precipitation, coagulation, disinfection, and corrosion control is pH dependent (Clesceri *et al.*, 1989). Many equilibria in water are pH-dependent and pH affects the mobilisation, speciation and toxicity of metals and other compounds (Wolff *et al.*, 1988). The degree of dissociation of weak acids or bases is affected by changes in pH. This effect is important because the toxicity of many compounds is affected by the degree of dissociation. Ammonia toxicity to fish increases as pH increases and cyanide toxicity increases as pH decreases (USEPA, 1986).

The instantaneous value of pH in a river does not, on its own, give very much information about the water's fitness for use unless the value is an extreme one. Exposure to both low or high pH water causes fish mortalities.

The pH of surface water will vary on:

- a daily cycle due to algal and aquatic macrophyte photosynthesis
- on a seasonal cycle due to runoff entering the river from different geological areas as well as bacterial decomposition, and
- on a random basis due to discharges from industrial and municipal effluents.

5.4.2 Equipment

- pH field instrument (*in situ* measurement), or
- 250 to 350 *ml* white polyethylene bottle with blue or white cap (for laboratory analysis)

5.4.3 Procedure

The pH of a water sample may be determined using (1) test-papers for an approximate determination, (2) colorimetric methods using a Lovibond comparator, (3) electrometric method using a glass electrode and meter (Harris *et al.*, 1992). The last *in situ* method is usually used by the IWQS in the field because it found to be most accurate and shows no interference for coloured or turbid water samples. Calibrate the instrument according to the manufacturers instructions. Always use two

buffer solutions (4.01 and 7.01) for calibration to assure accuracy of reading. If the sample pH is outside the buffer solution pH range by more than about 2-2.5 pH units, recalibrate using a different buffer solution combination. After calibration, record the pH readings of the two buffer solutions. It is critical that the pH probe is rinsed with distilled water when changing from one buffer solution to the other, from buffer solution to sample or from sample to sample. Allow time for the excess rinse water to drain off the probe before pH measurement. Collect a water sample in a small beaker (thoroughly rinsed with water) and lower the probe into the water and record the pH reading. Allow sufficient time for the probe to stabilize. After measurement, the probe must be rinsed in distilled water and covered in its protective rubber sleeve. Be careful to follow the manufacturers instruction on storing the pH probe.

Long intervals (exceeding 24 hours) between collection and measurement of water samples for laboratory analysis should be avoided, since the pH of a water sample will be modified due to biological activity or by carbon dioxide exchange with the air (see Standard methods). Sample bottles must be thoroughly rinsed with water and completely filled (no air gap) and preferably kept at below 10 °C in a cool-box (Bath, 1989).

Range: 0 - 14

5.5 ELECTRICAL CONDUCTIVITY

5.5.1 Background

Electrical conductivity (EC) is a quantitative measurement of the ability of an aqueous solution to conduct an electric current: the higher the conductivity, the greater the number of ions in solution. The highest values will be found in extremely saline or brackish water as in the Karoo region. This ability depends on the presence of ions, their total concentration, mobility, valence, and relative concentrations, and on the temperature during measurement. Since the electrical current is associated with the migration of ions, the conductivity bears a strong relationship to the total ion concentration, and consequently total dissolved salts (TDS) (Bath, 1989). As the conductivity can be readily measured, it is convenient to estimate the TDS from the conductivity measurement, by multiplying the conductivity by an empirically derived factor. This factor varies depending on the specific salts present in the sample:

TDS (mg/l) = conductivity (mS/m) * 6.6

Conductivity is reported as milli-Siemens per meter (mS/m) at 25 °C
where: (1 mS/m = 10 µmhos/cm)
where empirical factor: 6.6 (1 mS/m = 6.5 mg/*l* TDS + 0.1 meq/*l* total cation or anion)

5.5.2 Equipment

- WTW conductivity meter and electrode (*in situ* measurement), or
- 250 to 350 *ml* white polyethylene bottle with blue or white cap (for laboratory analysis)

5.5.3 Procedure

For *in situ* EC measurement connect the probe to the instrument, switch-on and rinse the electrode in distilled water and dry. Calibrate the instrument according to the manufacturer's instructions. Use the correct temperature correction as EC increases with temperature at a rate of *ca.* 1,9% / °C. Some instruments automatically make this correction, if not report conductivity to the corrected value at 25 °C. Collect a water sample in a small beaker and lower the probe into the sample, then

gently stir for about 30 seconds (Bath, 1989). Allow sufficient time for stabilization before taking the readings. If the incorrect conductivity range is selected the meter will show an overflow and in such case select a higher range. After a measurement, the electrode must be rinsed in distilled water, dried, and returned with the meter to the carrying-case.

Sample bottles must be thoroughly rinsed with water and completely filled (no air gap) and preferably kept at below 10 °C in a cool-box for EC or TDS analysis in the laboratory (Bath, 1989) (see Standard methods).

Range: 0 - 199 mS/cm

5.6 TURBIDITY

5.6.1 Background

Turbidity in water is caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble coloured organic compounds, plankton and other microscopic organisms that are held in suspension (Harris *et. al.*, 1992). Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample (Clesceri *et al.*, 1989). Particles that cause turbidity in water range in size from colloidal dimensions (approximately 10 nm) to diameters in the order of 0.1 mm (CCREM, 1987). The most common method measures the scatter of light in a sample in terms of nephelometric units (NTU). Because of the many advantages associated with low turbidity and the relative ease of continuous turbidity monitoring, it is often used as an indicator of potential water quality problems during treatment (Kempster and Smith, 1985).

The immediate visual effect of a change in turbidity is a change in water clarity. It gives an indication of the limit of visibility in water. An increase in turbidity or suspensoids affects light penetration, which may have far-reaching consequences for biota. Increase in turbidity can, and often do, result from anthropogenic processes, such as release of domestic sewage, industrial discharge (including mining, dredging, pulp and paper manufacturing) and physical perturbations such as road and bridge construction, dam construction, road use and reservoir management.

Turbidity is important in all five of the recognized water uses. In treatment of drinking water, turbidity is of great importance first because of the aesthetic considerations, and second because pathogenic organisms can adsorb to the tiny particles (CCREM, 1987). Water clarity is important in numerous industries producing materials destined for human consumption, such as the food and beverage industry and various manufacturing industries.

5.6.2 Equipment

- Nephelometric turbidimeter (*in situ* measurement)
- 250 or 350 *ml* green polyethylene bottle (for laboratory analysis)

5.6.3 Procedure

Calibrate the instrument according to the manufacturer's instructions and determine the turbidity of the sample. Report results as nephelometric turbidity units (NTU). If *in situ* measurement in the field is not possible, collect a sample in a 250 *ml* to 350 *ml* plastic bottle and store it in the dark. A samples may be kept for up to 3 weeks if stored in the dark. Agitate the water sample to resuspend any sediment that may have settled to the bottom of the sample bottle before being analysed (see Standard methods).

Range: 0 - 1000 NTU

5.7 LIGHT PENETRATION

5.7.1 Background

Clarity refers to the depth to which light can penetrate in a water body. Clarity is a important variable in eutrophication processes. In turbid waters light penetration is reduced (Kirk, 1985), leading to a decrease in photosynthesis. The resultant decrease is primary production reduces food availability for aquatic organisms higher up in the food chain. In clear waters with high nutrient concentrations, an increase in light penetration can stimulate eutrophic conditions. Impairment of clarity or increased turbidity is likely to impact upon judgement regarding the suitability of water for full-contact recreation. This relates to considerations of both safety and aesthetic enjoyment, although safety is probably the dominant aspect (DWAF, 1993).

Light penetration is normally measured either *in situ* by visual observation or using a light probe. There are two ways of determining the light penetration into the water column, namely using either a Secchi disc or a light meter.

5.7.2 Secchi disc

It is one of the easiest field instruments to use and is commonly used for the determination of the transparency of water. The Secchi disc is a round metal disc, 20 cm in diameter, with white and black painted quadrants (Figure 5). The Secchi depth is that depth at which the disc just disappears when lowered into the water and reappears when pulled back up. It is influenced by the natural absorbance of light by water, absorbance by dissolved matter, and absorbance and scattering by particulate matter in the water. The Secchi depth is the depth where approximately 10% of the surface light is still available in the water column. The application for this measurement is to determine the depth at which light is still available for photosynthesis.

5.7.2.1 Equipment

- Secchi disc

5.7.2.2 Procedure

Lower the secchi disc into the water on the shadow side of the boat until it vanishes out off sight, and record the depth. Pull the disc up until you can just see it reappears and record this reading too. The average of the two readings is the Secchi depth. Repeat the procedure twice. The extinction coefficient (k) of water can be determined from the Secchi depth (a more accurate measurement can be determined with a lightmeter):

$$k_{SD} = \ln 0.10 / z_{SD}$$

where: k_{SD} is the extinction coefficient of water (m^{-1})
where: z_{SD} is the secchi depth (m)

5.7.3 Light meter

The light meter measures the photosynthetically active radiation (PAR) available for plants for photosynthesis. Only wavelengths between 400 nm and 700 nm can be used for photosynthesis. The Quantum light meter measures the (PAR) per unit area per time. The units are micro Einstein $m^{-2}s^{-1}$.

5.7.3.1 Equipment

- Li-cor Quantum/Radiometer/Photometer.

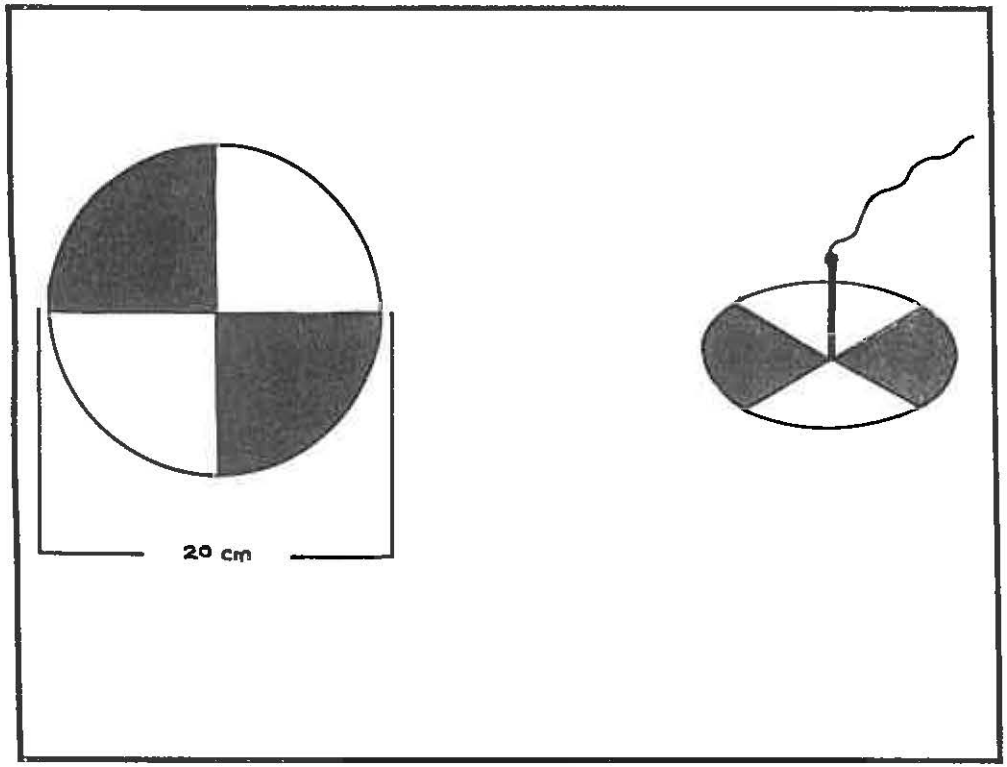

Figure 5: Secchi disc

5.7.3.2 Procedure

Connect the cable of the light sensor to the measuring unit. NOTE: The serial number of the "Calconnector" and the sensor must be the same. Switch-on the "BATT" switch and ensure that the battery voltage is above the minimum level, if not, replace battery with a 9 Volt battery. Turn the range switch to 1x104 range and "sensor in air". Determine the light intensity in the air, record reading. Turn switch to "sensor in water" and lower the sensor until it is just covered with water and measure the light intensity. Switch range switch to smaller range and record the light intensity reading. Measure the light intensity every 25 cm until the reading is 1% of the light intensity at the surface. Determine the extinction coefficient (k) of the water column as follows:

$$k = \ln (I_1) - \ln (I_2) / z$$

where: I_1 = light intensity at depth 1 (micro Einstein $m^{-2}s^{-1}$)

I_2 = light intensity at depth 2 (micro Einstein $m^{-2}s^{-1}$)

z = difference in depth between depth 1 and 2 (m)

Light meters are mainly used in primary production studies and the extinction coefficient (k) should be measured before and after the production studies. Always try and take the reading at the same time during the day, as the sun's elevation has an effect on the measurements.

6. SAMPLING TECHNIQUES FOR CHEMICAL ANALYSIS OF WATER, SEDIMENT, FISH AND RADIO NUCLIDES

This section deals with the collection of water, sediment, macrophytes, and fish samples for chemical analysis. Included are sampling for major ions, metals, organic compounds, THMs, and radio nuclides.

The sampling site must be chosen to represent a certain portion of a river or reservoir. In rivers, the sampling site is normally just below a ganging weir. This serves two purposes (Rossouw and Badenhorst, 1987):

- Flow data may be obtained simultaneously, which enables loads to be calculated, and
- the turbulence serves to mix the water and ensure a greater degree of homogeneity.

IMPORTANT: Always liaise with the appropriate laboratory when undertaking a sampling exercise. The laboratory can then supply the correct sample containers and preservatives, and will know when to expect the samples.

6.1 MAJOR INORGANIC IONS

6.1.1 Background

The determination of inorganic ions is necessary to characterize a water body for a specific use. It is also necessary to assess the need for specific treatment. The ions most commonly found in natural waters are the cations calcium, magnesium, sodium, and potassium, and the anions bicarbonate, carbonate, chloride and sulphate (Dallas & Day, 1993).

6.1.2 Equipment

- 250 to 350 *ml* polyethylene bottles (white or white with blue cap), precleaned
- 5 *l* plastic beaker (for surface samples)
- Hose pipe sampler(for integrated samples) (Figure 6)
- Van Dorn sampler (for depth samples) (Figure 7)
- Mercury(II) chloride ampoules (see section, 6.1.4.2 Precaution)
- Waterproof labels for bottles, and waterproof pen
- Cool bag

6.1.3 Procedure

Ensure that your hands and the outside of the bottle are clean. Rinse the bottle well with water from which the sample is to be taken. Always discard the rinse away from the sampling point in a downstream direction in a river. If sampling takes place from a boat, collect the sample in front of the boat to avoid contamination of the sample with oil and exhaust fumes. The type of sampling method will be determined by the specific requirements of the study.

6.1.3.1 Sub-Surface water sample

Collect a sub-surface sample using a plastic beaker to obtain water from approximately 20 cm below the surface. Rinse the beaker before collecting the sample. The sample can also be collected directly in the bottle by plunging the bottle neck down into the water (approximately 20 cm below the surface) and then tilt the neck up to face the incoming flow of water. Water should pass the neck of the bottle before it passes the hand of the sampler to prevent contamination.

A true surface sample, collected by just lowering the lip of the bottle below the surface and allowing the surface water, with the surface film, to flow into the bottle may differ significantly in chemistry from the sub-surface sample (approximately 20 cm below the surface). Particularly as trace metals and some organic compounds tend to be concentrated at the air-water interface layer.

6.1.3.2 Integrated water sample

To obtain an integrated sample between 0 and 5 m, use a 5 m hose pipe (Figure 6) adapted for this purpose. Lower the one end of the pipe, where the weight is fasten to, very slowly straight into the water until the other end (top end) is just above the surface of the water. Hold this end stationary and slowly pull up the end lowered first until the two ends are together (Figure 6). Lift the pipe out of the water and collect the sample in precleaned a plastic beaker.

6.1.3.3 Depth water sample

Use the Van Dorn sampler (Figure 7) or any other depth sampling device to collect samples at different depths in an impoundment. Empty the sampler into a plastic beaker that has been rinsed before transferring the sample to the sample bottle.

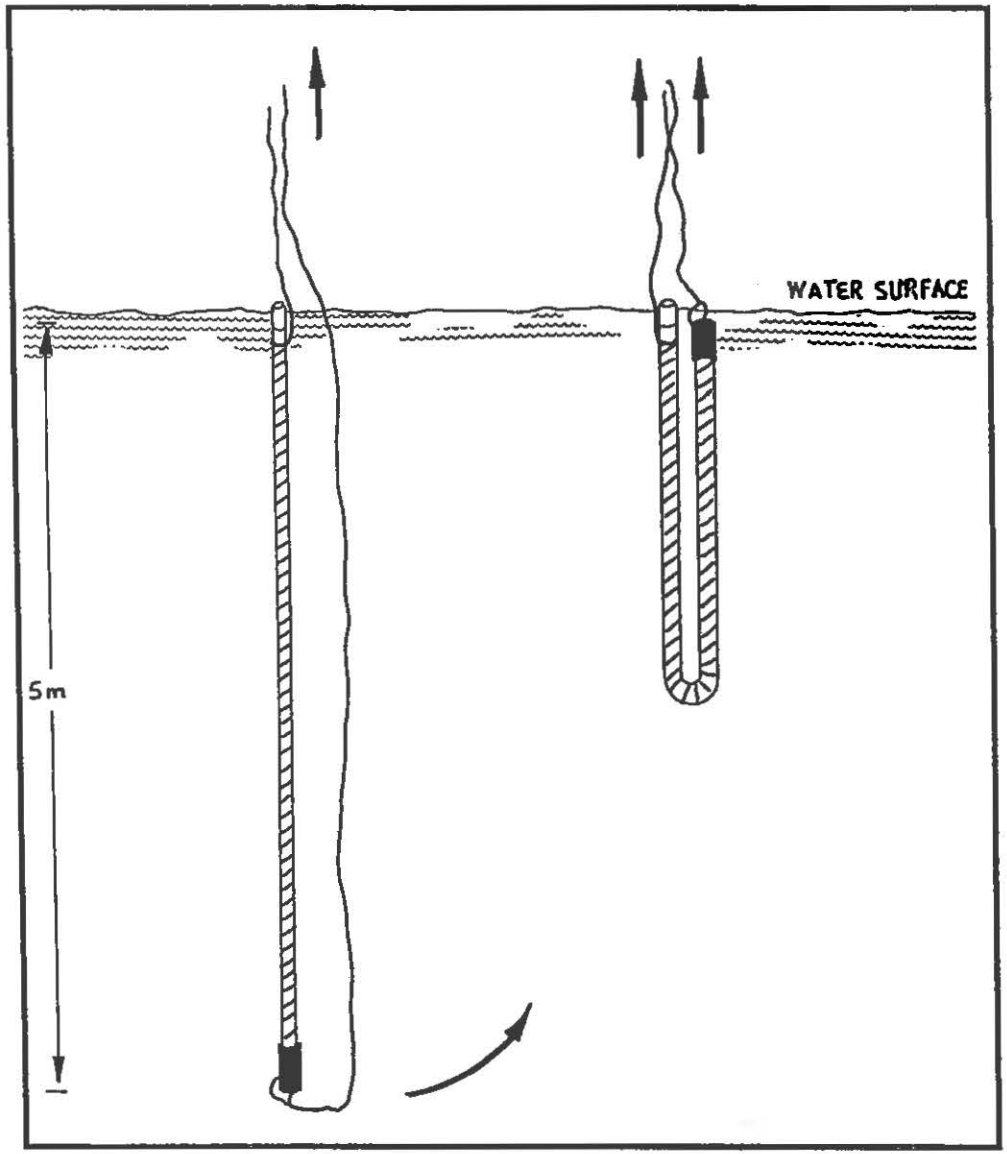

Figure 6: Hose pipe to collect an integrated water sample.

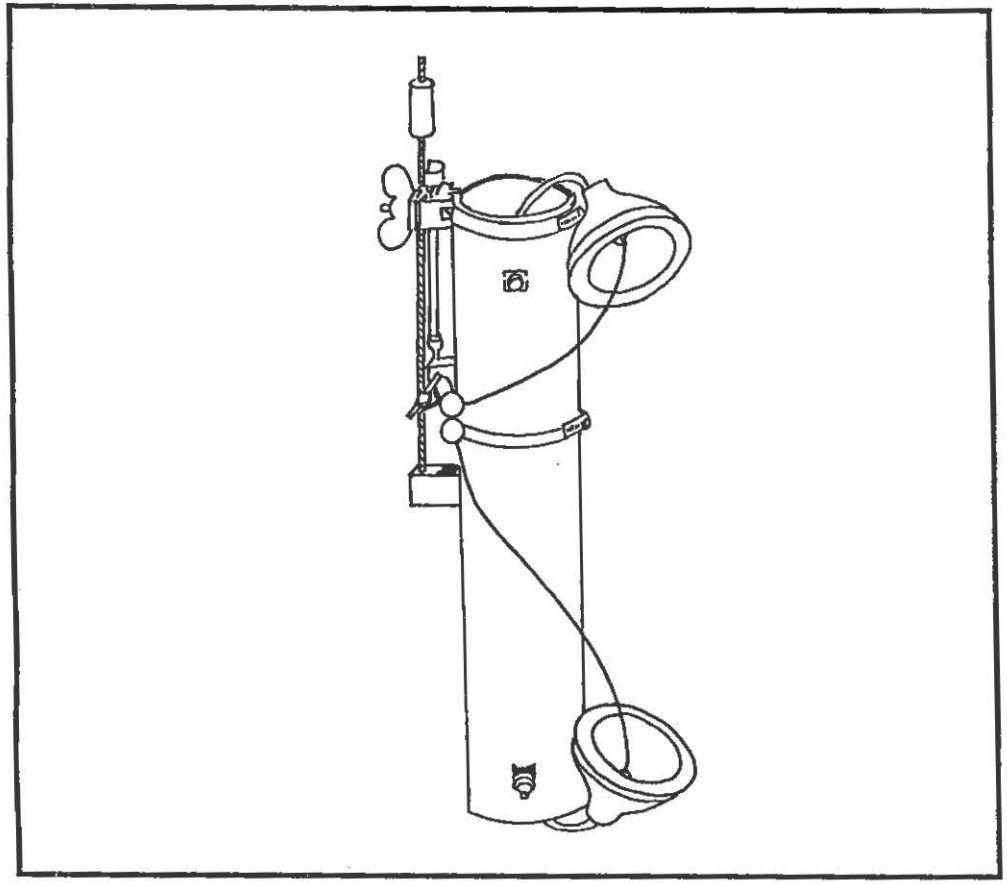

Figure 7: Van Dorn sampler to collect a depth water sample.

6.1.4 Instructions for the preservation of samples and precaution

6.1.4.1 Preservation of samples

If the sample is not to be preserved, fill the bottle completely. If the sample is to be preserved, fill the bottle within 0,5 to 1 cm of the upper rim of the neck of the bottle. Take one ampoule (1 *ml* mercury (II) chloride solution, and tap the bottom gently on a firm surface so that all the liquid flows to below the constriction. Hold the ampoule firmly upright. Place your thumbs on each side of the constriction in the neck and flex to break off the neck. Turn the ampoule upside down over the bottle containing the sample (the contents will not run out). Lower the ampoule with liquid carefully into the sample bottle. The broken off glass portion should also be lowered into the bottle. Screw the cap of the sample bottle on firmly and shake well. This will result in a final Hg (II) concentration of 6 mg/*l* in the sample. Do not open the bottle again. Clearly label the bottle with all the relevant information and store the sample in a cool, dark place. Transport the samples back to the laboratory as soon as possible. If a water body with a high salt content, e.g. sea water, is sampled it must be indicated clearly on the sample bottle.

6.1.4.2 Precaution

The ***mercury (II) chloride ampoules*** must be treated with care, as the liquid contents are ***extremely poisonous***. Each ampoule contains 1,8 mg mercury (II). Whole or broken ampoules or the liquid contents should under no circumstances be exposed to fire or heat. Should the liquid come into contact with the skin, the affected area should be thoroughly washed with soap and water. If ampoules are accidentally broken, the area should be thoroughly washed with water. Consult a doctor immediately if the contents are ingested by mouth.

FIRST AID: Drink a mixture of one raw egg-white and a glass of milk. To keep contamination of samples to a minimum the ampoules should be kept clean and handled as little as possible. Keep the ampoules in containers and store out of reach of unauthorised people, especially children.

6.2 HEAVY AND TRACE METALS

6.2.1 Background

Contamination of an aquatic ecosystem can be confirmed by examining the water, the sediment and the organisms. In water, metals are exposed to many different chemical reactions: thus analysis of this medium gives a transitory view of the metal loading. Soluble metals eventually reach the sediments, where they become bonded to various components of the sediment such as clay minerals or organic matter

Biological information is often more valuable than periodic physical and chemical monitoring in assessing the long-term effects of pollution (Dallas & Day, 1993). Organisms used to measure metal accumulation at the IWQS include macrophytes and fish.

The effect of metals on life in water and waste water range from beneficial to troublesome to dangerously toxic. Some metals are essential, whilst others may adversely affect water consumers, waste water treatment systems, and receiving waters. Some metals may be either beneficial or toxic, depending on their concentration.

Before collecting a sample, decide which fraction is to be analysed (Badenhorst, 1994):

- *Dissolved metals:* Those constituents (metals) of an unacidified sample that pass through a 0,45 µm membrane filter.
- *Suspended metals:* Those constituents (metals) of an unacidified sample that are retained by a 0,45 µm membrane filter.
- *Total metals:* The concentration of metals determined on an unfiltered sample after vigorous digestion, or the sum of the concentrations of metals in both dissolved and suspended fractions.
- *Acid-extractable metals:* The concentration of metals in solution after treatment of an unfiltered sample with hot dilute mineral acid.

Serious errors may be introduced during sampling and storage because of contamination from sampling devices, failure to remove residues of previous samples from sample containers, and loss of metals by adsorption and/or precipitation in the sample container caused by failure to acidify the sample properly. Failure to filter a sample properly prior to acidification will also result in falsely high concentrations for dissolved metals being obtained.

6.2.2 Water samples

The type of sample will depend on the information requirements of the study. The water sample can be either: (i) a *sub-surface sample* (grab)- collected with a beaker; (ii) an *integrated sample* (0-5 m)- collected with a hose pipe, or (iii) a *depth sample* - collected with a Van Dorn sampler.

6.2.2.1 Equipment

- 250 *ml* to 350 *ml* polypropylene- or polyethylene bottle with a polyethylene cap (red bottle), pre-cleaned. Borosilicate glass containers are used for mercury sampling. Containers must have been acid rinsed, before use.
- 5 *l* plastic beaker.
- Preservative (3 *ml* nitric acid (HNO_3)/ 300 *ml* sample) (consult with laboratory).
- Filter unit and membrane filters, 0,45 µm (Figure 8).

6.2.2.2 Procedure

(a) Standard trace metal sample

Collect surface samples with a beaker, integrated samples with a hose pipe sampler or depth samples with a Van Dorn sampler. Collect the

water sample in either a polyethylene bottle. Unless samples can be filtered efficiently in the field, it is preferable not to add any preservatives to any of the samples. As the addition of acid to metal samples tend to precipitate silica, with co-precipitation of some metals, it is preferable not to preserve metal samples in the field. Try to get the samples to the laboratory within 72 h. Samples should be kept cool.

Where longer time intervals elapse between sampling and collection, preservation can be considered, but only if it is certain that the preservative is metal free. The sample is filtered through a 0.45 µm membrane filter into a 350 *ml* pre-cleaned polyethylene bottle and polyethylene cap with no liner. The first 20 *ml* of filtered sample is discarded. Immediately after filtration the sample is preserved with 3 *ml* redistilled concentrated HNO_3, which has been sealed in the laboratory into small pre-cleaned glass ampoules for field use. The concentrated nitric acid preservative serves to decrease the pH value of the water to less than 2, and thus discourage adsorption / hydrolysis of metals.

(b) Trace metal sample for mercury (Hg) and cyanide

Samples for mercury analysis must be collected in glass containers. No filtration is necessary when mercury and cyanide analyses are to be done. Samples for mercury analysis are preserved with 3 *ml* concentrated sulphuric acid (H_2SO_4) or nitric acid (NHO_3) per 300 *ml* sample. Samples for cyanide analysis are preserved by raising the pH of the sample to pH > 12 with NaOH (0,1 M).

(c) Trace metal sample for Cr (III) and Cr (VI)

For Cr (III) or Cr (VI) special precautions are necessary depending on the analytical method used. Where concentrated nitric acid, 3 *ml* per 300 *ml* sample, is used to preserve samples for the other metals the acid must be metal free.

NOTE: Do not add the glass body of the ampoule to the water sample. The contents must be poured into the sample and the glass pieces kept separate in a container for disposal.

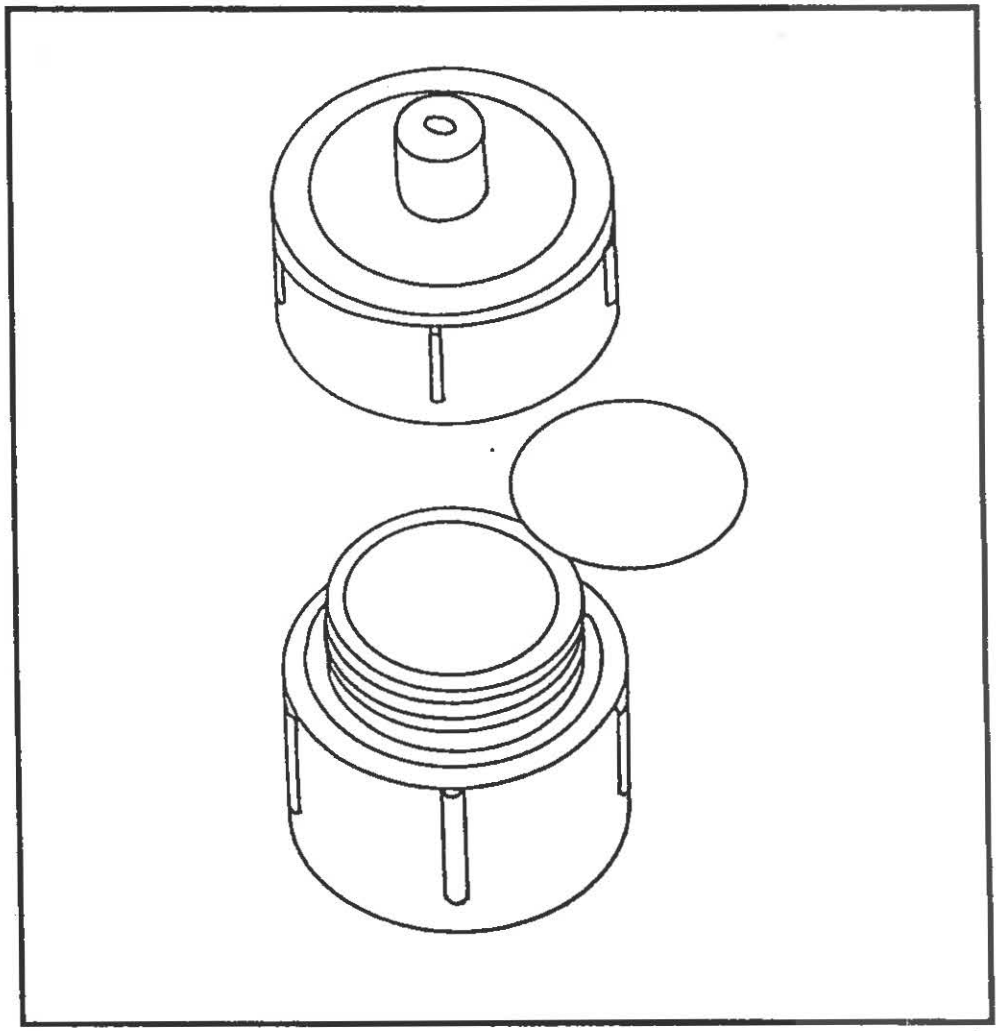

Figure 8: Filter unit for filtering samples for metal analysis.

6.2.3 Sediment samples

Metals can accumulate in the clay fraction of the sediment. Information gained by analysing these can give some historical data as well as an indication of long term exposure. The ideal location to collect sediment samples are in dams or upstream of large weirs, that act as sediment traps.

6.2.3.1 Equipment

- 1 ℓ plastic container
- Ekman grab sampler (Figure (9), or
- Petersen grab sampler (Figure 9), or
- Corer sampler (Figure 10)

6.2.3.2 Procedure

River or reservoir bottom sediment are usually collected using a grab sampler e.g. Ekman or Petersen grab (Figure 9). These methods allow grab collection of approximately the top 150 mm of the sediment. Always collect the most representative sample possible. Samples for metal analysis are collected in 1ℓ plastic containers. . The samples for metal analysis must be kept cool at 4°C, not frozen, prior to analysis.

(a) Ekman grap sampler

Open the clamps of the Ekman grap sampler and lower the grab slowly into the water, until it reaches the bottom. When the sampler hits bottom the valve spring of the clamps of the Ekman grab sampler are amputated with a messenger that slides down along the rope. The clamps shut and consequently a sediment sample is taken in the top layer centimeters of the bottom sediment.

(b) Petersen grap sampler

The Petersen grap sampler works with a scissors movement. The sampler is lowered into the water to the bottom with the clamps open. When the sampler hits the bottom, the scissors unlock and the clamps close just as the sampler is hauled up again.

Both samplers can be used in reservoir sampling, but the Petersen grap is more effective in flowing water seeing that it is much heavier. Both samplers are more fit for soft bottom sediment, since stones cause the clamps not to close properly.

(c) Corer sampler

If it is important that the sediment must be disturbed as little as possible and the stratification of the sediment should be kept retained, a Corer sampler (Figure 10) is used. The Corer sampler is slowly lowered to the bottom. The weights on the corer push it into the sediment. When it is hauled up, a clip in the upper part of the Corer shuts and prevent consequently that the column sediment does not fall out

6.2.4 Fish samples

The commonest causes of metal poisoning are the heavy metals: copper, lead, mercury, zinc, chromium, cadmium, magnesium and iron. Metals can accumulate in fish tissue, especially in the liver and fatty tissue. Information gained by analysing these can give some historical data as well as an indication of long term exposure. Better results will be obtained when fish species higher up in the food chain are sampled. It is important to determine the age of the fish, as well as the length and mass. The best places to collect fish are in dams or large weirs, which trap and limit the migration of fish along the course of the river.

6.2.4.1 Equipment

- Gill nets
- Polypropylene dissection board
- Buckets to handle fish
- Metric weighing scale
- Fish measuring board
- Foil
- Sharp knife
- Bone scissors
- Sharp and blunt point stainless steel dissection scissors and forceps (tweezers)
- Scalpels with spare blades
- Paper towel

6.2.4.2 Procedure

Collect fish using gill nets. The species to be collected will depend on the study. The purpose of the study will also determine the tissue to be collected e.g. liver or/and muscle tissue or muscle tissue with skin, etc.

IMPORTANT: Permission from the local Nature Conservation authority must be obtained before using gill nets to collect fish.

The mesh size of the nets to be used will depend on the size of the fish you intend to catch. The smallest individual collected and used for analysis should not be less than 75 % of the length of the largest individual. After collection of the fish, select at least 5 fish per species for analysis. Weigh and measure each fish, standard length and fork length. Cut the fish and remove the liver (Figure 11). If possible determine the gender of the fish. For age determination of species with scales, remove 10 to 15 scales 2 rows above the lateral line just underneath the dorsal fin. When the fish is scaleless, the first dorsal and/or pectoral spine can be removed and use for age determination

Remove the scales on one side of the fish (where applicable) and cut a fillet out of the tissue, preferably with the skin intact. The liver and fillet are used for metal analysis.

Wrap the liver, fillet, scales or dorsal spine, separately in aluminium foil. The dull side of the foil must be in contact with the samples. Clearly label all the samples with all the relevant information. Store the samples at -20 °C or freeze until analysis can be performed.

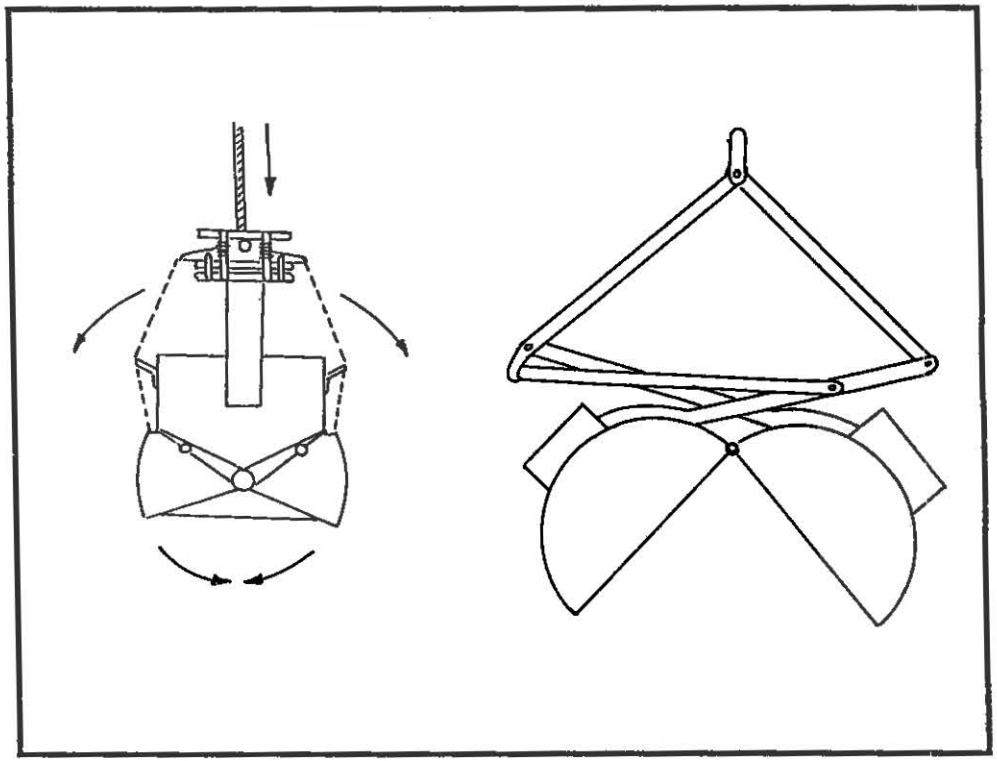

Figure 9: The Ekman grap (left) and Petersen grap (right) samplers for sampling of bottom sediment (Rossouw and Badenhorst, 1987).

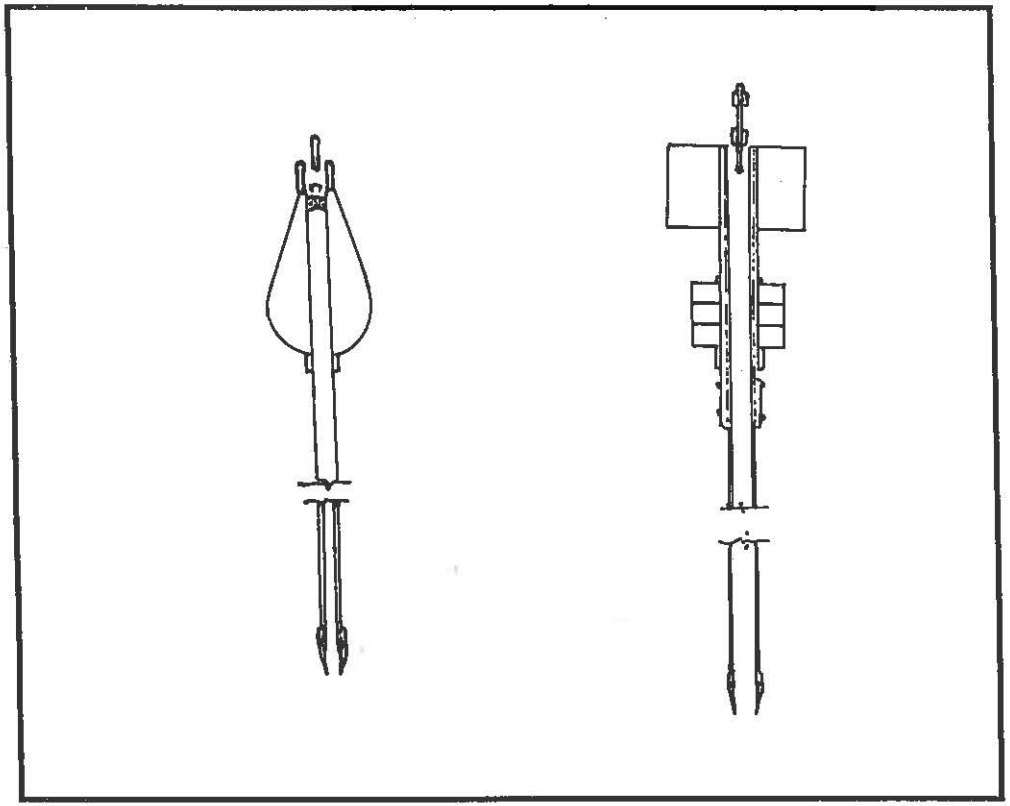

Figure 10: The Corer sampler for sampling bottom sediment (Rossouw and Badenhorst, 1987).

6.2.5 Macrophyte samples

Metals and alkalies are absorbed from the water by leaves and interstitial water and sediment by roots. Many elements are concentrated in the macrophytes in great excess of metabolic requirements. As a result, submersed and emergent plants are often suggested as scavengers of contaminants in surface waters and used in bioaccumulation studies (Wetzel, 1983).

6.2.5.1 Equipment

- Plastic bags
- Distilled water
- Large, small and fine point stainless steel dissection forceps (tweezers) for dislodging sediment particles
- Cool bag with ice blocks

6.2.5.2 Procedure

Samples of aquatic macrophytes are subdivided on site, in the case of floating macrophytes, into areal (leaf) and submerged (root) portions, washed with distilled water, placed in plastic bags. For analysis of organic compounds, wrap in foil. It is important to dislodge sediment particles from the submerged portions of an aquatic plant at the time of sampling to prevent contamination.

6.3 ORGANIC COMPOUNDS

6.3.1 Background

Organic compounds are those which include mainly carbon, but also usually hydrogen and oxygen, as the main components of their chemical structure. In natural water, most organic compounds are humic and fulvic acids. As a result of technological development, man-made organic compounds have also been introduced into the water environment. These include different pesticides used for agriculture as well as chemicals used in the manufacturing industry.

The impact of pesticides and/or trace metals on aquatic organisms can be either dramatic (e.g. acute fish kills), or insidious due to gradual accumulation in the body tissues of organisms. Biotas are not only influenced by toxicant concentrations in the water and sediment, but due

to the process of bioaccumulation, may be exposed to much higher concentrations. Concentrations of many toxic substances in water are magnified as they are passed up through the food web, via phytoplankton, zooplankton and fish. This results in high contaminant concentrations in top predators (Swackhamer and Eisenreich, 1991).

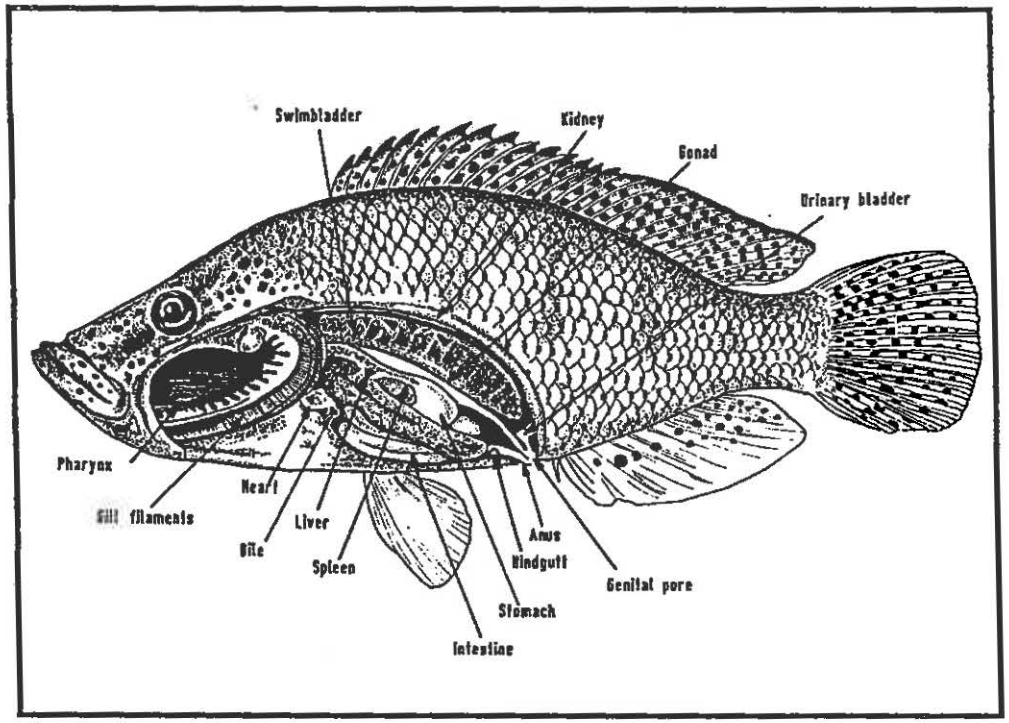

Figure 11: Schematic diagram of a fish (Skelton, 1993)

6.3.2 Water samples

Sub-surface samples (grab) are collected for organic analysis of water. The hydrophobic compounds are generally not present in water in measurable concentrations, therefore sediment or fish tissues are generally analysed for organic compounds.

6.3.2.1 Equipment

- 4.5 *ℓ* glass jar, pre-cleaned, with a teflon lined screw cap.
- 5 *ℓ* stainless steel bucket
- 100 *ml* measuring beaker
- Preserve with 4 *ml* conc. H_2SO_4 (1 ampoule), pH = 2), and/or
- 100 *ml* dichloromethane (preservative)
- Aluminium foil
- Cool bag

6.3.2.2 Procedure

Collect a sample directly in the pre-cleaned 4,5 litre bottle or use the stainless steel bucket. Do not rinse the bottle, and avoid contamination of the sample by the hands. Also avoid contact with rubber or plastic. If possible, take the samples back to the laboratory within 24 h. In this case no preservation is necessary. Fill the bottle completely so that no headspace exists, cover the sample with aluminium foil (dull side in contact with the sample) and replace the cap. Transport and store the sample in a cool, 4 °C, dark place.

If the sample cannot be taken back to the laboratory within 24 h, it must be preserved. Preservation is done by adding 100 *ml* dichloromethane, or 4 *ml* concentrated sulphuric acid, or both. The type of preservation depends on what compounds the sample will be analysed for. Always discuss the preservation technique with the laboratory personnel before going out into the field.

6.3.3 Sediment samples

Consequently, sediment is a net trap for various of chemicals, and concentrations are often considerably higher than in any other environmental compartment. Some organic compounds, such as herbicide paraquat, are incorporated into clay particles, while other or their degradation products are incorporated into the sediment organic matter (Verhoef and Gestel, 1995).

6.3.3.1 Equipment

- 2 ℓ tins with tight fitted lids, pre-washed and rinsed with acetone
- Ekman grab sampler (Figure (9), or
- Petersen grab sampler (Figure 9), or
- Corer sampler (Figure 10)
- Cool bag

6.3.3.2 Procedure

River or reservoir bottom sediment are usually collected using a grab sampler e.g. Ekman or Petersen grab (Figure 9). Always collect the most representative sample possible. Samples for organic compounds analysis are collected in 2 ℓ tins with tight fitted lids, pre-washed and rinsed with acetone. Use the Ekman grap, Petersen grab and Corer sampler as described in section 6.2.3 (sediment samples). The samples for organic analysis must be frozen if possible (- 20 °C) prior to analysis, or otherwise kept cool at 4 °C, until arrival in the laboratory. Upon laboratory arrival, samples must be placed in freezer promptly.

6.3.4 Fish samples

Some organic compounds (e.g. PCBs) can accumulate in fish tissue, especially in the liver and fatty tissue. Many organic compounds used in agriculture and industry are toxic for fish. Pesticides are chemical designed to destroy plant or animal life. The major source are runoff from treated farmlands, industrial and domestic sewage and spillage. Many are rapidly inactivated by microbial degradation or adsorption to benthic particulate matter but some, such as DDT and dieldrin, are highly resistant to degradation and are concentrated with little chemical alteration in the food web (Roberts, 1989).

6.3.4.1 Equipment

- Gill nets
- Polypropylene dissection board
- Buckets to handle fish
- Metric weighing scale
- Fish measuring board
- Foil
- Sharp knife
- Bone scissors
- Sharp and blunt point stainless steel dissection scissors and forceps (tweezers)
- scalpels with spare blades
- Paper towel

6.3.4.2 Procedure

IMPORTANT: Obtain permission from the local Nature Conservation authority before using gill nets to collect fish.

Collect fish using gill nets. The species to be collected will depend on the study. The purpose of the study will also determine the tissue to be collected e.g. liver or/and muscle tissue or muscle tissue with skin, etc.

The mesh size of the nets to be used will depend on the size of the fish you intend to catch. The smallest individual collected and used for analysis should not be less than 75 % of the length of the largest

individual. After collection of the fish, select at least 5 fish per species for analysis. Weigh and measure each fish, standard length and fork length. Cut the fish and remove the liver (Figure 11). Collect scales or pectoral spine for age determination as described in section 6.2.4 (fish samples). Fillet the muscle tissue of fish (first remove scales where applicable). The liver and fillet are used for organic compound analysis.

Wrap the liver, fillet, scales or dorsal spine, separately in aluminium foil. The dull side of the foil must be in contact with the samples. Clearly label all the samples with all the relevant information. Store the samples at -20°C or freeze until analysis can be performed. Upon lab arrival, samples must be placed in freezer promptly.

6.4 TRIHALOMETHANES (THMs)

6.4.1 Background

Trihalomethanes (THMs) are formed as by-products of chlorination in the water treatment process. Some of the THMs are suspected carcinogens. The amount of THMs formed depends on several factors, including the amount of dissolved organic material in the raw water.

6.4.2 Equipment

(a) Purification works

- 4 Glass bottles (25-30 *ml*) / monitoring point, pre-cleaned, only with teflon lined screw caps. (2 raw water, 2 final or product water)
- L-ascorbic acid (for preservation).
- Cardboard boxes fitted with polystyrene holders.

(b) Reservoirs

- 40 *ml* glass bottles, in duplicate/point, pre-cleaned, only with teflon lined screw caps.
- L-ascorbic acid (for preservation).
- Cardboard boxes fitted with polystyrene holders.

(c) Boreholes, and toxic waste sites

- 40 *ml* glass bottles, in duplicate/point or one 100 *ml* sample/point, pre-cleaned, only with teflon lined screw caps.
- L-ascorbic acid (for preservation).
- Cardboard boxes fitted with polystyrene holders.
- Beaker
- Hand gloves

6.4.3 Procedure

The majority of samples are grab samples. Use only glass sampling bottles. The volume depends on the type of sample needed. Prior to sampling from a tap, the tap should be turned on and the water allowed to flush out for at least 2 minutes. An extended flushing time will be required if the tap is situated at the end of a long distribution line. In case of bore hole samples, use a beaker. Sampling toxic waste sites, sample with hand gloves on. Fill the sample bottle in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle with no headspace and so that no air bubbles are entrapped in it. If the sample contains free or combined chlorine, L-ascorbic acid must be added to the bottle prior to sampling (0,15 g/25 *ml*). Keep the samples cool at 4 °C, and return them to the laboratory within 24 h. Analysis must be done within 7-14 days.

6.5 RADIOACTIVITY AND RADIO NUCLIDES IN WATER

6.5.1 Background

Water samples are collected for the analysis of metals associated with radioactivity. The physical measurements of radioactivity in water are also addressed.

Radioactivity exposure to man from water can occur by a number of routes. The most direct route is that arising from absorption of radio nuclides present in water which is used for drinking purposes. Other routes of exposure are for instance, via consumption of fish, milk, meat and edible crops (Kempster, 1997).

6.5.2 Equipment

- Polyethylene bottles, 250 to 350 *ml* (red bottle), precleaned.
- Polyethylene bottles, 5 ℓ, supplied by AEC.
- 5 *l* plastic beaker with nylon rope.

6.5.3 Procedure

Collect surface samples with plastic beaker for trace metal analysis. Care should be taken to ensure that the correct sampling point is identified. Fill the 5 ℓ polyethylene bottle and the 250-350 *ml* red bottle, leave no headspace. No preservatives are necessary if samples is returned to the laboratories within 24 h. Label the samples with the relevant information (sampling site number, sampling type, date and time). The sample can be held at room temperature for 24 hours before analysis.

The 5 ℓ polyethylene bottle samples must be delivered to the Atomic Energy Council (AEC) before 09:00 the following morning for physical analyses, while the 250-350 *ml* red bottles are returned to the IWQS laboratories for trace metal analysis.

At the AEC the physical analyses are performed. This includes the measurement of α- and β- activity and the determination of ^{238}U and ^{226}Rn.

7. SAMPLING TECHNIQUES FOR BIOLOGICAL AND BACTERIOLOGICAL ANALYSIS OF WATER

7.1 BACKGROUND

Biological methods used for assessing water quality include the collection, counting and identification of aquatic organisms, and biomass measurements. Bacteriological examinations are done to determine the sanitary quality of the water.

IMPORTANT: Always liaise with the appropriate laboratory when undertaking a sampling exercise. The laboratory can then supply the correct sample containers and preservatives, and will know when to expect the samples.

7.2 CHLOROPHYLL *a*

7.2.1 Background

Chlorophyll *a* is the primary photosynthetic pigment in all algae (except the so-called colourless algae) and blue-green bacteria, such as *Mycrocystis*, therefore a correlation between the chlorophyll concentrations and the algal density exists. Chlorophyll *a* is often used as an algal biomass indicator which measure the amount of planktonic algae present in a water body. Large values of chlorophyll *a* always indicate that the fitness for use of the water has been diminished (Harris *et al.*, 1992). Chlorophyll *a* constitutes approximately 1 to 2 % of the dry weight of organic material in planktonic algae.

Chlorophyll *a* is a major variable used in the quantification of eutrophication and the determination of the trophic status of the water body. The effect of excessive algal numbers are experiencing in problems with water colour, taste, and odour, resulting in increasing costs of water treatment; the water is less attractive for boating, swimming, and fishing; irrigation canals may become clogged; toxins produced by some of the algae may be fatal to livestock (Harris *et al.*, 1992).

7.2.2 Equipment

- Hand vacuum pump
- Plastic filtration unit
- Glass fibre filter paper
- 5 *l* plastic beaker
- Hose pipe or Van Dorn sampler
- 25 *ml* glass container with cap (with 9.8 ethanol solution)
- 1 *l* plastic bottle

7.2.3 Procedure

The type of sample will be determined on the information requirements of the study. The sample can be either: (i) a surface sample - collected with a beaker; (ii) an integrated sample - collected with a hose pipe (iii) a depth sample - collected with a Van Dorn sampler. Throw the collected water sample into the beaker and mix well. Using a filtration unit (Figure 12), filter 250 *ml* or more of the sample through a glass fibre filter paper. Start off by fill the top part of the filtration unit up to the

250 *ml* mark. Pull a vacuum with the trigger, up to 15 cm pressure, and let the water slowly filter through. Repeat this procedure until 250 *ml* or more water is filtered through the glass fibre filter paper. If it is not possible to pass 250 *ml* or more sample through the filter paper, record the exact amount that was filtered. If the sample will reach the laboratory within 6 hours, collect the sample in the 1 *l* plastic bottle and filtration of the water sample is not necessary.

After filtration, carefully remove the filter paper, without touching the surface. Loosely roll the filter paper with the entrapped algae on the inside and place it into the 25 *ml* glass container with 9,8 *ml* 91,8% (v/v) ethanol solution. Seal the tube and label it correctly. The type of sampling method, the depth sampled and the amount of sample filtered must be recorded. Samples must be stored in the dark at 4 °C and analysed within 24 hours.

The choice of the correct sampling spot is of critical importance. Relatively deep, slow-moving sections of the river will characteristically contain higher concentrations of algae than sections where turbulent flow occurs.

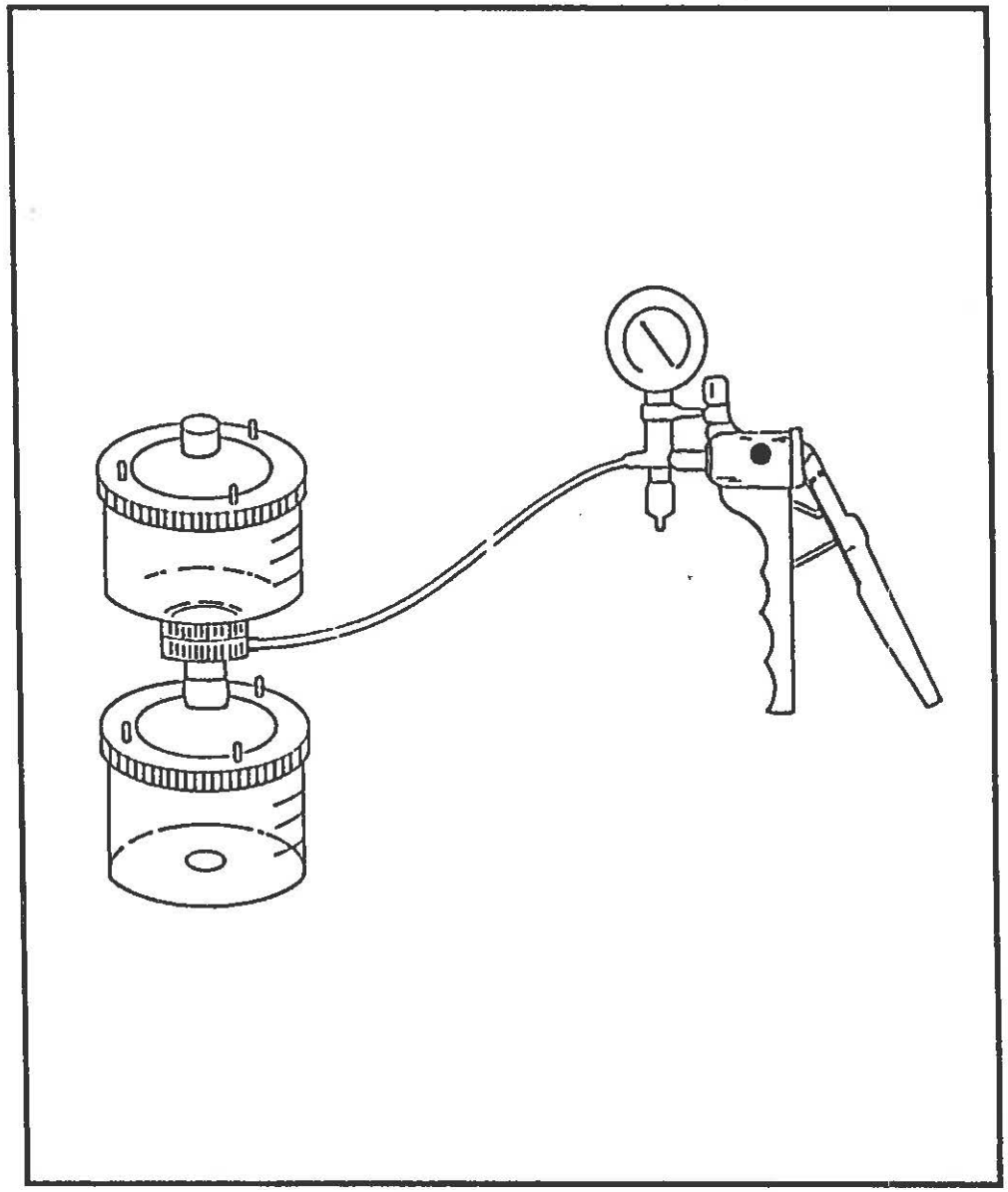

Figure 12: Filtration unit (Badenhorst, 1994).

7.3 SESTON DRY MASS

7.3.1 Background

The seston of an impoundment refers to the total suspended matter occurring in that impoundment. It includes the dead and alive phyto- and zooplankton, suspended solids and detritus. The amount of seston present influences the optical properties and ecology of the aquatic environment. In very turbid waters seston dry mass closely approximates the suspended sediment concentration.

7.3.2 Equipment

- Hand vacuum pump
- Plastic filtration unit
- pre-weighted glass filter paper in a petri dish
- 5 *l* plastic beaker
- Hose pipe or Van Dorn sampler
- 1 *l* plastic bottle.

7.3.3 Procedure

Collect surface samples with a beaker, integrated samples with a hose pipe sampler or depth samples with a Van Dorn sampler (see section 7.1 - chlorophyll *a* determination), depending on the type of sample needed. If the water sample can not reach the laboratory within 6 hours, the sample is filtered through a pre-weighed glass fibre filter paper and the amount of water filtered is recorded. Remove the filter and store in a Petri dish. Label the Petri dish with the relevant information, namely the date, time, location, depth of sample and volume filtered.

If the sample will reach the laboratory within 6 hours, collect the sample in the 1 *l* plastic bottle and filtration of the water sample is not necessary.

7.4 ALGAL IDENTIFICATION

7.4.1 Background

Algae is a collective term referring to a wide range of pigmented oxygen-producing photosynthetic organisms usually found in water (DWAF, 1993). Many types of freshwater algae (phytoplankton) are found, floating (no true roots, stems, and leaves) freely in the currents or attached to plants and other submerged substrates. The algae form the basis of the food chain and their productivity and ecology will influence other aquatic life. The presence of algae in surface waters is a function of the amount of nutrients (nitrogen, phosphorus and carbon), light and other physical constituents (DWAF, 1993). Excess algal growth can lead to taste and odour problems, while some algae produce toxins in water supplies, which may have an adverse effect on human health, and cause animal deaths. The impact of abundant growth of algae on the water quality of an impoundment is often dependent upon the species present, particularly upon whether the dominant forms are diatoms, green algae or blue-green bacteria. Because of their short life cycles, algae respond rapidly to environmental changes, and therefore the species composition may be an indicator of the quality of the water in which they are found.

7.4.2 Equipment

- 25 *ml* glass container with cap (with Lugol's solution)
- 5 *l* plastic beaker
- Hose pipe, or Van Dorn sampler

7.4.3 Procedure

Collect surface samples with a beaker, integrated samples with a hose pipe sampler or depth samples with a Van Dorn sampler (see section 7.1 - chlorophyll *a* determination). Throw the collected water sample into a beaker and mix well. Pour the sample into the 25 *ml* glass container (with Lugol's solution). Cap the sample bottle and label with all the relevant information, namely the date, time, location and depth of sample. The sample can be kept for at least one year if it is stored in a cool, dark place.

7.5 ZOOPLANKTON

7.5.1 Background

The term "plankton" refers to those microscopic aquatic forms having little or no resistance to currents and living free-floating and suspended in open or pelagic waters, because of their small size or insufficient mobility. Zooplankton are planktonic animals, where as phytoplankton are planktonic flora.

7.5.2 Equipment

- Zooplankton net (Figure 13) when an integrated sample of zooplankton in the whole water column is required
- Schindler-Patalas trap (Figure 14), Haney chamber or closing net when zooplankton species composition, concentration or biomass at a certain depth are required.
- 5 *ml* 4% formaldehyde
- 1 ℓ sample bottle

7.5.3 Procedure

The choice of sampler depends on the kind of study, e.g. distribution or productivity. To collect an integrated sample, lower the Zooplankton net vertically into the water column until it reaches the bottom or the given depth. Haul the net hand over hand with a steady, unhurried motion. If excessive algae is present, splash water against the net to wash everything into the cup. Remove the cup from the zooplankton net and carefully pour cup contents into the 1ℓ sample bottle. Rinse the cup to ensure that all organisms adhering to the sides of the cup are washed into the 1ℓ sample bottle. Preserve the sample by adding *ca.* 5 *ml* 4% (v/v) formaldehyde solution. Label the sample bottle and also record the date, time location and depth of the water column that was sampled. Determine the volume water filtered through the net, as follows:

$$V = \pi r^2 d$$

where: V = volume water filtered
r = radius of the net opening
d = depth of water column sampled

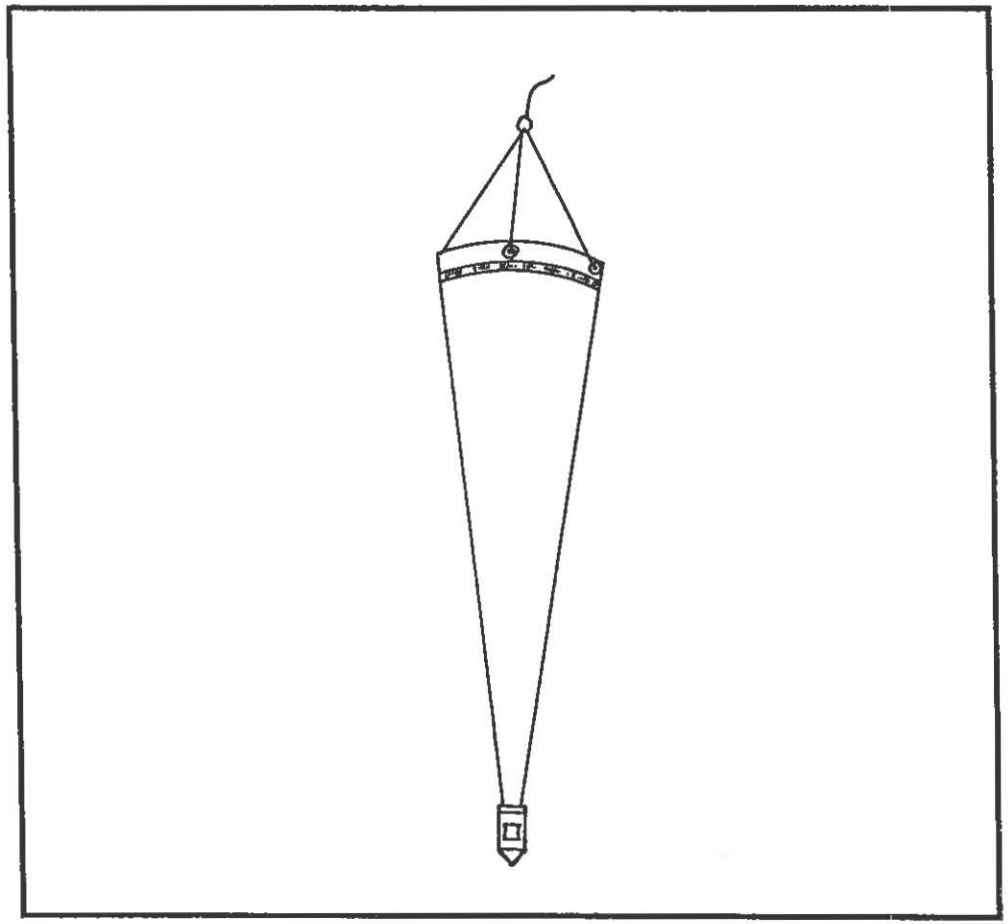

Figure 13: Zooplankton net (Badenhorst, 1994)

Take one sample at each sampling point. The sample is used for enumeration and species composition.

Always clean the zooplankton net and the cup properly before storage by rinsing it well with water.

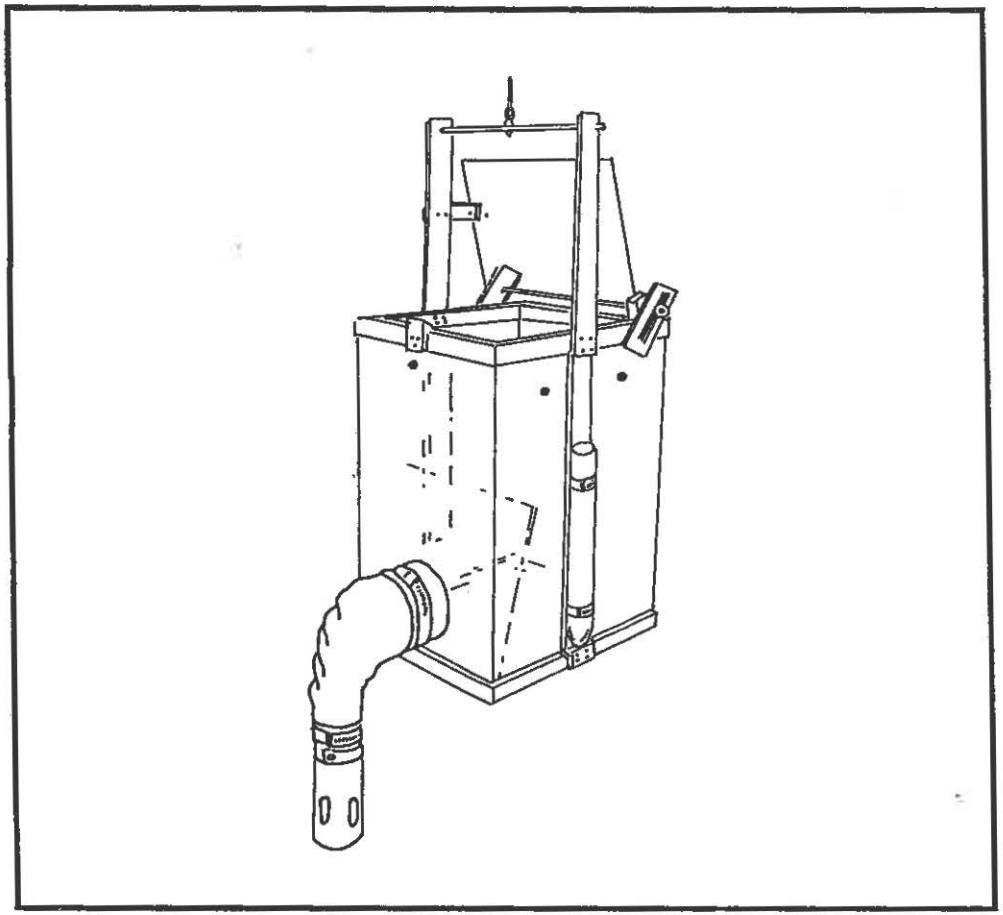

Figure 14:: Schindler Patalas trap (Badenhorst, 1994)

7.6 BACTERIOLOGICAL COUNT

7.6.1 Background

Bacteriological examinations of water are necessary to determine the sanitary quality of water. The presence of pathogenic, or disease-producing, microbes in water significantly reduces the fitness for drinking water, contact recreation, and the irrigation of the crops that are usually eaten raw. Most water-borne diseases (for example: gastroenteritis, amoebic dysentery, cholera and typhoid fever) are associated with water contaminated with human faeces (Harris *et al.*, 1992). Parasitic infections such as giardiasis, ascariasis, and bilharzia can also be transmitted by recreational water contact (Canadian Guidelines, 1992). Infection may occur by ingestion, inhalation or surface contact.

As a result of the low density of pathogens in environmental waters, the wide range of possible pathogens, and the expensive and time-consuming nature of many pathogen detection methods, it is common practice to monitor and control microbiological water pollution on the basis of levels of indicator organisms, rather than of pathogens themselves. Ideal indicator organisms should meet the following criteria (Canadian Guidelines, 1992);

- they should occur in sewage and polluted waters whenever the pathogens are present,
- they do not occur in unpolluted waters and when the pathogen are not present,
- they should be suitable for use in all types of water,
- they should be present in numbers that show correlation with the degree of pollution,
- they should be present in numbers much greater than the pathogen,
- they should not multiply in the aquatic environment and not itself be pathogenic to either humans or animals,

- they should survive for at least as long as the indicated pathogen, and preferably longer,
- they should respond to natural conditions and treatment processes in a manner similar to the pathogens, and
- they should be easily isolated, identified and enumerated in the laboratory.

Total coliform bacteria are frequently used to assess the general hygienic quality of water, while faecal coliforms are widely used as indicators of faecal pollution to evaluate the quality of wastewater effluents, river water, raw water, drinking water supply and recreational waters. The presence of *Escherichia coli* is used to confirm the presence of faecal pollution by warm-blooded animals (often interpreted as human faecal pollution). It is used to evaluate the possible faecal origin of total and faecal coliforms (Grabow, 1983). Faecal coliforms and *E. coli* are often used as indicators of all microbial pathogens, including viruses and parasites, and also of bacteria which cause surface (i.e. skin, eye, ear, etc.) infections and respiratory illness, rather than gastrointestinal disease (DWAF, 1993).

Before samples are collected, a reconnaissance of the area is generally advisable. In impoundment's the investigator should be aware of major point sources of pollution (e.g. sewage works effluent) as well as watering places for cattle and other sources of pathogenic bacteria. Small streams should be sampled close to the confluence with the main river stream. The sample should not be taken in stagnant water or at a point where the river may have been pushed up. (Rossouw and Badenhorst., 1987).

When sampling a river with the aim of appraising the general bacteriological quality, samples should be taken from a mid-stream position. Prior to sampling from a tap, the tap should be turned on and the water allowed to flush out for at least 2 min. Should the tap be situated at the end of a long distribution pipe and not been run for a long time, or from a deep borehole, an extended flushing time will be required (Badenhorst, 1994).

7.6.2 Equipment

- 250 *ml* or 500 *ml* sterilized glass bottles

7.6.3 Procedure

7.6.3.1 Environmental and effluent samples

The majority of samples are grab samples. Use only sterilized 250 *ml* glass sampling bottles. At the sampling point, remove the cap while ensuring that the interior surface of the bottle or cap is not touched. Plunge the bottle neck down into the water (approximately 30 cm below the surface) and tilt the neck up to face the flow of the water. The water should pass the neck of the bottle before it passes the hand of the sampler to prevent contamination. Ensuring that there is an air space (*ca.* 2,5 cm) in the bottle to facilitate mixing before analysis. Cap the bottle immediately. Samples should be analysed as soon as possible and kept below 10 °C but not frozen. Uncooled samples must be analysed within 6 h and cooled samples within 24 h.

7.6.3.2 Drinking water samples

If drinking water or chlorinated effluents are sampled, it is necessary to add a dechlorinating agent to the bottle. The dechlorinating agent employed is a 100 *g/l* solution of sodium thiosulphate, of which 0,1 *ml* for every 100 *ml* sample volume is added to the sample bottle prior to sterilization. After the tap has been flushed, adjust the tap to a gentle flow and fill the sampling bottle and store it as outlined above. Avoid taking samples from encrusted or badly corroded pipes. Residual chlorine measurements should be made at the time of collection of the sample.

8. SAMPLING TECHNIQUES FOR TOXICITY TESTING OF WATER

8.1 SAMPLING TECHNIQUE FOR TOXICITY TESTING

8.1.1 Background

These toxicity tests or bioassays generally refer to laboratory-based biomonitoring. These toxicity tests are necessary in water pollution evaluations because chemical and physical tests alone are sometimes insufficient to assess potential effects on aquatic biota. Toxicity tests are useful for a variety of purposes, including suitability of environmental conditions for aquatic life, effectiveness of waste treatment methods and compliance with water quality standards, effluent requirements and discharge permits.

Various test organisms can be used in acute (short term) and chronic (full life cycle or long term) tests, both to quantify the toxic effects of a single substance or multiple substances and to serve as biological indicators of effluent and receiving water quality.

Acute tests are used for routine monitoring and are valuable for quickly supplying an estimate of toxicity. Species used for toxicity testing at the IWQS include an algal bioassay, a microbial assay, fish and waterflea tests.

IMPORTANT: Always liaise with the appropriate laboratory before taking samples. The laboratory will supply the correct sample containers. Knowing when the samples will arrive will enable the personnel to prepare before hand to ensure that the sample will be tested immediately.

8.1.2 Equipment

- 4 ℓ glass bottles

8.1.3 Procedure

Samples are collected in precleaned glass bottles (minimum of 4 litres). Sample bottles must be filled completely. Remember to rinse the sample bottle with the sampling water and pour the water away in a downstream direction. When collecting the water the opening of the sample bottle must face an upstream direction. No chemical preservation must be performed. Preferably samples must be analysed within 6 h of collection. If this is not possible, store the samples at 4 °C and start analysis within 36 h of collection. Always store the samples in the dark.

8.2 SAMPLING TECHNIQUE FOR AMES BACTERIAL MUTAGENICITY TESTING

8.2.1 Background

A mutagen is a chemical or physical agent that can induce a permanent, transmissible change in the genetic material of a cell, usually a single gene. Mutagens are therefore important compounds as they can initiate irreversible illnesses such as cancer. Damage to DNA is likely to be a major cause of cancer and other diseases. The *Salmonella* Ames

Mutagenicity Test, is currently being used to survey substances for mutagenic activity.

During the last two decades, the Ames test has acquired the status as a benchmark assay to detect genotoxins (DNA-damaging substances), mainly in drinking water. It has become an accepted procedure to screen for chemicals that may have genotoxic potential and has the criteria for an established predictive test for carcinogens. The test measures back-mutation in several specially constructed mutants of *Salmonella typhimurium* (TA98, TA100, TA102, etc.) which increases specificity of the test. This test has been adapted for use in detecting chemicals which are potential human carcinogens or mutagens (Hill, 1995).

Modern industrialisation of our society has resulted in a dramatic increase in the production and use of new synthetic organic compounds and the concern relating to the possible pollution of the environment with chemicals which may have toxic, mutagenic and carcinogenic effects (Keith, 1976). It has also been found that advanced wastewater purification processes as well as conventional potable water treatment processes may not only fail to remove or deactivate mutagens but sometimes even lead to the formation of additional mutagens (Denkhaus, *et al.*, 1979).

8.2.2 Equipment

- 2 x precleaned 2.5 litre brown bottles

8.2.3 Procedure

Samples for the *Salmonella* Ames Mutagenicity Assay are collected in 2.5ℓ brown bottles which are precleaned according to certain procedures. Two 2.5ℓ samples are taken per sampling site (when requested). Rinse the sample bottle with the sampling water and pour the water away in a downstream direction. Plunge the bottle neck approximately 30 cm into the water and tilt the neck up to face the flow of the water in an upstream direction. No chemical preservation must be performed. Label the bottles with the relevant information (time, date sampled; sampling site). Samples must be kept cool during transportation. Preferably samples must be analysed within 1 week of collection.

Samples must be stored at 4°C at arrival at the IWQS and the relevant personnel in the Microbiology Laboratory must be informed so that the necessary arrangements for analysis can be made.

8.3 SAMPLING TECHNIQUE FOR DETECTION OF BLUE-GREEN BACTERIA TOXINS

8.3.1 Background

Toxicity in freshwater algae is associated with a specific group of organisms, the blue-green bacteria (cyanobacteria). In South Africa the common bloom forming species are *Microcystis aeruginosa*, but blooms of other *Microcystis spp.* together with *Anabaena spp.*, *Oscillatoria spp.*, and *Nodularia spumigena* have been reported. Many blue-green bacteria regulate their buoyancy to remain in the near surface waters where the light conditions are more favourable for growth. Cells may however become over buoyant, and form thick scums (Quibell *et al.*, 1995).

Algal or blue-green bacteria blooms are likely to be linked to water bodies which receive discharges or runoff containing high concentrations of nutrients such as nitrates and phosphates, and are of a high trophic status (Du Plessis, pers. communication, 1997). Excessive algal growth is a symptom of eutrophication and is the result of enrichment of a water body with plant nutrients (Du Plessis *et al.*, 1990). Blooms may contain both toxic and non-toxic strains. Toxin production is highest under very low or

high pH, and also increases with increases in light intensity. Removal of nitrogen results in a decrease in toxicity (Quibell *et al.*, 1995).

Blue-green bacteria toxins can have an effect on the nervous system, the liver, and primarily cause skin irritations. These bacteria have been associated with a number of livestock and game deaths, and human health problems.

8.3.2 Equipment

- 250 *ml* plastic or glass bottles for sampling scums
- 2 x 1ℓ plastic bottles for water samples
- beaker or hose pipe

8.3.3 Procedure

The type of sample will depend on the information requirements of the study. In impoundments collect (i) a surface water sample with a beaker, 50 meters from the dam wall; or (ii) an integrated water sample (0-5 meters) with a hose pipe at the discharge point. Surface water samples are also collected at canals, before purification works, and after treatment process. Pour the water sample in a 1ℓ bottle. Keep the sample cool at 4 °C, or freeze.

Scum can be collected as a surface sample with a beaker. Pour the sample in a 250 *ml* plastic or glass bottle if scum sample is directly taken to the laboratory. Keep the sample cool at 4 °C, do nor freeze glass bottle. The sample should reach the organic laboratory in 24 h for analysis.

The detection for blue-green toxins are usually done inherence with chlorophyll *a,* algal identification and seston dry mass.

9. SAMPLING TECHNIQUE FOR COD OF WATER

IMPORTANT: Always liaise with the appropriate laboratory before taking any samples. The laboratory will supply the correct sample containers. Knowing the arrival time of the samples will enable personnel to make the necessary preparations.

9.1 COD (Chemical oxygen demand)

9.1.1 Background

The COD is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant (DWAF, 1993). The COD therefore gives an estimate of the organic matter available as a carbon source in a water body or effluent for the micro-organisms such as bacteria, fungi and algae. The greater the COD of the water supply, the greater the potential for profuse microbial and algal growth.

The presence of organic matter in process and product water may have several deleterious effects and consequences in industrial processes.

9.1.2 Equipment

- 250 *ml* glass bottles
- preserve with cons. H_2SO_4 (pH ≤ 2)
- beaker
- hand gloves
- cool bag

9.1.3 Procedure

Samples are collected in clean 250 *ml* glass bottles. Surface water samples are collected at canals, rivers, impoundments, sewage works, etc., where ever there is an indication of organic pollution. Rinse the sample bottle with the sampling water and pour the water away in a downstream direction. Plunge the bottle neck approximately 30 cm into the water and tilt the neck up to face the flow of the water in an upstream direction. Remember to wear hand gloves. No chemical preservation has to be performed when sample will be back at the laboratory in 24 h. Label the bottles with the relevant information (time, date sampled; sampling site). Samples must be kept cool during transportation, store in cool bag on ice blocks or at 4 °C.

Unstable samples must be tested without delay. If delay before analysis is unavoidable, preserve sample by acidification to pH ≤ 2 using concentrated sulphuric acid (H_2SO_4). Maximum storage of 48 h with preservation is aloud.

10. SPECIAL TECHNIQUES

10.1 BACKGROUND

The majority of aquatic organisms have the unfortunate handicap of living downstream of humans. As a consequence, the integrity and biodiversity of aquatic ecosystems is highly dependent on the way humans manage the landscape (Karr, 1991).

The health of an ecological system is often reflected by the health of the organisms that reside in the system. Biological communities have been generally recognised as useful in assessing water quality and aquatic ecosystem integrity, because they are sensitive to low-level disturbances and function as continuous monitors (Chandler, 1970).

Numerous environmental stressors such as unfavourable or fluctuating temperatures, high or low water velocities and sediment loads, low dissolved oxygen concentrations, poor water quality, limited food availability, anthropogenic stressors such as contaminant loading and other types of episodic variables can individually or together impose stress on the physiological systems of aquatic organisms and impair their health (Wedemeyer *et al.*, 1984).

This has led to the introduction of aquatic biomonitoring, which makes use of aquatic organisms (macroinvertebrate and fish) in order to reflect the quality of the associated water and habitat conditions, and to determine the effect of changing environmental conditions (Roux, 1993).

10.2 FISH KILL INVESTIGATIONS AND SAMPLING PROCEDURE

10.2.1 Background

Fish mortalities result from a variety of causes, some natural and some man-induced. Natural fish kills are caused by phenomena such as acute temperature change, storms, decomposition of natural organic materials, salinity changes, spawning mortalities, and parasitic, bacterial, and viral epidemics. Man-induced fish kills may be attributed to municipal or industrial waste water, agricultural activities and water manipulations (Klemm *et al.*, 1992).

Temperature changes, either natural or the result of a heated water discharge will often result in fish kills. Long periods of very warm, dry weather may raise water temperature above lethal levels for particular species. Oxygen depletion and extreme pH variation can be caused in winter periods when plant and algae decomposition takes place because of insufficient light, or by respiration or decay of algae and higher plants during summer months in very warm weather. Kill resulting from such causes are often associated with a series of cloudy days that follow a period of hot, dry sunny days (Klemm *et al.*, 1992).

Occasionally fish may be killed by toxins released from certain species of living or decaying algae that reached high population densities because of the increased fertility resulting from organic pollution. Disease, a dense infestation of parasites, or natural death of weakened fish at spawning time must always be suspected as contributory factors in fish mortalities.

Dead fish are a strong indication that there is pollution on the loose in the lake or stream. If that body of water is also a source of potable water, it is important that the problem be tracked down quickly.

Success in solving a fish kill problem is usually related to the speed with which investigators can arrive at the scene after a fish kill begins, since fish disintegrate rapidly in hot weather and the cause of death may disappear or become unidentifiable within minutes.

10.2.2 Important factors to keep in mind

- Teams are desirable rather than a single individual, as a investigator.
- Generally, when an investigator arrived on site, the water mass in which the fish kill occurred has travelled to some unknown point downstream. If the investigator can estimate or knows when the fish kill occurred, he can calculate the distance the contaminated water travelled and intercept it at some downstream point, where he may be on site with a fish kill in progress.
- A kill may not occur until the pollutant is mixed across the stream, thus eliminating escape to save waters. For instance if the pollutant follows one bank for a great distance, the fish kill may not occur for several miles downstream from the discharge. The reason for this is that with most pollutants, fish will avoid the contaminated waters and thus will move from along the bank to better quality water in midchannel or along the opposite bank.
- The season of the year may have considerable impact on the magnitude and characteristics of the kill. In colder temperature, biodegradable materials may persist longer because of reduced decomposition rates. As a result, fish kills may extend far greater distances.
- In addition to the temperature effects on decomposition, the species of fish killed react very differently as to when and for how long they will be visible.
- Investigators should be constantly alert to the selective kills involving only a couple of species or one species, or certain sizes of fish. The habitat occupied by these species, may aid in determining what caused the kill or its mode of action. An excellent example of this would be in a lake containing both warm water and cold water fish.
- Frequently fish kills occur at a point remote from the pollution source because of the time involved for reactions to develop. An example of this may occur downstream from a reservoir that is discharging deep waters. These deep water may be void of dissolved oxygen and contain many materials that have a demand for oxygen such as organic materials, sulphates, ammonia, iron and manganese.
- Dual kills would most likely occur during periods of lowest stream flow and/or highest water temperature.
- One should also remain alert to multiple factors (synergism and antagonism) that may effect a fish kill. For example, the presence of ammonia will create the toxicity of water at low dissolved oxygen concentrations, though a bioassay would indicate that neither the ammonia nor the oxygen concentrations, tested separately, would be toxic.

10.2.3 Equipment

- Map and GPS
- Fish kill database questionnaire
- waterproof notebook
- waterproof pens
- pH meter
- Oxygen and temperature meter
- EC meter
- ½ *l* glass bottle for toxicity testing
- 250 *ml* polypropylene bottles (5 each) for inorganic and metal analysis
- 1 *l* glass bottle for bacteriological analysis (where applicable)

- glass bottles for organic compound analysis (where applicable)
- bucket
- sampling net
- dissection kit
- cool bag with ice chests
- camera
- waders
- aluminium foil
- paper towels
- fish kill sampling procedure and questionnaire forms (Appendix 1B & 2B)

10.2.4 Procedure

Gather all the necessary information from the informant. The location of observed kill, the time that the kill was first observed, the general kinds of organisms affected, an estimate of the number of dead fish involved, and any usual phenomena associated with the kill.

Inform the Director: IWQS and the Regional Office of the fish kill. Prior to the fish kill investigation, inform all the relevant laboratories and key personnel at IWQS and the pathologist on duty at Onderstepoort (Veterinary Institute).

Collect motor vehicle and relevant equipment.

10.2.4.1 On site action

- Locate the fish kill area, preferably meet the informant on the fish kill site.
- The location of sampling sites is very important. If there are no obvious reasons for a kill, sites should be selected inside and outside the apparent kill area. If there are possible polluters, each should be suspect and sampling sites must be selected inside and outside of the area of influence for each possible suspect. Select sampling sites in the affected area, downstream and upstream the affected area.
- Take *in situ* measurements (e.g. pH, temperature, DO, conductivity) and previously described in manual.
- Take water samples for chemical analysis (inorganic and metals), toxicity testing and where applicable take water or sediment samples for organic compounds and/or water samples for bacteriological analysis. (Collection methods previously described in this manual).
- The on-site study includes specific observations (Appendix 1B) that may be made on a Standard form (Appendix 2B). Record observation on questionnaire form (weather prior and during fish kill, odour, water flow, presence or absence of algal blooms, other vegetation, dead or alive fish or macroinvertebrates, actions of moribund fish, other aquatic life behaviour, other activities, water conditions both in and outside affected area, possible pollution sources, own opinion as to the cause, etc.).
- It is always best to collect moribund (dying) fish from the affected area. If none are available, freshly dead fish will have to be utilised. Unaffected fish from outside the affected area must also be collected. Collect 2-5 dying fish, and make external and internal observations and examinations (fungus, parasites, bleeding, erosion on fins or opercula, aberration, anomalies or damage on skin or eyes, severe mucus secretion on gills, discolouration of any tissues or organs, enlarge liver or spleen, extended intestine, etc.).
- Transport 2-5 dying fish in cool bag at 4 °C, and deliver the dying fish at Onderstepoort within 6-12 hours for histopathological and bacteriological examinations.
- Take numerous pictures of the overall area, specific problem area, dying fish, algal bloom, and water condition.
- Back at the IWQS, deliver water samples at appropriate laboratories for rapid analysis.
- Contact informant the next day to view the situation.

10.2.4.2 Report

- The magnitude of fish kill should be carefully documented. Essential elements of the report are: (1) introduction, (2) description of study area with hand drawn map of the general and specific location of fish kill, (3) Water users, (4) Description of all sampling methods and analysis, (5) results (photo's of affected fish and location of kill), (6) discussion, and (7) conclusion.

10.3 SOUTH AFRICAN SCORING SYSTEM (SASS) SAMPLING PROCEDURE

10.3.1 Background

The SASS protocol is based on the British Biological Monitoring Working Party (BMWP) method which has been adapted for South African conditions. It uses the occurrence of aquatic invertebrate families (insects, snails, worms, etc.) to determine the biological integrity of rivers and streams. The SASS protocol is designed not to be too academic. It is aimed at allowing field operators with minimal training in aquatic invertebrate taxonomy to be able to recognize these organisms at the family level with the aid of illustrations provided (Thirion *et al.*, 1995).

10.3.2 Equipment

The minimum amount of equipment needed are: a net, tray, forceps, and a scoring sheet. However, to make things easier, the following should be used: waders (or rubber boots), bucket, magnifying glass, towel, and a manual.

In order to collect and preserve samples, the following will be needed: tubes, waterproof bags or small bottles, formalin (or 80% ethanol), waterproof labels and a pencil or pen containing waterproof ink.

(a) Net: The net should be sturdy, 30 cm square, and have a 1000 micron mesh netting securely fixed to the frame. A long handle (e.g. a broomstick) is also preferred.

(b) Sampling Tray: The tray should be light coloured, fairly large (any size between 30 cm x 30 cm to 40 cm x 50 cm), and at least 5 cm deep (e.g. photographer's tray).

(c) Forceps: Pointed forceps are used for removing debris; stirring the water; and catching the organisms for closer examination to identify them.

(d) Scoring Sheet: A standard form for scoring. See example in Appendix C.

(e) Bucket: For convenience of carrying water, if the place chosen for examining the sample is not situated on the river bank.

(f) Magnifying Glass: It can be of any type: e.g. jewellers, hand-lens, etc.

(g) Towel: For drying hands, equipment, etc. or for sitting on.

(h) Manual: Contains methods to be used and sketches to aid identification of the benthic macroinvertebrates (Thirion *et al.*, 1995).

(i) Sample Tubes: Or other type of container. Must be fairly small and water-tight. For taking specimens back to the lab for positive identification or for your specimen collection.

(j) Formalin/Ethanol: Formalin kills and preserves organisms (this includes humans, so take care!). A 4% solution (volume/volume)

formalin or 80% (volume/volume) ethanol is recommended for preserving specimens taken back in sample tubes.

(k) Labels: Labels should be waterproof, and placed in the container with the sample. Date, locality and the name of the sampler should be noted using pencil or waterproof ink.

(l) Waders: As used by fly-fishermen, for protection when kicking stones and from Bilharzia, cold, etc. Rubber boots can be used instead of waders when working in shallow water especially in warm weather.

Other useful items would include a storage facility for samples taken, as well as maps, a hat and a set of dry clothes!

10.3.3 Procedure

10.3.3.1 Site Selection

A sampling site should preferably have a diversity of biotopes, not be too deep or fast flowing, and should at least have a regular flow. Biotopes are defined as follows:

(a) Stones in current (SIC): Moveable stones of at least pebble size (3 cm in diameter) to approximately 20 cm in diameter; within the slow and fast flowing sections of the river.

(b) Stones out of current (SOOC): Where the river is still, such as behind a sandbank or ridge of stones. These stones are often covered by a thin layer of silt.

(c) Sand: Sand should be out of current, either sand-banks within the river, or small patches of sand in hollows at the side of the river. Sand is often also found under, and between, the stones at the side of the river.

(d) Gravel: Gravel or shingle typically consists of smaller stones, ranging in size from 2 or 3 mm up to 3 cm. This biotope is less common than the others.

(e) Mud: Mud consists of very fine particles, usually as dark-coloured sediment in rivers and streams. It usually settles to the bottom in still or slow flowing areas of the river.

(f) Marginal Vegetation (MV): This is overhanging grasses, bushes, twigs and reeds from the river bank.

(g) Aquatic Vegetation (AQV): Rooted, submerged or floating water weeds such as *Potamogeton, Aponogeton* and *Nymphaea*.

NOTE: It is important that all biotopes must form part of the main river channel. Isolated pools should not be used.

A site should at least have stones, vegetation (this might be a problem in winter), and one of the smaller grain biotopes i.e. sand, gravel or mud; and preferably all of the above. All the biotopes present **must** be noted on the scoring sheet.

10.3.3.2 Sampling procedure

The various biotopes are sampled using different methods, and for different lengths of time, but using the same net. If preferred, the biotopes can be sampled and analysed separately. Although one person can do the sampling, it is easier if two people work together. One person does the kicking, while the other person handles the net. Sampling should always be done moving upstream so that the organisms are not disturbed before sampling.

(a) Stones in current: The net should be placed close to, and downstream of, the stones to be kicked, in a position where the current will carry the dislodged organisms into the net. The stones should be kicked over and against each other to dislodge the animals, at different places. This should continue for 2 minutes, if the stones are encrusted or difficult to move, sampling may continue for up to five minutes.

(b) Stones out of current: For this and all remaining biotopes, the net must be moved to catch the biota as they are dislodged. Approximately one square meter of these stones should be sampled by kicking the stones in the same manner as described for stones-in-current, whilst continuously sweeping the net in the disturbed area.

(c) Sand, Gravel and Mud: In all three cases stir the substrate by shuffling or scraping the feet for half a minute, whilst continuously sweeping the net over the disturbed area to catch the dislodged organisms.

(f) Marginal Vegetation: A total of approximately two meters of vegetation must be sampled, in one or more locations. The net must be held perpendicular to the vegetation, half in and half out of the water and then swept back and forth in the area being sampled.

(g) Aquatic vegetation: The net can be pushed against and amongst the vegetation under the water in an area of approximately one square meter.

Once sample collection is complete, the sample should be washed down to the bottom of the net, then carefully tipped into the tray of water. The net should be submerged, so that the whole sample can be transferred in this way. The net should then be checked by hand for any lingering specimens, which should be taken out and placed into the tray.

Before scoring can begin, leaves, twigs and other loose debris should be removed from the tray, shaken in the water and checked for biota, before being placed into the bucket for eventual return to the river, or thrown directly into the river. Upon completion of identification and scoring, the sample is returned to the river.

The SASS index is usually done together with a Habitat Quality Index (HQI) (Appendix C), *in situ* physical measurements and chemical characteristics.

10.4 IDENTIFICATION OF AQUATIC WEEDS

10.4.1 Background

Aquatic plants are large vascular plants that live in wet conditions. Aquatic plants (also called aquatic macrophytes) usually posses true roots, stems and leaves. They can be grouped into four types:

- *Emergent plants*

have a large portion of stems and leaves growing above (emerging from) the water surface; they are found in shallow water (less than 50 cm deep), or along the shoreline.

- *Rooted floating-leafed plants*

have leaves that float on or just above the water surface but are connected to the bottom by long, tough stalks.

- *Submersed plants*

have most of their leaves and stems below the water surface, often with flowering parts projecting above surface. They may be securely or loosely rooted in the bottom.

- *Free floating plants*

float near the water surface with root systems dangling in the water, but not connected to the sediment.

Our reservoirs, rivers and streams have been involved in a long, continuous process of evolution. As each system evolved and achieved a natural balance of its own, native species of aquatic plants and animals (flora and fauna) became uniquely connected. Native plant communities serve a variety of important functions in aquatic systems. These range from providing food, shelter and nesting sites for fish, waterfowl and other animals to protecting water quality and quantity and shoreline stability. Invasion of a system by foreign species, however, can quickly destroy the fine balance that took so many years to develop. Away from diseases and insects that serve as natural controls in their native regions, invader plants can grow and spread quickly. In doing so they can damage the structure and function of ecosystems by crowding out native plants and changing the habitat quality for fish and wildlife.

Determining whether a plant is a problem is not always easy. *A plant is considered an aquatic weed when it grows were it is not wanted and pose a nuisance condition to the user of the water.* Some uses of the water body that can be affected by excessive aquatic plant growth are:

- water loss due to excessive evapotranspiration
- provide habitat for agents of human diseases such as malaria, bilharzia, cholera and river blindness
- destroying wildlife habitat
- interfere with recreational activities such as fishing, boating and swimming
- limit access to water
- negative impact on the aesthetic value
- economic (affects the value of property)
- endanger livestock and human live
- endanger civil structures, obstructing and reducing water flow and increasing flood levels

It is important that the field worker or environmental scientist should become aware which plants are declared weeds. Four aquatic plants are declared as weeds according to the *Conservation of Agricultural Resources Act (Act No 43, 1983).* This act states that no person is allowed to keep, sell, exhibit or transport these weeds in any part of the Republic of South Africa; facilitate the dispersion of these weeds in any way and transport the weeds to another area. The act also forces riparian land owners to destroy declared weeds in water bodies bordering their property or weeds in water bodies on their property.

10.4.2 Aquatic Weeds of concern

The following plants are declared* or proposed declared weeds:

- Water Hyacinth (*Eichhornia crassipes*) [declared weed]
- Water Lettuce (*Pistia stratiotes*) [declared weed]
- Parrot's Feather (*Myriophyllum aquaticum*) [declared weed]
- Kariba Weed (*Salvinia molesta*) [declared weed]
- Red Water Fern (*Azolla filiculoides*) [proposed declared weed]

***Act No 43 of 1983.**

10.4.3 Reporting of aquatic weed infestations

In is expected of all Water Affairs personnel to report any aquatic weed infestation as soon as possible to ***The Departmental Committee for the Management of Aquatic Weeds in Water Systems***. The committee can be contact either directly by telephone or a memo to the Chief Engineer: Water Utilisation, or you can report the infestation to your directorate representative on the committee.

Contact persons:

- Chief Engineer: Water Utilisation (Att. HH Bosman, tel: 012-338 8067)
- IWQS representative: BJ Du Plessis (Tel: 012-808 0377 x 200)

The selling, moving, exhibition of aquatic weeds can also be reported directly to:

- The Director: Gauteng Nature Conservation (Tel: 012-323 3403)

10.4.4 Collecting samples of plant species

To collect and prepare an aquatic plant sample for verification follow the following steps:

STEP 1: Obtain a aquatic plant sample by dropping weighted rake to the bottom of the lake and pull up the vegetation snagged by the rake. Remove the plants from the rake, sorting out the different plant types. If a river is invested, plants can be collected by hand. To keep plants from drying out, sort them in a shallow pan filled with water.

STEP 2: Rinse a few healthy specimens of the plant types with water from the river or impoundment. Carefully lay the plant specimens between two double layers of paper towel, place them in a plastic bag and seal the bag securely. Label the bag with the following information:

- Date
- Name of water body
- Location (GPS co-ordinates will be helpful)
- Depth of sample
- Extent of infestation
- Your name and telephone number

STEP 3: Send or deliver the sample to your representative on the Departmental Committee for Aquatic Weeds in Water Systems or send it to:
Chief Engineer: Water Utilisation
Att. Mr HH Bosman
Department of Water Affairs and Forestry,
Private Bag X313,
PRETORIA
0001

10.4.5 Description of aquatic weeds of concern

10.4.5.1 Water Hyacinth (*Eichhornia crassipes*)

Description:

Water hyacinth is a free-floating or mud-rooted plant with round to oval leaves up to 80 mm in diameter, although smaller leaves are common. Leaves are bright green and shiny and held upright so they act like sails, which facilitates distribution of the plant. The leave stalk is spongy and thick and helps to keep the plant buoyant. A mass of fine roots hang in the

water column. Flowers are large and produced during the summer months. They are purple-blue or lilac coloured with a yellow spot (Figure 15).

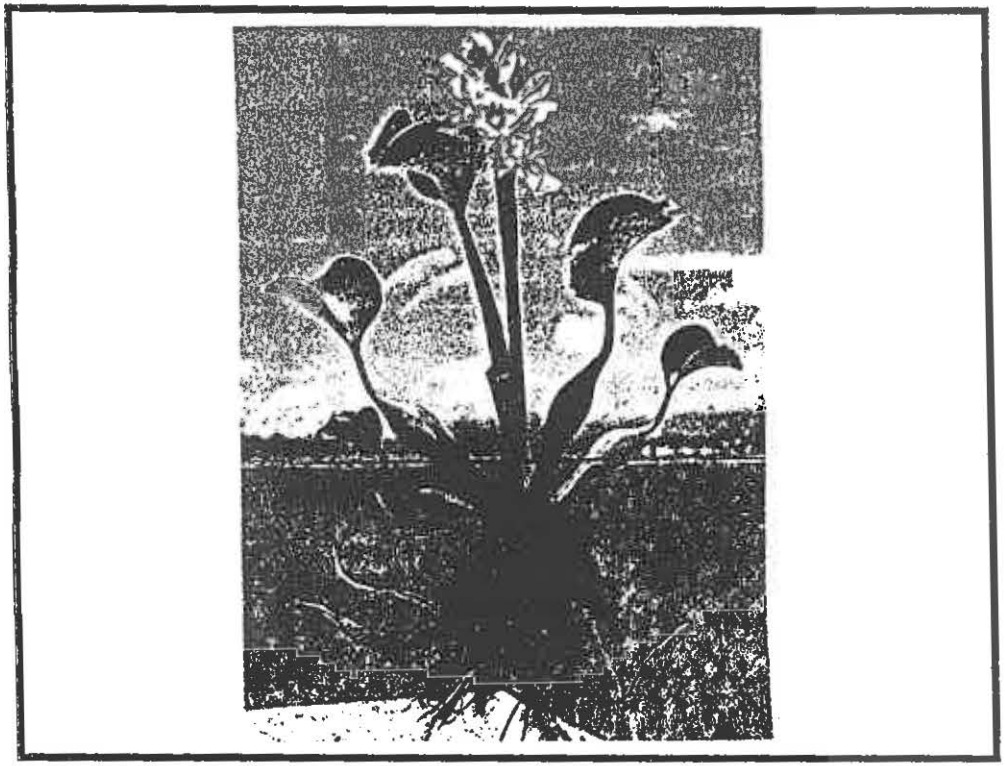

Figure 15: Water Hyacinth

Growth habitat:

Water hyacinth can form impenetrable mats of floating vegetation. Water hyacinth is a native plant to the American tropics, water hyacinth has spread to all the warmer regions of the world and is today one of the major aquatic weeds in the world. It was introduced to South Africa shortly before 1910 and is now well-established in all four provinces, especially in the eastern and southern regions of the country. Particularly large concentrations are found in the coastal regions of Natal and the Eastern Cape. A major management programme in the Vaal River is currently successfully controlling the weed in this river system.

Propagation:

Water hyacinth reproduces by seeds and vegetatively. Daughter plants form on rhizomes which can form dense beds of water hyacinth. In one study one plant produced 1200 daughter plants in a period of four months. Individual plants break off the mat and are dispersed by water currents. As many as 2000 seeds can be produced by a single plant. Seeds are eaten and transported by water fowl. The seeds sink to the bottom and may be viable for 15 years. Seedlings are common on mud banks exposed by low water levels (Hartbeespoort Dam, Leeuwkraal Dam, etc).

Legislation and control:

Water hyacinth is a proclaimed noxious weed throughout the Republic of South Africa. (Proclamations 170/1937, 161/1938, 199/1938, 45/1945, 50/1948 and 252/1956 of the Weeds Act No 42 of 1937, and the *Conservation of Agricultural Resources Act (Act No 43, 1983)*).

The Department of Water Affairs and Forestry is currently practising a integrated control strategy which involves the integration of chemical and biological control methods. The national aquatic weed management plan include close co-operation between the departments of Water Affairs and Forestry, Agriculture and Nature Conservation as well as various semi-private and private institutions in South Africa.

10.4.5.2 Water Lettuce (*Pistia stratiotes*)

Description:

Water lettuce is a perennial, mat-forming, aquatic plant. It is usually free-floating but it can survive in mud (Figure 16). Under tropical conditions, where plant nutrient levels are adequate, the water lettuce produce rapidly. The leaves are pale yellow-green, narrower at the base, rounded, straight or notched at their tips, ribbed, with many longitudinal veins radiating from the base. The leaves are also characterising hairy on both sides. The flowers are pale green or white and are produced from February to May (in some regions all year).

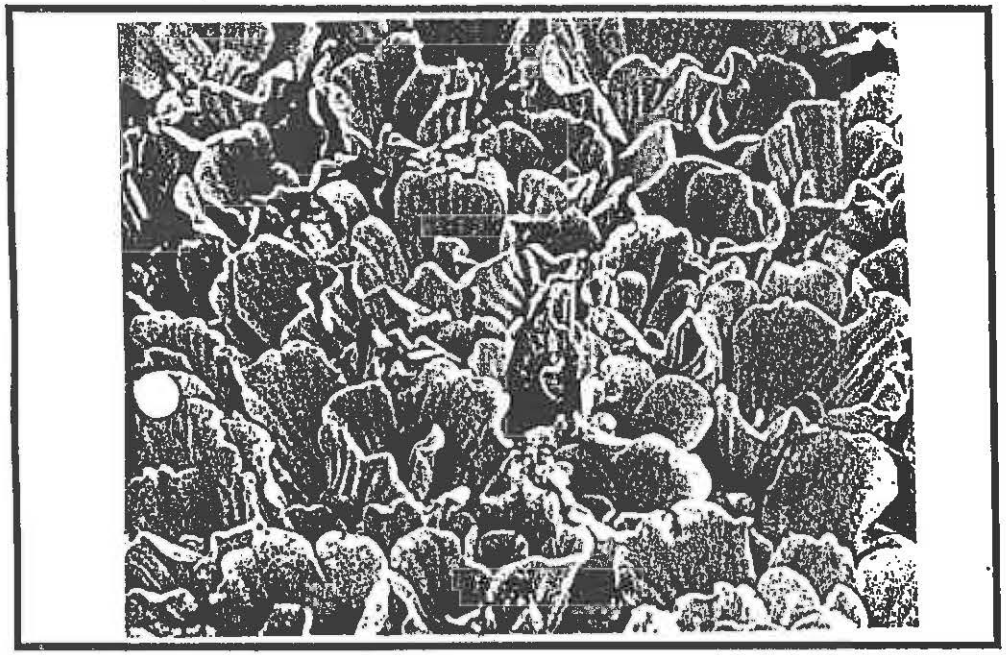

Figure 16: Water Lettuce

Growth habitat:

It invades dams, pans and slow moving rivers in subtropical regions.

Propagation:

Water lettuce originates from South America and are now distributed in the northern parts of the country. At this point in time the plant is localised and under control.

Legislation and control:

Water lettuce is a proclaimed noxious weed throughout the Republic of South Africa. (*Conservation of Agricultural Resources Act (Act No 43, 1983)*). Chemical and biological control measures are in place.

10.4.5.3 Parrot's Feather (*Myriophyllum aquaticum*)

Description:

Parrots feather is a rooted water plant with long shoots whose pale green tips emerge for about 0.2 to 0.5 m above the water surface. The plants has both emergent and submerged leaves. The submersed leaves are finely-dissected, and feathery, often with a reddish colour. The emergent stems are the most distinctive feature of parrot's feather. Emergent leaves form in whorls on the stem. Leaves are bright green and finely divided. In spring, very small, white, tuft-like flowers form where the emergent leaves attach to the stem (Figure 17).

Growth habitat:

Parrot's feather grows best when rooted in shallow water. In nutrient-enriched lakes it can grows as a free-floating plant. The emergent stems can survive on wet banks of rivers and lake shore, so it is well adapted to moderate water level fluctuations. The species, which often occurs in

dense, pure stands, is capable of invading the shallow parts of most water bodies in southern Africa.

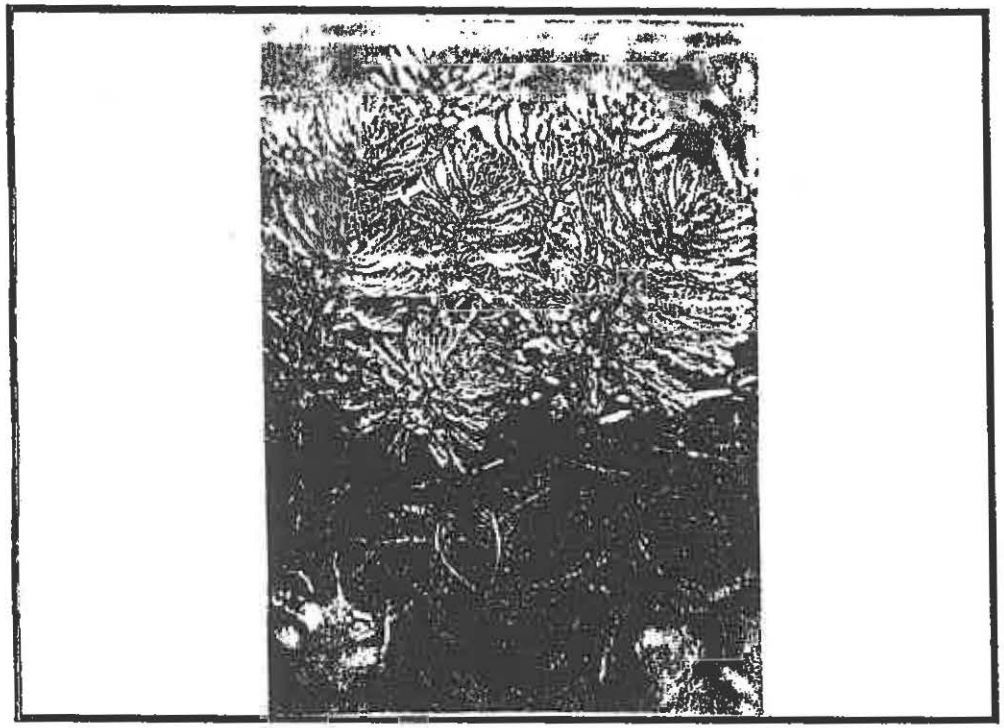

Figure 17: Parrot's Feather

Propagation:
Only female plants are known from southern and eastern Africa, therefore parrot's feather spreads only by plant fragments. Parrot's feather rhizomes are quite tough and can be transported long distances on boat trailers. Parrot's feather 's attractive green foliage make it a popular aquascaping plant, which has contributed to its distribution. The plant was introduced to South Africa before 1919 and has since spread throughout the country. It now grows in many dams and steams particularly in the south-western and eastern Cape, Natal and the southern and eastern Transvaal.

Legislation and control:
Parrot's feather is a proclaimed noxious weed throughout the Republic of South Africa. (Proclamation 252/1956, Act No. 42 of 1937 and the *Conservation of Agricultural Resources Act (Act No 43, 1983)*).

10.4.5.4 Kariba Weed (*Salvinia molesta*)

Description:
Kariba weed is a small, green to yellow-green, free floating aquatic plant. Its thin horizontal stem bears pairs of oval leaves, notched at the tip and varying in width from 10 to 60 mm. The leaves are unwettable and velvety due to a dense cover of hairs, each bearing a minute cage-like structure at the tip. These hair trap air bubbles and keep the plant afloat. Clusters of rounded, sterile fruiting bodies, about 1 mm in diameter, may be attached to the lower root-like leaves (Figure 18).

Growth habitat:
Kariba weed is native to South America. It is a free floating plant but it can survive in mud in the shoreline of a water body. Kariba weed forms thick impenetrable mats which over time develop in thick, floating islands. These have the capability to cause severe ecological alterations.

Propagation:
Kariba weed is today one of the most troublesome aquatic weeds widely distributed in tropical and subtropical countries such as India, Malaysia and Australia. African countries include Kenya, Zambia, Zaire, Zimbabwe, Angola, Mozambique, Eastern Caprivi, Botswana and South

Africa. In South Africa it is found in Transvaal, Natal and the southern and western Cape.

The plant reproduces vegetatively, by fragmentation of the main rhizome, and regenerates easily. Where high nutrient concentrations are available, and temperature and light conditions are favourable, it can double its mass in three to five days.

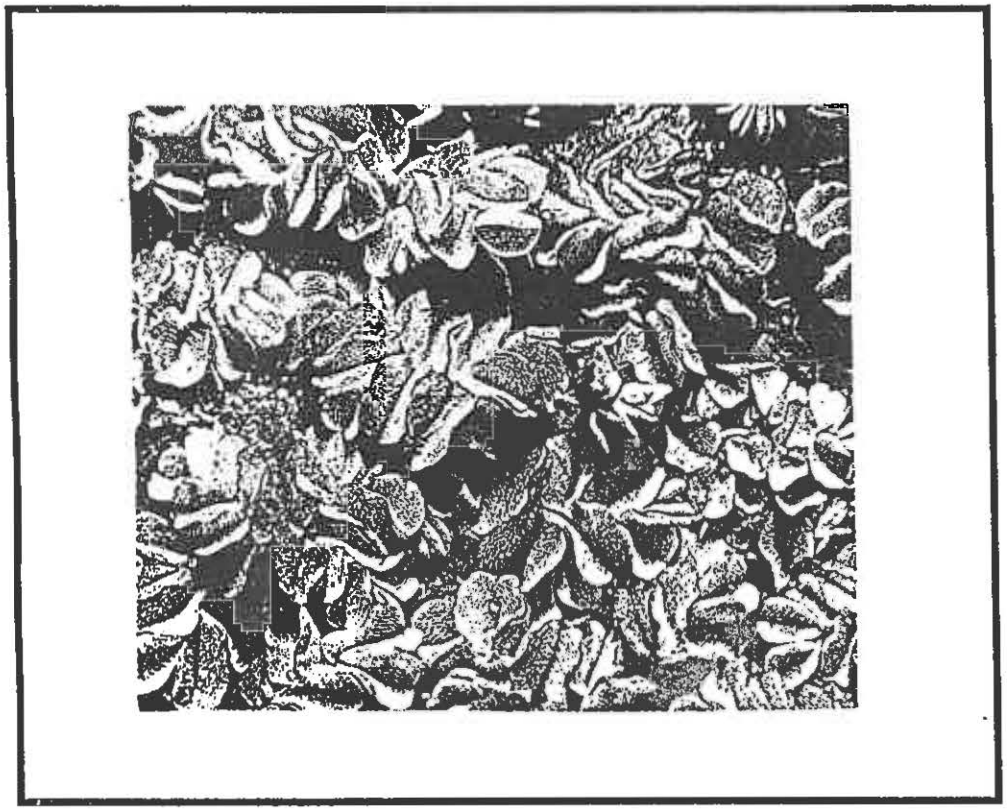

Figure 18: Kariba Weed

Legislation and control:

Kariba weed is a proclaimed noxious weed throughout the Republic of South Africa.(Proclamation 252/1956, Act No. 42 of 1937 and the *Conservation of Agricultural Resources Act (Act No 43, 1983)*). Due to its hairy leaves, chemical treatment is a difficult task. It can however be controlled by chemical means and bio-control agents are available.

10.4.5.5 Red Water Fern (*Azolla filiculoides*)

Description:

Red water fern is a small, green to reddish-brown or purplish, free-floating annual or perennial rarely longer than 25 mm. The short, branched stem bears roots and is densely covered with small leaves which are 1 to 1.5 mm long and overlap in a scale-like manner. Each leave is two-lobed. The upper lobe contains chlorophyll, while the lower one is colourless. A blue-green bacteria is present in the cavities in the upper lobe of each leaf (Figure 19).

Growth habitat:

Water fern grows within a wide temperature range and can withstand short periods of freezing. The growth is apparently inhibited by wind and wave action which accounts for their rather limited mass occurrence in large water bodies. There exist an association of symbiosis between water fern and the blue-green bacteria, *Anabaena azollae.* The blue-green is capable of fixing atmospheric nitrogen and to supply the plant of all its nitrogen needs.

Although not a declared weed as jet, red water fern is recognised as a major problem in South African rivers and smaller impoundments. Red water fern rapidly colonise an open water surface and can destroy the aquatic environment by reducing dissolved oxygen levels to zero.

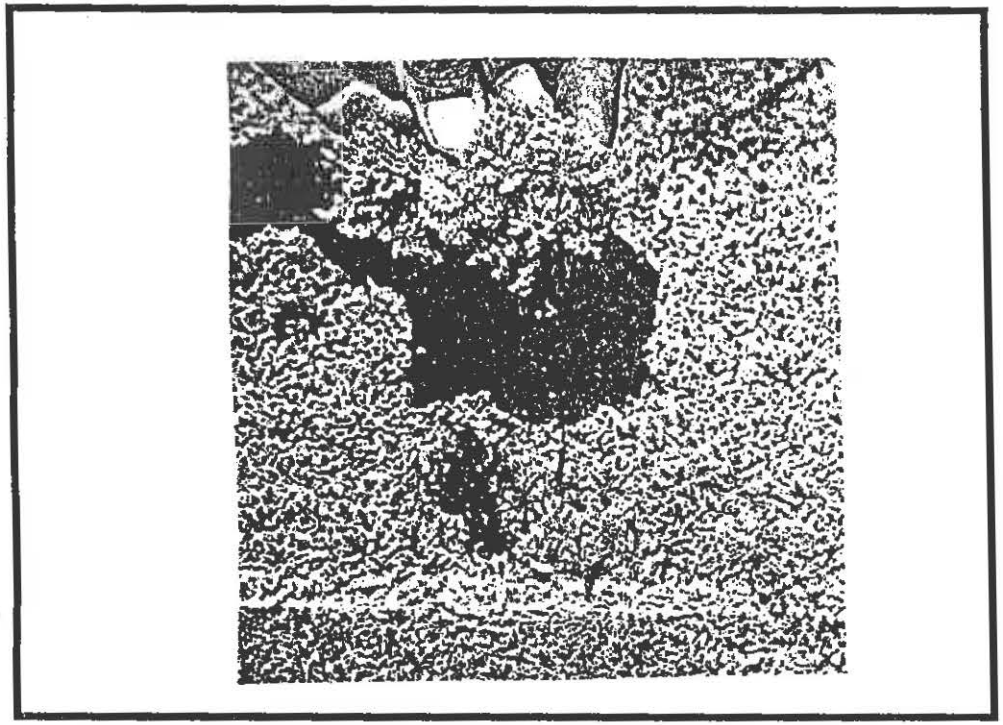

Figure 19: Red Water Fern

Propagation:
From October to February spores are produced in two types of very small, rounded fruiting bodies borne in the axils of leaves. Reproduction occurs via seeds and vegetatively.

Azolla plants has been used as green manure in rice paddies in South East Asia. In certain countries it is also used as pig and duck food. Red water fern is already distributed right through South Africa, from Cape Town to the Northern Province.

Legislation and control:
Legislation is on its way to proclaim red water fern as a noxious weed throughout the Republic of South Africa. No chemical is registered in South Africa at this point in time for the control of *Azolla*. Effective control is possible with both chemical and biological methods.

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APPENDIX A

TABLE 1: SUMMARY OF SPECIAL SAMPLING OR HANDLING REQUIREMENTS

Determination	Container	Minimum sample size (*ml*)	Preservation	Maximum storage without preservation	Maximum storage with preservation	Page References
Alkalinity	P, G	300	Refrigerate/ $HgCl_2$	24h	14d	25
Boron	P(red)	300	None required	14d	6 months	25
Carbon, organic, total	G	300	Analyze immediately or preserve with 3 *ml* HCl	7d	28d	
COD	P, G	300	Analyze immediately or preserve with 3 *ml* H_2SO_4	24h	14d	46
Chlorine, residual	P, G	500	Analyze immediately	0.5h	stat	25
Chlorophyll *a*	P, G	1000	Refrigerate/filter glass fibers/9.8 *ml* ethanol	24h	N.S	37
Conductivity (EC)	P, G	300	Refrigerate/ $HgCl_2$	14d	28d	21
Fluoride	P(whitel)	300	Refrigerate/ $HgCl_2$	14d	28d	25
Hardness	P, G	300	Refrigerate	6 months	6 months	25
Metals, general	P(A), G(A)	300	Refrigerate/ add 3 *ml* HNO_3	14d	6 months	25
Chromium (VI)	P(A), G(A)	300	Refrigerate	24h	24h	29
Mercury	P(A), G(A)	300	Add 3 *ml* HNO_3/refrigerate	24h	28d	29
Nitrogen:						
Ammonia	P, G	300	Add 1 *ml* $HgCl_2$/ refrigerate	7d	28d	25
Nitrate	P, G	300	Add 1 *ml* $HgCl_2$/ refrigerate	48h	48h (28d for chlorinated samples)	25
Nitrate and nitrite	P, G	300	Add 1 *ml* $HgCl_2$/ refrigerate	none	28d	25
Nitrite	P, G	300	Add 1 *ml* $HgCl_2$/ refrigerate	none	48h	25
Organic, Kjeldahl	P, G	300	Add 1 *ml* $HgCl_2$/ refrigerate	7d	28d	25

P = Plastic (polyethylene or equivalent), G = glass, G(A) or P(A) = rinsed with 1+1 HNO_3 , N.S = not stated in cited reference, stat = no storage allowed analyze immediately, Refrigerate = storage at 4°C, TFE = Teflon lined cap, F = aluminium foil lined cap, IS = *in situ*.

Organic compounds:						
Pesticides	G (F)	4000	4 *ml* H_2SO_4/ 4l Water, 100 *ml* DCM	24h	30d	32
Phenols	G (F)	4000	Add 4 *ml* H_2SO_4/ refrigerate	7d	7d	32
Purgeables by headspace	G, (TFE)	40	Refrigerate, add 6 g/*l* ascorbic acid if residual chlorine is present	7d	14d	32
pH	P, G / IS	300	Analyze immediately, *in situ*	24h	14d	20
Phosphate	G(A)	300	Add 1 *ml* $HgCl_2$/ refrigerate	14d	28d	25
Silica	P(white)	300	Refrigerate	14d	28d	25
Solids, suspended	P, G	300	Refrigerate	14d	28d	21, 25
Sulfate	P, G	300	Refrigerate	14d	28d	25
Sulfide	P, G	300	Refrigerate	14d	28d	25
Temperature	IS		*In situ*	none	none	19
Turbidity	P, G / IS	300	*In situ*, Refrigerate	24h	2d	22, 25
Radioactivity	P	5000	Refrigerate	24h	24h	36
SASS:	P					
Trace metals, (dissolved)	P(red)	300	Refrigerate	24h	14d	28
Macro	P(white)	300	Add 1 *ml* $HgCl_2$/ refrigerate	24h	14d	25
Turbidity	P (green)	300	Refrigerate	24h	14d	25
Algal ID	G	25	Lugol's solution		1 year	39
Microcystins	P	4000	Refrigerate/ Freeze	24h	7d	44
Toxicity	G	8000	Refrigerate	6h	36h	43
Bacteriological	G/ sterile	500	Refrigerate	6h	24h	41
Seston dry weight	P, G	1000	Refrigerate	6h	24h	38
AMES	G	8000	Refrigerate	24h	7d	43

P = Plastic (polyethylene or equivalent), G = glass, G(A) or P(A) = rinsed with 1+1 HNO_3 , N.S = not stated in cited reference, stat = no storage allowed analyze immediately, Refrigerate = storage at 4°C, TFE = Teflon lined cap, F = aluminium foil lined cap, IS = *in situ*.

APPENDIX B

a fish kill sampling procedure

Hotline (012) 808 0374

SITE HISTORY AND INFORMATION

RECORD THE FOLLOWING INFORMATION

- date
- time
- extent
- collect photographic evidence (photos/video)

(consult DATABASE QUESTIONNARE for guidance)

CAREFULLY STUDY THE ENTIRE ENVIRONMENTAL PICTURE, INCLUDING:

- weather
- water flow
- vegetation
- algal blooms
- pollution
- water chemistry
- fish behaviour
- other activities

ON SITE ACTION

TAKE ACTION AT THE FOLLOWING SITES

- upstream
- at site of kill
- downstream

OBSERVE BEHAVIOUR OF BIOTA

FISH

☐ rate of mortality
note whether:
- small fish died first
- large fish gulp for air
- fish swim erratically
- fish hyperexcited

OTHER

☐ Macro-invertebrates
dead, dying or absent

☐ Algae
alive, absent or dead

MEASURE

- pH
- temperature
- dissolved oxygen
- electrical conductivity

COLLECT RELEVANT SAMPLES
(use appropriate bottles and procedures)
Samples CAN include:

- toxicity (store at 4 °C)
- major inorganic (preserve)
- metal (4 °C)
- pesticide (preserve)
- bacteriological (4 °C)

IF A DISCHARGE IS SUSPECTED TO BE THE CAUSE OF THE FISH KILL

- Collect relevant samples according to appropiate procedures for possible legal action

COLLECT FISH THAT ARE AFFECTED BUT NOT DEAD

COLLECT FISH (2-5) OF AFFECTED SPECIES

FOR DIRECT DELIVERY TO ONDERSTEPOORT (<6 hours)

- keep cool (4 °C) in coolbox with ice

FOR DELAYED DELIVERY (>12 hours)

- Toxicological samples - frozen
- Pathological (post mortem) samples - preserve in 10% buffered formalin. Use glass containers with sealed lids

SUBMISSION OF SAMPLES

Water samples to:
(to relevant laboratory)

- SABS
- Onderstepoort
- CSIR
- Institute for Water Quality Studies

Fish samples to:
Onderstepoort (Tel: 012-52 99111)
Pathologist on duty - 24 hour sample receiving service
Request relevant analyses:
(Complete sample registration form)

- post mortem
- toxicological investigation
(specify - metals and/or pesticides)

PUBLIC AND MEDIA LIAISON

REMEMBER TO BE:

- friendly
- approachable
- courteous
- sympathetic

MEDIA LIAISON

Prepare and issue relevant press release in collaboration with Communication Services
(no speculation/be factual)

FISH KILL QUESTIONNAIRE

COMPLETE QUESTIONNAIRE AND SEND TO THE

DIRECTOR:
Institute for Water Quality Studies
Private Bag X313
PRETORIA
0001

FISH KILL DATABASE QUESTIONNAIRE

GENERAL INFORMATION

WHO IS THE INFORMANT?

NAME _____ TELEPHONE: _(____)_____

ADDRESS _____

_____

DATE AND TIME REPORTED _____

REPORTING SOURCE

NAME OF OFFICIAL/INVESTIGATOR: _____

ORGANISATION: _____

ADDRESS: _____

_____

TELEPHONE: (W) _(____)_____ (H) _(____)_____

DATE AND TIME OF INVESTIGATION: _____

SITE INFORMATION

Details of site of fish kill:

Type of water body: Stream ☐ Reservior ☐ Other ☐
Wetland ☐ Ocean ☐

Name of water body: _____

(latitude/longitude): _____

Has a fish kill or abnormality been observed at this site before?

Yes ☐ No ☐ Unsure ☐

Duration of the kill (First noticed and stopped) - Date and time _____

Extent of the kill: Area covered (kilometres of river or size of pond or reservoir). _____

_____

Approximate total number of fish affected

(10 - 100) ☐ (100 - 1000) ☐ (1000 - 10 000) ☐

Species affected (please specify)	*Number*	*Size (length - min and max)*

Behaviour of affected fish (indicate what is applicable)

Rate of mortality was abrupt and most fish died within 24 hours	☐	Jerky movements of fins	☐
Small fish died first	☐	Fish hyperexcited	☐
Large fish coming to the surface and gulping for air	☐	Fish swimming erratically	☐
Small fish alive and well	☐		

External appearance (abnormalities) of affected fish _____

_____

Own opinion as to the cause _____

_____

_____

_____

Known recent activities in the surrounding area (crop spraying, weather change, ect.) _____

_____

_____

Possible sources of pollution _____

_____

_____

Field measurements (samples collected):

	UPSTREAM	IN KILL AREA	DOWNSTREAM
Temp			
pH			
Conductivity			
DO			
Odour/Colour/Foam			

Collected sample information

Water sample (what type)			
Fish(frozen)			
Fish (formalin)			
Fish (fresh)			

General remarks

_____

_____

_____

_____

SEND COMPLETED QUESTIONNAIRE TO:

The Director: Institute for Water Quality Studies
Department of Water Affairs and Forestry
Private Bag X313
PRETORIA
0001

APPENDIX C

SASS4

River.....Date.....Time.....

Sampling point.....

Temp.....pH.....EC(mS/m).....

DO(mg/l).....% sat.....Turb.....

Biotopes sampled

SIC.....(Type./time.....)

Marg veg.....Dom.sp.....

Aq veg.....Species.....

SOOC.....Sand..... Mud..... Gravel.....

Other.....HABS1.....

Procedure Protocols

If SIC all kickable, sample for 2 min., otherwise for maximum of 5 min.
Gravel 1/2 min.
Marg/Aq veg, back & forward sweep 2 m.
SOOC kick +- 1m
Sand/mud stir with feet & sweep net over disturbed area for 1/2 min.
Any other biotopes -1/2 min.
Complete top of form.
Tip net contents into tray. Remove leaves, twigs & trash.
Check taxa present FOR THE LESSER of 15 minutes or 5 minutes since the last taxon was found.
Estimate abundance on scale: A 1-10; B 11-100 C 100 - 1000; D >1000
BEFORE LEAVING THE SAMPLING POINT CHECK THAT THIS FORM HAS BEEN FULLY COMPLETED!!!!
* Air breather

TAXON	SCORE	ABUN
Porifera	5	
Coelenterata		
Hydra sp.	1	
Turbellaria		
Planarians	5	
Annelida		
Oligochaeta	1	
Hirudinea	3	
Crustacea		
Amphipoda	15	
Crabs*	3	
Shrimps	8	
Hydracarina		
Hydrachnellae	8	
Plecoptera		
Notonemouridae	12	
Perlidae	12	
Ephemeroptera		
Polymitarcyidae	10	
Ephemeridae	15	
Baetidae 1 sp	4	
2 spp	6	
> 2 spp	12	
Oligoneuridae	15	
Heptageniidae	10	
Leptophlebiidae	13	
Ephemerellidae	15	
Tricorythidae	9	
Prosopistomatidae	15	
Caenidae	6	
Odonata		
Chlorolestidae	8	
Lestidae	8	
Protoneuridae	8	
Platycnemidae	10	
Coenagriidae	4	
Calopterygidae	10	
Chlorocyphidae	10	
Zygoptera juvs	6	
Gomphidae	6	
Aeshnidae	8	
Corduliidae	8	
Libellulidae	4	

Hemiptera	SCORE	ABUN
Notonectidae*	3	
Pleidae*	4	
Naucoridae*	7	
Nepidae*	3	
Belostomatidae*	3	
Corixidae*	3	
Gerridae*	5	
Veliidae*	5	
Megaloptera		
Corydalidae	8	
Trichoptera		
Hydropsychidae 1 spp	4	
2 spp	6	
> 2 spp	12	
Philopotamidae	10	
Polycentropodidae	12	
Psychomyiidae	8	
Ecnomidae	8	
Hydroptilidae	6	
Other movable case larvae:		
case types score fam		
1 8 1	8	
2 15 1	15	
3 20 1	20	
4 30 2	30	
5 40 2	40	
>5 50 3	50	
Lepidoptera		
Nymphulidae	15	
Coleoptera		
Dytiscidae (adults*)	5	
Elmidae/Dryopidae	8	
Gyrinidae (adults*)	5	
Haliplidae (adults*)	5	
Helodidae	12	
Hydraenidae (adults*)	8	
Hydrophilidae (adults*)	5	
Limnichidae	8	
Psephenidae	10	

Observations:

Diptera	SCORE	ABUN
Blepharoceridae	15	
Tipulidae	5	
Psychodidae	1	
Culicidae*	1	
Dixidae*	13	
Simuliidae	5	
Chironomidae	2	
Ceratopogonidae	5	
Tabanidae	5	
Syrphidae*	1	
Athericidae	13	
Empididae	6	
Ephydridae	3	
Muscidae	1	
Gastropoda		
Lymnaeidae*	3	
Melaniidae*	3	
Planorbidae*	3	
Physidae*	3	
Ancylidae	6	
Hydrobidae*	3	
Pelecypoda		
Sphaeridae	3	
Unionidae	6	
Sample score		
No. of families		
Score/taxon (ASPT)		
Air breathers fam.		
Air breathers score		

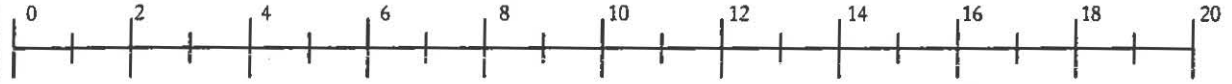
Other families present

HABITAT Q.I.

River: _____ Site: _____ Date: _____

V 2

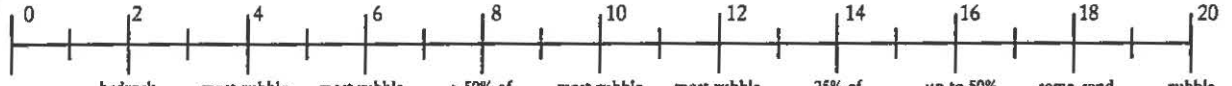
1: RIVER BED



0	2	4	6	8	10	12	14	16	18	20
d	b + c + > 50% d	> 90% b	> 10% a > 50% b + c / d	10% a > 50% b + c / d	30% a 30% b + c / d	50% a 30% b + c / d	a + d	75% a + c / d	75% a + b + c / d	> 80% a the rest c	> 80% a the rest b

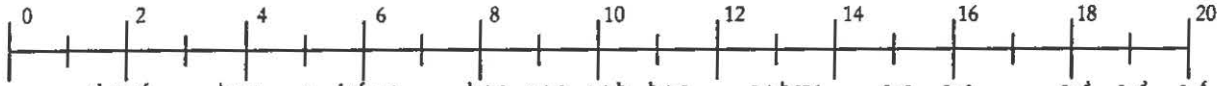
a - rocks : b - sand : c - mud : d - boulders or bedrock

2: EMBEDDEDNESS



0	2	4	6	8	10	12	14	16	18	20
bedrock	most rubble mostly covered by fines	most rubble covered with green or brown algae	> 50% of rubble covered with green or brown algae	most rubble unmoveable due to cementation	most rubble 1/2 covered with fines or river mostly sand	25% of rubble covered in green or brown algae	up to 50% of rubble unmoveable due to cementation	some sand or mud around rubble	rubble totally clear	

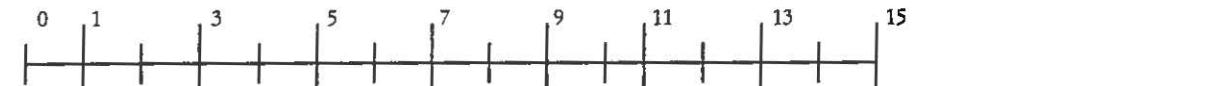
3: HABITAT SAMPLED



0	2	4	6	8	10	12	14	16	18	20
d, e or f	b or c	a	1 of a - c + 1 of d - f	b + c	a + c	a + b	b + c + 1 of d - f	a + b or c + 1 of d - f	a - c	a - c + e or f	a - d	a - d + e or f	a - f

a - stones in current : b - vegetation : c - sand : d - stones out of current : e - gravel : f - mud

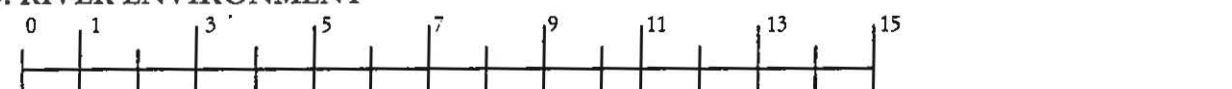
4: TYPES OF VELOCITY



0	1	3	5	7	9	11	13	15
e	1 of a - d + e or f	2 of a - d	3 of a - d + e or f	3 of a - d	3 of a - d + e or f	a - d	a - e	

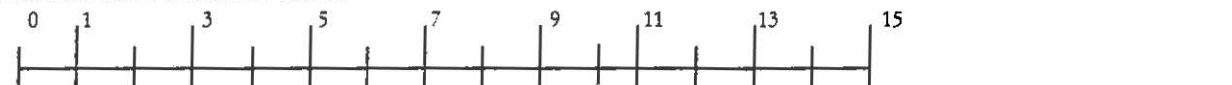
a - fast riffles : b - slow riffles : c - deep water or riffles : d - shallow water or riffles : e - pool : f - running water

5: RIVER ENVIRONMENT



0	1	3	5	7	9	11	13	15
litter - dumping site	smelly water	rural settlement runoff	evidence of close farming / agriculture	greenish water OR very silty from rain	within 20 m of weir OR brown silty water	within 50 m of weir OR water slightly discolored	clear fresh flowing water	

6: HABITAT POTENTIAL



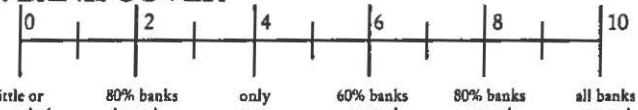
0	1	3	5	7	9	11	13	15
d or e	b or c + e / b or c + d	b, c	b, c, e	b, c, d	a	a, e	a, d	a, c, d OR a, b, e / a, b, d	a - c	a - c + e	a - d

a = riffles : b = hanging vegetation (leaves) : c = growing vegetation (stems) : d = pools : e = running water

7: BANK EROSION

0	2	4	6	8	10
both banks near vertical	1 bank near vertical	both banks steep and raw areas	1 bank < 30° other bank steep	all banks < 45°	all banks < 30°

8: BANK COVER



0	2	4	6	8	10
little or no rocks/ vegetation	80% banks is sand or soil	only one bank covered	60% banks covered by rocks/ vegetation	80% banks covered by rocks/ vegetation	all banks covered by vegetation

9: BANK VEGETATION

0	2	4	6	8	10
no cover at all	little cover bridge etc.	c	a - c	b	a
		b + c	a + c	a + b	

a - shrubs : b - trees : c - grass and reeds

INSTRUCTIONS:

Take each category and move up from the zero until you reach a number that best describes the sampling area. Circle the number and repeat for all nine categories. Add the total score, wich is out of a possible maximum of 135.

EXAMPLES:

In category 1, the river bed consists of > 80% rocks with sand and mud. You would give a score of 19. In category 5, a river with slightly discoloured water would score 13; and in category 8, a river consisting of one bank totally covered, and the other just sand would score 4.

TOTAL SCORE _____

R. WEST.