DEVELOPMENT OF A BIOMONITORING METHOD USING PROTOZOANS FOR ASSESSMENT OF WATER QUALITY IN RIVERS AND GROUND WATERS AND SEASONAL/EPHEMERAL WATERS

Report to the Water Research Commission

by

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EXECUTIVE SUMMARY

Protozoans are single cells that have evolved into some 30 000 species. They are of major ecological importance in that they consume bacteria and also in hat many can encyst or excyst according to the conditions in which they live. Protozoans live in both marine and freshwater conditions and there are four major groups: the amoebae, the flagellates, the ciliates and the sporozoans. The sporozoans are parasitic and, although of immense importance, form a separate field of study – in medical and veterinarian research. They are not dealt with here.

Few studies have been done on protozoans in South Africa, the major work having been done in Europe, the United Kingdom and the United States.

The first component of this report is a literature review that establishes the potential uses of biomonitoring and the systems that are currently in use in South Africa. The South African Scoring System version 4 (SASS4) is currently being used to identify the water quality conditions in a river using the presence or absence of macroinvertebrate taxa. In some parts of Europe and in the UK a similar system, the Biological Monitoring Working Party scoring system is used. Other biomonitoring systems that have been proposed for South African rivers include the Fish Assemblage Integrity Index and the Riparian Vegetation Index. All of these systems require the presence of water in the system in order to produce useful results. SASS4
cannot be used in seasonally dry rivers, ground water and temporary waters, so a system is needed where the permanent presence of water is not crucial.

It would clearly be useful to have additional biomonitoring systems such as

- a backup system to run in parallel with SASS4 in rivers
- means of identifying particular types of pollution (SASS4 merely identifies a general impairment in water quality)
- sytems equivalent to SAS4 for wetlands, non-perennial rivers, sediments and ground water.

The potential of protozoans as useful biomonitoring tools was explored by investigating the protozoan assemblages of a number of sites down the length of a small urban river, a well as a variety of wetlands and some borehole waters. The suitability of direct collection, artificial substrates and laboratory cultures for examining protozoan assemblages was investigated.

The first aim of this project was to investigate and identify those protozoans that could be used as biomonitoring tools and water quality indicators especially for seasonal/ephemeral rivers.

- Initially we undertook a literature review to establish the purposes of biomonitoring and look at the systems which are used in South Africa.
- Currently the South African Scoring System version 4 is being used to identify the state/condition of the river using the presence and or absence macroinvertebrate taxa to calculate a score which assesses pollution levels.
- In some parts of Europe and in the UK a similar system, the Biological Monitoring Working Party scoring system is used.
- Other biomonitoring systems have been proposed for South African rivers viz. the Fish Assemblage Integrity Index (biological), the Riverine Vegetation Index (biological), the Index of Habitat Integrity (non-biological), the Invertebrate Habitat Assessment System (non-biological) and the Geomorphology Index (non-biological).
- All these systems require water in able to be used. Seasonally dry rivers, borehole/subterranean sources and other temporary water sources cannot be tested with SASS. A system was needed where the presence of water was not crucial.
- The Protozoa are single cells which have evolved into some 30 000 species. They are of major importance in that they consume bacteria and that many can encyst or excyst according to certain conditions.
- Protozoa have many features of single cells (they are eukaryotic and have a system of differentiated areas defined by membranes) but they also live as complete individual organisms, moving, feeding, excreting, reproducing and respiring (Curds, 1992).
- There have been limited studies on Protozoans in South Africa. The major work has, and is, being done in Europe, the United Kingdom and the United States.
- The Protozoa live in both marine and freshwater conditions and there are four major groups. The amoebae, Rhizopoda; the flagellates, Mastigophora; the Ciliates and the Sporozoa. The latter group is parasitic and, although of immense importance, they form a separate field of study – in medical and veterinarian research.
- Protozoa have an outer cell membrane within which is the protoplasm which contains the nucleus. The nucleus is commonly species specific and in the Ciliates there are two types of nucleus in the protoplasm.

- Reproduction is generally asexual, but sexual reproduction does occur. Asexual reproduction is by cell division; longitudinal in the amoebae and flagellates and transverse in the ciliates. There are a number of variations to these systems.

The second aim of the project was to establish whether certain groups within the protozoans e.g. ciliates are particularly suitable for water quality assessment.

- In order to make biomonitoring methods accessible to non-biologists it was important to establish whether certain groups, species or populations were more relevant in biomonitoring.
- We decided to carry out four types of sampling which would, we hoped, indicate by their results whether they represented a viable biomonitoring method.
- Lotic sampling along the Liesbeek River, using sites close to those which are regularly sampled for water quality monitoring by the Cape Metropolitan Council, Scientific Services Branch.
- Lentic sampling at various disparate sites in the Cape Peninsula, from pristine to polluted.
- Soil sampling, where soil samples, varying from dry to waterlogged were collected then rehydrated and examined for protozoans present.
- The Liesbeek River is perennial, but water is abstracted from it throughout the year.

The third aim of the project was to establish whether local taxa are cosmopolitan or at least whether or not they respond to water quality variables in the same way that northern hemisphere taxa do or are specifically endemic.

- Our findings were that the major species, used for establishing the saprobic index, are indeed cosmopolitan.
- Using this finding on the species found at the sampling sites on the Liesbeek River we were able to ascertain that the river is mildly polluted from Site 2, the Kirstenbosch site. The pollution level increasing to strong pollution at Site 7, the Valkenberg site**.**

The fourth aim of the project was to establish preliminary methods for collecting protozoans for the biomonitoring of groundwaters.

- Protozoans were collected by means of direct sampling with a basting tube in the lotic sites, using Plastic Foam Units (PFU's) in the lentic sites, surface soil and litter collection for soil sample examination and baler samples direct from boreholes for borehole sampling.
- Lotic samples were examined directly but then stored for a minimum of one week in petri dishes in the laboratory. Lentic samples were squeezed out, examined and then stored in the laboratory in sterile sample jars for a minimum of one week. Dry soil and litter samples were held in sterile sample jars for six months before rehydration and examination. Waterlogged soil samples were examined straight after collection, but held in Petri dishes for a minimum of one week. Baled water samples were held for one week after sieving on arrival at the laboratory. All the samples retained their integrity (did not degrade)when held at ambient room temperature.
- Petri dishes and sample jars were initially examined using a dissecting microscope. Thereafter, individual species were removed with a Pasteur Pipette and examined using a concave slide on the compound microscope.
- Various methods, as suggested in the literature, were used to either impede movement and/or stain the species for further identification.
- In this project we rarely identified specimens which were > 50 μm in length. The exceptions to this had very specialised movement patterns and were identifiable because of this. We were unable to make use of Phase Contrast or Light and Dark Field microscopy.
- However, where water is lentic, underground or present only as a film on subsurface sediments, the use of protozoans as a biomonitoring tool, in tandem with the Saprobic System, may become of major importance in water-poor countries such as Africa.
- Simple but regular biomonitoring methods, such as SASS, which is already carried out by CMC's Scientific Services, should be able to track changes in the condition of the river.
- In the lentic study the effect of site-specific conditions on protozoan communities in wetlands was impossible to separate from the effect of the water quality itself.
- This study was conducted on a very small scale, but it did point to problems in the implementation of any kind of lentic biomonitoring system using protozoans. A new worker would have to become familiar with the identification of organisms and learn the special method of sampling.

- Soil and sediment sampling has a potential as a biomonitoring tool and a key to the identification of species would enable non-biologically trained personnel to undertake biomonitoring.

Conclusions

This study was conducted on a small scale but did point to problems in the implementation of any kind of lentic biomonitoring system using protozoans. Identification of protozoans is difficult and so it would be time-consuming to train biomonitoring technicians: quality assurance of identifications might be a problem.

Protozoans might be useful biomonitoring agents for ephemeral systems, although since many species are able to encyst under unsuitable conditions, results would have to be carefully interpreted.

Recommendations

Identification of protozoans

This project has developed considerable expertise in the identification of freshwater protozoans. In particular, numerous photographs have been taken and video recordings have been made of several taxa.

Independently of this project, Heeg (in press) has produced a brief guide to the identification of freshwater protozoans as part of the WRC-funded project to publish guides to the identification of all freshwater invertebrates. In order to make the best use of the protozoan information in both projects, it would be valuable to collate species lists, keys, photographs and video recordings into a single package for use by future workers on the group.

Use of protozoans in biomonitoring

The rather preliminary results of this project have indicated that protozoans do not offer an easy alternative to the existing SASS biomonitoring system, which uses macroinvertebrates for estimating impairment of water quality in rivers. Developing a similar system using macroinvertebrates for perennial wetlands is likely to be difficult because of the intrinsic differences in water chemistry and other environmental features

between wetlands: it is likely, from the work reported in this project, that this would be true of the use of protozoan assemblages too.

Nevertheless, the real possibility exists of using protozoans in the biomonitoring of various aspects of non-perennial systems and of ground water. The fact that protozoan cysts can persist for some time in a desiccated state offers the possibility that they can provide information on antecedent conditions in dry rivers and wetlands. Further their very rapid responses to inundation means that protozoans should be useful for estimating water quality conditions over relatively short periods of time in ephemeral systems. This aspect should be followed-up.

Protozoans in ground water

The fact that we were unable to find a method for collecting protozoans from ground water should not preclude attempts using a variety of techniques, including artificial substrata, which we did not use in our very brief study of borehole waters.
The National River Act of 1998 requires that a Reserve be calculated for such water resources, though, and we need to continue to investigate protozoans in this regard.

Method recommended for further work on protozoans

Based on the investigations detailed in the report, we offer the following tips for future work on protozoans in biomonitoring studies.

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CHAPTER 1

Introduction

South Africa is not "river rich" and many of our smaller rivers are subject to seasonal and sometimes to longer-term dry periods. Much dependence is placed on water supplied by boreholes from underground water sources and well points in seasonally dry river beds. With increased population growth, and basic amenities being supplied to formerly disadvantaged communities, in addition to the large use of water for agricultural purposes (Rawhani, 1991), water demand from these sources will increase.

One of the inevitable consequences of excessive use of water is pollution. SASS, the 'South African Scoring System', is a simple biomonitoring system that uses riverine invertebrates to reflect water quality and thus the extent to which a river's water has been polluted. SASS, which was developed and refined as a result of a project undertaken by the Water Research Commission (Chutter, 1998), can usefully be employed only in perennial streams and so an additional method of biomonitoring is required for other kinds of aquatic ecosystems such as wetlands, non-perennial rivers, and subsurface waters. The present project explores the usefulness of protozoans in biomonitoring of water quality, particularly for aquatic ecosystems where the SASS system is inappropriate.

The aims of this project, with a brief explanation of the intention of each, were:

i) To investigate and identify those protozoans that could be used as biomonitoring tools and water quality indicators especially for seasonal/ephemeral rivers. The intention was to develop a familiarity with the freshwater protozoan faunas of the rivers, wetlands and ground waters of the region so as to identify taxa that might display predictable differential distributions based on identifiable differences in physical or chemical conditions in their habitats.

ii) To establish whether certain groups within the protozoans (e.g. ciliates) are particularly suitable for water quality assessment. The intention was identify taxa that could be identified relatively easily and/or that are associated with particular physical or chemical features of the water in which they live.

iii) To establish whether local taxa are cosmopolitan or at least whether or not they respond to water quality variables in the same way that northern hemisphere taxa do.

It has been claimed that protozoans are largely cosmopolitan in distribution, which suggests that each identifiable species, wherever it occurs throughout the world, will

have identical (or at least very similar) habitat requirements. It may be, though, that morphologically identical taxa have been isolated from each other for so long (on different continents, for instance) that they have evolved different habitat requirements. Since virtually all existing data on habitat requirements of protozoans are based on European (and to a lesser extent on American) specimens, we need to know if we can extrapolate those data to the South African situation.

iv) To establish preliminary methods for collecting protozoans for biomonitoring of ground waters. We have remarkably little understanding of the biota of underground waters or of their habitat requirements. It has been suggested that protozoan assemblages might provide useful information in this regard.

CHAPTER 2

Literature Review

The biological assessment of water quality has gained increasing value since it has been realised that such assessment can be of critical importance in the understanding of environmental threats and also in prediction of water quality.

2.1 Biomonitoring: systems presently in use

Using bioindicators for the purposes of monitoring is not a modern concept. It was noted by Pliny the Elder (AD 23-79) that "the value of living organisms as indicators of specific sets of environmental conditions" was seen in Germany 2000 years ago where grazing wild animals selected specific pastures (Phillipson, 1983). Biomonitoring has been the subject of research and controversy ever since (Kneitz, 1983). Today biomonitoring systems commonly measure the presence of one or more types of plants and animals and compare the resultant figures with a prescribed and tested index in order to assess the degree of pollution or to track, and sometimes to predict, changes in the biotic integrity of a system.
Biomonitoring has been defined as "long term standardised measurement, observation, evaluation and reporting of the aquatic environment in order to define status and trends" Meybeck *et al* (1992)

In Europe and the United Kingdom, where very different river conditions exist from those in South Africa, the Biological Monitoring Working Party (BMWP) scoring system was developed and is used. The BMWP scoring system uses invertebrates as an overall indication of water quality.

According to Murray (1999) there are six environmental monitoring systems either being used or proposed for routine use in South African rivers. Three are biological: SASS, the Fish Assemblage Integrity Index (FAII), and the Riverine Vegetation Index (RVI). Three - the Index of Habitat Integrity (IHI), the Invertebrate Habitat Assessment System (IHAS) and the Geomorphology Index (GI) are non-biological. Only three of these, namely SASS4, the RVI and the IHI ,are "mature and well tested" biomonitoring methods (DWAF, 1999), the rest being prototypes, still undergoing tests. Importantly, all these biological or non-biological methods require the actual presence of water in the system being analysed, and most have been developed for rivers rather than for lentic systems. The underlying premise of the present project was that, since various species of protozoans are known to indicate particular forms of impairment of water quality, it might be possible to develop a protozoan-based biomonitoring system.

Further, since protozoans can survive desiccation, and are ubiquitous in wetlands as well as rivers, such a biomonitoring system might represent an expansion of the existing biomonitoring systems, like SASS, that can be used only in perennial rivers.

2.2 The Protozoa – history and research

Protozoans are a very important group ecologically. Wetzel (1975) estimated, for instance, that their consumption of bacteria was of major importance in energy transfer in lakes during times of bacterial blooms. The Protozoa is a huge group of organisms that occur in marine, estuarine and fresh waters. Their species number has been estimated at 30 000 (Curtis, 1968). But, more importantly for South African conditions, protozoans also occur in soils where there is only a microfilm of water. Protozoans are able to live even where water is severely restricted, and to encyst when water dries up completely, during droughts for instance. It is also easy to persuade them to excyst in the laboratory, so examining dry soil or wetland sediments for aquatic protozoans might allow one to track water quality conditions during a previous dry spell.

The monitoring and prediction of changes in pollution levels by identifying the free-living protozoans in water and soil have been used fairly extensively in the northern hemisphere. Specific communities of protozoans have long been used as part of the biota in sewage treatment plants, "treating" differing levels of polluted water and consuming specific kinds of bacteria, even in anaerobic conditions. Thus the presence or absence of particular protozoans reflects levels of bacterial and other pollutants in the water (Curds, 1982; 1983 1992; Foissner, 1996). The use of protozoans as bioindicators is discussed in detail by Foissner (1987).

Protozoa have many features of single cells (they are eukaryotic and have a system of differentiated areas defined by membranes) but they also live as complete individual organisms, moving, feeding, excreting, reproducing and respiring (Curds, 1992). They were first reported in the 16th century by Anton van Leeuwenhoek (Curtis, 1968), who is regarded by most protozoologists as the "father of protozoology" (Kudo, 1971). Many protozoologists undertake research on specific taxa within the protozoans (e.g. Kahl, 1930; 1931; 1932; 1935; Stout, 1967; Kudo, 1971; Foissner, 1987) . Corliss, (1979) lists many of the published reports on the ciliates, which have been studied more than any other free living protozoan group. Almost all of these researchers studied northern hemisphere species, although there have been isolated exceptions.

South African soil Protozoans were studied by Fantham (1921; 1922 ; 1923; 1924; 1929; 1931), who was Professor of Zoology at the University of the Witwatersrand, and also by Professor H Sandon, who was at the University of Cape Town in the late 1930s and early 1940s. Sandon's major work was a book on soil Protozoa (Sandon, 1927), although he also worked on South African endozoic ciliates (i.e. ciliates living in the guts of animals). Currently, research is being undertaken on parasitic protozoa by Profs Van As at the University of the Free State and Markus at the University of the Witwatersrand, and a small group of researchers at the CSIR laboratories in Stellenbosch in the Western Province. Some publications on African Protozoa are Hecky (1978; 1981), Dragesco & Dragesco-Kerneis, (1986) and (Bamforth, 1987).

2.3 The Protozoa – taxonomic position

Protozoa are unicellular eukaryotes which, with the algae and the flagellate fungi, have been placed in the kingdom Protista (Sleigh, 1989). The Kingdom Protista is a fairly new construct, and is not accepted by all protozoologists, although there is general consensus that this alliance of unicellular eukaryotes is a sensible one (e.g. Curds, 1992). Until a few decades ago many of the single-celled eukaryotes were placed in one phylum, the Protozoa, which were generally considered to have affinities with multicellular animals and were usually studied by zoologists. Corliss (1984), describing the newly erected Kingdom Protista, considered the kingdom to have 45 phyla ranging through amoebae to green (Chlorophyta) and red (Rhodophyta) algae, Sporozoa (parasitic protozoa) and Myxosporidia (parasitic on cold-blooded vertebrates) but the Protozoa no longer existed as a taxonomic unit. Thus the word 'protozoan' is now used mostly as a common name for the animal-like members of the Protista.

Various modern classification systems have been proposed (see Sleigh, 1989 and Curds, 1992) but for convenience we have adopted the somewhat simplistic one used by biologists for many decades before the erection of the Kingdom Protista. Four groups of protozoans are recognised, based on their means of locomotion. Originally the Protozoa was considered to be a single phylum and each of the major subgroups was given the taxonomic level of class. More recently, each of these classes has itself been elevated to the level of phylum.

The phyla are:

- -the **Sarcodina** (or Rhizopoda), which move for a major part of the life cycle by means of pseudopodia and which are represented mainly by the amoebas;
- -the **Mastigophora**, or flagellates, which move by means of flagella;

-the **Ciliata**, which may or may not be mobile, but which have cilia at some stage of their life cycles

-the **Sporozoa**, all parasitic, seldom with any form of locomotion (with exceptions in some parts of their life cycles).

Some species, having aberrant features, do not fit neatly into these four groups; they will not be discussed here.

Some members of each of the three free-living groups occur in marine, estuarine, freshwater and soil environments. All four groups, in whichever environment they occur, are the focus of a great deal of research. Particular attention is paid to those living in concentrated or ultra-bacterially rich situations (e.g. sewage works) , those useful in soil/water biomonitoring situations and those that are socio-economically important (mainly parasites such as species of the genus *Plasmodium*, which cause malaria). Most free-living species live on bacteria and are often species-specific feeders, which means that the presence of a predator is an important indicator of the presence of its prey. Not all protozoans feed on bacteria. Some eat other species of protozoans, sometimes many times larger than themselves (Curtis, 1968; Sleigh, 1989; Patterson, 1996).

2.4 The protozoans – morphology

Common to all protozoans is the protoplasm, contained within the cell membrane and composed of two sections, the cytoplasm and the nucleus. As there is a great range of morphological form in the Protozoa there is almost as great a range in size and type of nuclei, from small to large and from single to multiple. The cytoplasm varies from a rather dense gel-like ectoplasm at the edges of the cell to a more liquid endoplasm in the centre of the cell.

Sarcodina

In the freshwater testaceans (shelled amoebas) the decoration of the testa or shell is species-specific but the body form is flowing, as in the "naked" amoebas, although it is attached with cytoplasmic threads to the inside of the testa. There is only a single nucleus, one or a few contractile vacuoles, which control water balance within the cell, and food vacuoles containing digesting food, which is obtained by the flowing pseudopodia. The unusual naked freshwater amoebae of the genus *Pelomyxa* retain symbiotic bacteria, which enable them to live in anaerobic conditions (Berger *et al*, 1997). They also retain, within the cytoplasm, sand grains which render them dark and opaque.

To indicate the taxonomic complexity and the development of modern investigative techniques, *Pelomyxa* has recently been reassessed and described as an “amoeboid flagellate” (Griffin, 1988). Asexual reproduction is by binary fission and in the testate species the original testa may be shared by the offspring or an entirely new one developed by one of the partners (Barnes, 1980).

Mastigophora

The free-living freshwater flagellates often have chloroplasts within their cytoplasm. This has led to some confusion since botanists claim some flagellates, such as *Euglena* spp. and *Peranema* spp., because they are plant-like rather than animal-like (Paterson, 1996). Flagella are whip-like extensions of the cytoplasm. The flagella of a single flagellate cell are often of different length and their particular whip-like movement leads to the turning of the cell as it is propelled forward. A single flagellate will seldom have more than eight flagella and most species have no more than one or two. Flagellates reproduce by dividing longitudinally with the contents of the cytoplasm divided and shared (Curtis, 1968).

Ciliates

The phylum Ciliata is estimated to include about 8000 species. They all bear cilia, which are similar to flagella but occur in large numbers on an individual specimen. Most species have a stable body shape, although the construction of the cell in some species allows for a sinuous swimming movement. Most ciliates have a cytostome or cell mouth. The position of the cytostome, the presence or not of a vestibule (a “depression or invaginated area of the body, at either pole, leading directly to the cytostome”: Corliss, 1979), and associated compound groups of cilia, are species-specific. With rare exceptions all ciliates have two types of nucleus - the macronucleus and the micronucleus. The macronucleus is usually much larger than the micronucleus and is often of a distinctive shape important in identification to species. All ciliates have at least some external body cilia arranged in very species-distinctive patterns. One or more contractile vacuoles are usually present and many species have other distinctive organelles that become visible on staining (Corliss, 1979). All ciliates reproduce asexually by binary fission, but sexual reproduction does occur occasionally in many species (Sleigh, 1973).

Protozoans require some care in identification and various researchers use different methods, usually those that they particularly have found suitable. Jahn & Bovee (1949) is a good basic text and very full.

Quite complicated methods are described (mainly for ciliates) by Foissner (1992 a, b, c, d). Using a good quality dissecting and compound microscope we were able to identify many

protozoans > 50 μm in size. Methyl green pyronin is a stain available from Germany, but the specimen must be stained and observed with speed as the stain burst the cell within a short time. In some genera such as *Euplotes*, Methyl green pyronin is very useful for showing the shape of the nucleus, but in other specimens the results are disappointing. Foissner & Berger (1996) recommend the method of pressing specimens between slide and coverslip, but only one of us found this a reliable technique. We found the best key with good line drawings that by Foissner & Berger (1996), but this is for ciliates only. Patterson (1996) gives very good colour pictures, using different microscope techniques, and he shows a range of protozoans from amoebae to flagellates and ciliates. Keys for amoebae and flagellates are not, to our knowledge, readily available and much of the old literature is out of print. Ogden & Hedley (1980) published a book on British testate amoebae with excellent electron microscope photographs. Since many of these species are cosmopolitan it is a very useful guide. See Siemensma (1981) for Heliozoans, Page (1988) for Gymnamoebae, Corliss (1979) for recent ciliate taxonomic work and protozoan research history. Interestingly, the "forefather" of ciliate taxonomy worked with a very basic microscope in the 1920's and 30's and published four of the definitive works on ciliates, Kahl (1930; 1931a; 1931b; 1932) in German. Wolowski (1998) recently published his taxonomic and environmental studies on Euglenophytes. Kudo (1971) is a very good, although taxonomically dated, general protozoan book.

As already stated, one of the aims of the present project was to "establish whether local taxa are cosmopolitan or at least whether or not they respond to water quality variables in the same way that northern hemisphere taxa do or are specifically endemic". Using species numbers of ciliated protozoans to define the results of their experiments, Fenchel *et al* (1997) compared sediment core samples from both freshwater and marine sites. A minimum of 57% of both marine and freshwater species found were regarded as cosmopolitan and they concluded that "everything is everywhere" as far as microorganisms are concerned. In a study of ciliates found in soils in extreme conditions in Australia, of the 19 species which were fully identified only three were considered to be endemic to the area (Pomp & Wilbert, 1988).

Similarly, a study of the Protozoa found in two Kenyan lakes revealed an "abundance" of protozoans, most of which were cosmopolitan species (Bamforth *et al*, 1987). Conversely, Foissner (1987) considered that there was a definite geographical zonation in freshwater and marine ciliates with two probable zones, a northern zone comparable to the geological Laurasia and a southern zone comparable to the geological Gondwana.

Generally, up to very recently, the southern groups have been poorly studied, but there are some recent African research publications such as those by Dragesco & Dragesco-Kernéïs (1986) and also some publications from protozoan researchers in South America (Foissner,1987). The literature is controversial, with Finlay *et al* (1998) maintaining the view that global diversity of ciliates (the most studied group) is low and the species range well studied, against that of Foissner (1997b) who considers that at least 47% of soil ciliate species are as yet undescribed and therefore the generalisation of low global diversity unfounded. From this information it appears important that as much as possible of the more recent literature from the southern hemisphere should be obtained in order to successfully compare the species and their distribution. In many parts of the southern hemisphere, cyclic droughts are a normal occurrence and for this reason as much emphasis should be placed on describing soil protozoans as on species found in fresh water. This emphasis would fulfill another of the aims of the project: "to investigate and identify those protozoans that can be used as biomonitoring tools and water quality indicators especially for seasonal/ephemeral rivers". The use of protozoans is well established and there is a large body of literature available for their identification, not only as individual species, but also as pollution indicators (see Bick, 1972; Corliss, 1979; Curds, 1982; Curds *et al*, 1983; Foissner, 1987; Berger *et al*, 1997). Linked with the study of protozoans is their use as indicators of organic pollution. The Saprobic system is widely used in Europe, but is also a subject of some controversy.

The Saprobic System

The original publication proposing the Saprobic System was as a result of work done by the hydrologists, Kolkwitz & Marrson (1908)who applied its theories to plants. The following year, 1909, they published a paper proposing its use for macroinvertebrates. Basically it described four levels of organic pollution a river below a organic waste discharge point. From highly polluted to relatively clean waters they described four zones – polysaprobic, α-mesosaprobic, β-mesosaprobic to oligosaprobic. Unfortunately, this zoning was soon expanded and in 1966, Caspers and Karbe, were describing 10 saprobic zones (Curds, 1992). One of the major problems with the Saprobic System is its requirement for identification to species level and thus it requires skills not commonly found in field workers.

There is some argument as to what sample size is truly representative. It is argued also that it does not show inorganic or toxic waste pollution (Curds, 1962), but Foissner (1992c) considers this incorrect. According to Jeffries & Mills (1990) organic pollution represents the commonest form of freshwater "degradation". They state that the occurrence and number of bacteria and other fungi, protozoa and microbes is dependant on organic matter present and this overall "supply and demand" is termed "the saprobity of the system".

A large amount of literature has been published, both pro- and anti- the Saprobic System. A review by Washington (1984) looks at the many systems produced for ecological application. Many of the defenders are European ecologists (see Sladecek, 1973; 1977a; 1977b; 1978; 1979; 1981 a; b, 1986; 1988) although the publication by the German biologists, Caspers & Schultz concludes that the Saprobic System "does not give true value to heavily polluted waters". Sladecek (1978) in his defense of the Saprobic System probably did more harm than good since his extremely detailed descriptions of the Saprobic System are too complex. His 10 saprobity levels should be compared with those few as described by Harrison (1958) who worked on the Berg River in the Western Cape. Harrison further found that the Black River, into which the Liesbeek River flows was heavily polluted with a very slow flow and was "probably ideal for application of the Saprobic System".

In the United Kingdom the original Trent River Board Scheme was developed by Woodiwiss (1964) and this was further modified by later workers [see Graham (1965) and Chandler (1970)]. This scheme eventually became the BMWP in 1981 (Chutter, 1998). The SASS (South Africa) and RIVPACS (Australian) schemes were further developments of the BMWP arising for more localised assessment of pollution. A major factor which probably led to the discarding of the Saprobic System in the UK and the USA was the decline in the teaching of taxonomy at universities from the 1950's onwards (Prof. P. Linder, pers comm.) The publication by Hynes (1960) was very critical of the Saprobic System and it never gained much popularity in the United Kingdom after this. Friedrich (1990) considered that the Saprobic System was useful only in lotic situations, either where the flow was permanent or temporary. In the United States major studies on the protozoans and their ecological impact have been undertaken by Cairns. He pioneered the monitoring methods for the study of protozoans in lentic situations, investigated the evolutionary position of protozoans and continues to publish on recent research into the ecological importance of this group (see Cairns 1981a;b; 1991; 1993; 1999a, b, c and Cairms & Albaugh, 1976, Cairns & Beamer, 1971; Cairns & Kaesler, 1976; Cairns & Plafkin 1976; Cairns & Platt 1986 a, b; Cairns & Yongue 1973, 1974, 1977).

The negative publications on the Saprobic System are contradicted by Foissner and he and his workers have produced publications which not only simplify the identification of protozoans, but show, by in depth research that protozoans in the soil and in underground waters are of great importance for prediction of water quality and various pollutants, including toxic substances (Foissner, 1987). Publications such as "A user-friendly guide to the ciliates" (Foissner & Berger, 1996) and the 1997 publication by Berger, Foissner & Kohmann, which incorporates the Schizomycetes (bacteria), Mycophyra (Fungi), Rhizopoda

(amoebae), Flagellates and Ciliates into the Saprobic System indices, with photographs, line drawings and full descriptions can only serve to support the importance of this system. Admittedly, it has shortcomings and for ease of usage it cannot compete with SASS in the lotic situation. However, where water is lentic, underground or present only as a film on subsurface sediments, the use of protozoans as a biomonitoring tool, in tandem with the Saprobic System, may become of major importance in water-poor countries such as Africa.

2.5 Protozoans as Bioindicators

An aim of this project was to "establish whether certain groups within the protozoans (e.g. ciliates) are particularly suitable for water quality assessment". The literature certainly indicates that certain taxa are commonly used for this purpose. Bick (1972), for instance, was commissioned by the World Heath Organization to produce an illustrated guide describing the commonest species of ciliated protozoans that could be used as biological indicators; Bick's publication lists 135 suitable species. Similarly, Foissner & Berger, (1996) use only ciliated protozoa as indicators of water quality. These publications detail simple methods of examination and have keys specially constructed for ordinary biologists and water technicians. The recent publication by Berger *et al* (1997) is of importance as it also has simple keys and many illustrations, and identifies bacteria, fungi, rhizopods, and flagellates as well as ciliates to identify different types of water quality conditions. Unfortunately, this book is presently available only in German. From these and other publications it appears that the cilates are the most important protozoans for the assessment of water quality, although some of the testaceans have been shown as good indicator species, especially among the soil protozoans. Interestingly, both ciliates and the testaceans, are readily able to adapt to changing physical and biological conditions (Foissner, 1987).

In natural ecosystems, species assemblages of testaceans and cilates have been used to indicate soil conditions, eutrophication, pesticides, acid rain, oil (Foissner, 1987), effects of irrigation and fire (Fantham & Paterson, 1924), and radiation pollution (Foissner, 1987; Sinclair & Ghiorse, 1987; Sinclair *et al*, 1993). Sinclair *et al* (1993), investigating the effects of aviation-fuel-contaminated soil, showed that the increased amount of organic carbons released into the soil by the fuel spill led to an increase in the numbers of bacteria living on the organic carbons in the fuel. This in turn caused an increase in the number of protozoans which fed off the bacteria. In an examination of soil core samples taken at a pristine groundwater site in Oklahoma in the USA, Sinclair & Ghiorse (1987) concluded that the presence of protozoans in the subsurface samples indicated that there was a regulation potential for large bacterial growth.

The use of protozoans as indicators is gaining in importance as researchers describe conditions under which species are found and conduct laboratory experiments that reliably indicate the levels of pollution indicated by the presence and absence of certain species (Berger *et al*., 1997). In the past, though, possibly too much emphasis was placed on complicated and expensive staining techniques and on investigation of very small specimens beyond the taxonomic capabilities of the average biologist.

The increase of human populations and the associated increase in pollution levels have placed pressure on clean water resources worldwide. Infiltration of pesticides and noxious substances via the soil to rivers has underlined the importance of diagnosing soil conditions before they are transmitted to the sources of potable water. This may be particularly important in seasonal or drought-influenced systems such as those commonly found in South Africa and Australia. Furthermore, the discovery of endemic species, or of cosmopolitan species that form endemic communities indicating specific conditions, may lead to further developments in the monitoring and control of pollution.

2.6 The protozoans – methods of study

Most protozoans are extremely difficult to fix and preserve without distortion, so they have to be examined live. Furthermore, they range in size from 15 to 2000 m although the majority fall into the 40 to 200 m size range; many are colourless and some move at great speed. Thus methods of investigation require specimens to be examined live but it is necessary to slow them down, and often to stain them, so that their characteristic features can be seen. Thus a certain minimum of equipment is essential for a study of protozoans: simple stains, and dissecting microscope and a good compound microscope that magnifies to 200 times (e.g. (Jahn *et al*, 1949; Curds, 1982; Foissner, 1991; Patterson, 1996; Foissner *et al*, 1996; Berger *et al*, 1997). It is also necessary to be able to keep cultures alive, but this can normally be carried out fairly simply in ordinary laboratory conditions.

Many books and other publications list the methods suggested for the collecting and culturing of protozoans. Soil samples can be kept in their dry condition for many months and a simple petri dish method will provoke the ciliates and testate amoebae to excyst (Foissner, 1987). This method involves wetting approximately 30-50g of dry soil or sediment, either with distilled water or with sterilised water collected from the original site, sufficient to dampen the sample, but not flood it completely. Foissner (1987) estimates that average recovery rates for soil ciliates is 72%, for testate amoebae is 60% and for flagellates is 50%. More detailed examination, especially of the ciliates, requires complex staining techniques, specialised light microscopes and often a scanning electron microscope (see Jahn *et al*, 1949; Curds, 1982; Foissner, 1991; Anonymous, 1992).

Limiting the species used as bioindicators to those of a size easily visible under dissecting microscope (> 50 m in length - see Bick, 1972) would, however, resolve the many fears that these animals are too small to use as bioindicators.

CHAPTER 3

Lotic sampling

The Liesbeek River was chosen for sampling riverine protozoans as it is relatively short, it is supposedly perennial, and a fair amount of biological and physical data have been collected at sites down the length of the river for the last 14 years as least. Collections of invertebrates, or water samples for chemical analysis have been done by the Cape Town City Council, Scientific Services division (CTCSS), and also by students of the Zoology Department at the University of Cape Town (UCT) (Davies & Luger, 1993, 1994; Luger & Davies, 1993; Day, 1995).

The Liesbeek River has its headwaters in the Vaalkat stream in Nursery Ravine on south side of Table Mountain. It passes through the National Botanic Gardens of Kirstenbosch and then through the suburbs of Bishopscourt Estate, Newlands, Rondebosch, Mowbray, Rosebank and Observatory, being joined by numerous small first-order tributaries, which contribute to its perennial flow. It also benefits from orographic rainfall due to its geographic situation. Similar to many rivers in large cities all over the world, it declines in condition from almost pristine (Site 1 in this study) to extremely degraded (Site 7 in this study). It is degraded as a result of long stretches of canalisation (cement lining and alteration of original of flow path by earthworks), inflow of effluents and stormwater runoff, refuse dumping, water abstraction, invasion of alien plants and, in the past few years, markedly reduced rainfall in its catchment area. This decline in rainfall may be seen in Table 1. The Liesbeek flows into the now-larger Black River, approximately 3 km from Table Bay. The Black and Salt Rivers originally formed a large delta mouth which has been greatly altered, initially due to the construction of Table Bay Harbour and later with the development of the industrial area of Paarden Eiland, and the construction of roads and railways in the 1950s.

Biological and physical data shown in Day (1995), based largely on UCT student data, clearly indicate the degradation of the river from its headwaters (Site 1) downstream (Site 7). For the present study we selected sites approximately the same as, or close to, those used by the UCT students and the water quality sample sites of the CTCSS. Thus, although no studies had been made of protozoan fauna in the past we could compare our findings with the water quality conditions and faunal counts as found by the CTCSS and UCT. These sites were all on uncanalised sections of the river.

As mentioned by Davies & Luger (1993) and Day (1995), the negative impacts on the Liesbeek River have been slightly less than they might have been because its upper reaches

run through affluent areas and there are no obvious industrial impacts in this stretch of river. Although we have no data regarding the amount of water abstracted from the river, firstly by the Kirstenbosch Botanic Gardens above Site 1, and secondly at Rosebank just above Site 6 by a commercial nursery, the reduction in flow from virgin conditions must be significant, particularly in the drier months.

3. 1 Description of Sites on the Liesbeek River

Site 1 – Liesbeek River headwaters

Site 1 lies 20m above the intake pipe where Kirstenbosch Botanic Gardens (KBG) abstracts water for offstream storage and just below the meeting point of Vaalkatkloof and Nursery Ravine. Here the headwaters of the Liesbeek River fall down a steep rocky incline with large Table Mountain sandstone boulders, some >1 m in diameter. The stream bed is some 4m wide. The site is deeply shaded in summer with only dappled sunlight reaching the streambed.

On the first sampling occasion the river was dry except for a small rock pool with about 200mm of water but as bankside mosses were still slightly damp, water had probably been flowing until recently. Because criminal elements are active on Table Mountain, this site was only sampled twice when enough people were able to walk to the site.

Site 2 - Kirstenbosch Botanic Gardens

Site 2 lies just below the headwaters of the Liesbeek River at the entrance to the Kirstenbosch Botanic Gardens where the river has forged a steep, somewhat eroded, boulder-strewn course. Close to the sampling site the river flows under a road bridge. At its widest point, the stream bed was 5.2m wide. There was no vegetation in the river bed, but some marginal vegetation was present in the form of grasses and some mosses were seen on the overhangs of large boulders. Fairly dense indigenous riparian vegetation grows on both sides of the river. The river bed is mostly shaded by the overhanging canopy. This site was completely dry from mid-February to mid-May 2000 as a result of water abstraction by KBG.

Site 3 - The Hill

This site, approximately 200 m below the entrance to KBG, is at the upstream extent of a well established urban area. The natural river path has been disturbed by canalisation under a road and the river bed, which is about 7.3m wide, is deeply incised.

For the most part the riparian vegetation consists of introduced exotics. Most of the trees are oaks (*Quercus* sp.), thickly undergrown with exotic grass. Mosses grow on the sides of the boulders. The river bed is steep, deeply eroded and boulder-strewn with some coarse sand in the eddy areas. Direct sunlight reaches the river for most of the year. The site was dry from mid- December 1999 to mid-May 2000, a six-month period. Since site 2, just above, was dry from mid-February to mid-May, 2000, a three-month period, water abstraction might have been taking place between these two sites as well as above site 1.

Site 4 - Paradise Road

Site 4 is approximately 1 km below KBG and in an affluent urban area. The river bed is 3.7 m wide and the bed deeply incised. Although the bed and banks are more or less natural at this point, the river is canalised both up- and downstream. The steep-sided river banks are heavily overgrown with alien trees and shrubs; litter was noted on several sampling occasions. The close proximity of urban gardens probably contributes to the prominent growth of exotic garden species along the river banks. The river flowed constantly and fast throughout the year as a result of contributions of water from mountain tributaries joining the Liesbeek along this stretch. Dense fine root systems of willow trees (*Salix* sp.) contribute to some sandy substrate retention. Filamentous algae and some desmids (e.g. *Vaucheria* sp., S*pirogyra* sp. and *Cosmarium* sp.) were found in small amounts in most months. The width of the flowing water varied between 1 and 2m and water depth did not exceed 120mm.

Site 5 - Brewery

Site 5 is just below an outlet from the South African Breweries' brewery in Newlands, and the drainage outlet from Newlands Fedsure Western Province Rugby grounds. The river is partly canalised and heavily affected by human presence. The sampling site, which is not canalised, is cobble-strewn, the cobbles supporting small amounts of algae and some aquatic moss, *Fontanalis antipyrreticum.* Although the flow rate is fairly rapid there was occasionally a markedly furry epilithic growth of 'sewage fungus' on cobbles in some of the side eddies, presumably as a result of discharge of organically-enriched effluent a short distance upstream. The banks are steeply eroded and have been partly stabilised with gabions and concrete supports. The area is fairly heavily populated, highrise apartment buildings and small houses and gardens being common. The river bed is 9.5m wide but the width of the stream itself was always 5m, and the greatest depth 190mm. Flow velocity was fairly rapid in winter but less during the summer months, October 1999 to April, 2000.

Site 6 - Gordon's Institute

Site 6 is at the junction of a canalised section and an offstream artificial wetland below a weir. The canalised section is 20.1 m wide and the bed is covered with a thin layer of sand/silt to a depth of approximately 100mm. Bank-side samples were taken from the "wetland" area where the soil is not the normally occurring substrate and appears to have been introduced. A species of the alga *Cladophora* was visible in the wetland for most of the sampling period, as was the introduced aquatic snail, *Physa acuta*. River width during the sampling period averaged 3.6 m; the greatest depth recorded was 170 mm.

Plastic and other litter was visible throughout the year. The river flowed constantly throughout the sampling period although a decrease could be seen in the summer months. As flow was fairly rapid, samples were taken from side-eddies where there were small boulders and a muddy substrate. In April 2000, we were unable to sample this site as workers from the Cape Metropolitan Council (CMC) were bulldozing the river to remove vegetation encroaching as a result of decreased flow in the river because of offtake of river water to feed the artificial wetland.

Site 7 - Valkenberg

Site 7 is below the so-called 'Liesbeek lake' (a term used by municipal workers), which is in fact a man-made earth-dug canal in the river, ranging from 60 to 150 m wide. The 'lake' is markedly refuse-strewn and the sides are heavily overgrown with alien aquatic plants and thick algal mats. Many years ago the original path of the river was diverted and a new canal dug so that the river now appears to fork. A mesh grid (approx. 1.5 m wide) separates the path of the water in the canal from the original river bed; a drain from Groote Schuur Hospital also enters the river just upstream of this site. Our samples were taken from the original river bed close to where it passes under a road bridge with constant human and vehicular traffic. At this point the width of the river ranged from 3 to 8m; the greatest depth was approximately 300mm. Water flow was very slow and dwindled markedly in the summer months. Large quantities of litter were always present, with plastic bags, food containers and other evidence of human presence constantly seen. Exotic aquatic angiosperms such as *Myriophyllum aquaticum* (parrot's feather), *Ceratophyllum demersum* (hornwort) and *Eichhornia crassipes* (water hyacinth) were present throughout the sampling period, despite efforts of the CMC to clear the river once or twice during the year. The filamentous alga, *Spirogyra* sp., was also found in loose masses in the slow-flowing areas throughout the year.

3. 2 Methods

Except for Site 1, sites were sampled bi-monthly. Three replicate samples were collected on the river bed ('benthic' samples) from sand or gravel and very small stones where possible, and also from leaf detritus. Samples were collected using a basting tube. This enables a sample of substrate and water to be collected. Also any small pieces of detritus and algae/plant material were included. When water was not present at a site, dry samples, separately, of sediment and leaf detritus were collected from the river bed.

Electrical conductivity was measured, calibrated according to temperature, using a YSI Electrical conductivity meter. This instrument measures the water temperature initially and this was recorded. pH measurements were taken using Merck Spezialindikator paper strips of a range of pH values in the range from 4.0 to 10.0. Conductivity, temperature and pH measurements were made *in situ*.

The basting tube (which is normally used in cooking) allows for a specific sample to be taken at the water substrate interface. Each sample was placed in a clean screw top sample jar. Samples were taken back to the laboratory where each sample was poured into 10 cm plastic Petri dish. Mud or detritus remaining in the base of the sample jar was washed out into the Petri dish using distilled water. Each sample was examined on the day of collection and on the following two days. This was necessary as populations of protozoans may "develop" over a period of time and dominant species may change (Curds, 1982). If necessary to prevent desiccation, distilled water was added to the Petri dish but no extra food source was added.

The petri dish was initially inspected with a dissecting microscope and specimens seen in this way were transferred with the aid of a Pasteur pipette to a cavity slide for examination under a compound microscope. Closer examination of specimens was made using a stain called methyl pyronin, together with methyl cellulose, a viscosity-increasing medium (see Foissner, 1998). Because we did not have adequate access to phase contrast, differential contrast or bright field illumination, identification of specimens greater than 40 m was seldom made.

3.3 Results

Rainfall figures for the years 1995 to 2000 at the four collection points closest to the Liesbeek River were obtained from the South African Weather Bureau (Table 1).

Rainfall during the winter months of the sampling period, May-August 2000, was very low when compared to rainfall figures for the last five years.

Table 3.1: Monthly rainfall figures (mm) for Kirstenbosch (representative of site 2), the Newlands Forestry Station (representative of site 4), Groote Schuur (representative of site 5) and Cape Town Astronomical Observatory (representative of site 7) for the years 1995-2000. (Supplied by the S.A. Weather Bureau)

N/S = "not supplied"

Kirstenbosch (08H00)

	Jan.	*Feb.*	*March*	*April*	*May*	*June*	*July*	*August*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1995	40.4	10.2	5.3	25.9	72.6	162.4	374.1	172.2	54.7	182.7	19.4	33.5
1996	1.1	59.5	51.5	73.3	115.3	321.8	225.2	165.1	341.5	155.3	95.0	88.6
1997	25.1	11.3	21.0	75.6	161.5	302.4	47.8	312.7	20.2	24.7	107.8	34.1
1998	23.7	0.5	28.1	93.0	286.3	168.6	215.9	92.2	102.5	50.8	82.7	48.1
1999	17.7	4.0	1.6	102.0	109.9	251.7	159.3	222.5	201.9	3.4	50.3	11.0
2000	39.3	2.5	10.7	32.9	152.1	137.2	135.7	105.5	220.8	**N/S**	**N/S**	**N/S**

Nuwelandbosboustasie (08H00)

	Jan.	*Feb.*	*March*	*April*	*May*	*June*	*July*	*August*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1995	95.5	28.5	6.0	31.5	93.5	197.0	516.0	192.0	88.0	207.5	**N/S**	**N/S**
1996	**N/S**	**N/S**	**N/S**	**N/S**	188.6	331.5	284.1	218.0	489.5	169.0	107.5	98.0
1997	18.5	4.5	23.4	107.5	172.6	334.2	37.7	464.0	33.6	40.5	144.7	38.0
1998	23.3	2.8	53.1	139.4	391.5	264.5	270.0	96.5	146.5	98.0	95.5	**N/S**
1999	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**
2000	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**	**N/S**

Groote Schuur (08H00)

	Jan.	*Feb.*	*March*	*April*	*May*	*June*	*July*	*August*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1995	90.1	24.8	4.8	25.4	97.2	177.4	349.0	131.3	57.6	151.4	18.4	33.1
1996	0.0	44.9	53.9	84.7	93.1	229.5	229.5	129.5	312.4	109.0	77.0	92.2
1997	19.1	13.6	11.9	102.7	139.6	198.0	35.0	308.3	30.9	37.9	118.3	18.3
1998	17.5	2.7	46.8	110.8	316.2	186.3	184.2	72.3	86.5	77.3	75.6	49.6
1999	15.0	7.5	2.1	79.1	110.4	213.2	107.5	240.4	233.7	2.7	52.2	2.5
2000	41.3	0.0	7.2	24.7	214.6	184.1	135.0	145.0	178.5	**N/S**	**N/S**	**N/S**

Cape Town Astron Obs (08H00)

	Jan.	*Feb.*	*March*	*April*	*May*	*June*	*July*	*August*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1995	41.4	0.8	0.4	27.7	71.5	102.9	125.9	78.6	3.8	30.2	2.5	14.8
1996	0.0	29.6	27.6	41.1	37.1	105.9	119.8	88.1	91.2	51.4	36.2	27.2
1997	8.3	5.6	0.1	57.2	69.1	100.9	27.0	107.1	8.1	22.1	80.2	5.2
1998	11.3	0.0	17.8	47.6	145.0	71.6	96.8	50.0	27.8	22.1	60.3	50.5
1999	0.1	1.5	0.0	71.9	33.3	65.6	36.9	98.1	112.4	0.2	20.3	0.0
2000	13.7	0.0	6.0	10.2	30.9	83.2	67.9	56.4	58.7	**N/S**	**N/S**	**N/S**

Table 3.2: Electrical conductivity (µS/cm at 25°C) measured *in situ* during the sampling period. ns = not sampled

Date	Top	Kirsten-bosch	The Hill	New-lands	Brewery	Gordons	Valken-berg
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Sep-99	125	112	118	118	155	240	285
Oct-99		66.5	75	127.5	176	225	262.5
Nov-99	70	89	90	164.5	211.5	246	279
Dec-99		95	DRY	185	208	272	268
Jan-00		100	DRY	180	244	256	193
Feb-00		81	DRY	184.5	225	242.5	171.9
Mar-00		DRY	DRY	180.5	211	195	281.5
Apr-00		DRY	DRY	261	237	ns	287.5
May-00		DRY	DRY	200.5	190	87.15	185
Jun-00		72	72	164	183	204	221
Jul-00		65.5	79	146	173	194	183
Aug-00		70	61	151.5	190	267.5	297.5

Conductivity values increased markedly from site 1 to site 7 throughout the sampling period, but even at the Valkenberg site the highest value recorded was 297 µS/cm^{-1}. The greatest range of values between sites was recorded in August 2000 when conductivity at site 1 was 61 µS/cm^{-1} and at site 7 was 297 µS/cm^{-1}, almost a four-fold difference. Seasonal differences in conductivity were noticeable but not marked**.**

Table 3.3: pH values measured *in situ* during the sampling period. Values are monthly averages. Ns = not sampled

Date	Top	Kirstenbosch	The Hill	Newlands	Brewery	Gordons	Valkenberg
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Sep-99	6.1	5.5	5.1	6.1	6.1	6.5	6.5
Oct-99		5.4	5.5	6.4	6.5	7.1	6.5
Nov-99	4.2	5.6	5.8	5.9	6.5	6.9	6.2
Dec-99		5.1	DRY	5.8	6.5	6.5	6.6
Jan-00		5.5	DRY	6.1	6.8	6.5	6.3
Feb-00		4.7	DRY	6.4	6.8	7.2	7.1
Mar-00		DRY	DRY	5.9	6.6	6.6	6.7
Apr-00		DRY	DRY	6.8	6.5	ns	6.8
May-00		DRY	DRY	6.9	6.6	6.4	6.6
Jun-00		5.1	5.4	6.2	6.5	6.5	6.5
July-00		4.9	5.3	6.1	6.6	6.5	6.4
Aug-00		4.5	5.1	5.9	6.9	7.1	6.8

Throughout the sampling period pH values increased down the river from site 1 to site 7. The lowest value recorded was 4.2 at site 1 in November 1999 and the highest was 7.2 at site 6 in February 2000.

Table 3.4:. Temperatures (°C) measured *in situ* during the sampling period. Values are monthly averages.

Date	Top	Kirstenbosch	The Hill	Newlands	Brewery	Gordons	Valkenberg
	Site	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**
Sep-99	15	12.5	14	14	16	15	12
Oct-99		12.9	13.1	13.8	14.8	20	20.3
Nov-99	14.5	16.5	18.5	18.5	17.5	19.4	18.8
Dec-99		21.3	DRY	21	20.1	23	23.2
Jan-00		20.6	DRY	21.8	20.5	22.5	22.4
Feb-00		21.2	DRY	21.9	20.8	22.8	22.4
Mar-00		DRY	DRY	18.7	19.2	20.2	20.8
Apr-00		DRY	DRY	18.7	18.7	ns	20.5
May-00		DRY	DRY	12.6	13.2	12.7	16.6
Jun-00		11.7	14.1	13.1	13	14.2	12.5
Jul-00		11.6	11.8	13.1	13.9	14.3	14.4
Aug-00		13.6	13.5	15	14.8	16	14

As expected, water temperatures increased in summer all the sites. There was a difference of 2°C or less between sites 2 and 7 throughout the sampling period.

Table 3.5: List of protozoan taxa found on at least one occasion at each sampling site.

SPECIES	Top	Kirsten-bosch	The Hill	New-lands	Brew-ery	Gor-dons	Valken-berg
	Site1	Site2	Site 3	Site 4	Site 5	Site 6	Site 7
Amoebae							
Actinophrys sp.							
Actino-sphearium sp							
Amoeba proteus.		X		X	X		
Arcella sp.	X	X		X	X	X	X
A. vulgaris	X						X
Centropyxis sp.		X		X	X	X	X
Chaos sp.							X
Difflugia sp.		X	X	X	X	X	X
Nebela sp.		X		X	X	X	X
Pelomyxa palustis							X
Flagellates							
Anthphysa sp.		X	X	X			
Cryptomonas sp.		X	X	X			
Euglena oxyuris	X						X
Peridinium undulatum	X		X	X	X	X	X
Other small flagellates	X	X	X	X	X	X	
Ciliates							
Amphileptus procerus				X	X	X	
Bursaria truncatella							X
Campanella umbellifera							X
Coleps cf. spetai						X	
Coleps sp.			X				X
Colpoda cf. culcullus	X						
Colpoda sp.		X		X	X		
Dileptus margitifer		X			X	X	X
Epenardia myriophylli							X

SPECIES	**Top**	**Kirsten-bosch**	**The Hill**	**New-lands**	**Brew-ery**	**Gor-dons**	**Valken-berg**
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Euplotes eurystomas	X	X	X	X	X	X	X
E. patella					X	X	X
Euplotes sp.			X				
Frontonia elliptica							X
F. leucas				X			X
Frontonia sp.				X	X	X	
Halteria grandinella		X	X	X	X	X	X
Holostichia cf. monilata		X		X	X		
Holotrichia sp.			X				
Homalazoon vermiculare						1	
Kahlilembus attenuatus	X	X	X	X	X		X
Lacrymaria olor					X		
Lembadion bullinium					X		
L. lucens		X	X	X	X	X	
Litonotus lamella		X		X	X	X	X
L. Cygnus				X			
Litonotus sp.	X			X		X	X
Loxodes magnus							X
Loxodes sp.						X	
Loxophyllum sp.							X
L. melagris					X		
Nassula picta						X	
Opercularia articulata					X	X	
Oxytrichia sp.		X	X	X			

SPECIES (Contd.)	**Top**	**Kirsten-bosch**	**The Hill**	**New-lands**	**Brew-ery**	**Gor-dons**	**Valken-berg**
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Opercularia articulata					X	X	
Oxytrichia sp.		X	X	X			
O. ferruginea				X			
O. haematoplasma				X	X		
O. hymenostoma				X			
Paradileptus elephantinus						X	
Paramoecium aurelia			X	X	X		X
P. bursaria		X		X	X	X	X
P. caudatum		X	X	X	X	X	X
Paramoecium sp.					X	X	X
Paraurostyla viridis					X		
Plagiopohyla nasuta		X	X	X	X	X	
Prorodon sp.							X
Spirostomum ambiguum				X	X	X	X
S. caudatum						X	X
S. minus		X		X	X	X	
Stentor coeruleus				X			
S. niger		X					X
S. meulleri					X		
S. cf. polymorphus					X		
Trachelius ovum		X			X		
Urocentrum turbo		X		X	X	X	X
Uroleptus piscis				X			

SPECIES (Contd.)	Top	Kirsten-bosch	The Hill	New-lands	Brew-ery	Gor-dons	Valken-berg
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Vorticella cf. convalleria						X	
V. moniliata						X	
Vorticella sp.		X	X	X	X	X	X
Zoothamnium procerius						X	

Seventy-two species of Protozoa, not including a variety of unidentifiable flagellates, were found during the sampling period. Individuals of all species were >50 μm in length except for a few smaller ones that could be identified by a peculiar types of movement. A number, especially of ciliates, could not be identified to species. The largest number of taxa (greatest species richness) was found at the Brewery site with 38 taxa in total. Thirty-three, 32 and 32 different taxa were found at Site 6, Site 3 and Site 7, respectively.

The ciliate *Euplotes eurystomas* was found at all sites. The testate amoebae *Arcella* sp*.,* *Centropyxis* sp., *Difflugia* sp., and *Nebela* sp. were found at six of the sampling sites during the year. The flagellate *Peridinium undulatum* and a number of species of unidentified, very small flagellates were found at six of the sites. The ciliates *Halteria grandinella*, *Kahlilembus attenuatus*, *Paramoecium caudatum*, *Plagiophyla sp.* and *Vorticella* sp. were found at six of the sampling sites.

Conversely, the amoeba *Pelomyxa palustris*, the flagellate *Actinosphaerium* sp., the ciliates *Bursaria truncatella, Campanella umbellifera*, *Colpoda cf. culcullus*, *Coleps cf. spetai, Euplotes* sp., *Holotrichia* sp., *Homa*lazoon *vermiculare, Lacrymaria olor, Litonotus cygnus*, *Loxodes* sp., *Nassula picta*, *Oxytrichia ferruginea, O. hymenostoma, Paradileptus elephantinus, Pauraurostyla viridis*, *Prorodon sp., Stentor coeruleus, Uroleptus piscis, Vorticella cf. convalleria, V. moniliata* and Z*oothamnion procerius* were found only at one site. The flagellate *Euglena oxyuris*, was found only at the Valkenberg site and on one of the two sampling occasions at The Top site. Thirty-three species (49%) were found only at sites 4-7, from Newlands to Valkenberg, while three species (4%) were found only at the upper three sites. These sites were irregularly sampled and yet they supported a greater percentage (47%) of the total number of species found than did the lowest four sites.

Appendix I lists all the species found in the project and gives a short description for each.

3.4 Conclusions

The state of the river

In her analysis of the state of the Liesbeek River over 15 years or so, Day (1995) indicated that there was cause for concern, although the river did not seem to be particularly polluted, as urban rivers go. She did indicate, that SASS sampling (based on invertebrate assemblages) over several years had shown marked decrease in water quality down the length of the river. The measurements of conductivity, pH and temperature taken during the last year or so (Tables 3, 4, and 5), also do not indicate a marked degradation in the water quality of the Liesbeek River, nor do data for a number of other chemical variables (Appendix 2) provided by CMC Scientific Services and used with their permission. All indications are, then that the river is not particularly polluted chemically, even though there was a very noticeable increase in litter such as plastic bags, vegetable litter and other human debris from the top to the lower reaches of the river.

Important also was the marked drop in rainfall during the year and especially during the winter months (see Table 1) relative to the situation in most winters, and this, too, must have had a detrimental impact upon the river. Natural flushing is important as it removes anoxic mud and overgrown aquatic vegetation as well as diluting chemical pollutants. The reduction in flow due to abstraction has a major impact on the biota of the upper river, but we cannot quantify it since no records exist, to our knowledge, of the volumes of water abstracted from day to day or from month to month. Cessation of flow at the Kirstenbosch site (site 2) for three months, and at The Hill site (site 3) for six months, are cause for concern. The disparity in timing of cessation between these two adjacent sites is curious and appears to indicate some form of water abstraction downstream as well as upstram of the KBG. The volume of water abstracted near site 6 by the commercial nursery is also unknown; such abstraction is unlikely to condoned under current water laws. In the canalised sections of the river there is almost constant utilisation of the water for the washing of clothes and other ablution requirements. This latter usage by the vagrant community must impact upon river quality to some extent, particularly when flow is lowest in summer. The damaging effects of the canalised sections of the river on aquatic invertebrate communities has been recorded by Davies & Luger (1993).

What can the protozoan assemblage tell us about the river? Whereas one species, the ciliate *Euplotes eurystomas*, was ubiquitous, some appeared to be restricted to certain sites (e.g. the flagellates *Euglena oxyuris* and the amoeba *Pelomyxa palustris* to the Valkenberg site).

Euglena spp. are generally considered to prefer still waters and to be able to tolerate poor water quality (e.g. Leedale *et al*. 1965; Yongue *et al*. 1979; Wolowski, 1999) Although *E. oxyuris* was found at Site 1 when there was no flow. Similarly *Pelomyxa palustris* is a species well documented as occurring in organically enriched (alpha mesosaprobic) waters (Foissner, 1988, 1992; Berger & Foissner, 1997). The testate amoeba (*Arcella* sp.) was present at all sites and three other species of testate amoebae, *Centropyxis* sp., *Difflugia* sp and *Nebela* sp., were all found to be widespread. Although there is little ecological literature available for these amoebae, their ability to encyst and excyst according to prevailing conditions (Wagtendonk, 1999) probably accounts for their persistent occurrence. The distributions shown by these taxa suggest, then, that some - but not all - are, indeed, differential indicators of water quality.

Some species may be found in a wide range of conditions e.g. *Euplotes eurystomas*, and others are found in a narrow range e.g. *Pelomyxa palustris*. It may be seen from Table 2 in Berger et al. (1997) that species tend to occur in three out of the five saprobic zones in great or lesser number. The testate amoebae and other encysting forms of protozoans are probably well able to encyst and excyst as conditions deteriorate and improve. Although testate amoebae appear ubiquitous, occurring in all sites at most times it is probably due to this latter ability. The observation as to whether they are active (excysted) or not may be critical.

Conversely, if we consider the number of taxa recorded per site, relative to the number of sampling occasions, then the four downriver sites (Newlands to Valkenberg) appear to support a greater number of taxa than the three upstream sites do. The only chemcial variable that seems to very in roughly the same manner is Total Nitrogen. The apparent increase in nitrogen, and to a lesser extent in orthophosphate and total phosphorus, indicate pollution by nutrients or some form of organic matter. Either could be contained in effluents from the Brewery, and excessive quantities of nutrients might be produced in runoff from both the rugby fields in Newlands and from the extensive urban gardens in the immediate catchment of the river in its middle reaches. If the number of species found is actually a reflection of conditions at a site, and not the number of occasions in which each site was sampled, then we can postulate that the large numbers of bacteria that result from elevated nutrient levels might give rise to an increased number of protozoan taxa. It is important to note, however, that large numbers of single species of protozoans were not present on any one sampling occasion, except for *Euglena oxyuris,* which was the notably dominant species at the Valkenberg site throughout the year.

On one occasion *Paramoecium caudatum* was present in large numbers only at the Gordons site. This was evidently due to a large number of bacteria which were present on some rotting animal material. To complicate matters, the flagellate *Peridinium undulatum* was found at six sites, but only in September, while *Urocentrum turbo* was found at four sites, but only during the summer months.

Further issues need to be borne in mind. Although we always collected benthic samples, species were commonly observed moving towards and away from the surface. It may be that some species move in the water column and are differentially collected under different conditions of light or temperature. Light may, for instance, increase growth rates of photosynthetic organisms such as zoochlorellae, while temperature has been shown to alter responses of some species of *Euplotes* and *Frontonia* (Finlay *et al*., 1987). Collecting methods, and an ability to notice cryptic species, may also affect results. In this study, for instance, very few naked amoebae were found, probably because of their cryptic colouration and very slow movements. Issues relating to collecting methods are discussed in detail in section 4 below.

Use of the Saprobic system on the data for the Liesbeek River

With regard to the question of the cosmopolitan nature (or otherwise) of the local species, we can postulate the following. If the species identified from the Liesbeek River are physiologically as well as morphologically similar to those found in Europe, applying the Saprobic system (e.g. Berger *et al*., 1997) to our data should provide an indication of the extent of pollution, particularly organic pollution, in the Liesbeek River. Morphologically, the taxa do seem to be cosmopolitan and their occurrence may be compared with the results of research undertaken in Europe (e.g. Bick, 1972; Siemensma, 1981; Foissner & Berger, 1996; Berger *et al*, 1998), the United Kingdom (e.g. Ogden & Hedley, 1980; Curds, 1982; Curds & Gates, 1983; Sleigh, 1989), the United States (e.g. Corliss, 1979; Corliss, 1979), Australasia (e.g. Stout, 1967, 1973, 1980; Pomp & Wilbert, 1988) and Africa (e.g. Sandon, 1927; Fantham, 1929, 1931; Fantham & Paterson, 1923; Viljoen & van As, 1983; Dragesco & Dragesco-Kerneis, 1986; Nilsson, 1986).

In their Saprobic Index, Berger et al (1997) give exact saprobic values for each species. There are a very few exceptions e.g *Coleps* spp, *Cyclidium* spp, *Difflugia* spp. This underlines the importance of correct species identification. In much of the literature however (see Foissner 1991, Foissner *et al*, 1992 a,b; Foissner *et al*. 1994) the descriptions of species include “look-alikes”.

Berger *et al*. (1997) give a full list of saprobic indices for a number of species of Schizomycetes, Mycophyta, Rhizopoda (amoebae), Flagellata, and Ciliophora. These saprobic indices, for the most part, depend upon identification to species level. Table 6 (which is a free translation from the German) indicates the detailed nature of the Saprobic System. Using the Index numbers given by Berger et al. (1997) in their Table 4, we have calculated the range of greatest pollution levels, at a sampling site. These values are shown in Table 7 below. Not all of the saprobic value species occurred at the same sampling occasion, but their occurrence indicated their ability of presence. It must be bourne in mind that protozoan populations come and go and are often food driven. Thus, a bacterial "flood" would, within a very short time, result in protozoan species appearances which had been encysted prior to the "flood".

Table 3.6: Pollution indices using the idea of saprobity and the saprobity index (from Berger *et al* (1997).

Pollution Index	**Saprobity**	**Saprobic Index**	**Minimum O_2 level (mg/)**
Unpolluted to minimal affect	Oligosaprobic	1,0-<1,5	>8
Minimally affected	Oligosaprobic with β-mesosaprobic influence.	1,5-<1,8	>8
Strongly affected	B-mesosaprobic	1,8-<2,3	>6
VERY STRONGLY affected	β- to α- mesosaprobic border	2,3-<2,7	>4
EXTREMELY strongly affected	Mostly α - mesosaprobic	2,7-<3,2	>2
CRITICALLY affected	A - mesosaprobic with polysapbrobic influence	3,2-<3,5	<2
Completely polluted	Polysaprobic	3,5-4,0	<2

The protozoans, with their appropriate saprobic values, that we used to calculate the saprobic indices were :

Amoeba proteus = 1,8
Coleps sp. = 2,5
Difflugia sp. = 1,8
Dileptus margitifer = 2,1
Euplotes patella = 2,3
Halteria grandinella = 2,2
Homalozoon vermiculare = 1,9
Lacrymaria olor = 2,2
Litonotus Cygnus = 2,0

Litonotus lamella = 2,8
Pelomyxa palustris = 3,5
P. caudatum = 3,6
Plagiophyla nasuta = 4,0
Paramoecium bursaria = 2,5
Spirostomum ambiguum = 3,2
S. minus = 2,9
Stentor coereuleus= 2,5
Stentor polymorphus = 2,6
Trachelius ovum = 2,5

Table 3.7: Average annual pollution levels for sampling sites on the Liesbeek River, 1999 to 2000, calculated using the data as given in Table 1 Berger *et al* (1977).

Sample Site	Average Saprobic Index	No. of species	Average Saprobity	Average Pollution Index
1: Top	<1	Nil	Oligosaprobic	Unpolluted to minimal
2: Kirstenbosch	2,4	6	β- to α- mesosaprobic border	Very strongly affected affected
3: The Hill	2,5	3	β- to α- mesosaprobic border	Very strongly affected affected
4: Newlands	2,4	7	β- to α- mesosaprobic border	Very strongly affected affected
5: Brewery	2,5	13	β- to α- mesosaprobic border	Very strongly affected affected
6: Gordons	2,6	12	β- to α- mesosaprobic	Very strongly affected affected
7: Valkenberg	2,6	11	β- to α- mesosaprobic border	Very strongly affected affected

From Table 7 it can be seen that pollution indices calculated for our sampling sites on the Liesbeek River indicate that sites two (Kirstenbosch) to seven (Valkenberg) are possibly strongly to very strongly affected by pollution. The species used to calculate the saprobic indices did not occur throughout the year, however, and it is therefore assumed that pollution levels fluctuate throughout the year.
The level of bacterial(and assumedly organic pollution) probably fluctuates throughout the year.

These levels are dependant on organic input and with the variable of flow affecting pollution levels at a given site. Further, these results imply that the species of protozoans found in the Liesbeek River do respond to environmental conditions in the same ways that their European counterparts do, and therefore that they are physiologically as well as morphologically similar. This very preliminary assessment is in contrast to some of the results obtained for wetland protozoans, as described in section 4 below.

The Liesbeek River

Pollution of the Liesbeek River is a matter of serious concern. Water abstraction probably affects the river from the initial abstraction point just below site 1 to below site 3.

Canalisation, effluent inputs (both chemical and refuse), and human activities impact the river to a considerable degree. Rehabilitation is possible by planting appropriate endemic riparian vegetation and by controlling water abstraction and, dumping of refuse and

chemically polluted effluents. Simple but regular biomonitoring methods, such as SASS, which is already carried out by CMC's Scientific Services, should be able to track changes in the condition of the river.

CHAPTER 4

Lentic Sampling

Wetlands are standing water bodies and provide very different types of environments from those in rivers. The lack of flowing water in many wetlands affects both the chemical and physical nature of these systems and therefore also affects the biota that inhabit them. Results obtained from sampling protozoans in rivers cannot therefore be assumed to apply equally to wetlands. In addition, the most appropriate sampling methods applied in the two types of systems are likely to differ. For this reason, sampling was undertaken independently in wetlands and the results have been examined separately from those found in rivers.

One of the reasons for investigating the feasibility of using protozoans as bioindicators is their potential value in assessing freshwater ecosystems where conventional biomonitoring techniques such as the South African Scoring System (SASS: Dallas 1995) cannot be applied. Such systems include dry riverbeds as well as wetlands. For this reason, lentic sampling was carried out to assess the feasibility of using protozoans as bioindicators in wetlands. The specific aims of this part of the project were:

to investigate and develop methods for sampling protozoans in wetlands;

to assess whether protozoan assemblages (or specific indicator species) are effective indicators of water quality in wetlands;

to assess the feasibility of using protozoans as indicators of water quality in wetlands.

The second and third of the aims are equivalent to the aims listed for riverine protozoans (section 3 above). The first arose when it became clear that sampling protozoans effectively is not a trivial task and specific investigations of sampling methods were undertaken.

A number of procedures can be used to sample protozoans. Generally they are sampled either directly in sediment samples or through the use of artificial substrates such as Polyurethane Foam Units (PFUs). Most protozoans are found within the first few centimetres of the sediment in decaying vegetative material (Finlay *et al* 1988) and this makes direct sampling a very simple way of collecting these organisms. However, despite the advantage of being quick (direct sediment sampling involves only one site visit per sample), this method has a number of disadvantages. Firstly, because of the difficulties in collecting consistent proportions of water, sediment, vegetation and algae in each sample, the use of sediment samples is not quantitative. Secondly, there is great variation in both sediments and vegetation both within and between wetlands and this makes it difficult to isolate the effects of water quality, as opposed to physical habitat, on the structure of protozoan communities. It also means that many samples must be taken to account for habitat "patchiness" both within and between wetlands. As well as these theoretical problems, a number of practical disadvantages are also associated with direct

sampling. Generally sediment samples contain fewer species than samples from artificial substrates, and detritus samples are more difficult to work with in the laboratory due to the collection of detritus and sediment in water samples.

The use of artificial substrates was initiated by John Cairns (Cairns *et al* 1973, 1976; Henebry and Cairns 1980). Since then, such methods have been used by many protozoologists and have been further developed in China (Yunfen *et al* 1994). The use of artificial substrates has a number of advantages. Not only is substrate variability controlled for, but semi-quantitative sampling is possible, provided that the same-sized PFUs are used on each sampling occasion. The major disadvantage of this method is that it is more time-consuming because a site must be visited twice, once to put the substrate in place and the second time to collect it.

It was important in this study to determine which procedure would be most appropriate for use in wetlands. Once an appropriate sampling methodology had been developed, this could be used to ascertain firstly whether protozoan assemblages, or individual taxa, or both, are effective indicators of water quality and secondly, whether it is feasible to use protozoans as bioindicators in wetlands.

4.1 Description of sites

Sampling was carried out in a number of wetlands near Cape Town in South Africa. After initial sampling experiments in a small dam on University of Cape Town (UCT) property during May 1999, three areas were selected for further research: Sunset Park (a detention pond fed by the Lotus River in Grassy Park); Westlake wetland (a freshwater wetland feeding into Sandvlei near Muizenburg); and two wetlands, one permanent and one temporary, in the Cape Point Nature Reserve. Site characteristics, sampling dates and water chemistry are summarised in Tables 4.1.and 4.2. Temporary ponds on Rondebosch Common, as well as various wetlands near Betty's Bay, were also investigated. All of these wetlands were used to test various questions regarding sampling methodology.

Once a sampling methodology had been developed, further sites were selected for a more detailed analysis of the protozoan assemblages associated with a range of water qualities. Two of the sites used in the initial sampling procedure, namely the Westlake and Cape Point wetlands, were sampled again. Two further sites were sampled at Zeekoevlei and Rietvlei, the site at Rietvlei being a channel situated below an outlet pipe suspected of transferring stormwater runoff into the channel

Table 4.1: A summary of the site characteristics, sampling dates and type of samples (PFU or sediment) taken at each site during the pilot sampling program.

	Betty's Bay	**Cape Point**	**Cape Point**	**Rondebosch Common**	**Sunset Park**	**Westlake Wetland**
Seasonality	perm/temp	permanent	temporary	temporary	temporary	permanent
Disturbance	Probably affected by building and development	Un-isturbed	Un-disturbed	probably affected by roads and people	highly polluted, disturbed, nutrient enriched	nutrient enriched
June						Sediment
June						
July					Sediment	PFU
July		Sediment	Sediment	Sediment	Sediment	
August					PFU	
August		Sediment	Sediment	PFU	PFU	
September	Sediment					
September		PFU	PFU	PFU		
October						Sediment
October						Sediment
November		PFU	PFU			PFU
November						PFU

Table 4.2: Information on the known water chemistry of the sites sampled. Conductivity in μS/m, nutrient measures in mg/l; ~ = no available data

	Betty's Bay	**Cape Point**	**Cape Point**	**Rondebosch Common**	**Sunset Park**	**Westlake Wetland**
Conductivity	500	540	1150	~	1020	3630
pH	4.7-7.1	4.4	<4	~	8.4	7.1
Total Nitrogen	~	~	~	~	5.4	1.3
NOx	0.04	0.07	0.07	~	0.57	0.08
NH4	0.09	0.12	0.19	~	0.59	0.11
Total Phosphorus	~	~	~	~	0.64	0.09
Sol.Reac-tive Phosphorus						

In all, the sites were chosen to represent a range of water chemistry (Table 4.3), varying from the unimpacted, acidic Cape Point site to wetlands such as Westlake, which have higher levels of nitrogen and phosphorus and represent more eutrophic conditions. During the course of the project, three additional sites were selected for further sampling: two relatively unimpacted sites (one permanent and one temporary pond) at the Kenilworth Racecourse, and a third in a canal

connected to the Westlake wetland. The water chemistry in this final site is likely to closely resemble that of the Westlake wetland itself.

Table 4.3: Chemical data collected at each site. Values are averages taken over the period of sampling. W = Westlake; CP = Cape Point; Z = Zeekoevlei; KP = Kenilworth permanent pong; KT = Kenilworth temporary pond. WC = Westlake canal; P = permanent and T = temporary.

	W	**CP**	**Z**	**R**	**KP**	**KT**	**WC**
Seasonality	P	P	P	P	P	T	P
Temperature (^{o}C)	17.3	17.3	20	19	16.5	16.0	18
Conductivity (μS/m)	2217.5	450.8	1773	1956.7	385	125	480
pH	8	<4	9.2	8.5	5.5	5.8	7

4.2 Methods

During the pilot stage of the project, the aim was to experiment with different sampling procedures and to gain some understanding of the range and variability of protozoan assemblages and taxa associated with a range of wetlands. As such, no statistically valid experiments were set up. Sampling was carried out at different times and using different sampling methods. Table 4.1 summarises the sampling dates and types of samples taken at each site. These data were used to answer questions relating to field and laboratory sampling methodologies. Specific issues to be addressed included the feasibility of using PFUs, the number of PFUs required per site and the length of time for which they should be left in place in a wetland. Laboratory-related questions, such as the length of time for which the protozoan species assemblage of a sample remains unchanged in the laboratory, and the number of slides that need to be examined to adequately represent the protozoans present in the sample, were also addressed.

Having developed methods for sampling protozoans in wetlands, the next step was to determine whether either protozoan communities, or individual taxa, are effective indicators of water quality and to assess the feasibility of using protozoans as bioindicators. At Westlake, Cape Point, Zeekoevlei and Rietvlei, sampling was carried out every two weeks for a period of two months. In each wetland, three PFUs were positioned at the edge of the wetland, suspended about 100mm above the substratum. They were left in place for two weeks, removed and replaced with new PFUs. Towards the end of this sampling period, changes in environmental conditions at some sites resulted in fewer samples being taken. At Zeekoevlei, drawdown of the water level in anticipation of winter rainfall meant that PFUs were left high and dry and could not be used in the analysis.

At Cape Point, a dramatic increase in depth on one occasion meant that PFUs could not be located. As a result, four samples were analysed from Westlake and Rietvlei, whilst only three were examined from Cape Point and Zeekoevlei.

The permanent and temporary ponds at Kenilworth Racecourse, as well as the canal at Westlake, were sampled twice towards the end of the study period. The data collected from these sites were insufficient to allow comparison with the other four sites and were therefore used simply to test some of the ideas resulting from the initial data analysis. In addition to biological sampling, chemical measurements (temperature, pH and conductivity) were also recorded at all sites. The equipment and methods are discussed in chapter three for river sampling. One reading was taken for each of the variables on each sampling occasion.

In the laboratory, 2-3 slides were examined per sample and new genera encountered were recorded. No measure of abundance was estimated for any of the taxa, only presence-absence data being recorded. Where possible, organisms were identified to species level, but in most cases identifications were to genus only. Identification was mostly limited to those organisms >50μm in size, both because small, fast-moving protozoans are very difficult to identify without sophisticated equipment, and because any practical biomonitoring system would require the simplest of microscope equipment. The most useful protozoan identification guides were Jahn *et al*. (1979), Foissner & Berger (1996), and Patterson (1996).

The data were used to compile a list of the species and genera found at each site, to compare diversity between sites, to identify common taxa within sites and to identify taxa common to a number of sites.

Multivariate analysis by means of the Cluster and MDS modules in PRIMER (Clarke and Warwick 1994) was used to assess both within-site and between-site similarity of protozoan communities in order to determine whether distinctive communities could be identified for each site or for a particular type of water quality. Another PRIMER module, SIMPER, was used together with species lists for each site to identify potential indicator taxa for different water types. Multivariate analyses were carried out using the data set of common genera (i.e. excluded any genus found on only one sampling occasion at any one site). The reason for excluding rare genera was to reduce the importance of those groups that may have been misidentified as well as those groups that were seldom sampled, since one of the requirements of a good indicator species is that it should be numerically abundant (Johnson *et al* 1993). Species data were excluded from these analyses because of their potential unreliability and because most species were found only on isolated occasions.

4.3 Results

While sampling these wetlands, a single species list of protozoans collected by all sampling methods in this project was compiled (Appendix I). Between August and November 1999, a microscope slide collection was also produced and now contains 50 species. Further results of the data analysis are outlined below.

To investigate the benefits of using PFUs, the average number of taxa found in sediment samples and on PFUs was compared for sites at Cape Point, Sunset Park and Westlake. Sediment samples contained fewer protozoan taxa than samples from artificial substrates (Figure 4.1). In addition to the advantage of supporting a greater diversity, PFUs were also far easier to use in the laboratory since very little sediment or detritus was collected with the sample.

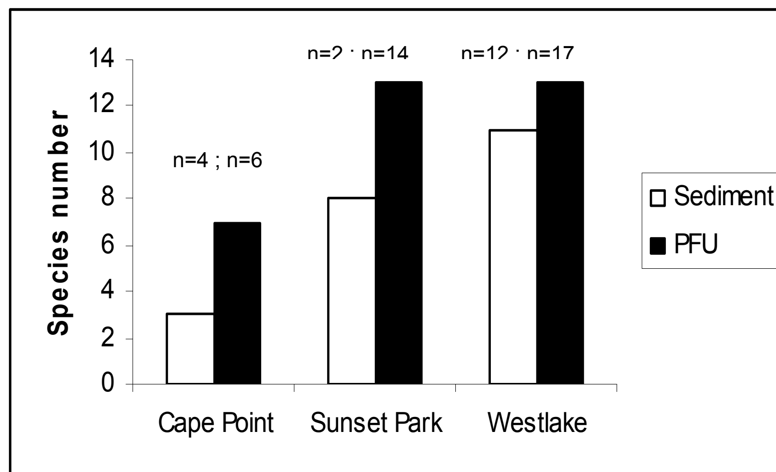

Figure 4.1: Comparison of average number of species on slides from PFU samples and direct sediment samples.

Deciding on an appropriate size of PFU involved experimenting with two sizes, 20 x 20 x 30mm, and 30 x 40 x 60mm. Cairns *et al* (1976) and Yunfen *et al* (1994) both found that larger foam units supported more diverse communities, although very large PFUs can be cumbersome and unsightly when left in place in the field. In our case, the larger PFUs also supported a higher number of taxa than the smaller ones did (Figure 4.2).

In addition, PFUs were left in the wetlands for varying lengths of time ranging from 7 days to 45 days. Community structure and species number remained little changed after 7-14 days. These results are supported by experiments carried out by Cairns *et al* (1973).

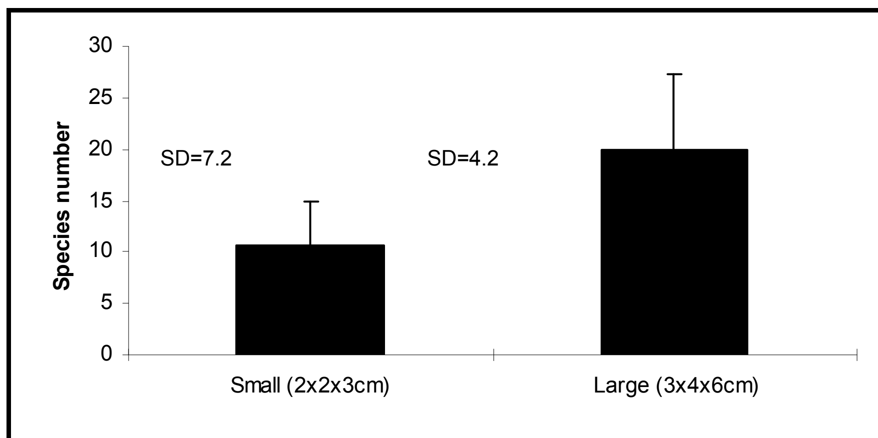

Figure 4.2: Comparison of average number of species between small and large PFUs (Date from Westlake wetland)
SD = standard deviation. n=3 for both small and large PFUs.

Using the results from samples already taken in developing the sampling methodology, a preliminary investigation was made of the variability in samples taken both within and between sites. The results are shown in Figure 4.3. The similarity between replicate PFUs was found to be high, although the similarity between PFUs from different sites in the same wetland was almost as low as the similarity between PFUs in different wetlands. The effect of depth and season on community structure was not adequately addressed, and needs further investigation.

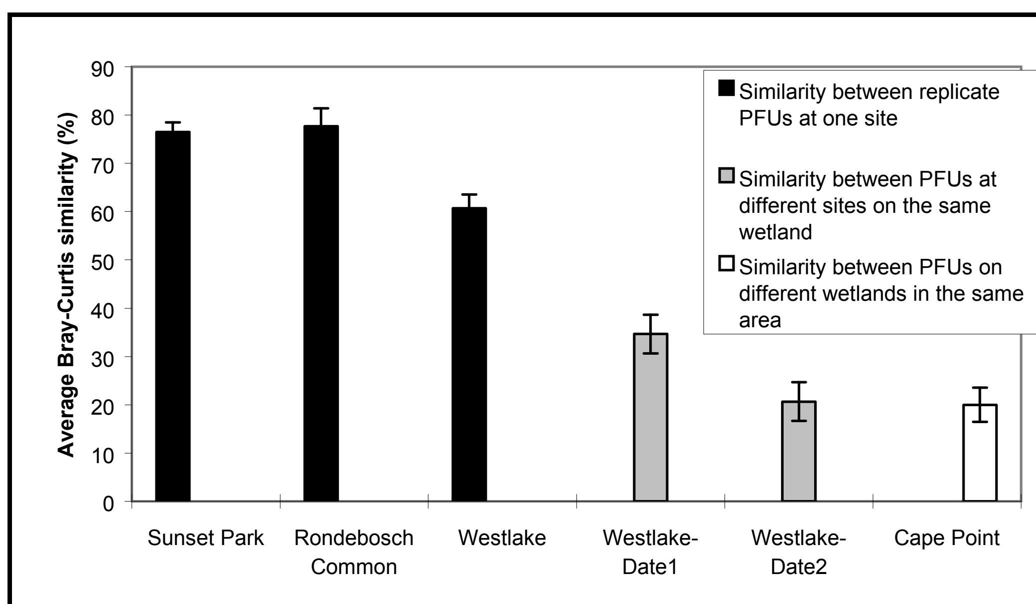

Figure 4.3:
Comparison of Bray-Curtis index of similarities for PFUs under various conditions. Similarity between replicate PFUs was fairly high, but the similarity between PFUs from different sites on the same wetland is almost as low as the similarity between PFUs in different wetlands.
At Sunset Park, n=6, standard deviation (SD)=4;
At Rondebosch Common n=3, SD=7.5; At Westlake n=3, SD=5.7;
Westlake (Date 1) n=3, SD=6; Westlake (Date 2) n=4, SD=6;
Cape Point n=6, SD=5.

When identifying protozoans, a few drops of water are placed on a slide and examined under a compound microscope. This process should ideally be continued until no new species are found. It is necessary therefore to determine the number of slides that need to be examined in order to represent all the species in a sample, in other words the number of slides above which, any new slides examined will reveal no new species. In the laboratory, experiments were performed to determine the acceptable number of slides required for adequate investigation of each sample.

Cumulative species number was plotted against slide number (Figure 4.4). Cumulative species number began to level off after three slides in most cases, and this was taken to be a sufficient number of slides for each sample. Usually 80% of the species in a sample can be recovered after three slides. Similar results have been found by Henebry *et al* (1980) and Yunfen *et al* (1994), who used 3 and 2 slides respectively.

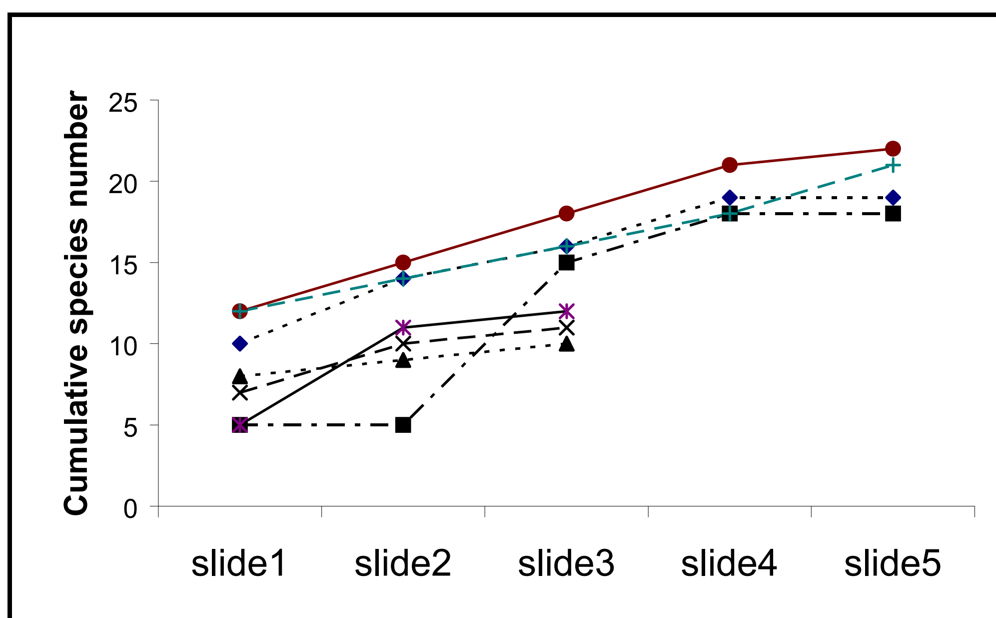

Figure 4.4: Cumulative species count with increasing number of slides viewed for seven different samples. After three slides the number of new species found with each new slide decreases quickly.

Extensive investigation was carried out to ascertain the extent of changes in protozoan communities with bioindicators. Protozoans are very delicate organisms and cannot be identified if they have been frozen or preserved in alcohol. They thus have to be examined as soon as possible after collection. Samples were kept in a cooler box in order to reduce the likelihood of individuals encysting or excysting before they could be examined and changes in community structure were then investigated over a number of days. Generally after day three, the community similarity with day one had dropped to below 60% (Bray-Curtis similarity measure), and the number of species had also declined drastically (Table 4.4). It would seem, then, that samples should not be kept longer than three days and should ideally be examined within a day of collection.

Table 4.4: Investigation of percentage similarity with time in the laboratory using the Bray-Curtis similarity measure. Numbers in parentheses are percentages of the number of species in the sample on day 1.

	Sunset Park A	Sunset Park B	Rondebosch Common	Cape Point
Day 1-Day 2	79% (100%)	72% (125%)		
Day 1-Day 2	72% (89%)	65% (94%)		
Day 1-Day 3	55% (73%)	43% (43%)	62% (44%)	
Day 1->Day 4	50%	53%	50% (122%)	31% (44%)

In looking at both the number of species and the number of genera at each site (Figure 4.5) it can be seen that biological diversity was greatest at Rietvlei and Westlake and lowest at Zeekoevlei.

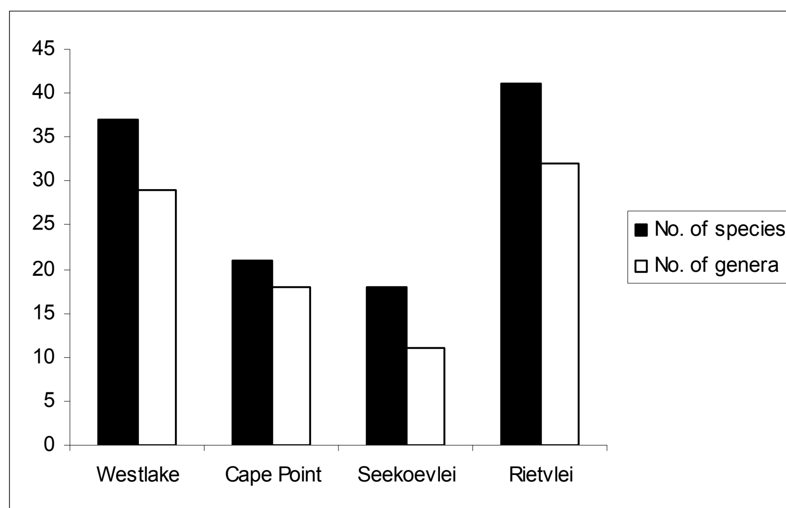

Figure 4.5: A comparison of biological diversity between the four sites sampled.

Figure 4.6 shows the number of times each genus and species was recorded in the Westlake wetland. A similar pattern was observed at all the sites. Most protozoans were recorded only once in the four sampling occasions and only three were recorded on every occasion. These results suggest either that there are very few common taxa at each site, or alternatively that not enough sampling was done to accurately represent all the taxa present at a site.

Diversity at Cape Point is higher than at Zeekoevlei but lower than at the remaining two sites. Since sampling at Kenilworth Racecourse and the Westlake canal was only carried out on one or two occasions, the data for these sites have not been included. It is worth noting, however, that diversity was higher in the temporary pool than in the permanent pond at Kenilworth Racecourse. This observation that may be worth investigating further.

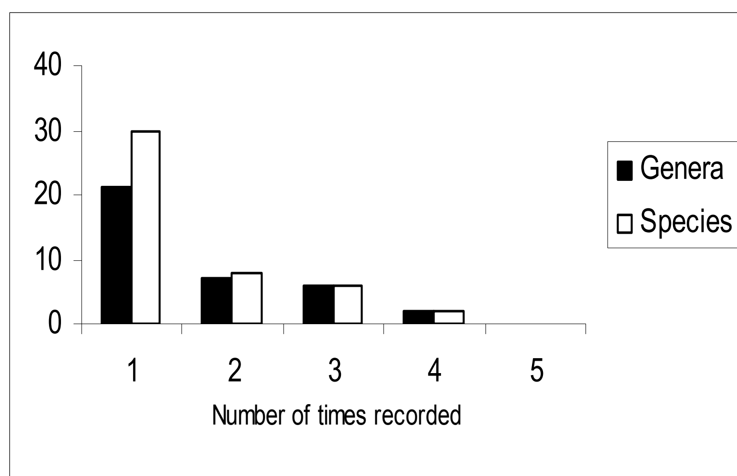

Figure 4.6: Frequency histogram showing the number of times each genus or species was recorded at Westlake.

In order to identify potential indicator taxa, the distribution of taxa across a range of water types was examined. Some genera were found to occur across a wide range of different habitats and were present at all sites. Others were found at a single site only. Table 4.5 provides a list of common genera (i.e. those that were found on more than one sampling occasion at a site) and the sites at which they were found. The results from the three sites (temporary and permanent pools in Kenilworth; Westlake canal) sampled on one occasion towards the end of the study period are also included to allow comparison with sites of similar water quality.

Aspidisca, Euplotes, Halteria and *Litonotus* were found at all four original sites and seem to be tolerant of a range of environmental conditions whilst other genera were restricted to a single site. *Chrysamoeba, Entosiphon* and *Ochromonas* were found only at Westlake, *Chlamydodon* and *Urocentrum* only at Rietvlei, and *Peridinium* and *Synura* only at Cape Point.

In the dendrogram and MDS plot (Figures 4.7a, b) only the common genera at each site were used, and a clearer pattern becomes evident in that similarities appear between samples from the same site. This within-site similarity, coupled with a greater dissimilarity between the sites, results in a clustering of sites separately from one another.

Table 4.5: The distribution of the more common genera across the sites sampled (Westlake=W; Cape Point=CP, Zeekoevlei=Z; Rietvlei=R; Kenilworth Racecourse permanent pond=KP; Kenilworth temporary pond=KT and Westlake canal=WC).

	W	CP	Z	R	KP	KT	WC
Anisonema	X				X	X	X
Arcella	X	X				X	
Aspidisca	X	X	X	X		X	
Chlamydodon				X			
Chrysamoeba	X						
Coleps	X		X	X			X
Entosiphon	X				X		
Euglena	X			X			X
Euglypha				X			X
Euplotes	X	X	X	X		X	
Gymnodinium	X					X	
Halteria	X		X	X		X	
Litonotus	X	X	X	X		X	X
Oxytricha	X	X					
Paramecium				X	X		
Peridinium		X			X	X	
Placus	X						X
Stentor				X		X	
Synura		X				X	

When the sites at Kenilworth Racecourse and Westlake canal were included in the analysis, the results were less clear. In the dendrogram, the permanent pond at the racecourse appeared to be an outlier from the rest, although the temporary pond and Westlake canal were most similar to the Cape Point and Westlake sites respectively. This pattern was not however, evident in the MDS plot of the sites. For this reason, and because sampling occurred only on once-off occasions at the Racecourse and Westlake canal sites, the results of this analysis have been excluded from this discussion.

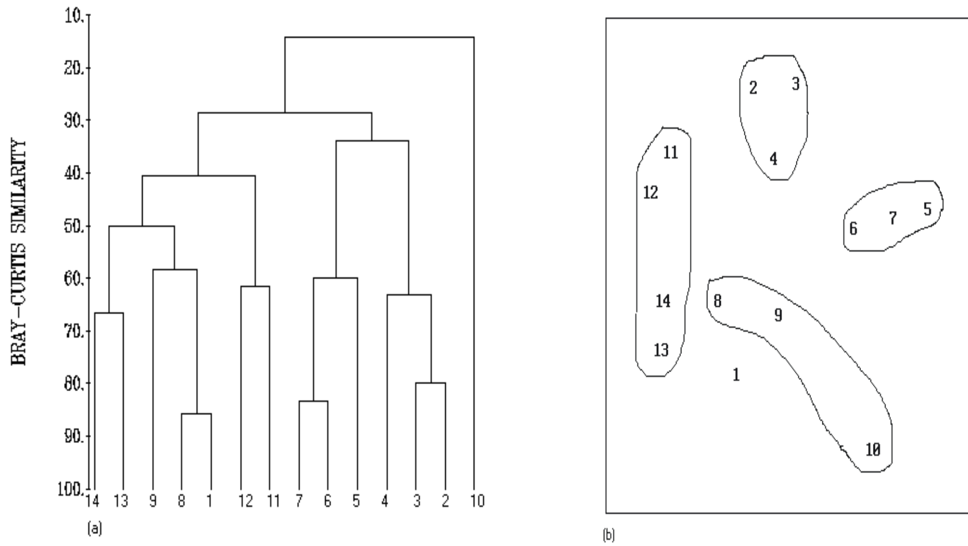

Figure 4.7: The dendrogram and MDS plot resulting from the PRIMER analysis using the common genera from each site.
On the dendrogram, numbers 1.1-1.4 represent samples taken from Westlake; 2.1-2.3 from Cape Point; 3.1-3.3 from Zeekoevlei and 4.1-4.4 from Rietvlei.
In the MDS plot, numbers 1-4 represent samples taken from Westlake; 5-7 from Cape Point; 8-10 from Zeekoevlei and 11-14 fom Rietvlei.

Within PRIMER, the BIOENV module was also used to assess whether there was any correlation between the biological and water chemistry data. This was not found to be the case for any of the environmental variables recorded (temperature, conductivity and pH). In examining the results of the SIMPER analysis (which identifies those taxa that contribute most towards the similarity/dissimilarity between sites), along with lists of taxa found at each site, some characteristics of the protozoan communities can be identified that may account for the dissimilarity between sites. These are summarised in table 4.6.

It should be noted that no two sites share the same characteristics, in other words whilst many of the sites contain similar genera, there are also unique characteristics of the protozoan community at each site that make them distinctive from one another.

	GENUS/GENERA	COMMENTS
Westlake	*Anisonema*	Common at this site
	Chrysamoeba and *Entosiphon*	Occur only at this site
	Coleps	More common at this site than at others
	Gymnodinium	More common at this site than at others
	Placus	More common at this site than at others
Cape Point	*Halteria*	Uncommon
	Coleps	Absent
	Peridinium and Synura	Only occur at this site
Zeekoevlei	*Tachysoma*	More common at this site than at others
Rietvlei	*Chlamydodon*	More common at this site than at others
	Euglypha	More common at this site than at others
	Paramecium	More common at this site than at others
	Stentor and *Urocentrum*	More common at this site than at others

Table 4.6: A summary of some of the characteristics of the protozoan communities at each site that may contribute towards the dissimilarity observed between sites. “Common” implies that the genus was found on many sampling occasions.

In the Western Cape, disturbed wetlands can differ from pristine wetlands in terms of organic enrichment, increased nutrient concentrations, presence of hydrocarbons and other urban pollutants, and change in pH. Protozoans have been considered as water quality indicators primarily in the context of organic enrichment (Curds 1992), although there is also information available on their salinity preferences (The effect of other aspects of water chemistry, and pH in particular, on protozoan communities has not been well studied.) To investigate the extent to which the data collected during this study correlates with findings in the international literature, we used Foissner's (1996) saprobic categories for each species. Although not all the sites have been included in the comparison, the results were similar for all sites sampled.

Table 4.7: A simplified description of the saprobic categories defined by Foissner and Berger (1996).

Key letter	Saprobity	Description (for more detailed descriptions see Curds 1992)
O	oligosaprobic	clean water (BOD 2.5)
B	β-mesosaprobic	purification zone BOD 5
A	α-mesosaprobic	purification zone BOD 10
P	polysaprobic	highly organically enriched BOD 50
I	isosaprobic	sewage (BOD 400)
M	metasaprobic	BOD 700

Table 4.8: The number of species in each saprobic category (Table 4.7) in each wetland studied.

	Betty's Bay	Cape Point	UCT dam	Rondebosch Common	Westlake	Sunset Park
O	0	0	0	0	0	1
Ob	2	2	1	1	3	1
B	2	0	1	2	8	0
Oba	0	0	0	0	1	1
Ba	9	12	4	5	26	10
A	1	3	1	1	4	3
Bap	1	4	0	1	4	3
Ap	2	1	1	0	2	1
P	0	0	0	0	3	1
Pi	1	2	0	2	4	1
Pim	0	0	0	0	1	0
obapi	1	1	1	1	1	1

In Table 4.8, saprobity increases down the rows and organic enrichment increases across the columns (Betty's Bay is the least enriched, while Sunset Park has the highest level of organic enrichment). One would therefore expect a large number of species of low saprobic index at sites with low levels of organic enrichment and vice-versa for richer sites but this was not the case. Sunset Park (the most organically enriched site) has the only oligosaprobic species, whilst Cape Point (one of the least organically enriched) supported several polysaprobic species. The data do not, therefore, fir in with the saprobic categories of Foissner and Berger (1996). It may be that organic enrichment is not the factor controlling species composition in the wetlands studied.

It is also possible, however, that the species in South Africa have different environmental requirements from the species in regions where this saprobic system was developed. This is in strong contrast to the findings for riverine protozoans in Chapter 3 above and is thus an issue that needs to be investigated further, before findings from the international literature are applied in South African systems.

4.4 Conclusions

4.4.1 Methods for sampling protozoans in wetlands

The use of PFUs seems to be the most practical way of sampling protozoans in wetlands. Polyurethane blocks of uniform size (30 x 40 x 60 mm) are tied to stones or weights using fishing line, so that the foam floats about 100mm above the sediment. While replicates are obviously desirable, time constraints may mean that only one sample can be analysed per site. The PFU should be left in the wetland for 2-3 weeks, after which it is squeezed into a sampling bottle. The sample should be kept cool both in the field and in the laboratory.

In the laboratory, a dropper is used to draw up water from the sediment at the bottom of the sampling bottle. Five drops are put on a slide, covered with a cover slip, and systematically searched under 100x magnification. This process should be repeated twice more (three slides in all) and all taxa recorded. All samples must be viewed within three days of collection, before community structure changes significantly.

The method described above for protozoan sampling in wetlands has proven to be effective and easy to implement. The problems and limitations arise in the identification of organisms and the way in which data are recorded. Problems relating to the size of organisms that can easily be identified, and the level of taxonomic identification required, have already been discussed. Another question that arises is whether it is sufficient to record the presence of each taxon or whether abundance should also be measured in the assessment of water quality. This could be particularly important when considering nutrient-enriched waters where one of the distinguishing characteristics is an increase in the abundance of organisms, together with a decrease in species richness (Curds 1992). After some preliminary attempts at assessing the abundance of protozoans however, the sampling strategy in this study was limited to recording presence/absence data only. This does not exclude the possibility of measuring the total biomass of protozoans in a sample, however. Yunfen *et al* (1994) take this idea one step further in their use of a heterotrophy index (HI). This is a measure of the total biomass of heterotrophs and autotrophs, divided by the amount of Chlorophyll a present. It thus reflects the quantity of heterotrophs in the system (more heterotrophs implies more polluted waters). Although this could be an effective measure, it provides some additional complexities such as identifying algae and measuring chlorophyll.

Other potential problems relating to both site and seasonal variability. Changes in communities within a single site, as well as over time, have not been adequately investigated and this may be an important consideration in the design and sampling strategy for any future projects.

4.4.2 Protozoan assemblages (or specific indicator species) as effective indicators of water quality in wetlands

Although the sampling conducted during this study was not very intensive, a number of general observations can be commented on. Greater protozoan diversity was observed at more eutrophic and polluted sites than at oligotrophic sites. Curds (1992) comments on the fact that increased nutrients usually result in a decreased number of taxa in freshwater systems, although overall biomass is often greater. The reason for the high diversity at eutrophic sites is uncertain, but may be due to some variable (such as salinity) that has not been examined in detail in the present work. The lower diversity found at Cape Point might be attributable to the low pH, which normally results in a decrease in species richness (Niederlehner and Cairns 1990).

Very few common taxa occurred at each site, most being sampled on only one or two occasions per site, implying that sampling may need to be more intensive if it is necessary to record all the taxa in a water body. This is a drawback in a biomonitoring context because of the time-consuming nature of the sampling itself, since a biomonitoring programme should, by nature, be quick and easy to use. In addition, it becomes difficult to identify potential indicator species because they simply are not encountered often enough in a standard sampling programme.

Many biomonitoring systems rely on the use of indicator species in their assessment of water quality. Some authors (e.g. Patrick 1973, cited in Stewart *et al.* 1987) have argued, however, that in some cases it makes sense to assess water quality on the basis of certain "indicator communities". Considering the lack of common taxa within sites, and the associated problems of identifying good indicator species, this suggestion may make sense in the context of protozoa and wetlands. The aim of the multivariate analyses was to assess whether the four sites sampled did indeed exhibit characteristic communities and whether these could be attributed to the water quality at that site. The results of these analyses have shown that, at least when only the common genera were used, each site does show distinctive community characteristics, and an attempt was made to suggest some of the taxa that contribute towards the distinctiveness of each site. It would seem however, that these distinctive community characteristics are the result of site-specific conditions, rather than of differences in water chemistry between the sites. This is reinforced by the fact that sites that would have been expected to show similar protozoan communities due to their similar water characteristics (e.g. Westlake and Rietvlei) are in fact no more similar to one another than they are to any of the other sites. Similarly Cape Point, which is clearly distinguishable from the other sites due to its low pH and unimpacted condition, does not, with the exception of one or two taxa, have a particularly distinctive protozoan community.

A similar situation pertained at the other three sites. In the Westlake canal, where one would expect the protozoan community to resemble the first Westlake site, this was not the case. Although some taxa occurred at both sites (*Anisonema, Coleps, Euglena, Litonotus* and *Placus)*, at least two of these were found commonly at most of the other sites and therefore provide little information on specific environmental conditions. In the same way, the temporary pond at Kenilworth Racecourse, despite sharing similar environmental characteristics with the site at Cape Point (i.e. unimpacted with low pH) showed few similarities in terms of its protozoan community. *Synura* and *Peridinium* for example, which were found only at Cape Point, were not encountered at the racecourse site. This may be attributable to the temporary nature of the pool, or to other environmental conditions not considered, but it highlights some of the difficulties in making predictions about which protozoan taxa will be present in a particular type of water quality.

The ecological characteristics and habitat preferences of many protozoans have been identified in the international literature (e.g. Foissner and Berger 1996) but the data generated in the present study did not always corroborate the information in the literature. Although some taxa did seem to have ecological requirements to those described in the literature for morphologically similar specimens ('species'), far more extensive sampling will be needed before assumptions can be made about the ecological characteristics of the South African wetland protozoans.

4.4.3 The feasibility of using protozoans as indicators of water quality in South Africa

Although some studies have attempted to correlate the distribution of protozoans in the field with various physical, chemical and biological variables (e.g. Stewart *et al.* 1987), it remains a difficult task. If protozoans are to be used as biomonitoring tools in the assessment of water quality in wetlands, it must be possible to identify certain taxa or communities that characterise particular aspects of water quality. In addition, the monitoring programme should ideally be quick and easy to implement. A number of problems arise in meeting these criteria for wetland biomonitoring. Firstly, the identification of both indicator species and indicator communities is extremely difficult. In this study, the effect of site-specific conditions on protozoan communities in wetlands was impossible to separate from the effect of the water quality itself. In addition, it seems that a far more extensive sampling programme would need to be implemented to adequately represent those taxa that do not occur commonly, to take into account both temporal and spatial variability in protozoan communities and to examine a greater range of habitats with different environmental conditions.

Although it is acknowledged that this study was conducted on a very small scale, it does point to some potential problems in the implementation of any kind of biomonitoring system using protozoans. Considering the time required for a new worker to become familiar with the identification of the organisms, and the time and restrictions associated with the actual sampling and storage of samples, the feasibility of implementing such a programme is questionable.

In summary:

- It is possible to identify South African protozoans using European and American taxonomic guides. Most species seem to be cosmopolitan in distribution;
- It takes at least three months for a new researcher to become adept at identifying protozoan species;
- Protozoan taxonomy is not complete and many inconsistencies occur, particularly at species level. It is recommended that only one guide be used for each group of protozoans to avoid taxonomic confusion.
- Because of confusion at species level, it is recommended that genus be used as the taxonomic level for any bioindicator system.
- Closer-to-pristine wetlands (Cape Point and Betty's Bay) probably support fewer species than do the disturbed wetlands, because only specialised organisms are able to survive under such low pH conditions.
- No taxa were found that could indicate low pH conditions but some deserve further investigation
- Species were found at undisturbed sites that were said to flourish in organically enriched environments, and species requiring clean water were found at the most organically polluted site. This is probably because the major factor controlling species distribution, at the sites studied, is not organic enrichment but pH and pollutants.
- Some protozoan species (*Peranema*, for example) are found only in permanent wetlands which suggests that it might be worthwhile to investigate the differences between temporary and permanent wetlands in more detail.

CHAPTER 5

Soil and Sediment Sampling

The importance of protozoans that occur in the soil is widely discussed by Foissner (1987). He defines soil as including dead and decaying organic matter " in which the vegetation takes root". In the South African context, soil protozoans were first studied many years ago. Prof. H.B.Fantham and his students at the University of the Witwatersrand collected a wide range of soil samples from sites in the then Cape Province, Orange Free State, Lesotho, Natal and Transvaal. They collected both "ordinary" and "waterlogged" soils at different times of the year (Fantham, 1928; 1931, Fantham & Taylor, 1921, 1922; Fantham & Paterson, 1923; 1924; Fantham & Porter, 1945). Sandon, who had previously published his book on protozoan fauna of the soil (Sandon, 1927) came to work at the University of Cape Town Zoology Department and continued his work on Protozoa (Sandon, 1941; 1965). Although mainly concentrating on ciliates, Foissner has conducted more recent research on soil protozoans from Gough and Marion Islands (Foissner, 1996) and from Africa, Australia and Antartica (Foissner, 1997). Papers on soil protozoans in other parts of Africa have been published by Drageso & Dragesco-Keneis (1986), Bamforth & Curds (1987) and Finlay & Curds (1987). Foissner (1987) considers that soil protozoans have the potential to disclose many ecological problems, *inter alia* the effects of sewage disposal, of soil compaction and of pesticides. Foissner (1987) concludes "the whole field is so understudied that its potentials and limitations cannot be seriously estimated".

5.1 Description of sites

Eight samples were investigated for the presence of soil protozoans.

- Firstly, samples of dry soil as well as leaf and other vegetative debris, were collected from the Liesbeek River sampling sites, Site 2 at Kirstenbosch and Site 3 at The Hill, when these were dry in summer; Thus, there were samples of leaf litter from Site 2 and Site 3 and a soil sample from Site 3. The latter collected when the river bed was dry. (Samples 5a, 5b and 5c. Shown in Table 5.1)
- Secondly, a sample of damp soil was collected from the Kenilworth Racecourse in summer, in an area surrounding a dried out vlei. (Sample 5d. Shown in Table 5.1)
- Thirdly, samples were collected at a Kenilworth temporary pond that had been totally dry for at least eight months. A sample was also collected from an earth-dug small dam at Kenilworth Racecourse. Two other waterlogged sand samples were collected, from an agricultural drainage canal at Phillipi on the Cape Flats, and from Lakeside Canal, an earth-dug canal intended as a drainage conduit from a nearby residential development. (Samples 5e, 5f, 5g and 5h. Shown in Table 5.2) The Kirstenbosch and Hill sites have been described in Chapter 3.

The dry samples were collected after they had had no water for two months. The sand samples collected at The Hill site were extremely coarse (1 to 5 mm diameter) and of a quartz origin.

The Kenilworth site lies in the middle of the Kenilworth Racecourse at Kenilworth in the Western Cape. Unfortunately, although previously considered as pristine, the site is now showing some signs of minimal human pollution. At the time of our sampling, the temporary pond had been dried out for a number of months. During years of "normal" rainfall the main vlei usually retains water for the entire year albeit at a very low level in summer. During the winter months of the period in which we sampled the area, the rainfall was below the average and the main vlei area no longer retained any water. A number of temporary vlei areas remained with a small amount of water for a short period at the end of winter and early spring. Waterlogged samples were also taken from the bankside of the small dam, probably used for irrigation water in the middle of Kenilworth Racecourse. The dry soil sample from Kenilworth Racecourse was taken close to a dried out vlei area.

The Phillipi site was in the middle of the Cape Flats where housing development has taken place in areas surrounding vegetable farming areas. The drainage canal was partly earth-dug, but with concrete sides. In the summer months the flow is very slow and probably ceases during prolonged periods of high temperatures. The area is fairly polluted and the drainage canal had some debris in it. There was no large leaf litter present and the surrounding area only has a medium cover of indigenous herbs and shrubs, typical of the Cape Flats vegetation type.

The Lakeside Canal has a very slow flowing current and the sides are covered with the exotic grass *Kikuyu* sp. and a a variety of invasive aquatic angiosperms and fern species, *Azolla* sp. and Kariba Weed.

5.2 Methods

The first two sets of samples (5a, 5b, 5c, 5d), from Kirstenbosch and The Hill and from Kenilworth were stored, in the sealed sample containers, for six months in the laboratory. Water was collected from the Kirstenbosch site just prior to the examination of the dry samples. This water was autoclaved and brought to a pH of 5.5. Dry samples of both sand and leaf debris were weighed and divided in equal amounts in Petri dishes. The actual mass per sample is shown in Table 5.2. Each sample was flooded with 30 ml of the autoclaved water. The actual mass per sample is shown in Table 5.2. These were flooded with 30 ml of distilled water.

All these samples, from the three conditions, were examined the day following flooding and twice thereafter at two day intervals.

Excess water from the waterlogged samples (5e, 5f, 5g and 5h) was tested for pH using Merck Spezialindikator paper strips or different values, ranging from 4.0 to 10.0. Electrical conductivity was measured, after calibrating for temperature, using a YSI Electrical conductivity meter.

The waterlogged samples were examined in the laboratory directly after collection and in the three following days.

5.3 Results

The dry soil, leaf litter and damp earth samples and the waterlogged samples from Phillipi had a far lower number of protozoans than the samples from Kenilworth and Lakeside Canal. The results of the investigations of these two different collections of soil protozoans are shown in tables 5.1 and 5.2, respectively.

Table 5.1: Protozoans found in soil and leaf litter samples (5a, 5b, 5c, & 5d) from Kirstenbosch, The Hill and Kenilworth sites.

Protozoans		**Kirstenbosch**	**The Hill**	**The Hill**	**Kenilworth**
		5 a	5b	5c	5d
Time after		**Leaf litter**	**Sand**	**Leaf litter**	**Soil**
		~ 0.03 g	**~4.00 g**	**~ 3.03 g**	**~1.01 g**
I day		Nil	Nil	Flagellates Ciliate sp. (≥20 μm)	Nil
3 days		Nil	*Colpoda* sp.	Flagellates *Colpoda* sp.	Flagellates *Colpoda* sp.
6 days		Nil	Nil	*Urosytla* sp.	Flagellates
					Colpoda sp. *Uroleptus* sp.

Table 5.2: Protozoans found in Kenilworth temporary vlei, Kenilworth Dam, Boundary Road and the Lakeside Canal (Samples 5e, 5f, 5g,& 5h).

SITE	Kenilworth	Kenilworth	Boundary Rd.	Lakeside Canal
	(temporary)	(permanent)	(temporary)	(permanent)
Sample	5e	5f	5g	5h
Conductivity	125 μS/cm^2	385μS/cm^2	1250μS/cm^2	480μS/cm^2
Temperature	16 °C	16.5°C	16°C	18°C
pH	5.8	5.5	7.4	7
	Actinosphaerum nucleofilum	*Euplotes patella*	*Euplotes eurystomas*	*Lacrymaria olor*
	Arcella sp.	*Halteria grandinella*		*Spirostomum*
	Halteria grandinella			
	Stentor sp.			
	Euplotes eurystomas			

5.4 Conclusions

There was a marked discrepancy between the protozoans found in dry soil and leaf litter. Since all the samples were flooded with the same autoclaved water we are at a loss to explain these differences. The pH selected was the same as that found in the regular site sampling and it is felt that this is probably not the cause of the lack of protozoans. Fantham's experiments in 1923 and 1924 were conducted with soils collected during the wet winter months and were, in fact, mostly enriched soils from the cultivated section of the Botanic Gardens. He did however, collect a sample from the "virgin soil" in the Silver Tree forest. This latter sample yielded the largest number of species. He maintained his samples in a "water culture" for a period of time during which various species of Protozoa were found. He maintained these cultures at a pH of 6.2 (Fantham, 1923) and 6.6 (Fantham, 1924). Our leaf litter samples were collected during a very hot dry period which had followed a relatively "dry" winter. Possibly our pH was too low and the length of the experiment too short.

The leaf litter samples from The Hill revealed a large number of flagellates and two ciliate species after 6 days of flooding with the same water. The leaf litter from The Hill had a greater mass, but not volume, than that of the Kirstenbosch site and the leaf litter contained fragments of exotic species e.g. *Quercus* sp. The Hill sand sample also yielded few protozoans. This site was also extremely dry and had not had any river water for a longer period than the Kirstenbosch site. Did the protozoans present retreat further down into the sand to avoid dessication? Possibly the leaf litter in the sample had not been on the dry river bed for as long a period of time.

The Kenilworth soil was slightly damp on collection and having been sealed in a sterile container did not dessicate. This sample produced the largest range of protozoan species although not in very large numbers. Whereas The Hill leaf litter sample had protozoans within one day, in all the other samples protozoans only appeared three days after flooding. Of note is the fact that no testate amoebae were found.

If soil cultures are made in the future it would be important to maintain them for a longer period of time e.g two weeks to a month, but under sterile conditions. Seasonal sample collections might yield a variation in species. Samples should be taken from the surface to 20 to 30 cm in depth. Seasonally compared this could reveal a migration of species. Careful note should be taken of the vegetation type and comparison between indigenous and exotic species might reveal some variation in protozoan populations.

Table 5.2 shows the results of waterlogged soil samples and actual water samples from Kenilworth, possibly a more "pristine" area. As with the dry soil and leaf litter samples the greatest diversity was found in the Kenilworth dry soil, temporary and permanent ponds. Boundary Road and Lakeside Canal had higher electrical conductivities and pH values. Both these sites were obviously polluted and with exotic aquatic plant species present. Both are also artificial situations.

But it can be seen that the Kenilworth permanent sample was also collected at an artificial site. However, the Kenilworth site was relatively unpolluted and surrounded by indigenous vegetation. Both Kenilworth sites had low pH (5.8 and 5.5 pH) and although the electrical conductivity at the Kenilworth permanent site was higher than that of the temporary site it was still comparatively low, < 400 $\mu S/cm^{2.}$ The temporary water had the larger number of species and this was not expected. The site had been completely dry for at least six months and there was a very sparse herb and grass cover. This temporary water body remained for only three weeks and has now reverted to it previously dry state. Not one of the protozoan species found has a saprobic indicator value of greater than 2.7 (Berger et al, 1997) revealing a pollution level below alpha-mesosaprobic.

It would appear that the protozoans in the temporary situations are ephemeral and able to adapt to transitory water availability. Do these species encyst or migrate up and down the soil moisture film controlled by water tension effects or some other environmental "trigger"? Ambient temperatures in the Western Cape very rarely drop below 5°C and it is assumed therefore that temperature fluctuation does not play a role in the presence of not of soil protozoans.

The small numbers of species found could be due to the relatively short period of sample examination and that our methods restricted the identification of species < 50 μm in length – *Halteria grandinella* being an exception as it is easy to identify having a peculiar "zig-zag" movement. Although one species of testate amoeba, *Arcella* sp., was found, the low species diversity was disappointing. However, it is interesting that Fantham and Paterson (1923) found a sharp decrease in protozoan diversity in waterlogged soils.

The sampling methods adopted were simple and can be carried out in a limited laboratory situation with only the use of a dissecting and compound microscope necessary. Maintaining the samples for longer periods of time and having facilities/equipment which could be used to identify small species would enhance any future research. Foissner (1987) stresses the change in protozoan populations over time. This change in dominant species would be understood with more frequent sampling. Identification of flagellate species is not easy as they are usually very small and the literature is difficult to obtain thus many of the small protozoans are not identified. According to Paterson & Larsen (1991) "few studies have been carried out on soil flagellates….and soil flagellates represent virtually uncharted territory".

Soil and sediment sampling has potential as a biomonitoring tool and a key to the identification of species would enable non-biologically trained personnel to undertake biomonitoring. It is unlikely that South African rivers and water bodies will receive increased water supply. It is more likely, with the probability of decreased rainfall, water demand and agricultural requirements that water flow will decrease. Soil and sediment sampling for protozoans would then become a powerful biomonitoring tool.

CHAPTER 6

Borehole Sampling

In a "dry" country such as South Africa, water is commonly obtained for household use, for stock and irrigation via underground accumulations which are often though of as "never ending" and unlikely to suffer from contamination. Both these misconceptions are unfortunate and, borehole water can be exhausted and may well be contaminated. Protozoans have been found in borehole water and may indicate bacterial accumulations/pollution of some sort. The Cape Town Municipality has sunk and services a number of boreholes in various parts of the Western Cape. Most importantly a large group of boreholes are regularly sampled and their water laboratory tested by the Scientific Services Branch of the Municipality. Appendix IV shows the Municipal boreholes at the Coastal Park refuse disposal site on the Cape Flats near Strandfontein beach. In this area the water table is high and monitoring of the water quality from these boreholes seeks to avert any seepage leaks from the Dump site into the water table in this area. We requested permission from the Scientific Services Branch to take water samples from specific boreholes to ascertain if there were any protozoa in the water.

6.1 Description of sites

Assisted by Mr John Stow of the Cape Town Municipality, Scientific Services Branch, we drew samples, using a baler, from four boreholes in December, 2000. The boreholes all lie on the westerly side of the Coastal Park refuse dump. We selected boreholes number, BH2; BH14; BH15; BH16 which lie directly beneath the base of the dump (see APPENDIX IV).

6.2 Methods

The boreholes were all 20m to 30 m deep and samples were taken about 4m from the soil surface. The water was clear with no offensive odour. The water was clear with no offensive odour. All the samples were collected in clean white plastic buckets which were sealed once the sample was obtained

In the laboratory we stored the samples in a cool room with approximate temperature of 10°C until they could be concentrated by filtering through a 44. μm mesh sieve. The residue remaining on the sieve surface was washed off with distilled water on to a sterile Petri dish plate.

6.3 Results

Examination of the samples under a dissecting and then a compound microscope revealed that not one of the borehole specimen samples had any Protozoa present. Samples from a second site visit obtained the same result.

6.4 Conclusion

Appendix IV shows the water quality findings from the borehole sites belonging to the Cape Town Municipality. Unfortunately, salinity levels are not tested, but it may be seen that chloride levels are high. The boreholes range in distance from the sea from 100 to 500 m. The lack of protozoans could possibly be due to a saline water backflow, but we were not able to test this hypothesis. The results were extremely disappointing and although the area is heavily polluted this would not have prevented the occurrence of protozoans. It would be helpful if boreholes that are not in such close proximity to the sea could be sampled.

CHAPTER 7

General Conclusions

Using protozoans as a monitoring tool is feasible in certain situations. Although it was found that the lotic sampling could be done on the SASS method and the lentic sampling required quite complex sampling methods there is definitely a place for soil and sediment sampling in those situations where SASS cannot be used.

All the protozoan species which were found are cosmopolitan and thus a ready comparison with the Saprobic values as in Berger *et al*, 1997 is possible, Identification to species level requires some training and microscope skills, but considering the fact that SASS cannot be used where there is no water, these skills should be taught.

Possibly a limited simple key to the protozoans could be produced. This should have line drawings and colour photographs.

In the Liesbeek River, regular sampling enabled the quantification of pollution levels in the river with ease. The Liesbeek is polluted and "strangled" by water abstraction. This abstracted water would otherwise enable a natural flushing to take place,

Sampling Methods

Reliable quantitative sampling of protozoans is not easy, largely because of the scale at which they occur.

- Firstly, during direct sampling important micro-biotopes may be missed and others over-represented, giving an inaccurate picture of the taxa present.
- Secondly, the ability of protozoans to reproduce very rapidly to respond quickly to change in environmental conditions, means that the number of individuals and the taxonomic composition of a sample can change after it has been collected and before examination under the microscope.
- Thirdly, protozoans can encyst and excyst rapidly, so that, unless conditions in the collateral samples are identical to those in the field, the suite of taxa evident *in* vitro in the laboratory may not necessarily be the same suite that was active *in vivo* in the field.

Protozoologists therefore recommend the use of artificial substrata to be placed in the field where it will be colonised by the entire suite of protozoan taxa, with the result that the effects of biotope patchiness can be negated. Although certain taxa might be entirely overlooked, this method does allow greater comparability from site to site and from time to time.

The utility of artificial substrata was investigated by the application of polyurethane foam blocks (PFUs). In the event that protozoans are used for biomonitoring, PFUs or similar systems of artificial substrata will be required to be left in place for a predetermined period.

Regardless of the sampling method, it is necessary to examine protozoans as soon as possible after collection, because satisfactory identification takes place only while they are alive. Some might encyst if conditions in the laboratory are unsuitable. Ideally, samples should be identified within a few hours.

Identification of protozoans

A good quality compound microscope with oil immersion is the minimum requirement for adequate identification. Some taxa cannot be identified without more sophisticated equipment such as dark field or phase contrast illumination, while some require specific staining, while a few cannot be identified without a scanning electron microscope.

Time and effort is necessary to identify small individuals and some identification might be incorrect. To avoid unnecessary errors it was decided not to deal with specimens that were smaller than 50µm in length and to identify only to generic level.

It may be argued that the physiological divergence of protozoans in southern Africa from their northern counterparts is much greater than physical divergence. If this was the case, What appears to be the same morphological species may respond differently from their northern counterparts. The significance of this argument in relation to the use of protozoans in biomonitoring is that, if local forms are physiologically different from northern counterparts, then their tolerances to water quality might also be different. This means that the existing body of knowledge concerning European and North American protozoans may not reflect aspects of local water quality.

This study produced conflicting results. The European Saprobic Index provided expected results on local riverine protozoans. By implication local riverine protozoans have comparable environmental requirements to European counterparts. However, local wetland protozoans provided a less clear picture and appear to have different requirements to the European counterparts. Further study will be needed to examine this aspect.

Assessment of the efficacy of protozoans in biomonitoring protocols

At present, the only biomonitoring tool in regular use in South Africa is SASS4, which is a rapid and field-based system that uses macro-invertebrates to assess water quality in perennial rivers.

It would be useful to have additional biomonitoring systems in order to

- o Provide a back-up system for rivers, running parallel with the SASS method
- o Identify particular types of pollution (as SASS identify general impairment of water quality)
- o Develop systems equivalent to SASS for use in wetlands, non-perennial rivers, sediments and ground water.

The advantages of using protozoans for any of these purposes have been confirmed by this project and include:

- o Rapid response to changes in water quality;
- o Some are able to survive desiccation, cysts are present even in the absence of water
- o Some protozoan taxa are represented in wetlands, non-perennial rivers, sediment and ground water
- o Different taxa respond differentially to impairment to water quality.

However, the disadvantages of using protozoans for water quality assessment are practical. These include:

- o Expertise is necessary for adequate taxonomic identification
- o Samples cannot be identified in the field, since good microscopic equipment is required
- o Samples cannot be preserved prior to identification and samples can change over time. Therefore, rapid identification is essential
- o A standard protozoan collection method for ground water has not been developed
- o Quantification is technically difficult

A biomonitoring system for water quality would be of greatest use if it could distinguish between pollutants of various kinds, such as organic waste, acid effluents, pesticides, heavy metals and salinity. The possibility exists that certain protozoan taxa might be useful in this regard, but further research is required.

Recommendations

Identification of protozoans

This project has developed considerable expertise in the identification of freshwater protozoans. Numerous photographs have been taken and video recordings have been made of several taxa. A brief guide to the identification of freshwater protozoans has been published by the Water Research Commission as part of a series to identify all freshwater invertebrates. It might be worthwhile to collate species lists and photographs into a single package.

Use of protozoans in biomonitoring

The preliminary results of this project indicated that protozoans do not offer an easy alternative to the existing SASS biomonitoring system, which uses macroinvertebrates for estimating the impairment of water quality in rivers.

From work done elsewhere (Day *pers. obs.)* it appears that developing a similar system using macro-invertebrates for perennial wetlands is likely to be difficult because of the differences in water chemistry and environmental features between wetlands.

A real possibility exists for using protozoans in biomonitoring for various aspects of non-perennial systems and ground water. The fact that some protozoan cysts can survive for some time in a desiccated state offers the possibility that they could provide information about antecedent conditions in dry rivers and wetlands. Further, their very rapid response to inundation means that protozoans should be useful for estimating water quality conditions over relatively short periods of time in ephemeral systems.

Although this project was unable to find a method for collecting protozoans from ground water, this should not preclude further attempts with a variety of techniques, including the application of artificial substrata. In South Africa, our understanding of the biota and of the environmental quality of ground water (particularly of the "deep" biosphere) is in its infancy. The National Water Act of 1998 requires that a Reserve be calculated for such water resources, and we need to continue to investigate protozoans in this regard.

Recommendations for further work

It is suggested that work on protozoans and biomonitoring should:

- Concentrate on species with large individuals (>50µm in length);
- Concentrate identification at the generic level, unless certain have very obvious characteristics
- Concentrate on the Ciliates (particularly when in doubt), as they are generally fairly easy to identify and for which good keys exist
- Use artificial substrata for ease and uniformity of collection
- Complete identification of each sample within as short a time as possible following collection – certainly no longer than a week
- Several samples should be taken from each collection in order to obtain a reasonable indication of the taxa present
- Keep a good reference collection of 35mm slides and/or video recordings

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APPENDIX I

Species list and descriptions of those protozoans found in this project.

Note: Where genera are listed as "spp." this usually indicates that species were found that were not confidently identifiable, or occasionally, that a generic group is commented on.

These protozoans have been arranged in the taxonomic orders according to Kudo (1971)

G. = Greek

L. = Latin

Phylum Protozoa

Class Mastigophora (Flagellates)

Order Chrysomonadida

Anthophysa spp. (G. *anthos* = flower; G. *physa* = bellows)

Considered as a flagellate by protozoologists (Kudo, 1971; Patterson, 1996) and as a chrysophyte alga (Fritsch, 1948) by botanists! This is an easily recognised genus whose members have a tree-like structure, the ends terminating in colourless/pale groups of flagellated organisms, 5 – 10 µm in diameter. Often termed "iron" flagellates because of the rusty colour of the "stems", which may or may not be attached to the substrate (Patterson, 1996).

Found at The Hill and Kirstenbosch sites in January, 2000.

Order Cryptomonadida

Cryptomonas spp. (G. *kryptos* = hidden ; G. *monas* = unit)

Very small (≤10 µm) flagellates commonly containing one to a number of chloroplasts. Fritsch (1948) placed the genus in the algal class Cryptophyceae, and commented on their characteristic "swaying" motion. *Cryptomonas* tend to be blue-green to olive-green in colour (Fritsch, 1948; Patterson, 1996).

Found at The Hill and Kirstenbosch sites in October, 1999.

Entosiphon spp. (G: *entos* = within; *siphon* = pipe, tube)

Oval or flattened with one trailing flagellum. The cytopharynx can be protruded.

Found at Westlake and Kenilworth permanent pond.

Order Chrysomonadida

Synura spp. (G: *syn* = together; o*ura* = tail)

Forms colonies, from two to larger number cells. Oval body with a covering of short bristles (Kudo, 1971)

Found at Cape Point and the Kenilworth temporary pond.

Order Euglenoidida

Anisonema spp (G*: anisos* = unequal)

Usually oval with a central, slit-like furrow; anterior flagellum often trailing and larger than the second flagellum (Kudo (1971).

Found at Westlake, Kenilworth permanent and temporary pond and Westlake Canal.

Euglena oxyuris (G. *eu* = well ; *glene* = eyeball ; *oxys* = sharp; ouros = tail)

Members of this genus are generally spindle-shaped with one obvious flagellum and a red eyespot. They usually contain starch-storing paramylum bodies which appear paler than the rest of the cell (Kudo, 1971). *E. oxyuris,* which may be up to 300 μm long, has a twisted posterior end with a marked point and a spirally striated pellicle (Wolowski, 1998).

Found throughout the sampling period at the Valkenberg site.

Order Dinoflagellida

Peridinium tabulatum (G. *peri* = around; *dinos* = rotation) (*tabulatum* = floor/storey)

This is an easily recognised freshwater species, individuals 20–50 μm in diameter and rounded or sub-spherical in shape. Usually a brownish-orange colour, which masks the chloroplasts within (Kudo, 1971). The theca (coat/outer covering) appears to be partially divided into four sections (Patterson, 1996).

Found at Gordons, Newlands and The Hill sites in September, 1999 and at Cape Point and Kenilworth (permanent and temporary ponds)

Class Sarcodina (Amoebae and their kin)

Order Amoebida

Amoeba proteus (G. *amoebe* = change; G. *proteion* = first)

Large amoebae between 220 μm and 760 μm, mainly polypodial but occasionally monopodial with a dark granulated cytoplasm; mononucleate. Often confused with the genus *Chaos,* whose members are generally smaller and multinucleate (Patterson, 1996; Page, 1976). These organisms may be more common than they seem to be, but they required more concentrated examination than was possible.

Found at the Breweries site in May 2000, and the Newlands site in July 2000.

Chaos spp.

One of the earliest described genera (Linnaeus, 1767). These amoebae are multinucleate with granular or ovoid nuclei. Some species retain zoochlorellae and most species are polypodial (Page, 1976). This genus is not always recognised as being separate from *Amoeba* spp. (Kudo, 1971).
Found at the Valkenberg site in October, 1999.

SuborderEuchrysomadina

Chrysamoeba spp. (G chrysos = gold*, amoebe* = change*):*
A naked amoeba found in standing freshwater (Kudo,1971)
Found at Westlake

Pelomyxa palustris (G. *pelos* = clay, *myxa* = slime, *palustris* = marshy)
Generally classed as an amoeba, this organism has recently been recognised as a flagellate (Griffin, 1988). It is usually pale to dark grey, opaque (due to granular contents), and seems at first appearance to be a lifeless amorphous lump, 1-5 mm in size. A slight prod will produce slow movement and the posterior uroid may be seen. Due to the fact that it commonly contains bacteria that enable it to live in anaerobic situations, it is often an indicator of alpha saprobic (highly polluted) conditions. It is cosmopolitan and can be found from running to still water conditions which are usually polluted **(**Berger *et al*, 1997).
Found at the Valkenberg site in October - December, 1999

Order Testacida

Arcella spp.(L. *arc* = arch; F. *cella[ciel* = canopy)
These amoebae have a proteinaceous circular or ovoid test with a central circular aperture. They range from colourless through yellow to brown with various types of decoration, indentations or basal collars which are used to define the species (Ogden & Hedley, 1980).
Found at the Valkenberg site from October 1999 to March 2000 and June to August 2000; at the Gordons site from November 1999 to January 2000 and April to June 2000; at the Breweries site in November 1999, January 2000, February 2000 and April-June, 2000; at the Newlands site in October-November, 1999, February 2000 and March-May, 2000; at the Kirstenbosch site from November, 1999 to January, 2000; at Westlake, Cape Point and Kenilworth temporary ponds

Arcella vulgaris (L. *vulgaris* = common)
As the name implies this is a cosmopolitan species. Usually yellow to red to brown in colour, looking rather like miniature condom with no obvious indentations or patterning. Individuals may be up to 150 µm in diameter; occasionally the pseudopodia may be observed extruding

from the shell in the feeding process. In large specimens, small diatoms may be observed in the interior cytoplasm.
At all sites throughout the year.

Centropyxis spp. (G. *kentron* = center; G. *pyxis* = box)
The test may be circular, hemispherical or ovoid with a rough surface, commonly with spines on the lateral margins. Viewed from the underside, this testate amoeba looks rather like a bedpan with the lateral, spiked edge, being slightly raised and the aperture at the opposite end, off-centre. Described as colourless, yellow or brown by Ogden & Hedley (1980), most specimens seen by us were colourless.
Found at the Valkenberg site in November and December 1999, January, February, May and June, 2000; at the Gordons site in December 1999, January, March, April and May 2000; at the Breweries site in November and December 1999, February, March and May, 2000; at the Newlands site in November-December 1999, February, April and May, 2000; and at the Kirstenbosch site in November 1999 and January-February, 2000.

Difflugia spp. (L. *dis* = away; *fluere* = flow)
The test may be circular but is generally amphora-shaped with "spikes" and a large range of decorations, which may be composed of bits of diatom or pieces of mineral. The aperture may be specifically ridged and the test is often constricted behind the aperture. Species names often refer to the type of material found on the shell, e.g. *D. bacillifera*, which accumulates bits of diatom (Bacilliariaceae) frustule on it; *D. corona* has a varying number of spines (L. *corona* = to wreathe, to crown with a garland) at the distal end of the shell.
Found at the Valkenberg site in November, 1999, January-February, April-July 2000; at the Gordons site in January, April and June, 2000; at the Breweries site in November-December 1999, April-May, 2000; at the Newlands site in April-June, 2000; at The Hill site in August 2000; and at the Kirstenbosch site in January, February and July 2000.

Euglypha spp. (G: *eu* = well; *glyphein* = to carve)
Test composed of overlapping silicaceous plates and usually with one or more spines on the test.
Found at Rietvlei and Westlake canal.

Nebela spp.

The tests are similar in shape to those of *Difflugia* spp. but do not have “spikes”. Most appear to be amphora-shaped with the lateral edges compressed or ridged. The apertures have species-specific features such as collars (Ogden & Hedley, 1980).
Found at the Valkenberg site in February and April 2000; at the Gordons site in December, 1999; at the Brweries site in November 1999 and April 2000; at the Newlands site in October and December 1999, February-March, 2000; at the Kirstenbosch site in July 2000.

Order Heliozoida

Actinophrys spp. (G. *aktis* = ray)
These are spherical organisms with axopodia radiating from the central, highly vacuolated body with a single nucleus. The axopodia are commonly "gekorreld" with tiny granules (Siemensma, 1981). Planktonic in oxygen-rich fresh waters.
Found at the Valkenberg site in December 1999, January and June 2000; at the Breweries site in December 1999

Actinosphaerium spp. (G. *aktis* = ray; G. *sphaira* = globe)
These are spherical organisms with obvious multivacuolated ectoplasm and numerous nuclei, which may be in both the endo- and ectoplasm. The axopodia, which are smooth, appear to be anchored within the centre of the cell (Siemensma, 1981). Planktonic in oxygen-rich freshwater. (May be listed as *Echinosphaerium* sp. in some references (Patterson, 1996).
Found at the Breweries site in February 2000.

Subphylum Ciliophora

Class Ciliata

Order Gymnostomatida

Amphileptus procerus
(G. *amphi* = both; G. *leptos* = small; G. *pro* = before; G. *cerus* = a horn)
Similar in appearance to *Litonotus lamella* and *L. cygnus*. Has a long narrow “neck” containing extrusomes (trichocysts) at its tip; length variable, 200 μm to 800 μm (Foissner, 1995). When extended, the “neck” is very noticeable and flexible and forms approximately two-thirds of the cell length. The lower section of the cell is spindle-shaped and a short “tail” is present. Identifiable by the approximately 20 contractile vacuoles in two rows along the spindle-shaped section. Generally in mesosaprobic conditions.
Found at the Gordons site in June 2000; at the Breweries site in September 1999; and at the Newlands site in January 2000
Coleps spp.

A barrel-shaped body that retains its shape (i.e. does not contract or have a sinuous movement). Cilia visible at the anterior (cyclostome) and posterior ends. Basket-like patterning commonly visible on the outer surface.
Found at the Valkenberg site in April and June 2000; at The Hill site in August 2000; and at Westlake, Zeekoevlei, Rietvlei and Westlake Canal.

C. spetai (*spetai* = named after a person called "Speta")
This species often contains zoochlorellae and has a markedly long caudal cilium at the posterior end of the cell .
Found at the Gordons site in November 1999.

Dileptus margitifer (G. *di* = two; *leptos* = thin; *margarites* = a pearl; ferein = to carry)
An elongate predatory ciliate (occasionally up to 1mm long) with a cytostome approximately one-third down the cell length and a proboscis, at the end of the upper third, which searches around for food. The movement is sinuous, the proboscis curling around objects in its surroundings. The proboscis contains extrusomes (trichocysts) that can be "shot" into prey (Kudo, 1971; Patterson, 1996; Berger *et al,*1997).
Found at the Valkenberg site in March 2000; at the Gordons site in January and May 2000; and at the Breweries site in January and May 2000.

Homalozoon vermiculare (L. *vermicularus* = worm-like)
This is a large (up to 1.5 mm long), worm-like ciliate with the large cytostome at the one end of the cell and the other end slightly tapered. Noticeably contractile; the often-visible macronucleus looks rather like a string of beads. Ciliation occurs on one side of the cell only. More common in flowing water in detritus close to the substrate (Kudo, 1971; Patterson, 1996; Berger *et al,*1997).
Found at the Gordons site in November 1999.

Lacrymaria olor (L. *lacrima* = a tear; *olor* = swan)
Long (up to 1mm), very flexible and retractible, spindle-shaped (Patterson, 1996) with the cell divided into three sections: an obvious cytostome, a long "neck" and a tear-shaped "body". Predatory but also consuming single-celled algae and detritus (Berger *et al*, 1997). This species is found in both marine and fresh waters (Kudo, 1971) and is also commonly found in ephemeral waters (Berger *et al*, 1997).
Found at the Breweries site in March and April 2000; and at the Newlands site in February 2000.
Litonotus spp. (L. *litus* = seashore; *notus* = familiar)

Sometimes called 'Lionotus' (Kudo, 1971; Patterson, 1976), which would be appropriate as these are predatory ciliates. The cell has an elongate pear shape, is flattened dorsoventrally, and is contractile with a flexible neck and a curved cytostome (sometimes almost hooked) at the top of the cell. The movement is sinuous. This genus, too, is found in both marine and fresh waters.
Found at Westlake, Cape Point, Zeekoevlei, Rietvlei and Kenilworth temporary pond.

L. cygnus (L. *cygnus* = swan)
An elegant species with a very long neck, the whole body very elongated and flexible, possibly up to 0.5 mm long (Berger *et al*, 1997). Acid-loving so more common at pH <6.
Found at the Newlands site in November, 1999.

L. lamella (L. *lamella* = small plate, sheet)
Individuals smaller than in the previous species, up to 300 µm long, which is longer than the greatest length (200 µm) quoted by Berger *et al* (1997). Moderately contractile. Regularly arranged extrusomes may be visible on the dorsal side of the cell (the side opposite to the neck curvature). Occurs in both marine and fresh waters.
Found at the Valkenberg site in October-November 1999, February, April and June, 2000; at the Gordons site in November 1999, January, April and May 2000; at the Breweries site in January, February and April 2000; at the Newlands site in December, 1999, January-April 2000; at the Kirstenbosch site in January 2000.

Loxodes spp. (G. *loxos* = oblique)
Flattened and lancet-like (Kudo, 1971), up to 700 µm long. These ciliates may be yellowish or brownish with an incurving cytostome area. They are often found in anaerobic conditions, mostly consuming bacteria and algae (Petterson, 1996)
At the Gordons site in April 2000.

L. magnus (L. *magnus* = large)
A definite brownish colour and with a well-defined incurving cytostome. The cell body is not retractile so they move with a slight sinuous wave from top to bottom of the cell. The largest of the species in the genus, up to 700 µm long and readily visible in a sample.
At the Valkenberg site in April, June and July 2000.

Loxophyllum spp. (G. *loxos* = oblique; *phyllon* = a leaf)

Similar in appearance to *Litonotus* spp. but with obvious small, wart-like protrusions on the same side as the curved cytostome. The cell is slightly contractile. This genus is found in both marine and fresh waters (Kudo, 1071).
Found at the Valkenberg site in April, June and July 2000 and at the Gordons site in April 2000.

L. maelagris
Individuals are up to 700 μm long, which is long for the genus. Predatory, commonly eating rotifers (Kudo, 1971). The macronucleus, which is sometimes visible without staining, is like a string of breads. Found at the Breweries site in November 1999.

Nassula picta (L. *nasus* = nose; *picta* = painted)
Round to oval-elongate with the cytostome approximately one-third from one end, often with an obvious basket-like vestibule (Kudo, 1971, Patterson, 1996)
Found at the Gordons site in May 2000.

Paradileptus elephantinus (G*. para* = beside ; *di* = two ; *leptus* = slender; L. *elephantus* = elephant-like)
A V-shaped cell with an elongated proboscis with an area (not readily visible) of trichocysts around the proboscis and cytostome at the top of the V-shape. Small contractile vacuoles occur around the cell and partly up the length of the proboscis (Kudo, 1971; Foissner, 1996). Found at the Gordons site in May 2000.

Placus spp*. (G:plax = a plate)*
Flattened, ellipsoid or oval with conspicuous spiral furrows. May be found in salt, brackish or fresh water (Kudo, 1971).
Found in Westlake and Westlake Canal.

Plagiopyla nasuta (G. *plagio* = oblique; *pylos*= a gate)(L. *nasutus* = large nosed)
A kidney-shaped ciliate with the peristome (entrance to the mouth) visible on the ventral side as a deep groove. Individuals may be up to 180 μm long but are usually shorter. Berger *et al* (1997) aptly describe this species of having a nose-like ridge surrounding the peristome. This cosmopolitan ciliate is found in anaerobic and hydrogen-sulphide-rich conditions. Found at the Gordons site in November 1999; at the Breweries site in November 1999; at the Newlands site in September and October 1999; at The Hill in August, 1999 and September 2000; at Kirstenbosch in November 1999; at the Top site in November 1999.
Prorodon spp. (L. *pro* = before; *rodere* = to eat)

Usually oval sometimes cylindrical, this ciliate is of the “primitive” type where the cytostome has a “basket” and is at the top of the cell. The macronucleus is usually large and there is a contractile vacuole at the lower part of the cell (Kudo, 1971; Foissner & Berger, 1996). Individuals of some species may reach a length of 300 µm (Patterson, 1996).

Occurrences not provided

Trachelius ovum (G. *trachelos* = neck; *ovum* = an egg)

Similar in shape to *Paradileptus* but the main body of the cell is rounded and the proboscis is not as pronounced. The cyclostome lies at the base of the proboscis and has the “basket” type of opening typical of this group (Kudo, 1971; Foissner & Berger, 1996; Patterson, 1996).

Found at the Breweries site in March 2000; at the Kirstenbosch site in January 2000.

Order Trichostomatida

Colpoda culcullus (G. *kolpos* = a fold; ? *cucullus* = a hood)

A kidney-shaped ciliate with the cytostome opening in the concave side of the cell, which probably gave rise to the species name (hooded). Small ciliates, not recorded as longer than 110 µm in length and those found in the present samples only 50 µm. This genus is similar in appearance to *Plagiophyla* but the cytostome is lower down the body of the cell than it is in *Plagiophyla* (Kudo, 1971; Foissner & Berger, 1996; Patterson, 1996). This common genus is also found in the soil (Foissner, 1987).

Found at the Breweries site in June 2000; at the Top site in November 1999.

Order Hymenostomatida

Epenardia myriophylli   (G. *myrios* = numberless; *phyllos* = a leaf, but probably because the species is associated with the plant *Myriophyllum*)

A genus erected by Corliss in 1971, this monotypic ciliate was formerly in the genus *Glaucoma.* This large ciliate (up to 200 µm long and 120 µm wide) has a laterally placed cytostome and a distinctive supraoral groove (Foissner *et al*, 1994). There is an undulating membrane to the right of the cytostome opening, but this is not readily visible.

Found at the Valkenberg site in March 2000.

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Frontonia spp. (L. *frontonis* = a broad forehead)

Oval to elliptical in shape, usually well rounded at the anterior end (hence the genus name *Frontonia*). The oral apparatus and a postoral groove are easily visible. Similar to the genus *Paramoecium,* these ciliates have trichocysts visible just under the pellicle (outer layer). Both fresh water and marine species are known (Kudo, 1971). Individuals of some species

are large (up to 600 µm long), with dark spots or whole-cell pigmentation and often with one contractile vacuole only (Foissner & Berger, 1996).
Found at the Gordons site in April-May 2000; at the Breweries site in March and May 2000; at the Newlands site in May 2000.

F. elliptica (G*. elleipsis* = falling short)
This species has two contractile vacuoles and could be mistaken for *Paramoecium* but the broad anterior shape distinguishes it. Common in running water (Foissner & Berger, 1996).
Found at the Valkenberg site in April 2000.

F. leucas (G*. leukos* = colourless/white)
There is no pigmentation in this species hence the name *leucas*. One of the larger species in the genus (up to 600 µm long) in the genus, its main food source is algae and sometimes amoebae.
Found at the Valkenberg site in April and May 2000 and at the Newlands site in May 2000.

Kahlilembus attenuatus (Kahl = after Kahl; L*. lembus* = boat*; attenuatus = thin)*
Formerly *Lembus attenuatus*, this species was been moved to various genera by different researchers but finally to the newly erected genus *Kahlilembus* (after the renowned protozoologist, Alfred Kahl) by Groliere and Couteaux in 1984 (Foissner & Berger, 1994). It is a small ciliate, slim and lancet–shaped, up to 80 µm long and 15 µm wide. It has a noticeable "tail" and is swift moving.
Found at the Valkenberg site in February and April 2000 ; at the Brewery site in March, May and June 2000; at the Newlands site in May-June 2000; at The Hill site in June and August 2000; at the Kirstenbosch site in August 2000.

Lembadion bullinium (L. l*embus* = boat-like; *bulla* = a bubble)
Oval, with a rounded dorsal side and a concave ventral side with an easily visible furrow. The cell is up to 200 µm long and 120 µm wide and posterior cilia (caudal cilia) form a visible "tail".
Found at the Brewery site in November 1999.

L. lucens (L. *lucens* = shining)
Individuals are small for the genus (up to 70 µm long) and readily identified by the bright and shining appearance.
Found at the Gordons site in May 2000.; at the Brewery site in February and June 2000; at the Newlands site in November 1999 and February, April and May 2000; at The Hill site in June 2000 and at the Kirstenbosch site in January 2000.

Paramoecium aurelia (G. *paramekes* = long in shape; L. *aurelia* = golden)
This is a common foot-shaped ciliate. Because of variance in some features it is now considered to form a species-group, *viz* the *P.Aurelia* complex (Foissner & Berger, 1994). A visible oral groove on the left side of the cell leads to the cytostome. The two contractile vacuoles are also easily seen, often contracting sequentially. Individuals are up to 180 µm long and 55 µm wide.
Found at the Valkenberg site in August 2000; at the Brewery site in June 2000; at the Newlands site in June 2000; at the Hill in June 2000.

P. caudatum (L. *cauda* = a tail)
Specimens may be up to 300 µm long and 80 µm wide and are obviously slipper-shaped. They are often found in large numbers throughout the year in standing or flowing water. Most commonly they consume bacteria, but algae are also eaten. They may be found in water with low oxygen levels and are considered as occurring from polysaprobic to alpha saprobic conditions (Kudo, 1971; Bick, 1972; Berger *et al,* 1997).
Found at the Valkenberg site in February, March and April 2000; at the Gordons site in November-December 1999, April-May and August 2000; at the Brewery site in November and December 1999 and March-April and June 2000; at the Newlands site in November 1999; at The Hill in July 2000 and at the Kirstenbosch site in November 1999 and January and February 2000.

Paramoecium sp.
Found at Rietvlei and in the permanent pond at Kenilworth.

Urocentrum turbo (G. o*ura* = a tail; *centrum* = centre; *turbo* = a whirl)
These are small ciliates, 50-80 µm wide, with a distinctive type of movement, looking like rolling *champignon* mushrooms or spinning tops with a short “tail” on the ventral surface which is, in fact, a tuft of cilia. Despite its rotund shape, this ciliate is closely related to *Paramoecium* (Kudo, 1971; Patterson, 1996).
Found at the Valkenberg site in May and June 2000; at the Gordons site from October 1999 to June 2000; at the Brewery site from October 1999 to June 2000; at the Newlands site from November 1999 to February 2000 and in May 2000; at The Hill in January 2000; at the Kirstenbosch site in January and February, 2000.

Order Heterotrichida

Bursaria truncatella (L. *bur*sa = purse; L. *truncatus* = cut off)
A U-shaped ciliate with a lateral crescent-shaped oral cavity, it could also be described as scoop-shaped. There are a large number of small contractile vacuoles around the perimeter

of the cell. Although it has been described as reaching 1.7 mm in length (Kudo, 1971; Foissner & Berger, 1996) all the specimens found in this project were considerably smaller (approximately 500 µm).
Found at the Valkenberg site in June 2000.

Spirostomum ambiguum (G. *speira* = a coil; *stoma* = mouth; L. *ambiguus* = wavering from side to side)
This is the largest of the three species of *Spirostomum*, able according to Kudo (1971) reach 3 mm in length although our specimens were approximately 2 mm in length. The body is elongated and cylindrical, but laterally compressed. The rows of cilia are visible even under a light microscope. Also noticeable is the posterior contractile vacuole, the "string of beads" mucleus and the entrance to the oral cavity, about two thirds from the anterior end. The cell is markedly contractile and movement is sinuous. They consume bacteria, algae and flagellates and are common in oligohaline conditions.
Found at the Valkenberg site in May-August 2000; at the Gordons site in November 1999 and February-May 2000; at the Brewery site in March- July 2000; and at the Newlands site in December 1999.

S. teres (L. *teres* = rounded)
Shorter and more rounded than *S. ambiguum*, up to 600 µm but more commonly 400 µm, and up to 75 µm wide, this ciliate is also highly contractile. The nucleus is ellipsoidal and the oral opening just under halfway down the length of the cell. Individuals are often found in water with low oxygen levels and may also be found in hydrogen-sulphide-rich sediments in fresh and brackish water (Kudo, 1971; Berger *et al*, 1997).
Found at the Valkenberg site in October 1999 and at the Gordons site in May 2000.

S. minus (L. *minus* = smaller)
Similar to *S. ambiguum*, but approximately half the length and width. The nucleus, like that of *S. ambiguum*, is beaded and contractile vacuole and ciliation are easily seen. The oral aperture is one-third to halfway down the length of the cell. Often found in alpha mesosaprobic waters (Kudo, 1971; Berger *et al*, 1997).
Found at the Gordons site in November 1999 and January and March 2000; at the Brewery site in November and December 1999; at the Newlands site in May 2000; at the Kirstenbosch site in February 2000.

Stentor spp.
These ciliates are trumpet-shaped. Some have a lorica (coat) and may be free-swimming or attached, depending on the species and the stage in the life cycle. The cell is very

contractile and the trumpet shape is not always obvious when the individual is free-swimming. A circle of long, obvious cilia on the anterior margin form a partial spiral leading into the peristome. Many species are identifiable by their colour (Kudo, 1972; Foissner & Berger, 1996).
Found at Rietvlei and the Kenilworth temporary pond.

S. coeruleus (L. *caeruleus* = blue)
This is a large easily identified species, up to 2 mm in length, an attractive sky-blue. Highly contractile, they may remain sessile whilst feeding but are also commonly seen swimming. They are easy to maintain in rice culture and are considered “an amenable experimental organism” (Patterson, 1996). They consume flagellates, algae, other ciliates, rotifers and each other. They are found in standing or slow-flowing waters and can survive for short periods under anoxic conditions (Kudo, 1971; Berger *et al*, 1997).
At the Newlands site in March, 2000.

S. meulleri (L. *meulleri* = named after Meuller)
Large undividuals, up to 3 mm long, generally colourless and often with a lorica (coat) (Kudo, 1971). The macronucleus is like a string of beads (Foissner & Berger, 1996). According to Berger *et al* (1977), this species had not yet been recorded in Africa.
At the Brewery site in February 2000.

S. niger (L. *niger* = black)
Individuals of this species are generally brownish in colour with an oval macronucleus and can be up to 350 µm in length (Foissner & Berger, 1996); Kudo (1971) states that they may be yellowish in colour.
Found at the Valkenberg site in November 1999 and August 2000.

S. polymorphus (G. *polys* = many; *morphe* = form)
Individuals are greenish in colour because of the presence of symbiotic algae. They are large, up to 2 mm long, and have a macronucleus like a string of beads (Foissner & Berger, 1996). Berger *et al* (1997) report that this species is very variable in size and that it is flexible and contractile. It may occur in a variety of conditions and has also been recorded in estuaries.
At the Brewery site in November 1999 and in February 2000.

Class Oligotrichida
Halteria grandinella (G. halter = a weight; L. grandis = large*)*

Individuals are small, up to 50 µm long, but with a very distinctive motion, erratically moving from place to place, probably because they have “jumping” bristles, a type of cilium (Foissner & Berger, 1996). They are round to ovoid and generally colourless; the “jumping” bristles can be seen using a compound microscope. Cosmopolitan and commonly found in still water, they can apparently encyst and may be found in damp soils (Berger *et al*, 1997). This was one of the most commonly found species in a variety of water conditions from still to fast flowing.

Found at the Valkenberg site in February-April and August 2000; at the Gordons site in October-November 1999 and July-August 2000; at the Brewery site in October-November 1999 and January and March-May, 2000; at the Newlands site in September-December 1999 and February-April and June 2000; at the Hill site in September- November 1999; at the Kirstenbosch site in September and November 1999 and February 2000; and at Westlake, Zeekoevlei, Rietvlei and the Kenilworth temporary pond.

Order Hypotrichida

Euplotes spp. (G. *eu* = well; *plotes* = floating)

A commonly occurring genus in our samples. Individuals are ovoid with a distinct ruffled “collar” of cilia ventrally. Viewed laterally, *Euplotes* spp. tend to resemble beetles with a rounded “carapace” and “legs” and are commonly found searching for food in detritus in either fresh or marine waters (Patterson, 1998).

Found at Westlake, Cape Point, Zeekoevlei, Rietvlei and Kenilworth temporary pond.

E. eurystomas (G. *eurys* = wide; *stoma* = mouth)

The larger of the two species of *Euplotes* found in our samples, *E. eurystomas* imay reach 230 µm in length. The macronucleus is a reverse “3” shape and shows up well with methyl green-pyronin stain (Foissner & Berger, 1996).

Found at the Valkenberg site in September 1999, February- July in 2000; at the Gordons site from November 1999 to May 2000 and August 2000; at the Brewery site in September, and November 1999 to June 2000; at the Newlands site in October-November 1999, February 2000 and August 2000; at The Hill site in November, 1999; at the Kirstenbosch site in January-February 2000.; and at the Top site in September 1999.

E. patella (L. *patella* = a small dish)

Individuals are smaller than those of *E .eurystomas,* up to 120 µm in length, with dorsal ridging that is not easily seen. The macronucleus is a reverse “C” shape which shows up well with methyl green-pyronin stain (Foissner & Berger, 1996). The species is considered to be a good mesosaprobic indicator by Berger *et al* (1997) but we found it on only a few

occasions, *E. eurystomas* being far more common in number and in range of sites and conditions.
Found at the Valkenberg site in March 2000; at the Gordons site in January, March, June and August 2000; and at the Brewery site in January and April 2000.

Holosticha spp. (G. *holo* = whole; *stichos* = row)
The ends of the cell/body are rounded, making it slightly cigar-shaped. Anterior and posterior cilia are visibly lengthened, with a curve of cilia leading to the cytostome on the ventral surface. Specimens may be up to 200 μm long (Patterson, 1998). Species identification is difficult.
Found at The Hill site in June 2000.

H. monilata (L. *monile* = a necklace)
Individuals are listed as ≤ 160 μm in length but with up to 23 macronuclear nodules (Foissner & Berger, 1996), which are not easily seen. Species identification is difficult and tentative here.
Found at the Brewery site in June 2000 and at the Newlands site in June 2000.

H. multistilata (L. *multi* = having many; *stila* = a writing instrument)
This species listed as having individuals ≤ 170 μm in length and with 100 macronuclear nodules (Foissner & Berger, 1996); yellowish-green granules can be observed and are illustrated in Patterson (1996).
At the Kirstenbosch site in June 2000.

Oxytricha spp. (G. *oxys* = sharp; *thrix* = a hair)
Ellipsoid and oviform in shape with a well-defined adoral zone (Kudo, 1971) and up to 260 μm in length (Foissner & Berger, 1996). The number of cirri and their positions define the species.
Found at the Newlands site in July 2000; at The Hill site in August 2000; at the Kirstenbosch site in August 2000; and at Westlake and Cape Point.

O. ferruginea (L. *ferruginus* = rusty)
As the name implies, individuals are rusty-brown colour; the marginal row of cirri is not superimposed (i.e. they lie parallel to one another at the posterior end) (Foissner & Berger, 1996).
Found at the Newlands site in June 2000.

O. haematoplasma (L. *haematoplasma* = blood-coloured)

This is a distinctively pinky-red coloured species. The marginal rows of cirri are superimposed (i.e. lie parallel to one another at the posterior end) (Foissner & Berger, 1996). At the Newlands site in August 2000.

O. hymenostoma (G. *hymen* = a membrane, skin; *stoma* = mouth)
Oval, but with flattened almost parallel sides. The caudal (tail) cirri are hardly longer than the cirri of the marginal row (Foissner & Berger, 1996).
At the Newlands site in February 2000

Paraurostyla viridis (G *para* = near; *ourose* = tail; *stylos* = a pillar; L. *viridis* = green)
May be listed in older literature as *Urostyla viridis*.
Up to 175 µm long, slim (width one-third or less of body length); two ellipsoid macronuclei and the contractile vacuole on the left side; the adoral region with a "collar" of cirri. The cytoplasm is full of symbiotic algae, hence the species name "*viridis*".
At the Brewery site in May 2000.

Uroleptus piscis (G. *ouros* = a tail; *leptos* = slender; L. *piscis* = a fish)
A very distinctively fish-shaped species, described as vermiform and flattened and up to 800 µm in length with a definite "tail"(Foissner & Berger, 1996)
Found at the Newlands site in March 2000.

Order Peritrichida

Campanella umbellaria (L. *campanella* = a little bell; L. *umbella* = a sunshade)
Stalked with a bell-shaped "body", more than three rows of adoral cilia, and an S-shaped oral vestibule half surrounded by the coiled macronucleus (Foissner *et al*, 1992). This species may form large colonies in polluted (usually still) waters (Berger *et al*, 1997).
Found at the Valkenberg site in November 1999.

Opercularia articulata (L. operculum = a lid*; articulatus* = jointed)
Colonial and stalked with a narrow bell-shaped "body". The stalk is smooth and contracts and extends regularly. The top of the bell appears to have a "lid" which is, in fact, feeding cilia that can be raised or lowered (Patterson, 1996).
Found at the Gordons site in March 2000 and at the Breweries site in March 2000.

Pseudovorticella moniliata (G: pseudo = false*; L. vortex = a vortex; L. monile* = a necklace*)*
Many tiny "blisters" can be seen on the outer part of the pellicle. Two contractile vacuoles are present (Foissner, 1996).
Occurences not provided

Vorticella spp.
Commonly colonial with a contractile stalk and a bell-shaped “body”. The only cilia are those around the top of the “bell”; these cause a visible current that brings food into the vestibule/buccal cavity. The macronucleus is a flattened coil (Patterson, 1996). These ciliates are sessile when mature, but after reproduction *via* division a mobile larva is produced. Threy can be found in a range of conditions but are generally more numerous in polluted areas. Bacteria are the main food source, but algae and detritus are also consumed (Foissner & Berger, 1996; Berger *et al*, 1997).
Found at the Valkenberg site in November 1999 and January 2000; at the Gordons site in May 2000; at the Breweries site in October-November 1999 and January, April and May 2000; and at the Newlands site in October-November 1999, January, May and June 2000.

V. convallaria (L. convallis = a sheltered valley shut in on all sides)
This species is part of a taxonomic complex exhibiting morphological differences within the existing "species". Individuals have a “J” shaped macronucleus, are up to 80 µm in length and have a bell-shaped “body” (Foissner & Berger, 1996).
Found at the Gordons site in November 1999.

Zoothamnium procerius (G*: zoo* = animal; *thamnos* = a bush; *pro* = before, in front of*; cereus* = waxy)
A colonial form of *Vorticella*-like cells, contracting or expanding as one. They may be found in marine or freshwater environments (Kudo, 1971; Foissner, 1996).
Found at the Gordons site in September, 1999.

APPENDIX II : Liesbeek Water Quality Data (1999 and 2000)

[Data source: Cape Metropolitan Council Scientific Services Department]

NR number = CMC station number m = missing data

Chemical Oxygen Demand (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	The Hill	**Newlands**	**Brewery**	**Valkenberg**
Sept-99	28	22	20	20
Oct-99	14	12	9	10
Nov-99	18	24	18	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	22	23	14	26
Mar-00	4	m	m	4
April-00	10	13	14	11
May-00	9	10	16	m
June-00	18	19	18	18
July-00	19	16	15	15
Aug-00	8	13	12	13

Dissolved Oxygen (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	TheHill	**Newlands**	**Brewery**	**Valkenberg**
Sept-99	7.8	8.8	8.9	8.1
Oct-99	8	7.3	7.6	7.6
Nov-99	7.6	5.5	7.7	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	7.7	7.1	7.6	4.5
Mar-00	10.2	8.1	8	6.1
April-00	13.4	11.6	11.6	8.5
May-00	5.2	6.5	3.4	m
June-00	6.3	5.7	5.7	4.6
July-00	8.9	8.2	7.8	6.1
Aug-00	10.1	9.2	10.1	9.2

Ammonia (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	The Hill	Newlands	Brewery	Valkenber g
Sept-99	0.03	0.03	0.04	0.07
Oct-99	0.1	0.154	0.058	0.123
Nov-99	0.11	0.142	0.1127	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	0.069	0.091	0.032	1.454
Mar-00	0.038	0.098	0.104	0.08
April-00	m	0.069	0.107	0.087
May-00	0.019	m	0.065	m
June-00	0.259	0.255	0.131	0.227
July-00	0.09	0.098	0.056	0.095
Aug-00	0.067	0.187	0.154	0.127

Nitrite+Nitrate (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	The Hill	Newlands	Brewery	Valkenberg
Sept-99	0.06	0.43	0.62	0.75
Oct-99	0.071	0.398	0.694	0.663
Nov-99	0.052	0.094	0.736	0.136
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	0.052	0.094	0.736	0.136
Mar-00	0.029	0.073	0.629	0.006
April-00	0.009	0.066	0.525	0.156
May-00	0.078	0.049	0.554	m
June-00	0.099	0.086	0.394	0.636
July-00	0.207	0.109	0.509	0.701
Aug-00	0.196	0.119	0.616	0.909

Orthophosphate (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	The Hill	**Brewery**	**Newlands**	**Valkenberg**
Sept-99	0.008	0.03	0.013	m
Oct-99	0.006	0.013	0.016	0.006
Nov-99	0.006	0.019	0.023	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	0.017	0.012	0.021	0.141
Mar-00	0.03	0.061	m	0.198
April-00	0.008	0.013	0.097	m
May-00	0.021	0.004	0.019	m
June-00	0.017	0.011	0.014	m
July-00	0.028	0.005	0.02	0.031
Aug-00	0.014	0.022	0.028	0.019

Total Phosphorus (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	The Hill	**Newlands**	**Brewery**	**Valkenberg**
Sept-99	0.275	0.036	0.023	0.039
Oct-99	0.01	0.029	0.025	0.029
Nov-99	0.03	0.033	0.052	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	0.031	0.013	0.026	0.153
Mar-00	0.122	0.349	0.036	0.055
April-00	0.041	0.509	0.142	0.063
May-00	0.099	0.099	0.103	m
June-00	0.018	0.029	0.024	0.064
July-00	0.028	0.013	0.046	0.047
Aug-00	0.016	0.022	0.045	0.019

Total Nitrogen (mg/ℓ)

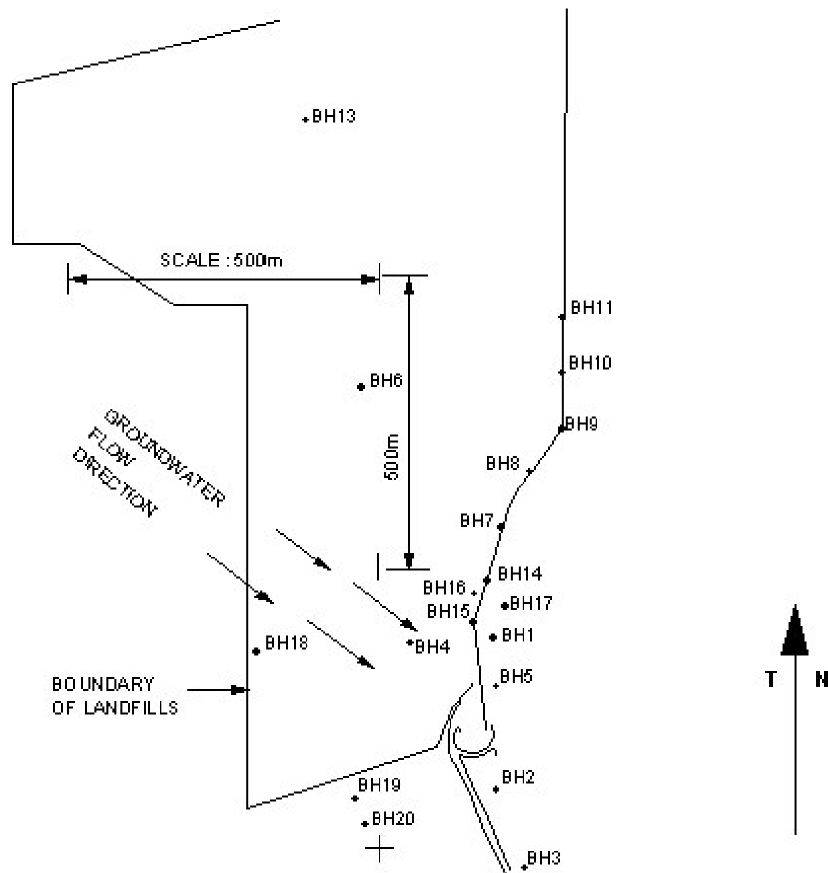
Date	NR23	NR22	NR12	NR08
	The Hill	Newlands	Brewery	Valkenberg
Sept-99	0.46	0.88	1.12	1.12
Oct-99	0.521	0.867	1.14	1.19
Nov-99	0.597	0.689	1.041	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	0.12	0.137	m	3.193
Mar-00	0.398	0.462	1.064	0.724
April-00	0.268	0.472	0.869	0.126
May-00	0.421	0.356	0.863	m
June-00	1.579	1.629	1.682	2.237
July-00	0.543	0.702	0.724	3.491
Aug-00	1.019	0.764	0.969	1.888

Total Suspended Solids (mg/ℓ)

Date	NR23	NR22	NR12	NR08
	The Hill	Newlands	Brewery	Valkenberg
Sept-99	6	3	2	6
Oct-99	1	1	m	4
Nov-99	10	2	2	m
Dec-99	m	m	m	m
Jan-00	m	m	m	m
Feb-00	m	3	m	59
Mar-00	7	2	2	8
April-00	m	39	8	15
May-00	10	m	m	m
June-00	1	1	2	7
July-00	m	m	9	3
Aug-00	7	5	8	14

APPENDIX III

Map of the Coastal Park waste disposal site with groundwater monitoring network of boreholes
(supplied by the Scientific Services Division of the Cape Metropolitan Council



APPENDIX IV : WATER QUALITY DATE FOR THE COASTAL PARK BOREHOLE SITES

Directorate: Water & Waste **Cape Metropolitan Council** **Scientific Services Department**
Lab. Ref. Nos.: 1202 – 1216 **File CB.3/S6.7**
On: 15 samples of borehole water taken at the Coastal Park Landfill Site, sampled on 12/6/2000, submitted by Head: Scientific Services Department

Sample Number		cp01	cp02	cp03	cp05	cp08	cp09	cp10	cp11	cp14	cp15	cp16	cp17	cp18	cp19	cp20
Solids																
Total Dissolved Solids @ 105ºC mg/l		1860	1544	1816	500	2124	2004	2388	460	2338	4716	2518	3586	942	2152	2116
Oxygen Demand																
COD	O mg/l	148	62	62	72	297	57	129	33	268	120	110	96	38	139	105
Nitrogen																
Ammonia	N mg/l	49.7	3.1	7.2	6.7	174.5	1.9	42.5	0.3	199.5	23.6	84.9	14.2	0.9	22.5	0.6
Nitrate & Nitrite	N mg/l	<0.1	<0.1	0.1	0.3	5.1	2.9	52.8	7.2	1.9	3.2	1.7	<0.1	0.2	<0.1	0.3
Physical																
pH		7.0	7.4	7.4	7.2	7.1	7.6	7.3	7.8	7.3	7.4	8.3	7.8	7.0	7.0	7.3
Conductivity @ 25ºC	mS/m	385	277	304	100	473	485	354	78	506	686	486	545	350	360	318
Mineral																
Chloride	Cl mg/l	517	637	725	108	596	1402	496	93	666	1729	1382	1671	273	557	717
Fluoride	F mg/l	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.1	0.3	0.3	0.2	0.3
Sulphate	SO_4 mg/l	72	6	71	39	46	100	159	44	59	70	10	173	70	164	191
Alkalinity	$CaCO_3$ mg/l	968	419	382	346	1299	373	599	166	1374	492	287	357	307	780	422
Sodium	Na mg/l	274	394	442	54	320	788	260	44	350	690	713	837	163	349	405
Potassium	K mg/l	138	14	18	8	206	42	191	6	216	99	33	36	5	40	5
Calcium	Ca mg/l	177	109	110	113	195	109	243	87	157	211	28	127	115	238	170
Magnesium	Mg mg/l	54	40	51	16	70	103	47	12	72	95	51	119	26	91	69
Chromium	Cr μg/l	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
Cadmium	Cd μg/l	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Lead	Pb μg/l	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
Mercury	Hg μg/l	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Boron	B mg/l	0.8	0.4	0.5	0.3	0.9	0.6	0.7	0.1	1.0	0.4	0.5	0.5	0.2	0.8	0.4
Carbon																
Cyanides	CN mg/l	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Phenolic Compounds	C_6H_5OH mg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
DOC	C mg/l	41	9	9	6	49	7	33	2	65	23	19	9	8	33	20