The South African Diatom Collection: An Appraisal and Overview of Needs and Opportunities

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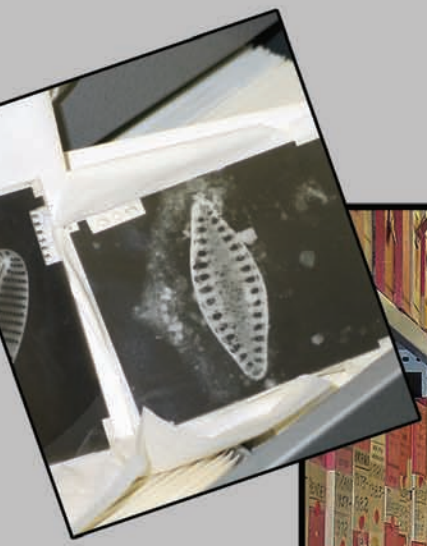

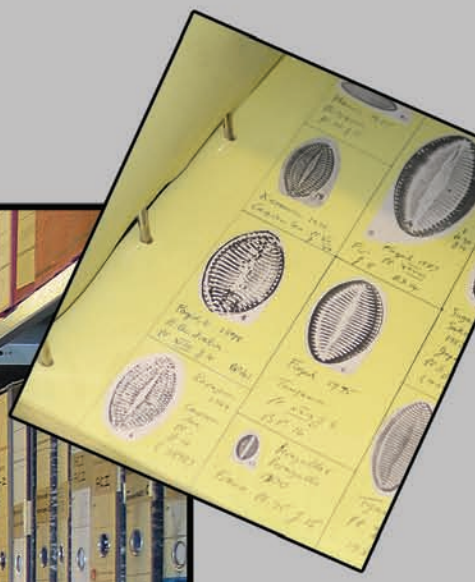

TT 242/04

Water Research Commission

THE SOUTH AFRICAN DIATOM COLLECTION: AN APPRAISAL AND OVERVIEW OF NEEDS AND OPPORTUNITIES

William R Harding[1], Colin GM Archibald[2], Jonathan C Taylor[3] and Saras Mundree[4]

Report to the
Water Research Commission

by

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WRC Report Number: TT 242/04

December 2004

Obtainable from:

Water Research Commission
Private Bag X03
Gezina
0031

The publication of this report emanates from a project entitled: *Reference conditions for surface waters: Diatom indicators (WRC Project No. K8/508)*

ISBN 1-77005-274-7
Printed in the Republic of South Africa

THE SOUTH AFRICAN DIATOM COLLECTION: AN APPRAISAL AND OVERVIEW OF NEEDS AND OPPORTUNITIES

FINAL REPORT

Water Research Commission Research Consultancy K8/508/2

"*To assess, collate and store data relating to unprocessed, un-collated information on diatoms in Southern African surface waters with a view to determining ecosystem and water quality reference conditions and for the augmentation of extant aquatic ecosystem assessment methodologies*"

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EXECUTIVE SUMMARY

BACKGROUND

This research consultancy was established with the following core aims and objectives:

1. To begin the process of transferring the pre-computer age information contained within the South African Diatom Collection into an electronic format;
2. To undertake a preliminary assessment of the value of the information contained in the Collection for the purposes of determining the historical "reference" water quality conditions of South African rivers and surface waters;
3. Assessing the value of the use of diatoms as a index-based water quality assessment method.

The South African Diatom Collection is a vast resource of documents, slides, unprocessed sample materials and various records and observations collected between 1950 and 1995. The information contained in the Collection was hitherto in a pre-computer age format and not readily accessible for general investigative use. This situation prevailed in South Africa at a time when, on the global stage, the use of diatom assemblages for river, lake and wetland assessments, paleolimnological and climate change studies is gaining ascendancy. It was accordingly deemed important that the information contained with in the Collection be subjected to a preliminary appraisal of its value for similar use in South Africa.

The findings of this short duration consultancy have clearly established the value of the South African Diatom Collection as a resource of historical water quality information and the applicability of diatom-based water quality assessment methods for South African use. The latter approach constitutes a high-confidence augmentation to the use of invertebrate-based assessments.

This consultancy not only achieved all of its goals but also completed a number of additional tasks pertinent to the Terms of Reference. In summary the project deliverables were:

1. An audit of the content of the three main components of the Collection, viz. the slide collections, the sample collection and the publications, reprints, notes and maps;
2. Transfer to electronic format of the content of the slide collections;
3. Extraction of all samples (within the slide collections) relevant in particular to South African rivers and South African surface waters in general;
4. Carrying out a number of case study analyses wherein the use and value of diatoms was demonstrated – one of these being a direct comparison with the application of SASS5;
5. Proving the relevance of the OMNIDIA software package for water quality assessment under South African conditions;
6. The production of four manuscripts for peer-review publication, including a position paper on the value of the diatom-based assessment approach;
7. The production of a poster presentation at an international diatom meeting;
8. The establishment of contacts with a large number of international diatom specialists, and the formulation of in-principle agreements for further cooperation;
9. The transfer to an electronic bibliography of the details of 7000 reprints pertaining to the use of diatoms in South and southern Africa.

CONCLUSIONS

The following Conclusions stem directly from the findings of this consultancy:

1. Diatoms embody considerable ecological importance that stems from their vital position at the base of the food chain. Notwithstanding their cosmopolitan occurrence specific species and assemblages occupy habitats described by specific physico-chemical and biological attributes. Communities change rapidly in response to changes in environmental exposure. These distinct environmental characteristics have wide-ranging practical applicability and

has particular relevance and value for assessing anthropogenic impacts on aquatic ecosystems;

2. The use of diatom-based indicators has clear and immediate relevance for providing information relevant to societal concerns about ecological condition; practical for short and long-term monitoring, as well as for historical assessments and use in extreme environments (acid drainage, elevated salinities); disaggregating noise from fundamental environmental drivers; and providing results that are understood by specialists, managers and stakeholders;
3. The use of diatoms as a diagnostic tool, and the value of the historical information contained in the SA Diatom Collection, can no longer be ignored. International precedents and local experience clearly indicate the versatility and durability of this protocol as a scientifically robust tool. It should be used in conjunction with the SASS invertebrate method as two independent indicator systems comprising a more comprehensive ecosystem health screening protocol in South Africa. If the ability to inform strategic water resource assessments is to be appropriately developed at a higher level of confidence, then any delays in attention to the diatom-based assessment methods would be both administratively and functionally negligent. Not least, a failure to do so would flout the considerable amount of effort that has been historically invested in the development of the resource base that is the SA Diatom Collection;
4. As stated above the SA Diatom Collection is a vast resource that contains materials not only from South Africa, but from many southern African and overseas collections. In addition to information on diatoms, the SA Diatom Collection contains a considerable amount of information on South African surface waters that is contained in a variety of *ad hoc* notes and observations made by the collectors. Not least are the scale diagrams of various phytoplankton species made during first encounters. In addition to continuing the process of cataloguing the data in the Collection, a process of identifying information for future "data mining" should be undertaken;
5. The Collection contains, or can yield, historical information from a wide array of South African rivers – several of which are currently being assessed in terms of their potential to sustain further development (the 'Ecological Reserve'). Others remain the focus of pollution studies for which the use of diatoms provides a fine level of resolution for determining impacts and recovery;
6. The diatom analysis sheets contained in the SA Diatom Collection constitute a valuable resource from which accurate inferences may be drawn concerning the past ecological status of the rivers and streams for which data exists in the SA Diatom Collection, and are likely to prove equally valuable resource for obtaining historical (baseline) data against

which present day and/or future environmental assessments may be compared, and provide a measure of either degradation of restoration since the time of original sampling. OMNIDIA proves to be both useful as a database and as a tool for calculating diatom index scores;

7. The application of diatom-based water quality assessment protocols has direct and immediate value for use in South Africa – as an 'added-value' assessment approach in addition to the use of SASS-based approaches;
8. The fact that the diatom sampling also has less restrictions in terms of habitat requirements than macroinvertebrates could facilitate its use in monitoring water quality in small tributaries, for instance mining and industrial effluent. Case studies that illustrate this applicability are contained in this report;
9. Most of the diatom indices are designed to give an indication of general water quality. The indices differ in respect to the diatom species included in the calculation and in the number of taxa included in the calculation. Several refinements have eventually culminated in the Biological Diatom Index (BDI), which incorporates 14 parameters of water quality. 70% of the variation in the scores of the BDI index can be explained using these 14 water quality variables. The remaining 30% of the variation is ascribed to physical factors such as light penetration, current speed and general habitat integrity. This illustrates the enormous potential of the diatom-based assessment approach;
10. The SA Diatom Collection requires the attentions of an appropriately-trained and experienced curator. The nature of the materials (glass slides, liquid samples, scanning electron microscope images on glass plates and hardcopy reports and reprints) is delicate and prone to permanent loss. The rapid and complete transfer of the complete resource to electronic format should be undertaken as a matter of urgency.

RECOMMENDATIONS

It is recommended that a further consultancy be commissioned to:

1. Develop a strategic research approach to the future use of diatoms as a water quality and aquatic ecosystem assessment tool in South Africa. *Inter alia* this approach should:
   - 1.1 establish collaborative working links with other researchers in this field, and further develop those that have already been established;
   - 1.2 identify all those already-available resources that would augment the use of diatom-based approaches in South Africa and offset any repetition (= "reinvention of wheels").

The application of diatom approaches is cosmopolitan and a vast number of resources already exist;

1.3 Refine the diatom sampling protocol for use in rivers and streams in parallel with SASS;

1.4 development of a Rapid Bioassessment Protocol, and associated testing protocol, along the lines of that used in the development of SASS, but fast-tracked based on international and local precedents and experience;

1.5 identify options for the local training of diatomologists and the establishment of Centre of Excellence for the analysis and interpretation of diatom samples;

1.6 identify diatom-based interest and initiatives already extant in South Africa, e.g. that of the North West University.

2. Construct a set of reference taxonomic images (already available from the Collection) into a hierarchical taxonomic key using the Lucid™ database software, and using the already-established Cyanobacterial and Chlorophyte keys as a type-example. This has international relevance and use, and an offer of collaborative support and interest has already been gained from Dr Martyn Kelly (UK). Furthermore the existence of such a key would form a widely distributable first-step to re-introducing diatoms to the South African aquatic sciences community;

3. Initiate the identification and calibration of locally-relevant diatom - water chemistry transfer functions (= ecological optima and water quality tolerances), i.e. the association of diatom samples and environmental data and the creation of related mathematical response (= transfer) functions. Current international development is focussed on diatom-pH, diatom-phosphorus and diatom-salinity relationships. This project has established contact with the European Diatom Database (ECRC) Team and is pursuing options for joint initiatives in this regard. A similar link is being pursued with the Australian diatom initiative driven from Monash University.

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This report includes 2 diskettes: Diskette 1 contains the spreadsheet details of the SA Diatom Collection as detailed in Section 2; Diskette 2 contains the text-format of the bibliography as at completion of this project.

SECTION 1

THE RELEVANCE OF DIATOMS FOR WATER QUALITY ASSESSMENT IN SOUTH AFRICA

A Position Paper

William R Harding, Colin GM Archibald & Jonathan C Taylor

Abstract

Water quality assessment protocols based on the use of diatoms are now well developed and their value substantiated at an international level. The use of diatoms is not designed or intended to be a "rapid" technology; this notwithstanding that the detailed level of information generated from the procedure outweighs perceived disadvantages of the additional time required for sample preparation and analysis to species level. The method is applicable across a wide range of aquatic ecosystem types: freshwater; brackish; acid; estuarine; and inclusive of both lentic and lotic environments, wetlands and their associated damp, marginal and littoral zones. Details provided by diatom assemblages support paleoecological investigations, historical reconstruction of water quality and the determination of prevailing water quality conditions. Deliberate determination of responses to management strategies or impacts arising from a variety of anthropogenic activities can be achieved via the simple expedient of retrieving living material from introduced artificial substrates. Previous studies in South Africa and elsewhere have shown that on a site-by-site basis the use of diatoms informs a fine level of diagnostic resolution of the causes underlying changes in water quality and environmental condition.

The South African Diatom Collection ('the Collection'), a repository of diatom specimens and records that spans the length and breadth of this country, contains an as-yet unutilized wealth of ecological and accurate taxonomic information. More importantly, it provides an insight into water quality conditions prevailing 40-50 years ago – in many cases prior to the 'development' of many of our rivers, streams and wetlands. The real value of its existence underpins the great potential for renewed attention to the value of diatom-based approaches to water quality assessments. In addition, the Collection provides a ready-made foundation on which a locally-relevant tool for water quality assessment may be established to augment the current use of invertebrate indicators.

It is now appropriate that the full potential of the use of diatoms in water quality assessments, and the information contained in the Collection, be developed and utilized for water quality assessment in South Africa.

Introduction: Assessing water quality using biotic indices

Few people involved with ecohydrology and water resource management doubt the value of water quality assessments derived from the use of biotic indices, i.e. assessments based on observations of the resident floral and faunal communities (Cholnoky, 1953, Chutter, 1972, Patrick, 1973, Schoeman, 1976, Descy, 1979, Kelly et al., 1995, Kelly, 1998a, Bate et al., 2002). Assuming requisite levels of ecological experience and taxonomic proficiency on the part of the assessor, such evaluations provide a description of the water quality that is often not achievable from elemental analyses alone. The value of an integrative biological response provided by the analysis of diatom associations offsets the inconsistency of rapid changes in water chemistries that render the use of conventional analytical approaches inadequate. A further potential advantage is that the diatom-based approach could obviate the need for additional and expensive toxicity testing protocols – particularly because of the attendant uncertainty of extrapolation to the real environment of the responses of selected single species testing gauged under laboratory conditions. Ecological risk assessments are more appropriately based on biological endpoints in the field than on measures of chemical constituents (Karr and Chu, 1997). Monitoring procedures based on the biota measure the health of a river and the viability of aquatic systems to support life, as opposed to simply characterizing the chemical and physical components of a particular system. This is the central purpose of assessing the biological condition of aquatic communities of a river (Barbour, 1997).

Karr and Chu (1997) have stressed that chemical criteria based on laboratory-derived dose-response curves for single toxicants cannot account for cumulative, synergistic or antagonistic interactions of the suite of chemicals found in a polluted river system. Comprehensive and accurate multimetric indices explicitly embrace several attributes of the sampled assemblage including taxon richness, indicator taxa and the health of individuals. In many cases, biotic indices provide an indication of the existence or absence of life in stream water where routine chemical measurements of 'indicator elements', even at the limits of analytical detection, are not definitive.

Cairns (1981) highlighted the need for standard methods for the bio-monitoring profession because he recognized that the condition of individual species and of communities of indigenous biota was one of the best measures of the heath of an ecosystem. In an effort to provide a reliable assessment tool within as short as possible a time frame, the search for rapid assessment technologies in South Africa centered largely on the use of aquatic insects, and protocols such as the South African Scoring System, now in its fifth iteration, were derived (Dickens and Graham, 2002). Subsequently the value and validity of rapid assessment biological methods have been challenged by Taylor (1997) and he contends that further refinements of these rapid techniques will be less useful than improving established methods. With

proficient use, SASS provides a great deal of insight regarding what may be happening in a particular riverine environment. Nevertheless there is also an inappropriate tendency, perhaps borne of the apparent absence of any alternative, to extend the use of SASS into other environments, such as wetlands. The diatom approach provides a viable and already-developed alternative protocol for the health assessments of wetlands.

The need to augment invertebrate-based protocols

The presently-preferred use and value of the aquatic invertebrate method is variably limited by: hydrology; substrate(s); habitat; food availability; seasonality; and distribution patchiness. The latter aspect places an obvious demand on the number of samples that need to be taken in order to produce a quantitative result within regional considerations and a host of anthropogenic factors – not least the major modifications in assemblages occurring downstream of dams and weirs (Dallas,1997). While invertebrate indices do not provide a reliable indication of eutrophication, the identification of diffuse- and point-source impacts have been ably identified previously by direct measurements of the diatom-associations found.in South African river systems (Cholnoky 1960 & 1968, Archibald, 1972, Schoeman, 1976, Kelly, 1998). More recently Charles (1996) stressed that the use of algae for monitoring rivers has increased because of limitations of benthic invertebrates and fish as indicators. Coupled with significant improvements in technologies for algal assessment that increases the information/cost ratio there is a realization and acceptance by water authorities of the value of biological monitoring, particularly where multiple groups of organisms (ie from different trophic levels) are included in the evaluation.

Justification for and recognition of the value of such a scientific management approach is demonstrable in the existence of the South African Diatom collection. The meticulous manner in which our former dedicated colleagues - Cholnoky, Giffen, Archibald and Schoeman - recorded sample localities, their results, and preserved the collected material, has provided an invaluable environmental legacy for South African aquatic systems. It has become a matter of concern therefore that since the late 1980s the real value of this material has gone unrecognized amongst water management authorities and aquatic scientists, if only because the information and its existence has not been sufficiently advertised.

While assessments such as SASS should undeniably remain in the toolbox of water quality assessment professionals, there exists a pressing demand for additional protocols that will confirm and/or augment the value of invertebrate-based evaluations. Ideal attributes for such additional tools would be: wide-ranging applicability across aquatic ecosystem types and their adjacent (damp) environments; permanence – the indicators should be retrievable under all hydrological conditions, including stagnant

and dry; and – most important – should lend themselves to forensic interrogation – i.e. paleoecological assessment. Furthermore the results obtained therefrom should be incontrovertibly linked to water quality.

The use of diatoms as an 'added-value' assessment tool

One group of organisms fulfils these core requirements, and more – the algal Class Bacillariophyceae (the 'diatoms', belonging to the Division Heterokontophyta). Diatoms comprise the major component of the microphytobenthos (bottom-dwellers), performing essential photosynthetically-driven, microbial functions at the base of the food chain (Cox, 1996). Accordingly they respond directly to growth stimulants (nutrients) and/or stressors such as toxicants, as well as to physical factors. They occur in all types of aquatic ecosystems, and extend into the more saline estuarine environments as part of the river continuum. Importantly, the ability to use diatoms to evaluate present and past conditions of water quality and environmental change in just about any aquatic environment has been recognized world-wide for many decades (Patrick 1973, van Dam (1974), Chessman 1986, Whitmore, 1989), but has been limited in South Africa – until now – by perceptions of seemingly onerous sample preparation and time-consuming dedication required to develop key taxonomic skills. State and regional river-monitoring programs for algae in the United States tend to rely solely on analysis of diatom assemblages (Charles, 1996).

The authors' personal experience from using the most recent and modern computer technology, supported by image analysis software, and informed by recently published literature, is that this has resulted in a marked reduction in time required and greater confidence in the results. Difficulties with the taxonomy and nomenclature of the diatoms can now be resolved through comparison of images with electronic keys and rapid e-mail communication with other experts – as is the common norm in many disciplines of scientific investigation. Kociolek and Stoermer (2001) hold a strong view that studies on accurate taxonomy and ecology of diatoms in the 21st Century will and must be linked. They also emphasize that this approach will be driven by integrated research programs (eg river health studies) and can now be facilitated by technological advances (eg computer toolkits and image analysis) that support both accurate taxonomy and improved ecological interpretation. They envision a research paradigm that closely integrates diatom taxonomy and ecology to develop conservation biology in which microbial communities (eg diatoms) can be used to define 'natural' habitats requiring of conservation.

Diatoms have for some time, and latterly increasingly so, been used for the assessment of short- and long-term environmental change (Dixit et al., 1992). Assessment approaches based on diatom indices

were developed in the lacustrine environment, and have recently been extended to encompass the riverine systems (Round, 1991a, 1991b, Stevenson and Pan, 1999 and Eloranta and Soininen, 2002). Diatom-based information can be gleaned not only from natural surfaces (sediments, stones-in-current and marginal vegetation) but also from just about any other substrate or surface type in an aquatic environment. The living component can also be deliberately gathered in a controlled fashion using the simple expedient of artificial substrates – a significant advantage in the formulation of stressor-:response models (Gold et al., 2002). High frequency, multi-parameter water quality monitoring programs are simply not cost effective in the present South African situation – and an alternative for assessing change over time is urgently required. The examination of living diatoms in sediments and on stones, together with the invertebrates, provides a method that combines two independent indicator systems at different trophic levels (Smol, 1992; Hofmann, 1996).

Diatoms provide the following essential suite of diagnostic attributes (e.g. de la Rey et al., - in press)

- they collectively show a broad range of tolerance along a gradient of aquatic productivity, and with individual species having specific water chemistry requirements;
- they have one of the shortest generation times of all biological indicators (~2 weeks). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration;
- they are sensitive to change in nutrient concentrations, supply rates and silica/phosphate ratios. Each taxon has a specific optimum and tolerance for nutrients such as phosphate and nitrogen, usually quantifiable to high degree of certainty. Moreover, whereas the use of historical water chemistry data are constrained by the level of analytical sophistication prevailing at the time, the associations of diatoms with water quality remain unchanged;
- their assemblages are typically species-rich – augmenting the information gained from a diversity of ecological tolerances. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, increasing the confidence of environmental inferences;
- they respond rapidly to eutrophication. Because diatoms are primarily photoautotrophic organisms, their growth response is directly affected by changes in prevailing nutrient concentrations and light availability;
- their rapid immigration rates and the lack of physical dispersal barriers ensure there is little lag-time between perturbation and response;
- diatom frustules, the silicaceous walls of the individual cells, demonstrate a lasting permanence in sediments, such that sediment cores provide details of changes in the quality of the overlying water for as far back as one is able to search. This attribute alone has significant and far-

reaching relevance for the determination of reference conditions, not only climatic but also the condition of the system prior to intrusion from cultural development;

- the taxonomy of diatoms is comprehensively documented. Species identifications are largely based on frustule morphology – an attribute readily identifiable with modern light microscopy and image analysis techniques, and not dependent on electron microscopic techniques as is commonly misconceived;
- they can be found on substrata in streambeds even when dry, so they can be sampled at most times of the year and still accurately reflect recent or prevailing conditions;

Additionally the use of diatoms is supported by the attributes identified by Schoeman and Hayworth (1984):-

- their ease of collection, preparation for observation, and storage (small sample volumes, no dessication risk) for reference purposes;
- the considerable amount of tried and tested ecologically-associative information already available, both nationally and world-wide;
- their suitability for diversity analysis;
- the availability of the OMNIDIA interpretive software package.

Concerns that the wealth of diatom-based information developed in the north-temperate zone might not be directly applicable to the southern latitudes have been comprehensively quelled by more recent findings that diatoms are 'subcosmopolitan', i.e. they occur anywhere where certain environmental conditions are fulfilled (Kelly, 1998b). This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities; but rather the specific environmental variables prevailing at a particular site (Gold et al., 2002). Comparison of South African examples of paired sets of diatom and water quality data confirms that several of the diatom indices are directly applicable locally in certain rivers (de la Rey et al., in press). In fact Cholnoky's South African work at the CSIR 30 years ago supported the identification of water quality and environmental change parameters at relatively fine spatial scales. More recently, an analysis of the Upper Hennops River revealed that use of the Lange-Bertalot method – or saprobian system in which diatom taxa are placed in 9 different classes according to their specific pollution tolerances was adaptable to South African conditions (Schoeman, 1979)

Importantly, the use of diatom-based approaches is now supported by a better understanding of the relationship between diatoms and environmental variables (Prygiel & Coste, 1993, Gomez, 1999,

Juttner et al., 2003). Predictive models exist that demonstrate their direct applicability for use in some South African rivers (de la Rey et al., in press, Taylor, unpublished data). Numerous case studies that provide clear guidance on the validity and strength of the approach abound worldwide (Prygiel et al., 1999, Stevenson and Pan, 1999, John, 2000, Wu and Kow, 2002). Moreover our work indicates that a significant amount of historical water quality information (= 'reference condition') is contained in the SA Diatom Collection (see below).

The aforementioned attributes have led to diatoms becoming firmly established, although not yet in South Africa, as important indicators of the present and past nature and condition of the aquatic environments in which they may be found. Such communities typically range from opportunistic tolerant species in areas of severe pollution; giving way to less tolerant and more competitively dominant species at the most distant location from the pollution source. Given that the types of diatoms dominant in nutrient-poor waters (oligotrophic) are distinct from those in enriched (eutrophic) environments and/or in potentially toxic conditions, the pattern of cultural eutrophication can be readily discerned (Cholnoky, 1960, Canter-Lund and Lund 1995). To quote Round , an eminent UK diatomologist, "*the value of using diatoms lies in the fact that the flora reflects rather precisely the water quality at any one point and, by monitoring changes in the flora, subtle changes in water quality conditions will be detected*."

The South African Diatom Collection

While many water resource managers and aquatic scientists may not realize it, South Africa possesses one of the most comprehensive collections of diatoms in the world. This substantial collection is currently housed at the offices of the Durban CSIR, and was considered to be the largest in the Southern Hemisphere. Information on this collection may be found at www.dhec.co.za/diatoms. During the early- to mid 20th centuries, two types of botanical collectors criss-crossed this country – *viz*. botanists and diatomologists. Were it not for the untiring efforts of these early 'explorers' much of what our natural history of aquatic systems was like would still be unknown. Notable amongst these early efforts is the work of diatom specialists such as Cholnoky, Giffen, Archibald and Schoeman. Between them they conscientiously collected samples from a wide range of South and southern-African aquatic environments – many of which have been subjected to infrastructural development such as dams, weirs, water-transfer and abstraction schemes. Diatom material was also obtained from many parts of the world. The availability of computerised diatom assessment procedures now enables re-working of these data and comparison with current conditions. This information will be invaluable in judging both the impact of man-made alterations to aquatic systems and the efficacy of remedial management strategies.

Some selected, and by no means exhaustive, examples of the coverages are:

Gauteng

- The Diatom Flora in the Vicinity of the Pretoria Salt Pan (Schoeman and Ashton, 1982)
- The Pretoria Salt Pan, A Unique Southern African Saline Lake Ecosystem (Ashton and Schoeman, 1985)
- Diatom Indicator Groups in the Assessment of Water Quality in the Jukskei-Crocodile River System (Schoeman, 1976)
- Diatoms of the Jukskei-Crocodile River System: A Preliminary Checklist (Schoeman, 1982)
- Ecology of Diatoms from Goedeverwachting and Lake Chrissie, East Transvaal (Cholnoky, 1965)
- Diatoms from the Vaal Dam Catchment Area, Transvaal (Archibald, 1971).

Eastern Cape

- Diatoms of the Swartkops Estuary (Eastern Cape) (Cholnoky, 1960)
- Diatoms of the Estuaries of the Eastern Cape Province (Giffen, 1963)
- The Diatoms of the Sundays and Great Fish Rivers in the Eastern Cape Province of South Africa (Archibald, 1983).

Western Cape

- New and Rare Diatoms of the Cape Province (Cholnoky, 1959)
- Algal flora of the Wemmershoek Dam near Cape Town (Cholnoky and Claus, 1961)
- An Account of the Littoral Diatoms from Langebaan, Saldanha Bay (Giffen, 1975)
- A Further Account of the Marine Littoral Diatoms of the Saldanha Bay Lagoon (Giffen, 1976)
- Marine Littoral Diatoms from the Gordon's Bay Region of False Bay (Giffen, 1971)

KwaZulu-Natal

- Contributions to our knowledge of the diatom flora of Natal (Tugela, Mooi, Mkomaas, Umgeni, Pongola, and Umfolozi rivers (Cholnoky, 1960)
- New and rare diatoms from Africa – Diatoms from the Tugela system (I, II and III) (Cholnoky, 1956, 1957)
- Diatom associations of St Lucia Lake (Cholnoky, 1968)

Other southern African areas

- Diatoms of the Okavango (Cholnoky, 1966a)
- Diatoms of Bechuanaland (Cholnoky, 1966b)
- Diatoms from the Harmony Gold mine, Welkom (Cholnoky, 1966c)
- Diatoms from Sewage Works in the Republic of South Africa and South West Africa (Schoeman, 1972a)
- A Further Contribution to the Diatom Flora of Sewage Enriched Waters in Southern Africa (Schoeman, 1972b)
- New and Rare Diatoms from South Africa (Archibald, 1966)
- Diatoms of Swaziland (Cholnoky, 1962)
- Diatoms from Southern Rhodesia (Cholnoky, 1954)
- Diatom flora of Lesotho (Schoeman, 1984).

Evaluation of the SA Diatom Collection

The findings of the aforementioned studies were documented and curated in slide collections, but have remained in almost permanent disuse since the early 1990s. More recently, new research efforts have contributed to the revival of interest in diatom associations as indicators of water quality in South African rivers (Watt, 1998 and Bate et al., 2002). The main aim of the WRC-funded work of the latter has been to survey benthic diatom flora and to relate the dominant taxa to the chemical water quality of selected river systems of the Eastern Cape, Western Cape and Mpumalanga. This study concluded that '*benthic diatoms have the potential to be used as biological indicators as they are ubiquitous members of riverine systems, react rapidly and predictably to water quality and their taxonomy has been well described*". Unfortunately, the historical value of the resources contained in the South African Diatom collection were not fully realised, perhaps leading to the comment that *" so far the use of benthic diatoms as indicators of river water quality in South Africa has been limited"*. They did conclude, however that *"diatoms appear to be very suitable biomonitoring organisms. They give an accurate indication of water chemistry within water quality classes"*.

Diatom assemblages: Indices and interpretive tools (e.g. Omnidia)

Within the last decade diatom-based indices have gained considerable popularity throughout the world as a tool to provide an integrated reflection of water quality, and in support of management decisions for rivers and streams (Kelly, 1998b; 2002 and Prygiel and Coste, 1993). Work on the applied use of

diatoms as bio-indicators has proceeded such that diatom indices have replaced those of invertebrates as the biomonitoring method of choice in certain situations e.g. canalised waterways (Prygiel and Coste 1993)

The bulk of the developmental diatom work has been carried out in French drainage basins – with testing on the scale of a territory as large, and as typologically diversified, as France; enabling the general application of these indices on the European continent (Prygiel and Coste 1993). In other examples diatoms have been integrated into a suite of testing methods required to support nutrient reduction directives (Kelly 2002). The design of software programmes such as OMNIDIA for the calculation of diatom indices has greatly enhanced the use of diatom-based methods (Le Cointe et al., 1993). A variety of diatom indices have been adopted and tested by many European countries including Finland (Eloranta, 1999) and Poland (Kwandrans et al., 1998).

The majority of the diatom indices are based on the weighted average equation of Zelinka & Marvan (1961) and have the basic form:

$$index = \frac{\sum_{j}^{n}=1 \quad a_j s_j v_j}{\sum_{j}^{n}=1 \quad a_j v_j}$$

where $\mathbf{a_j}$ = abundance (proportion) of species **j** in sample, $\mathbf{v_j}$ = indicator value and $\mathbf{s_j}$ = pollution sensitivity of species **j**. The performance of the indices depends on the values given to the constants **s** and **v** for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of **s**. Diatom indices differ in the number of species used (Table 1.1) and in the values of **s** and **v** which have been attributed after compiling the data from literature and from ordinations (Prygiel & Coste, 1993).

Diatom indices function in the following manner: In a sample from a body of water with a particular level or concentration of determinant (e.g. orthophosphate-phosphorus), diatom taxa with their optimum close to that level will be most abundant. Therefore an estimate of the level of that determinant in the sample can be made from the average of the optima of the pollution sensitivity ('s') of all the taxa in that sample, each weighted by its abundance ('a'). This means that a taxon that is found frequently in a sample has more influence on the result than one that is rare. A further refinement is the provision of an 'indicator value' ('v') which is included to give greater weight to those taxa which are good indicators of particular environmental conditions. In practice, use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean

of the pollution sensitivity of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (de la Rey et al., in press).

Concluding remarks

The use of diatoms as a diagnostic tool, and the value of the historical information contained in the SA Diatom Collection, can no longer be ignored. International precedents and local experience clearly indicate the versatility and durability of this protocol as a scientifically robust tool. It should be used in conjunction with the SASS invertebrate method as two independent indicator systems comprising a more comprehensive ecosystem health screening protocol in South Africa. If the ability to inform strategic water resource assessments is to be appropriately developed at a higher level of confidence, then any delays in attention to the diatom-based assessment methods would be both administratively and functionally negligent. Not least, a failure to do so would flout the considerable amount of effort that has been historically invested in the development of the resource base that is the SA Diatom Collection.

Acknowledgements

The authors would like to thank Brian Allanson, Brian Whitton (UK), Peter Ashton, Martyn Kelly (UK), Steve Mitchell and Jenny Day for their comments on the draft manuscript.

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SECTION 2

THE SOUTH AFRICAN DIATOM COLLECTION
A FIRST APPRAISAL

William R Harding, Colin GM Archibald & Jonathan C Taylor

1. INTRODUCTION

This project commenced in July 2003. It was conceived out of a realization of the great potential value contained within the South African Diatom Collection (SADC). The SADC was on the brink of being discarded, yet it is a resource which embodies a national record of the biological condition of many South African river systems during the period 1950 – 1995.

Present legislation governing water resource management in South Africa (Water Act 36 of 1998) now specifically requires that 'river health', and indeed aquatic ecosystem health, be scientifically assessed in order to determine user strategies and /or the impacts of new developments. In support of this requirement, river 'health' assessment protocols are being developed and refined as part of the implementation of the policy on Environmental Water Quality (Palmer et al., 2003). These protocols will inform the setting of permit conditions and associated requirements for compliance monitoring for individual water users.

The use of multi-disciplinary approaches to determine 'river health' at different trophic levels, rather than single measures at single trophic level (eg. invertebrates only], is a process advocated by many experienced aquatic ecologists (e.g. Cairns, 1991, Hofmann, 1996 and Kelly et al., 1995. Caution is also expressed by Taylor (1997) and others about the efficacy and wisdom of solely adopting rapid assessment procedures (the 'SASS' trend) on the basis that they compromise and trivialize good science in the desire to '*overcome the roadblocks of time and cost which purportedly prevented efficient monitoring of inland waterways with conventional approaches*'. It is of paramount importance that the methodology applied be applicable and pertinent to the level of assessment and more importantly, the degree of confidence with which long-term predictions of ecosystem change are made.

One of the long term goals of this project is to introduce and promote acceptance of **an added value** protocol for river health assessment by using diatom associations. Diatom assemblages

are robust, biologically-meaningful and sensitive measures of physico-chemical changes occurring in a wide range of aquatic environments. There is a relatively long history, world-wide, of the use of diatoms for such biological monitoring (e.g. Kelly et al., 1995). South Africa has fallen behind in the application of these tried and tested procedures because of a lack of appreciation of the information inherently contained in diatom-based indices. The loss of skilled expertise and simultaneous inertia in developing newly-skilled people, together with perceived taxonomic challenges, has also not helped the acceptance and adoption of this approach. The problem is furthermore exacerbated by the singular lack of formal algology courses in the curricula of any South African tertiary institutions. South Africa is almost globally unique in its lack of a Chair of Phycology at any of its Universities.

The South African Diatom Collection is currently housed at the CSIR (KwaZulu) laboratory in Durban. The wealth of information retained in this resource needs to be unleashed using modern technological advances in microscopy and computer software. The original vision was to resurrect the almost defunct SADC and thereby establish the groundwork for a renewed thrust involving the re-introduction of diatom communities as extremely useful and critical indicators of aquatic ecosystem health. Historical data sets in the SADC span the period 1950-1995, i.e. from the time that Dr BJ Cholnoky undertook the first investigations of South African rivers.

The information contained in the SADC may facilitate the reconstruction of historical water quality conditions, as well as support comparisons of the effect of changing land-use patterns in key development/impacted areas, using recorded diatom analyses and the application of modern software. Round (1991) successfully followed an approach of restricting identification to the most abundant and dominant species. With the benefit of modern technology, the original dominant specimens can now be captured with image analysis to provide a recognizable database of species which typify distinctive yet varying types of pollution eg. pulp and paper waste, sugar waste, acid mine drainage, and eutrophication from sewage outfalls.

In the longer term the real value of the SADC will be realised when a training program for new young SA diatomologists is developed, and a diatom (biological) procedure for aquatic ecosystem health assessments once again receives due recognition in South Africa. Collaboration with other diatomologists working with world-recognized collections will also be possible once the collection is resurrected and image analysis procedures are used as a communication and taxonomic identification tool (see Section 5.3.1). The value of using

diatoms as indices of water quality is now widely recognized (e.g. the draft European Guidance Standard for the Identification, Enumeration and Interpretation of benthic diatom samples from rivers for water quality assessment – currently a working document in preparation). This protocol broadly divides diatom assemblages into 5 water quality classes, namely:

Water quality classes as per the proposed European Guidance Standard

I: Excellent	II: Good	III: Moderate	IV: Poor	V: Bad
Achnanthes peragallii	*Acnanthes minutissima*	*Achnanthes conspicua*	*Actinocyclus normanii*	*Achnanthes delicatulum*
A. dauii	*C. sinuata*	*Amphora pediculus*	*Amphora romana*	*Amphora veneta*
A. subatomoides	*Cymatopleura elliptica*	*Cooconeis pediculus*	*Bacillaria paradoxa*	*Navicula accommoda*
Cymbella mesinaum	*Cymbella affinis*	*Cyclotella pseudostelligera*	*Cyclostephanos dubius*	*Navicula arvensis*
Denticular. mesodon	*Cymbella lanceolata*	*Fragilaria brevistriata*	*Cyclostephanos invisitatus*	*Navicula atomus*
D. tenuis	*Diatoma vulgaris*	*Fragilaria pulchella*	*Cyclotella atomus*	*Navicula cuspidate*
Diatom hiemale	*Fragilaria capucina*	*Gomphonema parvulum*	*Cyclotella meneghiniana*	*Navicula halophilia*
Eunotia exugua	*Frustulia vulgaris*	*Gomphonema truncatum*	*Gomphonema pseudoaugur*	*Navicula minuscule*
Fragilaria arcus	*Gomphonema olivaceum*	*Gyrosigma attenuatum*	*Hantzschia abundans*	*Navicula molestiformis*
Frustulia rhomboids	*G. acuminatum*	*Navicula capitatoradiata*	*Hantzschia amphioxys*	*Navicula pymaea*
Gomphonema oliviaceum	*G. minutum*	*Navicula viridula*	*Navicula goeppertiana*	*Navicula saprophilia*
G. rhombicum	*Navicula ignota*	*Rhoicosphenia abbreviata*	*Navicula hungarica*	*Navicula veneta*
Meridion circulare	*Navicula radiosa*	*Thalassiosira brahmaputrae*	*Navicula mutica*	*Nitzschia capitellata*
Pinnularia gibba	*Nitzschia dissipata*		*Navicula pupula*	*Nitzschia frustulum*
Stauroneis phoenicenteron	*Sellaphora bacillum*		*Navicula subminuscula*	*Nitzschia umbonata*
			Nitzschia filiformis	
			Nitzschia palea	
			Surirella ovalis	

Use of protocols such as the European Guidance Standard is increasingly being supported by the availability of allied resources such as DIATCODE – a dynamic list of diatom names and codes maintained by the Environmental Change Research Centre (ECRC), and the Diatom Paleolimnology Data Cooperative (DPDC) (Patrick Centre for Environmental Research, USA).

In addition a number of predictive and/or forensic (climate change) applied case studies that have been based on diatom assemblage analysis, e.g. Troeger, 1981, Birks et al., 1990, Fritz et al., 1991, Juggins, 1992, , Bennion et al., 1996, Cameron, 1997, Potapova and Charles (2002) and Battarbee et al., in press. Using diatoms as indicators of trophic status of rivers is becoming a popular tool. The Diatom Biological Index (DBI) is now a routine application in France (Cemagref) as is the European Diatom Database (EDDI)

2. INITIAL GOALS OF THIS PROJECT

The main purpose of this phase of the project, as described in the revised proposal submitted to the Water Research Commission, was focused on the following objectives:-

- *Processing, evaluating and collating the existing SA Diatom Collection, now housed at the CSIR, Durban, into a useable database* (NB. At the outset of this project all of the information contained in the SADC was in a pre-computer format)*;*

- *Testing the potential value of this historic collection for determining the reference (water quality) condition of South African rivers for which no historical water quality data exist.*

3. THE COMPOSITION OF THE SOUTH AFRICAN DIATOM COLLECTION [SADC]

The collection is referred to as the South African Diatom Collection (SADC) and is known world-wide by the international diatomologist community. It is comprised of several distinct components (Dr F Schoeman – pers.comm., January 2003). A great deal of the above information has now been captured in electronic format - as spreadsheets in preparation for conversion to a more comprehensive database (see Section 4):

The slide collection comprises the following:

- A number of **special slide series** donated to the former National Institute for Water Research of the CSIR by the British Museum (Natural History), e.g. Van Heurck, Peragallo, W Smith and others. **These are extremely valuable and often contain type specimens**;

- **SA Reference Slides**. These refer to the slides mentioned in the "Diatom Flora of Southern Africa." Many of these slides have ringed specimens. They are intended as reference material for the various taxa. These slides are referred to regularly in the Slide Index and Sample Index Manuals, with their appropriate reference numbers, i.e. Shelf and CSIR Serial Number;

- **M Giffen collection:** Slides from Kidd's Beach, Gulu, Amatola Mountains, West Coast and other locations (Professor Giffen was based at University of Fort Hare and donated his contributions (slide and documents) to the South African Diatom Collection before he died;

- **BJ Cholnoky collection:** An extensive series of slides of material collected by Dr Cholnoky during his country-wide investigations. (Details of this material are recorded in a catalogue by Schoeman and Meaton, 1982);
- **Miscellaneous External slides:** Contributions of numerous slides from colleagues abroad e.g. Carter, Barber, Hendey, Haworth (all from United Kingdom), H. van Dam (Netherlands) and others;

- **Schoeman and Archibald collection:** Probably the most extensive part of the collection. The information relating to these samples is recorded in the **Sample and Slide Indexes** where the **Shelf Number** and **Slide Serial Number** are given together with collection locality details. In addition the positions of many of the samples are mapped, and card indices exist for valuable diatom materials, e.g. Kützing, Ehrenberg and W. Smith.

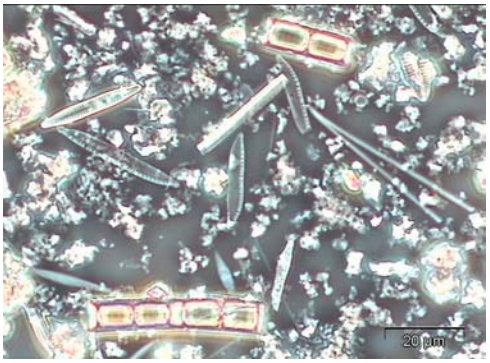
Various researchers, in particular Cholnoky, Schoeman and Archibald, produced and maintained records of diatom counts, and therefore species composition, in samples from some river systems. These records are invaluable because they provide critical information of accurate identifications of the species present and their relative dominance in the association. This data is very necessary and useful for application of the Omnidia software package and the reconstruction of water quality indices.

4. SADC SORTING AND CATALOGING

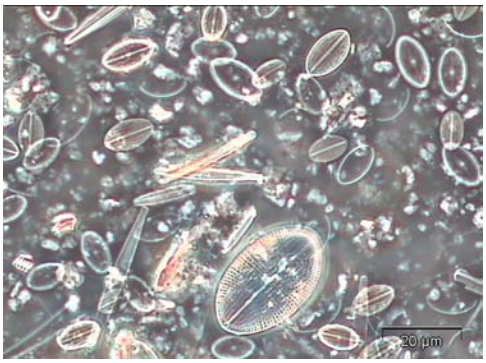
Task 1. **Information assessment, data sorting and collation on a geographical basis derived from 3 main resources (A, B and C).**

Resource A: **Hardcopy information in Sample Index and Slide Index Files is linked to Slides in several cabinets and boxes** (see Figures 1 & 2). Each cabinet holds several trays (20 slides each) referred to as a Shelf. Each slide is

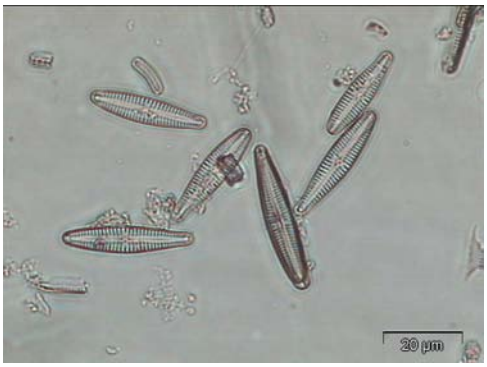
Figure 1: Selected views of the slide (LM and SEM) components of the South African Diatom Collection.



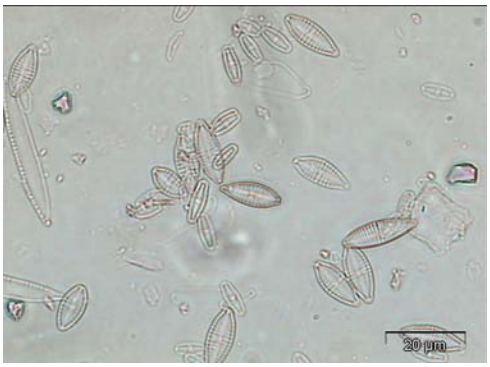
Shelf 237 Slide 436



St Lucia Shelf 174 / Slide 5461



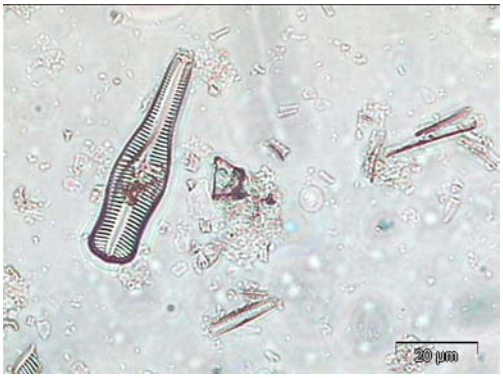
Tugela river Shelf 176/Slide 3517



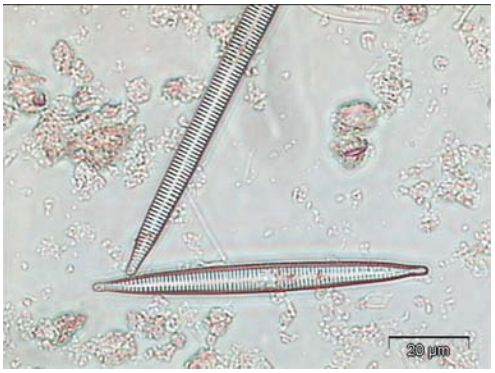
Vaal River Shelf 131 / 2616a

Fish River Shelf 363/ 7288a

Surirella Shelf 176 / Slide 3517



Gomphonema Shelf 598 / Slide 5



Fragilaria Shelf 237 / Slide 436

Figure 2 Examples of image analysis of diatom material from various slides in the SA Diatom Collection [SADC]. (x400 magnification)

numbered by Shelf/ CSIR Serial Number. These numbers correspond with additional information captured in the Sample Index and Slide Index files.

Activities Close scrutiny of checklists and CSIR Index Manuals describing the material in the collection;

Audit and scrutiny of the material (slides and documents) to determine what actually still physically exists in the collection;

Compilation of an inventory of South African river basin, sampling sites, survey date and associated data. (non-South African information will also be captured if time permits];

Scrutiny and assessment of the quality of the data sets *vis a vis* other immediate aims of this WRC project.

None of the data contained in the Slide and/or Sample Indexes had previously been captured into an electronic format because the bulk of the work on the collection was developed prior to the desk-top computer age. All the back-up records for the slides and stored diatomaceous material (in bottles) were therefore maintained in boxed records or document files. Material from more than 14 000 slides need to be verified at some future stage.

The completeness of the SADC cannot be determined as no records have been maintained of materials loaned or removed therefrom. Several shelves have cards indicating 'missing slides' but there is no record of who borrowed a slide or where a slide was sent for examination. To protect the integrity of the SADC it is recommended that:

a. In the context of modern computer/microscope technology, the present day curator should adopt a rigorous policy of not allowing historical permanent **slide material** to leave the Collection, but rather encourage interested diatomologists to visit the Durban laboratory and/or capture the material using image analysis and correspond through e-mail).

b. New slide material should always be made in triplicate for submission to the SADC and allowing for 'swapping' with other Diatom Collections.

The following summary deliverables have been generated from this phase of the project. Due to their size and complexity they have been included as electronic (Excel spreadsheet files) appendices:

1) A summary of the total spread of information covered in the **Slide Index** and **Sample Index**. Only the first and last row of data has been captured per collector (Appendix - Table 1 – Diskette 1);
2) A summary of the information pertinent to river systems in South Africa. This combines the information from the **Slide** and **Sample Indexes** (Appendix - Table 2 – Diskette 1)**.**

SA River systems covered by the SADC

The following major river systems have been surveyed over the years but the coverage is very variable in terms of space and time. This level of information will dictate the selection of the areas in South Africa which can be addressed in the latter stages of the project (See Task 3). In some instances aquatic resources, other than a river have been noted for the sake of completeness at this stage.

The listing follows the alphabetical format utilized in the SADC, and the original names have been used.

Barberton - Nelspruit/ White river
Bloukrans
Berg River
SW Cape Rivers
Buffalo River
Crocodile/ Sabie / Nelspruit
Vaal Harts
Fish River
F Series
G series - NCape
Grahamstown Palmiet
H Series Southern Cape
Hennops River

Jakkals
Jukskei
Jukskei-Crocodile
Lesotho/OFS Orange river
Modderfontein (Jukskei Toxicity)
Nonoti
Umzimkulu
Umhlatuze
Upper Thukela
N.Transvaal
Orange River
Upper Hennops
Pienaars
Pongola
Tugela
Umlazi
Usutu
Vaal

Resource B. Permanent slides / bottled material (see Figure 3)

Slides in cabinets

There are approximately 700 shelves (trays), with each shelf capable of holding 20 slides. An unofficial start was made to capture and record the details **on each slide** electronically. This information contained on the slide would allow for rapid location and determination of coverage of a river survey. (NB This exercise was subsequently discontinued temporarily in July 2003 in favour of direct data capture of **more comprehensive but** similar information from the Slide and Sample Index files.

Assessment of material on the slides

Random scrutiny of slides drawn from various surveys carried out on river systems in South African over the last 50 years shows that there is a huge disparity in the state of the material. In some cases the original diatom material must have been sparse, in others the

Figure 3: Selected views of the bottled sample material component of the South African Diatom Collection.

extraction/preparation procedure was not good and the diatom specimens are blurred or non-existent. Where the material is still in good condition it will be possible to capture pertinent images from the slides for comparison and confirmation of findings using image analysis.

A start has been made to the capture of images of the specific type species contained in the Van Heurck collection. The material on these slides was meticulously prepared and the images are particularly clear and distinct despite their age. Approximately 50 species specific images are captured with morphological variations. Many more images have been captured of other clear specimens of other unknown species. Identifications can be made at a later stage so that these can be included in a "Training Set' for future workers in the field.

Resource C. Reports, publications and documentation (see Figure 4)

General comments

Some data sets are scattered through the collection and can be re-sorted using the electronic medium into a more logical reference system. Some redundant provincial names have been retained for the present for clarity and ease of identification with the original material. Once the bulk of information is captured it is relatively simple to delete and rearrange the entire data set.

Although material is recorded alphabetically it is sometimes ordered by region rather than by river system and therefore studies on a river (eg. the Orange} appear more than once in the spreadsheet data.

Electronic capture of literature in the Collection

The published papers are stored in box files while sampled material is contained in bottles of dried material, and also as permanent mounts on glass slides. **It has been estimated that** there are several thousand (approx 15 000) reprints from all over the world, and approximately 350 text books of various descriptions. The titles of the books have previously been captured on a spreadsheet but the arrangement of the books has not been ordered in any special way.

Figure 4: Selected views of the reprint and publications components of the South African Diatom Collection.

The input template was designed to capture the following information.

It was estimated that there was information on approximately 10 398 papers to be captured and only sufficient funds were available to capture 6000. Accordingly it was agreed that the data from the set of papers highlighted in red - largely related to South African work - in Table 3 would be the first to be captured.

Table 3: Breakdown of estimated numbers of papers

Category	No. of Papers	No. of Boxes
1. Literature papers by box numbers	**5453**	196
2. Papers by general author names	**360**	12
3. Papers on ecological information	**300**	10
4. Papers on chemistry	**200**	8
5. Papers in Africa	**1365**	39
6. Papers by specific authors	**2000**	100
7. Reprints and general papers	**720**	36

Task 2. Interactions with national and international diatomologists

Links with other diatom specialists and curators of collections were **re-**established **between May 2002 – 2004** in order to advertise the revival of the SADC and attempt to set up collaborative agreements.

The following interactions were initiated:

National

UPE	**Prof Guy Bate** (Ex University of Port Elizabeth, Retired) **Ms Pat Smailes** Technical support
Interaction	Both individuals are effectively retired and their level of activity in 2003/04 remains one of professional interest. There is a proposal to undertake an investigation of Lake St Lucia because of the topical interest in the severe impact of drought on the flora and fauna of the lake. **Prof Janine Adams** - Supervisor for student diatom projects.
Rhodes University	**Val Meaton** ex CSIR Pretoria. Worked with the SADC as an assistant to Drs Schoeman and Archibald. Only person who knows the technicalities and set up of the original collection.
University of North	**Prof Braam Pieterse** Lecturer in General Algology, University of the North Potchefstroom campus. **Jonathon Taylor** MSc Student– University of North-West (NWU), Potchefstroom**.** Active participation in this project and analysis of test data using Omnidia software.

Work at the NWU is focused in the following areas:

1. Contributions to the taxonomic knowledge of the diatom flora of South Africa. As stressed before this project attempts to provide taxonomic knowledge and support to the other facets of the initiative while providing a contribution to the scientific knowledge of diatoms in such diverse habitats as the plankton, benthos and even extending into soil habitats;
2. Examination of the diatom flora from extreme and polluted to highly polluted habitats. This project will provide information on the ecological tolerance levels of diatoms occurring under extreme physical and chemical conditions, with an attempt to develop relationships between community composition and environmental variables;
3. Comparison of the diatom communities inhabiting the surface of rocks and those inhabiting the surface of aquatic macrophytes. This project aims to determine whether index systems developed for riverine environments can be transferred for use in wetlands utilising a different more common substrate, macrophytes as opposed to rocks, to that commonly used for sampling diatoms in rivers;
4. Taxonomy of specific diatom groups. A project is currently under way dealing with the *Cymbella s.l.* group, which has recently been split into several new genera. The project will undertake to update the taxonomy and nomenclature of this particular group.

Miscellaneous	**Dr Ferdiand R Schoeman** Deputy Director**:** Pretoria Zoological Gardens. Only living diatomologist of the previous South African era (circa 1965 – 1985).

International

Holland	**FAS Sterrenberg** Diatomologist and administrator
Interactions	Several e-mail discussions to map out a plan for reviving the SADC. Great moral support and encouragement. Was keen to search for potential funding sources to save the SADC.

Luxembourg	**Dr Luc Ector** Association of French-speaking Diatomists) 'CREBS' (Cellule de Recherche en Environement et Biotechnologes)
Interaction	e-mail discussion of problems relating to SADC. Introduction to obtaining Omnidia software.
France	**Prof. Karen Serieyssol** (European Editor of Diatom Research – Journal) The American University of Paris.
Interaction	Offer of support and use of the Database of Diatomolgists for advertising the revival and existence of the SADC
	Catherine LeCointe Developer of the Omnidia Software package.
	Commercial interest in expanding the use of Omnidia software in South African conditions.
Germany	**Dr Richard Crawford** Director of the Hustedt Collection in Bremerhaven in Germany. Co author of the Manual by Crawford, Round and Mann.
Interaction	E-mail correspondence to advise on the revival of SADC. Promoted our case at the last International Diatom Workshop held in Canada in 2002.
	Expressed support for funding '*maintenance of diatom collections' at the workshop.*
	Prof H. Lange-Bertalot Botanische Inst. Goethe University, Frankfurt. **–** personal contact and links to ensure that we get the best back-up on diatom identifications.
	Dr Regine Jahn: Botanical Museum, Free University Berlin. She has written to report that isotype material of Dr

	Cholnoky is held in the museum where Professor Gerloff (a friend/colleague of Dr Cholnoky) worked.
Austria	**Professor Dr Anna-Maria Schmid:** Salzburg. She was Dr Cholnoky's last student in Pretoria, after having spent nearly a year in Pretoria in 1971. She is a renowned diatom cell biologist but apparently is in poor health (Dr Jahn – pers.comm).
Poland	**Professor Andrzej Witkowski** Head of Department Institute of Marine Sciences, University of Szczecin, Poland.
*Interaction***:**	First high profile researcher to visit the SADC in Durban in March 2004, including a sampling trip to Lake St Lucia. The purpose was to examine all the material in Professor Giffen's marine littoral diatom collection. Images were captured and material was used for SEM outputs later. Note: Giffen never produced any photographs although his line drawings were good. Witkowski is attempting to document the best images. Funding: Providing funds for flight, accommodation to attend the next International Diatom Symposium in Poland. A 1-month training course on taxonomy of diatoms has also been arranged with Dr Horst Lange-Bertalot – the world's leading diatom taxonomist.
United Kingdom	**Dr Pat Simms** British Museum (retired in 2002)
Interaction	Retired and referred to Dr David Williams
	Dr David Williams Head of Cryptogamic Research (Botany Dept) of the Natural History Museum (London) his primary interest is diatom research.

Interaction	e-mail correspondence on the possibility of support and assistance in running a workshop on freshwater diatoms. A provisional offer was made to come to South Africa with no charge on time.
Brazil	**Dr Lezilda Carvalho Torgan** Museu de Ciencias Naturais, Porto Alegre, Brazil
Interaction	email query on taxonomy of *Surirella schweikerdtii*. Offers of assistance for taxomonic problems.
USA	**Dr Freda Reid (retired 2001)** Scripps Institute of Oceanography
Interaction	email discussion and passed information to Dr Lange
	Dr Carina Lange Expression of interest and willingness to be of assistance but this could be a costly exercise
	Anna Wachnick-Kosiorek South Eastern Environmental Research Centre Florida International University, Miami.
Interaction	email discussions on taxonomy of one St Lucia species. Very helpful. Ex-student of Professor Witkowski.
	Dr Gregory Ruiz Smithsonian Environmental Research Centre, Maryland, USA. Potential for ballast water investigations, specifically on 'phantom algae' and other invasive diatoms
Norway	**Dr Bjorg Stabell : Dept of Geological Sciences, University of Oslo.**

She is pursuing research interests using diatoms to date historical sediment records, specifically at St Lucia. Supervisor to student Mugabe (see below**)**

Mozambique **Mr Joao Mugabe –** Geology Department, University of Eduardo Mondane, Maputo. Email contact has thusfar proved unsuccessful.

Task 3. Identification of case studies and demonstration of the value of diatom associations as water quality indicators

3A Data pertaining to specific systems in South Africa

3A.1 Jukskei – Crocodile river system

This is a Gauteng river system which has been impacted by sewage effluent for several decades. The main river eventually discharges into the Hartbeestpoort Dam which has become renowned for highly eutrophic conditions with the attendant algal and water quality problems.

Considerable information is available in the diatom collection dealing with this system. The diatom associations have been extensively studied by Cholnoky, Schoeman and Archibald at various times, and applied by researchers such as BR Allanson. Physico-chemical characteristics and water quality data are well documented and the diatom analysis sheets are available. Many of the original slides are of good quality and can be used for checking and comparison of original identifications. The historical data contained in the SADC for the Jukskei/Crocodile was re-assessed, taxonomic updates applied and the data re-reprocessed using the Omnidia software package. The results are presented in Section 3.

3A.2 Upper Thukela river system

This set of data provides a baseline of the almost pristine conditions of the upper catchment of the largest and most important river system in KwaZulu-Natal. Cholnoky (1960) originally recognized the potential value of using diatoms to determine the oligotrophic status of rivers when he suggested that '*there are many oligotrophic systems in South Africa. A study of those*

'situated in the old sandstone deposits of higher plateaus' would be of great theoretical and practical importance.

It is now suggested that appropriate diatom studies should be undertaken before human development impacts mask the pristine conditions in the upper Mzimkulu, Mkomazi and Thukela systems. These are the rivers which drain high profile tourism areas in the Drakensberg. Cholnoky's early work on the upper Tugela sites should provide a comparison with impacts of general land-use changes and also changes that may have occurred over time in the upper catchments of all three of these key rivers. Diatom based work on the Thukela system is ongoing (CGM Archibald, unpublisjed data).

3A.3 Vaal River

Comprehensive diatom analysis sheets exist for several specific sites and studies on the upper reaches of the Vaal where major development impacts have been recorded, particularly where the river flow regime has been altered downstream of the Vaal dam.

3A.4 Mooi River

A comparative analysis of the use of SASS5 and a diatom-based approach on the Mooi River system is presented in Section 4.

3B. System descriptions - Impact by type of waste

There are several distinct diatom surveys of South African rivers which have been impacted by various wastes, in particular sugar waste, pulp and paper waste, acid mine drainage and research on the impact of high nitrogen content from sewage waste discharges. Other studies relate to demonstrating the effect of salinity changes in rivers (Sundays and Great Fish river surveys in the Eastern Cape)

3B.1 Impact of sugar mill waste on the Nonoti river

This is a small river on the KZN north coast sugar belt. This set of data pertains to a survey made by the CSIR (Archibald CGM et al., 1967) and contains water quality data of the river conditions above and below the Doornkop sugar mill. The diatom species identification and

community composition analysis was undertaken by Cholnoky and presented in his paper (Cholnoky, 1969). These data have now been subjected to the Omndia software protocol to test the interpretation of such a diatom assemblage using one or more of the index protocols available for diatoms. (see original project goals stated above).

The original analysis sheets of diatom species composition in the Nonoti river above and below the point of discharge of the Doornkop sugar mill. Matching water quality data at each site was also compiled from original river investigations made by CGM Archibald in 1968. Image analysis of the dominant species are to be made for addition to the electronic data base and 'training set' for future applications.

3B.2 Impact of Acid Mine drainage on the Tshoba river

This is a small tributary of the Umfolozi River and the zone of interest lies immediately downstream of a defunct coal mine dump**.**

This study was undertaken by the CSIR in 1998 following complaints by the public relating to the abandonment of coal mines and the consequent impact of seepage water (acid mine drainage) on the quality of the downstream river system.

Some historic CSIR data on the water quality of Northern Natal rivers exists: Kemp (1962, 1967); Oliff (1960, 1963); Oliff and King (1964); Oliff et al., (1965) and Archibald et al., (1969). Some other diatom material from sporadic sites was analysed by Cholnoky (1958) on the upper Umfolozi river but there does not appear to have been a well defined river system study.

This Tshoba river investigation was carried out more recently (Archibald and Fowles, 1998) with the diatom identifications and community composition analysis being undertaken by Dr REM Archibald. A paper on this specific study is in preparation with recent inputs from JC Taylor using Omnidia to test the water quality indices. This latter work, entitled "The role of diatoms and aquatic invertebrates as biological measures of river health: A case study of the impact of acid-mine drainage on the biota and water quality of the headwaters of the small Tshoba river, KwaZulu-Natal (South Africa)". A summary of the Tshoba River study is provided in Section 5.

3B.3 Impact of pulp and paper waste on lower Thukela river

Much diatom and water quality work has been done on various reaches of the Thukela river system over the last 30 - 40 years by Cholnoky et al. A more recent study was undertaken by the CSIR (Archibald and Fowles, 1997 and 1999) covering the lower Thukela river and estuarine zone downstream of the Sappi (Tugela mill) effluent discharge. Water quality, SASS5 and diatom analysis was made of this river reach at 5 sites. However formal permission from Sappi Management for the release of the data in order to demonstrate its value has been difficult to acquire. This work may therefore remain unpublished as a case history in the application of diatom associations until Sappi makes their position known.

Task 4. Applications of Omnidia

The Omnidia software has been applied to several data sets since the first WRC meeting of which some outputs are presented in this report (See Jukskei – Section 3 and Tshoba River – Section 5 - studies - This assessment is dealt with in Sections 3 and 4 of this report.. Other applications are in an advanced stage of preparation and processing but have not been fully addressed in this report i.e. Lower Tugela river study, the Nonoti sugar mill waste study, the Mbilo river sewage works study.

Further case studies need to be attempted on salinity problems in the Fish and Sundays rivers of the Eastern Cape and the impact of flow regime and water quality changes in the eutrophied Umhlanga and Umhlangane rivers.

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SECTION 3

DIATOMS AS INDICATORS OF WATER QUALITY IN THE JUKSKEI-CROCODILE RIVER SYSTEM IN 1956 AND 1957, A RE-ANALYSIS OF DIATOM COUNT DATA GENERATED BY DR. B. J. CHOLNOKY

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1. INTRODUCTION

Over many years the work of Dr. B. J. Cholnoky provided an invaluable contribution to the knowledge of the taxonomy and ecology of diatom species he encountered in a variety of southern African habitats. Cholnoky's ecological work attempted to provide a reflection of water quality based on the pollution tolerance limits of diatom species, and especially to nitrogenous compounds. In addition Cholnoky was one of the first people to predict pH of a water body based on its diatom community (Cholnoky, 1958). In essence his work was far ahead of its time as he could only relate several species to different pollutants; - later workers have had the luxury of using statistical techniques such as correspondence analysis to determine the relationships between the abundances of all diatom species encountered in a certain community and the chemical composition of their aquatic environment. Consequently direct tolerances can be assigned to diatom species for a whole range of water quality variables rather than just nitrogen or pH.

If Cholnoky's (1968) definitive work on the diatoms " Die Ökologie Der Diatomeen in Binnengewässern" is examined it can be noted that Cholnoky painstakingly dealt with all practical aspects relevant to diatom ecological studies. He first stressed that any person studying ecology should have a sound taxonomical background; secondly he carefully determined margins of error for diatom analysis. Most importantly he tested various counting procedures and determined whether different slides from the same site need to be counted to generate an accurate result, how many individual cells should be counted and the manner in which diatom cells should be counted. Cholnoky only employed methods yielding a margin of error of 2% or less. Thus, Cholnoky's diatom analysis sheets should provide an accurate reflection of the structure of the diatom communities that

he encountered. If Cholnoky's diatom community analysis is considered to be accurate then the ecological conclusions drawn from his data should be equally sound.

An explanatory note follows about working with diatom species encountered in South Africa: When diatom publications were written by various authors (Cholnoky, Giffen, Schoeman and Archibald) it was with the intention either to describe all diatom species encountered in a given sample, or to describe novel species from a particular locality. The method of illustrating these publications was with line drawings, which are both time-consuming and difficult to generate. Thus common species were usually not illustrated and the reader is most often referred to the works of Hustedt or other authors for illustrations of the species in question. Thus we have a large amount of South African literature that has few, or no, illustrations of common diatom species, only novel and rare species. Only later, workers such as Schoeman and Archibald in the late 1980's use photographic images to illustrate articles, such as the work done in Namibia at the Gross Barmen thermal springs (Schoeman & Archibald, 1988). In this work far more common species with their variations are illustrated using photomicrograph images.

Another obstacle encountered in relating older publications to current data and literature lies in the taxonomy and nomenclature of the diatoms. Internationally, diatom nomenclature has undergone several major upheavals and changes in the past 15 years. Since the publication of Round *et al.* (1990) "The Diatoms: Morphology and Taxonomy of the Genera" the taxonomical trend has been to split large genera into smaller groups, establish synonyms between con-specific taxa, and to generally rearrange the diatom species into more natural groupings. In addition, many of the species described by Cholnoky have been established as synonyms for taxa described from Europe, while on the other hand many of his species have been validated and found to occur in Europe. Cholnoky also described many 'African' forms of extant species, adding to taxonomical confusion. Schoeman (1973), writing after Cholnoky's death, comments " *transitional forms (of diatoms), linking certain species with their forms and varieties… clearly indicate that the demarcation into varieties or forms is often entirely superfluous and can serve no purpose at all*." This comment creates doubt about the validity of Cholnoky's 'African' forms.

The lack of illustration of common species together with vast changes in diatom taxonomy over the last decade has lead to misconceptions about diatom taxa encountered in South Africa. The vast majority of common diatom taxa found in South Africa are cosmopolitan both in distribution (see Krammer & Lange-Bertalot, 1986-1991), and environmental tolerances. There are a number of diatom species endemic to South or southern Africa (see Schoeman & Archibald, 1976-1980), but the dominant diatom species in a given community are well known, well documented cosmopolite species. This is illustrated in the present analysis where the majority of species occurring on

Cholnoky's analysis sheets were described from Europe. Neither lack of distribution data (diatom distribution changes according to water quality), nor lack taxonomic information should stand in the way of the application of diatoms in South Africa to environmental issues. The taxonomy and nomenclature of diatom species encountered in South Africa can be quickly updated using the wealth of modern literature and electronic database's such as OMNIDIA - as this study demonstrates.

South Africa is in possession of an enormous database of literature, diatom material (slides and preserved material) and most importantly diatom analysis sheets housed in the South African diatom collection at the Council for Scientific and Industrial Research (CSIR), Durban. To draw correct inferences about the water quality of a given river or stream using diatom analysis methods, several hours are needed behind a high power microscope to determine the relative species composition of the sampled community. In addition, to perform the diatom analysis the operative needs to have a very good knowledge of diatom taxonomy. However, the South African Diatom Collection benefits from the existence of the original diatom analysis sheets. Thus the most time consuming and painstaking part of using diatom indices has been completed. It now only remains to convert these diatom analysis sheets to digital format and then generate historical ecological information based on the diatom communities using modern diatom pollution indices that have been developed and tested over several decades in Europe and elsewhere.

Diatom indices function in the following manner: In a sample from a body of water with a particular level of determinand (e.g. salinity), diatom taxa with their optimum close to that level will be most abundant. Therefore an estimate of the level of that determinand in the sample can be made from the average of the optima of all the taxa in that sample, each weighted by its abundance. This means that a taxon that is found frequently in a sample has more influence on the result than one that is rare. A further refinement is the provision of an 'indicator value' which is included to give greater weight to those taxa which are good indicators of particular environmental conditions. In practice, use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998).

The diatom indices used in this analysis are known as Descy's index or DES (Descy 1979); the Generic Diatom Index or GDI (Coste & Ayphassorho, 1991); the Specific Pollution sensitivity Index or SPI (Coste in CEMAGREF, 1982); the Biological Diatom Index or BDI (Lenoir & Coste, 1996); the Artois-Picardie Diatom Index or APDI (Prygiel *et al*., 1996); Sládeček's index or SLA (Sládeček, 1986); Leclercq & Maquet's Index or LMI (Leclercq & Maquet, 1987); the Commission of

Economical Community Index or CEC (Descy & Coste, 1991); Schiefele and Schreiner's index or SHE (Schiefele & Schreiner, 1991); the Trophic Diatom Index or TDI (Kelly & Whitton, 1995); and the Watanabe index or WAT (Watanabe *et al*., 1986, 1990). In all cases except in the CEC, SHE, TDI and WAT index, the diatom indices are calculated using the formula of Zelinka & Marvan (1961). For all of the above indices, except TDI (maximum value of 100), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe *et al.*, 1993) indicates clean water.

Most of the diatom indices listed above were designed to give an indication of general water quality. The indices differ in respect to the diatom species included in the calculation and in the number of taxa included in the calculation. The first index to be developed was that of Descy. This index was followed by the Specific Pollution sensitivity Index (SPI), which has the broadest species base of all of the indices. Several refinements followed on the SPI index that eventually culminated in the Biological Diatom Index (BDI), which incorporates 14 parameters of water quality. 70% of the variation in the scores of the BDI index can be explained using 14 water quality variables. The remaining 30% of the variation is ascribed to physical factors such as light penetration, current speed and general habitat integrity. Several indices were designed to reflect eutrophication including the Eutrophication Pollution Index (EPI) and the Trophic Diatom Index (TDI). The calculation of correct scores for the TDI index is dependent on the percentage of pollution tolerant diatom taxa in the sample (%PT), more than 20% PT valves indicate organic pollution rather than eutrophication. Sládeček (SLA index) and Watanabe (WAT index) developed diatom indices which were designed to reflect degrees of organic loading.

The object of this short study is to demonstrate the value of historical diatom analysis sheets for use in drawing conclusions about the past condition of South Africa rivers.

2. METHODS

2.1 Study area

The Jukskei-Crocodile River system drains an area of 2046 km^2 between Johannesburg and the Hartbeespoort Dam at an altitude of between 1200 and 1800 m (see Fig. 1). Climatically this region is cold and dry in winter and warm to hot in summer. About 80 to 90 percent of the rainfall occurs in the six summer months i.e., between November and April (Allanson, 1961). The southern catchment area (northern Johannesburg) is densely populated and heavily industrialised, whereas the northern part consists mainly of agricultural areas. At the time of Cholnoky's work the Jukskei-Crocodile river system received effluent from many different sources including power station blow-down (mineralising effect), industrial and sewage effluent (Schoeman, 1976). The Crocodile River drained

what was then a predominantly agricultural area and accordingly contained water of a higher quality (Schoeman, 1982). The extant situation is such that a number of wastewater treatment plants discharge tertiary effluent and high levels of orthophosphate phosphorus into the Jukskei (Johannesburg Northern Works, 65 tonnes P per annum), and into the Crocodile (Randfontein and Percy Stewart Works, combined 42 tonnes P per annum).

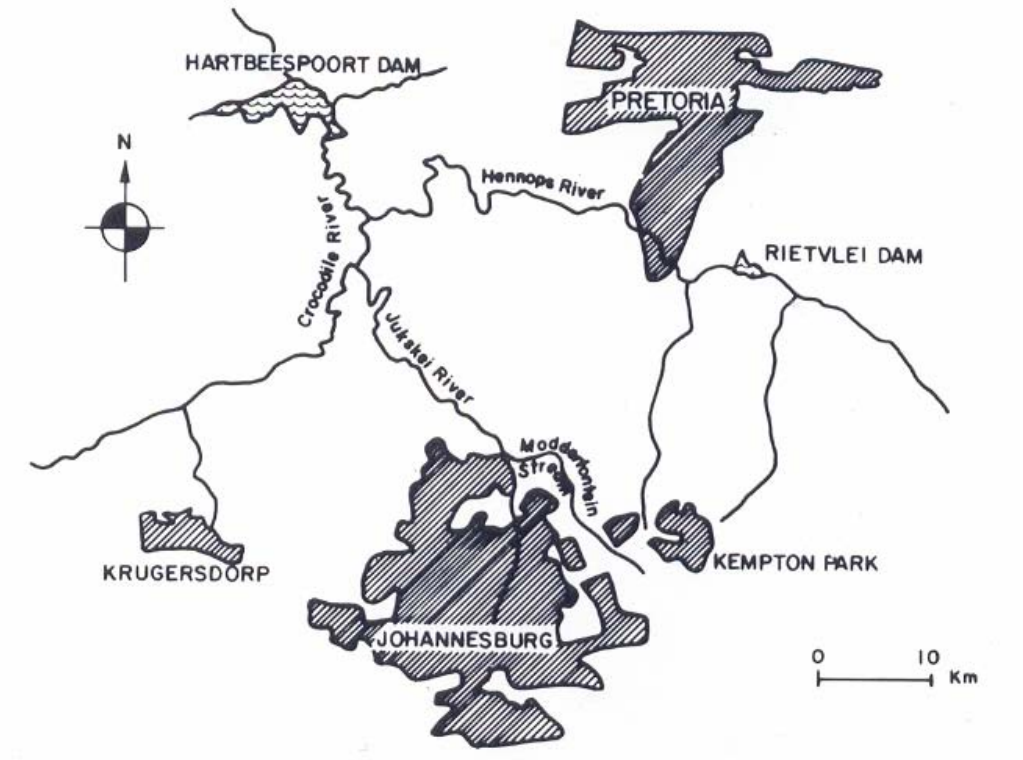


Figure 1: *Location of the Jukskei-Crocodile River catchment area (Schoeman, 1982).*

3. RESULTS

3.1 Introduction

The results of this study demonstrate the usefulness of historical analysis sheets. However, several problems are encountered in using these analysis sheets. Firstly the data sheets need to be converted to a digital format. In the present study this was achieved by entering the data first into spreadsheets and then into the OMNIDIA database. The first entry was into MICROSOFT EXCEL was necessary as the data had to be electronically transferred to the authors of the present article. However, if the person entering the data is proficient in the use of the OMNIDIA database the data can be directly entered without a first, time-consuming, entry into spreadsheets. Data entry into OMNIDIA only requires a species acronym together with absolute abundance of the relevant diatom species. From this data the program generates the full species name and relative abundance of the species in the community, and hence is far less time consuming than entering species data into spread sheets.

Results obtained from OMNIDIA are in the form of individual diatom analysis sheets together with site information, relative abundance of the species, population, diversity, evenness and a number of diatom index scores generated from the diatom community data. Alternatively diatom analysis sheets can be grouped together up to 20 at a time saving repetitive mention of species (see Appendix 2). These files can then in turn be exported to EXCEL or some other similar program.

It should be noted that the entry of diatom data of a historical nature using the acronym method poses several problems for the inexperienced user. The first complication that arises is whether the species name used by the original author of the analysis sheets is currently valid and recognized by the software? The validity of species names can be checked in OMNIDIA or, failing that, in a number of literature resources. If the name is no longer valid then the accurate synonym can be obtained in this fashion. Secondly, the relevant acronym for data entry needs to be identified. There is a printable list of acronyms in OMNIDIA for about 9000 species, or alternatively an electronic search may be conducted by typing the full species name into OMNIDIA. The acronym construction follows certain rules and once the operator is familiar with these rules most of the acronyms can many times be determined without resorting to either a manual or electronic search.

Once the data has been entered, the database (OMNIDIA) calculates the indices listed above in the introduction. In the following section the diatom index results for this analysis will be presented and discussed.

3.2 Index scores

The diatom index scores generated from Cholnoky's diatom analysis sheets are presented in Table 2, and should be interpreted using Table 1.

TABLE 1

Class limit values for diatom indices (Eloranta & Soininen, 2002)

Class	Trophy	Index score
high quality	oligotrophy	>17
good quality	oligo-mesotrophy	15 to 17
moderate quality	mesotrophy	12 to 15
poor quality	meso-eutrophy	9 to 12
bad quality	eutrophy	<9

The results as presented in Table 2 can be seen to give an accurate indication of a highly impacted river system (as per by Schoeman, 1976). Caution should be exercised, however, in interpreting the data yielded by those samples with the population number marked in red in Table 2. The number of

frustules counted in these samples is lower than the 350 minimum recommended by Cholnoky in his book (Cholnoky, 1968), and later by European authors (e.g. 300; Prygiel *et al*., 2002).

TABLE 2

Diatom index scores generated from diatom analysis sheets for the Jukskei-Crocodile River system in 1956/57

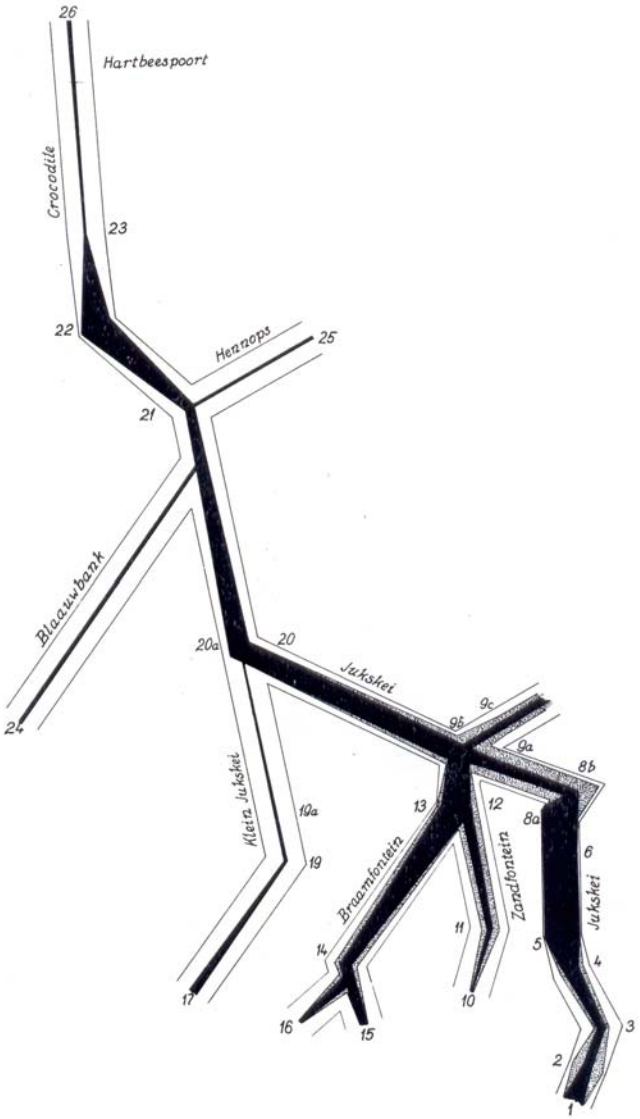
(Authored by Dr. B. J. Cholnoky)

Sample number	OMNIDIA ref number	Population	Number of species	SPI	DES	L&M	SHE	ROT	CEC	APDI	BDI	GDI	SLA	WAT	EPI	TDI	%PT
JK 1 STA 8A	141	565	17	1.2	1.1	5.8	1.3	6.7	1.6	6.6	6.7	1.3	7.7	1.7	7.5	75.6	96.5
JK 2 STA 8A	142	604	19	1.3	1.2	5.9	1.6	5.5	1.8	6.8	6.5	1.5	7.9	2.3	7	75.7	95.2
JK3 STA 8A	143	323	19	2	1.5	6	3.2	5.5	1.6	7.8	6.5	2.5	7.9	4.5	7.5	75.6	84.2
JK 5 STA 6	144	288	22	5	3.6	7.4	4.5	9.7	4.8	8.2	9.9	9	9.2	10.5	7.1	93.5	83
JK 6 STA 6	145	549	16	1.7	1.2	6.2	1.6	7	1.8	7.9	6.9	2	8.2	2.9	6.7	75	89.6
JK 7 STA 6	146	387	24	11.1	8.5	10.5	8.9	11.2	9.6	12	14	11.8	11.5	13.2	10.6	64.3	31.5
JK 8 STA 5	147	538	21	1.5	1.2	5.8	2.3	4.1	1.6	4.9	6.5	1.8	8	2.6	7.7	75.4	91.1
JK 9 STA 4	148	562	24	1.5	1.4	5.8	2	6	2.1	6.3	6.2	2	7.8	2.8	7.2	75.2	92.7
JK10	149	471	27	2.7	2.2	6.5	4.2	6.1	2.7	7.1	7.3	3.8	8.8	5.9	7.7	78.7	79.8
JK 11	150	471	27	2.1	1.7	6	3.9	5.1	2	7.5	6.5	2.7	8.1	4.9	7.7	77.4	83.9
JK 12	151	532	23	1.8	1.4	5.8	3.2	4.3	1.8	7.6	6.2	2.3	7.7	4.1	7.6	76.1	89.8
JK 13 STA 20	152	507	7	12.3	6.7	6.8	13	13.9	7.7	5.7	11	16.5	12.8	5.8	9.6	92.1	25.2
JK 14 STA 20 A	153	509	13	4.3	3.7	7.6	6.1	9.6	6.3	5.4	11.3	3.8	10.9	7.9	8.1	70.2	56.2
JK 15	154	532	10	11.2	14.8	10.5	17.8	18.6	18.3	9.6	8.8	14.3	11.7	19.8	8.8	10.4	0.4
JK 16	155	528	12	12.9	14.5	10.5	18.1	18.4	17.2	9.5	13.9	14.3	12.1	19.8	9.8	9.4	0.2
JK 17	156	562	15	16.9	15.4	14	18.1	18	18.3	10.4	14.6	18.4	15.3	18.8	12.6	5	2
JK 18	157	523	20	11.4	13.5	10.6	16.5	16.8	16.8	9.6	9.3	14.2	11.8	19.6	9.1	26.4	0.8
JK 19	158	533	13	11.8	13.7	10.5	18.4	18.9	17.2	9.5	10.3	14.4	11.9	19.6	9.1	12.1	1.3
JK 101 STA 1	159	265	14	4.9	5.7	8.3	8	11.9	6.1	9.4	11.6	3.1	10.9	10.5	12	77.2	54.7
JK 102 STA 2	160	282	14	2.9	4.9	6.4	4.8	6.6	5.2	2.2	10.1	7.9	12.5	4.6	11.7	94.4	50
JK 103 STA 3	161	247	19	6.8	9.9	7.2	5.4	7.8	5.9	5.4	10.9	9.2	9.3	8.9	10.6	93.3	62.3
JK 103B STA 3	162	268	30	5.1	7.1	8.1	6.4	8.8	4.2	5.2	9.7	7.8	12.1	9.2	8.8	87.5	48.1
JK 104 STA 4	163	325	25	2.9	4	6.5	4.2	7.4	3.9	4.8	7.9	6.9	10.4	8.1	8.8	90.9	79.7
JK 105 STA 5	164	349	14	5.7	6.2	6.2	5.4	5.7	4.4	5.4	9.7	11.5	8.2	10.7	9.2	95.1	88
JK 106 STA 8A	165	347	20	6.4	9.1	8.9	10.8	8.9	4.2	8.6	8.7	10.4	12.9	11.5	8	83.2	46.4
JK 106B STA 8B	166	258	19	4.5	6.7	6.7	7.7	9.5	4.2	7.5	9.3	6.5	12	10.8	9.9	85.1	65.1
JK 107A STA 9A	167	318	16	4.1	5.5	6	2.9	8.4	4.8	5	9.1	9.3	10.9	14	9.5	97.5	78.6
JK 107B STA 9B	168	265	24	4	7	7.6	7.3	9.3	5.2	6.6	9.3	9.5	11.4	9.8	8.9	93.4	70.2
JK 107C STA 9C	169	287	22	5	8.2	8	7	9.2	5.4	5.5	10.4	8	12.1	8.8	9.2	90	36.6
JK 108 STA 10	170	301	2	18.5	14.3	14.3	15.3	15.2	18.7	5.8	17	13.4	14.7	20	12.5	100	10.3
JK 109A STA 11	171	349	14	4.7	4.5	7.6	6.4	7.1	3.3	2.3	7.3	8.9	10.2	11	8.3	95.5	25.5
JK 109B STA 11	172	338	22	3.9	4.7	6.7	5.1	6.8	4.6	4.8	8	8.5	9.8	10.5	9.2	94.7	64.2
JK 109C STA 11	173	313	17	4.2	5.8	7.3	6.4	6.7	3.7	2.6	7.5	9.1	10.2	10.3	9.1	95.3	26.2
JK 110A STA 12	174	157	28	6.1	6.8	8.5	8.6	9.9	5.8	7.7	9.6	9.8	11.1	9.6	9.5	87.7	55.4
JK 110B STA 12	175	310	16	12.9	14.7	14.6	13.4	14	12	14.2	12.3	12.2	14.5	11	8.8	55.2	6.8
JK 111 STA 13	176	299	18	2.6	3.2	6.3	3.2	11.3	4.4	8.1	8.5	3.3	9.6	6.2	10.7	82.4	81.6
JK 112 STA 14	177	272	17	4	6.2	6.6	5.8	7.7	7.1	5.6	10.1	8.4	10.1	9.7	10.3	93.8	72.8
JK 113 STA 15	178	351	4	12	10.6	12.1	13.7	15.2	16.2	8.9	16.1	1.8	13.7	16.4	12.8	78.7	20.2
JK 114 STA 16	179	263	19	10.4	11.8	11.1	11.5	11.7	11.1	7.8	12.8	9.8	12.3	16.4	10.3	87.5	31.2
JK 115 STA 20	180	262	20	3.8	4.8	7.3	5.4	8.6	3.5	4.6	7.9	8.8	12.3	8.1	9.1	91.7	87.4
JK 116 STA 20A	181	259	12	1.5	2.2	5.9	2.3	5.5	3.9	4.1	8	9.6	10.3	2.7	9.2	95.1	94.6
JK 117 STA 21	182	234	32	7	7.3	8.2	9.6	12.5	7.1	7.9	10.6	9.5	11.8	10.8	11.2	75.3	43.6
JK 119 STA 8B	183	347	13	3.3	2.9	6.7	4.5	5.5	2.9	1.8	10.7	7.5	12.9	2.4	8	93.6	24.2
JK 120 STA 10	184	345	4	18.4	14.3	14.4	15.6	15.2	13.5	5.8	17.3	10.8	14.8	19.8	12.6	98.3	8.1
JK 122 STA 9A	185	267	11	2.8	2.1	6	2.9	5.2	4.6	1.7	9.7	7.2	12.5	3.9	9.3	92.7	43.4
JK 123 STA 17	186	297	34	14	13.5	12.3	14.9	15.3	16.2	12.4	13.3	8.6	13.8	15.7	11.6	46.6	6.1
JK 124 STA 19A	187	367	19	17.5	15.9	14	15.9	16.2	17.3	7.6	16.8	13.7	14.6	18.8	12.2	45.4	6.3
JK 125 STA 20	188	309	26	10.3	14	10.1	14.3	11.8	11.5	7.2	13	11.4	10.7	12.3	8.1	64.2	13.6
JK 126 STA 2	189	280	19	6.1	7	6.5	3.2	6.8	5.9	4.6	9.5	12.3	11.3	16.1	9.5	98.7	70
JK 127 STA 3	190	313	16	4.2	2.6	7.2	3.9	9.6	4.4	9.4	9.5	6.9	8.4	7.9	7.1	89.6	87.5
JK 128 STA 4	191	164	19	6.7	9.4	8.4	9.6	8.1	6.3	5.9	9.7	7	10.3	12.9	8	95.1	48.2
JK 129 STA 5	192	281	12	1.6	1.5	6.1	2.3	7.9	3.5	8.8	7.3	2.6	8	3.3	7.3	79.4	93.6
JK 130 STA 6	193	347	16	4.4	2.9	6.7	9.2	9	1.8	5.9	6.9	2	8.5	7.9	7	80.1	36.6
JK 131 STA 8B	194	347	18	3.2	2.9	6.5	4.5	7.5	3.9	7.4	7.8	5.5	8.5	7.8	8	87.1	82.1
JK 132 STA 12	195	301	12	6	13.1	8.9	12.7	10.5	4.4	5.9	7.2	2.1	9.3	10.6	6.8	85.7	17.3
JK 133 STA 16	196	308	32	10.8	13.5	10.9	11.8	12.8	11.1	8.9	10.7	9.3	12.3	13.7	8.9	63.7	18.5
JK 134 STA 17	197	313	32	15.1	14.1	10.9	16.2	14.2	14.9	11.3	13.1	14.3	12	16.8	10.8	59.5	3.5
JK 135 STA 19	198	375	31	15.3	14	12.3	15.3	17	16.4	11.5	15	15.2	14.7	17.8	12.6	45	5.3
JK 136 STA 20A	199	300	16	2	2	6.2	1.6	7	3.5	6.3	6.2	4.1	8.3	6.8	7.7	85.6	92.3
JK 137 STA 21	200	423	18	17.6	15	14.7	17.5	16.9	17.7	8	16.1	16.8	15.3	18.9	12.7	9.9	0.5
JK 138 STA 22	201	344	21	3.3	2	6.7	4.5	7	4.6	6.1	5.7	3.7	8	6.2	7.6	75.4	89
JK 139 STA 23	202	351	24	12.3	14.4	12.3	11.8	10.2	12.4	7.1	14.2	11	11.4	15.6	10.7	82.4	2.8
JK 140 STA 24	203	338	28	14.5	14.4	13	15.3	15.4	15.6	12.9	14.3	12.4	13.6	16.5	11.9	55.8	7.1
JK 141 STA 24	204	328	26	16.3	13.7	12.5	15.9	15.7	17	13.9	15.5	15.1	13.8	16.6	11.5	43.2	3.4
JK 142 STA 26(1)	205	431	29	13.3	13.3	12	14	13.4	14.3	12.2	16.5	11.4	12.6	16.1	11.6	62.2	9.5
JK 143 STA 26(2)	206	363	11	16	17.5	13.5	18.4	13.2	17	14.4	15.4	12.3	14.1	7.9	12.5	89.5	0.3
JK 144 STA 26 (3)	207	407	28	14.6	15.1	12.9	14.3	13.1	14.3	11.5	16.3	12.7	13.1	17.8	12.1	54.6	4.4

All of the species listed on Cholnoky's diatom analysis sheets could be entered into the OMNIDIA database with the exception of *Cymbella bengalensis*. There is no acronym for this species or any of its synonyms in OMNIDIA v3.1. However, *C. bengalensis* was present in only two samples (5 and 7 individuals respectively), and its absence from the index calculation is not considered to exert an influence on the final score in any way. J. Prygiel (pers. comm.) cautions that when dominant species are not included in the index calculation then one runs the risk of incorrect assessments, however, this does not hold true for sub-dominant species.

It is interesting to compare the diatom-index data with the diagram that Cholnoky drew of the Jukskei-Crocodile system, based on his diatom analysis at the same sampling stations he used in 1956/7 (see Figure 2). Cholnoky constructed the diagram based on the relative abundance of two diatom species, *Nitzschia palea* and *Sellaphora (Navicula) semminulum*, both species known for their tolerance to organic pollution (especially nitrogenous compounds). It is evident from a comparison of Figure 2 with Table 2 that the sampling stations on Cholnoky's diagram having the lowest percentage of *N. palea* and *S. seminulum* in the communities (hence higher quality water) have the highest scores generated from modern diatom indices. Cholnoky's diagram also agrees with the calculated percentage of pollution tolerant diatoms using the TDI index of Kelly (1995; Table 2).

Figure 2: *Abundance of the pollution tolerant diatom species* Nitzschia palea *(black) and* Sellaphora (Navicula) seminulum *(grey) in the Jukskei-Crocodile river system (Cholnoky, 1968).*



It can also be deduced from Table 2 that some of the sampling stations have a diatom-index score that is representative of pristine water quality. The author of this report considers this to be an erroneous assessment. If the samples classified as pristine (highlighted in bright green in the first column of Table 2) are related to the abundance sheets in Appendix 2 it will be noted that all these sites have high abundance of *Achananthidium minutissimum* (*Achnanthes minutissima*). At several of the sites with index scores indicating pristine conditions, there is a high abundance of *Gomphonema parvulum*. *G. parvulum* is known to be tolerant to several forms of pollution and indicates disturbed conditions, Cholnocky was later to add *G. parvulum* to his list of pollution tolerant species (Cholnoky, 1970). The occurrence of *G. parvulum* in a community dominated by *A. minutissimum* alerts one to the fact that there is at least moderate pollution at the site. How then can *A. minutissimum* be dominant as it is intolerant to even slight pollution? From a re-examination of the original material it can be seen that although *A. minutissimum* composed some portion of the diatom community the additional portion of the diatoms recorded as *A. minutissimum* are in fact *A. saprophilum* (*Achnanthes minutissimum* var. *saprophilum*). This species or variety cannot have been noted as separate to *A. minitissimum* in 1956 or 1957 as it was only described in 1982 (Kobayasi & Mayama, 1982). *A. saprophilum* was described originally from severely polluted rivers in the vicinity of Tokyo. The valve morphology of this taxon closely resembles the nominate variety (i.e. *A. minutissimum*). *A. saprophilum* has a very high tolerance to organic pollution and often occurs as the dominant taxon even in polysaprobic water. Schoeman (1973) found in a small number of his samples from Lesotho that *A. minutissimum* was present in large numbers (20-55%) together with a large number of nitrogen heterotrophic (i.e. pollution tolerant) species (20-46%). I would like to suggest that perhaps the species encountered by Schoeman in these samples was not *A. minutissimum* at all but rather the pollution tolerant *A. saprophilum*. If the TEM illustrations of *A. minutissimum* from Pretoria salt pan found in the work of Schoeman & Ashton (1982) are compared with those presented by Mayama & Kobayasi (1989), it can be seen that several of the photographs depicting *A. minutissimum* are undoubtedly *A. saprophilum* (see Fig. 3).

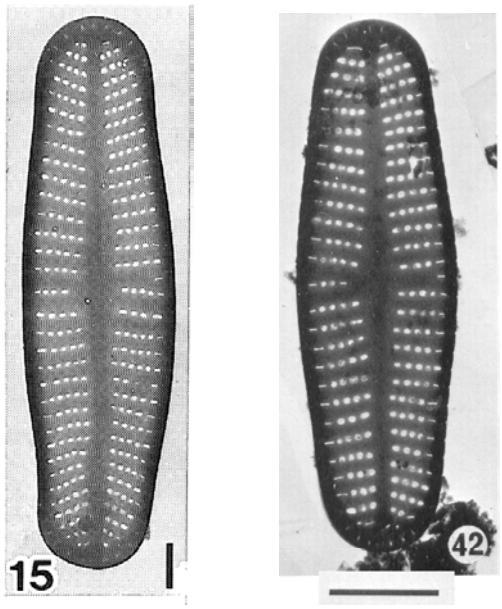


Figure 3: 15; Acananthidium saprophilum *(Mayama & Kobayasi, 1989), scale bar = 1µm.* 42; Achnanthidium minutissimum *(Schoeman & Ashton, 1982), scale bar = 2µm.*

The error of identification between the two species is very easy to remedy, in the samples where the identification is doubtful the ratio between *A. minutissum* and *A. saprophilum* needs to be calculated. Once the diatom analysis has been corrected in this way, the data can once more be used in accurate historical ecological assessments. It is important to do this when diatom indices are being used for assessment as the two species have different tolerance values in the diatom index equation. On re-counting the abundance of *A. minutissimum,* and finding that a percentage of these valves are in fact *A. saprophilum,* the resultant relative abundance when used in the diatom index calculation lowers the index score by several points and in some cases transfers the sample to a lower water quality class (Table 1) as demonstrated in Table 3.

TABLE 3

Diatom index scores before and after reclassification of *Achnanthidium minutissimum* and *A. saprophilum*

	Index score with all species as *A. minutissimum*				
Site	**SPI**	**SHE**	**BDI**	**WAT**	**ROT**
JK 141 STA 24	16.3	15.9	15.5	16.6	15.7
JK 143 STA 26 (2)	16	18.4	15.4	7.9	13.2
JK 144 STA 26 (3)	14.6	14.3	16.3	17.8	13.1
	Index score after splitting of *A. minutissimum* and *A. saprophilum*				
JK 141 STA 24	15.5	13.7	13.4	14.2	13.4
JK 143 STA 26 (2)	14.9	16.8	12	5.8	9.3
JK 144 STA 26 (3)	13.6	12.4	14.9	15.6	11.3

Of the specific indices, the Eutrophication and Pollution Index (EPI) shows that most of the sites are eutrophic, with several falling into the class meso-eutrophic and others which could be classified as mesotrophic, no sites warrant the classification of oligotrophic. The Trophic Diatom Index (TDI) is included for the %PT valves as the index itself was developed for monitoring sewage outfall (PO_4-P concentrations) and not organic pollution or general stream quality. The index cannot be used accurately if the %PT valves is above 20. The % PT valves does, however, demonstrate that for the most part the Jukskei-Crokodile system was subject (as it still is) to high loading with organic pollutants. The Generic diatom Index or GDI is separated from the other indices presented in Table 2, as it has a lower resolution being based only on the genus of the taxa composing the diatom communities. Although far simpler to use than indices that rely on species level identification it seems to yield comparable results in most cases.

The diatom index scores were correlated to the average water quality variables at 10 of the sites for which average annual data was available and the results are presented in Table4.

TABLE 4

Correlation between water quality variables at selected sites in the Jukskei-Crocodile system and diatom index scores generated from re-analysis of historical data sheets
Marked correlations are significant at p < 0.05
***n* =10 (Casewise deletion of missing data)**

	SPI	DES	L&M	SHE	ROT	CEC	APDI	BDI	GDI	SLA	WAT	EPI
pH	0.67	0.64	0.77	..	0.64	0.74	..	0.81	..	0.64	0.77	0.77
EC	..	-0.68	..	..	-0.67	..	-0.86	..	-0.81	..	..	..
Temp.	0.68	0.64	0.69	..	0.73	0.76	..	0.72	0.35	0.86	0.69	0.81
COD	..	..	..	..	..	..	-0.89	..	-0.65	..	..	..
NH_4-N	-0.84	-0.86	-0.86	-0.79	-0.84	-0.82	-0.78	-0.85	..	-0.69	-0.86	-0.76
NO_2-N	-0.78	-0.81	-0.76	-0.74	-0.79	-0.74	-0.85	-0.74	..	..	-0.77	-0.64
NO_3-N	..	..	..	..	..	..	-0.76	..	-0.73	..	..	..
TKN-N	-0.82	-0.85	-0.82	-0.79	-0.83	-0.78	-0.87	-0.78	..	-0.65	-0.82	-0.68
PO_4-P	..	..	..	..	..	..	..	..	..	..	..	..
Total P	..	..	..	..	..	..	..	..	..	..	..	..
Na^+	..	-0.65	..	..	-0.64	..	-0.87	..	-0.76	..	..	..
K^+	..	..	..	..	..	..	..	..	-0.75	..	..	..
Ca^{2+}	..	..	..	..	..	..	-0.80	..	-0.86	..	..	..
Mg^{2+}	-0.74	-0.75	-0.66	-0.74	-0.78	-0.70	..	-0.67	..	-0.69	-0.68	..
SO_4^-	-0.77	-0.80	-0.73	-0.74	-0.80	-0.73	-0.90	-0.68	-0.71	-0.66	-0.74	..
Cl^-	-0.69	-0.73	-0.66	-0.67	-0.73	-0.66	-0.90	..	-0.74	..	-0.67	..

Variables were measured in $mg.l^{-1}$ except for temperature ($^{\circ}C$), electrical conductivity ($\mu S.cm^{-1}$)

It is interesting to note that the strongest correlations are between nitrogen and the diatom index scores, with no significant correlation to either orthophosphate-phosphorus or to Total phosphate. This would suggest that the major impact in the system is from waste containing nitrogenous compounds. This finding is in agreement with Cholnoky's assessment at the time, showing that at some sites almost all of the diatom species encountered were tolerant to nitrogenous pollution. Other strong correlations exist between the diatom index scores and electrical conductivity and the major ions. This correlation between ionic compounds matches a descriptive assessment of the Jukskei-Crocodile system as being heavily impacted by industrial and agricultural run-off and effluents.

4. SUMMARY AND CONCLUSIONS

In general it can be concluded from the preceding sections that the diatom analysis sheets authored by Dr Cholnoky constitute a valuable resource from which accurate inferences may be drawn concerning the past ecological status of the rivers and streams for which data exists in the SA Diatom Collection. The classification of the various sampling stations carried out by Cholnoky yields similar results to those gained by using modern diatom indices. The use of diatom indices relies on information stored

in a database rather than the operative's own knowledge, and thus provides a relatively rapid assessment technique cf those employed by Cholnoky half a century ago.

The diatom analysis sheets contained in the SA Diatom Collection are likely to prove to be a valuable resource for obtaining historical (baseline) data against which present day and/or future environmental assessments may be compared, and provide a measure of either degradation of restoration since the time of original sampling. OMNIDIA proves to be both useful as a database and as a tool for calculating diatom index scores.

It has been demonstrated that the species listed on the diatom analysis sheets can be related to current nomenclature (synonyms) (see Appendix 1 and 2) when necessary, and entered into the electronic database OMNIDIA. The diatom analysis sheets provide enough data for the calculation of accurate diatom index scores. Results generated from diatom analysis sheets with a population count of less than 300 should be regarded with caution.

Besides the difficulties encountered caused by the identification of *Achnanthidium minutissimum,* the species data and identifications contained in the SA Diatom Collection are of a quality sufficient to support the generation of accurate, high confidence results that support the formulation of ecologically-based inferences on ecosystem condition.

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APPENDIX 1: OMNIDIA TAXON CODES

TAXON	ACRONYM
***Achnanthes amoena* Hustedt**	AAMO
Achnanthidium exiguum (Grunow) Czarn. ***Achnanthes exigua* Grunow *in* Cleve & Grun.**	AEXG
Achnanthidium exiguum var.*heterovalva* (Krasske) Czarn. ***Achnanthes exigua* var.*heterovalva* Krasske**	AEHE
Achnanthidium minutissimum (Kütz.) Czar ***Achnanthes minutissima* Kütz.**	ADMI
Achnathidium microcephalum (Kütz.) *vide* Rabenh. ***Achnanthes microcephala* (Kutzing) Grun**.	AMIC
***Amphipleura pellucida* Kütz.**	APEL
***Amphora coffeaeformis* (Agardh) Kütz.**	ACOF
Amphora montana Krasske ***Amphora submontana* Hustedt**	AMMO
***Amphora ovalis* (Kütz.) Kütz.**	AOVA
Amphora pediculus (Kütz.) Grun. **Amphora ovalis var. perdiculus (Kütz.) Van Heurk**	APED
***Amphora veneta* Kütz.**	AVEN
***Anomoeoneis sphaerophora* (Ehr.) Pfitzer**	ASPH
Aulacoseira granulata (Ehr.) Simonsen ***Melosira granulata* (Ehr.) Ralfs**	AUGR
Aulacoseira granulata var. *angustissima* (O.Müll.) Simonsen ***Melosira granulata* var. *angustissima* O.Müll.**	AUGA
Aulacoseira italica (Ehr.) Simonsen ***Melosira italica* (Ehrenb.) Kütz.**	AUIT
Brachysira vitrea (Grunow) Ross *in* Hartley ***Anamoneis exilis* (Kütz.) Cleve**	BVIT
***Caloneis aequatorialis* Hustedt**	CAQT
***Caloneis bacillum* (Grun.) Cleve**	CBAC
***Caloneis schumanniana* var. *biconstricta* (Grunn) Reichert**	CSBI
Caloneis silicula (Ehr.)Cleve ***Caloneis ventricosa* (Ehr. Donkin) Meister**	CSIL
***Cocconeis pediculus* Ehr.**	CPED
***Cocconeis placentula* Ehr.**	CPLA
Craticula ambigua (Ehrenberg) Mann *in* Round, Crawford & Mann	CAMB

***Navicula cuspidata* var*. ambigua* (Ehr.) Cleve**	
Craticula cuspidata (Kütz.) Mann *in* Round, Crawford & Mann Navicula cuspidata *Kütz.*	CRCU
***Cyclotella meneghiniana* Kütz.**	CMEN
***Cyclotella operculata* (Agardh) Kütz.**	COPE
***Cyclotella stelligera* Cleve *et* Grun. *in* Van Heurk**	CSTE
***Cymatopleura solea* (Bréb.) W.Smith** *Cymatopleura librile* (Ehenberg) Pantocsek	CSOL
***Cymbella amphicephala* Naegeli**	CAPH
***Cymbella amphicephala* var. *hercynica* (A.Schmidt) Cleve**	CAHE
***Cymbella aspera* (Ehr.) Cleve**	CASP
***Cymbella begalensis* Cleve**	
***Cymbella cistula* (Ehr.)Kirchner**	CCIS
***Cymbella kappii* Cholnoky**	CAFF
***Cymbella kolbei* Hustedt**	CKOL
***Cymbella turgida* Gregory**	CTUR
Diadesmis confervacea Kütz. ***Navicula confervaceae* (Kütz.) Grunow**	DCOF
Diadesmis contenta var. *biceps* (Grun. *ex* V.Heurk) Mann *in* Round, Crawford & Mann ***Navicula contenta* Grun.**	DCBI
***Diploneis ovalis* (Hilse) Cleve**	DOVA
***Diploneis smithii* var. pumila (Grun.) Hustedt**	DSPU
***Diploneis subovalis* Cleve**	DSBO
Encyonema minutum *(Hilse* in *Rabenhorst) Mann* in *Round, Crawford & Mann*	ENMI
Cymbella minuta Hilse *ex* Rabenhorst ***Cymbella ventricosa* Kützing**	
Encyonema muelleri (Hustedt) Mann in Round, Crawford & Mann ***Cymbella muelleri* Hustedt**	ENMU
Encyonopsis aequalis (W.Smith) Krammer ***Cymbella aequalis* W.Smith**	EAQL
Encyonopsis microcephala (Grun.) Krammer ***Cymbella microcephala* Grun**.	ENCM
Eolimna minima *(Grun.) Lange-Bertalot*	EOMI
***Navicula minima* Grun.**	
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	
Navicula subminuscula Manguin	ESBM
Navicula frugalis Hustedt	

***Navicula perparva* Hustedt**	
Fallacia pygmaea (Kütz.) Stickle & Mann *in* Round, Crawford & Mann ***Navicula pygmaea* Kütz.**	FPYG
Fragilaria capucina Desmazieres ***Synedra rumpens* Kütz.**	FCAP
***Fragilaria capucina* var. *acuta* (Ehr.) Rabenhorst**	FCAC
Fragilaria capucina var. *vaucheriae* (Kütz.) Lange-Bertalot ***Synedra vaucheriae* Kütz.**	FCVA
***Fragilaria construens* (Ehr.) Grunow**	FCON
Fragilaria delicatissima (W.Smith) Lange-Bertlot ***Synedra acus* var. *radians* (Kütz.) Hustedt**	FDEL
***Frustulia rhomboides* (Ehr.) De Toni**	FRHO
***Frustulia vulgaris* (Thwaites) De Toni**	FVUL
*Geissleria decussis (Ø*strup) Lange-Bertalot & Metzeltin *Navicula decussis Ø*strup *Navicula canoris* Hohn & Hellerman ***Navicula exiguiformis* Hustedt**	GDEC
***Gomphonema clavatum* Ehr.**	GCLA
***Gomphonema clevei* Fricke**	GCLE
***Gomphonema gracile* var. *subcapitata* Gandhi**	GGSC
***Gomphonema gracile* Ehr.**	GGRA
***Gomphonema gracile* var*. lanceolata* (Kütz.) Cleve**	GGLA
***Gomphonema parvulum* Kütz.**	GPAR
Gomphonema pumilum (Grun.) Reichardt & Lange-Bertalot ***Gomphonema intricatum* var. *pumila* Grun. *in* V.Heurck**	GPUM
***Gomphonema schweickerdtii* Cholnoky**	GSCH
***Gomphonema truncatum* Ehr.**	GTRU
Gomphonema truncatum var. capitatum (Ehr.) Patrick	GTCA
***Gyrosigma nodiferum* (Grunow) Reimer** *Gyrosigma spencerii* var. *nodifera* (Grun.) Cleve	GNOD
***Gyrosigma scalproides* (Rabenhorst) Cleve**	GSCA
***Gyrosigma spencerii* (Quekett) Griffith**	GSPE
***Hantzschia amphioxys* (Ehr.) Grun*. in* Cleve & Grun.**	HAMP
***Hantzschia amphioxys* var. *africana* Hustedt**	HAAF
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski ***Navicula capitata* Ehrenberg**	HCAP

Hippodonta hungarica *(Grunow) Lange-Bertalot & Metzeltin* in *Witkowski* ***Navicula hungarica* Grunow**	HHUN
Kobayasiella subtilissima (Cleve) Lange-Bertalot ***Navicula subtilissima* Cleve**	KOSU
Lemnicola hungarica (Grun.) Round & Basson ***Achnanthes hungarica* Grun. *in* Cleve & Grun.**	LHUN
Luticola mutica (Kütz.) Mann *in* Round, Crawford & Mann ***Navicula mutica* Kütz.**	LMUT
Luticola nivalis (Ehr.) Mann *in* Round, Crawford & Mann ***Navicula mutica* var. *nivalis* (Ehr.) Hustedt**	LNIV
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot *Navicula atomus* var. *permitis* (Hustedt) Lange-Bertalot ***Navicula muralis* Grunow**	NAPE
***Melosira varians* Agardh**	MVAR
***Navicula bryophila* Boye Petersen**	NBRY
Navicula capitatoradiata Germain ***Navicula cryptocephala* var*. intermedia* Grunow**	NCPR
***Navicula cincta* (Ehr.) Ralfs *in* Pritchard**	NCIN
***Navicula cryptocephala* Kütz.**	NCRY
Navicula cryptotenella Lange-Bertalot ***Navicula radiosa* var. *tenella* (Bréb.) Cleve & Möll.**	NCTE
***Navicula gregaria* Donkin**	NGRE
Navicula kotschyi Grunow ***Navicula grimmei* Krasske**	NKOT
Navicula lanceolata (Agardh) Ehr. ***Navicula viridula* var. *avenacea* (Bréb. *in* Grun.) V.Heurk**	NLAN
***Navicula menisculus* Schumann**	NMEN
***Navicula menisculus* var. *upsaliensis* Grun.**	NMUP
***Navicula minusculoides* Hustedt**	NMNO
***Navicula muticoides* Hustedt**	NMTD
***Navicula radiosa* Kütz.**	NRAD
***Navicula rhynchocephala* Kütz.**	NRHY
***Navicula rostellata* Kütz.**	NROS
***Navicula schroeteri* Meister**	NSHR
***Navicula tenelloides* Hustedt**	NTEN
***Navicula zanoni* Hustedt**	NZAN
***Neidium affine* (Ehrenberg)Pfitzer**	NEAF

***Nitzschia acicularis* (Kütz.) W.M.Smith**	NACI
Nitzschia acidoclinata Lange-Bertalot ***Nitzschia perminuta* (Grunow) M. Peragallo**	NACD
***Nitzschia amphibia* Grunow**	NAMP
Nitzschia capitellata Hustedt *in* A.Schmidt *et al*. ***Nitzschia allanssoni* Cholnoky**	NCPL
***Nitzschia clausii* Hantzsch**	NCLA
***Nitzschia communis* Rabenhorst**	NCOM
***Nitzschia debilis* (Arnott) Grun.**	NDEB
***Nitzschia denticula* Grun.**	NDEN
***Nitzschia desertorum* Hustedt**	NDES
***Nitzschia dissipata* (Kütz.) Grun.**	NDIS
***Nitzschia elliptica* Hustedt**	NELP
***Nitzschia epiphytica* O.Müll. *sensu* Hustedt 1949**	NEPI
***Nitzschia fonticola* Grun. *in* Cleve & Möll.**	NFON
Nitzschia frustulum (Kütz.) Grun. Nitzschia frustulum var. perpussila	NIFR
***Nitzschia intermedia* Hantzsch *in* Cleve**	NINT
***Nitzschia linearis* (Agardh) W.M.Smith**	NLIN
***Nitzschia microcephala* Grun. *in* Cleve**	NMIC
Nitzschia nana Grunow *in* V.Heurck ***Nitzschia ignorata* Krasske**	NNAN
***Nitzschia palea* (Kütz.) W.Smith**	NPAL
Nitzschia paleacea (Grun.) Grunow *in* V.Heurk ***Nitzschia bacata* Hustedt**	NPAE
***Nitzschia parvuloides* Cholnoky**	NPVL
Nitzschia pusilla (Kutzing)Grunow ***Nitzschia kuetzingiana* Hilse**	NIPU
***Nitzschia sigma* (Kütz.) W.M.Smith**	NSIG
***Nitzschia sinuata* var. tabellaria (Grun.) Grun.**	NSIT
Nitzschia solgensis Cleve-Euler ***Nitzschia interurupta* (Reichelt) Hustedt**	NSOL
***Nitzschia tropica* Hustedt**	NTRO
Nitzschia umbonata (Ehrenberg) Lange-Bertalot ***Nitzschia thermalis* (Kütz.) Auerswald**	NUMB
***Pinnularia eburnea* (Carlson) Zanon**	PEBU
***Pinnularia gibba* Ehr.**	PGIB
***Pinnularia gibba* var. sancta (Grun.) Meister**	PGSC

***Pinnularia interrupta* W.M.Smith**	PINT
***Pinnularia viridis* (Nitzsch) Ehr.**	PVIR
Placoneis dicephala (W.Smith) Mereschkowsky *Navicula dicephala* (Ehr.) W.Smith ***Navicula dicephala* var. *neglecta* (Krasske) Hustedt**	PDIC
Planothidium lanceolatum (Brébisson) Round & Bukhitiyarova ***Achnanthes lanceolata* (Brébisson)Grun.**	PTLA
***Rhopalodia gibba* (Ehr.) O.Müll.**	RGIB
***Rhopalodia gibberula* (Ehrenberg) O.Müll.**	RGBL
Sellaphora pupula (Kutzing) Mereschkowksky *Navicula pupula* Kütz. ***Navicula nyassensis* O.Müll.** ***Navicula nyassensis* var. *minor***	SPUP
Sellaphora seminulum (Grun.) Mann ***Navicula seminulum* Grunow**	SSEM
Stauroneis anceps Ehr.	STAN
Staurosira construens var. *venter* (Ehr.) Hamilton ***Fragilaria constuens* f. *venter* (Ehr.) Hustedt**	SCVE
Staurosirella pinnata (Ehr.) Williams & Round ***Fragilaria pinnata* Ehr.**	SPIN
***Stephanodiscus hantzschii* Grunow *in* Cleve**	SHAN
***Surirella angusta* Kutzing**	SANG
***Surirella ovalis* Bréb.**	SOVI
***Surirella tenera* Gregory**	SUTE
***Synedra ulna* (Nitzsch) Ehr.**	SULN
***Tabellaria fenestrata* (Lyngbye) Kütz.**	TFEN
Tryblionella apiculata Gregory ***Nitzschia apiculata* (Gregory) Grun.**	TAPI
Tryblionella hungarica (Grunow) Mann *in* Round, Crawford & Mann ***Nitzschia hungarica* Grunow**	THUN
Tryblionella levidensis W. Smith ***Nitzschia levidensis* (W.Smith) Grun. *in* V.Heurk**	TLEV
Tryblionella victoriae Grunow ***Nitzschia levidensis* var. *victoriae* Grun.**	TVIC

APPENDIX 2: JUKSKEI RIVER DIATOM (RAW) DATA

	JK 1 STA 8A	JK 2 STA 8A	JK3 STA 8A	JK 5 STA 6	JK 6 STA 6	JK 7 STA 6	JK 8 STA 5	JK 9 STA 4	JK10	JK 11
Achnanthidium exiguum						1			1	
Achnanthidium minutissimum				2		89				
Amphora montana	1		1				1	1		1
Anomoeoneis sphaerophora					1				1	
Caloneis aequatorialis				11						
Craticula ambigua							1	4		
Craticula cuspidata										2
Cyclotella meneghiniana									4	3
Cymbella kolbei						54				
Cymbella turgida						12			1	
Diadesmis confervacea										2
Encyonema minutum							1	1	1	
Eolimna subminuscula								1		
Fallacia pygmaea	3	4	1				2	2	2	
Frustulia vulgaris			1							
Gomphonema gracile							1			
Gomphonema parvulum	3	2	3	72	3	57	1	4	20	9
Hippodonta capitata	1							3		
Luticola nivalis			1							
Mayamaea atomus var. *permitis*						7			1	
Navicula capitatoradiata						4			3	
Navicula cincta	2	4	16	2	10	3	10	2	3	8
Navicula cryptocephala	3	1	14	6	6	18	1	1	9	14
Navicula gregaria	1	2	1	76		1	1	1	2	1
Navicula lanceolata		4		1			1		3	
Navicula menisculus				3				1	1	2
Navicula minusculoides	1	1		9	7	5	3	6	18	14
Navicula pygmaea										1
Navicula rostellata	1	4	2	4	13	64	7	4	20	9
Navicula schroeteri	1	5	3	13	8	3	7	1	11	7
Navicula subminuscula				2						
Nitzschia acidoclinata						1				
Nitzschia amphibia				1						1
Nitzschia clausii				1						
Nitzschia debilis		1								
Nitzschia denticula			1							
Nitzschia desertorum	8	26	28	1	32	1	14	27	53	35
Nitzschia elliptica										4
Nitzschia frustulum			1	1		5			2	
Nitzschia microcephala										1
Nitzschia palea	528	525	219	75	449	42	446	464	249	292
Nitzschia pusilla	5	7	6		7	7	9		17	14
Nitzschia tropica										2
Nitzschia umbonata	3	9	18	2	5	2	23	14	22	33
Pinnularia gibba var.*sancta*						1			1	
Placoneis dicephala								1		
Planothidium lanceolatum				1						
Sellaphora seminulum	2	1	4	3		6	6	3	5	4
Surirella angusta	1	2		1	3			2	8	3
Surirella ovalis			2		8		1	13	7	2
Synedra ulna						2				
Tryblionella apiculata	1	1		1	2	1		1		1
Tryblionella hungarica		4	1		1	1	1	4	6	5
Tryblionella levidensis		1			1					1

	JK 128 STA 4	JK 129 STA 5	JK 130 STA 6	JK 131 STA 8B	JK 132 STA 12	JK 133 STA 16	JK 134 STA 17	JK 135 STA 19	JK 136 STA 20A	JK 137 STA 21
Achnanthidium minutissimum	8		1	3		51	72	116		272
Amphipleura pellucida						21				
Amphora montana	4			1	1	1	11			
Amphora pediculus						2				
Amphora veneta						5				
Brachysira vitrea								1		1
Caloneis aequatorialis				1			1			
Caloneis bacillum							2	3		
Craticula ambigua						1				
Cyclotella meneghiniana									1	
Cymatopleura solea						1				
Cymbella amphicephala							5			
Cymbella amphicephala var.*hercynica*						1				
Cymbella aspera								1		
Cymbella cistula						9	6	24		
Cymbella kappii								24		
Cymbella kolbei						1	8	2		21
Cymbella turgida								6		
Diadesmis confervacea						4			2	
Diploneis smithii var.*pumila*										1
Encyonema minutum						7	89	7		7
Encyonema muelleri								1		
Encyonopsis microcephala							3	115		92
Eolimna subminuscula			6						6	
Fallacia pygmaea							5	1		
Fragilaria capucina								3		
Fragilaria capucina var.*vaucheriae*								1		
Fragilaria delicatissima							1			
Frustulia vulgaris						4				
Gomphonema clavatum						1				
Gomphonema gracile								2		
Gomphonema parvulum	33	13	8	63	2	30	3	2	57	2
Gyrosigma nodiferum							1			
Hantzschia amphioxys	2									
Hippodonta capitata			1	4		1				1
Hippodonta hungarica		5								
Lemnicola hungarica									8	
Luticola mutica							1			
Mayamaea atomus var. *permitis*	2									
Navicula cincta		1			1	1	9	1		
Navicula cryptocephala	7	2	5	8	3	14	40	24		5
Navicula cryptotenella										1
Navicula gregaria	15	12	3	16	23	1			7	
Navicula kotschyi							9			
Navicula lanceolata	2		1	4			1	1		
Navicula minusculoides	12	1		16	1					
Navicula radiosa							1			
Navicula rhynchocephala						3				
Navicula rostellata						1	2	1		
Navicula schroeteri						36			1	
Navicula zanoni								6		7
Neidium affine										1
Nitzschia acicularis	1							1		
Nitzschia amphibia	1									
Nitzschia capitellata			5			5			17	
Nitzschia clausii	3									
Nitzschia communis	6									
Nitzschia constricta										
Nitzschia frustulum	4	1	6		22		2	3		
Nitzschia linearis	1					65	1	1		
Nitzschia microcephala			1						5	
Nitzschia palea	154	232	102	173	2	5	4	8	179	
Nitzschia paleacea						2	2	5		2
Nitzschia pusilla	46		197	19	241	9	6			5
Nitzschia sigma					1					
Nitzschia sinuata var.*tabellaria*								4		
Nitzschia solgensis							10	5		1

	JK 12	JK 13 STA 20	JK 14 STA 20 A	JK 15	JK 16	JK 17	JK 18	JK 19	JK 101 STA 1	JK 102 STA 2
Achnanthidium exiguum		354						1		
Achnanthidium minutissimum	1	13	119	9	10	262	28	7	75	2
Amphora montana									10	3
Amphora pediculus					1	2	1			
Aulacoseira granulata				3			2	4		
Aulacoseira granulata var. *angustissima*				425	272	17	384	374		
Brachysira vitrea				6	8	40	1	8		
Cocconeis placentula							1			
Craticula ambigua	1									
Cyclotella meneghiniana	2		14		1		3	1		
Cyclotella operculata								3		
Cyclotella stelligera				80	206	2	72	109		
Cymbella cistula						9				
Cymbella kappii						2				
Cymbella kolbei									1	
Diadesmis confervacea		16								61
Encyonema muelleri				1		8	1			
Encyonopsis microcephala				4						
Encyonopsis microcephalaum					23	202	16	18		
Fallacia pygmaea	3									
Fragilaria capucina						2	1			
Fragilaria capucina var.*vaucheriae*							1		10	
Fragilaria delicatissima						3				
Frustulia rhomboides				1						
Frustulia vulgaris					1					
Gomphonema parvulum	7	112	1		1			1	9	19
Hantzschia amphioxys									1	
Hippodonta capitata	1								2	
Luticola mutica							2			
Mayamaea atomus var. *permitis*			46							
Navicula cincta	9									
Navicula cryptocephala	8			1	2	1			8	5
Navicula gregaria	2									
Navicula lanceolata	2		1							
Navicula minusculoides	9		12							2
Navicula rostellata	8									
Navicula schroeteri	4									
Navicula zanoni					1	1		1		
Nitzschia amphibia			1							
Nitzschia capitellata						5				
Nitzschia communis									6	
Nitzschia constricta										
Nitzschia denticula						6	1	4		
Nitzschia desertorum	42		1							
Nitzschia fonticola									12	13
Nitzschia frustulum									3	
Nitzschia linearis										1
Nitzschia palea	371		271				2		126	56
Nitzschia pusilla	6	2	19		2					
Nitzschia sinuata var.*tabellaria*				2				2		
Nitzschia tropica							1			
Nitzschia umbonata	38								1	3
Pinnularia eburnea							1			
Pinnularia gibba		9	17							
Pinnularia gibba var.*sancta*										3
Pinnularia viridis		1								
Planothidium lanceolatum	1		1				1			7
Sellaphora seminulum	1		6						1	106
Staurosirella pinnata										
Surirella angusta	2									
Surirella ovalis	7									
Synedra ulna							1			
Tryblionella apiculata	1									
Tryblionella hungarica	6									

	JK 103 STA 3	JK 103B STA 3	JK 104 STA 4	JK 105 STA 5	JK 106 STA 8A	JK 106B STA 8B	JK 107A STA 9A	JK 107B STA 9B	JK 107C STA 9C	JK 108 STA 10
Achnanthidium exiguum			1							
Achnanthidium minutissimum	11	7	5	2		7	4	4	2	270
Amphora montana	3		1			1				
Amphora pediculus		1						1		
Caloneis aequatorialis		1			10	2				
Caloneis bacillum	2									
Cocconeis placentula								1	14	
Craticula cuspidata							1		1	
Cymbella kolbei						1				
Diadesmis confervacea		4	40				2	71		
Encyonema minutum	4									
Fallacia pygmaea									1	
Gomphonema gracile								2		
Gomphonema parvulum	35	31	73	119	25	37	187	52	34	31
Gyrosigma nodiferum									1	
Gyrosigma scalproides					3					
Hantzschia amphioxys		1					2			
Lemnicola hungarica								2	1	
Luticola mutica						1				
Mayamaea atomus var. *permitis*	75	3	2	89	3				14	
Navicula cincta		1	1					3		
Navicula confervacea						4			27	
Navicula cryptocephala	12	24	5	11	40	9	3	13	12	
Navicula gregaria	1	1	1		14		1	1		
Navicula lanceolata		2	1		6			1	1	
Navicula minusculoides		11	14	73	67	38	2	26	15	
Navicula muralis						4				
Navicula radiosa		1								
Navicula rhynchocephala		3								
Navicula rostellata	1	1	3		1			2		
Navicula schroeteri	6	36	6	4	112	26	2	13	17	
Navicula seminuloides		1								
Navicula tenelloides								1		
Nitzschia amphibia	2	1	4	1	5		2	9	15	
Nitzschia capitellata		3	12	1		7		3		
Nitzschia clausii		1						3	1	
Nitzschia fonticola	13	7	6	1	5	32	16	12	33	
Nitzschia frustulum	20	18	8	7	22	9	2	1		
Nitzschia palea	20	53	103	15	22	68	53	28	39	
Nitzschia umbonata	3	1	5	3	1	5	3		1	
Pinnularia gibba			1							
Pinnularia gibba var.*sancta*	2		1		2				3	
Pinnularia interrupta									2	
Planothidium lanceolatum	5	3	2	1				4	1	
Sellaphora pupula		1								
Sellaphora seminulum	30	47	27	22	5	5	36	11	52	
Stephanodiscus hantzschii							2	1		
Surirella angusta	2	2	2		1					
Surirella ovalis		1	1							
Synedra ulna						1				
Tryblionella apiculata					1					
Tryblionella hungarica		1								
Tryblionella levidensis					2					

	JK 109A STA 11	JK 109B STA 11	JK 109C STA 11	JK 110A STA 12	JK 110B STA 12	JK 111 STA 13	JK 112 STA 14	JK 113 STA 15	JK 114 STA 16	JK 115 STA 20
Achnanthidium exiguum	2	3		3						
Achnanthidium minutissimum	4	11	3	6	6	6	23	278	102	2
Amphora montana		2		1						
Amphora pediculus					2				3	
Amphora veneta									9	
Caloneis aequatorialis					6					
Cocconeis placentula				2	243				8	
Cymbella kappii						1				
Diadesmis confervacea	9	43		27			52			38
Encyonema minutum				1		1			1	1
Encyonopsis microcephala				1						
Fragilaria capucina var. *vaucheriae*			1							1
Gomphonema gracile				1			1		1	
Gomphonema gracile var.*lanceolata*	3	7								
Gomphonema gracile var.*subcapitata*										5
Gomphonema parvulum	31	86	35	17	6	34	65	4	42	35
Hantzschia amphioxys var. *africana*				1			1			
Lemnicola hungarica	122	29	80							
Luticola nivalis			1				2			
Mayamaea atomus var. *permitis*		5	2	3	2	4	14			
Navicula cincta									3	2
Navicula confervacea			32							
Navicula cryptocephala	6	15	8	18	6	9	9	2	26	1
Navicula gregaria				1					3	2
Navicula minusculoides	16	4	12	8	6	1	5		6	23
Navicula muralis										60
Navicula radiosa						2				
Navicula rostellata		2		2					3	2
Navicula schroeteri				9	14	4			21	2
Navicula tenelloides				1						
Nitzschia amphibia	14	6	9	4	6	2	3			
Nitzschia capitellata		3	2	1						
Nitzschia clausii		4								
Nitzschia fonticola		4			1	19	11			
Nitzschia frustulum				4	5	2			16	15
Nitzschia intermedia					1					
Nitzschia linearis		1				1				
Nitzschia palea	26	59	18	23	2	201	52	67	14	54
Nitzschia parvuloides									2	
Nitzschia umbonata	7	13	13	3		2	10			1
Pinnularia eburnea				4						
Pinnularia gibba var.*sancta*	2			2	2		2			
Pinnularia interrupta				1						
Planothidium lanceolatum	1	1	4	5	2	5	1		1	1
Rhopalodia gibba						1				
Sellaphora pupula		1								
Sellaphora seminulum	106	38	89	6		4	17			15
Stauroneis anceps				2						
Staurosira construens var *venter*							4			
Staurosirella pinnata			2							
Stephanodiscus hantzschii		1	2							
Synedra ulna									1	1
Tryblionella apiculata									1	
Tryblionella hungarica										1

	JK 116 STA 20A	JK 117 STA 21	JK 119 STA 8B	JK 120 STA 10	JK 122 STA 9A	JK 123 STA 17	JK 124 STA 19A	JK 125 STA 20	JK 126 STA 2	JK 127 STA 3
Achnanthes amoena			1							
Achnanthes exigua var.*heterovalvum*					1					
Achnanthes microcephala							31	24		
Achnanthidium exiguum		9								
Achnanthidium minutissimum	1	22		317	1	118	246	17	10	2
Amphora coffeaeformis								12		
Amphora montana									1	
Aulacoseira italica		42								
Brachysira vitrea						5	18	1		
Caloneis schumanniana							1			
Caloneis silicula		3								
Cyclotella meneghiniana		3			1			1		
Cymatopleura solea						3				
Cymbella amphicephala		3				1				
Cymbella cistula						3				
Cymbella kappii							1			
Cymbella kolbei						3	1	53		
Cymbella microcephala		3				29		1		
Diadesmis confervacea	163	20						36	8	
Diadesmis contenta var. *biceps*							1			
Encyonema minutum	1	1						29		
Encyonopsis aequalis						1				
Eolimna subminuscula			2							
Fallacia pygmaea						2				
Fragilaria capucina							4		5	
Fragilaria capucina var.*acuta*									1	
Fragilaria capucina var.*vaucheriae*		2								
Frustulia vulgaris									2	
Gomphonema clevei						1				
Gomphonema gracile							11			
Gomphonema parvulum	19	14	3	23	36	3	20	4	191	49
Gomphonema truncatum							2			
Gyrosigma nodiferum								1		
Hantzschia amphioxys		1								
Hantzschia amphioxys var. *africana*									1	
Kobayasiella subtilissima						8				
Mayamaea atomus var. *permitis*	10	3	8							2
Navicula capitatoradiata		12				1				
Navicula cincta		2				2				
Navicula cryptocephala			13		3	20	12	34	2	15
Navicula gregaria									1	77
Navicula kotschyi						1		2		
Navicula lanceolata						1				1
Navicula minusculoides	2	9	4		1				2	1
Navicula radiosa						10				
Navicula rhynchocephala		1								
Navicula rostellata		1				1	1	2		1
Navicula schroeteri	1									
Navicula tenelloides		2				1		2		
Navicula zanoni		1								
Nitzschia acicularis						1		6		
Nitzschia amphibia	1	2							1	
Nitzschia capitellata						2				
Nitzschia clausii								1		
Nitzschia dissipata								1		
Nitzschia fonticola		2								
Nitzschia frustulum			5	1		4		2		
Nitzschia linearis						7				1
Nitzschia nana								2		
Nitzschia palea	51	54	63	4	79	7	2	14	1	143
Nitzschia paleacea						4	2			
Nitzschia perminuta							2			
Nitzschia pusilla			7			3	9	47	7	
Nitzschia sigma								13		
Nitzschia solgensis						43		1		
Nitzschia umbonata		2	1						1	1
Pinnularia eburnea		2								

	JK 138 STA 22	JK 139 STA 23	JK 140 STA 24	JK 141 STA 24	JK 142 STA 26(1)	JK 143 STA 26(2)	JK 144 STA 26 (3)	JK 145 STA 1	JK 146 STA 2	JK 147 STA 3
Achnanthidium exiguum	1	5					1			
Achnanthidium minutissimum	4	107	104	106	103	66	83	304	1	12
Amphipleura pellucida			1							
Amphora montana		2							3	4
Amphora ovalis		1								
Amphora pediculus					23	40	28			
Aulacoseira granulata var.angustissima		1			1		2			
Brachysira vitrea							2			
Caloneis bacillum		3	1							
Cocconeis pediculus		23	38	6	6	1	1			
Cocconeis placentula		1	12	2						
Cyclotella stelligera					81	6	47			
Cymbella amphicephala			1	4						
Cymbella bengalensis			7	5						
Cymbella cistula			2	2	1		4			
Cymbella cuspidata	2									
Cymbella kappii			22		2		15			
Cymbella kolbei	1		1	44	2		2			
Diadesmis confervacea										10
Diploneis ovalis		1								
Encyonema minutum			8	2						
Encyonema muelleri				2	1					
Encyonopsis microcephala	1	1	8	41	16		12			
Eolimna minima										1
Fallacia pygmaea	4	1								
Fragilaria capucina var.vaucheriae				2	53	10	122			
Frustulia vulgaris				6						
Gomphonema clevei			3	9	1					
Gomphonema gracile							1			
Gomphonema parvulum	3	2	19	7	5		2		62	41
Gomphonema pumilum			2							
Gomphonema schweickerdtii							1			
Gomphonema truncatum var.capitatum		1	1	1	1					
Gyrosigma scalproides			1	1						
Gyrosigma spencerii		2	1			1				
Hippodonta capitata		68								
Mayamaea atomus var. permitis	2							4	17	
Melosira varians							2			
Navicula bryophila			3	7						
Navicula cincta				1	12		1			
Navicula cryptocephala	1	40	9	38	7	2	6			8
Navicula gregaria	6									1
Navicula lanceolata			2							
Navicula menisculus var.upsaliensis					8		17			
Navicula minima				4						
Navicula minusculoides	18								2	9
Navicula muticoides										1
Navicula radiosa					1					
Navicula rhynchocephala					1					
Navicula rostellata	5	16			5		3			2
Navicula schroeteri			7	2						
Navicula subminuscula									2	20
Navicula zanoni			61	20	41	2	30			
Nitzschia amphibia		6					1			7
Nitzschia capitellata					2		1			
Nitzschia clausii										9
Nitzschia denticula					3	1	4			
Nitzschia desertorum										2
Nitzschia dissipata			1	2						
Nitzschia epiphytica						10	2			
Nitzschia fonticola	9	4	1		1					
Nitzschia frustulum		7								2
Nitzschia intermedia			6	5	3					
Nitzschia linearis			18							1
Nitzschia palea	169	1	3		25		7	33	61	118
Nitzschia pusilla					1					

	JK 148 STA 4	JK 149 STA 5	JK 150 STA 6	JK 151 STA 8E	JK 152 STA 9A	JK 153 STA 9E
Achnanthes lanceolata	3	1		1		1
Achnanthidium minutissimum	6	6		2		2
Amphora montana	8	1				
Caloneis ventricosa var.*truncatula*			1			
Cyclotella meneghiniana		2			1	
Cymatopleura solea		1		1		
Diadesmis confervacea	3	4		3		7
Diploneis subovalis						2
Fallacia pygmaea				3		
Fragilaria construens	3			2		
Frustulia vulgaris				1		
Geissleria decussis		1				
Gomphonema gracile				1		
Gomphonema parvulum	24	19	24	13	5	47
Hantzschia amphioxys var.*africana*	2					
Luticola mutica	1					
Mayamaea atomus var. *permitis*					3	1
Navicula cincta	5			3		1
Navicula cryptocephala	2	5	6	7	1	3
Navicula cryptotenella						
Navicula gregaria	5	1	152	1	1	1
Navicula lanceolata				1		
Navicula menisculus		2				
Navicula minusculoides	4	7	10	8		10
Navicula rhynchocephala	1					
Navicula rostellata	2	10	12	11		3
Navicula schroeteri	2	3	116	8		1
Navicula subminuscula	1	4	4	4		
Navicula tenelloides	1					
Navicula zanoni	2					
Nitzschia amphibia	2			3		
Nitzschia clausii		2		4		
Nitzschia constricta						
Nitzschia denticula				1		
Nitzschia desertorum	3	3		5		2
Nitzschia frustulum	2		1	1		
Nitzschia linearis	3			1		
Nitzschia palea	48	87	25	122	557	215
Nitzschia pusilla	2	8	2	8	2	5
Nitzschia sigma						1
Nitzschia tropica	7	4		5		
Nitzschia umbonata	4	19	2	17	3	4
Pinnularia eburnea	1					
Pinnularia gibba var.*sancta*					2	2
Placoneis dicephala		1		1		
Sellaphora seminulum	14		2	8	48	112
Staurosirella pinnata		1				
Surirella angusta	2	3		4		2
Surirella ovalis		8	1	3		1
Synedra ulna		1		3		1
Tryblionella apiculata			1			
Tryblionella hungarica				3		
Tryblionella victoriae			2			

SECTION 4

DETERMINING THE POSSIBLE APPLICATION VALUE OF DIATOMS AS INDICATORS OF GENERAL WATER QUALITY IN THE MOOI RIVER (NORTH WEST PROVINCE): A COMPARISON WITH SASS 5.

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Article I. Abstract

The applicability of a European numerical diatom index, the Specific Pollution sensitivity Index (SPI), was tested in the Mooi River system in the Northwest Province of South Africa. SPI scores were compared both to chemical water quality and to scores yielded by using a macro-invertebrate index of riverine health namely the South African Scoring System (SASS 5). This preliminary investigation shows that SPI reflects certain elements of water quality with a high degree of accuracy. Due to the broad species base of SPI, few problems are encountered when using this system in the Southern Hemisphere. It is concluded that SPI or a similar diatom index would provide a valuable addition to the suite of biomonitoring tools currently in use in South Africa.

Article II. Introduction

Section 2.01 Why Monitor Water Quality

South Africa has long recognized that water is one of its prime limiting natural resources (Department of Water Affairs, 1986; Huntley et al.*, 1987).*

We live on a subcontinent notorious for its unpredictable rainfall. South Africa is a semi-arid country, and the decline in the quality of available water is one of the biggest problems currently facing the country (Davies & Day, 1998). There are several factors that contribute to this decline, the most important being industry, bad agricultural practices and the population explosion.

The National Water Act 36 of 1998, repealed and replaced over one hundred previous acts dealing with water, so that we now have two consolidated Acts, the National Water Act and the Water Services Act 108 of 1997. The tenor of the democratic reform process and the underlying cornerstone of the government's water law reform process is encapsulated in a preliminary section of the Act, which states that the National Government is the public trustee of the nation's water resources and is to "…ensure that water is protected, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons in accordance with its constitutional mandate."

Under the National Water Act certain activities, which pollute or degrade water resources require a water use license from the Department of Water Affairs and Forestry. It is stipulated in the Act that an applicant may be required to provide "…an assessment by a competent person of the likely effect of the proposed license on the resource quality…", which can be subject to independent review. A license is not issued in perpetuity, but rather for a fixed period, which may not exceed 40 years. Provision is made for the periodic review of the license at intervals which do not exceed 5 years. Water quality monitoring forms an essential part of the conditions of many such water licenses.

Section 2.02 Biological monitoring of river waters

Biological monitoring techniques have been introduced as part of routine monitoring programmes due to certain shortcomings in standard physical and chemical methods. Because of the difficulty of chemically analysing every potential pollutant in a sample of water, and of interpreting results in terms of the severity of impact, it makes sense to turn to the monitoring of aquatic biota. Results given by biological monitoring are also more cost effective and results can be obtained more rapidly than an extensive chemical analysis. The main advantage of a biological approach is that it examines organisms whose exposure to water and any pollutants therein is continuous. Thus species present in riverine ecosystems reflect both the present and past history of the water quality at a particular point in the river, allowing detection of disturbances that might otherwise be missed (Eekhout *et al.*, 1996).

Biological communities reflect overall ecological integrity by integrating various stressors over time and thus providing a broad measure of their synergistic impacts. Aquatic communities (e.g. fish, riparian vegetation, macro-invertebrates) can integrate and reflect the effects of chemical and physical disturbances that occur in river ecosystems over extended periods of time. These communities can provide a holistic, and integrated measure of the integrity or health of the river as a whole (Barber-James, 2001; Roux, 2001).

Numerous methods have been developed for the bioassessment of the integrity of aquatic systems. Some of these are based on some or other aspect of a single species, but most are based on the attributes of whole assemblages of organisms such as fish, algae or invertebrates. Although some methods have been available for many years, biomonitoring has only recently become a routine tool in the management of South Africa's inland waters (Davies & Day, 1998).

Dixit *et al* (1992) lists the ideal characteristics of biological indicators: they should be simple; be able to quantify the rate of degradation (or recovery) in water quality; be applicable over large geographic regions; and furnish data on background or reference conditions.

(a) Aquatic Invertebrates

Invertebrate communities respond relatively quickly to localized conditions in a river, especially water quality, though their existence also depends on habitat diversity. They are common, have a wide range of sensitivities and have a suitable life cycle duration that indicates short to medium term impacts on water quality (Murray, 1999). For this reason a bioassessment technique was developed by Chutter (1998) called SASS (South African Scoring System) that is currently in its fifth revised form.

However, there are a few restrictions regarding the use of macroinvertebrates in biomonitoring and water quality assessment:

- the distribution and abundance of macroinvertebrates are affected by a wide range of factors other than discernible water quality effects (e.g. flow, nature of substrate, habitat and food availability),
- they may not show responses to certain types of water quality impacts, such as some herbicides,
- some species are naturally patchy in distribution, irrespective of suitable water quality conditions within a river system; this requires high numbers of samples to achieve reasonable estimates of population abundances if doing quantitative sampling, and if doing qualitative sampling, may result in the erroneous conclusion that such species are absent from an area,

- regional distribution patterns of many species vary considerably depending on a number or combination of abiotic factors such as temperature, altitude and latitude,
- the faunal composition of resident macroinvertebrate communities can vary extensively longitudinally down a river with changes in flow and habitat conditions (e.g. mountain torrent streams versus lowland meandering rivers), which can lead to problems where comparisons are required,
- the presence and abundance of certain species vary seasonally,
- in lotic water species may drift downstream to areas where they do not naturally occur,
- in South Africa, the taxonomy of many groups is poorly known and understood at genus and species-level while many new species are awaiting description,
- effective biomonitoring programmes require ecological knowledge of species involved, (which is a problem when many of the species being collected are new to science) (Barber-James, 2001),

Furthermore, the composition of the aquatic invertebrate community is always modified immediately downstream of dams and weirs. This is also often true downstream of bridges (Chutter, 1998). This decreases the potential uses of SASS.

(b) Diatoms

No single group of organisms is always best suited for detecting the diversity of environmental perturbations associated with human activities.

If the maintenance of ecosystem integrity is the aim of environmental management of a river system, the need to monitor the status of different taxonomic groups is vital. Diatoms provide interpretable indications of specific changes in water quality, whereas invertebrate and fish assemblages may better reflect the impact of changes in the physical habitat in addition to certain chemical changes (McCormick & Cairns, 1994).

The diatoms (Bacillariophyceae) comprise a ubiquitous, highly successful and distinctive group of mostly unicellular algae, with the most obvious distinguishing characteristic the possession of siliceous cell walls (frustules). As autotrophs diatoms contribute significantly to the productivity of such ecosystems, frequently forming the base of aquatic food chains (Cox, 1996).

Diatoms are abundant, diverse and important components of algal assemblages in freshwater bodies. They comprise a large portion of total algal biomass over a broad spectrum of trophic states (Kreis *et al*., 1985).

Diatoms are used as biological indicators for a number of reasons:

- they occur in all types of aquatic ecosystems, also extending into damp sub-aerial habitats,
- they collectively show a broad range of tolerance along a gradient of aquatic productivity, individual species have specific water chemistry requirements (Werner, 1977; Round *et al*., 1991),
- they have one of the shortest generation times of all biological indicators (Rott, 1991). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success,

- they are sensitive to change in nutrient concentrations, supply rates and silica/phosphate ratios (Tilman, 1977; Tilman *et al*., 1982). Each taxon has a specific optimum and tolerance for nutrients such as phosphate (Hall & Smoll, 1992; Reavie *et al*., 1995; Fritz *et al*., 1993; Bennion, 1994, Bennion *et al*., 1996) and nitrogen (Christie & Smol, 1993), which can usually be quantified to high degree of certainty,
- they assemblages are typically species rich. Considerable ecological information may be gained from this diversity of ecological tolerances. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, which increase confidence of environmental inferences (Dixit *et al*., 1992),
- they respond rapidly to eutrophication and recovery (e.g. Zeeb *et al*., 1994). Because diatoms are primarily photoautotrophic organisms, they are directly affected by changes in nutrient and light availability (Tilman *et al*., 1982),
- rapid immigration rates and the lack of physical dispersal barriers ensure there is little lag-time between perturbation and response (Vinebrooke, 1996),
- the taxonomy of diatoms is generally well documented (Krammer & Lange-Bertalot, 1986-91). Species identifications are largely based on cell wall morphology,
- diatoms can be found on substrata in streambeds even when dry, so they can be sampled at most times of the year (Stevenson & Pan, 1999),

Round (1993) lists numerous reasons why diatoms are useful tools of biomonitoring, amongst which the following bear especial relevance to the South African situation; methods are cost effective, data is comparable, techniques are rapid and accurate, and identifications and counts can be done by non-specialists with a biological background if they are provided with illustrated guides.

Concerns have been expressed as to the transfer and comparison of data between the Northern and Southern Hemisphere (Round, 1991). It is well known that some species have the same morphology, but questions still remain concerning the range of ecological tolerances of these various species. This is a valid concern when distance, climatic condition, and other environmental pressures are taken into account.

However, Kelly, (1998) introduced the concept that diatoms are 'subcosmopolitan', i.e. they occur anywhere certain environmental conditions are fulfilled. This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a specific site that determine this distribution.

Diatom indices may be able to provide answers to the problems involved in monitoring rivers for the inorganic nutrients which cause eutrophication, organic loading, ionic composition and dissolved oxygen (Kwandrans *et al*., 1998).

Section 2.03 Aim of Study

The aim of the study was to ascertain whether a numerical diatom index developed in Europe has a potential use for indicating general water quality in the North West Province. Bate *et al.* (2002), in a study on South African rivers, came to the conclusion that benthic diatoms could be a useful addition to the national biomonitoring programme as they give a time-integrated indication of specific water quality components. However, Bate *et al.* (2002) went on to state that the particular data set tested in their study that of Van Dam *et al*. (1994) could not be transposed directly to South African conditions. For this reason the current study investigates the potential use of another autecological diatom index developed in Europe (France).

A further aim of the study was to establish whether diatom species are subcosmopolitan as stated by Kelly, (1998), by determining the number of species actually used in the calculation of the chosen index.

SASS 5 was chosen for comparison as it is widely used in biomonitoring of river systems in South Africa and is currently considered as the industry standard for biomonitoring.

Article III. Materials and Methods

Section 3.01 Sampling Sites

Twelve sampling sites in the Mooi River in the North West province were chosen for this study. The study was conducted during May 2003. Study sites were chosen to represent a range of water quality and the impact of some of the tributaries entering the Mooi River. The study sites (Figure 1) extended from below Klerkskraal Dam (M1; 26°30,86' S, 27°07,40' E), downstream to the Prozetsky Bird Sanctuary in Potchefstroom (M5; 26°34,13' S, 27°06,03'E). The four tributaries that formed part of the study were the Wonderfontein Spruit (WFS), an unnamed tributary near Boskop Dam (T3), The Wasgoed Spruit (WS) in Potchefstroom as well as the Loops Spruit (LS) entering the Mooi River at the Prozetsky Bird sanctuary on the outskirts of Potchefstroom.

Land use in the upper reaches of the Mooi River catchment is mainly agricultural with activities such as peat and informal diamond mining occurring further downstream. Gold mining and sewage effluent enters the Mooi River through the Wonderfontein Spruit. The

unnamed tributary (T3) introduces water from a canal into the Mooi River just above Boskop Dam, from an unknown source. Effluents from heavy industry (e.g. a fertilizer manufacturer) as well as storm water drain into the Mooi River from Potchefstroom via the Wasgoed Spruit. The Loop Spruit is mainly influenced by agricultural activities.

The study also included samples above and below two major dams in the system namely the Boskop Dam and Potchefstroom Dam.

Figure 1

The Mooi River system (North West Province, South Africa) showing the location of the sampling sites used in the study

Section 3.02 SASS 5

Macro-invertebrates were collected and the SASS 5 and ASPT indices calculated according to standard methods as set out in Dickens & Graham (2002) and Chutter (1998).

Section 3.03 Diatoms

(i)

(ii) Sample collection

Three to five different boulders at any particular site (Round, 1993) were sampled from different positions within a defined 10m reach, as far as possible using riffles. As far as possible, boulders (>256 mm) free of filamentous algae and obvious siltation were selected.

The diatoms were removed to provide a composite sample. The diatoms were sampled from the upper surface of the boulder with a stiff toothbrush and the epilithon collected in a 250 ml sample bottle, suspended in distilled water (Kelly *et al*., 1995).

(iii)

(iv) Preparation and identification

Samples were allowed to settle for 24 h and the supernatant decanted. Samples were first examined live to establish if a considerable number of dead cells were present. This was done, as only living cells will be able to provide a reflection of recent water quality. The samples were then oxidised in a saturated solution of potassium permanganate. Carbonates were removed using concentrated (32%) hydrochloric acid (Pienaar, 1988). Samples were then rinsed with distilled water and collected by centrifugation, using five successive runs at 2500 rpm. Clean frustules were then mounted in Pleurax (Hanna, 1949).

Diatoms were identified under phase contrast using an oil-immersion lens at 1000x magnification. The nomenclature follows Krammer & Lang-Bertalot (1986-91). At least 400 frustules (400-500) were identified for each sample (Prygiel, 2002).

(v) Description of the SPI diatom index.

The index used is based on the weighted average equation of Zelinka & Marvan (1961) and has the basic form

$$index = \frac{\sum_{j}^{n}=1 \; a_j s_j v_j}{\sum_{j}^{n}=1 \; a_j v_j},$$

where a_j = abundance (proportion) of species j in sample, v_j = indicator value and s_j = pollution sensitivity of species j. The performance of the indices depends on the values given to the constants s and v for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of s. For SPI (Specific Pollution sensitivity Index; CEMAGREF, 1982), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe *et al.*, 1993) indicates clean water. SPI is a comprehensive index, with values of s and v available for over 1300 species (Coste *et al.*, 1991).

Section 3.04 Chemical Analysis

Chemical analysis was performed according to standard methods (American Public Health Assosiation, 1995) by accredited laboratories namely Mogale City local municipality water laboratory and the Agricultural Research Council: Institute for Soil, Climate and Water, Pretoria.

The following water quality variables were analyzed in the water quality laboratories:

total nitrogen (total N),

ammonia (NH_4),

total phosphate (total P),

chemical oxygen demand (COD),

five day biological oxygen demand (BOD_5),

sulphate (SO_4),

chloride (Cl),

Several variables were determined in-stream with a calibrated temperature/pH/conductivity/oxygen meter (YSI 556 MPS Multimeter, USA) at the time of sampling. These included:

temperature (temp.),

pH

electrical conductivity (EC,)

dissolved oxygen (DO_2),

turbidity.

The variables were chosen to represent general water quality according to the monitoring requirements for domestic and industrial wastewater release (DWAF 1999). The BOD_5 was added to this suit to provide an indication of organic load according to analysis list of Kwandrans *et al*., (1998).

(a)

Section 3.05 Data Analysis

Correlation and stepwise forward multiple regressions were carried out using STATISTICA version 6. Prior to statistical analysis, the distribution of the water quality data was analyzed for normality (STATISTICA version 6). Where the data showed a skewed distribution the data was $\log_{10}$ transformed. The SPI diatom index was calculated in the database OMNIDIA (Lecointe *et al.*, 1993).

In this study the ASPT value (7) for site M2C was deemed to be an outlier due to its exaggerated residual value in comparison with the other sites. According to Hair *et al*., (1998) the definition of an outlier, in strict terms, is an observation that has a substantial difference

between its actual and predicted values of the dependent variable (a large residual) or between its independent variable values and those of other observations. The objective of denoting outliers is to identify observations that are inappropriate representations of the population from which the sample is drawn, so that they may be discounted or even eliminated from the analysis as unrepresentative. For this reason M2C was neither used in the calculation of the correlation matrix or in the multiple regression for ASPT.

Article IV. Results and Discussion

Table 1 shows the values produced the various indices for the different sites in the Mooi River catchment. For the interpretation of the various indices limit classes are given in Table 2 and 3. The lowest SASS 5 and ASPT scores were recorded in the Wonderfontein Spruit (WFS) that shows major deterioration in water quality, while the diatom index showed the water to be of moderate quality. The lowest SPI score was recorded in the Wasgoed Spruit (WS) which displays a value that can be interpreted as bad water quality, while SASS 5 and ASPT for the same site show values that indicate only some deterioration in water quality.

TABLE 1

TABLE 2

TABLE 3

It is clear from these two sites (WFS & WS) that the various indices do not give the same indication of water quality. Table 4 shows the correlation matrix of the various indices together with physical and chemical parameters. From the matrix a significant correlation ($p<0.05$) can be observed between SASS 5 and ASPT scores. No such correlation was observed between the macroinvertebrate indices and the diatom index used. However, a

decline in all the indices can be observed from M1 to M5 as would be expected from studying the water quality data.

TABLE 4

The results of the water quality analysis are given in Table 5. When assessing the water quality data qualitatively, according to the variables tested, it appears that the lowest water quality was observed in the Wasgoed Spruit. The stream contained elevated levels of chloride, sulphate, ammonia and other nutrients and displayed the highest electrical conductivity in the system.

TABLE 5

The highest levels of biological oxygen demand and sulphate, as well as elevated levels of chemical oxygen demand, chloride and total nitrogen was observed in the Wonderfontein Spruit. The influence of the Wonderfontein Spruit on the Mooi River can be seen when comparing the chemical data from sites M1 and M2. Sulphate levels increased considerably from M1 to M2 due to the confluence of the Mooi River with the Wonderfontein Spruit. Increases in chemical oxygen demand, chloride and total nitrogen was also be observed the Mooi River after the confluence of these two streams.

The entire system displayed elevated levels of phosphate and could be described as being eutrophic (Walmsley, 2000). The general water quality in the Mooi River decreased steadily from M1 to M5 due to many point and non-point influences such as agriculture, mining and storm water drainage.

SPI displayed significant correlations ($p<0.05$) with several of the measured water quality variables (Table 4), these included negative correlations with electrical conductivity, chloride, and ammonium. A positive correlation was observed between the pH and the SPI score.

ASPT scores correlated significantly with the biological oxygen demand of the water as well as sulphate levels. Biological oxygen demand indicates the degree of organic loading of a stream (Viessman & Hammer, 1998). SASS 5 index scores did not show any significant correlation with water quality variables.

Multiple regressions were performed on the data to establish if there were any physical or chemical variables that influenced the indices other than the ones that showed clear significant correlations in Table 4. Forward stepwise regression was used for this purpose. This regression method takes the independent variable with the greatest contribution and adds it to the model first. Independent variables are then selected for inclusion based on their incremental contribution over the variable(s) already in the equation. Independent variables that are closely correlated in the correlation matrix may not all be included but rather other variables that also contribute to the variation in the index scores. For this reason this method can give important additional information about the factors that influence the various index scores over and above pure correlations. Adjusted R^2 values are used as indicators of the level of success with which the independent variables are able to explain the variation in the index values. This value was chosen as the Adjusted R^2 takes into account the sample size as well as the number of variables used (Hair *et al*., 1998). Since twelve sites can be deemed a relatively small sample size the value will give more reliable confidence values than the R or R^2 values.

Table 6 shows the regression results of the multiple regression performed on the physical and chemical variables and the SASS 5 index scores. From the results it can seen that five of the independent variables were used to account for the variation in the SASS 5 index values. sulphate, total phosphate and dissolved oxygen all contributed significantly to the variation in the data while COD and total nitrogen also contributed, but not significantly. From the Adjusted R^2 (Table 6) it is clear that the proposed linear model can successfully account for approximately 74% of the variation in the index values. This would mean that about 26% of the variation in the data could not be accounted for by the proposed model and might be accounted for by factors such as habitat.

TABLE 6

Figure 2 shows the predicted versus observed SASS 5 index values. The closer the observations are to the straight line the better the observations could be explained by the proposed multiple regression model. As can be seen from the graph the model was fairly successful in predicting the actual SASS 5 scores.

Figure 2

Predicted SASS 5 values versus observed SASS 5 values

Table 7 shows the multiple regression results for the ASPT scores and environmental variables. Four variables were taken into account by the multiple regression for the ASPT scores. Sulphate, total phosphate contributed significantly ($p<0.05$) to the variation in the

ASPT scores while ammonia and turbidity also contributed, but not significantly. The model predicted approximately 68% of the variation in the ASPT scores (adjusted R^2 of 0.678).

TABLE 7

The graph of predicted versus observed variables also shows that the model was also fairly effective (when compared to the SASS 5 model) in predicting the actual index scores.

i. Figure 3

Predicted ASPT values versus observed ASPT values

The Adjusted R^2 for the SPI multiple regression (Table 8) is very high with approximately 99% of the variation in the data explained by various water quality variables. The variables included in the regression model were chloride, pH, turbidity, chemical oxygen demand, sulphate and oxygen. All of the variables except oxygen contributed significantly ($p<0.05$) to the model.

TABLE 8

The graph of predicted versus observed SPI values (Figure 4) shows that the model was highly effective in predicting the SPI index values.

Figure 4

Predicted SPI values versus observed SPI values

A total of 112 species, representing 18 genera, were found in the 12 samples. Of the 112 species only 3 were not relevant to the calculation of the SPI index scores.

Conclusions

From Table 1 it would seem as though the diatom index (SPI) is more sensitive to the elevated physical and chemical parameters (that were measured for this study) in the Wasgoed Spruit than the two other indices tested. This would concur with Willemsen *et al*. (1990), who in a study of the impact of stormwater in the Netherlands, concluded that diatoms were more sensitive to these discharges than were bentic invertebrates; they attribute this to the inability of diatoms to migrate away from unfavorable conditions and to recolonize when conditions have improved.

SASS 5 showed a very low index value for the Wonderfontein Spruit that can be explained by the influence of organic pollution. According to Dallas and Day (1993) the enrichment of a water body with organic waste almost certainly results in a decrease in species richness, diversity and an alteration in the composition of biotic communities. Chutter (1998) also observed that SASS scores were very low in organically polluted water. SPI scores did not accurately reflect the degree of organic loading in the Wonderfontein Spruit. This can also be seen in the correlation matrix (Table 4) which shows that SPI has no significant correlation to biological oxygen demand.

According to our results, the diatom index is sensitive to changes in electrical conductivity, ammonia, chemical oxygen demand, chloride, sulphate and turbidity. Form this we can conclude that SPI gives a good reflection of general water quality. It would seem as though SPI is able to give a more accurate reflection of the ionic composition of water than the

macroinvertebrate index. This is indicated by the strong correlation between electrical conductivity and SPI. Chutter (1998) states that SASS is less sensitive to increases in total dissolved solids (total dissolved solids ≈ electrical conductivity x 6.5) than to other types of chemical change.

Form the correlation matrix (Table 4) and the multiple regressions (Tables 6-8) it can be deduced that diatom index is more closely influenced by water quality than the ASPT or the SASS 5 indices. It would seem as though the macroinvertebrate indices cannot be fully explained by the water quality variables used in this study and may also be affected by other factors such as habitat diversity.

There is therefore, still a need for a biological indicator (such as the diatom index used in this study) that can be indicative of specific water quality variables.

Round (1991) suggested that caution should be observed when transferring index data from the Northern hemisphere to the Southern hemisphere as some species may exhibit different ecological tolerances. However the fact that the SPI values can almost fully be accounted for by the physical and chemical variables in the Mooi River and tributaries (Table 5 & 8) should satisfy such a concern. In addition 97% of the diatom species encountered in this investigation were useful for SPI and hence cosmopolitan in nature.

The fact that the diatom sampling also has less restrictions in terms of habitat requirements than macroinvertebrates could facilitate its use in monitoring water quality in small tributaries, for instance mining and industrial effluent. This conclusion is strengthened by Round's (2001) statement that "…river diatoms can colonize massive rivers but also "rivers" millimeters deep and centimeters wide…"

From the results of this study it would seem fair to say that there is definite potential in the use of numerical diatom indices as indicators of general water quality and the usefulness of these indices should be verified by further studies that cover a broader geographical area and a broader range of variables.

Section 4.02

Section 4.03 Acknowledgements

The authors gratefully acknowledge the comments provided by Prof K.N. de Kock (School for Environmental Sciences and development, P.U. for CHE) as well as the assistance provided by Mrs T. de la Rey (School for Computer, Statistical and Mathematical Sciences, P.U. for CHE) with the statistical analysis.

Section 4.04

Section 4.05 References

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SECTION 5

THE ROLE OF DIATOMS AND AQUATIC INVERTEBRATES AS BIOLOGICAL MEASURES OF RIVER HEALTH : A CASE STUDY OF THE IMPACT OF ACID-MINE DRAINAGE ON THE BIOTA AND WATER QUALITY OF THE HEADWATERS OF THE SMALL TSHOBA RIVER, KWAZULU-NATAL (SOUTH AFRICA).

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(This section is an extract from a paper being prepared for publication by the above authors).

INTRODUCTION

Some of the earliest studies benchmarking the impact of drainage from coal mines on biota and river chemistry in KwaZulu-Natal was undertaken by Kemp (1962, 1967) and Oliff et al (1965) mostly in the upper reaches of the Thukela river. These investigations of invertebrates and/or water chemistry covered the upper catchments of river systems draining the active coal mining operations around the towns of Newcastle, Glencoe and Dundee in the north-west of the province. Cholnoky (1956, 1960) also investigated some diatom associations sampled from various sites in KwaZulu-Natal rivers, including those affected by drainage from coal mines.

Acidic drainage water generated by coal mining activities is derived from underground oxidation of pyrites (iron sulphide contained in the coal and associated strata) to ferrous and ferric sulphates (Kemp, 1962). Brick-red precipitates of these iron compounds are deposited on the stream bed in conjunction with high sulphate concentrations making these surface waters very acidic. The formation of such acidic conditions can be a natural process and is not necessarily a consequence of the mining activities *per se*, yet such operations accelerate the process by disturbance of mineral matter in the coal and geological strata. Kemp (1962) estimated that if the rate of coal output was maintained in these KwaZulu-Natal catchments, the pollution of rivers in Northern Natal would double in less than 40 years (i.e. circa 2000). This situation would seriously threaten the ecological and biological integrity of small streams rendering them unfit for various uses.

Over thirty years have now elapsed since the erstwhile Natal Town and Regional Panning Commission sponsored a provincial investigation of rivers in Northern Natal and Zululand when many coal mines were still in full operation (Archibald et al., 1969). These investigations essentially only covered the bacteriology and water chemistry of the principal rivers of the north-western parts of the province. The

quality of water at most of the river sites was found at the time to be Class II i.e. good water '*suitable for potable purposes and almost any other use"*. In some localities however, deterioration of the water quality was already evident and this was ascribed to two types of pollution namely, organic pollution and mineralisation from drainage out of coal mines in the headwaters of the Mkuze and Mfolozi rivers. Both these rivers flow through game reserves and finally drain into Lake St Lucia – a world heritage site.

Several coal mining operations in north-western KwaZulu-Natal have since ceased to operate because of an unfavourable economic situation but the flow of acidic drainage from coal mines does not cease when mining activities are discontinued (Kemp, 1962). Several small, unprofitable operations were simply abandoned without recourse to rehabilitation because there was no requirement or environmental protocol for measuring potential damage to the aquatic life in these rivers. However environmental management plans are now required by the Department of Minerals and Energy as part of a proper decommissioning process. This policy also pre-supposes that appropriate biological measures of river conditions are available and well accepted by the industry, water resource managers, the legal and scientific community.

This brief investigation was carried out in 1998 (more than 30 years after Kemp's work) to determine the impacts on stream integrity of a long term persistent flow of acid mine drainage. The study targeted the headwaters of the small Tshoba stream which has been exposed to acid drainage from ponds surrounding the base of a coal dump in the vicinity of Boomlaer. This study also forms part of an on-going strategy to establish the added value of bio-monitoring of a river in South Africa using two independent biological indicator systems at different trophic levels as recommended by Hofmann (1996). The first indicator system uses the diatom community which functions at the base of the food chain and is directly linked to the water chemistry as measured by conventional water quality indicators. The SASS4 rapid assessment procedure (now acknowledged by DWA&F as Version 5) was also carried out as the second indicator system to provide a comparison of river health using the invertebrates as the conventional measure of biological condition of the river.

STUDY AREA

Four distinct sampling areas were located on the upper Tshoba river which received acid drainage runoff from a defunct coal mine at Boomlaer near Hlobane (Latitude 27^{o} 44.28'S, Longitude 30^{o}58.20'E, some 10km to the west of the town of Vryheid). It is a small tributary at the headwaters of the White Mfolozi River which ultimately discharges into the St Lucia estuary. Since there are also several other coal mines (operational and defunct) in the upper Mfolozi catchment it is of ecological

concern and of environmental management importance to determine whether impacts from long term acid mine drainage can be detected using these biological procedures. This is a necessary precautionary measure to determine whether self-purification downstream has occurred and the environmental water quality has improved in the feeder streams of the Mfolozi River before it discharges into the estuarine area of Lake St Lucia.

The presence of the relatively extensive coal mine operation was the dominant land-use feature in the headwaters and the drainage from the dump formed the main supply of water feeding into the upper Tshoba River. A natural stream with a smaller steady flow of freshwater entered from the right bank of the Tshoba River immediately below the mine dump site. Dairy farming and cattle-rearing was the obvious agricultural practice in this area of the Tshoba catchment at the time of sampling. Open grassland formed the dominant natural vegetation cover interspersed with stands of maize as a subsistence crop.

River sites

The discernible stream channel was about 5 - 10m wide at Site 1 with marginal stands of *Phragmites australis* and *Typha capensis* forming a relatively small wetland at the base of the mine dump. The shallow stream meandered in channels over the sandy substrate and the water was no more than 5 – 10 cm deep except in the odd pool.

Site 1: This site was located at the headwaters of the Tshoba stream on a small private holding, some 300m below the diffuse surface drainage from the coal dump.

Sample 1a: A sample was taken from the sandy substrate of the drainage channel upstream of the confluence with the natural headwater stream. This was an acid stream draining out of ponds surrounding the base of a disused coal mine.

Sample 1b: A sample was taken from the sandy substrate of the natural stream draining the hillside, adjacent to the mine, and upstream of the confluence with the acid drain.

Site 2: **Tshoba stream**. This site was located below the confluence of inflows from 1a and 1b allowing for the first apparent visible mixing of both streams some 40 - 50metres downstream where channels were still visible. Slightly turbid water flowed over a

typical brick-red precipitate and fluffy 'fungus-like' growths of iron bacteria which together covered the sandy sediments.

Sample 2c:	Below confluence	Sandy substrate on left bank of main channel
Sample 2d:	Mixed stream	Sandy substrate at mid- point of main channel
Sample 2e	Mixed stream	Sandy substrate on right bank of main channel

Site 3: **Tshoba stream.** This site is approximately 3 - 5 kilometers downstream, beneath the bridge to the west of Vryheid on the main road to Louwsburg. Clear water flowing over sandy substrate on the margins and between rocks in mid-stream.

Site 4: **Tshoba stream.** The site is approximately 8 - 10km downstream of the mine dump beneath a bridge on a district road**.** Clear water flowing over a sandy substrate between rocks

FIELDWORK AND LABORATORY METHODS

Water quality

Water quality characteristics were determined for several key constituents which are typically used to characterize physico-chemical conditions and environmental water quality in a river (Palmer, 2002). Conventional analytical methods were used by an accredited CSIR (KwaZuluNatal) laboratory in the determination of pH, conductivity, suspended solids, turbidity, dissolved oxygen, nitrates, ammonia, and soluble phosphorus..

Diatom protocol

Since there were no rocks at the upper sites, diatoms were sampled from the sandy substrate at all river sites using a perspex corer (21 mm in diameter) to ensure consistency of approach. Several 1 cm surface cores were removed randomly from each site and stored in a cooler box for immediate extraction of the **living** component on return to the laboratory within a day after completion of the fieldwork.

Laboratory extraction of living diatoms was accomplished by 'floating' coverslips on the damp sediments of each sample (Round et al., 1993). Material from these coverslips was subjected to acid treatment and washings in the manner recommended by Welsh (1964) so that diatom frustules would be preserved on slides for observation and counting under oil immersion at 1 000x magnification.

Diatom identifications were made using the available literature with particular reference to works by Hustedt (1930,1959,1961), Krammer and Lange Bertalot (1986, 1988,1991) and Cox (1996).

SASS4 protocol

The river was sampled at the same 4 sites according to the recommended SASS4 protocol (Thirion et al 1995).

RESULTS

Environmental Water Quality

The water quality in the headwaters of the Tshoba stream was markedly different in samples taken from Sites 1a and 1b - the left and right feeder arms respectively to the main stream. The principal chemical impacts of the drainage from the dump (Site 1a) manifested as a very acidic water (pH of 2.6) (Table 1). Conductivity measurements and hence Total Dissolved Solids values were also elevated due mainly to higher calcium, magnesium, sulphate and chloride concentrations (Tables 1, 3).

The water sampled from Site 1b, however, was typically alkaline with the concentrations of most salts, except sodium and potassium lower than that of the acid drain. The water quality characteristics of this natural stream was considered to be the '*Reference Condition*' for comparison and assessment of downstream impacts, changes and river recovery (Tables 1, 3). Enrichment of the natural stream with nutrients was greater than that of the acid mine drainage. There was minimal organic nitrogen contamination from the mine at site 1a and the concentration of nitrates, ammonia and soluble phosphorus of the water at Site1b was higher than that of the acid drainage.(Table 2).

A set of three samples (Samples 2c , 2d, 2e) was taken across the channel (left bank, mid stream, right bank) at Site 2, some 50m downstream of the confluence. The configuration of the shallow stream (5 cm depth) at this point and the discoloration of the stream bed indicated that the physico-chemical conditions were markedly different across the stream bed even though it was only 15-20metres wide. The water quality at these three sites clearly demonstrated that the channels of water from the two main inflows had not mixed properly across the width of the stream. Concentrations of chemical constituents of the left channel (Site 2c) were consistent with the acid drainage conditions found at Site1a and constituents of the right channel (Site 2e) were consistent with conditions at Site1b in the natural stream (Tables 1-3).

The water quality at Site 3 located 3-5km downstream from the mine dump showed marked improvement in terms of reduced levels of chemical constituents. The pH value reverted to alkaline conditions and nutrient concentrations were reduced.

Concentrations of cations and anions were also much reduced and thus the conductivity and TDS values were lower than those at Sites 1 and 2. (Table 1, 3)

This trend was maintained at Site 4 which was the lowest point that was sampled on the Tshoba River where dilution from small side streams and in-stream self-purification processes had occurred. Concentrations of cations and anions were lower than those recorded at Site 1B - the reference site (Table 3). This Tshoba River survey was made during the summer wet season of 1998 and the steady flows in the river below the mine dump contributed to the rapid recovery of the quality of the water at Site 4 before its confluence with the White Mfolozi River. Some thirty years ago Archibald et al (1969) also reported similar physico-chemical characteristics from a site on the Tshobaspruit (X12) upstream of the confluence with the White Mfolozi. These water quality data indicated that there was no measurable physico-chemical impact from coal mine drainage at that time. (Table 1).

Biomonitoring using the SASS4 protocol for macroinvertebrates

The **SASS** (South African Scoring System) was originally developed as a rapid bio-assessment method by Chutter (1971) and later improved upon (Chutter,1994,,1998). The procedure attempts to measure the effects of changing river conditions on the bottom-dwelling invertebrate community**, although it does not necessarily identify the cause of the changes** (Palmer et al, 2002). . The composition of the communities present has been used as an indicator of the water quality and general river health due to the differing sensitivities of the organisms to water quality (some tolerant of poorer quality, others sensitive to it),.

The scores allotted to each family are related to the response of the families to water quality, with families most sensitive to pollution being scored 15 and those most tolerant of it 1. Families of intermediate tolerance are allocated intermediate scores. The scores for each of the four Tshoba river sites were summed to give a total sample score, number of taxa present and the average score per taxon and are presented in Table 8.

Results of assessments using this SASS methodology were interpreted according to the method described by Thirion et al (1995) : High total sample scores (eg >100) are associated with unpolluted

conditions, whilst low scores (eg < 50) reflect poor quality/polluted conditions, (assuming that habitat availability is not a limiting factor).

SASS data is only really meaningful when assessed together with the various factors that may influence the scores. Most important is a measure of habitat quantity, quality and diversity. The method developed by McMillan (1998) was used to assess the habitat at each of the sites sampled in conjunction with the habitat integrity index, which assesses the river as a whole with regards to habitat. The results from analyzing the macro-invertebrate and habitat scores, together with an evaluation of habitat and macro-invertebrate integrity are presented in Table 9.

Biomonitoring using Diatom Community Analysis

Site 1 Tshoba river above the confluence of the two main inflows

Analyses of the diatom associations and their associated index scores are displayed in Tables 4 – 6. It is evident from this data that the conditions at Site 1a and 1b support diatom communities that are distinctly different in terms of dominant species, percentage composition and species diversity (Table 4). The contrast in water quality between the two upper samples is reflected distinctly in the response of the diatom communities.

Sample 1a : Acid drainage 2 dominant species making up a total of 100% with only 3 - 4 other species which were not captured in the counting exercise.

Only two species are able to tolerate these conditions. The dominance of the acidophilic species *Nitzschia paleaeformis* (85,6%) and to a lesser extent *Stauroneis kriegerii* (14,4%) is almost complete in this association. The former has been recorded from sulphuric acid ponds associated with coal mining operations while the latter has a preference for circum-neutral water (Table 4).

Sample 1b : Alkaline stream 5 dominants out of a total of 17 species making a total of 81.1%

The species diversity and % composition of several co-dominants (*Anomoeoneis vitrea* 25,2%; *Achnanthes minutissima* 19,2% ; *Cymbella cesatii* 17,7% ; *Nitzschia nana* 9,6% ; *Navicula pseudohalophila* 9.4%; indicate the 'normal' concentrations of organically bound nitrogen, well-oxygenated, circum-neutral water. This association was regarded as a baseline or **reference community** characteristic of the water quality at Site 1b in the upper subcatchment of the study area.

Site 2: Tshoba river some 50m downstream of the confluence.

Examination of the respective diatom associations extracted from Samples 2c, 2d, and 2e confirmed that the 'integrity' of the water inflows from Site 1a and 1b was maintained. Markedly different diatom associations developed across the stream width of no more than 15metres. Distinctly acidophilous diatom components dominated on the left bank with a gradient through an intermediate midstream association to an alkalophilous diatom composition on the right bank, reflecting characteristics of the community found at the *'reference water'* site. (Table 4).

Sample 2c (Acid left bank component) 2 dominants made up 99 % of the population count.

The diatom species association was identical to that found in Sample 1a – the acidic feeder stream from the coal mine drainage. The acidophilic *Nitzschia paleaeformis (88.0%) and circum-neutral Stauroneis kriegerii* (11,6%) remained the dominant tolerant species (Table 4) This data confirmed the findings upstream that the very low pH value was the primary determinant of the species composition.

*Sample 2d (Mid-stream intermediate component***)** 6 dominants out of 9 species made up 95% of the population count.

The complete dominance of the acidophilous species was reduced at the midstream site. *Nitzschia paleaformis(40%)* was associated with the appearance of several other diatoms. There was a measurable shift towards a greater percentage of *Stauroneis kriegerii* (30%) which favours circum-neutral waters although the water was still somewhat acidic at a pH of 4.3. Species composition changes indicated sensitive diatom community responses to some mixing and dilution in mid-stream between the left and right component compared with the extreme conditions on the far left of the stream. (Tables 1 and 4).
The reduction of the acidophilous diatom species and the concomitant increase in diversity and abundance of at least four other diatoms is the first indication that dilution by the natural stream has an ameliorating affect on the harsh acid drainage within 50 m of the confluence. More species (9) were able to tolerate the 'mixed conditions' downstream (Table 4).

Sample 2e (right bank alkaline component) 7 dominants out of 21 species make up 73% of the population count.

The species composition of the diatoms taken from the sample at the right hand side of the main stream is consistent with that of the alkaline conditions recorded at Site 1b upstream of the confluence with the acid mine drainage**.** There was a marked reduction in the species shown to be tolerant of upstream

acidic conditions **i.e. (***Nitzschia paleaformis 4,1% and Stauroneis kriegerii 5,7%)***.** The dominance reverted to a diversity consistent with the upstream more alkaline reference condition (*Achnanthes minutissima 14,6%; Anomoeoneis vitrea 18,5% and Nitzschia nana 16,8%).*

Site 3: Tshoba river some 3 - 5km downstream from the coal dump

Sample 3: (marginal sediments) 6 dominants out of 32 species made up 69,2% of the population count.

The diatom ccmmunity reflected waters with a pH greater than 7.0, a low conductivity (<139mS/m) and a high oxygen content (>75%) with little organically bound nitrogen. The absence of *Nitzschia paleaeformis* and *Stauroneis kriegerii* in any significant numbers indicated some recovery from the impacts of acid mine drainage in the upper Tshoba river. Other co-dominant species such as *Nitzschia linearis* (38,3%), *Caloneis molaris* (10,1%) and *Nitzschia denticula* (9,9%) indicate that the water quality has improved downstream from the mine (Table 4).

Site 4 : Tshoba river about 8 -10km downstream from the coal dump

Sample 4: Marginal sediment : 5 dominants out of 21 species make up 67,2% of the count.

The diatom community again reflected fresh water with a low conductivity (< 139mS/m) and a pH value that was greater than 7. The presence of increased numbers of *Achnanthes minutissima* (50%) indicated conditions consistent with high oxygen saturation with small amounts of organic nitrogen. The latter condition is indicated by the presence of numbers of *Gomphonema parvulum* (7,6%) and *Nitzschia palea(1,5 %)* , and *Nitzschia palea var debilis (3.0%). (Table 4)*

Diatom Index Scores

The Diatom Index Scores for the Tshoba River were derived from diatom community analysis data (Table 4) which were processed using the Omnidia software for calculation and output of the index values (Table 5). The index scores presented in Table 5 give a comparison of various diatom indices under acronyms used in the Omnidia programme. The diatom index scores were calculated using the formula of Zelinka & Marvan (1961) in all cases except for CEC, SHE, TDI and WAT indexes. A maximum value of **5** (converted to a score of 20 by the Omnidia software package; Lecointe *et al.*,

(1993) indicates **clean water** for all of the above indices, except TDI which has a maximum value of 100.

Most of the diatom indices are designed to give an indication of general water quality. The indices differ in respect to the diatom species included in the calculation and in the number of taxa included in the calculation. . Several indices were designed to reflect eutrophication including the Eutrophication Pollution Index (EPI) and the Trophic Diatom Index (TDI). The calculation of correct scores for the TDI index is dependent on the percentage of pollution tolerant diatom taxa in the sample (%PT). A **value greater than 20% PT indicates organic pollution rather than eutrophication**. Sládeček (SLA index) and Watanabe (WAT index) developed diatom indices which were designed to reflect degrees of **organic loading**.

Application of Diatom Index Scores

In practice, use of diatom indices involves making a list of the taxa present in a sample along with a measure of their abundance (Table 4). The index is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998).

DISCUSSION

Biomonitoring using diatom protocols as a measure of water quality

The findings described in this approach demonstrate the ecological and management value of using biological associations at different trophic levels. In particular, the **diatom communities** are extremely sensitive to water quality changes and thus provide valuable information for river health assessment. The information supports the need for the re-introduction of diatoms as key biological indicators for the assessment of river health under varying conditions of water quality. The procedure is supportive of the imperatives and requirements of the new water law (Act 36 of 1998) which now promotes 'river health' assessments in terms of environmental water quality, in situ bio-monitoring and if necessary application of laboratory eco-toxicology protocols. (Palmer et al. 2003).

The object of these initial studies is to demonstrate the value of historical diatom analysis sheets for use in drawing conclusions about the past condition of South Africa rivers.

This paper is the first of three historical case studies demonstrating that diatom community responses can be used to indicate the specific quality of river waters subjected to acid mine drainage, pulp and paper waste discharges and sugar mill wastes. The three case studies and others (Harding et al., 2004 on the Jukskei River) promote the concept of biomonitoring using diatom protocols as added value for river health assessment. because diatoms occupy a key trophic level at the base of the food chain.
Ecological inferences can be drawn from a sound knowledge of the diatom communities and species preferences for a specific quality of water. The procedure does demand knowhow and accurate taxonomic identifications. Much of the substantive early taxonomic work was accomplished in South Africa by internationally recognized specialists (Drs BJ Cholnoky, REM Archibald, F.R. Schoeman and Giffen). The legacy left by their extensive, detailed and meticulous taxonomic studies of South African aquatic systems is accessible now in the South African Diatom Collection, the biggest collection in the Southern Hemisphere.

Image Analysis

Image analysis techniques give rapid access to the original material which is housed in the collection in the Durban laboratories of the CSIR – the present national custodian of the collection. It is axiomatic that future investigations will require intensive interaction between properly trained diatom taxonomists/ecologists to ensure a robust and valid interpretation (Kocioleck and Stoermer, 2001). Modern computer technologies and image analysis software programmes have now improved previously perceived constraints and deficiencies of the past relating to time-consuming taxonomic analysis procedures. These constraints can be discounted by rapid electronic interaction with diatom specialists around the world because of the accuracy of image comparisons of the dominant species. Images from previous slide material can be clearly captured and checked for accuracy so that ecological inferences are robust and valid both historically and for present day investigations.

The ultimate objective of characterising the environmental water quality with diatom associations of the river under consideration presupposes the use of standard sampling procedures to ensure the acquisition of good and appropriate material from the aquatic habitats frequented by **living** diatoms. As is the case with the SASS protocol, the preferred habitat is usually ‘stones in current’ ie the epilithic component. However the headwaters of the Tshoba were characterized by sandy substrate without any visible stones

and therefore the living episammic/epipelic component was extracted from the sediments of the stream bed.

The use of periphyton (diatoms attached to marginal vegetation) in the Tshoba stream study was discounted because of the rapid changes in habitat and downstream discontinuity in the spread of rooted aquatics characteristic of the small wetland at the base of the mine dump.

The most suitable habitat in the Tshoba river was the marginal shallow water sediments which were consistently present at each site, although in the reaches around Site 1 and 2 much of the original sediment was smothered with iron bacteria.. The similarity in particle size analysis (mostly medium to fine sand) at each site discounted the possibility that the sediment texture was a key determinant in diatom community changes and stressor responses (Table 7) .

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Table 1. Physical water quality characteristics of the upper Tshoba stream components. (CSIR summer survey data - 16/02/1998)

Tshoba stream (1998)	Physical Characteristics						
	Temperature	**pH**	**Conductivity**	**Alkalinity**	**Total Dissolved Solids**	**Suspended Solids**	**Turbidity**
Units	°C		mS/m @ 25 °C	mg $CaCO_3$ ℓ^{-1}	mgTDSℓ^{-1} @ 180 °C	mgSS ℓ^{-1} @ 105 °C	NTU
Sampling Sites							
1a. Acid mine drainage	25,2	2,6	459	-	5020	27	1.1
1b. Natural stream	21,3	7,7	277	62	2360	15	0.4
2c. Mixed (left bank)	21,7	2,6	468	-	5100	21	0.3
2d. Mixed (mid-stream)	21,7	4,3	289	-	2600	41	56
2e. Mixed (right bank)	21,7	7,0	281	56	2370	16	0.2
3. 3-5km downstream	24,5	7,1	178	48	1430	5	2,5
4. 8-10km downstream	22,3	8,1	53	110	352	26	24
White Mfolozi River (1969 Dry winter data)							
(X12) Tshoba tributary	9.8	8,3	31,8	162	202	- -	- -

Table 2. Oxygen and nutrient characteristics of the upper Tshoba stream components. (CSIR summer survey data - 16/02/1998)

Tshoba River (1998)	**Oxygen and Nutrient concentrations**					
	Dissolved Oxygen	**COD**	**NH_4-N**	**NO_3-N**	**Total Kjeldahl -N**	**Total Soluble-P**
Units	$mgO\ell^{-1}$	$mgO\ell^{-1}$	$\mu gN\ell^{-1}$	$\mu gN\ell^{-1}$	$\mu gN\ell^{-1}$	$\mu gP\ell^{-1}$
Sampling Sites						
1a. Acid mine drainage	6,7	<5.0	12	70	-	30
1b. Natural stream	8,7	16,0	40	2 300	1200	<10
2c. Mixed (left bank)		12,0	30	670	1100	30
2d. Mixed (mid-stream)	8,1	16,0	1000	2200	1000	<10
2e. Mixed (right bank)		8.1	110	2300	110	<10
3. 3-5km downstream	7,3	12,0	470	260	800	<10
4. 8-10km downstream	7,4	16,0	40	670	510	10
White Mfolozi River (1969 Dry winter data)						
(X12) Tshoba tributary	9,9	- -		60	-	80

Table 3. Inorganic water quality characteristics of the upper Tshoba stream components. (CSIR summer survey data - 16/02/1998)

Tshoba River (1998)	**Cation and Anion Concentrations**						
	Calcium	**Magnesium**	**Sodium**	**Potassium**	**Sulphate**	**Chloride**	**Iron**
Units	mgCaℓ^{-1}	mgMgℓ^{-1}	mgNaℓ^{-1}	mgKℓ^{-1}	mg $SO_4\ell^{-1}$	mgClℓ^{-1}	mgFeℓ^{-1}
Sampling Sites							
1a. Acid mine drainage	363	198	115	1.8	3100	31	151
1b. Natural stream	198	85	350	5.8	1540	8.7	0,03
2c. Mixed (left bank)	368	200	100	1.5	3720	31	133
2d. Mixed (mid-stream)	222	92	338	5.7	1650	11	0,24
2e. Mixed (right bank)	208	83	360	6.0	1530	9.2	0,01
3. 3-5km downstream	114	55	213	3.6	1030	12	0.04
4. 8-10km downstream	31	22	45	1.9	158	13	0,14
White Mfolozi River (1969 Dry winter data)							
(X12) Tshoba tributary	23,9	19,3	23,2	1,2	11,5	8,8	0

Table 4. Benthic diatom community analysis (%composition) at sites on the Tshoba river (CSIR Field Survey : 16-02-1998)

Sampling Sites 16/02/1998 Diatom Species List	**Site 1a** Acid drain	**Site 1b** Natural Stream	**Site 2c** Mixed Left	**Site 2d** Mixed centre	**Site 2e** Mixed right	**Site 3** 3-5km downstream	**Site 4** 8-10km dowmstream
Achnanthes minutissima		**19.2**		**7.0**	**14.6**	0.4	**50.0**
Amphora fontinalis						**3.7**	
Anomoenis vitrea		**25.2**		2.0	**18.5**		2.3
Caloneis bacillum						**3.7**	1.5
Caloneis molaris		0.4			0.6	**10.1**	2.3
Cap crucicula						2.1	
Cyclotella meneghiniana		0.8			0.6		
Cymbella affinis						1.2	**3.6**
Cymbella aspera						2.3	
Cymbella cesatii		**17.7**			4.8		
Cymbella gracilis							0.8
Cymbella kapii		1.1					
Cymbella microcephala		1.7			2.1	0.2	
Cymbella pusilla						1.9	1.5
Cymbella silesiaca						1.0	0.8
Diploneis puella						0.4	
Fragilaria fasciculata							2.3
Fragilaria nanana		4.1		1.0	2.6		
Gomphonema gracile				2.0			
Gomphonema parvulum		2.8			2.8		**7.6**
Hatzschia distinctepunctata						0.4	
Navicula elginensis						1.0	
Navicula erifuga						2.1	
Navicula heimansii					0.3		
Navicula libonensis						1.1	
Navicula pseudohalophila		**9.4**			2.1		
Navicula pupula						0.6	0.8
Navicula rostellata						2.7	
Navicula schroeteri		0.7			0.3	1.2	1.5
Navicula tenelloides					0.3	1.2	
Navicula vandamii						1.8	0.8
Navicula veneta						1.6	
Nitzschia angusteforaminata						0.2	
Nitzschia debilis							
Nitzschia denticula						**9.9**	1.5
Nitzschia dissipata						1.2	
Nitzschia linearis						**38.3**	1.5
Nitzschia microcephala							0.8
Nitzschia nana		**9.4**		**6.8**	**16.8**		
Nitzschia palea		2.4			**6.8**	**3.5**	1.5
Nitzschia palea v. debilis		0.4			3.1		**3.0**
Nitzschia paleaeformis	**85.5**		**88.0**	**40.0**	4.1	1.6	
Nitzschia reversa		0.8			**6.3**		0.8
Nitzschia tropica		0.9		**6.0**	**5.2**		
Nitzschia vitrea						0.4	
Rhopaloidia operculata							0.8
Stauroneis kriegerii	**14.4**		**11.6**	**30.0**	**5.7**		
Surirella angusta						1.2	
Surirella tenera						0.4	
Synedra ulna		2.8		**5.0**	1.2	0.2	**3.0**
Other species	0.1	0.2	0.4	0.2	0.9	0.4	6.8
Percentage Composition	100	100	100	100	100	100	100
No of frustules counted	532	500	517	517	579	516	132
No of species/site	2	17	2	9	21	32	21
No of dominants:Combined %	2 (99)	5 (81)	2 (99)	6(95)	7(73)	6(69,2)	5(67.2)

Dominant species data (> 3% of count) in bold

Table 5. A comparison of Diatom Community Index scores for each site generated from species data in Table 4 using Omnidia software.

SAMPLING SITES ▼	◄ DIATOM COMMUNITY INDEX SCORES ►							
	SPI	**SHE**	**WAT**	**EPI-D**	**ROTT**	**GDI**	**CEC**	**BDI**
Two separate inflows above the confluence								
1a. Acid drainage	11,7	10,5	0	15,3	15,9	2,7	7,3	1
1b. Stream	15,8	16,5	11,7	14,2	18,3	12,8	16,0	10,3
Conditions 50 metres downstream from the confluence								
Site 2c. (left edge)	11,5	10,5	0	15,3	15,9	2,4	7,3	1
Site 2d. (centre)	14,5	12,1	10,7	14,5	16,7	6,2	16,4	14,3
Site 2e. (right edge)	13,3	14,9	9.7	13,3	18,7	8,5	12,8	12,4
River recovery scores several kilometers downstream								
Site 3. 3-5km	12,1	12,7	10,7	8,9	15,8	8,0	6,5	10,2
Site 4. 5-8km	14,3	14,6	17,0	12,4	16,7	12,6	14,9	15,2

Table 6. Correlation matrix of water quality variables (ex Tables 1 – 3) and diatom index scores (ex Table 5) Tshoba river survey and diatom population analysis (16-02-1998)

Water Quality Variables	**Diatom Community Index Scores**				
	SPI	**SHE**	**WAT**	**GDI**	**BDI**
pH value	0,54	**0,86**	**0,86**	**0,92**	**0,82**
Suspended solids	-0,36	-0,58	-0,40	-0,57	-0,33
Turbidity	0,06	-0,24	0,32	0,08	0,34
Conductivity	-0,04	-0,30	-0,60	-0,53	-0,56
Total dissolved solids	-0,41	-0,47	-0,63	-0,65	-0,57
Temperature	-0,57	-0,44	-0,28	-0,28	-0,32
Chemical oxygen demand	0,49	0,37	0,59	0,50	0,57
Ammonium-Nitrogen	0.61	0,32	0,67	0,49	0,72
Nitrate-Nitrogen	0,56	0,54	0,54	0,46	0,59
Total soluble phosphorus	-0,74	**-0,80**	**-0,98**	**-0.95**	**-0.97**
Sodium	0,02	0,01	-0,05	-0,11	0,02
Potassium	0,44	0,41	0,42	0,34	0,48
Calcium	-0,37	-0,45	-0,60	-0,62	-0,54
Magnesium	-0,43	-0,50	-0,66	-0,68	-0,60
Sulphate	-0,04	-0,31	-0,60	-0.53	-0,57
Chloride	-0,68	**-0,73**	**-0,83**	**-0,85**	**-0,79**

NB: Bold/Shaded correlations are significant at p< 0,05 (N= 7 :Case wise deletion of missing data)

Table 7. Particle size analysis of sediments extracted from the Tshoba River. (16-02-1998)

Tshoba River Study (16/02/1998)	**Sediment particle size categories (% composition)**			
	Coarse sand to gravel	**Medium sand**	**Fine Sand**	**Mud**
Particle dimensions ►	(> 1mm)	(0,25 - 1mm)	(0,063 - 0,25mm)	(< 0,063mm)
Sampling Sites ▼				
1a	8,8	87,7	3,6	0,0
1b	0,0	9,5	45,5	45,0
2	14,3	71, 7	12,0	2,0
3	0,7	85,2	14,2	0,0
4	2,4	96,4	1,1	0,0

Table 8. SASS4 index scores for the Tshoba river study - (16/02/1998)

Tshoba river study	**◄ SASS Index scores ►**		
Sampling Sites ▼	**SASS4 Score**	**No. of Families**	**Score/Taxon (ASPT)**
1a.	7	2	3,5
1b.	66	13	5,1
2.	32	6	5,3
3.	25	5	5,0
4.	81	15	5,4

Table 9. **River condition c**ategories developed from Habitat Quality Index (HQI) SASS and ASPT (Average Score per Taxon) values:

HABITAT (HQI)	**SASS**	**ASPT**	**CONDITION**
>100	>140	>7	Excellent
80-100	100 - 140	5-7	Good
60-80	60 - 100	3-5	Fair
40-60	30 - 60	2-3	Poor
<40	<30	<2	Very poor

SECTION 6

COLLECTION OF DIATOM SAMPLES FOR MEANINGFUL ENVIRONMENTAL ANALYSIS: A METHODS SUMMARY

William R Harding, Colin GM Archibald & Jonathan C Taylor

1. INTRODUCTION

If the results of a diatom survey are to be used for the purpose of environmental classification the correct sample collection procedure needs to be observed. Collecting diatom samples is relatively simple, but a few key principles need to be observed in order to avoid confusion when interpreting the data yielded by the analysis of diatom communities. What follows is a summary of the procedural elements contained in the following two scientific publications:

Kelly MG, Cazaubon A, Coring E, Dell'umo A, Ector L, Goldsmith B, Guasch H, Hürlimann, J, Jarlman A, Kawecka B, Kwandrans J, Laugaste R, Linstrøm EA, Leitao M, Marvan P, Padisák J, Pipp E, Prygiel J, Rott E, Sabater S, Van Dam H and Vizinet J (1998) Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology* **10**:215-224.

Prygiel J, Carpentier P, Almeida S, Coste M, Druart J-C, Ector L, Guillard D, Honeré MA, Iserentant R, Ledeganck P, Lalanne-Cassou C, Lesniak C, Mercier I, Moncaut P, Nazart M, Nouchet N, Peres F, Peeters V, Rimet F, Rumeau A, Sabater S, Straub F, Torrisi M, Tudesque L, Van der Vijver B, Vidal H, Vizinet J, Zydek N. (2002) Determination of the biological diatom index (IBD NF T 90-354): Results of an inter-comparison exercise. *Journal of Applied Phycology* **14**:27-39.

Observations pertaining to the use of diatoms in river assessments have been sourced from:

Round FE (1991). Diatoms in river water-monitoring studies. *Journal of Applied Phycology* **3**:129-145.

2. SAMPLING EQUIPMENT

The required equipment overlaps largely with that required for SASS (aquatic invertebrate) sample collections (*) – making the concomitant collection of SASS and diatom samples a simple procedure:

- Waders*
- Toothbrush

- Sample storage bottle (150 ml), preferably HDPE (high density polyethylene)
- Plastic tray (preferably white)*
- Wash-bottle filled with distilled water (clean potable water is suitable if distilled water is not readily available)*
- 96% ethanol (approx 20-50 mℓs required per sample)
- Lugol's iodine (alternative to ethanol, 1.5 – 5 mℓ required per sample).

3. SITE SELECTION

As for the sampling equipment requirements, the site selection[1] also mirrors that common to SASS.

(i) The sample site should preferably be situated in a riffle – as close to the centre of the river as possible -see (ii);

(ii) In the case of wide/broad rivers - samples may be taken closer to the bank in the littoral zone.

Note 1: The collection of diatoms is possible from a wide variety of habitats (open water, sediments, plant surfaces, dry sand – see Appendix). The method described here, i.e. that for the epilithon, allows for standardization of the collection procedure with a high degree of confidence. Alternative approaches are described in the accompanying Appendix. For purposes of method reproducibility it is important that, in all cases, the conditions prevailing at the site sampled, and the method used (time, equipment, area covered) are carefully documented to enable the later comparison between sites, or between repeat visits to the same site (see also ***Note 2***).

4. SUBSTRATE SELECTION

(i) The preferred substrate in river monitoring studies is the epilithon (diatom communities on rocks);

(ii) Cobbles and boulders are the macro-substrate preferred for sampling;

(iii) The substrate must have been submerged (see Note) for at least six weeks prior to sampling[2];

[1] Site locations should be recorded using appropriate map or GPS referencing, and the site should be photographed and/or sketched to depict the main features and show conditions prevailing at the time of sampling.
[2] Duration of submergence should be recorded if known.

(iv) 5 to 10 cobbles or boulders (depending on size) should be selected in a 10 m reach;

(v) The substrate should have obvious diatom growth, either by their appearance (a brown film covering the substrate) or by feel ("slimy" or 'slippery' to the touch - caused by the mucilaginous excretions of the diatoms);

(vi) Attention should be paid to obtaining boulders free from thick layers of sediment or with visible growths of filamentous green algae. Both sediments and filamentous algae support unique diatom communities that differ from epilithic communities: this confuses the ecological-interpretation of the results. If no rocks are available other than those covered with filamentous algae, the filamentous algae should first be removed before sampling for diatoms[3].

Note 2: The issue of the 'submergence' characteristics of the site is an important yet arguably one that is likely to create controversy. This is a very important point, and not easy to determine. However there are several ways round it. Either when planning a study obtain flow data from DWAF or sample when water levels are receding. Winter is the best time to sample diatoms as they reach maximum biomass in the winter months. Also try to sample as close to the middle of the stream as possible, which helps with the assumption of constant submersion.

Significance of submersion time: It takes 4-6 weeks for communities to become established that accurately reflect the environmental conditions. In order to have correct and representative information from the site the substrate needs to have been exposed for this time period at least.

Unless this is the express purpose of the sampling exercise the sampling of rocks emerging from the water should not be undertaken under any circumstances – such rocks will have dead cells dried on to them from the previous communities; additionally the moist sub-aerial zone support a very unique aerophilous diatom community usually composed of *Luticola* and *Hantzschia* which will skew any index scores calculated from the site.

Note 3: Issues of habitat diversity: There are two approaches to the sampling of diatoms, and indeed other biota, in riverine environments. One approach is to gain an understanding of the complete diversity of diatoms whereas the other aims for standardized procedures for a single habitat type – in this case the submerged riffle zone. The alternatives that are provided in the Appendix hereto are for use in (a) the absence of a specified substrate or (b) when the specific intention is to sample such substrata. The alternatives, in order of preference, would be: submerged macrophytes; dead wood and lastly, sediments. It should be noted that employing a wide sampling regime, incorporating a variety of habitats, increases the noise level and decreases the confidence level.

[3] If algae or sediments have been removed the sampling record should be annotated accordingly.

In all cases it is most important to separate sediment samples from any assessments of the epilithon. The sediments are typically dominated by diatoms tolerant of anoxic conditions – resulting in skewed results (e.g. Kelly, 2003[4]).

5. SAMPLING PROCEDURE

5.1 Site and substrate description

Notes should be kept of the following details of the sampling site:

(i) sampling procedure followed (should special actions or considerations have been necessary);
(ii) sampling date and time;
(iii) substrate type;
(iv) the degree of bank-side shading[5];
(v) estimates of substrate composition at the site, cover of filamentous algae and other macrophytes;
(vi) a measurement or reasonable estimate the flow rate;
(vii) any other aspects deemed relevant to the conditions prevailing at the site.

5.2 Substrate cleaning and sample storage

(i) After the substrate has been selected it should be vigorously agitated in the stream to dislodge any sediment or any dead diatom cells
(ii) The substrate should then be brought to the shore and the exposed upper surfaces scrubbed clean with the toothbrush into the plastic tray;
(iii) The substrate can then be rinsed using the wash bottle to remove all diatom cells;
(iv) The toothbrush should also be well rinsed using the wash bottle;
(v) When the required number of rocks or cobbles (5 to 10) has been scrubbed into the tray and the toothbrush rinsed, the resulting brown suspension can be mixed by agitating the tray and then poured into the sample bottle.

[4] **Kelly, MG** (2003). Short term dynamics in an upland stream and implications for monitoring eutrophication. *Environmental Pollution* **125**:177-182.
[5] Although opinions vary as to the degree of impact, the flora present in some river reaches may be affected by the amount of shading from trees – especially in closed-canopy reaches.

(vi) 20 mℓ of 96% ethanol should be added to 100 mℓ of sample to preserve it and prevent cell division. An alternative preservative is Lugol's Iodine – Lugol's Iodine can be purchased in 100 mℓ volumes from most pharmacies and used at a concentration of 1% v/v. Under no circumstances should formalin be used as it dissolves the first siliceous phase of the diatom frustule over time;

(vii) At the next site the toothbrush and tray should be thoroughly washed in the river before taking the diatom sample to prevent cross contamination between samples.

(viii) In the laboratory the sample should be allowed to settle out, and the supernatant can then be poured off to concentrate the sample. Care should be taken not to loose any of the diatom material as this will influence the calculated community structure.

APPENDIX

DIATOM SAMPLING METHODS:

WORKING WITH DIFFERENT SUBSTRATES – OPTIONS FOR ENSURING COMPREHENSIVE SITE ASSESSMENTS

This section describes alternate methodologies if the riffle zone collection procedure described above is not feasible at a particular site. In all cases the same attention to the description of the site and the prevailing conditions as detailed above should be adhered to. It is extremely important that details of the method parameters as applied on site are carefully recorded for later inter-site or repeat-visit comparisons. As with any scientific procedure, the results are only as good as the degree of care and effort that was employed during the sample collection phase.

A1. HABITAT TYPES

A1.1 Planktonic

The diatom plankton (= seston) may be used for generalized assessments of river water quality and ecological condition. However the associations found in the plankton are especially prone to contamination from other habitats and bodies of water such as dams and weirs. Accordingly the plankton is not the preferred community for assessing the condition of rivers.

Using a plankton net – 20 μm to no greater than 50 μm mesh - wade into water where there is sufficient current and allow the water to pass through net for 5 minutes. In standing water move the net through the water in such a manner so as not to entrain and capture sedimentary material.

Collect 3 (three) samples from each sampling site. Collect from the left, centre and right of small rivers, otherwise collect 3 subsamples in total. Aggregate the sample by washing out net and store in a clean sample bottle of appropriate capacity. The sample must be preserved by adding 20% v/v 96% ethanol, or 1% v/v Lugol's iodine. If the sample is also to be examined fresh then the aggregated sample should be split 50:50 and one portion retained on ice for transport to the laboratory (see Section 2: Living and Dead Material).

A1.2. Microphytobenthos

This is the most commonly sampled component and the effort really depends on whether sampling is qualitative or quantitative, and what habitats are available in the river system under consideration. There are several rivers that lack rocky substrates or where the gradient is such that scouring does not occur and siltation has created a meandering, sandy river bed.

Field workers often ignore the fact that the microphytobenthos comprises four component habitats viz.

(i) The submerged surfaces of aquatic plants supporting the *epiphyton*;
(ii) The submerged or semi-submerged rock/cobble/stone surfaces within the light zone supporting the *epilithon*;
(iii) The damp sandy substrates on which the *episammon* diatom component grows, usually at the margins of the receding water level or on exposed sandbanks;
(iv) The silty material in which the *epipelon* is found.

Here it is important to note that the diatoms grow 'on' the first three, and within or amongst the particles of the fourth. This results in the latter environment generally supporting a rich community of motile species.

When collecting diatoms it is advisable to separate material collected from the different habitats and not to combine them (see Note x in the main section of this chapter).

A1.2.1 *Stones–in-current*

Qualitative procedure

Select stones with a flattish surface. By positioning a small plankton tightly against the rock scrape the surface hard with a toothbrush to remove the sediment and algal material. Move around the site to obtain coverage of different subsites and variations in stream velocity. If the toothbrush action is not strong enough use any harder material (eg scalpel or metal teaspoon/spatula) to ensure that material is removed from the rocks.

Artificial substrates

An alternative to the sampling of naturally-occurring substrates is to introduce artificial substrates into the environment and allow the diatoms to colonize these. This provides for a high degree of quantification of not only the speciation occurring, but also the temporal and spatial nature thereof. It also normalizes problems of debate concerning the effect of stone and cobble size, surface areas of sands, depth in the water, current velocities, seasonality, grazing and the like (for example small stones may be expected to be more prone to hydraulic movement and, accordingly, support less diatoms than larger cobbles).

A wide variety of artificial substrates have been used, from pre-cleaned stones and cobbles, to lengths of rope, to glass slides. Clay or cement roof tiles have shown very promising results. The method is not without being fraught with difficulties and requires considerable care in terms of standardizing the approach. Generally the maintenance of *in situ* field trials in rivers is difficult.

A1.2.2 ***Bottom sediments – epipelon component (often marginal or exposed sandbanks):***

Note: Diatoms can be found on dry substrata in streams. If there is no flow in the river/stream then sampling should focus on areas of undisturbed sediments.

Bottom sediments: Qualitative procedure

The commonly-used method[6] is to use 5 mm glass tubing about a meter long or more attached (splinted) to a rod (eg. a broomstick) for deeper water at the margin of a river. Place a finger over the top end of the tubing, insert the lower end under water and rest it on the sediment. Partially release finger pressure as the tube is drawn lightly and horizontally over the sediment surface for about a distance of approx. one meter - as if scraping a line on the surface of the sediment. The pressure of the water will push the sediment material (with diatoms) into the tube. Clamp the top of the tube with your finger - to prevent loss of sample - and carefully swing the tube out and transfer the collected material into a sample bottle.

An alternative to the use of this procedure in shallow water is to use a large syringe attached to the upper end of a flexible latex tube. A syringing action will draw up sediment and diatoms that may be discharged in a sampling bottle.

Bottom sediments: Quantitative procedure

Press the beveled lower end of a clear Perspex tube (approximately 50 cm in length and 20 mm diameter) into the sand and carefully section out a core of the sediment. Carefully remove and and push out the top 1 cm core using an extruder (i.e. from the lower opening upwards). The upper 1 cm surface core of the sediment sample usually retains its integrity as the sample is removed - unless the grains are very large and loosely compacted, or too dry. If the habitat is available this is the most suitable technique for comparison between sites and over time. Typically 5 cores should be collected across the site.

Note: Cores collected in this manner can be used for chlorophyll 'a' analysis if placed into a bottle containing 90% acetone (known volume = 25ml).

A1.3. Periphyton

The method used here is similar to that used for stones and cobbles, except that sections of the submerged stems of plants are used. Sections of reed stems of 0.01 m (10 cms) in length are cut 5-10 cms below the water line. The surfaces of the cut stems are then cleaned into the collection tray using a toothbrush.

A2. SAMPLE PROCESSING

The following is a summary of the essential sample processing steps required before the material may be examined.

A2.1 Separation of living from dead material

It is an essential part of the preparation to ensure that the **living component** of the sampled diatom population is extracted and separated from the dead frustules that may be derived from sites other than the sampled habitat. This is best achieved in the laboratory by spreading the fresh sediments over the bottom of a petri dish and allowing it to settle (eg. overnight). The following day the excess supernatant is drained from the petri dish until the moist sediment is exposed. Several coverslips are allowed to gently rest on the damp sediments for a 4 hour period of exposure to natural light. The coverslips are then carefully removed and placed on a clean slide for microscopic examination of fresh motile cells.

[6] **Round, FE** (1991) Diatoms in river water monitoring studies. *Journal of Applied Phycology* **3**:129-145.

If the original sample contains large sand grains it is advisable to place a small tissue paper between the coverslip and the sediment. This allows the passage of the motile diatoms on to the coverslip but prevents the transfer of unwanted sediment grains to the slide.

If the epilithon was sampled the sample will contain many attached and non-motile species which cannot be removed from the sample in the above manner. In this case it is recommended that, on returning from collecting samples, the samples are examined immediately under the light microscope. If upon examination of sub-sample a large proportion of the cells are seen to be dead (i.e. having no chloroplast or lipids) the sample should be discarded[7].

A2.1.2 Permanent slides

The extraction procedure of Round (1991) described above is the same for producing permanent material except that the coverslips can be submerged in the supernatant water in the petri dishes. After the 4 hour exposure period, the coverslips are removed and the undesirable larger sand particles gently wash off. The extracted living diatom material on the coverslips is placed in beakers for further treatment. The pretreatment and acid washing procedure followed that described by Welsh (1964) with variations depending on the source of the material (see Section A2.2 below). It should be noted that there are several variations of these procedures (eg Round, 1993)[8] but it is advisable to standardize on procedure for a given set of samples.

A2.2 Sample cleaning and preparation for mounting

The method described below here is that described and published by Welsh[9]. The procedure is suited for the simultaneous processing of multiple samples:

[7] **Bate GC, Adams JB and Van Der Molen JS** (2002) *Diatoms as Indicators of Water Quality in South African River Systems*. WRC Report No 814/1/02. Water Research Commission. Pretoria.
[8] **Round, FE** (1993) A Review of methods for the use of epilithic diatoms for detecting and monitoring changes in river water quality. HMSO, London.
[9] **Welsh, H** (1964) A method of cleaning diatoms and the preparation of permanent slides for ecological work. *Limnological Society of southern Africa Newsletter* **1**:39-47.

A2.2.1 *Sample cleaning*

(i) The preserved algal material, as collected in the field or extracted from petri dishes in the laboratory, is transferred to labeled 100 mℓ glass beakers and dried in an oven at a temperature of 80-90°C until dry;

(ii) A small portion of the sample is then checked for the presence of calcium (Ca) by adding a drop of concentrated hydrochloric acid (HCl). If a positive reaction is observed HCl is added to the whole sample to remove Ca, and the sample then washed with distilled water to remove the acid, centrifuged and re-dried. This step is necessary to avoid the formation of insoluble calcium sulphate;

(iii) Approximately 10 mℓ of concentrated sulphuric acid (H_2SO_4) is carefully added to each beaker and allowed to stand for 5-15 minutes, or until all the organic matter has broken down, dehydrated or charred;

(iv) 2-3 mℓ of concentrated nitric acid (HNO_3) is then added to each beaker in a fume cupboard/hood. Red fumes of nitrogen peroxide may be released during this step;

(v) The beakers are then placed on a laboratory hot plate and the acids gently boiled taking care to avoid any bubbling or spitting that might result in sample carryover between beakers. This process is continued for up to 10 minutes or until all the carbonaceous material has been oxidized (rendered as pale as possible). The process may be accelerated by using a Pasteur pipette to add a few drops of nitric acid. When the process is complete the beakers are removed from the hot plate and allowed to cool;

(vi) The samples are transferred into distilled water in 250 mℓ beakers – 60 mℓ of distilled water being added to the beaker prior to sample transfer. The contents of the smaller beakers containing acid and diatoms are then gently swirled by hand and poured into the distilled water in the larger beaker. The object of the swirling is to cause the heavier sand and other particulate matter to collect at the bottom of the small beaker – these heavier particles **should not be poured** into the larger beaker containing distilled water;

(vii) The contents of the larger beakers, ~ 75 mℓ, are then transferred individually to 100 mℓ centrifuge tubes, balanced, and centrifuged at 2000 rpm for 10 minutes. The supernatant is then carefully decanted or removed by siphoning and the pellet of diatoms and small sand particles loosened from the bottom of the centrifuge tube using a wash bottle. This procedure is repeated twice more (a total of three centrifuge cycles) to ensure removal of all the acids (blue litmus paper will no longer turn red);

(viii) After the final washing and centrifuge process the diatom samples are transferred into 10 mℓ specimen tubes with screw caps (commonly called 'specimen' or 'blood' vials/tubes). These tubes should be labeled and the labels firmly secured using clear adhesive tape;

(ix) If, at this point, the samples are to be stored for any length of time before mounting, it is advisable to add 2-3 drops of a 5% aqueous solution of phenol in order to inhibit algal and/or fungal growth.

Alternate method[10]: Hot HCl and $KMNO_3$.

(i) Homogenise sample, place 5 to 10 ml of thick suspension in a beaker.

(ii) Add 10 mℓ saturated potassium permanganate ($KMNO_3$) solution, and leave to stand for 24 hours.

(iii) Add 10 mℓ concentrated HCl, heat on a hot plate at 90°C for 1 to 3 hours until the solution becomes clear.

(iv) After oxidation of organic material with acid, 1 ml of hydrogen peroxide is added to check if the oxidation process is complete, in which case the hydrogen peroxide will not cause lasting foaming.

(v) When oxidation is complete, the samples are rinsed by centrifuging with distilled water at 2500 rpm for 10 min.

(vi) The supernatant is then decanted and the washing is repeated a further 3 times. Care should be taken not to lose any material.

(vii) The cleaned diatom suspension is placed in small vials (with sample information) in alcohol or distilled water.

A2.2.2 *Sample mounting*

(i) To ensure quality mounts the glass slides and cover slips must be scrupulously cleaned;

(ii) Slides: This is best achieved by using a mixture of concentrated sulphuric acid (H_2SO_4 – 250 mℓ) and potassium dichromate ($K_2Cr_2O_7$ – 100 gm) made up in 750 mℓ distilled water. After cleaning the slides are washed in tap water and rinsed using distilled water. Thereafter they are stored in absolute alcohol until required;

[10] **Hasle GR** (1978) *Some specific preparations: diatoms*. In Sournia, A (Ed). Phytoplankton Manual. UNESCO, Paris.

(iii) Cover slips: These are first rinsed in a strong solution of sodium hydroxide (NaOH), washed well in tap water and rinsed with distilled. They are then stored covered with acetic acid in a wide-mouth bottle – until required;

(iv) Immediately prior to use both the slides and cover slips are wiped dry with a clean, well-washed cotton cloth ('handkerchief') – which should be kept stored in a bottle for this purpose;

(v) To prepare the mount 3-6 drops of distilled water are placed on a perfectly dry No 1 coverglass (20 mm diameter – square coverslips are preferable for counting purposes) – using a dropper or Pasteur pipette. A single drop of the prepared diatoms is thoroughly-mixed with the water. The preparation is covered using an inverted beaker and left to dry at room temperature. Note: The drying area should be free of vibration in order to prevent the diatoms from forming clumps – this renders counting using the Thomasson method very difficult. Drying should not be accelerated using a hot-plate as this leads to the formation of concentric rings of diatoms;

(vi) After drying 2-3 drops of xylol are placed on the cover glass such that the whole surface is covered. Thereafter 1 drop of Hyrax (or other suitable mounting medium, e.g. Pleurax) is added. Note: The mounting medium must drip off a glass applicator rod – if not it should first be thinned with xylol. The preparations are then left for an hour to allow the xylol to evaporate and then dried in a 45°C oven overnight;

(vii) Following overnight drying clean slides are placed on a 60°C hotplate, and once heated to this temperature the cover slip is inverted onto the slide, positioned, removed and allowed to cool;

(viii) If desired the cover glass may be ringed with shellac cement to prevent the cover glass from falling off;

(ix) The slide is now ready for labeling. It is advisable to attach two labels to each slide: one giving the locality and reference number, and the name of the collector; and the other the name of the mounting medium and date of slide preparation.

A2.3 Counting

A variety of counting methods are available. An excellent procedure to follow is that of Schoeman[11].

[11] **Schoeman, FR** (1979) A Method for the quantitative and qualitative determination of planktonic diatoms. *Journal of the Limnological Society of southern Africa* **5**:107-109.

SECTION 7

ADVANTAGES AND PROBLEMS IN USING HISTORICAL DIATOM COMMUNITY ANALYSIS SHEETS FOR INFERRING PAST WATER QUALITY IN SOUTH AFRICA

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South Africa has a long legacy of diatom research. The eminent diatomist Dr. B. J. Cholnoky spent much of his working life examining and enumerating diatom communities found in Southern Africa. Most if not all of Cholnoky's collected diatom material in the form of mounted material on glass slides is stored in the South African Diatom Collection housed at the CSIR in Durban. Cholnoky's diatom community analysis sheets are likewise stored together with the original slides used by him and other researchers (e.g. Giffen, Archibald and Schoeman) to make their identifications. As he only employed enumeration methods yielding a margin of error of 2% or less, Cholnoky's results should provide an accurate reflection of the structure of the diatom communities that he examined. Cholnoky, in addition to being a leading diatom taxonomist, was also interested in the ecology of this group. By tracking changes of certain groups, e.g. *Nitzschia*, along a stream or river he drew conclusions about the levels of nitrogenous pollution. Species composition changes were tracked using carefully composed analysis sheets. It was the aim of this 2004 study to demonstrate the value of these historical diatom analyses for inferring water quality conditions using the diatom-based index method.

Data for the Jukskei-Crocodile River system were obtained from the South African Diatom Collection for the period 1956/1957. This river system lies in the heart of the industrialised Gauteng province (Johannesburg-Pretoria area) and has been receiving polluted effluent from various sources for decades. The nomenclature of the diatoms listed on Cholnoky's data sheets was modernised and the data then entered into OMNIDIA v3 - with the exception of a single species *Cymbella bengalensis* for which no acronym could be found

Diatom index scores generated from OMNIDIA v3 were in general in agreement with Cholnoky's own assessment of water quality (especially with reference to nitrogenous/organic pollution). Erroneous assessments using diatom indices were made because of *Achnanthidium saprophila* (only described in 1982) being included in the diatom analyses as *A. minutissimum* (*Achnanthes minutissima*). Evidence for this opinion will be presented. It is concluded that the diatom analysis records housed in the South African Diatom Collection constitute a valuable resource for the assessment of past conditions of rivers and streams.