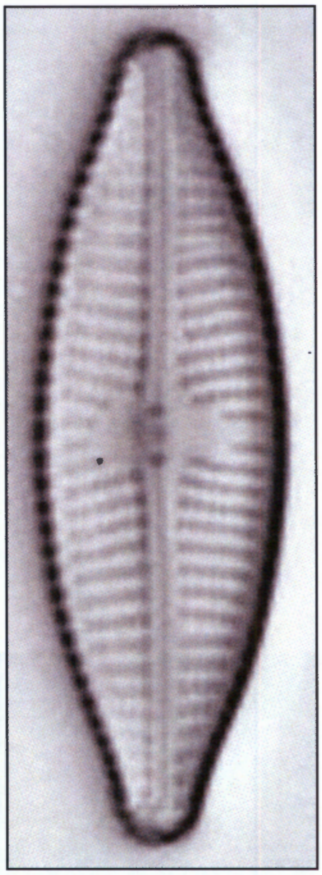
Princess Vlei: What diatoms can tell us about spatial and temporal heterogeneity in an urban wetland



Oliver Cowan

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Supervisors: Prof. M.E. Meadows & K. Kirsten

Abstract

Globally, wetlands are being degraded and destroyed largely as a result of anthropogenic activity. Monitoring and assessment are required to maintain functional ecosystems and the valuable services they provide. Surface sediment cores were taken from 4 points around an urban wetland (Princess Vlei) on the Cape Flats, Cape Town, South Africa. Diatoms were and used as a proxy for water quality variables to determine whether the diatom flora preserved in organic sediments accumulating in and around an urban wetland is a useful tool in determining the changes in water quality over time. In addition, the project attempts to establish whether there are spatial patterns in diatom flora within a small lake. Diatoms from three depths from each of the four sites were identified, counted and analyzed according to their trophic preferences and pollution tolerances which were acquired from a variety of resources. Results showed clear spatial differences between sites as a result of positioning in relation to effluent input, output and specific site characteristics such as reed stands. Differences between depths were evident although no significant trends were observed. In summation; spatial heterogeneity in the diatom assemblage of an urban wetland reveal that diatoms are suitable indicators of water quality, even within a small system, due to their niche specificity. The project also shows that Princess Vlei remains a eutrophic and polluted wetland, although not uniformly so. Continual monitoring is required to prevent the vlei from becoming a health hazard for the surrounding community and to maintain its ability to act as a buffering zone to protect the Rondevlei nature reserve, as well as acting as a functional ecosystem in an area threatened by urbanization.

Key Words: Princess Vlei; Diatoms; Water Quality; Eutrophication; Pollution

1. Introduction, aims and objectives

1.1 Introduction

Conservative estimates suggest that over 50 % of the worlds wetlands have been lost and, in certain regions, this number approaches 99% (van der Valk, 2006). Although wetland loss is the tragic endpoint, wetland degradation is a worldwide hazard as a result of direct and indirect anthropogenic effects such as increased nutrient loading, altered hydrologies and invasive alien species (van der Valk, 2006). Although the 'Flux of Nature' paradigm (Wu and Loucks, 1995) is widely accepted by conservation biologists, the acknowledgement that nature is in a constant state of flux driven by internal and external forces (Pickett *et al.*, 1992) does not equate to the acceptance that human activities do not threaten ecosystems.

Freshwater wetlands are particularly valuable to humans; the services they provide (see Zedner and Kircher, 2005, for a review) are estimated to be worth almost $5000 billion per year at 1997 prices (Costanza *et al.*, 1997). This alone is cause for their conservation and monitoring to be of paramount concern to all stakeholders. Added to this is their undoubted importance to promoting biodiversity (van der Valk, 2006); thus there exists an intrinsic value in their conservation and this in turn leads to the call to conserve wetlands for 'nature's sake' (McCauley, 2006). Regardless of which argument one uses, the importance of monitoring wetlands to aid in their conservation, restoration or rehabilitation is the common denominator.

1.2 Aims and Objectives

This study aims to determine whether the diatom flora preserved in organic sediments accumulating in and around an urban wetland is a useful tool in determining the changes in water quality over time. In addition, the project attempts to establish whether there are spatial patterns in diatom flora within a small lake. The project hypothesizes that diatom community structure varies through time as a result of urbanization effects on water quality and that it varies spatially with the characteristics of runoff at different locations around the wetland.

The aims will be achieved by:

- Identifying variations in the diatom flora at several locations and over depth
- Assessing Princess Vlei's water quality through an autoecological classification of the diatom assemblage based on eutrophication and pollution
- Determining the primary underlying mechanisms which affect the diatom assemblage; using principle component analysis to identify the characteristics of the assemblage
- Identifying the causes of observed trends by applying knowledge of the vlei's hydrology, biology and catchment characteristics

2. Literature Review

2.1 Monitoring and Assessing Water Quality

In broad terms, the monitoring of water bodies can be separated into two distinct divisions; namely, chemical monitoring and biological monitoring. Although chemical monitoring of water quality is a useful tool it has been argued that it is time-consuming, expensive and does not always provide a composite reflection of actual water quality (Taylor *et al.*, 2007*a*). Biological monitoring on the other hand examines organisms whose exposure to pollutants is continuous, thus aquatic communities reflect and integrate the effects of physico-chemical disturbances that extend over extended periods of time providing a holistic measure of the aquatic ecosystem health (de la Rey *et al.*, 2004). Invertebrates have been successfully used as biological indicators in many countries, including South Africa; however it has been argued that they struggle to monitor eutrophication, an increasing threat to, particularly, lentic water integrity (Taylor *et al.*, 2007*a*). An increasing emphasis has been put on algae as water quality indicators and monitoring tools worldwide (Whitton and Kelly, 1995; Rott *et al.*, 2003). The proceeding section focuses on diatoms and why they are ideal biological indicators in monitoring water quality.

2.2 Diatoms

Diatoms (Bacillariophyceae) are microscopic algae found in almost all aquatic and semi-aquatic environments. They are characterized my numerous features but are most easily recognized by their siliceous (opaline) cell walls, composed of two valves that together form a frustule (Stoermer and Smol, 1999). Diatom taxa can be identified by their cell walls which have a diagnostic size, shape and sculpturing. Due to the siliceous composition of the frustules, diatoms are often excellently preserved in palaeo-deposits and sediments (Stoermer and Smol, 1999). Although only effectively studied for the past 150 years, the number of diatom species has been estimated at between 10^4 and 10^5 (Mann and Droop, 1996). Due to their high species diversity and niche specificity, diatom community composition can be closely related to the biological and physico-chemical variables in their environment, thus they are justifiably considered excellent biological indicators (Fritz *et al.*, 1999). The ability of diatoms to act as a proxy for water quality was recognized shortly after their introduction into mainstream biology. According to Stephenson and Pan (1999) diatoms have been used to assess ecosystem health and diagnosing causes of stress to aquatic habitat for the past 50 to 100 years. Diatoms have one of the shortest regeneration times of all biological indicators. This high species turnover time allows them to respond rapidly to environmental change and act as early warning indicators for pollution, eutrophication or ecosystem restoration success (Rott, 1991).

Autecological indices use the relative abundance of species in assemblages and their ecological preferences, tolerances or sensitivities to infer environmental conditions in an ecosystem (Kelly *et al.*, 1995). Because of their niche specificity, many diatom-based autecological indices have been developed and are in widespread use. Environmental factors like eutrophication, organic pollution, heavy metals, salinity, pH, and pesticides can, and are, being modeled using indices (see Kelly *et al.*, 1995). Considerable debate exists over how many diatoms to count and to what level of taxonomic resolution is necessary to provide valuable autecological assessment (Pappas and Stoermer, 1996; Stephenson and Pan, 1999; Kelly *et al*, 1995); however Stephenson and Pan (1999) reason that the answer depends on the objectives and budget of the project.

There is a wealth of examples of applied studies using diatoms to assess water quality in the literature (Millie et al., 2004; Duong et al., 2007; Lane and Brown, 2007). Much of the work involves assessing the damages caused by anthropogenic actions. One of the main anthropogenic effects is eutrophication (predominantly nutrient inputs from domestic and industrial sewerage disposal and farming activity i.e. fertilizers in run-off and soil erosion), although it can also occur naturally such as after a fire (Davies and Day, 1998). Diatoms are intrinsically linked to nutrient loading as they are primary producers. Increased phosphorous loading stimulates diatom production and sedimentation. The bloom can reach appoint where silica, a component of the diatoms' cell walls, becomes the limiting factor in diatom growth. This can lead to cyanobacteria replacing diatoms in the water body which has consequences for the trophic structure and trophic efficiency of the water body (Schelske, 1999). Hall and Smol (1999) note that diatoms are excellent monitoring tools for eutrophication because individual species are sensitive to changes in nutrient concentrations, supply rates and ratios. Because each taxon has a specific optimum and tolerance for nutrients which can usually be quantified to a higher degree of certainty, indices have been developed for phosphorous (Fritz *et al.*, 1993; Bennion *et al.*, 1995) and nitrogen (Christie and Smol, 1993). A study on the effects of fish-farming on diatom assemblages by Camargo and Jimenez (2007) found that the organic pollution and increased nutrients from trout farm effluent resulted in an increase in diatom abundance with increased effluent but decreased diatom diversity. The authors also noted a significant shift in community composition but did not look at other water quality variables which are known to affect diatom community composition. Rasane et al. (2006), investigating whether eutrophication was a natural occurring phenomenon in certain European lakes, found diatoms an ideal proxy for eutrophication in the lake sediment. A review of European diatom eutrophication indices found that although variability existed between the various indices, in general they were suitable for assessing eutrophication (Besse-Lototskaya et al., 2010).

Closely linked to the problem of eutrophication is that of water pollution, both organic and industrial. Organic pollution is a particular concern in urban water bodies which are often the end point for sewerage of varying treatment – from raw to fully processed (Davies and Day, 1998). Diatoms can be found in even the most toxic of water and have been used throughout the world to gauge organic water pollution. Studies from the USA (Lane and Brown, 2007); Australia (Newall and Walsh, 2005); Asia (Wu and Kow, 2002; Wan Maznah and Mansoor, 2002); Morocco (Fawzi *et al.*, 2001), South America (Gomez, 1998) and Europe (Eloranta and Soininen, 2002; Kelly, 1997) have all successfully used diatoms to monitor and assess water pollution in urban rivers and wetlands.

In addition to the assessment of eutrophication and water pollution diatoms have also been successfully used to monitor and assess salinity (Denys and De Wolf, 1999); pH (Charles and Smol, 1988; Leira and Sabater, 2005); to gauge water levels (Wolin and Doutie, 1999); and in a more applied manner, as in the case of oil and gas exploration (Krebs, 1999) and even in forensic science (Peabody, 1999). It is due to their wide range of uses that the majority of studies using diatoms to assess water quality do not just focus on one water quality variable but rather a suite of variables. Turner and Rabalais (1991) used diatoms to assess water quality change in the Mississippi catchment and found compelling evidence showing environmental change due to human-related activities through the past century. Gomez (1998) worked on benthic diatoms to test how closely correlated they were to water quality in a river severely impacted by anthropogenic processes. The results showed a strong correlation and the author advocated the use of diatoms for similar studies. The study found that the distribution of diatom flora was influenced by human activities resulting in pesticide, phenols, heavy metals, organic matter and other industrial by-products entering the water.

2.3 Diatom use in assessing and monitoring water quality in South African freshwater systems

Diatoms have been studied in South Africa since the 1950's (e.g. Cholnoky, 1953) and indicate that species are either endemic or cosmopolitan i.e. species that are common to most other regions of the world (Bate *et al.*, 2002). According to Bate *et al.* (2002), both epilithic (attached to stones) and epipelic (attached to sand or mud) diatoms can be used as water quality indicators in South Africa. However, epilithic diatoms may integrate water quality over a shorter time period, whereas epipelic reflected long term integrated water quality patterns. Diatoms are suitable as biological indicators in South Africa as they are ubiquitous members of aquatic ecosystems, react rapidly and predictably to changes in water quality and their taxonomy has been well described (Bate *et al.*, 2002). A comprehensive study on diatom flora and water quality in rivers in the Western Cape, Eastern Cape and Mpumalanga showed that seasonal changes in the flora were not significant but community changes were evident along a pollution gradient. The results showed that changes in conductivity, nitrogen, pH and phosphorous can be effectively inferred from diatoms with a lower degree of variation than monthly monitoring of water chemistry due to the integration effects that changes in water quality conditions have on diatom assemblage composition (Bate *et al*, 2002).

Although efforts have been made in recent years to create a South African-specific diatom index for water quality, the project has been temporarily suspended due to a lack of funding (Harding, 2010). Fortunately, owing to the cosmopolitan nature of the majority of diatom species, Taylor *et al.* (2007) found that, although it may become necessary to develop an index unique to South Africa, European indices can be used to infer water quality in the country. Rott *et al.* (2003) warn that while European indices can be successfully reassigned to other continents, a preliminary examination of regional conditions is essential. Taylor et al. (2007*a*) found that 98% of the diatom species in a polluted section of the Vaal River were cosmopolitan, thus further strengthening the notion proposed by Kelly *et al.* (1998) that they may potentially occur anywhere in the world where a certain set of environmental conditions exist which favour the proliferation of a particular species.

The aforementioned examples reveal the potential for the use of diatoms in assessing and monitoring water quality in South African freshwater systems. Additionally, the importance of preserving functioning wetland ecosystems both for economic and biological reasons has been illustrated. The outline for the remainder of this project follows the standard format: a detailed description of the study site and its history is given before the methodology used is listed. The results are then presented and discussed before conclusions are drawn.

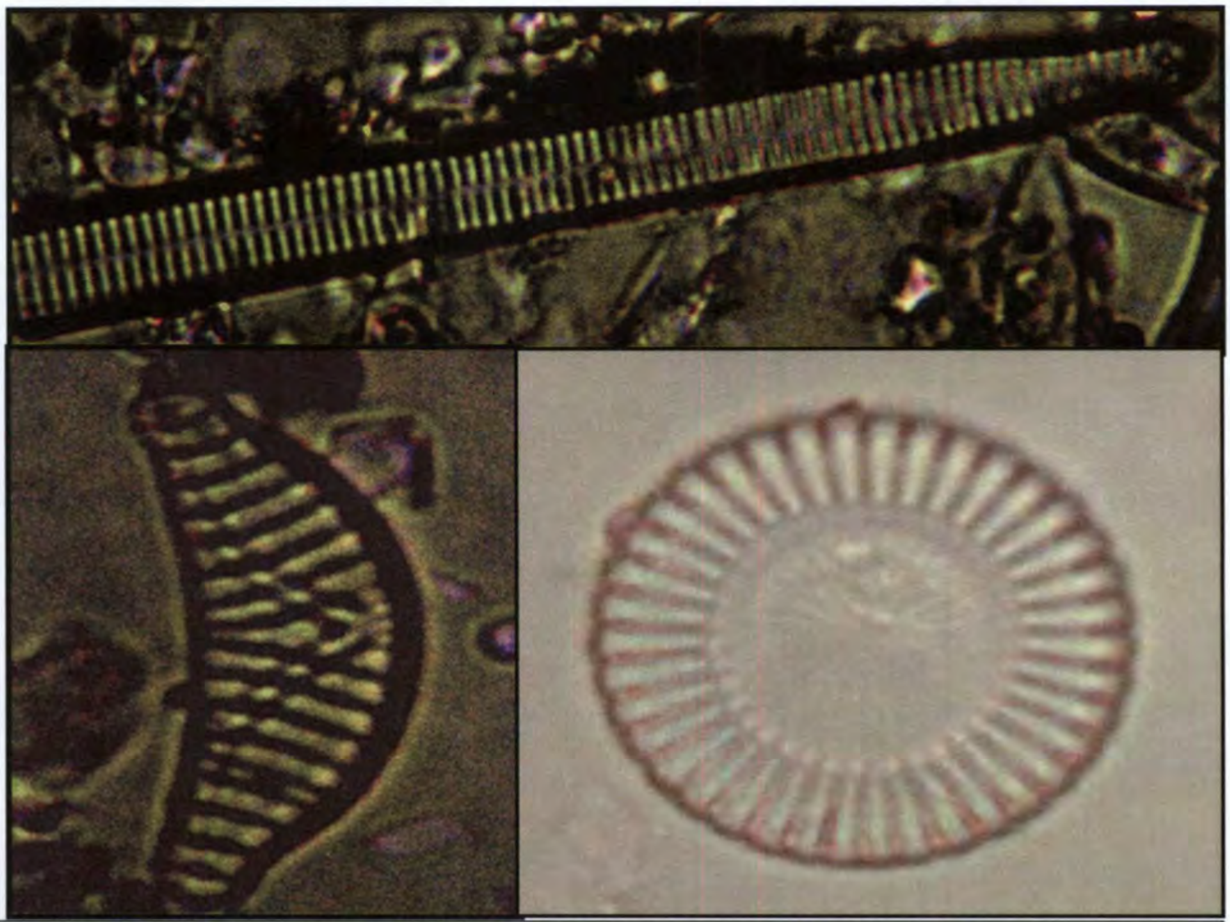

Figure 1: Photographs of three diatom species identified at Princess Vlei: (clockwise from top) *Fragilaria biceps; Cyclotelle meneghiniana; Epithemia sorex*

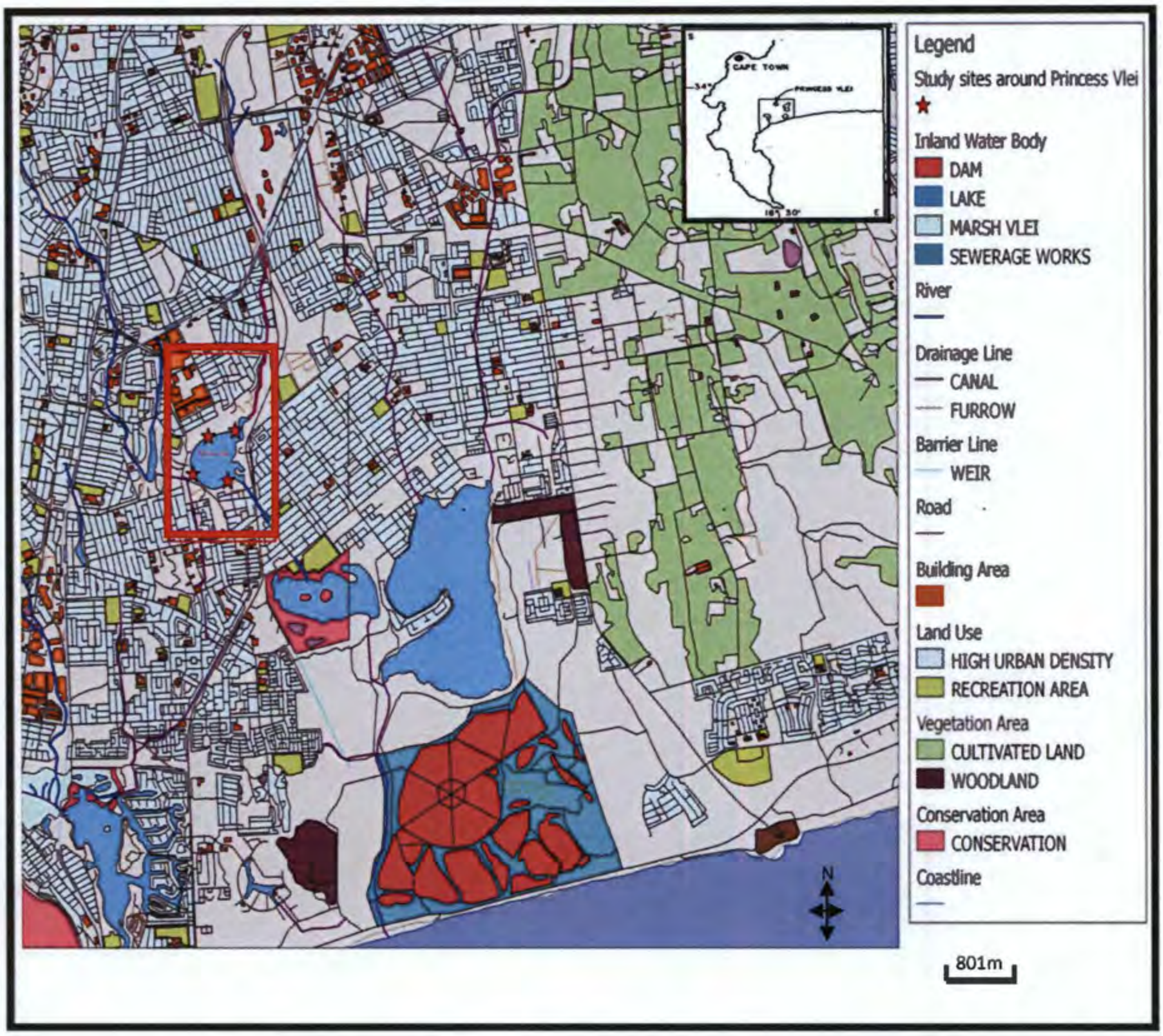

Figure 2: Land-usage in the Princess Vlei catchment (red square) and surrounding area along the Cape Peninsula (inset). (Data Source: Department of Land Affairs)

Princess Vlei is a small (29 ha), shallow, eutrophic freshwater coastal vlei (Harding, 1992) situated in the suburb of Southfield in the city of Cape Town, South Africa (34°03'S; 18°28'E). The region experiences a typically Mediterranean climate with mild, wet winters and dry, warm summers. Mean annual rainfall varies across the peninsula due to topography, although the Cape Flats (where Princess Vlei is situated) receives ~520 mm/pa (weathersa.co.za, 2010). Most of the rain occurs as strong north-westerly winds accompany cold fronts during the winter months. Rainfall events can be extreme and flooding is almost an annual occurrence on the Cape Flats. Mean maximum winter temperatures are 17. 5°C compared to mean summer maximum temperatures of 26.5°C. The warm summer conditions are frequently accompanied by strong south-easterly winds (weathersa.co.za, 2010).

Princess Vlei is one of numerous water bodies in the region (**Figure 2**). The primary input of water into the vlei is the Southfield Canal - a man made structure that drains an urban catchment of approximately 800 ha. An outlet weir joins Princess Vlei to Rondevlei (a nature reserve and bird sanctuary) via the Italian Road Canal (Harding, 1992). Adjacent to Rondevlei is the larger Zeekoevlei and south of Zeekoevlei lies the Strandfontein Wastewater Treatment Works. To the northwest of Princess Vlei are the Little Princess Vlei and the Diep River (Harding, 1992).

The vlei's perimeter is characterised by a mixture of different land-use. Princess Vlei is bordered to the north by urban housing and small-scale industry; to the east by middle-lower income urban housing and to the west by privately owned open space and the Little Princess Vlei; to the south by a combination of open public space and urban housing. The catchment (**Figure 2**) as a whole is characterized by middle to lower income-group urban housing and small scale industry. Agricultural land (market-gardening produce in the main) is found in the adjacent area of Philippi. The actual vlei is used for recreational activities including fishing although the vlei's popularity has waned recently in lieu of a much-publicised murder in 2000 (Cape Argus, 2000) and a more recent gruesome murder and robbery which occurred at the Vlei in early 2010 (Cape Argus, 2010).

The waterbodies of the Cape Flats are all situated above the Cape Flats Aquifer which has a potential of approximately 53 Mm^3/a; however, the water quality throughout the aquifer is variable. It has been determined that the vlei's in the region are groundwater features resulting from the shallow water table intersecting ground surface in areas of topographical lows (Parsons and Harding, 2002). The importance of groundwater recharge on Princess Vlei's water quality and level should not be underestimated. Mapping of groundwater flows in the Cape Flats was undertaken by Parsons and Harding (2002) who reported groundwater flow into Princess Vlei occurred on either side of Site 2, while groundwater out flow occurred between Sites 3 and 4 (see **Figure 3**).

2.1 History of Princess Vlei

Princess Vlei acquired its name from an ancient fable about a *Khoisan* princess who was abducted by Portuguese slave traders and whose tears allegedly formed the vlei (Brown and Magoba, 2009). Princess Vlei was described in the 1920's as alkaline and in the 1940's as alkaline and eutrophic (Harding, 1992). In 1983 the vlei was dredged to remove a shallow sand bar and in 1990 a bathymetric survey revealed sill from the central sand bar had been replaced by a central deepening and some redistribution of the sediment. The volume of the vlei was calculated as 715 000 m^3 with sediments contributing 21% (Harding, 1991). Although the vlei was previously linked to Little Princess Vlei and the Diep River, they are now in different catchments; however the link was restored in the 1990's so that Princess Vlei could act as a flood attenuation pond during heavy winter rains (Harding, 1992). In 1990 an outlet weir was constructed to drain the vlei into the adjacent Rondevlei, prior to this a temporary weir restricted the outflow. The majority of the outflow occurs between April and October during the winter rains. The Cape Town City Council has three sewerage pumps in the Princess Vlei catchment which are designed to flow into the Southfield canal during times of overloading or malfunction. These events occurred frequently in the winter months prior to 1985 whereafter improvements and modifications significantly reduced incidents (Harding, 1992) although they do still occur, as was the case towards the end of February 2006 when a regional power cut temporarily shut down the sewerage pumps re-routing raw sewerage into the stormwater drains and subsequently into the vlei (Cape Argus, 2006).

Figure 3: Princess Vlei, with arrows illustrating the primary input (Southfield Canal) and output (Italian Road canal) points, and groundwater inflow and outflow. Numbered balloons show the sites along the vlei's perimeter where sediment was sampled (Modified from Google Earth)

4.1 Sites

A preliminary, exploratory core was taken at Site 2 (sites were numbered in ascending order from proximity to Southfield canal). The 1.8m core was taken using the vibracorer (Baxter, 1996) and sub-sampled at the lab at 10cm intervals. The initial core revealed diatoms only in the organic layer (top

~20cms). It was then decided that sampling of this organic layer should be done at various other points around the Princess Vlei perimeter.

Four sites were selected around the perimeter of the vlei due to their accessibility and proximity to areas of inflow and outflow. Site 1 was located adjacent to the Southfield Canal and was in the vicinity of a small bay of lentic water. Site 2 was situated within a reed (*Typha capensis)* stand opposite the Italian Road Canal. The site was closest to the industrial buildings North-West of the vlei. Site 3 was sampled directly opposite the Southfield canal within a similarly dense reed stand. Site 4 was positioned adjacent to the Italian Road Canal, almost directly opposite to Site 2, just behind a reed stand.

The core tubing used in this study was standard, thin-walled aluminium irrigation tubing, which is available in 6 m lengths, with a diameter of 7.8cm (Baxter, 1996). For the purpose of this project, the tubing was manually cut into pieces 50cm long using a hacksaw. Using a file, one end of the tube was sharpened to allow for easier penetration. Surface sediments were retrieved by pushing the tubing into the ground by applying a hammer to a wooden plank placed over the top of the tube. When the bottom of the tube was estimated to have reached the base of the organic layer (~ 25cms) it was pulled up manually, labeled and transported back to the lab.

4.2 Diatom Analysis

The cores were split lengthwise at the lab, photographed and described using a Munsell colour chart. Each core was sub-sampled starting at a depth of 5cms and at subsequent 5cm intervals thereafter by scraping off the surface sediment and transporting a small portion into a labeled beaker or test tube. To achieve satisfactory slides for microscope analysis the sub-samples were treated with hot HCL to remove carbonates. They were then diluted with distilled water and allowed to settle overnight. Once excess supernatant liquid was removed via a pipette, samples were washed with 30% H_20_2 and heated to remove organic matter. Clays and silts were removed by swilling the liquid in a beaker, allowing the diatoms to sediment before the suspended clay was decanted. A final wash with de-ionised water was then performed and the samples were centrifuged. Three drops of the final diatom solution were pipetted onto cover slips, diluted with a few drops of de-ionised water and placed on a hot plate. Finally, after all the water had evaporated, the cover slips were mounted onto a microscope slide using Pleurax (R.I. = 1.73) (as adapted from Battarbee, 1986) (Note* A comprehensive, step-by-step guide can be viewed in Appendix I).

4.3 Diatom Counting

At least one slide was made for each depth (5, 10, 15cms) from each of the four sites around the vlei perimeter. Diatoms were identified and taxonomically resolved to species level where possible using a Zeiss Axiostar plus microscope at 1000x magnification. Identification of species was based on catalogues, particularly Taylor *et al.* (2007*c*) and Kelly *et al.* (2005). A count of 300 diatoms per slide was taken and recorded using the counting program *Polycounter* (Nakagawa, 2010). This is deemed sufficient to allow for the identification of ecologically important species not being obscured by mass occurrences of more common taxa (Battarbee, 1986). Once identified and counted, the diatom assemblages were displayed using the TILIA program (Grimm, 1997). A range of resources was used in assigning diatom species to their trophic preference (i.e. diatoms which prefer eutrophic, hypertrophic waters etc.) and their pollution tolerances (Taylor *et al.*, 2007c; Kelly *et al.*, 2005; Muscio, 2002; Carney, 1982; Price and Morel, 1991; Lysakova *et al.*, 2007). Due to the diversity in trophic categories amongst the different sources, it was decided to group certain trophic preferences together to create the four categories seen in the results. The 'Clean' category refers to diatoms which prefer oligotrophic to mesotrophic waters. The 'Nutrient Loading' category includes a variety of categories which have in common a preference for eutrophic conditions: Oligotrophic-Eutrophic; Mesotrophic-Eutrophic; Eutrophic; Polytrophic with a preference for Mesotrophic-Eutrophic; and Polytrophic with a preference for Eutrophic conditions. The 'Over Loaded' category contains diatoms found in Polytrophic and Polytrophic with a preference for Hypertrophic conditions. Where a species' trophic preference could not be obtained it was placed within the 'Unknown' category. Pollution categories were grouped according to Harding *et al.* (2007). A full list of diatom taxa and their trophic preferences and pollution tolerances can be viewed in Appendix II.

4.4 Statistical Analysis

Principle Component Analysis (PCA) was performed on the data using *Statistica V9.0*. PCA's aid in establishing relationships between environmental parameters as well as identifying the most influential environmental factors (Kirsten, 2009) and was thus deemed the most suitable analysis to determine what factors were contributing to the observed assemblage patterns.

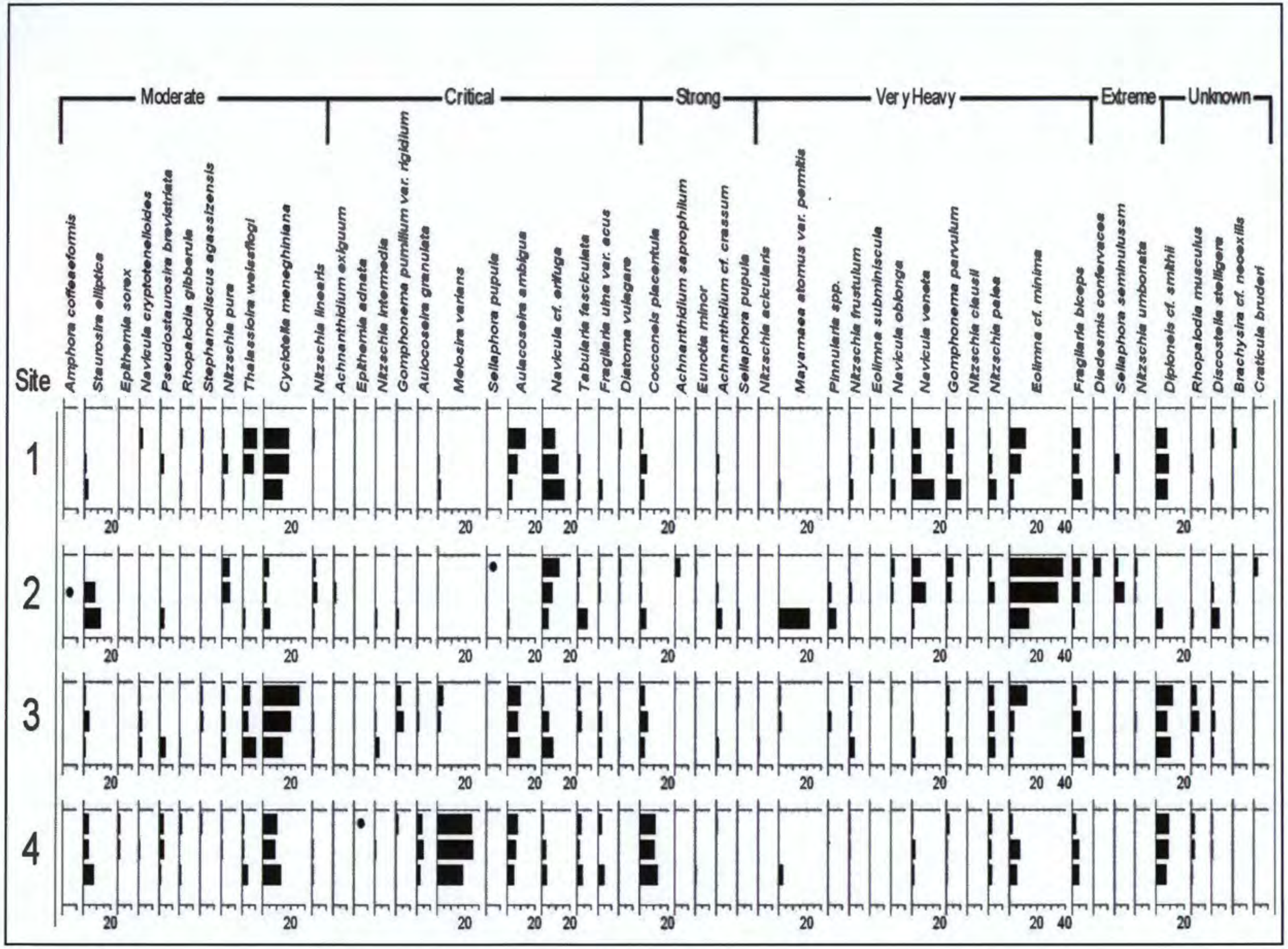

Figure 4: Tilia Graph indicating changes in diatom assemblages at four sites represented as percentage through three depths (5, 10, 15cms); diatoms grouped by pollution tolerance

The Tilia graph represented in **Figure 4** is a graphical representation of the diatom assemblages through 5, 10 and 15cm depths (from left to right on the graph) at the four sites. Black dots represent species presence but at insignificant amounts. 47 species of diatoms were identified in total. Diatoms were grouped according to their pollution tolerance to make it easier to spot trends in the assemblage. Visually, the graph shows the dominant species in the wetland as a whole and, more specifically, differences between species dominance within and between sites. Site 1 does not have a specific dominant *per se*, but rather numerous species which contribute significantly to the assemblage (*Cyclotella meneghiniana, Navicula cf. erifuga, Navicula veneta, Aulocoseira ambigua, Gomphonema parvulum, Fragilaria biceps, Diploneis cf. smithii)*. These species are diverse in their pollution tolerance, although *Cyclotella meneghiniana* can only tolerate up to moderate pollution levels and its relative abundance allows the site to be interpreted as less polluted than Site 2.

Site 2 appears the most dissimilar to the other 3 sites. The dominance of *Eolimna* cf *.minima*, a diatom which tolerates very heavy pollution levels, is evident. Other notable species include *Staurosira elliptica* (although it is absent from the 5cm sample), *Navicula veneta, Navicula* cf. *erifuga* and *Mayamaea atomus* var. *permitis* which is only present in the 15cm sample but is tolerant of very heavy organic pollution. At this depth it appears *Mayamaea atomus* var. *permitis* somewhat replaced *Eolimna* cf *.minima* which itself is a much reduced presence at 15cm.

Site 3 is dominated by *Cyclotella meneghiniana,* a species which favours moderately polluted waters although *Diploneis cf. smithii* (pollution tolerance unknown) is also evidently abundant. Various other species contribute to the diatom assemblages at this site (these include: *Aulocoseira ambigua, Fragilaria biceps* and *Thalassioira weissflogi*).

Site 4's dominant species through all three depths is *Melosira varians,* a diatom species which can tolerate up to critical levels of pollution. Other significant contributions to the diatom assemblage include *Cyclotella meneghiniana* and *Cocconeis placentula,* a species which is found in strongly polluted waters. A notable aspect of the site's assemblage is the stability of species percentage through the three depths.

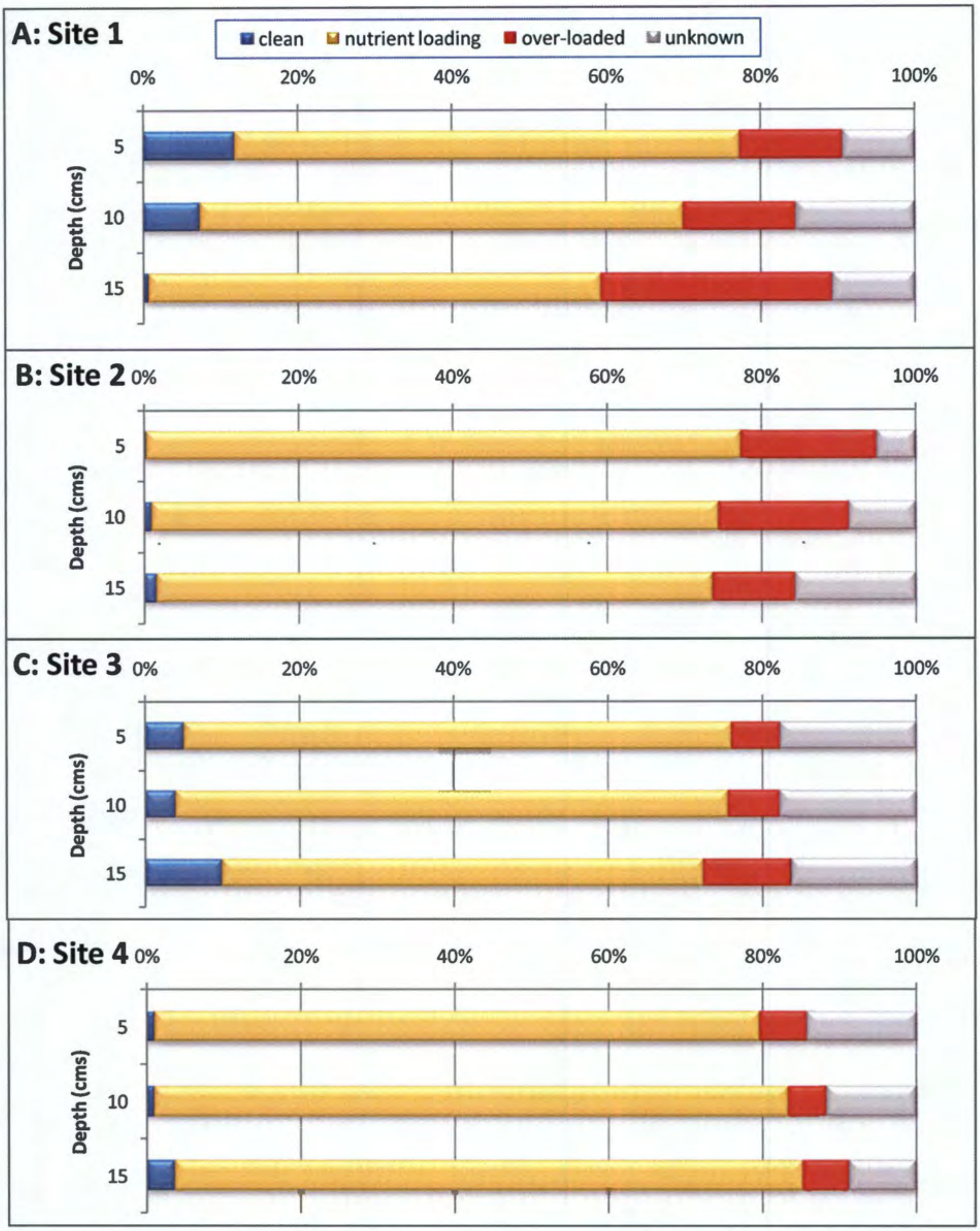

Figure 5: Stacked bar charts showing percentage of taxa classified according to their trophic preferences in the diatom flora from 4 sites around the perimeter of Princess Vlei at 3 different depths

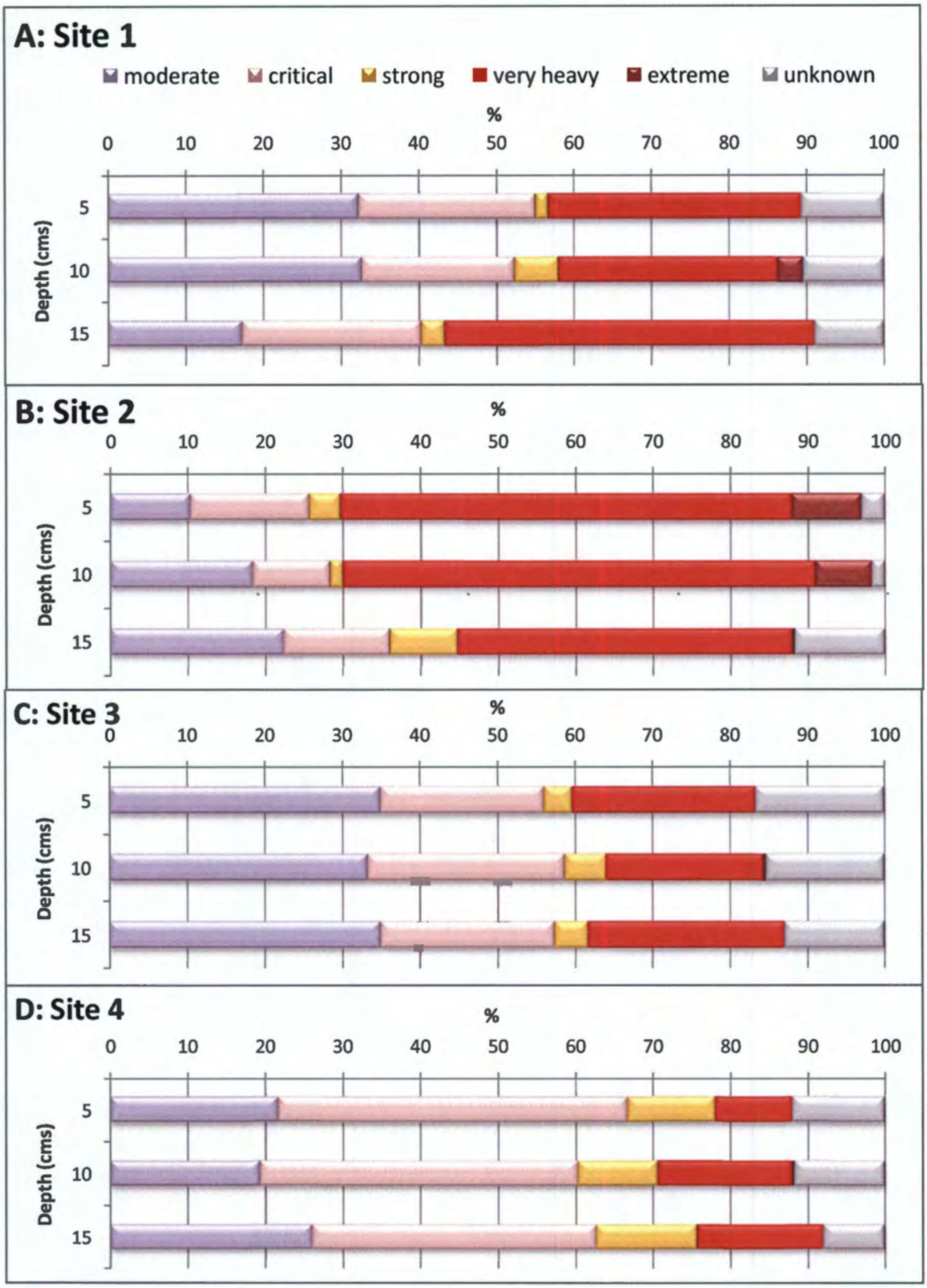

Figure 6: Stacked bar charts showing percentage of taxa classified according to their pollution tolerances in the diatom flora from 4 sites around the perimeter of Princess Vlei at 3 different depths

Figure 5 illustrates the trophic preference of the diatom assemblage at each of the three depths (5, 10, 15cms) at each of the four sites. The majority of species identified have a known trophic preference where they can be found, thus, by assigning individual species to a trophic preference (see methods section), a picture can be created of changes in trophic status both spatially and temporally.

At a broad scale the patterns look rather uniform: Unknowns comprise less than 20%; Nutrient-Loading provides the majority between 50-80% of the diatom assemblages; Clean is almost always below 10%; and Over-Loaded is more variable. However, looking more closely at the data it becomes apparent that changes are indeed evident, both temporally (i.e. through depth) within sites, and spatially between sites. Sites 1-3 appear to be rather more variable with depth in comparison to Site 4(Nutrient Loading comprising ~80% of the assemblage and Over-Loading ~5%). Sites 1-3, although their Nutrient Loading category remains fairly constant, exhibit far more variation within their Clean and Over-Loaded categories.

Figure 6 illustrates the change in pollution toleration of the diatom assemblages. Categories are grouped in ascending order with 'Moderate' species only tolerating the cleaner waters and 'Extreme' tolerating the most polluted waters. As in **Figure 5**, diatom niche specificity can be used to infer water quality variables. What is evident is the difference between Site 2 and the other sites. At Site 2 there is a much larger proportion of Very Heavy and Extreme pollution-tolerating diatoms. In addition, there is a significant decrease in Moderate pollution-tolerating diatoms and an increase in extreme pollution diatoms through time (i.e. depth). Site 1 displays a degree of variability as there is an increase of Moderate pollution-tolerating diatoms, while Extreme pollution-tolerating diatoms are only found at a depth of 10cm. Very Heavy pollution-tolerating diatom percentage decreased from ~45% to ~30% towards the surface. Sites 3 and 4 are more stable, exhibiting a far greater percentage of Moderate and Critical pollution tolerant diatoms than those seen at Site 2.

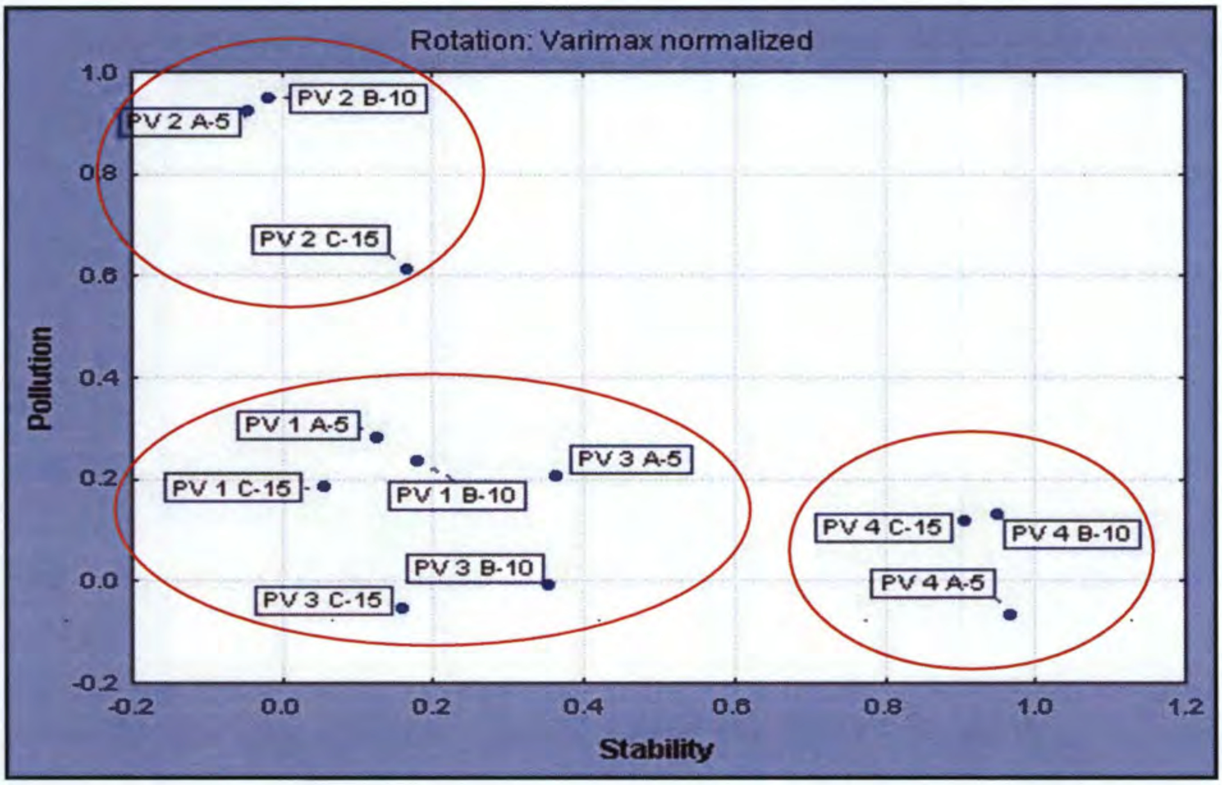

Figure 7: Principal Component Analysis (PCA) created using *Statstica V9.0,* plotting the study sites against two controlling factors which explained ~70% of the variation (Eigen value factor 1: 6.12; Eigen value factor 2: 2.26). For labeling purposes Site 1 - 5cm depth became PV 1 A-5 etc.

Figure 7, the Principle Component Analyses, groups the study sites by two factors which explain approximately 70% of the variation. Factor 1 was determined to be site stability though depth and Factor 2 was determined to be pollution by analyzing the data both visually and by analyzing the diatoms which accounted for most of the variation between sites. It is clear that each site's 3 depths are fairly close together in the PCA, thus spatial differences between assemblages seem greater than temporal differences. It is also noticeable that Site 2 and 4 are almost polar opposites on the PCA plot. Site 2 is more polluted and dynamic, while Site 4 is less polluted and more stable. Sites 1 and 3 fall somewhat in between, being less polluted than site 2 but less stable than Site 4.

Table 1: Table showing species which contributed significantly to the PCA distribution. Species were considered significant if their factor scores were either greater than 0.70 or less than -0.70.

Species Name	Factor 1 (Stability) Score	Factor 2 (Pollution) Score
Eolimna cf. minima	<0.70	6.15
Melosira varians	5.48	<0.70
Cocconeis placentula	2.40	<0.70
Cyclotella meneghiniana	1.24	-0.77
Diploneis cf. smithii	1.23	<0.77
Staurosira elliptica	0.99	1.13
Mayamaea atomus var. *permitis*	<0.70	1.11
Gomphonema parvulum	0.70	<0.70
Navicula veneta	-0.73	0.92
Navicula cf. erifuga	-0.72	0.99

Table 1 shows which diatom species caused the distribution witnessed in the PCA. Stability is best explained by *Melosira varians* (factor score=5.48) and *Cocconeis placentula*(factor score=2.40), while the largest factor score, by some distance, for pollution is *Eolimna minima* (factor score=6.15).

6. Discussion

The results clearly show that diatom assemblages in Princess Vlei vary spatially and temporally (if one follows the assumption that a change in depth is a proxy for a change in time). An inherent characteristic of Bacillariophyceae is their niche specificity and the results have shown that this niche specificity occurs even within a small (29 ha) water body. To explain the observed distribution of diatom assemblages one needs to consider the catchment as a whole and, more specifically, the dynamics of Princess Vlei. Overall, a total of 47 species were identified at the sites (see appendix II). Harding (1992), in his general survey of Princess Vlei, recorded 6 diatom genera (*Cocconeis, Cyclotella, Fragilaria, Nitzschia, Navicula, Melosira and Thalassiosira*) all of which were indeed identified in this study in 2010.

Princess Vlei was first described as a eutrophic lake in the 1940's and a subsequent overview study of the vlei in 1992 also found the vlei to be eutrophic (Harding, 1992). The results shown in **Figure 5** concur with these findings, although whether the system is in a 'stable' state or becoming increasingly nutrient-loaded is not clear from these data. The hydraulic flushing which occurs during the annual heavy winter rains (Harding, 1992; pers. obs.) may prevent the vlei from becoming hypertrophic and preventing cyanobacterial blooms. Flushing is well documented in decelerating eutrophication in lentic waters (Rippey *et al.*, 1997). The heterogeneity observed in the results deserves closer inspection and each of the four sites will be considered in an attempt to explain the trends observed.

Site 1

One might expect Site 1 to be the most polluted of the four sites due its proximity to the Southfield Canal, the primary input into the system. However, a closer inspection of the site shows that the sample was taken from sediment next to almost still water. The shape of the vlei, combined with the velocity of the flow, allowed much of the effluent to bypass Site 1. In addition, the sample was taken from a patch of clear ground outside of the reed stand, thus there was no physical stagnation of water occurring preventing the effluents from settling there. The direction of the groundwater inflow (**Figure 3**), although adjacent to Site 1, somewhat bypasses the site and probably adds to its pollution status. According to **Figures 6** and **7**, Site 1 is more polluted than Sites 3 and 4 which is not surprising when one considers its position relative to those sites in terms of input into the vlei. **Figure 5** graphically shows the exponential increase in 'clean' diatoms through time, which occurs concurrently with significant decrease in 'nutrient overloaded' diatoms. The site's shift to cleaner water through time is replicated in **Figure 6** where an increase in moderate diatoms is accompanied by a decrease in very heavy pollution tolerant diatoms through time. It is apparent that the site's assemblage is not very stable through time. The specific diatoms responsible for this trend are the moderate pollution-tolerant *Cyclotella meneghiniana* and *Thalassiosira weissflogi* which increase through time while very heavy pollution-tolerant species, *Navicula veneta* and *Gomphonema parvulum* decrease in numbers through time according to **Figure 4**.

Site 2

Site 2 is the most dissimilar site when compared to the other sites. As illustrated in **Figure 7**, it is separated from Site 4 due to its instability and pollution, while it is also separated from Sites 1 and 3 because it is more polluted. The trophic status of the site has increased through time with more nutrient over-loaded species in the assemblage (**Figure 5**). This is in contrast to the trend observed at Site 1 suggesting that an increase in velocity of effluent flow (perhaps as a result of catchment hardening) may be washing pollutants away from site 1, and towards site 2. This shift towards hypertrophic conditions has been accompanied by an increase in very heavy and extreme pollution-tolerant diatoms (**Figure 6**). The species primarily responsible for this according to **Figure 4** are *Eolimna* cf. *minima*, *Fragilaria biceps* and *Navicula veneta*. Indeed, the dominance of *Eolimna* cf. *minima* at Site 2 is the reason for it exhibiting a higher level of pollution compared to the other three sites where *Eolimna* cf. *Minima* is present, but in much lower numbers. Interpreting why this site is more polluted than the others requires examining the catchment as well as the positioning of the actual site in Princess Vlei. As mentioned previously, the velocity of the flow of influent from Southfield canal may bypass Site 1 and instead enter the dense reed stand of *Typha capensis* situated at Site 2 where the effluent may settle and remain for a period of time thus increasing in concentration. This property of reed stands in wetlands is well-known in the literature (van der Valk, 2006). Additionally, groundwater inflow into the vlei occurs at Site 2. That the groundwater flow makes its way directly below the industrial businesses (predominantly textile factories) behind the site can only contribute towards the increased pollution. Furthermore, the dominant summer wind direction (the infamous south-easter) may cause surface water to flow towards the *Typha* stands where pollutants may settle in the reeds. It is also conceivable that the hydraulic flushing which occurs during winter is somewhat counteracted by the dense *Typha* stands at Site 2.

Site 3

Site 3 is directly opposite the Southfield canal and some distance away from the groundwater inflow and the Italian Road Canal outlet weir. It is perhaps unsurprising then that it exhibits a more stable assemblage than Sites 1 and 2. Although still eutrophic, the site shows less nutrient overloading than Sites 1 and 2 while pollution at the site is substantially less than Site 2 but similar to Sites 1 and 4. Similar to Sites 1 and 4, Site 3 has a large proportion of *Cyclotella meneghiniana* which contributes to its less polluted status when compared to Site 2. *Cyclotella meneghiniana* is a planktonic species (Harding et al., 2007) and would have access to large parts of Princess Vlei where strong pollution would not prevent it from populating (i.e. site 2). Another species which is fairly abundant at the three less polluted sites but almost absent from Site 2 is *Diploneis* cf. *smithii*. Unfortunately, the biology of this species remains a mystery as numerous databases were unable to provide any information on the species' trophic preference or pollution toleration. The results of this project tentatively suggest that *Diploneis* cf. *smithii* does not tolerate strongly polluted conditions like those observed in Site 2.

Site 4

Site 4 was positioned closest to the Italian Road Canal, the outlet which joins Princess Vlei to the adjacent Rondevlei nature reserve. **Figure 7** shows the site to be the most stable through time. This is visually confirmed by **Figures 5** and **6** which reveal a non-dynamic assemblage dominated by moderate and critical pollution-tolerant diatoms and diatoms which flourish in nutrient loaded waters. The dominant diatom in the assemblage is clearly *Melosira varians* both visually (**Figure 4**) and numerically if one looks at its factor score for stability (**Table 1**). The species can tolerate up to critical levels of pollution, so why is not found at Sites 1 or 3 which have similar pollution levels? The answer may lie in its physical structure: Kelly et al. (2005) note that the species is filamentous and easily scoured off marginal surfaces. Perhaps the current towards the outlet in the vlei scours the *Melosira varians* off its habitat and it ends up in the reed beds adjacent to the outlet where it settles. The generally 'healthier' conditions found at this site shows that Princess Vlei is performing one of its fundamental functions. One of the reasons wetlands are so highly valued in conservation circles is their ability to act as natural water filters, purifying water (van der Valk, 2006). Indeed, the importance of Princess Vlei to purify water to some extent is doubly important as it is connected to the Rondevlei bird and hippopotamus sanctuary. The holding and cleansing function of Princess Vlei was evident in 2006 when a sewerage spill killed fish in the vlei but no deaths were reported in Rondevlei (Cape Argus, 2006).

6.1 Conclusion

The aims and objectives of this project were twofold: Firstly, to establish whether the diatom flora preserved in the organic sediment around an urban wetland could reveal information on the wetland's recent water quality history; and secondly, to determine whether diatom flora change spatially within a small lake due differential inputs and outputs. The hypotheses stated that increased urbanization would be visible in the sediments and differences would exist between the four sites around the vlei's perimeter. The data did not reveal significant trends through time, thus the hypothesis that an increase in urbanization through time would be visible in the sediments cannot be accepted. This can be explained by a number of factors. Firstly, the samples were not dated so it is unknown what timescale the three depths represented. If the 15cms represented a hundred years of sediment accumulation than one would expect to see changes in the sediment indicative of urbanization, whereas if the 15cms only encapsulated a decade or two then this trend might be less apparent. Secondly, increased effluent from urbanization could be countered by improvements in infrastructure within the catchment (i.e. sewerage pump improvements). The second part of the hypotheses can be irrefutably accepted: The results clearly show that diatom assemblages at the four sites are variable as a result of differences in site stability and levels of pollution. The results of this project show that diatoms are suitable indicators of water quality, even within a small system, due to their niche specificity. Ideally, a South African-specific diatom index will be developed so local water scientists would not have to rely solely on Northern Hemisphere indices. The project has shown that Princess Vlei remains a eutrophic and polluted wetland although not uniformly so. Continual monitoring is required to prevent the vlei from becoming a health hazard for the surrounding community and to maintain its ability to act as a buffering zone to protect the Rondevlei nature reserve, as well as acting as a functional ecosystem in an area threatened by urbanization.

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Appendix I

Steps to treat sediment in preparation of diatom analysis (adapted from Battarbee, 1986)

1. The sediments were initially treated with hot 10% HCl to remove all carbonates from the sample. This was achieved by placing a small quantity of sediment into a beaker, covering it with 10% HCl and heating gently for a minimum 15 minutes while swirling the contents. This was repeated until all carbonates were dissolved.

2. Following this, the residue was diluted with distilled water and allowed to settle overnight, with excess supernatant liquid removed through pipetting the following morning.

3. The sample was then washed in 20ml of 30% $H_2 O_2$ and heated gently in a water bath until all organic matter was removed, this was repeated several times to ensure complete removal of organic matter. Because all samples were taken from the organic layer, this was done for several hours.

4. To remove coarse organic matter (e.g. roots) and coarse mineral matter the residue was sieved through a 0.5mm screen.

5. The resultant residue was centrifuged and washed with distilled water at least three times.

6. The subsequent sample may have contained clays and finer mineral matter, by swilling the residue in a beaker, clays were removed by allowing the diatoms to sediment before decanting and discarding the suspended clay.

7. Following these steps, a final wash with de-ionised water was performed and the samples were centifuged before the excess fluid was decanted.

8. Following these steps, 3 drops of the diatom solution was pipetted onto a clean cover slip and diluted with a few drops of de-ionised water; the solution was then been allowed to settle.

9. The water in the solution was left to evaporate on a hot plate at a low temperature (~40°C).

10. Finally, after all the water had evaporated, the coverslip was mounted onto the microscope slide using a resin of high refractive index - for this purpose Pleurax (R.I. = 1.73) was utilised.

Appendix II

Table 2: Diatom species list and their trophic preference and pollution tolerance (continued overleaf)

Species	**Trophic Preference**	**Pollution Toleration**
Thalassioira weissflogi		moderate
Aulacoseira ambigua	eutrophic	critical
Cyclotella meneghiniana	eutrophic	moderate
Nitzschia linearis	polytrophic	moderate
Eolimna cf. minima	polytrophic (prefers eutrophic)	Very heavy
Navicula cf. erifuga	eutrophic	critical
Tabularia fasciculata	polytrophic	critical
Fragilaria ulna var. acus	meso- to eutrophic	critical
Diatoma vulagare	meso- to eutrophic	critical
Fragilaria biceps	polytrophic (prefers meso- to eutrophic)	Very heavy
Brachysira cf. neoexilis	oligo- to mesotrohic	Unknown
Craticula bruderi	polytrophic	Unknown
Achnanthidium saprophilum	eutrophic	Strong
Nitzschia palea	polytrophic (prefers eutrophic)	Very heavy
Sellaphora seminulussm	polytrophic (prefers eutrophic)	Extreme
Nitzschia umbonata	eutrophic	Extreme
Cocconeis placentula	meso- to eutrophic	Strong
Nitzschia clausii	polytrophic (prefers hypertrophic)	Very heavy
Navicula veneta	polytrophic (prefers hypertrophic)	Very heavy
Gomphonema parvulum	polytrophic	Very heavy
Nitzschia pura	unknown	Moderate
Diadesmis confervacea	polytrophic (prefers eutrophic)	Extreme
Navicula oblonga	polytrophic (prefers eutrophic)	Very heavy
Eunotia minor	unknown	Strong
Diploneis cf. smithii	unknown	unknown
Eolimna subminiscula	polytrophic (prefers hypertrophic)	Very heavy
Achnanthidium cf. crassum	unknown	Strong
Rhopalodia musculus	unknown	unknown
Melosira varians	eutrophic	critical
Stephanodiscus agassizensis	eutrophic	Moderate
Discostella stelligera	unknown	unknown
Rhopalodia gibberula	unknown	Moderate
Aulocoseira granulata	eutrophic	Critical
Epithemia sorex	polytrophic (prefers eutrophic)	Moderate
Gomphonema pumilium var. rigidium	meso- to eutrophic	Critical
Navicula cryptotenelloides	oligo- to eutrophic	Moderate
Pseudostaurosira brevistriata	olig-eutrophic	Moderate
Nitzschia intermedia	eutrophic	Critical

Sellaphora pupula	polytrophic (prefers hypertrophic)	Strong
Mayamaea atomus var. permitis	polytrophic (prefers eutrophic)	Very heavy
Pinnularia spp.	unknown	Very heavy
Nitzschia frustulum	polytrophic	Very heavy
Staurosira elliptica	unknown	Moderate
Nitzschia acicularis	polytrophic (prefers hypertrophic)	Very heavy
Amphora coffeaeformis	unknown	Moderate
Achnanthidium exiguum	Polytrophic (prefers eutrophic)	Critical
Epithemia adnata	meso- to eutrophic	Critical

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