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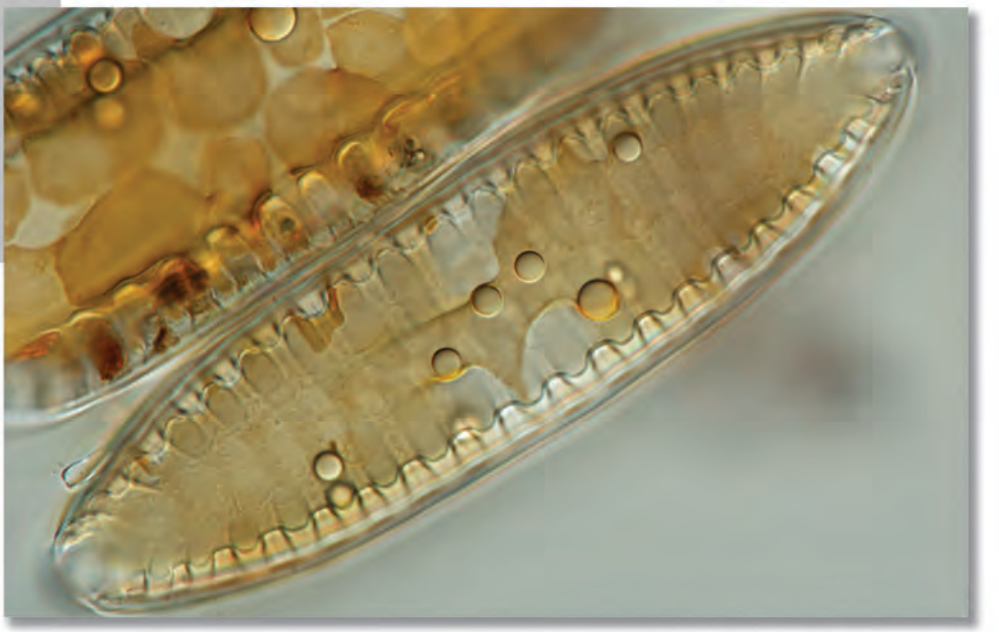
J.C. Taylor
C. Cocquyt



Volume 16 (2016)

AbcTaxa

the Series of Manuals
Dedicated to Capacity Building
in Taxonomy and
Collection Management

WITH THE SUPPORT OF
THE BELGIAN
DEVELOPMENT COOPERATION .be

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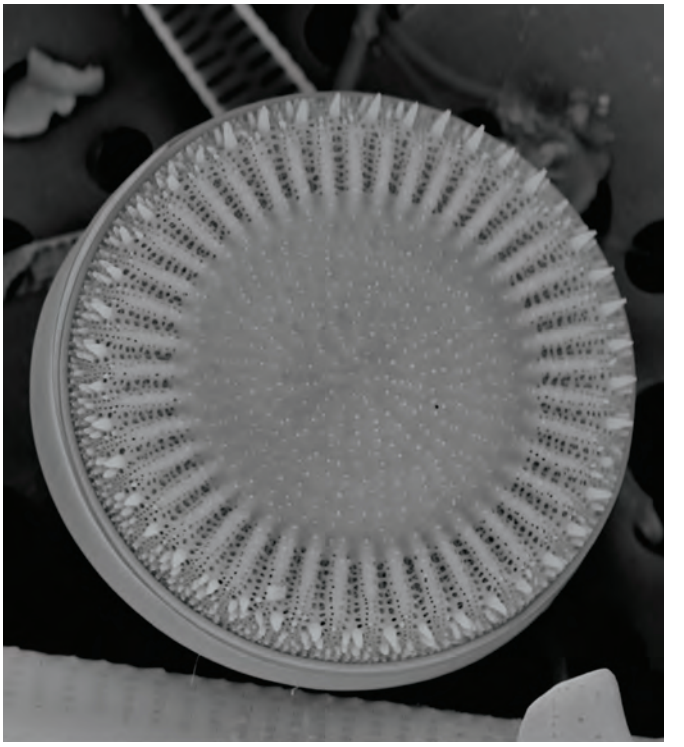
http://www.abctaxa.be

ISSN 1784-1283 (hard copy) / ISSN 1784-1291(on-line pdf)
ISBN 9789082641219 (hard copy) / ISNB 9789082641226 (on-line pdf)
NUR 910 D/2016/0339/5

# Diatoms from the Congo and Zambezi Basins - Methodologies and identification of the genera



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**Front cover:** *Surirella* Turpin

**Half-title page:** *Cyclotella meneghiniana* Kützing

## Preface

The Congo river basin is, after the Amazon, the second largest in the world and the Zambezi river is the fourth longest river in Africa. Both rivers and their catchments are of prime importance to millions of people. These human populations continue to increase. For example, the population in the Congo basin (c 777,000 square $km^2$) experiences an annual increase of c 1.7 million people. This creates rising demands (food, fuel shelter) at a great cost to the forest and to the river itself. As a result, wildlife and fish stocks rapidly decrease, mainly because of the largely uncontrolled trade in bush meat and because of overfishing. Moreover, waters in the catchments are experiencing rapid eutrophication, because of the countless domestic fires that are daily lit to cook food. Ashes are then washed into the water ways by torrential tropical rains. The fires themselves demand massive logging, which causes erosion and further degradation of water quality in these basins.

Measures need to be taken to stop the degradation of tropical river catchments in general, and those of the Congo and Zambezi rivers in particular, and many are already in place. Some of these measures deal with birth control, others impose sustainable hunting and fishing activities. Monitoring of biodiversity and water quality is urgently needed. Most of such interventions demands education, formation and training of local people.

One of the major drawbacks faced by programmes studying the biodiversity of tropical river catchments is the lack of taxonomic knowledge (also called the taxonomic impediment). Yet, estimations of fish stocks, identification of bush meat sold at local markets, the use of aquatic organisms to determine water quality, and many other monitoring activities that could provide scientifically underpinned recommendations to management, largely depend on good taxonomy.

Abc Taxa offers a welcomed forum to disseminate knowledge on a taxon that reveals itself as indicative to water quality: namely diatoms. Over the past decades, water quality monitoring research has produced a long set of (mostly locally applicable) Biotic Indices (BIs) using different biological groups. Such BIs have the advantage that they monitor the health of aquatic communities which are the result of time averaged effects of potential pollution events, that could easily be missed by point measurements of water chemistry, especially in flowing rivers. The first wave of BI largely dealt with fish, macrophytes and macro-invertebrates. Since about 20 years, however, there is an increased use of diatoms in water quality monitoring, mostly (of course) in the northern hemisphere where the taxonomy of these algae is much better known. The present book will remedy the taxonomic impediment of diatoms in the Congo and Zambezi catchments and will allow the start of new monitoring programs and the refinement of running ones.

The editors of Abc Taxa are to be congratulated for the production of this high - level monograph and so are the authors of this highly appreciated volume. May this series see light to many forthcoming issues that will relieve the taxonomic impediment in the Global South.

Koen Martens

Head of Research RBINS

Editor in Chief European Journal of Taxonomy

**Abstract**

Diatom research has historically been well established in Central Africa but more commonly directed towards the phytoplankton of large bodies of standing water. Recently there has been considerable international research interest on using diatoms as indicators of water quality. Usually attached diatoms originating from rivers and streams are used for this purpose. Diatom taxonomy has undergone considerable changes during the last 3 decades with many new diatom genera being established, these diatom genera are ecologically relevant in terms of establishing water quality conditions. This volume sets out to introduce researchers to the latest concepts in collection and preparation methodology as well as diatom taxonomy and nomenclature. This is achieved by illustrating and discussing methodological concepts, providing a fully illustrated glossary and illustrating, by a variety of means, the most common diatom genera occurring in the Congo and Zambezi catchment region.

**Key words** - *Bacillariophyta*, morphology, taxonomy, tropical Africa, water quality

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# 1. Introduction

## 1.1. Diatom research in Central Africa

The African Great Lakes, Tanganyika, Malawi and Victoria and surrounding regions attracted the interest of several phycologists at the end of the 19th century. The first publication appeared in 1880 written by Dickie who reported on attached algae on the aquatic phanerogams (seed-bearing plants) of Lake Malawi (also called Lake Nyasa or Lake Nyassa): he found a total of 38 taxa of which 31 are diatoms. The research on the diatoms of Lake Malawi/Nyasa and lotic ecosystems in the surroundings was continued by Müller (1897, 1903, 1904, 1905, 1910) resulting in the description of 126 new diatom taxa (species, varieties and forms). Müller also examined material from Lake Victoria from which he described another ten new diatom taxa, and one new variety from Mount Kilimanjaro. West (1907) reported on the algae of the three Great Lakes including a total of 58 diatom taxa, 26 from Lake Malawi, 19 from Lake Victoria and 37 from Lake Tanganyika. The publication included the description of nine new taxa, all from Lake Tanganyika. Several diatoms from this lake, new to science were depicted by Hustedt in Schmidt's atlas of diatoms (Schmidt 1914, 1922, 1925), followed later (Hustedt in Huber-Pestalozzi 1942) by an elaborated description. In addition to the studies of Müller the diatoms from Lake Victoria were studied by Ostenfeld (1908, 1909) who reported 15 and 9 taxa respectively, Schröder (1911) described a new *Rhizosolenia* species (now transferred to the genus *Urosolenia*), Virieux (1913) reported 25 taxa and one new variety, Woloszynska (1914) reported 34 species of which two were new species, one a new variety and one a new forma, and Bachmann (1933) mentioned 18 taxa including one new variety. A first overview of all taxa reported from the Great Lakes was published by Van Meel in 1954, followed by an updated checklist in 1993 (Cocquyt *et al.* 1993). Later the lacustrine and riverine algal diversity in the African Great Lakes area was discussed in Cocquyt (2006). Besides the Great Lakes, diatom investigation in the region was also carried out in rivers, ponds, etc., e.g., Bachmann (1938), Caljon (1987, 1988) and Mpawenayo (1996).

Diatom research in D.R. Congo, formerly the Republic of Zaire (1971-1997) and Belgian Congo (1908-1960) started with Zanon (1938) who studied the diatoms from the region of Lake Kivu. He reported 263 taxa belonging to 33 genera. Seventeen taxa were new to science of which ten were *Pinnularia* species; the others belonging to *Cocconeis*, *Cymbella*, *Eunotia*, *Neidium* and *Synedra*. In the mid-20th century Hustedt (1949) published a treatise on the diatoms of the Albert National Park in Belgian Congo, nowadays the Virunga National Park in D.R. Congo. Among the 55 new taxa, 25 belong to *Nitzschia*, the others are spread over 12 other genera. Some research was also done on the Congo River. In 1948 Kufferath reported 25 taxa in the plankton of the Congo River near Makanza, previously called Nouvelle-Anvers, midway between Kisangani and Kinshasa, including one new *Nitzschia* species (Kufferath 1956a). In 1956 he mentioned eight taxa near the isle of Mateba (Kufferath 1956a) and 44 taxa of which 10 were new

from Banana Beach (Kufferath1956b), both localities are close to the mouth of the Congo River in the Atlantic Ocean (Kufferath1956a) and include marine species. Not only the Congo River but its tributaries including the Lindi River, the Tshopo River and small rivers and ponds in Kisangani were studied by Golama (1996) who found 278 diatom taxa. A new *Gomphonema* was described from the Tshopo River (Compère 1995). Some years earlier a new *Stauroneis* was described from a fish pond in Kinshasa (Compère 1989). Cholnoky (1970) described three new species among the 93 taxa he observed in the Bangweulu swamps. In addition to the papers mentioned, there also exist a limited number of unpublished thesis studies conducted at universities in D.R. Congo.

Diatom research in Zambia started only recently, with exception of studies carried out in the Bangweulu swamps by Cholnoky (1970). Lake Kariba, located on the border of Zambia and Zimbabwe was also the subject of algal investigations. Thomasson (1965) reported ten diatom taxa, *Aulacoseira granulata* being the dominant species. Diatom communities and their seasonal succession were studied by Hancock (1979) as well as the epiphytic diatom community on underwater leaves of *Salvinia molesta* D.S. Mitchell (water fern) (Hancock 1985). Cronberg (1997) mentioned the presence of 155 algal species in Lake Kariba based on a study of 152 plankton samples collected between 1986 and 1990. Among these only thirteen taxa belong to the diatoms, seven are *Aulacoseira* species. A later study in this lake (Muzavazi *et al.* 2008) also reported twelve diatom species, of which only four could be assigned a species name.

### 1.2. Diatom research related to water quality in Central Africa

Attempts to use diatoms as a tool for water quality of rivers in Central Africa started only recently. Some small rivers and streams south of Gombe Stream National Park, Tanzania (Bellinger *et al.* 2006) have been the subject of such an investigation. A number of indicator species tolerant to eutrophication were found and the Trophic Diatom Index (TDI) (Kelly & Whitton 1995) values showed significantly higher impact in deforested than in forested streams.

Utete *et al.* (2013) studied the impact of aquaculture on the water quality in Lake Kariba based on physical and chemical variables, but not using diatoms. In East Africa some research was done over the last few decades on diatoms in relation to water quality, mainly through PhD theses in Kenya and Ethiopia (e.g., Lung'ayia 2002, Beyene 2010, Beyene *et al.* 2009, 2014).

This is in contrast to South Africa which has a long history of diatom research related to ecological research and water quality (Taylor & Cocquyt in press). Cholnoky can be considered as the founder of diatom studies in South Africa. His intensive and extensive studies on the taxonomy and ecology of the diatoms was also the start of the South African Diatom collection, nowadays owned by the South African Institute for Aquatic Biodiversity and housed at the North-

West University in Potchefstroom, South Africa. Cholnoky had little faith in only the chemical analysis of water quality and stated that "*the chemical and physical characteristics of a water body could be determined more reliably and easily through a study of the diatom associations found living in it*" (Cholnoky 1968). The application by Cholnoky of the Thomasson (1925) community analysis on benthic diatom community composition was a forerunner of modern autecological indices. The method allows comparisons between sites in the same river, or the tracking of changes at a single site, but only one aspect of the water chemistry is chosen. When we consider for example the amount of nitrogenous effluent, the sum of all specimens belonging to the genus *Nitzschia* is calculated as an abundance value relative to the total of the cells enumerated from a particular diatom community.

Why the genus *Nitzschia*? *Nitzschia* is a genus known generally to be nitrogen heterotrophic and be able to utilise organically bound nitrogen. Therefore the relative abundance of this genus in a sample gives a reflection of the amount of nitrogenous pollution at the site studied. A higher percentage indicates a higher degree of impact in terms of nitrogenous effluent. Another example is that a pH gradient can be tracked in a river system by using the abundance values of the diatom genus *Eunotia*. This genus is known to prefer acid environments, to be acidobiontic. Cholnoky (1968) obtained good results using this index. However, the user of the Thomasson analysis method needs to have an in-depth knowledge of the autecology of individual diatom genera and species to be able to draw accurate environmental conclusions based on diatom community composition. Several years later, Archibald (1972) and Schoeman (1976) attempted to develop better approaches using diatoms in water quality monitoring. Their development was parallel to the development in water quality monitoring in Europe. The first proved to be unsuccessful. Schoeman (1976) simplified the community analysis method used by Cholnoky: he divided the diatom associations into four groups, each with their own particular ecological requirements. The table of results is thus shortened compared to the long species tables used by Cholnoky.

Schoeman (1976, 1979) came to the conclusion that these diatom groupings (or associations) could be successfully employed to assess the quality of running waters especially in regard to their trophic status. Unfortunately the investigation of diatoms as indicator species in South African freshwater ecosystems was then interrupted to be restarted at the beginning of the 21st century. Bate *et al.* (2002) attempted to relate a descriptive index, based on a dataset for the environmental tolerances of diatom species found in the Netherlands (van Dam *et al.* 1994), to water quality in South Africa. The "van Dam *et al.* index" includes pH, conductivity, oxygen requirements, trophic status, saprobic status and habitat requirements of a selected number of diatom species found in waters of the Netherlands (van Dam *et al.* 1994). Bate *et al.* (2002) concluded that benthic diatoms could be useful for water quality investigation in South Africa and that they give a time-integrated indication of specific water quality components, but that the particular data set, generated in the Netherlands, could not be transposed directly for use under South African conditions.

Taylor (2004) and Taylor *et al.* (2007a, b) continued this investigation by testing several numerical diatom indices developed in Europe for indicating water quality in some of the most important river systems in South Africa. They concluded that in general these European indices could be used with success in South Africa but that there are, however, some potential problems (Taylor *et al.* 2007b). In particular, the list of taxa included in the indices needs to be adapted to the studied region. Although most European diatom indices may be used in many regions as they are based on the ecology of widely distributed or cosmopolitan taxa, special attention should be paid to taxa occurring in pristine waters and to endemic taxa, absent in the indices reference lists. When these taxa are abundant the inferred water quality may be misinterpreted.

Another problem that arises is the rapid changes in diatom taxonomy, especially at the genus level. Some European indices were erected in the seventies or in the eighties of the last century and have never been revised. The positive result in the study of Taylor *et al.* (2007b) is they demonstrated that many widely distributed diatom species found in South Africa have similar environmental tolerances to those recorded for these species in Europe and elsewhere.

### 1.3. Aim of the present diatom book

The aim of the present work on diatoms is twofold. On the one hand we want to encourage and facilitate the study of diatoms as a useful tool for water quality monitoring. On the other hand we want to give an overview of the most common diatom genera which can be observed in the Congo and Zambezi basins. Accurate identifications at this level form the basis for further taxonomic studies. Nomenclatural and taxonomic changes and the description of numerous new diatom species and genera during the past decades make the study of diatoms in tropical Africa complex. In the present work the recently accepted diatom taxonomy at genus level is illustrated for the most common genera of tropical Africa using schematic computer generated drawings. On these drawings the typical characteristics, or characteristics important for identification of species, are highlighted in red, often on a duplicate of the drawing. Moreover, light microscopic micrographs are presented from cleaned material, all from the Congo and Zambezi basins, to show the typical valve ornamentations on which identification is based. Where possible light microscopic micrographs from living material are given to show the plastid(s) structure typical for the genera; these micrographs however are mainly from Southern African material. Only if both authors of the present work were completely certain of the species identity the species name is added to the figure captions. For most genera the ultrastructures of the diatom valves are illustrated with photographs taken with a scanning electron microscope; the material used originates from the Congo and Zambezi basins. A scale bar is added to all micrographs to indicate the size of the valves and the ultrastructures. Light and scanning electron microscope investigations were performed at the North-West University, Potchefstroom, South Africa and at the Botanic Garden Meise, Belgium.

## 2. Definition of a diatom

Diatoms or Bacillariophyta are a major group (phylum) of microscopic eukaryotic algae, unicellular but often forming colonies. The cell wall, called a frustule, is highly differentiated and heavily impregnated with silica (hydrated silicon dioxide) and is composed of two valves connected by girdle bands (Fig. 1). The valves and girdle bands fit together very tightly preventing flux of material across the cell wall, which can only take place through openings (pores and slits) in the frustule. A thin layer of organic material (membrane) is also present on the outside of the cell wall. All diatoms probably secrete polysaccharides; some may diffuse in the surrounding environment while others may remain around the cell as stalks, pads, threads or even capsules. This thin organic layer obscures the details of the silica cell wall ornamentations which are used for identification. For this reason diatom cells must be cleaned (oxidation to remove the organic material) before making permanent light microscopic slides and before making preparations for scanning electron microscopic investigation.

The origin of the diatoms may go back to the early Jurassic period (201.3 Ma) or even before, although well-documented fossil records only extend to the middle Cretaceous (127-89 Ma). The diatoms found in the Upper Cretaceous sediments are all of marine origin; most genera are now extinct. Evidence for the presence of freshwater species is found from the late Eocene (38 Ma) and Miocene (23 Ma) onwards.

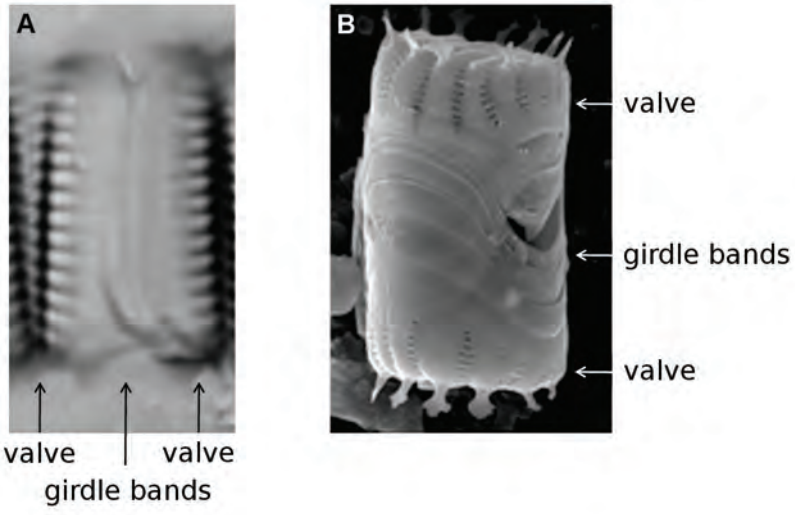


**Fig. 1.** Diatom frustules, composed of two valves connected by girdle bands. A. Light microcopy (LM). B. Scanning electron microscopy (SEM).

Algae traditionally formed part of botanical studies as they were considered in the past as plants: they are photosynthetic organisms, making their own organic material using sunlight (autotrophs). In the system of the five kingdoms (Whittaker 1969), they are part of the Protista (the other kingdoms are the Plantae, Animalia, Fungi and Monera or Bacteria); later the Monera were divided into the Eubacteria and the Achaebacteria (Woese & Fox 1977). A more recent system (Woese *et al.* 1990) divides all living organisms in three domains: Bacteria, Archaea and Eukarya or Eukaryota. The diatoms are part of the last domain as they possess a true nucleus encapsulated in a nuclear membrane.

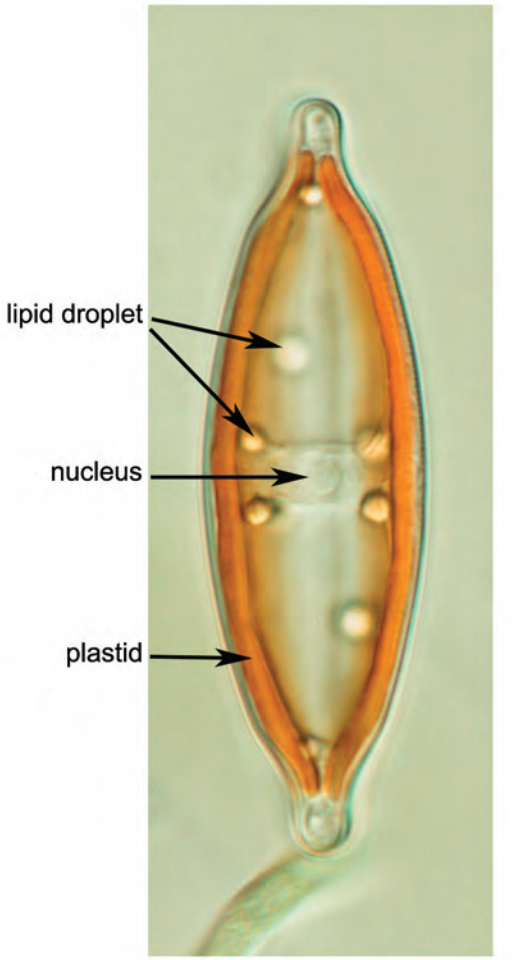


**Fig. 2.** Living diatom cell showing the plastid, lipid droplets and the nucleus.

## 3. Living diatoms

Diatoms are a major component of the primary producers in aquatic ecosystems. They can live free in the water column or be attached to a substratum. When they are free living, floating in the water column, they are called planktonic. When the entire life cycle takes place in the water column they are euplanktonic; when they become suspended in the plankton after being detached from the substratum they are known as tychoplanktonic. Tychoplankton occur mostly near-shore in the littoral zone of lakes. In rivers, suspended benthic organisms form part of what is known as the potamoplankton. Species that live on the bottom of a waterbody are called benthos. Benthic species can be attached to the bottom and are then sessile, or they can be motile (or both). The bottom can consist of a hard substratum such as stones, pebbles, boulders, rocks, or of loose sediments such as sand, mud, silt, clay. The term periphyton is used for species attached on submerged substrata; depending on the substratum we can have epiphytic species when they are attached to aquatic plants, epipelic when they are living on sediments and mud, epixylic when they are attached on wood, epilithic when they are living on stones, rocks, boulders, etc., and epipsammic when they are attached to sand grains.

## 4. Morphology of the diatom cell

Diatoms are unicellular algae with a siliceous cell wall, the frustule. Besides the photosynthetic pigments chlorophyll a and c and fucoxanthin they possess β-carotene, diatoxanthin, diadinoxanthin, violaxanthin, antheraxanthin, and zeaxanthin and storage products (oil/lipid droplets) (Fig. 2, previous page). Two main groups, based on cell symmetry can be distinguished: the radially symmetric or centric diatoms (Fig. 3) and the bilaterally symmetric or pennate diatoms (Fig. 4). In freshwater, the first are commonly found suspended in the water column while the second are more typical of benthic habitats or are temporarily re-suspended in the water column, although several *Nitzschia* (pennate diatom genus) are a typical component of the phytoplankton in tropical African lakes.

Other aquatic organisms can also possess siliceous structures which can be confused with diatoms. The first are structures called spicules, formed by sponges. They are composed of a solid silica body having a central empty canal almost as long as the entire length of the spicule. Several kinds of spicules exist, e.g., gemmasclere, microsclere and megasclere (Fig. 5).

The second type of siliceous structures are phytoliths, silica bodies formed in or between plant cells. Phytoliths are also solid silica structures with a very large diversity of shapes, often genus specific (Fig. 6). The solid silica structure can have ornamentations, such as cones which can be surrounded by smaller, satellite cones.

A third silica structure we can mention are cysts, formed by other algae such as Chrysophyta and Dinophyta. Cysts are characterized by an apical pore and the

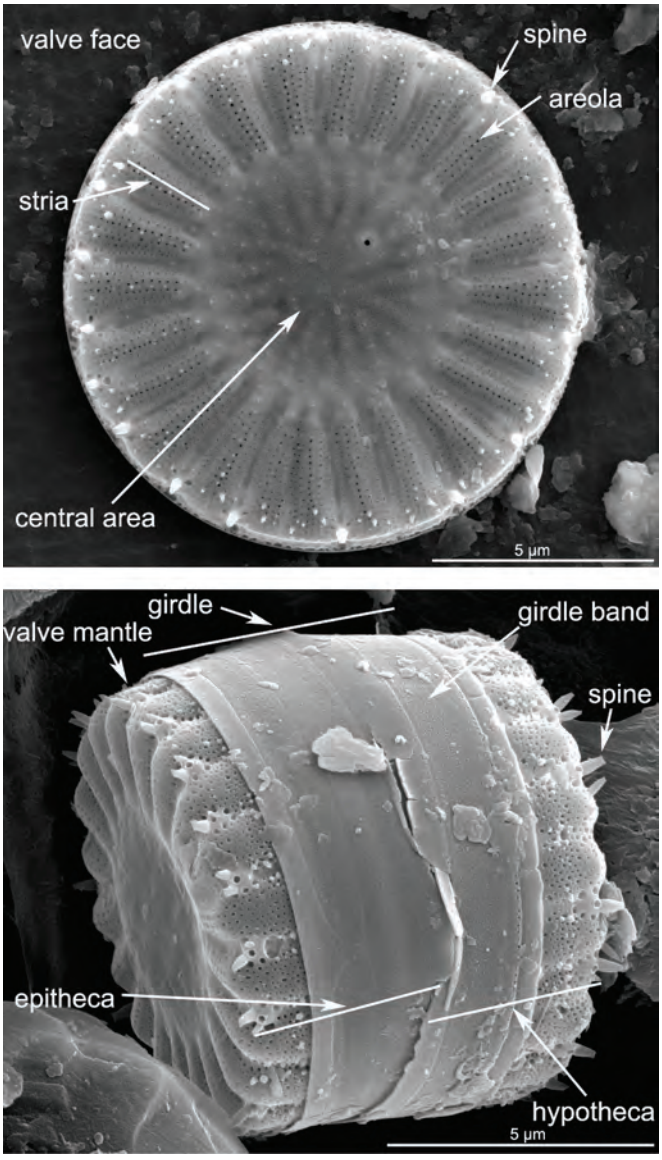


**Fig. 3.** Centric diatom showing the different structures. SEM. Top: valve view. Below: girdle view of an entire frustule composed of two valves and several girdle bands.

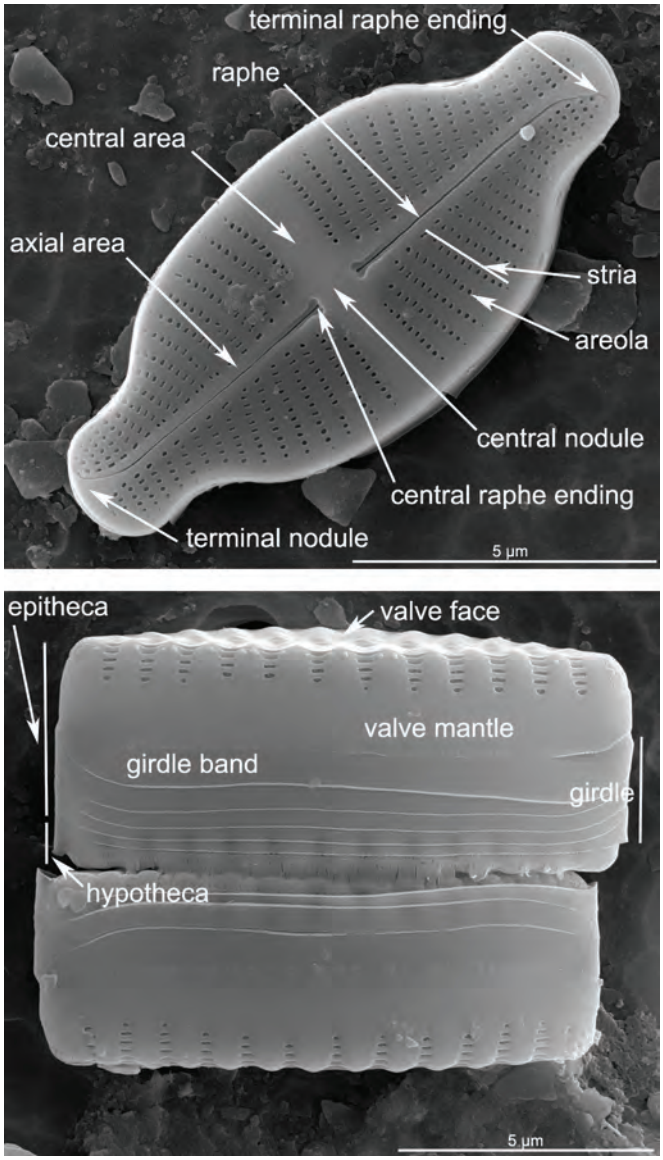


**Fig. 4.** Pennate diatom showing the different structures. SEM. Top: valve view. Below: girdle view of two entire frustules composed of two valves and several girdle bands.

wall can be smooth or ornamented (spines, verrucae, ridges) (Fig. 7). Another structure that can be confused with a diatom frustule is the lorica, a shell-like protection, of *Trachelomonas,* a genus within the Euglenophyta (Fig. 7). The wall

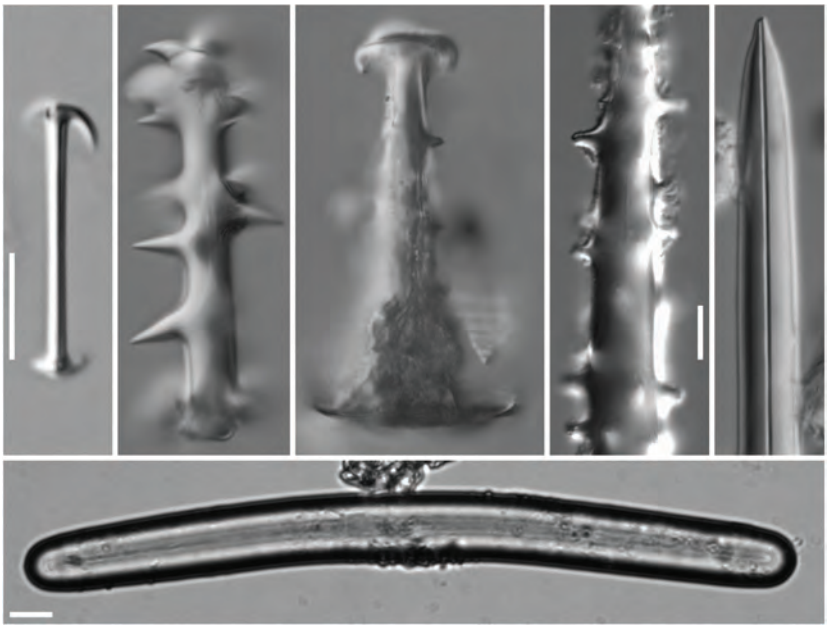

**Fig. 5.** Sponge spicules, different types. LM. Scale bars = 10 µm.

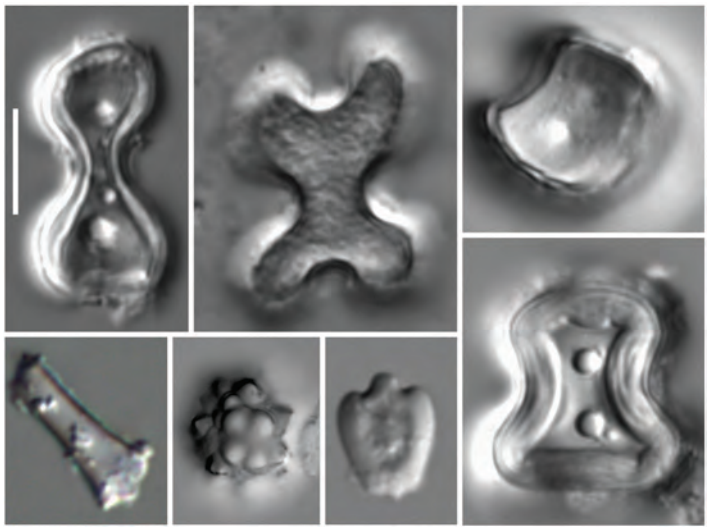

**Fig. 6.** Phytoliths, different types. LM. Scale bar = 10 µm.

of the lorica, in which silica can be incorporated, can be smooth or ornamented with small spines and is, like the mentioned cysts, characterized by an apical gap from which the flagellum protrudes.

Note that all the pictures presented of these silica bodies (sponges, phytoliths, cysts and lorica) are from material sampled in the Congo and Zambezi basins.

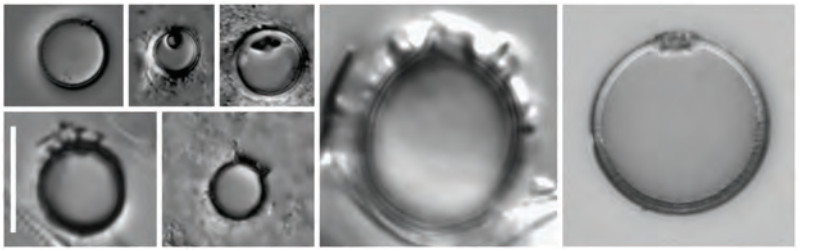

**Fig. 7.** Different types of Chrysophyte cysts on the left, a Dinophyte cyst in the centre, and the lorica of a *Trachelomonas* (Euglenophyte) on the right. LM. Scale bar = 10 µm.

## 5. How to recognize diatoms in natural environments

Periphytic (attached) diatom communities may be seen as a thin golden-brown film covering the substrata (Fig. 8). This film can be very thin but can also become thicker and much more obvious during certain times of the year when environmental conditions such as light, temperature and nutrient availability favour diatom growth. The film formed by the diatoms feels slimy or mucilaginous. Attached diatoms grow on all kinds of substrata including solid substrata such as pebbles, boulders and rocks and submerged stems and leaves of aquatic and/or submerged macrophytes (Fig. 8). Man-made objects such as paper, plastic bags and glass may be colonized by diatoms. Diatoms may even be found in soils, with several taxa adapted to survive desiccation. Diatoms form a component of the phytoplankton community where they live in suspension, or attached to other algae. An essential aspect when using diatoms to infer ecological conditions is to sample well colonized substrata. It takes several weeks for an uncolonized substratum to be fully colonized by diatoms. During that time a process of succession in diatom species and abundances can be observed.

The colonization of a substratum by diatoms takes place in various phases and by different diatoms during succession (Bijkerk 2014). Pioneer diatoms, often belonging to small *Achnanthidium* sp. (e.g., *A. minutissimum*), affix themselves to the substratum by small mucilaginous stalks. During succession they are overgrown by other species belonging to among others *Gomphonema*, *Encyonema*, *Rhopalodia* spp. These are attached also to the substratum by mucilaginous stalks but longer ones, or they are attached to the other diatoms or their stalks. The diatom film found

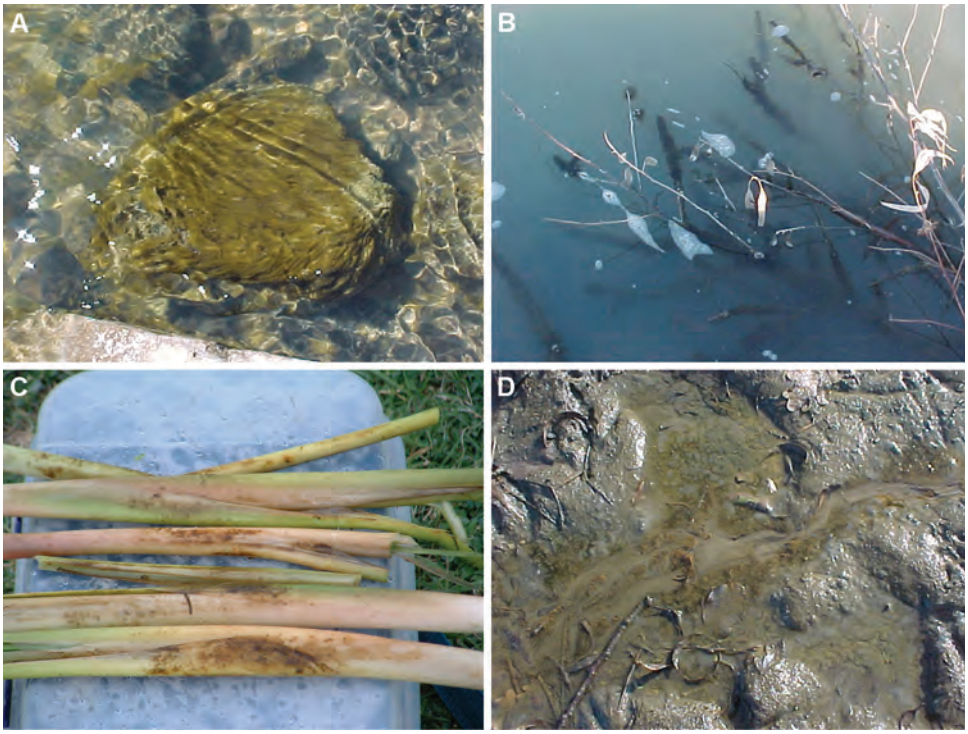


**Fig. 8.** Periphytic diatom communities on various substrata. A. Thick diatom layer (biofilm) attached to boulders. B. Thick diatom layer on submerged parts of tree branches. C. Thin diatom layer attached to submerged stems of *Phragmites australis* (Cavanilles) Trinius ex Steudel. D. Diatom layer on mud.

on a substratum can thus be compared to a forest with several layers such as the canopy, the understory, the shrub and the ground layer.

Diatoms can be attached in various ways to the substratum: mucilage stalks, mucilage tubes, mucilage pads (Fig. 9). Diatoms often form colonies, in particular this adaption allow planktonic species to remain suspended in the water column. The colonies can have the form of a chain, be stellate or zigzag (Fig. 10).

## 6. The role of diatoms in aquatic food webs

Diatoms are key organisms in aquatic ecosystems, together with representatives of the other micro-algae. They are autotrophic, making their own organic material from inorganic nutrients and sunlight through photosynthesis. Phosphorus (as dissolved orthophosphate) and nitrogen (as nitrate, nitrite or in the form of ammonium ions) are

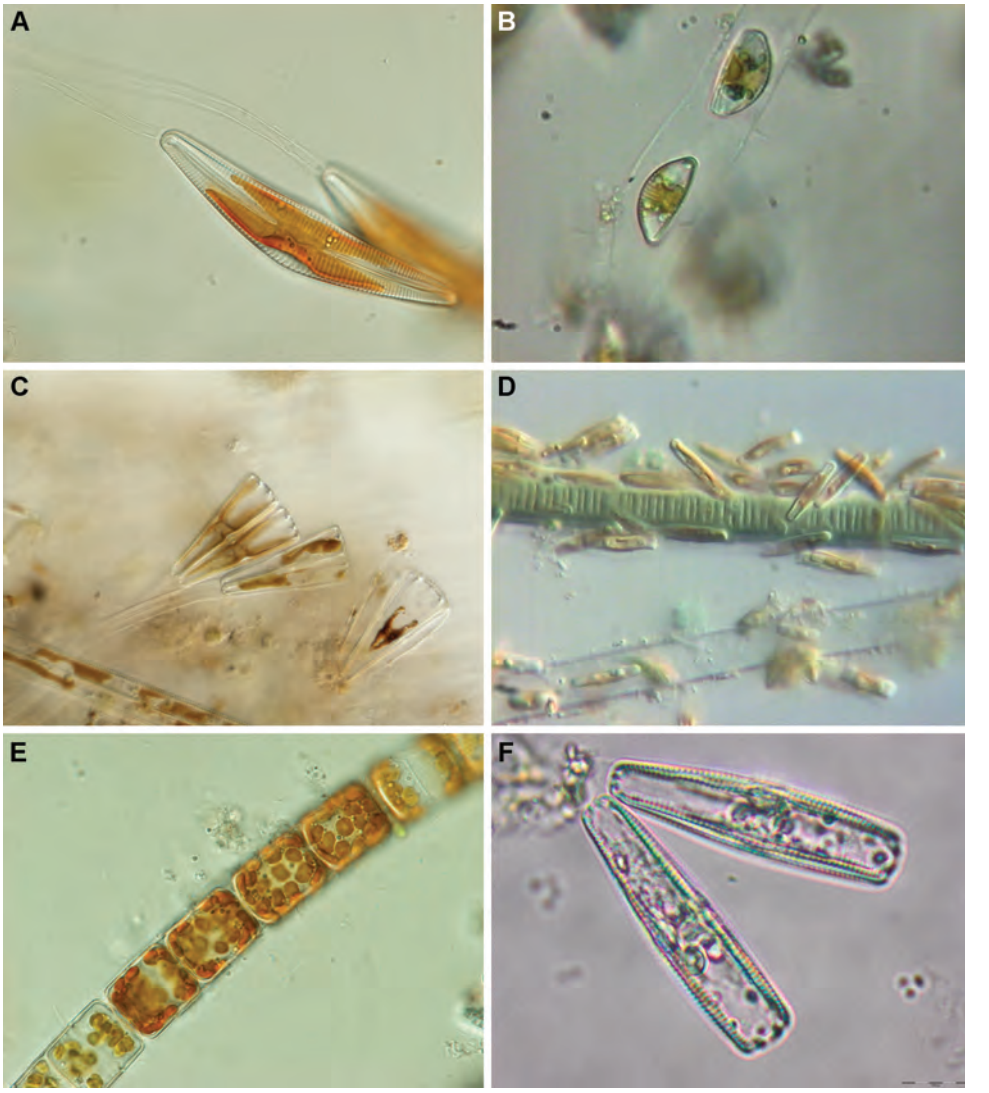


**Fig. 9.** Living diatom cells. LM. A. *Cymbella* sp. attached to a substratum with a mucilage stalk. B. *Encyonema caespitosum* inhabiting a mucilage tube. C. Cells of *Gomphonema* sp. with dichotomously branching mucilage stalks. D. Cells of *Achnanthidium minutissimum* attached to a *Lyngbya* sp. (filamenteous bluegreen alga) by means of short mucilage stalks. E. Chain forming cells of *Melosira varians* attached to each other by mucilage pads. F. *Afrocymbella barkeri* attached to a substratum with short mucilage stalks.

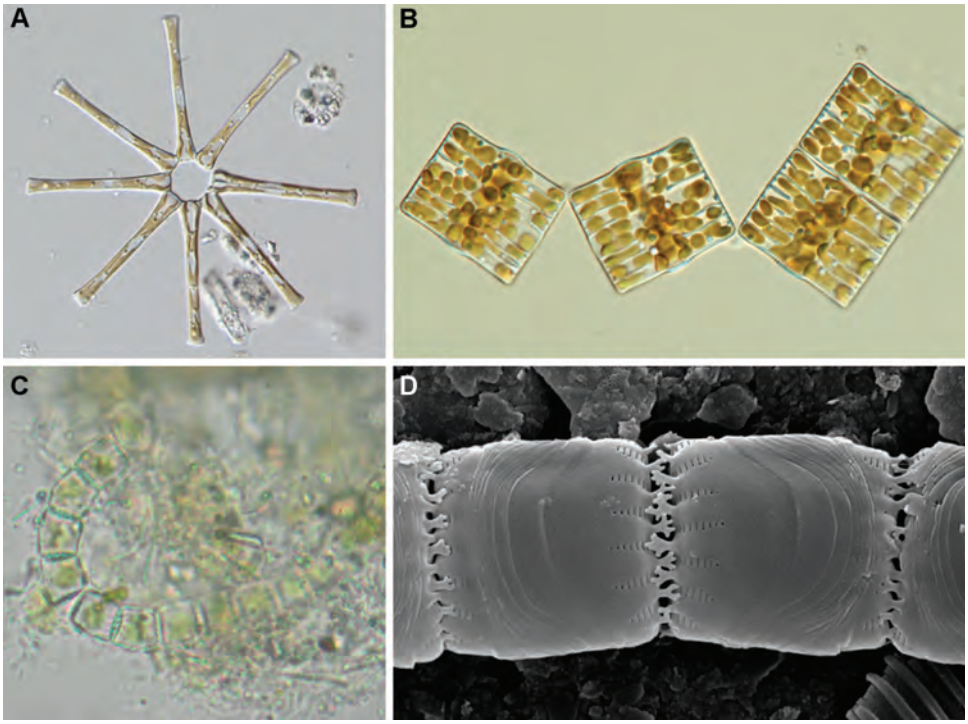


**Fig. 10.** Various types of colonies formed by diatom cells. A-C: LM, D: SEM. A. *Asterionella formosa* cells forming a stellate colony. B. *Tabellaria flocculosa* cells forming a zigzag colony; cells are attached to each other at the corners by mucilage pads. C. *Staurosirella* sp. cells forming a chain; cells are attached to each other, valve face to valve face, by connecting spines. D. *Staurosira* sp., detail of connecting spines.

among the most important nutrients for diatom growth, iron and other trace elements are also necessary. Being part of primary production they lie at the base of the food web and are consumed by heterotrophic organisms which are secondary producers. The consumers of diatoms range from microscopic ciliates to grazing molluscs and plankton filtering fishes. Changes in the environment will first become apparent at the base of the food web i.e., changes within the algae community including diatoms. For this reason diatoms are often used as bio-indicators in the study of water quality. The advantage of using diatoms in water quality analyses is that, besides the ease of sampling (section 7) they can be preserved for relatively long periods.

Identification of diatoms is based on the morphologic characteristics of the frustule/valve, a rigid structure (cell wall) composed of silica, and for this reason samples can be fixed in situ and stored with little concern that the cell wall will deform or rapidly

degrade. Immediate analysis is not required in contrast to phytoplankton analyses. However it is still worth examining the living communities and noting not only the other micro-algae and their interactions with the diatoms, but also the amount of dead diatoms (empty frustules). In lakes especially more than half of the observed diatoms can be empty frustules and sometimes only a minor portion are in fact living diatom cells.

## 7. Field collection methodology

### 7.1. Epilithon

The preferred substratum used for the diatom-based monitoring for water quality assessment in riverine environments comprises cobbles and small boulders or rocks. Most of the diatom indices developed and tested throughout the world can be applied using the diatom communities found on this substratum.

The main advantage with using the epilithon is that cobbles and small boulders are generally widely available throughout the entire length of a river or stream, from the source up to the mouth. Moreover the epilithic diatom community has been studied intensively around the world, their ecology is better known and the performance of the major diatom-based indices on this substratum is well understood at least in the case of Europe, North America and South Africa. In the absence of this substratum alternative man made substrates can be used such as bricks, pieces of concrete, bridge supports, pillars, channel walls, etc., and in the absence of these artificial substrata can be introduced. However, if this is the case sampling should only be done after these artificial substrates have been submerged for at least four weeks, allowing diatoms to colonize them. A disadvantage of this method is that one is not sure if the diatom community has already attained its climax structure after the four weeks and there is also the rather high probability that the substratum is removed by third party or animals before sampling can take place. Sampling from this substratum is achieved as follows. At least five cobbles or pebbles, of a size that can safely be picked up, are removed from the river or stream. The upper surfaces, those exposed to the flow of the river, are then firmly brushed with a small plastic brush such as a tooth or nail brush (or a knife) into a collection tray. All the substrata are pooled together and form a single sample. This sample is then well mixed and placed in a suitable labelled bottle. Other hard substrata which cannot be removed from the river may be sampled *in situ*. This is best achieved by scraping the substrata with a knife, spoon or with a tooth brush. The tooth brush tends to trap the biofilm rather well between its bristles and the brush can then be repeatedly rinsed into the sampling tray or other receptacle until sufficient material has been collected. Preservation in the field is recommended except when the cells are to be examined in their living state. Preservation is achieved using ethanol to a final concentration of 20% by volume. In the absence of ethanol formalin may be used but if unbuffered it is not suitable for long term preservation. After each sampling the apparatus used (tooth brush, knife, collection tray) must be thoroughly cleaned with distilled water before collecting a new sample in order to

avoid contamination. If distilled water cannot be carried into the field, the apparatus should be washed after the sampling event in the stream and then again in the stream at the new sampling site before collecting the sample.

### 7.2. Epiphyton

Although cobbles and small boulders are generally widely available, this is not the case for many rivers in D.R. Congo among which a prime example is the Congo River. Even if such a substratum is available the depth and velocity of flow of this river make obtaining them dangerous. An alternative is then to sample the diatom community growing on permanently submerged, usually rooted, parts of plants, belonging either to submerged macrophytes such as *Potamogeton* spp., *Ceratophyllum* spp., or emergent macrophytes, such as *Phragmites* spp., *Vossia cuspidata* (Roxburgh) Griffith, *Papyrus* spp. It is also possible to sample from *Eichhornia crassipes* (Martius) Solms and *Pistia stratiotes* Linnaeus but it is better to avoid these as they are floating plants and are easily transported and thus not representative of the sampling locality. In the European Integrated Water Policy *Phragmites* is often used as substratum. *Phragmites* was subjected to intensive studies, among others on the colonization by diatoms (e.g., STOWA 2014). In these studies it was found that maximal diatom growth on *Phragmites* stems was attained after five weeks and species diversity stayed more or less constant after seven weeks (Van Dam in STOWA 2014). Species quantity and composition is also dependent on the place the plant is growing in relation to the river bank. Therefore it is important that different parts of different plants are collected: at least 5 pieces of about 5 cm each on which a layer of diatoms/biofilm is clearly visible. These pieces are put together in a plastic zip bag with as little water as possible from the sampling locality. Close the bag and rub the bag with the plant pieces firmly between your hands so that the attached diatoms come loose from the substratum. A brown-greenish liquid will become visible, containing the diatoms. If the plant material is too dry, add a small amount of distilled water. Put the brown-greenish liquid in a bottle and fix immediately in the field with ethanol or formalin, see comments in section 7.1.

### 7.3. Epipsammon

Diatoms growing on sand grains, epipsammon, form colonies which are different from the epilithic and epiphytic communities. Sand as substratum is subject to abrasion as a result of movement of the grains where only strongly attached taxa can survive. Some typical taxa are *Cavinula lilandae* Cocquyt, M. de Haan & J.C. Taylor recently described from a stream in D.R. Congo and *Cymbellonitzschia minima* Hustedt, an endemic species of the East African Great Lakes. This habitat is not recommended for studies for biomonitoring, however it may form an important part of biodiversity studies. Sampling may be achieved in several ways. If the visible brownish biofilm is thick enough it can be gently scraped from the surface with a spoon or sucked up using a large pipette or syringe. Alternatively the very top layer of sand can be collected and rinsed to free the diatoms from the grains. Motile diatoms may be sampled by collecting the top layer of sand and returning this wet and unpreserved to

the laboratory. The sand is then placed in a Petri dish and either cover glasses or lens tissue is placed over the moist surface of the sand. The diatoms will migrate towards the light and in so doing stick to either the tissue or the glass. The tissue or glass can then be removed and rinsed in order to collect the diatom cells for examination.

### 7.4. Phytoplankton

Diatoms are one of the algal groups composing the phytoplankton. They can thus also be studied as part of a phytoplankton investigation. Sampling can be done in a quantitative way or, when only relative composition is needed, in a semi-quantitative way.

The quantitative method is mostly used to study the entire phytoplankton community, not only the diatoms. Depending on the trophic state of the water body the sample can be collected in a bottle which can range in volume from 1 l for oligotrophic waters to 50 ml for eutrophic waters. The preservation is done *in situ* with an alkaline iodine solution (Lugol's). When it is not possible to analyze the samples shortly after sampling (within a few days) formalin should be added to the sample in the field just after the fixation with Lugol's. Samples must be kept in the dark and preferably but not obligatory in a refrigerator or a cool box at around 4°C. Prior to analysis the sample is concentrated by settling for 24 to 48 hours for 1 l. To preserve the phytoplankton sample after analysis, buffered formalin must be added as in an alkaline environment dissolution of the diatom frustules is slowly taking place.

The semi-quantitative method is based on the sampling with a phytoplankton net. Best is to use a net with mesh size of 10 µm. Experience in tropical Africa has demonstrated that the diatom communities are composed of small cells, often smaller than 20 µm and even 10 µm. However, in eutrophic systems the meshes are quickly blocked preventing the water to be filtered; nets with larger mesh size can be used but the mesh size must never exceed 27 µm. One must be aware that the results of analyses based on such samples may not be representative of true algal communities as small organisms are not be retained in such a net sample. The phytoplankton net should be held in the stream of moving waters for a couple of minutes, avoiding suspension of benthic material. The concentrated sample at the bottom of the net should then be put in a storage bottle and fixed with ethanol or buffered formalin. In shallow standing waters the phytoplankton net should be dragged back and forth just below the surface. Again care should be taken that benthic material is not disturbed and included in the sample. For standing waters deeper than 10 m, such as lakes and dams, where vertical stratification can occur in the water column, a vertical haul with the phytoplankton net must be taken. The depth of the haul depends on the depth of the photic zone (up to where light penetration is sufficient for algal growth). In oligotrophic lakes such as Lake Tanganyika, the vertical haul may be up to 40 m depth; in other small oligotrophic deep crater lakes a haul of up to 25 m is recommended.

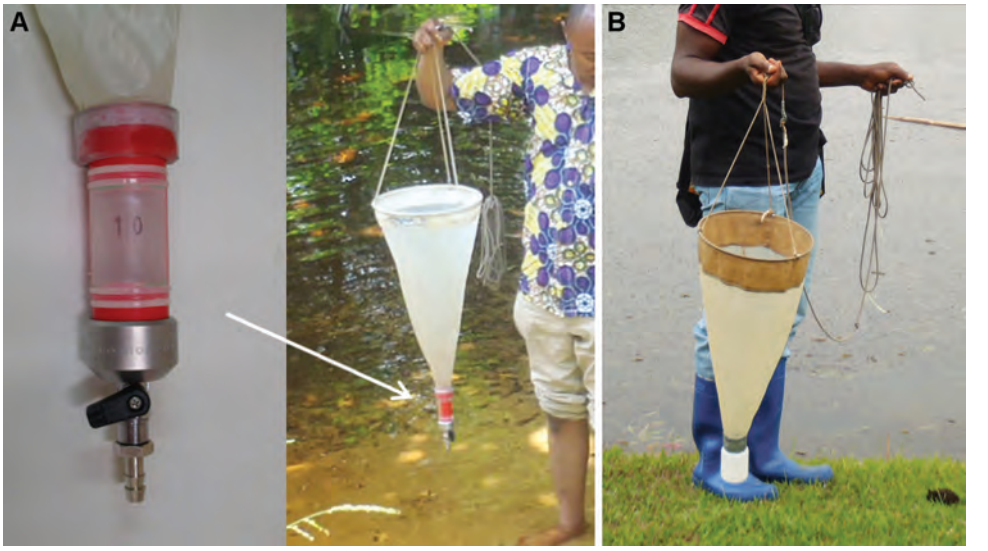


**Fig. 11.** Phytoplankton net. A. Commercial net (Hydrobios) with removable net bucket with a mesh screen which aids in concentration of the sample and a small tap at the bottom to decant the sample. B. Self-made net with screw thread adapted to a particular plastic bottle, in the depicted case a polyethylene bottle of 100 ml.

In order to aid the passage of water through the net it may be moved gently back and forth but care should be taken that none of the water is lost from the net.

The phytoplankton net is composed of a supporting ring made of stainless steel to which a conical net bag is attached. Usually the ring has a diameter of 25 cm and the bag is around 50 cm long. The sample is concentrated in a removable container or bucket on the end of the net with an opening covered by a mesh screen (Fig. 11 A), the sample is transferred to a bottle by opening a small tap. As the commercial nets (e.g., Hydrobios) are rather expensive, it is also possible to use homemade nets. Special material for phytoplankton nets with mesh size of 10 µm is available from specialized (web)shops. Make sure to use a stainless steel or aluminium ring to avoid corrosion. Instead of a concentration removable net bucket, an adaption piece to screw the bottles used for storing samples can be made (Fig. 11 B).

### 7.5. Terrestrial or soil diatoms

Soil diatoms, also called aerophilic diatoms, are a special group with many adaptations for the microclimate they live in which can be relatively arid compared to the permanently wet condition of aquatic environments.

Collecting can be done from moist sub-aerial, aerial and arid aerial habitats. It is recommended that six sub-samples of about 5 cm² should be taken within a radius

of 10 m to cover the local variability. To sample arid or dry soils remove carefully any detritus and other material covering the surface of the soil using a knife or a spoon. Collect the soil with a knife or spoon to a depth of about 1 cm. The six sub-samples thus collected should be placed in a paper envelope and not in a plastic bottle, this is to lessen the chances for the growth of bacteria and fungi due to the accumulation of moisture in a bottle. Dry or semi-dry rock faces and seep zones can also be sampled in a similar fashion. The biofilm is scraped from the surface of the rock using a spoon or knife. It is often not possible to cover a 10 m radius and thus several subsamples within the area covered by the biofilm should be collected. Depending on the amount of moisture present the sample can be stored in either paper envelopes or plastic bottles

### 7.6. Environmental parameters

During sampling, especially for water quality investigation, it is essential to note several characteristics of the water body as well as to measure some environmental parameters.

- Hydrological characteristics: stream velocity
  lake, river or channel depth
  river or cannel width

- Physical variables: water temperature
  turbidity
  sampling depth
  coordinates (collected using a GPS)

- Chemical variables: pH
  electrical conductivity (EC) also called specific electricity
  dissolved oxygen (DO)

- Nutrients: samples for measuring nitrogen:
  nitrates ($NO_3$-N)
  nitrites ($NO_2$-N)
  ammonium ($NH_4$-N)
  phosphorus:
  orthophosphate (PO4-N)
  total phosphate (TP)
  soluble reactive phosphorous (SRP)
  silica, iron, … is recommended if the equipment for measuring these parameters is available.

- Others: sampling site shaded or not

## 7.7. Annotations

It is essential to take notes during a sample collection trip. Besides the date and time of sampling all information mentioned in the previous section must be noted in the field during or just after the sampling. It is very difficult to remember everything on return back to the laboratory as most of the time collection trips have several sampling occasions and sampling sites.

How to take notes in the field? Do you have to use a field notebook or is it better to use prepared field record forms? Both have their advantages and disadvantages. Field record forms have the advantage that all information needed to be noted in the field is clearly indicated on a A4 sheet and have only to be filled in. It also allows for a standard data set to be collected at each site. The disadvantage is that separate sheets easily get lost. Forms must be clearly and logically composed. An example is given in Fig. 12. This example of a field record form can be adapted to the specific needs and type of sampling that will be conducted.

A notebook has the advantage that all the collected information from different sampling trips remains together. Notebooks are easy to store and must be kept with the relevant sample or slide collection if these are stored in a herbarium, museum, etc. A disadvantage of using a notebook is that one must be careful not to forget to include necessary information on the sample/sampling site, which may be the case for novice collectors; but once sampling becomes a routine, taking notes will also become routine.

## 7.8. Sample numbering and labeling

The correct numbering and labeling of the samples is also essential during a sampling trip.

Individual labels for each sample should be provided. It is recommended that sample information be written on self-adhesive labels with a pencil to avoid smudging if exposed to water. Once the label has been stuck onto the sampling bottle/vial, transparent tape can then be placed over the label and extend onto the bottle/vial to prevent damage such as abrasion and fading of the label during transport and storage (Fig. 13).

When a routine sampling is planned, labels can be made in advance in the office before starting the field work.

Field annotations in a field notebook or on a field record form together with the corresponding label on the sampling bottle are crucial for successful sampling and sample archiving. A sample without a label and without related field information is scientifically useless.

River: _____ Site: _____
Date: _____ Sample number: _____
Sample collected by: _____

---

**Physical records**

Width: _____ Depth: _____

**Substrate (record estimated percentage)**

| | | | |
|---|---|---|---|
| bedrock | □ | boulders/cobbles | □ |
| pebbles/gravel | □ | peat | □ |
| sand | □ | silt/clay | □ |

**Estimated percentage of boulders and cobbles covered by:**

Filamentous algae □ Other macrophytes □

**Shading (record estimated percentage)**

| | | | |
|---|---|---|---|
| Left bank | None □ | Broken □ | Dense □ |
| Right bank | None □ | Broken □ | Dense □ |

**Habitat**

Pool □ Run □ Riffle □ Slack □

**Water clarity**

Clear □ Cloudy □ Turbid □

**Bed stability**

Firm □ Stable □ Unstable □ Soft □

**Chemical records**

| | |
|---|---|
| pH: | type of meter used: |
| Conductivity (μS.cm$^{-1}$): | type of meter used: |
| Water temperature (°C): | type of meter used: |

**Photograph**

Facing upstream: Facing downstream:

Remark: It is important to include an immovable structure in a photograph as a reference for future comparison e.g. a bridge

**Fig. 12.** Example of a field record form to accompany a diatom sampling trip in a river or stream.


**Fig. 13.** Field collection methodology. A-B. Collecting samples with a phytoplankton net. C-D. Measuring of physical and chemical variables E-F. Taking notes in a field notebook and labeling of a sampling bottle.