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Protocols for Collection, Preservation and Enumeration of Diatoms from Aquatic Habitats for Water Quality Monitoring in India

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Diatoms have become important organisms for monitoring freshwaters and their value has been recognized in Europe, American and African continents. If India is to include diatoms in the current suite of bioindicators, then thorough testing of diatom-based techniques is required. This paper provides guidance on methods through all stages of diatom collection from different habitats of streams and lakes, preparation and examination for the purposes of water quality assessment that can be adapted to most aquatic ecosystems in India.

Keywords: Diatoms, Biomonitoring, Diatom-based indices, Water quality assessment, Indian rivers

Introduction

Diatoms constitute a fundamental link between primary (autotrophic) and secondary (heterotrophic) production and form a vital component of aquatic ecosystems. Cellular characteristics such as a siliceous wall (frustule), possession of unique photosynthetic pigments and specific storage products (oil and chrysolaminarin) make them unique amongst the algae. Two groups of freshwater diatoms are: 1) centric diatoms, which are in general circular in shape and adapted to live in the water column as part of the phytoplankton; and 2) pennate diatoms that live in benthic habitats but are often temporarily re-suspended in the water column.

Aquatic ecosystem monitoring has been carried out in India based on either chemical or biological analysis. The chemical approach is useful in order to determine the levels of nutrients, metals, pesticides, radioactive substances, etc., while the biological approach aids in assessing the overall effect of the chemical input on organisms. The chemical

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constituents of a waterbody exhibit enormous fluctuation, as certain compounds are broken down, absorbed or complexed over stretches of river which may vary greatly in length; other compounds are added in variable quantities from permanent or intermittent sources. However, both chemical and biological features are influenced by climatic factors, especially those affecting current speed and the associated dilution/concentration of nutrients Due to these factors, the interpretation of the 'chemical status' of a stretch of river becomes difficult except in crude terms In this context, biological monitoring relies on assessment of the changes in the biota to establish the relationships between the biological features and the overall chemical status and the response of the individual species to specific nutrients, pollutants, etc.

Water Quality Assessment Using Diatoms

Water chemistry variables are meaningful proxies for human disturbance in some cases: for example, when nutrient enrichment results from agriculture (McCormick and O'Dell, 1996; and Pan et al , 1996) For other disturbances, measures of chemical parameters may fail to capture changes associated with loss of in stream or riparian vegetation, increased sunlight or altered flow regime (Barbour et al , 1995; and Karr et al , 2000) Multiparameter water quality monitoring programs with a high sampling frequency are not cost-effective; this necessitates the use of an alternative method for assessing the integrity of water Consequently, other studies have taken a broader view of human influence and evaluated algal response to more direct measures of human disturbance, such as catchment land cover use and riparian disturbance (Kutka and Richards, 1996; Chessman et al , 1999; Pan et al , 1999; Carpenter and Waite, 2000; Hill et al , 2000; and Leland and Porter, 2000)

Algal monitoring evolved from the early indices of saprobity developed for European stream (Lange-Bertalot, 1979; Reid et al , 1995; and Lowe and Pan, 1996) Tolerance indices typically summarize the relative abundances of species weighted by their sensitivity to specific stressors (Prygiel and Coste, 1993; Kelly and Whitton, 1998 and Stevenson and Pan, 1999) Many studies have linked changes in algal assemblages, particularly diatoms, to changes in water chemistry such as pH, phosphorus and nitrogen (Carrick et al , 1988; Pan et al , 1996; and Winter and Duthie, 2000) The use of diatom tolerance values in water quality monitoring traces its history to Europe, where it has been used for a century (Kolkwitz and Marsson, 1908) and is currently considered, across the world, as important for biomonitoring (Schoeman and Haworth, 1986; and De la Rey et al , 2004) Diatoms have been shown to be reliable indicators of specific water quality problems such as organic pollution, eutrophication, acidification and metal pollution (Dixit et al , 1982; Tilman et al , 1982; Rott, 1991; and Cattaneo et al , 2004) as well as for general water quality (AFNOR, 2000) In India, the taxonomy of diatom flora has been studied since 18th century (Sarode and Kamat, 1984; Prasad and Misra, 1992; Gandhi, 1998 and Karthick et al , 2009); however, a study of the ecology and application of diatoms in water quality

monitoring has never been attempted. Although this paper is not intended as a motivation for the use of diatoms as bioindicators, it is perhaps important to mention the reasons why diatoms are useful organisms for biomonitoring (Round, 1993)

- Universal occurrence in lotic and lentic ecosystems;

- Field sampling is rapid and easy;

- Microscopic techniques are reliable;

- The ecological requirements of diatoms are better known compared to other groups of aquatic organisms;

- Shortest life cycle (~2 weeks) of all bioindicator organisms—primarily photoautotrophic organisms reproduce profusely and respond to environmental changes and provide early indications of both pollution impacts and habitat restoration;

- Sensitive to changes in nutrient concentrations—growth response is directly affected by changes in prevailing nutrient concentrations and light availability. Each taxon has a specific optimum and tolerance for nutrients such as phosphate and nitrogen, and this is usually quantifiable;

- Their assemblages are typically species-rich—a large number of taxa provide redundancies of information and important internal checks in datasets, increasing the confidence of environmental inferences;

- Their rapid immigration rates and the lack of physical dispersal barriers ensure that there is little lag-time between perturbation and response;

- Diatom frustules have a lasting permanence in sediments, such that sediment cores provide details of changes in the quality of the overlying water and also the past climatic changes;

- The taxonomy of diatoms is comprehensively documented largely based on frustule morphology—an attribute readily identifiable with modern light microscopy and image analysis techniques, and not, in most cases, dependent on electron microscopic techniques;

- Diatoms can be found on substrata and even in dry streambeds, enabling sampling throughout the year (Lane *et al.*, 2009);

- Availability of ecologically associative information worldwide (e.g., http://craticula.ncl.ac.uk/Eddi/);

- Permanent records can be made from every sample by means of strewn slides;

- Unlike invertebrates, diatoms do not have specific food requirements, specialized habitat niches, and are not governed to a major extent by stream flow; and

- The availability of interpretive software packages (e.g., OMNIDIA).

Assessment approaches based on diatom indices were developed in the lacustrine environment, and have since been extended to encompass the riverine systems (Round, 1991a; Round, 1991b; Stevenson and Pan, 1999; and Eloranta and Soininen, 2002). Diatoms can be collected not only from natural surfaces (sediments, stones and vegetation) but also from other substrate or surface types in an aquatic environment. The living component can also be collected in a controlled fashion using the simple expedient of artificial substrates (Gold et al., 2002). They collectively show a broad range of tolerances along a gradient of aquatic productivity, with individual species having specific water chemistry requirements. They respond directly and rapidly to many environmental parameters such as geology (Stevenson, 1997; and Pan et al., 2000), current velocity (Peterson and Stevenson, 1990) and nutrients (Potapova and Charles, 2007). These might vary according to species physiology and the species-specific sensitivity to parameters, which leads to a large panel of assemblage composition according to the river ecological conditions.

Although diatom-based water quality monitoring has many advantages, problems such as rapid changes in diatom taxonomy are encountered with the re-assignment of many taxa to new genera. Despite these problems, diatom-based indices of aquatic pollution have gained considerable popularity throughout the world. Much of the development and testing of diatom indices has been carried out in France, where that country's size and typological diversity enabled a more general application in Europe (Prygiel and Coste, 1999). Design and validation of OMNIDIA for the computation of diatom indices have further enabled diatom-based biomonitoring (Lecointe et al., 1993). Research of diatom species optima for nutrients and trophic status (Van Dam et al., 1994), as well as diatom tolerance to acidification (Van Dam et al., 1994), organic pollution (Lange-Bertalot, 1979 and Palmer, 1969), and sedimentation (Stevenson and Bahls, 1999) has helped in supporting the use of these indices. These, along with other measures of assemblage attributes (such as diversity and biomass), may yield a multimetric index that is both responsive to general environmental degradation and diagnostic of specific causes (Karr, 1993).

Many European countries, including Finland (Eloranta and Andersson, 1998), France (Prygiel et al., 2002) and Poland (Kwandrans et al., 1998), adopted and tested a variety of diatom indices. In recent years, diatom-based techniques have been incorporated in water quality monitoring in many countries, including Europe (Kelly et al., 1998; and Prygiel et al., 2002), Taiwan (Wu, 1999), South Africa (Taylor et al., 2007a), Malaysia (Wan Maznah and Mansor, 2002), Argentina (Gomez, 1999), Australia (John, 2000), Switzerland

(Hürlimann *et al*, 1999), Austria (Maier and Rott, 1988) and the United States of America (Stevenson and Pan, 1999). These countries are now either using diatoms as part of their routine monitoring programs or are in the process of developing the techniques necessary to do so.

European diatom indices were applied successfully in temperate regions, but there is little information regarding their application in the tropics and subtropics (Wu and Kow, 2002 and Taylor *et al.*, 2007b). This necessitates evaluation of these indices before they are adopted in warmer climates. Measurable relationships between water quality variables such as pH, electrical conductivity, phosphorus and nitrogen, and the structure of diatom communities as reflected by diatom index scores in South Africa showed that the diatom-based pollution indices may be good bioindicators of water quality in riverine ecosystems (De la Rey *et al*, 2004). However, it was found that the technique needed further testing with standard field and laboratory protocols across the country (Taylor, 2004). Such testing entails the standardization of techniques for collection, preparation and enumeration of diatom samples. Such standard methodology also aids in the evaluation and refinement of diatom-based water quality indices based on the deviations between reference and observed communities.

Kelly *et al.* (1998) strongly recommended the standardization of methods used for the sampling of benthic diatoms for water quality studies in Europe. Taylor *et al.* (2005) provided protocol for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa They emphasized the need for basic data collected in a robust and systematic manner to facilitate the evaluation of indices in different geographical areas and to enable individuals developing and refining indices to draw upon data from other regions in order to get a better idea of the environmental preferences of taxa. Recent studies, (Karthick *et al*, 2009) as well as studies in progress have identified diatoms as useful organisms to include in the suite of biomonitoring tools currently used in India both for the assessment of current water quality and for establishing historical conditions in rivers in India.

The focus of this paper is to present a set of standardized protocols based on methodological information from Indian, South African and European studies for field collection of samples and the preparation and enumeration of these samples in a manner yielding the most reproducible data. This protocol will aid those wishing to use diatoms in water quality monitoring studies in India

Recommendations

Timing of Surveys

In general, species diversity varies from season to season, and these patterns may also vary between substrates. However, flow-related factors override these patterns by washing away loosely attached species during spates and scouring floods (Stevenson, 1990 and

CEN, 2003) and the early recolonizers may not reflect water quality accurately. For these reasons, surveys should not be conducted for three to four weeks after a major storm. However, local knowledge is essential if the potential of the diatom-based monitoring is to be maximized. At least one sample per site per year is required for surveillance of water quality. This should be taken either at a time of low flow or when the highest concentration of pollution is expected. The rationale for the time of sampling will vary from region to region. In the peninsular part of India, diatom communities are at the peak of their development in winter to early summer (Karthick *et al.*, 2009). In peninsular and North-East hill regions, west-flowing rivers should be sampled during the post-South-West monsoon season from September to February, and east-flowing rivers should be sampled during post-North-East monsoon season from December to February. The Himalayan streams should be sampled during pre-monsoon, which is before June or post-monsoon during November to January to avoid the influence of monsoon rain on the stream diatom communities.

Site Selection

The number and location of sampling sites should be selected so as to provide representative samples, preferably where marked changes in water quality are likely to occur or where there are important river uses, for example, confluences, major discharges or abstractions. If sampling is intended to monitor the effects of discharges, sampling both upstream and downstream of discharge points should be carried out. Sampling should extend for an appropriate distance to assess the effects of pollution on the river (CEN, 2003; and Taylor *et al.*, 2005). Basically site selection can be considered at three different scales: (1) a very broad scale, concerned with location of the sampling sites within a catchment, (2) an intermediate scale concerned with selection of a site for sampling within a designated area, and (3) a fine scale concerned with determining the precise areas within a reach from where samples should be collected.

Where benthic diatoms are being added to pre-existing surveys, then sample sites are likely to be located close to existing survey stations to aid comparisons between diatoms and other types of environmental information. Such a process is likely to limit the choice of sampling station to a zone of approximately 100 m. Selection of an appropriate reach within this stretch will be determined partly by the choice of substratum. It is important to take notes on the site in standard format to aid subsequent data interpretation. Local situations and needs will determine the precise nature of the information that is required, but the following points should be borne in mind:

- A detailed description of the site is required on the first visit, on subsequent visits notes on unusual occurrences must be made.

- Details (including sketch maps) of the site location with map references from the Survey of India topographical sheets (preferably 1:25,000/1:50,000); this

information should include coordinates (Latitude, Longitude, altitude) and name of village/hamlet.

- Design of field record form to record all parameters at site; this includes: name of the sampler, measurements of channel width and depth, estimates of substratum composition, cover of filamentous algae and other macrophytes, extent of bank side shading, existence of small check dams in upstream and, if known, time since last spate or major rain event.

- A photographic record as an aid to data interpretation; on subsequent visits, records may be limited to major changes that have occurred since the previous visit, and any variations in sampling protocol employed.

Sites for stream biomonitoring should be in a 'riffle', where the water is flowing over stones (Round, 1993) with a current velocity >20 cm/s (CEN, 2003). However, 'runs' and 'glides' with suitable substrata are also suitable (DARES, 2009). Sampling in 'riffles' or areas of moderate or high water velocity ensures continuous exchange of the water surrounding the algae and prevents the buildup of a local chemical environment. Selection of cobbles from pools and ponded areas is to be avoided for sampling for the assessment of water quality (Kelly *et al* , 1998). The above recommendations have, however, been made for wadeable rivers and are not necessarily applicable to deep rivers. In deep rivers, it is often too hazardous to wade very far. A suitable sample may be collected near to the bank of such a river, provided the river is flowing over the substratum in question. This situation is typical of the lower reaches of all Indian westerly flowing rivers and the delta sections of the easterly flowing rivers, which are not wadeable; cobbles or other substrata may be collected close to the riverbank from 'riffles' with flowing water or where flow is >20 cm/s (Fore and Grafe, 2002). This is based on the assumption that the flowing water at the edge of the main stream (littoral zone) is of the same physical and chemical quality as that in the main stream.

The light regime and velocity of water are two physical factors which determine the diatom community in the streams. The light regime can affect both diatom community structure (Cox, 1984; and Kawecka, 1985 and 1986) as well as physiological processes (Guasch and Sabater, 1995) influencing organism's response to pollutants (Guasch *et al* , 1997). Samples that are to be compared should be collected from sites with similar light regimes. Heavily shaded areas are to be avoided, unless it a characteristic of the system under study. Areas very close to the bank should also be avoided because of possible varied water quality and an increased sediment influence on diatom community because of the lower velocity. Stream survey is done by wading, which in turn sets an upper limit on the sampling depth; performance of diatom indices is not affected up to 0.5 m depth (Elber *et al* , 1992) as long as this is still within the euphotic zone. Water velocity from

1 to 16 cm/s has no effect on indices (Antoine and Benson-Evans, 1982). Higher water velocity may often lead to changes in the growth rate and relative abundance of species (Antoine and Benson-Evans, 1982 and Wendker, 1992), and also a decrease in species diversity (Lindstrøm and Traaen, 1984; and Rolland *et al.*, 1997).

Choice of Substratum

Diatoms can be found growing on most submerged surfaces; however, the composition of the community varies, depending upon the substratum chosen. Ideally, a single substratum should be used at all sites included in a survey. According to Round (1993), diatoms form distinct assemblages that occur closely associated with particular microhabitats, e g , on sediments (epipelon), sand (epipsammon), gravel, stone and bedrock (epilithon) and macrophytic plants (epiphyton). Care needs to be taken not to contaminate the target community with species from other microhabitats when sampling, as each substratum has distinct diatom assemblages. Sampling well-colonized substrata could minimize the error in inferring ecological conditions. Diatom communities are detected on substrata by feel (slimy or mucilaginous) or seen as a thin golden-brown film covering substrata. In some conditions or at certain times of the year, this film may become thicker and much more noticeable. Diatoms also colonize artifacts and waste materials (e.g., plastic bags, pieces of wood). Samples may be collected from such substrata when all other alternatives are absent.

Diatom community structures are governed by substrata associations (Reavie and Smol, 1997). However, major influences on community composition are disturbance (mainly from floods), resource supply (mainly from inorganic nutrients) and, to a lesser extent, grazing (Biggs *et al.*, 1998). It is advisable to carry out an exploratory survey to gain knowledge about the flora growing in different habitats before initiating a detailed study. Stratified random sampling strategies will be appropriate for a site with abundant substrata for detailed investigations. For example, some workers do not recommend the flora living on silt ('epipelon') for routine monitoring, as its high organic content tends to favor taxa with a higher 'saprobity' than the prevailing water quality of the river (Vizinet, 1995);

Epilithon

Round (1993), Kelly *et al* , (1998), Prygiel *et al.* (2002) and Taylor (2004) consider cobbles and small boulders as the preferred substratum for monitoring diatoms in the riverine environment, promoting the universal applicability of diatom indices and for routine water quality monitoring. The flora at a particular site does not depend on the type of stone sampled (Kelly *et al.*, 1998), although broad aspects of catchment geology do have a pronounced influence on the flora (Lay and Ward, 1987; and Maier, 1994). In addition, this substratum type is preferred for the following reasons:

- Availability of epilithic substrata throughout the length of a river across all seasons (Kelly *et al*, 1998);

- Easy to collect; and

- Clear understanding of diatom ecology and the performance of major diatom-based indices on this substratum (Round, 1993 and Eloranta and Kwandrans, 1996).

It is preferable to collect samples from five or more cobbles ($>64 \le 256$ mm diameter) or small boulders (>256 mm) from a reach of at least 10 m (Kelly and Whitton, 1998) in the river or stream. Remove any loosely attached surface contamination (e.g., organic debris) by agitating the substratum briefly in the stream water. Place the substrata in a tray, along with approximately 50 mL of river water. Diatoms are removed by vigorously scrubbing the upper surface (the side exposed to flowing water) of the substratum with a small brush (e.g., toothbrush) to dislodge the diatom community. Only the upper side of boulders should be scrubbed to avoid contamination with sediment that might be present on the lower portions of the boulders. A knife or other sharp instrument can be more effective for removing firmly attached diatoms, but will be less efficient at penetrating crevices on rough surfaces, which may cause more damage to frustules and may lead to more rock particles being transferred to the sample. Care should be taken to avoid instrument contamination between sites by washing the toothbrush, knife and the plastic tray in clean river water and rubbing the toothbrush on a clean surface both before and after taking the diatom sample in order to minimize the contamination. However, it is unlikely that there will be any quantitative difference in the results obtained. Replace the substratum in the stream, and repeat the process for the other replicate substrata. Transfer the contents of the tray to the sample bottle of approximately 125 mL. The contents of the bottle should be brown and turbid due to the presence of diatoms. Label the sample bottle with details relevant to the sample.

Epiphyton

In the absence of cobbles or small boulders, emergent or submerged macrophytes — such as *Typha* sp., *Phyla* sp., *Phragmites* sp., *Nymphoides* sp., *Cyperus* sp., and *Eichhornia* sp.— may be sampled for diatoms. Habitat preference of diatom should be considered while sampling in aquatic vegetation. There are important differences in diatom flora between the epiphyton on bryophytes, emergent macrophytes and submerged macrophytes as well as spatial and temporal differences inherent in sampling a dynamic substratum.

In general, submerged macrophytes are preferred over emergent ones for routine monitoring purposes; however, there is considerable diversity in the manner in which the plants are subsequently treated, from digesting entire portions of the plant to scraping

or brushing stems, leaves and roots to squeezing or shaking the plant to dislodge the epiphyton (Porter *et al*, 1993); Comparative analysis of samples collected from macrophytes and from stones was done by Lenoir and Coste (1994) for rivers in the Rhin-Meuse basins in France. IPS (*Indice de Polluosensibilité*/Specific Pollution Sensitivity Index) (CEMAGREF, 1982) scores varied by less than 2 units on a scale of 1-20 for samples from stones, compared to those from submerged plants. A similar difference in scores was observed for trophic indices for diatom flora on epiphyton and epilithon from lakes in Germany (Hofmann, 1994) and rivers in Finland (Kwandrans *et al.*, 1998)

Sampling from macrophyte substrata: Sampling from emergent macrophyte substrata should be achieved as follows. The emergent macrophyte stem is cut above the water line. A plastic bottle is then inverted over the remainder of the stem and the stem is cut slightly above the point where it emerges from the sediment. The bottle is then inverted and brought to the bank. This procedure needs to be repeated until five stems have been collected (CEN, 2003). The scrubbing and removal of the diatom communities can then proceed in a similar fashion to that described above for solid substrata. Submerged macrophytes can be sampled by selecting replicates from five different plants growing in the main flow of the river. Each replicate, consisting of a single stem plus associated branches of the plant from the lowest healthy leaves to the tip, should be placed in a plastic bag together with 50 mL of stream water. Diatoms should be present as a brown film associated with the macrophytes. The plants should be shaken vigorously in the plastic bag and the resulting brown suspension poured into a sample bottle (Taylor *et al.*, 2005; and DARES, 2009).

Artificial Substrata

Artificial substrata may be introduced in the euphotic zone of deeper rivers and lakes (with fine silt and sand as substratum) for ecological studies and for bioindication (Iserentant and Blancke, 1986; Cattaneo and Amireault, 1992 and Lane *et al.*, 2003). The following points should be kept in mind when using artificial substrata:

- A minimum exposure time of four weeks is recommended (Cattaneo and Amireault, 1992; and Hürlimann and Schanz, 1993), although this period is dependent on the trophic status and other parameters like shade and temperature of the water (Guasch *et al.*, 1997). Exposure time must be constant for comparisons between sites.

- Although glass slides have been widely used in the past (Butcher, 1932; and Patrick *et al*, 1954), Cattaneo and Amireault (1992) highlight the need for near 'natural' surfaces such as unglazed tiles, (Coring, 1993), or polypropylene rope, frayed at the ends as a substratum and staked to the river bed (Salden, 1978; Snoeijs and Simenstad, 1995; and Goldsmith, 1997).

- Artificial substrata should not interfere with the activities of legitimate users of the river (e g., nearby habitants) or attract the attention of passers-by. Normally, artificial substrata are introduced in secluded stretches of river away from footpaths and bridges to minimize losses or tampering with substrata. Involving nearby schools and local people in monitoring programs helps in creating awareness and minimizing loss of substrata.

- The smooth surfaces of some artificial substrata require proper positioning to prevent sloughing off of the diatom film.

- Results from studies using introduced substrata cannot be interpreted unless full details of methods are reported.

- Care should be taken while interpreting to avoid any bias towards fast growing diatoms that are not a 'climax' community (Round, 1993).

Preservation of Diatom Material

If the sample is to be processed within a few hours, then no preservative is necessary as long as steps are taken to minimize cell division (i e., by storage in cool, dark place). For short-term storage, the sample can be stored either in refrigerator, or Lugol's iodine may be added. However, Lugol's iodine sublimates and is unsuitable for long-term storage of samples, for which Ethanol is recommended. Ethanol should be added to reach a final concentration of 20% by volume. Formalin is a preservative commonly used for algal samples, it should be avoided as it is carcinogenic and it damages the fine structure of diatoms (Kolbe, 1948; and Krammer and Lange-Bertalot, 2000). Riemann (1960) demonstrated that formalin—even in extremely low concentrations—causes silicic acid to be released from diatom valves. Appropriate health and safety guidelines must be followed when using these preservatives and the name of the preservative should be marked on the outside of the bottle.

Laboratory Treatment

Pre-Preparation Examination

On return to the laboratory, a quick examination of unpreserved samples should be performed to assess whether they consist predominantly of live cells (dead cells will form part of the bio-film and are not washed away, under normal conditions). If the majority of the diatoms are dead cells (empty frustules with no chloroplasts) the sample should be discarded, as it will not be possible to obtain a true reflection of recent water quality at the particular sampling site from this sample (Bate *et al*, 2002).

Cleaning Technique

Frustules may be cleaned with either acids or hydrogen peroxide. These procedures are modified from the techniques of Welsh (1964), Hasle (1978), Lohman (1982), McBride

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(1988), Krammer and Lange-Bertalot (2000) and Taylor *et al* (2005). Optimum conditions for Light Microscopy (LM) and Scanning Electron Microscopy (SEM) must be achieved as most structures of a frustule, used for identification, are fine and difficult to resolve. The organic components of the cell must, therefore, be removed. Diatom slides should meet the following criteria (DARES, 2009):

- Complete removal of organic matter in the sample;

- Foreign matter should be either absent or insufficient to cause problems during the enumeration or identification of the specimens;

- The distribution of valves on the cover-slip should not be clumped, but be evenly spread over the whole area of the cover-slip without edge effects;

- Ideally, there should be 5-15 valves, but not less than 1 and not more than 20 valves, per field of view when viewed at $1,000 \times$ magnification; and

- The mountant should be properly cured, without air bubbles, and should evenly spread right up to the edge of the cover-slip.

Contamination must be guarded against in all phases of preparation from the collection of the sample in the field to the final mounting of the sample on a glass slide. Only simple glassware, such as glass beakers, watch glasses and centrifuge tubes, are used as these are capable of being easily and thoroughly cleaned after each use with distilled water. As it is impossible to clean a pipette tip, it should be used only once (Lohman, 1982). A cheap alternative to a pipette is a plastic drinking straw.

Necessary precautions should be taken with all cleaning methods to avoid health hazards. The chemicals used for preparation of samples may be carcinogenic and corrosive, adequate health and safety precautions should be taken in each step. Familiarize yourself with the material safety data sheets for each chemical in question.

Acid oxidation is a common method of preparing diatoms slide. It effectively removes all organic parts of a cell, including the diatotepum covering membrane. It has the disadvantage that very delicate silica structures of the cell wall may be damaged. The acids dissolve one of the solid phases of the silicic acid of the cell wall, so that when studied at high magnification under SEM, the cell wall appears more or less jagged in structure. In LM studies, such damage is of little significance (Krammer and Lange-Bertalot, 2000). A series of techniques, including both acid and non-acid techniques, are described below. When material is required for SEM techniques, the use of acid oxidation should be avoided, and the more gentle method using hydrogen peroxide should be employed (Round *et al*, 1990), or the material should be left untreated (Taylor, 2003). With the exception of material from calcium deficient water, it is almost always necessary to dissolve

traces of calcium in the sample with hydrochloric acid and then to rinse the sample (Krammer and Lange-Bertalot, 2000) This is particularly important if further processing with sulphuric acid is needed, otherwise a calcium sulphate diatom precipitate will form, which will make subsequent identification of the valves difficult. In the absence of a fume cabinet, all methods employing boiling acids must be avoided.

In all these methods the original sample should be allowed to settle for 24 h and subsequently cleared of supernatant water without losing any diatom materials. A portion of the original sample should be retained throughout the preparation stages until the slide has been prepared and checked under a microscope. After cleaning, the final rinsing of the samples is essential to remove any remnant acid and also to prevent its reaction with the mounting medium when a slide is prepared (Round et al., 1990).

Decalcification

Decalcification is necessary if samples are to be later treated with Hot HNO_3/H_2SO_4 method, as in these methods both the acids combine with calcium, causing the formation of an insoluble precipitate This stage can be omitted if you are sure that the sample does not come from a site with any calcareous rock in the catchment or if using the Hot HCl and $KMnO_4$ method.

- Shake the sample well and pour 5 to 10 mL (depending on the concentration of the material) of thick suspension into a heat-resistant beaker.

- In a fume cabinet, add a few drops of dilute HCl (e g , 1 M) and agitate gently; the material should effervesce as the carbonates are reduced to CO_2 (if the sample does not effervesce on addition of HCl, there is not a significant amount of Ca in the sample and it is not necessary to continue with decalcification).

- Continue adding dilute HCl and agitate the beaker gently until effervescence stops

- Pour the solution into a centrifuge tube (10 mL) and add distilled water to 1 cm below the rim of the centrifuge tube and centrifuge to remove the acid

- The samples are rinsed by centrifuging with distilled water at 2,500 rpm for 10 min.

- After centrifugation, the supernatant is decanted and the washing is repeated further four times until the sample is circumneutral.

Hot HCl and $KMnO_4$ Method

This method is based on that of Hasle (1978) and adapted by Round et al. (1990). It has proved suitable for samples from India, as demonstrated by the ongoing ecological research at Indian Institute of Science. The procedure is as follows:

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- Shake the sample well and pour 5 to 10 mL (depending on the concentration of the material) of thick suspension into a heat-resistant beaker.

- Mark the beaker clearly with the sample number in several places.

- Add 10 mL saturated potassium permanganate ($KMnO_4$) solution, mix well and leave it for at least 48 hours

- Add 10 mL concentrated HCl (32%), taking care not to inhale the gasses released. Cover the beaker with a watch glass and heat on a hot plate at 90 °C for 1 to 3 h inside a fume cabinet, until the solution becomes clear and yellow in color. Do not allow the sample to boil. Care should be taken to avoid cross contamination of samples during violent bubbling while heating with acid (Welsh, 1964).

- After oxidation of organic material, carefully add 1 mL of hydrogen peroxide, one drop at a time, to check if the oxidation process is complete. In the absence of organic material, hydrogen peroxide will not cause lasting foaming

- When oxidation is complete, allow the sample to cool and transfer into a 10 mL centrifuge tube. Beakers must be vigorously swirled to re-suspend the diatoms and for settling of stone and heavier sand particles before transferring to centrifuge tubes

- Rinse the samples by centrifuging with distilled water at 2,500 rpm for 10 min, followed by washing

- During washing, supernatant should be poured off in a single movement without losing any diatom material. Then, diatoms and small particles of sand at the bottom of the tube are loosened by means of a jet of distilled water from a wash bottle. More water is then added till it reaches the required volume in the centrifuge tube.

- Decant the supernatant and repeat the centrifugation and further washing at least four times or until the sample is circumneutral.

After the last wash, the diatoms are again loosened by means of a jet of distilled water and then poured into a small glass storage vial bearing the necessary sample information. It is important to store diatom samples in glass as opposed to plastic vials, as glass releases silica, which counters the dissolution of diatom valves

Hot HNO_3/H_2SO_4 Method

- Check the sample for the presence of calcium and decalcify the sample if necessary (as per the decalcification procedure mentioned above).

- Mix the diatom suspension carefully and take a subsample, (~10 mL) into a beaker. The size of the sample depends on density, which is judged by the visible concentration of suspended material.

- Mark the beaker clearly in several places with the sample number
- Add 5 mL of the strong acid mixture ($HNO_3 + H_2SO_4$, 2:1) and place the beakers with a watch glass on a hot plate. Heat the samples at 90 °C for 2-3 h, depending on the amount of organic matter in the sample. Care should be taken to avoid mixing of samples during violent bubbling while boiling with acid
- Rinse the samples and test for organic material as in points 5-10 in the Hot HCl and $KMnO_4$ method

Hydrogen Peroxide Method

Hydrogen peroxide is much gentler than acid, as it is not as corrosive as in the former described methods. It is best used with samples that require little cleaning, and where corrosion should be limited, as in SEM studies (Krammer and Lange-Bertalot, 2000). The choice of technique (either hot or cold) depends on the availability of a fume cabinet. If one is available, the peroxide can be boiled, and if not, a cold method should be used, but only in a well ventilated room.

Hot H_2O_2

- Mix the diatom suspension and place 5 to 10 mL of the suspension in a beaker.
- Mark the beaker clearly in several places with the sample number.
- Add 20 mL H_2O_2 and heat on a hot plate at 90 °C for 1 to 3 h
- Add a few drops of HCl and leave to cool.
- Rinse the samples as in Hot HCl and $KMnO_4$ method.

Cold H_2O_2

- Mix the diatom suspension and place 5 to 10 mL of the suspension in a beaker.
- Mark the beaker clearly (preferably in several places) with the sample number.
- Add 20 mL H_2O_2 to the beaker and leave for a minimum of four days
- Rinse the samples as in Hot HCl and $KMnO_4$ method.

Bleach Method

- Rinse any preservative (e.g., ethanol) from the sample by centrifugation with distilled water (3 runs at 2,500 rpm).
- Mix the diatom suspension and place 5 to 10 mL of the suspension in a beaker
- Mark the beaker clearly in several places with the sample number.
- Add 5-10 g of commercially available bleach (5.25% sodium hypochlorite) to the beaker and leave for a minimum of one day (this depends on the amount of organic content in the sample).
- Rinse the samples five times using distilled water.

Preparation of Diatom Slides

Most of the ultra-structural details of diatoms lie at the limit of resolution of light. In addition, all mounting media generally used in cytology have a refractive index similar to that of diatom valves, with the result that slides with diatoms mounted in these media are too low in contrast for satisfactory investigation. Hence, diatoms are enclosed in a medium of higher refractive index than that of the diatom valves (Krammer and Lange-Bertalot, 2000). Three types of mounting media are generally used: 'Hyrax' r.i. (refractive index) 1.71 (Hanna, 1930); 'Naphrax' r.i. 1.69 (Flemming, 1954) and 'Pleurax', r.i. 1.73 (Hanna, 1949; refractive indices after Meller, 1985).[1]

Slides should be free of contamination by other diatomaceous material and should display an assemblage of diatoms that is as close as possible in terms of composition to that of the original sample. For this reason, strewn slides are used almost exclusively (Lohman, 1982) and can be prepared following the methods of Welsh (1964), described below (Note: It is always necessary to keep the sample well mixed or shaken, as the larger diatom cells, will tend to settle out of solution quicker than the smaller cells and thus the community counts will be skewed and unreliable):

- Slides and cover-slips should be thoroughly cleaned with detergent soap and stored in ethanol until used.

- Using a pipette, a portion is drawn from a well-shaken numbered vial of cleaned material. The cleaned diatom suspension is diluted until the sample appears slightly cloudy (to the naked eye).

- A single drop of ammonium chloride (NH_4Cl; 10% solution) is added for every 10 mL of diluted diatom suspension to neutralize electrostatic charges on the suspended particles and reduce aggregation (Mcbride, 1988).

- Using a pipette or straw, ~0.5-1 mL (depending on the size of the cover-slip) of this suspension is placed on a clean, dry cover-slip.

- The diatom suspension placed on the cover-slip is allowed to dry in a dust-free environment at room temperature. Care should be taken not to disturb until dry, as vibration causes clumping of the diatom valves.

- The drying of cover-slips on a hot plate is not recommended because the resultant convection currents form more or less concentric rings of diatoms. If more rapid drying is required, the samples may be dried under a 60 W light globe.

- After the water evaporates, diatom-coated cover-slips are placed on a hot plate at ~350 °C for 1 min to do away with the excess moisture and to sublimate the residual ammonium chloride.

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[1] 'Naphrax' is available at Brunel Microscopes, Ltd, Chippenham, SN14 6QA, England (http://www.brunelmicroscopes.co.uk/acatalog/Diatom_Mountants.html), while 'Pleurax' may be obtained from Dr. Jonathan Taylor, North-West University, South Africa (Jonathan.Taylor@nwu.ac.za).

- The cooled cover-slip can be examined under $400 \times$ magnification to determine if the concentration of diatoms in the solution was correct. At least 10, but not more than 40, valves should be visible per field. When the sample is finally viewed at $1000 \times$ magnification, there should ideally be between 5 and 15 but not more than 20 valves visible in each field. If the concentration is too high or low, steps 1-7 need to be followed again, using a more or less dilute suspension, before proceeding further.

- After the diatom-coated cover-slips have been allowed to cool, one or two drops of mountant are placed onto each by means of a glass rod or pipette.

- Heat the mountant on the cover-slips gently for 30 s to 1 min

- A previously cleaned glass slide is then lowered onto the cover-slip, inverted, and then heated at 90-120 °C on a hot plate until the mounting medium 'boils' and all the solvent evaporates

- The solvent of the mounting medium should be evaporated quickly. If this is not done, a ring of exuded medium will harden around the edge of the cover-slip, while the mounting medium under the cover-slip remains more or less viscous.

- Under no circumstances should the mounting medium be heated for too long, because it will then turn dark in color.

- Depending on temperature and the quality of the mounting medium, it is necessary to heat the slide on the hot plate for two to five minutes.

- After the mounting medium is boiled for sufficient length of time and while it is still viscous, the hot slide is quickly removed from the hot plate, and laid on the work bench

- The cover-slip is then adjusted into position. If this operation is not successful for the first time, the slide need only be re-heated for a few minutes and the positioning is repeated

- When the slide is thoroughly cooled, the mounting medium should be hard and brittle and capable of being easily chipped off with the point of a scalpel.

- Surplus medium, which has been exuded and has set round the edge of the cover-slip, may be carefully removed with the point of a scalpel, after which the slide is wiped clean with a soft rag soaked in the particular mounting medium's solvent (iso-propyl alcohol for 'Pleurax') and toluene (which is carcinogenic for 'Hyrax').

- The slide should be carefully labeled with sample details—date of collection, site location and coordinates, habitat, collector and type of mounting medium. The

slide should also be labeled with the date of preparation and the name of the technician.

- The slide is ready for microscopic examination and archiving.

Enumeration

Different conventions have been evolved for the enumeration of diatoms, using either valves or frustules as the basic unit, or not distinguishing between valves and frustules. The effect that such conventions have on the final results has not been evaluated, but is likely to be small. However, it is important that the convention used be specified in advance. In the case of small diatoms, such as some *Achnanthidium* and *Naviculoid* species, it may not be possible on all occasions to distinguish with certainty between intact frustules and isolated valves. CEN (2003) and Prygiel *et al.* (2002) recommended that the required number of individuals be counted, without any distinction between valves and frustules. The aim of counting diatom units is to produce semi-quantitative data for ecological conclusions. For this, it is important to know how many valves to count to get a reliable estimation of the relative species composition at a specific sampling site. The total number of valves to be counted for each sample varies according to the purpose of the analysis and according to the need to produce statistically good results. The statistical precision of percentage counting depends on the frequency of the taxon in the sample count in relation to the size of the sample count (Battarbee, 1986). In a study from South Africa by Schoeman (1973), a series of experimental counts—200, 300, 400, 500 and 800 valves per sample— were counted and their relative abundance calculated. When only 200 valves were counted, compared to when 800 valves were counted, the percentage differences of the relative abundances of individual species were often as high as 6-7%. However, the results obtained from counting 400 as opposed to 800 valves differed by only 1-2%, which would suggest that counting 400 valves was satisfactory for the calculation of relative abundance of diatom species. Similarly, Battarbee (1986) demonstrated that there were marked differences in the percentages between counts of 100 and 200 valves, while there was little difference between counts of 400 and 500. For this reason, he recommended that a count of 300 to 600 may be used for purposes of routine analysis. This range is supported by Prygiel *et al* (2002), who, in an inter-comparison exercise, found that diatom index scores were not affected at counts of 300 and above. Hence, it is recommended that for diatom community analysis, 400 diatom valves should be counted in each sample.

Suggested rules for counting diatoms, according to CEN (2004) are summarized below:

- Counts of diatom valves on slides should be made using a compound light microscope equipped with incident light and capable of $1,000 \times$ magnification ($100 \times$ oil immersion objective in combination with a 10x eyepiece). Phase contrast optics or differential interference contrast optics (DIC) may provide better contrast.
- The eyepiece graticule or other measuring equipment must be calibrated against a stage micrometer prior to the analysis to allow for measurement of dimensions and taxonomic features.

- Either the field of view or the grid of a graticule is used as the area defining the limits of the count. All diatoms visible in the field of view (or within the grid of a graticule) are identified and counted before moving along either a horizontal or vertical traverse to the next field, or selecting a new field of view at random.

- In cases where a diatom lies only partially inside a defined counting area, include taxa that are only partially visible at the upper but not the lower margin (in the case of vertical traverses), or the left but not the right margin (in the case of horizontal traverses).

- It is important that each subsequent traverse does not overlap with the previous one and multiple count of a single specimen is avoided. The distance that the stage is moved on each occasion must also account for any diatoms only partially visible in the field of view.

- If sample analysis is unlikely to be completed in a single session, then it is useful to record the position of each traverse to avoid overlap of traverses/counts in the subsequent session.

- Each individual specimen encountered is counted as a single unit, with no differentiation between a valve and frustules (Prygiel *et al.*, 2002). Girdle bands (copulae) should not be enumerated as being representative of diatom taxa.

- Occasional filaments should be recorded as the corresponding number of diatom units. If a large number of diatom units are found in filaments, a new preparation technique using a more aggressive mix of oxidizing agents is required.

- In order to eliminate the risk of including separate fragments of broken valves or frustules, valves should be counted only if approximately three quarters are present, or broken valves may be excluded altogether. The presence of many small fragments of diatoms may indicate that dead diatoms are being washed in from upstream sites.

- If many valves are obscured, then new slides should be prepared using more diluted suspensions. A diatom may not be identifiable for a number of reasons, including the presentation of a girdle view and the presence of overlying material obscuring the view.

- Some taxa are identifiable from girdle (side) views, either because the girdle view is particularly characteristic (e.g., *Rhoicosphenia curvata*), or because the girdle view can be assigned with confidence to a particular taxon by 'matching' it with corresponding valve views of taxa found in the sample. However, in cases of doubt, the analyst should record the girdle views at the lowest level to which they can be assigned with confidence (e.g., 'unidentified *Gomphonema* sp.', 'unidentified pennate girdle view').

- As most diatom indices presume that all taxa in a sample are identified, it is recommended that not more than 5% of the total count should comprise unidentifiable individuals. If a diatom unit cannot be identified for any reason, photographs, digital images or detailed drawings should be made. Notes should also be taken of the shape and dimensions of the diatom unit, striae density and arrangement (at the center and poles), shape and size of the central area, number and position of punctae and arrangement of raphe endings.

Identification

The most valuable recent flora or identification guide for Europe is that of Krammer and Lange-Bertalot (1986-1991). This flora can be used for the identification of many of the species occurring in India and for the confirmation of species identifications by other authors. Other taxonomic guides that may be consulted include Schoeman (1973), Schoeman and Archibald (1976-1980), Archibald (1983), Sarode and Kamat (1984), Gasse (1986), Round *et al.* (1990), Hartley (1996), Gandhi (1998), Prygiel and Coste (2000), Lange-Bertalot (2001), Krammer (2002), and Taylor *et al.* (2007).

Diatom taxonomy has recently undergone many changes and is currently in a state of flux. This is mainly due to the splitting of large genera such as *Navicula* and *Nitzschia*. There is now consensus amongst diatom taxonomists that the diatom genus *Navicula* is restricted to the section Lineolatae (Lange-Bertalot, 2001). This has led to the creation of new genera by encapsulating species that used to belong to the genus *Navicula*. Examples of these new genera are *Luticola* (Mann, in Round *et al.*, 1990), *Fallacia* (Sickle and Mann, in Round *et al.*, 1990) and *Microcostatus* (Johansen and Sray, 1998).[2]

Possible Sources of Error in Diatom Community Analysis

When implementing monitoring programs based on assessments of diatom community composition, earlier studies in Europe would aid in identifying the likely sources of error. Several sources of error, at all stages of analysis, have been highlighted by Prygiel *et al.* (2002) in an inter-laboratory comparison exercise. For example, when the sampling protocol is not strictly followed, then variability due to sampling can be very high. Errors include sampling from exposed substrata—from areas subjected to water level change and from areas of low-velocity flow, as compared to other parts of the river—and sampling from stones covered by abundant filamentous algae. Laboratory and counting errors may include the use of high temperatures when drying slides (leading to clumping of diatom valves) and the settling out of large taxa during the preparation of consecutive slides from

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[2]   For revised nomenclature, works such as Lange-Bertalot (2001), Krammer (2002) and Kellogg and Kellogg (2002) or resources such as AlgaeBase (http://www.algaebase.org) can also be consulted.

a single sample. The main source of variability is, however, in the identification ability of individuals (Prygiel *et al.*, 2002). That is why biological quality controls focus mainly on counts and misidentification (Kelly, 1999). Diatoms are suitable for such controls, and proposals relating to quality control have been made by Kelly (1999). Prygiel *et al.* (2002) made recommendations which should be seen as the way forward for India in terms of quality control and the validation of diatom analysis data. Most of the variability due to sampling and slide preparation can be avoided by organizing comparisons between different studies. Such comparisons are very useful because, with field and laboratory approaches, they make operators aware of the consequences of not following protocols. They are also useful because they highlight some taxonomic problems. Diatoms are good subjects for photomicrography and therefore many diatomists make use of the diatom archives such as (http://keisou.hp.infoseek.co.jp/mokuji.html#m1, http://craticula.ncl.ac.uk/Eddi/, http://www.math.ualberta.ca/~bowman/diatom/) to check problematic identifications. This approach should be further encouraged by formalizing expert-practitioner exchanges, by creating iconographic databases, and by organizing regular workshops to allow updating of knowledge. The archiving of permanent slides also facilitates the creation of reference collections, which are particularly useful for the identification of difficult species.

Diatom-Based Indices

Diatom-based indices are used for a variety of practical purposes in ecological assessment throughout Europe, North America and Africa. In each case, it is important that surveys are designed in such a way that data collected can be translated into information useful for management purposes. Autecological indices use the relative abundance of species in assemblages and their ecological preferences, sensitivities or tolerances to infer environmental conditions in an ecosystem. The sensitivity and tolerance of diatoms to a number of environmental characteristics, such as eutrophication, organic pollution, heavy metals, salinity, pH and pesticides, are known to differ among species (Stevenson, 1996). These species-specific sensitivities and tolerances can be used to infer environmental conditions in a habitat (Lange-Bertalot, 1978). Many diatom autecological indices of water pollution in rivers have been developed and are in widespread use (Table 1). Diatom autecological indices can infer specific or general environmental conditions. Most are indicators of organic pollution of water (Palmer, 1969; Descy, 1979; Lange-Bertalot, 1979; Sládeček, 1986; and Watanabe *et al.*, 1986) and are reviewed by Coste *et al* (1991) and in Whitton and Kelly (1995). The indices can be based on the detailed characterization of assemblages with many species—Prygiel (1991) used 1,550 species—or they can be simplified to only identify genera or a few species for use by non-specialists (Rumeau and Coste, 1988 and Round, 1993).

Table 1: List of Diatom Autecological Indices for Water Pollution Monitoring

Index	Inference	Reference
DESCY – Descy's pollution metric	Pollution level	Descy (1979)
The taxonomic and autecological analysis	Classification	Lange-Bertalot (1979)
The index B method	pH	Renberg and Hellberg (1982)
Saprobity index (Sládeček's index or SLA)	Organic pollution	Sládeček (1986)
Watanabe index or WAI (Diatom community index)	Pollution level	Watanabe et al. (1986)
Specific pollution sensitivity index	Pollution	CEMAGREF (1987)
IPS – specific pollution sensitivity metric	Pollution level	Coste(1987)
LMI – Leclercq and Maquet's index		Leclercq and Maquet (1987)
SHE – Steinberg and Schiefele trophic metric		Steinberg and Schiefele (1988)
Lake acidification	Acidification	Eloranta (1990)
GDI – generic diatom index		Coste and Ayphasshorho (1991)
Commission for economical community index	Organic pollution	Descy and Coste (1991)
Autecological index	Stressor levels in habitats	Denys (1991)
Schiefele and Schreiner's index or SHE		Schiefele and Schreiner (1991)
pH Classes	pH	Hakansson (1993)
Trophic diatom indices	Trophic status	Hofmann (1994)
DAIpo index	Trophic status	Van Dam et al. (1994)
IDI – trophic diatom index	Organic pollution	Kelly and Whitton (1995)
%PT – % pollution tolerant taxa	Pollution level	Kelly and Whitton (1995)
EPI – D eutrophication/pollution index	Saprobic, trophic levels, halobic levels	Dell'Uomo (1996)
BDI – biological diatom index		Lenoir and Coste (1996)

Table 1 (Cont)

Index	Inference	Reference
Artoise-picardie diatom index		Prygiel et al (1996)
Rott's index or ROT		Rott (1999)
IDP – pampean diatom index		Gómez and Licursi (2001)
WAI – Watanabe index	Pollution metric	Lecointe et al (2003)
IDG – generic diatom metric	Pollution metric by genus	Lecointe et al (2003)
IDAP – indice diatomique artois picardie		Lecointe et al (2003)

Indicator lists for some of the important ecological variables, such as salinity, trophy, nitrogen metabolism types, pH, and oxygen requirements, have been published (Van Dam et al , 1994). Diatom indices have gained considerable popularity throughout the world during the last two decades as a tool to provide an integrated reflection of water quality, which can form the basis of management decisions. Once the sample has been counted in the correct manner the data can be entered into a computer database, OMNIDIA (Lecointe et al , 1993), from which several calculations can be made using a specific equation or using the sum of the water quality optima of all the species in the sample The design of OMNIDIA (http://clci.club fr/omnidia_english.htm) for computing diatom indices has further facilitated the use of diatom-based biomonitoring. This software has an inbuilt large number of dataset for each species, which comprise complete name, references, family, type, sensibility and indicative values for the calculation of the indices A variety of diatom indices based on comprehensive database in OMNIDIA have been adopted and validated by many countries across hemispheres. In most of the indices, diatom species used in the calculation/equation is assigned two values; the first value reflects the tolerance or affinity of the diatom to a certain water quality (good or bad), while the second value indicates how strong (or weak) the relationship is. These values are then, in addition, weighted by the abundance of the diatom in the sample, i.e , how many of the particular diatom in the sample occurs in relation to the total number counted. Most of the diatom indices are based on the formula of Zelinka and Marvan (1961):

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

where a_j = abundance (proportion) of species j in sample, v_j = indicator value and s_j = pollution sensitivity of species j. The performance of the indices depends on the values given to the constants s and v for each taxon, and the values of the index ranges from 1 to an upper limit equal to the highest value of s.

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Conclusion

Although the techniques and procedures recommended here are superficially very simple, it is essential that the persons collecting diatom samples for water quality monitoring are properly trained. They need to be able to recognize a diatom community (both by coloration and feel) and understand the factors that may influence its composition. They also need hands-on training in sample collection. This paper proposes a set of guidelines that can be adapted to most circumstances. The above represents a minimum set of conditions that need to be followed if reliable data for assessment of water quality are to be collected, and there may be situations where more stringent standards are required for particular purposes. There are also several points where we highlight a need for decisions on sampling to be taken in the light of local knowledge, and some preliminary sampling may be required in order to establish these conditions. However, the techniques described here should allow diatoms to be collected from most aquatic habitats on the Indian subcontinent. █

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