Diatoms

Diatoms are unicellular microalgae belonging to the class of Bacillariophyceae that represent an important part of biodiversity in rivers and lakes. They constitute an extremely diverse phylum, encompassing about 100,000 taxa (Mann & Droop, 1996) and several hundred new taxa are discovered every year. Diatoms are characterised by siliceous (SiO2 x nH2O) cell walls (Round *et al.* 1990), which are also called frustules. The frustule is essentially bipartite structures, with an older and a younger half (valve); each with a series of linking bands (girdle bands), often comparable to a petri dish or soapbox. In the two halves of the frustule, the hypotheca (the bottom portion in a soapbox) and the epitheca (the overlapping lid of the soapbox). The epitheca consists in turn of two parts, viz. a flat upper part with down-turned edges, called the epivalve (epivalva), and a ring or hoop like side wall, the epicingulum (upper girdle). Similarly, the hypotheca consists of a hypovalve (hypovalva) and a hypocingulum (lower girdle). The epicingulum and epivalve are separated by a suture, as are the hypocingulum and hypovalve (Figure 1). Moreover, the frustule shows rigid silicate structure with an amazing array of sizes, shapes and ornamentation. These features and symmetry of the frustules are most important for taxonomy. The size of diatom valves ranges between 2 micron to 200 mm. Due to their siliceous frustules, diatoms are preserve well in many sedimentary environments.

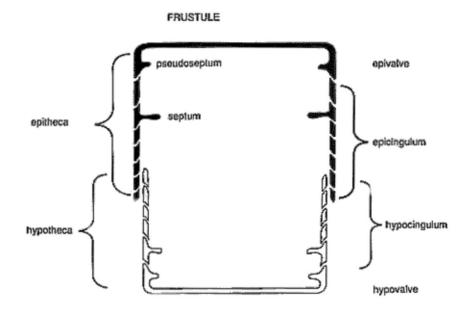


Fig. 1. Diagram shows the main morphological structure of the diatom frustule.

Diatoms ecology

Diatoms are the most diverse and important group in any aquatic ecosystem and are of immense ecological importance (Falkowski et al., 2004). They are distributed in almost all waters; they are abundant in the phytoplankton and phytobenthos of marine, brackish and fresh waters, at all latitudes (Round et al., 1990), as well as in moist soil, on wet surfaces, in hot springs or acidic environments; ice brine canals etc (Mann, 1999). Diatoms in the ocean tend to dominate in a number of oceanographic settings that offer both the high nutrient and turbulence conditions they require for growth (Ragueneau et al., 2001). In the ocean, as the diatoms dominate the productive zones, it is estimated that they contribute with up to 45% of the total oceanic primary production (Mann, 1999) and 20-25% of the worldwide net primary production (Werner, 1977). Besides of the large amount of oxygen that they produce, they are great primary producers, as they serve as food for many organisms (Tillmann, 2004). Diatoms can also be found in hypersaline environments. Several studies describe their distribution and salinity tolerance in this kind of habitat (e.g., Compère, 1994; Gell and Gasse, 1994). Generally, they are found forming microbial mats in the benthos. Their ecological diversity is reflected by their occurrence in almost all aquatic habitats, where they play an important role as primary producers and in geochemical cycling of various naturally occurring elements in particular C and Si (Round et al. 1990).

Diatoms have specific salinity, temperature, light, pH, and other environmental requirements, which affect their abundance and distribution (Zalat & Servant Vildary 2005). Several authors (e.g., Hustedt, 1957; Simonsen, 1962; Ehrlich, 1973 -1979) had classified the diatoms according to their salinity preferences into three groups: Polyhalobous (marine taxa), mesohalobous (brackish water taxa) and oligohalobous (freshwater taxa). According to their habitat, diatoms can be classified into six categories; it found as floating on the water surfaces (Planktonic), adhering to the floor of water bodies (Benthic), adhering to plants and macroalgae parts (Epiphytic), adhering to fauna such as mollusks and fishes (Epizoic), attached on rocks that submerged under water (Epilithic) and found between clay particles (Epipelic). Most diatoms live as single cells or as chains of cells, and are generally found drifting within water, however, some live colonially within different substrates (Zalat & Servant-Vildary, 2005).

Diatoms as indicators of environmental change

Environmental change due to climatic influence and anthropogenic impacts has affected many freshwater ecosystems (Steinberg & Wright 1994, Smol 2002, Welch & Jacoby 2004. Zalat 2015). The diatoms frustule is resistant to many ecological changes (e.g. pH, salinity and temperature and other environmental factors). They preserve well in many types of sediment due to the siliceous composition of their cell walls (Dixit et al., 1999). They can maintain its form during long periods to form fossils. Its geological record is back to Cretaceous age about 130 millions of years ago (Round et al., 1990; Zalat, 2013). In the Quaternary, diatoms are dominant in the most of sedimentary environments, including marine, lacustrine and lagoonal sediments. Due to their siliceous wall, abundance, species richness, wide geographical distribution, ease of collection and preservation, relatively well known taxonomy and their central role in aquatic ecosystems, diatoms have been commonly used as bioindicators for a large range of applications in the environmental and earth sciences (Stoermer & Smol 1999). They have been used in paleolimnology as indicators of environmental changes over time (e.g. Anderson, 1990; Fritz et al., 1991; Kashima, 1994; Chavez and Haberyan, 1996; Stoermer, 1998, Zalat & Servant Vildary 2005, 2007). The community composition reflects and responds to environmental conditions such as water depth, aquatic pH, nutrient availability, salinity and curent conditions, etc. (Stoermer and Smol, 1999; Zalat & Servant Vildary 2005, 2007). Many studies on lakes and ponds have applied paleolimnological techniques. The most important investigations have addressed the two most common environmental problems affecting aquatic ecosystems, eutrophication referring to the enrichment by inorganic nutrients such as phosphorus and nitrogen (Mason 1991), and acidification resulting from the deposition of acidifying pollutants such as oxides of sulphur and nitrogen (Steinberg & Wright 1994). Both can cause considerable change in biochemical cycles and biological assemblages including changes of the food web structure, decrease in diversity or the disappearance of entire organism groups (Tilman et al. 1986). Many diatom species have specific optima and tolerance ranges for nutrients as well as pH and have therefore been used successfully in studies of eutrophication of lakes (Fritz et al. 1993, Dixit et al. 1993; Hall and Smol, 2010). Their responses to anthropogenic disturbances have been observed for a long time (e.g. Butcher 1947). Diatoms reproduce and divide rapidly and quickly react to water quality changes (e.g. Round 1991). This prompted water managers to select the benthic diatoms as one of the tools for assessing a wide range of water quality (Stevenson & Pan 1999). Now, diatom indices are used routinely in different European countries to assess the biological quality of running waters (Prygiel et al. 1999).

Some studies have included large-scale monitoring projects for regional assessments of the lake environment and its water quality to identify problem areas where lakes have been most severely affected (Dixit & Smol 1994). Most investigations in standing waters have used diatom assemblages from sediment cores, while there are fewer investigations on periphytic diatoms from the littoral areas of the lakes. The response of littoral diatom assemblages to changes in water quality such as increased nutrient loading can be rapid (Hawes & Smith 1992), and this can be beneficial to detect localised or short to medium term changes for example pollution resulting from the release of sewage. A number of studies have shown that diatoms from littoral areas can indicate major environmental gradients. They reflected changes in pH, salinity as well as concentrations of nutrients, calcium and silica in the lakes (Lim et al. 2001, Douglas & Smol 1993, 1995).

The modem relationships between the diatom assemblages and the environmental variables are modeled statistically and the resulting function is used as a transfer function to transform fossil assemblage data into quantitative estimates of the past environmental variables (Hall and Smol, 1999; Zalat & Servant Vildary, 2005). Many diatom-based transfer functions derived from weighted-averaging regression (WA). The validity of using diatom-based WA inferences to reconstruct paleoenvironmental variables has been tested by comparison to actual historical records (e.g, Bennion et al., 1995; Lotter, 1998; Zalat & Servant Vildary, 2005, 2007).

Fossil diatoms in lake sediments can provide sensitive high-resolution records of salinity and lake level changes, which are mainly associated with fluctuations in climatic conditions such as precipitation and evaporation rates. Thus, diatoms have been used as proxy indicators to reconstruct the paleoenvironmental and climatic changes through the Holocene on every continent. Numerous studies have used fossil diatoms to track past changes in lake water salinity (e.g., García-Rodríguez et al. 2004; Bracco et al. 2005). Others have used changes in the relative abundance of planktonic versus littoral and benthic diatoms, as well as other diatom changes, to reconstruct lake-level changes and other climate related variables (e.g., Zalat 2000, 2002; Kuwae et al. 2004; Stager et al. 2005; Moos et al. 2005). Furthermore, recent diatom studies have used multivariate techniques and qualitative assess-ments to reconstruct past climate variables either directly or indirectly, such as Pienitz et al. (1995), Fritz et al. (1999), Korhola et al. (2000), Rosén et al. (2000), Taylor et al. (2001), Bigler and Hall (2002), Bigler et al. (2003), Bradbury et al. (2002), Bloom et al. (2003), Bradbury et al. (2004), Antoniades et al. (2005) and Karst-Riddoch et al. (2005). Although there are many paleoclimatic studies from Africa and North America, and very limited studies were done on the inland lake sediments in Egypt.

Nanotechnology research

The deposition of silica by diatoms may also prove to be of utility to nanotechnology. Diatom cells repeatedly and reliably manufacture valves of various shapes and sizes, potentially allowing diatoms to manufacture micro- or nano-scale structures which may be of use in a range of devices, including: optical systems; semiconductor nanolithography; and even vehicles for drug delivery. With an appropriate artificial selection procedure, diatoms that produce valves of particular shapes and sizes might be evolved for cultivation in chemostat cultures to mass-produce nanoscale components. It has also been proposed that diatoms could be used as a component of solar cells by substituting photosensitive titanium dioxide for the silicon dioxide that diatoms normally use to create their cell walls. Diatom biofuel producing solar panels have also been proposed.

Materials and Methods of study

The present work is based on 10 samples collected from Kafr El Zayat – Nile branch and representing different habitats and environments. The sediments were collected in plastic bags. All samples were stored in ice box, transferred to laboratory and stored at the refrigerator at 4°C until use.

Diatoms were extracted from 2 g of dry sediments by the two following treatments according to (Zalat, 2002):

Treatment with 30% HCL: (to remove the carbonate fraction) •

Approximately 20 ml of HCL was added to the dry sample and then boiled on a hotplate for 30 min. until all carbonate material removed. Distilled water added to the samples and left 24 hours to settle down diatoms. The supernatant was carefully siphoned using a siphon droplet system (5 mm in diameter) till final volume became 100 ml.

Treatment with 35% H₂O₂: (to remove organic matter) •

About 20 ml of 35% hydrogen peroxide was added to the final previous suspension and boiled for about 20 min. to eliminate the organic matter. The sample rinsing in distilled water several time to remove all acids and the suspension become neutral. Coarse particles were removed by further decantation (settling for 5 seconds). The sample was concentrated and stored in final volume of 50 ml vial.

Preparation of permanent slides:

A single drop of ammonium chloride (10% solution) or ethanol (70%) was added for 10 ml of the cleaned diatom suspension to neutralize electrostatic charges on the suspended particles and reduce aggregation. Approximately one drop or 0.1 ml of the final suspension was distributed over a clean, dry coverslip (22 x 50 mm) using a pipette and dried at low temperature or the coverslip left to dry at room temperature in a dust free environment. Diatom-coated cover-slips were placed on a hot plate at ~100°C for 5 minutes to remove the excess moisture and the residual ammonium chloride or ethanol. One or two drops of mounted media such as Canada balsam or DPX with refractive index[®] (R.I = 1.67). were

placed onto a clean slide then the cover slip lowered onto the glass slide, inverted, and then heated at 80-90°C. Sample material and the diatom slides are now part of the permanent diatom collection of the Geological Department, Faculty of Science, Tanta University, Egypt.

Pictures explained the places from which the studied samples were

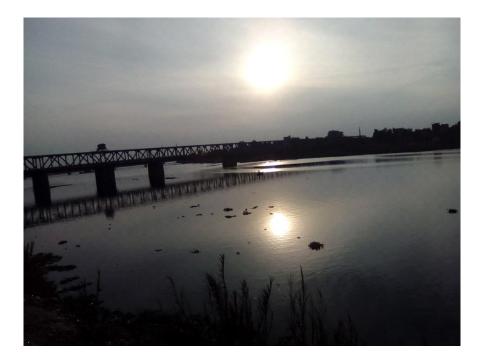
obtained.













Diatom Taxonomy and classification

Taxonomy of diatoms is almost exclusively based on the shape and structure of the siliceous frustules and species are described essentially by

morphological characters. Two major groups of diatoms are generally recognized: The centric diatoms, which are exhibit radial symmetry and are circular, triangular or irregular in shape. They are most abundant in the ocean and sea, with very few genera are found in fresh and brackish waters. The pennate diatoms, which are elongated, bilaterally symmetrical and are most often, found in shallow areas of seas and freshwaters. In the last few decades, the taxonomy of diatoms has undergone a revolution, with many new taxa (particularly at the genus level) being proposed on ultrastructural characteristics. The classification proposed here after Round et al., 1990.

System: Vitae - living organisms

Kingdom: Chromista Cavalier-Smith, 1981

Subkingdom: Chromobiota Cavalier-Smith, 1991

Infrakingdom: Heterokonta (Cavalier-Smith, 1986) Cavalier-Smith, 1995

Phylum: <u>Heterokontophyta</u> (Phylum Ochrophyta (Cavalier-Smith, 1986) Cavalier-Smith, 1995)

Subphylum: *Diatomeae* (Dumortier, 1821) Cavalier-Smith, 1995 -

Class: Coscinodiscophyceae Round & Crawford, 1990

Class: <u>Bolidophyceae</u> L. Guillou & Chrétinnot-Dinet in Guillou et al., 1999

Class: Fragilariophyceae Round, 1990

Class: Bacillariophyceae Haeckel, 1878

Class: Coscinodiscophyceae Round & Crawford in Round et al. 1990

Order: Aulacoseirales Crawford in Round et al. 1990

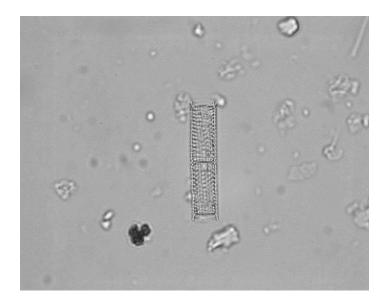
Family: Aulacoseiraceae Crawford in Round et al. 1990

Genus: Aulacoseira Thwaites 1848

Synonym: Gallionella Bory 1823; Discosira Rabenhorst 1853; Melosira C. Agardh 1824

Diagnosis: Valves cylindrical, rarely elliptical or discshaped, bound in chains of frustule; margin mostly denticulate, and two halves of different frustules attached together by spines; punctate, the puncta coarse or fine, arranged in longitudinal, or spiral rows. The surface microstructures comprising spines, mantle areolae and sulcus are important diagnostic characters in the taxonomy of this genus. These features are distinctly revealed by the SEM and their value in the taxonomy of the genus confirmed.

Type species: Aulacoseira crenulata (Ehrenberg) Thwaites 1848

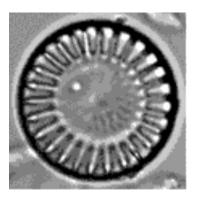


Genus: Cyclotella Kützing 1833

Diagnosis: Frustules short, drum-shaped, cylindrical in girdle view, and the valves appearing to be undulate. Valves circular in valve view; valve face is either a tangential or concentric undulate; the undulation more evident in the middle area. Central area often ornamented with granules or punctae and sometimes smooth. Disci with marginal radial ribs, short or long, thick or thin. Fultoportulae present in a ring near the valve margin and also found over the centre of the valve. Cells usually solitary, but sometimes united in short chains.

Type speceis: Cyclotella operculata (Agardh) Brébisson 1838

This genus contains over 100 species (Van Landingham 1969). Several species of this genus appear almost similar, if not identical in the light microscope, and their morphological characteristics are not always easy to discern. Nowadays, with the help of scanning electron microscope, the finest details of the morphological features of the taxa can be distinguished, e.g. shape and structure of the central area, the morphology of the marginal striations, position and number of the valve face and mantle fultoportulae and their satellite pores, and features of the rimoportulae. Such morphological characters alone are generally insufficient to differentiate between taxa; all of these features are needed in combination. However, there is still very little known about the variability of many taxa during the life cycle. There are also different opinions about the importance of such morphological characters for differentiating taxa of centric diatoms (Håkansson 1996).



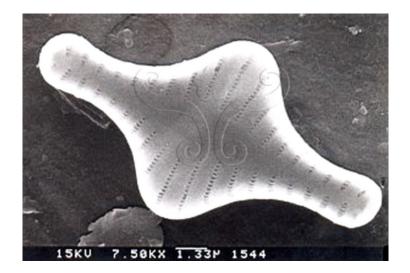
Class: Fragilariophyceae Round in Round *et al.* 1990 Subclass: Fragilariophycidae Round in Round *et al.* 1990 Order: Fragilariales Silva 1962 Family: Fragilariaceae Greville 1833

Genus: Fragilaria Lyngbye 1819

Diagnosis: Valve linear, lanceolate to elliptic, Frustules forming filaments; septa absent; valve symmetrical to the transapical and apical axes. In a few species the valves are tripolar rather than bipolar, without marked transapical costae; pseudoraphe is distinct, may be narrow or form a broad lanceolate space; the central area is variable in structure, and may be absent; fine transapical striae are arranged on both sides of the valve. Valve face has complex spines, which are located on the costae between the rows of areolae. These spines may be very simple or expanded in a plate-like manner and then interlocking to form a firm join

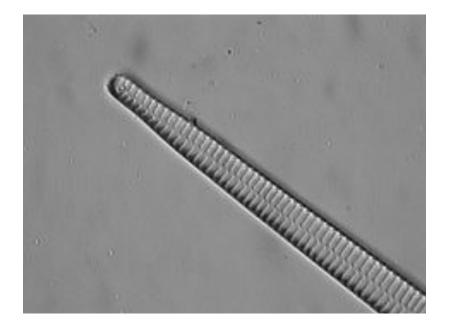
between adjacent cells. Cells with three isopolar axes, mostly straight.

Type species: Fragilaria pectinalis (Müller) Lyngbye 1819



Genus: Synedra Ehrenberg 1830

Synedra is a genus which falls into a section of the araphid diatoms characterized by the lack of any form of linking spine along the edge of the valve face, such as occur in Fragilaria. Hustedt (1959) divided the genus into three subgenera, Belonastrum, Eusynedra and Ardissonia. Cleve-Euler (1953) rejected Belonastrum but maintained another subgenus Toxarium for the marine forms with irregular punctation. The species in this genus are characterized by a valve face-mantle junction ties totally devoid of spines, hence there is no colony formation by juxtaposition of the valve faces in this genus (Round 1982). Lange-Bertalot (1980) unites the subgenus Synedra of Synedra with Fragilaria under the latter name and supports his procedure on the presence of the interlocking spines, and the similar morphological forms in both genera. Studies by Poulin et al. (1986) seem to confirm the proposal by Lange-Bertalot (1980) and suggest the alternate system of considering Fragilaria with two subgenera Fragilaria and Synedra, which in turn facilitates these u of the "traditional" binomial names.



species:gomphonema

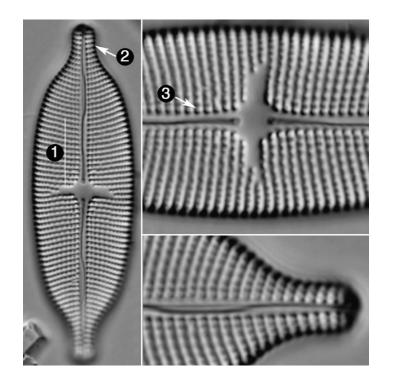
Valves are elliptical-clavate with the headpole produced slightly and broadly rostrate and footpole rounded. Axial area is straight, expanded slightly due to a shortened stria on either side of the central area. A single stigma is present in the central area. The internal opening of the stigma opening is offset from the external opening. The raphe is lateral and weakly undulate. External proximal raphe ends are dilated slightly. Striae are punctate, parallel to radiate, number 9-11 in 10 μ m and are strongly radiate at the footpole. An apical pore field is present at the footpole. Septa and pseudosepta are present at the poles.



Genus::Mastogloia pseudotuscula

- Striae 12-16 in 10 μm $\,$ \bullet
 - Apices protracted •
- Areolae evenly arranged •

Valves are lanceolate with protracted apices. The axial area is relatively straight, expanded in the center to form an unequal, irregular rectangular central area. Areolae are small, evenly arranged and nearly square, to dash-like.

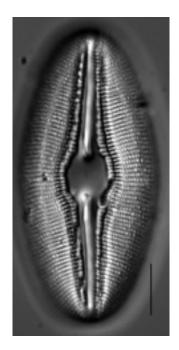


species: diploneis

Valves are elliptic to oval with broad, round apices. The longitudinal canals are narrow, the width of one to three areolae, and follow the margin of the axial and central areas. The axial area is occupied almost completely by a broad raphe. The central area is large and orbicular, 4.9-8.5 µm wide. Striae are uniseriate and radiate throughout. Areolae are large, blocky and number 16-20 in 10 µm.

- Valves elliptic-oval •
- Longitudinal canals narrow
 - Striae uniseriate •
 - Central area large •

Valves are elliptic to oval and up to 33 μ m wide. The longitudinal canals are narrow. Striae are uniseriate and radiate throughout. The central area is large and orbicular, up to 8.5 μ m wide.



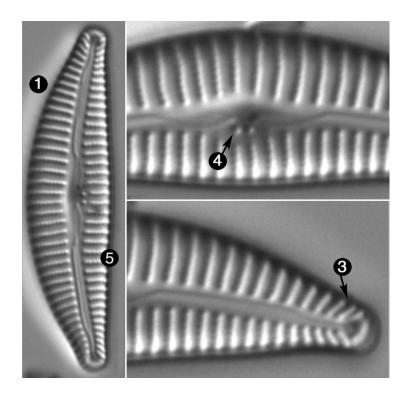
Genus: Cymbella affiniformis.

Valves are strongly dorsiventral with narrow rostrate to subrostrate apices. The dorsal margin is strongly arched and the ventral margin is weakly convex. The axial area is narrow and linear. The central area is very small. Two, rarely 1 or 3, stigmata are present at the proximal ends of the middle ventral striae. The raphe is lateral, becoming filiform near the indistinct terminal fissures. Proximal raphe ends are reverse-lateral and deflected to the ventral side. Striae are slightly radiate, becoming strongly radiate near the apices. Areolae number 26-30 in 10 μm.

- Valves strongly dorsiventral
 - Valves 7.2-9.2 µm wide •
 - Apices narrow subrostrate
 - Stigmata two

Ventral margin weakly convex •

Valves are strongly dorsiventral and 7.2 to 9.2 μ m wide. Apices are narrow and rostrate to subrostrate. Two isolated stigmata occur at the proximal ends of the median ventral striae. The ventral margin is weakly convex.



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