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Diversity of freshwater diatoms from few silica rich habitats of Assam, India

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ABSTRACT:

Diatoms are a ubiquitous class of phytoplankton of extreme importance for the biogeochemical cycling of minerals such as silica. Few places of Nagaon district of Assam, India viz., *Jiajuri*, *Borhola*, *Thanajuri* and *Chapanala* have been recognized as the highest silica zones by Geological Survey of India. No any research has been conducted to explore the diatom diversity at this important silica rich habitat. In the present investigation, the morphology and diversity of freshwater diatom species were investigated during May 2012 to April 2013. The samples were subjected to acid wash treatment followed by microscopic observations. Altogether 103 species of diatoms belonging to 20 genera were recorded. Occurrence of diatom varied in all the four different study sites. The dominant genera includes: *Stauroneis, Kobayasiella, Eunotia, Pinnularia, Nitzschia, Gomphonema, Frustulia, Surirella, Achnanthes, Rhopalodia, Navicula, Synendra, Encyonema, Achnanthidium, Cymbella, Hippodonta, Tabularia, Actinella, Encyonopsis and Luticola.* Notably, all the diatom species belonged to pennate type.

Keywords:

Freshwater diatoms, silica rich soil, diatom diversity, Geological Survey of India.

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INTRODUCTION

Diatoms belonging to the class Bacillariophyceae are the major group of single-celled photosynthetic eukaryotic algae which can be found in almost all aqueous and humid environments. Diatoms are an important component of phytoplankton in freshwaters. There are over 250 genera of diatoms with more than 100,000 species (Gurung *et al.,* 2012, Van Den Hoek *et al.,* 1997), which includes both marine and the freshwater environments. These microscopic autotrophic microalgae possess highly ornamented cell wall composed of glass silica (SiO2) called frustules which provide a variety of shapes from nano to micro-scale structures. Diatoms can occur in large amounts, either solitary or in colony and is cosmopolitan in distribution. A major constituent of the plankton family, diatoms are free floating, planktonic or attached to a substrate and benthic forms (Werner, 1977). Diatoms are important from the point of the biogeochemical cycling of silica. Diatoms play a very significant ecological role by fixing about 25% carbon globally. The diatoms of North East region of India are still largely unexplored and unexploited. Friable quartzite's belonging to the Shillong groups of rocks occur sporadically along eastern most part of the Nagaon district. *Borhola, Chapanala, Jiajuri* and *Thanajuri* are some of the important places where friable quartzites are found abundantly. About 75% of the glass sand may be recovered from this friable quartzite by using different methods of beneficiation (Goswami, 2006).

The Geological Survey of India (GSI) has found significant reserves of silica deposits in the *Jiajuri* region between the district of Nagaon and Karbi Anglong in Assam (Borpuzari, 2012). The area is located about 30kms South-East from Nagaon and is adjacent to Jiajuri Tea Estate. The deposit is bounded by latitude 26° 18′ 0″ to 26°19′ 0″ N and longitude 92°52′ 55″ to 92°54′ 15″ E. *Jiajuri* hill covers an area of 2.9 km^2 and the possible friable quartzite is about 7.4 million tones. *Chapanala* (26°20′10″ N latitude and 92°51′30″ E longitude) deposits occur friable quartzite covering an area of 0.373 km^2 and possible reserve is 3.5 million tones. *Thanajuri* hill (26°12′ 35″ to 26°13′10″ N latitude 92°48′40″ to 92°50′35″ E longitude and) is situated in the northern part of Karbi-Anglong plateau and southern part of Nagaon district. The possible reserves of glass sand is about 1.788 million tones. Friable quartzite occurs in *Borhola* (26°26′ 15″ N latitude and 92°56′45″ E longitude) covering an area of 0.595 km^2 and the possible reserve of glass sand is about 1.25 million tones. Till date, there is no any extensive work on the detailed investigation of diatom diversity in these silica rich regions of Assam. Set in this backdrop, the present investigation is assessed for the exploration of diatom, having the genetic ability to deposit natural silica over their cell surface in characteristics nanoporous forms.

MATERIALS AND METHODS

Sample collection and growth conditions

Samples were collected from aquatic and semi-aquatic habitats of the four study sites- *Jiajuri, Borhola*, *Thanajuri* and *Chapanala* from May 2012 to April 2013 (Fig.1). The freshly collected samples were immediately transferred to Diatom Medium (DM) proposed by Beakes *et al.,* (1988) which was standardized with slight modifications and the composition of stock (per 200ml) includes- $Ca(NO_3)_2$. $4H_2O$ – 4g, KH_2PO_4– 2.48 g, $MgSO_4.7H_2O$ - 5 g, $NaHCO_3$- 3.18 g, EDTAFeNa- 0.45g, $EDTANa_2$ – 0.45g, H_3BO_3 – 0.496g, $MnCl_2$. $4H_2O$ –0.278g, (NH_4) $6Mo_7O_{24}.4H_2O$ – 0.20g, Cyanocobalamine - 0.008g, Thiamine HCl – 0.008g, Biotin – 0.008g and $Na_2SiO_3.9H_2O$ – 22.8g.

One ml of each stock solution was added to make the final volume of 1L with distilled water, and adjusted to pH 6.8. For solid medium, 1.5% agar was added. The cultures were allowed to grow at 3K light at 18-20°C for 20-22 days. Repeated sub-cultures were done on the solid medium to obtain pure cultures of diatom species.

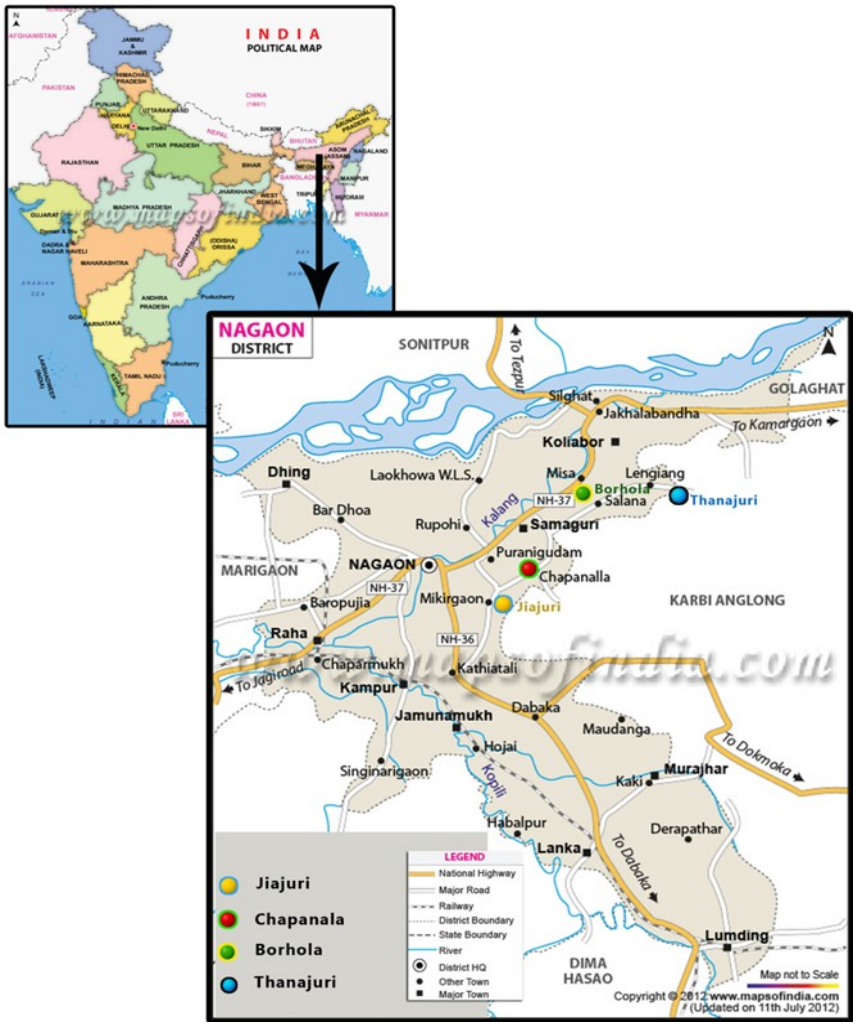

Figure 1: Map showing the four study areas (source: www.mapsofindia.com).

Cleaning diatom frustules by acid wash method for microscopic analysis

In order to analyze the diatom frustules for microscopic studies, a cleaning procedure was needed that removed the external organic matrix covering the frustules. Plankton samples were subjected to acid wash method according to the protocol of Hasle and Fryxell (1970) before light microscopic observations. About 20ml of liquid cultures were transferred into a beaker and treated with equal quantity of concentrated H_2SO_4 and agitated gently. Freshly prepared $KMnO_4$ was added to the sample until the sample had a purple tint. Then freshly prepared oxalic acid $(COOH)_2$ was added to obtain clear solution. The sample was centrifuged at 2500 rpm for 15 min and then rinsed with distilled water until the cell suspension become less acidic. To confirm the complete removal of organic matters, a drop of cleaned samples was observed under the microscope.

For light microscopy (LM) observation, the slides were prepared by evaporating drops of the cleaned diatoms suspended in distilled water onto cover-slips and the mounting was done by using Naphrax (a specific diatom mountant with refractive index 1.74). The slides were examined carefully under 1000x magnification and the diatom images were documented in Nikon ECLIPSE E200 with photo micrographic attachment.

Identification of diatoms

The diatoms obtained through laboratory pure cultures were identified by consulting various literatures and monographs (Gandhi, 1955; Husted, 1959; Hendey,

1964; Patrick and Reimer 1966; Prescott, 1975; Desikachary, 1989; Round *et al.,* 1990; Nautiyal *et al.,* 1996; Anand, 1998; Gurung *et al.,* 2013).

RESULTS AND DISCUSSION

During the present investigation, a total of 103 species of freshwater diatoms belonging to 20 genera of class Bacillariophyceae were reported from the silica rich soils of Nagaon district of Assam i.e. *Jiajuri, Borhola, Thanajuri* and *Chapanala*. The prominent genera in terms of its abundance and frequency were *Nitzschia* (25), *Gomphonema* (17), *Navicula* (15), *Pinnularia* (14), *Eunotia* (5), *Stauroneis* (4), *Cymbella* (4), *Frustulia* (3), *Synendra* (3), *Achnanthes* (2), *Achnanthidium* (2) and single species of the following diatoms: *Actinella, Luticola, Encyonema, Hippodonta, Surirella, Tabularia, Encyonopsis, Kobayasiella* and *Rhopalodia.* Pure cultures of diatoms obtained in this study were identified upto their genus level (Fig. 3-9). Morphological descriptions of the diatom isolates obtained in pure culture were enumerated.

Out of 103 diatoms species obtained in pure cultures, 25 diatoms were found to be of different species of *Nitzschia* representing 24.3% of the total diatom flora. Further, there were 17 different species of *Gomphonema*, 15 different species of *Navicula*, 14 different species of *Pinnularia* and 5 different species of *Eunotia* representing 16.5%, 14.6%, 13.6% and 4.9% respectively. There were four different species of *Stauroneis*, *Cymbella* (3.9% each), followed by *Frustulia* and *Synendra* (2.9% each) and *Achnanthes* and *Achnanthidium* (1.9% each). The remaining diatoms viz. *Surirella, Tabularia, Encyonema, Actinella, Encyonopsis, Rhopalodia, Luticola, Hippodonta* and *Kobayasiella* were represented by only one species showing 8.7% out of the total diatoms identified in pure cultures (Fig. 2).

Taxonomic account:

Taxonomic description of the 20 pennate freshwater diatom genera obtained in the four silica rich

Diversity of diatom flora

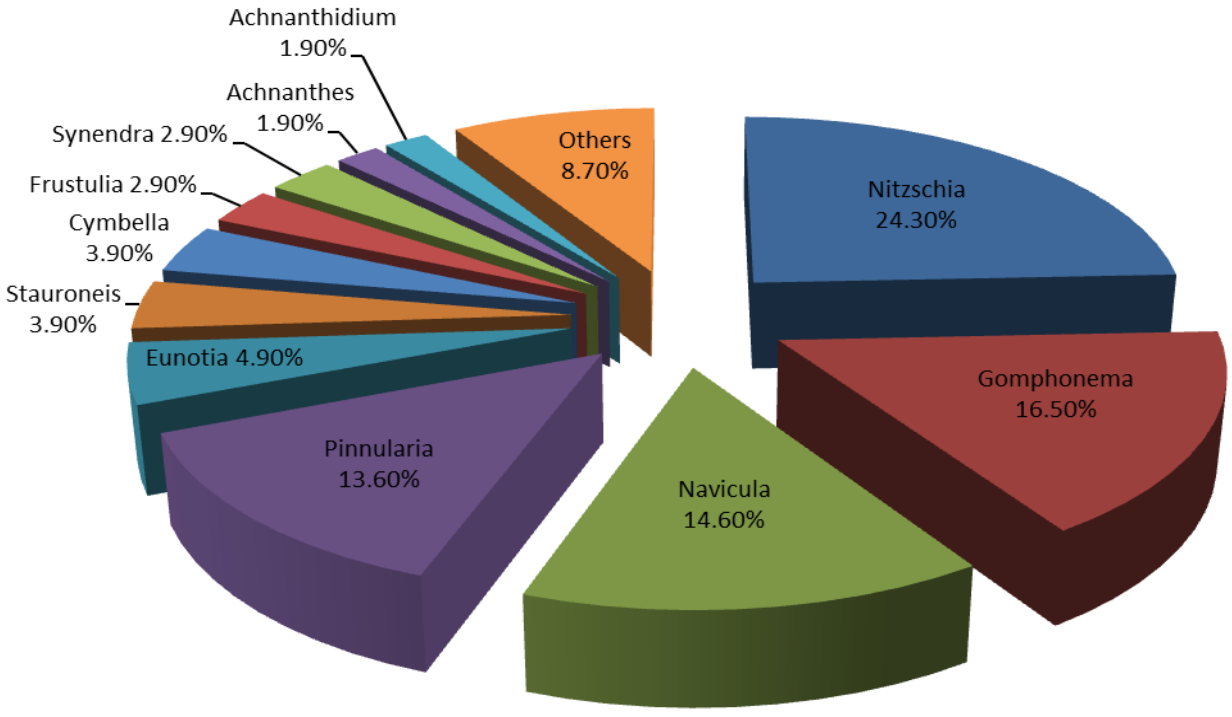

Figure 2: Representation of diatom flora diversity.

sites during the study period are described below:

Class: Bacillariophyceae

Order: Bacillariales

Family: Naviculaceae

Genus: ***Navicula*** Bory 1822, Cleve 1894

Navicula sp. (Fig. 3 A-O)

Valves 36 µm long, 14 µm broad, broadly elliptical with convex margins; ends slightly produced, slightly capitate rounded; raphe thin, straight; central nodules distinct; axial area narrow, linear; central area somewhat obliquely rectangular; striae 23 in 10 µm, very fine.

Class: Bacillariophyceae

Order: Naviculales

Family: Pinnulariaceae

Genus: ***Pinnularia*** Ehrenberg 1843

Pinnularia sp. (Fig. 4 A-N)

Valves 53 µm long, 11 µm broad, linear, more or less parallel margins with slightly tapering, broadly rounded ends; raphe thick, straight, placed on one side with distinct, unilaterally curved central nodules and curved terminal fissures; axial area distinct, linear; central area large reaching the sides; striae 7 in 10 µm, coarse, 2-4 middle striae short and thick, radiate in the middle, convergent towards apices.

Class: Bacillariophyceae

Order: Cymbellales

Family: Gomphonemataceae

Genus: ***Gomphonema*** C.A. Agardh 1824

Gomphonema sp. (Fig. 5 A-L, 6 M-Q)

Valves 45 µm long and 8 µm broad, clavate with capitate head pole and slightly capitate foot pole; axial area linear, narrow, and widening into a small circular central area with an isolated pore on the primary side of the central nodule; raphe straight with distinct central nodules; striae 10-11 in 10 µm, punctate and slightly radiate, wider at the centre of the valve.

Class: Bacillariophyceae

Order: Naviculales

Family: Amphipleuraceae

Genus: ***Frustulia*** Lange-Bertalot

Frustulia sp. (Fig. 6 A-C)

Valves 71-160 µm long and 15.3-30.2 µm broad, rhombic-lanceolate, narrowing sharply to the rounded apices. Axial and central areas narrow but distinct. Transverse striae perpendicular to the raphe at

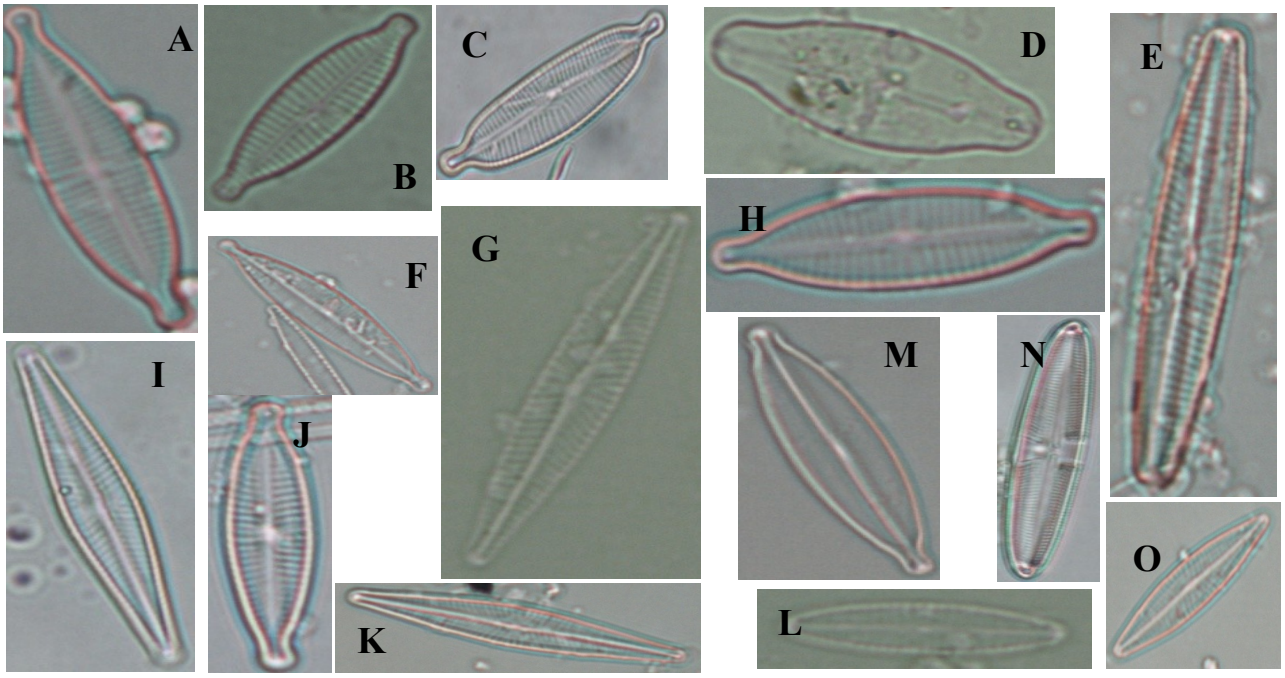

Figure 3(A-O): ***Navicula.***

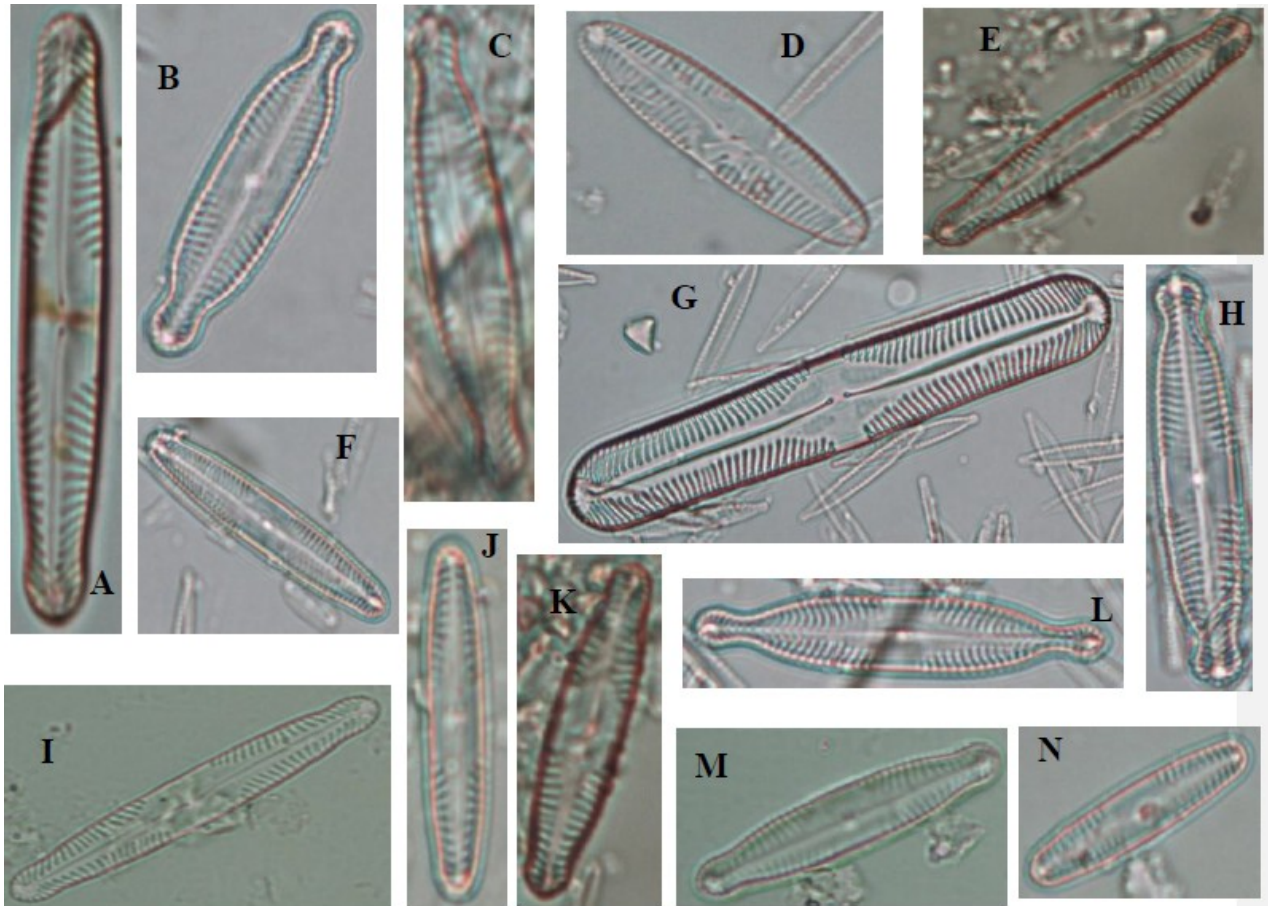

Figure 4: (A-N) *Pinnularia*

the center of the valve, sometimes becoming slightly convergent towards the ends of the valve, but radiate at the apices, striae 20-30 in 10 µm.

Class: Bacillariophyceae

Order: Cymbellales

Family: Cymbellaceae

Genus: ***Encyonema*** (Berkeley) Kutzing

Encyonema sp. (Fig. 6 D)

Valves 37-91 µm long and 15-30 µm broad, robust and broadly dorsiventral and symmetrical to the transapical axis. Dorsal margin normally arched, ventral margin biarcuate to convex. Valve apices bluntly rounded. Raphe straight with central endings deflected dorsally and apical ends deflected ventrally, striae coarse and 8-21 in 10 µm.

Class: Bacillariophyceae

Order: Naviculales

Family: Diadesmidaceae

Genus: ***Luticola*** (Ehrenberg) D. G. Mann, 1990

Luticola sp. (Fig. 6 E)

Valves 12-24 µm long and 7-9 µm broad, linear to linear-elliptical. Transapical striae radiate throughout, composed of two to four rounded areolae. Largest areolae near the valve margins. One isolated, circular stigma present, striae 18-20 in 10 µm.

Class: Bacillariophyceae

Order: Cymbellales

Family: Cymbellaceae

Genus: ***Encyonopsis*** (Grunow) Krammer, 1997

Encyonopsis sp. (Fig. 6 F)

Valves 21-25 µm long and 5.1-6.3 µm broad, cymbelloid with dorsal margin strongly curved and straight ventral margin. Axial area narrow, straight and without a central area. Small central nodule. A stigmoid

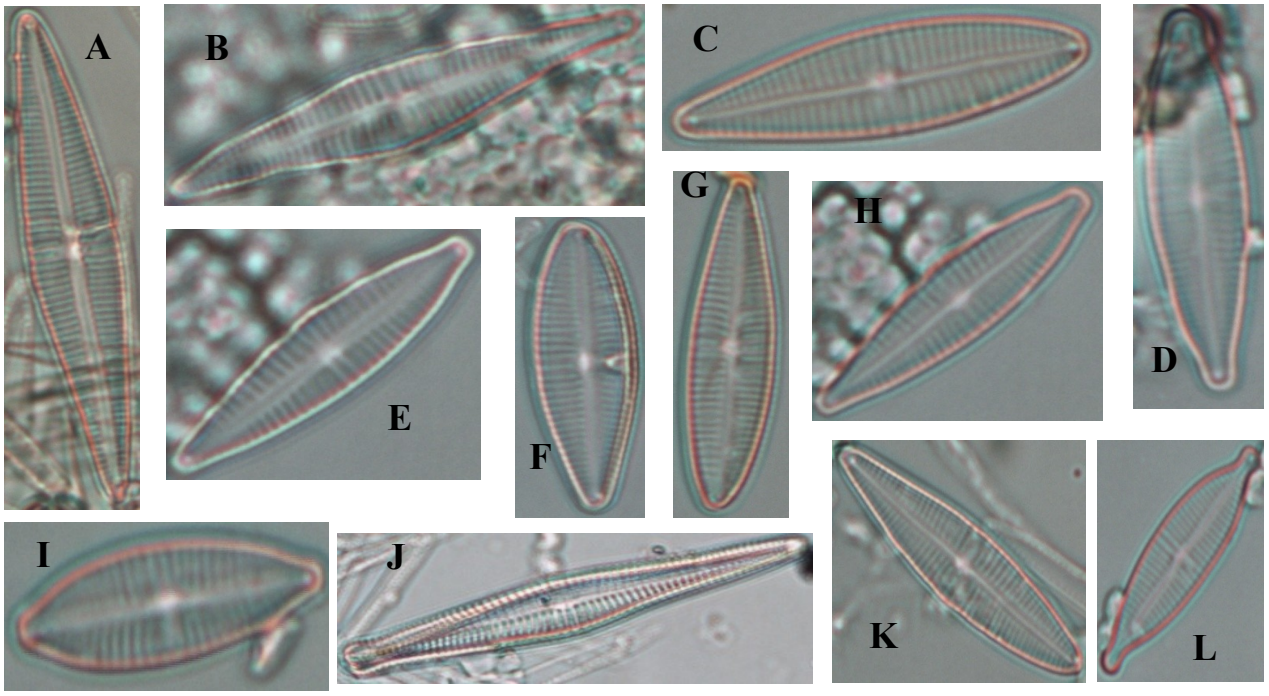

Figure 5 (A-L):*Gomphonema.*

Presented near the dorsal central striae, striae 14.2-16 in 10 µm.

Class: Bacillariophyceae

Order: Rhopalodiales

Family: Rhopalodiaceae

Genus: ***Rhopalodia*** Otto Muller, 1895: 57

Rhopalodia sp. (Fig. 7 A)

Valves 21-30 µm long and 6-9 µm broad, isopolar and dorsiventral, lanceolate-elliptical in shape, acute apices. The dorsal margin curved and straight at the ventral margin. Striae composed of a single row of puncta composes. Fibulae radiate, striae 14-20 in 10 µm.

Class: Bacillariophyceae

Order: Naviculales

Family: Naviculaceae

Genus: ***Kobayasiella*** Lange-Bertalot, 1999

Kobayasiella sp. (Fig. 7 B)

Valves 22-26 µm long and 5-7 µm broad, linear-lanceolate with convex sides and short, capitate apices. The axial area is narrow and nearly linear. The central area is small and elliptical and bordered by alternately long and short striae, striae 35-40 in 10 µm.

Class: Bacillariophyceae

Order: Eunotiales

Family: Eunotiaceae

Genus: ***Actinella*** Lewis, 1864

Actinella sp. (Fig. 7 C)

Valves 76-140 µm long and 5.7-8 µm broad, arcuate, asymmetrical to both the apical and transapical axes. External distal raphe ends extending slightly to the valve face on both ends. Striae parallel, striae 13-19 in 10 µm.

Class: Bacillariophyceae

Order: Achnanthales

Family: Achnanthaceae

Genus: ***Achnanthidium*** Kutzing, 1844

Achnanthidium sp. (Fig. 7 D and E)

Valves 6.2-14 µm long and 2-3.7 µm broad, linear-elliptic, slightly or more elongated near the end, and with bluntly rounded poles. Striae slightly radiate and often a shortened striae near the small central area, axial area narrow, striae 19-21 in 10 µm.

Class: Bacillariophyceae

Order: Bacillariales

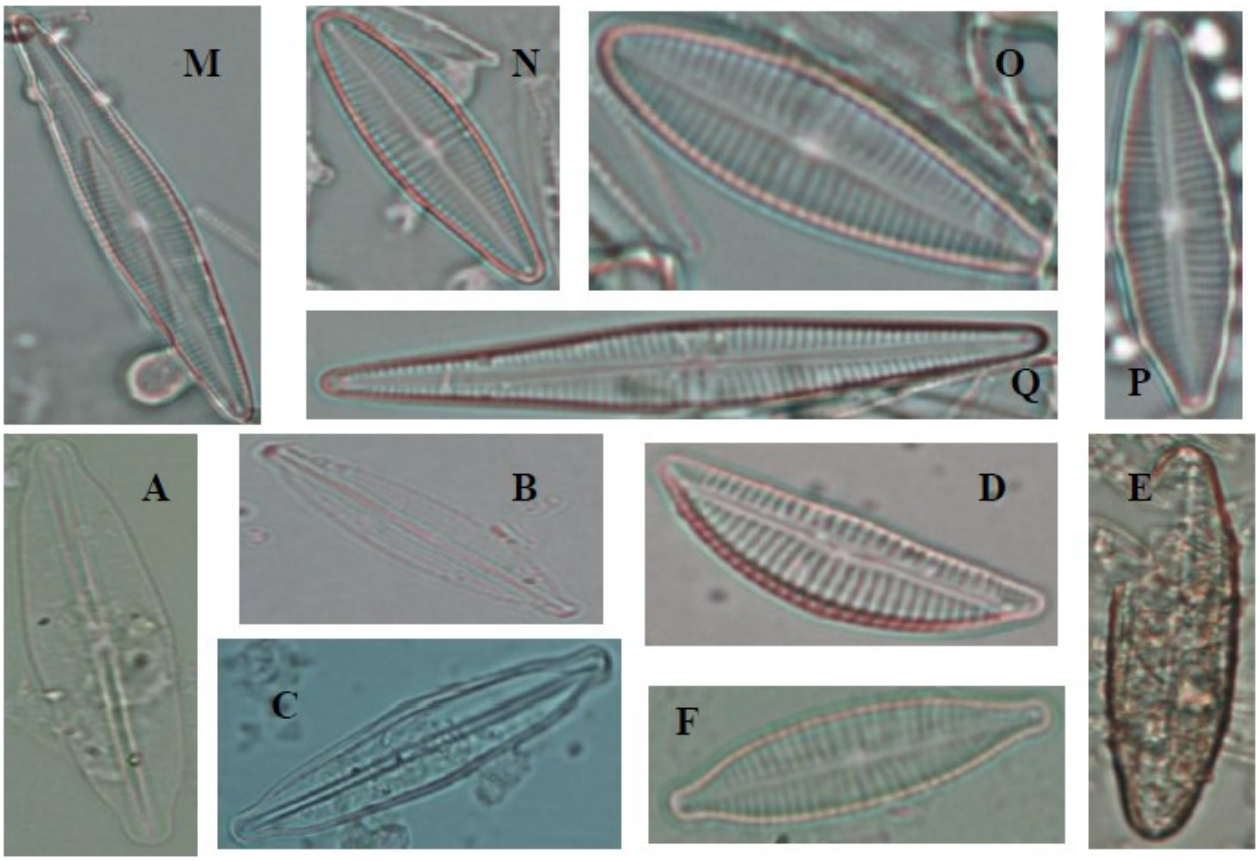

Figure 6: (A-C) Frustulia, D– Encyonema, E-Luticola, F-Encyonopsis, (M-Q) Gomphonema.

Family: Eunotiaceae

Genus: ***Eunotia*** Ehrenberg 1837

Eunotia sp. (Fig.7 F-J)

Valves 68µm long, 12 µm broad, slightly arched, dorsal margin convex with two wavy ridges at the middle, gradually narrowing towards the ends, ventral margin concave; ends slightly constricted on the dorsal side, slightly produced, rounded; raphe thin; polar nodules distinct, on the ventral side near the apices; striae 13 in 10µm, coarse, lineate, parallel, somewhat radiate and closely placed near apices.

Class: Bacillariophyceae

Order: Fragilariales

Family: Fragilariaceae

Genus: ***Synendra*** Ehrenberg 1832: 87

Synendra sp. (Fig. 7 K-M)

Valves 44 µm long and 3.2- 3.8 µm broad, linear with narrow and capitate ends. The central area reaches the margins. Pseudo raphe linear and broad. Striae strong and distantly placed, striae 13 in 10 µm.

Class: Bacillariophyceae

Order: Bacillariales

Family: Bacillariaceae

Genus: ***Nitzschia*** Hassall, 1845: 435

Nitzschia sp. (Fig. 8 A-Y)

Valves 27-30 µm long and 5.2-6.7 µm broad, linear with concave sides and wedge shaped, constricted produced ends, striae very fine, almost indistinct, striae 31-35 in 10 µm.

Class: Bacillariophyceae

Order: Naviculales

Family: Naviculaceae

Genus: ***Hippodonta*** (Ehrenberg)

Hippodonta sp. (Fig. 9 A)

Valves 20.2-29 µm long and 5.5-8 µm broad, elliptic-lanceolate, ends subcapitate to capitate. Raphe straight, filiform, central pores fairly close. Striae

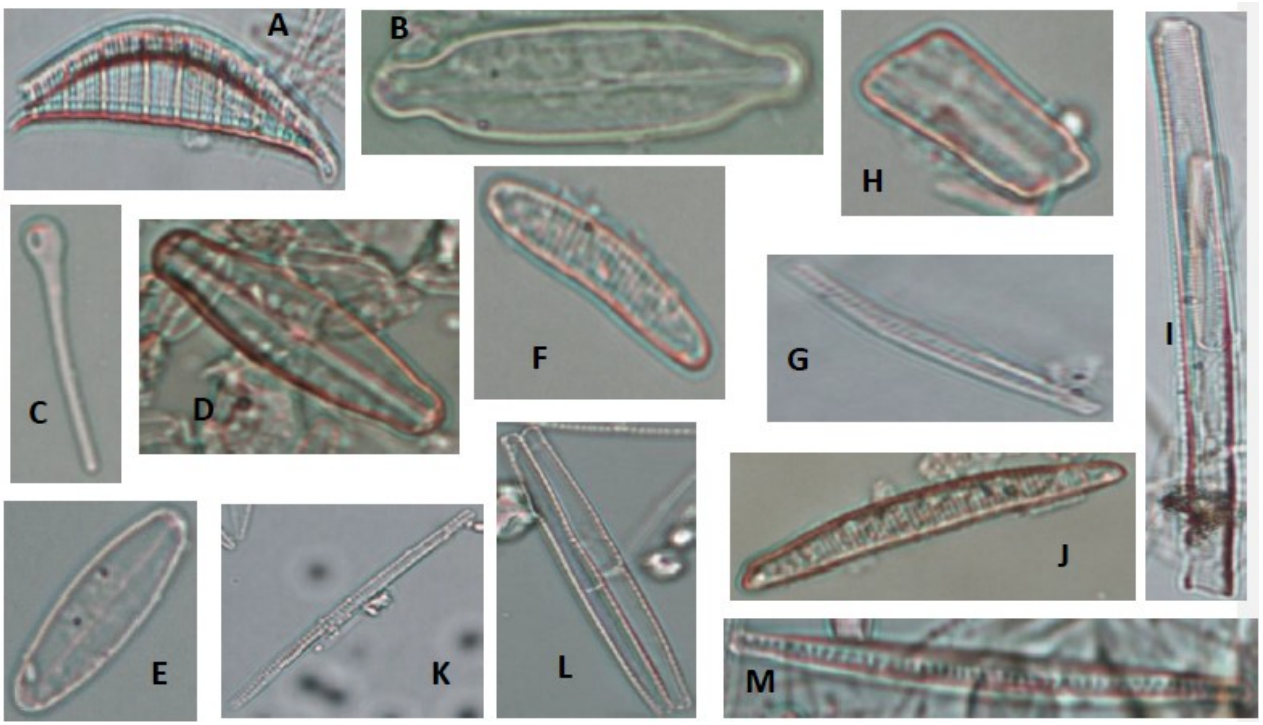

Figure 7 A: *Rhopalodia,* B- *Kobayasiella,* C- *Actinella,* D and E- *Achnanthidium,* (F-J) *Eunotia,* (K-M) *Synendra.*

noticeably broad, radiate in the middle, convergent at the ends, striae 9-11 in 10 µm.

Class: Bacillariophyceae

Order: Surirellales

Family: Surirellaceae

Genus: ***Surirella*** Turpin 1828

Surirella sp. (Fig. 9 B)

Valves 55-65 µm long and 30-34 µm broad, heteropolar, ovate with broad rounded ends. Middle line absent. Middle field linear-lanceolate. Striae very thick, widening towards the middle, set at unequal distances, Striae 11-16 in 10 µm.

Class: Bacillariophyceae

Order: Achnanthales

Family: Achnanthaceae

Genus: ***Achnanthes*** C.A. Agardh (1824)

Achnanthes sp. (Fig. 9 C & D)

Valves 12.5-16 µm long and 5-7 µm broad, rectangular-elliptical to almost quadrate in the middle portion, constricted at the ends which are rostrate. Axial area narrow and central area linear reaching the margins.

Class: Bacillariophyceae

Order: Fragilariales

Family: Fragilariaceae

Genus: ***Tabularia*** (C. Agardh) D.M. Williams and Round

Tabularia sp. (Fig. 9 E)

Valves 21-400 µm long and 3.1-5.3 µm broad, elliptic or elongate and variable in outline, from narrowly linear to linear- lanceolate or lanceolate valves with rounded or capitate ends, striae 7.4-25 in 10 µm.

Class: Bacillariophyceae

Order: Cymbellales

Family: Cymbellaceae

Genus: ***Cymbella***, C.A. Agardh 1830

Cymbella sp. (Fig. 9 F-I)

Valves 118 µm long, 24 µm broad, ventricose, curved, asymmetric, dorsal side convex, ventral side slightly concave with middle inflation; ends slightly constricted, produced rounded; raphe thick, arcuate, excentric with ventrally curved central nodules; axial area not narrow; central area elliptical with 3-4 isolated

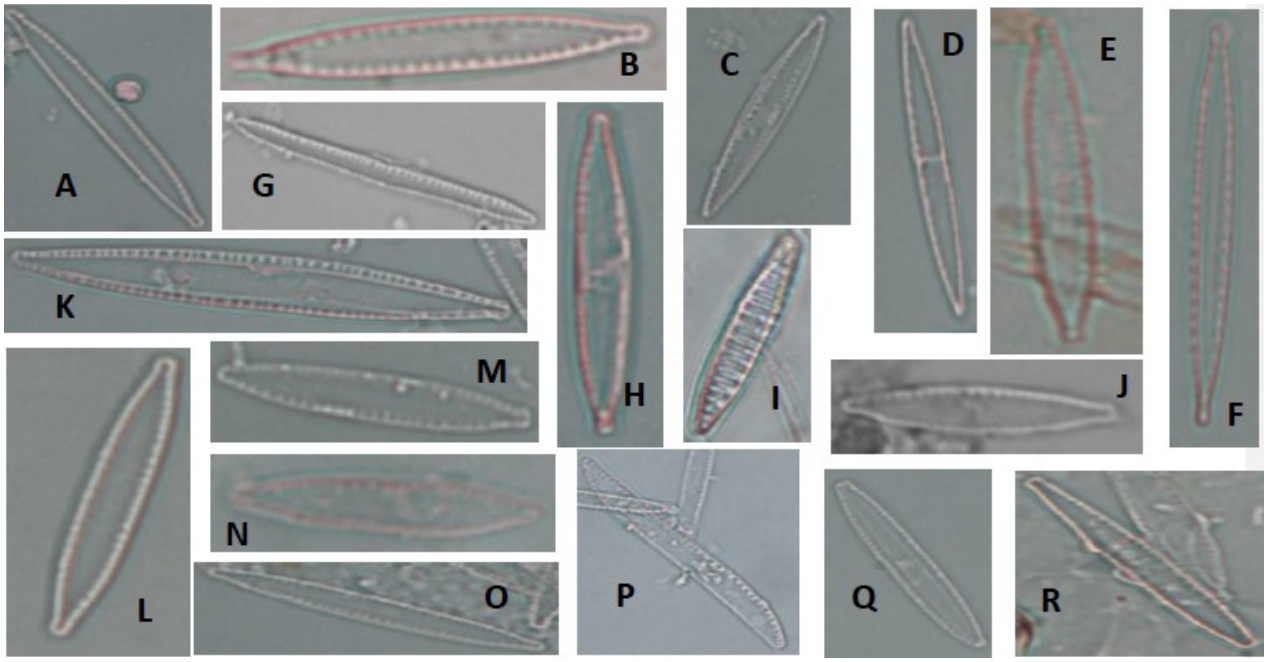

Figure 8(A-R):*Nitzschia*

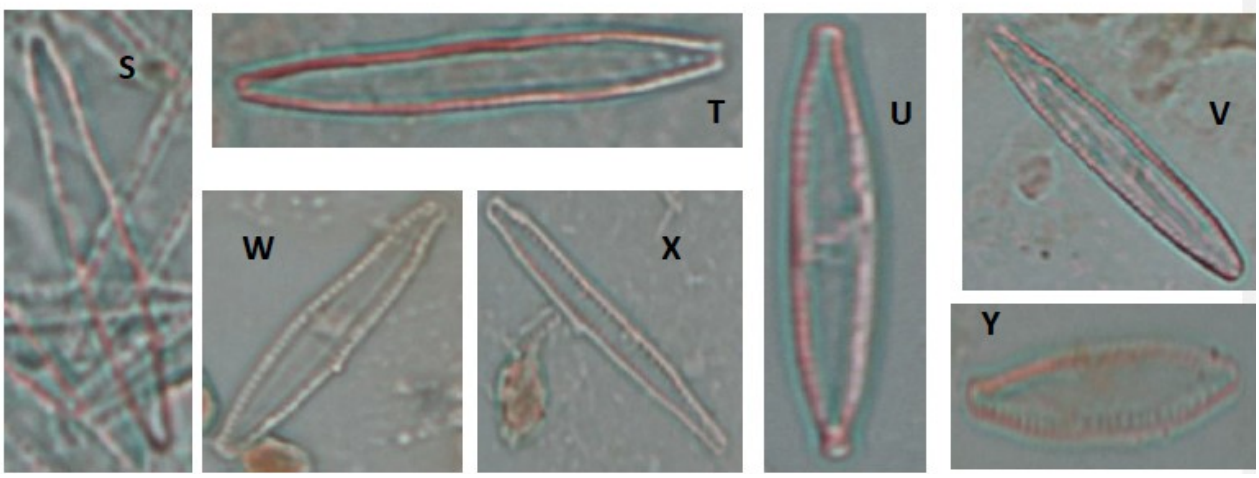

Figure 8(S-Y):*Nitzschia*

stigmata at the ends of the middle ventral striae; striae 8-10 in 10 µm, punctate, radiate.

Class: Bacillariophyceae

Order: Naviculales

Family: Stauroneidaceae

Genus: ***Stauroneis*** Ehrenberg, 1843

Stauroneis sp. (Fig. 9 J-M)

Valves 62-66 µm long and 15-18 µm broad, lanceolate with abruptly constricted, somewhat produced capitate ends. Raphe thick with slightly unilaterally bent central pores and curved terminal fissures. Axial area moderate, linear or slightly widened between the middle and ends: Striae radial, striae 20-22 in 10 µm.

It is interesting to note that all the diatom taxa belonged to pennate type. No centric forms of diatom were found in all the four sampling sites. Majority of the forms were solitary and colonial forms were absent. The dominant genera includes- *Gomphonema, Nitzschia, Stauroneis, Navicula, Frustulia, Eunotia* and *Pinnularia* which were common in all the sampling sites in all the

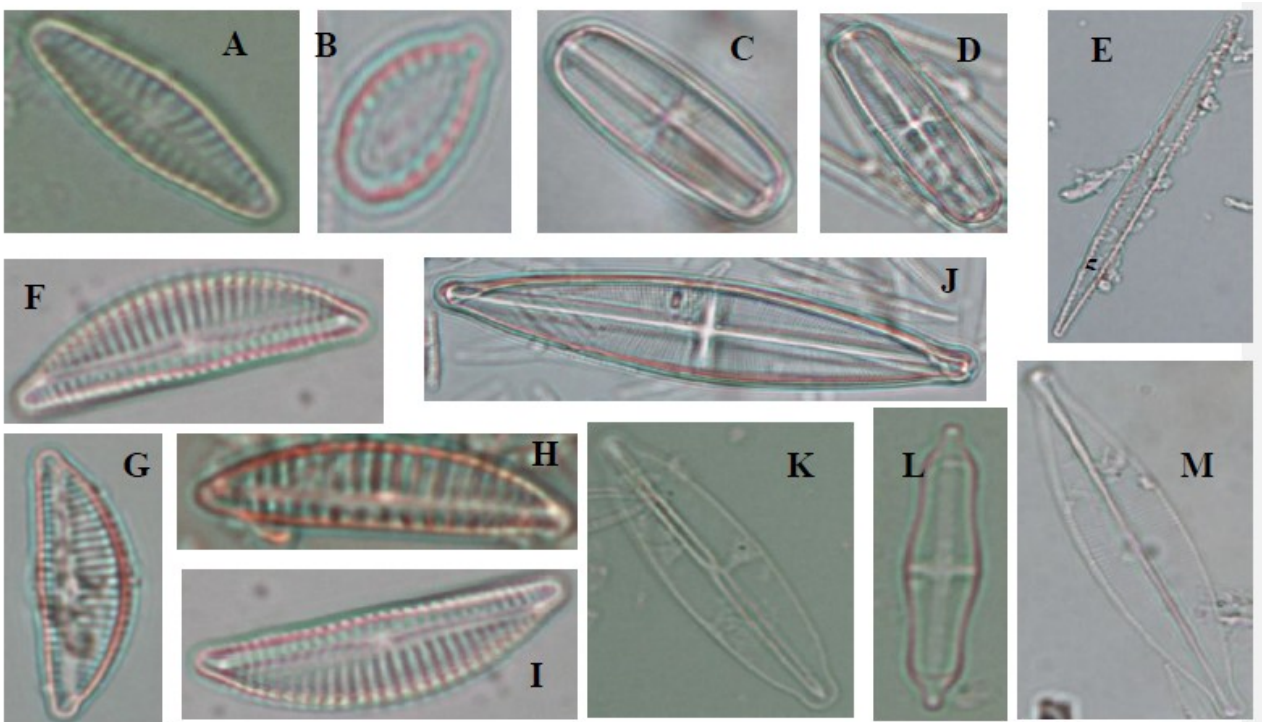

Figure 9. A- ***Hippodonta*****, B-** ***Surirella,*** **C and D-** ***Achnanthes*****, E-** ***Tabularia,*** **(F-I)** ***Cymbella,*** **(J-M)** ***Stauroneis.***

seasons throughout the year. *Kobayasiella, Cymbella, Synendra, Achnanthidium* and *Tabularia* were abundant only in *Chapanala* while *Luticola, Encyonema* occurred in *Borhola*. Pennate diatoms like *Achnanthes*, *Encyonopsis, Hippodonta, Actinella* and *Rhopalodia* were found only in *Jiajuri.* Only pennate diatom *Surirella* was found in *Thanajuri*.

CONCLUSION

Silica rich soils *Jiajuri, Borhola, Thanajuri* and *Chapanala* of Nagaon district of Assam harbours rich assemblage of various forms of diatoms; many of which are new to the region. As detailed taxonomic investigations on the diatom flora of North- East India is very limited, the present basic information of diversity and distribution of diatoms would form a useful tool for further monitoring and ecological assessment of these silica rich soils of Assam. Further, the diversity of freshwater diatoms could also be used as a resource database for future applications.

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