Seasonal Variation Investigations on Diversity of Some Freshwater Diatoms of Kumaon Region, Uttarakhand State in India

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The aims of present studies is to document species of diatoms in water bodies of Kumaon Himalayas and the effect of seasonal variations on population and community dynamics of these microscopic photosynthesizers in aquatic ecosystems. Our studies have documented a total of 98 taxa belonging to class Bacillariophyceae Coscinodiscophyceae and Fragilariophyceae. The taxa have been described along with morphotaxonomic status, locality, and collection number and with date of collection. The maximum taxa have been reported from class Bacillariophyceae (19 genera and 69 species) followed by class Fragilariophyceae (4 genera and 10 species) and Coscinodiscophyceae (3 genera and 4 species). During this study it has been observed that some genera of diatoms dominate in winter season while others in summers. Diatoma De Candolle, Sellaphora Mereschkowsky, Rhopalodia Muller, Staurosira Ehrenberg, Gomphonema C.A. Agardh, Cymbella C.A. Agardh, Reimeria Kociolek and Stoermer, Brachysira Kuetzing, Navicula Bory; emend. Cleve, Surirella Turpin, and Fragilaria Lyngbye; Rabenhorst have frequently been found during summers while Cyclotella Kuetzing, Aulacoseira Thwaites, Cocconeis Ehrenberg; Grunow, Epithemia Brebisson, Synedra Ehrenberg, Achnanthes Bory, Gyrosigma Hassall emend. Cleve and Amphora Ehrenberg dominate in winters. The study will help us to understand the diversity of diatoms in the Kumaon region: It will help us to document the magnitude of biodiversity, understand the population and community dynamics and seasonal variation of phytoplankton. The study may also help us to understand the effect of changing environment on aquatic ecosystems.

> **Key words:** *Bacillariophyceae, Coscinodiscophyceae,* Fragilariophyceae, Diatoms, freshwater, Kumaon region

Diatoms are unicellular, eukaryotic, microscopic algae and are distributed throughout the World in aquatic, semi-aquatic and moist habitats. The diatoms comprise a highly successful and distinctive group of unicellular algae, with the most obvious distinguishing characteristic the possession of siliceous cell walls., Although account for only about 1% of the Earth's photosynthetic biomass, these microscopic algae are responsible for about 45% of our planet's annual net primary productivity (Field *et al.*, 1998). They are extremely important for the biochemical cycling of Silica and as contributors to global fixed Carbon (Willey *et al.*, 2008). The main storage compounds of diatoms are lipids (TAGs) and β -1, 3-linked carbohydrate known as chrysolaminarin. They are sunlight driven cell factories that convert carbon dioxide to potential biofuels, foods and feeds (Walter *et al.*, 2005). Diatoms are known to synthesize a silicified cell wall (frustules) through

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the intracellular transport of silicic acid (Hecky and Kilham, 1988). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success. They are sensitive to change in nutrient concentrations, (Pan et al., 1996). Each taxon has a specific optimum and tolerance for nutrients such as phosphate (Hall and Smol, 1992; Bennion, 1994, Bennion et al., 1996), which can usually be quantified to a high degree of certainty. They respond rapidly to eutrophication and recovery (Zeeb et al., 1994). Diatoms have the peculiar predicament that every time they divide vegetatively, they decrease in cell size. This is due to their unique construction of having two rigid silicon thecae that compose the diatom frustule. When the diatom cell reaches a threshold size, it becomes physiologically capable of undergoing sexual reproduction to produce auxospores which then develop into large initial cells (Round et al., 1990; Mann 1993). Diatoms are traditionally divided into two orders: centric diatoms (Centrales), which are radially symmetric and pennate diatoms (Pennales), which are bilaterally symmetric. The former are paraphyletic to the latter. A more recent classification (Round and Crawford 1990) divides the diatoms into three classes: centric diatoms (Coscinodiscophyceae), pennate diatoms without a raphe (Fragilariophyceae), and pennate diatoms with a raphe (Bacillariophyceae). Species identifications are largely based on frustule morphology. Diatoms can be found on substrata in streambeds even when dry, so they can be sampled at most times of the year (Stevenson and Pan, 1999).

In the present work, systematic studies based on morpho-taxonomy of freshwater diatoms of Kumaon Region have been carried out. Diatom taxonomy is solely based on the morphology of the opaline frustule, its shape, its fine structure, the presence-absence of special elevation, spines, and processes (Schutt, 1896, Round *et al.*, 1990, Hasle and Syversten 1997). Although diatomists speak of vegetative cell division regulating in high degree of fidelity in diatom morphology, variability occurs within and between cell lines in a population and between populations. It has important implications for the identifications of diatoms at the species and sub species levels. The effect of cell division and the distribution of resulting sizes, variability in size, shape and ornamentation may occur within and between descendants of one or more individuals. Diatom taxonomists usually distinguish taxa based on a lack of intermediates between ranges of variability. Differences in seasonal conditions can alter taxon.

MATERIALS AND METHODS

Study sites

Samples were collected from different sites as follows-

Nainital

District Nainital lies in Kumaon division of the state falling in the North-West of Uttar Pradesh. It is located in between 80° 14′ to 78° 80′E longitude and 29°00′ to 29° 05′ N latitude with an area of 3000 km² approximate.

Bheemtal

It lies in Lower Himalya to North-East of Haldwani, about 22 km from Nainital town, at an altitude of 1370 m a.m.s.l. The Lake itself has an elevation of 4,500 feet above sea level, lying in between latitude 29°21′N and longitude 75°34′E. Naukuchiatal

The nine cornered Lake is situated in the Nainital district about 26 km away from Nainital city. The lake is as big as Bheemtal and is deepest Lake of the region.

Kosi River

This River flows through Garampani at an altitude of 900 m and 30 km away from Nainital. **River Jataganga, Jageshwar**

A fast flowing River flows just through Jageshwer touching the temple of Lord Shankar. Dhobighat Stream, Ranikhet

It is located at Ranikhet in the form of small stream in the forest tracks.

River Gagas, Ranikhet

A small River 16 Km away from Ranikhet. The River is swift flowing and contains little amount of water.

Saryu and Gomati Rivers

These two Rivers are fast flowing hilly River full of pebbles, confluence at Bageshwar. Sample collection

Diatom samples were collected by Random Sampling Technique from different geographical localities of Kumaon region during December 2004 to April 2007. Generally epiphytic forms were collected by squeezing submerged plants and planktonic forms with the help of planktonic mesh net having pore size $(10\mu m)$ in the plastic bottles (250 ml.). All these collections were fixed in 3-4% formalin immediately in the field and the collection number, date and localities were also marked.

Preliminary Identification

The microscopic preliminary examination of all the samples was carried out in the field with the help of field microscope.

Preparation of slide and Identification

Diatoms were studied after cleaning their frustules following modified conc. H_2SO_4 - $K_2Cr_2O_7$ method of Patrick and Reimer (1966). For this purpose, the collection tube called 'Mixgen' was shaken vigorously and two drops of algal suspension were dropped on one side of a slide and then heated gradually over a heater so that the water evaporated leaving behind the material dry. When the slide was completely dried, a drop of conc. H_2SO_4 was dropped on the material and

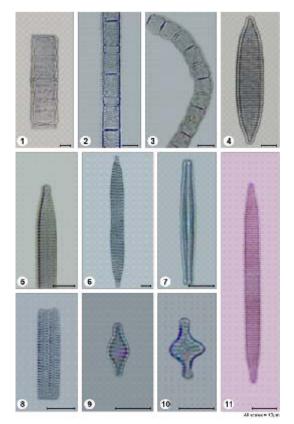


Fig. 1. General morphological structure of Diatoms (LM Pictures) 1. Melosira varians Agardh, 2. Aulacoseira ambigua (Grun. in Van Heurck) Simenson, 3. Aulacoseira ambigua (Grun. in Van Heurck) Simenson f. curvata Hustedt, 4. Synedra dorsiventralis Muller, 5. Synedra ulna (Nitzsch) Ehr. var. amphirynchus (Ehr.) Grunow, 6. Synedra ulna (Nitzsch) Ehrenberg, 7. Fragilaria crotonensis Kitton, 8. Fragilaria virescens Ralfs 9. Staurosira construens (Ehrenberg) Williams and Round, 10. Staurosira construens (Ehrenberg) Williams and Round var. indica Gandhi, 11. Synedra ulna var. contracta Øttr.

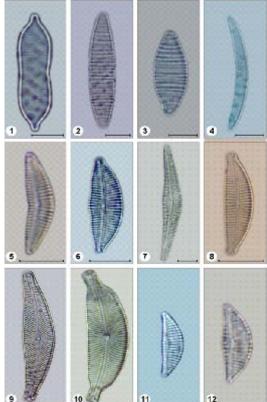
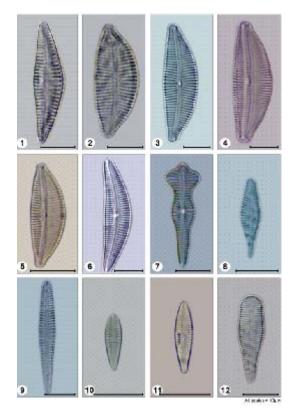


Fig. 2. General morphological structure of Diatoms (LM Pictures)1.Synedra sp.,2. Diatoma hiemale (Lyngbye) Heiberg 3. Diatoma vulgare Bory,4. Eutonia lunaris (Ehrenberg) Grunow, 5. & 6. Cymbella affinis Kuetz. 7. C. cymbiformis (Kuetzing) Brebisson 8. C. obtusa Greg. 9. & 10. C. tumida (Brebisson) Van Heurck, 11. C. ventricosa Kuetz.. var. minuta (Hilse) Van Heurck, 12. C. ventricosa Kuetzing

heated again till the residue became brownish then it was decanted gently. In the final step 3-4 drops of conc. H_2SO_4 was dropped on the material and slightly heated then few crystals of $K_2Cr_2O_7$ were added and again heated till the solution became yellow homogeneously. The traces of acid $K_2Cr_2O_7$ were washed with distilled water and heated till it became dry. A drop of DPX (Distyrene Pthalate Xylol) was put on the dried and cleaned diatom material and covered carefully with cover slip. Several permanent slides were made for each compositive sample and some of them representing maximum taxa were chosen for the study. Photomicrographs of the studied taxa were clicked by Nikon Labophot - II microscope in Phycology Laboratory of Botany Department of University of Lucknow, Lucknow. All measuring scales given for diatom photographs (LM images) are equal to 10 μ m. Diatom classification depends to a great extent upon the intricacies of pore structure, and the arrangement of the wall organelles (ocelli, portulae, raphes, etc.).

These anatomical features of the valve can now be studied in all its detail using scanning electron microscopy (SEM). For SEM, thoroughly washed material was dried onto the Aluminum SEM stubs (here cover glass method is used). When the cover glass with material is completely dried,



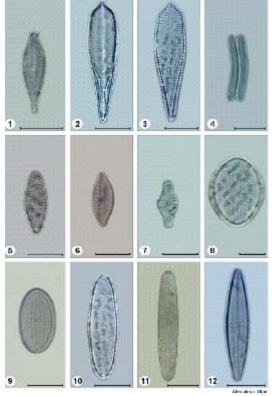


Fig. 3. General morphological structure of Diatoms (LM Pictures) 1. Cymbella turgida Gregory 2. C. reinhardti Grunow, 3 & 4. C. sumatrensis Hustedt 5. C. tumescens Cleve 6. C. parva (Wm. Simth) Cleve 7. Gomphonema acuminatum Ehrenberg 8. G. intricatum Kuetzing 9. G. montanum Schumann var. subclavatum Grunow 10. G. olivaceum (Lyngbye) Kuetzing 11. G. parvulum (Kuetzing) Grunow 12. G. constrictum Ehrenberg

Fig.4. General morphological structure of Diatoms (LM Pictures) 1. Gomphonema sphaerophorum Ehrenberg, 2. Gomphonema sp.1, 3. Gomphonema sp.2 4. Achnanthes brevips Ag. var. intermedia (Kuetzing) Cleve, 5.A. lanceolata (Brebisson) Grunow, 6. A. lanceolata (Breb.) Grun. var. elegans Cleve, 7. A. lanceolata (Breb.) Grun. var. rostrata Hustedt, 8. Cocconeis pediculus Ehrenberg, 9. C. placentula Ehrenberg, 10. Caloneis alpestris (Grunow) Cleve11. Pinnularia gibba (Van Heurck) Boyer 12. Navicula feuerborni Hustedt

thin metal coating of Gold/Palladium is applied to glass cover and specimen by sputtering using a sputter coating machine. The pictures are taken including scale bar and other magnification information of the image.

Examination of specimen

The diatom taxa have been studied by examining their morphological characters and identifications have been confirmed by crosschecking with the authentic illustrations and descriptions of related monographs and journals available. For the purpose of taxonomic enumeration, diatoms have been arranged according to Round *et al.* (1990).

Data collection basis

District wise distribution of diatom taxa and frequency of occurrence have been given on the basis of identified taxa. Each genus has been shown on the microscopic visual basis and classified into four categories: (a) abundant (dominant), occurring homogeneously in low number in a locality, (b) frequent, occurring homogeneously in large number in a locality; (c) rare, occurring in low number in a locality; and (d) absent not occurring at all.

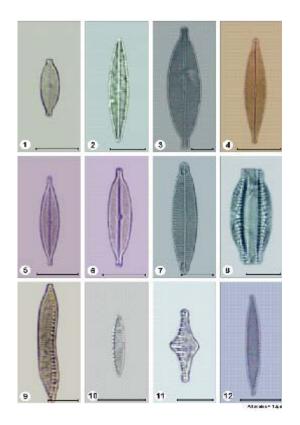


Fig.5. General morphological structure of Diatoms (LM Pictures)1. Navicula elginensis (Greg.) Grunow, 2.N. radiosa Kuetzing,3. N. cuspidata Kuetzing var. ambigua (Ehr.) Cleve,4.N. radiosa var. tenella (Breb.) Grunow,5. N. salinarum Grun. var. intermedia (Grun.) Cleve,6.N. subrhynchocephala Hustedt, 7.N. viridula (Kuetz.) Ehr.,8. Amphora exigua Greg.,9.Hantzschia amphioxys (Ehr.) Grunow,10. Nitzschia frustulum (Kuetz.) Grunow,11.N. sinuata (Wm.Smith) Grun. var. tabellaria Grunow,12.N. chauhanii Gandhi

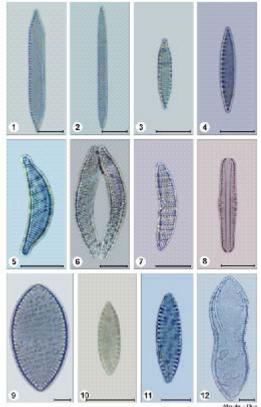


Fig. 6. Morphological structure of Diatoms (LM Pictures)1. Nitzschia commutata Grunow, 2. N. denticula Grunow,3.N. microcephala Grunow,4.N. clausii Hantzsch,5.Epithemia sorex Kuetzing,6.E. zebra (Ehr.) Kuetzing,7.E. zebra (Ehr.) Kuetz. var. saxonica (Kuetz.) Grunow,7. Rhopalodia gibba (Kuetzing) Muller,8.Surirella ovalis Brebisson,10. &11. S. apiculata Wm. Smith,12. Cymatopleura solea (Breb.) Wm. Smith

RESULTS AND DISCUSSION

The present work deals with the study of diatom flora of Kumaon region of Uttarakhand State. Geologically the region is recent formation and is composed of middle and inner Himalaya gifted with several tectonic and glacial lakes, swift flowing streams and Rivers. Nainital region has few large and prominent lakes like Bheemtal and Naukuchiatal. From Almora region samples were collected from Jageshwar, Dhobighat Stream of Ranikhet and Gagas River. From district Bageshwar samples were collected from Saryu River and Gomati River. The present study revealed a total of 98 taxa belonging to classs Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae. The taxa have been described along with morphotaxonomic status, locality, and collection number and with date of collection. Traditionally, Caloneis Cleve is treated here as separate genus. The maximum taxa have been reported from class Bacillariophyceae (19 genera and 69 species) followed by class Fragilariophyceae (4 genera and 10 species) and Coscinodiscophyceae (3 genera and 4 species). The sub-class Bacillariophycidae of class Bacillariophyceae represents maximum taxa by representing 18 genera and 67 species. Only one forma has been reported from class Coscinodiscophyceae. In Naukuchiatal, Nainital, the species of Cymbella C. A. Agardh; C. parva (Wm. Smith) Cleve, C. cymbiformis (Kuetz.) Brebisson, C. obtusa Gregory and C. turgida Gregory are very frequently found during summer season. Gomphonema montanum Schumann var. subclavatum Grunow dominates during summer while Gomphonema constrictum Ehrenberg found

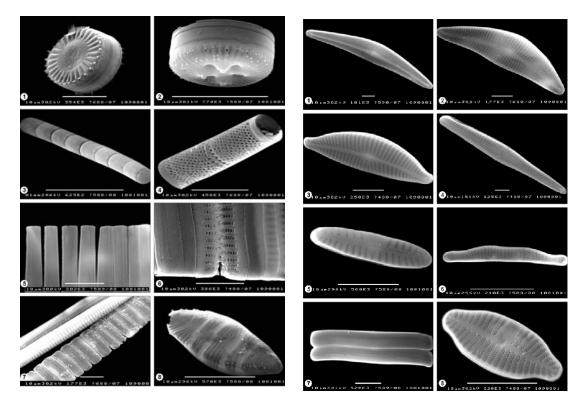


Fig. 7. Morphological structure of Diatoms (SEM Pictures)1. *Cyclotella meneghiniana* Kuetzing,2. C. *ocellata* Pantocsek 3.& 4. *Aulacosiera ambigua* (Grun.in Van Heurck-) Simenson 5. & 6. *Fragilaria* sp., 1 7. *Fragilaria* sp.2, 8. *Diatoma vulgare* Bory

Fig. 8. General morphological structure of Diatoms (SEM Pictures)1. Cymbella cymbiformis (Kuetz.) Brebisson, 2. C. helvetica Kuetzing, 3. Cymbella sp., 4. Gomphonema montanum, . Schumann var. subclavatum Grunow, 5. Reimeria sinuata (Gregory) Kociolek and Stoermer, 6. Eunotia arcus Ehrenberg, 7. Achnanthes brevips Ag. var. intermedia (Kuetz.) Cleve, 8. A. lanceolata (Breb.) Grun. var. elegans Cleve

in abundance during winters. Bheemtal Lake of this region is rich in Gomphonema montanum Schumann var. subclavatum Grunow. Brachysira vitrea (Grunow) Ross and Cymbella parva (Wm. Smith) Cleve are found in summers in Bheemtal Lake, Cyclotella ocellata Pantocsek is commonly found in winters in this huge lake, Gyrosigma sp., and Aulacoseira ambigua (Grunow in Van Heurck) Simenson flourish during winters. In this huge Lake Eunotia arcus Ehrenberg is rarely found. The blooms of Cymbella cymbiformis (Kuetz.) Brebisson, Epithemia sorex Kuetzing, and of Amphora exigua Gregory are found in Naukuchiatal in summers. Being tourist spots the ecology of these lakes is going to be disturbed. The presence of Cymbella helvetica Kuetzing and Cocconeis placentula (Ehrenberg) var. euglypta (Ehrenberg) Cleve eutrophic habitat loving species in Bheemtal is an indicator of this situation. The increasing eutrophication can lead to dominance

of a few genera and extinction of others. In Khaira of Nainital region, the bloom of Diatoma vulgare Bory is found in summers. Epithemia sorex Kuetzing is found in abundance in Kosi River in Khairna in winter season. In Dhobighat Stream of Ranikhet Melosira varians Agardh and Fragilaria crotonensis Kitton are found in abundance during winters. Here, genus Synedra ulna (Nitzsch) Ehrenberg dominates in winters while Fragilaria virescens Ralfs and Navicula viridula (Kuetz.) Ehrenberg are very much common in summers. Surirella apiculata Wm. Smith and Pinnularia gibba (Van Heurck) Boyer are rare taxa of the locality which are seen in summer season. Being oligotrophic, water bodies of Jageshwar are poor in diatom flora. Cymbella turgida Gregory, Cymbella obtusa Gregory, Navicula subrhynchocephala Hustedt, and Cocconeis placentula Ehrenberg are frequently found in Kosi River in Almora during winter season. In Bageshwar

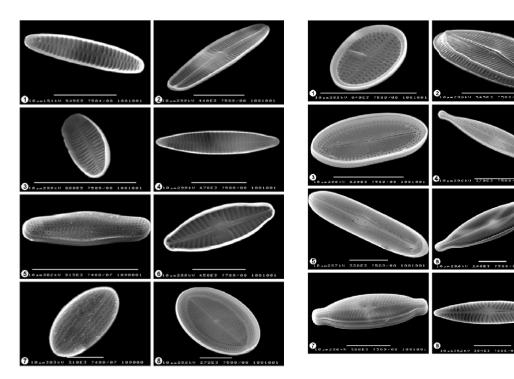


Fig. 9. Morphological structure of Diatoms (SEMPictures)1. *Achnanthes* sp.2, 2. *Achnanthes* sp. 3,3.*Achnanthes* sp. 4,4.*Achnanthes* sp.5,5.*Achnanthes* sp. 6,6.*Achnanthes* sp.1, 7.*Cocconeis placentula* Ehr. var. *euglypta* (Ehr.) Cleve ,8. C. *distans* Gregory

Fig. 10. General morphological structure of Diatoms (SEM Pictures)1. *Cocconeis* sp., 2.C. *scutellum* Ehrenberg, 3.C. *placentula* Ehrenberg,4. *Brachysira vitrea* (Grunow) Ross,5. *Sellaphora pupula* (Kuetzing) Mereschkowsky,6.*Navicula capitatoradiata* Germain,7.N. *subrhynchocephala* Hustedt,8.N. *edrigiana* Carter.

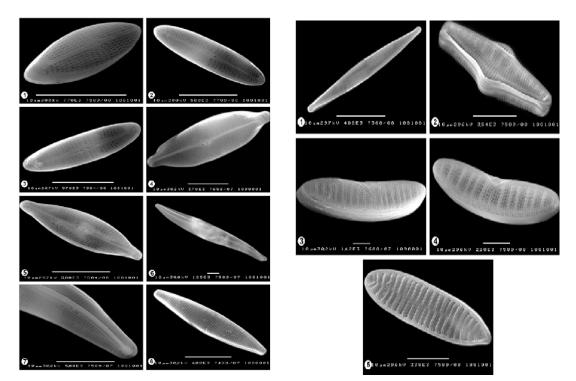


Fig.11. Morphological structure of Diatoms (SEM Pictures)1. *Navicula* sp.1,2. & 3. *Navicula* sp.2,4. *Navicula* sp.3,5. *Navicula* sp.4,6. & 7. *Gyrosigma* sp.,8. *Nitzschia* sp.1.

region Saryu River is rich in diatom flora because it is eutrophic and rich in niche diversity. During winters Synedra dorsiventralis Muller, Diatoma vulgare Bory, Fragilaria varians Ralfs, and Achnanthes lanceolata (Brebisson) Grunow are frequently found in this River. Navicula radiosa Kuetzing and Nitzschia microcephala Grunow are found in abundance in summers. In the River the bloom of Achananthes lanceolata (Brebisson) Grunow are found in summer. Gomati River of Baijnath (Bageshwar) is rich in diversity but members are poorly distributed. Cymbella ventricosa Kuetzing and Cymbella tumida (Brebisson) Van Heurck are commonly found taxa of this River. Reimeria sinuata (Gregory) Kociolek and Stoermer is frequently found in winter in this River. It is a tourist place and human disturbances are very common in this region therefore, the locality always experiences selection pressure. This may be one of the reasons that make the habitat rich in diatom flora. Morphological variations are seen in many diatoms which belong to the same J PURE APPL MICROBIO, 9(1), MARCH 2015.

Fig.12. Morphological structure of Diatoms (SEM Pictures) 1.*Nitzschia* sp.2,2.N. *sinuata* (Wm.Smith) Grun.var. *tabellaria* Grunow,4. *Epithemia adnata* (Kuetzing) Brebisson 5. *Surirella ovata* Kuetzing.

species. The shape of Cymbella affinis Kuetzing, for example, varies in the same species. These variations may be due to effect of phenotypic plasticity imposed by certain environmental changes. During this study it has been observed that some genera of diatoms dominate in winter season while others in summers. Diatoma De Candolle, Sellaphora Mereschkowsky, Rhopalodia Muller, Staurosira Ehrenberg, Gomphonema C.A. Agardh, Cymbella C.A. Agardh, Reimeria Kociolek and Stoermer, Brachysira Kuetzing, Navicula Bory; emend. Cleve, Surirella Turpin, and Fragilaria Lyngbye; Rabenhorst have frequently been found during summers while Cyclotella Kuetzing, Aulacoseira Thwaites, Cocconeis Ehrenberg; Grunow, Epithemia Brebisson, Synedra Ehrenberg, Achnanthes Bory, Gyrosigma Hassall; emend. Cleve, and Amphora Ehrenberg dominate in winters. This floristic work on diatoms will help us to understand the diatom flora of the Kumaon region of Uttarakhand State.

It will help us to document the magnitude of biodiversity, understand the population and community dynamics of phytoplankton. The study may also help us to understand the effect of changing environment on aquatic ecololgy of Kumaon region.

CONCLUSION

The present studies revealed a total of 98 taxa belong to class Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae. The taxa have been described along with morphotaxonomic status, locality, and collection number and with date of collection. The maximum taxa have been reported from class Bacillariophyceae (19 genera 69 species) followed by class and Fragilariophyceae (4 genera and 10 species) and Coscinodiscophyceae (3 genera and 4 species). During this study it has been observed that some genera of diatoms dominate in winter season while others in summers. Diatoma De Candolle, Sellaphora Mereschkowsky, Rhopalodia Muller, Staurosira Ehrenberg, Gomphonema C.A. Agardh, Cymbella C.A. Agardh, Reimeria Kociolek and Stoermer, Brachysira Kuetzing, Navicula Bory; emend. Cleve, Surirella Turpin, and Fragilaria Lyngbye; Rabenhorst have frequently been found during summers while Cyclotella Kuetzing, Aulacoseira Thwaites, Cocconeis Ehrenberg; Grunow, Epithemia Brebisson, Synedra Ehrenberg, Achnanthes Bory, Gyrosigma Hassall emend. Cleve and Amphora Ehrenberg dominate in winters.

The study will help us to understand the diversity of diatoms in the Kumaon region: It will help us to document the magnitude of biodiversity, understand the population and community dynamics of phytoplankton. The study may also help us to understand the effect of changing environment on aquatic ecology of Kumaon region.

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