ISSN 0973 – 6921 ; E – ISSN 2319 – 5983 *J. Environ. Res. Develop.*
Vol.13No. 04,April-June2019
Journal of Environmental Research And Development **Vol. 14 No. 01, July-September 2019**

EXPLORING THE DISTRIBUTION, AND DIVERSITY PATTERN OF DIATOMS FROM PAVANA RIVER, PUNE, INDIA

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ABSTRACT

In an attempt to evaluate the spatio-temporal variation in diversity of diatoms and associated water quality, we established 3 stations on the river Pavana based on point and non point sources of pollution. Three stations namely upstream, middle stream, and downstream were selected and sampled every month for physicochemical parameters and Microscopic Diatom analysis. Diatoms are composed of siliceous cell wall making it a reliable tool for monitoring water pollution. The relationship between water variables affecting species diversity in 3 site stations were analyzed by multivariate analysis using Canonical Correspondence Analysis method. We observed species richness higher in undisturbed water flow *i.e.* at the source of the river (upstream), indicating the importance of diatom diversity in water quality analysis. This also suggests water chemistry as a controlling factor to diatom variation. Diatoms like *Phinnularia*, *Cymbella*, *Navicula*, *Nitzschia*, and *Fragilaria* were observed in all the three stations. *Epithemia* diatom, a fresh water diatom was abundant upstream and *Nitzschia* observed in highly polluted water was abundant in downstream water flow. Our result indicates the importance, and a correlation of stream flow harboring a unique diatom community. Thus this study will aid to understand the factors affecting the distribution pattern of diatoms and predict the water environment which may also help to redeem the water quality in the future.

Key Words : Phytoplankton, Epilithic diatoms, Bioindicators, Water quality, Diversity pattern, Physicochemical parameters

INTRODUCTION

Diatoms have received much attention as one of the most diverse, and ecologically important phytoplankton groups as they contribute around 20% of global primary productivity[1]. They are single-celled silicified algae of size as small as 5 µm to as large as 1mm. Structurally they are observed as filaments, chains, arrange within mucilage tubes that are solitary or attached to any single substratum [2]. The structural elements (siliceous cell walls) allow for reliable taxonomic determination at specific, and sub-specific levels[3]. Diatoms species are very particular about the water chemistry in which they live. They have a distinct range of pH and salinity where they grow. Diatoms also have a range of tolerance for other water variables, including nutrient concentration, suspended sediments, flow regime, elevation, and different types of human disturbance. Such studies are performed in much detail in different countries like France, Continental US, because of the increased frequency of pollution level in the water[4-6]. As a result, diatoms are used extensively in water assessment, and as a monitoring tool[7]. This property can be explored to study the effect of water quality and diversity of diatoms to determine appropriate management actions[4]. Pavana River in Maharashtra, Pune is known to experience disturbances, like the discharge of industrial effluents, and sewage water discharge in the water body[8,9]. The taxonomic study of diatoms in Pavana River is carried out in the last few years and several new species of

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diatoms are recorded from Pavana River. *Cymbella pavanaensis* was a newly recorded species from this site[10]. Diatoms are used as a biological indicator and can reproduce rapidly, therefore, it helps in observing the rapid changes in water quality[11]. This study will provide insights into the factors that affect the distribution pattern of diatoms. This may also help to generate a river and diatom map with a change in water quality.

Hypothesis : Water Parameters influences the Diversity of diatoms

AIMS AND OBJECTIVES

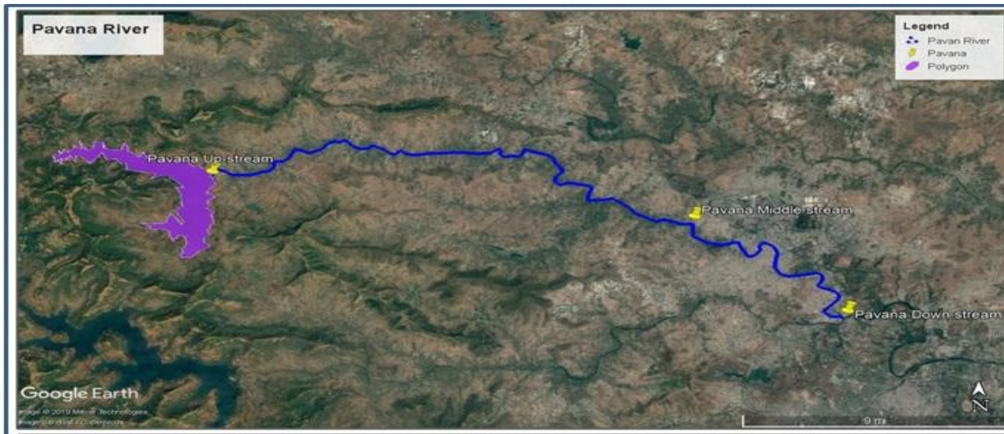
1) Exploring species diversity of diatoms from the study area.
2) Evaluation of Water parameters influencing diatoms.

MATERIAL AND METHODS

Study area and sample collection

The study area covered a 60 km Pavana River that originates from south of Lonavala including a small part of Western Ghats and the river ends to Mula River in Pune city. The river was divided in 3 site area 20 Km each termed as upstream, middle stream, and downstream. The latitude and longitude of the sample site is shown in **Fig. 1.**

Diatom materials were collected every month from at least five cobbles, and small boulders from a reach of at least 10 m in the river from each site location.



Sr. No	Site Location	Latitude	Longitude	Land Use
1	Upstream	18 ° 37' 26" N	73°46' 06" E	Agricultural
2	Middle stream	18° 66' 33" N	73° 49'16" E	Domestic Use
3	Downstream	18° 57' 39" N	73° 83' 17"E	Domestic Use

Fig. 1 : Location of the sampling site of Pavana River drawn using Google Earth software

Diatom observation and water analysis

The Diatom frustules were cleaned using the wet purification method given by Rosa Trobajo[12]. In the collected sample concentrated Hydrochloric acid (HCL) was added for clearing the calcium salts from the sample then treated with the concentrated H_2SO_4, $NaNO_3$, HNO_3, and $K_2Cr_2O_7$ for 24 hrs. Diatoms were observed using Trinocular microscope, Olympus CX31 model no-CX31RBSFA and Binocular micros cope, Lawrence & Mayo, model no-LM-52-1710. The collected materials were preserved using DPX mountant[13-14]. Diatoms were identified using several databases Diatom Image Database (DID), and Diatom Base and identify ication key by Krammer & Lange-Bertalot, Lange-Bertalot & Krammer and Gandhi H.P also be used[2, 15-18].

Water analysis: The water samples were collected with the diatom sample every month in clean and sterilized 1 Liter of a plastic bottle from each site from the depth of 20-50 cm in water during the day. The water variables were analyzed which include both the physicochemical characteristics of water, and habitat description We determined water temperature, pH, Dissolved Oxygen (DO), Biological Oxygen Dem, and (BOD), Total Dissolved Solids (TDS), Total Phosphate (TP), Free Chlorine using a thermometer, pH meter, Aquasol DO Kit, Titrimetric method and Colorimeter method using standard procedure[16,19-23].

Diatom Diversity Analysis

Species and evenness and abundance were calculated for diatom species from each sampling site[20,24-27]. The Multivariate Canonical correspondence analysis (CCA) was performed to establish the relationship between diatom species distribution, and water parameters using PAST version 3.1 statistical software.

Shannon diversity (H) and Shannon evenness (E_H), are calculated as shown below:

1. Shannon Diversity index (H), $H = -\sum_{i=1}^{S}[(pi) \times In(pi)]$

2. Evenness (E_H), $Evenness = \frac{H}{In\ S}$

Where pi is the proportion of number of i^{th} individual species (n) found, S = Number of species

RESULTS AND DISCUSSION

Species richness and abundance of diatoms

Total 251 epilithic diatoms were recorded from the study site during three visits representing 17 genus that includes *Phinnularia, Triceratium, Navicula, Cymbella, Gomphonema, Fragillaria, Nitzschia, Melosira, Rhopalodia, Pleurosigma, Nitrschia, Gyrosigma, Eunotia, Secletonema, Epithemia, Biremis*, and *Diatoma* with wide range of community composition and species distribution. The unidentified diatom species were grouped into one category as OTU species in which the similar-looking diatoms were marked as OTU 1 and OTU 2 as shown in **Fig. 2.** It was observed that *Phinnularia* diatom was the most abundant during all the visits followed by *Navicula*, *Nitzschia*, and *Cymbella*. Diversity indices were calculated using Shannon Diversity (H), and evenness Index (E_H) from Upstream river site H= 3.68; E_H=0.80. This value indicates richness in the diversity. Indices for downstream site was H= 1.68; E_H=0.5, indicates low richness and high abundance. *Nitrschia*, *Cymbella*, *Epithemia* and *Phinnularia* sp are most abundant species reported from the Upstream. Whereas *Navicula* and *Fragillaria* sp. are most abundant species reported from the Middle stream. *Nitzschia* and *Phinnularia* were the most abundant from the downstream station. The highest number of diatoms from per slide per sample was approximately 10. *Phinnularia viridis* found to be the sensitive species was the most commonly observed in the undisturbed area, and *Nitzschia kittlii* which found to be the most tolerant group of diatom was the commonly found species in the disturbed area. A pollution tolerant species *Nitzschia kittli*, and *Gomphonema sp.* were highly tolerant to water parameters especially pH, Temperature, and Phosphate. Likewise, other species also provide the information with the help of other diatom diversity indices which represents the effect of anthropogenic activities on diatoms **(Fig. 3)**.

Interestingly *Triceratium* genus a triangular marine water diatom was observed in the upstream of the river for the first time, further characterization may reveal the species.

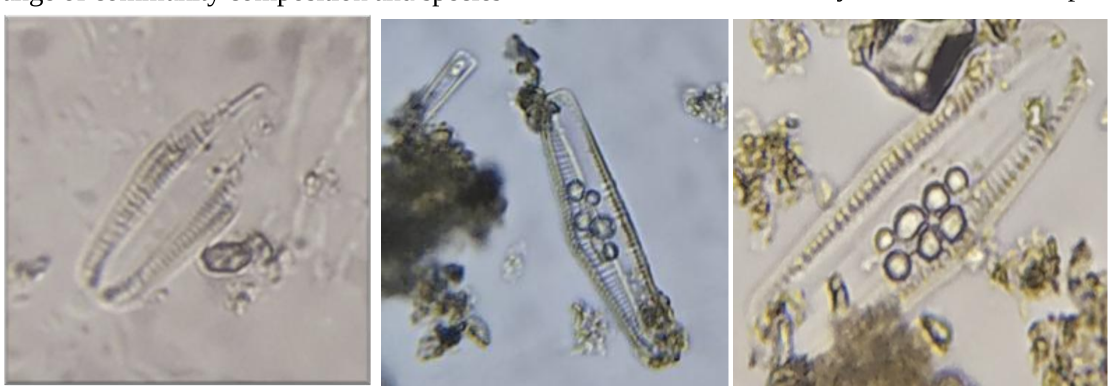


Fig. 2 : Operational taxonomic under (OTU) of diatioms

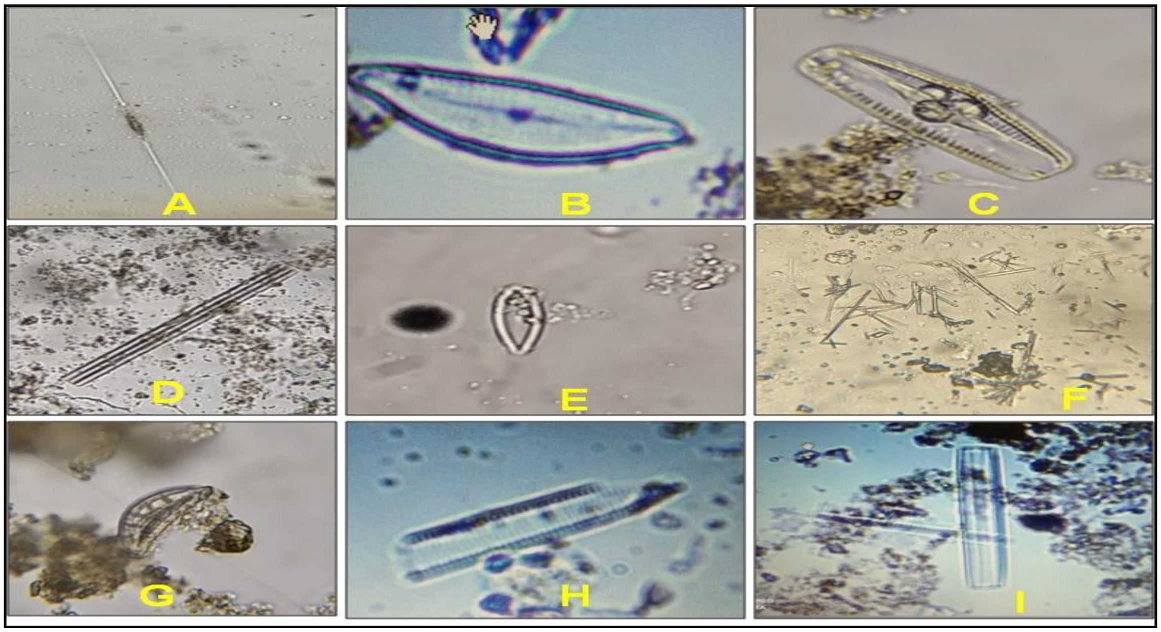


Fig. 3 : Photo-representation of diatom species diversity. (A = *Nitreschia sp*.; B= *Cymbella sp*.; C = *Rhopalodia sp*.; D = *Fragillaria sp*.; E = *Navicula sp*.; F = *Asterionella sp*.; G= *Cymbella sp.;* H = Diatoma sp.; I = *Epithemia sp.*)

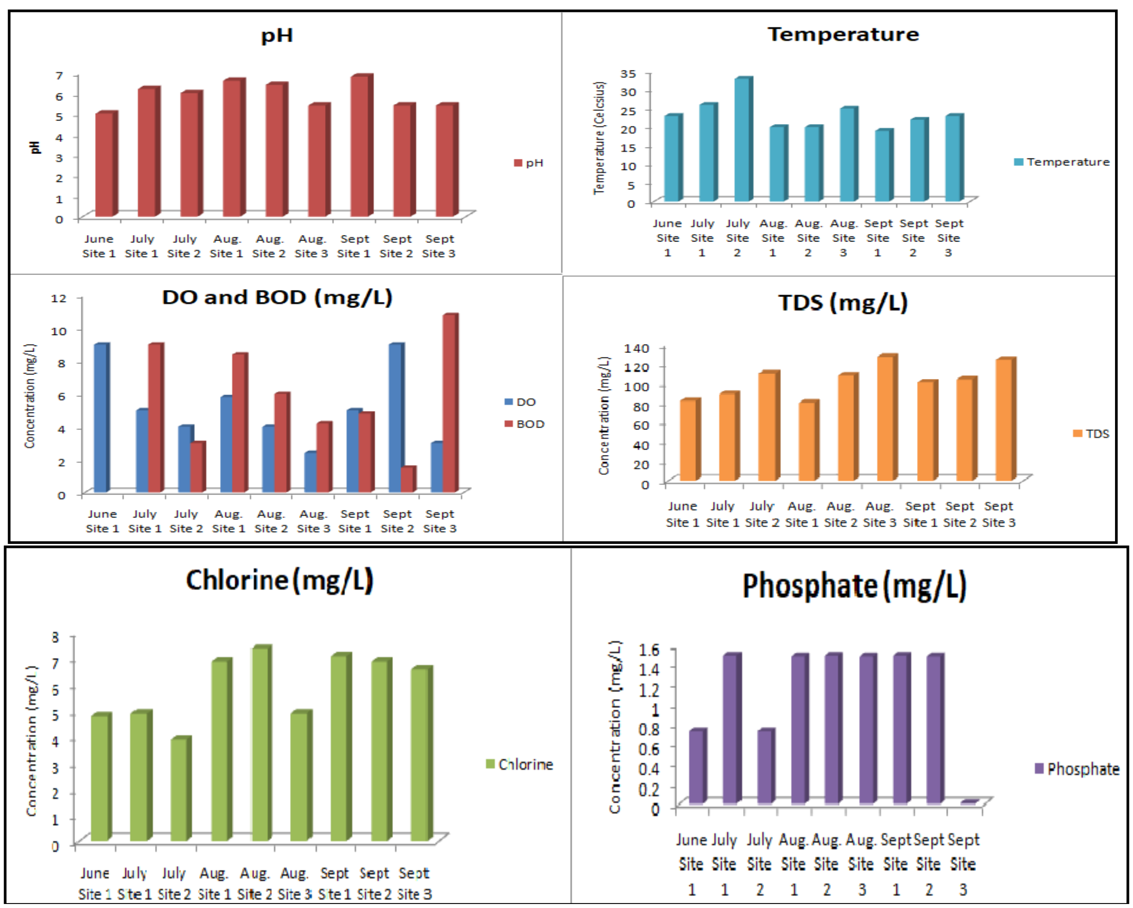


Fig. 4 : Variation of physicochemical variables in River sample

With various disturbances like discharge of Industrial waste, sewage, and animal washing the river has experienced several anthropogenic pressures. The villagers staying nearby river celebrate a festival called *'Pavanai'* in this period no one is allowed to use the water for domestic purpose. In the month of August and September *i.e.* during Navrati festival, they follow the ritual of washing all the clothes, utensils in the river. They follow the custom as a taboo, that the river will purify the house. Therefore, in the mentioned months the pollution level is thought to be more and indicated in the **Fig. 4**. The BOD values were higher in the middle and downstream in the month of August, and September. According to the observations, there was a decline in the Phosphate concentration during the month of August in Upstream, and Downstream due to heavy monsoon which may have also affected the species diversity.

Relationship between the water parameters and diatoms occurrence

The Canonical Correspondence Analysis (CCA) indicates the patterns within species , and sites based on the influencing water variables [21]. Eigen value of first two axes were x-axis=0.165 (92), y-axis=0.07 (3%). In the CCA with three water station sites and water variables, diatoms observed were retained in the resulting ordination map. Species, and sites are represented by points, and water variables are represented by arrows. The placement of a species in the CCA represented the water variable optima for the particular species relative to the other taxa shown in the **Fig. 5**. *Nitzschia*, *Fragillaria*, and *Epithemia* diatom species are found in the area with high Phosphate concentration, with high BOD DO and in acidic pH. *Cymbella sp* was observed in the water site with high pH, temperature, and BOD. *Phinnularia and Navicula* diatom was observed when the concentration of chlorine and high concentration of TDS. In June-upstream (2a), July downstream (3c), and August-upstream (4a) diatom diversity was rich with *Epithemia Nitzschia*, *Fragillaria* and *Triceratium* species. Of all the water parameters tested Temperature and chlorine was found to be least considered by the species for richness or abundance.

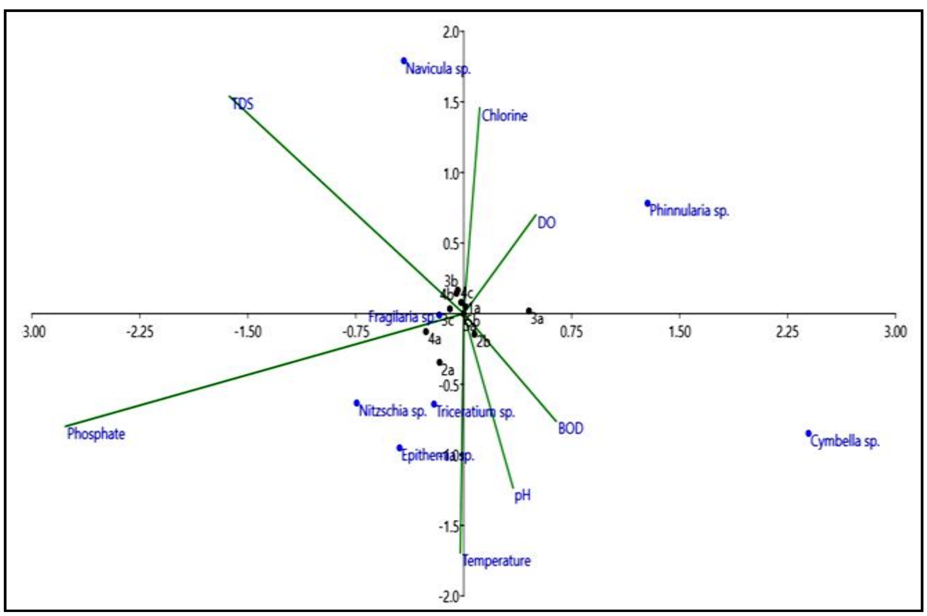


Fig. 5 : Distribution of diatom species from site station. Canonical correspondence analysis (CCA) analyzed using site location, diatom observed, and water parameter. The sampling sites were: 1a= Visit 1 Upstream; 2a= Visit 2 Upstream; 2b= Visit 2 Middle stream; 3a= Visit 3 Upstream; 3b= Visit 3 Middle stream; 3c= Visit 3 Downstream; 4a= Visit 4 Upstream; 4b= Visit 4 Middle stream. Water variables were pH, Temperature, TDS, BOD, Chlorine, DO, and Phosphate

Distributions and preferential pattern in diatoms were related with the sampling sites, and physicochemical characteristics. Richness of the diatom community was associated with water parameters (pH, Temperature, BOD, and Phosphate concentration) and site station. It was observed that anthropogenic activities could affect both the water quality and habitat of the diatoms species. Anthropogenic activities like the influence of industrial waste and sewage discharge were prominent on the site station selected. Our result indicates the importance in the correlation of stream flow harboring a unique diatom community.

CONCLUSION

It was observed that diatom community distribution changes according to the water parameters in the selected sites. Important factors that affect the diatom community were water temperature, pH level, BOD, and Phosphate concentration, which are influenced by the sampling station and human interference. The significance of water quality on diatoms along the sampling sites was determined by CCA which results in larger species richness in an undisturbed area which was upstream of the Pavana River, and larger abundance of tolerant diatom species in the disturbed area which is downstream due to pollution by the anthropogenic activities. Diatoms *Phinnularia*, *Cymbella*, *Fragillarila*, and *Navicula* genus were abundant in all the sites. Characterization of the OTUs may aid to understand the complete distribution pattern. This is first reported study on the distribution pattern of diatoms in Pavana River. In nut shell, this study will aid to understand the factors affecting the distribution pattern of diatoms and predict the water environment which may also help to redeem the water quality in the future.

ACKNOWLEDGEMENT

The authors would like to acknowledge Dr. Ankur Patwardhan, Head of the Biodiversity Department and Dr. D.G. Naik for their valuable comments. The authors also like to thank Dr. Dhanashree Paranjpe for extending help on Trinocular microscope and Dr. Tejaswini Pachpor for guiding in statistical analysis.

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