Indexed in
Scopus Compendex and Geobase Elsevier, Chemical Abstract Services-USA, Geo-Ref Information Services-USA, List B of Scientific Journals, Poland, Directory of Research Journals

www.cafetinnova.org

International Journal of Earth Sciences and Engineering

April 2015, P.P.417-423

ISSN 0974-5904, Volume 08, No. 02

Freshwater Diatoms as Proxies of Assessing Environmental Changes in Reservoirs of Sri Lanka

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Abstract: Proxy indicators are used worldwide to understand the changes in the environment we live in. Among them, diatoms are well known for their species specific responses to environmental variables. Therefore this study was carried out to determine whether reservoir diatoms can be used to understand the changes in the environment. Six reservoirs selected for the study included, Bathalagoda, Bowatenne, Kurunegala and Nalanda, representing the Intermediate Zone and Kandy and Dambarawa reservoirs representing the Wet Zone of Sri Lanka. For environmental measurements of six study reservoirs, both onsite measurements and laboratory analysis were carried out at monthly intervals to understand the changes of pH, temperature, conductivity, dissolved oxygen, nitrate and phosphate for the study period (February-July 2013). For species data, plankton and sediment samples were collected using plankton net, a dipnet and a gravity corer. Relative abundance of present day diatoms and subfossil remains of diatoms were used to understand whether diatoms are preserved in sediments and whether they are sensitive to measured environmental variables. Twelve diatom species were identified from six reservoirs during the study period. Aulacoseira granulata was the most abundant diatom in all six sites. Bathalagoda, Dambarawa and Nalanda reservoirs supported a high diversity of diatoms while the lowest diversity was observed in Kandy Lake. Although a monthly variation of relative abundance of species was detected in different reservoirs, sediment analysis showed that they are well preserved at reservoir bottoms (Pearson correlation values obtained between the present day diatoms and subfossil diatoms Kurunegala-0.996, Bathalagoda-0.994, Nalanda-0.983, Bowatenne-0.999, Damabarawa–0.841 and Kandy –1.000). According to the statistical analysis we found those species specific responses to measured environmental variables and the species data helps to discriminate study reservoirs showing the importance of diatoms in tropical reservoirs for environmental predictions.

Keywords: *Diatoms, Subfossil diatoms, Environmental changes, Reservoirs*

1. Introduction

Due to high demands of human population worldwide, the environment we live is subjected to constant changes. Among these, aquatic systems are more affected as several pollutants end up especially in standing water bodies. In this regard freshwater systems associated with human communities are recognized as more vulnerable to pollution [30, 33, 39].

Diatoms are diverse eukaryotic unicellular micro-organisms characterized by siliceous cell walls [11, 26]. 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 Carbon and Silicon. Because of the short life cycle, species richness as well as species specific response to environmental factors such as nutrient concentrations [9], acid-base status [31],salinity [32], dystrophy , light [21], temperature [13], hydrological conditions [8,17], substrate character [10]and grazing [34], diatoms play a major role as biological indicators in environmental assessments [35,36].

Agricultural and domestic water requirements of 21 million human population of Sri Lanka have been fulfilled by 103 natural river basins and thousands of reservoirs since the ancient time. Water quality of many of the reservoirs is known to be affected negatively due to human activities [41]. As a result water quality of some of the reservoirs has already become seriously affected especially in highly populated urban areas. The alterations in the limnological state of these reservoirs should result in changes of their aquatic communities [40]. Already, diversions of rivers into reservoirs connecting the Wet Zone and the Dry Zone landscape have resulted in major limnological changes [12].

Because of the importance of understanding the water quality, limnological studies have been promoted by the government, industries and individuals of Sri Lanka, especially during the past two decades [12, 41]. These studies were designed mainly to understand current limnological conditions and the impacts of dams and fish introductions [12]. However, few studies have been carried out to assess whether the aquatic communities could be used to understand the water quality changes in Sri Lankan systems [40, 41, 42]. As long-term monitoring data are not available in Sri Lankan systems, indirect measurements would be the only viable option. Thus, the uses of biological indicators to predict past environmental changes have started to draw attention of environmental scientists.

The main reason for the lack of applications of diatoms as environmental indicators could be related to less availability of taxonomic data of diatoms and the lack of knowledge of the potential of preservation of diatoms in reservoirs in Sri Lanka. As reservoirs are structurally and functionally different from natural lakes it is essential to better understand the taxonomy of diatoms and their potential of preservation in reservoir environments. In addition many of the studies on diatom as indicators are based on temperate, subarctic and arctic regions with few studies on mountain natural lakes. Thus the tropical diatoms and the applications of environmental predictions will definitely help to fill gaps in the knowledge about environmental indicators. Thus our study aims to understand whether reservoir diatoms could be used as proxy indicators to assess environmental changes in Sri Lanka.

2. Materials and Methods

2.1. Study Sites

Six reservoirs, Bathalagoda(BG) (7^{o}35'60"N 80^{o}04'60"E), Bowatenne (BW) (7°40'16"N 80°39'23" E) ,Dambarawa(DM) (7^{o}20'00"N 80^{o}58'00"E), Kandy(KL) (7^{o}17'47"N 80^o 38'06"E) , Kurunegala(KG) (7^{o}29'12"N 80^{o}21'53"E), and Nalanda(NL) (7°40'00"N 80°37'00"E) were used in the study, representing two different climatic zones of the country: Kurunegala Bathalagoda, Nalanda and Bowatenne located in the intermediate climate zone (Mean annual rain fall = 1.5 to 2 m) and Kandy and Dambarawa are situated in the Wet Zone (Mean annual rain fall = 2 to–2.5 m).

2.2. Collection of biological and environmental data

Sampling was carried out monthly for a period of six months from February to July 2013. At each sampling event, five sampling sites were selected from each reservoir to represent littoral and limnetic regions of the water body. Two plankton net (34µm pore size) samples and two dip net (34µm pore size) samples were collected from each site. For sediment sampling, a gravity corer with 6cm diameter core tube was used for each reservoir. Physicochemical parameters (Temperature, pH, Conductivity, Dissolved Oxygen (DO), Phosphate and Nitrate) of each reservoir were measured for each sampling event using field instruments and laboratory analysis.

2.3. Preparation of plankton and sediment samples

Plankton samples collected, using the plankton net and dip net was filtered using a filter with pore size of 34 µm and transferred to 25 ml vials. The final solution was brought up to approximately 10 ml. These samples were preserved by adding 2 drops of Lugol's Iodine and stored under 4^{o}C until analysis. The sediment samples collected were stored at -20^{o}C in 250ml containers until analysis. Each sediment sample was divided in to three sub samples and was processed using standard acid digestion technique [25]

2.4. Identification of diatoms

A pre-prepared special cover slip and a glass slide were used to count the diversity and abundance of different diatom genera/species in the plankton samples and sediment samples. For each sample, diatom valves were identified and enumerated along the marked transects of the cover slip using a research microscope (OLYMPUS CX 31) equipped with phase contrast optics. Relative abundance for each diatom species/genus was calculated using the following formula.

Relative abundance = (number of diatom valves of a particular species/genus * 100)/Total number of diatom valves.

Identification keys prepared by Fernando (1990), Abeywickrama (1979) and Patrick & Reimer (1966, 1975) were used to identify diatoms to possible taxonomic levels. In addition photo micrographs of diatoms available in international data bases [2, 3] were also used for confirmation of the identification.

2.5. Statistical Analysis

Statistical analyses of the data obtained were carried out using Minitab (v.14) Systat (v.9) and Canoco for windows (v.4) statistical packages. The relative abundance of each diatom species/genus in water was compared with physicochemical variables and with the relative abundance of preserved taxa in sediments in the six reservoirs using Pearsons correlation coefficient. A principal component analysis (PCA) was carried out to understand the variation of sites according to the environmental variables. Canonical Correspondence Analysis, a constrained ordination technique, was used to identify the relationships among environmental variables and the diatom species [37, 38]. Finally the data was used to discriminate diatoms according to their preservation ability and then the potential of using them as indicators for environmental assessment.

3. Results

3.1. Diversity of diatoms in the six study reservoirs

Twelve diatom species were identified during the six month period (February to July, 2013) in the six study reservoirs, and among them *Aulacoseira granulata* was the dominant diatom species in all the sites. Bathalagoda, Dambarawa and Nalanda reservoirs supported a high diversity of diatoms, having 8 species out of the total of 12 species recorded during the study. Only two species of diatoms were observed in the Kandy Lake (Table 1).When considering the abundance of diatoms the highest abundance of diatoms was observed in the Nalanda reservoir.

3.2. Variation of the relative abundance of diatoms in the six study reservoirs

The most abundant diatom species observed during the study period in the six reservoirs was *Aulacoseira granulata, Surirella elegans, Cyclotella stelligera, Fragillaria* sp. and *Navicula gregaria*. *Gyrosygma* sp. was also identified and had a low relative abundance (~1%) only in some reservoirs.

Table 1: *Relative abundance of diatom species in the six reservoirs during the six months*

	KG	BG	NL	BW	DM	KL
Aulacoseira granulata	87.07	94.09	82.53	90.47	93.36	99.73
Surirella elegans	1.00	4.26	0.50	0.29	3.99	0
Synedra ulna	0	0.19	0.41	0	0.31	0
Cyclotella stelligera	6.52	0.19	1.70	3.89	0.47	0.26
Fragillaria sp.	0.62	1.02	12.59	1.19	0.39	0
Navicula gregaria	4.26	0.11	1.75	2.20	0.55	0
Cymbella lanceolata	0	0	0	0.27	0	0
Gyrosygma sp.	0	0.03	0.39	1.66	0	0
Pinnularia sp.	0	0	0.10	0	0.23	0
Amphora sp.	0	0.076	0	0	0	0
Frustulia sp.	0.50	0	0	0	0	0
Nitschia sigmoidea	0	0	0	0	0.63	0

However a variation of the relative abundance of these diatom species were observed in different reservoirs during different time periods.

3.3. Variation of the relative abundance of diatoms in the sediment

Table 2: *Relative abundance of diatoms in the sediment*

	KG	BG	NL	BW	DM	KL
A.granulata	100	76.19	84.74	100	45.83	100
S. elegans	0	4.76	10.16	0	8.33	0
S.ulna	0	6.34	1.694	0	25	0
C. stelligera	0	1.58	0	0	0	0
Fragillaria .sp.	0	1.58	1.694	0	8.33	0
N.gregaria	0	6.34	0.847	0	12.5	0
C.lanceolata	0	0	0	0	0	0
Gyrosygma sp.	0	0	0.847	0	0	0
Pinnularia sp.	0	0	0	0	0	0
Amphora sp.	0	0	0	0	0	0
Frustulia sp.	0	0	0	0	0	0
N.sigmoidea	0	0	0	0	0	0

Similar to the high diversity of diatoms in water in the Nalanda reservoir during the six months (Table 1) the diversity of diatoms in sediments was also high in Nalanda. *Aulacoseira granulata* had a 100% relative abundance in the sediment in Kurunegala, Bowatenne and Kandy (Table 2).

3.4. Variation of measured environmental variables of six study reservoirs

Among the six reservoirs distributed in the Wet Zone and Intermediate Zone of Sri Lanka a clear variation in the environmental variables (pH, Temperature, Conductivity, Phosphate, Nitrate, DO and Nitrate/Phosphate ratio) were observed (Table 3).

Table 3: *Average values of measured environmental variables of the six reservoirs during six month study period*

	Cond µs/cm)	pH	Temp (°C)	PO_4^{3-} (ppb)	NO_3^- (ppm)	DO (mg l^{-1})	NO_3^- / PO_4^{3-}
KG	154.50	6.09	29.67	51.61	0.38	8.40	7.28
BG	148.33	6.33	29.83	88.74	0.27	7.58	2.75
NL	118.67	5.85	28.17	42.46	0.26	8.19	6.13
BW	91.33	5.31	27.33	33.26	0.73	8.18	22.08
DM	239.33	5.98	28.33	78.51	0.49	8.28	6.10
KL	206.33	5.89	28.17	64.28	1.37	6.35	20.59

3.5. Variation of the relative abundance of diatoms according to environmental variables

Since *Aulacoseira granulata* was the most abundant diatom species in almost all the reservoirs during the study period and seem to have a high tolerance to environmental variables, a correlation was carried out for other abundant diatom species. According to the Pearson correlation matrix obtained for other dominant diatom species, a significant positive correlation between the relative abundance of *Surirella elegans* and the amount of phosphate was detected (Table 4).

Table 4: *Pearson correlation values on measured environmental variables and relative abundance of dominant diatoms species except Aulacoseira granulata of the 6 study reservoirs.*

	pH	Temp	Cond	PO_4^{3-}	NO_3^-	DO
Surirella elegans	0.082	0.035	0.14	0.728	0.077	0.022
Cyclotella stelligera	-0.202	0.039	0.227	0.066	-0.213	-0.222
Navicula gregaria	0.107	0.171	0.025	-0.046	-0.253	-0.505
Fragillaria sp.	0.03	-0.15	0.024	0.176	-0.448	0.041

Canonical Correspondence Analysis (CCA) explains the variation of species of diatom along the environmental gradients and also helps to separate the sites using the measured environmental variables (Figure 1).

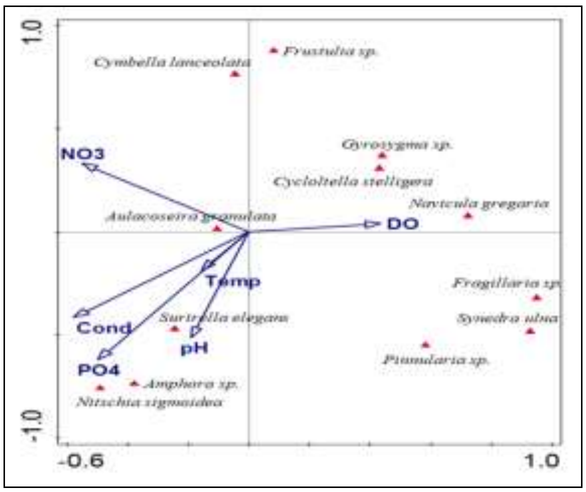

Figure1: *CCA ordination diagram of the relative abundance of diatom species according to the environmental variables.*

The relative lengths of the vectors indicated that the amount of phosphate, conductivity and nitrate were the most important environmental factors that determine the abundance of the diatom species. Temperature indicates to be the less determining factor of the relative abundance of diatoms. The position of each taxon approximates its environmental optimum relative to other taxa along the environmental gradient. The CCA of the species data yielded eigen values of 0.1828 and 0.0545 for the first two axes respectivey.CCA axis 1 and axis 2 together explained the variation of the ordination 78.98%. *Gyrosygma* sp. and *Cyclotella stelligera* plot in the right upper quadrant of the CCA plot (Figure 1) suggests that these taxa prefer low conductivity, phosphate and pH levels. In contrast *Surirella elegans, Amphora* sp. and *Nitschia sigmoidea* plot in the left lower quadrant seem to prefer reservoirs having high conductivity, phosphate and pH levels such as Bathalagoda and Dambarawa. The amount of dissolved oxygen appears to play a role in the distribution of diatom taxa. As DO mainly contribute to the first CCA axis, taxa that positioned towards the right hand quadrants of the CCA ordination likely prefer high DO levels. *Navicula gregaria* prefer high levels of DO in contrast to *Aulacoseira granulata* which prefer low levels of DO. *Fragillaria* sp., *Synedra ulna* and *Pinnularia* sp. seem to prefer reservoirs such as Nalanda having low amounts of nitrate while *Aulacoseira granulata* indicates a preference for reservoirs such as Kandy having high nitrate levels.

3.6. Relationship between diatoms in the water body and the sediment

Aulacoseira granulata in the water body was well represented in the sediment in all reservoirs. *Surirella elegans* in the water body was well represented in the sediment in Bathalagoda and Dambarawa reservoirs. A significant positive correlation was observed between the present day taxa in water and sub fossilized taxa in the sediment in all the six reservoirs, Pearson correlation value > 0.5 (Table 5)

Table 5: *Pearson correlation values for the diatoms in the water body and sediment in the six reservoirs*

Pearson correlation values	
KG	**0.996**
BG	**0.994**
NL	**0.983**
BW	**0.999**
DM	**0.841**
KL	**1.000**

4. Discussion

The six reservoirs studied vary according to their location in the climatic regions of Sri Lanka as well as according to the level of urbanization and impact of anthropogenic interferences. Despite the great historical and cultural significance, potential economic value and importance for conserving aquatic biodiversity, fresh water reservoirs are subjected to pollution and habitat degradation. Similar to terrestrial systems, changes of environmental conditions can affect biological communities in aquatic systems both negatively and positively.

The study recovered 12 species of diatoms known to be sensitive to various environmental conditions in the six reservoirs studied (Table1). The most abundant species encountered in our study was *Aulacoseira granulata* which is known to respond to trophic conditions and appear in blooms in eutrophic freshwaters around the world [20, 22]. It forms resting stages preserved in sediments for several years are capable of starting carbon fixation within 1-8 hours of exposure to moderate light [29].

Similar to the high diversity of planktonic diatoms in Nalanda ,Bathalagoda and Dambarawa a high diversity of diatoms was observed in the sediment in the same three reservoirs indicating that the conditions in those three reservoirs supported preservation of diatoms in the sediments as well. When considering these reservoirs all three of them are located in sub urban areas and therefore may have a less disturbance in the sediment. In addition bathymetry of the reservoir may also have contributed to the preservation of diatoms where all three reservoirs have funnel shape bottoms and bottom anoxia, a condition that do not support biological degradation [40]. On the other hand poor preservation of diatoms exists as a result of silica dissolution and valve fragmentation in both freshwater and saline systems in the presence of alkaline conditions under tropical climate in shallow systems, when opaline silica becomes susceptible to dissolution [5, 6, 15, 19, 28]. Since all of our reservoirs contained water with pH less than 7, the conditions for preservation of diatoms should be ideal.

The statistical analysis of diatom species (present day and subfossil) and the measured environmental variables did not show any significant correlations during the study period. As *Aulacoseira granulata* was the most abundant species in the six study reservoirs during 6 months of study period which is known to have a wider tolerance to various environmental conditions, a correlation between species and environmental variables can hardly be expected, especially when a limited number of environmental variables are measured. The situation was similar for other dominant taxa, *Cyclotella stelligera*, *Navicula gregaria* and *Fragillaria* sp. Though a correlation was not observed between the taxa and environmental variables in our study, diatoms are identified as the best biological indicators to address various worldwide trends of environmental conditions including eutrophication acidification and climate in broad scale.

Although the correlation analysis was not able to find a significant correlation between taxa and measured environmental variables the multivariate approach was able to find the directions of species along the gradients of environmental variables. In this context Canonical Correspondence Analysis (CCA) showed a distribution of diatom species (Figure1). *Surirella elegans* seems to prefer environmental conditions that exist in Bathalagoda and Dambarawa reservoirs in which excessive amounts of PO_4^{3-} and conductivity were recorded. According to Ruhland (2003), *Surirella elegans* is common in eutrophic waters in even high arctic. So the study also detected similar signals of *Surirella elegans* and therefore can be suggested as environmental proxies to detect cultural eutrophication. Unlike in temperate countries very few studies have been carried out in tropical countries including Sri Lanka about diatoms as bioindicators and the related environmental conditions. Therefore, this study can be considered as a primary approach of understanding the importance of tropical diatoms in developing models to understand the temporal and spatial variation of environmental changes.

5. Acknowledgements

My sincere gratitude goes to the Department of Zoology, Faculty of Science, University of Peradeniya for providing me facilities in every way to complete my research work.

I also thank Prof. Herman Van Dam, consultancy of water and nature, Amsterdam, Netherlands for providing me with a coded check list and ecological indicator values of fresh water diatoms.

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