Environment and Ecology 37 (3B) : 1061—1066, July—September 2019
Website: environmentandecology.com ISSN 0970-0420

Study of Foraminifera Using Foldscope

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Received 2 April 2019; Accepted 6 May 2019; Published on 29 May 2019

Abstract Foraminifera are single-celled organisms inhabiting marine environment. They have shells or tests mostly composed of calcium carbonate. The biodiversity of Foraminifera is widely used in environmental studies for sea level variations, biostratigraphy, to indicate marine pollution, nature and extent of any anthropogenic or natural impacts. Foldscope is an optical paper microscope developed by Dr. Manu Prakash. It is light-weighted and easy to carry and handle making it a revolutionary invention in microscopic world. In the current study, Foldscope was used as an onsite microscopic tool to identify Foraminifera on the beach itself. Sand samples were collected from four beaches of Mumbai for further studies including pH profiling and species density analysis of Foraminifera. The samples collected from all the sites were found to be silty and sandy in nature with pH ranging from 7.5 – 8.0. The study hows density of Foraminifera was highest in the samples collected from Aksa beach. Foraminifera belonging to 21 genera were recorded most common from the sand samples. The authors are thankful to Department of Biotechnology,Government of India for the Foldscope Research Grant and the Foldscopes used to carry out this study.

Keywords Biodiversity, Bioindicators, Foldscope, Protista, Foraminifera.

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Introduction

Biodiversity studies play a vital role in surveying and monitoring ecological systems. Foraminifera are unicellular testate rhizopods constituting the most diverse group of shelled microorganisms (Rao et al. 2016). They are abundant in occurrence, durable as fossils, easy to collect and separate from sand samples. The act as ideal bioindicator organisms as they are very sensitive to geological/hydrological changes and leave a microfossil record which has stratigraphic applications. Several physico-chemical parameters of the aquatic environment, anthropogenic activities and pollutants influence the distribution and abundance of forminifera (Frontalini and Coccioni 2011, Gadi and Patil 2012, Musco and Mazzola 2017).

Foldscope is a portable and versatile microscope which is constructed by folding paper. It magnifies the wonders of the microscopic world, without the bulk and expense of a conventional research microscope. Foldscope is designed to bring microscopy out of

science laboratories and into the hands of people around the world. It has borosilicate lens with 140x magnification with nearly 2 micron resolution. It has features of focus locking and field of view locking. By adjusting illumination, Foldscope can be used in bright-field, dark-field, and pseudo-phase contrast modes (Cybulski et al. 2014). This study is a preliminary analysis which aims to assess the biodiversity of Foraminifera from four sites along the Mumbai beach. The objectives of this study are to use Foldscope as a tool for identification of Foraminifera on the beaches and to collect sand samples from specific locations along four main beaches of Mumbai and analyze their pH and grain size to identify, enumerate and determine the density of Foraminifera from the samples.

Materials and Methods

Sand samples were collected from four main beaches of Mumbai viz., Aksa, Dadar, Girgaon and Marve beaches. From each site, three sub-samples were collected from three different locations. Temporary mountings of the sand samples on slides using cellotapes were done and the slides were observed through Foldscope. Sand samples were brought to the laboratory and the grain size characteristic of the soil was determined sieve analysis. 100 g of sample was sieved through a nest of test sieves stacked one above the other. The particles of size larger than the mesh size of each sieve size were retained on the sieve. The cumulative weight of all material larger than each sieve size was determined and the percentage calculated to arrive at the sand profile. The pH of the sand samples were analyzed microscopically for foraminiferal diversity (Devi and Rajashekhar 2009). The foraminiferal density was standardized to 1 as total foraminiferal number (TFN) (Nigam and Chaturvedi 2000). The Foraminifera were then kept on slide individually, sealed with coverslips and observed under Foldscope & microscope. Images were clicked using mobile phone.

Results and Discussion

The samples collected were found to be sandy/fine sandy in nature. The silt content varied from 0.3% to 0.8%. The grain size analysis of the soil samples collected from the four sample sites is shown in Figure 1. The pH of the samples were found to be as follows-Aksa-8.0, Dadar & Marve-7.5, Girgaon-7.8. The TFN (number g^{-1}) of Foraminifera ranged from 2500 to 17000 (Fig. 2). The density was highest in the sample collected from Aksa beach followed by Marve and Dadar. The least density was observed in the sample collected from Girgaon.

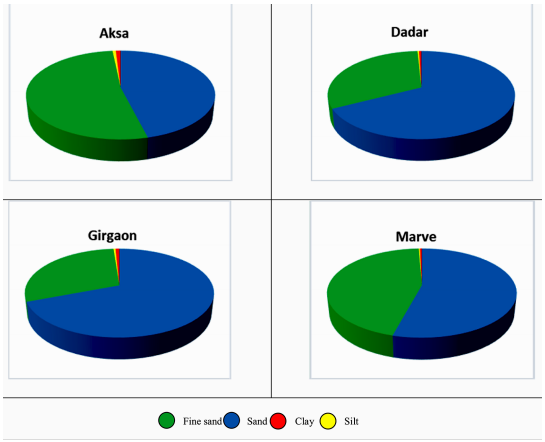


Fig. 1. Sand profiling of the samples collected from Mumbai beaches.

Foraminifera classified under the two large multi-chambered clades namely Globothalamea and Tubothalamaea were found in the study. The Foraminifera identified in the samples belonged to five different orders viz. Rotaliida, Miliolida, Robertinida, Endothyrida and Astrorhizida. Foraminifera belonging to twelve families of order Rotaliida, two families of order Miliolida, one family each of orders Robertinida, Endothyrida and Astrorhizida were identified (Pawlowski et al. 2013). The distribution of the major specified Foraminifera as determined in the study is tabulated in Table 1. On field observation of

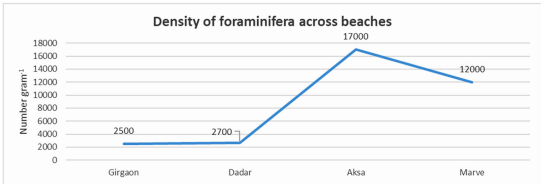


Fig. 2. Density of Foraminifera across Mumbai beaches.

Table 1. Foraminifera identified in the sand samples.

Order	Family	Genus	Aksa	Dadar	Girgaon	Marve
	Amphisteginidae	*Amphistegina* spp.			✓	
	Rotalidae	*Ammonia* spp.	✓	✓	✓	
	Calcarinidae	*Calcarina* spp.	✓			
	Cibicididae	*Cibicides* spp.	✓	✓	✓	✓
	Cymbaloporidae	*Cymbaloporetta* spp.	✓		✓	
	Discorboidae	*Discorbis* spp.		✓		✓
Rotaliida	Elphidiidae	*Elphidium* spp.	✓		✓	
	Eponidida	*Eponides* spp.	✓	✓	✓	✓
	Glabratellidae	*Glabratella* spp			✓.	
	Alabaminidae	*Oridorsalis* spp.	✓			
	Calcarinidae	*Pararotalia* spp.	✓		✓	
	Planarbulinidae	*Planorbulinella* spp.	✓			
	Rosalinidae	*Rosalina* spp.	✓		✓	
`	Rotaliidae	*Rotalia* spp.	✓	✓	✓	✓
	Siphoninidae	*Siphonina* spp.	✓			
Miliolida	Cornuspiridae	*Cornuspira* spp.		✓		
		Quinqueloculina spp.			✓	
	Hauerinidae	*Triloculina* spp.			✓	
Robertinida	Conorboididae	*Conorboides* spp.	✓	✓		
Endothyrida	Endothyridae	*Endothyra* spp.		✓	✓	
Astrorhizida	Hyperamminidae	*Hyperammina* spp.			✓	

the common Foraminifera frequently occurring was done using Foldscope and the images captured using mobile phone camera. The slides were then viewed under compound microscope in the laboratory and the images were clicked using mobile phone camera (Fig. 3).

The earliest record of Foraminifera dates back to the 5th century B. C. Various characteristics of Foraminifera can be used to evaluate sea level changes, monitoring of pollution, dating of strata, reconstruction of sedimentary environments, ecotoxicology studies, paleo environmental studies (Rao et al. 2016, Suokhire et al. 2017).

Foldscope, also known as pocket microscope, is a very handy tool which can be used for field studies. Foldscopes have magnification ranging from 140x to 1000x. The Foldscope used in this study have magnification of 140x. The collected sand samples were mounted on the slides using cellotape to check for the presence of Foraminifera. Foraminifera can be observed in dark field except few which can be observed in dim light thus, observing the sand samples through Foldscope required a little patience. Foldscopes were a convenient tool because of instant slide preparation method and handling. A very small amount of sand sample was spread on the slide to facilitate easy observation of Foraminifera. Due the boon of focus locking, images were captured using mobile camera through Foldscope for both dark and light field observation. On field, observation of the common Foraminifera occurring more than 2-3 times on the slide was convenient to identify.

Studies on relationship between natural and artificial substrate and colonization of Foraminifera have shown that Foraminifera shows substrate preferences (Burkett et al. 2016). Sediment characteristics including organic matter determine the abundance of Foraminifera. Sediments that are rich in fine sand and clay are reported to have higher diversity and density of Foraminifera. Benthic Foraminifera were reported to be more affected by sewage slude than macrofauna (Frontalini and Coccioni 2011, Mojtahid et al. 2008). The highest number of Foraminifera was found in Aksa beach sample could be correlated to its fine sandy nature. In the current study, sample from Girgaon had maximum species variety but the density was less. Gadi and Patil (2012) reported that diversity of Foraminifera was not related to TFN (g^{-1}). They have correlated a very high abundance and

diversity of Foraminifera at Juhu beach in Mumbai to the presence of high amounts of fine sand and clay in sediment composition.

pH, salinity of coastal water due to seasonal changes, fresh water influx, high concentration of polychlorinated biphenyls, iron, zinc, lead and copper in the marine environment causes decrease in foraminiferal density and diversity (Bergamin et al. 2009). For the same reason they are used as bioindicators. Ocean acidification resulting in decrease in pH may pose a threat for extinction of benthic Foraminifera (Uthicke et al. 2013). High tidal currents, high concentration of suspended matter and high tidal water influx are responsible for poor assemblage of living foraminiferal fauna. In the current study, the highest

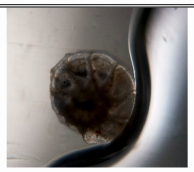
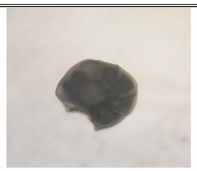
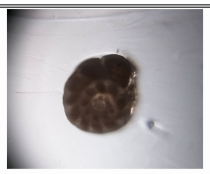
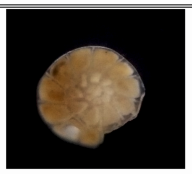
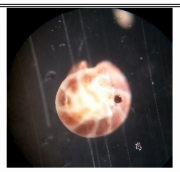
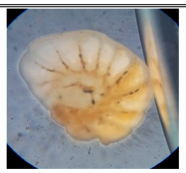
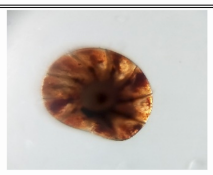
Classification	Foldscopic Image	Microscopic Image
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Calcarinoidea Family : Calcarinidea Genus: *Pararotalia*		
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Rotalioidea Family : Rotaliida Genus : *Ammonia*		
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Rotalioidea Family : Rotaliida Genus : *Rotalia*		
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Chilostomelloidea Family : Alabaminidae Genus : *Oridorsalis*		
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Discorboidea Family : Discorbidae Genus : *Discorbis*		
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Planorbulinoidea Family : Cibicididae Genus : *Cibicides*		

Fig. 3A. Images of Foraminifera captured through foldscope and compound microscope.

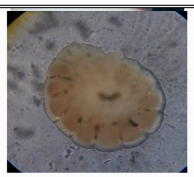
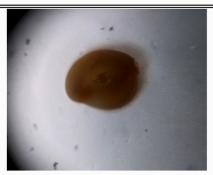
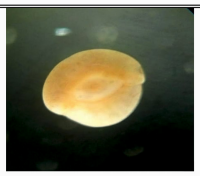
Classification	Foldscopic Image	Microscopic Image
Phylum: Foraminifera Class : Globothalamea Order : Rotaliida Superfamily : Rotalioidea Family : Elphidiidae Genus : *Elphidium*		
Phylum: Foraminifera Class : Tubothalamea Order : Miliolida Superfamily : Milioloidea Family : Houerinidae Genus : *Triloculina*		

Fig. 3B. Images of Foraminifera captured through foldscope and compound microscope.

density of Foraminifera was found in Aksa beach where pH was 8.0. In the other three sites, though the pH ranged between 7.5-7.8, there was a considerable decrease in the foraminiferal density. Godi and Patil (2012) have reported a density of 18900 (number g^{-1}) in Juhu beach of Mumbai - genera *Ammonia* being the most dominant. In the current study, *Ammonia beccarii* (Linne) was the dominant species in Girgaon, *Cibicides lobatulus* (Walker and Jones) in Dadar, *Rotalia translucens* (Phleger and Parker) in Aksa and *Oridorsalis umbonatus* (Reuss) in Marve beach. *Cymbaloporetta bradyi* (Cushman), *Planorbulinella larvata* (Clarke), *Elphidium jenseni* (Cushman), *Triloculina insignis* (Brady), *Amphistegina madagascarensis* (d'Orbigny) were found to be less frequent in their occurrence. The study of Foraminifera is used as a major tool to provide insight on the environmental conditions including marine pollution that affect their distribution and can play a role in integrated coastal zone management (Romano et al. 2008) . Use of Foldscope as microscopic tool for *in situ* observation of Foraminifera has been explored in this study. The images got through Foldscope were comparable to those from compound microscope using 10x objective and 10x cyepiece. The study will serve as a preliminary analysis providing a base line data which could be used for continuous and comparative study in the following years.

Acknowledgement

The authors thank the Department of Biotechnology, Government of India for the Foldscope Research Grant and Foldscopes used to carry out this study. The authors acknowledge the support given by the Principal & Staff, Department of Zoology, The D.G. Ruparel College of Arts, Science and Commerce, Mumbai.

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