

Unveiling the Ancient: Reconstruction of Paleoenvironment of the Mio-Pliocene Sediments through Micropaleontological Analysis

Syeda Jesmin Haque¹, Kazi Munsura Akther¹, and Asma Huque²

¹Deputy Director, Geological Survey of Bangladesh, 153 Pioneer Road, Segunbagicha, Dhaka 1000, Bangladesh. (juthi_n@yahoo.com)

²Director, Geological Survey of Bangladesh, 153 Pioneer Road, Segunbagicha, Dhaka 1000, Bangladesh

Abstract

This study aims to reconstruct the paleoenvironmental condition by studying the taxonomy and ecology of palynomorphs and diatoms and their dominance-diversity of the Mio-Pliocene sediments of Shahbazpur gas field, Bhola, Bangladesh. Seven sediment samples from different well depths have been collected and prepared for micropaleontological analysis following standard methodology. After observing the temporary slides of palynomorphs under the transmitted light microscope, all the imaged samples have been identified and an inventory has been prepared with all the taxonomical, morphological characteristics and ecological records. Data has been scrutinized for paleoenvironment and Alpha diversity by using POLPAL Diagram, and PAST 4.03 software respectively. The study found that the sediment samples contained 43 identified species of varying ecology, indicating, a fresh to brackish water marshy-estuarine, marginal area of land and sea of the transitional environment to a very early stage of the formation of the delta, tidal flat, mangrove swamp, terrestrial and freshwater floodplain environment in humid climates. In addition, evidence of grazing mammals was found at depths of 2593.21m to 2593.23m and 2591.95m to 2591.98m. Furthermore, evidence of grazing mammals and high production of herbaceous plants due to heavy monsoon rain were identified at depths of 2591.52m to 2591.55m. The ecological complexes of microfossils indicate shallow marine to fluvial-deltaic conditions with the indication of continuous shoreline regression towards the sea, developing mangrove swamps with some terrestrial flood plain area. Statistical analysis expresses shallow marine to the transitional environment in the deeper part of the well and transitional to a fluvial-deltaic condition in the shallower part of the well. Dominance is low to moderately high; the Simpson diversity index exhibits a low to moderately high degree of diversity and the Shannon-Wiener diversity index designates very low diversity. Therefore, the study describes the paleoenvironmental situation of the Shahbazpur area during the Mio-Pliocene time.

INTRODUCTION

Justification

Micropaleontological research performs a key role in the field of stratigraphy and biostratigraphy discipline for reconstructing depositional history, paleoenvironment, climate change and also the presence of hydrocarbon.

Palynological research is based on the study of spores, pollen and related organic-walled microfossils (palynomorphs), their dispersal and distribution in sediments. This is a powerful and useful tool to reconstruct the Quaternary environment (Birks and Birks, 1980).

Palynomorphs are derived from four kingdoms: protocista, fungi, plantae and animalia. These kingdoms are produced Embryophytic spores, Pollen, Dinoflagellates, Acritarchs, Microforaminiferal inner tests, Chitinozoans, Microscopic colonial algae and Scolecodonts groups of palynomorphs.

On the other hand, Diatoms are Protista or protocista types of microorganisms that are composed of silica. These organisms contain circular, elongate, or irregular valves of aquatic origin, less than 200 μm in size with a geological range of Jurassic to Recent. Similar to palynomorphs, fossil diatoms are also useful tools for paleoenvironmental and biostratigraphical analysis.

Paleoenvironmental studies provide additional information about the unique conditions associated with deposition and diagenetic processes in the past. A thorough understanding of paleoenvironments enhances our ability to recover valuable resources. In particular, paleoenvironmental considerations have been an essential component in our exploration of fossil fuels (Kennedy, 1998). Furthermore, the climatic conditions also can be identified from this type of study.

These interpretations, particularly paleoclimatic, depend on the principle of uniformitarianism, the doctrine that the present is the key to the past and therefore that ancient and modern environment can be compared by analogy. The reconstruction of paleoenvironments is in high demand, not only for the understanding of past environmental, climatic changes and past ecology but also to provide reference data to aid the assessment of the ecosystem responses to changing climate modes and conditions in the future.

This study focuses on the identification of microfossils (palynomorph and diatom), delineates the paleoenvironment, dominance-diversity of the microspecies.

The core rock samples of the reservoir strata were collected from the Chittagong Regional Office of Bangladesh Petroleum Exploration & Production Company Limited which belongs to Shahbazpur gas field (well 2), Bhola district.

Objectives of the Study

The objectives of the Study are:

- To identify palynomorph and diatom species on the basis of morphological characteristics.
- To interpret the paleoenvironment and depositional environment of the samples by emphasizing the identified species.
- To compare paleoenvironmental situations among the different layers of the well.
- To analyze the dominance, diversity and evenness of different species.

Study Area

The Shahbazpur gas field in Bhola Island is one of the commercial gas fields of Bangladesh. It is the southernmost on-shore gas field located in island district Bhola under Barisal division and in the exploration Block no. 10 of Bangladesh. The gas field area belongs to Borhanuddin Upazilla which is about 34km from Bhola town and 150 km south from the capital Dhaka (Bapex, 2016; Rahman *et al.*, 2016) (Figure-1). The Shahbazpur structure is located in the central part of the Bhola Island amid the southern part of the platform slope and the northern margin of Hatiya trough of Bengal Foredeep of the Bengal Basin (Rahman *et al.*, 2016). This island is surrounded by mighty Meghna to the North and East and Tetulia River to the West, while the island is open to Bay of Bengal to the south.

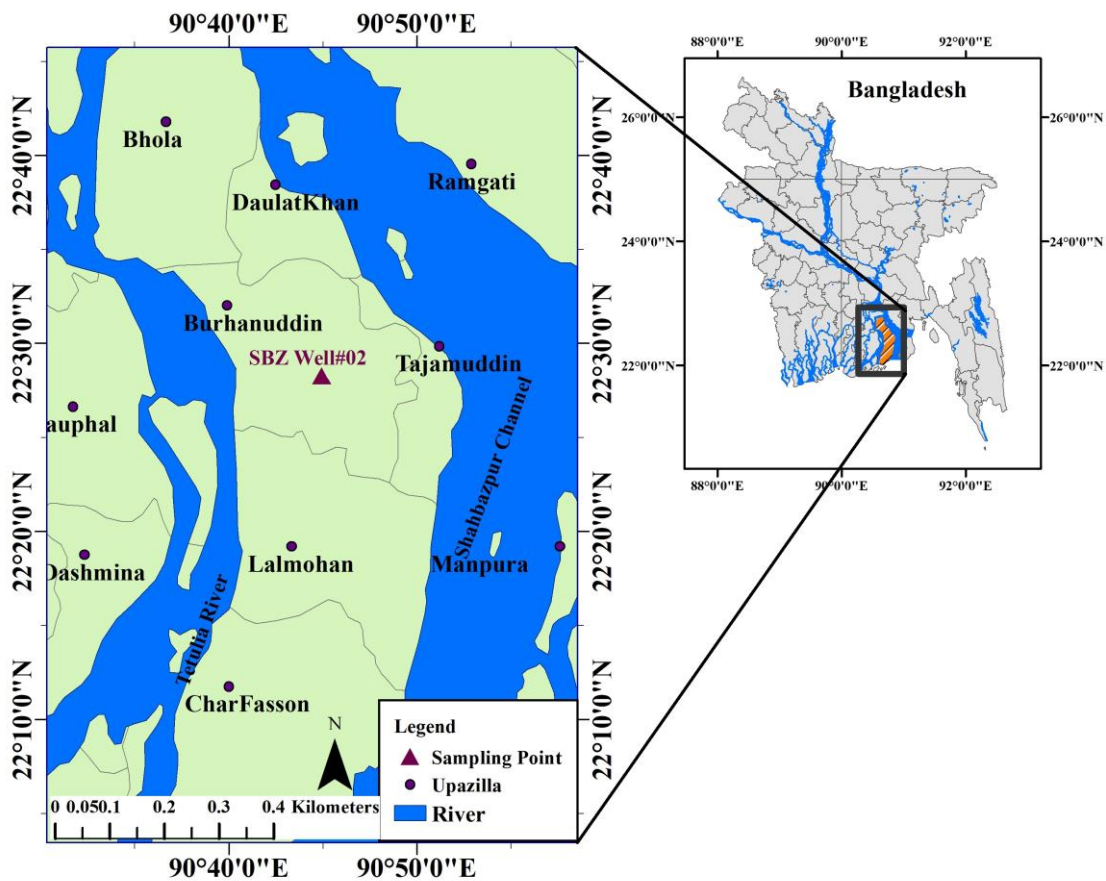


Figure-1. Map showing the location of the study well in the Shahbazpur Gas Field, Bhola districts.

Stratigraphically the fluvial-deltaic Surma Group of the Bengal Basin is generally considered the reservoir rocks of the Shahbazpur structure (Hasan *et al.*, 2020; Rahman *et al.*, 2011). The age of these reservoir rocks probably extends from Late Miocene to Early Pliocene (Rahman *et al.*, 2011; Alam *et al.*, 2003). This study mainly focuses on the paleoenvironmental implication identified from the recovered microfossils (palynomorph and diatom) of this group

(Table-1). Figure 2 shows simple litho-log of SBZ well 2 and Table-2 describes the Lithological condition of the collected samples.

Table-1. Stratigraphy and lithology of the Shahbazpur area (Collected from Rahman *et al.*, 2018; Modified after Mondal *et al.*, 2009, Roy and Roser, 2012).

Depth (m)	Age	Group	Lithology	Depositional Environment
0-480	Recent	Alluvium	Loose unconsolidated sand with occasional clay	Fluvial Plain
480-1505	Pleistocene	Dupi Tila	Shale with occasional occurrence of interbedded sandstone and calcareous siltstone	Deltaic to Fluvial plain
1505-2010	Pleistocene-Pliocene	Tipam	Shale and sandstone	
2010-2750	Plio-Miocene	Surma	Sandstone and shale	Deep sea to shallow marine
2750-3631	Miocene		Sandstone and shale	

Table-2. Lithological description of the collected samples (Core# Core Number).

Depth (m)	Sediment Characteristics
2591.52m-2591.55 (C#1)	Light to dark grey color, moderately consolidated, Silty sandstone unit with Bluish grey color, hard and compact, laminated, slightly calcareous shale.
2591.95m-2591.98 (C#1)	
2593.21m-2593.23m(C#1)	
3259.80m (C#2)	Light grey to grey color, moderately consolidated, calcareous, sandstone unit with thin grey to dark grey, well laminated, moderately hard and compact, calcareous shale bed.
3260.6m-3260.62m (C#2)	
3265.66m-3265.63m (C#2)	
3268.25m-3268.28m (C#2)	

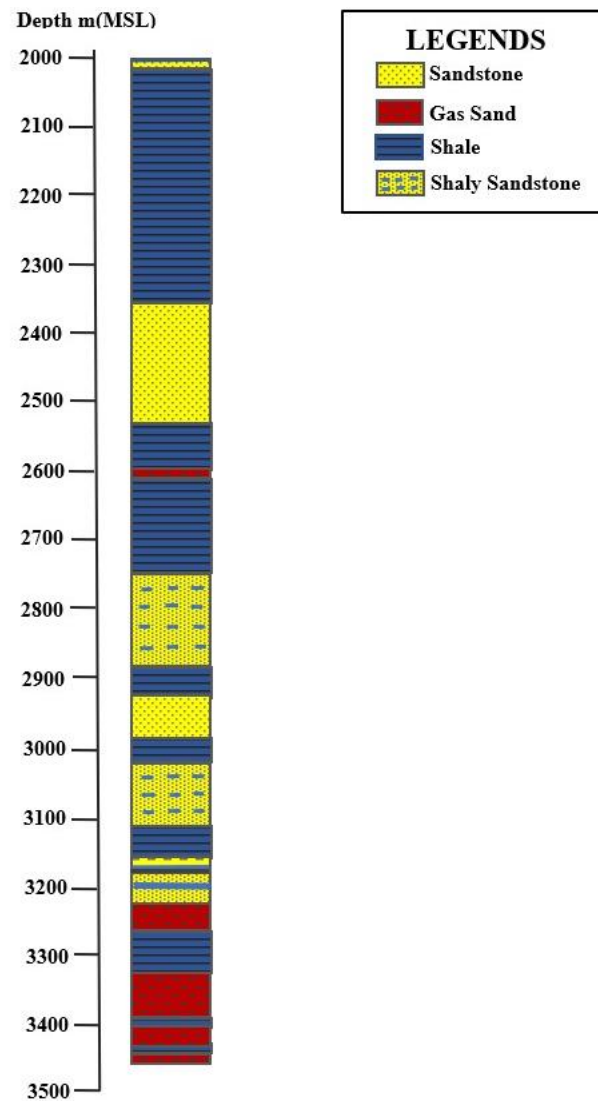


Figure-2. Litholog of the SBZ Well 2 (Source; Bapex, 2016)

METHODOLOGY

Methods of the study are divided into several heads on the basis of different procedures:

1. Field Techniques.

a) Sample Collection.

2. Laboratory Techniques.

a) Sample Processing.

b) Slide Preparation.

c) Microscopic Observation.

3. Identification and Interpretation.

a) Identification.

b) Paleo-environmental analysis.

c) Statistical analysis.

1. Field Techniques.

a. Sample Collection

All the samples of the wells have been collected from BAPEX zonal office Chattagram with relevant data and literature. Shale or clay samples from the reservoir strata are collected from different depths of the well log to identify the micropaleontological diversity. Samples are stored in zip-lock poly bags to avoid unnecessary contamination with appropriate labeling.

2. Laboratory Techniques.

a) Sample Processing

Seventeen samples of two wells with different depths have been prepared in the laboratory by following standard methodology (Traverse, 1988; Traverse, 2007a; Traverse, 2007b; Bercovici and Vellekoop, 2017). One gm of each sample has been collected and heated with KOH, then centrifuged, and decanted until the water is clean. Treatment of liquid samples with HF and HCl (10%) has been carried out at high temperatures to ensure the removal of carbonates and silicates respectively. Following each action with HF and HCl, the samples are washed thoroughly with distilled water in a centrifuge machine and then decanted respectively. To avoid processing-induced bleaching of the polymorphs, no oxidation (Acetolysis treatment or nitric acid treatment) was performed (Pross *et al.*, 2007). After the process alcohol has been added to remove water and then added 1ml of toluene. To avoid the staining of samples no staining method has been followed. After then dry the samples by placing them into a fumer for some time. Finally, processed samples are stored in small vials with glycerol gel for slide preparation.

To conduct Diatom analysis, the samples were processed using hydrochloric acid (10% HCl) and hydrogen peroxide (30% H₂O₂). The samples were heated for the reaction and then allowed to cool for a few minutes. Finally, the samples were washed thoroughly with distilled water (Renberg, 1990).

b) Slide Preparation

Stored processed samples have been used to prepare palynological slides for optical microscopy observation. The temporary mounts have been made by using glycerol jelly as a mounting medium.

c) Microscopic Observation

After slide preparation, all the samples have been observed at magnifications of $\times 400$ and $\times 1000$ using Carl Zeiss Axio Lab. A1 biological microscope with fluorescence attachment. Photographs of the palynomorphs and diatom species have been captured with an Axiocam 105 colour camera attached to the microscope and processed with the help of ZEN 2.3 lite (blue edition) software. Different species are counted and all the observed information with morphological characteristics has been documented in a data sheet.

3. Identification and Interpretation.

a) Identification

Species of different palynomorphs and diatoms are identified on the basis of visual morphological characteristics by following different kinds of literature and available catalogues. Identified species of palynomorph and diatoms are categorized on the basis of their family, types (like; fern, herb, shrub, tree, fungi, algae, dinoflagellate, foraminifera, diatom) and habitat disclosed from different kinds of literature and online sources. An inventory has been prepared with these characteristics along with the morphological characteristics of the species.

b) Paleoenvironmental analysis

A checklist of taxa considering Palynological and diatom related ecological complexes has been prepared and delineated the depositional environment, paleoclimate considering the sample depth and lithological composition. Species Composition has been calculated by following the formula below:

Species Composition:

$$\text{Composition (\%)} = (n/T) * 100$$

(n= Number of individual species, T= Total number of species)

All the calculated data has been plotted on a diagram (pie chart), in terms of palynomorph type and diatom, and frequency diagram (bar diagram) of different samples, has been constructed with the support of MS Excel application. A comparative study has been done by drawing the concentration of the identified palynomorph and diatom in the MS Excel bar

diagram of different depths of two wells. A simple Profile diagram of palynomorph and diatom presenting temporal changes of species has been constructed by using the online free software POLPAL Diagram (v. 10.12) (Nalepka and Walanus, 2003).

c) Statistical analysis

The statistical analysis of the data collected from SBZ well 02 has been done by the online free software PAST 4.03. Alpha diversity has been described in this analysis. Alpha diversity defines the species diversity or species richness in an ecosystem. It is a small-scale measure. Near about fifteen parameters are calculated by the software but for the target of a simple interpretation, six parameters have been described.

Diversity Measures:

Richness: Number of species at a site

Evenness: Relative abundance of species at a site.

Diversity indices:

Dominance Indices: Dominance indices are weighted toward the abundance of the commonest species.

A commonly used dominance index is Simpson's diversity index. It considers both richness and evenness.

Simpson's diversity indices

The Simpson's index (D) (introduced by Simpson, 1949)

$$D = \sum p_i^2$$

Where, p_i is the proportion of individuals in the i -th species.

The formula for calculating Simpson's index (D) is:

$$D = \sum (n/N)^2 \quad (\text{where, } n = \text{the total number of individuals of each species and } N = \text{the total number of organisms of all species.})$$

or,

$$D = \sum (n_i * (n_i - 1)) / (N * (N - 1)),$$

where: n_i — Number of individuals in the i -th species; and

N — Total number of individuals in the community.

Simpson's index is one of the most popular and strong ways to measure diversity in a community; as D increases, diversity decreases.

Simpson's index of diversity formula:

According to the original formula proposed by Simpson, a higher D value suggested a community with low biodiversity. This is neither intuitive nor logical, as typically, a high diversity index should suggest a more diverse community. Hence, the formula usually express Simpson's diversity index as $1 - D$, i.e.,

Simpson's index of diversity = $(1 - D) = 1 - [\sum(n_i * (n_i - 1)) / (N * (N - 1))]$

The Simpson index of diversity score varies between 0 and 1 (Table-3). As the number of different species increases and the population distribution of species becomes more even, the diversity index increases and approaches one.

Table-3. Guidelines for Interpreting Simpson Diversity Index Scores (Simpson, 1949).

Simpson Score	Interpretation
0.00	Absence of diversity (homogeneity)
0.01-0.40	A low degree of diversity/heterogeneity
0.41-0.60	A moderate degree of diversity/heterogeneity
0.61-0.80	A moderately high degree of diversity/ heterogeneity
0.81-0.99	A high degree of diversity/ heterogeneity
1.00	Absolute (perfect) diversity/ heterogeneity

Shannon diversity index

The Shannon diversity index (the Shannon–Wiener diversity index) is a popular metric used in ecology. It's based on Claude Shannon's formula for entropy and evaluations species diversity.

Shannon diversity index Formula:

The index considers the number of species living in a habitat (richness) and their relative abundance (evenness) (Shannon and Weaver, 1949).

$$H = -\sum[(P_i) * \log (P_i)],$$

where: H - Shannon diversity index;

p_i - Proportion of individuals of i-th species in a whole community;

$$p_i = n / N,$$

where: n - individuals of a given type/species; and

N - total number of individuals in a community,

Σ - Sum symbol; and

log - Usually the natural logarithm, but the base of the logarithm is arbitrary (10 and 2 based logarithms are also used).

Table-4. Classification scheme for the Shannon Diversity Index (Fernando, 1998 (Unpublished)).

Relative Values	Shannon–Wiener diversity index (H)
Very high	3.50 and above
High	3.00-3.49
Moderate	2.50-2.99
Low	2.00-2.49
Very low	1.99 and below

The higher the index, the more diverse the species are in the habitat. If the index equals 0, only one species is present in the community (Table-4).

MAJOR FINDINGS

Identification of Species

Mio-Pliocene sediment samples collected from two cores of SBZ Well 02 contain about 43 species with some broken and unidentified non-pollen palynomorphs. Plate A and B shows some images of the identified microfossils.

Identified palynomorphs from the analyzed sample at the depth of 3268.25m to 3268.28m of core 2, belong to Amaranthaceae, Cyperaceae family pollen; Trichosphaeriaceae, Botryosphaeriaceae family fungi; Pleurosigmales family diatom and Zygnemataceae family freshwater algae. The collected sample from the depth of 3265.63 m to 3265.66m contains pollen and spores belonging to six families; they are Cannabaceae, Arecaceae family pollen; Trichosphaeriaceae, Periconiaceae, Coniochaetaceae family fungi; Pteridaceae family fern and some unidentified diatoms and fungi. Analyzed sample from the depth of 3260.6 m to 3260.62m of core two shows limited palynomorphs, only from two types of species which are reported from Trichosphaeriaceae family fungi and Zygnemataceae family algae. Sample at the depth of 3259.8 m of core two contains very small palynomorphs which are identified from Arecaceae family pollen and Coniochaetaceae, Trichosphaeriaceae family fungi.

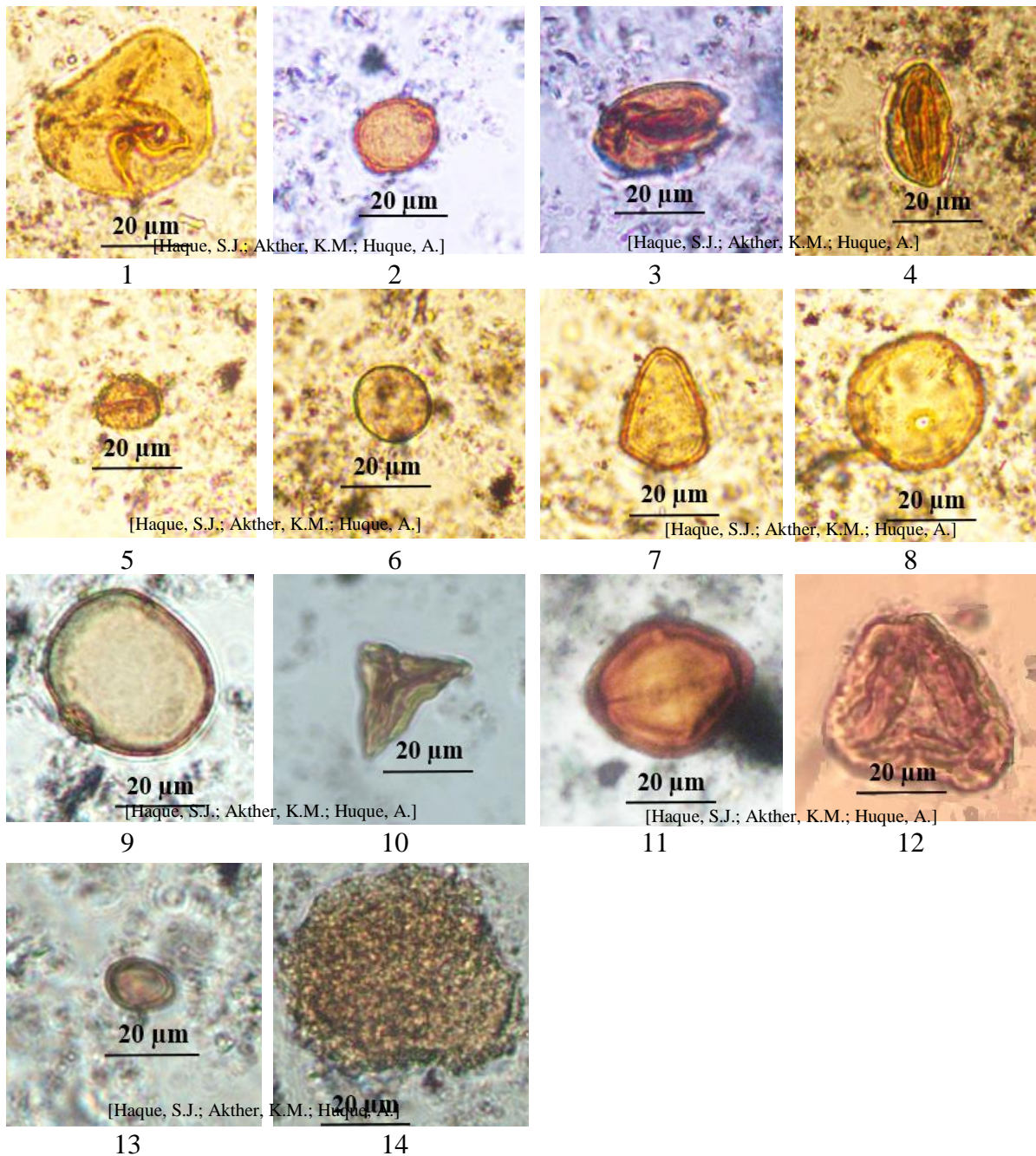


Plate A: Name of the Identified species with Family (Under Transmitted Light Microscope).
Miospores 1. Family: Poaceae; 400X; 2. *Morus alba* (L.) Vent.; 400X; Family: Moraceae.; 3. *Larix* sp.; 400X; Genus: *Larix* Mill.; Family: Pinaceae. 4. *Phoenix* sp.; 400X; Genus: *Phoenix* L.; Family: Arecaceae. 5. *Nenga pumila*; 400X; Family: Arecaceae. 6. *Amaranthus spinosus* L.; 400X; Family: Amaranthaceae. 7. *Carex* sp.; 400X; Genus: *Carex* L.; Family: Cyperaceae. 8. *Oryza* sp.; 400X; Genus: *Oryza* L.; Family: Poaceae. 9. *Triticum sativum* Lam.; 400X; Family: Poaceae. 10. *Ceratopteris* sp.; 400X; Genus: *Ceratopteris* Brongn; Family: Pteridaceae. 11. *Terminalia catappa* L.; 400X; Family: Combretaceae. 12. *Adiantum* sp.; 400X; Genus: *Adiantum* L.; Family: Pteridaceae. **Freshwater Algae:** 13. *Zygnema* sp.; Zygosporae; 400X; Genus: *Zygnema* C.Agardh, 1817; Family: Zygnemataceae. 14. Plant stomata; 400X.

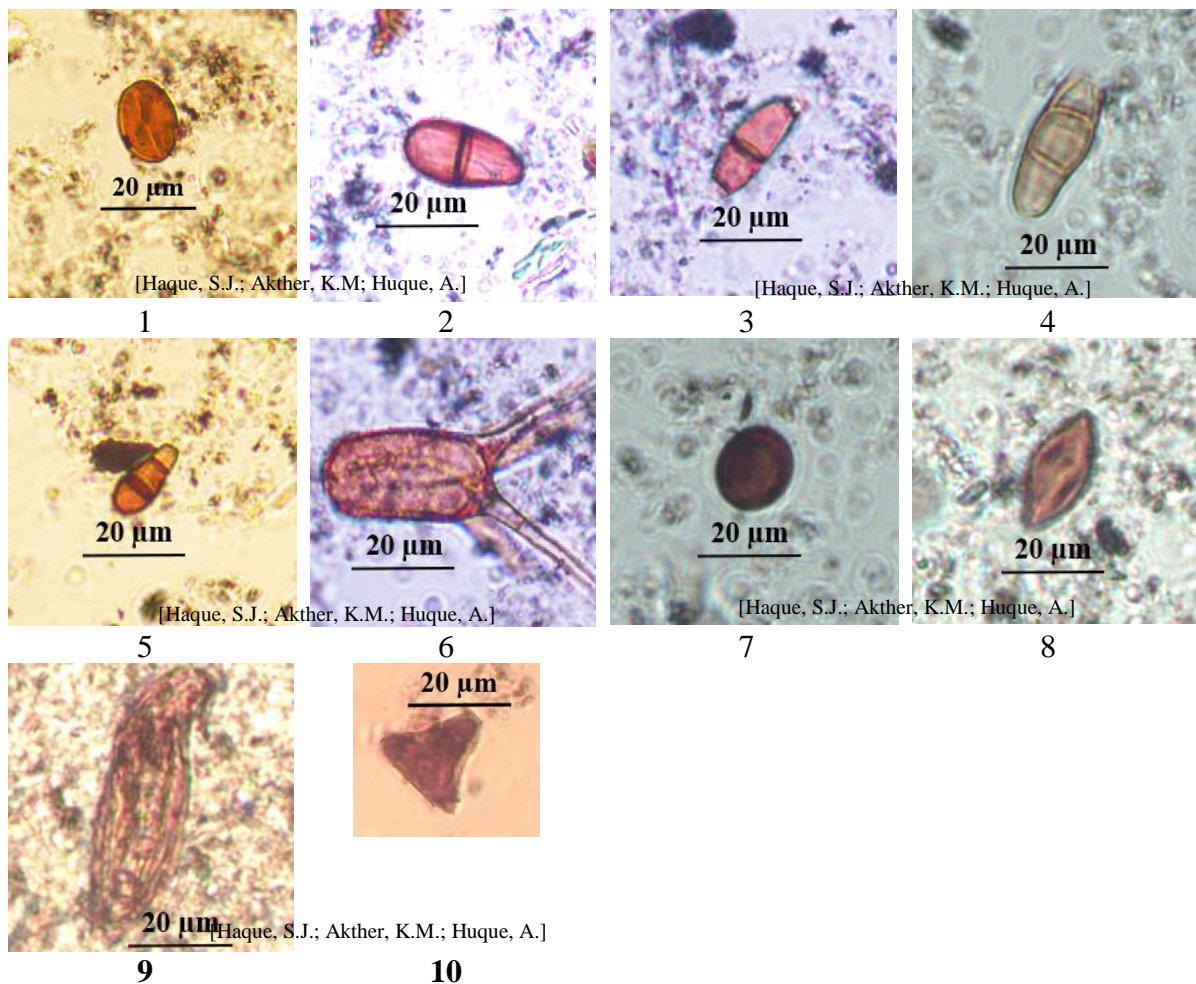


Plate B: Name of the Identified species with Family (Under Transmitted Light Microscope).

Fungi: 1. *Coniochaeta sp.*; 400X; Genus: *Coniochaeta* (Sacc.) Cooke; Family: Coniochaetaceae. 2. *Cookeina Meliola*; 400X; Family: Sarcoscyphaceae. 3. *Cladosporium sp.*; 400X; Genus: *Cladosporium* Link, 1816; Family: Davidiellaceae. 4. *Curvularia sp.*; 400X; Genus: *Curvularia* Boedijn; Family: Pleosporaceae. 5. *Alternaria sp.*; 400X; Genus: *Alternaria* Nees ex Wallroth, 1816; Family: Pleosporaceae. 6. *Tetraploa sp.*; 400X; Genus: *Tetraploa* Berk. & Broome, 1850; Family: Tetraplosphaeriaceae. 7. *Nigrospora sp.*; 400X; Genus: *Nigrospora* Zimm; Family: Trichosphaeriaceae. 8. *Arniium sp.*; 400X; Genus: *Arniium* Nitschke ex G.Winter; Family: Lasiosphaeriaceae. **Diatom:** 1. *Gyrosigma sp.*; 400X; Genus: *Gyrosigma* A.H.Hassall, 1845; Family: Pleurosigmatataceae. 2. Unidentified diatom; 400X.

Samples collected from core one at the depth of 2593.21 m to 2593.23m contain a very limited amount of palynomorphs that are belonging to the Arecaceae, Cyperaceae, Moraceae family pollen and Trichosphaeriaceae, Sordariaceae, Sarcoscyphaceae family fungi. A comparatively remarkable amount of palynomorph is revealed at the depth of 2591.95 m to 2591.98m. The species are identified from Combretaceae, Apiaceae, Piperaceae, Rhizophoraceae family (pollen), Trichosphaeriaceae, Sarcoscyphaceae, Coniochaetaceae, Lasiosphaeriaceae,

Pleosporaceae, family (fungi), Pteridaceae family (fern) and Zygnemataceae family (algae). Abundant palynomorphs with diverse species are observed in the sample at the depth of 2591.52m to 2591.55m of core one. Pollen samples are identified from Combretaceae, Moraceae, Pinaceae, Arecaceae, Amaranthaceae, Poaceae, Apiaceae, Cyperaceae and Rubiaceae families. Fungi from Sporormiaceae, Periconiaceae, Coniochaetaceae, Sarcoscyphaceae, Davidiellaceae, Pleosporaceae, Botryosphaeriaceae, Roussoellaceae, Tetraplosphaeriaceae family, fern from Pteridaceae family and algae from Zygnemataceae family are also being identified.

Paleoenvironmental Reconstruction

Ecological complexes related to palynomorphs-diatoms and a checklist of taxa from subsurface sediments of the study well have been arranged with collected information (Table-5). Complete paleoenvironmental delineation has been done with the help of this checklist.

Depth: 3268.25m to 3268.28m of core 2,

- Contains a rich amount of palynomorph and organic debris concentration.
- Dominant Amount of fungal remains (91%) and a very sparse amount of herb, fern and algae (with diatom) present in the sample (Figure-3a, Figure-3b).
- Floodplain herbs of Cyperaceae and Amaranthaceae (*Amaranthus sp.*) family, freshwater fern *Zygnema sp.* (zygospore) with fresh and brackish water diatom *Gyrosigma sp.* exist in a very few amounts. Decaying plant material and soil habitat *Nigrospora sp.* is very common in this sample.

All the evidence suggests **a very warm and wet climate in a fresh to brackish water marshy land (few terrestrial deposit).**

Depth: 3265.66m to 3265.63m

- Contain a reasonable amount of palynomorph and amorphous organic debris.
- A very limited number of trees (6%), shrubs (4%) and fern (2%) with diatom (2%) (Figure-3c) have been identified. Concentration of fungi is nearly 86% (Figure-3d).
- Along with fungi tree pollen from Arecaceae and shrub pollen from Cannabaceae family are common. Few amounts of *Trema orientalis and Arecaceae* (tropical, terrestrial habitat) pollen with moist soil fern *Adiantum sp.* is identified from this sample. Lots of *Nigrospora sp.* together with a few *Periconia byssoides* and *Coniochaeta sp.* with some unidentified brackish water diatom shows the **indication of land and near-shore environment with tropical, wet climate.**

Table-5. Palynological and diatom related ecological complexes and a checklist of taxa from subsurface sediments of Shahbazpur Well 02.

Freshwater-Floodplain	Freshwater	Inland plant complex	Shoreline plant complex	Mangrove
Poaceae; <i>Carex sp.</i> ; <i>Oryza sp.</i> ; <i>Triticum sativum</i> ; <i>Saccharum officinarum L.</i> ; <i>Dentella repens</i> ; <i>Peperomia sp.</i> ; Cyperaceae;	<i>Zygnema sp.</i> ,	<i>Phoenix sp.</i> ; <i>Amaranthus spinosus</i> ; <i>Eryngium foetidum</i> , <i>Terminalia arjuna</i> ; Plant stomates; <i>Ficus sp.</i> ; <i>Trema orientalis</i> ; <i>Amaranthus sp.</i> ;	<i>Morus alba</i> ; Arecaceae (Unidentified)	<i>Phoenix paludosa</i> ; <i>Carex sp.</i> ; <i>Ceriops decandra</i> ; Rhizophoraceae;
Brackish water/littoral	Fungal complex	Fern complex	Diatoms	Transported
<i>Nenga pumila</i> ; <i>Terminalia catappa</i> ;	<i>Sporormiella sp.</i> ; <i>Periconia byssoides</i> ; <i>Coniochaeta sp.</i> ; <i>Cookeina Meliola</i> ; <i>Cladosporium sp.</i> ; <i>Curvularia sp.</i> ; <i>Alternaria sp.</i> ; <i>Diplodia magnoliigena</i> ; <i>Neoroussoella leucaenae</i> ; <i>Tetraploa sp.</i> ; <i>Nigrospora sp.</i> ; <i>Cookeina sp.</i> ; <i>Arniium sp.</i> ; <i>Sordaria sp.</i> ;	<i>Ceratopteris sp.</i> ; <i>Adiantum sp.</i> ; Monocolpate, bean shape spore; Unidentified, Broken Fern;	Unidentified diatom, <i>Gyrosigma sp.</i> ;	<i>Larix sp.</i> ;

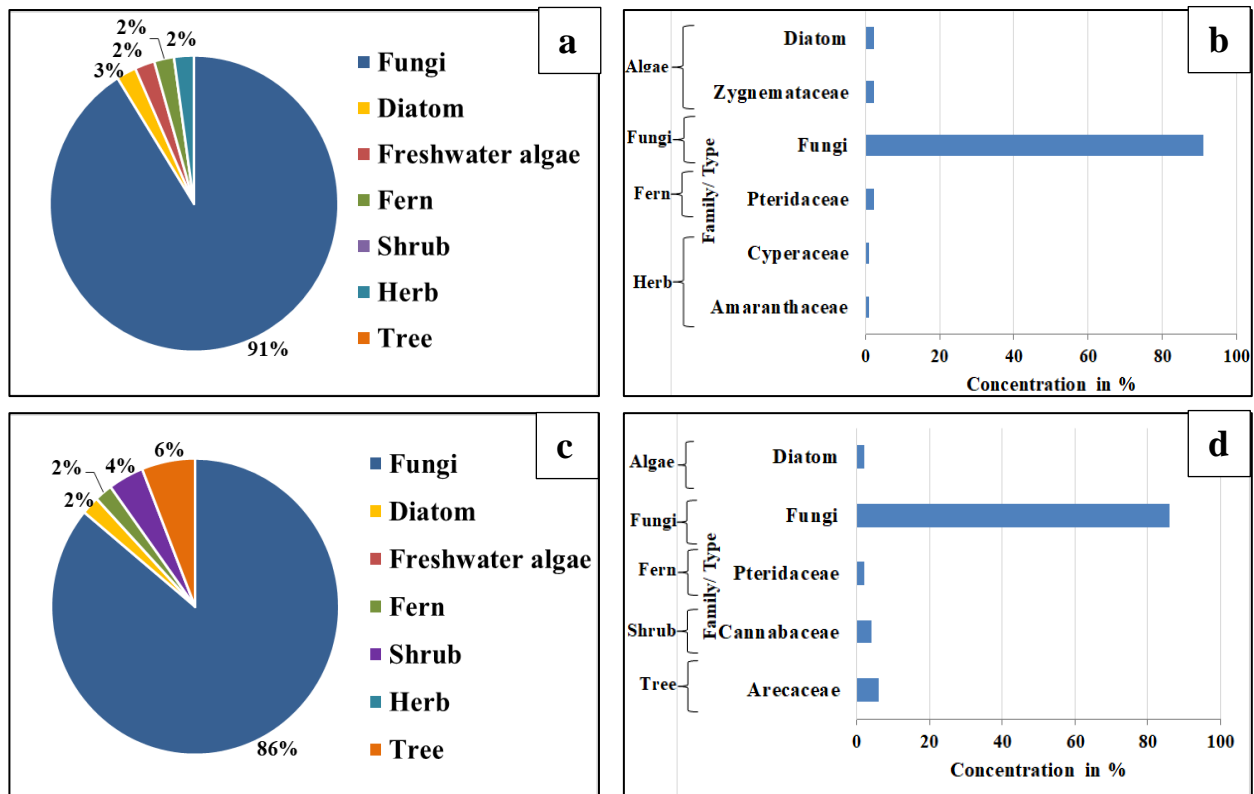


Figure-3. Species Composition (pie chart), in terms of palynomorph type and diatom, and frequency diagram (bar diagram) considering species family of SBZ Well 02, Core 2, depth 3268.25-3268.28m (a, b) and 3265.66m-3265.63m (c, d).

Depth: 3260.6m to 3260.62m,

- Contains a very limited amount of palynomorphs.
- Among them, a large number of fungi (97%) and a sparse number of freshwater algae (3%) indicate a sudden change in biota at this depth (Figure-4a and Figure-4b).
- Evidence of terrestrial plants along with floodplain pollens is absent at this depth. The abundant concentration of freshwater algae *Zygnema sp.* (zygospore) and decaying plant fungi *Nigrospora sp.* indicates **a freshwater to nearly brackish water estuarine environment of deposition with a hot and humid climate.**

Depth: 3259.80 m core-2

- A very small amount of palynomorphs have been identified.
- About 80% of fungal remains and a sparse amount of terrestrial tree (Arecaceae) pollen have been recognized (Figure-4c and Figure-4d).
- An abundant amount of dung fungi *Coniochaeta sp.* and *Nigrospora sp.* which are grown on decaying plant material and soil reveals the initial stage of land development with a very tiny amount of terrestrial plant *Phoenix sp.* (Arecaceae). At this stage, the change of biota is noticeable which indicates **a marginal area of land and sea with a hot and humid climate.**

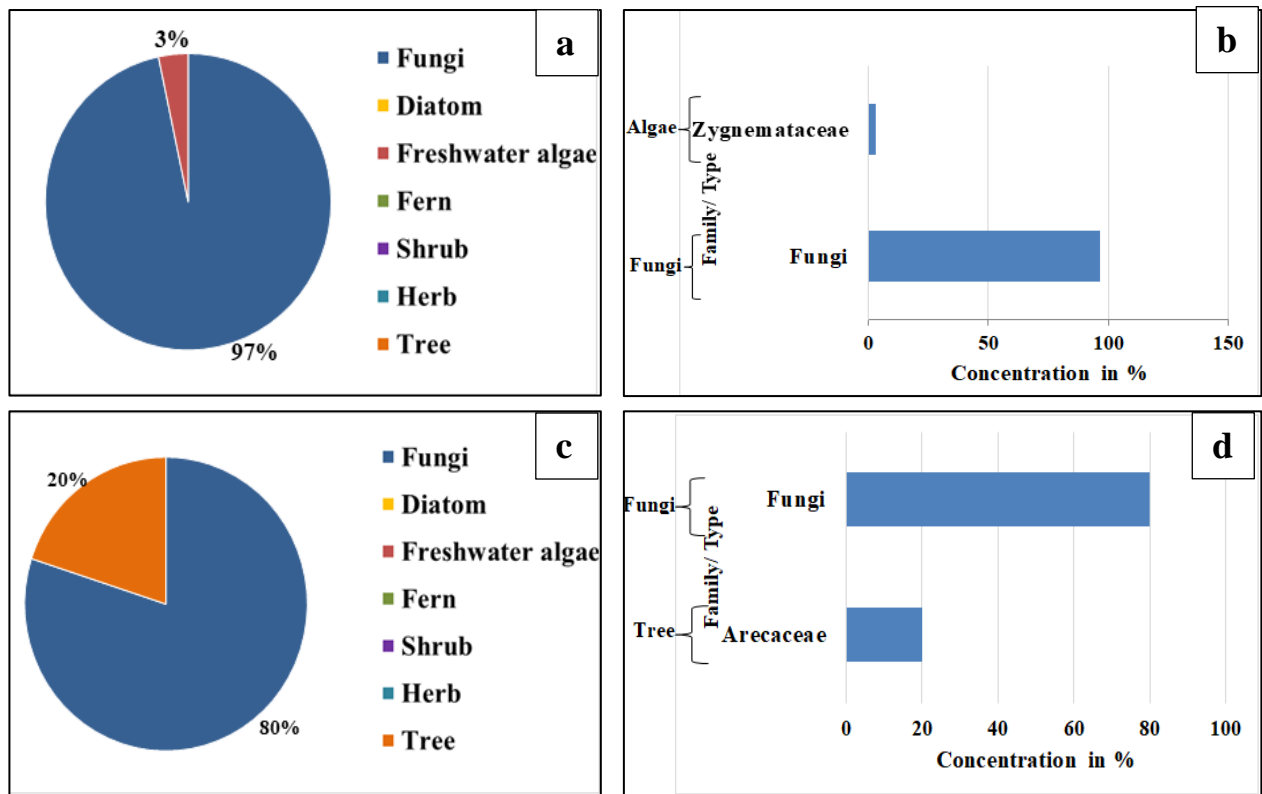


Figure-4. Species Composition (pie chart), in terms of palynomorph type and diatom, and frequency diagram (bar diagram) considering species family of SBZ Well 02, Core 2, depth 3260.6-3260.62m (a, b) and 3259.80 m (c, d).

Depth: 2593.21m to 2593.23m of core 1

- A small amount of palynomorphs is recovered from this depth.
- A huge accumulation of fungal remains (62%) and a very small amount of tree (13%), herb (13%) and shrub (12%) pollens have been identified in samples from the sample (Figure-5a). In the frequency diagram (Figure-5b) the highest frequency of palynomorphs is fungi.
- Identified tree-type pollen belongs to Moraceae (13%) family and shrub-type pollen only in the Arecaceae (12%) family. Weed-type herb pollen is also recognized from the Cyperaceae family. A small amount of Cyperaceae and *Ficus sp.* indicate an early stage of floodplain formation. *Phoenix paludosa* designates the very initial stage of mangrove swamp formation. Coprophilous fungi *Cookeina Meliola*, *Sordaria sp.* and the presence of *Nigrospora sp.* show the presence of animals. Identified species suggest the very **initial stage of a transitional environment which contains delta, tidal flat and mangrove swamp.**

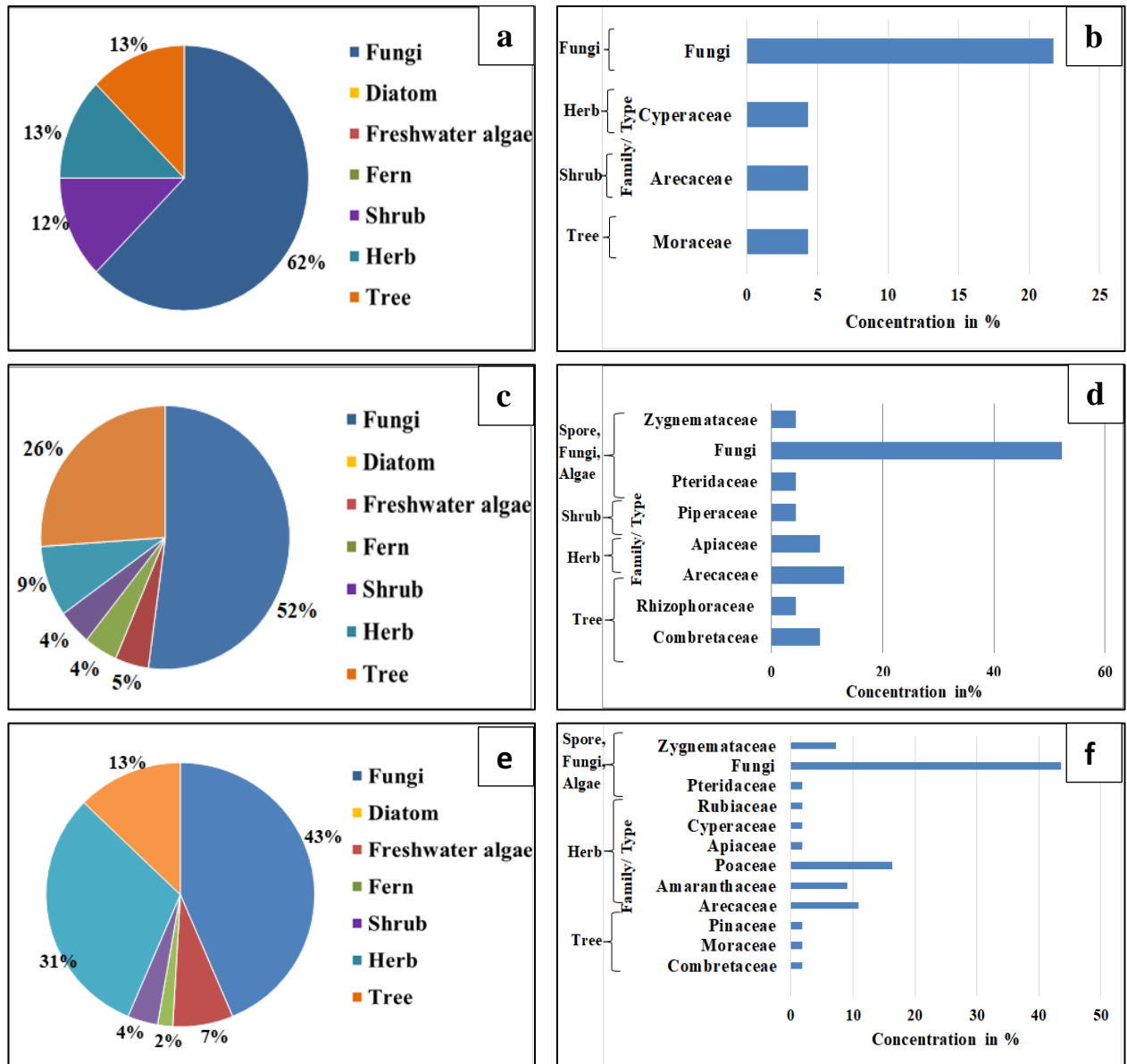


Figure-5. Species Composition (pie chart), in terms of palynomorph type and diatom, and frequency diagram (bar diagram) considering species family of SBZ Well 02, Core 1, depth 2593.21m to 2593.23m (a, b), 2591.95m to 2591.98m (c, d) and 2591.52m to 2591.55m (e, f).

Depth: 2591.95m to 2591.98m,

- Remarkable amount of pollen and spore are available in this sample.
- the collected sample contains a huge accumulation of fungal remains (52%) and tree pollens (26%) (Figure-5c). The concentration of herb pollen is 9%. A very sparse number of shrubs (4%), fern (4%) and freshwater algae (5%) have also been available. In the frequency diagram (Figure-5d), the highest frequency of palynomorphs is fungi. Arecaceae (13%) and Combretaceae (9%) tree pollen is abundant, as well as Apiaceae (9%) herb pollen, is also frequently available. A scant amount of shrub

pollen Piperaceae (4%), fern pollen Pteridaceae (4%) and freshwater algae Zygnemataceae (5%) exists in the sample.

- Floodplain weeds *Peperomia sp.* and *Eryngium foetidum* are evidence of fluvial deposits. Terrestrial plant *Terminalia arjuna* (Combretaceae), Arecaceae plants and some plant stomates indicate nearly stable landforms. Small amount of *Ceriops decandra* is a sign of the early stage of mangrove swamp formation. Aquatic or semi-aquatic fern *Ceratopteris sp.* (Pteridaceae) and still freshwater algae *Zygnema sp.* (zygospore) (Zygnemataceae) are the indications of the preliminary stage of the transitional environment. Some coprophilous (dung loving) fungi *Coniochaeta sp.*, *Cookeina Meliola* as well as fungi growing on decaying plant material and soil *Curvularia sp.*, *Nigrospora sp.*, *Arnium sp.* are also available. All the identified palynomorph and organic remains suggest **the early stage of the transitional environment from brackish to freshwater with the formation of mangrove swamps, terrestrial and sparse amount of freshwater floodplain input. Climatic condition reveals hot and humid.**

Depth: 2591.52m to 2591.55m

- contains comparatively good preservation of palynomorphs with some degraded amorphous dark organic matter.
- In the Composition diagram (Figure-5e), fungi are abundant (43%) compared to other palynomorphs. Except for fungi, the sample is rich in herbs (31%) and trees (13%) pollen. Very small percentages of freshwater algae (7%), shrub (4%) and fern (2%) have also been present. Among the herb pollen Poaceae (16%) and Amaranthaceae (9%) pollen is abundant compared to other herbs (Figure-5f). Arecaceae pollen is abundant (11%) compared to other tree pollens. Except for fungi, freshwater algae Zygnemataceae (7%) are also common in the sample.
- Freshwater floodplain herbs like Poaceae, *Oryza sp.*, *Triticum sativum*, *Dentella repens*, *Saccharum officinarum* and *Carex sp.* are abundant. Inland plant *Phoenix sp.* *Amaranthus spinosus* (grows on roadside and wet places as weed), *Eryngium foetidum*, and some plant stomates indicate stable floodplain areas. Shoreline indicator *Morus alba*, rainforests and the landward edge of mangrove plant *Nenga pumila*, *Terminalia catapp*, as well as mangrove plants *Phoenix paludosa*, *Carex sp.* are indications of intertidal regions of the tropical and subtropical coastlines, where freshwater mixes with seawater and swamp, near, or at the edge of a river in places affected by the tide. Still, freshwater algae *Zygnema sp.* (zygospore) and freshwater marshy pteridophytes *Ceratopteris sp.*, are frequent. Abundant coprophilous fungi (dung-loving fungi) like *Sporormiella sp.*, *Coniochaeta sp.* *Cookeina Meliola*, are types of saprobic fungi that grow on animal dung that also indicate the presence of different animals. Fungal remain grows on the decaying plant material and soil like

Curvularia sp., *Alternaria sp.*, *Periconia byssoides* and *Nigrospora sp.* are also abundant. The presence of *Diplodia magnoliigena* (host genus Magnolia); *Neorousoella leucaenae* (host genus Leucaena) indicates the growth of Magnoliaceae and Fabaceae plants. Identified palynomorph and organic remains specify **transitional environments from brackish to fresh water with salt swamp (mangrove), freshwater floodplain and terrestrial deposits. Climatic condition reveals hot and moist.**

Comparative Analysis of Different Layers

- The upper part of the well contains pollen of trees, herbs, shrubs, freshwater algae and fungi. The concentration of tree pollens is comparatively rich in the upper area (Figure-6).
- The lower part also contains a very poor concentration of trees, herbs, shrubs, freshwater algae, diatoms; and a very rich amount of fungi.
- The middle part of the well contains only a high concentration of fungi with some trees and shrubs content (Figure-6). The percentage of fungal content shows an increasing trend with depth and contrary the tree content shows a decreasing trend with depth.

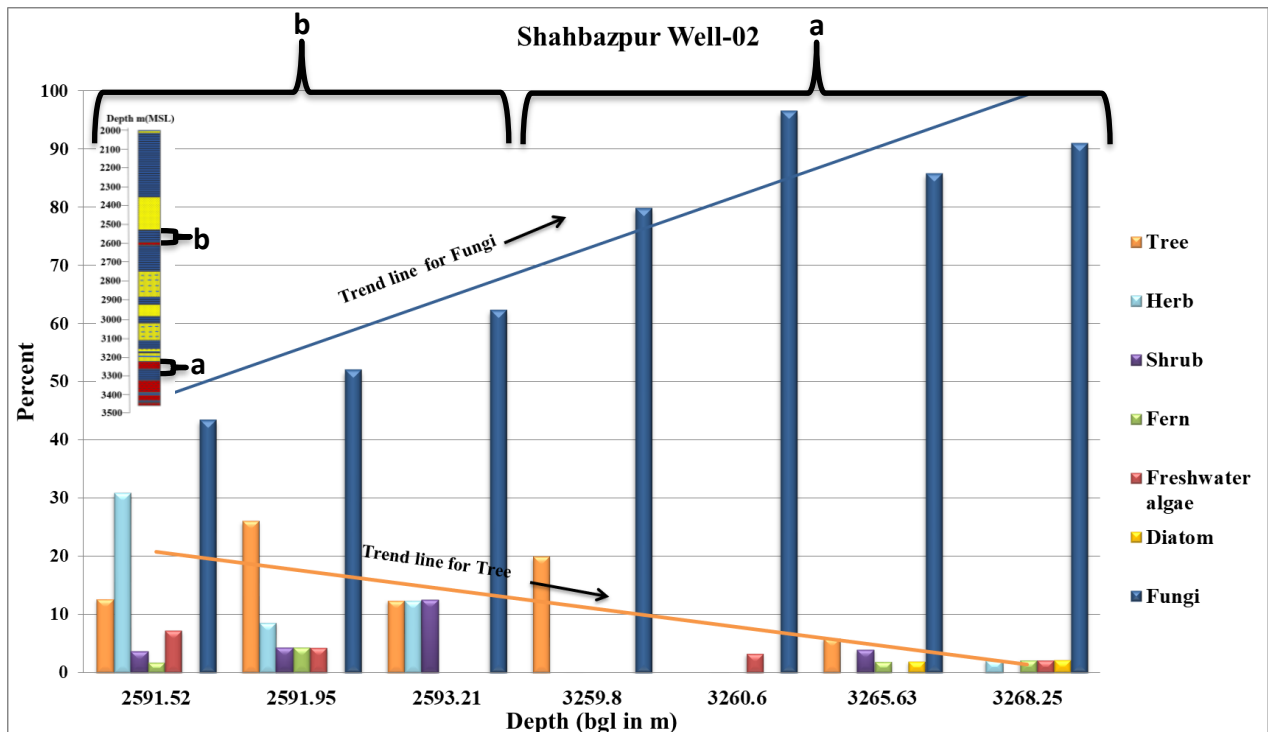


Figure-6. Concentrations of the identified palynomorphs and diatoms in different depths of the reservoir rock of the Shahbazpur Well 02.

- All the information reveals that the paleoenvironment of the area is in the **shallow marine to fluvial-deltaic condition. The shoreline is continuously regressed towards the sea leaving behind a mangrove swamp with some terrestrial floodplain deposits.**

- Between the sampling zone “a” and “b” the log shows a thick layer of sand, shale and sandy shale alteration that reveals a short period of sea transgression with a medium to low energy zone (Figure-6). The climatic condition is **hot and humid as well as rainfall is medium** to heavy.

According to the profile diagram,

Depth **2593.21m to 2591.52m**, all three samples contains a very high concentration of fungal remains which indicate very **wet and humid climatic condition** (Figure-7). At the depth of 2593.21m, the minute concentration of terrestrial tree Moraceae, shrub Arecaceae (mangrove) and herb Cyperaceae with a huge accumulation of fungal remains imply tropical swampy areas.

Herb pollens like Amaranthaceae, Poaceae and Rubiaceae suddenly increase above the depth of 2591.95m (Figure-7). **This suggests that there has been a high production of herbaceous plants due to heavy monsoon rain.**

At the depth of **2591.52m** presence of Poaceae and Amaranthaceae herb pollen with Arecaceae, Combretaceae and Moraceae tree pollen supports that situation also (Figure-7). Mangrove plant of the Arecaceae and Cyperaceae family shows the development of the swampy area. Fern spore Pteridaceae and freshwater algae Zygnetmataceae decrease below the depth of 2591.52m which indicates an increasing trend of **water salinity with depth** (Figure-7).

The profile diagram implies a very ample concentration of fungal remains throughout the time, from depth **3268.25m to 3259.8m**. A very sparse number of trees, herbs and shrubs along with fresh water algae is noticeable (Figure-8).

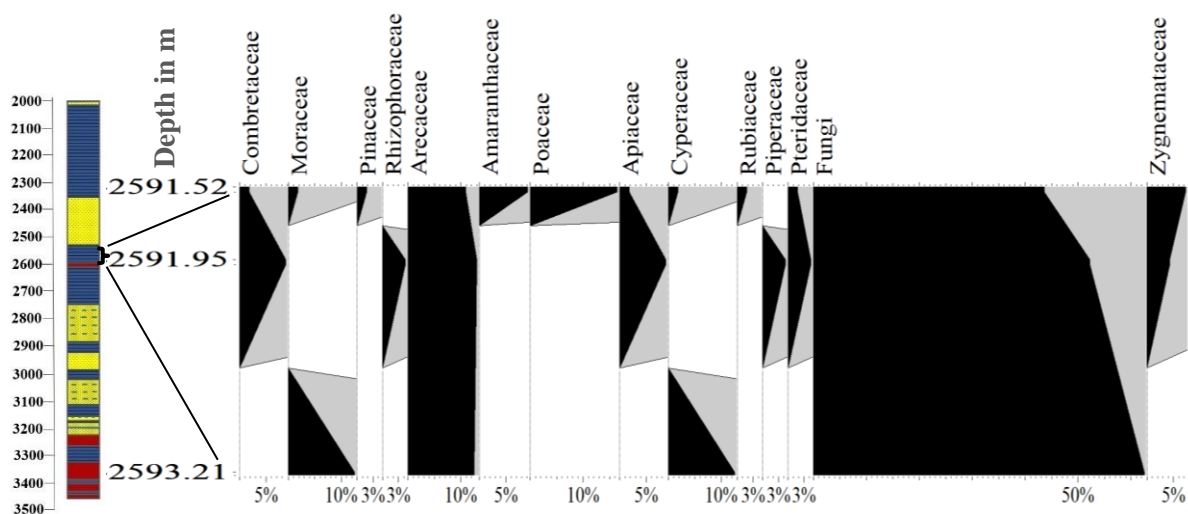


Figure-7. Profile diagram showing temporal changes of palynomorph in SBZ well 02, from depth 2593.21m to 2591.52m.

The absence of mangrove pollen and the occurrence of few fresh to brackish water diatom indicate a very **early stage of the sea regression process**. Some herb (Amaranthaceae, Cyperaceae) pollen with ferns (Pteridaceae) and freshwater algae (Zygnemataceae) at the depth of **3268.28 m** specify **marshy land of fresh and brackish water environment** (Figure-8).

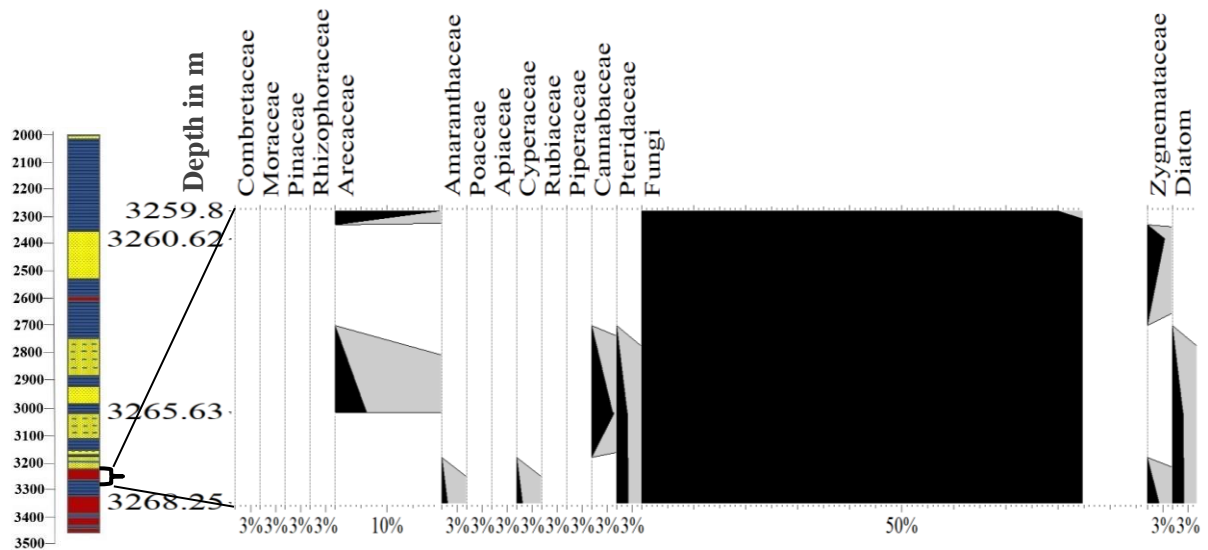


Figure-8. Profile diagram showing temporal changes of palynomorph in SBZ well 02, from depth 3268.25m to 3259.8m.

At the depth of **3265.63m**, **marshy** land gradually becomes a stable floodplain with an indication of tree (Arecaceae) and shrub (Cannabaceae) plants.

But at the depth of **3260.62 m**, only fungi and fern spores are available and after that, fungi and a few terrestrial plant pollens are available. All the evidence suggests a **marginal area of the land and shoreline of the sea**.

Statistical Analysis: Dominance and Diversity

The calculated alpha diversity indices of SBZ well 02 shows in Table-6. The taxa diagram shows a gradually increasing trend from the depth of 3260.6m (Figure-9a). But because of the combination of fresh and brackish water species, the taxa curve shows an increasing trend below this depth. The individual curve shows a similar phenomenon but the curve line is sharply increased from 3259.8m to 3268.25m (Figure-9b).

Table-6. Dominance-Diversity chart of Shahbazpur well 02 calculated by using the PAST software.

Depth in m	2591.52	2591.95	2593.21	3259.8	3260.6	3265.63	3268.25
Taxa_S	12	8	4	2	2	5	6
Individuals	55	23	8	5	60	50	90
Dominance_D	0.231	0.2806	0.3571	0.6	0.9345	0.7404	0.83
Simpson_1-D	0.769	0.7194	0.6429	0.4	0.06554	0.2596	0.17
Shannon_H	1.918	1.727	1.261	0.6004	0.1545	0.6237	0.4664
Evenness_e^H/S	0.5675	0.7032	0.8823	0.9114	0.5835	0.3732	0.2657
Brillouin	1.567	1.237	0.7271	0.3219	0.1246	0.4892	0.3733
Menhinick	1.618	1.668	1.414	0.8944	0.2582	0.7071	0.6325
Margalef	2.745	2.233	1.443	0.6213	0.2442	1.022	1.111
Equitability_J	0.772	0.8307	0.9097	0.8662	0.2229	0.3876	0.2603
Fisher_alpha	4.733	4.352	3.184	1.235	0.3983	1.383	1.447
Berger-Parker	0.4364	0.5217	0.625	0.8	0.9667	0.86	0.9111
Chao-1	32.62	9.913	6.625	2	2	5.49	6.247
iChao-1	32.62	12.5	6.625	2	2	7.317	6.659
ACE	24.68	12	12.57	3.125	2	6.787	7.667

The dominance graph displays an increasing curve line from a shallow depth to a deeper depth. The high value implies a clumping of individuals in a few species as well as the low value indicates a more uniform distribution of species (Figure-9c). The Simpson diversity index graph displays a gradual decreasing line from shallow depth to higher depth (Figure-9d). Low degree diversity exists at the depth of 3268.25m, 3265.63m, 3260.6 m and 3259.8m. On the other side, a moderately high degree of Simpson diversity exists in the shallower depth. The Shannon-Wiener diversity index graph also shows the same trend and all the value indicates very low diversity (Figure-9e). The evenness graph shows a gradually increasing trend from the deeper to the middle part of the log and again decreasing trend to the shallower depth (Figure-9f). All the calculated data and plotted graphs of the SBZ well 02 imply the **diversity of species is low to moderate and the paleoenvironment contains two different types of environmental conditions. The deeper part of the well shows a shallow marine to the transitional environment and the shallower part shows a transitional to fluvial-deltaic condition.**

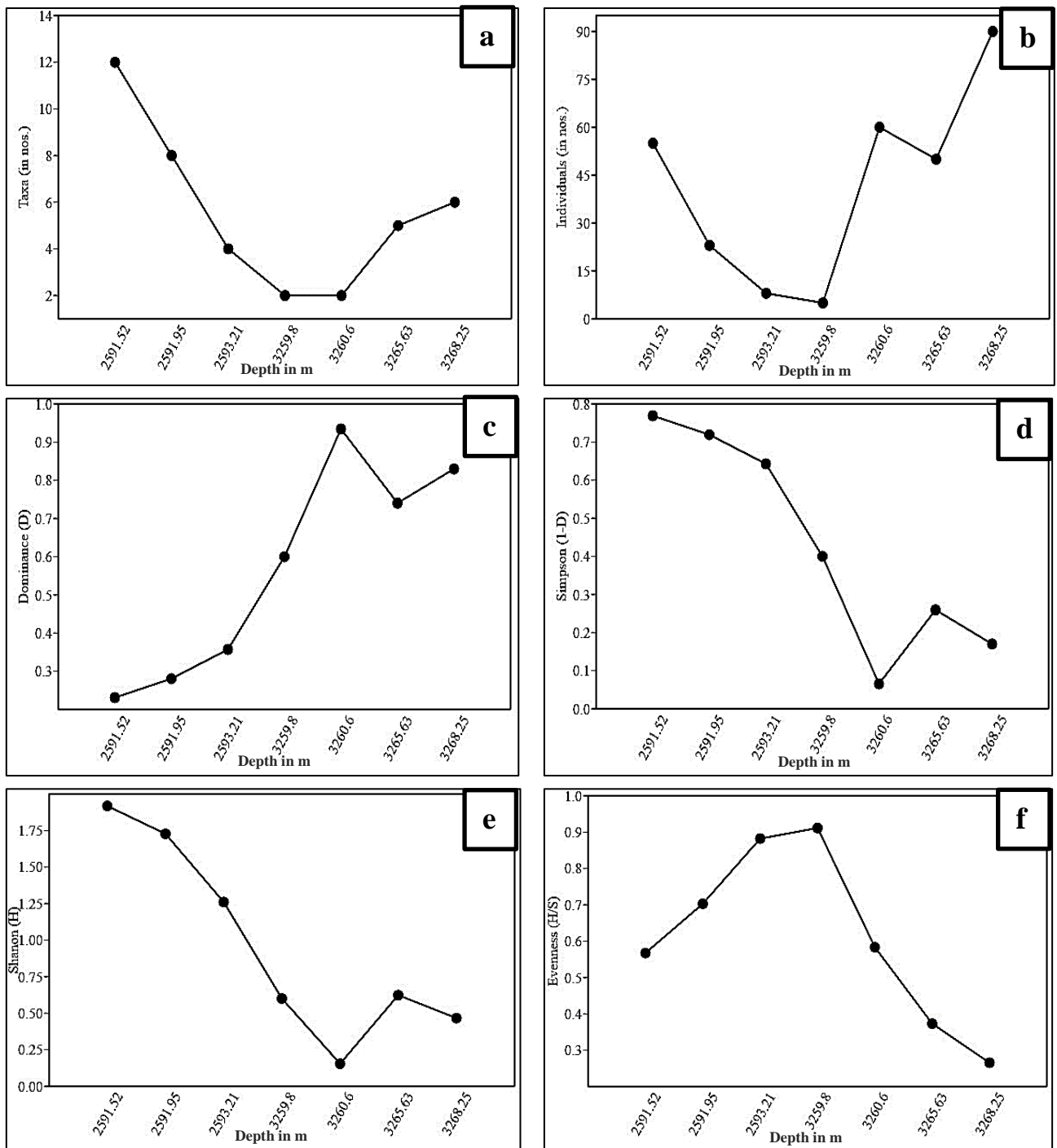


Figure-9. Diagram of calculated Taxa (a), Individuals (b), Dominance (c), Simpson Diversity index (d), Shannon Diversity index and Evenness (f) of recovered palynomorphs and diatoms of the Shahbazpur well 02.

CONCLUSION

Mio-Pliocene sediments from different depths of the SBZ well 02 contains about 43 species. Recovered microfossils (palynomorphs and diatoms) from depth of 3268.25m to 3268.28m the disclosed paleoenvironment is fresh to brackish water marshy land in a very warm and wet climate. Land and the near-shore environment with a tropical, wet climate have been unveiled

at a depth from 3265.66m to 3265.63m. Moreover, freshwater to a nearly brackish water estuarine environment of deposition in hot and humid climatic conditions has been identified at a depth from 3260.6m to 3260.62m. A sudden change of biota has been discovered at the depth of 3259.80m, which indicates a Marginal area of land and sea in a hot and humid climate. Early-stage of the transitional environment from brackish to freshwater with the formation of the delta, tidal flat, mangrove swamp, terrestrial and sparse amount of freshwater floodplain input has been disclosed in hot and humid conditions from 2593.21m to 2593.23m and 2591.95m to 2591.98m with evidence of the presence of grazing mammal. A Transitional environment from brackish to fresh water with salt swamp (mangrove), freshwater floodplain, and terrestrial deposits in hot and moist climatic conditions disclosed at the depth from 2591.52m to 2591.55m. A significant increase in the production of herbaceous plants due to heavy monsoon rain has also been present. The presence of grazing mammals has also been identified at this depth.

Comparing paleoenvironment of different depths of the well reveal shallow marine to fluvial-deltaic condition with the indication of continuous shoreline regression towards the sea and developing mangrove swamps with some terrestrial flood plain area. At the middle of the well, very few amounts of pollen with ample fungal remains indicate transgression of the sea in a short period with medium to low energy conditions. Evidence of grazing mammals have also been identified from the middle to the upper part of the well.

Dominance is low to moderately high from shallow depth to a deeper depth of the well. The Simpson diversity index exhibits a low degree of diversity at a deeper depth and a moderately high degree of diversity at a shallower depth. The Shannon-Wiener diversity index indicates very low diversity. Two different types of paleoenvironmental conditions are identified from the statistical analysis of Mio-Pliocene sediments of the well, the deeper part of the well shows a shallow marine to the transitional environment and the shallower part shows a transitional to fluvial-deltaic condition.

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Important Links:

Australian Pollen and Spore Atlas, The Australian National University, Canberra
<https://apsa.anu.edu.au>.

Diatoms of North America-The source for diatom identification and ecology,
<https://diatoms.org>

Eol-Encyclopedia of Life, <https://eol.org>

Eurofins EMLab P&K, <https://www.emlab.com>

GBIF Secretariat. GBIF Backbone Taxonomy. Checklist
dataset <https://doi.org/10.15468/39omei>, <https://www.gbif.org>.

Global pollen project, <https://globalpollenproject.org>

PalDat - A palynological database. <https://www.paldat.org>.

Pollen-Wiki, Stebler Th., <https://pollen.tstebler.ch/MediaWiki/index.php?title=Pollenatlas>

Fungi Outer Hebrides, <https://www.outerhebridesfungi.co.uk>

Phycokey-an image based key, <http://cfb.unh.edu/phycokey/phycokey.htm>