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Research Article

Isolation and Analysis of the Palynomorphs from Khadro Formation Exposed at Ranikot Fort Area Sindh, Pakistan

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Abstract

Seventeen (17) palynomorphs were found from the Khadro Formation comprises of sandstone, shale, and limestone exposed at Ranikot Fort area Sindh, Pakistan. Palynomorphs are microfossils, possessing organic-walled microscopic organisms, their size range is from 5-500 µ (micrometers). Majority of the samples contained angiospermic and algal microfossils. Algal remains indicated the prevalence of sub-tropical to tropical climate, in which marine environmental species were *Chaetomorpha akineta, Zygnema terrestre* and *Synechococcus aeruginosus* and also including fresh water species such as, *Pinnularia denticulata, Oscillatoria vizagapatensis* and *Genecularia* sp. Arecaceae & Nymphaeaceae also indicated the fresh water vegetation. The fungal counterparts viz,; *Dicellaesporites minutus* inferred the deposition time of Khadro Formation and the environmental conditions was humid, hot, and with heavy rain fall. Presence of the Pteriodophytic spores (*Laevigatosporites* sp.) indicated that environment was deltaic, swampy and being estuarine. From Palynomorphs of different groups based on distribution pattern, confirms that during deposition time of Khadro Formation at early environmental condition was hot.

Keywords: Angiospermic; Algal and fungal Palynomorphs; Pteriodophytic spores; Khadro Farmation; Sindh; Pakistan.

Introduction

The research of the palynomorphs which are found buried in sedimentary rocks and are of great importance in paleo-botanical studies, which tells us various evidence, regarding past vegetation distributions, and evolution of Plants, through passage of time. It also

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improves and develops the natural phenomena of Plant taxonomy. Knowledge of vegetation changes during the Tertiary provides valuable information for tracing the history of species, plant groups, or ecosystems (Graham, 1999; Singh *et al.*, 2023). In this regard, paleopalynological and palaeobotanical studies help to understand the migrations, interchange of paleoclimate, endemism. The present contribution deals with the palynological component of the Khadro Formation, which corresponds to the Paleocene age with aim of reconstruction of past vegetation history and paleoclimatology.

Microfossils were isolated from rock samples of Khadro Formation belongs to Tertiary periods (early Paleocene Epoch) (Figure 1-2). The area under investigation being part of lower Indus basin lies between the Latitude: 250 53' 11" N and Longitude: 670 55' 18"E. (Survey of Pakistan). Systematic description of the examined Palynomorphs including identification, terminology appearance and mode of setting have been implemented from Sahito *et al.* (1988); Kar and Saxena (1974); Naskar and Baksi (1976); Khan *et al.* (2017); Medeanic (2006); Rao *et al.* (2013); Leghari *et al.* (2001); Randhawa (1938); Dutta and Sah (1970). In this research work, the past vegetational history was taken from the fossil pollen found in rock. Recent work is strived to discover the Tertiary strata from the Sindh Province with respect to Palynomorphs mainly consisting of Angiospermic pollen, algal, Fungal and pteridophtyic spores from the rock samples of Khadro Formation, Pakistan.

Geological Set up

This formation has been described by Blandford as "Cardita beaumunti beds" because due to the presence of Mollusks animals Cardita beaumunti in 1878 (Jones, 1961), but later Williams (1959), renamed it Khadro Formation which now includes the basal parts of the "Karkh", "Gidar Dhor" and "Jakhar Group".

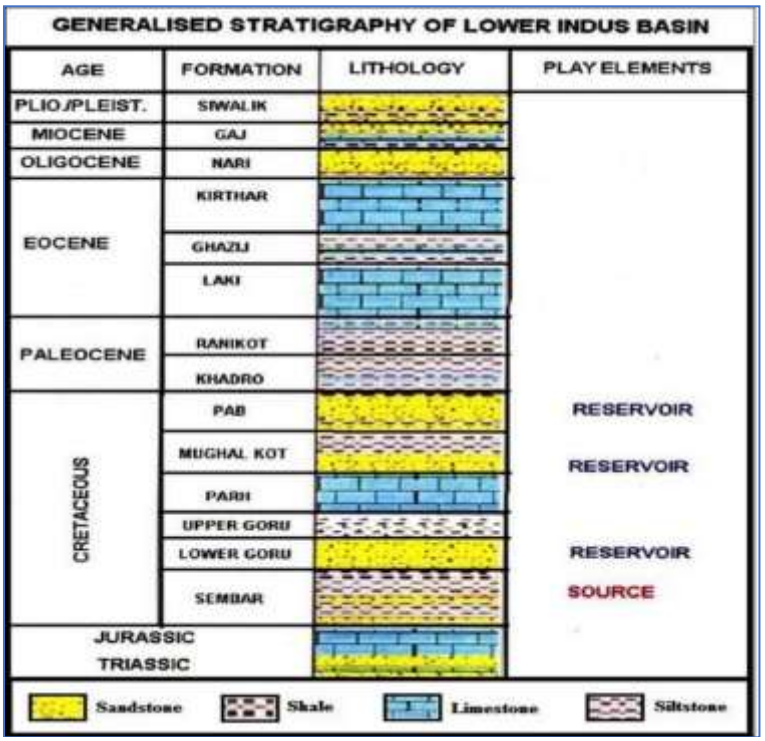

Figure 1. Stratigraphic column of lower Indus basin (Qadri and Shuaib, 1986).

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According to "Hunting Survey Corporation", in 1961 (Jones, 1961), named "Bed Kachu" and Thar formation. The Formation comprises sandstone, and shales (Figure 1). The discoloration of sandstone and shale's such as yellowish brown, grey and green consist of soft, medium grain, ferruginous and calcareous while the shale's discoloration is olive, pale, bluish grey, chocolaty and reddish brown and Gypsiferous which contain grey to brown argillaceous thin interbeds. Both limestone and sandstone bear fossils such as oyster and reptile's bones (SanFilipo). In the basal part of unit, various "basaltic flows" which are partly massive and partly amygdalodoidal, brecciated and weather back found in the unit. Volcanic flow is found on the top of the unit (SanFilipo).

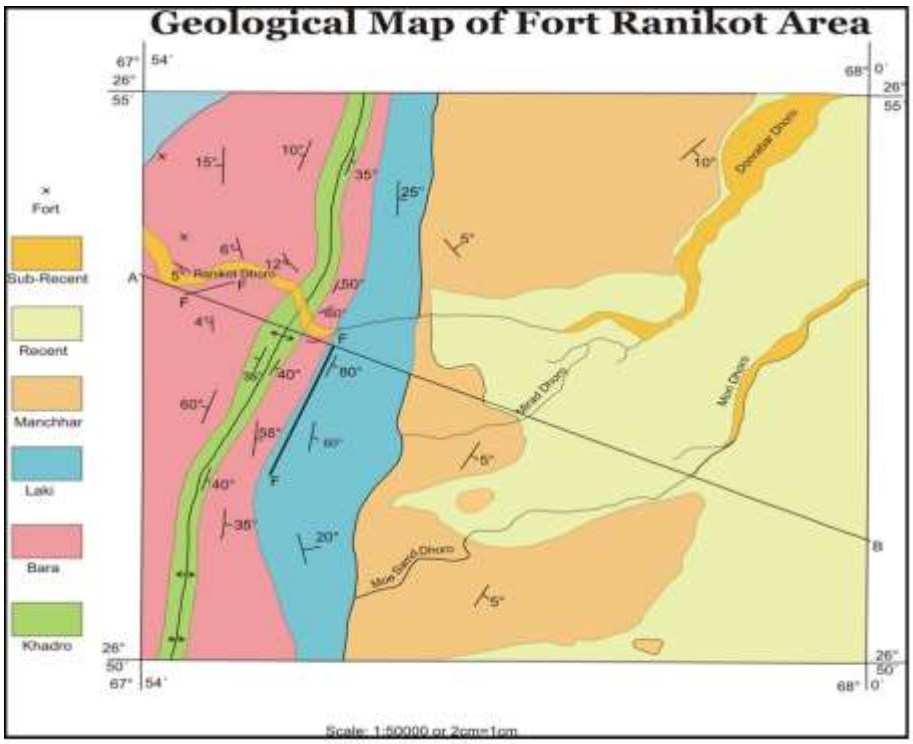

Figure 2. Geological Map of Ranikot Fort Area with Khadro Formation (Khan *et al.*, 2017).

Methodology

Palnomorphs isolation was carried out by collecting twenty eight (28) rock samples from the outcrop of exposed portion of the Khadro Formation. Collected materials were subjected to standard preparatory techniques adopted from **Phipps** and **Playford (**1984). For the maceration of samples, following steps were facilitated (Figure 3).

1) **Pre-maceration** 2) **Reactivity Test** 3) **Maceration procedure**

Pre-maceration: 200g of material were washed with distilled water and physically crushed with pastel & mortar

Reactivity Test: In order to determine the reactivity test of the collected material with different chemicals were used that is 50% HCl, 50 % HF and 50% HNO3 each of which was added, and reaction rate was observed on glazed porcelain tile in order to check the reaction of the sample with concentrations of various acids and the whole process has been performed in the fume hood.

Maceration procedure: By using (Inorganic) HCl (Hydrochloric Acid), HF (Hydro Fluoric

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Acid) and HNO_3 (Nitric Acid), the samples were disintegrated and carbonates, silicates, pyrites were removed out respectively. Samples were neutralized subsequently after each acid treatment. Further for the recovery of Palynomorphs, preliminary microscopic observation was processed. After acid digestion, palynomorphs were processed for oxidation. In this process, different quantities of HNO_3 were used at different times. After oxidation 1 % KOH was added for 4-to-5 minutes to observe discoloration if it was turned into coffee color indicating that palynomorphs were oxidized. Inorganic remains were further removed through centrifugation at speed of 500 rpm in a heavy liquid (preferably $ZnCl_2$). Palynomorphs were later on mounted in Canada Balsam or glycerin jelly mountants. Palynflora was then taxonomically identified and systematically evaluated. Prepared permanent slides were placed at Palaeobotany Laboratory, Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. Based upon findings of the reactivity test, further maceration of the sample was designed (Table 1).

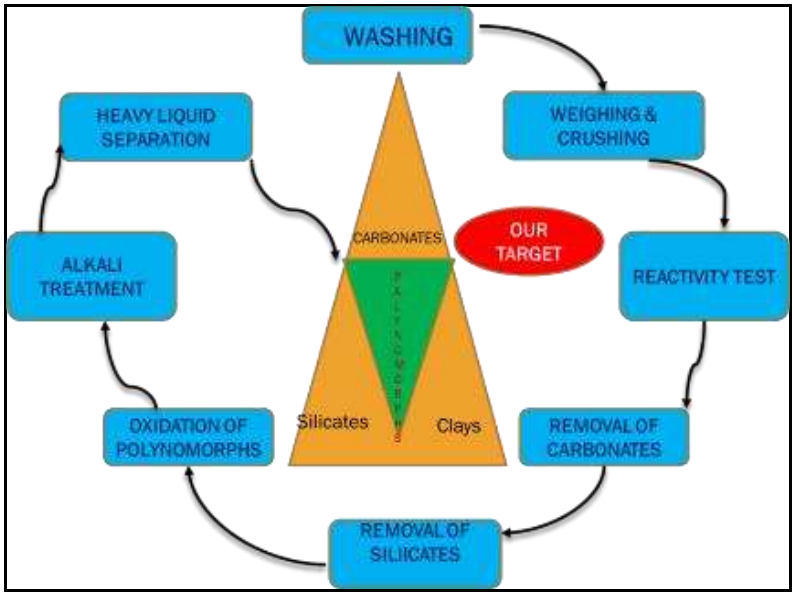

Figure 3. Laboratory Techniques chart includes all steps which were employed for the isolation of palynomorphs.

Table 1. Showing the results of Reactivity test and major lithology of samples from Khadro Formation.

Sample No.	Lithology	HCl 50%	HF 50%	HNO_3 50%
KH1	Soft, shale, brown	VFR	FR	BVR
KH2	Sandy shale, grey, brown	FR	FR	VSR
KH 3	Calcareous, shale, yellowish brown	VSR	VSR	VSR
KH4	Limestone, Yellowish white	VSR	NR	NR
KH5	Calcareous shale, yellowish brown	VSR	VSR	VSR
KH6	Limestone, Yellowish white	VSR	NR	NR
KH7	Soft, shale, brown	VFR	FR	BVR
KH8	Sandy shale, grey, brown	FR	FR	VSR
KH9	Calcareous shale, yellowish brown	VSR	VSR	VSR
KH10	Soft, shale, brown	VFR	FR	BVR
KH11	Soft, shale, brown	VFR	FR	BVR

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KH12	Sandy shale, grey brown	FR	FR	VSR
KH13	Calcareous shale, yellowish brown	VSR	VSR	VSR
KH14	Soft, shale, brown	VFR	FR	BVR
KH15	Sandy shale, grey brown	FR	FR	VSR
KH16	Calcareous shale, yellowish brown	VSR	VSR	VSR
KH17	Soft, shale, brown	VFR	FR	BVR
KH18	Limestone, Yellowish white	VSR	NR	NR
KH19	Calcareous shale, yellowish brown	VSR	VSR	VSR
KH20	Calcareous shale, yellowish brown	VSR	VSR	VSR
KH21	Limestone, Yellowish white	VSR	NR	NR
KH22	Soft, shale, brown	VFR	FR	BVR
KH23	Sandy shale, grey brown	FR	FR	VSR
KH24	Soft, shale, brown	VFR	FR	BVR
KH25	Sandy shale, grey brown	FR	FR	VSR
KH26	Soft, shale, brown	VFR	FR	BVR
KH27	Soft, shale, brown	VFR	FR	BVR
KH28	Soft, shale, brown	VFR	FR	BVR

Legend: NR No Reaction; VSR Very Slow Reaction; SR Slow Reaction; FR Fast Reaction; VFR Very Fast Reaction; BVR Bumping Violent Reaction

Results and Discussion

The palynological analysis from Khadro formation yielded significant palynomorphs the exceptional concentration of palynomorphs were confirmed by high resolution sampling, and fossil algae, pollen, spores all indicate an extraordinary set of environmental condition during the time of deposition.

Systematic Description

1) *Dicellaesporites minutus* (Kar and Saxena, 1974)

Description: Spores are bicellate, Oval in aperture, Individual cells among similar in size, Septa separate, Spore wall thick up to 1.5 µm, 23-33×7-12 µm in size (Plate 1, Figure 1).

Remarks: This specimen, found from the Khadro Formation of Ranikot is similar than that diagnosed by Kar and Saxena (1974) from Matanomadh Formation (Paleocene), Kutch, India.

Diagnosis: Spores (fungi)

Sample No. KH. 3, 5 & 7

Plate No.1. Fig.No.01

2) *Pinnularia denticulate*, (Sahito *et al.*, 1988)

Description: Phytoplankton found in fresh water, frustule rectangular, Inner side board forming dental structure in straight, the size of specimen is measuring from 9.78X23.85µm to 39.20X22.12µm (Plate 1, Figure 2).

Remarks: The present result is based on a specimen recorded from the Khadro Formation of Ranikot, the epoch with Paleocene resemblances (Sahito *et al.*, 1988), Sonda coal, Thata, Sindh. The specimen had a board structure and an inner dental structure with a rectangular shape.

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Diagnosis: Algal microfossils

Sample No. KH. 22 & 23.

3) *Arecipites punctatus* (Naskar and Baksi, 1976; Khan *et al.*, 2017)

Description: The color of the Pollen grains was observed as dark brown, Monosalcate pollen, amb spherical-elliptical, 18.89 x 50.29 µm in size, sulcus 30µmlong and 3.5 µm wide, extending end to end of the narrow and tapered at both ends. Exine 3µm thick and psilate (Plate 1. Figure 3).

Remark: The present pollen grains are smaller in size than that defined by Khan *et al.* (2017) from Bara Formation (Middle Paleocene) exposed at Ranikot Fort area Sindh, Pakistan (Naskar and Baksi, 1976). Reported from Akli Lignite of Paleocene – Eocene age from Rajistan, India.

Diagnosis: Arecaceae, (Angiosperm)

Sample No. KH. 2

4) *Constructipollenites ineffectus* (Ola and Adeoti, 2017)

escription: The pollen grains from Khadro Formation, isolated them and were studied with characters such as dark brown color, amb sub circular shape, 32 µm in long and thickness is 2 µm, of exine, the spines are uniformly distributed all over the surface of the pollen grain.

Remark: The discovered pollen grains are larger in size than described by Ola and Adeoti (2017). from the First Down-Hole Shale, Occurrence in Three Contiguous Basins, in Nigeria. Implication on the K-Pg Boundary.

Diagnosis: Arecaceae, (Angiosperm)

Sample No. KH.16

Plate 1. Figure 4

5) Asteraceae Senecio type (Medeanic, 2004)

Description: The type of pollen grain which was examined from Khadro Formation, Sindh, Pakistan was light brown in color, amb circular, thin walled and having blunt raised vesicles on their surface, 141.27 x 139.16 µm in size, and the size of exine is 7.4 µm.

Remark: This fossil pollen grain is larger in diameter than described by Medeanic (2004) from Brazil.

Diagnosis: Asteraceae (Angiosperm)

Sample No. KH. 15

Plate 1. Figure 5

6) *Polygonum hydropiperoides* (Medeanic, 2004)

Description: Pollen grains are oblate–spherical, polyporate with many pores, Exine reticulate with 3.51 µthick, 69.08 µm in diameter 70.91µm in height.

Remark: In the Present result, the specimen recorded from Khadro Formation of Ranikot, the epoch of Paleocene resemblances (Medeanic, 2004).

Diagnosis: Polyporate grains/periporate (Polygonaceae and Angiosperm).

Sample No. KH.14

Plate 1. Figure 6

7) Poaceae type (Medeanic, 2004)

Description: Monoporate grain which contains thick and spherical wall with one pore,

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but the pore was not clear; color of the pollen is brown measuring 19.96 x 20.51μm in size and the size of exine ranging in-between 0.71-3.95 μm.

Remark: The Palynomorphs, we have found and observed from Khadro Formation, exposed at Ranikot shows great affinities with the species that have been studied by Medeanic (2004) from Brazil.

Diagnosis: Poaceae type Pollen grain (Angiosperm)

Sample No. KH.19

Plate 1. Figure 7

8) *Retimonocolpites ellipticus* (Rao *et al.*, 2013)

Description: Monocolpate/ Monosulcate pollen grains having one furrow or colpus, shape: prolate, exine ornamentation: reticulate which was not clear and the aperture: elongated.

Remark: The pollen grains found and observed from the Khadro Formation exposed at Ranikot show great affinities with the species studied by Rao *et al.* (2013).

Vastan Lignite Mine (Early Eocene), Gujarat, western India.

Diagnosis: Angiospermopsida

Sample No. KH.23

Plate 1, Figure 8

9) *Laevigatosporites* sp. (Ola and Adeoti, 2017)

Description: Kidney shaped, monolet spore with scare, bright yellow in color, equatorial view: amb, laesurae measuring 66 μmin length, covering up to the 2/3 of the spore circle, thickness of exine measuring 1.5 μm foveolate distal surface was psilate while proximal surface sculpturing.

Remark: Existing variety (spore) is a little large than monolete spores, described by Peter S. Ola1 and Blessing Adeoti in 2017 from Ola and Adeoti (2017) the First Down-Hole Shale occurrence in three Contiguous Basinsin Nigeria.

Diagnosis: Polypodiaceae (Pteropsida)

Sample No. KH.24

Plate 1, Figure 9

10) *Couperipollis brevispinosus* (Khan *et al.*, 2017)

Description: The color of the Pollen is shady brown, amb sub circular, measuring 51.23 x 50.56 μm in size, Pollen Monocolpate grain, and colpus unclear, spreading nearly from one end to the other very thin and 30 μm long in measurement. Exine: 1.3 μm thick, spines 3-4 μm long echinate.

Remarks: The present pollen grain is larger and thicker than described by Khan *et al.* (2017) from Bara Formation, Ranikot Fort area Sindh, Pakistan.

Diagnosis: Arecaceae (Angiosperm palms)

Sample No. KH.1

Plate 1, Figure 10

11) *Grandispora spinosa* (Playford *et al.*, 2001)

Description: The color of spore is black, Clavate, amb: sub circular to rounded triangular, Laesurae different, Exoexine consists of irregular spines and cones.

Remarks: The present spore from the Khadro Formation (Paleocene) is bigger in size

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than that explained by Playford *et al.* (2001). This specimen encompasses spines and cones and morphologically is spherical.

Diagnosed: Lycopsida

Sample No. KH.10

Plate 1, Figure 11

12) *Nymphaecidites clarus* (Khan *et al.*, 2017; Dutta and Sah, 1970)

Description: The color of pollen is white to brown,monoporate grains contain thick and spherical wall with one pore, position of sitting equatorial, external sculpturing psilate while measurment 67 x 70 μm.

Remarks: The present recovered pollen grains from the Khadro Formation (Paleocene) are larger in size than described by Khan *et al.* (2017) and Dutta and Sah (1970) from Bara Formation, Sindh, Pakistan and from Tertiary deposits of Assam, Lower Eocene of Shillong Plateau, India.

Diagnosis: Nymphaeaceae (Angiosperrm)

Sample No. KH.17 & 25

Plate 1, Figure 12

13) *Oscillatoria vizagapatensis* (Leghari *et al.*, 2001; Sahito *et al.*, 1988)

Description: Discovered filament: unbanked no septate, found in fresh water, calculating 10,5 x 12.5 μm in diameter, elongated tube-shaped in structure, at each boarder the elongated cells are placed in chain like structure. Figure 1 of plate 6 is same but figure 2 elongated fibrous like in structure and golden in color, granular like structure are apparently seen in the filament.

Remarks: The existing specimen is larger and chain of cells are present at both border as defined by Leghari *et al.* (2001) and Sahito *et al.* (1988) from Sonda Coal deposits, Thata district, Sindh, Pakistan.

Diagnosis: Algal microfossils

Sample No. KH. 11, 12 & 13

Plate 1, igure 13

14) *Synechococcus saeruginosus* (Nägeli, 1849; Sahito *et al.*, 1988)

Description: Marine cell of recovered Algal is moderate in size, little bit constricted at the mid of the cell with border as well as rounded poles and the apex has greater width, exact size of cell, 16 to 18 μm in diameter and thickened wall with dark brown in color.

Remarks: The found palynomorphs observed from Khadro Formation, exposed at Ranikot Fort shows great affinities with the species that have been studied by Nägeli (1849) and Sahito *et al.* (1988) in comparison to the palynomorphs, it is larger.

Diagnosis: Algal microfossil (Cyanophyceae)

Sample No. KH. 26

Plate 1. Figure 14

15) *Zygnematous terrestre* (Sahito *et al.*, 1988; Randhawa, 1938)

Description: Fresh water terrestrial filament looks like single flattened layer, measures about 25 x 130 μm in diameter, the length wise cells are shorter than 7.5x30.5μm.

Remarks: The present specimen found from Khadro Formation is similar to the species of Sahito *et al.* (1988) from Sonda Coal deposits, Thatta district, Sindh, Pakistan (Sahito *et al.*,

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1988; Randhawa, 1938), but the present specimen is larger and posterior uplifted and with some curved like bow.

Diagnosis: Algal microfossil (Zygnematophyceae)

Sample No. KH. 14

Plate 1, Figure 15

16) *Chaetomorpha akineta* (Sahito *et al.*, 1988)

Description: Marine filament: long and cylindrical, cross wall is present in the filament cubical in shape, measuring about 7.5-15 x 7.5-10 µm wide-ranging in diameter, cell just like akinete.

Remarks: The present specimen, found from Khadro Formation is similar to the species of Sahito *et al.* (1988) from Sonda Coal deposits, Thatta district, Sindh, Pakistan. This specimen is larger and change in color asdepicted from the work of Sahito *et al.* (1988).

Diagnosis: Algal microfossil (cladophoraceae)

Sample No. KH.8, 13 & 14

Plate 1, Figure 16.

17) *Genecularia* sp. (Sahito *et al.*, 1988)

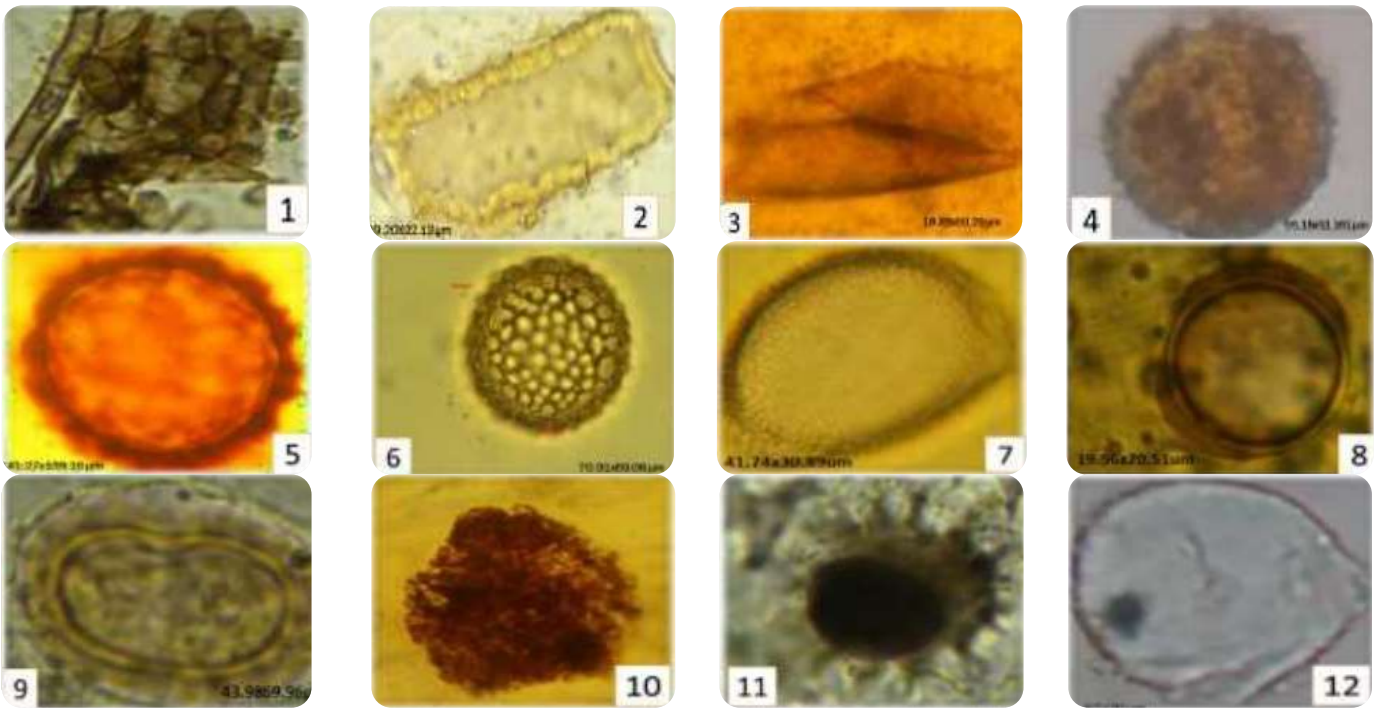
Description: Filament is cylindrically long, without any branch, black color spots visible in each filament, calculating 28-35 x 280-297.5 µm in diameter.

Remarks: The existing variety found from Khadro Formation is alike to the species of Sahito *et al.* (1988) from Sonda Coal deposits, Thatta district, Sindh, Pakistan. This specimen is larger and change in color than described by them.

Diagnosis: Algal microfilament Fossil

Sample No. KH.8, 13 & 14

Plate 1, Figure 17



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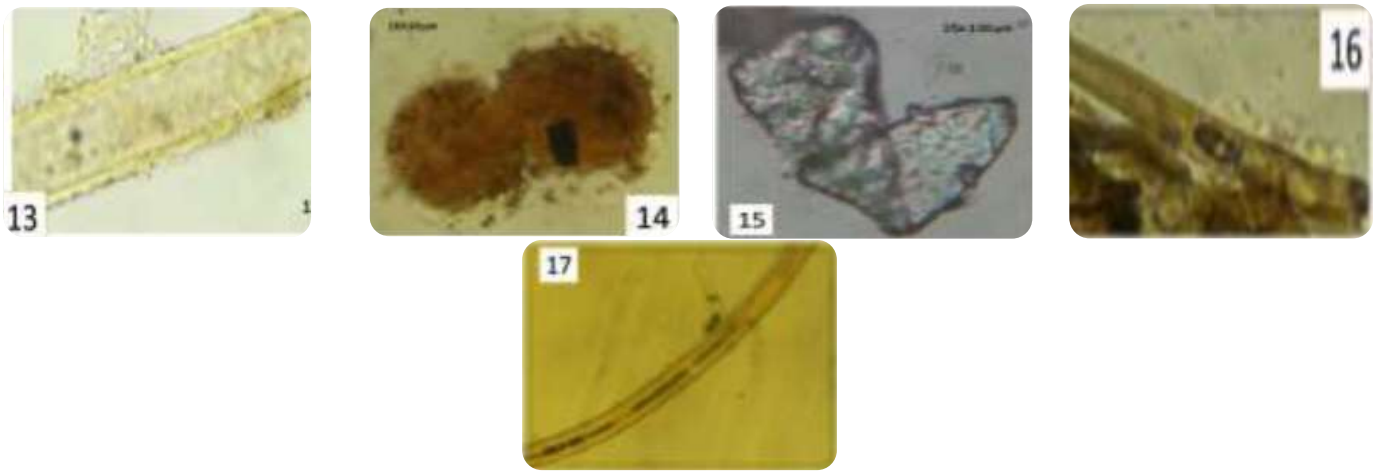

Plate 1. Figure 1-17 Palynomrphs

1. Dicellaesporites minutus (Kar and Saxena, 1974)
2. Pinnularia denticulate (Sahito *et al.*, 1988)
3. Arecipites punctatus (Naskar and Baksi, 1976; Khan *et al.*, 2017)
4. Constructipollenites ineffectus (Ola and Adeoti, 2017)
5. Asteraceae Seneciotype (Medeanic, 2004)
6. Polygonum hydropiperoide stype (Medeanic, 2004)
7. Retimonocolpites ellipticus (Rao *et al.*, 2013)
8. Poaceae type (Medeanic, 2006, 2004).
9. Laevigatosporites sp. (Ola and Adeoti, 2017)
10. Couperipollis brevispinosus (Khan *et al.*, 2017).
11. Grandispora spinosa (Playford *et al.*, 2001; Khan *et al.*, 2017; Dutta and Sah, 1970)
12. Nymphaecidites clarus (Rao *et al.*, 2013; Leghari *et al.*, 2001; Sahito *et al.*, 1988)
13. Oscillatoria vizagapatensis (Nägeli, 1849; Sahito *et al.*, 1988)
14. Synchronous aeruginosus (Sahito *et al.*, 1988; Randhawa, 1938)
15. Zygnema terrestre (Sahito *et al.*, 1988)
16. Chaetomorpha akineta (Sahito *et al.*, 1988)
17. Genecularia sp.

The micromorphs isolated from early Paleocene, Khadro Formation, Sindh, Pakistan exposed at Ranikot Fort, contains of 51 form genera and 46 form species attributable to Fungal spores (08 genera and 08 species), Algal remains (19 genera and 16 species), angiosperms (21 genera and 21 species), pteropsida spores (2 genera) and the Lycopsida spore consist of (1 genus and 1 species) (Figure 4). At Ranikot Fort, the Khadro Formation was explored, and twenty-eight (28) samples were collecte.. Fifty-one Palynomorphs were isolated, among those mostly had been found morphologically similar. With only seventeen Palynomorphs with scientific names and families. The deposition of Palynomorphs were found poor to moderate productive in the most samples and found that Palynomorphs were in deformed, some filaments overlapped and cracked. Besides these findings, very few other samples were also found in good productive status. Our findings align with those of `Avav and Ugese (2009), who discovered that the Paleocene region of the core is nearly devoid of vegetation but does contain exceptionally high relative abundances of angiosperm pollen. Recovered Palynomorphs were taxonomically evaluated with already published articles from different parts of the world by different

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scientists. The Palynomorphs were compared by the morphological characters with modern plant taxa. Following families were identified which were deposited during the deposition of Khadro Formation as follows: -Polygonaceae, Asteraceae, Arecaceae, Poaceae, Polypodiaceae and Nymphaeaceae. The isolated palynomorphs exhibited occurrence of Palynomorphs relations belonging to varied ecological groups *viz.*; Fresh Water aquatic Members, marine, Wetland and Water-Edge Members (Table 2). The palynomorphs composition inferred a warm moist tropical - subtropical environment. Moreover, Algal microfossils occurred during the whole depositional period of the Khadro Formation along with angiosperm, both were preserved in dominant state. No age diagnostic Palynomorphs were found. However, the collective existence of *Dicellaesporites minutus, Retimonocolpites ellipticus ,Laevigatosporites sp.* and algal remains roughly recommend Early to Late Paleocene of Khadro Formation, Ranikot Fort.

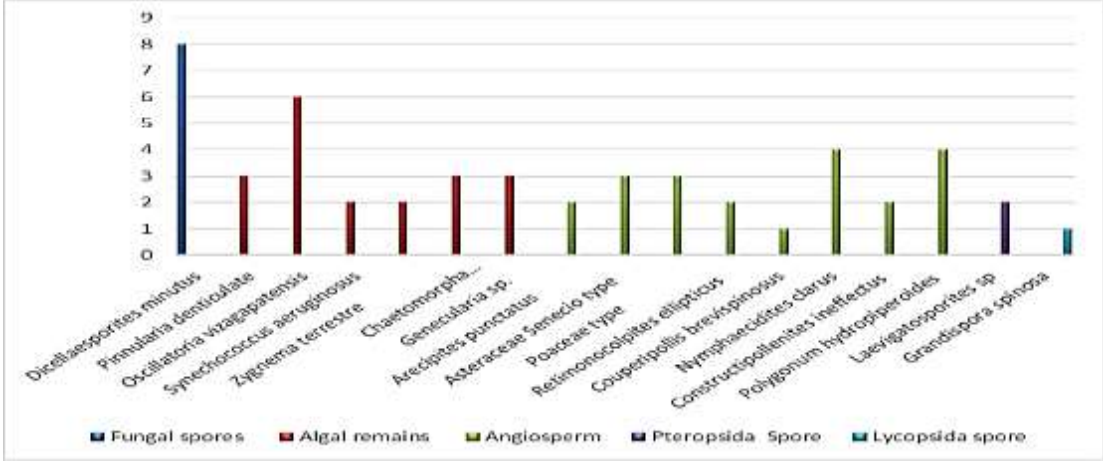

Figure 4. Frequency of palynomorphs record.

Table 2. Palynomorphs diversity across the Khadro Formation.

Palynomorphs	Identified Specimens	Qty.
1) Fungal spores	*1) Dicellaesporites minutus*	*08*
	Total Numbers	*08*
2) Algal remains	*2) Pinnularia denticulate*	*03*
	3) Oscillatoria vizagapatensis	*06*
	4) Synechococcus aeruginosus	*02*
	5) Zygnema terrestre	*02*
	6) Chaetomorpha akineta	*03*
	7) Genecularia sp.	*03*
	Total Numbers	*19*
3) Angiosperm	8) *Arecipites punctatus*	02
	9) Asteraceae Senecio type	03
	10) Poaceae type	03
	11) *Retimonocolpites ellipticus*	02
	12) *Couperipollis brevispinosus*	01
	13) *Nymphaecidites clarus*	04
	14) *Constructipollenites ineffectus*	02
	15) *Polygonum hydropiperoides*	04
	Total Numbers	21

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4) Pteropsida Spore	16) *Laevigatosporites sp.*	02
	Total Numbers	02
5) Lycopsida spore	17) *Grandispora spinosa*	01
	Total Numbers	01

Conclusions

Fifty-one microfossils were isolated. Among those mostly had been found morphologically similar. Seventeen palynomorphs with scientific names and families were identified. Nineteen algal remains, two pteropsida and one lycopsida were recovered. Formation was dominated by angiosperms. Palynomorphs relations belonged to varied ecological groups viz; Fresh Water aquatic Members Marine, Wetland and Water-Edge Members. The palynomorphs accompanying shows a warm, moist tropical to subtropical environment.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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