Palynological evidence for the Neogene environment analysis of the Thakkhola Graben, Nepal

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ABSTRACT

The Thakkhola Graben, a north-south graben, lies in central Nepal Himalayas consisting of Neogene sediments. The presence of pollen in these sediments provides a unique natural laboratory to understand the paleoenvironment during the formation of the graben. This study provides a detailed description of the pollen collected from the Tetang and Thakkhola formations with the help of the Light Microscope and Scanning Electron Microscope. A variety of pollen assemblages from the Thakkhola Graben explains that the sediments contain dominant alpine trees with some steppe vegetation. Presence of evergreen subtropical and temperate deciduous broad-leaved forest, needle-leaved element, and high altitude taxa show a mixed pollen assemblage in fluvial-lacustrine sediments of the graben. The presence of evergreen subtropical and temperate deciduous broadleaf forest (Quercus, Betula, Juglans, Alnus), needle-leaved element (Pinus, Tsuga) and high altitude taxa (Picea, Abies) with Artemisia, Chenopodiaceae, Poaceae, Rosaceae show mixed pollen assemblages indicating warm and humid paleoclimate. The coniferous pollen indicates the altitude was higher during the deposition time and the presence of Betula, Quercus, and Juglans suggests temperate forest. Domination of Artemisia and Chenopodiaceae shows a strong influence of the Himalayan topographic barrier during the sediment deposition.

Keywords: Thakkhola graben; Pollen assemblage; Scanning Electron Microscope; Paleoenvironment

INTRODUCTION

The paleoenvironmental study can be done by various proxy such as pollen, carbon and oxygen isotopes (Fort et al., 1982; Yoshida et al., 1984; Garzione et al., 2003; Paudayal and Ferguson, 2004; Adhikari and Paudayal, 2012). The late Cenozoic Asian environment is one part of the long-term paleoenvironmental studies in the High Himalaya and Tibetan plateau to understand the complex relationship between climate change and tectonic activities (Molnar and Tapponnier, 1978; Kroon et al., 1991; Yin and Harrison, 2000; Zhisheng et al., 2001; Garzione 2008; Xu et al., 2012; Dhital, 2015). The upliftment of the Himalayan orogenic belt is a resultant of the collision between Indian and Eurasian Plates at the Cenozoic time (Rowley, 1996) and this tectonic activity affected the Asian monsoon. The spatio-temporal distribution of paleoprecipitation δ¹⁸O in Central to East Asia remained constant since 50 Ma, suggesting that Tibetan topography continuously blocked southerly moisture incursion (Caves et al., 2015). The East Asian Winter Monsoon (EAWM) was active since as long as 20 Ma but was very weak until 13-12 Ma and then it again intensified (Tada et al., 2016) till glacial periods (Sun et al., 2010) representing different types of topographic circulation.

The Thakkhola Graben (83°50’-84°east and 29°-28°50’ north) is north-south trending graben in the southern Tibet and the Himalaya that was formed (10-11 Ma) during the East-West extension of Tibet (Molnar and Tapponnier, 1978; Fort et al., 1982; Adhikari and Wagreich, 2011) (Fig.1). Palynological studies are very scanty in this graben and with the help of Light Microscope (LM) some previous paleoenvironmental studies (Yoshida et al., 1984; Adhikari and Paudayal, 2012) have reported some pollens such as Pinus, Alnus, Abies, Quercus, Tsuga, and Betula in some horizons of this graben. However, a detailed description of pollen morphology using a Scanning Electron Microscope (SEM) is fundamental because it gives a better resolution to visualize the fossil pollen grains and outer layers in an enlarged scale. This information can be used to identify and trace the phylogenetic origin of the functional features of pollen grains of different geological time period (Stelleman, 1978; Ferguson et al., 2007). Therefore, this study aims to investigate and report the pollen horizons of the graben sediments by means of Light Microscope (LM) and Scanning Electron Microscope (SEM). Moreover, it provides a detailed morphological description for the interpretation of the paleoenvironment in the Neogene Period.
GEOLOGICAL SETTING

The upliftment of the Tibetan Plateau is considered as a response to the convective removal of the lower portion of the thick Asian Lithosphere (Platt and England, 1994). This plateau experienced the Cenozoic extensional tectonic phase resulting in many north-south grabens. Some of the major grabens from east to west are: Burang, Thakkhola, Gyirong, Kungo, Pum Qu, and Yadong grabens. The horizontal graben spacing decreases from south to north, representing a northward decrease in the crustal thickness (Yin, 2000). The Thakkhola Graben is a part of the series of north-south trending graben systems in the Himalayan arc and the southern Tibet (Fort et al., 1982; Coleman and Hodges, 1995; Mark Harrison et al., 1997; Adhikari and Wagreich, 2011). This graben is bounded by Indus-Tsangpo Suture Zone.

Fig. 1: Location map of the Thakkhola Graben (a) Regional geological map of Nepal Himalaya showing study area (Amatya and Jnawali, 1994) (b) Geological map of the Thakkhola Graben showing sample locations, basement geology and graben fill sediments (modified after Adhikari, 2009 and Dhital, 2015). HHC: Higher Himalayan Crystalline; STDS: South Tibetan Detachment System.
Palynological evidence for the Neogene environment analysis of the Thakkhola Graben, Nepal

The Thakkhola Graben is a thick and nearly continuous Lower Paleozoic to Tertiary marine sedimentary succession of the Tethyan Sedimentary Series. The Mustang Mugu leucogranite massif of 17.6 ± 0.3 Ma age are well-exposed in the northern part of the graben (Le Fort and France-Lanord, 1994; Mark Harrison et al., 1997). The Tethyan Sedimentary Series were deposited in the northern continental margin of Indian Plate, which were crumpled, stacked, and deformed as a consequence of the collision between Indian Plate and Eurasia Plate in the early Eocene (Garzanti et al., 1987; Searle et al., 1987). The sedimentology and stratigraphy of the Thakkhola Graben have been studied by numerous researchers (Bordet et al., 1971; Fort et al., 1982; Colchen, 1999; Hutardo et al., 2001; Garzione et al., 2003; Adhikari, 2009) and explained the evolution of the graben and its sedimentary environment. This graben is filled with more than 850 m thick Plio-Pleistocene sediments that are divided mainly into Thakkhola and Tetang formations. The older Tetang Formation is well exposed around the Tetang village and divided into four distinct sequences: basal member, interbedding of conglomerate with sand and silt layers, sand dominated sequences, and fine siltstone with carbonate layers (Adhikari and Wagreich, 2011). The basal member consists of a massive conglomerate, imbricated conglomerate, and sand beds. This basal member of the Tetang Formation is overlain by sand dominated sequences with siltstone and carbonate beds. The siltstone and carbonate beds were deposited in the fluvio-lacustrine environment. The Thakkhola Formation overlain the Tetang Formation by a low angle (~5°) angular unconformity and consists of conglomerate, alteration of imbricate conglomerate bed with sandstone and siltstone, fine-grained sediments, upper imbricated conglomerate, and sandy layers (Adhikari and Wagreich, 2011). Numerous growth faults are widespread in the graben fill sediments and at the Tange area it displaces sediments of the Thakkhola Formation and at the Dhakmar area it displaces the massive conglomerate about 50 cm (Adhikari, 2009).

MATERIALS AND METHODS

The twenty six samples of silty clay and clay (300-400g) collected from the Tetang and Thakkhola formations (Fig. 2) were crushed by hand with a mortar and pestle. Then, the procedure developed by Ferguson et al. (2007) and Zetter (1989) was followed for pollen analysis. The Tetang Formation consists of fine sand, silt, and clay layer over limestone bed where clay layers consist of plant fossils and pollen (Fig. 2a). Pollen bearing layers of the Thakkhola Formation consists of gray to black siltstone beds with bioturbation and root fragments (Figs. 2 b&c).

The crushed samples were cleaned by scraping with a knife to avoid the contamination from the recent pollen on the surface and boiled in hydrochloric acid (HCl) to remove carbonate contain. Carbonate free samples were treated with hydrofluoric acid (HF) in a copper pan and boiled for half an hour to remove silicate materials. The samples were kept in a large polyethylene jar containing three to four liters of water for the separation of sediments. The settled...
samples were boiled with concentrated hydrochloric acid (HCl) to avoid the fluorite. Samples then washed with distilled water three times, centrifuging it at 1500 rpm before the acetolysis. The samples were then transferred to a test tube and glacial acetic acid (\(\text{CH}_3\text{COOH}\)) followed by of freshly prepared solution of saturated sodium chlorate (\(\text{NaClO}_3\)) and few drops of conc. HCl. Then, samples were centrifuged at the rate of 2000 rpm for 2-3 minutes to remove the finer fragments. Liquid zinc chloride (\(\text{ZnCl}_2\)) was used to separate organic and inorganic residue. The remaining organic residue was washed two times and mixed with glycerin for LM and SEM study. Possible pollen grains up to 400 were counted by moving the slides in the LM and interested pollen grains were brushed to the edge of the glycerin with hair needle and transferred to another glass slide and photographs were taken with the help of a binocular microscope. Glycerin was removed from the selected grains and placed SEM stub, and the gold coating was done by BIORAD Sputter Coater. The SEM stub was placed inside the SEM and pollen grains were examined with Jeol 6400 SEM at 10 kV with different magnitude and orientation at the laboratory of the Department of Sedimentology and Geodynamics, University of Vienna, Austria. High-resolution photographs were taken in the desired magnitude after careful analysis of the pollen grains.

**RESULTS**

The preservation of palynomorphs was very poor, hence the construction of pollen diagram was not possible because pollens were present in a single layer but absent in both the upper and lower horizons. More than 19 families and genera of pollen were identified from the samples where a high percentage of grassland taxa and a low percentage of temperate forest taxa characterized the pollen assemblages (Fig. 3).

**Gymnosperms** (*Tsuga* sp.) (Plate. 1a-c)

LM: Monosaccate, oblate, circular in polar view; Equatorial axis 76 µm. SEM: Corpus verrucate to regulate, foveolate and echinate. Saccus are echinate and folded.

**Angiosperms Fagaceae** (*Quercus* sp.) (Plate. 1d-f)

LM: Prolate, circular, and lobate in polar view; 22 µm in polar axis and 21 µm in equatorial axis. SEM: Aperture is tricolporate, Colpi relatively broad; Exine is 1-1.25 µm thick, tectum consists of very small (<1 µm), randomly oriented and uniformly distributed rods.

**Fagaceae** (*Fagus* sp.) (Plate. 1g-i)

LM: Lobate in polar view; 33µm in polar axis and 24 in polar view. SEM: Aperture is tricolporate, Colpi relatively broad; Exine is 2-2.5 µm thick tectum consists of very small (<1 µm), randomly oriented and uniformly distributed rods.

**Juglandaceae** (*Juglans* sp.) (Plate. 1j-l)

LM: Oblate, (sub spheroidal to spheroidal) and circular; 41-43 µm. SEM: Aperture is Pantoporate, pores aspidate, circular rarely oval, 2-3 µm; Exine is 2µm thick, sexine is thicker than nexine, tectum is microechinate.

**Betulaceae** (*Alnus* sp.) (Plate. 2 a-c)

LM: Oblate, penta-angular in polar view, 35-40 µm in size. SEM: aperture is pentorate, porivestibulum type, neighboring pori connected by arcs or bands of nexinous thickening; Exine is 1.5 µm, tectum consists of irregular regulate with very small spinules. Sexine slightly thicker than nexine.

**Betulaceae** (*Betula* sp.) (Plate 2d-f)

LM: Oblate, triangular in polar view and 29 µm. SEM: aperture is triporate occasionally tetraporate, pores circular and vestibulum type, sexine with
Plate 1: LM and SEM of pollen grains. (a-c). *Tsuga* sp.: (a) Polar view LM X 600; (b) Polar view, SEM X 1400 as same grain in (a); (c) Detail view of Tectum, SEM X 4300 as same grain in (a). (d-f). *Quercus* sp., (d) Equatorial view, LM X 600; (e) Polar view, SEM X 3000 of grain (d); (f) Detail view of Tectum, SEM X 14000 of grain (d). (g-i). *Fagus* sp., (g) Equatorial view, LM X 600; (h) Equatorial view, SEM X 2200 of grain (e); (i) Detail view of Tectum, SEM X10000 of grain (e). (j-l). *Juglans* sp., (j) LM X 600; (k) SEM X 1900 of grain (j); (l) Detail view of Tectum, SEM X 10000 of grain (j).
tectum thickest at the apex, vestibulum forming a well-defined annulus; Exine is 2 µm, sexine consists of irregular regulae with very small spinules.

**Tiliaceae (**Tilia** sp.) (Plate 2g-i)**

LM: Triangular to rounded in the polar view and oblate in the side view and 35 µm in size. SEM: aperture is tricolporate; Exine is three very short oval furrows, each containing an elliptical pore, which is surrounded by coarse areas. A network pattern tapers to the equator and there are small polar meshes.

**Salicaceae (**Salix** sp.) (Plate 2j-l)**

LM: Prolate, reticulate, and 20-25 µm in size. SEM: aperture is tricolporate; the reticulation decreases in size near the furrow, often producing a psilatemargo parallel to the furrow. The lumina generally decrease in size at the poles.

**Aceraceae (**Acer** sp.) (Plate 3a-c)**

LM: Prolate shape with 26-30 µm. SEM: aperture is trocolpate, exine is rugulate-striate sculpture.

**Oleaceae (**Fraxinus** sp.) (Plate 3d-f)**

LM: Prolate, circular in polar view, polar axis 17 µm and equatorial axis 14 µm. SEM: aperture is tricolporate, colpi are long, small circular endoaperture. Exine is 1 µm, sexine thicker than nexine, reticulate, lumina are heterobrochate, triangular to polygonal in shape, muri with a fine ring-like ornamentation.

**Oleaceae (**Ligustrum** sp.) (Plate 3g-i)**

LM: Sub prolate, polar axis is 22 µm and equatorial axis 20 µm. SEM: aperture is tricolporate, exine is 1.2 µm, sexine is thicker than nexine, reticulate, heterobrachate, muri are smooth, lamina with rudimentary columellae.

**Caryophyllaceae (Caryophyllaceae gen. indet.) (Plate 3j-l)**

LM: Spheroidal, 28-30 µm in size. SEM: aperture is pantoporate, pori circular, diameter ca. 2 µm, exine is 2 µm, sexine as thick as exine, sexine perforate, spinulate, pore membrane spinulate.

**Rosaceae (**Rosaceae** gen. indet.) (Plate 4a-c)**

LM: Shape is oblate spheroidal, triangular in polar view with polar axis in 23 µm and equatorial axis is 16 µm in size. SEM: aperture is tricolporate, colpi long, exine is 1-1.5 µm, sexine is thicker than nexine, striate, striations sometimes joined with each other and variable in size.

**Vitaceae (Parthenocissus sp.) (Plate 4d-f)**

LM: Shape is prolate, semi angular in polar view with polar axis is 38 µm and equatorial view is 29 µm. SEM: aperture is tricolporate, and exine is 1.5 µm, sexine thicker than nexine, microreticulate to perforate and sparsely covered by granules.

**Chenopodiaceae (Chenopodiaceae gen. indet.) (Plate 4g-i)**

LM: Spheroidal with 28 µm in size. SEM: aperture is pantoporate, pore circular, exine is 1 µm, sexine thicker than nexine, sexine spinulate, perforate, mesosporium raised above pori, pore membrane spinulate, granulate.

**Plantaginaceae (**Plantago** sp.) (Plate 4j-l)**

LM: Spherical shape with 20 µm in size. SEM: aperture is pantoporate, diameter of pore 2-3 µm, exine is 1 µm, Pores are surrounded by a thickened, projecting ring known as annulus, and with an operculum. Network of small hills and valleys, with large irregular verrucae.

**Poaceae (Poaceae gen. indet.) (Plate 5a-c)**

LM: spheroidal with 21 µm in size. SEM: aperture is ulcerate, pore annulate, diameter of pore 2-3 µm, exine is 1 µm, sexine as thick as nexine, microverrucate with small spiny elements especially at the edges of the microverrucae.

**Compositae (Compositae gen. indet.) (Plate 5d-f)**

LM: Sub-prolate to prolate with 25 µm in size. SEM: aperture is tricolporate, exine is 5-6 µm (with spines), sexine thick, sexine perforate and echinate, the basal part of spine broadly perforate with perforations of various shape and sizes, surface granulate, spines 2-3 µm in length.

**Asteraceae (**Artemisia** sp.) (Plate 5g-i)**

LM: Prolate circular in polar view, lobate with 22 µm. SEM: aperture is tricolporate, colpi nearly as long as polar axis, exine is 3 µm, sexine much thicker than nexine, distinctly stratified, sexine in mesocolpium thicker than in the colpi area forming margo, sexine microechinate and granulate, granules uniformly distributed between the spinules.

**Undetermined (Plate 5j-l)**

LM: Spheroidal in equatorial view, subtriangular in polar view, size is 25 µm in polar axis, equatorial view is 22 µm. SEM: aperture is tricolporate, exine is 1-2 µm thick, tectum is granules uniformly distributed between the spinules.
Plate 2: LM and SEM of pollen grains. (a-c). *Alnus* sp.: (a) Polar view LM X 600; (b) Polar view, SEM X 3700 as same grain in (a); (c) Detail view of Tectum, SEM X 14000 as same grain in (a). (d-f). *Betula* sp., (d) Polar view, LM X 600; (e) Polar view, SEM X 4000 of grain (d); (f) Detail view of Tectum, SEM X 14000 of grain (d). (g-i). *Tilia* sp., (g) Equatorial view, LM X 600; (h) Equatorial view, SEM X 2000 of grain (e); (i) Detail view of Tectum, SEM X 10000 of grain (e). (j-l). *Salix* sp., (j) Equatorial view LM X 600; (k) Equatorial view SEM X 3300 of grain (j); (l) Detail view of Tectum, SEM X 17000 of grain (j).
Plate 3: LM and SEM of pollen grains. (a-c). *Acer* sp.; (a) Polar view LM X 600; (b) Equatorial view, SEM X 3000 as same grain in (a); (c) Detail view of Tectum, SEM X 14000 as same grain in (a). d-f, *Fraxinus* sp., (d) Polar view, LM X 600; (e) Polar view SEM X 2500 of grain (d); (f) Detail view of Tectum, SEM X 14000 of grain (d). g-i, *Ligustrum* sp., (g) Equatorial view, LM X 600; (h) Equatorial view, SEM X 3300 of grain (e); (i) Detail view of Tectum, SEM X10000 of grain (e). (j-l). Caryophyllaceae, (j) LM X 600; (k) SEM X 4300 of grain (j); (l) Detail view of Tectum, SEM X 10000 of grain (j).
Plate 4: LM and SEM of pollen grains. (a-c). Rosaceae, (a) Polar view LM X 600; (b) Equatorial view, SEM X 3300 as same grain in (a); (c) Detail view of Tectum, SEM X 14000 as same grain in (a). (d-f) *Parthenocissus* sp., (d) Equatorial view, LM X 600; (e) Polar view SEM X 2000 of grain (d); (f) Detail view of Tectum, SEM X 8500 of grain (d). (g-i). Chenopodiaceae, (g) LM X 600; (h) SEM X 3500 of grain (e); (i) Detail view of Tectum, SEM X 14000 of grain (e). (j-l). *Plantago* sp., (j) LM X 600; (k) SEM X 3500 of grain (j); (l) Detail view of Tectum, SEM X 10000 of grain (j).
Plate 5: LM and SEM of pollen grains. (a-c). Poaceae, (a) LM X 600; (b) SEM X 3500 as same grain in (a); (c) Detail view of Tectum, SEM X 10000 as same grain in (a). (d-f). Compositae, (d) Equatorial view, LM X 600; (e) Polar view SEM X 2300 of grain (d); (f) Detail view of Tectum, SEM X 2300 of grain (d). Artemisia sp., (g) LM X 600; (h) SEM X 4500 of grain (e); (i) Detail view of Tectum, SEM X14000 of grain (e). (j-l). Unidentified, (j) Polar LM X 600; (k) SEM X 2500 of grain (j); (l) Detail view of Tectum, SEM X 14000 of grain (j).
DISCUSSIONS

Paleoenvironmental analysis based on pollen indicates that the interaction between tectonism, provenance and surrounding environment influenced the depositional sedimentary history and paleo-flora in this graben. The tectonic subsidence in this graben was controlled by the activation of Dangardzong Fault and STDS (Hutardo et al., 2001; Chamlagain and Hayashi, 2006). These faults primarily controlled the basin formation process and the source area of the pollen but the present study suggest that paleo-flora in this graben was mostly determined by regional monsoon intensification during the deposition time. The provenance analysis of the graben-fill sediments indicates sediments were mostly transported from the north to south (Adhikari and Wagreich, 2010). The sedimentation pattern in this graben was affected by both the depositional phases and the surrounding environment. The sediments were deposited in braided river channel, fluvial and lacustrine environments. The layer with the fine-grained sediments get deposited in the lacustrine environment and consists of pollen in poorly preserved condition. The percentage of Non-Arboreal Pollen (NAP) (90%) is very high as compared to Arboreal Pollen (AP), which shows that most of the pollen grains are deposited in these sediments were transported from the surrounding environment except some coniferous pollen (10%). The presence of evergreen subtropical and temperate deciduous broadleaf forest (Quercus, Betula, Juglans, Alnus), needle-leaved element (Pinus, Tsuga) and high altitude taxa (Picea, Abies) with Artemisia, Chenopodiaceae, Poaceae, Rosaceae show mixed pollen assemblages. This pollen assemblage is comparable to the Gyirong Basin (Xu et al., 2012) and broadleaf mixed forest was widespread in the southern Tibet during the north-south trending graben development (10-11 Ma) further indicating a warm and humid environment. Pollen grains of Coniferous plants dominate the upper horizon of the Tetang Formation and the δ¹⁸O study confirmed that the altitude was higher during the depositional time at 11-9.6 Ma than the present time (Adhikari, 2009).

The higher percentage of Ephedra spores also suggests that the dry climate was present during the deposition of Tetang Formation (Yoshida et al., 1984). The presence of Betula, Quercus, and Juglans suggests that temperate forest increased significantly implying a humid climate (Sun et al., 1996) (Table 1). The upliftment of the Himalayas acted as a barrier and exerted an intense effect upon the atmospheric circulation and environmental change in Asia (Ruddiman and Kurzbach, 1989; Li and Fang, 1999). So, the water vapor carried by the south-west monsoon could not reach the Tibetan Plateau leading to the decrease of rainfall and gradual vegetation change to arid grassland in this valley. Moreover, a high positive value of δ¹³C from this graben (Adhikari and Wagreich, 2013) indicates low plant respiration rates and predominance of C₄ plants as compared to the Siwalik (Harrison et al., 1993).

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CONCLUSIONS

The palynological investigation from the graben fill sediments of the Thakkhola Graben reports nineteen families and genera of plants with the help of Light Microscope and Scanning Electron Microscope. The pollen assemblages from the Tetang and Thakkhola formations are represented by broadleaf forest, needle-leaved, and high altitude taxa. The abundances of Coniferous pollen indicate the altitude was higher during the depositional time of graben-fill sediments. The presence of Betula, Quercus, and Juglans suggest that temperate forest increased significantly implying a humid environment. Similarly, the upliftment of Himalaya also acted as a topographic barrier and exert an intense effect upon atmospheric circulation so that more steppe vegetation i.e. Artemisia, Chenopodiaceae were dominated during the sediment deposition.

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AUTHOR’S CONTRIBUTIONS

B. R. Adhikari carried out fieldwork, data analysis, conceptualization, and manuscript preparation. K. N. Paudayal carried out pollen analysis. Both authors discussed and approved the manuscript.

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