**An Acad Bras Cienc (2024) 96(4): e20231241 DOI 10.1590/0001-3765202420231241**
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

PALEONTOLOGY

# Palynological Investigations of the Miocene sediments from Murree formation of Pakistan: Evidence for Palaeoenvironment and Palaeoclimate interpretations

SHABIR AHMAD, MUSHTAQ AHMAD, MUHAMMAD ZAFAR, SHAZIA SULTANA, MOHAMED FAWZY RAMADAN, SYED NOHMAN GILANI, MARYAM ANJUM ABBASI & SHAISTA JABEEN

**Abstract:** The present study was carried out to investigate the palynoflora of Murree formation using microscopic techniques to understand the climatic changes and vegetation's evolution. In this palynological study, 31 samples were collected, analyzed, and then identified as palynomorphs using different previous published literature. The results of this study will be described in terms of the evolutionary history of plants and the depositional environment of the reported taxa in the study area. The botanical affinities and systematic description of the taxa were examined using light and scanning electron microscopy. The floral record identified Asteraceae as the dominant family and Pinus as a genus. Most of angiosperms i.e Fabaceae and Poaceae, have a poor fossil record but have an abundant palynological record in the study area. The highest polar diameter (75.75 µm), colpus length (34.5 µm) and colpus width (31 µm) were examined for the *Convolvulus*. Most of the taxa explained here had a wide geographical occurrence in Southeast Asia and show the abundance of angiosperms in the Miocene epoch. The palynological record of Holocene samples is important to know about the vegetation's origin and environmental fluctuations in the study area.

**Key words:** Palynoflora, Miocene, microscopy, climate, taxonomic resolution.

## INTRODUCTION

The Miocene Epoch is of considerable importance for the evolution of plants and animals. It is further classified into 3 groups based on lithology for palaeo-environment and palaeo-climate changes, i.e., early, middle, and late Miocene epochs. Based on the proxy's data different paleo-climatic conditions existed that were quite different from the present climate. Considering the palynological data from the study area, we investigate the paleoflora of the proposed era through pollen analysis from Murree formation. In this epoch, significant climatic and environmental variations occurred in many parts of the world especially Asia (Chen et al. 2019, Sun & Wang 2005). Pollen grains and spores preserved in rock sediments serve as a proxy to indicate the previous vegetation and environment of the study area. The knowledge of palaeoflora from sedimentary strata of the Miocene has produced a rich record of fossils, including animals and plants (Flynn & Jacobs 1982, Shah 1977) while most of the studies of Miocene palynomorphs from all over the world were reported based on light and scanning electron microscopy. In this paper, we have compiled the data from the sedimentologic, stratigraphic, and systematic point of view to

know about the vegetation's evolution and depositional environment of the Miocene from the Murree formation of Pakistan (Ahmad et al. 2023).

The Murree formation has strong tectonic affinity regarding the structural evolution with the Himalayan orogeny. The formation has been named Murree due to the locality of Murree Hills in the district of Rawalpindi, Pakistan. It has expanded in Islamabad, Rawalpindi, Kohat, Potwar, and Kashmir. The strata are comparatively widely distributed to the Oligocene. Palynological study of the Miocene was unexplored and had a great interest in determining the paleoenvironment of the study. It provides important information to find out how the fossil plant pollen record in the CMB relates to the Indo–Asian tectonic collision and the environmental change preceding the Miocene-Eocene Transition (Strother et al. 2017, Yar et al. 2017, Ahmad et al. 2023).

The present study area is located in Islamabad, in the foothills of Margalla. This research work is very important and interesting to the palynologists in South Asia (particularly in India) and around the world. The sediments of Murree formations were deposited (23.0–3.33 Ma) in the fore deep of Margalla hills. Its section thickness varies in 9–3030 m range. In northern salt ranges the thickness of the formation ranges between 180 and 600 m. It consists of dark-red, purple color sandstones, intraformational conglomerates, reddish mudstones, purple-gray-dark shales, and siltstones. The lower formation predominantly consists of mudstones, siltstones, and shale's while the upper Murree formation consists of arenaceous lithology. The Murree formation was classified into 11 facies based on the sedimentological features. Near Ghori between Kuldana and Murree formation, there exists a fossil record of foraminifera (Mughal et al. 2018). It has formed a unique fossil record of mammalian bones, fish, frogs and silicified woods (Shah 1977). In Fateh Jang of this formation, many mammalian bones have been recorded, i.e *Hemimeryx* sp, *Brachyodus giganteus* and *Teleoceras fatehjangensis*. In Potwar and Kohat, it reveals a fluvial while in Kashmir-Hazara, it shows a shallow marine environment (Bossart & Ottiger 1989). In the Kashmir zone dispersed forms of sediments were recorded from this formation (Singh et al. 2000). It has been further classified into two types lower and upper Murree horizons (Wynne 1874). In the Siwalik sedimentary strata of Darjeeling Himalaya the sections were exposed and enriched with fossil palynomorphs. For the eastern Himalaya, the Siwalik group and India of the Miocene epoch humid environment were investigated (More et al. 2016). Our study also showed river meandering depositional environments in the study area (Kundu et al. 2012).

The Himalayan foreland basin in Pakistan's Miocene–Pliocene Siwalik Group reflects changing river conditions (Garzanti et al. 1996). In the Potwar Plateau of Siwalik range many faunal variations have been recorded due to climatic changes and geographical factors such as formation of land bridge between Asia and Africa (Barry 1986). Some of the Siwalik Group's sediments are entirely fluvial, having been laid down by extensive river systems that were once part of the western Indus and Ganges Rivers and their tributaries. Based on the mammalian record the Siwalik range of Pakistan has poor preservation of plant remains or fossils to compare with the palaeo-environment reconstructions (Quade et al. 1995). The fossil wood were studied from the Miocene Manchar formation, Sindh which provide evidence for the existence of fossil plants in different strata of Pakistan (Soomro et al. 2021)

The main questions in the present study are focused on plant taxonomy and biogeography. We ask, when did the first vegetation's record appear, and what is its correlation with the modern plants found in the study area? How did species biodiversity change over time? To answer these questions, we process the rock sediments and investigate the pollen morphology of fossil plants along with their occurrences. Based on this data, we have identified pollen taxa and investigated how plant biodiversity changed in the study area. These results are very vital to knowing how past vegetation's responded to the climate and how to challenge it in the future in the study area.

Various research works have been done on the geological aspects of the Miocene (Yar et al. 2021), but palynological records to interpret the paleoclimate and paleoenvironment were performed in Pakistan. Our study was based on pollen analysis found in the Murree formation, using pollen quantitative data and taxonomic approaches to answer the above questions. The study provides systematic linkages about the existence of fossil floral records. Paleo-environmental analysis helps in determining the physical, biological, and chemical conditions of the environment. It helps in the identification of the microfossil plants and determines the estimated age of the rocks. The study helps to find out regional paleoclimate and climate changes in the deposition area during the Miocene epoch. It helps to update the pollen profile by examining the morphological characteristics of fossil plant pollen (Ahmad et al. 2023).

## MATERIALS AND METHODS

The field trips were carried out to different regions of the Margalla hills to collect the sediments of the Miocene, Murree formation of Pakistan. The pollen grains were extracted from the shales and mudstone of the sedimentary rocks of the Miocene age. The pollen grains were identified based on morphological features and compared with the regional palynoflora and other previous published literature. The botanical affinities and taxonomic features of the pollen grains were determined following the published literature of (Miao et al. 2011, Grímsson et al. 2012, Jiménez-Moreno et al 2010). During the field survey the grain color, size, rainfall, temperature, latitude, and longitude of the study area were noted. The Miocene Murree formation has distinguished features based on lithology, bed thickness, grain structure, size, and color. It consists of sandstones, mud stones, shales and calcareous stones. Based on phylogeny, i.e., fossil mammalian records, fish, and plants remain, the study area is considered to be from the Miocene epoch.

### Samples collection

In the field survey 31 samples were collected from blackish shales to grey shales and siltstone units along the 70-meter-thick Murree section from Murree formations, Islamabad Margalla foothills and then processed it for palynological purposes. The samples were photographed, collected in polythene bags, and then brought to the laboratory for further processing. The rock sediments were collected carefully to avoid contamination (Figures 1, 2 and 3).

### Palynological analysis

For palynological processing, 30 g of each sample were taken and crushed into segments of 2-4 mm in size. The samples were washed with distilled water to remove dust particles and processed according to the standard techniques (Jaramillo et al. 2014, More et al. 2016, Traverse 2007). The samples were reacted with 10% Hydrochloric acid (HCl) for the removal of carbonates, and then treated with water to


**Figure 1.** (a) Hammer placed on the rock for cutting the rock sediments (b) Samples collection from the study area (c) Samples collected and placed in polythene bag for palynological purposes (d) View of study area

neutralize it. The samples were again reacted with 40% HF for at least 1 day for the removal of silicate particles and treated with distilled water for the purpose of neutralization. For further palynological processing, the samples were centrifuged at 2500 rpm to separate heavy and light particles. Heavy liquid ($ZnCl_2$) with a specific gravity of (1.9–2.0) was treated with samples and centrifuged again at 2500 rpm for 5 minutes. With a 150-µm mesh, the samples were then sieved to remove plant fragments and coarse debris. The obtained pollen residue was filtered using a 10 µm sieve and kept in distilled water until mounted into glycerol jelly on slides for pollen analysis. The supernatants were then retained after centrifugation. Samples were then put on a slide with the help of a camel brush and kept drying for a few minutes. For staining purposes, glycerin jelly was put on the slide, prepared according to the standard protocol (Meo & Khan 2005). Paraffin Max was used for the preparation of permanent slides. In this study, qualitative and quantitative features of palynomorphs were studied, and photographs were taken with a Meiji Infinity 1 camera fixed to a Leica microscope.

## Taxa identifications

The palynomorphs were identified using pollen atlases and previously published literature ( Miao et al. 2011, Wang et al. 1996, Xu et al. 2008, Grímsson et al. 2022, Jiménez-Moreno et al. 2010). The distinguishing characteristics of pollen grains, i.e., size, shape, diameter, aperture, colpi, and exine ornamentations, were very helpful for taxa identification in the field of plant taxonomy. The identification was carried out up to the family, genera, and somewhat species level (Ahmad et al. 2023).

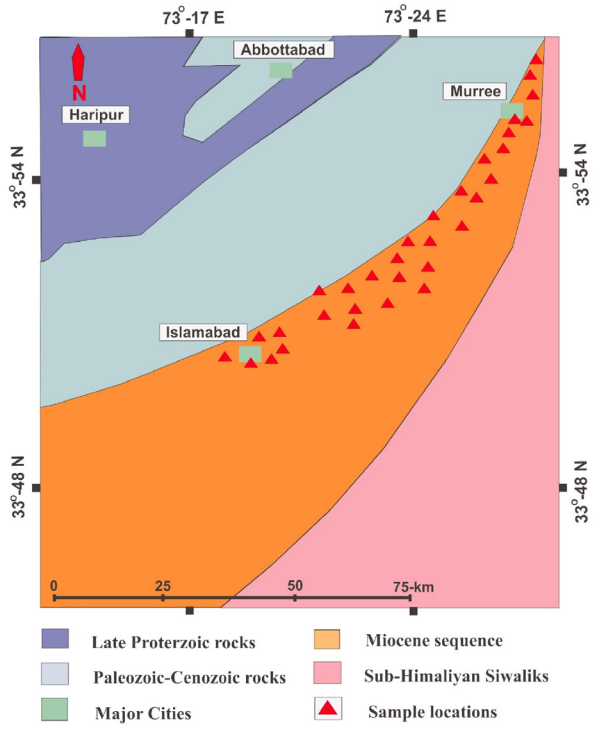


**Figure 2. Map of the study area showing the sample sites (Ahmad et al. 2023).**

## RESULTS

Using light and scanning electron microscopy, the diagnostic characters of palynomorphs were recorded, belonging to diverse families (Supplementary Material - Figure S3). The pollen terminology was described using previously published literature (Punt et al. 2007, Faegri 1966). In this study, a total 65 sporomorphs were reported, out of which 32 were identified up to the species, genera, and then family level. Qualitative and quantitative features of palynomorphs were recorded, which helps in accurate species identification, as shown in Supplementary Material - Tables SI and SII, and their micrographs are shown in Figures 4, 5, S4, and S5. The morphological characters of palynomorphs were examined, which are very important to study regarding paleoclimate, paleoecology, and biogeography. Palynomorphs' morphological features were investigated using

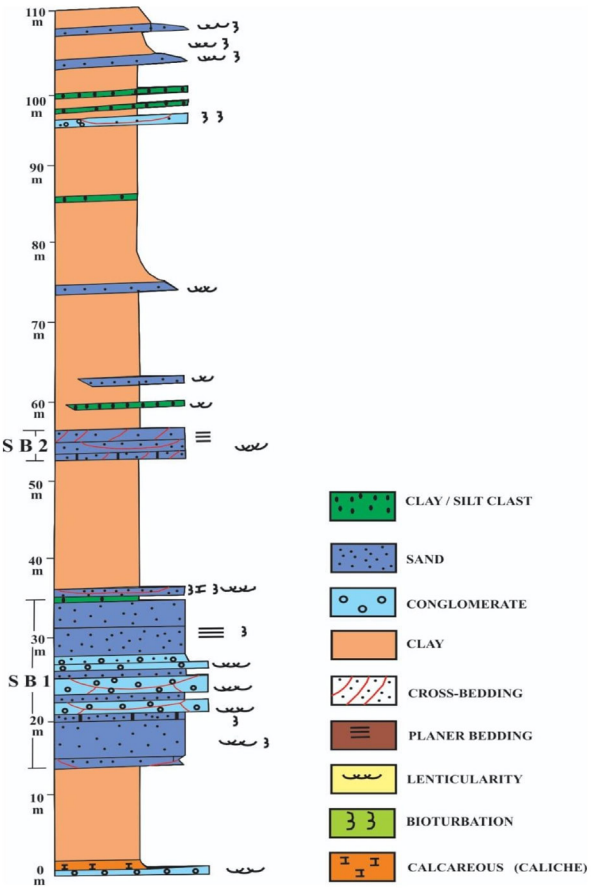


**Figure 3. Lithological column of the Murree Formation, Pakistan (Ahmad et al. 2023).**

qualitative and quantitative pollen characters to determine the paleoclimate of the past environment and further described along with the section near the Murree Formation. Pollen grains were described using the basic terminology of (Halbritter et al. 2018, Hesse et al. 2009, Erdtman et al. 1992).

### Asteraceae

*Artemisia* pollen was circular with psilate ornamentations, an exine thickness of 1.75 µm, and a pollen diameter of 17.5 µm. Chichorioideae pollen was prolate-spheroidal, tricolpate, psilate-echinate ornamentations, exine thickness 2.25–4.75 µm, pollen diameter 13.00–13.25 µm, colpi length 2.5–5.5 µm, colpi width 9.00 µm, spine length 6.00 µm, and spine

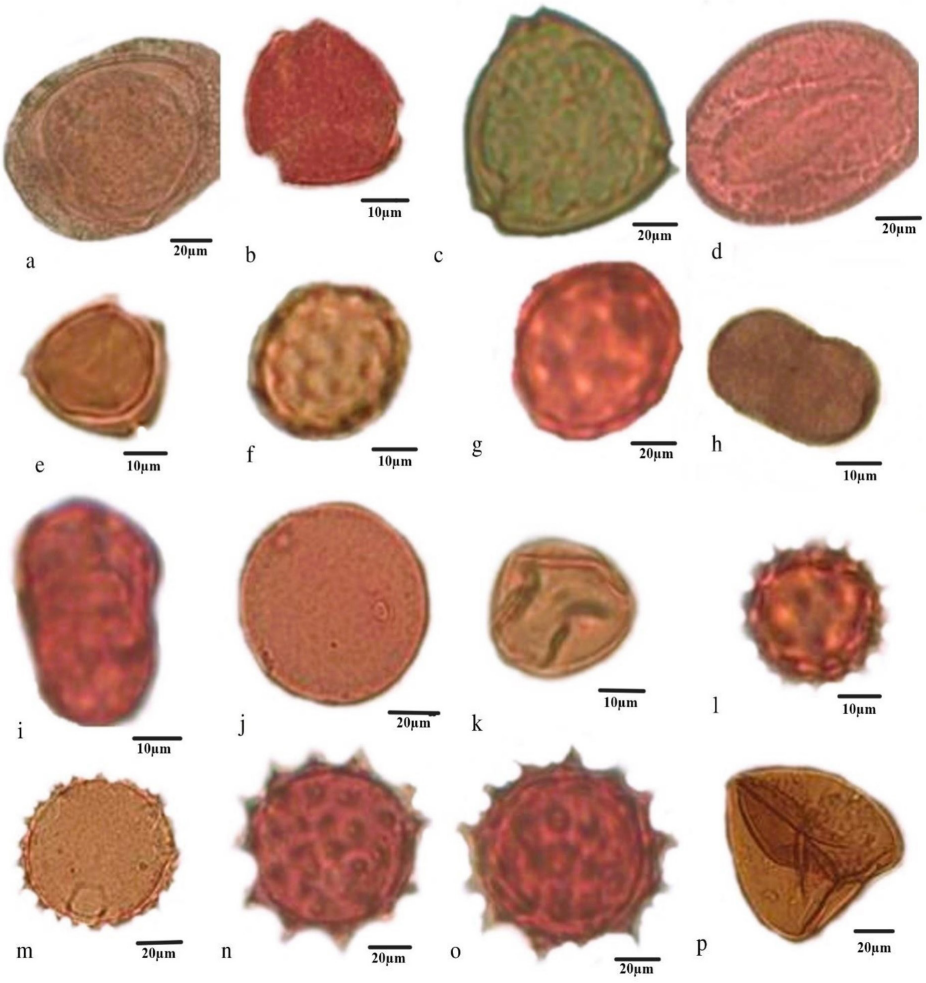


**Figure 4.** Light microscopy pollen micrographs. (a) Pinus (b) Fabaceae (c) *Corylus* (d) Arecaceae (e) Solanaceae (f-g) Amaranthaceae (h-i) Apiaceae (j-k) Poaceae (l-o) Asteraceae (p) *Triplanosporoites.*

width 5.00 µm. While the rest of the pollen was circular with psilate ornamentations, the exine thickness was 1.4–2 µm, the pollen diameter was 16.25–31.75 µm, the spine length was 3.25–6.75 µm, the spine width was 3.5–4.75 µm, the pores length was 5.0–6.25 µm, and the pores width was 2.75–5.00 µm.

### Pinaceae

*Pinus* pollen was bissacate, prolate- semi-angular, tricolpate, reticulate ornamentations having exine thickness was 1.25-4.75 µm and pollen diameter was 30.75-6125 µm,

### Poaceae

Unidentified genus and species belonging to Poaceae. Pollen grains were noted to be circular with psilate ornamentations, an exine thickness of 2.00 µm, and a pollen diameter of 16–30 µm.

### Apiaceae

Unidentified genus and species belonging to Poaceae. Pollen prolate-peroblate reticulate ornamentations have an exine thickness of 2–2.5 µm and a pollen diameter of 17.5-20.0 µm.

### Nyssaceae

*Nyssa* pollen was semi-angular-circular, triporate-tricolpate, reticulate ornamentations, exine thickness 2.25-2.75 µm, pollen diameter 28.5-35.00 µm, colpi length 2.5-11.75 µm, and colpi width 3.25 µm.

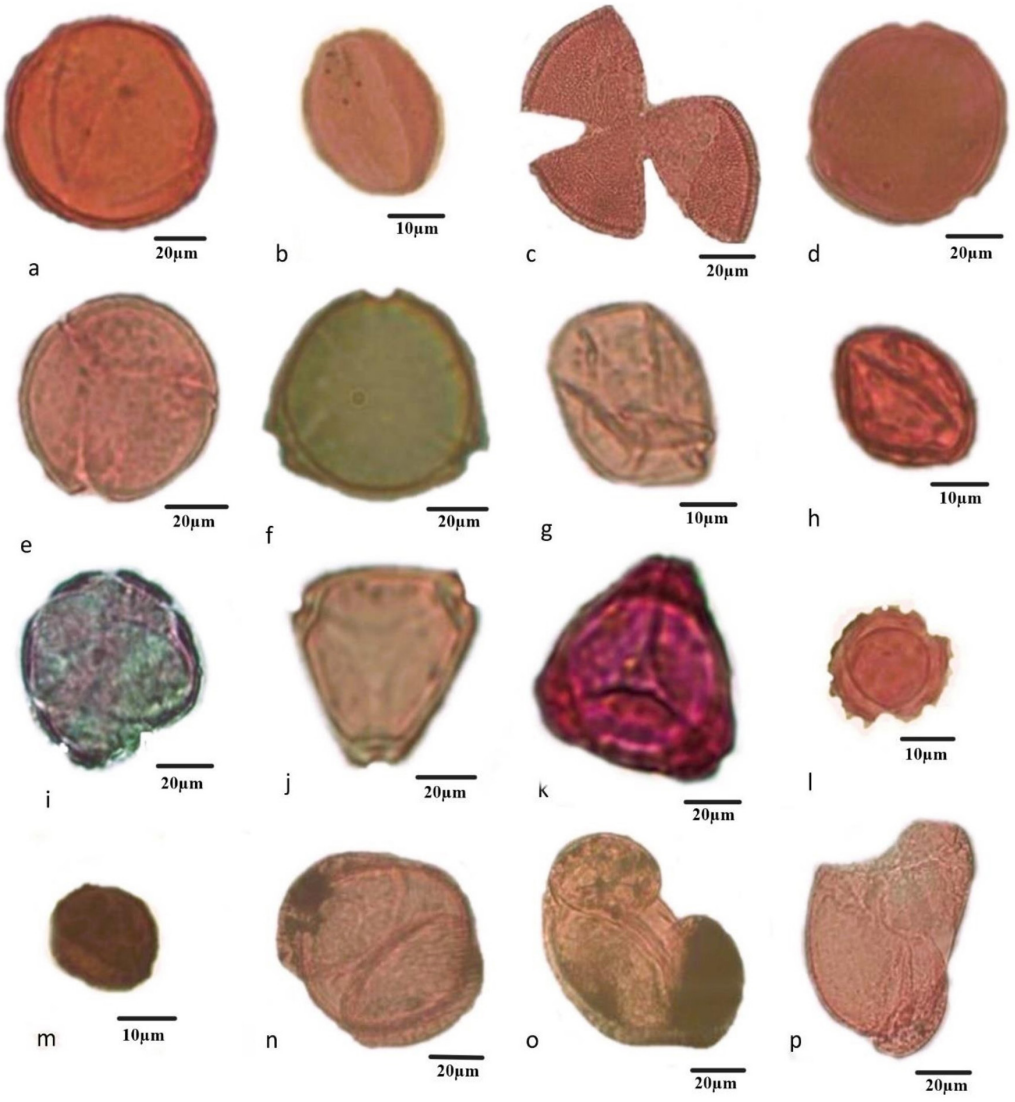


**Figure 5.** Light microscopy pollen micrographs. (a) *Sanguisobra minor* (b) *Artemisia* (c) *Convolvulus* (d) Carya (e-f) *Nyssa* (g-h) *Quercus* (i-j) *Myrtus* (k) Myrtaceae (l-m) Chichorioideae (n-p) *Pinus*.

## Fabaceae

Unidentified genus and species belonging to Fabaceae. Pollen has semi-angular, tricolporate, and psilate ornamentations; exine thickness is 1.75 µm; pollen diameter is 22.5 µm; colpi length is 13.25 µm; and colpi width is 5.5 µm.

## Fagaceae

The *Quercus* pollen was prolate, triporate-tricolpate, with reticulate ornamentations, an exine thickness of 2.75–3.25 µm, and a pollen diameter of 19.5-23.25 µm.

## Myrtaceae

*Myrtus* pollen was sub-angular, tricolporate, psilate ornamentations, exine thickness 2.00 µm, pollen diameter 17.5 µm, colpi length 5.25 µm, colpi width 9.75 µm, pores length 5.25 and pores width 6.25 µm. While the rest of the pollen noted were lobate, tricolporate, and psilate ornamentations, the exine thickness was 1.75 µm, the pollen diameter was 19.5 µm, the colpi length was 13.25 µm, the colpi width was 3.5 µm, the pores length was 6.00 µm, and the pores width was 9.00 µm.

### Rosaceae

*Sanguisobra minor* pollen grains have circular, psilate ornamentations, an exine thickness of 5.0 µm, and a pollen diameter of 42.0 µm.

### Juglandaceae

*Carya* pollen is semi-angular, tricolpate, with reticulate ornamentations; the exine thickness is 2.5 µm; the pollen diameter is 31.25 µm; the colpi length is 2.75 µm; and the colpi width is 6.75 µm.

### Solanaceae

Unidentified genera and species belong to the Solanaceae. The pollen had angular, triporate, reticulate ornamentations, an exine thickness of 2.25 µm, a pollen diameter of 13.25 µm, a pore length of 4.75 µm, and a pore width of 4.25 µm.

### Amaranthaceae

Unidentified genera and species belong to the Amaranthaceae. Pollen circular, polyproate triporate, reticulate ornamentations, exine thickness 3.00 µm and pollen diameter 3.75 µm,

### Arecaceae

Unidentified genera and species belong to the family the family Arecaceae. Pollen was peroblate, reticulate ornamentations, exine thickness was 3.75 µm, and pollen diameter was 55.75 µm.

### Betulaceae

The *Corylus* pollen had semi-angular, tricolporate, and psilate ornamentations, an exine thickness of 2.25 µm, a pollen diameter of 37.5 µm, a pore length of 6 µm, and a pore width of 1.25 µm.

### Lamiaceae

Pollen was spheroidal, hexacolpate, with reticulate ornamentations; exine thickness was 2.5 µm; pollen diameter was 35.5 µm; colpi length was 6.75; and colpi width was 35.5 µm.

### Convolvulaceae

The *Convolvulus* pollen was inter-semi-angular, tricolpate, with reticulate ornamentations, an exine thickness of 4.0 µm, a pollen diameter of 75.75 µm, a colpi length of 34.5 µm, and a colpi width of 31.0 µm.

### Spore

*Triplanosporoites* pollen was angular, with psilate ornamentations, an exine thickness of 2.25 µm, and a pollen diameter of 32.5 µm.

## DISCUSSION

In the present study, 32 palynomorphs were examined, out of which 31 contained pollen belonging to 16 different families and 1 was spore. The sporomorphs contained 27 angiosperms, 4 gymnosperms, and 1 pteridophytic spore. Asteraceae has been recorded as the dominant family, followed by Pinaceae, Poaceae, Apiaceae, Nyssaceae, Fagaceae, Myrtaceae, Amaranthaceae, Solanaceae, Rosaceae, Juglandaceae, Convolvulaceae, Arecaceae, Lamiaceae, and Betulaceae. The sculpture of Asteraceae was investigated following the published literature of Wodehouse (1929a) and Wodehouse (1929b). Similarly, the Chichorioide tribe and genus, i.e., *Pinus*, *Artemisia*, Nyssa, and *Quercus,* have been investigated. The angiosperms contain 27 pollen grains belonging to 15 different families: Asteraceae (*Artemisia*, Cichirideae), Rosaceae (*Sanguisobra minor*), Fagaceae (*Quercus*), Nyssaceae (*Nyssa*), Convolvulaceae (*Convolvulus*), Myrtaceae (*Myrtus*), Juglandaceae (*Carya*), Apiaceae, Poaceae, Fabaceae, Solanaceae, Amaranthaceae, Arecaceae, and Lamiaceae. In the 31 samples analyzed, 15 were barren and 16 contained various palynomorphs.

Qualitative and quantitative features of palynomorphs observed through scanning and light microscopy using palaeobotanical techniques proved very helpful for species identification in the fields of paleobotany and plant systematics. Pollen grains examined through light microscopy were hexacolpate, tricolporate, tricolpate, triporate, and polyporate in nature. Numerous shapes of the pollen grains were studied, i.e., circular, prolate, peroblate, angular, inter-semi-angular, subangular, lobate, spheroidal, and prolate-spheroidal. As very little research has been conducted to investigate the palynoflora of the Miocene, due to incomplete reference databases and complex taxonomy, the species were just identified up to the genera and family level (Ahmad et al. 2023).

Three different types of exine ornamentations were studied i.e psilate, reticulate and echinate which are very vital for differentiations and identifications of closely related species. Variations were examined in quantitative and qualitative characters of exine thickness, pollen diameter, spine length, spine width, pores length and pores width (Figures S1, S2 and S3). Highest exine thickness was seen in Cichorioideae 4.75 µm and lowest for *Pinus* 1.5 µm. Highest pollen diameter was recorded for *Convolvulus* and lowest for Chichorioideae. Highest colpus length was recorded for *Convolvulus* 34.5 µm and lowest for Chichorioideae 2.5 µm. Maximum colpus width was noted for *Convolvulus* 31 µm and minimum for *Nyssa* 3.25 µm. Highest pores length was recorded in Asteraceae 6.25 µm and lowest in Solanaceae 2.5 µm. Similarly, the highest pores width was examined for Fabaceae 9.5 µm and lowest for *Corylus* 1.5 µm. Colpi were noted just in *Convolvulus*, *Carya*, *Nyssa*, *Myrtus*, Cichorioideae, Lamiaceae and Fabaceae.

The shapes of pollen grains were measured using Halbritter et al. (2018). The *Pinus* pollen observed in the previous study were bisaccate, monad, oblate, acci nearly circular with its narrow attachment alveolate structuring (LM), corpus fossulate, verrucate, rugulate, and sacci perforated in nature (SEM), while the pollen grains observed in this study were bisaccate, prolate-semi angular, and psilate-reticulate ornamentations (Grímsson & Zetter 2011). *Quercus* pollen grains were prolate, eutectate, sculpturing perforate, micro verrucate, micro regulate (SEM), scabrate (LM), and basic sculpturing units are rod-shaped (Bouchal et al. 2016, Denk & Bouchal 2021). The *Carya* pollen were observed in the previous study to be oblate, eutectate, triporate, aperture alignment was found on the equator, and pores were sunken, sculpturing perforate and nanoechinate (SEM), and scabrate (LM), while in our study it was noted to be semi-angular, triporate, and psilate (Jiang & Ding 2008). Apiaceae pollen were noted as prolate, fossulate, microregulate (SEM), and scabrate (LM), while in this study prolate and psilate exine ornamentations were studied (Bouchal et al. 2017). The Cichorioideae pollen grains were examined as polygonal, spheroidal, tricolporate, perforate, echinate (SEM), and echinate sculpturing lophate (LM), while in this study we observed circular and echinated pollen grains (Zavada & de Villiers 2000). The Fabaceae pollen were monad, circular to irregular, eutectate, tricolporate, sculpturing scarbate (LM), perforate-fossulate, and regulate (SEM) (Grímsson et al. 2015). The Betulaceae pollen grains were seen to be oblate, eutectate, annulus present, tetra- to hexaporate (SEM), nanoechinate, and sculpturing scabrate (LM). Amaranthaceae: pollen, spheroidal, outline circular, pollen diameter 20–25 µm (LM, SEM); eutectate, exine 0.8–1.2 µm thick (LM), nexine thinner than sexine; pantoporate, pori diameter 0.7–1.0 µm (SEM), pori sunken, porioperculate, operculum ornamented with

two to six nanoechini; sculpture (Feakins 2013). The morphological features observed through microscopic techniques proved very useful for the identification of species (Ullah et al. 2019). The taxonomic identifications of accurate taxa were difficult to identify due to a lack of modern analogs for extinct data. The stratigraphic correlation and interpretation of different environmental parameters made it challenging to determine the vegetation dynamics and depositional environment of the study area (Ahmad et al. 2023).

Palynoflora investigated in the present study belongs to diverse families and is shown as an indicator of the environment in the depositional phase of the Murree formation. The assemblage of some families like Arecaceae and Amaranthaceae indicates the coastal placement of vegetation's, and Nymphaeaceae indicate the freshwater environments of the study area (Kern et al. 2013). The overall sporomorphs showed that vegetation was recorded in the coastal area or near it. The study revealed that most of the pollen belongs to angiosperms, some gymnosperms, and one pteridophytic spore. The past palynological record showed that a wetter climate and subtropical evergreen forests existed in China (Zhou 2000). It has been declared that the Miocene climate of China was similar to the modern one, and a similar case was reported in this study in Pakistan (Guo et al. 2008). The abundance of Fagaceae members, *Quercus,* shows that vegetation exists in drained soil. Among herbaceous taxa, some of the plants show that vegetation exists in rocky or sandy soil in their depositional environment (Bouchal et al. 2016). Due to global climatic changes in the late middle to early Miocene *Quercus*, *Carya* decreased while conifers increased. Higher angiosperm species (*Fagus, Betula, Quercus, and Carya*) were dominant in the study area as deciduous types. Until the late Neogene, herbaceous plants were not dominant. The palynological assemblage for *Artemesia* was reported from the Late Miocene–Pliocene in the Taibei Depression, a shelf basin of the East China Sea (Yunfa et al. 2011). *Carya* and *Nyssa* pollen were described as being triporate in nature, while in our study, *Carya* was triporate and *Nyssa* was triporate-tricolporate (Xu et al. 2008). The appearance of *Carya* and *Nyssa* as deciduous elements suggests that the climate was temperate during the cold season (Xu et al. 2008, Yu-Shu 2001). The Arecaceae pollen grains were studied to have peroblate, monocolpate, and reticulate exine ornamentations. Palynological studies of fossil plants from the Miocene Murree Formation of Pakistan were dominated by angiosperm pollen. This study investigated the past vegetation's record, its correlations with the past environment, and its reconstructions. The fossil records of Betulaceae, Fagaceae, Juglandaceae, and Arecaceae have also been documented (Klaus 1984). The dominance of families, i.e., Asteraceae, Poaceae, Pinaceae, Nyssaceae, and Fagaceae, in the study area is in accordance with the previous study carried out (Yang et al. 2018). The study provides detail about the vegetation variations that occurred in the Miocene epoch through the compilation of published research articles and a comprehensive literature review (Behrensmeyer et al. 2007, Strömberg 2011).

Paleoenvironmental changes in Asia happened primarily during the Miocene period. The current study emphasizes a wide range of geological or stratigraphic variations, demonstrating the diversity of vegetation over geological epochs (Ahmad et al. 2023). In this study, we explored the past vegetation record and how it interlinks with the environment and climate in the study area by studying the palynological investigations of microflora from the Miocene epoch. Three different palynological

successions were established in the study area: the early Miocene-prominent period for Pinaceae; the middle Miocene-predominant angiospermic plants; and the late Miocene-period of dominant herbaceous taxa. The presence of *Pinus* showed a temperate climate, while that of *Quercus* revealed a warm temperate climate. Poaceae, Fabaceae, Asteraceae, Pinaceae, and Myrtaceae were investigated from Siwalik sediments in Uttar Pradesh and support our finding (Sarkar et al. 1993). Based on the mutual interactions between evolutionary trends in vegetation and climatic changes, along with the geographical history of important species, i.e., *Artemisia,* these established connections can be linked with palynological assemblages in this study (Jiang & Ding 2008). The study provides details about the vegetation variations that occurred in the Miocene epoch through the compilation of published research articles and a comprehensive literature review. The morphological features of sporomorphs were identified as being preserved with a fair status in sedimentary rocks in the Miocene epoch of Pakistan. The study revealed that Angiosperm's taxa were comparatively dominant to gymnosperms and other lower vascular plants in the Miocene strata of Murree Formation (Ahmad et al. 2023). It has been concluded that the present palynological studies have some similarities with the previous published literature in different areas of the world.

## CONCLUSION

In the present study, a total of 32 palynomorphs were identified from the Miocene Murree formation, providing a detailed account of the climatic and vegetation variations within the study area. Based on the palynological analysis of 31 samples, it has been concluded that climatic fluctuations occurred in the late Miocene. The floral record of the Miocene Murree Formation in Pakistan provides an evolutionary history of rich plant diversity and responses to global climatic changes and species extinctions within the study area. The highest exine thickness was recorded for Asteraceae at 5 µm and the lowest for Pinus at 1.5 µm, which play a key role in the identification of species. With the help of the present study, the evolutionary record of past vegetation's extinction events, diversifications, and reconstructions of climate can be determined. Using LM and SEM, a catalogue of palynomorphs from the Miocene Murre Formation is an urgent need with its taxonomic affinities, standardizing nomenclature, and documenting the palynological assemblages of fossil plants. Based on these data, we concluded that the Miocene palynoflora shows similarity to modern vegetation, having warm tropical genera along with temperate elements. This research will contribute to the evolutionary record of past vegetation, its extinction events, diversifications, and reconstructions of climate. Further studies in the future are recommended to identify the vegetation's species and genus level through advanced microscopic techniques like transmission electron microscopy (TEM).

### Acknowledgments

The authors provide special thanks to the Herbarium of Pakistan, Quaid-i-Azam University, Islamabad, Pakistan (ISL) for providing the laboratory facilities. The authors also offer thanks to Prof. Dr. Carlos Jaramillo from the Smithsonian Institute of Panama for his continuous guidance and support thoroughly.

## REFERENCES

AHMAD S, AHMAD M, FAWZY RAMADAN M, SULTANA S, PAPINI A, ULLAH F & ZAFAR M. 2023. Palynological study of fossil plants from Miocene Murree Formation of Pakistan: Clues to investigate palaeoclimate and palaeoenvironment. Agronomy 13(1): 269.

BARRY J. 1986. A review of the chronology of Siwalik hominoids. Primate Evol 93-106.

BEHRENSMEYER AK ET AL. 2007. The structure and rate of late Miocene expansion of C4 plants: Evidence from lateral variation in stable isotopes in paleosols of the Siwalik Group, northern Pakistan. Geol Soc Am Bull 119(11-12): 1486-1505.

BOSSART P & OTTIGER R. 1989. Rocks of the Murree Formation in northern Pakistan: Indicators of a descending foreland basin of late Paleocene to middle Eocene age. Eclogae Geol Helv 82(1): 133-165.

BOUCHAL JM, MAYDA S, ZETTER R, GRÍMSSON F, AKGÜN F & DENK T. 2017. Miocene palynofloras of the Tınaz lignite mine, Muğla, southwest Anatolia: taxonomy, palaeoecology and local vegetation change. Rev Palaeobot Palynol 243: 1-36.

BOUCHAL JM, ZETTER R, GRÍMSSON F & DENK T. 2016. The middle Miocene palynoflora and palaeoenvironments of Eskihisar (Yatağan basin, south-western Anatolia): a combined LM and SEM investigation. Bot J Linn Soc 182(1): 14-79.

CHEN C, BAI Y, FANG X, GUO H, MENG Q, ZHANG W, ZHOU P & MURODOV A. 2019. A late miocene terrestrial temperature history for the northeastern Tibetan plateau's period of tectonic expansion. Geophys Res Lett 46(14): 8375-8386.

DENK T & BOUCHAL JM. 2021. New Fagaceous pollen taxa from the Miocene Søby flora of Denmark and their biogeographic implications. Am J Bot 108(8): 1500-1524.

FAEGRI K. 1966. Some problems of representivity in pollen analysis.

FEAKINS SJ. 2013. Pollen-corrected leaf wax D/H reconstructions of northeast African hydrological changes during the late Miocene. Palaeogeogr Palaeoclimatol Palaeoecol 374: 62-71.

FLYNN LJ & JACOBS LL. 1982. Effects of changing environments on Siwalik rodent faunas of northern Pakistan. Palaeogeogr Palaeoclimatol Palaeoecol 38(1-2): 129-138.

GARZANTI E, CRITELLI S & INGERSOLL RV. 1996. Paleogeographic and paleotectonic evolution of the Himalayan Range as reflected by detrital modes of Tertiary sandstones and modern sands (Indus transect, India and Pakistan). Geol Soc Am Bull 108(6): 631-642.

GRÍMSSON F, MELLER B, BOUCHAL JM & ZETTER R. 2015. Combined LM and SEM study of the middle Miocene (Sarmatian) palynoflora from the Lavanttal Basin, Austria: part III. Magnoliophyta 1–Magnoliales to Fabales. Grana 54(2): 85-128.

GRÍMSSON F & ZETTER R. 2011. Combined LM and SEM study of the middle Miocene (Sarmatian) palynoflora from the Lavanttal Basin, Austria: part II. Pinophyta (Cupressaceae, Pinaceae and Sciadopityaceae). Grana 50(4): 262-310.

GRÍMSSON F, ZETTER R & LENG Q. 2012. Diverse fossil Onagraceae pollen from a Miocene palynoflora of north-east China: early steps in resolving the phytogeographic history of the family. Plant Syst Evol 298: 671-687.

GUO ZT ET AL. 2008. A major reorganization of Asian climate by the early Miocene. Climate of the Past 4(3): 153-174.

HALBRITTER H, ULRICH S, GRÍMSSON F, WEBER M, ZETTER R, HESSE M, BUCHNER R, SVOJTKA M & FROSCH-RADIVO A. 2018. Illustrated pollen terminology. Springer.

HESSE MH, HALBRITTER R, ZETTER M, WEBER R, BUCHNER A FROSCH-RADIVO S & ULRICH. 2009. Pollen terminology: An illustrated handbook. Erdtman, G, Nilsson ST, Praglowski J, Arremo Y. 1992. Erdtman's handbook of palynology. (No Title).

JARAMILLO CA ET AL. 2014. Palynological record of the last 20 million years in Panama. Paleobotany and biogeography: A festschrift for Alan Graham in his 80th year.

JIANG H & DING Z. 2008. A 20 Ma pollen record of East-Asian summer monsoon evolution from Guyuan, Ningxia, China. Palaeogeogr. Palaeoclimatol Palaeoecol 265(1-2): 30-38.

JIMÉNEZ-MORENO G, FAUQUETTE S & SUC JP. 2010. Miocene to Pliocene vegetation reconstruction and climate estimates in the Iberian Peninsula from pollen data. Rev Paleobot Palynol 162(3): 403-415.

KERN AK, HARZHAUSER M, REUTER M, KROH A & PILLER WE. 2013. The Miocene coastal vegetation of southwestern India and its climatic significance. Palaeoworld 22(3-4): 119-132.

KLAUS W. 1984. Zur Mikroflora des Unter-Sarmat am Alpen-Südostrand.

KUNDU A, MATIN A & MUKUL M. 2012. Depositional environment and provenance of Middle Siwalik sediments in Tista valley, Darjiling District, Eastern Himalaya, India. J Earth Sys Sci 121(1): 73-89.

MEO AA & KHAN MA. 2005. Pollen morphology of invasive species Parthenium hysterophorus L. (Heliantheae-Asteracea) from Islamabad and Rawalpindi, Pakistan. SJA (Pakistan), p. 227-230.

MIAO Y, FANG X, HERRMANN M, WU F, ZHANG Y & LIU D. 2011. Miocene pollen record of KC-1 core in the Qaidam Basin, NE Tibetan Plateau and implications for evolution of

the East Asian monsoon. Palaeogeogr Palaeoclimatol Palaeoecol 299(1-2): 30-38.

MORE S, PARUYA DK, TARAL S, CHAKRABORTY T & BERA S. 2016. Depositional environment of Mio-Pliocene Siwalik sedimentary strata from the Darjeeling Himalayan Foothills, India: A palynological approach. PLoS ONE 11(3): e0150168.

MUGHAL MS, ZHANG C, DU D, ZHANG L, MUSTAFA S, HAMEED F, KHAN MR, ZAHEER M & BLAISE D. 2018. Petrography and provenance of the Early Miocene Murree Formation, Himalayan Foreland Basin, Muzaffarabad, Pakistan. J Asian Earth Sci 162: 25-40.

PUNT W, HOEN P, BLACKMORE S, NILSSON S & LE THOMAS A. 2007. Glossary of pollen and spore terminology. Rev Palaeobot Palynol 143(1-2): 1-81.

QUADE J, CATER JM, OJHA TP, ADAM J & MARK HARRISON T. 1995. Late Miocene environmental change in Nepal and the northern Indian subcontinent: Stable isotopic evidence from paleosols. Geol Soc Am Bull 107(12): 1381-1397.

SARKAR S, BHATTACHARYA AP & SINGH HP. 1993. Palynology of middle siwalik sediments (late miocene) from Bagh Rao, Uttar Pradesh. J Palaeosciences 42(1-3): 199-209.

SHAH SI. 1977. Stratigraphy of Pakistan: Geological Survey of Pakistan Memoir.

SINGH B, ANDOTRA D & KUMAR, R. 2000. Provenance of the lower Tertiary mudrocks in the Jammu Sub-Himalayan Zone, Jammu and Kashmir State (India), NW Himalaya and its tectonic implications. Geosciences 4(1): 1-9.

SOOMRO N, MANGI JU, PANHWER M, JATOI GH, KHUHRO SA, KHOKHAR Q, KHAN SA, MENGAL AN & SHAIKH N. 2021. Anatomical characteristics of fossil wood collected from the Manchar Formation (Miocene), Thano Bula Khan, Sindh, Pakistan. Ital Bot 11: 1-8.

STRÖMBERG CA. 2011. Evolution of grasses and grassland ecosystems. Annu Rev Earth Planetary Sci 39: 517-544.

STROTHER SL, SALZMANN U, SANGIORGI F, BIJL PK, PROSS J, ESCUTIA C, SALABARNADA A, POUND MJ, VOSS J & WOODWARD J. 2017. A new quantitative approach to identify reworking in Eocene to Miocene pollen records from offshore Antarctica using red fluorescence and digital imaging. Biogeosciences 14(8): 2089-2100.

SUN X & WANG P. 2005. How old is the Asian monsoon system? Palaeobotanical records from China. Palaeogeogr Palaeoclimato Palaeoecol 222(3-4): 181-222.

TRAVERSE A. 2007. Paleopalynology. Springer Science & Business Media, Vol. 28.

ULLAH F, NASAR SHAH S, ZAMAN W, ZAFAR M, AHMAD M, SOHAIL A & SAQIB S. 2019. Using palynomorphological characteristics for the identification of species of Alsinoideae (Caryophyllaceae): A systematic approach. Grana 58(3): 174-184.

WANG F, CHIEN N, ZHANG Y, YANG H. 1996. Pollen flora of China. Aerobiologia 2(12): 153.

WODEHOUSE RP. 1929a. The origin of symmetry patterns of pollen grains. Bulletin of the Torrey Botanical Club, 339-350.

WODEHOUSE RP. 1929b. Pollen grains in the identification and classification of plants. III. The Nassauvinae. Bulletin of the Torrey Botanical Club, 123-138.

WYNNE A. 1874. Notes on the geology of neighbourhood of Mari hill station in the Punjab. India Geol Survey Mem Rec 7(2): 64-74.

XU J-X, FERGUSON DK, LI C-S & WANG Y-F. 2008. Late Miocene vegetation and climate of the Lühe region in Yunnan, southwestern China. Rev Palaeobot Palynol 148(1): 36-59.

YANG Y, WANG W-M, SHU J-W & CHEN W. 2018. Miocene palynoflora from Shengxian Formation, Zhejiang Province, southeast China and its palaeovegetational and palaeoenvironmental implications. Rev Palaeobot Palynol 259: 185-197.

YAR M, ARIF M, AFRIDI AK, SAEED M, ZIAD M & ALI A. 2017. Petrographic and mechanical properties of sandstone from Murree formation, Jena Kor area, Peshawar Basin. A case study. Pakistan J Eng Appl Sci.

YAR M, HANIF M & SAJID M. 2021. Lithofacies and petrography of Miocene Murree Formation, Peshawar basin, NW Pakistan: implications for provenance and paleoclimate. Arab J Geosci 14(8): 1-14.

YU-SHU W. 2001. Palynoflora at Late Miocene? aEarly Pliocene from Leijiahe of Lingtai, Gansu Province, China. J Integr Plant Biol 43(7): 750.

YUNFA M, QINGQUAN M, XIAOMIN F, XIAOLI Y, FULI W & CHUNHUI S. 2011. Origin and development of Artemisia (Asteraceae) in Asia and its implications for the uplift history of the Tibetan Plateau: a review. Quatern Int 236(1-2): 3-12.

ZAVADA M, DE VILLIERS S. 2000. Pollen of the Asteraceae from the Paleocene-Eocene of South Africa. Grana 39(1): 39-45.

ZHOU Z. 2000. On the Miocene Xiaolongtan flora from Kaiyuan, Yunnan Province. The Evolution of the Late Cretaceous–Cenozoic Floras in China. Science Press, Beijing, 64-72.

# SUPPLEMENTARY MATERIAL

**Figures S1, S2, S3, S4, S5.**
**Tables SI, SII.**

**How to cite**
AHMAD S, AHMAD M, ZAFAR M, SULTANA S, RAMADAN MF, GILANI SN, ABBASI MA & JABEEN S. 2024. Palynological Investigations of the Miocene sediments from Murree formation of Pakistan: Evidence for Palaeoenvironment and Palaeoclimate interpretations. An Acad Bras Cienc 96: e20231241. DOI 10.1590/0001-3765202420231241.

*Manuscript received on November 17, 2023;*
*accepted for publication on May 20, 2024*

**SHABIR AHMAD**[1]
https://orcid.org/0000-0002-3901-4798

**MUSHTAQ AHMAD**[1]
https://orcid.org/0000-0003-2971-2848

**MUHAMMAD ZAFAR**[1]
https://orcid.org/0000-0003-2002-3907

**SHAZIA SULTANA**[1]
https://orcid.org/0000-0004-5911-4548

**MOHAMED FAWZY RAMADAN**[2]
https://orcid.org/0000-0002-5431-8503

**SYED NOHMAN GILANI**[3]
https://orcid.org/0009-0007-3670-5853

**MARYAM ANJUM ABBASI**[1]
https://orcid.org/0009-0008-1554-6774

**SHAISTA JABEEN**[1]
https://orcid.org/0009-0002-9601-4505

[1]Department of Plant Sciences, Quaid- i- Azam University Islamabad, 45320, Pakistan

[2]Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Kingdom

[3]Institute of Geology, University of Azad Jammu and Kashmir, Muzaffarabad 13100, Pakistan

Correspondence to: **Shabir Ahmad**
*E-mail: shabir@bs.qau.edu.pk*

## Author contributions

Shabir Ahmad - Original Manuscript write up. Mushtaq Ahmad – Supervision. Muhammad Zafar- Editing and reviewing. Shazia Sultana - Methodology. Mohamed Fawzy Ramadan -Software. Syed Nohman Gilani - Field survey. Maryam Anjum Abbasi - Manuscript writing. Shaista Jabeen - Experiments.