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Comparative Mineralogical Analysis of Epigeal and Arboreal Termite Nests using Modern Analytical Techniques

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Abstract

Termites are generally known to be destructive to woody materials and are of benefit to agricultural sector as they improve the fertility of soil and recycle organic matter. Termite nests such as arboreal, epigeal, hypogeal amongst others are being built by termites and have received great attention due to its diverse complex architectural structures and its utilization by researchers for diverse applications. This study investigates the characteristic features of epigeal and arboreal termite nests by modern analytical techniques such as the scanning electron microscopy (SEM), transmission electron microscopy (TEM), Energy dispersive spectroscopy (EDS) and x-ray diffraction (XRD). The SEM results presented the morphology of the samples, indicating the degree of density of the termite nests due to the observed variance in crystallite shape, size and color of the nests. TEM analysis showed that the plane arrangements of epigeal termite nest are polycrystalline in nature with more than one lattice plane orientation in the grains, whereas the arboreal termite nest showed planes that have multiple layered crystallite boundaries at some diffractions, with grain lattice planes being single crystallite. EDS of both samples indicate diverse elemental composition in the termite nest, with carbon and oxygen being the predominant elements. XRD confirmed the presence of quartz (SiO_2), kaolinite ($Al_2(Si_2O_5)(OH)_4$) and microcline ($KAlSi_3O_8$) in the epigeal termite nest samples, while the arboreal termite nest consists of quartz (SiO_2) and kaolinite ($Al_2(Si_2O_5)(OH)_4$) only. The intensity

of quartz of the arboreal termite nest indicated a higher concentration of quartz compared to the epigeal termite nest. The features of the termite nest offer valuable insights into the construction practices involved in their formation.

Keywords: epigeal termite nest; arboreal termite nest; SEM; TEM; EDS; XRD

1.0 Introduction

Termites are economically important biological species that inhabit within and/or on the earth surface. Termites majorly feed on lignocellulose of wood, the most abundant biopolymer on earth, with a wide ecological spread in tropical regions. Premised upon the evolutionary history and current abundance, termites are among the dominant insects on earth (Legendre and Grandcolas, 2018). However, some termite species are soil feeders, or litter-foragers (Moreira *et al*., 2008). They are eusocial cockroaches (Inward *et al*., 2007), and demonstrate cryptic behaviours allowing them to camouflage (Oberst *et al*., 2020). Termites are voraciously destructive pest of wood material like furniture. They are an important component of pedofauna and are mainly distributed in subtropical and tropical areas (López-Hernández, 2023). They are one of nature's architect and ecosystem engineers. Termites cooperatively execute building instructions and makes necessary adjustments of parameters to construct diverse complex structures, which are underground nests, soil protruding mounds and nests on trees (Mizumoto and Bourguignon, 2020; Oberst *et al*., 2020; Perna *et al*., 2017).

Termite nests (termitaria) morphological structures differ. However, they may be classified into being either hypogeal (subterranean, below ground), epigeal (protruding above soil, above ground) or arboreal (tree-nest) (Oberst *et al*., 2020). Fig. 1 shows the habitat classification of termite nest. Termite arboreal nests could be within a trunk or branch cavity or on the exterior of a trunk or branch. Also, termites may either be soil nesting (epigeous and subterranean termites) or non-soil nesting termites (such as arboreal termite) (Lind *et al*., 2023). The large hillock termite mounds are constructed by the *Macrotermes* species, and the small termite mounds are built by the *Cubitermes* species in the DR Congo (Mees *et al*., 2021). Both species are common termite species that construct the epigeal termite mounds. The *Microcerotermes* species are common builders of the arboreal termite nests in DR Congo. These three species have different feeding behaviours. The *Macrotermes, Cubitermes,* and *Microcerotermes* species are fungus-growing, soil-feeding, and wood-feeding, respectively (Donovan *et al*., 2001; Mees *et al*., 2021).

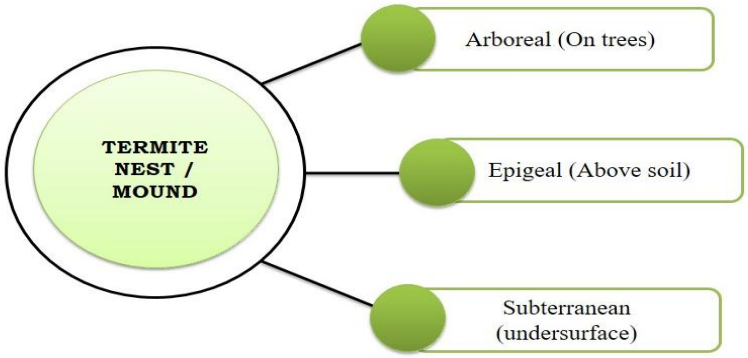


Fig 1: Habitat classification of termite Nest

Termites are involved in the construction of tunnels, galleries, mounds, and nests leading to changes in the physicochemical and biological characteristics of the soil. These bio-structures have distinct characteristics when compared to the surrounding topsoil. There is accumulation of nutrient in termite mounds, which has necessitated its exploitation in the use for soil nutrient improvement for farming in rural Africa and Asia societies (López-Hernández, 2023). Termite mounds are visibly seen features in the savanna region of the world. It protects the termites from negative impact of elements of weather like sun, rain and temperature, predators, and growth of symbiotic fungi. Termite mounds are biogenic materials which may be classified as bare and grassy termite mounds (Lind *et al*., 2023). The bare termite mounds have lower water permeability compared to the grassy termite mound that is variably permeable to water. Researchers have suggested that during rainfall in the savanna area, the bare mound is more likely to disperse water laterally through runoff due to their compacted soil surface texture while the grassy mounds with a loose soil surface are, more likely to concentrate water *in situ* through infiltration. Arboreal nests are made up of lignocellulosic matter (plant materials and faeces) and inorganic matter in a lesser amount (Eggleton, 2011; Jouquet *et al*., 2004; Ke *et al*., 2012). Epigeal mounds and hypogeal nests are closely similar, being that they are made of clay and lignocellulosic materials. The external wall layer of epigeal mounds is built from soil components which includes clay, silts, clay silicates, and sands, with smaller proportions used to construct for their nursery (Jouquet *et al*., 2002, 2004; King *et al*., 2015; Oberst *et al*., 2016, 2019).

Termite nests are a representation of a typical ecosystem designed to create a solid, long-lasting habitat where individuals can remain safe from sunlight, rain, and predators while being able to maintain the necessary humidity and temperature levels. In order to accomplish this, termites move and transform soil as a distinct pedogenic force, exposing it to variables related to soil genesis that can change over time (Salvucci *et al*., 2023). It has been reported that termites nest vary from locality to locality (Mahamat *et al*., 2021; Salvucci *et al*., 2023; Thorne, 1980). These variations are linked to their feeding strategies which significantly include; fungus-growing, soil-feeding and wood-feeding species respectively. The technique that termites synchronize their foraging operations enables them to build mound/nests, find food, and then return to their nests(Ugbomeh *et al*., 2019).

The large diversities/similarities seen among termite nest in one/different geographical locations and species have made a comparative study challenging (Akoth *et al*., 2022; Echezona *et al*., 2012; Thorne *et al*., 1996). Termite nest has been researched for a variety of reasons, including geochemistry, sociobiology, biote weathering into titanium and zirconium, or pedogenesis process (soil formation) (Shelke *et al*., 2023). In the epigeal nests, inhabited mainly by the primary species of fungus-growers, known as Macrotermes, oral pellets function as the fundamental components (Hindi *et al*., 2023; Mees *et al*., 2021).

However, these pellets mostly appears in cases of recent fillings and in specific scenarios where contrasting materials are present in significant portions of the nests (McAuliffe, 2023). Nest construction carried out by the primary soil-nourishing species (Cubitermes) is accomplished by the deposition of fecal material, displaying a confined presence of depositional characteristics resulting from either insufficient contrast or subsequent uniformization (Diehl *et al*., 2005; Mees *et al*., 2021). However, it should be noted that additional elements introduced through alternative modes of conveyance are also apparent. The arboreal dwellings of the prevailing wood-consuming species (Microcerotermes) consist of finely fragmented botanical remains, demonstrating a distinct divergence in the manner in which excrement is deposited during the initial and subsequent phases of septa formation of nest (Mees *et al*., 2021; Scheffrahn *et al*., 2005). Porosity, composition, and nutritional qualities of the soil in ground termite nests and ant nests were systematically studied by Shelke *et al*., (2023) using atomic absorption spectrophotometry and inductively coupled plasma mass spectrophotometry, the analysis revealed elements such as zinc, selenium, lead, cadmium, nickel drought, and chromium in the ant and termite nest samples. When same soil samples were subjected to microscope-energy dispersive analysis x-rays and scanning electron microscope, the samples sizes were 25.56 nm and 27.77 nm, respectively. The EDS analyses of the different composition of termite nests reveal the presence of calcium in large quantities whereas silicon, iron, and aluminium were found in small quantities (Mahamat and Azeko, 2018). Hindi *et al*., (2023) studied Najdian termite nest prevalently infecting timber trees. Analysis using Fourier transform infra-red spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses confirmed the presence of both microcrystalline cellulose and nanocrystalline cellulose. It was reported that termite naturally synthesize cellulose through their enzymatic action excreted by the termites and their symbiotic fungal communities inhabiting the nests (Shelke *et al*., 2023; Thorne *et al*., 1996; Vesala *et al*., 2023).

Generally, the termite nest structures perform varying survival roles, which are (i) nest, nursery and food storage, (ii) thermoregulation and climatisation, (iii) defence, shelter and refuge, (iv) termite clay as building material and foraging tool, and (v) stigmergy and communication channel. Micromorphological analysis is also a common technique for the characterization of features of sections of the termite nests (Erens *et al*., 2015; Mees *et al*., 2021; Mujinya *et al*., 2011, 2013). Thus, the present study employs SEM, TEM, EDS and XRD to compare the characteristics features between epigeal and arboreal termite nests in Nigeria, to explore the peculiarities and differences for environmental studies.

2.0 Materials and methods

2.1 Chemical reagents and termite nest samples

The termite nest samples were obtained at Onibueja, Osogbo Local Government of Osun

State, Nigeria at Latitude $7^0$46'13.34"N, Longitude $4^0$29'16.38"E. The samples were sun dried and were further dried in an oven at 60^{o}C for 24 h. They were ground with mortar and pestle and were sieved to smooth powder with 45 µm sieve.

2.2 Characterization of termite nests

The crystallinity and mineral phase of the termite nest sample were considered by XRD (Siemens D8 Advance Bruker XRD). The SEM (Nova Nano SEM 230) and TEM (Tecnai G^2 20) were used to analyze the surface morphology. EDS was used to qualitatively determine the elemental composition of the samples.

3.0 Results

3.1 Scanning Electron Microscopy

The SEM micrographs of epigeal termite nest shown in Figure 2 at different magnification viz., x10000 and x50000. It depicts an agglomerated structure with uncorrelated spherical and flower-like surface. This could be attributed to the organic matter that dominated the building constituent. The SEM micrographs of arboreal termite nest presented in Figure 3 at magnifications x10000 and x50000 showed a loosely packed structure with pores. This may be attributed to the nature of soil sample in the location. This is in consonance with the report of Shelke *et al*. 2023.

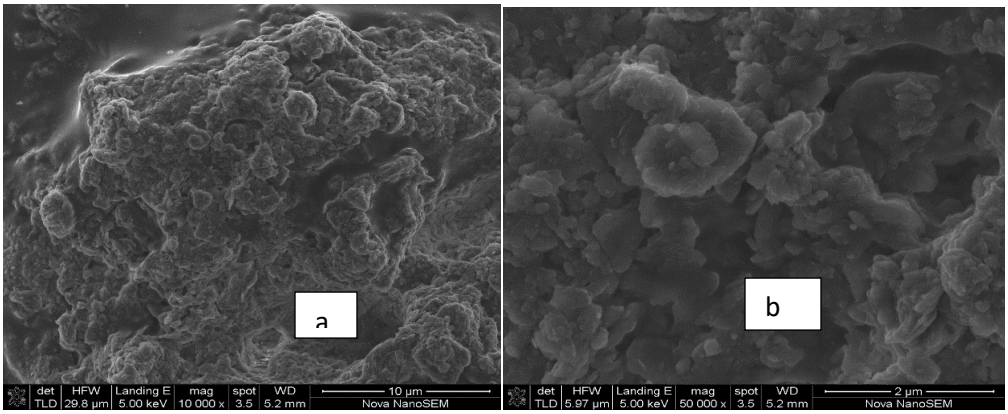


Fig 2: SEM of epigeal termite nest at (a) 10 000x mag. (b) 50 000x mag.

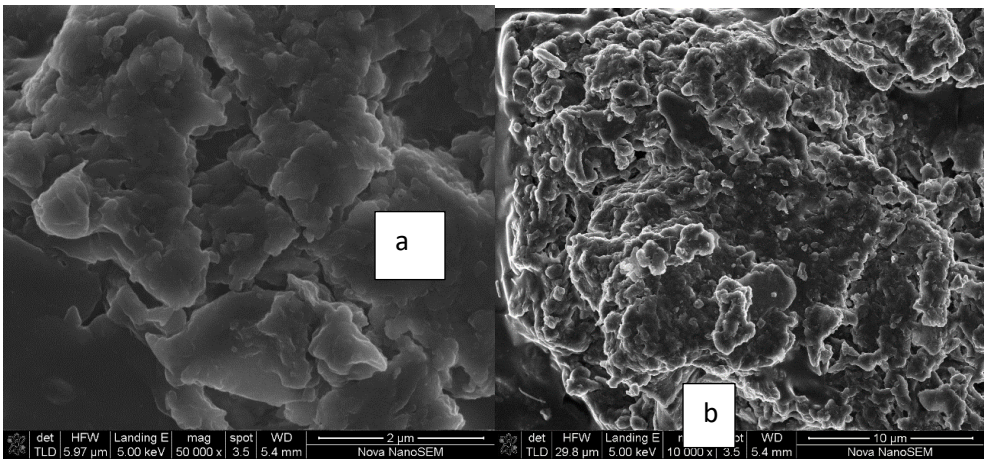


Fig 3: SEM of arboreal termite nest at (a) 10 000x mag. (b) 50 000x mag.

3.2 Transmission Electron Microscopy

The epigeal termite nest had mainly grains with agglomerate. The crystallite boundaries of each grain is clearer and also long. The plane arrangements are polycrystalline in nature with more than one lattice plane orientation in the grains. However, most of the grains have no plane arrangement in them and could be described as being amorphous. It could be observed that the epigeal termite nest showed a clearer TEM grain at a low magnification of 50 nm (Figure 4a) when compared to the arboreal termite nest clear result obtained at 100 nm (Figure 5b). However, at higher (200 nm) magnification, the epigeal termite nest showed compacted and sharp grain arrangements which is presented in Figure 4b. The TEM results for the grains in the arboreal termite nest presented in Fig. 5b were more clustered, organized and clearer at high magnification (100 nm) when compared with the TEM results at lower magnification (50 nm) as shown in Figure 5a.

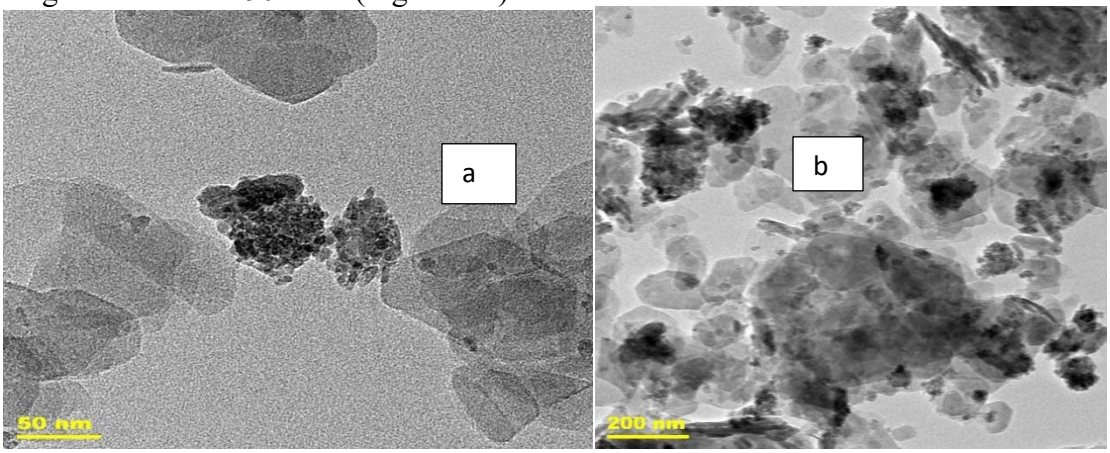


Fig 4: TEM of epigeal termite nest at (a) 50 nm (b) 200 nm

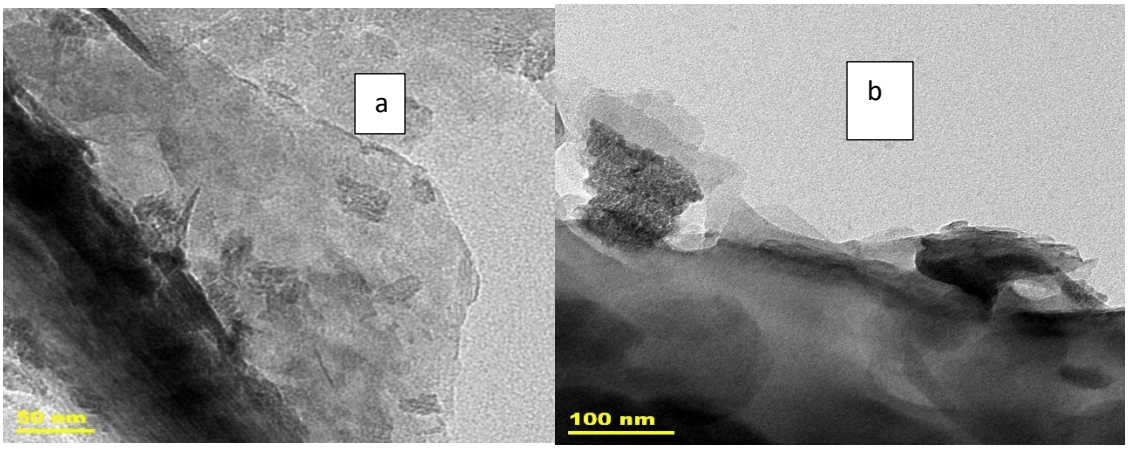


Fig 5: TEM of arboreal termite nest at (a) 50 nm (b) 100 nm

3.3 Energy Dispersive Spectroscopy

The EDS analysis of epigeal termite nests in Figure 6(a) revealed valuable insights into its elemental composition. The average values and standard deviations for key elements in epigeal termite nest sample were as follows: carbon (C): 23.35±3.93, oxygen (O): 51.29±1.78, aluminum (Al): 6.95±1.53, and silicon: 12.76±4.83. Additionally, the EDS pattern reveals the presence of potassium (K), titanium (Ti), and iron (Fe) in the termite soil, with the following values: K – 0.66±0.15, Ti – 0.48±0.23, Fe – 4.52±0.69. These results indicate a diverse elemental composition in the termite nest, with carbon and oxygen being the predominant elements (Shelke *et al*. 2023). The spectrum results of the arboreal nest in Fig. 6(b) show peaks at specific energy levels, corresponding to different elements present in the termite soil. By comparing these peaks to a database, the elements and their concentrations were identified. The composition of arboreal termite nest is primarily composed of carbon (C=51.84±10.12) and oxygen (O = 39.43±3.57), followed by traces of silicon (Si = 5.04±5.64), aluminum (Al = 1.59±0.96), iron (Fe = 1.22±0.93), calcium (0.42±0.21), potassium (K = 0.27±0.07), and sulfur (S=0.19±0.06). The EDS pattern derived from the arboreal termite next is illustrated as follows, highlighting its predominant elements: Carbon (C 51.84%), Oxygen (O 39.43%), and Silicon (Si 5.04%).

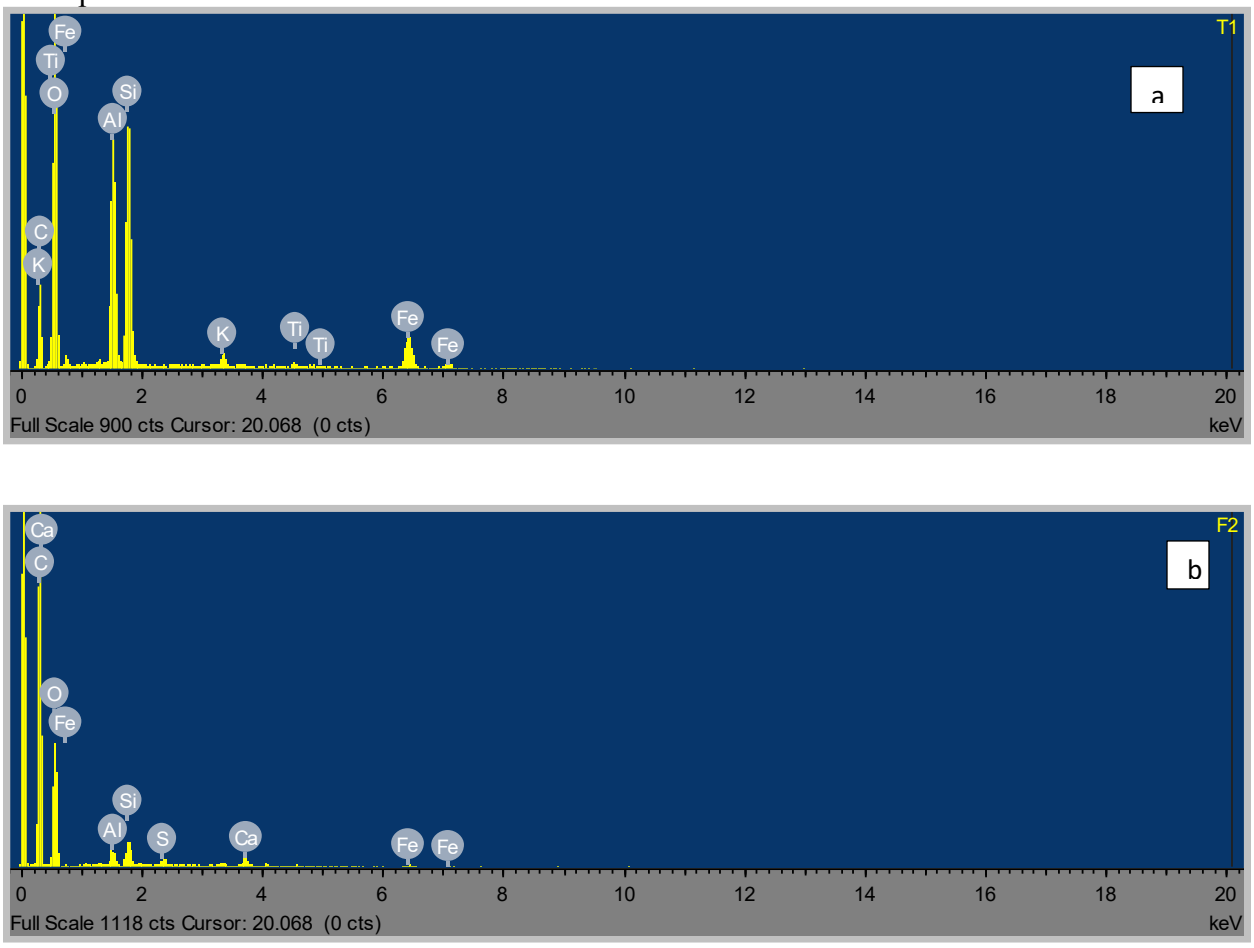


Fig 6: EDS of epigeal (a) and arboreal (b) termite nests

3.4 X-ray diffraction analysis

The XRD analysis is a powerful technique used for identification and characterization of mineralogical composition of various environmental and geological samples. In this study, samples were collected from arboreal and epigeal termite nests. For epigeal termite nest shown in Fig. 7(a), the result revealed several prominent peaks. The longest peak was observed at 28 º 2θ, which correspond to (101) plane of quartz and ascribed to JCPDS (joint committee on powder diffraction standard) Card No. 89-5574 (Yusuff & Ajayi, 2022). The intensity of the peak was approximately 8,000 Lin counts indicating a significant presence of quartz (SiO_2) in the termite mound. This agreed with previous findings that termite mound often contain minerals like quartz and kaolinite, which are derived from the local soil during nest construction (Adamou *et al*., 2023; Clarke *et al*., 2022; Ngoy *et al*., 2023). The high content of quartz also revealed that it is the most abundant mineral in the earth crust (Saeed *et al*., 2022; Wang *et al*., 2022). Other several peaks were observed at various positions of 2θ which correspond to quartz and kaolinite with only one of montmorillonite. The presence of kaolinite ($Al_2Si_2O_5(OH)_4$) in the termite nest, is consistent with previous studies, that kaolinite clay mineral signifies termite activity in some soils (Fruett *et al*., 2023).

For arboreal termite nest, the XRD analysis (Fig. 7b) also revealed a strong presence of quartz with a longest peak at 28º 2θ, which also correspond to (101) plane of quartz and ascribed to JCPDS card No. 89-5574 (Yusuff & Ajayi, 2022). It had an intensity of approximately 11,000 Lin counts indicating a higher concentration of quartz compared to the epigeal termite nest. Furthermore, additional peaks were also observed at various positions of 2θ which also correspond to quartz and kaolinite but not as many as that of epigeal termite nest. The distinct peak at 21 º 2θ in both the epigeal and arboreal termite nests also correspond to the (211) plane of quartz in the JCPDS card No. 96-901-4196 (Mees *et al*., 2021). The kaolinite peaks can be ascribed to JCPDS Card No. 96-900-8367 (kaolinite, (001)) (Shelke *et al*., 2023). There is absence of montmorillonite peak in the arboreal termite nest. It is an established fact that well-crystalline and pure minerals produce higher intense XRD peaks, as they are more easily detectable (Columbu *et al*., 2023).

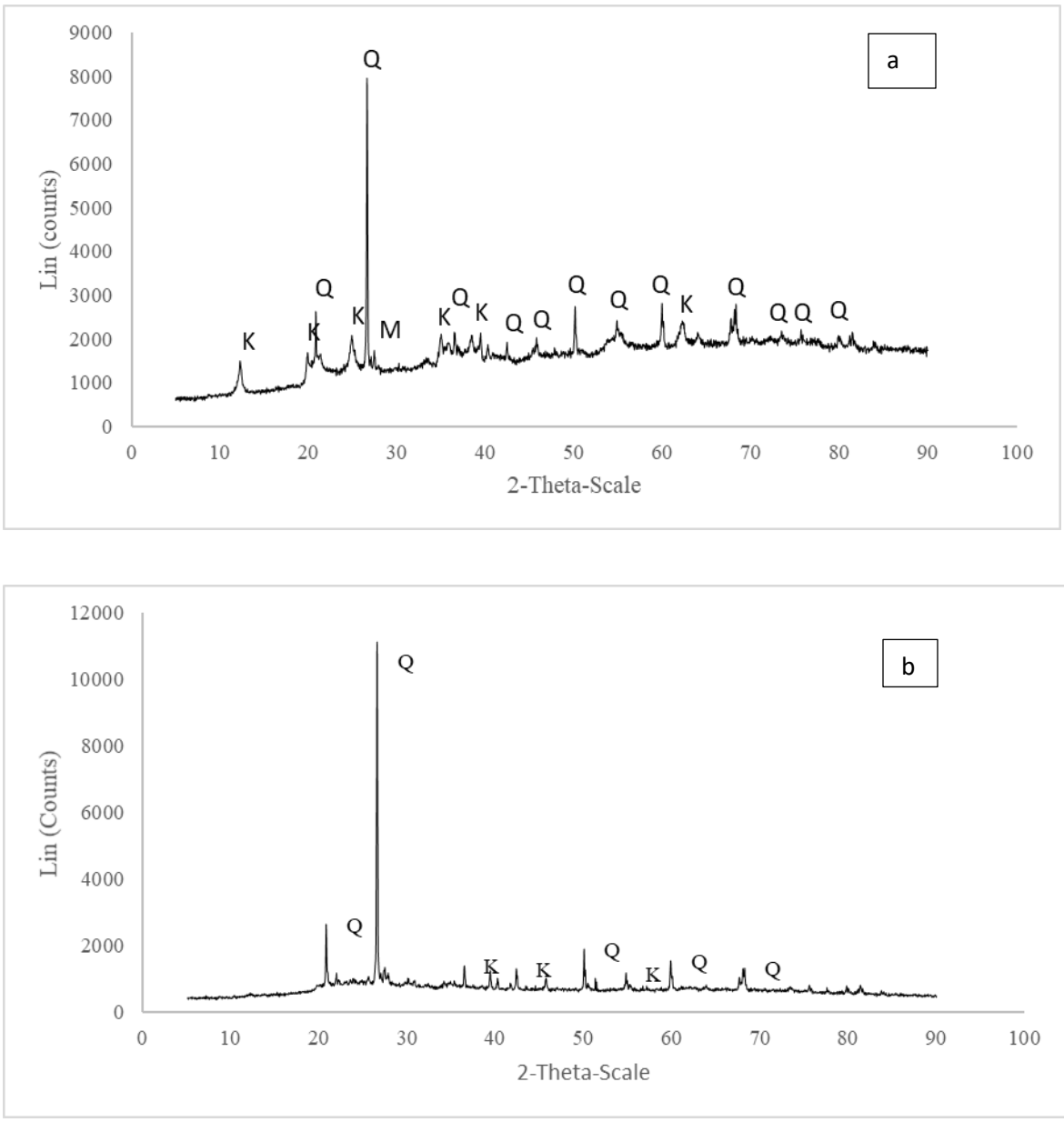


Fig. 7: X-ray diffractograms of epigeal (a) and arboreal (b) termite nests

Q – Quartz (SiO_2); K – Kaolinite ($Al_2(Si_2O_5)(OH)_4$); M – Microcline ($KAlSi_3O_8$)

4.0 Discussion

This study investigated the micro morphological characterization of arboreal and epigeal termite nests at different magnifications. It was observed that resolution sizes (magnification) have a great effect on the morphological and structural results generated during SEM and TEM analysis. The SEM micrographs of the termite nests differ as a result of the micro location, type of minerals present in the soil and mechanical factor of the environment (Shelke *et al*. 2023). The TEM analysis of arboreal termite nest showed planes that have multiple layered crystallite boundaries at some diffractions, with grain lattice planes being single crystallite based on

the plane orientation. Most of the grains are overlapping with each other and are meta-particulate in nature. From the EDS result, the presence of aluminum, silicon, potassium, titanium, and iron add further complexity to the soil's chemical makeup. The standard deviations highlight the variability in these concentrations, providing a comprehensive overview of the termite nest's elemental profile. The spectrum of the epigeal termite nest also displayed significant presence of silicon (12.8%) which confirms its ground location in contrast to the arboreal nest with just 5% silicon. Another major element in both spectra is aluminium, with 7% and 2% in the epigeal and arboreal nest, respectively. Interestingly, while the epigeal nest has iron, titanium and potassium as trace elements, the arboreal exhibits calcium and sulphur in addition to iron and potassium (Klimeck *et al*. 2022).

The XRD of the epigeal termite nest contains more peaks of quartz and kaolinite minerals, the arboreal termite nest shows significantly higher intensity of the quartz mineral. The results can be best explained using various environmental and geological factors that affect the mineral composition of these termite structures (Liu *et al*. 2019). The epigeal termite nest may have particles which are coarser and clustered, leading to fewer crystal planes resulting in fewer XRD-detectable crystalline structures (lower intensity). This might be as a result variation in the termite construction mechanisms. Conversely, the higher intensity observed in arboreal termite nest may be as a result of smaller well-distributed finely dispersed crystals. This increases the counts of the XRD signals, even though the overall content of the minerals is low.

Another important consideration is the purity and type of minerals used in the construction of the structures. It is very possible that the minerals used by the arboreal termite nest are more crystalline and purer compared to those used by epigeal termite nest. Finally, the local environmental conditions, differences in termite species, and feeding habits of the different termite species, in the epigeal and arboreal termite nests can also play a great role. These can determine the differences in the mineral distribution and the composition of the structures formed, as each species may select and process minerals in different ways.

5.0 Conclusion

Various techniques have been utilized to study termite nests. The examination of micromorphological features of termite-built structures can offer valuable insights into the construction practices involved in their formation. Termite nests can differ depending on their geographical location, mineral distribution as evidenced by variations in shape, size, and color observed in different regions. The architecture of these nests plays a significant role in the regulation of nest temperatures, with distinct types of nests exhibiting different temperature regimes. The shapes of termite nests are influenced by environmental factors such as solar irradiance and wind, leading to the formation of cone-shaped structures in areas exposed to sunlight and vertical domes in shaded areas. The nesting processes of termites have been found to have a considerable impact on soil microbial communities, resulting in disparities in microbial diversity and community compositions between termite nests and bulk soils. This study is concerned with a comprehensive characterization of epigeal and arboreal termite nests, to offer further insights into the composition and structure of the termite nests.

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Availability of data and materials

All data generated during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Competing interests

The authors declare that they have no competing interests.

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