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Chapter 20

Investigation of Subterranean Termites Nest Material Composition, Structure and Properties

Petr Ptáček, Jiří Brandštetr, František Šoukal and Tomáš Opravil

Additional information is available at the end of the chapter

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1. Introduction

Termites (Isoptera[1]) are colourless or white insect sometimes called "white ants[2]" but they are intimate relatives of cockroaches. The organization of termite community definitely belongs to the most complicated in the nature hence termites are described as the eusocial[3] insect. Termites are distributed all over the world from the 47° Northern latitude to the 47° Southern latitude, but they are extremely abundant in the tropical rainforest[4]. Until today there are recognized about 3 000 termite species in the 9 families but two of them became extinct (Fig. 1).

Only species of the genera *Archotermopsis*, *Hodotermopsis*, *Zootermopsis* and *Reticulitermes* can be found also in the temperature regions. Only two species[5] are native of the temperature region of Europe. Besides the known kinds of termites an existence of other more than 1000 species is supposed [1-6].

The foundations of thermite taxonomy were established by Holmgren [7-9]. The following studies provided by Snyder [10], Grasse [11] and Emerson [12] define seven families of termites which are today generally accepted. Many of the conventional phylogenetic reconstructions

1 Isoptera is Greek name that means "equal wings" referring to the similar shape and size of four wings.

2 This name is widespread mainly in Australia. However ants belong to the order hymenoptera.

3 Eusocial behaviour is the highest level of social organization with specialized individuals (the developed system of castea). The expression is consisting of Greek words "eu" (real) and "social". Besides Isoptera (termites), the social Hymenoptera insect such as ants, bees and wasps is another example of eusocial insect but the social system of termites is the oldest known.

4 Each square meter of tropical rainforest may contain hundreds of termite individuals.

5*Reticulitermes lucifugus* and *Reticulitermes flavipes*, the cold climate restricts the dissemination of termites into Europe to the Atlantic coast of France and Hamburg in Germany, respectively.

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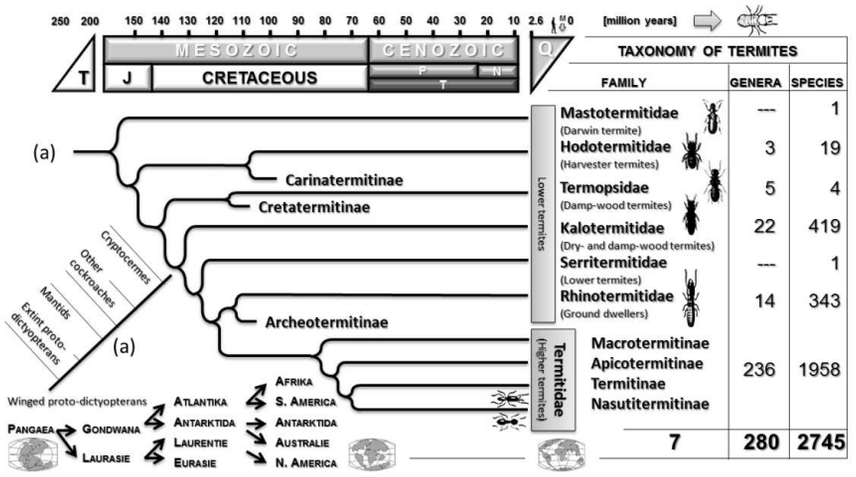


Figure 1. Taxonomy and phylogeny of termites: Triassic (***C***), Jurassic (***J***), Palaeogene (***P***), Neogene (***N***), Tertiary (***T***), Quaternary period (***Q***) and appearing of modern human (***M***).

are based only on the limited subset of characters [13]. For example the hypothesis of Ahmad [14] is based on the worker-imago mandibles, the classifications of Johnson [15] and Noirot [16] use the worker gut and the classification of DeSalle [17] is based on DNA. Others looked at the relationships within as well as between particular families [16, 18, 19]. The hypothesis about morphological phylogenetics of termites is given in the Fig. 1.

The Hodotermitidae are generally considered as the basal group for all other families, except the Mastotermitidae which are the most primitive and closest to the cockroaches. The positions of Kalotermitidae, Termopsidae, Rhinotermitidae and Serritermitidae are less certain. Termopsids living in small colonies inside decaying wood are assumed the most primitive with regard to their caste differentiation and eusociality in general[6]. The family of Termitidae (higher termites) represents about two thirds of all the described termite species [6, 13, 20, 21].

1.1. Evolution of termites and evolution of eusocial behaviour of termites

Molecular [22] and fossil evidence [23] suggests that during late Jurassic termites evolved from subsocial wood-feeding Cryptocercus cockroaches after which a period of rapid radiation and spreading followed [24]. This foundation is in agreement with previous hypothesis of termite origin [25] that was established on the similarity in nesting and feeding habitats as well as the presence of cellulolytic protozoa in their hindguts. The fossil records of termites are known from early Cretaceous deposit [24, 26] although structures from late Triassic and lower Jurassic were described as fossilized termite nests. Those support the hypothesis which explains the worldwide distribution of the social insect including termites by their early Mesozoic origin (Fig. 1), prior to breakup of the Pangaea [27-28].

Comparative studies of the social biology of the spectrum of solitary through eusocial species of bees and wasps elucidated the evolution of complex societies but parallel research on

6 For example relictual Himalayan termite *Archotermopsis wroughtoni*.

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termites or ants is impossible hence all living species are eusocial. The two ways are generally accepted for origin of social groups:

- **Subsocial route** when social groups originate from familial units initially composed of parents and offspring;
- **Semisocial route** when social groups are formed by association of individuals of the same or different generation.

Therefore workers and soldiers of termites are specialized juveniles[7] and there is no evidence for exchange of reproductives between different established colonies, termites eusocial behaviour is probably not formed via semisocial route [20].

1.2. Termite social system

Termite eusocial society consists of specialized casts including reproductives[8], sterile workers, soldiers, and immature individuals. The differentiation of individual caste phenotypes is affected by the complex interaction of extrinsic and intrinsic factors. Although experimental data suggest a genetic component to termite caste differentiation, environmental and social condition and are still considered the major trigger that initiates the development of different caste phenotypes [30].

The two types of reproductive species are recognised – primary and supplementary. The primary reproductives are king, queen and fully developed winged adults. Their role is in production of eggs and distribution by colonizing flights. The queen lives up to 25 years and lays about 3000 yellowish-white eggs per day. The eggs are hatch after 50–60 days of incubation. Termite eggs are genetically identical hence the differentiation into each of the different castes is controlled by intrinsic and extrinsic factors that modulate caste-regulatory and developmental gene expression [30, 31]. The lifecycle of termite colony is schematically drawn on Fig. 2(a).

Queen also produces pheromones[9] which have important functions in social regulation of termite society. These substances influence the behaviour and physiology of colony members, for example maintain sterility of colony members or the induction of special worker care. Primer pheromones which cause a psychological change in the receiver are considered the type of extrinsic caste-regulatory factor [30, 32].

The sterile castes, the workers (2 – 15 mm) and the soldiers (up to 20 mm), are wingless and usually lack eyes. Workers (Fig. 2(b)) mature in a year and live up to 3–5 years. Soldiers also mature within a year and live up to 5 years. The colony reaches its maximum size in approximately 4–5 years and it may include 60 000 to 200 000 workers. Workers construct the nest

7 Label not fully grown or developed young individuals such as larva or another form before the adult stage is reached.

8 Only the limited numbers of individuals are fertile and fecund in eusocial society while the others are sterile or have reduced reproductive capacity.

9 Pheromones are volatile chemicals (chemical signalling molecules) released by one individual which can have behavioural or physiological effects on other individuals. Using pheromones for communication is common phenomenon for insect.

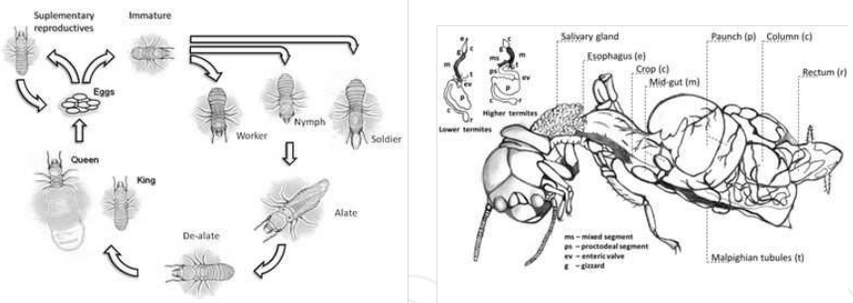


Figure 2. The life cycle of termite colony (b) and segments of a typical termite worker gut (b).

(Fig. 3), distinctive shelter tubes and collect food to feed the young and other members of the colony. Soldier termites guard and defend the colony [3, 31, 35].

The role of the workers in termite defence should not be underestimated. First, they are always the most abundant caste and are fully responsible for the construction and repair of the passive defensive structures. Second, they indeed actively participate in defence [33, 34]. The research on aggression of the termite worker indicates the flexible mechanisms of defence of colony which depend on the social context. The workers show only limited aggression against intruders (ants) if the soldiers are present but they attack the enemy if soldier isn't on the site. [35, 36]. The soldier caste is fully devoted to defensive activities. An impressive variety of defensive adaptations including heavily sclerotized head and the mandibles can be found [37, 38]. As a complement to the mechanical weapons, the chemical defence occurs in soldiers [30, 39, 40].

1.3. Termite nest

The nest built by the termite society refers to the complexity of their social organization, diet, biology and environmental factors on the site but there is the large scale of variations. Some of the termite species create only simple galleries in wood or ground while others build the nest of large dimensions and complexity [5].

From this point of view, the two species can be recognised:

- The wood dwellers;
- The ground dwellers (subterranean termites).

The wood dwelling and eating termites live in the fresh (damp-wood termites) or dead (dry-wood termites) wood in which they also build the nest. The connection of nest with ground is not required. On the contrary the subterranean termites (ground dwellers) live in soil and connection with the ground is necessary for the normal life and breeding of the colony.

The nests of subterranean termites are certainly the most admired natural structures which can reach 6 m of height and 4 m in the diameter of the base but towers built by some of the African Macrotermes species can be even 9 m high. The nest consists of galleries, chambers of different size and shape, system of tunnels and ventilation shafts. The nest of subterranean

termites is supported by extend underground structure that ensures proper temperature and humidity. The tunnels built by termites can be tens of meters long and often reach the level of ground water or the bedrock. The scheme of nest of subterranean termites is shown in Fig. 3.

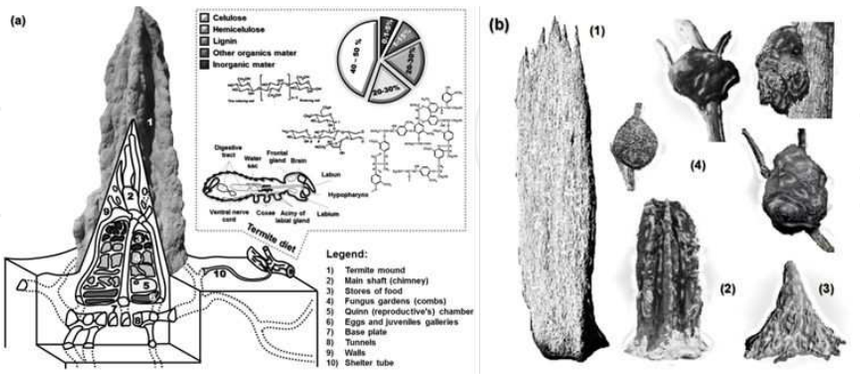


Figure 3. Simplified structure of termite nest with basic building block (a) and examples of ground-dweller (1, 2, 3) and wood-dweller nests (4) of different shape (b).

1.4. The diet of termites

The diet of termites as a group is quite diverse, but it is basically rich in cellulose, hemicellulose and lignin or lignin derivatives (Fig. 3). Termites digest lignocellulosic compounds due to the cooperation of their own enzymes and exogenous enzymes from microorganisms. According to the diet termites are divided into feeding groups i.e. wood feeding, dry-wood feeding, wood and litter feeding, soil feeding, fungus growing and grass feeding termites. The termite gut (Fig. 2(b)) provides a very distinct ecological environment which accommodates and promotes very specialized cellulolytic and hemicellulolytic microorganisms. The association between certain xylophagous termites and their hindgut protozoa is the fascinating and frequently cited example of nutritional symbiosis [5, 13].

On the other hand, Slaytor [41] found cellulose activity in the salivary glands and predominantly in the foregut and midgut of termites (Fig. 2(b)) and cockroaches. These parts are normal site of secretion of digestive enzymes and are devoid or have very limited content of microorganism. Therefore there is no evidence that bacteria are involved in cellulose digestion of termites.

The lower termites produce numerous endogenous enzymes (such as beta-glucosidases, exoglucanases, endoglucanases, chitinases) secreted in particular by the salivary glands and foregut. The higher termite species which do not possess symbiotic microorganism in the gut no longer feed wood. They prefer wood that is partially decayed by fungi or cultivate fungi in elaborate gardens for use as a nutrient source (fungus-growing termites). Fungus-growing termites which are abundant in the African and Asian tropics live in a unique mutualism with basidiomycete fungi of the genus *Termitomyces*.

The symbiotic fungi grow on a special culture within the nest maintained by the termites and called "fungus combs or fungus garden" (Fig. 3) which are made from partly digested foraged

plant litter passing rapidly through the termite's gut. The resulting faecal pellets are pressed together to make a comb-like matrix. As the comb matures, mycelium develops and produces conidial nodules, which together with older senescent comb are consumed by workers [5, 42-45].

Since the cellulose is encrusted with the lignin (lignocellulose) in the higher plants, which prevents its digestion, the lignin must be disrupted for the enzyme accessibility. Grassé and Noirot [46] proposed the "lignin degradation hypothesis" that the symbiotic fungi have the ability to degrade lignin, which makes cellulose more easily attacked by the termites' own cellulase. This hypothesis was confirmed by the foundation of Hoyodo at al [43].

1.4.1. Degradation of cellulose

Termites utilize wood as a source of food[10] and feeding experiments with wood show that cellulose and hemicellulose are subjected to degradation with efficiency from 59 to 99 %. Termites consume more than 90% of dry wood in some arid tropical areas. Cellulose is fermented anaerobically by the protozoa or directly by the termite own cellulolytic enzymes to acetate, CO_2 and H_2. Acetate is subsequently absorbed by the termites and used as their major oxidizable energy source [5, 41].

The hydrolysis of cellulose is a complex process that requires the participation of three cellulolytic enzymes at least [5, 47]:

- **Endo-*β*-(1, 4)-glucanase** (EG, endoglucanase or carboxymethyl cellulase) enzyme (EC 3.2.1.4) that hydrolyses *β*-1, 4-bonds of cellulose chains;
- **Exo-*β*-(1, 4)-cellobiohydrolase** enzyme (CBH, EC 3.2.1.91) that releases cellobiose from the non-reducing ends of cellulose;
- ***β*-glucosidase** enzyme (EC 3.2.1.21) that hydrolyses cellobiose or longer cellulose chains and releases glucose.

1.4.2. Degradation of hemicelluse and other polysacharides

Hemicellulose is digested by termites with high efficiency ranging from 49 to 78 %. Hence Xylans as polysaccharides made from units of xylose are the basic building blocks of hemicellulose. The degradation of beta-1, 4-xylan into xylose by xylanase breaks down hemicellulose chain. The process requires catalysis by enzyme endo-1, 4-*β*-xylanase (EC 3.2.1.8). In addition, termites can possess different kinds of carbohydrases, such as sucrase, maltase, trehalase and raffinase. In *Neotermes bosei* the chitinase activity could be found, which can be produced by the microbial symbionts. Chitin digestion plays a role during the cannibalism at the times of food shortage [5, 45].

1.4.3. Degradation of lignin

Lignin is a phenolic polymer that makes up 15 to 36% of wood biomass. It serves several functions in the extracellular matrix of plants [48]:

10 Digestion of cellulose is also found in other insect groups such as the Thysanura, Orthoptera, Coleoptera and Hymenoptera.

- Lignin gives the cell walls mechanical support;
- Lignin serves as a barrier against microbial attack;
- Lignin acts as a water impermeable seal for the xylem vessels of the plant vasculature.

Therefore the environment in the termite gut is predominantly anaerobic and the natural anaerobic mechanism of degradation of lignin is not known, the mechanism of degradation is still uncertain [5]. White rot fungi can degrade lignin by oxidative enzymes faster than any other organisms and they are responsible for most of the lignin decomposition in the nature. The degradation of lignin by ligninase (the group of oxidative extracellular fungal enzymes) occurs aerobically inside the fungal cells. The most studied lignin-modifying enzymes of white-rot fungi are [49]:

- 1, 2-bis(3, 4-dimethoxyphenyl)propane-1, 3-diol:hydrogen-peroxide oxidoreductase (LiP, EC 1.11.1.14) often termed as ligninase peroxidase or ligninase I;
- Mn(II):hydrogen-peroxide oxidoreductase (MnP, EC 1.11.1.13) also known as manganese peroxidase or peroxidase-M2.
- Laccases (EC 1.10.3.2), i.e. group of copper-containing oxidase enzymes.

As mentioned above the "lignin degradation hypothesis" assumes that the symbiotic fungi have the ability to degrade lignin, which enables the digestion of the cellulose by the termites' own cellulase [43, 46]. The hypothesis is also supported by the fact that no microorganism breaking down the lignin has been observed in the termite gut yet as well as no specific site for degradation of lignin is known [5].

1.5. The role of termites in the ecosystem

Termites are the dominant invertebrates in the tropical ecosystem where they can make up to 95% of the soil insect biomass. Therefore the termites play the important and irreplaceable role in the ecosystem. The main effect of termites in ecosystem is in mineralization of biomass and humification (enrichment) of soils [42, 50, 51].

In general termites are considered the most important soil ecosystem engineers with influence on [52, 53]:

- Distribution of soil organic matter (SOM);
- Hydraulic properties of soil;
- Erosion;
- Microbial diversity;
- Vegetation diversity and growth.

The termite nests and biomass are characterized as "sinks" as they withdraw large quantities of litter and soil organic matter from the "normal" decomposition pathways. Except for the case of fungus-growing termites, the SOM, Ca^{2+}, Mg^{2+} and K^{+} content is usually higher in

termite mounds and in the surrounding soil than in the soil unaffected by termites. The consumption of organic matter by termites is utilized in the production of termite biomass and in the building of nest-constructions. The return input of organic matter and mineral nutrients to the soil environment occurs via faeces, salivary secretions, corpses and predators. Mortality, particularly from ant predation, and mound erosion are the important contributors to the turnover and redistribution of the organic matter and mineral nutrients in the ecosystem.

The role of termites in water infiltration and runoff is closely related to the importance, structure and arrangement of their subterranean biogenic structures, which comprise foraging and storage galleries, feeding chambers and communication channels, in addition to chambered colony centres (in whole underground species) and the foundations of epigeal mounds. The termite biostructures act therefore as a network of horizontal and vertical macropores influencing bulk density, aeration, water infiltration and runoff, then capturing overland flow and determining the hydrological characteristics of watersheds. The termite mounds can also influence water infiltration and nutrient leaching, although this effect likely depends on the age and whether the mounds are inhabited by termites or abandoned.

The erosion of termite mound would take ten years. The process is very rapid in the first years after the abandonment but then the rate subsides gradually until it is more or less negligible due to the stabilization effect of vegetation growth on the remains of the structure. Temporal scale is important: some termite mounds persist in the landscape for periods of a decade or more, and their individual influence on soil profile development during that time may be negligible.

However, over 100s or 1000s of years, the importance of termites in the turnovers of both mineral materials and organic matter in the landscape and in the whole soil profiles may be much greater, such that long-term pedogenesis is mediated by their activity. The abandoned termite nest does not lack purpose hence it can be occupied by other animal, insect or it can provide substrate for the seeds of trees and plants.

Termites modulate the availability of resources for other species, such as soil macro- and microfauna and microorganisms, and create habitats which can be used by a remarkable number of organisms for nesting, roosting or accommodating stages of their life cycles.

The concentration of SOM and nutrients in termite nest structures exaggerates resource patchiness and the structures can be considered as “fertile islands” which are beneficial resources for plants [52, 54-57].

1.6. Termite control

Therefore the termites are the excellent decomposers of cellulose; they become a serious menace to both the plants and the wood structures. Termites cause significant losses to the annual and perennial crops and damage the wooden components in buildings, especially in the semi-arid and subhumid tropics [3]. The methods developed in order to control termites are shown in Fig.4.

The physical barriers are the most popular methods used against subterranean termites to prevent wooden structures from their attack. The barriers are of two types - toxic and nontoxic.

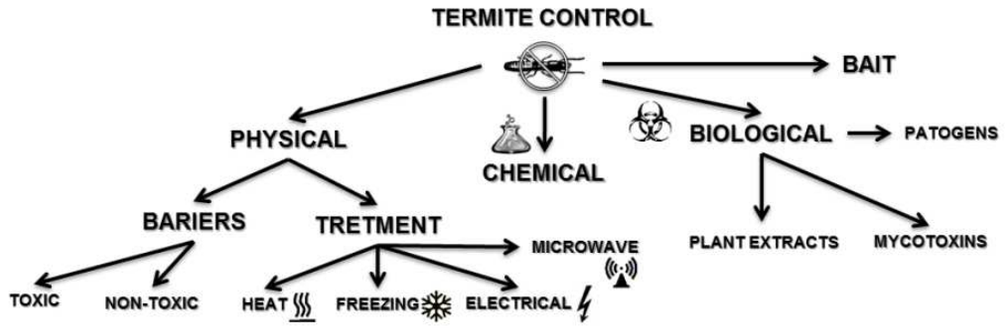


Figure 4. Method developed for termite control.

Toxic physical barriers include the use of chemical termiticides in the soil around the structure. Nontoxic physical barriers are substances (e.g., sand or gravel aggregates, metal mesh or sheeting) which exclude termites because they are impenetrable, thus act as physical/mechanical barriers preventing termite penetration and damage to the building. Other physical methods include heat, freezing, electricity, and microwaves. Chemical control is a successful method of preventing termite attack, but the effects of these chemicals are of concern as they create problems to our health and the environment.

Baiting is the method of termite pest control that is more environmentally friendly as it uses very small amounts of insect toxicants. In bait technology the termite colonies can be eliminated by the use of toxic or nontoxic baits. Bait is a wood or a cellulose matrix favoured by termites that is impregnated with a slow-acting toxic chemical or nontoxic substance such as fungal spores, mycelium (that grows through termite cuticle and utilizes entire termite body) and infective stages of nematodes (corynebacterium which produces toxins lethal to termites). The termite workers are exposed to the lethal dose of desirable food bait inside the bait stations.

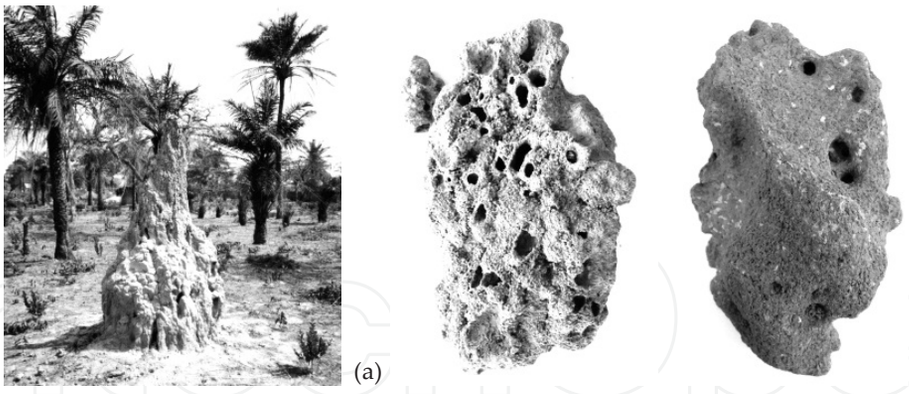
Plant-derived natural products and biological control agents are promising replacements of chemical methods for termite control. These techniques utilize insecticidal activity of essential oils, extracts and resins, parasites and various pathogens (bacteria and fungi) [3]. Although many studies are overly optimistic the more than 50 years of the development of biological control of termites using pathogens demonstrated that this technology is not successful [58]. The high carbon dioxide and naphthalene contents and secretions of termites may also inhibit the growth of pathogens in termite nests or galleries, and therefore the termite control with pathogens stays unsuccessful under the field conditions [59].

2. Experimental

2.1. Sample and sample treatment

The nest of subterranean termites (ground dwellers) from Gambia was used for investigation of the material composition and properties in this work (Fig.5).

The four portions (samples) were prepared from termite nest material:



(a) (b)

Figure 5. Termite mound (a) and sample of termite nests analysed in this work (b).

- **Sample A**: untreated material;
- **Sample B**: finely dispersed (milled) material of the nest;
- **Sample C**: sand grains rich fraction ≥63 μm;
- **Sample D**: clay minerals rich fraction ≤63 μm.

The sand grains and clay minerals rich fractions were prepared via careful dispersion of the sample in the porcelain dish using a force insufficient to crush the grains of sand. The ground material was divided into both fractions by sieving.

2.2. Analysis of samples

The composition and properties of the material were investigated by scanning electron microscopy (SEM), x-ray diffraction analysis (XRD), infrared spectroscopy (IR), thermal analysis (TA) techniques including simultaneous termogravimetry and differential thermal analysis (TG-DTA), effluent gas analysis (EGA) and the high-temperature x-ray diffraction analysis (HT-XRD). other tests and analyses (Table 1) including the assessment of humidity, loss on ignition, pH, etc., were performed according to the standards.

2.2.1. Scanning electron microscopy

The scanning electron microscopy was performed with the microscope JEOL JSM-7600F. The samples were coated by the gold layer. The energy dispersive x-ray fluorescence spectroscopy (EDX) was used for the assessment of the sample composition.

2.2.2. X–ray diffraction analysis

The quantitative Rietveld phase analysis of phase composition was performed using x-ray diffractometer (PANanalytical Empyrean) with Cu(K_α) radiation at 40 kV and 40 mA. For the high temperature x-ray diffraction analysis (HT-XRD) the sample was inserted into the heating chamber (Anton-Paar HTK16) and heated on the platinum pane at the heating rate 10 °C•min^{-1} up to temperature 1250 °C.

2.2.3. Infrared spectroscopy

The infrared spectra were collected within the mid-IR region by means of the KBr pellets technique using FT-IR spectrometer iS10 (ThermoScientific) using the sample to KBr ratio of 1:100.

2.2.4. Thermal analysis

Thermal analysis was carried out using TG-DTA analyser Q600 (TA Instruments) connected to measuring cell (TGA/FT-IR Interface, Thermo Scientific) of FT-IR spectrometer Nicolet iS10 (Thermo Scientific) through heated capillary (200 °C).

2.2.5. Particle size distribution

The particle size distribution was determined by the sieve analysis and the particle size analyser Helos (Sympatec). The specific surface area was calculated from the obtained data.

3. Results and discussion

The basic properties of investigated sample from termite nest are listed in Table 1.

Feature		Value	Description
Humidity	[%]	0.5±0.1	Performed on sample B by drying to constant weight at temperature 105 °C.
Loss on ignition	[%]	3.1±0.1	Performed on the dry sample B. Burning of organic matter and dehydroxylation of clay.
Pour density	[g·cm^{-3}]	2.4±0.1	Weight of granular materials that related to volume unit.
True density	[g·cm^{-3}]	2.6 ±0.1	Volumetric technique. The value corresponds to both the main components (kaolinite and quartz)
pH		6.4±0.1	Sample D in the suspension with re-boiled (dissolved CO_2 free) distilled water.
Zeta potential	[mV]	–17±1	Electrokinetic potential of particles ≤ 63 μm (Sample D).
Conductivity	[W(m K)$^{-1}$]	0.7±0.1	Coefficient of thermal conductivity measured on material of Sample A (TCI, C-Therm).

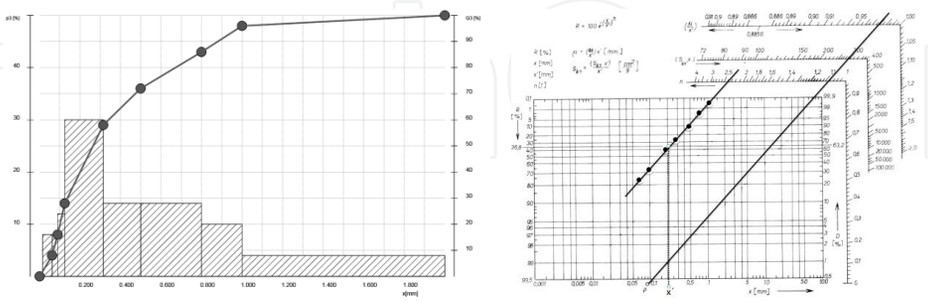
Table 1. The basic properties of the sample of termite nest.

3.1. Granulometry

The particle size distribution was assessed by the sieve analysis for the rough grains of sand (Sample C) and by laser analyser for the fraction of particles ≤ 63 μm (Sample D).

3.1.1. Sample C

The results of the sieve analysis including histogram of log-normal particle size distribution and cumulative particle size distribution curve are shown in Fig. 6.



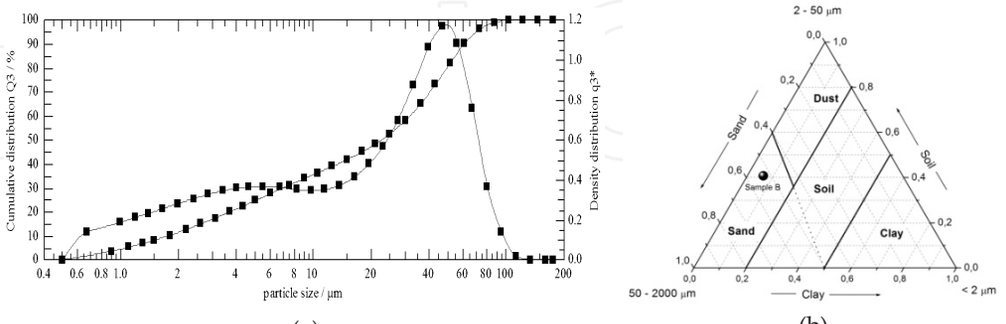
(a) (b)

Figure 6. The sieve analysis of the Sample C (a) and RRSB diagram (b)

Using RRSB diagram[11] the granulometry of the sample is determined by the value of exponent n = 1.08. The value is close to one and that corresponds to the rounded particles of termites applied sand (please referee to Fig. 5). The characteristic size of grain (**x**′) was assessed to 0.2 mm[12]. The specific surface area of 1450 $cm^2 \bullet g^{-1}$ is resulting from these results.

3.1.2. Sample D and B

The particle size distribution in the fraction ≥63 μm is shown in Fig.7 (a). The median of particle size is 22.9±0.3 μm. Combination of the results of both samples leads to Fig.7 (b) hence the material of sample B should be described as sandy clayish (in this case kaolinite) sand.



(a) (b)

Figure 7. The granulometry of the Sample D (a) and total granulometric composition (b).

11) The acronym for the Rosin-Rammler-Sperling-Benett diagram.

12) The characteristic value for sand is typically higher (~1.4). The x′ corresponds to R = 36.8 %.

3.2. Electron microscopy

The structure of the termite nest wall is shown in Fig.8. Termites build their nest from the sand grains and use the clays and faeces as the binding material. The parts of plants serve as the reinforcement. The detail shows that smaller grains fill the spaces between the larger and reinforcing plants material. The surface of sand grains is treated by layers of clay (Fig.9). It is obvious that the resulting composite structure of termite nest shows excellent mechanical properties which enable to build structures of several meters of height / structures several meters high.

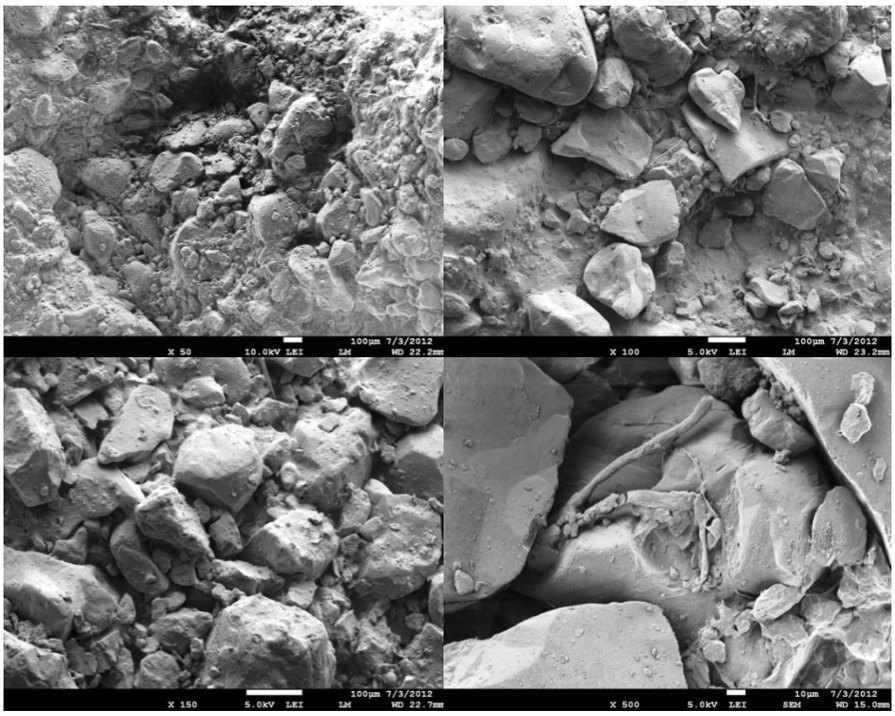


Figure 8. Termite nest microphotography (Sample A).

While the structure inside of the wall is rough the surface of tunnels is polished by the mixture of clay (Fig.10) and faeces (Fig.11). The clay phase loses plasticity via evaporation of water and formation of new weak (van der Walls) bonds between lamellar particles (Fig.10) which leads to the formation of hard aggregates fixing the silica grains on their positions. It stands to reason that the silica particles as the phase of high Young modulus serve as the reinforcement as well as the filling material. The conceived particle size distribution of grains of "aggregate" (packing density[13] of ~92 %) and mechanical and saliva activation clay phase (mainly kaolinite) form very solid structure that is analogical to the ceramic green body.

Hence the structure of termite nest is tailored well, and it is possible that the role of organic matter (SOM) and faeces [52] often discussed in literature do not increase mechanical proper-

13 Defined as the ratio of pour and true density (Table 1).

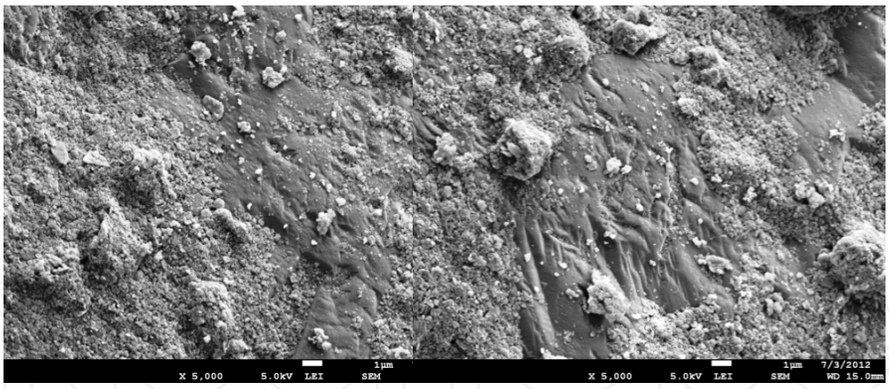


Figure 9. Microphotography of surface of clay layer treated sand grain (Sample C).

ties of material but more probably prevent spreading of pathogenic organism [2] and support fungi inside fungus garden [5] of fungus growing termites.

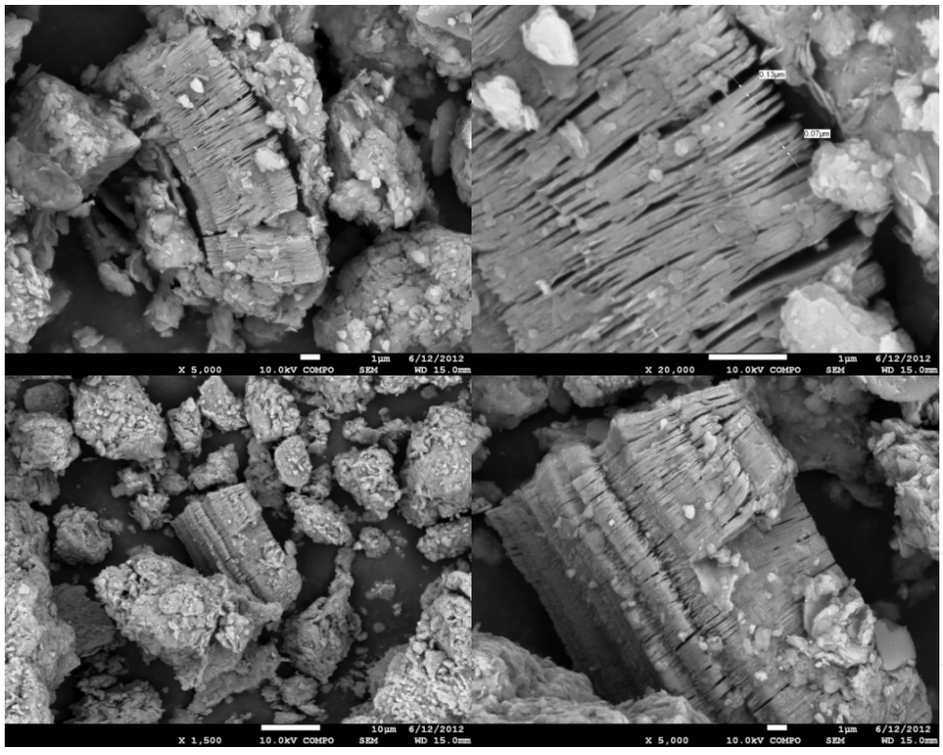


Figure 10. Clay from termite nest (Sample D).

It is supported by the foundation that the highest concentration of elements such as phosphorus, potassium and nitrogen coming from termite diet is located near the surface of tunnels. Hence the faeces are used for the treatment of surface (and to provide plasticity to shelter tubes of course) but the main part of the weight of mould bearing material does not contain these elements and content of SOM is in common level[14].

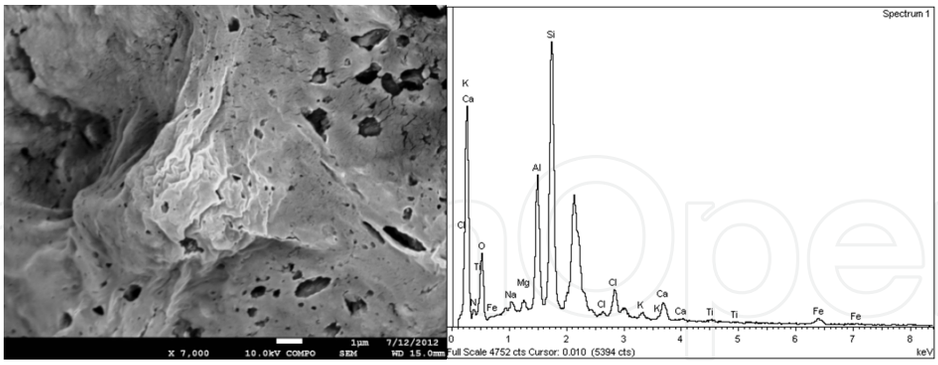


Figure 11. The faeces rich layer covering the surface of tunnel in termite nest.

Therefore the secret of ground dweller termite material engineering lies more probably in the well-tailored structure analogical to the dry ceramic body than in their diet or enzymes in their digestive tract.

Site	Si	Al	Mg	Fe	Ti	Ca	K	Na	N	Cl
Surface	46.5	18.6	1.6	6.9	1.4	6.3	2.0	1.6	12.7	2.5
Inside	67.1	24.4	0.3	4.6	1.1	0.2	0.7	0.7	0	0.9

Table 2. The composition of termite nest material inside and on the surface of wall of tunnel.

The fact that the faeces of herbivore animals increase the plasticity and reduce the drying shrinkage of clays has been known well. This foundation is used by many cultures over the world in the construction of clay houses from prehistoric until today[15]. There is the possibility that the role of faeces rich layer on the surface of galleries and tunnels may indirectly contribute to the strength as it slows down the rate of drying (reduce the drying shrinkage) and weathering. Considering the previous applied comparison to the dry ceramic body the layer can serve as the glaze on the surface but its thickness lower than 1 μm is not enough to reach a significant influence on the mechanical properties of the material.

3.3. X–ray diffraction analysis

The x-ray diffraction patterns of the sample B and D are shown in Fig. 8 (a) and (b), respectively. The kaolin and quartz were identified as the main constituents of the sample. Furthermore small amount of clay mineral halloysite was identified.

14 Various clays used by ceramic industry contain even higher amount of organic matter what can lead to failure known as "black core" which is formed if fired under improper forint regime ("firing curve").

15 For example clay pyramids known as "Temples of the sun" built by Mochica culture, in Europe were clay houses built from the Stone Age through Medieval to industrial revolution (in some countries even later), Massai houses, etc. Nowadays probably more than ½ of human population lives in clay houses.

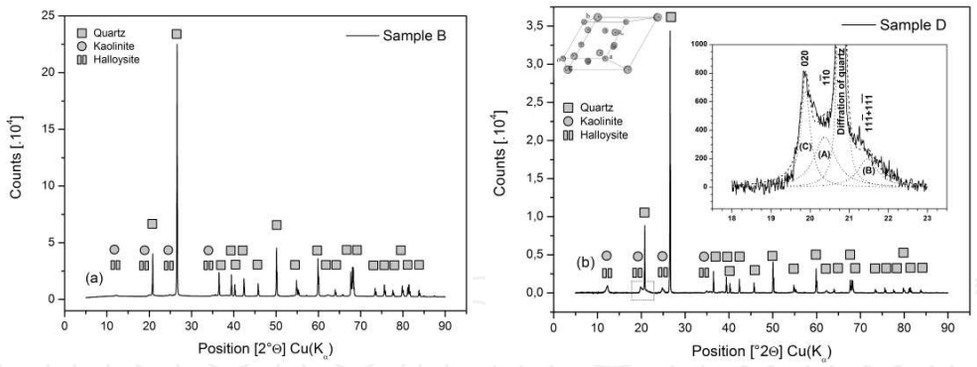


Figure 12. The XRD pattern of Sample B (a) and Sample D (b).

The structure refinement performed on the Rietveld analysis data shows slightly deformed triclinic kaolinite structure with lower parameter b and angle α. Table 1 shows the sample composition according to the results of quantitative Rietveld analysis.

Sample	Quartz [%]	Kaolinite [%]	Halloysite [%]
Sample B	97, 4	2, 6	---
Sample D	70.3	29.5	0, 2

Table 3. Phase composition of the thermite nest material.

In order to estimate the effect of termite treatment or activation of clays the structure order of the kaolinite was evaluated using weighed ratio intensity index[16] (WIRI) that is based on weighed intensity of (02, 11) diffractions and calculated as follows [60]:

$$AGFI = (Ia + Ib) / 2Ic \tag{1}$$

$$WIRI = 1 - \exp\left[\frac{-w_1^{-1} I(1\bar{1}0) + w_2^{-1} I(11\bar{1}) + w_3^{-1} I(1\bar{1}\bar{1})}{w_4^{-1} I(020)}\right] \tag{2}$$

$$HI = 0.1 + 1.44\, WIRI \tag{3}$$

where w is half-width of the diffraction line. The results of the XRD pattern fitting procedure are shown in Fig.12. The values of HI, AGFI and WIRI were calculated to be 0.40, 0.92 and 0.21 corresponding to the kaolinite of the poor level of structure order.

16 The WIRI index ranged from zero to one while for other often applied indexes including HI (Hinckley Index) and AGFI (Aparicio-Galán-Ferrell Index) the value higher than one can be reached.

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The sequence of transformations of the Sample D during thermal treatment was investigated by high temperature X-ray diffraction analysis (Fig.9) up to temperature 1250 °C.

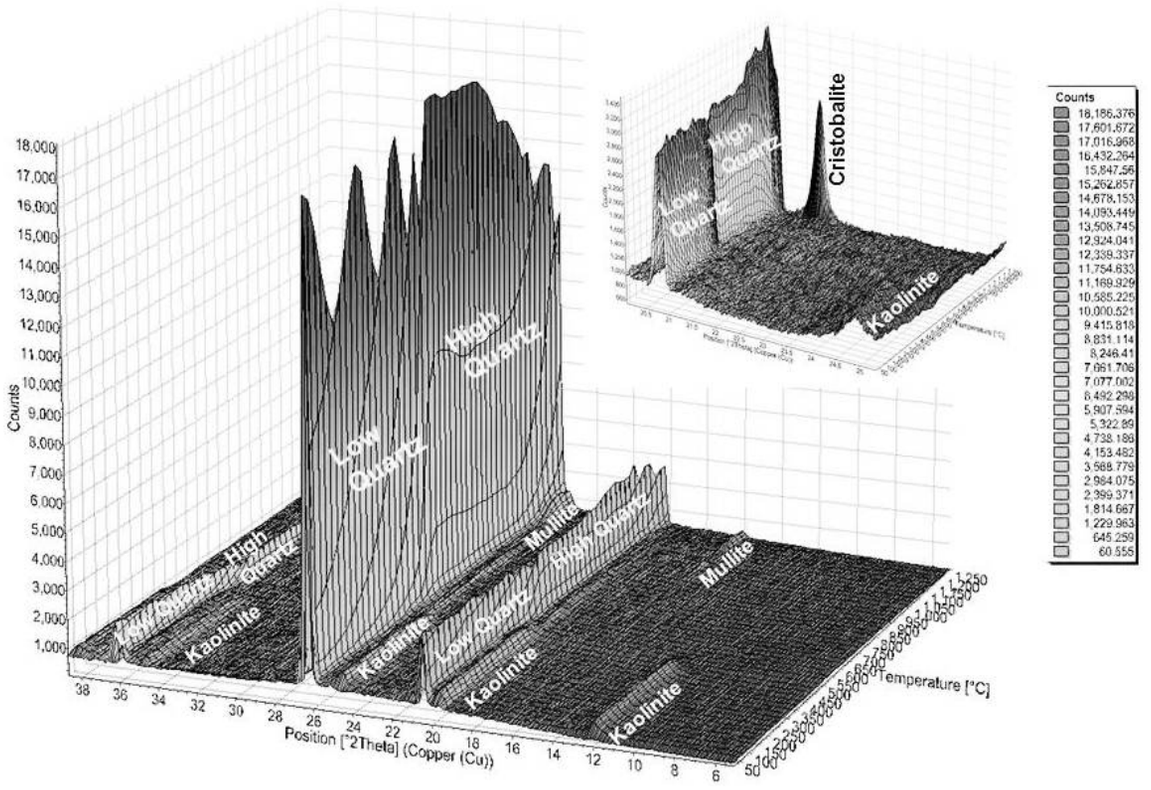


Figure 13. HT-XRD analysis of the Sample D heated to 1250 °C using heating rate 10 °C•min^{-1}.

The following process takes place in the sample:

- Dehydroxylation of kaolinite and formation of roentgen-amorphous metakaolinite (Eq.4) leads to disappearing of diffractions of kaolinite (K) from the HT-XRD plot.
- Transformation of low quartz to high temperature phase leads to the shift of diffraction to lower position. For example the most intensive diffraction line at 26.31 is shifted to 26.23 °2Θ.
- Formation of mullite (Eq.6).
- Formation of cristobalite (Eq.7) from the amorphous silica phase that is formed during the synthesis of cubic Al-Si spinel phase[17] and mullite as well.

3.4. Infrared spectroscopy

The infrared spectrum of the sample C and D is in Fig.14 (a) and (b), respectively. The results show the typical pattern of quartz and kaolinite.

17 While the process shows sharp exothermic effect on DTA curve (please see Fig.15 and discussion) the response in XRD diagram is low with uncertain interpretation in the literature. Some of the works bring evidence on the formation of mullite or γ-Al2O3 but the final consensus has not been reached yet.

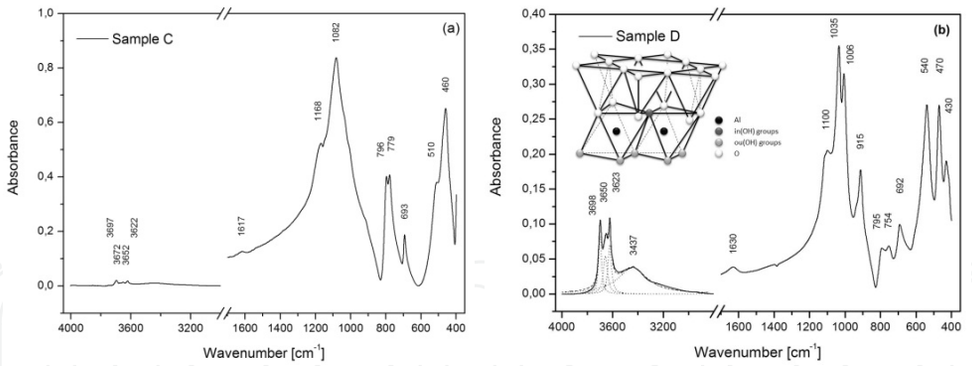


Figure 14. The infrared spectrum of the Sample C (a) and Sample D (b).

The adsorbed water shows broad band of stretching and deformation at 3440 - 3430 and 1630 – 1610 cm^{-1}, respectively. The interpretation of spectra is given in Table 4.

Sample C – Typical spectrum of quartz			Sample D – Typical spectrum of kaolinite		
Wavenumber	**Fragment**	**Note**	**Wavenumber**	**Fragment**	**Note**
3700 - 3620	AlO-H from kaolinite bound to the silica surface.		3698, 3672, 3650, 3623	AlO-H	ν_1(ou), ν_2(ou), ν_3(ou), ν_4(in)
1168	Si-O	LO part of ν_{as} mode	1100	Si-O_{ap}	ν(Si-O) of apical oxygen
1082		TO part of ν_{as} (TO_3) mode	1035, 1006	Si-O-Si	ν_{as}, ν_s in layer of tetrahedra
776, 779		Doublet of bending (TO_2)	935, 915	AlO-H	δ_2(ou), δ_2(in)
693		Perpendicular mode	795, 754	AlO-H	Translation modes
510		(LO_1) rocking mode	692	Si-O	Perpendicular mode
460		(TO_1) rocking motion	540	Al-O	ν(AlO_6) in the gibbsite layer
LO (longitudinal-optic), TO (transverse-optic) , ν_{as} and ν_s (antisymmetric and symmetric stretch), δ (deformation)			470, 430	Si-O	Deformation modes

Table 4. Interpretation of spectrum of samples C and D.

3.5. Thermal analysis

The results of thermal analysis of the sample including the simultaneous TG-DTA (a) and EGA (b) are shown in Fig. 15. The evaporation of water causes the first broad endothermic effect. The SOM mater begins to burn at temperature higher than 200 °C. The formed carbon dioxide is well visible in the plot of EGA. The dehydroxylation of kaolinite to metakaolinite takes place

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at 571 °C. The EGA is shown by increasing intensity of water vapour in the gas phase. The transformation of low quartz (trigonal) into the high quartz (hexagonal) shows very sharp endothermic peak[18] at temperature 571 °C. The sharp exothermic peak of the formation of spinel phase[20] appears at 975 °C.

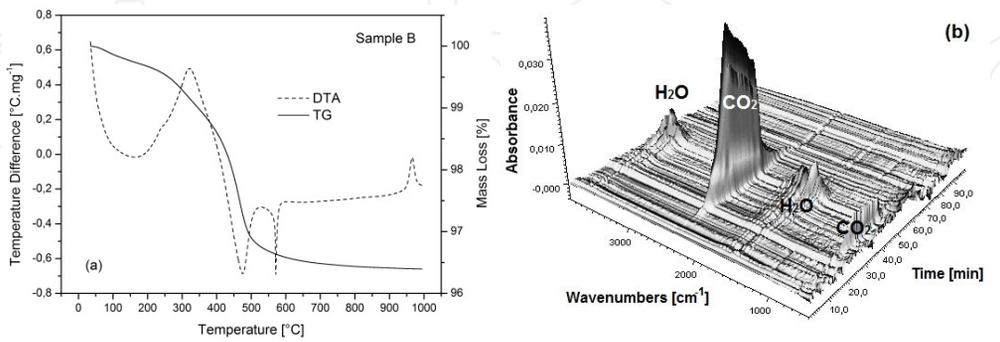


Figure 15. Thermal analysis of the sample B: TG-DTA (a) and EGA (b).

Therefore it is difficult to distinguish between individual steps of TG curve. The changes in the mass of the sample during evaporation of water, burning of the SOM and dehydroxylation were evaluated via the series of isothermal steps (Fig. 16(a)).

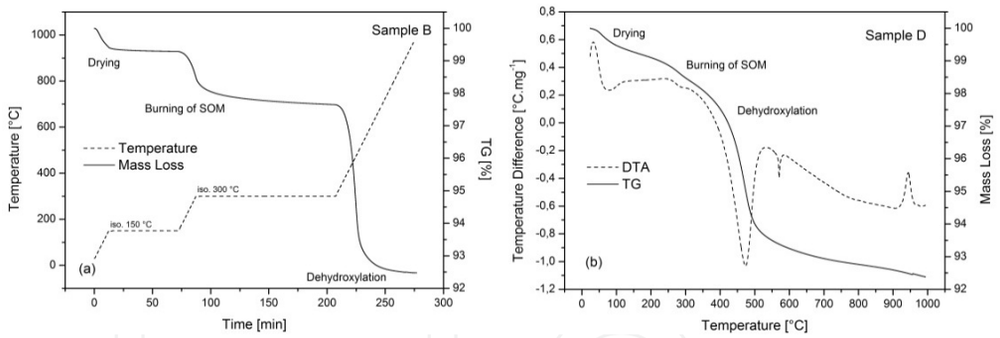


Figure 16. Evaluation of the SOM and kaolinite content in the Sample B (a) and thermal analysis of the sample D (b).

The sequence of thermal transitions for the sample B should be identified in the same way for the sample D (Fig. 16 (b)).

3.6. Thermal transformation of the clay from termite nest

Thermal transformations of kaolinite as the main part of clay phase of the termite nest material include the following processes [61-63]:

- Dehydroxylation of kaolinite into metakaolinite[19];

18) Transformation is independent on heating rate.

$$Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O \xrightarrow{400-700°C} Al_2O_3 \cdot 2SiO_2 + 2H_2O(g) \quad (4)$$

- Formation of the Al-Si spinel phase [20];

$$2\,(Al_2O_3 \cdot 2SiO_2) \xrightarrow{925-1050°C} 2Al_2O_3 \cdot 3SiO_2 + SiO_2(amorphous) \quad (5)$$

- Formation of the mullite;

$$3\,(2Al_2O_3 \cdot 3SiO_2) \xrightarrow{\geq 1050°C} 2\,(3Al_2O_3 \cdot 2SiO_2) + 5SiO_2(amorphous) \quad (6)$$

- Formation of the cristobalite.

$$SiO_2(amorphous) \xrightarrow{\geq 1200°C} SiO_2(cristobalite) \quad (7)$$

The kinetics triplet, i.e. apparent activation energy ($\boldsymbol{E}_a$), frequency factor ($\boldsymbol{A}$) and mechanism of the process and thermodynamics of these processes are the objective of numerous works [64-66]. The processes, especially dehydroxylation, are affected by the structural changes in kaolinite. In order to estimate the influence of potential treatment or activation of clay by the termites, the kinetics of the dehydroxylation and formation of spinel phase was investigated via Kissinger kinetic equation [67]:

$$\ln\left[\frac{T_m^2}{\Theta}\right] = \ln\left[\frac{AR}{E_a} n(1-\alpha)^{n-1}\right] - \frac{E_a}{RT_m} = const. - \frac{E_a}{RT_m} \quad (8)$$

The T_m denotes the peak temperature of thermal analysis (DTA, DTG, DSC EGA...), Θ is the heating rate, n is the empirical reaction order (kinetic exponent), α is conversion degree (fractional conversion) and R is the universal gas constant. The constant term is equal to $\ln(AR/E_a)$ for the pseudo first-order type of reaction. The plot of $ln[\Theta/T_m^2]$ on reciprocal temperature $1/T_m$ (Kissinger plot) provides the straight line with slope of E_a/R.

The mechanism of the investigated process can be estimated from the shape of TDA peak. The peak parameters of TA curves enable to determine the value of kinetic exponent (n) as follows [68-70]:

$$n = \frac{2.5\,R\,T_m^2}{w_{1/2}\,E_a} \quad (9)$$

19 The course of dehydroxylation is affected by the burning of organic matter (actually there is feedback between both processes) hence The formation of water during pyrolysis of SOM decreases equilibrium constant of the process [71].

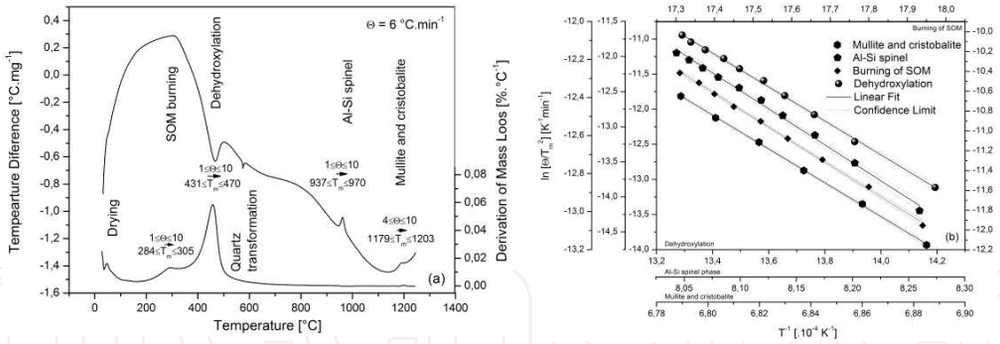


Figure 17. The shift of DTA effect with heating rate (a) and Kissinger plot (b).

The DTA results of sample D and Kissinger plot are shown in Fig. 17 (a) and (b), respectively. The formations of mullite (Eq.4) and cristobalite (Eq.5) show compound inexpressive effect on DTA hence the determined value is related to both processes. The overview of kinetic results including activation energy, frequency factor and the most probable mechanism are listed in Table 5.

Process	E_a [kJ•mol^{-1}]	A [s^{-1}]	*n*	Description
Burning of SOM	203	$3.23 \bullet 10^{16}$	0.80	Process limited by the rate of diffusion.
Dehydroxylation	201	$6.26 \cdot 10^{11}$	1.06	Random nucleation.
Al-Si spinel	852	$7.17 \cdot 10^{33}$	2.52	Diffusion controlled growth; const. or increasing nucleation rate.
Mullite and cristobalite	677	$5.85 \cdot 10^{21}$	3.79	Decreasing nucleation rate.

Table 5. The kinetics of thermal transformations of kaolin from termite nest.

The most frequently published value of the activation energy for dehydroxylation process of kaolinite ranges from 140 to 250 kJ•mol^{-1} and formation of Al-Si spinel shows E_a of 856 kJ•mol^{-1} [72]. The results indicate that the mechanism and kinetics of investigated process are not changed hence there is no supporting evidence for an activation process that would have a significant effect of termites on the structure of clay minerals.

The correlation between kinetic and thermodynamic parameters of the investigated process results from the combination of Arrhenius with Eyring or Wertera and Zenera laws related to the temperature dependence of rate constant ($\boldsymbol{k}(\boldsymbol{T})$) [73]:

$$k(T) = A \exp\left[-\frac{E_a}{RT}\right] = \frac{k_B T}{h} \exp\left[\frac{\Delta S^{\#}}{R}\right] \exp\left[-\frac{\Delta H^{\#}}{RT}\right] = \nu \exp\left[-\frac{\Delta G^{\#}}{RT}\right] = \nu K^{\#} \tag{10}$$

where $\boldsymbol{k}_B$, $\boldsymbol{h}$, $\boldsymbol{\nu} = \boldsymbol{k}_B\boldsymbol{T}/\boldsymbol{h}$ and $\boldsymbol{K}^{\#}$ are Boltzmann, Plank constant, vibration frequency and equilibrium constant of formation of activated complex, respectively.

The thermodynamic parameters of activated complex, including free energy ($\Delta G^{\#}$), enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) of process were calculated using Eyring equations:

$$\Delta H^{\#} = E_a - RT \tag{11}$$

$$\Delta S^{\#} = R\left[\ln\left(\frac{h\,A}{k_B T}\right) - 1\right] \tag{12}$$

$$\Delta G^{\#} = \Delta H^{\#} - T\,\Delta S^{\#} = -RT\ln K^{\#} \tag{13}$$

Table 6 shows the overview of average values of thermodynamic results calculated for activated complex according to Eq. 10 – 13 for interval of ΔT according to Fig.17.

Process	$\Delta H^{>\#}$ [kJ·mol^{-1}]	$\Delta S^{>\#}$ [J·(mol·K)$^{-1}$]	$\Delta G^{>\#}$ [kJ·mol^{-1}]	$K^{>\#}$
Burning of SOM	201	63	183	$1.96 \bullet 10^{-17}$
Dehydroxylation	195	-35	220	$2.60 \bullet 10^{-16}$
Al-Si spinel	844	385	475	$8.87 \bullet 10^{-21}$
Mullite and cristobalite	667	152	486	$4.79 \bullet 10^{-18}$

Table 6. Thermodynamics of thermal transformation of kaolin from termite nest.

The negative value of entropy ($\Delta S^{\#} < 0$) indicating formation of more ordered transition state during dehydroxylation is out of the usual findings for thermal decomposition of kaolinite. Since pure well defined sample of kaolin shows mostly $\Delta S^{\#} > 0$, SOM interacting with clay phase are responsible for this behaviour.

The assessed kinetic triplet of combustion of SOM shows that the activation energy of the process is close to the activation energy for dehydroxylation but the burning of SOM proceeds faster due to the higher A and the lower n. The gas products of burning of SOM (Fig.15(b)) diffuse through the kaolinite aggregates and increase the pressure of water vapour affecting the thermodynamics of dehydroxylation via the equilibrium constant (Eq.13; the $\Delta G^{\#}$ increases due to negative value of $\Delta S^{\#}$)[20]. The temperature dependence of $\Delta G^{\#}$ determined by extrapolation of the values calculated from the experiment is shown in Fig.18 for both processes. From the kinetic point of view which is given by Eq.10, the rate constant of dehydroxylation with temperature increases more slowly than for the combustion process of SOM. In the other words, increasing pressure

20 It must be pointed that combustion of SOM is strongly affected by the content of individual kind of humic substances and the both processes (combustion of SOM and dehydroxylation) show mutual relationship. For example, intensive origin of water vapor slows down diffusion of oxygen, leads to reducing conditions, changing composition of product and slowing down the rate of process [74].

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of water vapour slows down the rate of decomposition of activated complex into product and the rate of dehydroxylation process decreases.

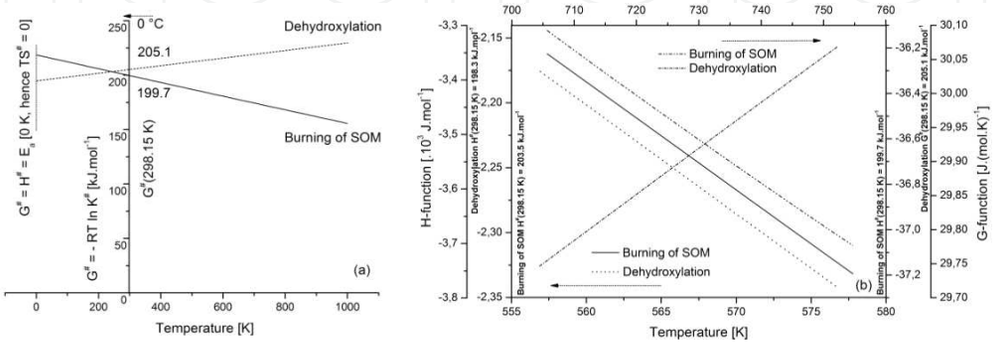


Figure 18. Temperature dependence of $\Delta G^{\#}$ for burning of SOM and dehydroxylation (a) and temperature dependence of $G^{\#}$-function and $H^{\#}$-function.

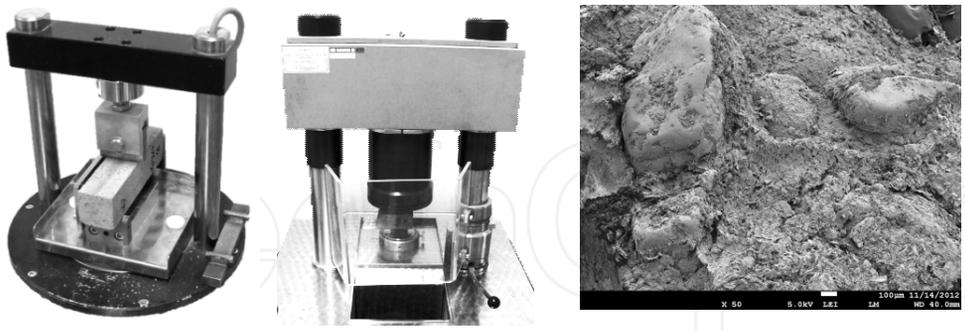
In Fig. 18(b) the temperature dependence of $\Delta H^{\#}$ and $\Delta G^{\#}$ is expressed in usual form of $G^{\#}$-function and $H^{\#}$-function:

$$G^{\#} - function = \frac{G^{\#}(T) - H^{\#}(298.15\ K)}{T} \tag{14}$$

$$H^{\#} - function = H^{\#}(T) - H^{\#}(298.15\ K) \tag{15}$$

3.7. Properties of artificial termite nest material analogue

The material with the particle size distribution (Fig. 7(b)) and the composition analogical to the termite nest material was prepared from the mixture of sand and kaolin in order to evaluate material properties. The determination of dry bending strength (Fig. 19(a)) and crushing (compressive) strength were performed using 40×40×160 mm test pieces. The microphotography of fracture planes (Fig. 19(b)) shows the grains of sand surrounded by the clay phase like in the termite nest material. The mechanical properties and some other investigated material properties are listed in Table. 7.



(a) (b) (c)

Figure 19. Examination of bending strength (a) crushing strength (b) and fracture plane (c).

Property		Value	Description
Pour density	[g•cm^{-3}]	2.3± 0.1	Weight of granular material related to volume unit.
Volume density		1.9 ± 0.1	The weight of volume unit of porous material.
True density		2.6 ± 0.1	Volumetric technique.
Packing density	[%]	88	The ratio of the pour and true density.
Drying shrinkage	[%]	1.6 ± 0.3	Shrinkage of dry material with regard to original dimensions.
Dry bending strength	[MPa]	0.52 ± 0.05	Measured on the dry material using testing hydraulic press with device for testing of bending and crushing strength.
Dry compressive strength		1.23 ±0.02	
Thermal conductivity	[W(m K)$^{-1}$]	0.5±0.1	Coefficient of thermal conductivity (TCI, C-Therm).

Table 7. Properties of artificial termite nest material analogue.

The material shows a small drying shrinkage as the consequence of high content of aggregates. The lower value of pour density is reached in comparison to the termite nest material (Table 1). That means a lack of fine particles of sand in the material and gives the reason for measured poor value of compressive strength. The results indicate that an optimal moisture and weighted granulometry is necessary to reach the good material properties while the role of SOM that usually increases plasticity of clay minerals pastes is of peripheral importance for the materials with as high content of aggregates as in the investigated one.

4. Conclusion

Behind all of the presented experimental data there is one significant foundation. The building engineering of soil-dwelling termites shows us that the relationships between the particle size distributions, particle shape, packing density of aggregate grains and the plasticity as well as the amount of binding phase (clays) are sufficient to cover the surface of the aggregates by the continuous thin layer as well as the amount of mixing water has the same relevance for the construction of clay buildings as for the preparation of concrete structures. Therefore the research that finds out and describes the mutual influence of these factors and condition of treatment on the resulting properties of clay based material (similarly as for preparation of concrete mixtures) is required.

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Author details

Petr Ptáček*, Jiří Brandštetr, František Šoukal and Tomáš Opravil

*Address all correspondence to: ptacek@fch.vutbr.cz

Brno University of Technology, Faculty of Chemistry, Centre for Material Research, Purkyňova, Brno, Czech Republic

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