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The Influence of Geographical Location of the nest on Chemical Composition of Termite *Macrotermes Bellicosus* Fungus Comb Collected in Côte d'Ivoire

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Abstract: ***Macrotermitinae grow Termitomyces spp. in their mounds on a substrate called fungus comb or fungus garden, which is elaborated with plant material. The combs are an important food source for these termites and a substrate for the Termitomyces. When the fungus garden fails to be created by the termites, they eventually run out of food and are unable to survive. This study aims to determine the influence of the geographical area on the chemical composition of fungus comb of the Macrotermes bellicosus termite of Côte d'Ivoire. Fungus comb of M. bellicossus were collected from termite mounds at sites in ten different localities in Côte d'Ivoire. The results obtained showed that the humidity levels of fungus comb are between 46.73 ± 0.7% to 48.73 ± 1.59%. In addition, fungus comb of the nest of the M. bellicosus termite are acidic and the pH values tend towards 4.1 regardless of the origin of the samples. Considering the major compounds determined in the fungus comb, namely cellulose, lignin, proteins and ash, their rate varies from one site to another. The cellulose rate varies from 26.04 ± 1.46% to 28.33 ± 1.34%, while that of lignin is between 15.59 ± 0.98% to 18.64 ± 1.13%. However, the fungus comb is devoid of fat. The principal component analysis (PCA) of the compounds made it possible to distinguish three groups of fungus comb. The first group is made up of fungus comb of the localities of Abidjan (ABJ), Abengourou (ABG), Adiaké (ADK), Duékoué (DUE) and Méagui (MEA). The second group comes from Korogho (KRG), Niakaramadougou (NIA), Toumodi (TMD) and Bondoukou (BDK). The last group comes from the locality of Bouaké (BKE). These different groups are distinguished by their geographical location characterized by vegetation, climate and soil.***

Keywords: Temite, fungus comb, chemical composition, localities, Côte d'Ivoire

1. Introduction

Termites are widespread in tropical and subtropical regions of the world. The greatest diversity is found in tropical forests, where termites constitute the largest insect biomass [1]. Termites of the Macrotermitinae subfamily particularly, are fundamental organisms in the functioning of tropical ecosystems. They have a very significant influence on their environments. Through their construction activity, they considerably affect the physical and chemical properties of the soil [2]. With the assistance of their symbiotic fungi, Macrotermitinae termites are very active in the process of decomposition of plant matter [3] [4]. Indeed, Macrotermitinae termites farm symbiotic fungi of the genus Termitomyces in their nests [5]. The nests act as shields against predators and help maintain the temperature and humidity conditions that allow the colony to develop [6] The termitomyces fungi also benefit from optimal growth conditions inside termites mounds [7]. Fungus farming termites of genus Macrotermes are giant mounds builders [2]. *Macrotermes bellicosus* that belong to the Macrotermitinae subfamily (Termitidae), builds impressive cathedral - like mounds [8] [9]. This termite is widespread in Côte d'Ivoire (West Africa). For example, [8] noted an abundance of *M bellicosus* in the Comoé National Reserve. In other parts of the country, the presence of this termite has been reported by [10] and [11]. [12] noted the strong presence of *M. bellicosus* mounds inside and outside the Lamto Reserve (Côte d'Ivoire). *M. bellicosus* nest is structured and the main part of the population lives in the central habitat. Large quantities of fungus combs, also called fungus gardens, are stored in chambers inside the central habitat and can reach up to 30 kg dry weight [13]. To process the fungus comb, old termite workers harvest and bring plant debris into their nest. Young termite workers ingest and predigest this plant material [7]. During this quick gut transit, Termitomyces spores are inoculated into the plant substrate, which is excreted as primary faeces. These faeces are used to build fungus comb [4]. As they grow, Termitomyces fungi enhance comb digestibility and nutritional value [14]. Fungus combs are an important food source for Macrotermitinae termites [15] and serve as substrate for Termitomyces [16]. When the fungus comb fails to be created by the termite colony, they eventually run out of food and are unable to survive [17]. In view of to the crucial importance of fungus gardens for the survival of Macrotermitinae termites and their symbiotic fungi, some researchers have studied the process of fungal comb formation [16]. Other works have focused on the fungus garden [18] [19] [20] to determine the involvement of microflora (other than fungal flora) in the symbiotic life of Macrotermitinae termites. Often, studies about termites investigate the impact of these insects on their environments. However, environment seems to have a significant influence on termites, as demonstrated by [21]. Their studies allows us to understand the adaptation of termites to different ecosystems, such as forest and savannah. Environment also has an impact on termites diet. [22] analysed Macrotermitinae termites' diets. They observed that these diets were related to local plant composition, although termites do have food preferences. However, in Côte d'Ivoire, there is no scientific work on the influence of the geographical location of the Macrotermitinae termite nest on the chemical composition of fungus combs. This study is based on the physico - chemical analysis of *Macrotermes* *bellicossus* fungus combs collected in ten different localities of Côte d'Ivoire. The aim is to highlight the influence that these different harvest areas could have on the chemical composition of the fungus combs.

2. Material and methods

2.1 Study area

Côte d'Ivoire is located in West Africa and is among the countries of the Gulf of Guinea. It has an area of 322, 462 km^2. The sampling was carried out in ten localities of Côte d'Ivoire from May to August 2016. The localities are Abidjan in the south, Adiaké in the south - east Abengourou in the east, Bouaké in the centre, Toumodi in the centre - east, Niakaramandougou and Korogho in the north, Bondoukou in the north - east, Duékoué in the west and Méagui in the south - west. For the sake of simplicity, the names of the localities have been abbreviated to: ABJ, ADK, ABG, BKE, TMD, NIA, KRG, BDK, DUE, and MEA respectively. The southern part of the country is covered with forest due to the equatorial, hot and humid climate. The northern part is made up of savannahs, more or less wooded, due to the drier tropical climate. The cold and humid mountain climate is located in the west of the country. Its territory is under the influence of three major types of climate and is covered by two types of vegetation, forests and savannahs.

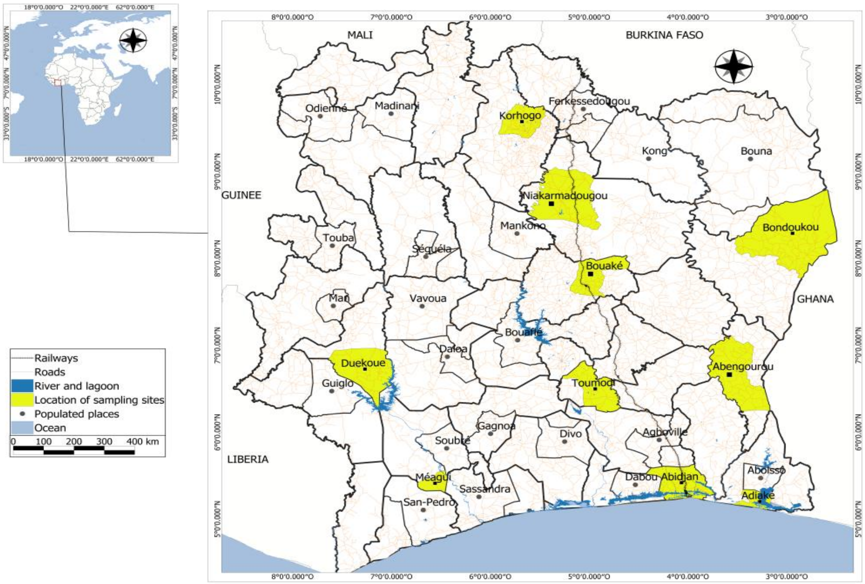

Figure 1: Map of Côte d'Ivoire showing in green sampling site locations within the country

2.2 Biological material

In the present study, the biological material studied consists of fungus combs of termite *Macrotermes bellicosus*. They were collected from termite mounds and were used for physico - chemical analysis.

2.3 Sampling methods

At each sampling site, fungus combs of *M. bellicosus* were collected from six termite mounds. The selected mounds were opened with a pickaxe and the combs were collected with ice tongs. The collected combs were stored in bottles that were sealed, placed in a cooler with ice and transported to the laboratory. The fungus combs were crumbled by hand and then dried in a ventilated oven (SELECTA) at 50° C for 3 days. After drying, the samples were ground in a grinder (CULATTI MFC^R). The dried grinding samples were packaged in airtight bottles for further analysis.

2.4. Physico - chemical analysis

Determination of pH
The pH of fungus combs was measured by the potentiometric method [23]. Ten grams (10 g) of fresh sample were homogenized into 100 ml of distilled water. The pH was measured directly using a HACH SensionTM+ MM340 pH meter.

Determination of moisture content
Moisture content was determined by method [23]. In practice, three grams (3 g) of fresh termite fungus combs samples were oven - dried at 103±2°C during 4 hours to constant weight. The moisture content was calculated as the difference between fresh and dry weight.

Determination of ash content
Ash was determined gravimetrically according to the official method [23]. Three grams (3 g) of dried fungus combs sample were incinerated in a muffle furnace at 600°C for 4 hours. At the end of the process, the resulting ash is removed from the furnace, cooled in a desiccator and weighed with a SARTORIUS balance.

Détermination of Crude protein content
Crude protein was determined from the determination of total nitrogen, according to the Kjeldhal method [23]. Total nitrogen was determined after sulphuric mineralisation of 1 g of dried fungus comb, in the presence of Kjeldhal catalyst. The nitrogen content was multiplied by 6.25 (nitrogen to protein conversion factor).

Détermination of fat content

Fat content was determined by the soxhlet extraction method using hexane as solvent [24]. Five grams (5 g) of dried fungus comb was extracted with 200 ml of hexane for 6 hours using Soxhlet. When the extraction process was completed, the solvent was removed using Heidolph Laborota 4003 rotary evaporator. The evoporating flask was dried in an oven at 105 °C for 30 minutes. The difference in weight of the flask was the sample fat content.

Détermination of cellulose content

The cellulose content was assessed by using sodium chlorite and acetic acid treatment at 70°C according to the method described by [25] to remove lignin from the sample. After filtration and washing, the non - cellulosic polysaccharides were removed from the residues by hydrolysis with NaOH [25]. The insoluble residues finally obtained by filtration were dried at 105°C. The weight loss resulting from the incineration of the residues corresponds to the amount of cellulose contained in the sample.

Détermination of lignin content

The determination of lignin content was carried out according to the Klason method as described by [26]. The sample was mixed with 72 % sulphuric acid and hydrolysed at 20°C. The mixture was then diluted to reduce the acid concentration to 5 % and post - hydrolysed under reflux boiling. The lignin recovered by filtration was quantified gravimetrically.

Détermination of total sugars and reducing sugars content

Prior to the analysis, soluble sugars were extracted from 1g of sample following the protocol described by [27]. Subsequently, to estimate the amount of total soluble sugars, the method described by [28] has been adopted. It uses phenol and concentrated sulphuric acid. The reducing sugars were determined by the method of [29] using 3, 5 dinitrosalicylic acid.

Détermination of total phenolic compound content

The procedure employed by [30] was used to extract phenolic compounds from samples. These compounds were then quantified according to the method of [31], using the Folin - Ciocalteu reagent.

3. Statistical Analysis

The results presented in this work are the averages of the analyses performed with the corresponding standard deviations for each parameter. The statistical analysis ANOVA One - Way and Duncan's post hoc test were used to find significant differences between the means. The threshold for a significant difference was set at 5%. The results were also assessed by principal component analysis (PCA). The applications of these statistical analyses were carried out using STATISTICA 7.1 software.

4. Results

4.1 Chemical composition of termite fungus combs

4.1.1 Moisture and Dry Matter

The humidity levels of fungus combs from the nests of the *M. bellicosus* termite collected in Abengourou, Abidjan, Adiaké, Duékoué, Méagui and Toumodi are identical to the 5% threshold. They are between 46.73 ± 0.7% and 47.32 ± 0.74%. The moisture content of fungus combs harvested in Bondoukou, Korogho and Niakaramadougou are identical at the 5% threshold and vary from 47.57 ± 0.59% to 47.96 ± 0.95%. The moisture content of fungus combs harvested in Bouaké is the highest. It is 48.73 ± 1.59%. In addition, the dry matter rates of fungus combs harvested in Abengourou, Abidjan, Duékoué, Méagui and Toumodi are identical at the 5% threshold and vary from 52.74 ± 0.61% to 53.26 ± 0.70%. The dry matter rates of fungus combs harvested in Adiaké, Bondoukou, Korogho and Niakaramadougou are identical at the 5% threshold. They are respectively 52.67 ± 0.74%, 52.22% ± 0.95, 52.04 ±1.21% and 52.42 ± 1.2%. These dry matter rates of fungus combs are at the threshold of 5% lower than those of fungus combs from the same termite harvested in Abengourou, Abidjan, Bondoukou, Duékoué, Méagui and Toumodi. The dry matter content of fungus combs harvested in Bouaké is the lowest. It is 51.26 ± 1.59% (Figure 2).

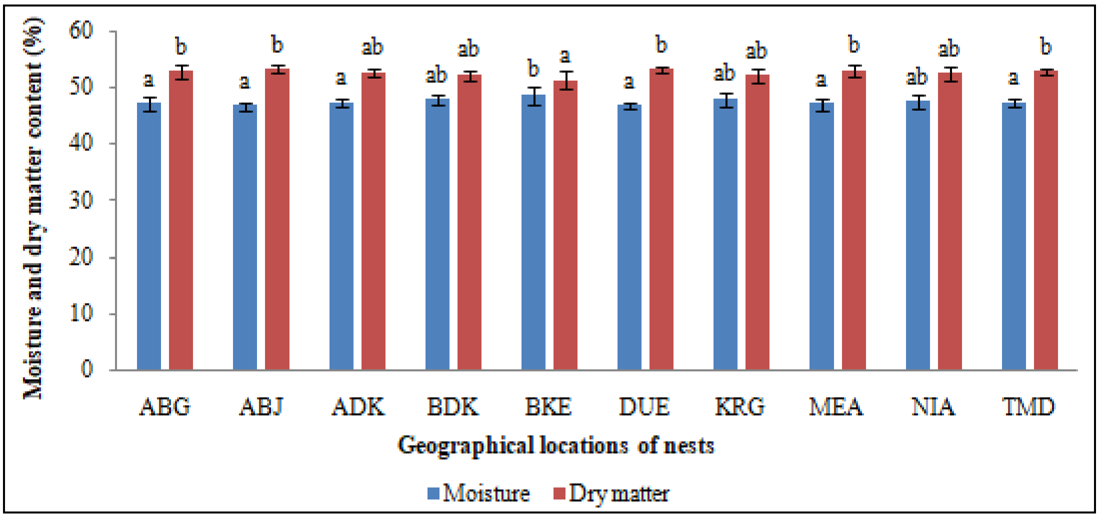

Figure 2: Influence of the geographic location of the nest on the humidity and dry matter rates of the *M. bellicosus* termite fungus combs harvested in Côte d'Ivoire. Abengourou (ABG), Abidjan (ABJ), Adiaké (ADK), Bondoukou (BDK), Bouaké

(BKE), Duékoué (DUE), Korogho (KRG), Méagui (MEA), Niakaramadougou (NIA) and Toumodi (TMD). The histograms of the same color and assigned the same letter have values that are not significantly different at $p < 0.05$.

4.1.2 Cellulose and lignin

The cellulose levels of fungus combs from the nests of the *M. bellicosus* termite collected in Korogho and Niakaramadougou are statistically identical at the 5% threshold. The same is true for the cellulose rates of fungus combs harvested in Abengourou, Adiaké and Toumodi. The cellulose content of fungus combs harvested in Bondoukou is the lowest 5% threshold with 26.04 ±1.46%. However, the highest cellulose rate is that of fungus combs harvested in Duékoué with a rate of 28.33 ± 1.34% (Figure 3). The lignin levels of the fungus combs from the nests of the *M. bellicosus* termite collected in Abidjan, Adiaké, Bondoukou, Duékoué, Korogho, Niakaramadougou and Toumodi are statistically identical at the 5% threshold. They are respectively 16.83 ± 1.55%, 16.94 ± 1.46%, 17.03 ± 0.90%, 16.59 ± 0.74%, 16.98 ± 0.86%, 17.52 ± 0.89% and 16.29 ± 0.83%. The lignin levels of the fungus combs from the nests harvested in Abengourou and Mégui are statistically at the 5% threshold the lowests among all the lignin levels obtained. They are respectively 15.59 ± 0.98% and 16.05 ± 0.90%, whereas the lignin level of fungus combs from the nests of the *M. bellicosus* termite collected collected in Bouaké is the highest 5% threshold. It is 18.64 ±1.13% (Figure 3).

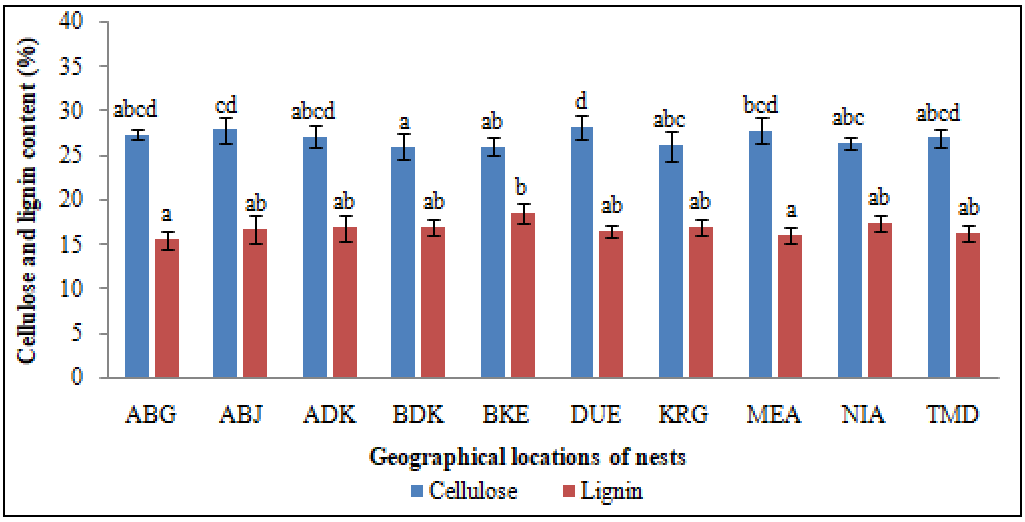

Figure 3: Influence of the geographical location of the nest on the cellulose and lignin levels of fungus combs of the *M. bellicosus* termite harvested in Côte d'Ivoire. Abengourou (ABG), Abidjan (ABJ), Adiaké (ADK), Bondoukou (BDK), Bouaké (BKE), Duékoué (DUE), Korogho (KRG), Méagui (MEA), Niakaramadougou (NIA) and Toumodi (TMD). The histograms of the same color and assigned the same letter have values that are not significantly different at $p < 0.05$.

4.1.3 Protein and ash

The protein levels of fungus combs of the nests of the *M. bellicosus* termite vary from 7.74 ± 0.46% to 9.53 ± 0.66%. The protein levels of fungus combs from the nests of Abengourou, Abidjan, and Duékoué are identical at the 5% threshold. Similarly, those collected in Bouaké, Niakaramadougou and Toumodi are identical at the 5% threshold. The protein level of fungus combs harvested in Méagui is the highest threshold of 5% (Figure 4). The ash content of fungus combs varies from 5.67 ± 0.65 % to 13.55 ± 0.35%. That of Adiaké is the lowest threshold of 5%, while the rate of ash from fungus combs harvested in Korogho is the highest. The ash content of fungus combs harvested in Abengourou, Duékoué and Toumodi are identical at the 5% threshold. The same is true for the rates of ash of fungus combs collected in Abidjan and Méagui. The ash levels of fungus combs harvested in Bouaké and Bondoukou are also identical at the threshold of 5% (Figure 4).

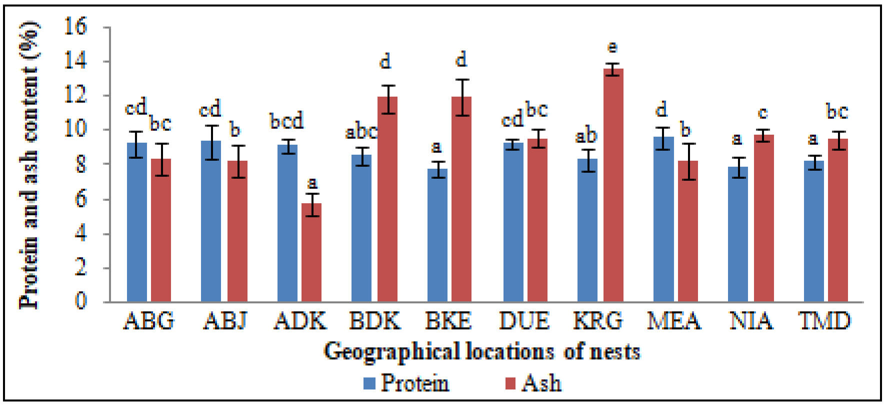

Figure 4: Influence of the geographical location of the nest on the protein and ash levels of fungus combs of the *M. bellicosus* termite harvested in Côte d'Ivoire. Abengourou (ABG), Abidjan (ABJ), Adiaké (ADK), Bondoukou (BDK),

Bouaké (BKE), Duékoué (DUE), Korogho (KRG), Méagui (MEA), Niakaramadougou (NIA) and Toumodi (TMD). The histograms of the same color and assigned the same letter have values that are not significantly different at $p < 0.05$.

4.1.4 Fat, total phenolic compounds and pH

The rate of total phenolic compounds in the fungus combs from the nests of the *M. bellicosus* termite collected in Méagui is 1.25 ± 0.1%. It is at the threshold of 5% the highest among the levels of total phenolic compounds determined. On the other hand, the levels of total phenolic compounds of fungus combs of the nests of the same termite collected in Bondoukou, Bouaké, Korogho and Niakaramadougou are at the same threshold the lowest. They are respectively 1.01 ± 0.11%, 0.99 ± 0.05%, 1.02 ± 0.12% and 0.99 ± 0.04%. The levels of total phenolic compounds in fungus combs harvested at Adiaké and Toumodi are identical at the 5% threshold. They are respectively 1.03 ± 0.03% and 1.08 ± 0.09%. Similarly, the levels of total phenolic compounds in fungus combs harvested in Abengourou and Duékoué are statistically equal to the 5% threshold. They are respectively 1.18 ± 0.10% and 1.17 ± 0.19% (Figure 5).

The pH of fungus combs from the nests of the *M. bellicosus* termite collected in Abengourou, Abidjan, Adiaké, Duékoué, Korogho, Niakaramadougou and Toumodi are identical at the 5% threshold. They are respectively 4.15 ± 0.70%, 4.13 ± 0.70%, 4.16 ± 0.04%, 4.14 ± 0.04%, 4.19 ± 0.08%, 4.17 ± 0.05%, 4.16 ± 0.09%. The pH of fungus combs from the nests of the *M. bellicosus* termite collected in Méagui is 4.10 ± 0.10%. It is at the 5% threshold the lowest among the determined pHs, while those of fungus combs harvested in Bondoukou and Bouaké are the highest at the 5% threshold. They are respectively 4.21 ± 0.08% and 4.22 ± 0.06%. They are at the 5% threshold identical (Figure 5).

The fat content of fungus combs of the nests of the *M. bellicosus* termite harvested in Abidjan is the highest while that of fungus combs harvested in Niakaramadougou is the lowest. These fat levels are respectively 0.76 ± 0.09% and 0.51 ± 0.10%. Those of fungus combs harvested in Abengourou, Adiaké, Duékoué and Méagui are at the threshold of 5% identical. They are respectively 0.67 ± 0.10%, 0.68 ± 0.10%, 0.71 ± 0.09% and 0.72 ± 0.08%. Similarly, the fat content of fungus combs harvested in Bondoukou, Bouaké, Korogho and Toumodi are identical at the 5% threshold. They are 0.62 ± 0.04%, 0.60 ± 0.09% 0.61 ± 0.11% and 0.64 ± 0.05% (Figure 5).

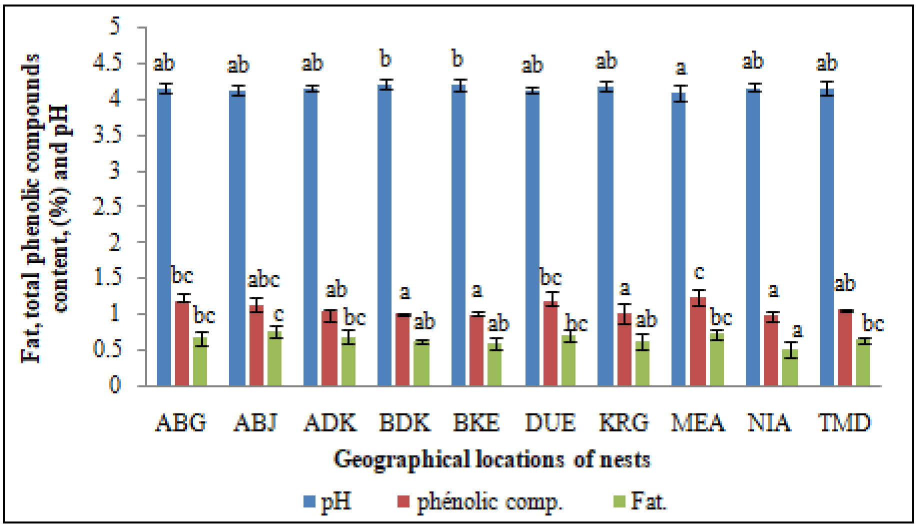

Figure 5: Influence of the geographical location of the nest on the pH, the levels of total phenolic compounds and the fat content of fungus combs of the *M. bellicosus* termite harvested in Côte d'Ivoire. Abengourou (ABG), Abidjan (ABJ), Adiaké (ADK), Bondoukou (BDK), Bouaké (BKE), Duékoué (DUE), Korogho (KRG), Méagui (MEA), Niakaramadougou (NIA) and Toumodi (TMD). The histograms of the same color and assigned the same letter have values that are not significantly different at $p < 0.05$.

4.1.5 Total sugars and reducing sugars

The total sugar levels of fungus combs from the nests of the *M. bellicosus* termite collected in Abengourou, Abidjan and Duékoué are at the threshold of 5% the highest. They are respectively 0.85 ± 0.08%, 0.87 ± 0.09% and 0.86 ± 0.06%. On the other hand, the total sugar content of fungus combs harvested in Bondoukou is at the lowest level of 5%. It is 0.71 ± 0.05%. The total sugar levels of fungus combs harvested in Adiaké, Korogho, Niakaramadougou and Toumodi are respectively 0.79 ± 0.05%, 0.77 ± 0.04%, 0.77 ± 0.06% and 0.77 ± 0.07%. They are at the 5% threshold identical (Figure 6).

The reducing sugar content of fungus combs harvested in Bondoukou is statistically at the 5% threshold the lowest among the reducing sugar content determined, whereas that of Duékoué is the highest. They are respectively 0.38 ± 0.06% and 0.49 ± 0.03%. The reducing sugar levels of fungus combs from the nests of *M. bellicosus* collected in Abidjan and Méagui are at the threshold of 5% equal. They are respectively 0.46 ± 0.06 and 0.47 ± 0.05%. The same is true for the reducing sugar levels of fungus combs harvested in Bouaké, Korogho and Niakaramadougou. They are respectively 0.41 ± 0.05%, 0.41 ± 0.07% and 0.42 ± 0.03% (Figure 6).

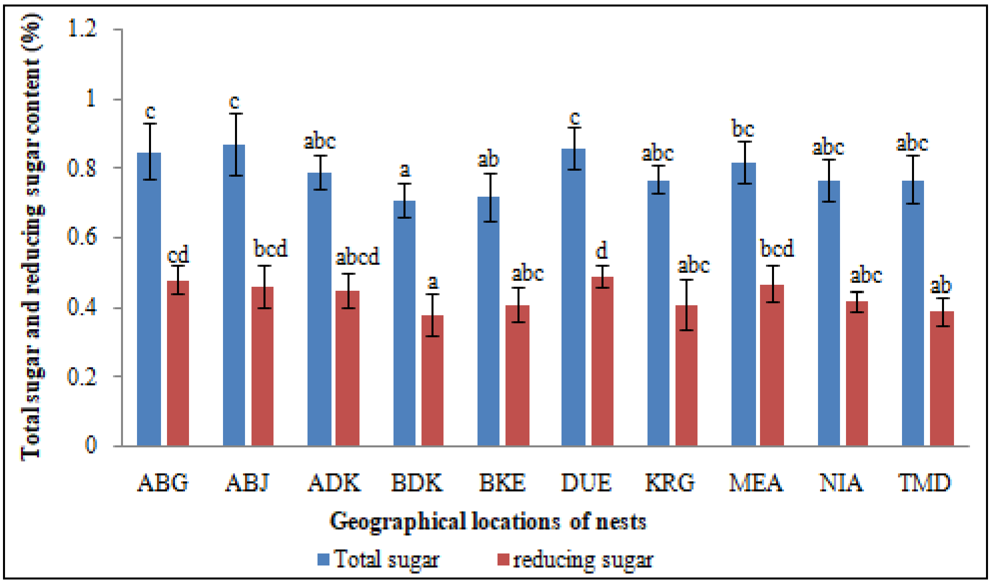

Figure 6: Influence of the geographic location of the nest on the levels of total and reducing sugars in fungus combs of the *M. bellicosus* termite harvested in Côte d'Ivoire. Abengourou (ABG), Abidjan (ABJ), Adiaké (ADK), Bondoukou (BDK), Bouaké (BKE), Duékoué (DUE), Korogho (KRG), Méagui (MEA), Niakaramadougou (NIA) and Toumodi (TMD). The histograms of the same color and assigned the same letter have values that are not significantly different at $p < 0.05$.

4.2 Principal component analysis (PCA)

The Principal component analysis (Figure 7) was used to visualise the projection *of Macrotermes bellicosus* fungus combs (A) and chemical components (B) in a factorial plane (F1 - F2). The PCA shows that the F1 - F2 factorial design accounts for 82.29 % of the total variance, which is sufficient for an unbiased interpretation of the results. The F1 axis accounts for 75.99 % of the dispersion of the variables while the F2 axis explains 6.30 %. In addition, the structuring highlighted in this factorial design shows three groups of *Macrotermes bellicosus* fungus combs from the ten defined geographical areas. The first group consists of ADK, ABG, ABJ, DUE, MEA. The second group of TMD, BDK, KRG and NIA and the third group of BKE. This discrimination reveals that individuals belonging to the same group have similar characteristics that differ from those of other groups.

In table I, the correlation coefficients are shown between the different pairs of variables. The results of the analysis showed the existence of positive or negative relationships between the variables. These linear relationships vary from good to strong correlation. However, positive linear relationships were observed, with positive Pearson correlation coefficients, between the variables protein - fat, cellulose - fat, cellulose - protein, lignin - ash, reducing sugar - fat, reducing sugar - protein, reducing sugar - cellulose, total sugar - fat, total sugar - protein, total sugar - cellulose, total sugar - reducing sugar, phenolic compound - fat, phenolic compound - protein, phenolic compound - cellulose, phenolic compound - reducing sugar, phenolic compound - total sugar, moisture - ash, moisture - lignin, pH - ash, pH - lignin and pH - moisture. Also, negative linear relationships were observed between the variables in the design shows three groups of *M. bellicosus* fungus combs from the ten defined geographical areas. These results show three groups of *M. bellicosus* fungus combs from the ten defined geographical areas.

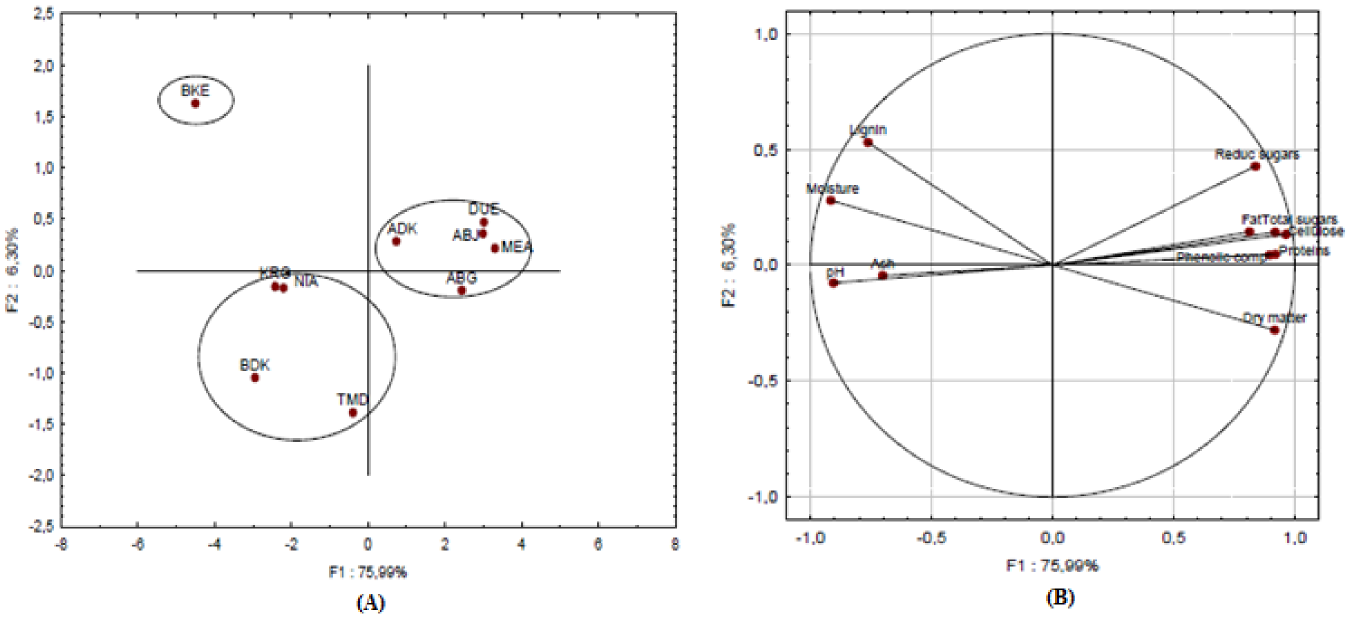

Figure 7: Projection of fungus combs from different localities (A) and chemical components (B) in the plane formed by the factorial axes F1 and F2

Table I: Matrix of correlation coefficients for chemical components of termite fungus combs of *M. bellicosus*

	Ashes	Fatty mat	Protein	Cellulose	Lignin	reduc Sugars	Total sugars	Phenol comp	Dry matter	Humidity	pH
Ashes	1.00										
Fatty mat	- 0.502	1.000									
Protein	- 0.637	0.873	1.000								
Cellulose	- 0.662	0.799	0.839	1.000							
Lignin	0.443	- 0.523	- 0.713	- 0.611	1.000						
reduc Sugars	- 0.590	0.600	0.779	0.839	- 0.473	1.000					
Total sugars	- 0.537	0.712	0.783	0.907	- 0.630	0.878	1.000				
Phenol comp	- 0.464	0.735	0.837	0.861	- 0.758	0.770	0.792	1.000			
Dry matter	- 0.657	0.676	0.791	0.884	- 0.759	0.643	0.837	0.719	1.000		
Humidity	0.659	- 0.676	- 0.790	- 0.8845	0.757	- 0.642	- 0.835	- 0.719	- 0.999	1.000	
pH	0.648	- 0.657	- 0.758	- 0.882	0.655	- 0.764	- 0.826	- 0.887	- 0.781	0.781	1.000

5. Discussion

M bellicosus fungus combs collected in ten localities in Côte d'Ivoire were analysed. The determination of the chemical parameters of these samples allowed us to observe small variations in humidity levels, ranging from 46, 73 ± 0, 7 % to 48, 73 ± 1, 59 %. These values are very different from those of *Macrotermes gilvus* fungus combs. Indeed, for the latter, the resulting humidity content ranges from 19.08% to 19.94% [17]. In contrast, the moisture content of *M bellicosus* fungus combs are closer to that of the Macrotermitinae (46.92% to 52.80%) determined by [32] and also by [33] (48.5% to 54.6 %). To maintain sufficient humidity to ensure the growth of mushrooms, termites water the fungus gardens with their excretions [32]. Other factors can also contribute to keeping the water content of fungus combs relatively stable and sufficient for the growth of Termitomyces. This is the humid atmosphere of the termite mound. This one keeps the relative humidity above 98% [34]. There is also the high water - holding capacity of the fungus comb - chambers walls, designed with fine clay particles, carefully selected by termite workers. This material acts as a buffer in regulating the humidity of the termite mound including that of fungus garden [35] [9]. Cellulose levels in fungus combs of the termite *M. bellicosus* range from 26.04 ± 1.46% to 28.33 ±1.34%. This difference in cellulose content would first be due to the nature of the plant debris harvested by the termite, then to the action of the symbiotic fungus Termitomyces on the fungus combs. These values are higher than cellulose amounts in fungus combs analysed by Kasseney [36]. The fungus combs as a food resource, provides the termite with the nutrients it needs to survive. The crude protein content of *M. bellicosus* fungus combs from the Bouaké locality (7.74 ± 0.46 %) is the lowest. While the protein content of Meagui fungus combs is the highest (9.53 ± 0.66 %). The differences in the protein levels of the fungus combs would be due to several factors including the plant material used to make the fungus combs, the growth of termitomyces and the termite that ingests growing termitomyces. In addition, a much lower protein content of 7% was determined in fungus combs of *M bellicosus* collected in Nigeria [37]. The proteins in the fungal gardens are thought to come mainly from *Termitomyces* fungi. According to [38], the latter play an important role in termite nutrition as they are a source of nitrogen. Also, [39] and [40] suggest that, these fungi mobilise enzymes that facilitate the exploitation of plant cell wall constituents. Indeed, proteins are essential macronutrients for any living organism, as they are the major constituents of biologically active compounds. They also participate in tissue synthesis, used for repair and growth of the organism [41].

As far as fat is concerned, termite fungus combs are practically fat - free. The proportions of 0.51± 0.10% to 0.76± 0.09% quantified are not significant. These low values suggest that plant debris collected in natural environments and used to make fungus combs are also very low in fat.

Paradoxically, termites are edible insects very rich in lipids [42], [43]**.** These insects would probably developed strategies to convert sugar from the degradation of cellulose and lignin into lipids.

M bellicosus fungus combs are acidic and pH values tend to be around 4.1 regardless of sample origin. An acidic pH has also been observed in fungus comb, but to a lesser degree of 4.5 [44]. Low pH levels are thought to be one of the strategies used by termites to limit the growth of bacteria while promoting the development of fungi. Indeed, the latter are acid - tolerant so they would be able to multiply under such conditions [45]. On the contrary, acidic pHs inhibit the growth of bacteria, most of which thrive at pHs near neutrality [46].

The levels of total phenolic compounds were recorded in a range of values from 0.99 ± 0.05% to 1.25 ± 0.10%. The presence of these compounds in fungus combs could be justified by the fact that they are naturally present in plants [47]. These compounds could also come to some extent from the saliva of termite workers. Indeed, [48] noted the presence of phenolic compounds in the saliva of termite workers and according to [49], salivary gland secretions are incorporated into the fungus combs to preserve them from invasion by fungi antagonistic to Termitomyces. Finally, the phenolic compounds could be derived from the degradation of lignin, because Termitomyces fungi mobilise enzymes for this purpose to make the fungus combs more digestible, during its maturation [50], [51]. But by the way, several studies indicate that aromatic compounds have an inhibitory effect on xylanase enzymes [52], cellulase [53] and many other digestive enzymes [54]. Consequently, there would probably be a mechanism within the fungus combs to regulate the degradation of lignin so as not to hinder the action of the digestive enzymes of the termite and Termitomyces. The inorganic fraction represented by the ash is relatively high and shows a great disparity. The average ash values range from 5.67 ± 0.65 % to 13.55 ± 0.35%. These two extreme values were recorded at the Adiaké and Korogho sites respectively. These values are much higher than those determined in experimental fungus combs of the termite *Odontotermes formosanus*, (around 2.50% - 4.96%), made from stems of Quercus palustris [55]. On the other hand, they are close to the ash levels assessed in fungus comb of *Macrotermes gilvus* at different stages of maturity [56]. Given that the soil consists mostly of mineral particles and that termite mound soils, in particular, are relatively richer in mineral salts [57], [58]. The high ash content that characterises fungus combs would be due to their permanent contact with the ground. Indeed, plant debris used to make fungus combs is harvested from the ground [59]. Before use, this harvest is stored in the termite mound. Once elaborated, the fungus combs are stored in galleries in the termite mound before being consumed by termites [22]. With regard to total sugars and reducing sugars, the quantified contents are of the order of (0.71 ± 0.05% to 0.87 ± 0.09%) and (0.38 ± 0.06% to 0.49 ± 0.03%) respectively. This result is much higher than that of [44] who estimated at 404.8 µg/g or about 0.04% the quantity of reducing sugars contained in the termite *M bellicosus* fungus comb harvested in a locality of Ethiopia. The differences observed would be due to the nature of the plant debris collected, which is linked to the vegetation, to the symbiotic fungus (Termitomyces) and to the termite. Reducing sugars and total sugars are present in plant tissues, but in varying proportions depending on the plant organ considered [60]. In the case of termite fungus combs, these sugars could also be hydrolysates of parietal carbohydrates under the action of glycosidases from Termitomyces fungi. The latter have cellulases and hemicellulases allowing them to exploit the carbohydrate compounds in plant fibres [61], [50]. Reducing sugars and total sugars being more simplified forms of carbohydrates, their presence in the fungus combs would serve as nutrition for Termitomyces which cannot use lignin alone as a source of carbon for growth [56].

The principal component analysis of the fungus combs carried out by considering all the biochemical parameters made it possible to segregate these fungus combs collected in several localities. The principal component analysis (PCA) of the compounds made it possible to distinguish three groups of fungus comb. The first group is made up of fungus comb of the localities of Abidjan (ABJ), Abengourou (ABG), Adiaké (ADK), Duékoué (DUE) and Méagui (MEA). The second group comes from Korogho (KRG), Niakaramadougou (NIA) Toumodi (TMD) and Bondoukou (BDK). The last group comes from the locality of Bouaké (BKE). The first group is distinguished by its relative richness in protein and cellulose from the group formed by KRG, TMD, BDK, and NIA which is relatively richer in ash. The positive correlation observed between cellulose, protein and fat, in contrast to that between them and lignin, suggests that these compounds are evolving in the opposite direction to lignin. The fungus comb is indeed a dynamic structure. This structural dynamism could impact the composition of the fungus comb.

6. Conclusion

The aim of this study was to determine the impact of the geographical situation on the chemical composition of fungus comb of the termite *M. bellicosus*. At the end of this work, we note that fungus combs are acidic foods with high amounts of ash, cellulose and protein but with no fat. There is quantitative variability in these compounds. The principal component analysis (PCA) of the compounds made it possible to distinguish three groups of fungus comb. The first group is made up of fungus comb of the localities of Abidjan (ABJ), Abengourou (ABG), Adiaké (ADK), Duékoué (DUE) and Méagui (MEA). The second group comes from Korogho (KRG), Niakaramadougou (NIA) and Toumodi (TMD). Bondoukou (BDK). The last group comes from the locality of Bouaké (BKE). The geographical location of the termite mound therefore has an impact on the chemical composition of the fungus comb in Côte d'Ivoire.

As the fungus comb is a wet substrate containing nutrients, a survey of its microflora will be interesting for the detection of germs with biotechnological potential.

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