Morphometrics of *Macrotermes bellicosus* (African mound termite) (Blattodea:Termitidae) and the Impact of its Saliva Amylase on the Strength of Termitarium Soil

Popoola K.O.K and Opayele A.V

Entomology unit, Department of Zoology, University of Ibadan, Oyo State.
taiwo_kok@yahoo.com

Abstract: The aim of this study was to investigate the morphology of *Macrotermes bellicosus* present on some parts of the University of Ibadan and to determine the effect of the saliva of termites on the strength of termitarium. Termites were collected from 8 termitaria on Parry Road, University of Ibadan and characteristics morphometrics were measured using stage graticules (10mm) on Microscope. Amylase analysis was conducted to determine the activity of the saliva content in the termitarium soil using Phadebas® α-amylase test method. Bricks were molded from the termitarium soil while pressure gauge was used to measure the strength via cracking of molded brick from the soil. The studies revealed that the length of head capsule for workers ranged from 0.16mm to 0.24mm, while those of soldiers ranged from 0.42mm to 0.68mm. The body length of workers ranges from 0.5mm to 0.7mm and that of soldiers ranged from 1.1mm to 1.4mm this confirmed that the soldiers were bigger. The amylase analysis showed that termitarium soil contained α-amylase while it was undetected in the control soil. The α-amylase activities for the termitarium soil was 41 unit per liter, 47 unit per liter and 56 unit per liter at dilutions 10^{-1}M, 10^{-2}M and 10^{-3}M respectively. The bricks molded from the termitarium soil and the control soil all cracked at a pressure less than 1MPa; however bricks molded from clayey and mature termitarium showed higher strength of materials. It may be concluded that the modification of the termitarium soil's physical properties and the selection of clay particles during construction activities by termites had more contribution to strength than the presence of α-amylase in the termitarium soil.

[Popoola K.O.K and Opayele A.V. **Morphometrics of *Macrotermes bellicosus* (African mound termite) (Blattodea:Termitidae) and the Impact of its Saliva Amylase on the Strength of Termitarium Soil.** *N Y Sci J* 2012;5(12):207-216]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 34

Keywords: *Macrotermes bellicosus,* University of Ibadan, morphometrics, amylase, termitarium.

1. Introduction

Termites are a group of eusocial insects that, until recently, were classified at the tasonomic rank of order Isoptera, but are now accepted as the epifamily Termitoidae, of the cockroach order Blattodea (Wikipedia, 2012). Termites comprises over 2,700 species and are of global importance as decomposers of lignocellulose material (Kambhampati and Eggleton, 2000; Konig *et. al*., 2006**).** Known species are mainly from tropical to warm temperate areas, though a few species are found in cool temperate climates such as those of southern Europe, southern and western North America. The greatest continental termite diversity is in Africa, where there are over 1000 species. Polar continents have none, and North America with 50 species and Europe with 10 species are intermediate in termite diversity (Resh and Carde, 2003). Termite diversity, abundance and biomass have been found to decrease with increasing latitude (Eggleton and Bignell 1995, Eggleton 2000). Termites are small to medium-sized insects that are cryptic in habit. All species live in eusocial colonies. Mature termite colonies contains individuals of remarkably different form and function (Kambhampati and Eggleton, 2000). Each group of individuals that perform the same function is known as a caste. In most species three castes occur: reproductive, soldier, and worker. Immature stages of all castes may also be present in the colony along with (occasionally) intercastes (Gillott, 2005).

Termite families traditionally were categorized as lower or higher. However, this categorization may change soon as newer classification systems are adopted. Lower termites (families Mastotermitidae, Kalotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae, and Serritermitidae) have symbiotic intestinal protozoa and bacteria. Higher termites (Termitidae) have intestinal bacteria. Recently, most researchers advocate retaining the termites as Termitoidae, an epifamily of the cockroach order, which preserves the classification of termites at family level and below.

Termites are herbivores, fungivores (i.e., plant or fungus feeders), and humivores. They feed on cellulose, directly from plants, dead or alive, or indirectly from fungus arising from decaying plant material within mounds. (Resh and Carde, 2003). Dead wood and withered leaves and grass are mostly composed of plant cell-wall material (lignocellulosic matter), which is primarily made up of two types of plant carbohydrate polymers, cellulose and lignin.

Vertebrate animals in general cannot derive sufficient nutrient from lignocellulosic matter, and hence almost none, except ruminant ungulates, utilize this abundant matter as food. However, a few groups of insects have evolved as successful detritivores (Kambhampati and Eggleton, 2000; Konig *et. al.,* 2006), in all cases by coevolving symbiotic relationships with microbial organisms such as bacteria, protozoa, or fungi (Breznak, 2000; Brune, 2006). The main groups of insect detritivores are cockroaches, termites, crickets, flies, and beetles. Termites are the only insect detritivores that are social, leading to a higher level of coordinated foraging and increased foraging reach, and therefore to an extraordinary level of lignocellulosic processing power (Grzimek, 2003).

Saliva is a watery, enzyme-containing fluid that serves to lubricate the food and initiate its digestion. The traditional view is that termites rely on intestinal gut microorganisms for cellulose digestion. However, there is evidence that termites also use their own enzymes for cellulose digestion (Resh and Carde, 2009). In termite saliva there are cellulose-digesting enzymes: a β-1-4-glucanase that brings about the initial splitting of the polymer, and β-glucosidase that degrades the resulting cellobiose to glucose (Nakashima *et al.*, 2002; Tokuda *et al*, 2002). Perhaps, the most fundamental and ubiquitous function of saliva in insects is lubrication of the mouthparts and lubrication of the food bolus to assist its transport through the foregut (Klowden, 2007). Lubrication can be achieved primarily by water, the most abundant constituent in saliva. The most common class of organic constituents of saliva consists of digestive enzymes, such as amylase, invertase, various proteases, and lipases (Resh and Carde, 2009).

Carbohydrates found in nature occur as polysaccharides, Homopolysaccharides contain only a single type of monomer; heteropolysaccharides contain two or more different kinds. Some homopolysaccharides serve as storage forms of monosaccharides that are used as fuels; starch and glycogen are homopolysaccharides of this type. Other homopolysaccharides (cellulose and chitin, for example) serve as structural elements in plant cell walls and animal exoskeletons (Lehmann, 1998). Amylase are carbohydrate digesting enzymes present in the saliva of some insects (Gillot, 2005). There are two categories of amylases, denoted alpha and beta, they differ in the way they attack the bonds of the starch molecules. Alpha - amylase is widespread among microbial, plant and animal kingdoms. Beta - Amylase are present in yeasts, molds, bacteria, and plants, particularly in the seeds (Encyclopedia Britanica, 2009).

Termites have a soft cuticle and are easily desiccated; they live in nests that are warm, damp, dark, and sealed from the outside environment. During the course of the mound construction, the termites transport, repack, and cement the soil particles together with their saliva and/or excreta. Hence, the physical and chemical properties of these biologically reworked soils are different from their surrounding areas from where the materials are derived for mound construction (Reddy and Raju, 2003). Earlier studies have shown that the mound-building termites have a considerable influence on many soil properties (Lee and Wood, 1971). Chemical changes are brought about by the incorporation of organic matter while physical changes appear to be due to selection and sorting of certain particles resulting in a change of structure and particle size distribution (Malaka, 1977a, b) The changes in texture brought about by redistribution of mounds and other structures in the surface is likely to be accompanied by chances in physical properties such as structural stability, bulk density, infiltration rate, permeability and water holding capacity (Wood and Sands, 1978). Termitarium soil has been utilized in various constructions; it has been used to build rammed antbed tennis courts, footpaths, bricks, drive ways, rammed antbed floors e.t.c. (Morrow, 2002)
This research work was aimed at determining the morphology of *Macrotermes bellicosus* present on some parts of the University of Ibadan and to know the impact of *Macrotermes bellicosus* saliva amylase on the strength of termitarium soil which will give an insight into its suitability as a good candidate for construction of facilities like rammed antbed court, antbed bricks, driveways, footpaths e.t.c. These aims were achieved through the following objectives:

- Survey and sampling of termitaria on Parry Road, University of Ibadan, Oyo state Nigeria.
- Morphometric measurement of the different caste members collected from the survey termitarium Amylase analysis of the termitarium soil, termite saliva and control soil.
- Cracking of bricks molded from termitarium and control soil under pressure gauge to determine their strength.

2. Materials and Methods

The study was carried out within the campus of the University of Ibadan located in Ibadan Oyo state on Latitude $7^{o}26^{1}$ to $37^{o}08^{1}$North and Longitude $3^{o}53^{1}$ to $36^{o}89^{1}$East with an altitude of 229m. Termites and termitarium soil were collected at the end of the dry season in the month of February 2012 from Parry road, an undisturbed area in the campus.

The area falls in equatorial rainforest or tropical rain forest.

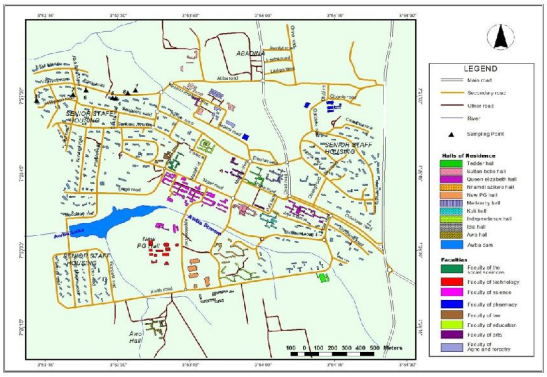

Figure 3: Map of University of Ibadan, Ibadan Nigeria showing the study site
Courtesy: Department of Geography, University of Ibadan.

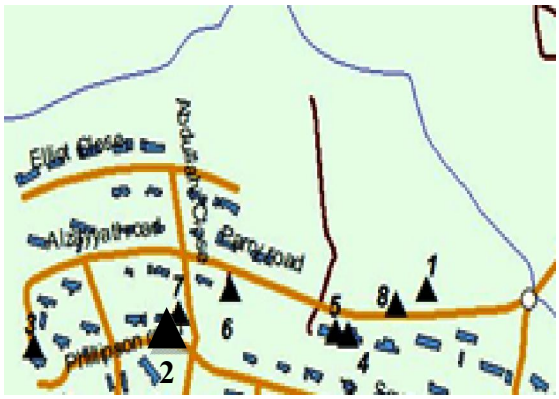

Figure 4: An Enlarged Map of Parry Road University of Ibadan, Ibadan Nigeria showing the sampling site

The materials used include termitarium soil and control soil without termites for block molding and strength test. Termite saliva which was obtained from the salivary gland region of the foregut by sectioning of the prothoraxic region was used in amylase analysis.

Hulger was used to break the termitarium, while hand trowel was used to pack the soil into polythene bags labeled, and transported to the Laboratory. Measurement of the termitarium diameter and height were done using meter rule.

. Stage graticles (10mm) was used to take measurement on the termite during morphometrics. Other equipment used was weighing balance, which was used to weigh the salts during amylase analysis. Gallenkemp oven was used for heating of the bricks made from the termitarium and control soils. The enzyme action was facilitated during analysis in incubator at 37^{o}c. Spectrophotometer which used to determine absorbance, concentration and % transmittance values of the amylase test solution.

For the amylase analysis, 50 Phadebas tablets, 210g NaCl, 9g $CaCl_2$ and 6g NaOH were the reagents used.

Furthermore, 1500ml of distilled water was employed, 100ml and 250ml beaker, 10cm^3 pipette and 1L volumetric flask were used for measurement, filter paper was used during analysis to filter the Phadebas residue from the amylase test solution, the solution was then kept in cuvette of Spectrophotometer , mortar and pestle was used to grind termitarium soil, Laboratory test sieve BS410 of 31.8 mm aperture was used to sieve soil particles during brick moldings, plastic mould of 13 x 9 x 4cm and 12 x 12 x 6cm, used to mould bricks.

Soil samples were collected from eight termitarium, the termitarium were of different sizes ranging from small to medium to large, some were newly being formed as active workers were found in large numbers during soil collection while some were already stable and old as only soldiers were seen in large numbers responding to the termitarium disturbance, in two of the medium sized termitarium, winged reproductive were observed to be flying off as the termitarium soil was being collected. The dimensions of the different termitarium surveyed are shown below.

Table 1: Sizes of Surveyed Termitarium at the study site

S/No of Termitaria	Termitaria measurement	
	Diameter	Height
1	62	74
2	135	93
3	76	52
4	183	114
5	104	72
6	64	85
7	247	118
8	127	62

Soil collection: The soil was collected after an hulger was used to break the termitarium, only the soils from the depth of 5cm and more were collected as the effect of erosion may have affected the original quantity of amylase in the surface soil samples, Hand trowel was used to pack the soil samples into labeled polythene bags, about 20kg of soil samples were taken at each termitarium. Soil sample was also collected from control soil with no termitarium and transported to the laboratory for analytical work.

Caste collection: Various caste members of termite species, *Macrotermes bellicosus* were collected from each termitarium into specimen bottles. Caste collections included workers, and soldiers with the exception of the king and queen which are not easy to

come by unless termitarium is destroyed completely. The specimen bottles were sealed and labeled according to sites of collection and transported to the laboratory where their saliva was collected for amylase analysis and morphometrics was carried out.

Eight morphological characteristics namely: total body length, length of antenna, length and width of head capsule, length and width of thorax and length and width of abdomen were considered (Mayr, 1969b). Soldiers and workers of *Macrotermes bellicosus* was used in this analysis. The length and width readings taken on the termites were measured through a stage graticule (10mm) mounted on the microscope taking into consideration all the morphometric characters mentioned above.

Amylase analysis: This was done according Meikle, (2007). However, the method was modified by reduction of the Phadebas to half.

Block molding: The termitarium soil was pounded into bits and sieved with a laboratory test sieve with 31.8mm aperture, the fine soil particles were soaked with water and no other additives was added, it was then poured in Plastic moulds of (13 x 9 x 4) cm^3, the plastic moulds were shaken and the surface were compressed to ensure even distribution and spread out of the soaked soil particles in the plastic moulds, the soil was left to dry in the open for 5 days under sun after which it was removed, turned over, and left to dry in the open for 3 days more. The dry bricks were then heated in the oven at 120°C for two hours to improve its strength. Another set of larger bricks were remolded again from four clayey termitarium with a plastic mould of larger length, breadth and height of dimension (12 x 12 x 6) cm^3 and allowed to dry for the purpose of strength comparism. These dry bricks were also heated in the oven at 120°C for two hours to improve its strength as already done to the smaller bricks

The dry bricks were subjected to pressure using a pressure gauge (TP FW-4A) to test their strength in mega Pascal (MPa), 1MPa=1,000,000 pa; the pressure gauge had a circular contact area of diameter 4cm above and 7cm below. The pressure was increased carefully and slowly until the bricks cracked and the pressures at the point when crack occurred were recorded.

3. Results

The mean, Standard deviation (SD) and range values of morphometric measurements for worker and soldier caste of termites from the study site (Parry road, University of Ibadan) is shown in the tables 2 below.

The α-amylase activities on termitarium soil are shown on table 3. The serial dilutions at three different concentrations revealed values on absorbance, concentration and % transmittance (Vide table 3). The effect of absence of α-amylase revealed different results on parameter as compared to the soil with termites. (Vide table 4)

Table 2: Mean ± SD and range values for morphometric analysis of worker and Soldier termites in parry road, university of Ibadan.

	Caste	Length of antenna (mm)	Length of head capsule (mm)	Width of head capsule (mm)	Total body length (mm)	Width of abdomen (mm)	Length of abdomen (mm)	Length of thorax (mm)	Width of thorax (mm)
Mean±SD	**Workers**	O.14±0.03	0.20±0.02	0.13±0.02	0.60±0.08	0.18±0.02	0.25±0.03	0.16±0.03	0.15±0.14
Range		0.09-0.19	0.16-0.24	0.09-0.15	0.50-0.70	0.13-0.21	0.21-0.30	0.11-0.21	0.10-0.90
Mean±SD	**Soldiers**	0.30±0.06	0.53±0.07	0.36±0.04	1.27±0.09	0.25±0.04	0.44±0.06	0.27±0.04	0.22±0.03
Range		0.19-0.43	0.42-0.68	0.29-0.47	1.10-1.40	0.20-0.32	0.33-0.58	0.18-0.35	0.15-0.28

*Each mean values are replicated 30 times

Table 3: Mean ± SD values of Absorbance, Concentration and % Transmittance values in termitarium soil from parry road, university of Ibadan.

Dilutions (M) of Termitarium soil	Absorbance (nm) (Mean±SD)	Concentration(mg/l) (Mean±SD)	% Transmittance (Mean±SD)
10^{-1}	0.036±0.018	0.285±0.273	92.510±3.776
10^{-2}	0.041±0.028	0.241±0.321	91.430±5.893
10^{-3}	0.049±0.018	0.336±0.337	89.760±3.981

*Each dilution has 3 replicates for each termitarium sampled

Table 4: Mean ± SD values of Absorbance, Concentration and % Transmittance in control soil (Zoology department, University of Ibadan).

Dilutions (M) of Control soil	Absorbance (nm) (Mean±SD)	Concentration(mg/l) (Mean±SD)	% Transmittance (Mean±SD)
10^{-1}	0.009±0.008	0.533±0.503	97.700±1.778
10^{-2}	0.016±0.003	0.667±0.577	96.367±0.551
10^{-3}	0.015±0.010	0.667±0.577	96.533±2.312

*Each serial dilution of soil treated with Phadebas had 3 replicates
α-amylase activities was also revealed to be very little in termite saliva. (Vide Table 5)

Table 5: Mean ± SD values of Absorbance, Concentration and % Transmittance in termite saliva from parry road, university of Ibadan.

Dilutions (M) of Termite Saliva	Absorbance (nm) (Mean±SD)	Concentration(mg/l) (Mean±SD)	% Transmittance (Mean±SD)
10^{-1}	0.013±0.003	0.233±0.208	97.200±0.700
10^{-2}	0.015±0.004	0.333±0.306	96.633±0.929
10^{-3}	0.019±0.006	0.300±0.265	95.767±1.286

*Each serial dilution of saliva treated with Phadebas had 3 replicates

Table 6: Absorbance values and the corresponding α-amylase activity (u/l) in Termitarium soil, Control soil and Termite saliva from Phadebas® Standard curve.

Treatment	Dilutions (M)	Absorbence (nm)	α-amylase activity(u/l)
Termitarium Soil	10^{-1}	0.036	41
	10^{-2}	0.041	47
	10^{-3}	0.049	56
Control Soil	10^{-1}	0.009	Undetected*
	10^{-2}	0.016	Undetected*
	10^{-3}	0.015	Undetected*
Termite Saliva	10^{-1}	0.013	Undetected*
	10^{-2}	0.015	Undetected*
	10^{-3}	0.019	Undetected*

*Each soil dilution has 3 replicates
*Undetected due to very low values obtained which cannot be interpreted on the graph

Bricks were molded from the termitarium soils with mould size of 13 x 9 x 4cm^3, the bricks were heated at 120°C for two hours; some bricks were noticed to be broken after heating. Others were subjected to pressure from a pressure gauge. The readings are shown in table 7.

Table 7: Table showing pressure readings in megapascal (MPa) at which cracking occurred on bricks with dimension 13 x 9 x 4cm^3

S/N of surveyed termiteria	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
1	<1	-	<1	<1	-	-
2	<1	<1	<1	<1	<1	<1
3	-	-	<1	<1	-	<1
4	<1	<1	<1	<1	<1	<1
5	<1	<1	<1	<1	<1	<1
6	<1	<1	<1	<1	<1	<1
7	<1	<1	<1	<1	<1	<1
8	<1	<1	<1	<1	<1	<1
Control soil	<1	<1	<1	<1	<1	<1

<1: Indicates that the bricks cracked before reading 1MPa
- : The bricks broke after being heated at 120°C in the oven

Bricks were again moulded with mould size 12 x 12 x 6cm^3 from the clayey termitaria where none of the bricks broke after the first heating process; the readings are shown in table 8.

Table 8: Pressure readings (MPa) at which cracking occurred on bricks with dimension (13 x 9 x 4) cm^3

S/N of surveyed termiteria	R1	R2	R3	R 4	R5	R 6
2	<1	<1	<1	<1	<1	<1
4	<1	<1	<1	<1	<1	<1
7	<1	<1	<1	<1	<1	<1
8	<1	<1	<1	<1	<1	<1
Control soil	<1	<1	<1	<1	<1	<1

R= REPLICATE

<1: Indicates that the bricks cracked before reading 1MPa

4. Discussions

The result on morphometric characteristics showed that soldier termites of *M. bellicosus* were larger than workers. The length and width values for the head capsule of the soldier termites were particularly noted to be much larger than those of workers.

Termites are ideal food source for other animals; they are relatively slow moving, soft-bodied and rich in fat and protein (Capinera, 2008). The larger values obtained from soldier termites compared to the worker termites was not surprising as the soldiers have the role of protecting the termitarium from their enemies, this large head capsule are the structural adaptations of the soldier termites to carry out their defensive role, the big head is necessary to house the needed muscles to operate the powerful biting mandibles during colony defense. The antennae length was also noted to be longer than those of the workers, the antennae helps in performing sensory roles as Soldier termites are usually blind (Wikipedia, 2012). The function of the antenna is of particular importance to the blind soldiers to sense their environment and respond to enemies appropriately. The larger measurement taken on both the thorax and abdomen is also expected. The thorax and abdomen houses the muscles and other visceral organs that are necessary to produce energy, coordination and general physiological functions for the good operation of the legs and other body organs all to make the soldiers live up to their responsibility of being the colony defenders. A study on morphometrics which involves the quantitative analysis of form of living organism is useful in detecting any changes on the morphology of organisms. The information generated on the present body form of the termites present on Parry road, University of Ibadan, can be used as a basis of comparison in future when morphometrics work are done on these termites.

Table 3, shows the values obtained from the spectrophotometer for Absorbance, Concentration and % Transmittance of the eight termitarium soils analyzed for α-amylase activities. The experiment was conducted with three dilutions of 10^{-1} M, 10^{-2} M and 10^{-3} M to monitor the trend of α-amylase activities among the various dilution gradients.

Spectrophotometric techniques are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer. The absorbance is the fraction of the light absorbed by the sample. The transmittance is the fraction of light in the original beam that passes through the sample and reaches the detector while concentration is the number of molecules of a solute in a given solution. There is an inverse relationship between Absorbance and % Transmittance and there is a direct relationship between the light absorbed (absorbance) and the concentration of the solute in the solution. The stronger the concentration of a solution, the stronger the ability of the solution to absorb light particles and the lesser its ability to transmit light particles becomes.

Phadebas® α-amylase test is a water insoluble, cross-linked starch polymer carrying a blue dye. The tablet was hydrolyzed by α-amylase to form water-soluble blue fragments. The absorbance of the blue solution is a function of the α-amylase activity in the sample thus α-amylase activities of the solution can be measured from the transmittance or absorbance of a solution. However the standard curve that gives the value of α-amylase activities in this experiment was constructed from absorbance readings. The various absorbance readings from the spectrophotometer were interpreted on the Phadebas® α-amylase standard curve provided by the manufacturer (Table 6)

From the summary done on table 6, it was obvious that the termitarium soil at dilution of 10^{-1} M, had an α-amylase activities of 41 unit per liter, at dilution of 10^{-2} M, it had an α-amylase activities of 47 unit per liter and at dilution of 10^{-3} M had an α-amylase activities of 56 unit per liter. From these results, it can be concluded that at all dilution gradients, α-amylase activities were detected and it was highest at the 10^{-3} M dilution. The α-amylase activities recorded is suspected to be a contribution of the rich micro biota present in the termitarium soil.

Paul and Verma (1992) reported that a rich collection of microbiota which include bacteria, actinomycetes, protist, fungi and other forms of microbes were prersent in termite nest as well as other environment associated with their living habitat than adjacent soils. α-amylases (1, 4-glucan–glucanohydrolase) are widely distributed in microorganisms (Shareghi and Arabi, 2008) where they assist to hydrolyze alpha bonds of large alpha-linked polysaccharides like starch to provide simple sugars that can be utilized by an organism's cell. The α-amylase activities noticed may have been contributed by these microbes. (Vivers *et.al.*, 2002) also reported the detection of α-amylase activities in the salivary glands of *Mastotermes darwiniensis* during the hydrolysis of amylase; α-amylase may have accumulated in the termitarium soil since the construction of termitarium involves the use of saliva to hold soil particles together by worker termites. Over time saliva and its α-amylase may be concentrated in the termitarium soil as a result of contribution from many workers.

Termitarium construction entails excavation in soil and mass shifting of the excavated soil to the surface and the soil is cemented together with chewed up partially digested wood, saliva and faeces (Longair, 2004), another microbial contribution to the level of α-amylase may result from the microbes that resides in the Gastrointestinal tract of the termites since one of the building materials includes partly regurgitated wood, intestinal microbes that have the ability to produce α-amylase may accompany the regurgitated material in the process of termitarium construction. Faeces of termites are also component of the termitarium soil. Faeces are composed of undigested food, microorganisms and other components; this too may be another contributor to the α-amylase presence in the analyzed termitarium soil.

Equally from the result shown in table 4 and the corresponding interpretation on table 6, the control soil as expected showed a very low or undetectable value of α-amylase activity after analysis. This is expected as there may not be enough microorganisms that produce α-amylase in the control soil when the soil sample was collected.

Amylase analysis was also conducted on the termite saliva, the values obtained from the spectrophotometer for Absorbance, Concentration and % Transmittance are recorded on table 4 and the corresponding match for amylase activities are recorded on table 6. Surprisingly α-amylase analysis conducted on the termite saliva had an absorbance value that was too low to be detected when matched with values from the Phadebas® α-amylase standard graphical curve that was sold with the Phadebas® tablet.

Termite saliva has been discovered to have cellulose-digesting enzymes: a β-1-4-glucanase that brings about the initial splitting of the polymer, and β-glucosidase that degrades the resulting cellobiose to glucose (Nakashima *et. al.*, 2002; Tokuda *et. al*, 2002). The low value of α-amylase present in termite saliva may be due to the fact that the substrate which its saliva acts on mainly was lignocellulolytic materials which include wood, dead foliage and detritus. Lignocellulolytic materials contains cellulose, hemicelluloses lignin and small amount of extracts (Baeza and Freer, 2001) and they represent over 90% of dry weight plant cell. Cellulose is a homopolysaccharide composed entirely of D-glucose linked together by β- 1, 4 -glycosidic bonds and with a degree of polymerization of up to 10,000 monomer molecules or higher. α-amylase can only break down alpha-1, 4-glycosidic bonds as present in starch but it cannot hydrolyse β-1, 4-glycosidic bonds as present in cellulose therefore there is a very little or undetectable presence of α-amylase in the analysed saliva. However termites have the ability to utilise starchy foods like tubers crops, since they are sometimes pests on farms, the contribution of the α-amylase in the breakdown of these starchy food materials may be made by the salivary gland, intestinal micro biota or may result from other secretions from the midgut and hindgut. Another reason for the low or undetectable value of α-amylase activity in the solution may be due to the fact that the Phadebas® test tablet is hydrolysed by only α-amylase to form water-soluble blue fragments. The test substrate (Phadebas® tablet) is absolutely resistant to hydrolysis by exo-enzymes such as β-amylase which may also be present in the solution.

The quantity of saliva obtained may be very small compared to that present in the termitarium soil where it may be concentrated over time by the large numbers of workers that have deposited their saliva in the process of moistening and assemblage of the reworked soil used in the process of termitarium construction.

Table 7 shows the result of the cracking done on the bricks molded from the termitarium soil and control soil. It was observed that the bricks of dimension 13 x 9 x 4cm^3 molded from the termitarium soil with the presence of α-amylase and those molded from the control soil without the presence of α-amylase cracked at a force less than 1MPa=1,000,000pa.

In the production of ant bed Bricks, heated bricks are more weather resistant (Morrow, 2002) and showed more strength. However, termitarium 1 and 3 were observed to have disintegrated on their own after it was heated in the oven. Termitarium 1 and 3 during collection were observed to be young

and was just being newly formed, and when the termitarium was being broken during collection, only workers were seen, there were no soldiers found responding to nest damage neither were there winged reproductive seen as obtained in other older and more stable termitarium, it can be deduced that the termitarium was still very young as there were no soldiers and secondary reproductive yet because of the newness of the termitarium. The termitarium wall was also observed to be composed of humus materials as the colour of the termitarium was dark, Leaf litters, plant stems and other materials that characterizes the top soil layer was also seen in the termitarium wall. The heating process was suspected to have burnt the organic components of the termitarium soil that held its parts together, this could be the reason for the observed disintegration in 3 of the 6 bricks molded from termitarium 1 and 3 respectively, the other bricks that did not disintegrate were observed to have become soft and could not withstand any force from the pressure gauge.

Bricks molded from termitarium 2, 4, 5, 6, 7, 8 and the control soils were all intact through the heating process. They were then subjected to pressure from the pressure gauge, they were all noted to break at a force less than 1MPa, though they provided more resistance to the force applied, all the termitarium in this category were observed to have clayey texture; they were obtained from more mature and stable termitarium. Soldiers were seen responding to nest damage and in some of these termitaria, winged reproductives were also seen flying off during termitarium soil collection suggesting the stability and maturity of the termitarium, there were no leaf particles, branches of trees and other organic matter that usually characterize the top soil in the termitarium wall. The major feature observed was the fine clayey nature of the termitarium soil which is excavated from the sub soil. Longair (2004) stated that Construction of termitarium entails excavation in soil and mass shifting of the excavated soil to the surface. The soil is cemented together with chewed up partially digested wood, saliva and faeces to produce a more durable building material these reworked soils are stable than surrounding ground mass. Jouquet *et al* (2004) noted that excavation activities by termites also modify the clay mineral composition of these soil materials. According to Kemp (1955), *Cubilermes* species mounds had 67.2% clay and 26.5% sand compared with 30.8% clay and 63.0% sand for the surrounding soils, Watson (1962) also reported that the termite mound had 94% fine material (clay + silt) compared to 52% clay and silt in adjacent soil in West Africa. Debruyn and Conacher (1987) also found that the mounds had significantly higher clay content than the surface surrounding soil, Ekundayo and Orhue (2002) observed that the various termite mound soil had higher values of silt and clay and lower values of sand in relation to bulk surrounding soil from which the mounds were constructed. Ghilarov (1962) describe the termite mound of *Anacanthotermes* species to be strongly cemented with clays. All these findings suggest that the strength seen in the termitarium soil was a result of the modification of the soil physical properties and selection for clay particles by the termites. Reddy and Rapu (2005) reported that the bulk density of surface mound were higher than that of the surrounding soils. Grasse (1984) noted that particle size of mounds of *Macrotermes* species in Africa showed that their composition is close to that of subsoil, but there is some selection in favour of fine size fraction in soils that are not rich in clay or against the finer fraction in soils rich in clay, these properties exhibited by the termitarium soil also contributed to their strength and the actual contribution of α-amylase in the soil may have very little contribution to the termitarium soil as both the termitarium with α-amylase and control soil without α-amylase all broke at a force less than 1MPa, the termitarium 1 and 3 soils without a balanced fraction of clay from the sub soil further supports this fact as they disintegrated without any force application despite the fact that they contained α-amylase"

.

Considering the fact that bricks with high levels of organic matter which are a substrate for α-amylase action broke much easily and α-amylase is an enzyme that contributes to the separation of bonds in starchy materials, its presence in large amount may not be favourable to holding materials in termitarium soils together, this may be a reason why as termitarium soils gets more mature, they have their walls built from clay particles which originates from the sub soil.

Bricks were again moulded with plastic moulds with a larger dimension of 12 x 12 x 6cm^3 from termitarium 2, 6, 7, 8, and control soil. These termitaria were chosen because they were all clayey and stable in appearance and the result is shown in table 8. It was again noticed that they all broke at a force less than 1 MPa despite their expected increase in strength due to their larger size. This shows that although termitarium soils are stronger than their neighboring soils, they can't still withstand a very large force as proved from this research work, however the exact force that cracking occur can be discovered if a pressure gauge with a lower force sensitivity like hectopascal (1HPa=100pa) and Kilopascal (1KPa=1000pa) were used for the cracking process.

This limitation restricts the use of termitarium soil to man-made structures that won't have to face high pressure and traffic as Morrow (2002) has reported that when it was used in the construction of footpath it did not last long and the surface started to peel off after some time and the floor had to be replaced with a concrete floor eventually. In cases where it has been used to make tennis courts it usually requires a lot of regular maintenance as the court needs to be re- rolled with equipment like a ride-on roller every time it is used otherwise its surface will become uneven and start to crack or breakup. It is however still possible to improve the strength of termitarium soil when they are to be utilized through the addition of additives like cement, its limitation of being prone to cracking can also be overcome by the addition of sand to it. Considering the above stated challenges, it is suggested that its use be limited to structures that would not face much pressure, large traffic or even experience much force application as it has proved inadequate for these purposes without the addition of additives or coatings to protect its surface, however rather than having termitarium soil as obstruction on farms and other landscape where it could be a source of damage to agricultural equipment e.t.c, it can be used for filling the floors of buildings, before a layer of cement or tiles is used to finish it up, and with appropriate additives it can still be put to other use

In conclusion, this study revealed the present form of *M. belicosus* found on parry road, University of Ibadan and may provides a basis of comparison with future morphometric works. It was also concluded that the contribution of α-amylase to the strength of termitarium soil may be minimal compared to the nature of the termitarium soil as its clayey nature had a higher contribution to strength. Both control soil without α-amylase and termitarium soil with α-amylase broke at a force below 1MPa.

For future, research should be designed to know the exact pressure that cracked bricks made from different termitarium soils by using pressure gauge with smaller calibration like hectopascal (1HPa=100pa) and Kilopascal (1KPa=1000pa).

To investigate the different contributors to the strength of termitarium soil, research could be done to know if homopolysaccharides like cellulose and chitin which serves as structural elements in plants cell walls and animal exoskeleton have any contribution to termitarium strength since they are all carbohydrate derivatives and carbohydrates are one of the major food sources for termites and they use regurgitated food materials in the process of termitariun construction.

All complements of α and β amylase, cellulolytic enzymes with other digestive enzymes utilized by termites is also worth investigating as this may provide more information on the ways we can be able to reutilize the large amount of lignocellulosic biomass that we generate as agricultural and forestry waste in our daily activities since they have the potential to become a major source of fermentable sugars for biofuels

Research should be designed to know the role lignin and other component of termite faeces plays in termitarium strengthening.

Corresponding Author:
Dr. Kehinde Popoola
Department of Zoology
University of Ibadan, Nigeria
E-mail: taiwo_kok@yahoo.com

References REFERENCES

1. Abe, T., Gignell, D. E., and Higashi, M. (eds.) (2000). "Termites: Evolution, Sociality, Symbioses, Ecology." Kluwer Academic, Dordrecht.
2. Baeza, J and Freer, E.J. (2001) 'Chemical characteristics of wood and its components', Wood and Cellulose Chemistry, Second Edition. Pp275-384
3. Breznak, J.A. (2000). Ecology of prokaryotic microbes in the guts of wood- and litter-feeding termites. In *Termites: Evolution, Sociality, Symbioses, Ecology* (T. Abe, D.E. Bignell, and M. Higashi, Eds.), pp. 209–231. Kluwer Academic Publishers, Dordrecht, Netherlands.
4. Brune, A. (2006). Symbiotic associations between termites and prokaryotes. In *The Prokaryotes, Volume 1: Symbiotic Associations, Biotechnology, Applied Microbiology* (M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, Eds.), pp. 439–474. Springer, New York.
5. Capinera, J. (2008). Encyclopedia of entomology. 2nd ed. Springer, Netherlands.
6. Debruyn, L.A.L and A.L. Conacher, (1987). Soil modification by termites in the central wheat belt of *Western Austealia .J. Soil Res*., 33:179-193
7. Eggleton P. (2000). Global patterns of termite diverstiy. In: *Termites: Evolution, Sociality, Symbioses, Ecology* (Abe T., Bignell D.E. and Higashi M., Eds.). Kluwer Academic Publishers, Dordrecht. pp 25-54.
8. Eggleton P. and Bignell D.E. (1995). Monitoring the response of tropical insects to changes in the environment: Troubles with termites. In: *Insects in a Changing Environment* (Harrington R. and Stork N.E., Eds.). Academic Press, London. pp 473-497.
9. Ekundayo, E.O. and E.R. Orhue, (2002). Properties of termite mounds soil in the Niger Delta region of Southern Nigeria. *J. Sci. Technol. Res*., 14: 15-21.
10. Ghilarov, M.S., (1962). Termites of the USSR, their Distribution and Important: Termites in the Humid Tropic. UNESCO, Paris, pp: 131-135.

11. Gillott, C. (2005). *Entomology*. 3rd ed., Springer, Netherlands.
12. Grasse´, P. P., 1982–1986, *Termitologia*, Vols. I–III, Masson, Paris.
13. Grzimek Bernhard, (2003).*Grzimek's Animal Life Encyclopedia,* 2nd edition. Volume 3, *Insects,* edited by Michael Hutchins, Arthur V. Evans, Rosser W. Garrison, and Neil Schlager. Farmington Hills, MI: Gale Group.
14. Jouquet P; Tessier D; Lepage M. (2004): The soil structural stability of termite net: role of clays in *Macrotermes bellicosus* (Isoptera, Macroterrmitinae) mound soils. *Eur. J. Soil Biol*.,40 : 23-29.
15. Kambhampati, S., and Eggleton, P. (2000). Chapter 1—taxonomy and phylogeny of termites. In *Termites: Evolution, Sociality, Symbioses, Ecology* (T. Abe, D.E. Bignell, and M. Higashi, Eds.), pp. 1–24. Kluwer Academic Publishers, Dordrecht, Netherlands.
16. Kemp, P.B., (1955). The termites of North-eastern Tanganyika: Their distribution and biology. *Bull. Entomol. Res*., 46: 133-135
17. Klowden M.J (2007). Physiological systems in Insects. *2nd ed*. Academic Press New York.
18. Konig, H., Frohlich, J., and Hertel, H. (2006). Diversity and lignocellulolytic activities of cultured microorganisms. In *Intestinal Microorganisms of Termites and Other Invertebrates,* 1st ed. (H. Konig and A. Varma, Eds.), pp. 271–301. Springer, Berlin.
19. Lee, K.E. and Wood, T.G. (1971) Termites and Soils. Academic Press, London, 251p.
20. Lehmann, J. (1998) *Carbohydrates: Structure and Biology,* G. Thieme Verlag, New York.
21 Longair R.W. (2004): Tusked Males, Male Dimorphism and Nesting Behaviour in a Sub-Social Afro Tropical Wasp, *Synagris cornuta*, and weapons and Dimorphism in the Genus (Hymenophera: vespidae: eumeniae).*Journal of the Kansas Entomological Society,*77(4):528-557
22. Malaka S.L.O., (1977a). A study of the chemistry and hydraulic conductivity of mounds materials and soil from different habitants of some Nigerian termites. *Aust. J. Soil Res*., 15: 87-92.
23. Malaka S.L.O., (1977b). A note on bulk density of Termite mounds. *Aust. J. Soil Res*., 15: 93-94.
24. Mayr .E (1969a): Principle of systematic Zoology. MC Graw-Hill, New York 428pp.
25. Mayr .E (1969b): Population, species and Evolution. Harvard University press, Cambridge 453 pp.
26. Miekles, M. (2007) Proteins in Food Science *α*-amylase analysis *www.**meikles**fc.com.au/media/file/study/20/f/.../**amylase-analysis**.pdf*
27. Morrow T, 2002. Rammed antbed courts – construction and maintenance. Centre for appropriate technology, Alice Springs NT
28. Nakashima, K, Watanabe, H., Saitoh, H., Tokuda, G., and Azuma, J.-I., (2002), Dual cellulose-digesting system of the wood-feeding termite, *Coptotermes formosanus* Shiraki, *Insect Biochem. Molec. Biol.* **32**:777– 784.
29. Paul J and Varma A.K. *(1992)* Glycoprotein components of cellulase and xylanase enzymes of a Bacillus sp. Biotechnology Letters vol. 14, 207-212
30. Reddy K.S and Raju A.N (2003) The Physical and Textural Characteristics of Termite Mounds from Podili and Talupula Areas, Andhra Pradesh. *GEOL. SOC.INDIA* Vol.61, pp.693-698
31. Reddy, K.S. and A.N. Rapu, (2005). The physical and textural characteristic of termite mounds from Podili and Talupula Areas. Andhra Pradesh, http://www.bestindia.com/ygsi/sinu.
32. Resh V.H and Carde R.T (2003). *Encyclopedia of Insects*. Academic Press New York.
33. Resh V.H and Carde´R.T (2009). *Encyclopedia of Insects*. *2nd ed.* Academic Press New York.
34. Shareghi, B and Arabi, M, (2008). Thermal denaturation of α -amylase from bacillus amyloliquefaciens in the presence of sodium dodecyl sulphate. *Iranian Journal of Science & Technology,* Transaction A, Vol. 32, No. A2
35. "termite." Encyclopædia Britannica. Encyclopædia Britannica 2009 Student and Home Edition. Chicago: Encyclopædia Britannica, 2009.
36. "Termites" Wikipedia, The free encyclopedia. Accessed in May, 2012.
37. Tokuda, G., Saito, H., and Watanabe, H., (2002), A digestive β-glucosidase from the salivary glands of the termite, *Neotermes koshuensis* (Shiraki): Distribution, characterization and isolation of its precursor cDNA by 5_- and 3_-RACE amplifications with degenerate primers, *Insect Biochem. Molec. Biol.* **32**:1681–1689.
38. Uys V. (2002). *A guide to the termite genera of southern Africa*. Plant Protection Research Institute, Handbook No. 15, Agricultural Research Council, Pretoria. 116 pp.
39. Veivers P.C, Anna M. Musca, R.W. O'Brien, M. Slaytor, (1982). Digestive enzymes of the salivary glands and gut of *Mastotermes darwiniensis*, Insect bichem. **12**, 35–40
40. Watson, J. A. L., Okot-Kotber, B. M., and Noirot, C. (eds.), (1985) *Caste Differentiation in Social Insects*, Pergamon Press, Elmsford, NY.
41. Woods, T.G. and W.A Sands (1978), *The role of Termites In Ecosystems.*In : Production Ecology of Ants and Termites. Brain, M.W. (ED), International Biological Program 13. Cambridge University Press, pp: 245-392.

11/25/2012