Comparative microbial analysis and clay mineralogy of soils eaten by chimpanzees (Pan troglodytes schweinfurthii) in Tanzania

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Abstract

The abundance of culturable microorganisms in Tanzanian termite mound soils similar to those eaten by chimpanzees (Pan troglodytes schweinfurthii) was compared with corresponding non-eaten soils using a serial dilution plating method. Additionally, the particle size distribution and clay mineralogy of the soils were examined. The microbiology of these soils was examined as a preliminary step to investigate the hypothesis that this behaviour relates to self-medication with antibiotics. Significantly higher \( P < 0.037 \) numbers of filamentous bacteria (actinomycetes), were detected in termite mound soils relative to control soils, but numbers of non-filamentous bacteria and fungi did not differ between the two groups. The clay content was significantly higher \( P < 0.007 \) in eaten soils, but the mineralogy of the less than 2 \( \mu \)m fraction was similar for all soils. This study suggests that if chimpanzees selectively consume soils based on their content characteristics, the importance of the type of clay minerals may be secondary to the overall clay content.

Keywords: Chimpanzees; Geophagy; Gombe; Mahale; Microorganisms; Termites

1. Introduction

Geophagy, or earth-eating, occurs worldwide in a range of ethnic, religious, and social groups and has been documented as early as 1398 (Parry-Jones and Parry-Jones, 1992). Throughout history, humans have eaten soil for a variety of reasons that include medicinal purposes, use during pregnancy, as a famine food, as a food additive or condiment, and for religious or ceremonial purposes (Laufer, 1930; Anell and Lagercrantz, 1958; O’Rourke et al., 1967). Geophagy in non-human primates has received increasing attention in the past two decades, and it has been documented in various species, including gorillas (Gorilla gorilla), chimpanzees (Pan troglodytes), and macaques (Macaca mulatta) (Hladik and Gueguen, 1974; Mahaney, 1993; Mahaney et al., 1993).

Given the long history and widespread occurrence of geophagy in both animals and humans, it is reasonable to hypothesize that the practice confers selective benefits. However, a conclusive biological explanation for the practice is lacking. Mason (1833) postulated that the consumption of soil might be a method of self-medication, and not a manifestation of disease, because of the presence of useful elements in soil, such as iron and alkalis. Similarly, mineral supplementation has been suggested as a reason for geophagy in red leaf monkeys (Presbytis rubicunda) and moustached tamarins (Saguinus mystax) (Davies and Baillie, 1988; Heymann and Hartmann, 1991), as well as a means by which to adsorb ingested dietary toxins (Oates, 1978; Gilardi et al., 1999). Vermeer and Ferrell (1985), Mahaney (1993) and Knezevich (1998) have all called attention to mineralogical similarities between eaten soils and pharmaceutical preparations commonly used for the treatment or prophylaxis of gastrointestinal upset or diarrhea in humans. Although these comparisons must be interpreted cautiously, they present a potential area for further research.

Termite mounds are a common source of soils eaten by animals and humans. Termites belonging to the subfamily Macrotermitinae are widespread throughout Africa and their mounds form a conspicuous part of the African landscape. These termites have evolved a symbiosis with a basidiomycetous fungus that they cultivate and eat (Darlington, 1994). The insects play an important role in the soil
ecosystem by moving and rotating large quantities of soil. A small number of studies have investigated the microorganisms present in these mounds. Most work has focused on microorganisms present in the fungal garden, or those occurring in the gut or on the exoskeleton of the insect. Few studies have investigated the microorganisms present in the outer casing of termite mounds, which are the areas most often ingested by primates.

Chimpanzees (Pan troglodytes schweinfurthii) in the Mahale Mountains National Park and Gombe National Park, Tanzania, have been observed to eat soil, as have chimpanzees in Gabon and Uganda (Hladik and Gueguen, 1974; Mahaney et al., 1996, 1997; Goodall, personal communication). In Mahale and Gombe, chimpanzees eat soil from termite mounds, but this behaviour appears unrelated to the “fishing” and eating of termites by chimpanzees. Chimpanzees of all ages engage in geophagy; a piece of soil approximately 2.5 cm³ is broken off from the top of the mound and is often rolled around in the mouth for a few minutes before swallowing.

Many soil microorganisms produce compounds with antimicrobial and/or antiparasitic activity. Among the prokaryotes in soil, filamentous bacteria (actinomycetes) are responsible for about three-quarters of all known medicinal antibiotic compounds (Kutzner, 1981). The present study used a serial dilution plating method to enumerate culturable microorganisms in termite mound soils eaten by chimpanzees and non-eaten, non-termite mound control soils. The microbiology of these soils was examined as a preliminary step to investigating the hypothesis that this behaviour relates to self-medication with antibiotics. Additionally, the particle size distribution and clay mineralogy of the soils were examined. The hypotheses were that termite mound soils would differ quantitatively in microbial content from control soils, and that the total clay content and mineralogy of the soils would be different between termite mound and control soils.

2. Materials and methods

2.1. Study sites

The Mahale Mountains National Park in western Tanzania (6°7′S, 29°55′E) and Gombe National Park (4°40′S, 29°38′E) are two field stations in East Africa where long-term study of chimpanzees has been conducted (Nishida et al., 1983; Goodall, 1986). Both parks contain groups of the same chimpanzee subspecies, Pan troglodytes schweinfurthii, and border Lake Tanganyika, one of Africa’s three largest lakes. Both areas have a single rainy season from November to April, and average temperatures range from a minimum of 18.4 to 21°C to a maximum of 25 to 30.5°C (Goodall, 1986; Collins and McGrew, 1988). Soils in both parks tend to be relatively young, stony and porous, and in areas containing an organic horizon it is usually 1–2 cm deep (Collins and McGrew, 1988).

Mahale has numerous tall peaks and Nkungwe, the tallest, is 2462 m above sea level and composed of granitic gneissess and schists (Collins and McGrew, 1988). Mist and cloud development often occur in Mahale as a result of damp air blowing from the lake (Nishida, 1990). This dampness sustains a concentration of gallery forests which can be found at the north-western foot of the mountains from 780 to 1300 m, providing food and shelter necessary for the survival of the chimpanzees (Nishida, 1990). In Gombe the highest land points are about 1500 m, and composed of hard quartzite and some gneiss and sandstone (Collins and McGrew, 1988).

2.2. Sample collection

Soil samples were collected from the outer parts of Macrotermiteinae mounds in Mahale Mountains National Park and Gombe National Park in October, 1996, using an alcohol-flamed spade. At each site, a sample of 200 to 600 g of soil was collected in a Ziploc bag. Soil samples were also collected approximately 5 m from each termite mound to serve as controls. All samples were double bagged and shipped in an insulated box to protect against temperature extremes during shipment. All samples were received in Toronto 10 days after collection.

Five termitarium soils (TM) and their corresponding control soils (C) were analysed: four samples were from Mahale Mountains National Park (1, 2, 6 and 10), and the fifth was from Gombe National Park ("G"). An additional sample from Mahale (11) was analysed for particle size distribution.

2.3. Soil pH and particle size distribution

The pH of the soil samples was determined by glass electrode, using a soil:water ratio of 1:5. For determination of particle size distribution, sand (63–2000 μm) was separated by wet sieving, and silt (2–63 μm) and clay (<2 μm) were determined by hydrometer (Gee and Bauder, 1982). Mineralogical analyses of the clay fraction were on oriented mounts prepared by centrifugation, and X-rayed with a Toshiba ADG-301H XRD using CuKα radiation and a scan rate of 1° 2θ per minute (Whittig, 1965).

2.4. Serial dilution plating

A serial dilution plate method (Waksman, 1927; Johnson et al., 1959) was used to enumerate microorganisms in the soils. Dilution series were prepared for five subsamples of each soil. For the enumeration of fungi, 1 ml aliquots of dilution series were transferred individually into sterile petri plates and mixed with molten dextrose–peptone yeast-extract agar (Papavizas and Davey, 1959) supplemented with streptomycin, chlorotetracycline, and penicillin G (50 μg/ml of each). Starch–casein agar (Küster and Williams, 1964) was used to enumerate actinomycetes, and tryptic-soy agar (Martin, 1975) was used to enumerate...
non-filamentous bacteria. Both media were supplemented with cycloheximide and nystatin (50 μg/ml of each). Plates were incubated at 20°C, and colonies were counted 4 days after inoculation.

2.5. Statistical analyses

Colony forming units per gram of dry weight soil (CFU/g) were determined for actinomycetes, non-filamentous bacteria, and fungi for each of the five subsamples of five termite mounds and their five corresponding control soils. Statistical analyses of microbiological data were performed using log10 transformed data to correct for heteroscedasticity, and on pooled samples to obtain statistically valid replicates. The mean counts of microorganisms in termite mound and control soils and the percent clay in these soils were compared using a one-tailed pair-wise t-test (Rosner, 1990).

3. Results

Termite mound soils had significantly higher numbers of actinomycetes than control soils (Table 1; \( t = 2.42, \text{df} = 4, P = 0.037 \)). There was no significant difference in the number of non-filamentous bacteria detected in termite mound and control soils (Table 1; \( t = 1.40, \text{df} = 4, P = 0.12 \)), although the number of non-filamentous bacteria enumerated from termite mounds 1 and 10 was higher by an order of magnitude than control soils. Levels of culturable fungi were not statistically different between termite mound and control soils (Table 1; \( t = 1.84, \text{df} = 4, P = 0.070 \)).

Termite mound soils were generally more alkaline (pH 4.7–6.7) than corresponding control soils (pH 4.4–5.4) (Table 2). The clay content of termite mound soils ranged from 30 to 68% and was significantly higher (Table 3; \( t = 4.12, \text{df} = 4, P = 0.007 \)) than that of control soils. The mineralogy of the less than 2 μm fraction of all soils was dominated by metahalloysite, illite, and illite-smectite (Table 4). The relative amounts of 1:1 (Si:Al) clay minerals, such as kaolinite, halloysite, and metahalloysite, and 2:1 clay minerals such as illite, illite-smectite, and smectite, were similar between termite mound soils and control soils.

4. Discussion

Our findings suggest that termite mounds soils are notable in the numbers of actinomycetes they contain. Boyer (1955) and Meiklejohn (1965) reported higher numbers of certain types of bacteria in Macrotermiteinae mounds compared with control soils in the Ivory Coast and Zimbabwe, respectively. Similarly, higher levels of bacteria, actinomycetes, and fungi as compared with control soils were reported from a mound of *Macrotermes bellicosus* in Nigeria (Amund et al., 1988). However, the numbers of actinomycetes in termite mound and control soils were lower than those detected in the present study (3000 and 220 CFU/g respectively). Zoberi (1979) found numbers of bacteria ranging from \( 3 \times 10^6 \) to \( 1 \times 10^7 \) CFU/g and numbers of fungi ranging from \( 4.5 \times 10^5 \) to \( 2.03 \times 10^7 \) CFU/g in the upper layer of a mound of *Macrotermes natalensis* in Nigeria. However, filamentous and non-filamentous bacteria were considered together in this study, and the surrounding soil was not analysed for comparison.

The percentage of clay-sized particles in termite mound soils was higher than in control soils, supporting our preliminary hypothesis that similarity in content of clay-sized minerals would be different between termite mound and control soils. This finding is comparable with other studies. Nye (1955) suggested an upper limit to the size of particles used to construct mounds of *Macrotermes* in the Ibadan area of Nigeria: termites preferred particles less than 2 mm, and Nye (1955) suggested an upper limit to the size of particle that the termites could carry of 4 mm. Arshad et al. (1982), investigating mounds of *Macrotermes* in Kenya, found the outer casing of a mound of *M. subhyalinus* to be 36% clay and the outer casing of a mound of *M. michaelensi* to be 43% clay, whereas the adjacent soil contained from 22 to 49% clay, depending on depth. *Macrotermes* mounds in Zimbabwe were also found to have a higher clay content.

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**Table 1**

Concentration (CFU/g) of actinomycetes, non-filamentous bacteria and fungi in termite mound and control soils with standard errors of the means

<table>
<thead>
<tr>
<th>Group</th>
<th>Termite mound</th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycetes (( \times 10^6))</td>
<td>11 ± 6.5</td>
<td>1.7 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Non-filamentous</td>
<td>15 ± 7.9</td>
<td>5.3 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Bacteria (( \times 10^6))</td>
<td>14 ± 2.6</td>
<td>32 ± 9.2</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

pH of soils

<table>
<thead>
<tr>
<th>Sample</th>
<th>Termite mound</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.4</td>
<td>5.4</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>6.2</td>
<td>5.0</td>
</tr>
<tr>
<td>10</td>
<td>6.1</td>
<td>5.3</td>
</tr>
<tr>
<td>G</td>
<td>4.7</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Table 3**

Distribution of sand, silt, and clay in termite mound and control soils

<table>
<thead>
<tr>
<th>Sample</th>
<th>Termite mound</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
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<td>28</td>
</tr>
<tr>
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<td>10</td>
<td>59</td>
<td>45</td>
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<td>11</td>
<td>74</td>
<td>34</td>
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<td>G</td>
<td>68</td>
<td>74</td>
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<td>1</td>
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<td>10</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>G</td>
<td>74</td>
<td>74</td>
</tr>
</tbody>
</table>

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relative to adjacent soils, but only when the mounds occurred in areas with minimal rainfall (Watson, 1975). McComie and Dhanarajan (1993) reported a significantly larger percentage of clay ($P < 0.05$) compared with adjacent soil in a mound of *M. carbonarius* in Malaysia, and Stoops (1964) reported a higher clay content in mounds constructed by *Cubitermes* (Termitidae: Termitinae: Cubitermes) compared with subsoil in Congo.

The types of clay minerals present in termite mound and control soils were similar. All soils were dominated by metahalloysite, illite, and illite-smectite. If chimpanzees selectively consume soils based on their content characteristics, our findings suggest that the importance of the type of clay minerals may be secondary to the overall clay content. The presence of certain clay minerals may indirectly influence the microbial composition of soils (Stotzky, 1986). However, regardless of the actual mechanisms and extent of this effect, it may be of secondary importance to the hypothesis that the high clay content and actinomycete numbers in termite mound soils are sufficient to reinforce geophagy.

There is growing evidence that chimpanzees selectively consume certain plant species for their medicinal value. Evidence in support of this hypothesis includes: (1) ingestion does not confer nutritional benefits (Wrangham and Nishida, 1983); (2) ingestion of certain plant taxa is correlated to illness at the time of consumption (Huffman and Seifu, 1989); (3) certain plant species are preferentially ingested during periods of high risk of parasitic infection (Kawabata and Nishida, 1991; Huffman et al., 1997); and (4) the relative amount of consumption of these plant species is considerably lower than other plant taxa common to the chimpanzee diet (Wrangham and Nishida, 1983; Huffman and Seifu, 1989). Moreover, antimicrobial properties have been demonstrated in some of these plants (Huffman, 1997). If chimpanzees are selectively consuming these plants for their medicinal value, then it can be hypothesized that they engage in geophagy for similar reasons. Data on the relation between health and geophagy in chimpanzees are limited. However, Mahaney and colleagues (1996) reported five episodes of geophagy by three chimpanzees, with four episodes being clearly linked to severe diarrhea resulting from parasitic infection.

The data presented in this study indicate that soils selected for ingestion differ quantitatively in microbial and clay content from other soils present in the chimpanzees’ habitat. Further studies are required to determine the therapeutic impact of the ingestion of these soils and the relation between health and geophagy in chimpanzees.

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