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UNDERSTANDING GEOPHAGY IN ANIMALS: STANDARD PROCEDURES FOR SAMPLING SOILS

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Abstract—Geophagy or soil ingestion is a multidisciplinary phenomenon that has attracted the attention of many researchers in recent years; who have sought to understand why a large number of animals consume natural earths. To find out why animals ingest soils, it is of paramount importance to establish standard methods to analyze comestible soil. Researchers have used different methods to examine soils ingested by animals, often with incomplete or inconclusive results. To make meaningful comparisons among studies, it is necessary to perfect research designs and establish standard methods to evaluate and analyze geophagy in animals.

Key Words—Soil sampling, soil eating, chemical and mineral analysis, geophagy soil analysis.

INTRODUCTION

Soil ingestion or geophagy is a phenomenon that has attracted the attention of a number of researchers in recent years. Although geophagy is not exhibited everywhere, this phenomenon is widespread in many animals such as elephants (Weir, 1969; Ruggiero and Fay, 1994; Klaus et al., 1998), ungulates (Kreulen and Jager, 1984; Mahaney and Hancock, 1990; Mahaney et al., 1996a), carnivores (Schaller, 1967), primates (Hladik and Gueguen, 1974; Hladik, 1977; Oates, 1978; Mahaney, 1987; Davies and Baillie, 1988; Mahaney et al., 1995a; Krishnamani and Mahaney, 2000 and the references therein; Wakibara et al., 2001), birds (Diamond

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et al., 1999; Gilandi et al., 1999), humans (Laufer, 1930; Halstead, 1968; Danford, 1982; Vermeer and Ferrell, 1985; Aufreiter et al., 1997; Wiley and Katz, 1998) etc. Kreulen (1985) reviewed the benefits and banes of soil consumption, and Robbins (1983) cautioned about the adverse effects of geophagy. However, the benefits far outweigh the limitations and, hence, animals engage in geophagy for a number of reasons that are nonexclusive.

Geophagy possibly evolved because of an animal's innate ability to explore and taste the chemically hostile environment. Since geophagy always has some medicinal value (Krishnamani and Mahaney, 2000), it is akin to self-medication in animals (for a detailed review see Huffman, 1997, 2001; Wakibara et al., 2001; Engel, 2002). Although naturalists have known geophagy for a number of years, only now are wildlife biologists trying to understand this phenomenon. Earlier, in a seminal paper, Krishnamani and Mahaney (2000) outlined the possible reasons that primates might engage in geophagy. They emphasized the need for a coordinated effort between soil and behavioral scientists to understand the proximate and ultimate causes of geophagy. Without quantitative and comparable measures to explain geophagy in a variety of species, our understanding of the selection pressures on geophagic animals will necessarily be incomplete. Although previous authors attempted to explain the nature of geophagy in a variety of animals, each paper brought out seemingly different approaches and problems by estimating and analyzing the extent and kinds of soils ingested (Table 1). In short, different research designs and methods were employed in attempting to study the phenomenon.

The lack of standardized sampling protocols for analyzing and describing geophagic soils has certainly impeded our understanding of the reasons why animals consume soil (Table 1). Benefiting from the diversity of approaches in understanding geophagy, we outline here a new synthesis of the conceptual basis and methods required to evaluate and analyze this phenomenon in animals. Our paper has three specific goals: (1) to develop and standardize methods to understand geophagy; (2) to illustrate shortfalls in previous reports on geophagy, and ways and means to rectify them; and (3) to help coordinate future studies on geophagy between soil and behavioral scientists.

WHAT IS GEOPHAGIC SOIL?

To determine why an animal consumes soil, it is important to identify causal factors including the soil physical–mineral–chemical composition. The geophagic soil contains high quantities of clay and fine silt with little sand; it is often clay (>40% clay) or silty-clay to clay-loam (27–40% clay) that indicates a high degree of weathering and great age. The principal colloids in geophagic soil are similar to most soils and include clay minerals, viruses, bacteria, and fungi. Research on consumed natural soil shows that animals have been observed to ingest clay with minimum quantities of sand and silt (for a brief synopsis on geophagic soils see Krishnamani and Mahaney, 2000).

TABLE 1. ANIMAL SPECIES ENGAGING IN GEOPHAGY AND INSTRUMENTS USED TO STUDY GEOPHAGIC SOILS FOR EACH STUDY

Species	Instruments used to analyze minerals	Particle size analysis done?	Major/minor elements analyzed?	Conductivity-pH measured?	Uningested soil analyzed?	SEM and/or EDS techniques used?	Source
Mongoose lemur and Brown lemur, *Eulemur mongoz* and *Eulemur fulvus rufus*	AAS	No	Major	No	Yes	No	Ganzhorn, 1987
Moustached tamarin, *Saguinas mystax*	XRF	No	M and M	No	No	No	Heymann and Hartmann, 1991
Masked Titi monkey, }it Callicebus personatus melanochir	Colorimetry, XRF	Yes	M and M	Yes	Yes	No	Müller et al., 1997
Red howler monkey, *Alouatta seniculus*	AAS, FP	No	M and M	Yes	Yes	No	Izawa, 1993
Guereza monkey, *Colobus guereza*	FAAS, XRD	Yes	M and M	Yes	No	No	Oates, 1978
Japanese macaque, *Macaca fuscata*	XRD, INAA, MA	Yes	M and M	No	Yes	No	Mahaney et al., 1993

TABLE 1. CONTINUED

Species	Instruments used to analyze minerals	Particle size analysis done?	Major/minor elements analyzed?	Conductivity-pH measured?	Uningested soil analyzed?	SEM and/or EDS techniques used?	Source
Pigtailed macaque, *M. nemestrina*; Assamese macaque, *M. assamensis*; Long-tailed macaque, *M. fascicularis*; Rhesus macaque, *M. mulatta*	XRD	No	M and M	No	No	No	Eudey, 1978
Rhesus macaque, *M. mulatto*	None	No	None	No	No	No	Knezevich, 1998
	XRD, INAA	Yes	M and M	Yes	Yes	Yes	Mahaney et al., 1995b
Bonnet macaque, *M. radiata*	XRD, INAA	Yes	M and M	No	Yes	Yes	Voros et al., 2001
Hybrid macaques, *M. mulatta* × *M. fuscata* × *M. fascicularis* × *M. thibetana* × *M. nemestrina*	ICP-MS, XRD	Partly	M and M	Yes	Yes	No	Bolton et al., 1998
Red leaf monkey, *Presbytis rubicunda*	FP, AAS	No	Major	Yes	Yes	No	Davies and Baillie, 1988

Mountain gorilla, *Gorilla gorilla berengei*	INAA, MA	No	M and M	No	No	No	Mahaney et al., 1990
	XRD, INAA	No	M and M	No	No	No	Mahaney et al., 1995b
Chimpanzees, *Pan troglodytes troglodytes*	Colorimetry, FP, AAS	No	M and M	No	No	No	Hladik and Gueguen, 1974
Chimpanzees, *Pan troglodytes schweinfurthii*	XRD, INAA,	Yes	M and M	No	No	Yes	Mahaney et al., 1996b
	XRD, INAA, EM	Yes	M and M	No	Yes	Yes	Mahaney et al., 1997
	XRD, INAA, EM, TA, OA	Yes	M and M	Yes	Yes	Yes	Mahaney et al., 1999
	XRD, MB	Yes	No	Yes	Yes	No	Ketch et al., 2001
Orang-utan, *Pongo pygmaeus and Pongo abelli*	XRD, INAA, OA	Yes	M and M	Yes	Yes	Yes	Stambolic-Robb, 1997
Human, *Homo sapiens*	XRD, INAA, TA,	Yes	M and M	Yes	Yes	Yes	Mahaney et al., 2000
	XRD, INAA	Yes	M and M	Yes	Yes	Yes	Aufreiter et al., 1997
African Savannah elephant, *Loxodonta africana africana*	MA	Yes	Major	Yes	Yes	No	Ruggerio and Fay, 1994

TABLE 1. CONTINUED

Species	Instruments used to analyze minerals	Particle size analysis done?	Major/minor elements analyzed?	Conductivity-pH measured?	Uningested soil analyzed?	SEM and/or EDS techniques used?	Source
African Forest elephant, *Loxodonta africana cyclotis*	POL	Yes	M and M	Yes	Yes	No	Klaus et al., 1998
Birds	MA, XRD	Yes	M and M	Yes	No	No	Diamond et al., 1999
Primates, ungulates, carnivores etc.	MA	No	Major	Yes	Yes	No	Moe, 1993
Ungulates	MA	No	Major	No	No	No	Kreulen and Jager, 1984
	XRD, INAA	Yes	M and M	Yes	Yes	No	Mahaney et al., 1996a,b

Note: The instrument column is arranged according to different groups abbreviated as follows: FP = Flame Photometer; XRD = X-Ray Diffraction; INAA = Instrumental Neutron Activation Analysis; XRF = X-Ray Fluorescence; AAS = Atomic Absorption Spectrophotometer; FAAP = Flame Atomic Absorption Spectrophotometer; ICP-MS = Inductively Coupled Plasma-Mass Spectrometer; SEM = Scanning Electron Microscope; PO = Polarography; EDS = Energy Dispersive Spectrometry; EM = Electron Microprobe; TA = Toxin Analysis by Gas and Liquid Chromatograph; MB = Microbiological Analysis; MA = Mechanical Analysis or field texture. Mechanical analysis refers to simple textural analysis whereas particle size refers to a grain size curve where reliable estimates of sand, silt and clay are given to a tenth of a percent. INAA provides a chemical matrix including REEs (rare earth elements), which are important in determining the uniformity of the consumed material and in particular the clay minerals (where the bulk of the REEs reside). XRF and ICP-MS also provide a chemical matrix, the latter a complete chemical composition of the sample. The EM is particularly important in the analysis of thin sections where the chemical composition of mineral grains and matrix material is necessary. In some cases microtransects can be established to analyze variations in particular chemical elements including Na, Mg, Al, Si, P, S, K, Ca, Ti, Mn, Fe and Ba, with P, S, K, Fe and Ca being of particular importance to geophagy (see Mahaney et al., 1999). PO or polarography quantitatively or qualitatively measures the redox potential (oxidation or reduction capability) of a sample; almost 30 metals can be analyzed down to ppb levels. TA is achieved using combined gas and liquid chromatography (see Mahaney et al., 1999) to test for the concentrations of the alkaloids lupanine, sparteine, quinine, and atropine. MB, only rarely carried out in geophagy investigations, is particularly useful in assessing the role of fungi and bacteria in soil consumption (Mahaney et al., 1999; Ketch et al., 2001). OA refers to oxalic acid digestion of a sample that is used in gut simulation experiments (Mahaney et al., 1999).

Clay minerals are primarily crystalline hydrous aluminosilicates, the component silicon oxide tetrahedra and aluminum hydroxide octahedra sheets, which are associated either in a 2:1 ratio (Si—Al—Si) or a 1:1 ratio (Si—Al). In these associations, the unit layers, are held together tenaciously by oxygen–hydroxyl bonds (e.g., kaolinite–serpentine group), or are only weakly associated through van der Waals forces (e.g., smectite-vermiculite group). Intermediate and amorphous forms also exist. Since geophagy is mostly a tropical–subtropical phenomenon (for details see Krishnamani and Mahaney, 2000), soils are so well leached that 2:1 (Si—Al—Si) clay minerals are mostly eradicated or exist in small concentrations, making it difficult to form soil structures that rely on adherence of sticky or expandable clay (Krishnamani and Mahaney, 2000).

Geophagic soil may be segregated into biotic and abiotic components. The biotic component contains microorganisms, including viruses, bacteria, fungi, algae and protozoa, and chemical elements such as nitrogen and carbon derived from the nitrogen and carbon cycles. The abiotic components include inorganic minerals/chemicals originating from chemical weathering processes such as oxidation, hydrolysis, chelation, solution, and hydration. The organic component is composed of three distinct fractions: a macroscopic component represented by particulate plant, animal, and microbial debris in the early stages of disintegration and decay; a soluble component (e.g., carbohydrates, proteins) arising from degradation of materials such as plant, animal, and microbial debris; and a complex, dark-colored component, largely aromatic, of humic acids, fulvic acids, and humins, which form from the breakdown of lignin (Stotzky and Burns, 1982).

GEOPHAGY LANDSCAPE MODEL

In every geophagy study, there is one or more sites where natural earths (soils) are ingested (Figure 1). Surface soils or controls which animals have not been reported to ingest are also collected. These samples consist of a high percentage of coarse particles and/or light yellow-red colors, the hallmark of immature soils or soils that are only approaching a mature stage in their development. These controls often lack appreciable clay, or they may contain different species of clay minerals from ingested soils.

Ingested soils are usually situated on older, stable landscape sites (Mahaney, 1999) where they have had ample time to reach maturity and even old age, passing through many stages of weathering where primary minerals have been transformed into secondary (clay) minerals. At the same time, oxidation has affected the iron minerals in the soil to the extent that Fe^{+2} is transformed into Fe^{+3} giving the soils a bright reddish color that may or may not be accompanied by shades of brown, indicating the downward movement of organic compounds. In ancient soils on the landscape, red colors persist, whereas brown colors eventually fade as a result of hydrolysis and conversion of organic matter to carbon dioxide in the carbon cycle.

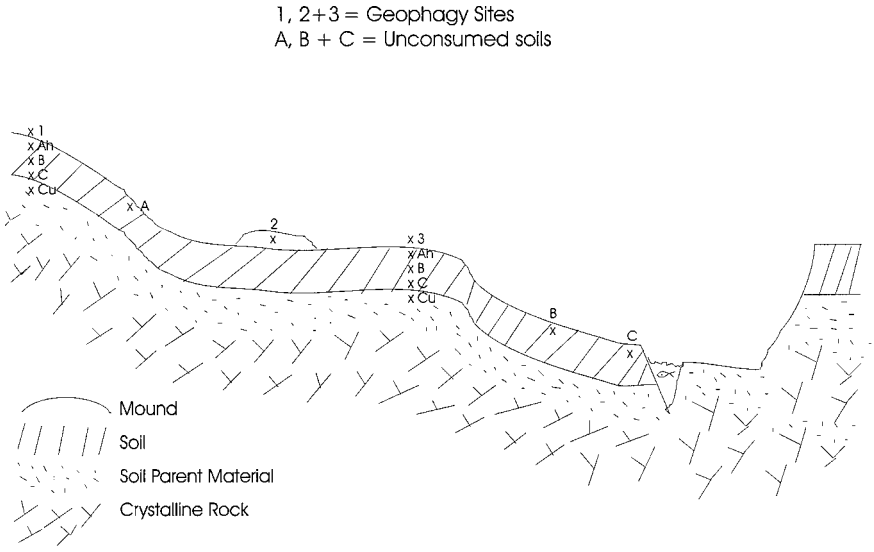

FIG. 1. Landscape model showing possible geophagy/control sites.

In collecting soils for study in a geophagy experiment, one is faced with the knowledge that a certain species of animal consumes a particular natural earth or earths. These sites must be carefully described and samples collected from the exact spot where they are ingested. In the model shown in Figure 1, soils are ingested only at sites 1 and 2 despite the occurrence of several adjacent sites where soils are available for ingestion along slopes or in cuts exposed by erosion.

The ingested soils are on higher, well drained, and presumably older segments of the landscape where they have been exposed to the subaerial atmosphere for long periods of time. One site (X1) exposes surface soil horizons and subsurface horizons, but only the B horizon is ingested. A second site is on a mound that might have been constructed by termites or ants and only the upper portion of the mound is ingested. The mound is certain to be a complex entity, so much so that it will have to be cut and sectioned in order to assess the degree of homogeneity of the ingested material. In collecting samples for laboratory analysis, some thought must be given to replicates, as single samples can produce false impressions and differences from the controls might be simply a matter of chance. It is absolutely essential to collect at least three, and possibly six, samples of the ingested material, to provide replicated data either from one site or several sites frequented by geophagic animals.

Geophagic animals do not consume control soils found at lower elevations and along slopes. In order to determine why these materials are not consumed, samples should be collected on the basis of color and particle size. The nonconsumption of

certain soils could have something to do with grain size, mineralogy, chemistry, and/or microbiology that can only be determined after detailed laboratory analysis. It is important to remember that the laboratory analysis is only as good as the field work and the field investigations must include relevant behavioral data indicating how often animals frequent a site to ingest a particular earth. The approximate mass of material ingested should also be noted.

Controls should be collected after analysis of different shades of color in the field using soil color chips (Oyama and Takehara, 1970) and by paying close attention to texture (particle sizes). Other relevant information includes soil structures—granular, blocky, and prismatic—as these often relate to texture. Soil consistency, along with plasticity and stickiness, is a function of clay content and provides an approximation of percent clay, providing important ancillary information that should not be neglected (Krishnamani and Mahaney, 2000).

If several controls are available for analysis, the researcher should select soils close to the geophagy sites as these, although not consumed, offer possibilities to ingesting animals. It is incumbent on the behaviorist to note which soils have been consumed and which ones are rejected or not consumed by geophagic animals. In such circumstances, it is important to fully analyze these avoided soils to determine the possible reasons for nonconsumption.

The site or sites need to be sketched out with respect to the full landscape. The ingested soils need to be fully described within the soil profile and individual horizons collected to compare the ingested material with refused earth. Once this is done, it is a matter of collecting multiple samples of the ingested material and a sufficient number of control samples to provide sufficient statistical data that differentiate comestible from noncomestible earth.

FIELD ANALYSIS

When collecting samples in the field, it is critical to do a complete soil description including the topsoil, subsoil, and parent material (unweathered substrate) with special attention to the particular horizon(s) that are consumed. The sites need to be sketched out with respect to the full landscape, the ingested soils fully described and differentiated from other soils in the same general area. Once this is done, it is a matter of collecting multiple samples of the ingested material and a sufficient number of control samples for statistical analysis that will help explain why animals opt for certain sites and consume earth.

The consumed horizon should be replicate sampled paying special attention to horizon thickness. If material in the ingested horizon is in a thin layer, it should be collected along the horizontal axis to insure that any variations in mineralogy and chemistry will be discovered. If the horizon is thick, multiple samples could be collected from top to bottom along a vertical transect. *Great care should be taken*

to ensure that the samples collected are the soils consumed by an animal. Within a thick soil horizon, it may be possible that a consuming animal might concentrate on just one part of it; for example, near the top where organic input might be slightly greater, or near the bottom where it might be less. These differences might only be detectable by close observation of the behavior of consuming animals in the field, i.e., from the senses they may detect slight variations in the composition of the soil that could be missed by the soil scientist.

The soil profile, when described in its entirety, may be expected to yield important information not only on the consumed material, but also on the surrounding material that is available, but not consumed (control samples). This unconsumed soil is as important as the ingested soil in geophagy studies because it must contain ingredients that are not beneficial to the consuming animal and hence it is important to determine precisely the constituents of the control samples.

Geophagy soils should be described according to the normal practice in pedology using color and particle size as the main criteria. Profile thickness and the thickness of individual horizons should be noted and the generic soil linked to the evolutionary history of the landscape in which it is formed. In addition, presence or absence of soil structures should be noted along with grade of consistency, plasticity, and stickiness, and such ancillary data as presence of pebbles, weathered state of pebbles, root systems, transitional or sharp contacts among horizons, and any other physical features considered relevant to understanding the geomorphic history of the soil. For a discussion of soil properties, see Soil Survey Division Staff (1993).

Controls should be collected after analysis of different shades of color in the field using soil color chips (Oyama and Takehara, 1970) and by paying close attention to texture (particle sizes). Other relevant information includes soil structures that are forms of granular, blocky, prismatic geometries, as these often relate to texture, although in tropical climates even mature/ancient soils often lack structure. Soil consistency is a function of clay content, and together with plasticity and stickiness, give an indication of clay content and provide important ancillary information that should not be neglected (Krishnamani and Mahaney, 2000)

The presence of datable material, when present, should be noted as it may provide valuable information as to the age of the profile. If, as is usually the case, the soil is open to the subaerial atmosphere, and still active in a weathering sense, it is important to determine its maximum age. It is possible to do this by obtaining organic material that can be radiocarbon dated in the soil or in the parent material from which it formed. If volcanic ash (tephra) is present, it may provide a means of absolute dating using potassium–argon concentrations or fission track methods (Mahaney, 1990). If various horizons of the soil contain fine and very fine sand, it may prove possible to use Optically Stimulated Luminescence (OSL) to obtain absolute age determinations (Mahaney et al., 2001). These dating methods provide ages that will assist in tracking the evolutionary history of the soil, a point

that is often important in geophagy investigations (Mahaney, 1999). Indeed, soils themselves may also provide relative age determinations simply by analysis of the primary and secondary mineral assemblages. The primary mineral suites may provide microscopic data that will aid in determining just how highly weathered the profile is and give some idea of the length of time it has been forming in its topographic setting.

In every geophagy study documented in the literature (Krishnamani and Mahaney, 2000), there is no evidence that animals, as diverse as mountain gorillas and birds, ingest surface horizons (topsoil). It may be that the microbial component in organic-rich soils contains bacteria and/or fungi that are toxic to the ingesting animal, or perhaps the A-horizon group is bereft of minerals that provide neither the raw chemical material or secondary weathering products that are the prime targets in geophagy. Whatever the reason, we have yet to document animals eating material in the zone of maximum biological activity i.e., the A-horizon (Figure 2).

Either by inference from published studies (Davies and Baillie, 1988), or from rare soil descriptions (Mahaney et al., 1990, 1995a,b, 1996a,b), the B-horizons, which occupy the zone of maximum chemical alteration in the soil (see Figure 2), are the prime targets of animals engaging in geophagy. As documented by Mahaney (1987), African Cape buffalo (*Syncerus caffer caffer*) always sought out

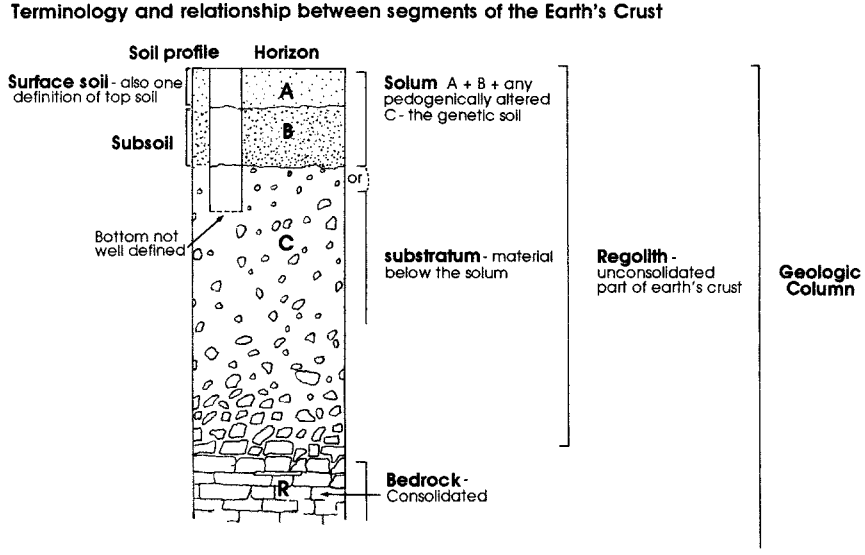

FIG. 2. Generalized soil profile showing individual horizons and relation to the regolith. Sampling of geophagy soils should include horizons not consumed by the animals since they are important and act as controls.

well-weathered, reddish B horizons on Mount Kenya over 12 years' observations of geophagic activity during the Mount Quaternary History Project (1976–1987). Buffalos always engaged in geophagy below the limit of the Last Glacial Maximum (LGM) where ancient soils (paleosols) were present (Mahaney, 1990). These soils provide clay-rich material containing kaolinite and metahalloysite, together with appreciable quantities of iron as secondary weathering products of goethite and hematite.

Evidence from Mt. Kenya (Mahaney, 1990) suggests a minimum age of ~100,000 years for soils to approach a weathering state sufficient to provide nutrients of interest to ingesting animals. Taking Eurasia and North America, for example, the analogous situation is that sites suitable for geophagy lie south of 35° north latitude in locations that escaped glaciation. Whether in mountains or in lowland continental positions, animals engaging in geophagy seek out older ancient soils (paleosols) that lie beyond the range of recent glaciation where weathering proceeded unimpeded, releasing active chemical ingredients and allowing the genesis of high concentrations of clay minerals.

Below the B-horizon in the soil, the thickness and state of the altered C-horizon group is important to describe and analyze, because it often contains material similar in kind to what is in the B, but in a less weathered state. Below the altered C-horizon is the parent material, usually termed the Cu horizon (u = unweathered). In some cases, the entire soil may form from this material over a certain length of time. In other cases, the soil may have several parent materials resulting from the additions of fluvial, aeolian (wind blown), glacial, and or mass wasted (gravitational) sediment. With respect to the latter scenario, analysis of the soil becomes more complex, and the investigator must consider the different chemical and mineral compositions of these diverse materials as they may influence a choice of geophagy soils.

Once the soil is described, the investigator will have collected multiple samples of the ingested material as well as several samples of material made available to, but not ingested by, the geophagic animals. If other geophagy sites exist in the same study area, the investigator should study them paying close attention to similarities and differences between sites as well as differences with the control soils.

LABORATORY ANALYSIS

In the laboratory, soil samples should be transferred from plastic bags to newspapers and left to air dry, a process that takes from a few hours, to several days depending on the clay content. Sand, for example, will dry quickly within a matter of hours, whereas clay-rich material may take several days to dry out fully. Once air dried, samples may be subjected to particle size analysis, a process that

involves fractionation of various grade sizes of material. First, pebbles, if present, need to be separated from the sand–silt–clay fraction by dry sieving using 2.0 mm brass sieves. The coarse fraction, consisting of pebbles (4–64 mm) and granules (2–4 mm), may be stored in paper bags for later analysis as dictated by additional laboratory analysis. The sand–silt–clay fraction may also be stored in paper bags and it is this fraction that will be continually used to perform analyses most closely related to geophagy (see Table 1, many studies have not done particle-size analysis, which means the ratios of sand/silt/clay are largely unknown).

The methods employed here are closely related to standard sedimentological methods previously described by Mahaney (1990, 2002) in the pursuit of lithostratigraphical and pedological problems. The normal procedure is to calculate the air-dried equivalent of 50 g oven-dried soil, a weight that is achieved by taking 50 g air-dried soil and heating to 110°C overnight, or for 15 hr. The weight loss is due to water loss, and the moisture factor is calculated from the ratio of weight of the air dried sample over the oven-dried sample. This quotient multiplied by 50 gives the weight of the air-dried sample in grams. Use of the air-dried equivalent weight avoids heating the sample to obtain 50 g oven-dried soil for particle size analysis (Day, 1965). Heating the sample is a procedure that alters the clay mineral components and requires the investigator to prepare two separate sample splits: one for particle size and another for clay mineralogical analysis.

Following this procedure, the <2 μm fraction can be drawn off from hydrometer jars after 24 hr sedimentation, stored in 1 l bottles, and later equilibrated by shaking for 1 hr. A 48-hr separation is also possible if one requires analysis of the <1 μm clay fraction (determination of fine vs. coarse clay might yield data important in understanding why certain soils are selected for ingestion). The agitated slurry may then be placed in centrifuge cups, fitted with special aluminum supports holding ceramic tiles of a size close to $45 \times 15 \times 2$ mm, and centrifuged at 2500 rpm for 15 min to obtain oriented mounts. After drying, the clay fraction may then be X-rayed using line diffraction equipment outfitted to produce Cu-K-alpha radiation. Normally, air-dried traces are required, followed by treatment with ethylene glycol, and heating in a furnace at 300°, 500°, and 550° (see Mahaney, 1981, 1990 for an explanation of the method).

Once the particle size analysis is complete, sand weights must be multiplied by 2 to give percentage values; these are then added together to give cumulative weight percents. The <63 μm fraction is analyzed using a hydrometer. Values are calculated from the sedimentation rate at intervals of 2, 5, 10, 40, 140, and 1440 min, and converted to phi diameters and cumulative weight percents according to procedures outlined by Day (1965) and Mahaney (1990). Once the phi diameters are known and correlated against percentages, they may be used to construct grain size curves (see Mahaney et al., 1995b and Figure 3). These curves are especially useful as they provide a visual image of the size–shape of the consumed

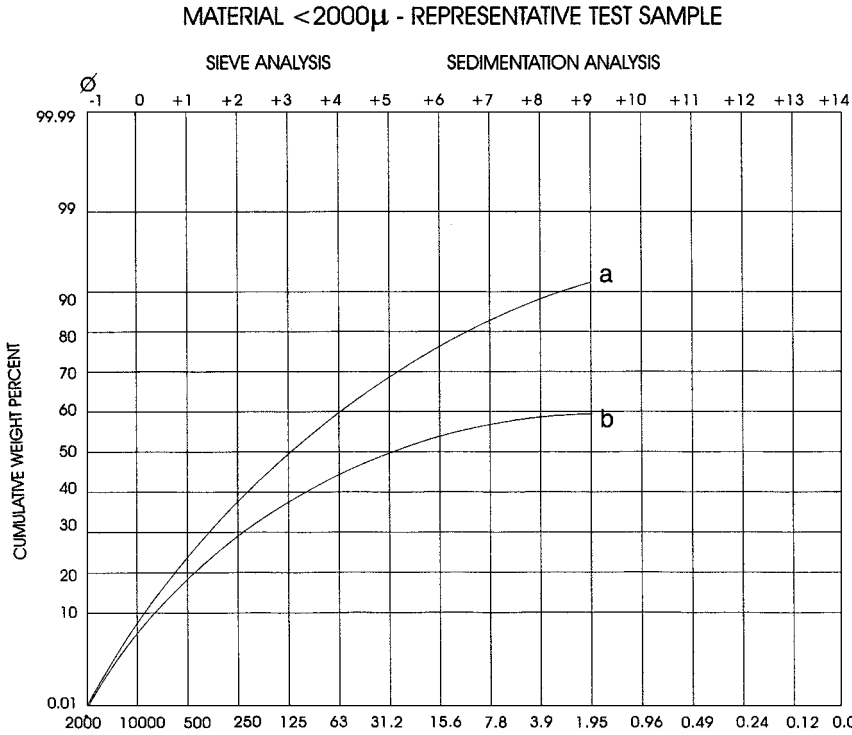

FIG. 3. Generalized grain-size curves showing hypothetical distributions of sand (2000-63:m), silt (63-2:m) and clay (<2:m) for geophagy soil (b) and control soil (a).

sediment/material. The left hand side of the curve gives the amount of sand present and the right hand side the amount of clay. The lower the percentage value on the right hand side, the greater the amount of clay present. Since the percentage of clay is one of the key factors in geophagy investigations, calculation of a precise grain size curve should be considered one of the cornerstones of soil ingestion studies.

The dry colors of the samples should be taken either under sunlight or warm fluorescent bulbs to approximate field conditions. Samples may then be loaded into plastic cups and weighed out to 20 g each for pH and electrical conductivity analyses. After adding 100 ml distilled H_2O and achieving a ratio of 1:5 (20 g soil: 100 ml H_2O), samples are placed in styrofoam plates, shaken for 1 hr, allowed to set for 30 min, after which pH and conductivity levels may be noted. Normally pH values of one tenth of a unit are sufficient and conductivity levels should be low in the range of 0–500 $\mu S/cm^{-2}$ (see Table 1, most studies have not reported pH and conductivity values of the ingested soils).

The organic constituents of the consumed and control material should be fully analyzed so that some estimate of the importance of carbon and nitrogen can be made. Nitrogen may be determined by Kjeldahl methods and carbon by ignition at 400°C overnight for 15 hr to give organic carbon. If automated equipment is available, carbon and nitrogen can be determined by use of a Leco apparatus, which gives total carbon and nitrogen. If no carbonate is present in the samples, the total carbon is the organic carbon. Normally, the value of organic carbon is considered to be 62% of total organic matter.

At some point, it is preferable to consider obtaining a chemical matrix on the consumed and control samples that will provide data on the distribution of macro- and microelements that are important in nutrition, dietary supplementation, zoopharmacognosy, and in assessment of parent material uniformity. One of the most reliable analytical methods is Instrumental Neutron Activation Analysis (INAA), which is used to bombard representative samples with a neutron stream, thus yielding a chemical matrix of some 35 chemical elements. Macroelements, such as calcium, sodium, magnesium, potassium, and iron, are of major importance in geophagy (Mertz, 1981; Robbins, 1983; Krishnamani and Mahaney, 2000). Concentrations of these elements can be obtained from the <2 mm fraction of the consumed and control material. Other microelements, such as arsenic, bromine, and copper, can be assessed for differences in concentration between the consumed and control groups. The concentration of aluminum, while not directly important in geophagy *per se*, yields important differences in clay mineral content, as usually the aluminum increases along with the clay content. If cadmium-lined vessels are used in the analysis, it is possible to obtain concentration levels of silicon to parallel the aluminum distributions, although it is a time consuming process. The rare earth elements (REEs), often considered to have little to do with nutrition, dietary supplementation, and/or zoopharmacognosy, are an important byproduct of INAA, and provide information on parent material uniformity and clay mineral variations (e.g., increases in REEs parallel increases in clay mineral content). As pointed out by Mertz (1981), many trace elements may prove to have an important role in human and animal nutrition, and their essentiality in primate physiology may eventually be shown by future research.

Close scrutiny of the collected material might reveal clumps of fine structures that form granules, blocks, or prisms. These soil structures contain a record of the evolutionary history of the soil. They also may contain clues as to why animals are ingesting the material. Collected as whole entities, they should be put aside in plastic bags for later analysis by Scanning Electron Microscope (SEM) and Energy Dispersive Spectrometry (EDS) to determine the chemistry of the enclosing material as well as the material in the nucleus of the structures.

Analysis of the sand fraction by SEM–EDS (Mahaney, 2002) is particularly useful for providing data on the weathered state of sands, and on coatings that

often envelope sand particles in consumed soils. While the sands themselves provide little if any benefit to animals ingesting earth, coatings on grain surfaces provide clues about the weathering history and lithology of the soil, and they may provide iron and other nutrients to consuming animals. Unlike the clay fraction that is mobile within the soil and prone to downward movement, sands >100:m are more or less fixed in the soil profile and provide a measure of the mineral and chemical uniformity of the parent material. The presence of iron, calcium, and silicon coatings precipitated on sands record the soil weathering history (Mahaney, 2002) and may prove important in assessing the chemical composition of soil water and the relative importance of iron and calcium to nutrition–zoopharmacognosy stimuli. Silicon, which may be important in bone connective tissue and skeleton breakdown, is also important in determining rates of leaching that affect clay mineral recrystallization. Because one of the important clay mineral combinations for alleviating diarrhea is kaolinite/metahalloysite or smectite, it is important to determine the ratio of silicon and aluminum (Si:Al).

The SEM provides imagery (Figure 4) that shows the weathered state of the sand fraction in comestible material, while EDS provides a chemical spectrum (example in Figure 5; and in Mahaney, 2002) useful in assessing the build up and loss of chemical elements. Analysis of the microbiological constituents in consumed soils suggest that animals may benefit from fungal components such as *Penicillium* spp. (Ketch, 1998; Mahaney et al., 1999; Ketch et al., 2001). Certainly, from what is known at present, antibiotic-rich earth might provide one of the main causes of geophagy, stimulating animals to ingest it and providing soil and behavioral scientists with a fertile area of interdisciplinary research.

CONCLUSIONS

While advances in geophagy investigations have been made on the behavioral side, physical–chemical–mineralogical–pharmaceutical methods have generally not been employed by investigators to search for causal relationships that might explain the behavior. Ostensibly, this results from the training of behavioral investigators, which is generally heavily slanted towards biology. The main conclusion offered here is that geophagy is fully a multidisciplinary problem that requires a team effort, usually a research group that is coordinated to undertake detailed field and laboratory methods.

It would be beneficial, and the investigator would do well to empathize with the consuming animal, to closely observe the field site to determine the likely exposures where geophagy might occur. How many geophagy sites exist? How many different kinds of soil are exposed to animals, and what are the differences among this group? Is it possible for animals to “mine” soil (Mahaney, 1990), and how do they discover mining sites? Most geophagy sites are on flat and stable

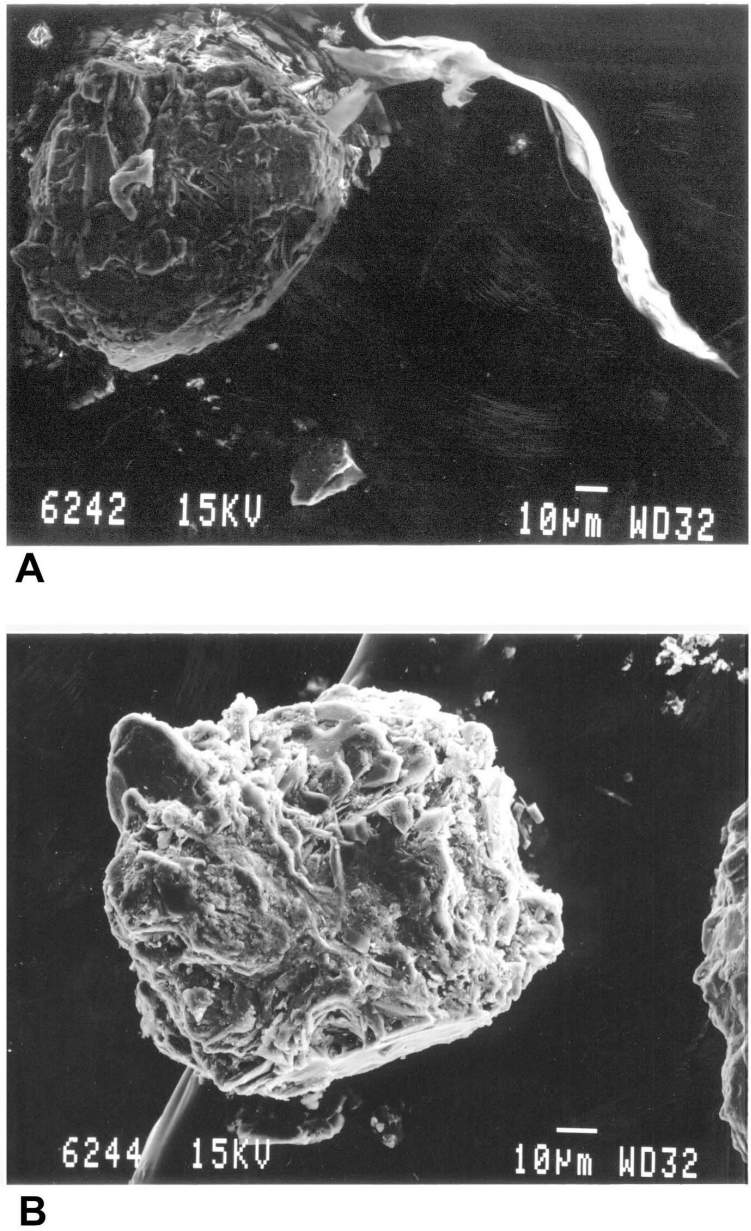

FIG. 4. Examples of SEM imagery. (A) Rutile (TiO2) with attached root and slight coating of Fe (see Figure 5 for chemistry) from site Suci6 (Stambolic-Robb, 1997); (B) Orthoclase (K-feldspar) sand with Fe coating (site Suci6; Stambolic-Robb, 1997).

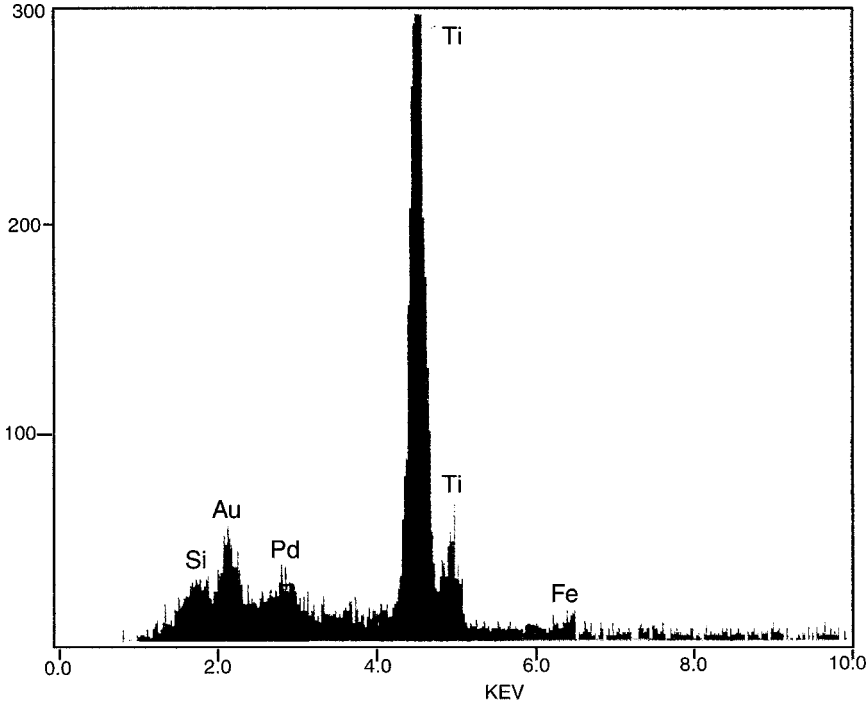

FIG. 5. Energy-dispersive spectrum for grain in Figure 4A showing thin coatings of Si and Fe on rutile. Gold (Au) and Palladium (Pd) are coatings applied to make the surface conductive.

surfaces, at the far end of the landscape evolutionary spectrum, and mostly of ancient age (e.g., older than the last glaciation or >100 ka). What are the differences between these sites and others on slopes or at different elevations? These are some of the questions that will aid finding answers to the perplexing question of why animals consume natural earths.

Organic-rich or even fully organic-depleted materials appear not to be eaten by animals engaging in geophagy. Yet, this should not discourage researchers from analyzing organic components in comestible soils, especially those with minute amounts of carbon that might provide an energy source for microbial populations, especially fungi such as *Penicillium.* If possible, a microbiologist should be part of a geophagy research team.

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