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ORIGINAL PAPER

Soil eaten by chacma baboons adsorbs polar plant secondary metabolites representative of those found in their diet

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Abstract Geophagy, the deliberate consumption of earth materials, is common among humans and animals. However, its etiology and function(s) remain poorly understood. The major hypotheses about its adaptive functions are the supplementation of essential elements and the protection against temporary and chronic gastrointestinal (GI) distress. Because much less work has been done on the protection hypothesis, we investigated whether soil eaten by baboons protected their GI tract from plant secondary metabolites (PSMs) and described best laboratory practices for doing so. We tested a soil that baboons eat/preferred, a soil that baboons never eat/non-preferred, and two clay minerals, montmorillonite a 2:1 clay and kaolinite a 1:1 clay. These were processed using a technique that simulated physiological digestion. The phytochemical concentration of 10 compounds representative of three biosynthetic classes of compounds found in the baboon diet was then assessed with and without earth materials using high-performance liquid chromatography with diode-array detection (HPLC–DAD). The preferred soil was white, contained 1% halite, 45% illite/mica, 14% kaolinite, and 0.8% sand; the non-preferred soil was pink, contained 1% goethite and 1% hematite but no halite, 40% illite/mica, 19% kaolinite, and 3% sand. Polar phenolics and alkaloids were generally adsorbed at levels 10× higher than less polar terpenes. In terms of PSM adsorption, the montmorillonite was more effective than the kaolinite, which was more effective than the non-preferred soil, which was more effective than the preferred soil. Our findings suggest that HPLC–DAD is best practice for the assessment of PSM adsorption of earth materials due to its reproducibility and accuracy. Further, soil selection was not based on adsorption of PSMs, but on other criteria such as color, mouth feel, and taste. However, the consumption of earth containing clay minerals could be an effective strategy for protecting the GI tract from PSMs.

Keywords Plant toxin adsorption · Simulated digestion · HPLC–DAD · Soil eating · Detoxification · Pica · Methods

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Introduction

Geophagy, the regular and deliberate consumption of natural earth materials, is a common behavior in humans (Abrahams 2013; Johns and Duquette 1991; Young et al. 2011), nonhuman primates (NHPs) (Ferrari et al. 2008; Krishnamani and Mahaney 2000), and other animals (Klaus et al. 1998; Matsubayashi et al. 2007; Young et al. 2011). However, its etiology remains poorly understood (Young 2012).

There are two major adaptive hypotheses about geophagy. The first is that it provides essential elements (Kreulen 1985); the second is that it is protective against gastrointestinal (GI) distress (Young et al. 2011). Clay minerals often found in geophagic materials may protect by either directly adsorbing harmful plant secondary metabolites (PSM), parasites, and pathogens or by reinforcing the luminal epithelium of the GI tract reducing its permeability (Gilardi et al. 1999; González et al. 2004; Mahaney et al. 1993; Said et al. 1980; Young et al. 2010).

There are three major types of PSMs relevant to human and nonhuman primate diets, alkaloids, phenolics, and terpenes. Alkaloids are a diverse collection of small molecular weight bioactive compounds containing a nitrogen heterocycle and are relatively polar (water soluble). They are often intensely bitter, neurologically active, and potentially toxic (Lounasmaa and Tamminen 1993; Meyerhof et al. 2010; Schober et al. 1978). Phenolics are derived from shikimic acid, contain unsaturated six-membered rings with OH groups, and are moderately polar. While simple phenolic compounds are often mild to taste, even aromatic, more complex phenolics like condensed or hydrolysable tannins are highly astringent, cause mouth pucker, and in high concentration are considered toxic (Esaki et al. 1977; Horowitz and Gentili 1969; Vidal et al. 2004). Terpenes are often the most lipophilic (least soluble). Small molecular weight terpenes are pungent, while larger molecular weight compounds are often bitter or resinous in flavor (Kubota and Kubo 1969).

PSMs in wild plants are well known to be feeding deterrents against both insect and mammalian herbivores and are an investment by plants to reduce feeding damage (Arnason and Bernards 2010). While cultivated plants have been selected over many generations for lower amounts and less noxious classes of compounds, wild plants remain vigorously defended and animals must contend with these chemical defenses, which may less frequently be a problem in human diets.

However, in human diets, where secondary metabolite concentration is high, there is evidence supporting the protection hypothesis in humans, especially the pioneering work of Johns (1986). He observed that clay sauces were eaten with potatoes by Aymara and Quechua peoples in the high Andes of Bolivia and Peru. The sauce reduced stomach upset and was shown to adsorb toxic glycoalkaloids, which were abundant in high-altitude potatoes that were hybridized with wild relatives to achieve frost resistance. Subsequent work concluded that edible clays used traditionally by humans adsorbed tannic acid present in acorns and helped make these nuts palatable (Johns and Duquette 1991). The science of geophagy in humans has advanced, and an in vitro model was used to confirm that kaolin can reduce the bioavailability of PSMs found in the human diet under physiological conditions (Dominy et al. 2004).

Research has also been conducted on detoxification in nonhuman primates. Studies have assessed the capacity of geophagic materials to adsorb PSMs eaten by golden-faced saki monkeys (*Pithecia pithecia chrysocephala*) (Setz et al. 1999), golden bamboo lemurs (*Hapalemur aureus*) (Jeannoda et al. 2003), Japanese macaques (*Macaca fuscata*) (Wakibara et al. 2001), and chimpanzees (*Pan troglodytes schweinfurthii*) (Aufreiter et al. 2001; Mahaney et al. 1999). The study objectives for these five studies varied as the PSMs tested were based on toxins commonly found in a specific NHP diet. These studies demonstrated that clay minerals adsorb tannins (Setz et al. 1999; Wakibara et al. 2001) and alkaloids (Aufreiter et al. 2001; Mahaney et al. 1999; Wakibara et al. 2001), but do not adsorb cyanide (Jeannoda et al. 2003). Few studies compared clay minerals and their ability to adsorb different PSMs. Of those that did, one found that bentonite had a higher adsorptive capacity than kaolinite for tannins (Setz et al. 1999) and another found that alkaloids were better adsorbed than tannins (Wakibara et al. 2001). Three studies compared eaten with "control" (non-eaten) samples (Aufreiter et al. 2001; Mahaney et al. 1999; Wakibara et al. 2001) and one only tested soil eaten by lemurs (Jeannoda et al. 2003). These studies demonstrated that both eaten and non-eaten soils adsorbed PSMs; however, the non-eaten soils were topsoil that contained organic carbon. Different methods were used, and none tested for terpenes, a PSM that is common in many NHP diets. Since these studies were published, the science has stalled and an accurate, reproducible, method has not been identified.

Some nonhuman primates possess a sacculated fermenting chamber (i.e., "forestomach"), which aids in the detoxification of PSMs. Herbivorous mammals having these types of specialized stomachs can break down some, but not all, PSMs using a bacterial and protozoan microflora requiring an anaerobic and alkaline environment. Other NHPs have saliva that binds tannins (Espinosa Gómez et al. 2015; Mau et al. 2011). Like humans, baboons have simple acid stomachs and hamadryas baboons possess salivary proline-rich proteins, which bind tannic acid (Mau et al. 2011).

To date, no detoxification research has been conducted on chacma baboons (*Papio ursinus*). During an 18-month study, a troop of chacma baboons was studied in the Western Cape, South Africa. Baboons are omnivores and were observed eating a variety of plant, animal, and non-food items. Included were plants containing phenolics (tannins) (*Quercus robur* L. acorns), alkaloids (glycoalkaloids) (*Solanum aculeastrum* Dunal flowers), and terpenes (asiaticoside) (*Centella asiatica* L. Urban leaves). Additionally, the study troop frequently ate earth, "preferred soils" and this behavior was monitored at fixed locations with camera traps (Pebsworth et al. 2012a).

The first aim of this study is to investigate whether the soil preferred by these baboons protected their GI tract from PSMs found in their diet, and which soil properties affected adsorption. We made the following hypotheses: (1) preferred soils would be more effective at adsorbing PSMs than non-preferred soils, (2) polar alkaloids and phenolics would be more readily adsorbed than lipophilic terpenes, and (3) 2:1 clay minerals like montmorillonite, having a greater surface area, would be more effective at adsorbing PSMs than 1:1 clay minerals like kaolinite. Second, because there is no recommended method to assess geophagic materials adsorption of PSMs, we sought to identify a method that yielded highly accurate and reproducible results.

Methods

Earth materials used

To test our hypotheses regarding baboon geophagy, we used behavioral observations and dietary and soil analyses collected at Wildcliff Nature Reserve, Western Cape, South Africa. Baboon behavior was documented and analyzed using focal animal observation (Altmann 1974), video camera traps images (Pebsworth et al. 2012a), and geographic information systems (GIS) (Pebsworth et al. 2012b). Based on video images and presence/absence and intensity of teeth and nail marks, we identified soil samples that were eaten/preferred and uneaten/non-preferred. Using a clean trowel, approximately 120 g of soil were collected and placed in a sealable polyethylene bag. Samples were labeled and photographed. Each sample was subsequently homogenized and quartered. One-quarter was shipped to the James Hutton Institute in Scotland, one-quarter was shipped to the University of Ottawa, Canada, and one-half was archived in South Africa. All soils were imported under permit, license no. IMP/SOIL/5/2015 from the Scottish Government and Permit No. P-2016-00713 from the Canadian Food Inspection Agency.

To test our hypotheses regarding soil properties, we used two clay minerals from Professor Stephen Hillier, James Hutton Institute. Montmorillonite was selected to represent 2:1 clay minerals because this clay mineral has been shown to alleviate GI distress in humans and animals (Dupont and Vernisse 2009; Song et al. 2012; Williams et al. 2009). Kaolinite was selected to represent 1:1 clay minerals as it is widely found in nature and has also been used therapeutically (Mahaney et al. 1997; Vermeer and Ferrell 1985; Williams and Hillier 2014). These samples had previously been characterized for mineralogical composition by quantitative X-ray diffraction.

Soil analyses

We measured soil pH, particle size, and cation-exchange capacity at the University of Ottawa, Canada. We measured pH using a Fisher Scientific AB15 pH/mV/°C meter, which was calibrated daily. We added approximately 10 g of soil to 100 mL of deionized water. We estimated particle size using the Microtrac S3500, a laser diffraction particle size analyzer. Prior to this analysis, we dispersed samples of clay minerals and soil in a 5% sodium hexametaphosphate solution for three days. We then agitated, vortexed, and added a representative sample to deionized water. Soil cation-exchange capacity (CEC) was determined using the $BaCl_2$ compulsive exchange method (Gillman and Sumpter 1986).

We characterized the mineralogical composition of the clay minerals and of the soil samples using X-ray diffraction at the James Hutton Institute. Preparation and analytical techniques are as previously described (Hillier 1999; Omotoso et al. 2006).

Phytochemicals used

Ten PSMs (Fig. 1) were selected from a library of pure phytochemical compounds maintained by Professor Arnason's laboratory at the University of Ottawa. Purity was assessed at $> 95\%$ by HPLC. These 10 compounds were selected to represent compound classes of PSMs encountered by baboons and found in other nonhuman primate diets.

Digestion procedures

We modified and used a simulated physiological digestion method (Gilardi et al. 1999; Klein et al. 2008). One at a time, all four earth materials (1 g) were suspended in 100 mL of digestive fluid. Five-milliliter aliquots were drawn and treated with varying concentrations of each compound. Samples were incubated under agitation (250 rpm) at 37 °C for 1 h with simulated gastric fluid (SGF) and then 2 h with simulated intestinal fluid (SIF). Simulated digestive fluids were prepared according to USP specifications; however, pepsin and pancreatin were not used because stomach enzymes do not metabolize pure compounds (US Pharmacopeia 2017). Digestion times mimic intestinal biochemistry and reflect the use of pure compounds and SGF and SIF. The pH was adjusted to 5.5–6.0 using 1.0 M $NaHCO_3$ prior to the addition of SIF. After incubation, samples were centrifuged for 15 min at $2000\times g$ and the supernatants were quantified using high-performance liquid chromatography with diode-array detection (HPLC–DAD).

HPLC–DAD quantification

HPLC–DAD is considered the best analytical instrument to assess adsorption of PSMs because unlike other techniques, which can only analyze one class of compound it can analyze a variety of PSMs. Furthermore, this modern standard method provides highly accurate and reproducible results that typically have a coefficient of variation of repeated analyses that is $< 5\%$. Quantification of samples was performed on an Agilent 1100 series HPLC system comprising a quaternary pump, a degasser, an auto-sampler with 100-μL loop, a column thermostat, and a diode-array detector (DAD). The identification of compounds found in PSMs was corroborated by comparing the retention time and maximum UV absorption values with authentic commercial standards (Sigma-Aldrich, St. Louis, MO, USA). For each sample, 5 μL was injected onto a Synergi 4μ-Hydro-RP column (250 mm × 2 mm, 4 μm particle size) with column temperature set at 55 °C and a flow rate of 0.5 mL/min. DAD was set to monitor maximum absorbance wavelengths specific to each compound. Standard curves were obtained by injecting different concentration of commercially available compounds. See Table 1 for specific optimal conditions for individual compounds.

The concentration of phytochemicals was quantified via HPLC–DAD after simulated digestion. After optimization trials were completed, for each soil sample tested (preferred, non-preferred, kaolinite, and montmorillonite), at least three concentrations per compound were tested. Quantitation for these methods was based on three technical replicates (i.e., triplicate injections), which were modified from previously validated and published methods (Harris et al. 2007).

Results

The earth materials used in these experiments are characterized in Tables 2 and 3. The preferred soil, like the clay minerals, contained very little sand. Both soils contained similar amounts of silt and clay, but the proportion of clay size fraction varied. The preferred soil contained more illite/mica, while the non-preferred soil contained more kaolinite (Table 3).

In terms of bulk soil minerals, the preferred soil contained more halite than the non-preferred soil

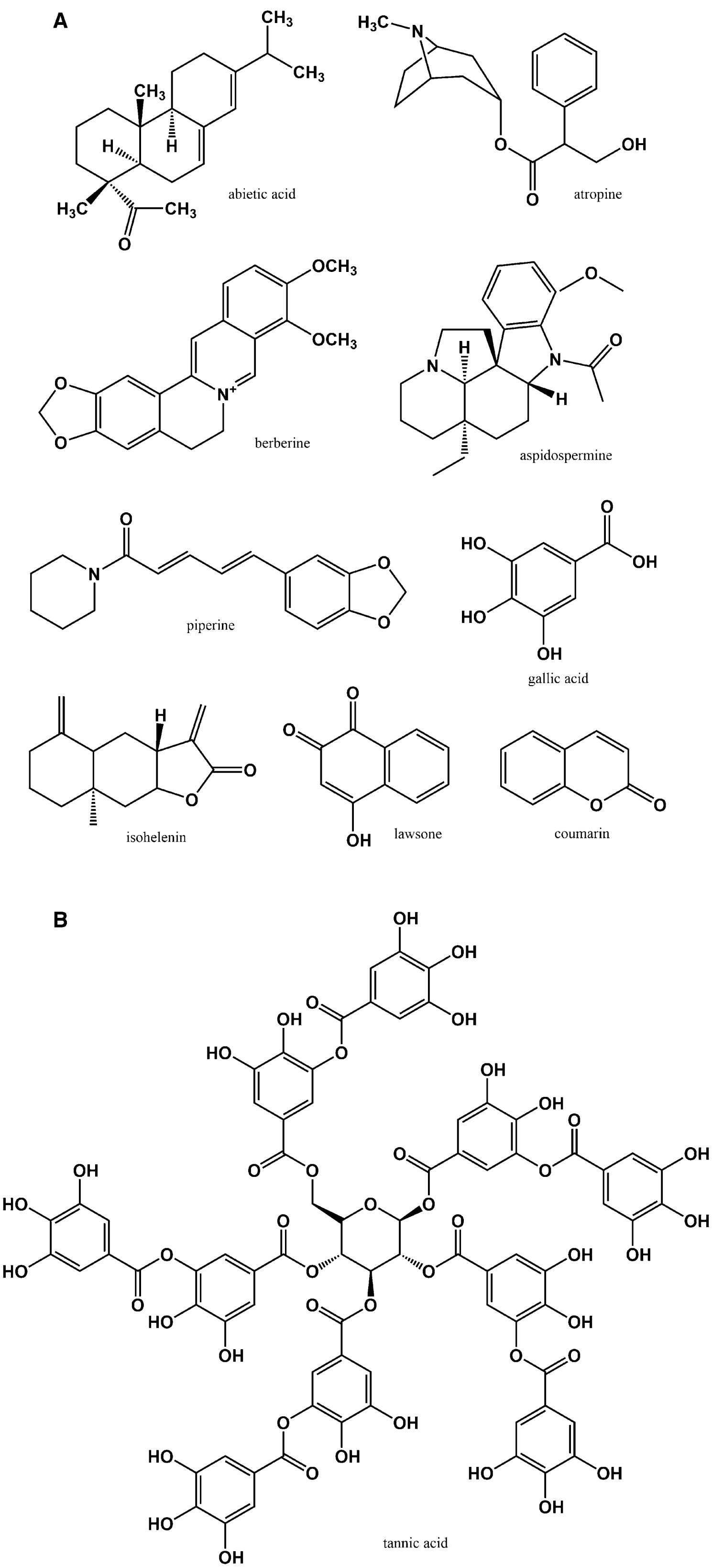

Fig. 1 Structures of plant secondary metabolites used in this study

Table 1 HPLC–DAD conditions for studied plant secondary metabolites

Compound	Mobile phases	Gradient[a]	UV detection (nm)	Range of quantitation (μg/mL)
Aspidospermine	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	40% B to 100% B in 6 min	255	5–20
Atropine	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	20% B to 100% B in 8 min	225	50–200
Berberine	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	30% B to 100% B in 7 min	255, 350	25–100
Piperine	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	40% B to 100% B in 6 min	350	6.25–25
Coumarin	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	30% B to 100% B in 7 min	280	100–400
Gallic acid	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	5% B to 100% B in 6.5 min	280	100–400
Lawsone	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	50% B to 100% B in 6 min	250	6.25–25
Tannic acid	A: H_2O + 0.1% TFA; B: ACN + 0.1% TFA	5% B to 50% B in 8 min; 50% B to 100% B in 2 min	280	100–400
Abietic acid	A: H_2O; B: ACN	85% B to 100% B in 8 min	240	2.5–10
Isohelenin	A: H_2O; B: ACN	60% B to 100% B in 7 min	250	2.5–10

[a]In all methods, the column was washed with 100% B for 3 min, equilibrated for 5 min, then set back to initial conditions. R^2 values for the standard curves were at least 0.9995

[b]Tannic acid was quantified using peak sum

Table 2 Physicochemical properties of earth materials tested

Soil sample	pH	CEC (meq/100 g)	Composition		
			% Sand	% Silt	% Clay
Preferred	8.9	6.2	0.8	79.4	19.8
Non-preferred	10.4	7.1	3.6	79.9	16.5

Table 3 X-ray powder diffraction bulk mineralogy (% weight)

Soil sample	Quartz	Plagiociase	Halite	Rutile	Goethite	Hematite	Illite/mica	Kaolinite	Montmorillonite	Total
Preferred	39	0	1	1	0	0	45	14	0	100
Non-preferred	39	0	0	1	1	1	40	19	0	100
Montorillonite	2	2	0	0	0	0	0	0	96	100
Kaolinite	1	0	0	0	0	0	4	96	0	100

(Table 3). The non-preferred soil contained the iron oxides hematite and goethite that were lacking in the preferred soil.

Formal validation of the HPLC–DAD in Professor Arnason's laboratory at the University of Ottawa is periodically tested. Prior to these analyses, the coefficient of variation of repeated analyses for similar methods was $< 5\%$. Adsorptions of PSMs by the four earth materials used in these experiments are summarized in Figs. 2, 3, and 4. When adsorption of pure

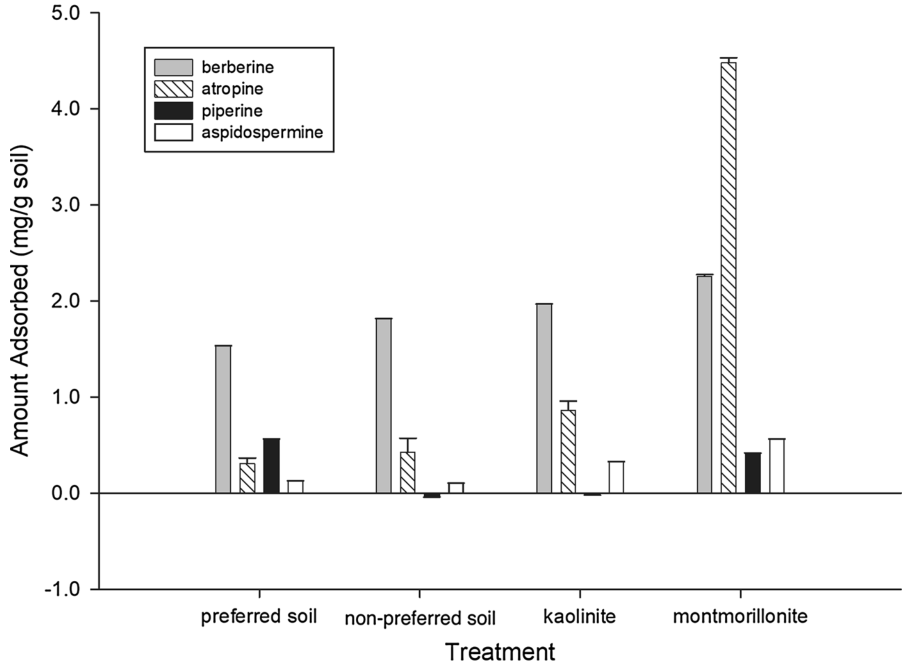

Fig. 2 Adsorption of representative alkaloids by four types of earth materials under simulated digestion. Means ± standard deviations are presented ($n = 3$). Quantitation of phytochemicals was performed via HPLC–DAD. Where no error bars are seen, standard deviation was very small

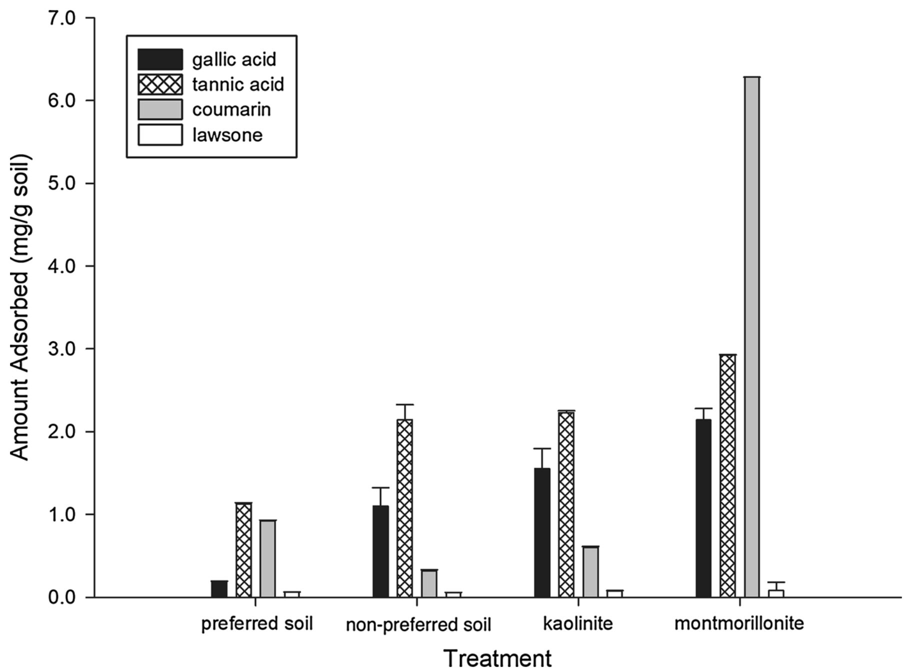

Fig. 3 Adsorption of representative phenolics by preferred and non-preferred soils in comparison with control clays under simulated digestion. Means ± standard deviations are presented ($n = 3$). Quantitation of phytochemicals was performed via HPLC–DAD

PSMs was compared (Figs. 2, 3, 4), the overall trend was that larger quantities of phenolics and alkaloids were absorbed (10×) than terpenes. In the alkaloid group (Fig. 2), across all types of earth materials, berberine was the most adsorbed (4 mg/g montmorillonite, 0.86 mg/g kaolinite, 0.42 mg/g non-preferred, 0.21 mg/g preferred) by all samples with the exception of montmorillonite, followed by atropine, piperine, and aspidospermine. In the phenolic group (Fig. 3), tannic acid was most adsorbed

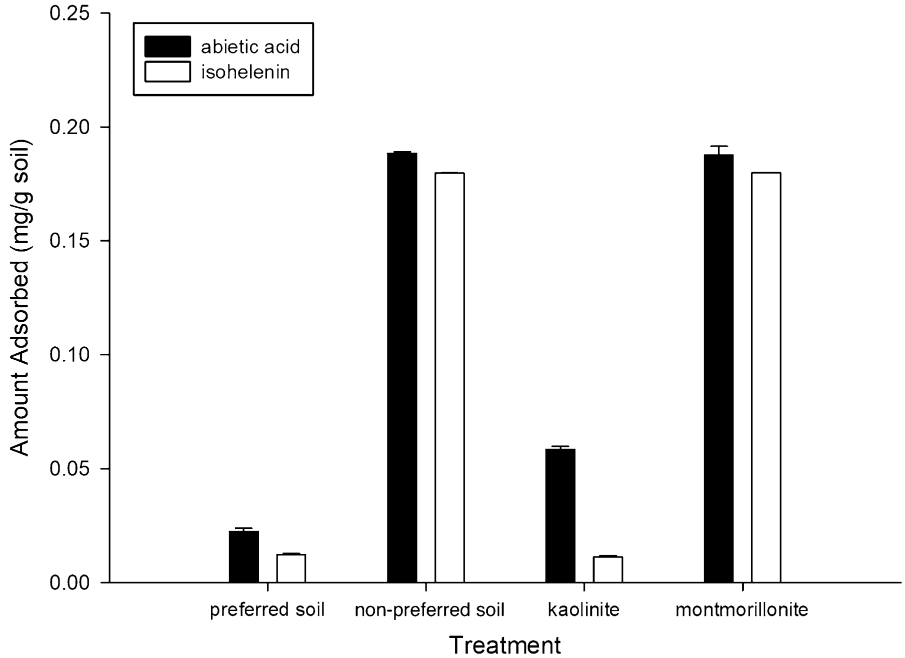

Fig. 4 Adsorption of representative terpenes by preferred and non-preferred soils in comparison with control clays under simulated digestion. Means ± standard deviations are presented ($n = 3$). Quantitation of phytochemicals was performed via HPLC–DAD

(2.92 mg/g montmorillonite, 2.23 mg/g kaolinite, 2.15 mg/g non-preferred, 1.12 mg/g preferred) except for montmorillonite followed by gallic acid, coumarin, and lawsone. In the terpene group (Fig. 4), abietic acid was more absorbed (0.19 mg/g montmorillonite, 0.06 mg/g kaolinite, 0.19 mg/g non-preferred, 0.02 mg/g preferred) than isohelenin, but the values for all four types of terpenes were poorly adsorbed.

When comparing clay minerals, montmorillonite (a 2:1 clay mineral) was generally more adsorbent of PSMs than kaolinite (a 1:1 clay mineral) (Figs. 2, 3, 4). Preferred and non-preferred soil samples were comparable to kaolinite and were less adsorbent than montmorillonite. As expected, the pure clay minerals were better at adsorbing PSMs than preferred and non-preferred soil in part because the latter contained $< 20\%$ clay-sized material and a clay mineral content not exceeding about 60%, if both illite/mica and kaolinite are counted as clay minerals (Tables 2, 3).

Discussion

The objective of this paper was to investigate which PSMs are adsorbed by earth materials and secondarily which soil properties affect adsorption. In general, we found that soil eaten by baboons, which contained clay minerals, can partially adsorb many potentially toxic PSMs from simulated digestion. Specifically, the more polar the compound or compound class, the more readily it is adsorbed to clay minerals and the 2:1 clay mineral montmorillonite was more effective at adsorption than the 1:1 clay mineral kaolinite. These results support our second hypothesis that polar alkaloids and phenolics would be more readily adsorbed than lipophilic terpenes and that clay surface area improves adsorption. Our results would be stronger if we had measured the surface area of all materials used in this study. We, therefore, suggest that future studies determine specific surface area, which is most commonly measured by nitrogen gas adsorption using the Brunauer–Emmett–Teller (BET) method (Heister 2014).

These results are in agreement with those reported by previous studies of geophagic material's adsorptive ability for alkaloids and tannins using other methods such as colorimetry (Wakibara et al. 2001), radial diffusion (Setz et al. 1999), gas chromatography (Wakibara et al. 2001), and spectrophotometry (Gilardi et al. 1999; Johns 1986). Gilardi et al. (1999) reported the adsorption of quinine and tannic acid, while Johns (1986) reported the adsorption of tomatine, a glycoalkaloid. Setz et al. (1999) and Wakibara et al. (2001) also documented the adsorption of tannins by geophagic soils of other nonhuman primates. Wakibara et al. (2001) also showed that geophagic soils containing moderate amounts of kaolinite and illite better adsorbed alkaloids (atropine, sparteine, quinine, and lupine) than tannins. While humans and nonhuman primates have a finely tuned taste system to detect PSMs in foods, it may be challenging for primates to acquire and retain knowledge of specific toxins in foods and which particular geophagic soils were most effective at adsorption. It remains to be determined whether geophagy is used more frequently in plants bearing alkaloids and phenolics and less against plants containing terpenes.

A surprising result was that in general, the non-preferred soil was more effective at adsorbing polar compounds such as berberine and tannic acid and nonpolar compounds such as abietic acid than the preferred soil (Figs. 2, 3, 4). As previously mentioned, both compound classes were found in this troop's diet. These results do not support our hypothesis that baboons prefer soil that is more effective at adsorbing PSMs.

We suggest that the non-preferred soil is better at adsorption of phenols and alkaloids than preferred soil because it contained higher kaolinite content and iron oxides. Both goethite and hematite are forms of iron oxides, which are known to have adsorptive properties (Cornell and Schwertmann 2006). In addition to influencing adsorption, iron oxides even in trace amounts strongly affect the color. The non-preferred soil was pink, while the preferred soil was white. Baboons belong to the subdivision Catarrhini and all members possess trichromatic vision—the ability to discriminate red–green colors (Strier 2007), such that color could also have influenced choice.

It would appear that other factors such as texture, mouth feel, and taste play an important soil selection role as opposed to their detoxification capabilities. Indeed, the preferred soil also contained halite which would impart a salty flavor which in laboratory settings baboons prefer (Laska and Hernandez Salazar 2004). The hydrologic/pedologic reason why the preferred soil contained some halite but the non-preferred soil did not has not been established. Future studies could explore what factors control the distribution of halite in soils.

In conclusion, eating these particular earths could be an effective strategy for assuaging GI distress caused by PSMs often found in human and animal diets. We encourage researchers to monitor soil consumption and characterize consumed soil, so that we can compare patterns of consumption between dietary types (e.g., folivores, frugivores, omnivores) and between various types of digestive systems (e.g., monogastric, ruminant, hind gut fermenter). We also encourage the use of HPLC–DAD as the results are reproducible and accurate. Future studies should also assess the timing of geophagy vis-à-vis PSM ingestion. Collectively, these types of data will help us understand this enigmatic behavior that is common among so many species.

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