



The Roles of Soil Characteristics and Toxin Adsorption in Avian Geophagy

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ABSTRACT

Geophagy, the intentional ingestion of soil, is a widespread phenomenon whose function is still debated. Recent studies suggest that consumed soils adsorb dietary toxins and provide sodium. However, quantification of soil choice has been absent from most studies, prohibiting the direct comparison of soil use and characteristics. To determine correlates among bird use and soil characteristics, we analyze physical structure, mineral composition, and alkaloid adsorption (14 soil characteristics in total) for 22 soil samples from a riverbank ‘clay lick’ used by macaws and parrots (family Psittacidae) in Amazon lowland forests in the Tambopata region of southeastern Peru. We quantified bird preferences through photographic mapping. Mineral concentrations were determined using analyses designed to approximate the mineral availability in the vertebrate stomach. The sodium levels in the soil were much higher than in food plants consumed by parrots in this region. Both adsorption of quinine, the model alkaloid used, and sodium concentration correlated significantly with bird use. Sodium explained a much higher percentage of the variation in bird use than quinine adsorption. However, the analyses were complicated by the high degree of correlation among soil variables: clay percentage and sodium concentration were highly correlated with each other and with bird use, making it impossible to determine which of these may have the greater contribution to bird preference. The results suggest that the soils consumed provide an important source of dietary sodium and adsorb alkaloid toxins.

Abstract in Spanish is available at <http://www.blackwell-synergy.com/loi/btp>.

Key words: clay; diet; nutrients; Peru; Psittacidae; sodium; soil; toxicity.

GEOPHAGY, THE INTENTIONAL CONSUMPTION OF SOIL, is practiced by hundreds of taxa on all continents except Antarctica (Kreulen 1985, Diamond *et al.* 1999). Geophagy has been studied extensively for decades and is usually attributed to the animal’s search for minerals that are otherwise missing in their diets (*e.g.*, sodium, calcium, iron), with sodium the most commonly cited reason for geophagy (Kreulen & Jaeger 1984, Jones & Hanson 1985, Knight *et al.* 1988, Mahaney & Hancock 1990, Klaus & Schmid 1998). Geophagy reportedly protects animals from dietary toxins (Oates 1978, Wink *et al.* 1993, Mahaney *et al.* 1995, Gilardi *et al.* 1999), treats ionic imbalance (Jones & Hanson 1985, Wachirapakorn *et al.* 1996), stabilizes gut pH (Oates 1978, Kreulen 1985), treats intestinal parasites (Knezevich 1998), and helps prevent diarrhea (Vermeer & Ferrell 1985). While avian use of grit for grinding seeds has been known for decades (Meinertzhagen 1954, Pendergast & Boag 1970, Gionfrido & Best 1996), recent studies find most parrots consume fine particle soils, useless as grinding aids (Diamond *et al.* 1999, Gilardi *et al.* 1999, Brightsmith & Aramburú 2004, Symes *et al.* 2006). Most studies of geophagy do not quantify animal use, making direct correlation between soil use and soil characteristics impossible.

In the western Amazon basin, large groups of parrots, macaws, and parakeets (Psittacidae: Aves) consume soil rich in sodium and toxin-adsorbing clays (Emmons & Stark 1979, Gilardi 1996, Burger & Gochfeld 2003, Brightsmith 2004, Brightsmith & Aramburú 2004). While most parrots probably do not use clay licks, those

that do make a substantial investment of time and energy in this behavior (> 2 h and many kilometers of travel; Munn 1992). In addition, birds appear nervous when approaching the licks, as the risk of death by predators and landslides is real (Burger & Gochfeld 2003; D. J. Brightsmith, pers. obs.). This suggests that they are driven to geophagy by strong physiological forces or dietary needs. Because these Peruvian soils also are consumed by other birds (Caraciiformes, Columbiformes, and Passeriformes), mammals (Perissodactyla, Artiodactyla, Rodentia, and Primates), and insects (Homoptera, Heteroptera, Lepidoptera, Diptera, and Hymenoptera including Apoidea, Vespoidae, and *Atta*), the soils may play a larger role than previously thought in the tropical ecosystems where they occur (Kyle 2001, Brightsmith 2004, Brightsmith & Aramburú 2004; D. J. Brightsmith, pers. obs.).

We document soil use by parrots and soil characteristics in the Tambopata region of Peru to determine if soil physical characteristics, nutrient composition, and toxin-binding capacity are driving soil choice. We compare soil and food plant nutrient concentrations to determine if the soil is providing nutrients missing from the birds’ diets. As geophagy can simultaneously provide multiple benefits (Davies & Baillie 1988, Izawa 1993), we hypothesized that birds consume soil high in sodium concentration and toxin-binding capacity.

METHODS

STUDY SITE.—We studied avian geophagy at a clay lick near the Tambopata Research Center (13°07′ S, 69°36′ W; 250 m asl) on

Received 18 November 2007; revision accepted 8 March 2008.

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the border of the Tambopata National Reserve (275,000 ha) and the Bahuaja-Sonene National Park (537,000 ha) in the department of Madre de Dios in southeastern Peru. The study area lies at the boundary between tropical moist and subtropical wet forest, (rainfall 3200 mm; Tosi 1960, Brightsmith 2004). The clay lick is a 500 m long, 25- to 30-m high dirt cliff along the western edge of the upper Tambopata River, formed by the river's erosion of uplifted Tertiary age alluvial sediments (Räsänen & Salo 1990, Foster *et al.* 1994, Räsänen & Linna 1995). A total of 28 bird species have been seen eating soil from this lick, and the lick may be visited by up to 1700 psittacines of 17 species per day (Brightsmith 2004; D. J. Brightsmith, pers. obs.).

This study focused on the most frequently used section of the clay lick, a 13 m wide by 27 m tall area located near the center of the region used by the birds (spot 2C). This area was chosen because it could be monitored in great detail and provided a natural buffet experiment with a wide range of soil characteristics and extreme differences in bird use. By concentrating on such a small area, we guaranteed that all of the soils under study were available to the birds during each foraging bout. Had we studied a larger area, our results would have been confounded by poorly understood factors such as the birds' sensitivity to disturbance, vegetative cover, and social factors (Burger & Gochfeld 2003).

BIRD USE.—To quantify soil usage, we photographed birds on the clay lick once every minute during feeding bouts on 13 early mornings (sunrise–07:30) and seven late mornings or afternoons (07.30–17.00 h) between 14 December 2002 and 16 February 2003. Due to temporal correlation in bird use, we did not analyze all of the photos. Instead, we randomly chose a single photo from each 'feeding bout'. To be eligible for analysis the photo had to be taken > 9 min after the beginning of the day's clay lick activity, > 4 min from the beginning of the feeding bout, and have > 49 birds (for early AM) or > 19 birds (for late AM or PM). Photos taken in the first 9 min of day's lick use and the first 4 min after the beginning of the bout were excluded because the birds often landed on parts of the lick that were higher and presumably safer, but not necessarily preferred (D. J. Brightsmith, pers. obs.). The cut off values for numbers of individuals were set so that there would be enough birds on the lick to ensure use of at least some collected soils, but not so many birds as to force competition for most desired soils. The late morning and afternoon threshold was lower because at this time the lick was used only by large macaws whose total numbers are much lower than the diverse early morning parrot assemblages (Brightsmith 2004). The day's first feeding bout started when the first bird(s) landed on the clay lick and each bout was considered over (and new bouts began) when > 85 percent of the birds flew from the clay lick.

To quantify bird use, we mapped the positions of the 22 soil samples on to each photo and counted the number of birds within a 30-cm radius of the point where each soil sample was collected. The 30-cm radius was used because soil characteristics were visually similar within this distance and because if we counted birds that were directly over each sample site, we would have had a very small and potentially biased sample of bird use. Three measures of bird use were calculated for each of the 22 soil samples: (1) the total

number of birds within 30 cm; (2) the total number of photos in which birds were within 30 cm; and (3) the total number of days on which birds were within 30 cm. All three measures were highly correlated ($r > 0.92$ for all combinations). The outputs from these three measures of bird use (total birds, no. of photos, and no. of days) were ranked and the ranks averaged to produce a unitless composite total bird use value for each soil. For the sake of this paper, we assume that all the bird species are consuming soil for the same reasons, and therefore lump the results from all bird species.

SOIL ANALYSES.—We collected 22 soil samples from the main section of the clay lick (spot 2C) on 30 and 31 January 2003. We sampled the visually distinct soil types in areas covering the range from low to high bird use. Soil analyses were performed at the Laboratorio de Análisis de Suelos in the Universidad Nacional Agraria La Molina, Peru.

We determined the percent dry weight of three different size categories; clay (< 0.002 mm), silt (0.002–0.05 mm), and sand (> 0.05 mm), by dry filtering and weighing the sand fraction. The remaining silt and clay fractions were suspended in dilute sodium hexametaphosphate and sampled at a fixed depth and time to determine the clay content (Tan 1996). The silt fraction was then calculated by subtraction. Cation exchange capacity (CEC) is a measure of a soil's ability to exchange positively charged ions with the surrounding solution (Bache 2002) and of the soil's ability to attract and retain cations including, in theory, the positively charged alkaloids (Boul *et al.* 1997). As a result, CEC is sometimes considered a surrogate measure for toxin binding (Diamond *et al.* 1999). The CEC per 100 g of soil was calculated using the ammonium acetate method (Tan 1996). Results are presented in milliequivalents per 100 g of soil. The pH for each sample was determined in the lab by suspending 20 g of soil in 20 ml of distilled water, agitating 30 min then letting settle 30 min and reading the solution's pH (Tan 1996).

We analyzed a single sample that was highly used by birds (sample U, the second most used of the 22 samples collected; Table S1) to determine the types of clays present (*i.e.*, clay mineralogy). The sample was separated into sand (2.0–0.5 mm), silt (0.5–0.002 mm), coarse clay (0.002 mm–0.2 μ m) and fine clay (< 0.2 μ m) by centrifugation (Jackson 1969). We performed X-ray diffraction using standard methods and treatments on the whole unfractionated soil, and on the silt, coarse clay and fine clay fractions (Whittig 1965, Klug 1974, Rich & Barnhisel 1977). For the heat treatments on the X-ray diffraction, the slides were placed in a furnace overnight at 300°C or 550°C.

Most geophagy studies use traditional soil analyses to measure the concentrations of soil nutrients (Jones & Hanson 1985, Symes *et al.* 2006). However, such analyses were designed to estimate the nutrients available to plants and the fertilization requirements for agriculture (Dellavalle 1992, Tan 1996). As a result, these analyses are probably inadequate for estimating the soil minerals available to vertebrates (Appendix S1). We employed analyses that approximate the pH and temperature of the vertebrate stomach (Hunter & De Kleine 1984, Diamond *et al.* 1999). While this does not simulate what minerals may become available during passage through the

entire vertebrate gut, it should provide a better approximation than traditional soil analyses or analyses of total mineral concentrations in the soil (Diamond *et al.* 1999, Gilardi *et al.* 1999). For these analyses, we added 1 g of powdered soil to 15 ml aqueous HCl at pH 2.0 and mixed for 1 h at 38° C. We centrifuged the resulting mix and read the concentrations of Cu, Zn, Mn, Fe, K, Mg, Na, and Ca, using atomic absorption spectrophotometry. Results are presented in parts per million.

TOXIN BINDING.—Wild parrots face a wide array of alkaloid toxins in their diet of seeds, unripe fruit, ripe fruit, and other plant parts (Coley & Barone 1996, Gilardi 1996, Cipollini & Levey 1997). The best evidence to support the toxin-binding hypothesis for geophagy comes from Gilardi *et al.* (1999) who studied: (1) clay binding of a model toxin (quinine); (2) clays' reduction of toxin absorption in live parrots (*Amazona aestiva*); and (3) the correlation between surrogate measures of toxin binding (CEC and clay %) and bird use. However, the authors did not directly compare soils' toxin binding and bird use. For this reason, we used quinine as the model alkaloid toxin to compare each samples' toxin binding to its level of total bird use, despite the fact that quinine is not known to be found in wild parrot diets.

The adsorption of quinine was calculated for all 22 soil samples and three commercially available pure clays to determine how the binding capacities of our samples compared to clays of known chemical structure. The commercially available soils were kaolinite (Freeport Kaolinite SP-33, Kaiser Chemicals), mica (muscovite, Engelhard Corporation lot #RS-15542, 870-157-2), and smectite (Na Montmorillonite SWy-2, U. of Missouri Source Clay Minerals Repository). Kaolinites are clays with low CEC (2–10 meq / 100 g clay), low surface area $6\text{--}39 \times 10^3 \text{ m}^2/\text{kg}$, small interlayer distances, little swelling capacity, and relatively low quinine binding (Borchardt 1989, Brady & Weil 2002, this study). Micas are nonswelling clays with intermediate CEC (20–40 meq/100 g of clay), low surface area ($70\text{--}120 \times 10^3 \text{ m}^2/\text{kg}$), small interlayer distances, and relatively low quinine binding (Dixon 1989). Smectites are swelling clays with high CEC (110 meq/100 g of clay) large surface area ($800 \times 10^3 \text{ m}^2/\text{kg}$), large interlayer distances and high toxin-binding capacities (Borchardt 1989, Gilardi *et al.* 1999, Plaster 2003, this study). We also tested heat collapsed smectite (the same Na Montmorillonite SWy-2) and the fine clay, coarse clay, and silt fractions of sample U. The heat-collapsed SWy-2 smectite was prepared by heating to 200°C for 30 min. and then heating the sample at 800°C for 1 h (Grant & Phillips 1998). This was done to collapse the clay and interlayer to help determine where the alkaloid bound to the clay.

All solutions and clay suspensions were prepared in 0.1 N NaCl pH 2 (HCl) to imitate gastric fluid (Hunter & De Kleine 1984, Gilardi *et al.* 1999). Eleven different dilutions of quinine (1, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 ppm) were prepared in glass tubes with a total volume of 5 ml. Fifty microliters of a 2 mg/ml soil suspension was added and they were placed on a shaker at 1000 rpm for 2 h. The tubes were centrifuged at 2000 rpm for 20 min and the supernatants read on a UV-Vis spectrophotometer at 347 nm. Adsorption values were compared to standard curves to calculate

the amount of quinine bound. For each sample, we calculated the average amount of quinine bound.

FOOD RESOURCES.—We compiled information on the Ca, Fe, K, Mg, Na, Mn, and Cu concentrations of seeds, whole fruits, pulp, flowers, leaves, stems, and bark consumed by macaw and parrot in southeastern Peru (Gilardi 1996: $N = 73$ plant parts from 50 spp; D. J. Brightsmith pers. obs.: $N = 22$ plant parts from 15 spp). For these data, the plant parts were dried and analyzed for mineral content following the procedures in Gilardi (1996). These data are presented to show the range of available mineral concentrations in food resources for comparison to the mineral concentrations of clay lick soils and are not weighted by frequency with which birds use each plant resource. The data are presented as average \pm standard deviation, and sample size (number of distinct plant parts).

DATA ANALYSIS.—A total of 14 characteristics were measured for the 22 soil samples. Total bird use deviated from the normal distribution so this variable was arcsine transformed (Sokal & Rohlf 1995). For each characteristic we used univariate linear regressions with the arcsine transformed total bird use as the dependent variable to determine which soil characteristics varied significantly with bird use. The small number of data points and the high level of correlation between soil characteristics precluded simultaneous use of all variables in a single analysis of variance model (Whittingham *et al.* 2006), so we used principal component analysis to extract four independent composite variables (PC 1–4) from the 14 soil characteristics and the resulting components were tested against the arcsine transformed total bird use using linear regression (Sokal & Rohlf 1995). The mean and variance of the variables under study spanned four orders of magnitude. Under such circumstances the PCA is driven by the variable with the largest variance, so the values for each variable were standardized by subtracting the mean and dividing by the standard deviation before the inclusion in the PCA. This transformation was used to allow the comparison of the variables with different units and measurement scales (Rummel 1970).

The soil samples were classified as 'used' or 'unused' based on the natural break in the bird use data (Table S1). We used Student's *t*-tests to compare used and unused soils for each soil characteristic (Sokal & Rohlf 1995). We used Student's *t*-tests to compare the mineral concentrations between geophagy soils and food items consumed by macaws and parrots. The means and standard deviations of used and unused soils for each soil characteristic are presented to facilitate comparison with the literature.

RESULTS

BIRD USE.—We recorded 514 psittacines (parrots, parakeets, and macaws) of eight species at the soils we collected ($N = 38$ photos from 18 different days). Mealy parrots (*Amazona farinosa*) dominated, with 55 percent of all individuals. The other species recorded were red-and-green macaw (*Ara chloropterus*, 9% of all individuals), chestnut-fronted macaw (*A. severus*, 7%), scarlet macaw

TABLE 1. Correlations among characteristics of soils consumed by parrots in Tambopata, Peru. Values shown are Pearson product moment correlation coefficients r . ^{NS} = not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Sand%	Clay%	CEC	Quinine binding	K	Na
Clay	-0.75***					
CEC	-0.84***	0.97***				
Quinine binding	-0.43*	0.68***	0.68***			
K	-0.58**	0.61**	0.60**	0.36 ^{NS}		
Na	-0.61**	0.88***	0.83***	0.63**	0.36*	
Fe	-0.55**	0.56**	0.53*	0.34 ^{NS}	0.68***	0.65***

(*A. macao*, 7%), blue-and-yellow macaw (*A. ararauna*, 7%), red-bellied macaw (*Orthopsittaca manilata*, 5%), blue-headed parrot (*Pionus menstruus*, 5%), white-eyed parakeet (*Aratinga leucophthalmus*, 4%), and unidentified small macaws (*A. severus* or *O. manilata*, 2%). Birds were recorded at 20 of the 22 soils. The maximum bird use for a single soil site was 72 birds in 29 different bouts on 16 different days (Table S1). The bird use data showed a natural division between those soils that were frequently used (41 ± 18 birds, 20 ± 7 bouts, 11 ± 3 d; $N = 12$) and those that were rarely or never used (4 ± 3 birds, 3 ± 2 bouts, 2 ± 1 d; $N = 10$).

CORRELATIONS OF SOIL CHARACTERISTICS.—Clay percentage, CEC, Na, and quinine binding were all significantly positively correlated (Table 1). K and Fe were also significantly positively correlated with all these variables except quinine binding. Sand percentage was negatively correlated with all the preceding except quinine binding (Table 1).

QUININE BINDING.—Most of our soil samples bound more quinine than pure mica or kaolinite, but much less than pure smectite (Fig. 1). Among the fractions of the highly used soil, the fine clay fraction bound the most quinine (38 mg/g clay) followed by the bulk soil (27 mg/g clay), coarse clay (20 mg/g clay), and silt (13 mg/g clay; Fig. 2). The quinine-binding capacity of the heat collapsed smectite was much less than that of normal smectite and similar to the binding capacity of the mica and kaolinite (Fig. 1).

CLAY MINERALOGY.—In sample U, the identifiable minerals in the sand and silt fractions were quartz, kaolinite, and mica. Minerals in the coarse clay fraction were mica, kaolinite, smectite, chlorite, goethite, and quartz. The fine clay fraction contained mica, kaolinite, smectite, and goethite.

BIRD USE VS. SOIL CHARACTERISTICS.—Used soils differed significantly from unused and rarely used soils for five of the 14 soil characteristics, (t -test, $N_{\text{used}} = 12$, $N_{\text{unused}} = 10$, $P < 0.05$; Table 2). Used soils had greater clay percentage, CEC, quinine binding, Na, and lower sand percentage. Seven of the 14 soil characteristics were significantly correlated with bird use (univariate linear regressions,

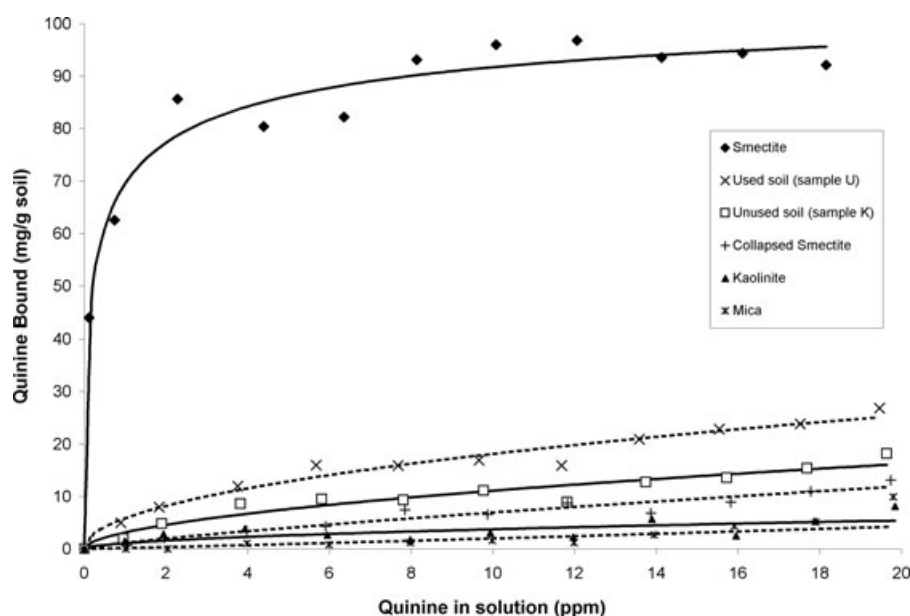


FIGURE 1. Quinine adsorption isotherms of pure clay minerals and soils from a geophagy site in the Tambopata region of southeastern Peru. The heat collapsed smectite is the same smectite heated at 200°C for 30 min, then 800°C for 1 h. See Methods for detailed descriptions of the pure clay minerals used. The 'used soil' (sample U) was the second most commonly consumed by the parrots and macaws (Table 2; see Appendix S1). The 'unused soil' (sample K) was collected from about 10 m away on the same river edge cliff, but was not eaten by the birds.

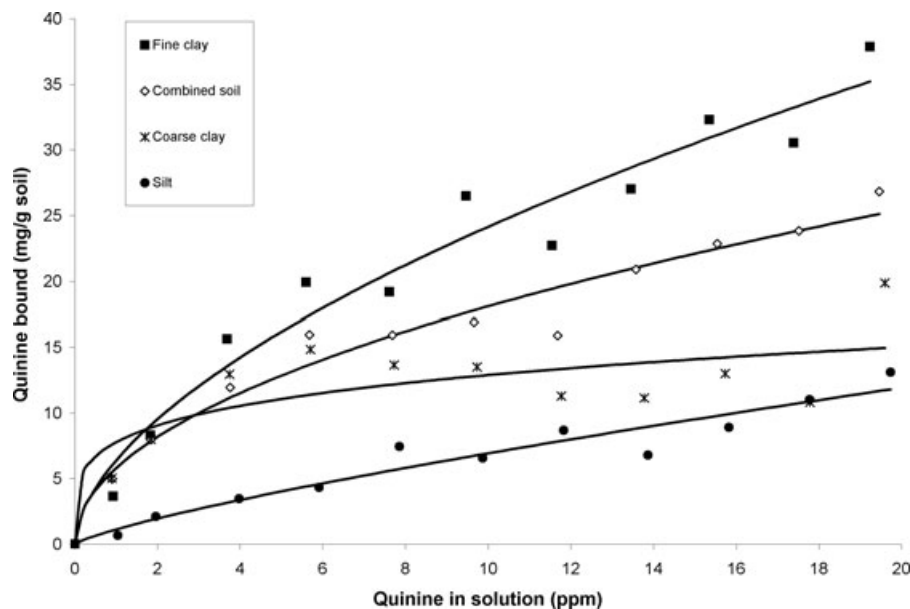


FIGURE 2. Relative quinine binding of soil fractions from a soil consumed by parrots in southeastern Peru (sample U). The curves represent the binding capacities of different soil fractions: silt (0.5–0.002 mm), coarse clay (0.002 mm–0.2 μ m), and fine clay (< 0.2 μ m). Bulk soil is the complete soil as collected and includes all particle sizes. This soil had insufficient sand to analyze as a separate fraction.

$N = 22$ soil samples, $R^2 = 0.24$ to 0.67 , $F_{1,20} > 6.30$, $P < 0.05$; Table 2). The characteristics that varied significantly with bird use in both analyses were: clay percentage, sand percentage, CEC, Na, and quinine binding. Clay percentage was most strongly correlated with bird use (univariate linear regression: clay % vs. arcsine transformed total bird use, $R^2 = 0.67$, $F_{1,20} = 40.8$, $P < 0.00001$; Table 2). The first principal component explained 40 percent of the variation in the data set and varied significantly with bird use (linear regression: $F_{1,20} = 25.4$, $R^2 = 0.56$, $P = 0.0001$) showing that the soils used most by the birds had more clay percentage, CEC, Na, K, Fe, quinine binding, Mg, and less sand percentage (Table 2). None of the other principal components varied significantly with bird use.

MINERAL CONCENTRATIONS IN SOIL AND FOOD.—For Ca, K, Mg, Mn, and Cu, the available concentrations in the consumed soils are significantly less or indistinguishable from the concentrations in the available plant resources (Table 3). Iron concentrations in the soils (119 ± 46 ppm, $N = 12$) are twice as great as they are in the average plant foods in southeastern Peru (66 ± 48 ppm, $N = 12$, t -test: $P = 0.002$; Table 3). However, 11 percent of the 90 plant resources studied have iron values greater than the average consumed soil (Gilardi 1996; D. J. Brightsmith, pers. obs.). The available sodium concentration in consumed soils (1360 ± 462 ppm, $N = 12$) is nearly 40 times higher than in the average plant resource (35 ± 32 ppm, $N = 89$, t -test unequal variance: $P < 0.0001$) and nearly eight times higher than the highest concentration found among the 89 plant resources tested (Gilardi 1996; D. J. Brightsmith, pers. obs.).

DISCUSSION

As hypothesized, parrots used soils with over twice as much sodium as unused soils (Table 2) and soil use by birds was strongly positively correlated with sodium concentration (Table 2). Birds consumed soils that bound more of the model alkaloid quinine. However, this relationship was much weaker than the correlation between bird use and sodium concentration (Table 2). Many of the key soil characteristics measured in this study, (sodium concentration, CEC, quinine binding, and clay %) were significantly correlated, hampering our ability to differentiate among hypotheses about what factors drive bird soil choice.

CLAY MINERALOGY.—Smectite occurred in only small amounts in our samples but apparently contributed the most to quinine binding, as smectites bound about 25 times more quinine than micas and kaolinites (Fig. 1). Other soils consumed by parrots in Peru and New Guinea had higher proportions of smectites and three to five times greater quinine-binding capacities (Diamond *et al.* 1999, Gilardi *et al.* 1999). These differences in binding properties of clay types explain how Tambopata soil samples with similar clay percentage and CEC can differ by up to 300 percent in their toxin-binding ability (this study) and may explain why low clay and CEC geophagy soils from New Guinea bind quinine better than higher clay and CEC geophagy soils from Manu, Peru (Diamond *et al.* 1999, Gilardi *et al.* 1999).

When we collapsed the interlayer spaces of the smectite through heating, we produced a clay similar in structure to mica and this heat-treated smectite bound quinine similar to mica and

TABLE 2. Soil properties from an avian geophagy site in the Tambopata region of southeastern Peru. Used soils are those regularly consumed by psittacines (N = 12 soil samples), while 'unused soils' are those soils where we did not record birds (N = 2) or where we rarely recorded birds (N = 8; see Appendix S1 and Methods). We compared used and unused soils using *t*-tests. The univariate regression was performed using the arc-transformed measure of total bird use (see Methods; N = 22 soil samples). Only the first orthogonal component from principal component analysis (PC1) explained a significant proportion of the variation in total bird use using linear regression, so its loading values are presented. Large positive loading values indicate that frequently consumed soils had higher values for that variable. Quinine binding is presented as milligram bound per gram of soil, CEC is presented as milliequivalents per 100 g of soil, and mineral concentrations are in parts per million (ppm). NS indicates not significant (i.e., $P > 0.05$).

Variable	Used Mean SD	Unused Mean SD	<i>t</i> -test <i>P</i>	Univariate regression			PCA loading <i>PC1</i>
				<i>R</i> ²	<i>F</i>	<i>P</i>	
Quinine binding	19.1 ± 8.9	14.4 ± 4.1	0.052	0.31	9.18	0.007	0.28
Sand %	11 ± 4	0.38 ± 0.24	0.006	0.41	14.0	0.001	-0.32
Silt %	29 ± 12	0.38 ± 0.18	NS	0.08	1.65	NS	-0.11
Clay %	60 ± 11	0.24 ± 0.13	0.000	0.67	40.8	0.000	0.39
CEC	17.9 ± 0.2	9.8 ± 3.7	0.000	0.65	37.4	0.000	0.38
pH	8.7 ± 0.6	8.3 ± 0.7	NS	0.01	0.24	NS	0.04
Cu	0.73 ± 0.57	1.05 ± 0.95	NS	0.08	1.79	NS	-0.04
Zn	2.30 ± 1.21	3.48 ± 4.01	NS	0.00	0.05	NS	0.15
Mn	16.9 ± 7.5	28.9 ± 23.8	NS	0.03	0.56	NS	-0.02
Fe	119 ± 46	85 ± 48	NS	0.24	6.40	0.020	0.31
K	140 ± 24	113 ± 43	NS	0.24	6.30	0.021	0.31
Na	1360 ± 462	551 ± 381	0.000	0.47	17.9	0.000	0.37
Mg	259 ± 55	218 ± 87	NS	0.16	3.87	NS	0.27
Ca	427 ± 209	359 ± 90	NS	0.06	1.31	NS	0.04

kaolinite. This suggests that the majority of the quinine is binding in the interlayer of the smectite particles, and not to the outer surface (see Huebner *et al.* (1999) and Phillips (1999) for a similar system for smectites binding aflatoxins). As a result, the amount of toxin the smectites can bind should be strongly dependent on the size of the toxin molecules: only those that fit well in to the interlayer spaces should bind in large quantities. Further research involving additional toxins should be conducted to determine if results found with quinine are generalizable to the array of alkaloids found in tropical psittacine diets.

BIRD USE VS. SOIL CHARACTERISTICS.—All three of the statistical analyses identified clay percentage as most strongly associated with bird use, followed by CEC, Na, and quinine binding (in this order). K and Fe concentrations positively associated with bird use in two of three analyses. Sand percentage was the only variable negatively associated with bird use in all three analyses. The potential biological importance of these soil characteristics will be discussed in turn.

Clay percentage was the variable most strongly associated with bird use. The birds can probably detect clay percentage, as soil scientists have simple field tests for clay percentage based on the texture and behavior of wetted soil when manipulated (Plaster 2003). Clay is thought to be important to geophagous vertebrates because it is the clay minerals that bind most readily to alkaloid toxins (Oates 1978, Borchardt 1989, Gilardi *et al.* 1999). However, clay percentage explained only about 46 percent of the variation in quinine

TABLE 3. Comparison of available mineral contents of soils and plants consumed by psittacines in southeastern Peru. Plant nutrient data from Gilardi (1996) and D. J. Brightsmith, pers. obs. NS = not significant (i.e., $P > 0.05$).

Mineral	Consumed soils (ppm)		Plant resources (ppm)		<i>P</i> -value
	Mean SD	<i>N</i>	Mean SD	<i>N</i>	
Ca	427 ± 209	12	5233 ± 7512	90	<0.0001
Fe	119 ± 46	12	66 ± 48	90	0.002
K	140 ± 24	12	16,977 ± 15,543	90	<0.0001
Mg	259 ± 55	12	2786 ± 2050	90	<0.0001
Na	1360 ± 462	12	35 ± 32	89	<0.0001
Mn	17 ± 7.5	12	67 ± 125	33	NS
Cu	0.73 ± 0.57	12	10.7 ± 7	50	<0.0001

binding in this study, suggesting that toxin binding may not be the driving factor for preferring high clay soils. Clays may also provide gastrointestinal cytoprotection, protecting the gut from damage by dietary phenolics, alkaloids, and other toxins (Gilardi *et al.* 1999). Due to the many correlations among soil characteristics in our study, birds choosing high clay soils in Tambopata apparently receive many benefits including high sodium, relatively high toxin binding, and clay to protect the gut lining.

CEC was the second most strongly associated with bird use after clay percentage. CEC *per se*, should not provide direct benefits to birds, but in theory should correlate with the soil's overall ability to bind positively charged toxins. CEC explained 46 percent of the relationship with quinine binding in our study. Of note, CEC was highly positively correlated with biologically relevant variables including clay percentage, Na, K, Fe, and negatively correlated with sand percentage ($F_{1,20} > 7.7$, $R^2 > 0.27$, $P < 0.05$). Some combination of these other variables could be driving the relationship between bird use and CEC. More work with additional toxins is needed to elucidate the relationships among clay percentage, CEC, clay type, and toxin binding for geophagy soils. However, until this is completed, we suggest that investigators should not use clay percentage or CEC as surrogates for toxin binding (Oates 1978).

The consumed soils had nearly 40 times more sodium than the average plant food and had much more sodium than any of the 89 plant resource tested, suggesting that clay lick soil is an extremely important source of dietary sodium for these birds (Brightsmith & Aramburú 2004). Sodium is the most commonly cited reason for mammalian geophagy (Jones & Hanson 1985, Klaus & Schmid 1998) and this mineral is needed for a wide variety of animal functions including muscle contraction, nerve impulses, and homeostatic balance (Randall *et al.* 1997). Contrary to the suggestions of some authors, sodium need not be found as NaCl (salt) to be biologically useful (Mahaney *et al.* 1996, 1997). Sodium is scarce in many ecosystems and leaches easily from most soils in humid environments (Klaus & Schmid 1998). In addition, sodium is not needed by the vast majority of plants, so is not actively taken up by most vegetation (Klaus & Schmid 1998). Where plants do accumulate sodium, this often leads to targeted herbivory (Oates 1978, Ohlson & Staaland 2001, Rothman *et al.* 2006). All the soil samples analyzed in our study had Na concentrations greater than 245 ppm (> 6 times the Na in the average parrot food plant in this area). When many available soils are above the Na requirement threshold, these birds may choose based on other soil characteristics (see also Brightsmith & Aramburu 2004).

Each gram of consumed soil bound about 20 mg of quinine, while the best soil bound about 40 mg/g of soil (this study). In studies where *Amazona* parrots were given 50 mg of quinidine (a less toxic stereoisomer of quinine), 2 g of geophagy clay reduced adsorption of quinidine by an average of 60 percent, suggesting quinidine adsorption of 15 mg/g of soil (Gilardi *et al.* 1999). This shows that the clays do adsorb toxins *en vivo* and this may have direct impacts on the health of the bird. So while our study did not find strong evidence that birds choose soils based on their toxin-binding capacities, they apparently adsorb biologically relevant quantities of alkaloids (Gilardi *et al.* 1999).

The concentration of potassium was positively correlated with bird use in the univariate regression and PCA, but two lines of evidence suggest that this may not be biologically relevant: (1) K is significantly positively correlated with clay percentage, CEC, Fe, Na and negatively correlated with sand percentage ($F_{1,20} > 5.1$, $R^2 > 0.20$, $P < 0.05$; Table 1); and (2) plant resources, including some available to the birds during this study, contain about 100 times

more K than the soils (Table 3). However, if future research shows the bioavailability of K in these foods is very low, the clay could still be acting as an effective supplement.

The concentration of iron was positively correlated with bird use in the univariate regression and PCA. Consumed soils had nearly twice as much iron as the average plant resource (Table 3). However, more than 10 percent of the available plant resources had iron concentrations higher than the average consumed soil. The soil could be providing an iron supplement, but alternative sources of iron are apparently available in the diet. The availability of iron is highly dependent on the manner in which it is bound in the plants and soils consumed, and its absorption in the gut is dependent upon the concentrations of vitamin A, vitamin C, cysteine, histidine, and lysine in the diet (McDonald 2006). As a result, further research is needed to elucidate the importance of clay lick soil as a supplemental iron source.

The sand percentage was negatively correlated with bird use, as has been found in similar studies in southeastern Peru (Gilardi *et al.* 1999, Brightsmith & Aramburú 2004). The birds apparently do not use these soils as grit for mechanical aid to digestion; instead, they may avoid sandy soils because of direct negative effects of sand or because of the higher mineral contents and toxin binding of high clay soils (Brightsmith & Aramburú 2004).

Our work shows that psittacines in the southwest Amazon basin consume soils that adsorb alkaloid toxins and provide an important source of dietary sodium. Clay percentage correlated most closely with bird use, suggesting a possible causative link, but key variables (CEC, sodium, quinine binding, and clay %) were significantly correlated, making it difficult to determine which factors drive soil selection in this system. Both adsorption of toxins and sodium supplementation remain as plausible reasons for geophagy in this system. Additional work is needed to tease out which of these stimuli birds can detect and which stimulus or set of stimuli drive the birds to consume soil. However, there is evidence that most of the world's parrots, including many in southeastern Peru, do not consume soil. So any truly comprehensive theory of parrot geophagy will have to explain not only why these birds consume soil, but why so many do not.

ACKNOWLEDGMENTS

We would like to thank U. Valdez for help with photography and soil collection and to R. Aramburú, D. Susanibar, R. Bazan, for soil analyses. The field research was funded by the Earthwatch Institute, Raleigh Durham Caged Bird Society, and rainforest Expeditions and conducted with permission of the Peruvian government's Instituto Nacional de Recursos Naturales (INRENA). Lab work was funded by USAID grant LAG-G-00-96-90013-00 to TP. G. Vigo helped with abstract preparation. This manuscript was improved by the comments of T. White, D. J. Levey, and an anonymous reviewer.

SUPPLEMENTARY MATERIAL

The following supplementary material for this article is available online at: www.blackwell-synergy.com/loi/btp

Table S1. *Bird use of 22 soil samples at Tambopata Research Center in southeastern Peru.*

Appendix S1. *Comparison of soil analyses with traditional and simulated gastric methods.*

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