SHORT COMMUNICATION

Differentiation in mineral constituents in elephant selected versus unselected water and soil resources at Central African bais (forest clearings)

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Abstract Natural forest clearings (bais) in the Central African rain forest attract large numbers of mammals. Little is known about the factors influencing bai use by forest species, though geophagy and hydro-mineral resources are assumed to be important attractants. In the present study, clay and mineral concentrations in water and soil were examined at 15 bais. Water samples from elephant excavated pits showed significantly higher concentrations of most minerals sampled relative to surface waters. But mineral portfolios varied markedly between bais. Geophagy sites were less differentiated from control soil samples, leading to the interpretation that geophagy may not structure bai visitation. Monthly sampling of pit water at one bai suggested higher dry season mineral concentrations, which may relate to seasonal wildlife visitation patterns. The complexity and variability in bai-specific mineral resources suggest there is not a single determining factor (or mineral) driving bai use. The protection of bai mosaics should be a conservation priority in order to ensure access to the portfolio of minerals likely required by endangered species such as the African forest elephant Loxodonta africana cyclotis.

Keywords African forest elephant \cdot Geophagy \cdot Limiting factors \cdot Nutrition \cdot Spatial heterogeneity

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Introduction

Mineral availability is thought to strongly shape wildlife distributions and densities (Milewski 2000). Geophagy is critical for the nutritional (mineral) budgets of numerous species in a variety of environments worldwide (Klaus 1998). It is also thought to be driven by gastro-intestinal disorders alleviated through clay ingestion (Ayotte et al. 2006).

The Central African rain forest natural clearings, locally known as bais, are considered to be mineral-rich sites attracting large numbers of African forest mammals, including elephant *Loxodonta africana cyclotis* (Turkalo and Fay 1995; Gessner et al. 2013). Relatively few studies have been conducted on the mineral properties of these features (see Klaus 1998). Mineral concentration in water holes at Central African bais has received even less attention, though elephants spend most of their time (60–90 %) at bais consuming water (J. MS, pers. observation). Consumed water resources are acquired from upwelling water (springs) in the bottom of holes that they dig in or nearby streams in contrast to geophagy where soils are the target of consumption. Magliocca (2000) reported high mineral concentrations in pit water at three bais, though sampling was limited.

Here, we compared mineral concentrations and clay content of soil and water consumed by elephants with those of random locations at 15 bais in Central Africa. In addition to comparison between used and available sites, our design provides insight to the relative nutritional importance of soil versus water resources. Furthermore, seasonal changes of mineral concentrations in three water holes at Dzanga bai are investigated.

Material and methods

Soil and water samples were collected from April 2011 to March 2012 at a forest stream and 15 clearings, five in the Lobeke National Park (Cameroon), nine in the Nouabale-Ndoki National

Park (Republic of Congo), and its surrounding area as well as the clearing Dzanga (Central African Republic) (Fig. 1).

Water samples were taken once in the rainy and again in the dry season from upwelling sources at the bottom of at least three holes dug by elephants (elephant holes) and one surface source at 11 bais and a forest stream. At Dzanga bai samples were taken once a month from April 2011 to February 2012 in three pits that were identified in collaboration with Andrea Turkalo and one surface water sample. A total of 100 samples from water upwelling at the bottom of elephant holes and 35 surface water samples were taken and analyzed.

Geophagical sites in bais were identified by recent feeding traces of elephants. Soil samples (of approximately 300 g dry weight) were collected at geophagy sites (n=20) within bais that had been used by elephants over longer periods (0–70 cm deep), at randomly selected non-geophagy control sites (n=27) within the bai (0–10 cm deep) and at three randomly selected non-geophagy sites (n=27) in the surrounding forest (5–10 cm deep). Samples were air-dried in the field and stored in plastic bags until analysis.

In the laboratory of the University of Oldenburg, sand, silt, and clay fractions were determined according to the method described by Schlichting et al. (1995). The pH was measured in a 0.01 M CaCl₂ suspension. The chloride concentration was determined with an electrode sonde (Mettler Toledo DX235-Cl, reference electrode inLab302) in a volumetric 1:10 soil/ distilled water suspension. Mineral concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, and Zn in soil samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction following the method of Ayotte et al. (2006). The iodine concentration of soil samples was analyzed in the laboratory of the Institute of Geoecology, Technical University Braunschweig, according to the method applied by Gilfedder et al. (2007).

The pH, conductivity, and the chloride concentration of water samples were measured by the respective electrode sonde. Due to logistic constraints samples had not been acidified and filtered ($0.45 \ \mu m$) in the field and consequently were pre-treated previous to further mineral analysis as follows: to a



Fig. 1 Map showing the 15 bais surveyed in the Lobeke National Park (Cameroon), Nouabale-Ndoki National Park (Republic of Congo), and Dzanga National Park (Central African Republic). The three National

Parks together with the Ndoki National Park and the Dzanga-Sangha Special Reserve constitute the Trinational de la Sangha area

25 ml aliquot of each sample, 32 % HCl was added until a pH of 2.0–2.5 was reached. This was left for 24 h, then sonicated for 1 h and passed through a 0.45-µm filter. Afterward, cations were determined by ICP-OES, and the iodine concentration was analyzed photometrically.

The average mineral (and clay) concentrations of bai-specific geophagical samples and control samples within and on the periphery (forest) of each bai were compared using paired Wilcoxon tests. Samples from each site associated with each bai were averaged to avoid problems with pseudo-replication. Similarly, mineral concentrations from bai-specific elephant hole and surface water samples were averaged and examined using paired Wilcoxon tests, where seasonal effects were also examined by comparing within and between seasons. Correlations between conductivity and concentrations of different minerals (except I) as well as between monthly mean conductivity and precipitation (data provided by A. Turkalo) were assessed using non-parametric methods. Non-parametric tests in SPSS version 20 were performed since normality was not reached after data transformation. Monthly conductivity data from Dzanga were log transformed and analyzed by univariate ANOVA and subsequent post hoc Tukey-B test. Statistical significance was set at $\alpha \leq 0.05$ for all analysis.

Results

The mean concentrations of minerals measured were higher in elephant hole water relative to surface water across all 11 bais and the forest stream (Fig. 2). Pair-wise comparison from averaged elephant hole water samples and surface samples in the respective bais collected during two seasons showed significantly higher concentrations in water samples from the bottom of elephant holes for all minerals except Al, Cu, and I (Table 1).

Significantly positive correlations were found between conductivity and all mineral concentrations (Table 1). The conductivity measured in elephant hole water samples at Dzanga bai varied significantly from April 2011 to February 2012 with a major peak from November to January and a minor peak in July (ANOVA and post hoc Tukey-B, d.f. = 10, F = 9.00, P < 0.05; Fig. 3). Both peaks coincide with a decrease in rainfall, though there was no significant correlation between conductivity and rain (Spearman correlation, n = 11, $R_s = -0.273$, P > 0.05).

The highest concentrations of minerals were found at bais in Cameroon where higher concentrations than the mean of elephant hole water were found for 15 (Djaloumbe) and 11 (Djangui) minerals, and mean conductivity of elephant hole water samples was more than 20 times higher than at the other bais (Fig. 4). Geophagical samples at all Cameroonian bais showed higher concentrations in 5–12 minerals relative to the mean concentration from geophagical and control samples. In



Fig. 2 Mean concentrations (with standard errors) of **a** Ca, Cl, K, Mg, Na, S, and Si; **b** Al, Cu, Fe, Mn, P, Sr, and Zn in elephant hole water samples (*black bars*) and surface water samples (*white bars*) collected in 11 bais and a forest stream

the remaining bais, no more than four minerals with high concentrations were found.

Significantly higher concentrations of Na and S were found in geophagical samples in comparison to control forest samples, while higher concentrations of I were found in control forest samples relative to geophagical samples. Comparing geophagical samples and control samples within bais, significant differences were only found for S (Tables 1 and 2).

Discussion

Mineral access and geophagy have long been assumed to be the driver of bai visitation, yet our knowledge of what attractants bring elephants to a bai has not been adequately addressed. In view of the high effort and large amount of time elephants spend acquiring water from the bottom of deep water holes while in bais, we assumed these water sources would be richer in nutritionally important minerals relative to surface water. Supporting this prediction, elephant hole water showed significantly higher concentrations of most minerals analyzed in comparison to surface water. The largest differences found were for Cl, Na, S, and Zn, though Ca, Mg, Mn,

Table 1 Pair-wise comparison (Wilcoxon test) of mineral and clay concentrations between geophagical and control soil samples within bais (n=10), between geophagical and control forest samples (n=10), and

bai-based pair-wise comparison of mineral concentrations in water hole (n=70) and average control surface water (n=22) samples from 11 bais

	Geophagical–control bai Wilcoxon test		Geophagical–control forest Wilcoxon test		Water surface–ground Wilcoxon test		Conductivity–minerals Spearman correlation	
	Z value	P value	Z value	P value	Z value	P value	R value	P value
Al	1.78	0.074	0.66	0.508	0.438	0.661	0.224	0.009
Ca	0.76	0.445	1.07	0.285	3.847	0.000	0.969	0.000
Cl	1.78	0.074	1.78	0.074	3.847	0.000	0.763	0.000
Cu	1.48	0.139	0.26	0.799	0.674	0.500	0.326	0.000
Fe	1.07	0.285	0.15	0.878	2.419	0.016	0.384	0.000
Ι	0.15	0.878	1.89	0.059				
Κ	0.87	0.386	0.26	0.799	3.393	0.001	0.739	0.000
Mg	0.15	0.878	0.663	0.508	3.815	0.000	0.958	0.000
Mn	1.27	0.203	1.58	0.114	2.711	0.007	0.356	0.000
Na	0.66	0.508	2.29	0.022	4.042	0.000	0.875	0.000
Р	0.56	0.575	1.38	0.169	2.127	0.033	0.465	0.000
S	2.37	0.018	2.37	0.018	3.718	0.000	0.876	0.000
Si					3.652	0.000	0.752	0.000
Sr	0.46	0.646	1.68	0.093	3.815	0.000	0.937	0.000
Zn	1.26	0.208	1.07	0.285	2.482	0.013	0.314	0.000
Clay	1.58	0.114	0.66	0.508				

Spearman rank correlations between conductivity and measured mineral concentrations in 135 ground and surface water samples are also listed. Significant findings are noted by *italics*

Fe, and Sr demonstrated tenfold higher concentration in the water from the bottom of elephant holes. Sodium as well as Cl is critical to regulatory functions in the body such as the transmission of nerve impulses and gastric acid. Rode et al. (2006) assumed that Na may be a cause for crop raiding by elephants due to low availability of alternative sources. Dietary recommendations of sodium for savannah elephants (forest elephant specific recommendations are not available) are 1, 000 ppm (Ullrey et al. 1997) or approximately 45 g day⁻¹ for a 5,000-kg elephant (Holdo et al. 2002). This could easily be met by the consumption of elephant hole water or alternatively bai soil, but not surface water or forest topsoil.

High concentrations of Na, Cl, and S in lick water have also been reported by Riesenhoover and Peterson (1986) and Clayton and MacDonald (1999) in North America and Indonesia. Like Na, S may function as an indicator of mineral rich sites for elephants due to its strong odor. As a potential parasiticide and fungicide (Mattson et al. 1999) and as an important element in rumen microbial communities (Ayotte et al. 2006), S may be an important mineral attractant. Regarding daily requirements of sulfur for elephants (1,500 ppm; Ullrey et al. 1997) the mean concentrations found in sampled substrates during the present study appear rather low, yet daily needs could be met in certain bais such as Djaloumbe (up to 346 ppm in elephant hole water), Djangui (up to 148 ppm in elephant hole water), and Ndangaye (up to 2,120 ppm in geophagical soil).

Further underlining the differences in mineral concentrations between bais, I concentrations at Djaloumbe (up to 172 μ g l⁻¹) were higher than the mean of all bais (5.6 μ g l⁻¹) and surpassed the range of 0.01-70 μ g l⁻¹ for drinking water reported by Edmunds and Smedley (1996). The fundamental role of iodine especially for the brain and reproduction of elephants has been emphasized by Milewski (2000).



Fig. 3 Mean monthly conductivity measured in three water holes at Dzanga bai and the respective monthly precipitation measured at Dzanga camp from April 2011 to February 2012. On the secondary axis, the number of days between sample collection and the last rain is displayed



Fig. 4 Number of minerals in bai-specific ground water samples with higher concentrations than the mean of all ground water (*black bars*) and the mean of geophagical soil samples (*white bars*) at different bais. At Mabale, no minerals showed higher concentrations than the mean of geophagical soil samples; for the remaining bais, zero indicates that no heavily used geophagical sites were found. Bais in Lobeke National Park (Ndangaye, Boulou, PetiteSavane, Djangui, and Djaloumbe) show highest concentrations of minerals

The role of Mg and Ca in terms of bai use is unclear as Holdo et al. (2002) suggested that maintenance requirements of these two minerals might be met by elephants through forage alone. Concentrations of Zn, Fe, and Mn measured in water sources were negligible in comparison to dietary recommendations for elephants (40 ppm; Ullrey et al. 1997).

Blake (2002) suggested that an increased requirement for minerals in the dry season might drive aggregations around bais during this time. In potential support for this hypothesis, our results demonstrated that mineral concentrations were seasonal, with a major peak of mineral concentrations in the dry season at Dzanga bai. The driver of this seasonal fluctuation was speculated to relate to seasonal ebbing of spring water flow.

Mineral rich soil has also been hypothesized to be an attractant for elephants to bais and, in particular, clay or Na may be major drivers for geophagy. Tropical rain forests are in general rich in digestion impeding secondary plant compounds such as tannins that may be absorbed by clay minerals (Klaus 1998). Yet, clay is not likely driving bai use since no significant differences in clay concentrations were found between used and unselected elephant sites. Significantly higher concentrations of Na were found in geophagical soil compared to random forest soils, yet not in comparison to random non-geophagy bai soil, suggesting this as well is not an attractant to bais. In contrast, significantly higher concentrations of S were found in geophagical soil samples relative to control samples both within and around bais. Another important driver of geophagy may be I since its retention in the soil is correlated to organic matter and clay (Hu and Moran 2010). Findings of elephant feeding traces at the roots of trees that may reduce leaching of I may support the importance of this micronutrient for elephants as suggested by Milewski (2000).

Altogether, mineral consistencies varied markedly across sites, rendering the interpretation of factors driving geophagy difficult. The presence of elephant geophagical sites (licks of several square meters) throughout the forest in the study area and the lack of heavily used geophagical sites at several bais frequented by elephants suggest that geophagical reasons may not be the predominant driver of visitation. Geophagy, however, certainly seems a reasonable driver of elephant visitation to bais like Petite Savane and Boulou where

Element (ppm), clay (%)	Geophagical soil within bai Mean ± S.E.	Control non-geophagical bai Mean \pm S.E.	Control non-geophagical forest Mean ± S.E.
Clay	16.59±2.24	10.45±1.63 ^c	15.61±1.56 °
Al	245.27±36.07	139.39±15.84 °	240.46±21.01 °
Ca	456.29±107.84	458.08±111.92	324.75±137.90
Cl	596.26 ± 225.88	441.36±201.31	37.77±6.53
Cu	$2.57 {\pm} 0.66$	2.56±0.61	$1.84{\pm}0.44$
Fe	237.21±45.64	$155.77{\pm}20.38$	167.00 ± 31.28
Ι	1.63 ± 0.30	1.93 ± 0.40	3.16±0.37
K	35.30±7.33	29.00 ± 5.77	40.94±11.63
Mg	98.32±18.87	116.93±27.09	81.96±19.26
Mn	23.55±5.19	14.07 ± 4.27	11.59±4.35
Na	435.34±142.23 ^b	$345.99{\pm}144.6$	31.26 ± 12.54^{b}
Р	$9.95 {\pm} 2.20$	$8.20{\pm}0.87$ ^c	12.84±3.15 °
S	313.19±123.08 ab	50.13±18.21 ^a	$5.75 {\pm} 1.84^{b}$
Sr	$2.48 {\pm} 0.48$	2.17±0.54	1.49 ± 0.69
Zn	$2.95 {\pm} 0.58$	$1.66 {\pm} 0.34$	1.35 ± 0.43

Table 2Mean concentrations ofclay and minerals in geophagicalsoil (20 sites), non-geophagicalsoil within bais (27 sites, control),and forest soil (27 sites) at ten bais

The respective samples from each site (n=20 or 27) associated with each bai (n=10) were averaged for pair-wise comparison

^a Significant differences (Wilcoxon) between geophagical and control bai samples

^b Significant differences (Wilcoxon) between geophagical and control forest samples

^c Significant differences (Wilcoxon) between control bai and forest samples no water holes were found. And, in support of this baispecific mechanism, these bais showed the highest numbers of above average mineral concentrations (11 and 12, respectively, out of 14) among geophagical samples (Fig. 4).

In conclusion, water from the bottom of elephant holes appeared to represent an important source of minerals for elephants while geophagy was interpreted as playing a minor role at most bais surveyed. Sodium, S, Cl, and I are suggested as important attractants. Other large mammal species such as forest buffalo and bongo antelope regularly visit bais and use elephant excavated sites, likely attracted to the same resources. Results emphasized differences between bais and seasons regarding concentrations of various minerals in water and soil. Differences in mineral concentrations between and within sites (Magliocca 2000; Ayotte et al. 2006) and seasons (Kreulen 1985) might explain the 'failure' to define a single mineral driving geophagy and bai use by forest elephants and other mammal species (though methodological differences between studies is also a hindrance). In fact, these differences between sites indicate that the heterogeneity in bai systems as well as other mineral sites worldwide, e.g., licks in other tropical systems, may be critical to the survival and wellbeing of forest species. Consequently, the protection of multiple bais should be a conservation priority in order to avoid the exclusion of important or unique sites from protection planning. Studies on the mineral availability and concentrations at bais are needed over a broader region of Central Africa and in respect to the diversity of species using bais in order to provide insight regarding reliance on these features by at risk forest species.

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