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COMMUNICATION

PALYNOLOGICAL ANALYSIS OF FAECAL MATTER IN AFRICAN FOREST ELEPHANTS *LOXODONTA CYCLOTIS* (MAMMALIA: PROBOSCIDEA: ELEPHANTIDAE) AT OMO FOREST RESERVE, NIGERIA

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PLATINUM OPEN ACCESS



Okwong John Walter 10, Olusola Helen Adekanmbi 20 & Omonu Clifford 30

- ^{1,2} Department of Botany, Faculty of Science, University of Lagos, Akoka, Lagos, 100213, Nigeria.
- 1,2 Center for Biodiversity Conservation and Ecosystem Management, University of Lagos, Akoka, Lagos, 100213, Nigeria.
- ³ Forestry Research Institute of Nigeria, P.M.B 5054, Jericho, Ibadan, Nigeria.
- ¹okwong56@gmail.com (corresponding author), ²helen_olu@yahoo.com, ³clifford.omonu@gmail.com

Abstract: The factors affecting African Forest Elephants include food availability, demand for ivory and changes in land-use. In order to survive, they tend to traverse considerable distances in search of food; on such occasions they are trapped and killed for their ivory. This present study is aimed at assessing the faecal matter of elephants, and at providing information on the season of ingestion and foraging preferences of these elephants. Faecal matter was collected at nine different locations for one year before being processed and subjected to standard palynological laboratory procedures. The analyses showed that the samples had moderately abundant and diversified palynomorphs. A total of 27 palynomorphs belonging to 22 families with a total count of 2,895 accounting for 94.34% were found to be eaten, while other plant fragments (epidermal cells, xylem vessel elements, and seeds) accounted for 5.66%. The wet and dry seasons accounted for 73.26% and 26.74% respectively. Epidermal cells and xylem vessel elements recorded (70.76%) and (29.2%) during the dry and wet seasons, respectively. In the palynological analysis, pollen of *Balanites wilsoniana*, *Desplatsia subericarpa*, *Chrysophyllum albidum*, among others were recovered in the faecal matter. Pollen analysis of faecal matters provided no information about the quantitative composition of the natural vegetation of elephants, but rather valuable information about their diet. It is recommended that these preferentially foraged parent plants should be cultivated on a large scale. This would potentially reduce competition for food and movement of these animals to other greener areas, consequently leading to poaching.

Keywords: Diet, ivory, palynomorph types, pollen, sampling, southwestern Nigeria, vegetation

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Author details: MR. OKWONG JOHN WALTER is a PhD Student at the Department of Botany, University of Lagos, Nigeria. He is also a scientist at Botanical society of Nigeria, Palynological association of Nigeria and Center for Biodiversity Conservation & Ecosystem Management, University of Lagos, Akoka, Lagos, Nigeria. DR. OLUSOLA HELEN ADEKANMBI is a Senior Lecturer, working on environmental management and pollution monitoring and is the research adviser to number of PhD students. She is also the Deputy Director Research & Innovation at the University of Lagos. MR. CLIFFORD OMONU is a Research Officer at the Forestry Research Institute of Nigeria. He is currently heading an out station in Omo Biosphere Reserve, Ogun State. A UNESCO site managing the Strict Nature Reserve SNR. His research interest include general wildlife management, protected area community advocacy and environmental education with focus in Elephant conservation in Nigeria.

Author contribution: OJW designed and conducted the present study. CO assisted in the collection of samples, while OHA supervised the research work with technical inputs as research adviser.

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INTRODUCTION

The studies of faecal matter allows one to determine a wide range of biological information about a creature, including its diet, environment, health, and infections such as tapeworm. In combination with the analysis of pollen, an abundance of information about diet, disease, and general health can be acquired from such archaeological materials (Reinhard 1994). Not all faecal matter have the same potential information due to taphonomic conditions. Seasonal changes in the diets of African Forest Elephants Loxodonta cyclotis are difficult to quantify because of the difficulty of observing the same animal regularly on a long-term basis. Using their trunks, elephants are skilled at manipulating food and show strong preference for specific plant parts (i.e., bark, roots, leaves, and fruits; McKay 1973). This selection is driven by factors like palatability, nutrient composition, secondary and toxic plant compounds, physical defenses, and handling time (Sukumar 1989). Understanding the diet of elephants requires detailed information on the specific plants they consume, but this information is rarely reported. This ideology instigated the study on how pollen grains and other macro elements of plants could be used to reveal the foraging preferences and period of ingestion of plants by African Forest Elephants. These will provide significant insights into ecological requirements relevant for the management of wild elephant populations and their habitats and for the mitigation of human-elephant conflicts, since their existence has been threatened as a result of increased human population and anthropogenic activities. They are sometimes the only disperser of some tree species, such as Desplatsia subericarpa and Balanites wilsoniana. The rate of seed germination of many forest plant species increases significantly after passage through an elephant's gut (Barnes et al. 1991). It is worth stressing that the pollen in elephant coprolites gives a regional perspective of the palaeo-environment since these animals travel long distances in search of food. Elephants' coprolites reflect more regional pollen sources than sediment analysis. Also, elephants play a significant role in seed dispersal and maintaining plant diversity (Campos-Arceiz & Blake 2011). In spite of their uniqueness and key role in forest ecosystems, African forest elephant populations have depleted over the years owing to a number of factors including ivory poaching and trade across the globe, habitat loss through the conversion of land to agriculture and increasing competition for resources with growing human populations (Maisels et al. 2013). In southwestern

Nigeria, *Loxodonta cyclotis* have become in danger of extinction in many ecological zones while the remnant fragile population remains at risk of been endangered. As the human population increases more rapidly, the elephant kingdom is currently being broken down into smaller units traversed by roads, human settlement and infrastructures, thereby bringing elephants into conflict with humans. This is with a view to assisting people in management to make strategic decisions for the effective conservation of our forest reserves and animals. A better understanding and knowledge of grazing behaviour and foraging preferences of some plants by elephants will make it possible to develop a coherent strategy for the conservation and management of the forest reserve.

MATERIALS AND METHODS

Description of the Study Area

Omo Biosphere Reserve is located between 6'35'09.90 –7'05'04.94 °N and 4'19'21.28–4'40'21.16 °E (Fig. 1) in the south-west of Nigeria, about 135km northeast of Lagos, about 120km east of Abeokuta and about 80km east of Ijebu Ode (Ola-Adams 1999). The reserve shares a common boundary in its northern part with Ago Owu and Shasha forest reserves in Osun State. It also has a common boundary with Oluwa Forest Reserve in Ondo State (Karimu 1999); and covers 130,500ha of land (Ojo 2004).

Vegetation

The study locations are currently open vegetation that comprise tree species such as Celtis zenkeri, Diospyros dendo, Cleistopholis patens, Anthonathia macrophylla, Ficus exaserata, Canarium scheveinfurthii, Brachystegia eurycoma, Albizzia ferruginea, Cola negrica, Aistonia boonei, Ricinodendron heudelotti, Cordia millenii, Diospyros nigerica, Desplatsia subericarpa, Terminalia superba, Humeria umbellate, Musanga cecropioides, Entandrophragma angolense, Diospyros monbuttensis, Celtis brownie, Khaya ivorensis, Ficus mucuso, Macaranga barteri, Celtis mildbraedii, Pycanthus angolense, Paspalum viginatum, among others. The study area is also composed of herbs, climbers, epiphytes, stranglers, saprophytes and parasites (Adamson 1996). The reserve is in the mixed moist semi-evergreen rainforest zone. The northern parts of the reserve are relatively dry with typical species such as Sterculiar hinopetala. Nauclea diderrichii and Balanites wilsoniana are common in the wetter central parts (Ola-Adams 1999). As a result of continuous human activities, mainly logging and

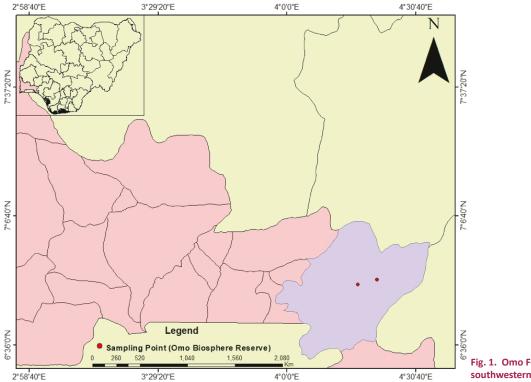


Fig. 1. Omo Forest Reserve in southwestern Nigeria.

farming for almost a century, the vegetation pattern in Omo has changed remarkably. The reserve now carries monocultures of *Gmelina arborea*, *Tectona grandis*, *Theobroma cacao*, and arable farmlands. Natural forests of varying degrees of disturbance and a 460-ha strict nature reserve (SNR) which was established in 1946 as an inviolate plot but later designated an SNR also exist. The SNR is in Area J1 by Omo River, just a few kilometers south of the confluence of Omo and Owena rivers in the north-central part of the reserve.

Sample collection

One-hundred-and-eight faecal matters collected monthly, from nine locations for a period of 12 months. The faecal matter was collected by removing the upper surface as well as the lower surfaces to form a thin section, thereby reducing the number of contaminants. The fresh faecal matter was then placed into sterilized plastic bags and then weighed and stored in tightly closed sterilized containers with a small amount of alcohol (70%) to avoid microbial growth and then frozen in the laboratory. This sampling period was divided into the wet and dry seasons. The strongest and the first wet period lasts between April and July while the second and weaker wet period between September and November. In between these wet periods is a relatively dry period in August to September commonly referred

to as the 'August Break'. The main dry season lasts from December to March and is usually characterized by harmattan winds from the north-east trade winds during November.

Laboratory analysis

Samples were placed into 10ml test tubes and washed using distilled water by centrifugation and decantation to remove alcohol. Potassium hydroxide (KOH) treatment was conducted for 10g of each faecal sample to remove humic materials and soften the other organic material. About 10ml of 5% KOH was added to each sample in the beaker and heated for several minutes. Samples were then sieved with a 1mm mesh to remove large organic particles that were then discarded. The remaining sample was placed in 15ml plastic screwtopped centrifuge tubes and centrifuged. The sample was then rinsed with distilled water and centrifuged multiple times. The residue was subjected to acetolysis method (Erdtman 1969). The acetolysis mixture of nine parts of acetic anhydride and one part of sulphuric acid (H₂SO₄) was prepared. This mixture was then poured into the tubes containing the residue and, boiled in a beaker at 100 °C for five minutes and was stirred occasionally with a glass rod. After cooling, the mixture was centrifuged at 2000rpm for five minutes. The liquid supernatant was then decanted leaving only the

palynomorphs in the tube. Samples were then stored in 100% glycerin to prevent the palynomorphs from drying out. From stock mixture, samples were collected and mounted on slides and studied under 40x objective lens magnification using an Olympus BX43 light microscope. A micropipette was used to pipette two drops of the prepared residue into a well-labeled slide and was stirred with the tip of the micropipette for even distribution. The coverslip was placed gently on the residue in a way to prevent the formation of air bubbles. The slide was sealed using a commercial nail lacquer and to make a semi-permanent slide. The prepared semi-permanent duplicated slides were then studied qualitatively and quantitatively using an Olympus BX43 light microscope under (400x magnification).

Taxonomic Identification

The identification of palynomorphs was made by comparing with some pollen albums, relevant journals (Sowunmi 1995; Gosling et al. 2013), and pollen reference slide collection in the Palynology and Palaeobotany Laboratory, Department of Botany, University of Lagos. Photomicrography of some of the identified palynomorphs was taken with the aid of a Motic 2300 digital camera. Pollen was identified up to the family level and where possible up to the species level. Those that were unidentifiable due to their broken nature were referred as indeterminate.

RESULTS AND DISCUSSION

Analyses showed that the samples have moderately abundant and diversified palynomorphs. The elephants consumed different plant species with varying degrees of preferences. Twenty-seven palynomorphs belonging to 22 families accounting for 94.34% were found to be eaten (Table 1). These Families include Zygophyllaceae which accounts for 882 (32.2%), Poaceae 410 (14.93%), Tiliaceae 295 (10.8%), Fabaceae 162 (5.91%), Irvingiaceae 135 (4.94%), Amaranthaceae 108 (3.95%), Lamiaceae 99 (3.62%), Asteraceae 85 (3.11%), and Calophyllaceae 69 (2.48%) while the least families are Sapindaceae 5 (0.18%) and Mimosaceae 4 (0.14%). Epidermal cells and xylem vessel elements recorded (70.76%) and (29.2%) during the dry and wet seasons, respectively. This has provided information on the season during which this large terrestrial mammal debarks trees more. Seeds were recovered in the faecal matter during sample preparation. The seeds recorded all-time high during the dry season (November–March), but few were also

recorded during the end of the wet season (August–October). The occurrences of these palynomorphs are displayed on a Tilia $^{\text{TM}}$ graph (Fig. 2).

Elephants browse and graze on a variety of plants but the time spent foraging and the proportions of the plants consumed vary depending on the season and availability (Fig. 2). The ratio of recovery of pollen grains in the faecal matter between Poaceae (grass), herbs (Tridax procumbens), and higher plants suggest that Loxodonta cyclotis spent more time browsing than grazing despite the availability of grass in both the wet and dry seasons (Fig. 2). Also, the occurrences of Mangifera indica, Desplatsia subericarpa, Balanites wilsoniana among others in their faecal matter conforms to the assertion made by Amusa et al. (2017) in which he reported that the African Forest Elephant is mostly a browser and frugivore rather than the grazing and browsing habit exhibited by the Savanna Elephant. The drastic increase observed in Poaceae, Asteraceae, Zea mays, and Tridax procumbens, however, indicate an obvious change in feeding preference with grazing becoming more important during the wet season. Browsing materials such as Zea mays, Parkia biglobosa, Mangifera indica, Chrysophyllum albidum, among others were therefore abundant in the dry season compared to the wet season (November-March).

The pollen analysis revealed that fruit was an important component of the diet, while Balanites wilsoniana is the most preferred plant for forage by forest elephants especially in the dry season, despite its unpleasant smell. Its recovery in these studies may suggest that at this time of the year a maximum number of plant species are flowering, while some are fruiting. Fruits such as Irvingia gabonensis, Chrysophullum albidum, Parinari excelsa, among others are characterized by firm, dry, dense flesh, and are therefore rich in lipids and proteins (Mckey 1975). Furthermore, the analyses revealed that during the second half of the main dry season, the lean season for most animals, there was a peak in fruiting of animal-dispersed tree species. Throughout this period forest elephants move from their preferred secondary vegetation, as well as the logging sites in which the pollen of Zea mays (March-May) and Treculia africana (July-December), ELaeis guineensis (Febuary-May) were used as a pointer, to denote the period at which the African Forest Elephants encroaches on farmlands where fruiting trees and crops of favoured species are to be found. This assertion is also supported by Merz (1986), Barnes et al. (1991), and White (1994). According to Chapman et al. (1992) the tree species, Balanites wilsoniana substantiated this hypothesis with

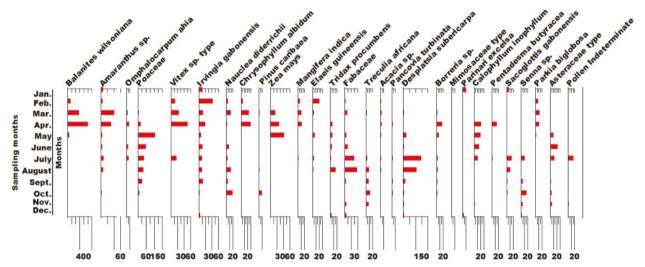


Figure 2. Pollen calendar of palynomorphs recovered from African Forest Elephant faecal matter at Omo Forest reserve.

germination trials on fresh and ingested seeds of eight trees and one species eaten by forest elephants. Such research has led to the understanding that several tree species could become extinct in the absence of forest elephants.

The family Poaceae were the second largest group that were found to be eaten. Poaceae abundances can be attributed to its ubiquitousness and their ability to produce in large quantities. The clear preference for grass over browses exhibited by the elephant in the wet season (April-October) is probably related to the protein content of the food available, as a result of high nutrient content, low toxins, and fibre contents (Lindsay 1994), it also provides a return per unit time feeding that is higher than browse. It lacks certain essential nutrients, however, and at maturity its nutrient content becomes very low. Their low recovery in the dry season could be linked to the reduction in moisture content which becomes more fibrous and abrasive, hence causing increased wear on teeth and a decline in digestive efficiency. This supports the view of (McCullagh 1969) that there is a decrease in the digestibility of protein when the protein content of a food item is low and the fibre content high. These support the claim made by Sinclair (1975) that this is apparently a limiting factor in any herbivore/resource relationship. This may account to some extent for the large amounts taken, as the elephant at the start of the wet season, may have been choosing the component with the highest protein level. The elephants graze throughout the year but grazing activity becomes unimportant when grass becomes dry and coarse. It could also be argued that elephants are primarily grazers because large quantities of grass

are eaten even when large quantities of browse are available. It does not seem that elephants have been forced to adopt a primarily grazing habit, as asserted by Sikes (1971), but have in fact always been grazers, and will always graze when large enough quantities of grass are available; yet factors such as digestibility, quality of food and ease of gathering also have to be taken into consideration. Laws (1970) stated that elephants when feeding on large quantities of young grass take bark as a form of roughage. The increasing number of epidermal cells and xylem vessel elements recovered in the month of May and (November-February) supports the assertion made by Laws (1970). The elephants debarked trees both in wet and dry seasons, but more particularly during the dry season, possibly because of the increased translocation of food substances from the roots to the new flushing leaves.

Desplatsia subericarpa Bocq. was the third most foraged species and was found to be abundant during the end of the wet season and the beginning of the dry season. It bears large fruits with a hard seed coat. The pollen analysis revealed that the African Forest Elephants are attracted to large fruits, as a result of their body size. Furthermore, there was a positive correlation between herbivore body size, the size of food intake and dietary breadth, but still dependent on the diversity and composition of the plants available. These highly frugivorous diets make them particularly formidable dispersers of seeds which could be referred to as 'megafauna-syndrome', i.e., plants with large fruits and seeds that may have evolved to attract large herbivores to consume and disperse them.

Crops which include Amaranthus sp., Zea mays,

Table 1. The frequency and percentage composition of palynomorphs recovered from faecal matter of *Loxondata cyclotis*.

	Palynomorph types	Family	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	%
1.	Amaranthus sp.	Amaranthaceae	6	5	41	31	3	4	8	6	1	2	1	0	108	3.95
2.	Mangifera indica L.	Anacardiaceae	0	6	10	12	0	0	1	0	0	0	0	0	29	1.06
3.	Elaeis guineensis Jacq	Arecaceae	0	21	4	0	7	0	3	2	1	0	0	0	38	1.39
4.	Tridax procumbens L.	Asteraceae	0	0	0	6	5	4	1	16	0	0	0	3	35	1.28
5.	Asteraceae type	Asteraceae	0	0	0	0	9	22	11	0	1	3	4	0	50	1.83
6.	Calophyllum inophyllum L.	Calophyllaceae	0	0	0	21	19	15	11	0	0	0	2	0	68	2.48
7.	Parinari excelsa Sabine	Chrysobalanaceae	8	0	0	0	0	0	0	0	0	0	0	2	10	0.36
8.	Pentadesma butyracea Sabine	Clusiaceae	0	0	0	14	0	0	2	0	0	0	0	0	16	0.58
9.	Parkia biglobosa(Jacq) R. ex Don-H.C	Fabaceae	0	6	11	9	1	0	0	0	0	0	0	0	27	0.98
10.	Acacia sp.	Fabaceae	0	1	2	2	1	0	1	1	0	0	0	0	8	0.29
11.	Senna hirsuta L.	Fabaceae	0	0	0	0	0	0	10	0	4	15	4	1	34	1.24
12.	Fabaceae	Fabaceae	0	1	7	0	4	8	28	36	2	0	3	4	93	3.40
13.	Sacoglottis gabonensis (Baill.) Urb.	Humiriaceae	6	0	0	0	0	0	15	11	2	2	0	0	36	1.31
14.	Irvingia gabonensis (Aubry Lecomte)	Irvingiaceae	10	42	12	19	6	10	9	9	12	2	1	3	135	4.94
15.	Vitex sp. type	Lamiaceae	0	11	23	50	0	0	15	0	0	0	0	0	99	3.62
16.	Mimosaceae type	Mimosaceae	0	0	0	0	0	0	0	1	1	1	0	1	4	0.14
17.	<i>Treculia africana</i> Decne.	Moraceae	0	0	2	0	0	0	3	8	7	12	7	0	39	1.42
18.	Pinus caribaea Mor.	Pinaceae	0	0	1	0	0	0	0	1	0	8	0	0	10	0.36
19.	Poaceae	Poaceae	0	2	5	18	125	55	42	38	30	9	3	0	327	11.9
20.	Zea mays L.	Poaceae	0	0	15	26	42	0	0	0	0	0	0	0	83	3.03
21.	Borreria sp.	Rubiaceae	0	0	1	15	5	0	2	3	1	1	0	0	28	1.02
22.	Nauclea diderrichii (De Wild. & Th. Dur.)	Rubiaceae	0	4	12	1	0	7	2	12	4	18	0	0	60	2.1
23.	Pancovia turbinata Radlk.	Sapindaceae	0	0	0	1	0	0	1	0	2	1	0	0	5	0.18
24.	Chrysophyllum albidum G.Don.	Sapotaceae	0	5	24	29	0	1	0	1	0	0	0	0	60	2.19
25.	Omphalocarpum ahia A.Chev.	Sapotaceae	0	0	3	7	0	4	5	0	0	0	0	0	19	0.69
26.	Desplatsia subericarpa (Bocq)	Tiliaceae	0	0	1	0	21	1	145	104	15	4	3	1	295	10.8
27.	Balanites wilsoniana Dawe & Sprague	Zygophyllaceae	1	65	271	494	32	0	0	2	6	4	5	2	882	32.2
28.	Pollen Indeterminate	Pollen Indeter.	0	0	0	0	0	0	15	0	0	0	1	0	16	0.58
		Total pollen count	31	169	445	755	280	131	330	251	89	164	34	52	2731	94.3
29.	Xylem fibers	Plant Fragment	13	16	0	0	20	0	0	0	0	0	17	11	78	2.84
30.	Seeds	Plant Fragment	1	7	4	0	0	0	0	9	3	13	11	19	53	2.82
Total plants fragments		14	23	4	0	20	0	0	9	3	13	38	30	154	5.66	

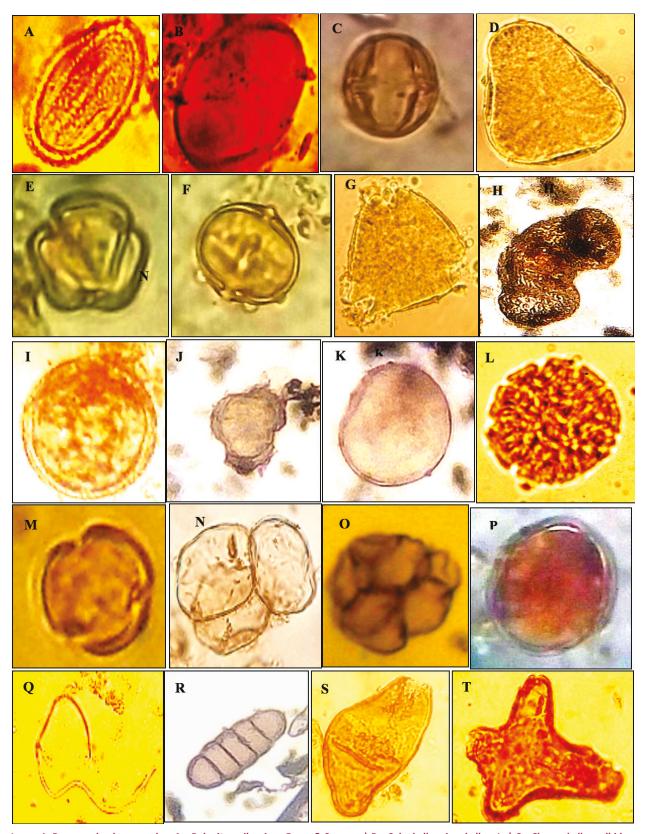


Image 1. Recovered palynomorphs: A—Balanites wilsoniana Dawe & Sprague | B—Calophyllum inophyllum L. | C—Chrysophyllum albidum G.Don. | D—Elaeis guineensis Jacq. | E—Nauclea diderrichii (De Wild. & Th. Dur.) | F—Omphalocarpum ahia A. Chev. | G—Parinari excelsa Sabine | H—Pinus caribaea Mor. | I—Senna hirsuta L. J—Tridax procumbens L. | K—Zea mays L. | L—Borreria sp. | M—Vitex sp. | N—Poaceae | O—Mimosaceae type | P—Fabaceae Q—Ascaris worm | R—Fungi spores | S—T—plant fragment (scale=10μm).

Mangifera indica, Elaeis guineensis, and Fabaceae were also found to be eaten and intensively used by the rural population. Their abundances in the faecal matter indicate farmland incursion by the African Forest Elephants during the months of February–May. Despite the large array of plants available for forage in the reserves, forest elephants still consume and destroy farm produce. This attests to the fact that the African Forest Elephants are species specific when it comes to their foraging preferences. Therefore, it should come as no surprise that elephants have an attraction for crops which are found to be nutritious and palatable, thus resulting in human-elephant interactions and sometimes death for both parties. Habitat fragmentation, however, increases the contact between elephants and agriculture, and the intensity of crop-raiding is usually higher in more fragmented habitats.

Pollen grains of Callophyllum inophyllum, were also recovered, this plant has been reported by several authors as toxic. Their presence in the faecal matter signifies that the African Forest Elephants take in toxic plants to balance their nutritional requirements. Therefore, detailed studies should be carried out on the nutritional value vs toxicity, and the mechanisms behind these choices. Some plants appeared to be fed on only incidentally, perhaps when the species was being browsed. The plants—Acacia sp., Pancovia turbinata, Mimosaceae, Parinari exelsa, Pinus caribaea, Pentadesma butyracea, and Omphalocarpum ahia were less than one percent in representation. These were mostly the smaller sized fruits which were commonly eaten by other animals, thus they may form a valuable supplement to the diet, and also mainly for their water content. This usually happens when they traverse a considerable distance in search of food in areas where there is little or no presence of water.

The presence of xylem fibers in the samples supports the claim made by Sukumar (1989) that the consumption of bark helps to cover the calcium needs of elephants, and may consequently serve more than just for satisfying hunger. Furthermore, there was an abundance of fungal spores recovered. This denotes that the Feacal matter may have retained some moisture during defecation by the African forest Elephants which favours fungal growth. The high relative humidity in the study location may have allowed for greater moisture retention, making it ideal for fungal invasion.

The information obtained from the faecal matter using the palynological method helped to describe the diet of *Loxodonta cyclotis* and to understand the paleoenvironment of the study location. Therefore, these

selected plants should be cultivated on a large scale in our forest reserves to restrict the movement of African Forest Elephants in search of food, which can lead to poaching as well as human-elephant interactions. Our forest reserves without the African Forest Elephants would be less in species richness and less structurally diverse. Likewise, the African Forest Elephants affect the complexity of the forest by spreading ingested seeds during defecation and help maintain open areas for the reflection of high light intensity near the forest floor, leading to the proliferation of grasses, climbers, shrubs, and young trees to spring up. Nevertheless, conservation concepts based on strictly intellectual or aesthetic values understandably may have little meaning to local villagers who have to struggle for their existence (Nepal & Weber 1995). In order to end the negative interactions between African Forest Elephants and the human populace in rural areas, multidisciplinary approaches must take into account the requirements needed for both elephants and humans so as to achieve sustainable forest management goals.

CONCLUSION

Feeding and nutritional ecology is key to elephant conservation. There is a need for the conservation and management of certain species of trees and grasses for the continued existence of forest elephants in the concerned forest reserves. It is recommended that these preferentially foraged parent plants should be cultivated on a large scale. This would potentially reduce competition for food and movement of these animals to other greener areas, consequently leading to poaching. Also, for a qualitative and quantitative assessment the habitat characteristics, techniques and data from many different disciplines must be combined; however, there is a need for continuous sensitization, support and empowerment of members of the host communities through community social responsibility initiatives to make sure they take part in the conservation and afforestation. Furthermore, nature conservation issues must be dealt with by considering also the needs of humans, since the existence of a native human population in any place involves complex interactions of ethnic, social, economic, political, historical, and biological aspects that exceed a strictly ecological approach. These will pose a positive outcome for the African Forest Elephants in the days ahead.

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