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Phytolith and Calcitic Spherulite Indicators from Modern Reference Animal Dung from Mediterranean Island Ecosystems: Menorca, Balearic Islands

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Abstract: This study illustrates the contribution of plant and faecal microfossil records to interdisciplinary approaches on the identification, composition, taphonomy and seasonality of livestock dung materials. The focus is on the taphonomy of opal phytoliths and calcitic dung spherulites embedded within modern faecal pellets collected from pasture grounds and pens from a range of animals, including cattle, sheep and pigs from three different farms and seasons of the year in Menorca (Balearic Islands, Spain) declared a Biosphere Reserve by UNESCO. Modern reference materials provide comparative plant and dung microfossil indicators on factors affecting the formation, composition, preservation and decay of animal faeces, as well as on the diverse environmental and anthropogenic aspects influencing these. The reported results show relevant changes in phytolith and spherulite composition according to animal species and age, livestock management, seasonality, and grazing and foddering regimes. Both microfossil records provide fundamental information on taphonomic issues that are understudied, such as the variation in the digestibility among different species, including under investigated animals such as pigs, as well on the seasonality of plant and faecal microfossils that are excreted with dung as an important material for reconstructing human-environment interactions which is commonly overlooked in archaeology.

Keywords: Mediterranean; Balearic Islands; livestock dung; modern dung reference collection; phytoliths; dung spherulites

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1. Introduction

Over the last few decades varied archaeobotanical and geoarchaeological methodological approaches have highlighted the fundamental importance of livestock dung as it embeds critical information on diverse ecological and past cultural ways of life ([1,2] and references therein). A range of studies conducted on modern dung materials including phytolith and dung spherulite records, have investigated herbivore faeces and stabling floors, primarily from herds of sheep, goats, and cattle, although studies on other important animals in many areas of the world such as suids as well as commensals are still underdeveloped [3–12]. Most of the basic processes of formation, composition, morphology and taphonomy of both opaline phytoliths [13–21] and calcitic dung spherulites [1,3,4,22–25] are well understood. However, critical information on taphonomic issues, such as the variation in their digestibility and durability among different animal producers, including less investigated species such as pigs, linked to dietary practices, age/sex-based aspects, as well on seasonality of these plant and faecal microfossils that are excreted with dung have not yet been fully investigated.

The current study builds on previous work on modern dung, primarily from ovicaprines and cattle, to address livestock management within the local landscape and to elucidate links between animal and plant resources through the study of the phytoliths and calcitic spherulite records embedded within animal dung deposits from Mediterranean inland ecosystems [9,10,26–29]. This study expands the modern reference records with new modern dung materials from Menorca (Balearic Islands, Spain). The island is home to a remarkable diversity of ecosystems and rural landscapes, as well as to rich archaeological late Bronze and Iron Age (Talayotic) heritage. The archaeofaunal records indicate that the main domestic species widespread across the Mediterranean, sheep (*Ovis aries*), goat (*Capra hircus*), cattle (*Bos taurus*), pig (*Sus domesticus*), as well as other domestic species such as dog (*Canis familiaris*) and rodents (house mice, rats), were introduced to the island along with the first human populations during the III millennium cal BC [30–34]. The faunal assemblages from later Talayotic Iron Age sites such as Cornia Nou in Mahon, located in our research area on the eastern side of Menorca (Figure 1), have revealed insights into ovicaprine herding (*Ovis*, *Capra*), and to a lesser extent from cattle (*Bos taurus*) and suids (*Sus domesticus*), as well as pollen assemblages indicative of herbaceous ruderal and nitrophilous vegetation related to husbandry [35,36]. Further direct evidence from dung remains come from the Talayotic settlement of Torre d'en Galmés (Alaior), in the southern portion of the island, where the identification through soil micromorphology of spherulithic ashes within domestic contexts from building areas points to the use of animal dung a source of fuel [37,38].

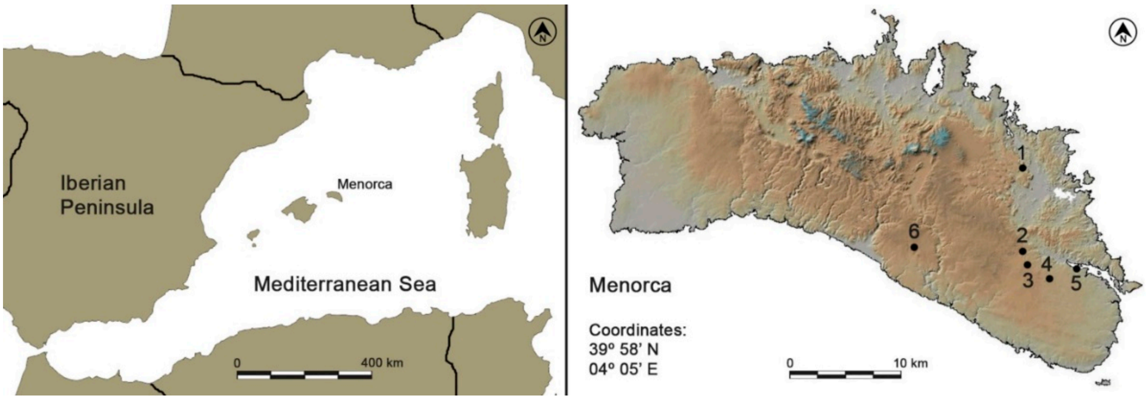

Figure 1. Localities in Menorca cited in the text. (1) Es Capell de Ferro (Mahon); (2) Algendaret Nou (Mahon); (3) Talatí de Dalt (Mahon), (4) Cornia Nou (Mahon); (5) Museum of Menorca (Mahon); (6) Torre d'en Galmés (Alaior).

In light of these previous studies, the focus in the current work is on the phytolith and calcitic dung components of modern faecal pellets collected from pasture grounds and pens from diverse adult and young animals, including cattle and sheep in addition to the lesser investigated pigs [9]. Dung samples correspond to three neighboring farms located in Mahon, displaying varied agricultural and livestock management strategies and animal diets. Furthermore, an innovative aspect of the reported research is the sampling strategies, focusing on the collection of a range of dung materials from different periods of the year to evaluate variations on seasonality of phytolith and calcitic spherulithic microfossils that are excreted with dung, as well as differences in their composition according to animal species and age, livestock management, and grazing and foddering regimes. The main objective is to contribute a better understanding of both microfossil records, in order to evaluate their implications for the identification of dung assemblages as fundamental reference frameworks for the interpretation of these important materials for reconstructing human-environment interactions which are commonly overlooked in archaeology.

Study Area

The study area is located on the southeastern edge of Menorca (Balearic Islands) close to the port of Mahon, considered one of the largest natural harbors in the Western Mediterranean (Figure 1). The area has a typically temperate Mediterranean climate with mild winters and warm, dry summers, and considerable fluctuations in rainfall. This area receives 650 mm of annual rain on average, characterized by a high annual variability, with rainfall mostly concentrated during the seasons of spring and autumn [39]. The total rainfall registered in the Mahon Airport weather station (B893) during the sampling field seasons (from September 2018 to June 2019) was 225 mm (Table 1). The research area is placed on a chromic cambisol in the Miocene platform that occupies the southern half of the island of calcarenite allowing seasonal flowing to seep through to the water tables with its height close to the sea level. Cambisols are suitable for agriculture as they provide available nutrients and depth for optimal growth, and these are the home of most Talayotic settlement sites [40]. The current vegetation in the area displays *Oleo-Ceratonion* communities and is dominated by sclerophyllous plants, whereas in deeper soils the *Cyclamini-Quercetum ilicis* communities are widespread, and its degradation allows the occurrence of large areas with Aleppo pine (*Pinus halepensis*) and mastic (*Pistacia lentiscus*) [41].

Table 1. Total monthly precipitation and average relative humidity records from Mahon Airport weather station, Menorca. Data obtained from the State Meteorological Agency (AEMET).

Month/Year	Precipitation (mm)	Humidity (%)
September 2018	57.4	73
October 2018	43.1	77
November 2018	25.8	82
December 2018	11.3	83
January 2019	46.3	75
February 2019	3.8	77
March 2019	4.6	74
April 2019	28.4	73
May 2019	4.1	61
June 2019	0.2	60
Total precipitation/average humidity	225	73.5

At present, cattle and dairy farming in particular play a key role in the island, with a production characteristic of the Western Mediterranean region, traditionally dry land in winter and with a use based on grazing [42]. The Balearic Islands have a total of 213 dairy farms, with an annual quota of 84 million kilos of milk (2011 data in [42]). Most of these farms are located in Menorca, which represents around 73% of the total milk production of the islands, intended mainly for the production of cheese of the Mahon-Menorca protected designation of origin (Mahon-Menorca PDO). In addition to livestock farming of cattle, sheep and pig, the latter for meat and minorcan pork sausage (locally called *sobrassada*), many of these farms on the island produce fodder (mainly barley and oat) and use manure on their own farmland as fertilizer. The widespread traditional system of parceling and division of the land comprise a wide mesh of drywalls, namely *pedra seca* or dry-wall constructions. UNESCO declared the island a biosphere reserve in October 1993, which is characterized by a rich diversity of Mediterranean natural habitats and resources, but also by a remarkable tradition of rural landscapes [43–46].

2. Materials and Methods

2.1. Modern Dung Material Sampling

The modern materials examined in the current study (cow, sheep and pig dung pellets) belong to three neighboring farms, Algendaret Nou, Talatí de Dalt, and Es Capell de Ferro, located in the eastern portion of Menorca close to the port of Mahon and many Talayotic archaeological sites (Figure 1). Previous experimental work in one of these settings of Mahon, Algendaret Nou, reported on the plant microfossil records from barley crop production (phytoliths and pollen grains) manured with their own farmland cattle dung, to assess the impact on cereal processing through grain cleaning and grinding into flour using Talayotic stone tools [47]. The present work enlarges modern reference studies and focuses in particular on livestock management and grazing/foddering records, with the sampling of new faecal materials from penning areas and pasture grounds, including informal interviews and photography within three farms using a questionnaire following ethical protocols, and with full permission. The aim in collecting modern dung remains was to study different grazing and foddering regimes, according to three different animal species and age, livestock management, and grazing and foddering regimes, including less investigated animals such as pigs, as well on seasonality of plant and faecal microfossils that are excreted with dung. This study focused on fresh dung samples primarily from ruminants (cattle and sheep), as these are prolific producers of dung spherulites [4], in addition to herbivorous pigs.

The dung samples collected in the current study reported below come from animals kept either in enclosures or semi-free-ranging herds within parceled pastoral grounds during most of the year. Cattle and pigs display a grass-rich diet based on grazing and supplementary fodder, primarily barley or oat chaff from the household's own production (in the case of pigs either barley grains or barley flour), in contrast with sheep which are grazers with no supplementary fodder. Diet regimes are diverse according to the different farm choices and resource availability across the different periods of the year (Figure 2). Sample collection took place in three different seasons: early autumn (end of September 2018), winter (middle of February 2019), and late spring-early summer (middle of June 2019). Table 2 summarizes the main livestock and crop management and production characteristics along with the number of animals kept, the secondary use of dung as fertilizer from the farm's own livestock for crops (cattle dung) or gardens where fruit and vegetables are cultivated for food and fodder (either cattle or pig dung), penning and grazing/foddering within their own farm crop fields and pastoral parceled grounds. Livestock grazing and foddering regimes are then summarized according to the three main seasonal periods reported in the present study–early autumn, winter, and late spring-early summer (Table 3). All samples were stored at the Museum of Menorca in Mahon prior to exportation to the Autonomous University of Barcelona for microfossil analyses. A total number of twenty-eight dung samples were selected for integrated phytolith and calcitic dung spherulite studies and served as modern reference standards in this study.

Figure 2. Livestock management and seasonal grazing and foddering at the study area, Mahon (Menorca). AN: Algendaret Nou; TD: Talatí de Dalt; CF: Es Capell de Ferro. (**a**) cattle penning, AN, February 2019; (**b**) sheep grazers, AN, September 2018; (**c**) pigs kept in enclosures, AN, September 2018; (**d**) cattle eating oat straw fodder, TD, February 2019; (**e**) sheep grazers within the pasture ground, TD, February 2019; (**f**) daily cattle grazing within the pasture grounds, CF, September 2018; (**g**) pigs kept in an open-air enclosure, CF, February 2019; (**h**) pig dung and chewed palm leaves (arrow) within the same enclosure CF, September 2018.

Table 2. Livestock and crop management practices. C: cattle, S: sheep, P: pig, Ch: chicken, R: rabbit.

Animal and Crop Management	Algendaret Nou (26 Ha)	Talatí de Dalt (64 Ha)	Capell de Ferro (95 Ha)
Number of animals	C: 9 adults (1 male) and 7 calves S: 15 P: 11 Ch: 70	C: 11 adults (1 male) and 10 calves S: 70	C: 19 adults (1 male) and 12 calves P: 21 Ch: 87 R: 1
Livestock production	Meat, cheese, eggs	Meat	Meat, Minorcan pork sausage (*sobrassada*)
Crops (rotation)	Barley (2 Ha) and oat (2 Ha) for livestock fodder	Oat for livestock fodder (18 Ha)	Oat (8 Ha) and barley for fodder (6 Ha), sulla (*Hedysarum*) for silage (5 Ha)
Fertilizer	Dung from own livestock, seaweed (macroalgae, salt), purchased fertilizer	Dung from own livestock during grazing, purchased fertilizer	Dung from own C during grazing, purchased fertilizer; P dung from penning cleaning used to fertilize the vegetable garden
Penning	C/S separately adults and calves, but S/P non penned	No	P/R separately adults and calves (3 palms within the enclosure), but C non penned
Grazing/ foddering	Grazing within the farm fields; C: straw, own barley/oat grains, purchased feed; P: barley grains, whey from own cheese manufacture	Grazing within the farm fields; C: straw, own oat straw and grain cultivated and processed at the farm	C grazing within the farm fields; C: straw, own oat grain, sulla (*Hedysarum*) silage made from own crops; P: own barley flour produced at the farm; purchased feed for Ch/R

Table 3. Seasonal grazing and foddering with indication of supplementary feed (SF). C: cattle, S: sheep, P: pig.

Season	Animal	Algendaret Nou	Talatí de Dalt	Capell de Ferro
Early Autumn	C	Grazing within the farm pasture fields (calves close to the house), fallow crop fields (rotation), own straw barley, purchased feed (barley, wheat, oat for adults and barley flour for calves) (SF)	Grazing within the farm pasture fields close to the house, fallow crop fields (rotation), straw, own oat grains in late summer (SF)	Grazing within the farm pasture fields (changing locations), fallow crop fields (rotation), straw own oat and sulla (*Hedysarum*) silage (SF)
	S	Grazing within the farm pastures and fallow crop fields	Grazing within the farm pastures and fallow crop fields	n/a
	P	Grazing within the farm pasture fields (changing locations), own barley grains, barley flour only in late autumn (SF)	n/a	Own barley flour processed at the farm
Winter	C	Grazing within the farm pasture fields (calves close to the house), own straw, purchased feed (barley, wheat, oat and barley) (SF)	Grazing fields close the house, own oat straw (SF)	Grazing within the farm pasture fields (changing locations, rotation), own oat straw, own sulla (*Hedysarum*) silage (SF)
	S	Grazing within the farm pastures	Grazing within the farm pastures	n/a
	P	Grazing within the farm pasture fields (changing locations), whey from own cheese production, purchased barley and flour (SF)	n/a	Own barley flour processed at the farm
Late Spring/Early Summer	C	Grazing within the farm pasture fields (calves close to the house), straw, purchased feed (barley, wheat, oat), green leaves (SF)	Grazing within the farm pasture fields close to the house, straw, own oat (SF)	Grazing within the farm pasture fields (changing locations, rotation), straw, own oat (SF)
	S	Grazing within the farm pastures	Grazing within the farm pastures	n/a
	P	Grazing within the farm pasture fields (changing locations), purchased barley, own barley in late spring, no flour (SF)	n/a	Own barley flour processed at the farm

2.2. Phytolith and Dung Spherulite Analyses

Dung materials including cow, sheep and pig dung pellets were dried and ashed in laboratory-controlled conditions (at 500 °C for 4 h using a muffle furnace). Phytolith analyses followed the methods of Katz and colleagues [48]. A weighed aliquot of 40 mg of ashed material was treated with 50 µL of a volume solution of 6N HCl. Phytoliths were then concentrated with 450 µL 2.4 g/mL of sodium polytungstate solution [$Na_6(H_2W_{12}O_{40})$]. Aliquots of 50 µL of material were mounted on microscope slides using 24 × 24 cover slips. Phytoliths were examined in random fields at ×200 and ×400 magnification using an BX-43 optical microscope. Photomicrographs were captured with a Color View (Olympus) camera. A minimum of 200 phytoliths with diagnostic morphologies were counted from each sample whenever possible. Morphological identification was based on modern plant reference collections from the Mediterranean area [10,17,49–51] and standard literature [15,16,52–56]. Where appropriate, the terms used to describe phytolith morphologies follow the International Code for Phytolith Nomenclature–ICPN 2.0 [57].

For dung spherulite analyses, samples of 40 mg of ashed material were treated with 450 µL 2.4 g/mL of sodium polytungstate solution. Similarly to the phytoliths, microscope slides were also mounted with aliquots of 50 µL of sample. A minimum of 200 spherulites were counted from each sample whenever possible at ×200 magnification, with occasional examination at ×400, under the optical microscope with cross-polarized light (XPL).

Samples were then compared to phytolith and spherulite ethnoarchaeological and experimental burning records of modern livestock dung materials that have followed a similar quantitative approach [9,10,25–27,29,58]. Principal component analysis (PCA) was conducted in IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY, USA) to investigate the relationships between the ratios of grass inflorescences to leaves/stems with the species (cow, sheep, pig), farm (and therefore foddering practice) and season. Scatterplots were generated to visually identify and highlight groupings.

3. Results and Discussion

3.1. Phytolith Composition, Taphonomy and Seasonality

A major aim in the current study was to assess variations in phytolith distributions and morphotype composition and whether these may be related to different animal defecators and livestock management, grazing and foddering practices, as well to seasonality of these particular plant microfossil assemblages that are embedded within the dung. The estimated phytolith numbers are based on abundances per weight of ashed dung material (Table 4). The relative abundances of the main consistent morphotypes identified in the whole assemblages are expressed as averages of percentages of the total identifiable phytoliths (Figures 3–5). Those phytoliths which were unidentifiable because of pitting and etching were recorded as weathered morphotypes. Ratios between individual (single-celled phytoliths) and multicellular phytoliths (multi-celled or in anatomical connection) were also calculated. Both the weathered and multicellular records are widely used as indicators of the state of preservation of phytolith assemblages, as well as regarding the extent of silicification of plant cells e.g., [10,17,27,50]. In modern fresh dung standards it is recognized that phytolith morphological integrity and preservation may also be affected by either the extent of silicification of plant cells ingested, as well as their solubility during digestion and excretion and variation in gut and bowel conditions [25], closely related to ecology and variability in dietary practices, seasonality, animal producers and age-based aspects, among others.

Table 4. Main quantitative phytolith results obtained from modern dung samples with indication of animal producer and known age (based on data from informants). Provenance (farm): AN: Algendaret Nou; TD: Talatí de Dalt; CF: Es Capell de Ferro. Month: S: September 2018; F: February 2019; J: June 2019.

Sample n.	Location-Month	Phytoliths 1 g of Ashed Material (Million)	Phytoliths Weathering (%)	Multicelled Phytoliths (%)	Ratio Individual/Multicelled Phytoliths	Grass Phytoliths (%)	Ratio Grass InfloresCences/Leaves-Stems	Description
1	AN-S	0.7	5.3	9.6	9.4	88.1	0.53	Cow (15 days)
6	AN-S	12.6	1.6	55	3.6	96.3	1.48	Cow
7	AN-S	10.9	2.1	13.2	6.5	93.8	0.33	Sheep
8	AN-S	7.1	3.6	4.5	21.2	97.4	8.76	Pig
11	TD-S	10.2	0.8	6.3	14.9	94.6	0.48	Cow
12	TD-S	9.9	3.6	11.7	7.6	92.4	0.37	Cow (1 month)
13	TD-S	12.6	3.3	3.7	26	91	0.38	Sheep
18	CF-S	7.5	4.8	13.5	6.4	91.3	5.25	Pig
22	CF-S	8.4	1.9	5.8	16.1	95.4	8.2	Pig
23	CF-S	6.6	0.9	4.7	20.4	98	8.6	Pig (15 days)
26	CF-S	11.9	0.4	12.1	7.2	95.5	0.63	Cow
28	TD-F	16.4	2.1	10.3	8.7	84.5	0.04	Sheep (1 month)
29	TD-F	7.3	3	12.3	7.1	86.3	0.04	Sheep
30	TD-F	18.6	0.8	8.7	10.5	95.9	0.28	Cow
34	AN-F	0.8	5.5	3.9	24.4	87.1	0.35	Pig
35	AN-F	6.3	3.4	10.6	8.4	87.8	0.04	Sheep
38	AN-F	16.2	2	19.5	4.1	95	0.39	Cow (3–4 months)
39	AN-F	23.2	1.2	8.5	10.7	95.9	0.66	Cow
47	AN-J	4.1	1.8	6.8	13.8	95.9	7.63	Pig
49	AN-J	12.8	3.2	8.2	11.2	91.1	0.61	Sheep
50	AN-J	10.4	3.6	7.5	12.3	91.8	1.51	Sheep (1 month)
54	AN-J	9.3	3.2	15	5.7	92.1	0.43	Cow (1 month)
56	AN-J	11.2	3	15.8	5.3	94.5	0.78	Cow
57	TD-J	10.4	2.3	6.6	14.2	92.1	0.22	Cow
60	TD-J	9.3	2.9	24.4	3.1	90	1.26	Sheep
62	CF-J	17.9	0.8	8.9	10.3	93.3	0.16	Cow
65	CF-J	3.4	1.3	12.4	7	93.3	6.25	Pig (2–3 months)
66	CF-J	6.6	0.5	16.6	5	98.2	6.65	Pig

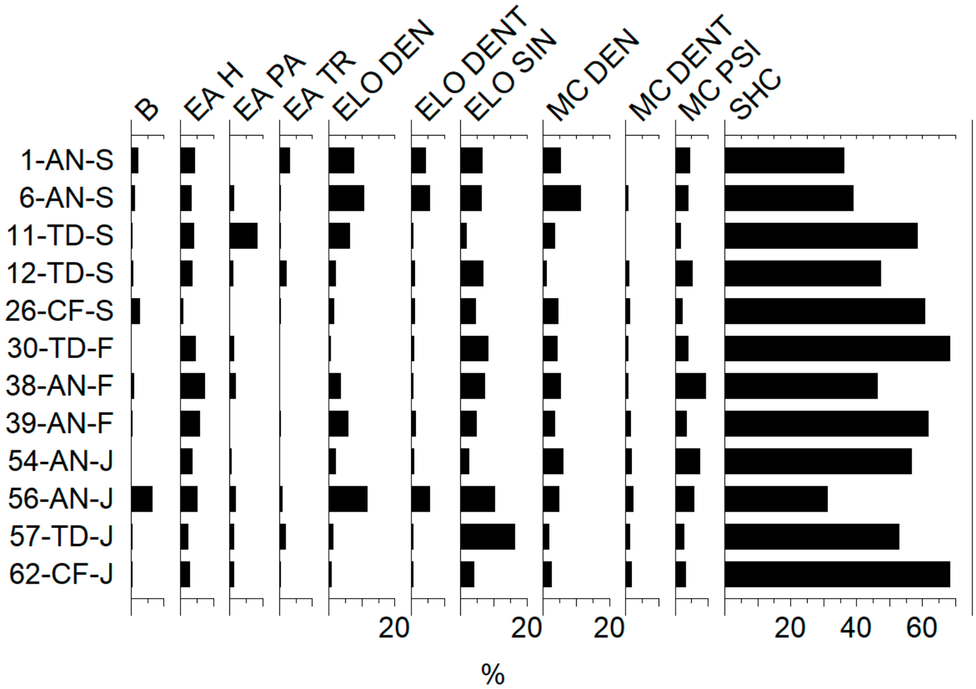

Figure 3. Relative abundances of main phytolith morphotypes obtained from cow dung samples with indication of sample number-farm-month. B = bulliform flabellate/blocky, EA H = Epidermal appendage hair, EA PA = Epidermal appendage *papillate* (*papillae*), EA TR = Epidermal appendage trichome (acute bulbosus), ELO DEN = Elongate dendritic, ELO DENT = Elongate dentate, ELO SIN = Elongate sinuate entire (psilate), MC DEN = Multicelled phytoliths of elongate dendritics (silica skeleton with dendritic/*papillate*/short cells/stomata), MC DENT = Multicellular structure of elongate dentates (silica skeleton with dentates/with *papillate*/short cells), MC PSI = Multicellular structure of elongate psilate (smooth), SHC= Short cell (rondel/trapeziform/bilobate).

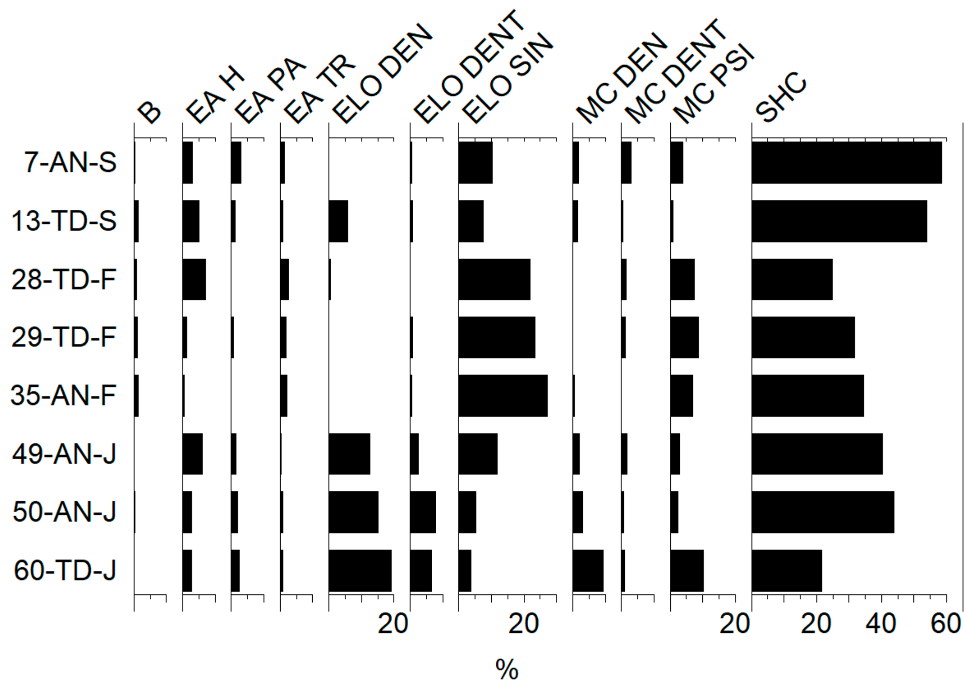

Figure 4. Relative abundances of main phytolith morphotypes obtained from sheep dung samples with indication of sample number-farm-month.

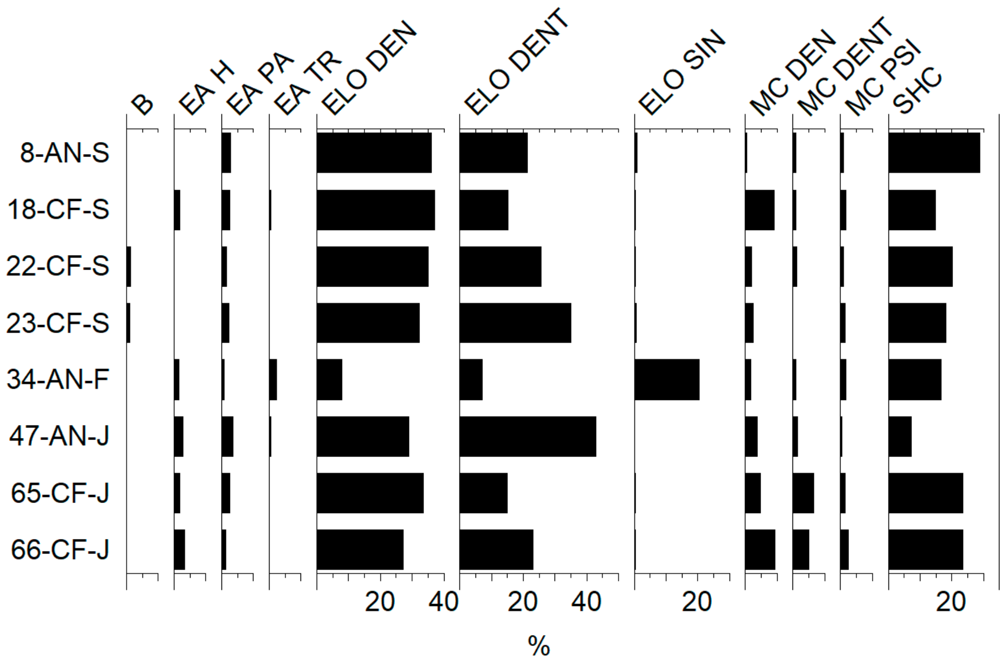

Figure 5. Relative abundances of main phytolith morphotypes obtained from pig dung samples with indication of sample number-farm-month.

Phytolith amounts range between 0.7 and 23.2 million per 1 g of ashed dung (Table 4). The low proportions of weathered phytoliths (below 6%), together with the presence of multicellular phytoliths (anatomically connected) in most of the samples (up to 55%, cow sample 6-AN-S), points towards generally good preservation conditions of the phytolith records. In general, cow dung samples contained more phytoliths than sheep and pig pellets in most of the assemblages. This is consistent with previously reported quantitative phytolith records from modern dung remains from ruminant herds from Mediterranean areas [9,10,26,27,29]. In the current study, this is particularly true for dung remains derived from adult or mature cattle, whereas for young animals around one month-old, phytoliths were noted in relatively lower amounts. This is clearly the case for the dung sample from a young cow collected from the property indoor enclosure of the household of Angendaret Nou (Figure 2a) whose diet was mostly based on lactation, ca. two weeks old (sample 1-AN-S, 0.7 m $\times$ 1 g/ashed dung, Table 4). Furthermore, the variable phytolith concentrations recorded among the sheep dung pellets, which are the only free-ranging animals that do not require any supplementary fodder and are more prone to browsing on the leaves and bark of shrubs and trees (Figure 2b), may be related to the differential production of phytoliths within dicotyledonous plants in comparison to prolific phytolith production of monocotyledonous plants, despite the similarities recorded on phytolith morphotype assemblages reported below (Figures 3–5). Also of significance are the greater proportions of multicellular or anatomically connected phytoliths within the cattle dung (up to 55%, Table 4), with a diet based on crops from the property for fodder, barley in Algendaret Nou and oat in Talatí de Dalt and therefore represent a consistent and repetitive dietary pattern based on supplementary fodder (Tables 2 and 3). Conversely, the fresh dung pellets from suids represent generally lower phytolith multicellular proportions (between 4–16%, Table 4). These variations could be explained in part due to possible differential digestive process of suids, but particularly to the known fodder which is based on complete barley grains in Algendaret Nou and barley flour in Es Capell de Ferro (Tables 2 and 3). Further research is needed to evaluate aspects of digestibility and preservation of phytoliths and other remains excreted with dung (e.g., pollen, non-pollen palynomorphs-NPPs), to better establish possible differences between ruminants and suids.

Although variations in the species of animal producers, dietary habits and grazing/foddering patterns through the seasons of the year may explain variations on the main phytolith morphotype records, grass short cells are by far the most dominant, although to a different extent. Short cells represent between 30–70% or more of the total grass phytoliths in the cow samples and around 20–60% in the sheep dung pellets, whereas short cells represent up to only 30% within the pig assemblages in this study (Figures 3–5). The short cell morphologies comprise mostly rondels (Figure 6a), although bilobates and polylobates from the Pooideae grass subfamily are also common in variable amounts, along with short cell towers (elongate) which are commonly produced in the Hordeum genus [10] and are also present in the pig assemblages representing human manipulation of fodder (barley complete grains and/or grinded into flour). Grass inflorescences were represented mainly by decorated elongate dentate (echinate) and dendritics in addition to epidermal cells such as papillate and hairs (Figure 6b,c). Epidermal appendages produced by grass leaves and culms, including stomata, acute bulbosus (trichomes), and bulliforms, were also common in all the samples in variable amounts (Figures 3–5). Furthermore, these diagnostic morphotypes derived from the floral parts of grasses were abundantly noted among the cow and pig samples through the different periods of the year, whereas these are almost absent among the sheep dung samples collected in winter (coded as AN-F and TD-F samples, Figure 4). In addition to grass phytoliths, mostly short cells, polylobates, and elongate sinuate or psilate (smooth), characteristic morphotypes produced by dicotyledonous plants were also common among the winter sheep dung pellets, including epidermal appendages such as hairs and their bases, as well as irregular multicellular jigsaw phytoliths (Figure 6d,e), therefore indicative of diet regimes more prone to browsing on the leaves and bark of shrubs and trees. Although all of these variations and changes in morphotype composition are relative, it is clear that the phytolith records from dung pellets from free-ranging grazers, represented by sheep in the current study, could be potentially used as seasonality indicators.

Of particular note is the identification of diagnostic spheroid echinate phytoliths produced by the leaves of palms (Arecaceae) among the pig dung samples from both indoor and outdoor penning areas in Es Capell de Ferro (Figure 6f). During all the sampling seasons, we observed how adult pigs pluck and chew the leaves of three palms (*Phoenix canariensis*) naturally growing on the stabling floor, indicating the ingestion of the palm phytoliths through the mouth with their saliva (Figure 2g,h). According to our informants, the reason for chewing the leaves of palms remains unknown. Although pigs eat calorie-dense foods their ability to extract energy from cellulose digestion is less efficient than in ruminant guts, oil-rich fibrous palm residues are reported as energy source in pig feeding fattening diets [59–61]. This particular case illustrates the non-deliberate inclusion within dung of other ingested plant microfossil remains rather than fodder.

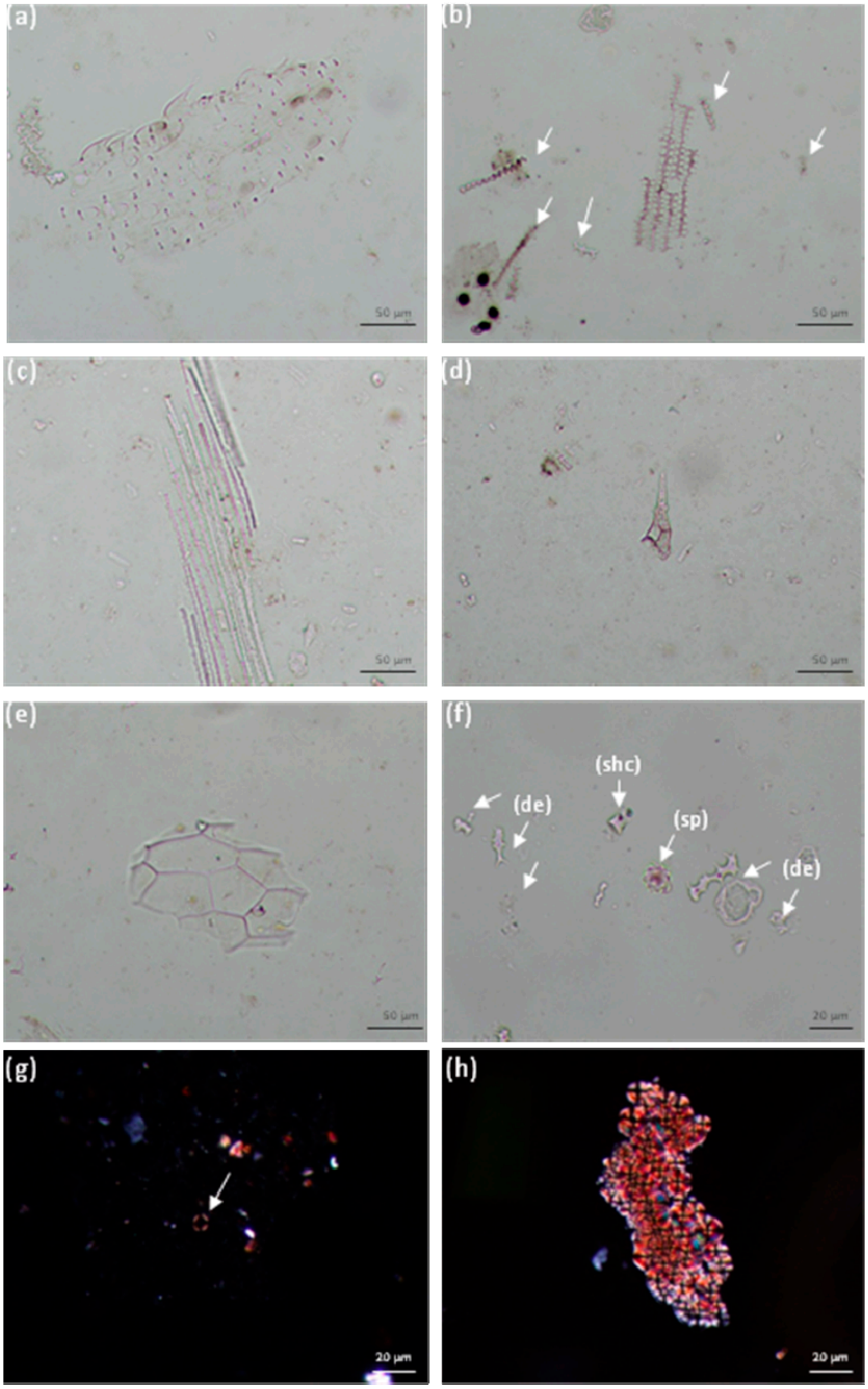

Figure 6. Photomicrographs of phytoliths and dung spherulites identified in the samples (200× or 400×). (**a**) multicelled (articulated) elongate psilate (smooth) phytoliths with short cell rondels and epidermal appendage acute bulbosus (trichomes) from the leaves of Pooideae grasses, sheep dung, sample 7-AN-S; (**b**) multicellular (articulated) dendritics with *papillate*, along with disarticulated dendritics (arrows) from grass inflorescences, pig dung, sample 18-CF-S; (**c**) multicellular (articulated) dendritics, cow dung, sample 6-AN-S; (**d**) epidermal appendage hair from dicotyledonous leaves, sheep dung, sample 29-TD-F; (**e**) epidermal appendage hair base from dicotyledonous leaves, cow dung, sample 6-AN-S; (**f**) spheroid echinate phytolith (sp) along with fragmented dendritics (de) and a short cell (shc), pig dung, sample 22-CF-S; (**g**) darkened dung spherulite at XPL, cow dung, sample 56-AN-J; (**h**) cluster of dung spherulites at XPL (none darkened), sheep dung, sample 29-TD-F.

Overall, the morphological results indicated that most of the phytoliths derived from monocotyledonous plants and particularly grasses (around 90% of all the counted morphotypes, Table 4). The only exceptions were the sheep dung samples collected in winter from both farms, Algenderet Nou and Talatí de Dalt (samples coded as AN-F and TD-F, ca. 85%). This difference may be due again to the dietary regime of these flocks of herds, which graze in semi-freedom without any supplementary fodder. Interestingly, the same sheep dung samples have delivered the lowest ratios in terms of inflorescences/leaves and stems of grasses (Table 4). In contrast, animals with a diet based on cereal grains and flour from their own farms yielded the largest concentrations of phytoliths from the floral parts of cereal crops, barley in the case of pigs from Algenderet Nou (AN samples, ratios between 7.6–8.7) as well as from Es Capell de Ferro (CF samples, 6.2–6.6, Table 4 and Figure 7). Therefore, these markers characterized by relatively high proportions of diagnostic phytoliths from the floral parts of cereals within dung can be used as indicators of livestock supplementary fodder such as cereal grains and flours. An additional potential indicator for the use of crop flours as fodder would also be the index of multicellular or anatomically connected phytoliths, which is associated with the state of preservation produced by the mechanical degradation of multicelled morphologies during the grain cleaning and the grinding processes, as demonstrated through experimentally produced records on barley processing from Algenderet Nou [47], as previously argued. In summary, the modern dung reference standards obtained in the current study demonstrate that particularly the ratios of phytoliths produced by the inflorescences vs the leaves and stems of grasses, as well as ratios of individual (single-celled) vs anatomically connected (multicellular) phytoliths may serve as indicators of human manipulation in the feeding of livestock animals.

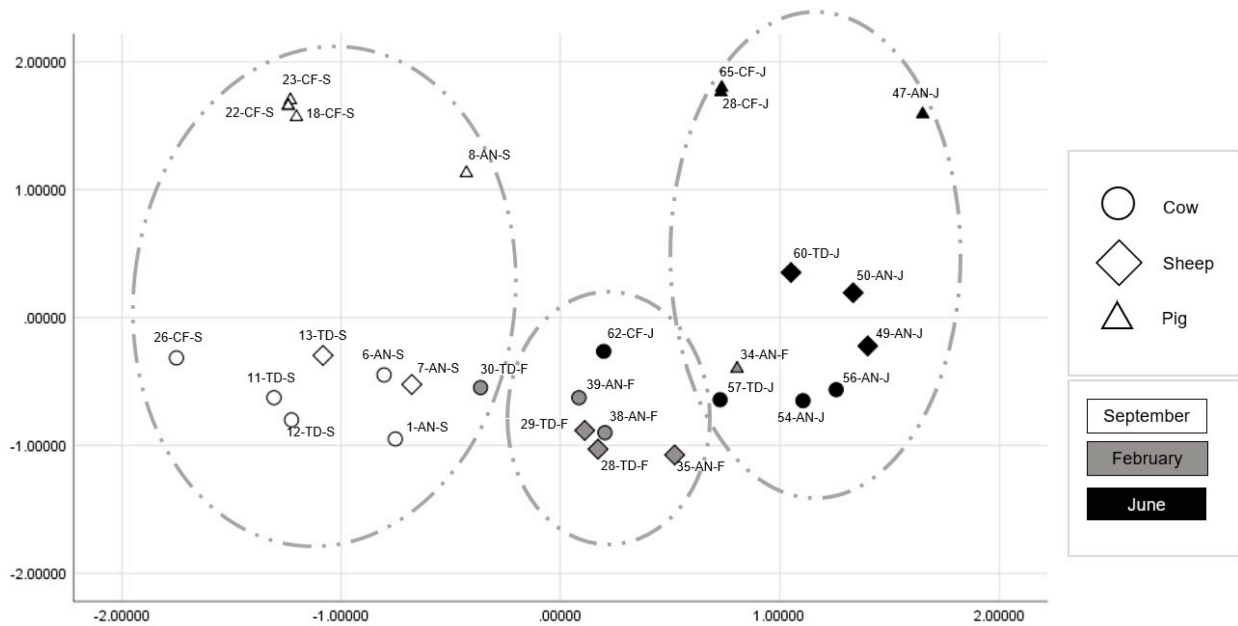

Figure 7. Principal component analysis (PCA) of grass inflorescences to leaves/stems ratios obtained from dung samples in relation to animal producers (cow, sheep, pig), farm (AN: Algendaret Nou, TD: Talatí de Dalt, CF: Es Capell de Ferro), and month (S: September, F: February, J: June,). Note that most of the samples tend to cluster according to animal producers, seasonality, and farm, particularly pigs (represented by triangles) with a diet based either on cereal grains or flour composed of large concentrations of grass inflorescences, and sheep grazers (in diamonds) with no supplementary fodder.

3.2. *Dung Spherulite Composition, Taphonomy and Seasonality*

Similarly to the phytolith quantitative analyses, the estimated dung spherulite numbers in Table 5 is based on abundances per weight of ashed dung material. The average percentages of total numbers of these calcitic microfossils were recorded for darkened or altered spherulites produced by ashing following the classification standards by Canti and Nicosia [24]. In those calcitic microremains the darkening spread across almost the whole spherulite and covers half or more of the overall diameter with a small clear fringe at its perimeter (Figure 6g). Darkened spherulites have been reported archaeologically in penning burnt spaces within caves and rock-shelters as well as in built environments as the result of the burning of dung including fuel remains [3,58,62–66]. The spherulite darkening has also been experimentally produced under increased heating laboratory-controlled combustions, occurring within a range between 500–700 °C, with a maximum production at 650 °C under reducing conditions [24,25]. In the current study these are the result of the sample extraction whereby the organic matter is removed by ashing in the muffle furnace, as a common extraction procedure for phytolith and other calcitic microfossil integrated studies from modern dung and plant materials (at temperatures from 500–550 °C for 4 h) e.g., [9–11,25–27,29,67].

Table 5. Main quantitative calcitic spherulite results obtained from modern dung samples with indication of animal producer and known age (based on data from informants). Provenance (farm): AN: Algendaret Nou; TD: Talatí de Dalt; CF: Es Capell de Ferro. Month: S: September 2018; F: February 2019; J: June 2019.

Sample n.	Location–Month	Spherulites 1 g of Ashed Material (Million)	Darkened Spherulites (%)	Description
1	AN-S	0	0	Cow (15 days)
6	AN-S	21	0	Cow
7	AN-S	13.5	0.4	Sheep
8	AN-S	0.3	0.4	Pig
11	TD-S	4.8	0	Cow
12	TD-S	4.7	0	Cow (1 month)
13	TD-S	5.6	1.3	Sheep
18	CF-S	0	0	Pig
22	CF-S	0	0	Pig
23	CF-S	0	0	Pig (15 days)
26	CF-S	0	0	Cow
28	TD-F	0.8	0	Sheep (1 month)
29	TD-F	5.7	0.8	Sheep
30	TD-F	4.5	0	Cow
34	AN-F	0.6	0	Pig
35	AN-F	10.9	0	Sheep
38	AN-F	0.5	0.4	Cow (3–4 months)
39	AN-F	7.3	0	Cow
47	AN-J	0	0	Pig
49	AN-J	9.1	0	Sheep
50	AN-J	0.04	0	Sheep (1 month)
54	AN-J	0.08	0	Cow (1 month)
56	AN-J	5	0	Cow
57	TD-J	8.7	0	Cow
60	TD-J	19.2	0	Sheep
62	CF-J	5.2	0.4	Cow
65	CF-J	0	0	Pig (2–3 months)
66	CF-J	0	0	Pig

These calcitic microfossils were recorded isolated individually or in clusters of dung spherulites (Figure 6h). As expected, significant differences were noted in the current modern dung assemblages in calcitic spherulite distributions depending on the species of animal producers as well as their age e.g., [4]. Dung spherulite concentrations range between 0.04 and 21 million per 1 g of ashed dung, with the exceptions of all the pig dung samples, as well as the young two week old cow from Algenderet Nou (sample 1-AN-S) and an adult cow from Es Capell de Ferro (26-CF-S) where these calcitic microfossils were absent (Table 5). The heavy rain reported in the study area a few days before the sampling (end of September 2018) may explain the absence of dung spherulites in the sample from a non-fresh dried pellet by an adult cow found in the pasture grounds (Figure 2f), as these calcitic microremains are known to dissolve in acidic environments even in neutral pH conditions, including by rain water [1,3,4]. Of particular note are the comparatively relatively lower spherulite abundances recorded in dung fresh pellets from young animals around one month old (up to 0.8 m per 1 g spherulites/g of ashed dung), with the only exception of one of the adult cow remains from Talatí de Dalt (sample 12-TD-S, 4.7 m per 1 g spherulites/g of ashed dung). In general, these microscopic remains were noted in good state of preservation, and only five of the samples displayed morphologies altered by effects of controlled combustion in the laboratory in relatively low proportions (between 0.4–1.3% of the total counted spherulites, Table 5). Interestingly, there are no significant differences in relative distributions across different periods of the year either (Figure 8), although more detailed research is needed in order to assess possible seasonal changes or variations based on age/sex aspects on dung spherulite production and composition. Issues on spherulite production by ruminants in particular may therefore need to focus on grazers with any supplementary fodder that may interfere with the ingested material linked to spherulite formation in herd guts such as sheep and goat, as spherulite preservation may relate in part to the organic composition and porosity of the dung pellets, and cattle dung may be more fibrous and porous than ovicaprine pellets [68,69]. In addition, laboratory extraction ashing procedures must be taken in account, as the gaseous exchange inside the furnace oven in the case of oxidative heating with unpredictable burning conditions points to spherulite decrease and complete loss in burning experimental studies conducted on both cattle and ovicaprine pellets [24,25]. Modern dung reference standards will be expanded further to assess the variability in microfossil signatures as a result of exposure to fire under open-air conditions in experimental situations.

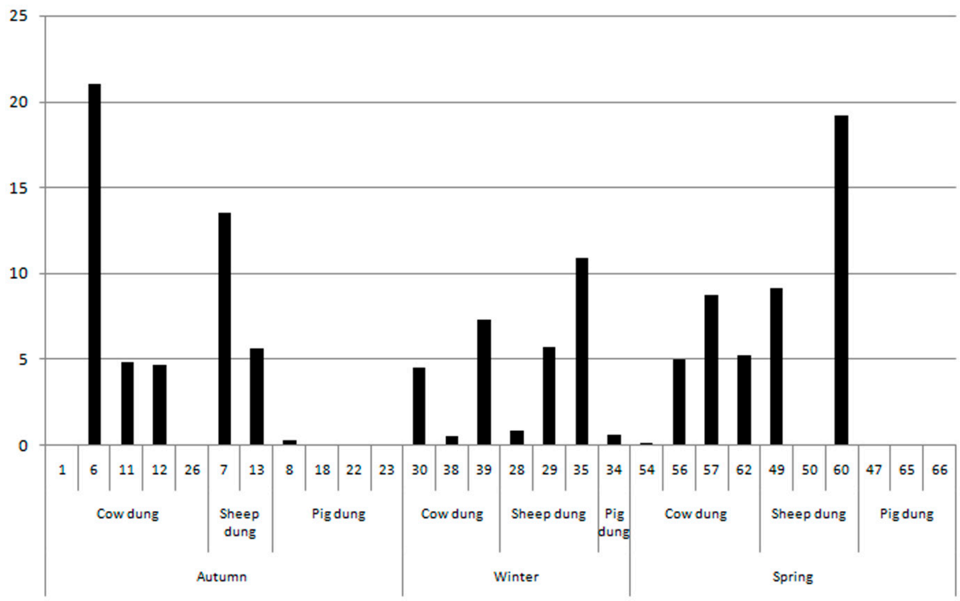

Figure 8. Plot showing average concentrations of dung spherulites obtained from dung samples.

4. Conclusions

This study contributes to our understanding of critical questions concerning dung origin, composition, taphonomy, and seasonality focusing on key parameters such as animal ecology, species and age, livestock management, grazing and foddering regimes and dietary habits, which have important implications for the identification and interpretation of plant and faecal microfossil archaeological records especially in agricultural and agro-pastoral archaeological contexts.

The phytolith records from the dung standards from ruminants and suids from the study area point towards clear changes in morphotype composition, but particularly regarding variations in the ratios of phytoliths produced by the inflorescences vs the leaves and stems of grasses, as well as on the ratios of individual (single-celled) vs anatomically connected (multicellular) phytoliths as potential markers of seasonality as well as foddering activity and human manipulation in livestock feed. Other factors affecting the composition of the phytolith records therefore may not be directly linked to foddering regimes, such as the inclusion of ingested plant remains from the leaves of palms chewed by pigs, illustrated in the current study.

In contrast, changes and variations on calcitic dung spherulite production and composition are not seen in relation to the type of feeding or on the seasonality. In fact, their production depends not only on the type of animal, with ruminants as the main producers, but also on its age, as pointed out in previous studies, although the available records regarding digestibility patterns according to age and sex patterns is still limited, to better establish possible differences between ruminants and suids for example. Further work is currently being carried out to assess aspects of digestibility and preservation of these and other remains excreted with dung (e.g., pollen grains, non-pollen palynomorphs-NPPs), as well as under increased heating (burning) open-air experimental situations.

Therefore, the modern dung reference datasets and the patterns reported in this study can be applied to compare and contrast microfossil dung assemblages from archaeological records, including the Balearic Islands and other inland contexts across the Mediterranean.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the University of Reading Research Ethics Committee for the research with humans and domestic animals; the University of Reading Data Protection Officer confirmed that this research does not involve the collection/nor processing of personal sensitive data as defined under the UK Data Protection Act 1998 and Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Details on all data supporting the reported results can be found in Tables 1–5 and Figures 1–8, this original manuscript.

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