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STUDIES ON MORPHOLOGICAL DIVERSITY AND FREQUENCY OF PHYTOLITHS OF UNDERUTILIZED GRASS SPECIES OF CYPERACEAE

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ABSTRACT

The phytoliths are micrometric particles protect the plant from various stresses; in past provides mechanical support and durability to the plant. Phytoliths from grasses are well documented and broadly applied in some archaeo-botanical studies, whereas sedge phytoliths have received less attention. Since sedge phytoliths appear to be morphologically distinct from those in grasses, they may be of taxonomic importance. Meticulous research towards phytoliths particularly in Underutilized grasses in scanty therefore the present study is aimed at to the find out the morphology and distribution of phytoliths present in eight Cyperaceae grass species by using wet-oxidation method. The results revealed that the most frequent types were the Elongate and Bilobate structures with highest frequency and measurements. Based on these observations it is concluded that the phytoliths produced by Cyperaceous grass species posses taxonomic values and major role in past environmental reconstructions.

INTRODUCTION

Silicon (Si) is the second most abundant element in the earth's crust after oxygen (28% vs. 47%). In nature Si exists mostly as silicon dioxide and is available to plants silicic acid. Large accumulations of Si are found in the Poaceae and Cyperaceae plant families (Currie and Perry, 2007). Silicon represents a major mineral constituent of plants, and is present in plants in concentrations similar to that of the other macronutrients. At 0.1 percent, Si is equivalent to the levels of macronutrients, Ca, Mg, P and S; while the upper levels of 10 percent exceed the concentrations of the mineral nutrients like K and N (Epstein, 1999). Cyperaceae is a family of monocotyledonous graminoid flowering plants known as sedges, which superficially resemble grasses. The family is large, with 5,500 species described. In about 109 genera worldwide although estimates of numbers vary greatly due to differing taxonomic concepts of individual researches and because modern sedge floras are available for only a few countries. Sedges occur primarily in the tropics and subtropics, but may be locally dominant in some areas like the subarctic regions. Sedges have featured in literature since antiquity. Phytoliths are found in many plant families though not all, but their production is most abundant in grasses, possibly 20 times that in other plant opal-producing families (Albert and Weiner, 2001). Phytoliths (Greek, *phyto*= plant, *lithos*= stones) or Plant stones are amorphous form of Silicon dioxide (known as "opal") deposition found in many plants (Mazumdar and Mukhopadhyay, 2009a). Opal phytoliths are created when hydrated silica dissolved in ground water is absorbed through the roots of a plant and carried throughout its vascular system. The term "phytolith" refers only to microscopically recognizable shapes, not amorphous pieces or traces of silica detectable only by microchemical methods that would not be recognized as discrete types (Piperno, 2006). As some plants were found to produce distinguishable or characteristic or "diagnostic" shaped phytoliths, they are well established as useful tools in archaeology, angiosperm taxonomy (Piperno, 1988) and recently for Nanotechnology (Neethirajan *et al.*, 2009). The occurrence of silicon as silica bodies or phytoliths in many plants has been correlated with mechanical support, stiffness, protect the plant from various biotic and abiotic stresses, reduction of water loss, protection from herbivores, prevention of the entry of pathogens, protection from metal toxicity, root elongation, increased capture of light for photosynthesis and in the cooling of leaves (Mazumdar, 2011). Silica bodies make some plants distasteful or give their tissues a prickly texture (Skinne and Jahren, 2004). Silica bodies also conserve water during moisture stress or drought (Hodson,

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2005) and they have been shown to influence stomata movement on the plant leaf epidermis and reduce the transpiration rate of water in maize. Plant silica bodies promote cell elongation in the growing zone and decrease cell-wall extensibility in the basal zone of stellar tissues in the roots and thereby enhance root elongation of plants (Hattori, 2003). Silica bodies also improve plant tolerance to fungal diseases, and metal toxicity (Hodson, 2005). Cells filled with silica bodies allow the plant to capture more light, thus aiding photosynthesis (Yoshida, 1959), perhaps in the manner of light piping that has been hypothesized for colonial diatoms (Gordon, 2009). At longer wavelengths, in the infrared, silica bodies aid cooling of leaves (Wang, 2005). In summary, silica helps a plant to survive many a biotic stresses, such as salt, metal toxicity, nutrient imbalance, drought, radiation, high temperature, freezing and ultraviolet (Ma and Yamaji, 2006) and reduce the impact of plant predators.

Phytoliths deposits, commonly referred to as opal or silica phytoliths, are found in many plant taxa but are most abundant and morphologically diverse in the monocotyledons, particularly the families Poaceae and Cyperaceae (Twiss *et al*., 1969). The size of the silica bodies that are deposited in the plant tissues mostly ranges between 10 and 30 μm and is occasionally up to 200 μm (Wilding, 1971). Phytoliths helps the formation of muscles in the animals. The cell-wall deposits of silica often replicate the morphology of the living cells. The objective of the present study was to find out the occurrence and morphology of phytoliths in eight Cyperaceae grass species which used as feed for livestock.

MATERIALS AND METHODS

Eight Underutilized grass species of Cyperaceae distriabution South India in wastelands *Bulbostylis barbata* (Rottb.) C.B. Clarke, *Cyperus difformis* L., *Cyperus rotundus* L., *Fimbristylis cymosa* R. Brown., *Fimbristylis monostachya* (Burm. F.) J. kern, *Kyllinga monoceps* Rottb, *Kyllinga triceps* Rottb and *Scleria lithosperma* (L.) Sw. were collected from different meadows during March to September 2010 to 2014 and all the grass species were uprooted by digging the soil and preserved in plastic bags. The samples of each grass species were immediately pressed in paper bags for herbarium specimen and the species were identified with the help of Gamble (1915-36) and compared, authenticated with the specimens of BSI (Coimbatore, Tamil Nadu).

Phytoliths were extracted by wet oxidation method of Mazumdar and Mukhopadhyay (2009a). All the collected plant samples were cleaned with distilled water in an ultrasonic water bath to remove adhering particles. Leaves of each species were placed in 20 ml of saturated nitric acid for one night to oxidize organic materials completely. The solutions were centrifuged at 2000 rpm for 10 min, decanted, and then boiled in 10 % Hydrochloric acid in water bath to remove calcium, then, washed with distilled water many times to remove the acid. The processed materials were then centrifuged with acetone at 2000 rpm for 10 minutes each time and dried with Acetone. The phytolith sediments were transferred to storage vials. The residual subsamples were mounted onto microscopic slides in Canada balsam medium for photomicrography and in liquid (glycerol) medium for counting and line drawing. A minimum of 350 phytolith grains were counted in each sample. Slides were observed under light microscope and photographed using Olympus digital camera attached with Olympus triangular microscope. Observations and photography were taken under oil immersion objective (400x). Measurements were taken from surface view of phytoliths using Motic Image Analyzer software. Various features of phytoliths noted including length, width and shape. In addition to measurement, frequency of phytolith assemblages was also noted. About 1000 phytoliths from each species were counted and frequency determined. For both frequency and measurement range, average and standard error were calculated. Measurements were made along the longest axis of the phytoliths all the dimensions of phytoliths were studied for taking measurements surface view of phytoliths were considered.. Phytoliths morphotypes were classified and described using the International Code of phytolith nomenclature (Madella *et al*., 2005). Silicon content estimated through the ICP- OES (Perkin Elmer 7000DV, USA).

RESULTS AND DISCUSSION

The phytoliths of each species were measured and shown in Table 2; frequency assemblages in table 3; and distribution in table 4; Photograph of different types of phytoliths are given in figure 1. The data obtained from the frequency of phytolith assemblages as well as measurements were utilized for the preparation of an identification key for grasses upto species level. Length and width dimensions and frequency of about 1000 phytoliths were measured from each species. The results of present study on phytoliths applies the rules of International Code for Phytolith Nomenclature. Various types of phytoliths observed and their abbreviations used are given in Table 1.

Table 1. Different types of phytoliths observed and their abbreviations

S.No.	Type	Abbreviation
1	Bilobate short cell (dumbbell)	BSC
2	Trapeziform short cell (rectangle)	TSC
3	Cylindrical polylobate (polylobate)	CP
4	Elongate echinate long cell (elongate and spiny)	ELC
5	Cuneiform bulliform cell (fan shaped)	CBC
6	Parallepipedal bullifrom cell (bulliform)	PBC
7	Acicular hair cell (point shaped)	AHC
8	Cross	C
9	Rondel	R
10	Saddle	S

Bilobate phytoliths is represented in the species of *Cyperus difformis*, *Cyperus rotundus*, *Kyllinga monoceps*, *Kyllinga triceps* and *Scleria lithosperma*. Whreas highest frequency and measurements in *Scleria lithosperma* and *Kyllinga monoceps*. The dumbbell (lobate) phytoliths originates from the epidermal cells (short cell) of Panicoideace and oryzoideae, some Arundinoideae, Chloridoideae subfamilies (Lu and Liu, 2003). Trapeziform short cell phytoliths are absent in all selected cyperaceae grass species. These are phytoliths with three or poly equal lobes. Their margins may be concave or flattened. Cylindrical polylobate phytoliths are in *Fimbrisylis monostachya*, *Kyllinga monoceps* and *Kyllinga triceps*. Highest frequency and measurements in *Kyllinga triceps* and *Fimbristylis monostachya*. Elongate structures are silicified long cells of the epideumis with echinate or sinuate walls. Elongate shaped phytolihs characteristrises the family of cyperaceae were forund to be the dominant type in all the

Table 2. Diversity in structures and Measurements of phytolith (µm) of Cyperaceae grass species

Name of the species	Shape Dimension		BS	TS	CP	ECL	CBC	PBC	AHC	C	R	S
		Average	-	-	-	34.2 ± 2.7	-	-	-	-	-	-
Bulbostylis	L	Range	-	-	-	19.2 to 41.5	-	-	-	-	-	-
barbata		Average	-	-	-	9.6 ± 0.7	-	-	-	-	-	-
	W	Range	-	-	-	6.9 to 13.3	-	-	-	-	-	-
		Average	13.8 ± 0.8	-	-	42.5 ± 5.2	-	-	-	-	-	-
Cyperus	L	Range	8.3 to 16.7	-	-	23.7 to 59.7	-	-	-	-	-	-
difformis		Average	10.9 ± 0.7	-	-	11.4 ± 0.4	-	-	-	-	-	-
	W	Range	6.9 to 13.6	-	-	6.3 to 13.6	-	-	-	-	-	-
		Average	13.5 ± 0.8	-	-	38.7 ± 1.9	15.1 ± 0.4	-	-	-	12.4 ± 0.8	12.4 ± 1.7
Cyperus	L	Range	7.6 to 16.9	-	-	26.7 to 43.1	12.4 to 16.7	-	-	-	3.2 to 18.6	7.6 to 16.4
rotundus		Average	8.5 ± 0.6	-	-	11.8 ± 0.8	14.1 ± 0.7	-	-	-	9.2 ± 0.5	7.9 ± 0.2
		Range	5.9 to 12.6	-	-	7.6 to 16.3	9.7 to 16.3	-	-	-	3.1 to 14.1	5.7 to 9.7
		Average	-	-	-	47.2 ± 3.5	-	-	15.8 ± 1.7	-	10.8 ± 0.8	15.2 ± 0.9
Fimbristylis	L	Range	-	-	-	22.4 to 56.3	-	-	8.6 to 19.2	-	4.7 to 16.3	5.3 to 26.4
cymosa		Average	-	-	-	9.5 ± 0.1	-	-	8.9 ± 1.4	-	9.5 ± 0.2	12.5 ± 1.2
	W	Range	-	-	-	5.9 to 12.4	-	-	5.7 to 12.7	-	3.6 to 13.7	4.9 to 19.7
		Average	-	-	14.6 ± 0.8	41.8 ± 4.7	-	-	-	-	-	14.3 ± 0.7
Fimbristylis	L	Range	-	-	6.3 to 24.3	14.2 to 63.6	-	-	-	-	-	6.3 to 20.2
monostachya		Average	-	-	7.5 ± 0.2	9.5 ± 0.1	-	-	-	-	-	8.7 ± 0.5
	W	Range	-	-	3.3 to 10.7	6.3 to 13.7	-	-	-	-	-	5.2 to 11.3
		Average	14.2 ± 0.1	-	15.2 ± 0.4	47.2 ± 2.8	-	-	-	-	8.5 ± 0.4	-
Kyllinga	L	Range	10.3 to 16.4	-	7.6 to 19.3	17.3 to 63.6	-	-	-	-	3.1 to 12.4	-
monoceps		Average	11.2 ± 0.5	-	9.6 ± 0.5	14.1 ± 0.5	-	-	-	-	5.9 ± 0.1	-
		Range	8.7 to 13.3	-	6.1 to 12.3	7.9 to 16.1	-	-	-	-	2.6 to 7.1	-
		Average	15.8 ± 1.6	-	13.8 ± 0.5	39.5 ± 1.8	-	-	16.5 ± 1.3	-	9.6 ± 0.5	-
Kyllinga	L	Range	6.3 to 24.7	-	5.7 to 21.3	19.1 to 51.7	-	-	10.3 to 23.1	-	3.9 to 13.6	-
triceps		Average	9.5 ± 0.4	-	11.4 ± 1.3	8.5 ± 0.7	-	-	11.2 ± 0.5	-	8.7 ± 1.4	-
	W	Range	4.7 to 13.9	-	4.3 to 15.8	5.6 to 12.3	-	-	8.3 to 12.7	-	2.9 to 12.3	-
		Average	14.8 ± 1.9	-	-	52.3 ± 4.1	-	9.1 ± 0.4	11.2 ± 0.5	-	-	-
Scleria	L	Range	7.3 to 19.6	-	-	23.2 to 74.1	-	6.1 to 14.6	7.2 to 13.3	-	-	-
lithosperma		Average	11.4 ± 1.4	-	-	11.7 ± 0.8	-	9.8 ± 0.6	7.9 ± 0.5	-	-	-
	W	Range	6.1 to 13.7	-	-	6.3 to 14.7	-	5.3 to 12.3	5.6 to 10.1	-	-	-

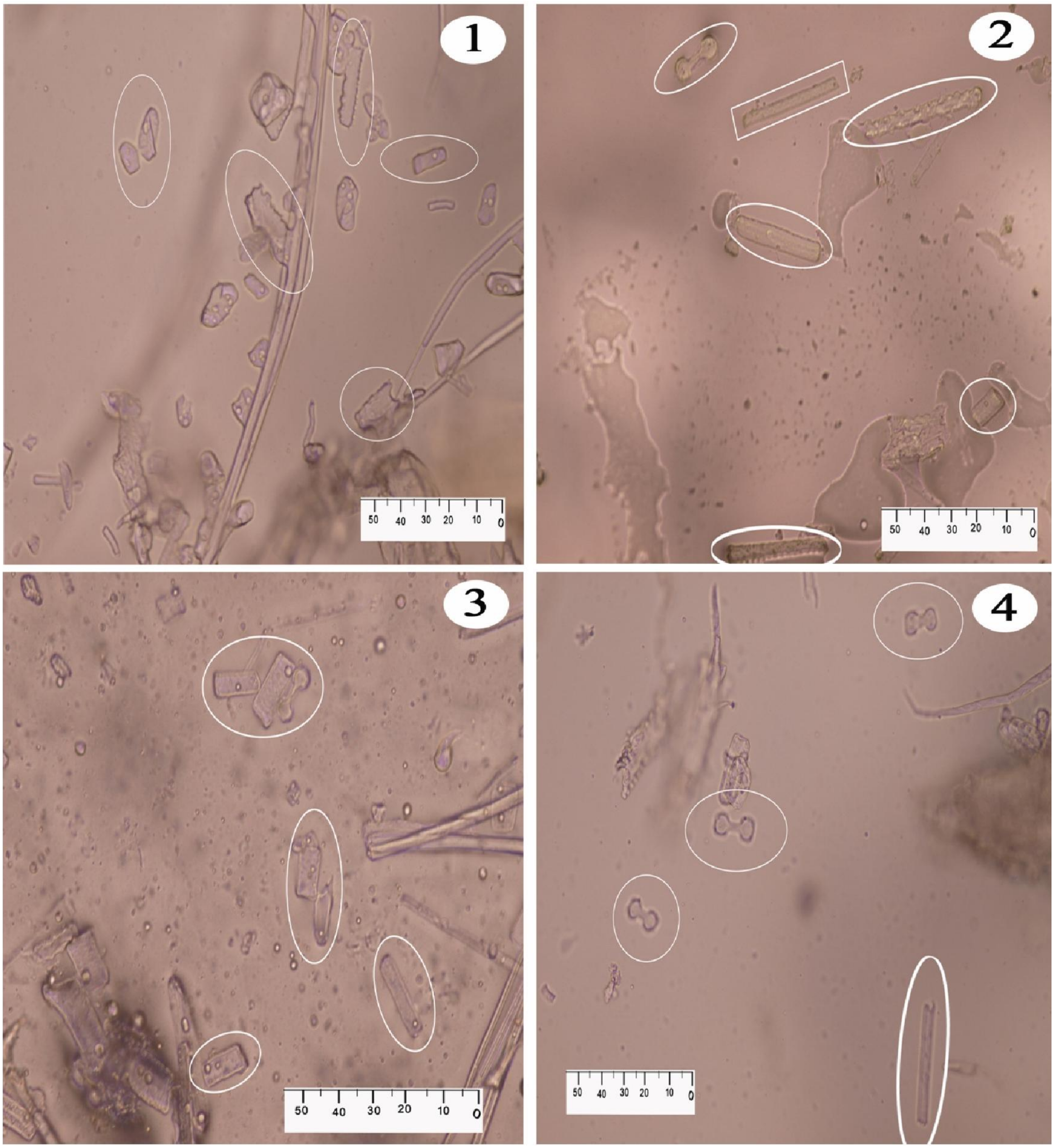
Table 3. Frequency (in percentage) of phytolith in eight Cyperaceae grass species

Structure of Phytolith	*Bulbostylis barbata*	*Cyperus difformis*	*Cyperus rotundus*	*Fimbristylis cymosa*	*Fimbristylis monostachya*	*Kyllinga monoceps*	*Kyllinga triceps*	*Scleria lithosperma*
BS	-	10.7	4.3	-	-	8.9	6.2	12.4
TS	-	-	-	-	-	-	-	-
CP	-	-	-	-	8.3	32.6	36.3	-
ECL	100	89.3	33.5	40.6	59.1	58.4	50.1	85.7
CBC	-	-	0.6	-	-	-	-	-
PBC	-	-	-	-	-	-	-	1.8
AHC	-	-	-	12.3	-	-	2.6	0.1
C	-	-	-	-	-	-	-	-
R	-	-	29.5	37.5	-	0.1	4.8	-
S	-	-	32.1	9.6	32.6	-	-	-

Table 4. Distribution of Phytolith types in grass species of Cyperaceae

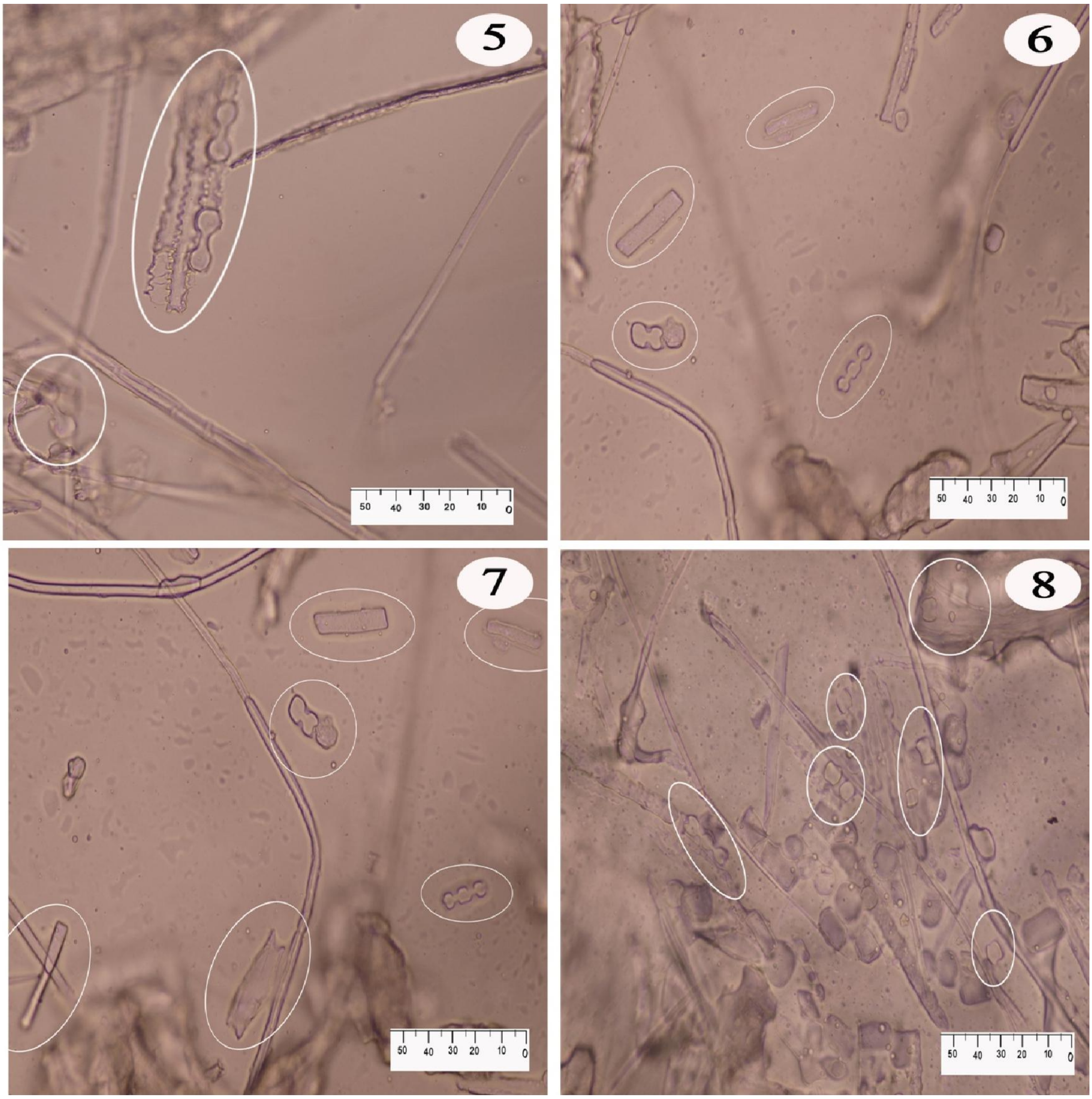
Taxon	BS	TS	CP	ECL	CBC	PBC	AHC	C	R	S
Bulbostylis barbata				A						
Cyperus difformis	C			A						
Cyperus rotundus	R			A					C	C
Fimbristylis cymosa				A			R		C	R
Fimbristylis monostachya			R	A						C
Kyllinga monoceps	R		C	A					R	
Kyllinga triceps	R		C	A			R		R	
Scleria lithosperma	C			A		R	R			

A, abundant; C, common; R, rare.



1. *Bulbostylis barbata* 2. *Cyperus difformis* 3. *Cyperus rotundus* 4. *Fimbristylis cymosa*

Figure 1. Phytoliths studies of eight cyperaceae grass species



5. *Fimbristylis monostachya* 6. *Kyllinga monoceps* 7. *Kyllinga triceps* 8. *Scleria lithosperma*

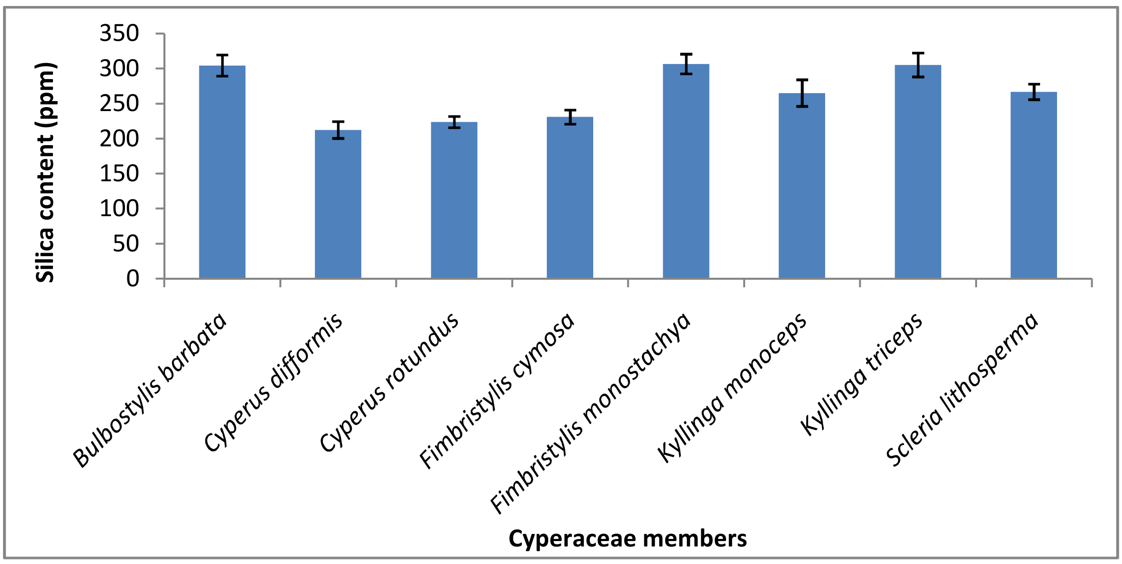


Figure 2. Silica content of Cyperaceae grass species (ppm)

selected species. The highest frequency and measurements were observed in *Bulbostylis barbata* and *Scleria lithosperma*. Elongate phytoliths forms are well preserved. These phytoliths are flat rectangular plates, some with pitted and others with smooth surfaces. Fan shaped phytoliths are silicified bulliform cells of the epidermis which were found in *Cyperus rotundus* only. These cells are usually found in the epidermis of the leaves and they are larger than the typical epidermal cells and characteristic of the family cyperaceae and other monocotyledons. Some workers hypothesized that bulliform cells have an important role in the opening of the leaves from the bud, whereas others suggested that they are involved in the rolling and unrolling of mature leaves.

Fahn (1953) proposed that bulliforms act as water storage cells. Parallepipedal bulliform cell phytoliths are silicified bulliform cells, these are observed in *Sclera lithosperma* only. Acicular hair cell phytoliths are in *Fimbristylis cymosa* and *Kyllinga triceps*; highest frequency and measurements in respectively. Cross phytoliths are obsent in selected cyperaceae grass species. Rondle shape phytoliths are observed in *Cyperus rotundus*, *Fimbristylis cymosa*, *Kyllinga monoceps* and *Kyllinga triceps*. Highest frequency and measurements in *Fimbristylis cymosa* and *Cyperus rotundus*. Saddle shaped phytoliths are arranged vertically on the epidemis of *Cyperus rotundus*, *Fimbristylis cymosa* and *Fimbristylis monostachya.* Highest frequency and measurements in *Fimbristylis monostachya* and *Fimbristylis cymosa*. Highest silica content in *Fimbristylis monostachya* followed the *Kyllinga triceps*, and *Bulbostylis barbata*. Whereas lowest content observed in *Cyperus difformis*. (Table.5)

Table 5. Silica content in leaves grass species of Cyperaceae (ppm)

S.No	Name of the species	Silica content ppm
1	*Bulbostylis barbata*	304.4 ± 15
2	*Cyperus difformis*	212.4 ± 12
3	*Cyperus rotundus*	223.6 ± 08
4	*Fimbristylis cymosa*	230.9 ± 10
5	*Fimbristylis monostachya*	306.6 ± 14
6	*Kyllinga monoceps*	265.1 ± 19
7	*Kyllinga triceps*	305.2 ± 17
8	*Scleria lithosperma*	266.8 ± 11

Frequency of phytolith assemblages and measurements are found to be consistent with in a species and have been useful for developing the key of identification. Even though phytolith multiplicity and redundancy occur in grasses, frequency assemblages reveal that a particular morphotype dominate over the other in a given species. The widespread production of phytoliths in leaves of grass species can therefore be most helpful for identifying plant species in archaeological or geological sediments, provided a reference collection of the surrounding area is available of the eight leaf samples analysed in this study, *Cyperus rotundus* and *Kyllinga triceps* contain the highest concentrations of phytoliths. Silicon dioxide may ameliorate the toxic effects of aluminium and other heavy metals, such as manganese, which are ingested by plants along with other substances in the ground water (Sangster *et al*., 2001). Protective functions have to do with an increased resistance to herbivores and pathogenic fungi that consume plant tissue or cause various diseases. It appears that these kinds of protection constitute some of the most important functions of phytoliths. The discovery of single genes that regulate the production of phytoliths adds a great deal to our understanding of this process (Piperno *et al.,* 2002). In areas where dung accumulated, large amounts of phytoliths can be expected, provided that the animals were fed the whole plant. If only the stems of certain cereals and wild grasses that contain relatively few phytoliths were used as fodder, this would deplete the phytolith record significantly. In addition dung from goats, animals that prefer leaves in their diet, may also contain small amounts of phytoliths given that many dicot leaves do not contain large amounts of phytoliths. A recent study by Madella *et al.* (2009) also explored the possibility of using phytoliths as indictors of past water availability. Grasses highly mineralise parts of their cells and bodies with opaline silicates. These so-called phytoliths are considered to be a mechanical defense against herbivory by abrading mammalian tooth enamel and dentine (McNaughton and Tarrants, 1983).

However, their effectiveness to do so has not been resolved conclusively to date and there are ongoing discussions about the hardness of phytoliths compared to enamel (Sanson *et al.,* 2007). Silica bodies in plants serve a variety of purposes, including lending the plant structural rigidity by supporting the shoot (Kaufman, 1979), giving lodging (falling over) resistance (Ma, 2006) and giving mechanical strength and rigidity to leaves (Namaganda, 2009). Their hardness deters obvious predators (Piperno, 1988) and, owing to their ability to wear down tooth enamel, they might even provide some (indirect) resistance to mammals, such as Homosapiens (Fox, 1996). The evolution of horse dentition correlates with increased phytolith content of grasses (MacFadden, 2005). A brief survey of eight cyperaceae species has proved the usefulness of phytolith in the identification of grasses. The present paper represents only a preliminary study towards developing an identification key for all south Indian grass based on the morphological phytolith characteristics. Hence it can be assumed that presence of phytolith in cyperaceae grass species may help protect the plants to some extent by ameliorating the toxic effects of heavy metals. Outcome of the study it an evident that although phytolith produced by cyperaceae grass species posses a major role in environmental reconstruction and may help in future phytolith studies of the deltaic environments.

Conclusion

The phytolith record is the most robust and palential source of information available on the botanical archaeological record. Representative members of family cyperaceae produce considerable phytolith types, taxonomically some of the phytolith types have been found to serve as diagnostic types at different levels of taxonomic hierarchy and hence are useful to resolve taxonomic problems because taxonomists have been regularly using characters of silica bodies of grasses classification of angiosperms. Present systematic study will bring further enhancement to this knowledge and will also be helpful to palaebotanists and archaeologists in reconstructing the vegetation of plants. Silicon plays an astonishingly large number of diverse role in plants and does so primary when the plants are under stressful conditions and benign conditions its role is often minimal or even nonexistent. The quantitative and morphological analysis of phytoliths from grass species highlights some of the strengths and weaknesses when decoding the archaeological phytolith record. This information is much enhanced by the availability of a reference collection of new grass species that includes information on the morphotypes as well as the numbers of phytoliths weight of the grass species. Apart from their meticulous role in muscles in livestock the *Fimbristylis monostachya*, *Kyllinga triceps* and *Bulbostylis barbata* mitigate the heavy metal toxicity of drinking water.

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