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Quantification and Identification of Phytolith in Surface and Sub-surface Soils of Intensively Cultivated Rice and Sugarcane Crop Sites

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ABSTRACT

The present study was undertaken to quantify and identify the phytoliths distribution in rice and sugarcane soils of Karnataka, South India. The quantity of phytoliths was higher in sugarcane than in rice soils at the surface. In the sub-surface soil, higher amounts of phytoliths were detected in rice soils, whereas, it decreased in sugarcane soils. The dominant phytolith morphotypes observed in rice soils belongs to rod-shaped, irregular trapezoidal, bilobate and bulliform. The dominant phytolith morphotypes in sugarcane soils mainly belongs to bulliform, papillae, elongate sinuous, elongate smooth, polylobate, bilobate, elongate echinate and irregular trapezoidal. The microscopic analysis will also reveal the status of preservation of phytoliths in the soils versus that of aquatic organisms like diatoms and sponges.

Keywords : Phytoliths, rice, sugarcane, bulliform, bilobate

PHYTOLITHS are produced as a result of biological and physical processes in certain plant groups and deposited as solid silica in intercellular or extracellular locations after absorbing silica in soluble form as monosilicilic acid (H_4SiO_4) (Shakoor *et al.,* 2014). When plant dies, these pieces of silica are deposited into soils and sediments as discrete, microscopic particles of varying sizes and shapes, where in it can either persists for a longer periods or dissolve in the soil solution. The term phytoliths was proposed by Ruprecht (Shakoor *et al.,* 2014). It is composed of two Greek words 'phyton = plant' and 'lithos = stone' meaning plant stone. This term has been used to indicate all forms of mineralized substances deposited in higher plants, either siliceous or calcareous in nature. Phytoliths are generally put under a more generalized term, as 'biogenic silica' or simply 'bioliths'. They help to distinguish silica derived from living system from the silica of inorganic, mainly pedogenic origin. However, Sommer *et al.* (2006) defined phytoliths *sensu stricto* as silicon precipitates of plant origin with a diameter > 5µm to separate them from 'undefined phytogenic silicon < 5µm'.

Plants are considered as silicon (Si) accumulators when the tissue Si concentration is >10 g kg^{-1} and an excluder when they have <5 g kg^{-1} (Ma and Takahashi, 2002). Seven (sugarcane, rice, wheat, barley, sugar beet, soybean and tomato) out of the ten most important crops ranked by global production are classified as Si accumulators (Guntzer *et al.,* 2012). Rice and Sugarcane are major silicon (Si) accumulator crops. The uptake by rice and sugarcane of 200-400 kg Si ha^{-1} yr^{-1} is well above that normally absorbed in natural system (20-100 kg Si ha^{-1} yr^{-1}) and consequently the bulk of this phytogenic Si or biogenic Si (Phytolith) is removed from the system at harvest rather than being returned to the soil (Haynes *et al.,* 2014). Desplanques *et al*. (2006) measured an exportation of Si from a rice field in the Camargue, France by grain and straw as 270 kg Si ha^{-1} yr^{-1} and calculated that if phytoliths was considered the only source of Si for plants then the stock of available Si would be exhausted only after 5 years.

Sommer *et al.* (2013) observed considerable dissolution of phytogenic Si pool in forest soils due to disequilibrium caused by loss of Si-rich grasses during canopy closure and logging of tress. For agriculture, disequilibrium is mainly attributed to the high uptake of Si by some crops, especially rice and sugarcane, and the annual removal of much of this Si in the harvested product (Haynes *et al.,* 2014). However, according to Cornelis *et al.* (2011), the importance of phytogenic cycling of Si will be highly dependent on the extent to which mineral weathering has taken place. In a humid tropical climate, strong depletion of

litho-pedogenic Si is generally noticed and thereby phytogenic cycling of Si will supply the bulk of soil solution Si which will be taken up by the plants.

There is no database on systematic description of phytoliths in intensively cultivated rice and sugarcane soils in India. Hence, the present study aims to quantification and identification of phytoliths for soil samples collected from the surface and sub-surface of rice and sugarcane crop sites from Karnataka, South India.

Material and Methods

Two soil profiles each from rice and sugarcane, where rice and sugarcane were being intensively cultivated for more than a decade, representing four different agro-climatic zones *viz.,* central dry zone, southern dry zone, southern transition zone and coastal zones of Karnataka were collected (Table I and II). Collected soil samples were from the southern transition zone with precipiations >1000 mm yr^{-1}, the southern dry zone with precipitations <700 mm yr^{-1}, the central dry zone with precipitations <600 mm yr^{-1} and the coastal zone with precipitations >2500 mm yr^{-1}. In general, eight profiles each from rice and sugarcane sites were collected. The soil samples collected from the upper two depths (surface and subsurface) of all the profiles were mainly used for extraction of phytoliths.

Collected soil samples were processed and physical separations of phytoliths were performed on surface and sub-surface soil sample using a gravimetric separation method. The principal steps for the sediment processing, prior to the actual removal of the phytoliths include (1) removal of carbonates to disperse mineral fraction and prevent secondary reactions, (2) removal of organic matter to aid clarity during observation under microscope and (3) removal of clays to aid clarity and overestimation of phytolith.

Air-dried, sieved (<2 mm) soil was treated with 10 per cent HCl to remove carbonates. Soils were then put in a sand bath (50°C) with 50-100 ml of 30 Per cent H_2O_2 in order to remove organic matter. After rinsing with deionised water, samples were shaken with 1 per cent sodium hexametaphosphate solution, followed by sedimentation technique to separate the clay from the silt and clay fraction. The removal of clays is, however, essential as a high concentration of these particles can obscure the phytoliths and seriously affect the quality of the data recovered.

Phytoliths were obtained by placing a subsample of either sand or silt in a 50 mL centrifuge tube along with a heavy liquid sodium polytungstate ($Na_6H_2W_{12}O_{40}\cdot H_2O$) solution (density 2.30 g cm^{-3}) (Vandevanne *et al.,* 2015). Sodium polytungstate is a non-toxic, high density separating compound which generates an almost neutral solution (pH 6) of relatively low viscosity. After centrifugation, floating phytoliths were decanted in a clean centrifuge tube. To avoid water evaporation and consequently crystallization of the sodium polytungstate, filtration is accelerated by the use of a vacuum pump. The specific gravity of the resultant, filtered heavy liquid solution is then adjusted by evaporation of water and stored for subsequent analysis. Additional stirring and centrifugation steps were repeated until negligible phytolith yield was obtained. The centrifuge tube was then rinsed with deionised water, and small amounts of clays, organic matter, and carbonates were removed by repeating the same cleaning steps. Sample solution was finally poured over a 5µ polycarbonate filter (Whatmann), and phytoliths were dried on the filter paper overnight and stored in plastic eppendorf tubes. In order to reduce the accidental loss of phytoliths by adherence to processing vessels only three items of glassware (a beaker, a centrifuge tube and an eppendorf vials) were used during the processing of each sample.

A subsample of the dried phytoliths was prepared by using Benzyl benzoate as mounting medium (Novello and Barboni, 2015) for microscopic examination under Lawrence and Mayo face contrast reverse microscope (TC5600). Wherever possible phytoliths were described by their shape according to International Code for Phytolith Nomenclature (Madella *et al.,* 2005).

Results and Discussion

Initial properties of the rice and sugarcane soils are briefly explained in Table I and II. In case of rice soils, coastal zone and southern transition zone soils were acidic in nature. Soils of central dry zone recorded

TABLE I

Initial properties of the rice soils

Agro-clinmatic zone	Location	Latitude (N)	Longitude (N)	Depth (cm)	pH (1:2.5)	EC (dS m^{-1})	OC (g kg^{-1})
Coastal zone	Brahmavara	13°25'59.0"	74°44'09.0"	0-31	5.92	0.24	16.6
				31-41	6.78	0.19	7.1
	Mandharthi	13°29'46.3"	74°49'20.9"	0-34	5.52	0.04	50.7
				34-61	5.73	0.01	10.3
Central Dry Zone	Kathalagere	14°16' 01.2"	75°49'43.5"	0-15	6.90	0.21	13.9
				15-23	7.35	0.15	9.1
	Kamalapura	14°26'19.2"	75°43'32.5"	0-25	8.14	0.14	2.0
				25-57	7.81	0.01	4.0
Southern Dry Zone	Mandya	12°34'05.6"	76°49'49.7"	0-18	6.56	0.04	5.3
				18-28	7.67	0.04	3.7
	Bellatha	11°57'17.5"	77°05'24.3"	0-18	6.40	0.02	11.1
				18-36	7.26	0.83	7.9
Southern	Shimoga	13°54'13.1"	75°40'07.8"	0-19	4.96	0.07	12.3
Transition Zone				19-39	5.58	0.03	9.5
	H N Pura	12°48'13.1"	76°16'38.0"	0-15	5.50	0.27	8.0
				15-32	6.98	0.71	7.2

TABLE II

Initial properties of the sugarcane soils

Agro-clinmatic zone	Location	Latitude (N)	Longitude (N)	Depth (cm)	pH (1:2.5)	EC (dS m^{-1})	OC (g kg^{-1})
Coastal zone	Bennekudru	13°27'54.0"	74°44'01.6"	0-16	5.41	0.17	15.0
				16-37	5.40	0.06	7.1
	Pejamangur	13°27'00.8"	74°49'34.2"	0-29	5.67	0.03	23.0
				29-50	5.56	0.02	5.5
Central Dry Zone	Kariganur	14°18' 36.0"	75°52'28.5"	0-15	8.63	0.42	12.3
				15-34	8.39	0.39	2.8
	Harihara	14°27'47.1"	75°43'59.4"	0-23	8.02	0.21	10.7
				23-70	8.67	0.20	2.0
Southern Dry Zone	Mandya	12°34'11.1"	76°49'14.1"	0-10	7.10	0.08	11.1
				10-20	7.28	0.10	9.1
	Honnur	12°04'56.3"	77°01'01.3"	0-13	8.66	0.27	7.6
				13-43	8.82	0.24	6.0
Southern	Shimoga	13°55'00.0"	75°40'24.1"	0-22	5.33	0.11	9.1
Transition Zone				22-42	5.91	0.14	7.5
	Valambige	12°48'47.4"	76°20'52.7"	0-15	6.85	0.03	7.9
				15-40	7.03	0.59	5.5

near neutral to slightly alkaline reaction, whereas soils of southern dry zone showed slightly acidic to near neutral reaction. Irrespective of the agro-climatic zones, soils were normal in electrical conductivity. In almost all the soils, organic carbon content was higher in surface soils and decreased in the sub-surface soils (Table I and II).

Sugarcane soils of coastal zone were acidic in nature, whereas, acidic to near neutral in soil reaction in southern transition zone. Central dry zone soils were alkaline, whereas, soils of southern dry zone recorded near neutral to alkaline reaction. Sugarcane soils were normal in EC irrespective of the zones under study. The highest organic carbon content was recorded in surface soils of rice and sugarcane representing coastal zone (Table II).

Phytoliths could only be detected in the silt fraction of the soils. Negligible phytoliths remained after physical separation from the sand fractions. The quantity of phytoliths was higher in sugarcane than in rice soils at the surface (Table III and IV). The highest amount of phytoliths in the surface soils were observed in southern dry zone followed by southern transition zone (Table IV).

In the sub-surface soil, higher amount of phytoliths were detected in rice soils, whereas, it decreased in sugarcane soils (Table III and IV). However, in some of the rice soils, phytolith content decreased with increase in depth. In most of the sugarcane soils, sugarcane trash was burnt and mixed in the soil which might be attributed to higher phytolith content in surface soils of sugarcane compared to rice soils. In the Bore site of the Gilgel-Gibe catchment in south-western Ethiopia, Cornelis *et al.* (2014) found very high content of amorphous silica (12-38%) quantified by the gravimetric separation which can be of geogenic (volcanic glass), pedogenic (opal) or biogenic (phytoliths and micro-organism remains) origin. Since Si is taken from the soil as silicic acid and deposited in the above ground biomass of crops as biogenic Si or phytolith and thereby the higher amount of phytoliths were observed in surface soils of sugarcane. Similar observations were also made by Sommer *et al.* (2006).

TABLE III

Phytolith content of rice soils

Agro-clinmatic zone	Location	Crop	Depth (cm)	Phytolith (g kg^{-1})
Coastal zone	Brahmavara	Rice	0-31	0.84
			31-41	3.72
	Mandharthi	Rice	0-34	33.19
			34-61	35.69
Central Dry Zone	Kathalagere	Rice	0-15	6.43
			15-23	35.85
	Kamalapura	Rice	0-25	1.89
			25-57	4.03
Southern Dry Zone	Mandya	Rice	0-18	9.10
			18-28	9.73
	Bellatha	Rice	0-18	15.67
			18-36	9.07
Southern Transition Zone	Shimoga	Rice	0-19	19.50
			19-39	10.70
	HN Pura	Rice	0-15	5.50
			15-32	8.70

TABLE IV

Phytolith content of sugarcane soils

Agro-clinmatic zone	Location	Crop	Depth (cm)	Phytolith (g kg^{-1})
Coastal zone	Bennekudru	Sugarcane	0-16	11.62
			16-37	4.10
	Pejamangur	Sugarcane	0-29	26.13
			29-50	35.69
Central Dry Zone	Kariganur	Sugarcane	0-15	21.23
			15-34	5.15
	Harihara	Sugarcane	0-23	2.35
			23-70	0.50
Southern Dry Zone	Mandya	Sugarcane	0-10	78.33
			10-20	9.34
	Honnur	Sugarcane	0-13	0.48
			13-43	8.97
Southern Transition Zone	Shimoga	Sugarcane	0-22	37.00
			22-42	26.70
	Valambige	Sugarcane	0-15	6.40
			15-40	2.50

In case of rice soils, lower amount of phytoliths in surface soils could be attributed to lower incorporation of straw residues after harvesting of the crop which leads to depletion of Si from soil and consequently decreased the phytolith content of soils. For rice, retention of straw on the field rather than straw removal is an important management tool for recycling Si and ultimately phytogenic Si. This is mainly due to the fact that a large proportion of the aboveground Si uptake (about 80%) is retained in the straw rather than the harvested grain (Haynes *et al.,* 2014). Keller *et al.* (2012) also revealed that on a long term trial in Bolgneville, France, a period of 12 year of wheat straw removal resulted in a reduction of 41-64 per cent in phytogenic Si in the surface soil.

The dominant phytolith morphotypes observed in rice soils belongs to rod-shaped, bilobate, irregular trapezoidal and bulliform (Fig.1). Haribabu *et al.* (2015) reported that the most frequent types of phytoliths of the underutilized grass species were the elongate and bilobate structures. But in case of sugarcane soil there was wide variation in phytolith morphotypes. Novello and Barboni (2015) revealed that the phytolith diversity was some what higher in the grass leaves than in the grass inflorescence of wild cereals from sub-Saharan of Africa. The dominant phytolith morphotypes were

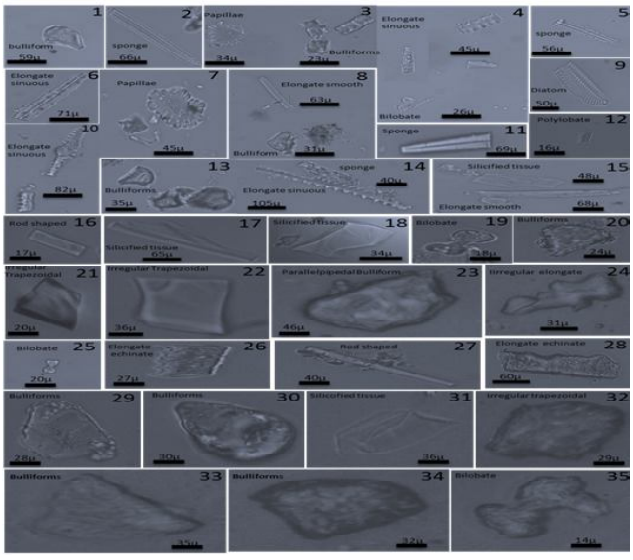

Fig.1: Different phytolith morphotypes of rice and sugarcane soils, Bar represents size of the phytolith. Resolution - 4*100 X. No. 1-15 and 25-31 are dominant morphotypes of sugarcane soils, whereas, 16-24 and 32-35 are dominant morphotypes of rice soils.

bulliform, papillae, elongate sinuous, elongate smooth, polylobate, bilobate, bulliform, elongate echinate and irregular trapezoidal (Fig.1). Roschel *et al.* (2006) reported that poaceae cuneiform bulliform cell, parallelepiped bulliform cell and elongate smooth long cell type dominant up to 80 cm in the Brazilian cerrado soil, whereas, cyperaceae rondel concave type showed the inverse trend being dominant in the upper part of the profile. In the Bore site of the Gilgel-Gibe cathment in south-western Ethiopia, Cornelis *et al.* (2014) noticed that the light silt fraction of the bleached and vertic horizons was mainly composed of phytoliths with elongated and globular granulate morphologies, which are very similar to the phytoliths shape in grass. However, the cropland soils of the Belgium loess belt, where maize, wheat and fodder beats were grown, mainly showed trapezoid sinuate, polylobate and bulliform morphotypes (Vandevanne *et al.,* 2015).

The quantity of phytoliths was higher in sugarcane soils than in rice soils at the surface. Large variation in phytolith morphotypes in sugarcane soils might be due to higher amount of phytolith present in sugarcane soils.

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