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Morphological Analysis of Opal Phytoliths for Soil Discrimination in Forensic Science Investigation

REFERENCE: Marumo, Y. and Yanai, H., **"Morphological Analysis of Opal Phytoliths for Soil Discrimination in Forensic Science Investigation,"** *Journal of Forensic Sciences*, JFSCA, Vol. 31, No. 3, July 1986, pp. 1039-1049.

ABSTRACT: A morphological analysis of opal phytoliths in soils was applied to forensic science soil discrimination. To examine a variation of opal phytolith composition with a difference of land use or topography, a region was chosen in a range of 5 km covering from diluvial plateau to alluvial plain. Opal phytolith compositions in soil samples collected from the same site were similar. On the other hand, there were obvious differences in the compositions between soils from different sites. In the region examined, contents of Chloridoid, Bambusoid, Fan-shape, and Elongate classes varied with sampling sites allowing us to discriminate the samples from different sites. Festucoid and Point-shape class were small in the amounts to be compared for the soil discrimination. Morphological analysis of opal phytoliths was effective to discriminate soil samples that came from different land use, even though they were indistinguishable by their mineralogical characteristics. About 2 mg of opal phytolith fraction, 10 to 200 μm in diameter, less than 2.3 in specific gravity, was a sufficient amount for the morphological analysis.

KEYWORDS: forensic science, soils, soil discrimination, opal phytoliths, gramineae plants

Soils contain various materials, for instance, primary minerals, clay minerals, humus, microorganisms, amorphous oxides, and exchangeable ions. Concerning these complexity of soils, many forensic scientists have developed techniques for discrimination of soil samples. Major techniques generally used in forensic science laboratories, however, were petrological ones [*1-3*], and frequently, only simple techniques such as color comparison or density gradient distribution are used in small forensic science laboratories because of lack of well-trained staffs. Certainly, the petrological techniques are important, since major constitutents of soils are mineral particles. But the petrological techniques are not always applicable. If soil samples come from different sites and have similar mineralogical compositions, they cannot be distinguished by the petrological techniques. Such a problem often arises in a region covered with volcanic ash or fluvial deposit that usually have uniformity in mineralogical compositions spread over a wide area. It is necessary in this case to examine an independent fraction from the geological materials in soils. An enzymatic analysis proposed by Thornton [*4*] is one of the most effective techniques for this purpose.

In this paper, the authors propose to apply a morphological analysis of opal phytoliths as a technique to resolve this problem. Because opal phytoliths originate from plant leaves, they principally depend on vegetation and are independent of geology. This analysis is so simple,

Received for publication 28 Sept. 1985; accepted for publication 22 Nov. 1985.

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only a microscope is required as an analytical instrument, and it is so easy that it can be used even in small laboratories.

Surface soil horizon contains biogenic silica particles called opal phytoliths, plant opals, silica bodies, opaline silica, or kieselkörper. Opal phytoliths, used herein, are formed through the progressive silicification of plant cells, showing distinct morphological types dependent on the shapes of cells. Because opal phytoliths tend to be produced more abundantly and to show more varied systematic morphological forms in monocotyledonous grasses, they have been investigated with a view of taxonomy of gramineous grasses by botanists [*5–10*]. Twiss et al. [*11*] classified opal phytoliths into four groups on the basis of their morphological examination of 17 species of gramineous grasses in addition to the initial worker's results: Festucoid class, Chloridoid class, Panicoid class, and Elongate class. Each class but Elongate relates to the respective subfamily; Elongate class does not possess any characteristics of the subfamilies. Kondo and Sase [*12,13*], furthermore, developed a morphological classification to add the following three groups to the above four groups: Fan-shape class originating from bulliform cells [*7*], Point-shape class originating from prickle hairs [*9*], and Sasoid class abundant in jenera *Sasa*, originating from short cells. Afterward, Kondo [*14*] established "Bambusoid class" instead of the Sasoid on the basis of the result of the study on 130 species of gramineous grasses that this type exists not only in jenera *Sasa* but also in Bambuseae generally. Opal phytoliths of tree origin had been reported by Rovner [*15*] in several conifers and recently detailed morphological investigation was conducted by Kondo et al. [*16–18*]. They classified opal phytoliths of gymnosperm and monocotyledonous angiosperm trees into six groups, and opal phytoliths of dicotyledonous angiosperm trees into 8 groups on the basis of the results of their experiment on tree leaves of 196 species from 55 families.

Having high durability, opal phytoliths have accumulated in surface soil horizon with lapse of time, and then they begin to show an important significance as a plant fossil system. Therefore, morphological analysis of opal phytoliths in soils provides valuable information for reconstruction of paleoenvironment [*15*]. Although soil scientists have attempted to solve this problem [*10,13,19–22*], their studies were conducted over very long distances. Variation of opal phytoliths with difference of land use or topography within short distances, which is important in forensic science investigation, has not yet been studied. As composition of the morphological class of opal phytoliths in soils varys depending on vegetal history, it must be different between soils where vegetations have been changed artificially in different ways, even though they are not far away. The morphological analysis of opal phytoliths will probably be a valuable technique for the discrimination of soil samples, especially when the samples have similar mineralogical characteristics. The present investigation was undertaken for the purpose to ascertain the above.

Materials and Method

Soil Samples

The region examined in this investigation is in Saitama Prefecture, a neighboring district of Tokyo, covering from diluvial plateau to alluvial plain in a range of about 5 km. Figure 1 shows locations of sampling sites, and topography and land use of the region. A contour line of 10 m above the sea level runs along the boundary between the diluvial plateau and the alluvial plain, which are separated by a cliff, a scarp, or a gentle slope. The plateau is covered with volcanic ash from Mt. Fuji, showing a rise gradually toward the southwest. Volcanic ash soil [*23*] develops on the plateau. The alluvial plain has been developed by fluvial sedimentation with regression after Jomon transregression, about 6000 years ago, spread within a level of 7- to 8-m altitude. Alluvial soil [*23*] is distributed on the plain.

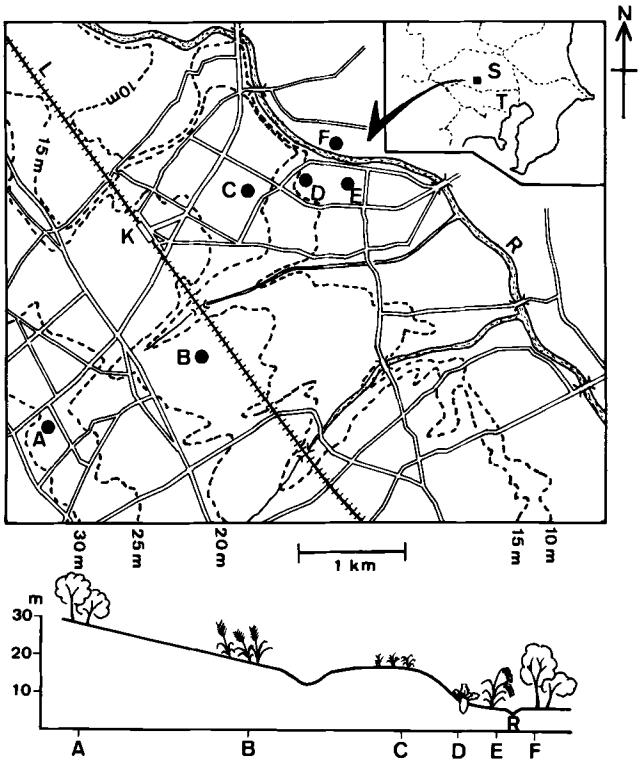

FIG. 1—*Sampling sites (*A *to* F*) and cross section along the sampling sites.* K, *Kamifukuoka Station;* L, *rail way (Tobu Tojo Line);* R, *Shingashi River;* S, *Saitama Prefecture; and* T, *Tokyo.*

Sampling Sites A to C are on the diluvial plateau. Site A is under a natural forest consisting of deciduous broad leaves (*Quercus serrata, Quercus acutissima*, and *Stylax japonica*). Site B is a field where corn and barley are cultivated in June to September and October to May, respectively. Site C is vacant land which was an army arsenal site until 1945, the end of World War II, and since then it has been left as a wasteland covered with annual herbs such as *Setaria viridis, Digitaria adscendens, Artemisia vulgaris*, and *Poa aeroleuca*. Sampling Sites D to F are on the alluvial plateau. Site D is a field of vegetables of carrot, radish, spinach, or Chinese cabbage, located at the foot of the boundary slope between the plateau and the plain. Site E is a paddy field. Site F is a forest of *Zelkova cerrata* where it is a part of a temple site built early in the 17th century.

Soil samples were collected from surface horizons (A horizon) twice at each site (A to F) at an interval of ten months, July 1979 and May 1980. The samples were symbolized alphabetically parallel to their sites in Fig. 1, and the samples collected formerly were numbered (1) and ones collected later were numbered (2) as Sample A(1), which was collected at Site A in July 1979. Samples A, B, and C, collected from the diluvial plateau, could not be distinguished from each other by comparison of mineralogical compositions. In addition to these, Sample D showed similar mineralogical characteristics with those of the above three, while it was collected from the alluvial plain. This is probably because deposition of soil materials were driven from the plateau by erosion. Samples E and F, collected from the alluvial plain, were also indistinguishable mineralogically.

Plant Samples

Mature leaves were collected to prepare reference specimens for morphological identification of opal phytoliths in soil samples. The plant samples collected are common in the region examined, 21 species of gramineous grasses and 12 species of trees, including cultivated plants in gardens. In addition to the above, six species of opal phytoliths samples that contain eminently Festucoid class were supplied from Dr. R. Kondo. Table 1 shows classification of the Gramineae plant samples according to Tateoka's system [*24*].

Extraction of Opal Phytoliths from Soil

Decomposition of Organic Matter—An air-dried fine earth (<2 mm) was placed into a 300-mL tall beaker and 5 mL of hydrogen perioxide was added followed by covering with a glass dish. When the reaction of peroxidation was too violent, the beaker was cooled in water and floats broken with water jets lest the contents should overflow. After the reaction calmed, 5 mL of hydrogen peroxide was added and the beaker was warmed at 100°C with a hot plate for 30 min. This procedure was repeated one more time.

Removal of Iron Oxides—Hydrochloric acid, about equal volume to the contents of the beaker, was added and the beaker was warmed at 100°C with a hot plate for 30 min. The contents were transferred into a 50-mL glass centrifuge tube and centrifuged at 2000 rpm for 10 min. The deposit was washed with water and centrifuged, the washing procedure was repeated more five times.

Separation of Size Fraction of 10 to 200 μm—After the deposit was wet-sieved with a 200-μm (67 mesh) sieve, the suspension, passed through the sieve, was replaced into its original centrifuge tube and subjected to sonic treatment to disperse the particles for 10 min with a sonic dismembrator (Model 150, Artek System Corporation). After the suspension stood for 15 min, the water layer within 5 cm from the surface was siphoned off to remove the particles

TABLE 1—*Classification of Gramineae plant samples.*

Species	Tribe	Subfamily
Pleioblastus chino	Bambuseae	Pharoideae
Shibataea kumasaca	Bambuseae	Pharoideae
Sasa albo-marginata	Bambuseae	Pharoideae
Oryza sativa	Oryzeae	Pharoideae
Phragmites communis	Arundineae	Arundoideae
Dactylis glomerata	Festuceae	Pooideae
Bromus japonicus	Festuceae	Pooideae
Bromus catharticus[a]	Festuceae	Pooideae
Lolium multiflorum	Festuceae	Pooideae
Elymus mollis	Triticeae	Pooideae
Triticum aestivum	Triticeae	Pooideae
Agrostis palustris	Agrosteae	Pooideae
Eragrostis megastachya	Chlorideae	Eragrostoideae
Eragrostis ferruginea	Chlorideae	Eragrostoideae
Zoysia japonica	Lappagineae	Eragrostoideae
Setaria viridis	Paniceae	Panicoideae
Digitaria adscendens	Paniceae	Panicoideae
Echinochloa crus-galli var. *hispidula*	Paniceae	Panicoideae
Panicum crus-galli var. *echinata*	Paniceae	Panicoideae
Miscanthus sinensis	Andropogoneae	Panicoideae
Coix lacryma-jobi	Maydeae	Panicoideae

[a]*Bromus unioloides*, according to Makino [*25*].

less than 10 μm. This procedure was repeated until the water layer within 5 cm from the surface was no longer turbid, adding an equal volume of water to that siphoned off each time. The residue in the centrifuge tube was filtered with a glass filter followed by washing with acetone.

Separation of Opal Phytolith Fraction with Heavy Liquid—The size fraction of 10 to 200 μm was placed into a separatory funnel (Squibb type or more slimmer type), and 10 mL of heavy liquid (specific gravity = 2.3) prepared from bromoform and ethanol was added followed by shaking for 30 s. After the funnel stood for 15 to 30 min, deposit in the funnel was removed by dropping through a stopcock, and this procedure was repeated twice more. The particles floating at the surface of the heavy liquid were filtered with a glass filter followed by washing with acetone. The fraction obtained here was examined with a microscope.

Extraction of Opal Phytoliths from Plant Leaves

About 0.5 g of air-dried plant sample was placed into a 100-mL conical beaker and 10 mL of acid mixture (sulfuric acid: nitric acid: perchloric acid = 1:10:4) was added. After covering with a glass dish, the beaker was heated at 200°C with a hot plate until the residue became white and fumes of perchloric acid appeared. The contents were transferred into a 10-mL glass centrifuge tube and centrifuged followed by washing with water five times. After 10 mL of water was added, the residue was subjected to sonic treatment and removal of < 10-

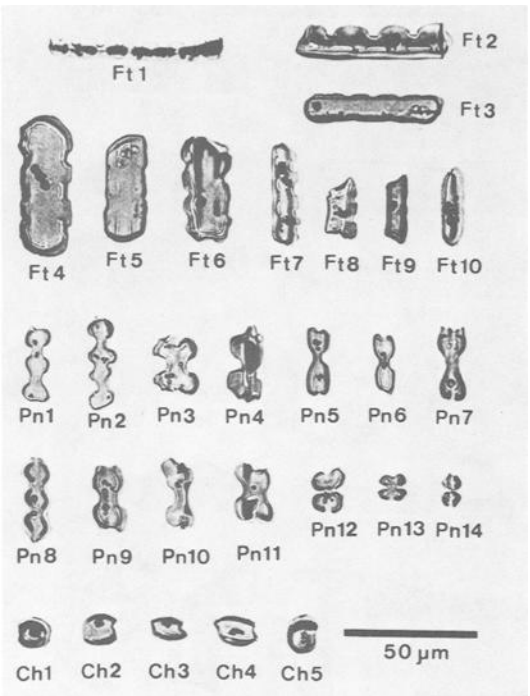

FIG. 2—*Opal phytoliths in the Gramineae plant samples.* Ft 1, *Dactylis glomerata;* Ft 2, *Agrostis palustris;* Ft 3, 8, 9, *and* 10, *Lolium multiflorum;* Ft 4, *Triticum aestivum;* Ft 5, *Elymus mollis;* Ft 6, *Bromus catharticus;* Ft 7, *Bromus japonicus;* Pn 1, 2, *and* 3, *Miscanthus sinensis;* Pn 4 *and* 5, *Setaria viridis;* Pn 6, *Eragrostis megastachya;* Pn 7, *Digitaria adscendens;* Pn 8 *and* 9, *Panicum crus-galli var. echinata;* Pn 10 *and* 11, *Coix lacryma-jobi;* Pn 12, 13, *and* 14, *Oryza sativa;* Ch 1, 2, *and* 3, *Eragrostis megastachya;* Ch 4, *Eragrostis ferruginea; and Ch 5, Zoysia japonica.*

μm fraction by siphoning in a same manner as that for the soil samples, mentioned previously.

Viewing of Opal Phytoliths

About 1 to 2 mg of opal phytolith fraction obtained from the soil or the plant sample was mounted on a microscope slide with Canada balsam. The specimen was examined under a petrographic microscope using magnifications of 100 to 400. For the soil samples, abundances of each class of opal phytoliths were recorded.

Classification of Opal Phytoliths

Opal phytoliths were classified morphologically on the basis of Kondo and Sase's classification system [*11-13,15-17*] as follows:

Festucoid Class—Festucoid class opal phytoliths originate from epidermis cells of Pooideae. Among Festucoid class shown in Fig. 2, Ft 4, 5, and 10 correspond to "oblong or elliptical" reported by Twiss et al. [*11*], and the others correspond to "trough- or boat-shaped" reported by Parry and Smithson [*10*].

Panicoid Class—Dumbbell-shaped opal phytoliths belong to the Panicoid class (Pn 1 to 14

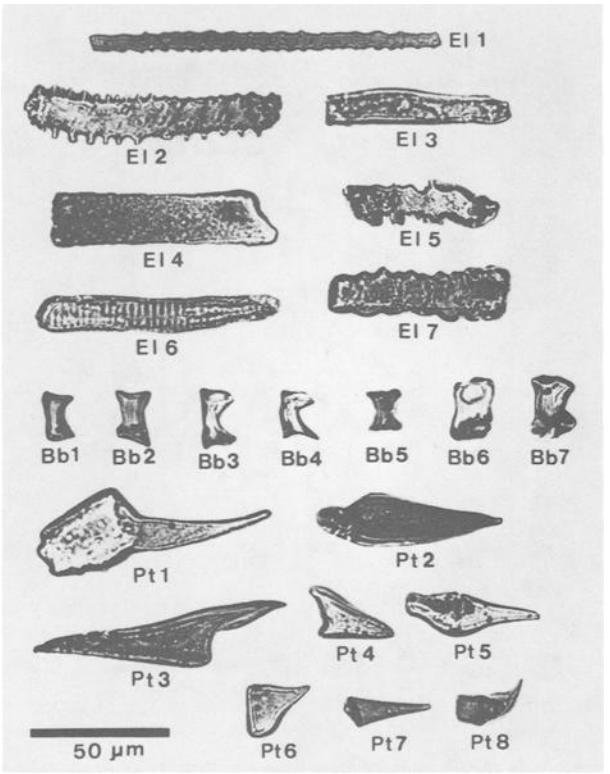

FIG. 3—*Opal phytoliths in the Gramineae plant samples.* El 1, *Setaria viridis;* El 2, *Pleioblastus chino;* El 3, *Digitaria adscendens;* El 4, *Elymus mollis;* El 5, *Miscanthus sinensis;* El 6, *Eragrostis ferruginea;* El 7, *Panicum crus-galli var echinata;* Bb 1, 2, 3, 4, *and* 5, *Pleioblatus chino;* Bb 6 *and* 7, *Shibataea kumasaca;* Pt 1, *Lolium multiflorum;* Pt 2 *and* 3, *Eragrostis megastachya;* Pt 4, *Panicum crus-galli var. echinata;* Pt 5 *and* 6, *Digitaria adscendens;* Pt 7, *Oryza sativa; and* Pt 8, *Setaria viridis.*

in Fig. 2). They originated from epidermis cells of Panicoideae, Oryzeae, and some of Arundoideae and Eragrostoideae.

Chloridoid Class—Saddle-shaped opal phytoliths, which had been reported by Plat [5] as battle-axes with a double edge, belong to the Chloridoid Class (Ch 1 to 5 in Fig. 2). They originate from epidermis cells to Eragrostoideae and Bambuseae.

Elongate Class—Elongate class opal phytoliths originate from long cells of leaves. They do not possess any subfamily or tribal characteristics (El 1 to 7 in Fig. 3). They are present not only in gramineous grasses but also in other monocotyledonous grasses such as Cyperaceae.

Bambusoid Class—Acutely angled opal phytoliths originating from short cells of Bambuseae and some of Arundoideae belong to the Bambusoid class. They usually show unsymmetric forms as Bb 1 to 4 and 7 in Fig. 3, but sometimes show symmetric rectangular forms with a variety of viewing angles as Bb 5 and 6 in Fig. 3.

Point-Shaped Class—Opal phytoliths, originating from prickle hairs, show point shape (Pt 1 to 8 in Fig. 3). They do not possess any subfamily or tribal characteristics.

Fan-Shaped Class—Opal phytoliths originating from bulliform cells showing fan-shape or rectangular outlines are classified into this class (Fig. 4). Their shape vary with family, genus, or species.

Opal Phytoliths of Tree Origin—Although Kondo et al. [*16-18*] classified opal phytoliths of tree origin into 14 groups, they were put into 1 group as "tree origin" for simplification in this paper, shown in Figs. 5 and 6.

Diatoms and Opal Sponge Spicules—Diatoms and opal sponge spicules are silica bodies

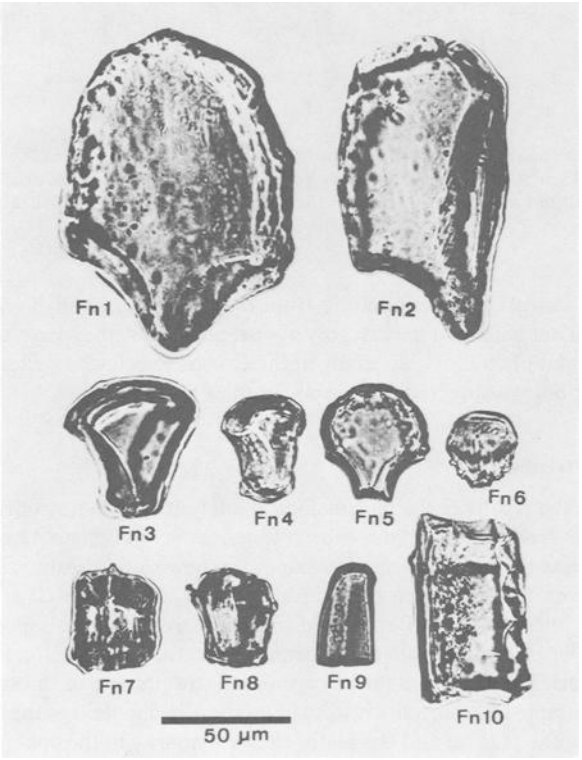

FIG. 4—*Opal phytoliths in the Gramineae plant samples.* Fn 1 *and* 2, *Phragmites communis;* Fn 3 *and* 7, *Pleioblatus chino;* Fn 4, *Bromus catharticus;* Fn 5, *Oryza sativa:* Fn 6, *Sasa albo-marginata;* Fn 8, *Shibataea kumasaca;* Fn 9, *Panicum crus-galli var. echinata; and* Fn 10, *Echinochloa crus-galli var. hispidura.*

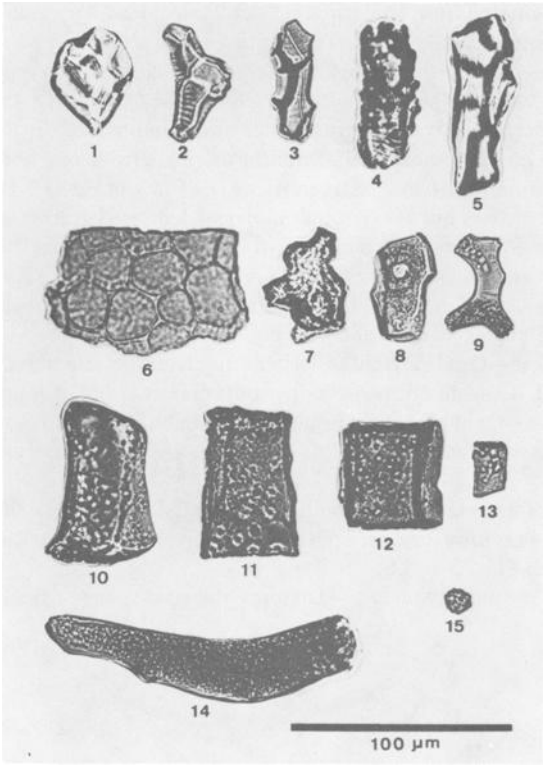

FIG. 5—*Opal phytoliths in the tree samples.* 1 *and* 5, *Shiia sieboldii;* 2 *and* 9, *Aesclus turbinata;* 3, *Magnolia kobus;* 4 *and* 14, *Magnolia obovata;* 6 *and* 7, *Zelkova serrata;* 8, *Morus bombycis;* 10, *Eriobotrya japonica;* 11 *and* 12, *Pinus densiflora;* 13, *Quercus serrata; and* 15, *Trachycarpus excelsa.*

showing various morphology, originating from *Bacillariophyta* and *Porifera*, respectively. Although they do not belong to the category of opal phytoliths, they are often observed in the examination of opal phytoliths, especially in the case of soils under wet condition. Figure 7 shows their morphology observed in the soil Samples D to F.

Results and Discussion

Table 2 shows the results of the morphological analysis of opal phytoliths in the soil samples. The samples from different sites show differences in opal phytolith compositions from each other, whereas the samples from the same site show similar results. There are obvious differences between the soils from the diluvial plateau (A, B, and C) and those from the alluvial plain (E and F) in that Bambusoid class and tree origin are more abundant, and Fan-shape class is less abundant in the plateau than in the plain. Diatoms and opal sponge spicules, which originated from aquatic organisms, are present in the soils of the alluvial plain (D to F). Sample D, whose site is located on the alluvial plain at the foot of the boundary slope between the plateau and the plain, shows similarity in the opal phytolith composition to the soils on the plateau, but it contains diatoms and opal sponge spicules. Apparently, these are caused by deposition of soil materials from the plateau by erosion and humid soil condition at low land, respectively. Comparison of opal phytolith composition enables us

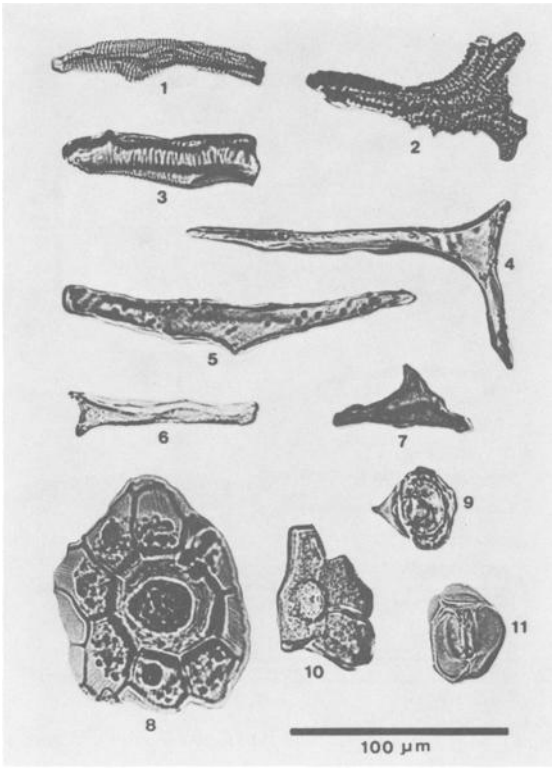

FIG. 6—*Opal phytoliths in the tree samples.* 1, *Shiia sieboldii;* 2, *Zelkova serrata;* 3, 4, *and* 5, *Magnolia grandiflora;* 6 *and* 7, *Quercus myrsinaefolia;* 8, 9, *and* 10, *Morus bombycis; and* 11, *Aesculus turbinata.*

to discriminate the soil samples from different sites, even though they have similar mineralogical characteristics. In the region chosen here, Chloridoid, Bambusoid, Fan-shape, and Elongate class, and tree origin are effective for the discrimination, as they vary with sampling site. Festucoid and Point-shape class, however, showed less effectiveness because of their paucity in this region.

The amount of the opal phytolith fraction required for morphological analysis is 1 to 2 mg, and the contents of this fraction in the soil samples used here were about 6% (A to D) and about 2% (E and F). Presumably, it is a sufficient amount to compare opal phytolith composition, if 100 mg of soil sample is obtained in the case of surface soil (A horizon) that has not been removed artificially.

Acknowledgments

The authors are grateful to Dr. R. Kondo, assistant professor of Obihiro University of Agriculture and Veterinary Medicine for his kindness with advice and in supplying Festucoid opal phytolith samples, and Dr. M. Kojima, Professor of Chiba University, and Drs. Y. Oba and S. Nagatsuka, professor and assistant professor of The University of Tsukuba, respectively, for their continued guidance.

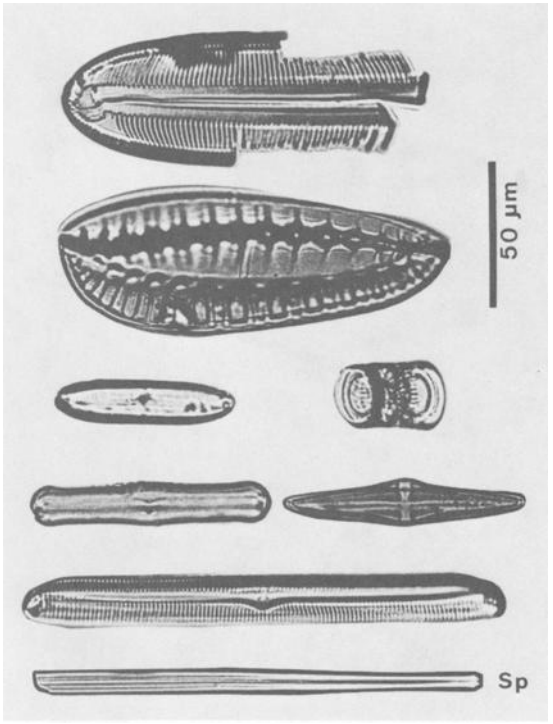

FIG. 7—*Diatoms and opal sponge spicules* (Sp) *found in the soil Samples D, E, and F.*

TABLE 2—*Morphological compositions of opal phytoliths in the soil samples (% by grain-counting).*[a]

Sample	Ft	Pn	Ch	El	Bb	Pt	Fn	Tr	Dm	Others	Total Grain Counted
A(1)	0.6	2.3	5.9	5.8	12.2	0.8	31.6	12.1	0.0	28.7	781
A(2)	0.4	1.7	5.9	6.3	12.6	1.0	33.2	11.7	0.0	27.2	709
B(1)	1.0	4.7	12.9	9.2	10.4	1.5	21.7	7.6	0.0	31.1	598
B(2)	1.0	3.5	11.9	10.6	8.9	0.6	23.3	6.6	0.0	33.6	629
C(1)	0.7	2.9	9.6	10.3	16.9	0.9	27.6	3.4	0.0	27.6	544
C(2)	0.8	3.0	12.2	10.2	15.9	0.8	27.9	2.4	0.0	26.9	502
D(1)	0.8	2.3	2.3	10.3	18.7	1.0	28.7	5.2	0.6	30.2	582
D(2)	1.5	1.5	2.7	11.2	17.4	1.1	30.0	4.5	0.4	29.7	651
E(1)	1.4	2.6	7.0	13.1	3.8	0.6	39.9	0.3	2.1	29.2	520
E(2)	0.5	1.8	5.3	9.6	3.8	2.3	43.2	0.8	1.5	31.2	581
F(1)	1.2	1.0	11.0	11.0	6.0	0.5	36.7	3.2	1.9	27.5	517
F(2)	1.0	2.1	13.1	9.8	5.2	0.8	38.4	2.2	2.1	25.3	584

[a]Abbreviations—Ft, Festucoid class; Pn, Panicoid class; Ch, Chloridoid class; El, Elongate class; Bb, Bambusoid class; Pt, Point-shape class; Fn, Fan-shape class; Tr, tree origin; and Dm, diatoms and opal sponge spicules.

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