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PRELIMINARY STUDY ON PHYTOLITHS IDENTIFICATION IN TWO MAJOR RIVERBANKS OF SELANGOR (GOMBAK AND KLANG RIVERBANKS)

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ABSTRACT

Phytoliths are plant fossils found within the plant cells of embryophytes and it can be deposited into the uppermost horizon of the soil when embryophytes die and decay. This will cause the phytoltihs is released from its organic matrix and chemisorbed into the soil particles. This study aimed to determine the presence and morphology of phytoliths found in two major riverbanks of Selangor (Gombak and Klang riverbanks). Composite soil samples were collected from the top two cm of the soil of Gombak and Klang riverbanks with each composite consists of five subsamples. The distance between each subsample is two m apart. The composite soil samples were subjected to extraction process before phytoliths isolation which involved deflocculation using 5% of sodium hexametaphosphate, decarbonation using 10% hydrochloric acid, organic material removal using 65% nitric acid and potassium chlorate, clay removal via centrifuge sedimentation and, organic matter and humic colloids removal using 10% potassium hydroxide. Heavy liquid zinc bromide/hydrochloric acid was used to isolate phytoliths. The presence and morphology of phytoliths between the two riverbanks were identified and counted using comparison microscope. The results were compared between the two riverbanks to determine if discrimination of soil in different site is possible.

Key words: Phytoliths, forensic science, soil, plant fossils

INTRODUCTION

Phytoliths are opal silica particles manufactured in and between the cells of embryophytes (Madella *et al*., 2005). The word phytolith is derived from the Greek origin where *phyto* means plant and *lithos* means stones (Hart, 2015). Almost all of the embryophytes found in the world produce such plant stones. They include flowering plants (angiosperms), seed-producing plants (gymnosperms) and vascular plants, which reproduce and disperse via spores (pteridophytes). Because phytoliths are produced in almost all of the embryophytes, it is safe to conclude that such phytoliths can be found in abundance in soil (Hart, 2015).

There are many uses of phytoliths in the field of science, from archaeology to forensic. In archaeology, phytoliths are used to identify local vegetation, reconstruct past climates and to identify diet of extinct fauna. In forensic science, phytoliths can be used to discriminate soil samples from different sites and to correlate suspect(s) and crime scene(s). Marumo & Yanai (1986) conducted a research titled Morphological Analysis of Opal Phytoliths for Soil Discrimination in Forensic Science Investigation, which showed that soil samples from same site have same phytoliths composition and soil samples from different sites have different phytoliths compositions.

Soil is composed of both physical and chemical properties that can be utilized by forensic scientists to make comparison between known and unknown samples. Soil analysis, however, has less value in crime scene investigation unless there is the presence of impression evidence such as shoe prints and tire tread marks on the surface of the soil. These investigations, however, are focused on the impression evidence and does not involve the analysis of the soil (Houck & Siegel, 2010).

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MATERIALS AND METHOD

Sample Selection

The research was based on convenience sampling. The soil sampling method used was adapted from Guidance Document 11 – Soil Sampling Guidance (2014) while the general sampling procedure was adapted from Pirrie & Ruffell (2012).

The author used composite soil sampling method to identify the presence of phytoliths in two major riverbanks of Selangor. The objective in using composite soil sampling was to represent the average presence of phytoliths in the sampled body of material (soil in this case).

Two composite soil samples were collected from two different riverbanks of Selangor, namely Gombak and Klang riverbank. Soil samples collected were from the top 2 cm of the soil and each composite soil sample consists of 5 subsamples. The distance for each subsample is 2 m along the downstream of the river, making the distance covered for each composite soil sample to be 10 m.

Sample collection

About 2 spatula of the soil sample with depth of 2 cm from the first soil subsample was collected. The soil sample was placed inside a clean mason jar. The following soil subsample was collected at 2 m apart from the first soil subsample until a distance of 10 m had been covered, using the same mason jar. The mason jar was then covered and inverted several times to thoroughly mix the soil together. The step was repeated until all the soil samples from two different riverbanks of Selangor are covered.

Sample extraction

The sample extraction protocol was adapted from Aleman *et al* (2013) with reference guide from Piperno (2006) and Zhao & Pearsall (1998). The soil volume used for all of the composite soil samples were 5 cm^3. The soil samples were not dried to prevent grinding that can distort the shape of the phytoliths. 10 mL of 1 N hydrochloric acid was added into the sediments and the contents were vortexed. The contents were placed in a water bath at 70ºC for 1 hour. This was to remove carbonate (mineral) and iron and aluminium oxide clays. Presence of is indicated by bubbling of the sediment. When bubbling ceased, the contents were centrifuged at 3000 rpm for 2 minutes and the supernatant was discarded. HCl was added and this step was repeated until no reaction was observed when more HCl was added. The contents were then centrifuged at 3000 rpm for 2 minutes and the supernatant was removed. The tubes were then filled with distilled water, shaken and again centrifuged at 3000 rpm for another 2 minutes and was repeated twice to rinse the contents.

5% of sodium hexametaphosphate was added into the contents and was shaken overnight via an automatic shaker to disaggregate the minerals and organic constituents present in the soil samples. The sediments were then centrifuged at 3000 rpm for 2 minutes and the supernatant was removed. To rinse the sediments, the tube was filled with distilled water, shaken and centrifuged at 3000 rpm for another 2 minutes and was repeated twice. 30 mL of 65% nitric acid was added into the sediments and was then heated at 70ºC using a water bath for 1 hour to remove organic material. A watch glass was used to prevent evaporation and splashing. A pinch of strong oxidizer such as potassium chlorate was added to speed up the process and to make the removal more efficient. The contents were rinsed by centrifuging them at 3000 rpm for 2 minutes when bubbling ceased and the supernatant became either clear or bright yellow in colour and does not have the colour of reddish or reddish-brown hue. The supernatant was removed. Distilled water was added and the content was centrifuged again at 3000 rpm for another 3 minutes and was repeated twice.

Clay was removed from the samples by adding distilled water to the residue to a height of 10 cm and centrifuge the content at 3000 rpm for 2 minutes. The supernatant was removed and new distilled water was added and this process was repeated until the supernatant became clear in colour. Humic colloids and excess organics were removed by adding 30 mL of 10% potassium hydroxide and was heated at 70ºC for 10 minutes in a water bath. The samples were then centrifuged at 500 rpm for 4 minutes. The supernatant was discarded. Distilled water was added to rinse off the potassium hydroxide and the content was centrifuged again at 3000 rpm for 2 minutes. The rinsing process was repeated twice. 5 mL of absolute ethanol was added to the sediment after rinsing. The contents were shaken gently and were centrifuged at 3000 rpm for 2 minutes. The supernatant was then discarded and the process was repeated once. After the supernatant was discarded, the ethanol was left to evaporate for 20 minutes at 30ºC using an oven.

Phytoliths isolation

10 mL of heavy liquid (zinc bromide/ hydrochloric acid) was added into the samples and the samples were placed in a vortex to ensure uniform mixing. The contents were then centrifuged at 3000 rpm for 2 minutes. The floating phytoliths in the form of fine white layer floating on the dense liquid was transferred to a clean 15 mL centrifuge tube. This step was repeated for few more times to ensure most of the phytoliths were isolated.

The heavy liquid was rinsed by adding distilled water to heavy liquid at a ratio of 2.5:1 to ensure the phytoliths sink to the bottom. The contents were inverted before being centrifuged at 3000 rpm for 10 minutes. This step was repeated for at least 4 more times to ensure all of the heavy liquid was removed. The phytoliths were then dried inside an oven at 50ºC.

Phytoliths count and identification

A pinhead size of the phytoliths was placed onto the microscopic slide followed by a drop of absolute ethanol to segregate it. The ethanol was left to evaporate and a cover slip was placed over the microscopic slide and was viewed under 400X magnification in random using comparison microscope to facilitate phytoliths count. Five microscopic slides were prepared for each soil samples and at least 200 phytoliths were collectively counted for each soil sample (Albert & Weiner, 2001).

A drop of immersion oil was added onto the microscopic slide after counting to facilitate 3 dimensional observations. The morphological structure(s) of the phytoliths were identified and recorded at 400 X magnification.

RESULTS AND DISCUSSION

Based on the International Code for Phytoliths Nomenclature 1.0 (Madella *et al*., 2005), the most similar phytoliths morphology observed under comparison microscope for soil samples taken from Gombak riverbank (Fig. 1) were oblong, volcaniform, scutiform, trilobate, bilobate and tabular while Klang riverbank (Fig. 2) consists of rectangle, ovate and quadra-lobate phytoliths.

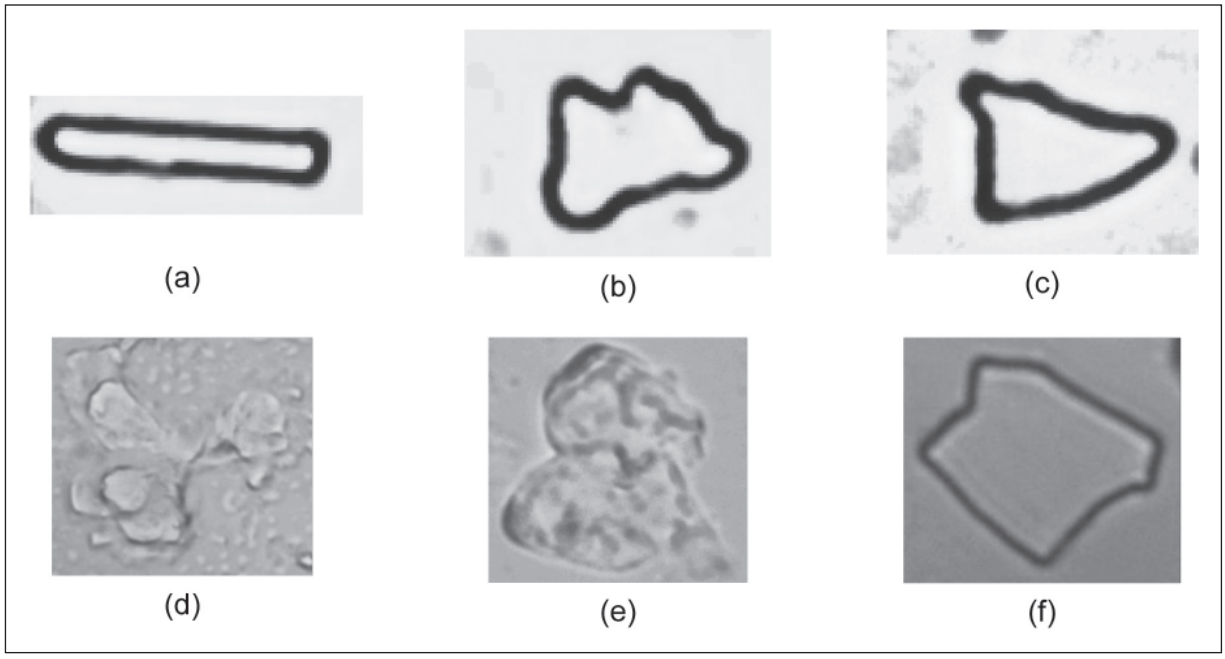

Fig. 1. Phytoliths present in soil sediments obtained from Gombak riverbank; (a) oblong, (b) volcaniform, (c) scutiform, (d) trilobite, (e) bilobate and (f) tabular.

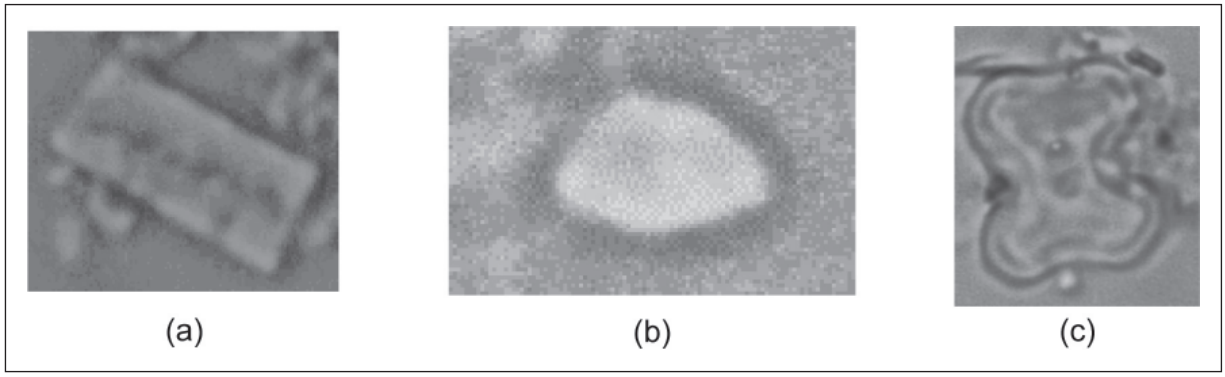

Fig. 2. Phytoliths present in soil sediments obtained from Klang riverbank; (a) elongate, (b) ovate and (c) quadra-lobate.

The morphology of the phytoliths were named based on the first descriptor which is based on 3-dimensional and 2-dimensional shapes.

Based on the International Code for Phytoliths Nomenclature 1.0, elongate is descripted as phytoliths that are longer than their widths while bilobate and quadra-lobate are phytoliths with two lobes and having four lobes with double mirror symmetry respectively. Oblong phytoliths is classified as longer than broad and with nearly parallel sides while ovate is shaped like an egg, oblong but broader at one end. Scutiform phytoliths has the shape of a shield while tabular phytoliths is thin and flat like that of a table.

The type of plants present is identified through phytoliths identification. Twiss *et al* (1969) divided the morphology of grass phytoliths into four classes, namely Festucoid (Class 1), Chloridoid (Class 2), Panicoid (Class 3) and Elongate (Class 4) classes. By comparing the types of phytoliths found in Gombak and Klang riverbanks with the classification of grass phytoliths from Twiss *et al* (1969), it was found that both Gombak and Klang riverbanks were composed of Festucoid and Panicoid grass. The presence of elongate class does not indicate specific grass because this class was found in all of the samples in the research of Twiss *et al* (1969). Ebigwai *et al* (2015) also documented grass-originating phytoliths in their study which was present in the soil sediment studied in this present study.

Ball *et al* (2005) mentioned that volcaniform is a type of phytoliths produced by banana plants, which suggest that there were banana plants planted in Gombak riverbank.

Results of the present study showed that both Gombak and Klang riverbanks have different types of phytoliths with the only exception in which both of the sites contain quadra-lobate type of phytoliths. The presence of different types of phytoliths in different site area allows forensics to discriminate soil samples and to correlate between suspect(s) and crime scene(s). For an example, finding a quadra-lobate, trilobite and oblong types of phytoliths in a given soil sample may indicate that the soil may have come from Gombak riverbank while finding an elongate, ovate and quadra-lobate types of phytoliths may indicate that the soil may have come from Klang riverbank.

This finding however, only provide an indication as to where the soil sample may have come from but it does not act as a confirmatory test because further research is needed to determine the species of plants based on the shape and size of the phytoliths.

CONCLUSION

Phytoliths had been widely studied in the field of archaeology because these particles can withstand extreme environment condition without affecting the structure and it is used by archaeologists to identify local vegetation, to reconstruct past climates and to identify the diet of extinct fauna. Besides archaeology, phytoliths are getting famous in the field of forensic science because it has the possibility to discriminate soil samples from different site as different location will have distinguishable phytoliths.

This can be seen from the research in which only one phytoliths morphological structure were similar when compared with Gombak and Klang riverbanks. The results from this research has high potential to be utilized by forensics to discriminate soil samples from different sites and to correlate suspect(s) and scene of crime(s).

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REFERENCES

Albert, R.M. & Weiner, S. (2001). Study of opal phytoliths in prehistoric ash layers using a quantitative approach. In H. Meunier & F. Coline (Eds), *Phytoliths: applications in earth sciences and human history.* The Netherlands: Balkema, 251-366pp.

Aleman, J.C., Jean, A.S., Leys, B., Carcaillet, C., Favier, C. & Bremond, L. (2013). Estimating phytolith influx in lake sediments. *Quatenary Research,* **80**: 341-347.

Ball, T., Vrydaghs, L., Van Den Hauwe, I., Manwaring, J. & De Langhc, E. (2006). Differentiating banana phytoliths: wild and edible *Musa acuminat*a and *Musa balbisiana*. *Journal of Archaeological Science,* **22**: 1228-1236.

Ebigwai, J.K., Nyannayo, B.L., Egbe, A.E., Asuquo, E. & Aniekebo, I.H. (2015). Variation of Phytolith Morphotypes of Some Members of Cucurbitaceae Juss. *Journal of Pharmacy and Biological Sciences,* **10(6)**: 100-115.

Guidance Document 11- Soil Sampling Guidance. (2014). Retrieved from Minnesota Department of Agriculture: http://www.mda.state.mn.us/chemicals/spills/incidentresponse/guidelist/gd11.aspx

Hart, T.C. (2015). Phytoliths: The Storytelling Stones Inside Plants. *American Scientist,* **103(2)**: 136-143.

Houck, M.M. & Siegel, J.A. (2010). *Fundamentals of Forensic Science* (2nd ed.). United States of America: Elsevier.

Madella, M., Alexandre, A. & Ball, T. (2005). International Code for Phytolith Nomenclature 1.0. *Annals of Botany,* **96**: 253-260.

Marumo, Y. & Yanai, H. (1986). Morphological analysis of opal phytoliths for soil discrimination in forensic science investigation. *Journal of Forensic Sciences,* **31**: 1039-1049.

Piperno, D.R. (2006). *Phytoliths: A Comprehensive Guide For Archaeologists and Paleoecologists.* United Kingdom: Alta Mira Press, 89-102pp.

Pirrie, D. & Ruffel, A. (2012). Forensic Geology and Soils. In N. Marquez-Grant, & J. Roberts (Eds.), *Forensic Ecology Handbook: From Crime Scene to Court.* United Kingdom: John Wiley & Sons, 183-201pp.

Twiss, P.C., Suress, E. & Smith, R.M. (1969). Morphological Classification of Grass Phytoliths. *Soil Science Society of America Proceedings,* **33(1)**: 109-115.

Zhao, Z. & Pearsall, D.M. (1998). Experiments for Improving Phytolith Extraction from Soils. *Journal of Archaeological Science,* **25**: 587-598.