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Unlocking the Science behind the Soil Phytolith Extraction: New Insights on Terrestrial Carbon Reservoirs

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Abstract

Climate change is accelerating carbon cycle disruptions that necessitate exploration of stable terrestrial carbon pools. Phytoliths, silica-based microstructures bring a resilient mechanism for long-term carbon stabilization. These structures encapsulate organic carbon, producing PhytOC, which remains chemically protected within the phytoliths. Unlike volatile soil organic carbon, PhytOC resists microbial decomposition and environmental leaching, contributing to persistent carbon storage in soils and sediments. Phytoliths extraction from soil involves the removal of carbonates and organics through oxidative digestion then to heavy liquid flotation using zinc bromide. Further, centrifugation enables density-based separation, allowing phytoliths to float and be decanted for morphotype characterization. Recent advancement in high-resolution imaging and stable isotopic tracing techniques have improved the characterization of phytolith spatial distribution, carbon occlusion and ecosystem level sequestration potential. Incorporating phytolith dynamics into terrestrial carbon evaluations can improve the estimation of phytolith contributions to global carbon budgets and support nature based climate mitigation strategies.

Keywords: Carbon sequestration, Heavy liquid flotation method, Soil phytolith, Terrestrial ecosystem

Introduction

Phytoliths are microscopic, amorphous silica structures synthesized intra- and inter-cellularly in plants through the biochemical processes of silica precipitation and polymerization, collectively termed biosilicification. In higher plants, biogenic silica is deposited intracellularly within the cytoplasm and vacuolar compartments, while intercellular accumulation of phytoliths occurs extensively across all anatomical structures including the subterranean (roots), axial (stems), foliar (leaves), reproductive (fruits and inflorescences) and other specialized tissues. Phytoliths are remarkably resilient, making it possible for them to remain intact long after a plant dies and decomposes, as they are built from inorganic silica that can resist breakdown from environmental forces and get preserved in terrestrial deposits and marine layers, making them valuable indicators in the fossil record. The increasing emissions of greenhouse gases are major causes of global warming and intensifying extreme weather events with increasing severe risks to ecosystem resilience and sustainability. Moreover, studies have demonstrated that phytoliths sequester trace elements, notably PhytOC, through incorporation into their biogenic silica matrices. In contrast to soil organic carbon, which is susceptible to environmental losses, PhytOC remains stable within the silica structure, thereby serving as a long-term carbon reservoir in terrestrial ecosystems.

Techniques and Processes in Phytolith Extraction

The principle behind isolating phytoliths from soil is followed with the heavy liquid flotation method slightly modified from Han *et al.* (2018). This method is widely recognized for its efficacy in isolating soil phytoliths because of its effectiveness to produce highly purified siliceous residues with minimal organic and mineral contamination, thereby enhancing morphotype visibility and reducing impurities during microscopic examination. By utilizing density-based separation, this technique helps in selective extraction

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with improved recovery efficiency relative to combustion based methods such as dry ashing or wet oxidation, which often suffer from selective loss or distortion of phytolith morphotypes. This technique involves the following four steps of deflocculation, elimination of organic fraction, decarbonation, density based phytolith extraction.

Deflocculation

Deflocculation plays a crucial role in heavy liquid flotation method for phytolith extraction. This process involves the use of chemical dispersant like calgon solution to break flocculated clay aggregates and enhances particle dispersion. This ensures that phytoliths can be efficiently separated based on density without interference from fine mineral fractions (Amarjargal and Tasdemir, 2023).

Decomposition of Organic Residues

In the context of heavy liquid flotation for soil phytolith extraction, the decomposition of organic residues is an essential step that enhances the purity and visibility of siliceous particles. This is typically achieved through oxidative digestion using reagent such as hydrogen peroxide (H_2O_2), which effectively break down humic substances and other organic matrices with the cessation of bubbles. Such treatments reduce interference and increase the reliability of morphotype identification (Andriopoulou and Christidis, 2020).

Decarbonation

Decarbonation is a key preparatory step involves the chemical dissolution of carbonates typically using dilute hydrochloric acid (HCl) to eliminate inorganic carbon compounds with termination point of color change from greenish to yellowish. According to Kooyman (2015), decarbonation prior to flotation enhances the efficiency of phytolith isolation by reducing mineral impurities and facilitating cleaner separation of silica bodies from soil matrices.

Phytolith Extraction

After the removal of carbonates and organic matrices through the above steps, the residual soil appeared ash-like material is subjected to heavy liquid separation using zinc bromide ($ZnBr_2$), adjusted to a specific gravity of 2.3. This density closely matches that of phytoliths (1.5-2.3), allowing for their selective flotation (Figure 1). Upon centrifugation,

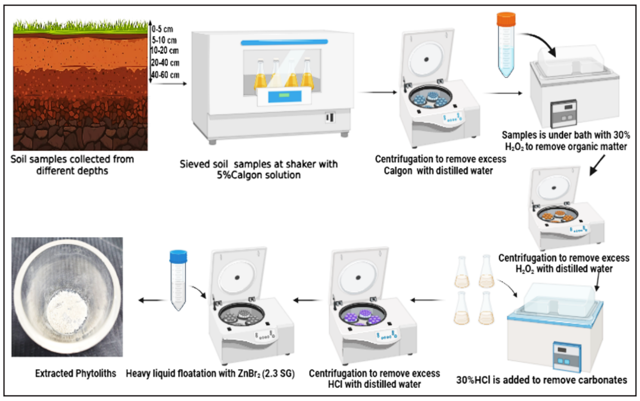

Figure 1: Pictorial representation of phytolith extraction from soil

phytoliths remain to float or suspend on the $ZnBr_2$ medium. The supernatant containing the phytoliths is carefully decanted, rinsed to remove residual $ZnBr_2$ and subsequently analysed for morphotype classification under microscopy (Sarret *et al.*, 2022). Table 1 represents the sequential steps of phytolith extraction mechanism.

Table 1: Sequential steps of phytolith extraction mechanism

Sl. No.	Steps	Chemicals/ techniques	Mechanism
1	Deflocculation	Calgon solution (Sodium hexa-metaphosphate)	Breaks soil aggregates to fine particles.
2	Decomposition of organic residues	Hydrogen peroxide	Removes organic residues from soil.
3	Decarbonation	Hydrochloric acid (HCl)	Extracts carbonates left in soil.
4	Phytolith extraction	Heavy liquid (*i.e.*, $ZnBr_2$)	Heavy liquid with specific gravity detaches phytolith from treated soils.
5	PhytOC estimation	CHNS analyser	Analyses elemental composition of carbon occluded in phytoliths.
6	Microscopic analysis	SEM/ light microscopy	Characterizes morphotypes of phytoliths.

Conclusion

Recent studies in phytolith research have harnessed cutting-edge imaging and isotopic tracing techniques to resolve the distribution and carbon binding properties of these phytoliths. Once considered passive residues of plant tissue, phytoliths are now recognized as active participants in terrestrial carbon dynamics, with growing evidence of their contribution in long-term sequestration. This emerging framework encourages researchers to witness phytoliths as not just ancient plant life, but also act as active participants in sequestering the terrestrial carbon to mitigate climate change.

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