

# Optimization of extraction method for quantitative analysis of Si/Al in soil phytoliths

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**Abstract** Elemental information carried by phytoliths plays a crucial indicative role in geochemical research. For instance, it serves as an indicator of the carbon pool effect in phytoliths and aids in the elucidation of silicon sources. However, early extraction methods for soil phytoliths primarily focused on obtaining their morphological and quantitative information, lacking efficient techniques for quantitative elemental analysis. In this study, we aimed to extract Si/Al information from soil phytoliths. Considering the need for complete extraction of phytolith, six extraction methods were developed and further by alkaline dissolution to determine Si/Al. Six methods were compared in terms of enrichment capacity, the weight of extracted phytoliths, and Si/Al differences. The results indicated that the addition of Ammonia-Catechol in the commonly used heavy liquid flotation method effectively improved phytolith extraction capability and the accuracy of Si/Al results. Additionally, the inclusion of an acetic acid step before alkaline dissolution further removed surface-adsorbed impurities and enhanced the analytical quality of Si/Al in phytolith. The comparison of the data in this study with other published data shows that our method is relatively robust. The improved method proposed in this study can provide a new idea for the quantitative analysis of other elements in soil phytoliths.

**Keywords** Soil phytolith · Extraction method · Silica-aluminum ratio · Ammonia-Catechol

## 1 Introduction

Phytoliths are amorphous silica particles formed by the precipitation of Mono-silicic acid in plant cells and surrounding areas in terrestrial plants (Cooke & Leishman 2011). It is estimated that 60–200 T mol Si yr<sup>-1</sup> of phytoliths are formed in plants, with the phytoliths stored in soils reaching 500–1000 times the current biomass (Conley 2002). Consequently, phytoliths play a crucial role in the regulation of Earth's surface silicon cycling (Tran et al. 2019). Accordingly, elements contained in phytoliths exhibit coupled cycling patterns. Therefore, the elemental information in phytoliths can serve as an indicative tool for geochemical processes. For instance, soil phytoliths may have a significant carbon pool effect; The Ge/Si of phytoliths can be used to distinguish the sources of fluvial dissolved silicon (Derry et al. 2005; Parr & Sullivan 2005; Ran et al. 2017). However, accurately obtaining the elemental information in phytoliths is very demanding for the extraction method of soil phytoliths. For example, the accuracy of carbon content in soil phytoliths and radio-carbon dating results are influenced by the efficiency of soil organic carbon removal during the extraction (Han et al. 2018; Zuo et al. 2019).

The methods used for extracting phytoliths from the soil primarily include heavy liquid flotation (HLF), combustion, and microwave digestion (Knox 1942; Parr 2002; Powers & Gilbertson 1987). Combustion may lead to clay aggregation (Lombardo et al. 2016), while microwave digestion (0.25 g/batch) has a lower sample processing

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capacity compared to HLF (5 g/batch) (Parr 2002). The drawback of the lower phytolith enrichment efficiency of both methods partially offsets their advantages in terms of safety and cost (Lentfer & Boyd 1998). Currently, HLF is commonly employed for extracting phytoliths from soil (Lombardo et al. 2016). Initially, this method was mainly used to obtain morphological and quantitative information about soil phytoliths (Carbone 1977; Twiss et al. 1969), but in recent years, it has been increasingly used to study elemental information in phytoliths (Li et al. 2021).

However, there are still limitations in the quantitative analysis of elemental composition in soil phytoliths. Factors such as clay minerals, iron–aluminum oxides, soil organic matter, and adsorbed ions on the phytolith surface can interfere with the quantification of phytolith elements (Calegari et al. 2013; Han et al. 2018). For instance, Clymans et al. (2015) attempted to differentiate silicon components between volcanic glass and phytoliths using Si/Al (Clymans et al. 2015). However, impurities within the samples impede the feasibility of the application. Additionally, the solubility of phytoliths is positively correlated with their Si/Al (Bartoli 1985). These impurities also hamper the exploration of the geochemical stability of soil phytoliths. On the other hand, chemical reagents during extraction to remove contaminants, such as  $H_2O_2$  combined with  $H_2SO_4$  or sodium dithionite-citrate-sodium bicarbonate (DCB), can cause phytolith dissolution (Parr & Sullivan 2013; Vander Linden et al. 2021). Therefore, the method used to analyze elemental information in phytoliths should fulfill the following criteria: (1) minimize interference from environmental impurities, and (2) minimize the loss of elements in phytolith during extraction.

In this study, an optimized extraction method for obtaining Si/Al information from soil phytoliths was developed. This provides a new approach to improve the quantitative analysis of elemental data in soil phytoliths. Simultaneously, obtaining relatively accurate Si/Al ratio data from phytoliths enriches our understanding of their inherent geochemical stability. First, to effectively remove impurities and improve the recovery efficiency of phytolith from soils, we tested six extraction methods. Second, the optimal protocol was selected based on the results from the extraction and subsequent alkaline dissolution stages. Finally, the published data were collected and compared with the data in this study to explore the effectiveness of the proposed method. Furthermore, a comparison between data from plant phytoliths and soil phytoliths reveals an intriguing trend. During the migration of phytoliths from plants to soil, sections with relatively lower Si/Al ratios appear to exhibit a higher propensity for storage.

## 2 Material and method

### 2.1 Sample origin

In this study, 22 soil samples were collected from the vicinity of the Xin'anjiang Reservoir ( $118^{\circ}20' E$ – $119^{\circ}20' E$ ,  $29^{\circ}11' N$ – $30^{\circ}02' N$ ) located in Chun'an County, Zhejiang Province, China. All samples were collected from the soil's surface layer (3–10 cm). One soil sample was selected for method optimization, while the remaining 21 soil samples were analyzed for Si/Al using the optimized method. The soil pH in this region ranged between 5.5 and 6.0 (Wang 2020), with the prevalent soil type identified as Ao (Orthic Acrisols) according to the FAO classification system.

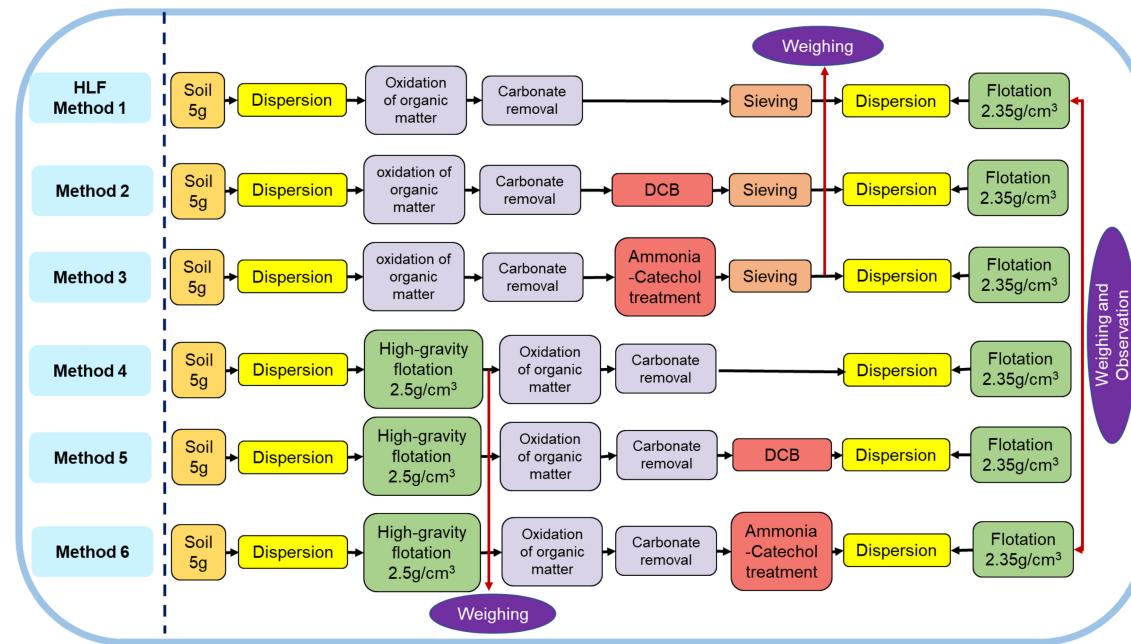
### 2.2 Phytolith extraction

In this study, HLF with combined ultrasound and centrifugation techniques was used as the standard extraction method (Method 1) (Han et al. 2018; Lombardo et al. 2016; Zhao & Pearsall 1998). The extraction involved clay dispersion, oxidation of organic matter, removal of carbonates, 250  $\mu m$  sieving, and a second clay dispersion; and the density difference between phytoliths ( $1.5$ – $2.3 \text{ g cm}^{-3}$ ) and clay minerals ( $2.5$ – $2.7 \text{ g cm}^{-3}$ ) was utilized to separate the phytolith samples by using a heavy liquid with a density of  $2.35 \text{ g cm}^{-3}$ . Methods 2 and 3 incorporated the addition of DCB and Ammonia-Catechol during the extraction to eliminate interference caused by iron–aluminum oxides in the sample (Prodromou & Kalovoulos 1994). Methods 4, 5, and 6 replaced sieving with a  $2.5 \text{ g cm}^{-3}$  high specific flotation step (high-gravity flotation) before oxidation to obtain intact-sized phytoliths. This is mainly due to phytoliths exhibiting wide size distribution ( $< 2$  to  $2000 \mu m$ ) (Nawaz et al. 2019), and sieving may result in the loss of large-sized phytoliths ( $> 250 \mu m$ ). The process of the six methods is shown in Fig. 1, and the details of the operation steps are shown in Table 1.

In the extraction experiments, extracted phytoliths were dried and weighed to compare the effect of different methods on soil dispersion. In addition, in methods 1, 2, and 3 and methods 4, 5, and 6, we compared the pre-enrichment effects of sieving and high-gravity flotation. Finally, optical microscopy at  $40 \times 10$  and scanning electron microscopy (SEM) were used to observe the morphology and clarity features of the extracted phytoliths.

### 2.3 Chemical analysis

Due to the small density difference between phytoliths and some clay minerals, along with the effect of clay hydration,



**Fig. 1** Process of different extraction methods

**Table 1** Detailed description of the steps for phytolith extraction from soil

Steps	Operation details
Sample handling	5 g of soil that has been ground and sieved through 2 mm in a 50 mL plastic tube;
First dispersion	Add 45 mL of 5% Calgon, place in an ultrasonic bath for 10 min, centrifuge to remove the supernatant (3000 rpm, 2 min), then add deionized water and wash by centrifugation 3 times (3000 rpm, 2 min);
Organic matter removal	Add 30% H <sub>2</sub> O <sub>2</sub> , place in a constant temperature water bath at 70 °C, and wash 3 times after the reaction is complete;
Carbonate removal	Add 15 mL of 30% HCl, place in a constant temperature water bath at 70 °C, and wash 3 times after the reaction is complete;
Sieving	Sort the sample with a 250 µm sieve, take the sieve down, dry, weigh, and place in a 50 mL plastic tube;
Second dispersion	Add 45 mL of 5% Calgon, place in an ultrasonic bath for 10 min, and wash 3 times;
Flotation	Add 10 mL of 2.35 g cm <sup>-3</sup> ZnBr <sub>2</sub> , place in an ultrasonic bath for 10 min, and then centrifuge (3000 rpm, 5 min) to collect the supernatant and repeat 2 times. Place the supernatant in another 50 mL plastic tube, add deionized water until the specific gravity of the solution in the tube is less than 1.5 g cm <sup>-3</sup> , remove the supernatant by centrifugation (3000 rpm, 10 min), wash three times, and then dry the resulting phytolith sample and weigh
DCB treatment	Add 40 mL of 0.3 mol L <sup>-1</sup> sodium citrate solution and 5 mL of 1 mol L <sup>-1</sup> sodium bicarbonate solution, then add 1 g of low sodium sulfite pellets (pH = 7.3 of the mixture), place the reaction in a constant temperature water bath at 80 °C for 15 min with shaking, then add saturated sodium chloride solution and acetone to remove the supernatant and wash it 3 times
Ammonia-Catechol treatment	Add 50 mL of 0.1 mol L <sup>-1</sup> catechol solution, then add 1 mL of 5% ammonia solution (pH = 8.67 of the mixture), seal the mouth of the tube, put it in a constant temperature water bath at 80 °C for 10 min after shaking, and then wash it 3 times
High gravity flotation	Add 10 mL of 2.5 g cm <sup>-3</sup> ZnBr <sub>2</sub> , place in an ultrasonic bath for 10 min, centrifuge (3000 rpm, 5 min) to collect the supernatant, and repeat 2 times. The supernatant was placed in another 50 mL plastic tube, deionized water was added until the specific gravity of the solution in the tube was less than 1.5 g cm <sup>-3</sup> , the supernatant was removed by centrifugation (3000 rpm, 10 min), and the residual sample was washed 3 times was dried and weighed, and used for subsequent processing

it is difficult to achieve complete separation of phytoliths and clays (Vander Linden et al. 2021; Zhao & Pearsall 1998). Therefore, the above-extracted phytoliths were

further analyzed using continuous extraction with alkali, to correct for the interference of clay minerals. This method was developed based on the principle that biogenic silica

dissolves faster than silicate minerals, also known as the multi-point method (DeMaster 1981). The extracted phytoliths obtained from the six extraction methods were placed in 50 mL centrifuge tubes. Subsequently, 50 mL of 0.1 mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> (pH = 11.44) was added to each tube. The tubes were then placed in a constant temperature shaking water bath at 85 °C. At specific time intervals (1, 5, 10, 15, 30, 60, 90, 120, 180, 240, and 300 min), the supernatant was collected and filtered. The silicon content in the extraction solution was determined using the silicon-molybdenum blue colorimetric method to generate a dissolution curve. Biogenic silica rapidly dissolves, whereas coexisting clay minerals dissolve slowly and exhibit a linear release pattern over time. Consequently, when the extracted amount of silicon shows linear growth over time, the intercept of this linear curve on the Y-axis can be employed to eliminate the influence of mineral silicon. The Y-axis intercept of the curve was considered as the silicon content in the phytoliths, referred to as PhytSi.

When the number of samples is large, the multi-point method analysis is time-consuming (Feng et al. 2019; Mortlock & Froelich 1989). To improve efficiency, an optimized single-point method was employed in this study to analyze the elemental information in phytoliths from 21 soils. Biogenic silica can dissolve rapidly in a short time (Barão et al. 2015; Kamatani & Oku 2000), so in this study, the PhytSi content at 15 and 30 min was selected for single-point analysis. The extracted phytoliths were dissolved using 0.1 mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> in a constant temperature oscillating water bath at 85 °C. Subsequently, the supernatant was collected and filtered at 15 and 30 min, respectively. The dissolved silicon in the solution was quantified via the silicon molybdenum blue colorimetric method, and a comparative analysis was performed with the results of the multi-point method to determine the optimum method.

During HLF extraction, impurities adsorbed on the surface of phytoliths may not be effectively removed. To address this concern, acetic acid washing was chosen to explore its impact in this study (Liu et al. 2019). 50 mL of 0.1 N acetic acid was added to the centrifuge tubes containing the extracted phytoliths, followed by shaking for 72 h. The control group underwent the same operation using 50 mL of deionized water. After treatment, the extracted phytoliths were dissolved using the optimized single-point method, and the dissolved aluminum content was determined using the chromium azure S spectrophotometric method, while the dissolved silicon content was determined using the silicon molybdenum blue colorimetric method. Six selected extraction methods and subsequent chemical analyses included three repetitions.

### 3 Results

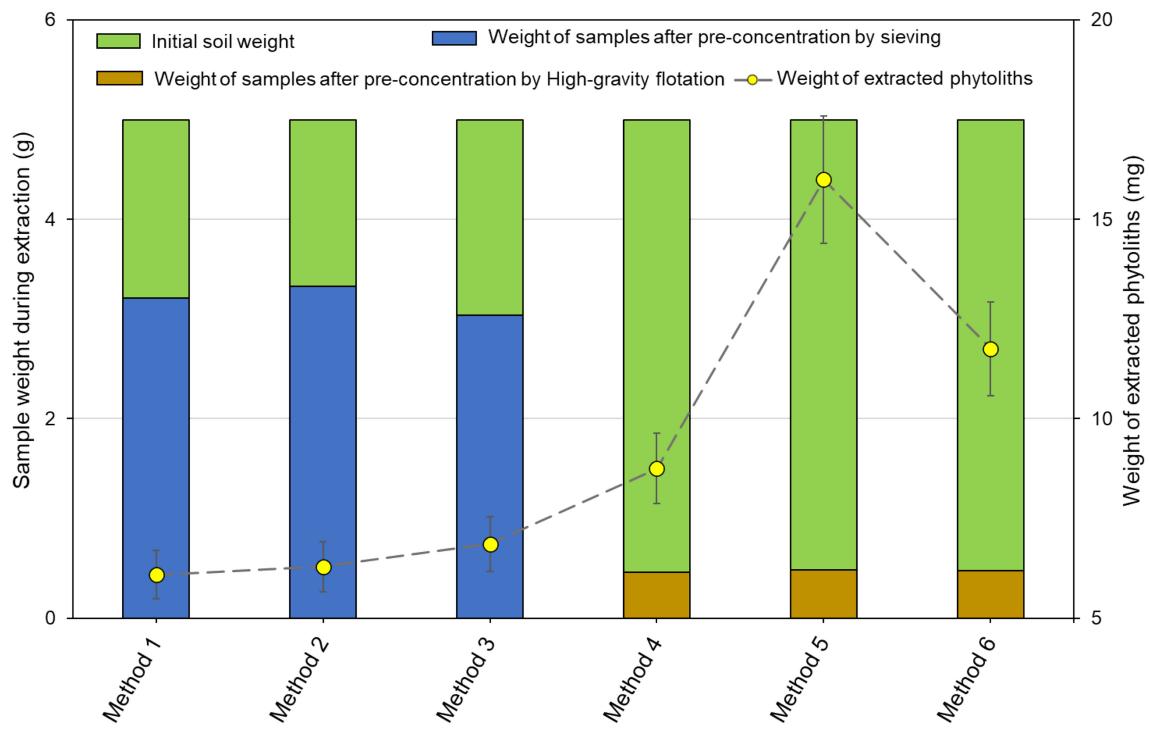
#### 3.1 Extraction results of the six methods

The results showed significant differences in the pre-concentration effect of the sieving and high gravity flotation, with significant differences in the weight of the corresponding extracted phytoliths (Fig. 2). The pre-concentrated sample amount in methods 4, 5, and 6 was smaller than that in methods 1, 2 and 3, while the weight of the extracted phytoliths obtained in methods 4, 5 and 6 was greater than that in methods 1, 2 and 3. This observation implies that the high gravity flotation, as opposed to sieving, significantly reduces the subsequent sample amount for processing, improves the liquid-to-solid ratio between chemical reagents and processed samples, and mitigates the interference of impurity particles on flotation. Furthermore, the weight of phytoliths extracted using methods 1 and 4 was lower than that obtained through other methods, indicating that the standard HLF failed to disperse the samples effectively.

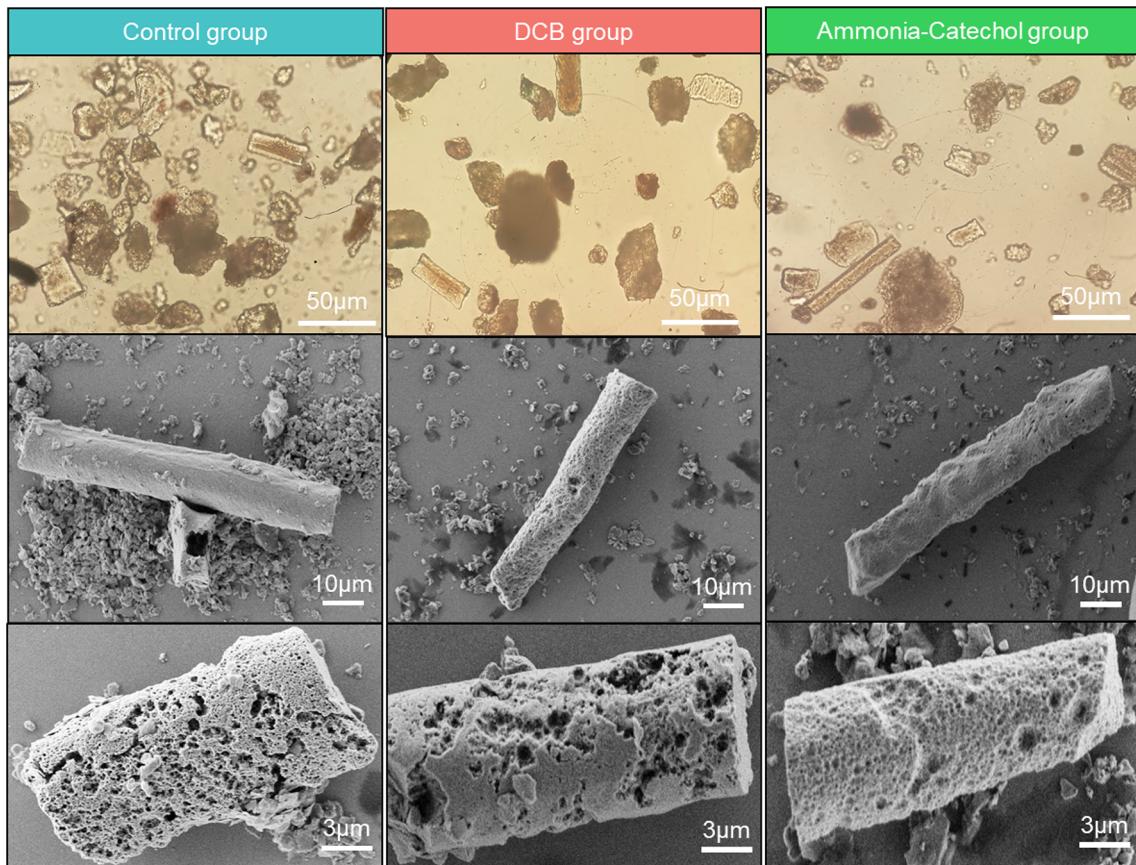
By comparing the microscopic features, it was found that the methods using sieving and high gravity flotation exhibited consistent results, whereas distinctions were observed when comparing the methods treated with DCB and Ammonia-Catechol and the control method (Fig. 3). SEM examination of the phytoliths' morphological characteristics revealed no significant differences among the different treatment groups. Surface features such as adhesion, roughness, porous corrosion, and fractures were observed on all phytolith particles. However, the DCB and Ammonia-Catechol groups displayed a clearer field of view around phytoliths and less particle aggregation (compared to the control group), indicating that the added chemical treatment improved sample dispersion.

#### 3.2 Alkaline dissolution analysis of the extracted phytoliths

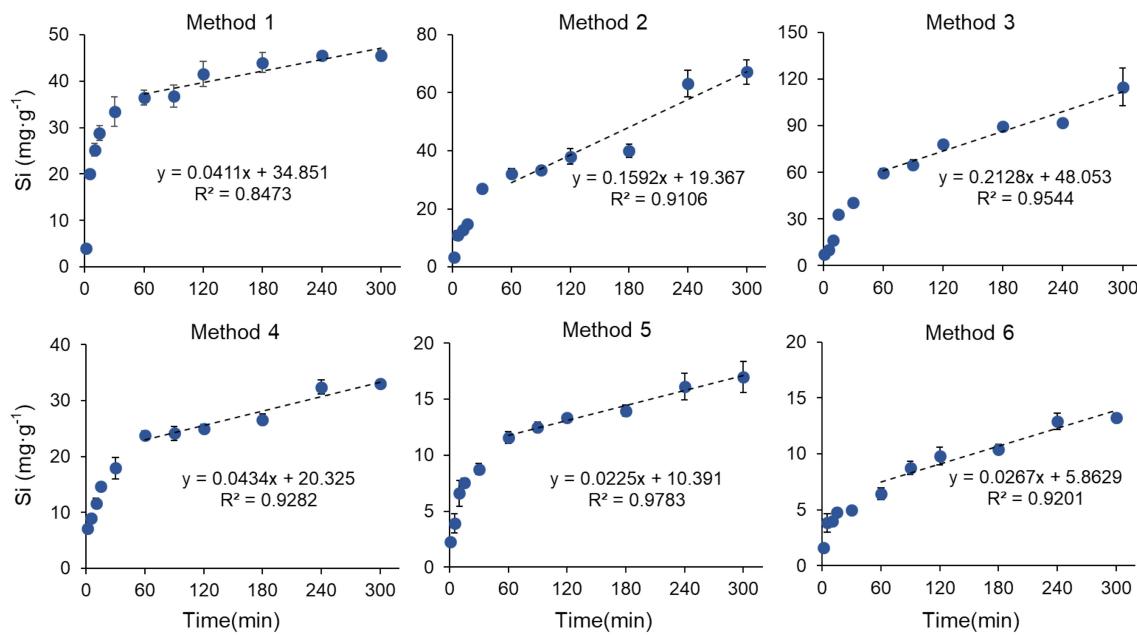
When the extraction time was less than 60 min, the extraction curves of the six methods were non-linear and had obvious slope changes. After 60 min, the extraction curves changed linearly (Fig. 4). This is because biogenic silica dissolves rapidly in a short time and the subsequent silicon is mainly controlled by clay mineral dissolution (Barão et al. 2015). PhytSi content based on the extraction curve's intercept showed significant differences among the six methods. The PhytSi content obtained by methods 4, 5, and 6 is lower than that by methods 1, 2, and 3, respectively, indicating that the removal efficiency of clay minerals by high gravity flotation is insufficient. Methods 2 and 5 exhibited lower PhytSi content than methods 1 and 4,



**Fig. 2** Pre-concentration effect of sieving and high gravity flotation on samples and the comparison of the final extracted phytoliths



**Fig. 3** SEM and optical micrographs of phytoliths; Results of methods 1,4 for the control group; methods 2,5 for the DCB group; and methods 3,6 for the Ammonia–Catechol group



**Fig. 4** Extracted silicon curves ( $\text{mg g}^{-1}$ ) obtained with the continuous analysis in  $0.1 \text{ M Na}_2\text{CO}_3$  for extracted phytoliths from six methods

indicating that DCB destroyed phytoliths to some extent. Notably, the PhytSi content of method 3 is 38% higher than that of method 1, suggesting that Ammonia–Catechol effectively enhances the enrichment of phytoliths.

Furthermore, comparing the results of the single-point method with the multi-point method (Table 2), it was found that the deviation of the PhytSi content measured by the 30 min single-point method was relatively small compared to that of the 15 min single-point method. Therefore, in this study, the 30 min single-point method in  $0.1 \text{ M Na}_2\text{CO}_3$  at  $85^\circ\text{C}$  was used to analyze the elemental information of soil phytoliths.

### 3.3 Si/Al differences in phytoliths obtained from the six methods

Si/Al ratios of the extracted phytoliths obtained by the six methods showed significant differences (Fig. 5). All Si/Al ratios were greater than 5, indicating that the components

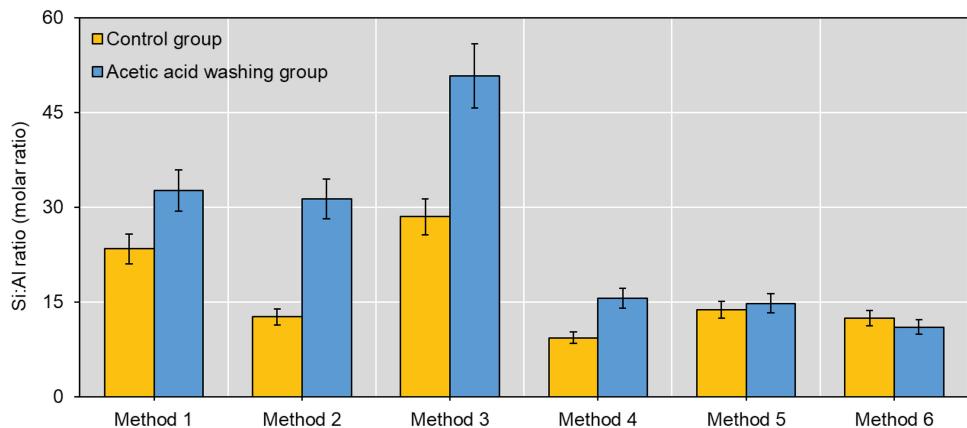
obtained from the alkali-soluble extracted phytoliths mainly originated from phytolith dissolution. This is due to the aluminum content in biogenic silica is much lower than that in clay minerals (Ragueneau et al. 2005), and when  $\text{Si}/\text{Al} > 5$  and relatively higher, it indicates a larger proportion of phytoliths in the extracted phytoliths (Barão et al. 2015). Noteworthy, the  $\text{Si}/\text{Al}$  ratio of method 3 is 22% higher than that of method 1. This finding suggests that the Ammonia–Catechol obtained more phytoliths while reducing impurity interference. Moreover, except for method 6, acetic acid treatment increased the  $\text{Si}/\text{Al}$  ratios, indicating that acetic acid treatment can effectively remove the ions adsorbed on phytoliths.

**Table 2** Analysis of silicon content ( $\text{mg g}^{-1}$ ) of extracted phytoliths and its relative deviation by single-point method and multi-point method in  $0.1 \text{ M Na}_2\text{CO}_3$

Analysis/extraction method		Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Single extraction at 15min	Si ( $\text{mg g}^{-1}$ )	27.17–29.58	12.75–15.97	29.71–33.22	12.17–16.91	5.97–7.95	3.75–5.96
	RD* (%)	15.11–22.03	17.55–34.18	30.86–38.17	16.82–40.14	23.48–42.54	1.7–36
Single extraction at 30min	Si ( $\text{mg g}^{-1}$ )	31.55–34.94	18.75–20.54	38.15–41.87	16.75–19.84	8.15–9.71	4.77–5.57
	RD* (%)	0.26–9.47	3.2–6.04	12.86–20.6	2.4–17.6	6.54–21.56	4.94–18.6
Multi-point method	Si ( $\text{mg g}^{-1}$ )	34.85	19.37	48.05	20.33	10.39	5.86

\*RD refers to the deviation of the silicon content measured by the single-point method relative to the content measured by the multi-point method

**Fig. 5** Si/Al in phytoliths obtained by six methods



## 4 Discussion

### 4.1 The effect of iron–aluminum oxide removal and acetic acid treatment on phytolith information

Samples used in this study were sub-tropical soils, which contain Fe–Al oxide clays that carry a positive charge under acidic conditions. The aggregation of anions caused by this charge reduces the dispersion efficiency, which is dependent on the hydration of  $\text{Na}^+$  (Calegari et al. 2013; Zhao & Pearsall 1998). Moreover, Fe–Al oxides on the surface of phytoliths affect the morphological information, elemental analysis, and dissolution properties (Calegari et al. 2013; Song et al. 2016). Therefore, the removal of Fe–Al oxides can effectively improve sample dispersion, reduce interference, and increase the dissolution of phytoliths in the alkaline soluble analysis. The Ammonia–Catechol caused the formation of Al-catechol water-soluble complexes in the samples under high temperatures ( $80^\circ\text{C}$ ) and ammonia conditions, which resulted in the removal of Al compounds (Prodromou & Kalovoulos 1994). DCB caused the reduction and chelation of Fe oxides in the samples by sodium citrate, while also removing small amounts of aluminum compounds (Aguilera & Jackson 1953; Prodromou & Kalovoulos 1994). Consequently, both treatments enhance the dispersibility of samples, resulting in improved weight and microscopic clarity of the extracted phytoliths (Figs. 2 and 3). This is consistent with the findings of Calegari et al. (2013) (Calegari et al. 2013). By comparing the weight of extracted phytoliths, PhytSi content, and Si/Al ratio (Figs. 2, 4, and 5), it was observed that the addition of Ammonia–Catechol to HLF was more effective than DCB. This may be attributed to the more effective removal of Al compounds by Ammonia–Catechol compared to DCB (Fig. 5), as catechol complexes are more stable than citrate complexes (Prodromou & Kalovoulos 1994).

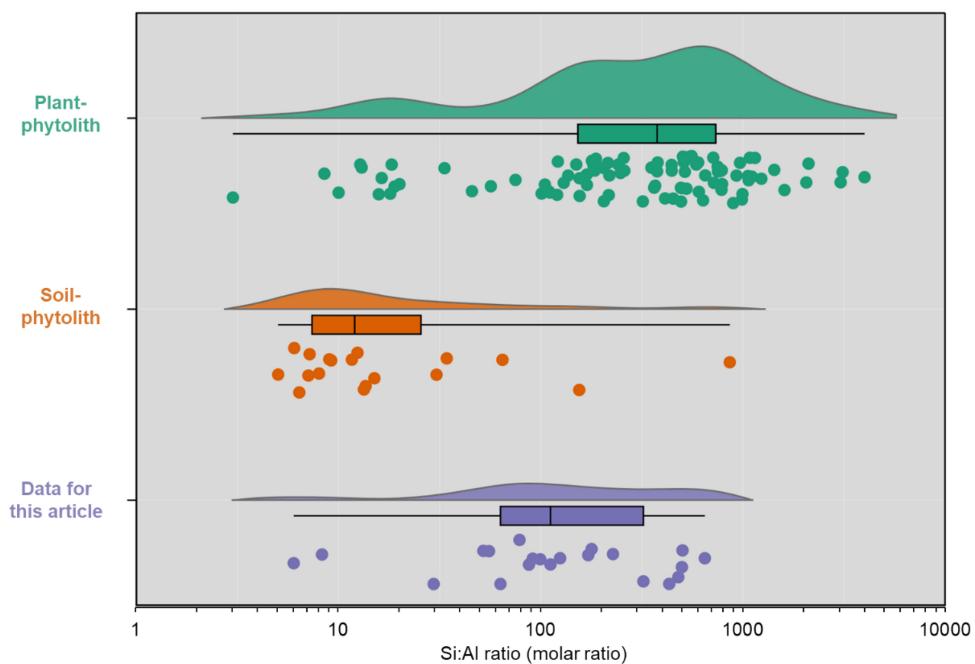
Due to their small particle sizes and relatively large specific surface area, phytoliths are prone to adsorb metal ions (e.g.,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ) (Ngoc Nguyen et al. 2013). Among these ions,  $\text{Al}^{3+}$  is more easily to adsorb to phytolith surface and consequently decreases its dissolution (Haynes 2017). Acetic acid can remove not only  $\text{Al}^{3+}$  but also eliminate some of the adsorbed soluble silica (Sauer et al. 2006). Furthermore, the removal of adsorbed ions may enhance phytolith dissolution during the alkaline solution process. Therefore, HLF combined with Ammonia–Catechol and acetic acid treatment is beneficial in reducing impurity interference with the Si/Al ratio of soil phytoliths.

### 4.2 Chemical damage to phytoliths

The silica–oxygen structure of phytoliths can disrupt the Si–Si bond by attracting electron transfer from water molecules, making a higher pH ( $> 9$ ) an important factor in driving phytolith dissolution (Ngoc Nguyen et al. 2013). Furthermore, the properties of chemical reagents can also damage phytoliths. For instance, the oxidative capacity generated by the combined use of  $\text{H}_2\text{O}_2$  and  $\text{H}_2\text{SO}_4$  can result in carbon loss in phytoliths (Parr & Sullivan 2013). Therefore, it is crucial to consider the potential influence of chemical reagents employed during extraction.

In this study, we focused on the possible damage of DCB and Ammonia–Catechol to phytoliths. Methods 2 and 5, which involve the addition of DCB, yielded higher extraction weights and clarity compared to Method 1 (Figs. 2, 3, and 4). However, their PhytSi content and Si/Al ratio were lower than those of Method 1 (Figs. 4 and 5). As Vander Linden et al. (2021) pointed out, DCB can cause damage to phytoliths (Vander Linden et al. 2021), which partially offsets the advantages it offers. Consequently, DCB may be suitable for acquiring morphological information about phytoliths but not for quantitative analysis of elements (Calegari et al. 2013).

**Fig. 6** Si/Al in phytolith; where Plant-phytolith represents phytolith data extracted from plant tissues; Soil-phytolith refers to phytolith data obtained from soil extraction; Data for this article represents phytolith data measured from the soil around Xin'anjiang River in this paper. Data from (Barão et al. 2015; Bartoli 1985; Bartoli & Wilding 1980; Cheng 2019; Georgiadis et al. 2015; Kameník et al. 2013; Li et al. 2014; Lisztes-Szabó et al. 2019; Nguyen et al. 2019; Wen 2021; Xinyue 2021)



Ammonia-Catechol can effectively remove Fe-Al compounds (Prodromou & Kalovoulos 1994), but the impact on phytoliths needs to be further evaluated. Method 3 demonstrated superior results compared to method 1 by comparing the PhytSi content and Si/Al ratio of the extracted phytoliths. Conversely, method 6, which also utilized amino-catechol, yielded poorer results. This difference can be attributed to the use of high-gravity flotation instead of sieving in method 6. The better pre-concentration effect of high gravity flotation compared to sieving reduced the buffering capacity of samples (Fig. 2), thereby increasing the aggressiveness of chemical reagents to phytoliths. For example, it has been observed that low pH (< 3) HCl intensifies phytolith dissolution in carbonate-free soils (Calegari et al. 2013). Consequently, it appears feasible to mitigate the chemical reagent's aggressiveness by adjusting sample quantity and treatment intensity following an exploration of its effects.

#### 4.3 Comparison with published Si/Al data

We compared the Si/Al ratios of soil phytoliths in this study with published data (Fig. 6). The results demonstrated that the measured Si/Al ratios (6–648) in this study were consistent with the range of Si/Al ratios found in published soil phytolith data (5–861), confirming the robustness of the optimized method. However, significant differences between the two datasets were observed ( $p < 0.01$ ), which could be attributed to the spatiotemporal heterogeneity of the samples and variations in the methodologies employed. Furthermore, the measured Si/Al

ratios exhibited a relatively low coefficient of variation ( $CV = 0.96$  for this study compared to  $CV = 2.84$  for published soil phytolith data). The primary distribution range of the data in this study (50% of data points falling within the range of 59–377) was closer to the Si/Al ratios (150–750) reported for plant phytoliths rather than the published soil phytolith data (7–31). These findings indicate that the optimized method effectively improved the accuracy of the data. Moreover, a comparison revealed that the range of Si/Al ratios in soil phytoliths may be smaller than that in plant phytoliths. Aside from sample and method effects, the dissolution of phytoliths, influenced by their Si/Al ratio, appears to be another crucial factor. Phytoliths with higher Si/Al have higher dissolution (Bartoli & Wilding 1980), suggesting that phytoliths extracted from soils may represent a selectively retained fraction.

## 5 Conclusion

Based on the comparison of the analytical methods for phytoliths in this study, the following conclusions can be drawn:

- (1) The utilization of high gravity flotation in the HLF instead of sieving, or the inclusion of DCB, intensifies the reagent's aggression towards phytoliths.
- (2) The incorporation of Ammonia-Catechol in the HLF, enhances sample dispersion and promotes phytoliths dissolution during alkaline dissolution analysis. This resulted in a 38% increase in phytolith

- enrichment capability and a 22% improvement in Si/Al accuracy for Method 3 (compared to Method 1).
- (3) The introduction of an acetic acid washing step before the alkaline dissolution analysis of extracted phytoliths can further enhance the accuracy of Si/Al results by removing impurities adsorbed on the phytolith's surface.

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## Declarations

**Conflict of interest** We declare no conflict of interest.

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