



Pretreatment method for the analysis of phosphate oxygen isotope ($\delta^{18}\text{O}_\text{P}$) of different phosphorus fractions in freshwater sediments

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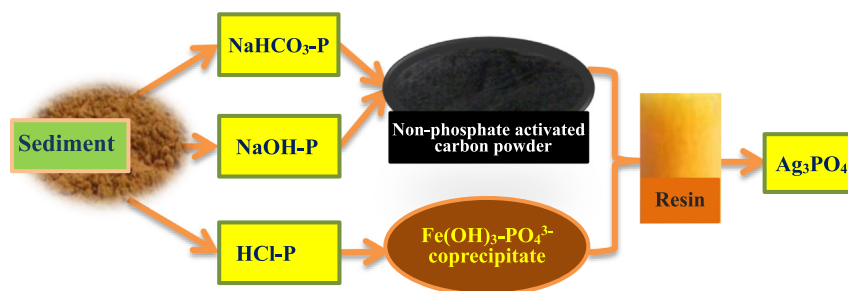
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HIGHLIGHTS

- A five-step method for the analysis of $\delta^{18}\text{O}_\text{P}$ of different P fractions in freshwater sediments was established.
- Use of non-phosphate activated carbon powder can remove organic matter in $\text{NaHCO}_3\text{-P}$ and NaOH-P extracts more easily.
- Coprecipitation of $\text{Fe}(\text{OH})_3\text{-PO}_4^{3-}$ can remove most of metals and Cl^- in HCl extract efficiently at the beginning.
- We provide a powerful tool for investigating the sources and biogeochemical cycle of P in freshwater sediments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 21 March 2019

Received in revised form 16 May 2019

Accepted 16 May 2019

Available online 20 May 2019

Editor: Dr. Damia Barcelo

Keywords:

Pretreatment method

Oxygen isotopic in phosphate

Different phosphorus fractions

Freshwater sediments

Eutrophication

ABSTRACT

Accurate measurement of the oxygen isotopic composition of dissolved phosphate ($\delta^{18}\text{O}_\text{P}$) of different phosphorus (P) fractions in lacustrine sediments is very difficult because of the influence of large amounts of impurities. In this study, we developed a five-step method for obtaining high purity Ag_3PO_4 sample for the analysis of $\delta^{18}\text{O}_\text{P}$ of different P fractions in freshwater sediments. Sedimentary P was divided into $\text{NaHCO}_3\text{-P}$, NaOH-P and HCl-P by chemical sequential extraction. Pretreatment procedures for different sedimentary P fractions were improved in the following respects: 1) abandonment of the magnesium-induced coprecipitation method to avoid the introduction of impurity ions, such as Mg^{2+} and Cl^- , and reduce the loss of P; 2) use of a small amount of non-phosphate activated carbon powder to efficiently remove organic matter in extracts of $\text{NaHCO}_3\text{-P}$ and NaOH-P , and reduce the loss of P; 3) adjustment of the HCl-P extract pH to 4 in order to form $\text{Fe}(\text{OH})_3\text{-PO}_4^{3-}$ coprecipitate, thereby removing most of metals and Cl^- . This method reduces the pretreatment steps, simplifies the operation and increases the recovery of phosphate (90.98%–96.69%). The high purity Ag_3PO_4 sample can be obtained and the repeatability and accuracy of measured $\delta^{18}\text{O}_\text{P}$ is better than 0.3%, demonstrating high reliability and accuracy. This new method was used to analyze the $\delta^{18}\text{O}_\text{P}$ of different P fractions in sediments of a eutrophic lake in southwestern China. The preliminary results indicated that the $\delta^{18}\text{O}_\text{P}$ in the sediments can be used to identify different P sources, and provide new insights into sedimentary P cycling. The method established in this study provides a powerful tool for investigating the sources and biogeochemical cycle of P in freshwater sediments.

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1. Introduction

Eutrophication is one of the most serious environmental problems in freshwater ecosystem, which is especially prominent in developing

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countries. Phosphorus (P) is the limiting factor of water eutrophication (Schindler, 1977; Conley et al., 2009). Understanding the P sources and its geochemical cycle has an important significance for clarifying the eutrophication mechanism and formulating rational countermeasures against eutrophication in freshwater lake.

It is well known that the P release from lake sediments can intensify eutrophication, especially in lakes with high internal P load (Jin et al., 2006; Ahlgren et al., 2011; Chen et al., 2018; Kwak et al., 2018). Sedimentary P can be divided into different fractions, which have different bio-availabilities (Ruttenberg, 1992; Kaiserli et al., 2002; Lukkari et al., 2007; Ni et al., 2016; Cavalcante et al., 2018). The source and migration-transformation of different P fractions in sediments have attracted more and more attention (Jaisi and Blake, 2010; Goldhammer et al., 2011a; Jaisi et al., 2011; Jaisi, 2013; Joshi et al., 2015; Granger et al., 2017b; Paytan et al., 2017; Mingus et al., 2019). However, methods of identifying quantitatively the P sources are limited.

P has only one stable isotope (^{31}P), which makes it impossible to carry out stable isotope analysis of P for identifying the P sources. Fortunately, P exists mainly in phosphate (PO_4^{3-}) in water, and bonding between the P and oxygen (O) atoms are very strong in phosphate (Longinelli et al., 1976; Blake et al., 1997). O has three stable isotopes (^{16}O , ^{17}O and ^{18}O). Therefore, the phosphate oxygen isotope ($\delta^{18}\text{O}_\text{P}$) is thought to be a potentially effective tool to analyze the sources and geochemical cycle of P (Longinelli et al., 1976; Blake et al., 1997, 2005, 2010; Colman et al., 2005; Gruau et al., 2005; Liang and Blake, 2006, 2009; McLaughlin et al., 2006a, 2006c, 2013; Paytan and McLaughlin, 2007; Jaisi et al., 2011; Tamburini et al., 2012; Granger et al., 2017a; Gooddy et al., 2015, 2018).

So far, the $\delta^{18}\text{O}_\text{P}$ analysis method for water samples has been established and applied to study P cycling in oceans and freshwater lakes (Colman, 2002; Vennemann et al., 2002; McLaughlin et al., 2006b; Elsbury et al., 2009; Young et al., 2009; Goldhammer et al., 2011b; Li et al., 2011; Gross et al., 2013; Granger et al., 2017b; Tonderski et al., 2017; Gooddy et al., 2018). Compared with water body, sediments are a mixture of natural and anthropogenic P sources that have accumulated in the basin over time. The $\delta^{18}\text{O}_\text{P}$ composition of different P fractions in sediments contains abundant information about the P source as well as its migration and transformation. However, accurate measurement of the $\delta^{18}\text{O}_\text{P}$ of different P fractions in lake sediments is very difficult because of the influence of impurities.

The traditional pretreatment method for $\delta^{18}\text{O}_\text{P}$ analysis usually transforms phosphate into high purity Ag_3PO_4 through a series of separation and purification processes (Colman, 2002; McLaughlin et al., 2004; Gruau et al., 2005; Tamburini et al., 2010; Zohar et al., 2010; Tamburini et al., 2010; Goldhammer et al., 2011b; Weiner et al., 2011; Granger et al., 2017a; Jiang et al., 2017). Meanwhile, it ensures efficient removal of impurities, reduces the loss of phosphates as much as possible and avoids the fractionation of oxygen isotopes in phosphates. McLaughlin et al. (2004) and Zohar et al. (2010) have successfully established the pretreatment methods for analyzing $\delta^{18}\text{O}_\text{P}$ (McLaughlin et al., 2004; Zohar et al., 2010). However, these methods suffer from difficulties when applied to lacustrine sediments. For example, the method established by McLaughlin et al. (2004) is effective for seawater samples with low organic matter content (McLaughlin et al., 2004), but it lacks a sufficient removal efficiency of organic matter for lacustrine sediment samples enriched in organic matter. The pretreatment method for analyzing $\delta^{18}\text{O}_\text{P}$ of different soil P fractions, established by Zohar et al. (2010), lay an important foundation for the analysis of $\delta^{18}\text{O}_\text{P}$ in lacustrine sediments (Paytan et al., 2017). However, sediments contain large amounts of impurities, such as various kinds of heavy metals and organic matter. The Zohar et al. (2010) method is quite time consuming, even unsuccessful when treating lacustrine sediment samples. On the one hand, the precipitation was subdivided to wash Cl^- multiple times during CePO_4 formation and removal of Cl^- step (Zohar et al., 2010), which is time consuming. On the other hand, a

part of P might be lost in the process of removing organic matter by adjusting the pH, especially for sediments with high organic matter content (Hiradate et al., 2006; Tamburini et al., 2010). In addition, the large change of pH by strong oxidative acids might lead to the oxidation and hydrolysis of organic P, further creating a risk of oxygen isotope fractionation (Tamburini et al., 2010). Tamburini et al. (2010) established a $\delta^{18}\text{O}_\text{P}$ pretreatment method for soil HCl-P by adopting DAX-8 Amberlite resin to remove the dissolved organic matter (Tamburini et al., 2010), and other studies have adopted different resins to remove dissolved organic matter (Colman, 2002; Jaisi and Blake, 2010; Goldhammer et al., 2011a, 2011b; Granger et al., 2017b; Paytan et al., 2017; Jiang et al., 2017; Pistocchi et al., 2017; Gooddy et al., 2018). However, the removal of organic matter using resins usually requires the pH to be adjusted to 5 (Hiradate et al., 2006), which will lead to precipitation of some organic matter components and the loss of P (adsorbed by precipitated organic matter) (Tamburini et al., 2010), especially for sediments enriched in organic matter. H_2O_2 and NaClO were also used to remove organic matter in the bioapatite samples (Wiedemann-Bidlack et al., 2008; Elsbury et al., 2009; Grimes and Pellegrini, 2013). Similarly, intensive oxidation might cause mutual transformation of P fractions and fractionation of oxygen isotopes (Tamburini et al., 2010; Zhang et al., 2017).

In this study, sediment samples were collected from Aha Lake, which is a eutrophic water body in southwestern China (Fig. 1). A sequential chemical extraction method was used to separate sedimentary P into NaHCO_3 -P, NaOH -P and HCl -P fractions, and their $\delta^{18}\text{O}_\text{P}$ were determined. The aims of this study were: 1) to establish an effective, reliable and convenient pretreatment method for the analysis of $\delta^{18}\text{O}_\text{P}$ of different P fractions in freshwater sediments; and 2) to explore the potential of using the $\delta^{18}\text{O}_\text{P}$ of different sedimentary P fractions to trace the P sources in freshwater lake, with Aha Lake as an example.

2. Experimental section

2.1. Materials and reagents

250 mL centrifuge bottles (Nalgene, America), 50 mL centrifuge tubes (light (Corning, Germany) and black (Greiner, Germany)), non-phosphate activated carbon powder (NT-WLT, Zhen Zhou Nourishment Technology Company, China), cerium (III) nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, Sigma-Aldrich, America), silver nitrate (AgNO_3 , Alfa Aesar, America), nitric acid (HNO_3 , Guaranteed Reagent (GR), Sinopharm Chemical Reagent Company, China), acetic acid (HAc, GR, Sinopharm Chemical Reagent Company, China), potassium acetate (KAc, GR, Aladdin, China), hydrogen peroxide (H_2O_2 , GR, Sinopharm Chemical Reagent Company, China), ammonium hydroxide (NH_4OH , GR, Sinopharm Chemical Reagent Company, China), sodium bicarbonate (NaHCO_3 , GR, Sinopharm Chemical Reagent Company, China), sodium hydroxide (NaOH , GR, Sinopharm Chemical Reagent Company, China), hydrochloric acid (HCl, GR, Sinopharm Chemical Reagent Company, China), the cation exchange resin (Biorad AG 50W-X8, America), pH meter (Mettler Toledo, Germany), ultra Milli-Q water (Milli-Q Bioce, Millipore, America), filtration device with a glass and sand core (250 mL and 2000 mL; Lantian, China), centrifuge (L550, Cence, China) and an oscillator (HY-2, Jinxin, China).

2.2. Sequential extraction of P fractions in sediments

Aha Lake, located in southwestern China, is a P limited and a mildly eutrophic deep lake. Surface sediment was collected at two sites, Jinzhong (JZ) and Youyu (YY), in November 2017 (Fig. 1). Sedimentary pollution at these two sites is serious and has obviously different sources. YY, located at the southwest of the lake, is mainly influenced by coal mine wastewater over multiple years. JZ, located at the north-east of the lake, is mainly influenced by municipal wastewaters.

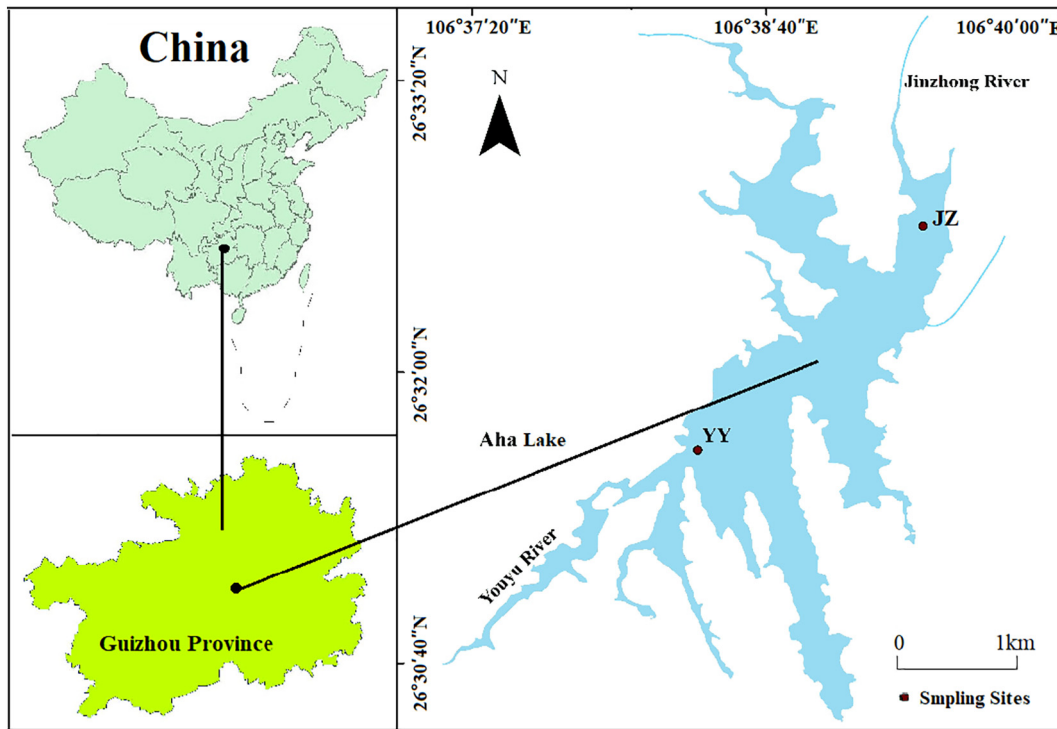


Fig. 1. The map of the sampling sites.

Water samples were synchronically collected at the JZ and YY sites, and were filtered through filter membranes with a pore size of 0.45 μm . P was obtained using the magnesium-induced coprecipitation (MagIC) method (Karl and Tien, 1992) and the water $\delta^{18}\text{O}_\text{P}$ pretreatment was carried out according to the method of McLaughlin et al. (2004). At the same time, a small amount of the water samples were filtered through a 0.22 μm filter membrane into a 5 mL centrifuge tube (avoid air entry) for analysis of $\delta^{18}\text{O}_\text{w}$. The *in situ* water temperature was measured using a water quality parameter instrument (YSI6600V2, YSI Inc., America).

The sediment was processed after freeze-drying, grinding, and screening through 200 mesh sieve. The sediments were mainly separated into $\text{NaHCO}_3\text{-P}$, NaOH-P and HCl-P fractions (Fig. 2) using sequential chemical extraction (Hedley et al., 1982). In brief, 3.3 g of sediment sample was weighted and placed into a 250 mL centrifuge bottle. Next, 200 mL of extractant (H_2O , 0.5 M NaHCO_3 , 0.1 M NaOH-P and 1 M HCl) was added and then the mixture was centrifuged after oscillating for 16 h. The supernatant was passed through a 0.45 μm filter membrane using a filtration device with a glass and sand core to obtain the extract.

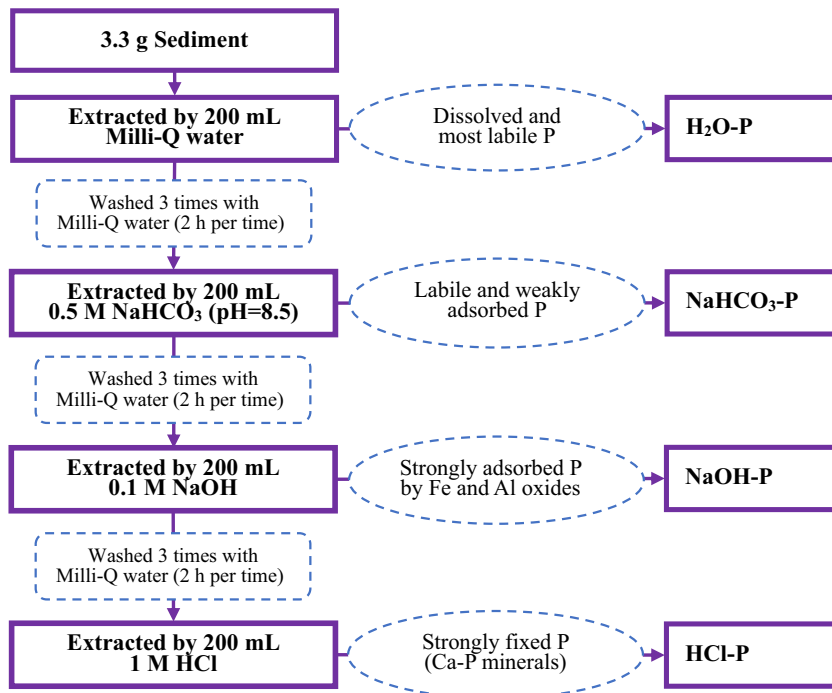


Fig. 2. Sequential extraction of different P fractions in sediments (Hedley et al., 1982).

After each extraction, the residual sediment was washed with 200 mL pure water using an oscillator for 2 h, which was continuously repeated 3 times. Since the concentration of $\text{H}_2\text{O-P}$ in the sediment was low (usually <5 mg/kg) or undetected (Zhu et al., 2015), we cannot obtain the Ag_3PO_4 samples finally using former methods (McLaughlin et al., 2004; Zohar et al., 2010). The $\delta^{18}\text{O}_\text{P}$ pretreatment method in $\text{H}_2\text{O-P}$ is not mentioned in this study.

2.3. Pretreatment of extract $\delta^{18}\text{O}_\text{P}$

Based on the methods of McLaughlin et al. (2004) and Zohar et al. (2010), a series of procedures was optimized. A $\delta^{18}\text{O}_\text{P}$ pretreatment method of different P fractions in freshwater lake sediments was established (Fig. 3) as follows:

1) The $\delta^{18}\text{O}_\text{P}$ pretreatment method of $\text{NaHCO}_3\text{-P}$. The $\text{NaHCO}_3\text{-P}$ extract was brown and weakly alkaline ($\text{pH} = 8.5$), with a relatively high content of P and organic matter. Details of the $\delta^{18}\text{O}_\text{P}$ pretreatment method of the $\text{NaHCO}_3\text{-P}$ extract sample are described as follows:

Step-1 Removal of organic matter. The pH of the extract was adjusted to 7 using 10 M HNO_3 (~1 mL), 1–3 g non-phosphate activated carbon powder was then added (the darker the extract color, higher the addition). After 30 min oscillation, the mixture was filtrated with a 0.45 μm filter membrane to remove organic matter. The color of the obtained filtrate was transparent and clear at this moment.

Step-2 Removal of HCO_3^- . 10 mL 10 M of HNO_3 was added to the filtrate, and the pH value dropped to approximately 1. Gentle magnetic stirring was needed during this process to prevent spillover, allowing HCO_3^- to volatilize in the form of CO_2 until complete removal.

Step-3 Formation of cerium phosphate (CePO_4) precipitate and removal of Cl^- . The pH of the above solution was adjusted to 5.8–6.0 using 10 M NaOH . A $\text{Ce}(\text{NO}_3)_3$ solution (0.5 mg $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ dissolved in 2 mL pure water) was added. CePO_4 precipitate gradually formed and the pH of the solution decreased at this moment. The pH was adjusted to 5.5 using 1 M KAC solution. An obvious CePO_4 precipitate formed as phosphates were completely transformed to

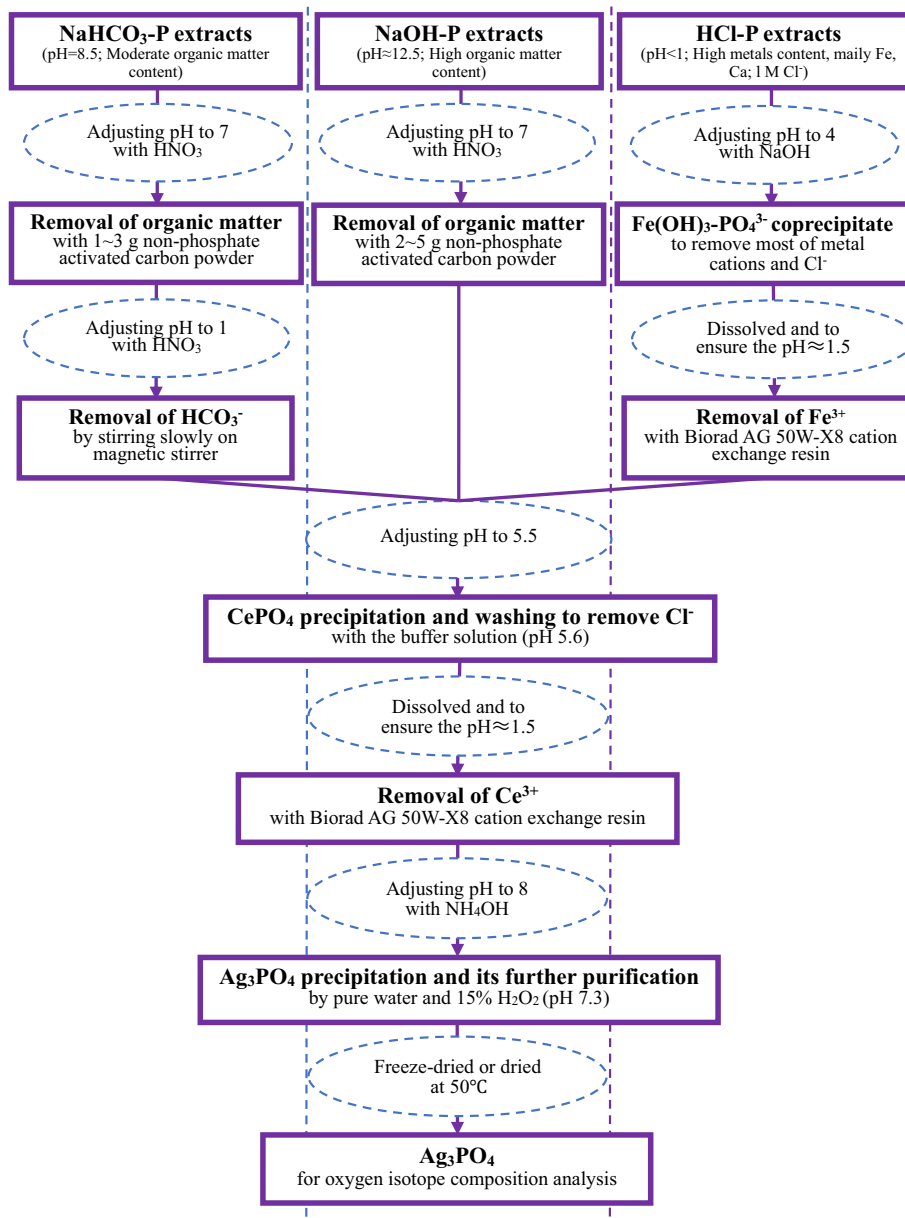


Fig. 3. The $\delta^{18}\text{O}_\text{P}$ pretreatment method of extracts with different P fractions in sediments.

CePO₄. After standing for 3 h, the mixture was transferred to a 50 mL centrifuge tube and centrifuged (4390g for 15 min) to collect the CePO₄ precipitate. The CePO₄ precipitate was then washed with 10 mL buffer solution (the pH of pure water was adjusted to 5.6 using trace KAc and HAc) and centrifuged (4390g for 15 min) to remove Cl⁻ attached on it. The above operation was repeated 3 times to completely remove the Cl⁻. Dilute AgNO₃ was used to determine if Cl⁻ was present in the supernatant. It should be noted that the separation of CePO₄ precipitate and the supernatant carefully avoided the loss of a small amount of CePO₄ after each centrifugation. In fact, in this procedure, there were few Cl⁻ attached on the CePO₄, which was hardly detected (see below discussion).

Step-4 Removal of Ce³⁺ using Biorad AG 50W-X8 cation exchange resin. CePO₄ without Cl⁻ was dissolved in a small amount of 1 M HNO₃ and pure water (volume ratio 1:4) to ensure the pH of the solution after dissolution was approximately 1.5. Biorad AG 50W-X8 cation exchange resin (~5 mL) was added into the dissolved solution and then the mixture was continuously oscillated overnight to completely remove the Ce³⁺ in the solution. The solution was filtrated through a 0.45 μm filter membrane and the filtrate was directly added into a 50 mL black centrifuge tube. The resin was washed with a small amount of pure water and filtered together into the centrifuge tube for the purpose of reducing the loss of P.

Step-5 Formation of silver phosphate (Ag₃PO₄) and its further purification. The pH of the above filtrate was adjusted to 8.0 using 2.8% NH₄OH solution. An AgNO₃ solution (0.5 g AgNO₃ dissolved into 2 mL pure water) was added and the bright yellow Ag₃PO₄ precipitate gradually formed. After standing overnight, the mixture was centrifuged (3040g for 10 min) and the supernatant was carefully removed and discarded using a disposable plastic dropper. The Ag₃PO₄ precipitate was washed using 10 mL pure water and then the mixture was gently shaken. Next, the mixture was centrifuged (3040g for 10 min) and the supernatant was discarded using a disposable plastic dropper. The above operation was continuously repeated 5 times. 5 mL 15% H₂O₂ solution (adjusted the pH to 7.3 using a small amount of 2.8% NH₄OH solution) was added to the Ag₃PO₄ precipitate. The reaction container was open and kept standing for over 5 h. After centrifugation, the supernatant was carefully discarded using a disposable plastic dropper. This operation was repeated 3 times. Ag₃PO₄ precipitate was then washed 5 times using 10 mL pure water. Finally, Ag₃PO₄ was freeze-dried or dried at 50 °C.

- 2) The δ¹⁸O_p pretreatment method of NaOH-P. The NaOH-P extract was dark brown and strongly basic (pH > 12) with a high level of phosphate and organic matter. The detailed δ¹⁸O_p pretreatment method of the NaOH-P extract sample was as follows:

Step-1 Removal of organic matter. The pH of the extract was adjusted to 7 using 10 M HNO₃. Next, 2–5 g non-phosphate activated carbon powder was added (the darker the extract color, the higher

the addition). After 30 min oscillation, the mixture was filtered through a 0.45 μm filter membrane, thus obtaining the transparent and clear NaOH-P filtrate.

Step-2 Formation of CePO₄ precipitate and removal of Cl⁻. The pH of the above solution was adjusted to 5.8–6.0 using 3 M HNO₃. The following pretreatment protocol was similar to that of NaHCO₃-P. In short, a Ce(NO₃)₃ solution was added to form CePO₄ precipitate. The pH was adjusted to 5.5 using a 1 M KAc solution to completely turn all phosphates into CePO₄. The mixture was centrifuged (4390g for 15 min) and the supernatant was discarded. The CePO₄ precipitate was washed 3 times with the above mentioned buffer solution (pH 5.6) to completely remove Cl⁻ attached on the surface of the CePO₄. The content of Cl⁻ in this procedure was very low.

Step-3 Removal of Ce³⁺ using Biorad AG 50W-X8 cation exchange resin. The operation is the same as the procedure described in Step-4 for the NaHCO₃-P sample.

Step-4 Formation of Ag₃PO₄ precipitate and its further purification. The operation is the same as the procedure described in Step-5 for the NaHCO₃-P sample.

- 3) The δ¹⁸O_p pretreatment method of HCl-P. The HCl-P extract was bright yellow and strongly acidic (pH < 1), containing high concentrations of phosphate, Fe³⁺, Ca²⁺ and Cl⁻ and a very low concentration of organic matter. The detailed δ¹⁸O_p pretreatment method of the HCl-P extract sample is as follows:

Step-1 Coprecipitation of Fe(OH)₃-PO₄³⁻ and removal of most metal cations and Cl⁻. The pH of the extract was adjusted to 4 using 10 M NaOH. During the pH adjustment process, a large amount of reddish brown Fe(OH)₃ precipitate was formed. The Fe(OH)₃ adsorbs phosphates and forms an Fe(OH)₃-PO₄³⁻ coprecipitate during this process. After intensive stirring and standing for 30 min, the mixture was centrifuged (3040g for 10 min) and the supernatant was discarded. Most metal cations and Cl⁻ were simultaneously removed.

Step-2 Dissolution of Fe(OH)₃-PO₄³⁻ coprecipitate and removal of Fe³⁺. Fe(OH)₃-PO₄³⁻ coprecipitate was dissolved using a small amount of 5 M HNO₃. Pure water (~100 mL) was added and the pH was adjusted to 1.5. Biorad AG 50W-X8 cation exchange resin (~10 mL) was added and the mixture was continuously oscillated overnight, followed by filtration with 0.45 μm filter membrane to remove the Fe³⁺ in the solution. A small amount of water was used to wash the resin surface. After washing, the aqueous solution was filtered into the filtrate to prevent the conservation of phosphates in resin gaps and the loss of P.

Step-3 Formation of CePO₄ precipitate and removal of Cl⁻. This operation is similar to Step-3 for the NaHCO₃-P extract. In short, the pH of the filtrate was adjusted to 5.8–6.0 using 10 M NaOH. A Ce(NO₃)₃ solution was added to form CePO₄ precipitate, then the pH was adjusted back to 5.5 using 1 M KAc to completely convert all

Table 1
Phosphate recovery in the key step of the pretreatment method (mean ± SD, %).

Name	Removal of organic matter	Removal of Cl ⁻	Removal of Ce ³⁺	Total recovery	n	
NaHCO ₃ -P	100.99 ± 3.95	97.17 ± 3.04	98.53 ± 6.61	96.69	6	
NaOH-P	98.83 ± 3.06	97.42 ± 2.47	98.86 ± 3.85	95.18	6	
Name	Fe(OH) ₃ -PO ₄ ³⁻ coprecipitate	Removal of Fe ³⁺	Removal of Cl ⁻	Removal of Ce ³⁺	Total recovery	n
HCl-P	98.58 ± 3.04	97.88 ± 3.92	96.72 ± 4.51	97.49 ± 4.66	90.98	6

phosphates into CePO_4 precipitate. The mixture was transferred to a 50 mL centrifuge tube and centrifuged (4390g for 15 min). It was then washed 5 times using 10 mL of the above buffer solution (pH 5.6). The supernatant was carefully separated from the CePO_4 precipitate each time. It should be noted that the HCl-P extractant (1 M HCl) introduces a large amount of Cl^- . Therefore, this procedure required multiple washes to completely remove the Cl^- .

Step-4 Removal of Ce^{3+} using the Biorad AG 50W-X8 cation exchange resin. The operation is the same as Step-4 above for the $\text{NaHCO}_3\text{-P}$ sample.

Step-5 Formation of Ag_3PO_4 precipitate and its further purification.

The operation is the same as Step-5 above for the $\text{NaHCO}_3\text{-P}$ sample.

2.4. Analyses and test

X-ray diffraction (XRD, Empyrean, PANalytical B.V., Holland) was used to detect the purity of Ag_3PO_4 samples. This analysis was performed in the State Key Laboratory of Ore Deposit Geochemistry in Institute of Geochemistry, Chinese Academy of Sciences. An elemental analyzer-stable isotope ratio mass spectrometer (Flash EA 1112 HT-MAT253, Thermo Scientific, America) was performed in the Third Institute of Oceanography, State Oceanic Administration to analyze the $\delta^{18}\text{O}_\text{P}$. The isotope value of $\delta^{18}\text{O}_\text{P}$ was standardized using the Vienna standard ocean water dissolved $^{18}\text{O}/^{16}\text{O}$, with an analytical precision

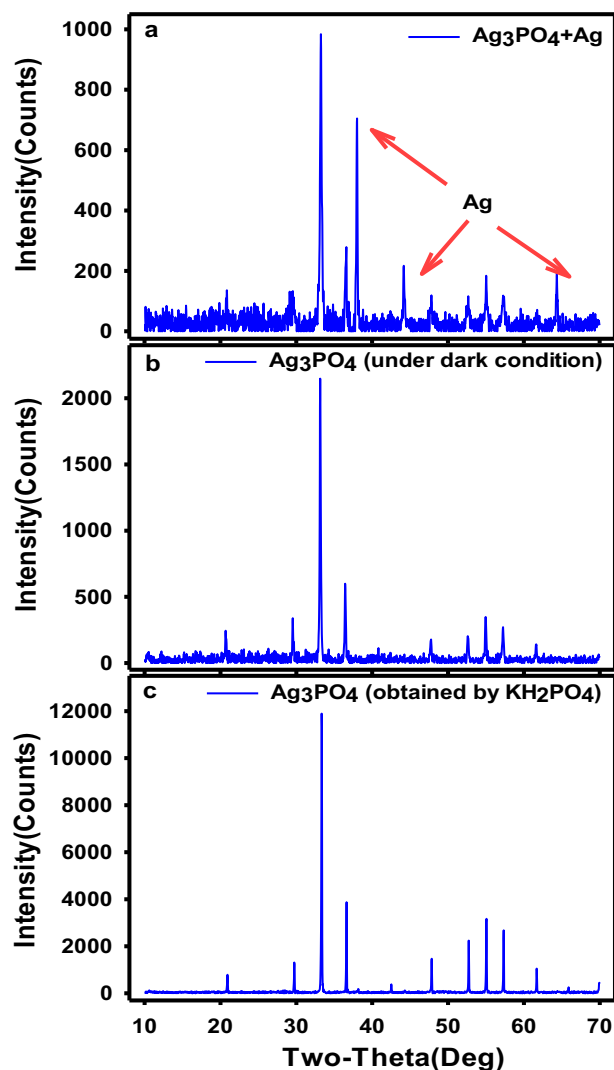


Fig. 4. XRD analysis of the Ag_3PO_4 samples.

Table 2

Validation of oxygen isotope fractionation (mean \pm SD).

Name	$\delta^{18}\text{O}_\text{P}$ (‰)	n
Ag_3PO_4 obtained from KH_2PO_4 and AgNO_3 by chemical reaction	11.20 ± 0.26	3
Ag_3PO_4 obtained from KH_2PO_4 by this improved method	11.34 ± 0.30	3

higher than 0.3‰. Gas isotope mass spectrometry (MAT253, NexION 300 X ICP-MS, PerkinElmer, America) was performed in the State Key Laboratory of Environmental Geochemistry in Institute of Geochemistry, Chinese Academy of Sciences (IGCAS) to measure the $\delta^{18}\text{O}_\text{w}$. The soluble reactive P (SRP) in the solution was measured using the Mo-Sb-Vc colorimetric method (Murphy and Riley, 1962). The concentrations of anions and cations in the sediment extracts were measured using ion chromatography (ICS-90, DIONEX, America) and atomic emission spectrometry (Vista mpx, VARIAN, America), respectively. Dissolved organic carbon in the sediment extracts was measured using a total organic carbon/nitrogen analyzer (High TOC II, Elementar, Germany).

3. Results and discussion

3.1. Recovery of phosphate in the key step

In the $\delta^{18}\text{O}_\text{P}$ pretreatment method, phosphate recovery is a key indicator. High phosphate recovery could reflect the reliability of the method and the accuracy of the $\delta^{18}\text{O}_\text{P}$ data, especially for samples with a low P content. Phosphate recovery from the key step in this method is high, with the total phosphate recoveries of $\text{NaHCO}_3\text{-P}$, NaOH-P and HCl-P being 96.69%, 95.18% and 90.98%, respectively (Table 1), significantly higher than the total recovery of 61.03% obtained by Xu et al. (2018). These results indicate that the established pretreatment method is reliable and efficient.

3.2. Purity test of the Ag_3PO_4 samples

The purity of Ag_3PO_4 precipitate is an essential indicator for evaluating the reliability of this method. The existence of oxygen-containing groups, especially organic matter, might contribute to distortion of the oxygen isotope. Therefore, it is necessary to guarantee that the purity of Ag_3PO_4 sample is very high. The XRD results indicated that the high purity Ag_3PO_4 sample can be obtained by this method (Fig. 4). However, in some Ag_3PO_4 samples, a small amount of elementary Ag was detected (Fig. 4a), which mainly includes two reasons. Firstly, during the formation process of Ag_3PO_4 precipitate, a small amount of Ag^+ from AgNO_3 that is attached on the surface of the Ag_3PO_4 precipitate might be reduced to elementary Ag due to the presence of NH_4OH (Colman, 2002; McLaughlin et al., 2004). Secondly, Ag_3PO_4 is a photo-sensitive

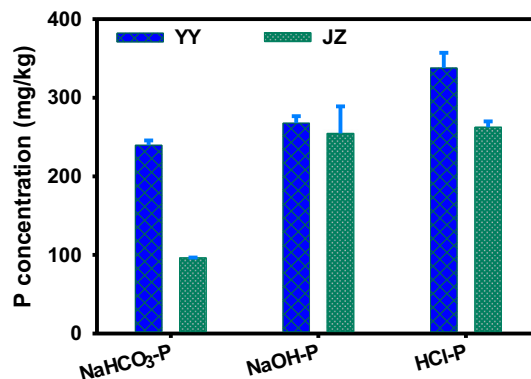


Fig. 5. Concentrations of different P fractions in sediments at two sites in Aha Lake (mean \pm SD, n = 3).

Table 3

Concentrations of main anions and dissolved organic carbon (DOC) in sediment extracts from two sites in Aha Lake (mg/L).

Site	Name	F ⁻	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻	DOC
YY	NaHCO ₃ -P	0.52	7.74	0.75	2.30	732.47
	NaOH-P	0.26	0.29	–	3.67	2476.73
	HCl-P	5.82	35,500	8.85	346.86	11.27
JZ	NaHCO ₃ -P	1.42	8.76	4.18	149.13	728.27
	NaOH-P	0.71	2.29	2.13	4.28	3028.23
	HCl-P	2.44	35,500	3.91	665.85	9.82

Note: “–” means undetected.

material. In the presence of water and natural light, it is easily degraded to grey-black elementary nano-silver on the surface (Martin et al., 2015). The phenomenon of Ag₃PO₄ containing a small amount of elementary Ag has been previously observed (Colman, 2002; McLaughlin et al., 2004). Fortunately, a small amount of Ag impurity will not lead to changes of the oxygen isotope value. In other words, it will not influence the accuracy of the validation results. Our study found that forming Ag₃PO₄ precipitate under dark condition could prevent the formation of Ag impurities (Fig. 4b). However, throughout the entire operation, including the subsequent sample storage, it is difficult to strictly avoid light. As a consequence, this difficult problem should be taken into consideration in future studies.

3.3. Fractionation validation of the oxygen isotope in Ag₃PO₄

In order to validate whether the pretreatment operation contributed to the isotope fractionation, Ag₃PO₄ was formed by the following two pathways. Firstly, potassium dihydrogen phosphate (KH₂PO₄; GR; Tianjin Guangfu Fine Chemical Research Institute, China) solution was directly reacted with the AgNO₃ solution to form Ag₃PO₄. Secondly, Ag₃PO₄ was obtained by using the separation and purification steps in Fig. 3. Results show that the oxygen isotope values in Ag₃PO₄ obtained by the two different pathways were 11.20 ± 0.26‰ (n = 3) and 11.34 ± 0.30‰ (n = 3), respectively (Table 2). This indicates that the pretreatment method did not cause fractionation of the oxygen isotope, which is consistent with the conclusion that the P–O bond in phosphate is resistant to inorganic hydrolysis and does not exchange oxygen with water without biological mediation (Longinelli et al., 1976; Blake et al., 1997).

3.4. Improvement and applicability analysis of this method

This method was developed to carry out a phosphate oxygen isotope pretreatment of different P fractions in freshwater sediments. As mentioned above, the components of the sediment extracts are complicated with high concentrations and variations of impurities; a multistep separation and purification protocol is often necessary, while which easily reduces the phosphate recovery. Therefore, the operation steps should be simplified as much as possible to reduce the loss of P. Moreover, an efficient and gentle removal method is necessary for sediment sample with high concentrations of organic matter, Cl⁻ and other oxygen-containing impurities in order to ensure high purity of Ag₃PO₄ sample and no oxygen isotope fractionation. The concentrations of different P fractions in sediments, the main anions and cations and the dissolved

organic carbon in the sediment extracts are shown in Fig. 5, Tables 3 and 4. The following measures and improvements are proposed:

- 1) Phosphate accumulation using the MagIC method was canceled to avoid the introduction of Cl⁻, Mg²⁺ and other impurity ions. The purpose of the MagIC method is the accumulation of phosphates in water bodies with low concentrations of P (Karl and Tien, 1992). There are enough phosphates in the extracts of the three P fractions in sediments (~200 mL), thus use of the MagIC method to accumulate P is unnecessary. The experimental steps could be simplified if the MagIC method is canceled. This not only prevents the loss of P caused by the MagIC process (Karl and Tien, 1992), but also avoids introducing a large amount of Cl⁻, Mg²⁺ and other impurities, especially the presence of Cl⁻ can easily form AgCl that could be mixed with the final Ag₃PO₄ product. Thus, the amount of Cl⁻ in NaHCO₃-P and NaOH-P could be largely decreased without using the MagIC method, which reduced washing times required in the CePO₄ step (Fig. 6).
- 2) Use of non-phosphate activated carbon powder could remove organic matter in the extract without leading to the loss of P. The sediment samples had a high back ground of organic matter; thus, the organic matter concentration was very high especially in the NaHCO₃-P and NaOH-P extracts, and the color of the extract was usually brown. Previous researchers removed organic matter, such as humic acids and fulvic acids, by adjusting the pH to <1 and equal to 5 for precipitation (Zohar et al., 2010); however, this would easily lead to an obvious loss of P in the sediment extract. In addition, the pH of strong alkaline extracts containing rich organic matter was abruptly adjusted to <1. This vigorous chemical reaction could possibly trigger hydrolysis of organic P oxidized by a strong acid, and cause the potential fractionation of oxygen isotope (Tamburini et al., 2010). Furthermore, although DAX-8 Amberlite resin is good at removing organic matter, the time spent to remove the organic matter is relatively long (12 h) and the cost is high. It was also operated at pH 5 (Zohar et al., 2010; Tamburini et al., 2010; Paytan et al., 2017), which will easily cause the precipitation of organic matter and the loss of P (Hiradate et al., 2006). The non-phosphate activated carbon powder is an efficient decolorant, which could achieve decolorization of the solution (indicating removal of organic matter) without loss of P (Fig. 7). The price of non-phosphate activated carbon powder is very cheap (40 UDS/kg). It was found in the experiment that at a neutral pH, a small amount of non-phosphate activated carbon powder could effectively remove organic matter in the extract within 30 min.
- 3) It is necessary to remove most metal cations and Cl⁻ using the coprecipitation of Fe(OH)₃-PO₄³⁻ method. The concentrations of Fe³⁺, Ca²⁺ and Cl⁻ in HCl-P extract were very high (Tables 3 and 4). If these impurity ions cannot be removed, they will contribute to the formation of a large amount of mixed precipitates during CePO₄ formation. The mixed precipitate needs to be divided into several subsamples and then wash Cl⁻ multiple times (Zohar et al., 2010). Furthermore, it was difficult to remove Cl⁻ completely after washing multiple times, and which caused the loss of P. As a result, we initially tried to adjust the pH of the HCl-P extract to 1.5 and removed the metal cation using Biorad AG 50W-X8 cation exchange

Table 4

Concentrations of main metal cations in sediment extracts from two sites in Aha Lake (mg/L).

Site	Name	Al	Ca	Fe	K	Mg	Mn	Na	Si	Sr
YY	NaHCO ₃ -P	–	6.66	2.03	3.55	2.90	–	11,500	1.66	0.08
	NaOH-P	43.62	0.30	7.59	3.59	1.49	–	2300	69.11	–
	HCl-P	61.28	3513.27	213.99	10.08	937.27	11.46	195.80	82.56	8.59
JZ	NaHCO ₃ -P	0.08	5.24	1.83	3.53	2.54	–	11,500	2.76	0.04
	NaOH-P	56.14	1.36	15.00	4.64	1.87	–	2300	89.75	–
	HCl-P	145.31	2103.40	654.12	7.44	343.47	54.40	251.61	126.37	5.53

Note: “–” means undetected.

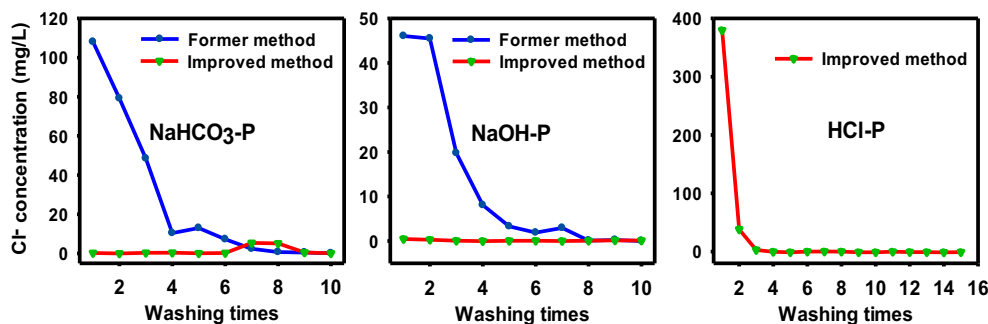


Fig. 6. Concentrations of Cl^- in the washing solutions by both former method (Zohar et al., 2010) and improved method during the removal of Cl^- process.

resin. However, a large amount of resin was needed to effectively remove these metal cations and partial P was lost due to the residual residue on the resin surface. It was found in the following experiment that, at an HCl-P extract pH of 4, a large amount of $\text{Fe}(\text{OH})_3\text{-PO}_4^{3-}$ coprecipitate formed; no phosphate was left in the supernatant after centrifugation; and most Ca^{2+} , Cl^- and other metal ions could be removed together. Finally, a small amount of Biorad AG 50W-X8 cation exchange resin was used to remove Fe^{3+} . Results indicated that precipitation was obviously reduced in the subsequent CePO_4 formation step. It was unnecessary to subdivide the precipitate to wash Cl^- , which dramatically decreased the washing times.

- 4) A buffer solution formed by adjusting the pH of pure water to 5.6 using trace KAc and HAC has been used to replace a previously proposed KAc solution to wash Cl^- . The main purpose of this improvement was to make the detection of Cl^- more convenient. KAc solution is weakly alkaline, which reacts with dilute AgNO_3 solution and forms $\text{Ag}(\text{OH})_2$ precipitate (further turning into AgO precipitate). This will complicate accurate verification of the existence of Cl^- . However, a weakly acidic buffer solution could avoid this problem. Thus, a buffer solution obtained by adjusting the pH of pure water to 5.6 using trace KAc and HAC was utilized. This buffer solution does not lead to the dissolution of CePO_4 precipitate and the loss of P in subsequent Cl^- washing steps.

In addition, forming Ag_3PO_4 precipitate under dark conditions could prevent photolysis. Washing of Ag_3PO_4 precipitate is a key operation. Firstly, washing the Ag_3PO_4 precipitate 5 times with pure water to completely remove Ag^+ , NO_3^- and NH_4^+ left on the surface. Secondly, using the 15% H_2O_2 (pH 7.3) to remove organic matter residue completely. Finally, the precipitate was washed 5 times using pure water to completely remove solute residue and NH_4^+ produced during the decomposition process of H_2O_2 . After washing Ag_3PO_4 , a direct

decant of the supernatant after centrifugation will cause loss of Ag_3PO_4 . Therefore, use of a disposable plastic dropper is suggested to gently remove the supernatant, efficiently decreasing the loss of Ag_3PO_4 . These details play an important role in purifying Ag_3PO_4 .

The pretreatment method established in this study is applicable for $\delta^{18}\text{O}$ analysis of different P fractions in sediments of freshwater lakes, rivers, reservoirs and other water bodies. For P fraction components of <100 mg/kg in the sediment, the final sample size of Ag_3PO_4 is too small to meet the required measurement because it is unfavorable for collection and weighting. In particular, it becomes a possible treatment method to solve this problem for the sediment samples with low P fraction content, such as $\text{H}_2\text{O}-\text{P}$, by conducting multiple groups of parallel samples and mixing the solution or using the MagIC method for P enrichment before the formation of Ag_3PO_4 precipitate.

3.5. Source tracing of sediment P in Aha Lake sediments using $\delta^{18}\text{O}_\text{P}$

The $\delta^{18}\text{O}_\text{P}$ values of P in water and different P fractions in sediments at two sites in Aha Lake are shown in Fig. 8. The $\delta^{18}\text{O}_\text{P}$ values of the water body at the YY and JZ sites were 13.6‰ and 15.0‰, respectively, which is both within the expected equilibrium value ranges (12.52–15.54‰ (YY) and 12.59–15.61‰ (JZ)), implying strong bio-recycling of P in the water body.

The $\delta^{18}\text{O}_\text{P}$ values of different P fractions in surface sediments at the two sites were different and different from the expected equilibrium value. The deposition rate of Aha Lake is relatively high (mean: 0.78 cm/a; $n = 26$, unpublished data), therefore, the $\delta^{18}\text{O}_\text{P}$ values of different P fractions in sediments mainly represent different sources of P. Among them, the $\delta^{18}\text{O}_\text{P}$ value of different P fractions in surface sediments at the JZ site is within 17.17–17.75‰. This is close to the mean value (17.5‰) of wastewater $\delta^{18}\text{O}_\text{P}$ from urban wastewater treatment plants (16.6–18.1‰) investigated by Gruau et al. (2005), indicating

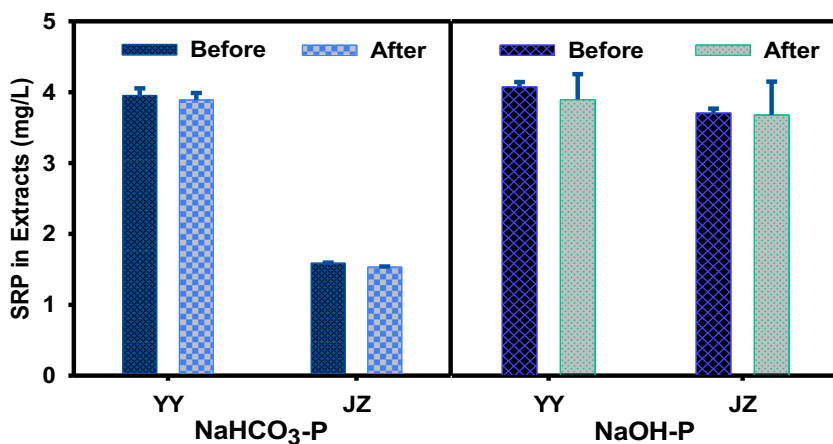


Fig. 7. Concentrations of SRP in extracts before and after the addition of non-phosphate activated carbon powder (mean \pm SD, $n = 3$).

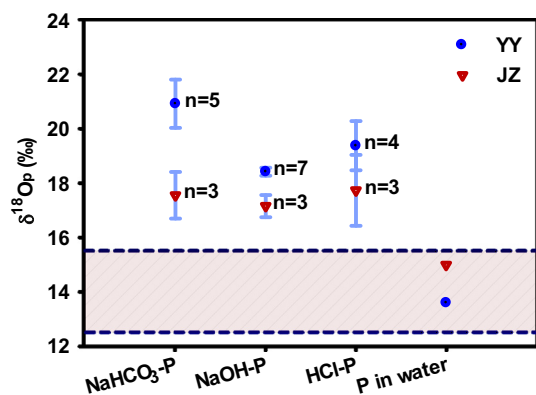


Fig. 8. The $\delta^{18}\text{O}_p$ values of sedimentary P fractions and P in water of Aha Lake (mean \pm SD). Note: The gray areas of blue dashed line represents the expected equilibrium range of the lake, the equilibrium is estimated using the temperature equilibrium formula of Longinelli and Nuti (1973): T ($^{\circ}\text{C}$) = $111.4 - 4.3 \times (\delta^{18}\text{O}_p - \delta^{18}\text{O}_w)$, where the temperature is determined according to the maximum (22°C) and minimum (9°C) annual temperatures of the lake, and the average values of $\delta^{18}\text{O}_w$ at YY and JZ are -8.27‰ and -8.20‰ , respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that it may represent P sourced from urban wastewater. The $\delta^{18}\text{O}_p$ values of different P fractions in surface sediments at the YY site were within $18.44\text{--}20.95\text{‰}$, which are close to the $\delta^{18}\text{O}_p$ values of sedimentary rock fertilizer or its ore as reported in previous studies ($18.2\text{--}21.6\text{‰}$) (Gruau et al., 2005; Young et al., 2009; Shemesh et al., 1983; Tamburini et al., 2014), could represent the emission of P from mine wastewater. This information coincides with the fact that the JZ and YY sites are mainly influenced by municipal wastewater emission and mine wastewater input.

The $\delta^{18}\text{O}_p$ values of different P fractions in sediments at the YY site are largely differentiated. Combining the general flow direction of Aha Lake from the southwest to the northeast, this might more reflect the source characteristics of different P fractions in the sediment. The smaller difference in the $\delta^{18}\text{O}_p$ values of P fractions at the JZ site might imply that three P fractions were notably influenced by the same effects, such as the re-distribution of mineralized organic P in microbes or enzymes in sediments (Zohar et al., 2010). This is consistent with the fact that this site is close to the lake outlet and the biological activity of the sediment is high. This also provides a research perspective for future studies concerning P cycling by microorganisms, as well as microbial degradation or enzymatic hydrolysis of organic P in sediments.

4. Conclusion

This study established a reliable pretreatment method for the phosphate oxygen isotope of different P fractions in sediments. This could be applied to study the composition of the phosphate oxygen isotope of freshwater sediment samples with high content of organic matter and complicated chemical constituents. The improved method effectively improved the removal of Cl^- , organic matter, cations and other impurities, and the removal of impurities is simple and efficient. Meanwhile, this improved method guarantees high phosphate recovery. The phosphate oxygen isotope pretreatment method of different P fractions in sediments, as established in this study, provides a powerful tool for studies seeking to trace P sources and its recycling processes in sediments from freshwater lakes, rivers and reservoirs; mineralization of organic P in sediments; and migration and transformation of P at the sediment-water interface.

Acknowledgements

This study was sponsored jointly by the National Natural Science Foundation of China (No. 41773145 and U1612441), the CAS

Interdisciplinary Innovation Team of China, the National Key Research and Development Project by MOST of China (No. 2016YFA0601000), and the Youth Innovation Promotion Association of the Chinese Academy of Sciences.

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