Research progress on the application of phosphate oxygen isotope in environmental science - tracing sources and reaction mechanisms identification

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Abstract. Phosphorus is a vital element in the ecosystem, which plays an important role in ecosystem. Organic phosphorus compounds are widely distributed in soil and water bodies. As many organic phosphorus compounds are pollutants, they have potential threat to the environment. Therefore, the study of the cycling of organic phosphorus compounds in the environment plays an imperative role in the prevention and control of phosphorus pollution. As a geochemical tracer, the oxygen isotope in phosphate has been widely used in recent years to study the cycling process of phosphorus in nature. This article summarizes the application principle of phosphate oxygen isotope and its application in the tracing phosphorus sources and reaction mechanisms identification of organic phosphorus compounds. This work provides a reference for the future researchers.

1. Introduction

Stable isotope technology is an extremely effective tool for tracking the cycling process of elements in nature. Phosphorus has one stable isotope ($^{31}$P) and two radioisotopes ($^{32}$P and $^{33}$P). Its radioisotopes are often used to analyze the dynamic characteristics of phosphorus in plant-soil, but due to environmental conditions and safety constraints, the application of radioisotopes in the natural environment is greatly restricted [1]. Since there is only one stable isotope of phosphorus, the stable isotope of phosphorus cannot be used to track the cycle of phosphorus.

In nature, phosphorus is mainly +5 valence, most of which exist in the form of orthophosphate (PO₄). Although phosphorus has only one stable isotope $^{31}$P, oxygen has three stable isotopes ($^{16}$O, $^{17}$O, $^{18}$O). In most biogeochemical cycles, phosphorus is always closely connected with oxygen [2, 3]. The P-O bond is very stable at most surface temperatures ($\leq 80^\circ$C), and under the condition of non-biological participation, the oxygen isotope in the phosphate hardly exchanges oxygen isotope with the surrounding water [4]. At $10^\circ$C, it takes 6000 years for 10% of the oxygen isotope in the phosphate to exchange with the surrounding water. At $2^\circ$C, it takes 35,000 years for every 10% of the oxygen isotope to exchange with the surrounding water [5]. With the participation of biological activities, oxygen isotopes in phosphate will rapidly exchange with that in the water under the catalysis of enzymes. The oxygen isotope exchange characteristics between phosphate and water make the $\delta^{18}$O value in phosphate an important indicator of the rate and scale of the biogeochemical cycle of phosphorus in nature [6]. At present, the oxygen isotope in phosphate has been applied in the research of soil [7], aquatic system, and paleoclimate. The relative abundances of $^{16}$O, $^{17}$O, and $^{18}$O in the earth...
spheres are 99.762%, 0.038%, and 0.2%, respectively, and their δ¹⁸O values range from -55‰ to 40‰. The expression method of δ¹⁸O is as follows:

$$\delta^{18}O (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$ (1)

where $R_{\text{sample}}$ is the $^{18}$O/$^{16}$O ratio of the test sample; $R_{\text{standard}}$ is the $^{18}$O/$^{16}$O ratio of the oxygen isotope standard sample, and the oxygen isotope standard is generally referred to Vienna Standard Mean Ocean Water (VSMOW).

2. Application of phosphate oxygen isotope in tracing phosphorus source

The sources of phosphorus in nature are mainly divided into man-made sources and natural sources. The amount of natural sources is around 0.1kgP/(hm²·a), which generally only accounts for a small part of the total phosphorus content in the water body. Its main sources are fish migration, atmospheric subsidence, rock and soil erosion, and nearshore plants, etc. Anthropogenic sources are mainly divided into point sources and non-point sources. Point sources are divided into domestic sewage and industrial sewage, and this type of sewage generally has fixed drainage outlets and drainage pipes. Compared with point sources, non-point sources have no fixed discharge points, and are mainly divided into agricultural and urban non-point source pollution. The main agricultural sources are from aquaculture and animal manure in farms, as well as pesticides and fertilizers remaining in the soil. The urban source is mainly surface runoff of rainwater that washes urban roads.

It is an important prerequisite for using phosphate oxygen isotope as source tracer that there are significant phosphate oxygen isotope differences between different sources. Young et al. [8] studied δ¹⁸Oₚ of different phosphate sources (soil, sewage treatment plants, animal manure, phosphate rock, etc.), and found that although there is overlap between different sources, their δ¹⁸Oₚ values are still significantly different with large interval spans (8.4‰~24.9‰). The δ¹⁸Oₚ values of various phosphate sources are listed below as table 1.

| Sample group     | Mean δ¹⁸Oₚ     |
|------------------|--------------|
| Chemical fertilizers | +19.3       |
| Fertilizer ore    | +20.1        |
| All fertilizers   | +19.8        |
| Aerosols          | +20.5        |
| Detergents        | +16.8        |
| Vegetation        | +16.9        |
| WWTP water        | +11.2        |
| Guano             | +20.7        |

Tian et al. summarized the δ¹⁸Oₚ composition in different ecosystems based on previous studies, which provide a reference for future phosphate source analysis. Mclaughlin et al. [9] observed that the δ¹⁸Oₚ value in the deep water layer of the Atlantic Sargasso Sea was close to the equilibrium value, indicating that the utilization rate of dissolved organic phosphorus was so low that the inorganic phosphate was fully metabolized. The δ¹⁸Oₚ value of the surface seawater was lower than the temperature-dependent equilibrium value, which indicates that extracellular enzymes catalyze the mineralization of dissolved organic phosphorus. In the euphotic layer, the mineralization of organic phosphorus may be caused by the massive utilization of phosphorus by phytoplankton.

Colman et al. [10] studied the δ¹⁸Oₚ of Pacific and Atlantic waters and found that on the depth profile, δ¹⁸Oₚ showed a temperature-dependent isotopic equilibrium fractionation between inorganic phosphorus and seawater. In the thermocline, δ¹⁸Oₚ has a small deviation. Below the thermocline, δ¹⁸Oₚ is 1.5‰ lower than the equilibrium value. This phenomenon may be caused by enzyme-catalyzed organic phosphorus decomposition because phosphohydrolase has a great influence on the δ¹⁸Oₚ fractionation. In the deep sea, δ¹⁸Oₚ closing to equilibrium fractionation may be due to slow
microbial activity or the oxygen isotope exchange between inorganic phosphorus and water catalyzed by extracellular pyrophosphatase.

Zheng found that the $\delta^{18}O_p$ value in the estuary area has well correlation with salinity and deviates from the equilibrium value in the Jiulong River Estuary. It indicates that phosphate in the estuary area has not been fully utilized by microorganisms, and there may be input of other phosphorus sources.

It can be seen from the above that the analysis of the fractionation value of phosphate oxygen isotopes provides a basis for tracing the phosphorus cycling process. It can be used to distinguish the different degradation pathway of organic phosphorus, such as photolysis, enzymatic and microbial degradation, which provides provide a theoretical basis for tracing source of phosphorus and studying its cycling process.

3. Application of phosphate oxygen isotope in the degradation of organophosphorus

Organophosphorus undergoes oxygen isotope fractionation under different degradation pathways, which makes clarifying tracing its sources more complicated. In water bodies, organophosphorus will undergo various degradation pathways [11, 22], mainly including microbial degradation, ultraviolet degradation, and photocatalytic degradation. Therefore, studying the phosphate oxygen isotope fractionation mechanism of organophosphorus in different degradation pathways is of great significance to trace its source.

3.1. A subsection Phosphate oxygen isotope signature of microbial degradation of organic phosphorus

Microbial degradation of organophosphate involves different enzymes. In nature, microorganisms preferentially use inorganic phosphorus, however, the total dissolved phosphorus in water bodies is dominated by organic phosphorus, accounting for 80-99% of total amount [11]. In contrast, there is very low concentration of inorganic phosphorus about 1~10 μM in surface soil, and 1~3 μM in sea water. Therefore, in most water bodies, organic phosphorus is the main phosphorus source for microorganisms. Under the natural temperature and pH, the P-O bond can hardly break down except by biological enzymes catalysis.

Inorganic phosphate is generated by organic phosphorus decomposition and utilized by organisms. Catalyzed by intracellular pyrophosphatase (PPase), the P-O bond breaks [12] and exchanges oxygen atoms with surrounding water, which gradually achieves equilibrium fractionation. The microbial cycling process of phosphorus is shown in figure 1. Longinelli [13] first established the equilibrium fractionation equation of phosphate and oxygen isotopes listed below.

$$T (^\circ C) = 111.4 - 4.3 (\delta^{18}O_p - \delta^{18}O_w)$$

Where $T$ is the temperature, $\delta^{18}O_p$ is the oxygen isotope value of phosphate, and $\delta^{18}O_w$ is the oxygen isotope value of the surrounding water.

![Figure 1. The microbial cycling process of phosphorus in water bodies. [12]](image-url)
Blake et al. [6] has studied phosphate oxygen isotope signature of microbial degradation of organophosphorus compounds in the laboratory. It was found that $\delta^{18}O_p$, the produced phosphate was greatly changed by microbial degradation, which is dominated by equilibrium fractionation rather than kinetic fractionation. It was proposed that the equation for the equilibrium fractionation of bacterial catalytic oxygen isotope as follows:

$$T (^{\circ}C)=155.8-6.4(\delta^{18}O_p-\delta^{18}O_w)$$  \hspace{1cm} (3)

Blake et al. [14] used glucose-1-phosphate as the only carbon and phosphorus source to cultivate Klebsiella aerogenes and found that the $\delta^{18}O_p$ of produced phosphate did not reach equilibrium fractionation. But the produced phosphate and ambient water still underwent massive oxygen exchange.

Liang et al. [15] studied RNA degradation process by Escherichia coli, the linear fitting slope of $\delta^{18}O_p$ of produced phosphate and $\delta^{18}O_w$ of water was 0.51 (±0.07), which indicated that 51% oxygen in phosphate produced by RNA degradation originated from water. Joint with other lab research, it was concluded that the microbial degradation of RNA by Escherichia coli was catalysed by phosphodiesterase and 5'-nucleotidase.

3.2. Phosphate oxygen isotope signature of enzyme degradation of organic phosphorus

Of various intracellular enzymes, the inorganic pyrophosphatase (PPase) plays an important role in the process of phosphorus metabolism in organisms. PPase is widely distributed in bacteria, fungi and archaea [16]. PPase can catalyzed P-O bond breakage so that $\delta^{18}O_p$ approaches oxygen isotope equilibrium fractionation in the cell.

Blake et al. [12] used PPase to catalyse KH$_2$PO$_4$ and found that the oxygen in KH$_2$PO$_4$ rapidly exchanged with the surrounding water, gradually approaching the temperature-dependent equilibrium fractionation, consistent with Longinelli’s equation. In aquatic ecosystems, alkaline phosphatase (Alkaline Phosphatase), 5'-nucleotidase (5'-Nucleotidase), etc are common extracellular enzymes, leading to kinetic fractionation.

Liang et al. [3] studied the phosphate oxygen isotope signature of monoesterase-catalysis degradation of phosphomonoester. The slopes of the linear fitting lines of $\delta^{18}O_p$ and $\delta^{18}O_w$ by different alkaline phosphatase are between 0.23-0.28, which proves that alkaline phosphatase-catalysis degradation of organophosphate, around 75% of the oxygen in the produced phosphate is inherited from the original substrate, and other 25% comes from the ambient water. The kinetic fractionation value of generated phosphate is -30 ± 8‰, which showed that the $^{16}$O in water is more likely to be incorporated into the generated phosphate.

Liang et al. [15] studied that phosphodiester (DNA and RNA) is catalysed by diesterase (deoxyribonuclease, phosphodiesterase) and phosphomonoesterase (5'-nucleotidase, alkaline phosphatase). By linearly fitting the $\delta^{18}O_p$ and $\delta^{18}O_w$, it was found that the slope of all the experimental groups of DNA (combination of different enzymes) is 0.45 (±0.02), indicating that approximately two oxygen of water are combined in the generated PO$_4$. It was found that diesterases (deoxyribonuclease and phosphodiesterase) produced the same isotope fractionation effect, while different monoestersases (alkaline phosphatase and 5'-nucleotidase) produced different isotope fractionation effects. The oxygen isotope fractionation value of phosphodiesterase degrading DNA and RNA are -20‰ and +20‰ respectively, which shows that the reaction mechanism of phosphodiester degrading DNA and RNA is different. Therefore, phosphate oxygen isotope provides a new possibility for the identification of degradation pathways and mechanisms.

C. von Sperber et al. [17] studied the phosphate oxygen isotope signature of phytase and acid phosphatase-catalysis degradation of phytic acid. The linear fitting slope of phosphate oxygen isotope $\delta^{18}O_p$ and $\delta^{18}O_w$ is 0.23-0.24, indicating that one of the four oxygen atoms in the generated PO$_4$ comes from water.

It was mentioned above that pyrophosphatase can catalyse phosphate to exchange oxygen with ambient water, therefore gradually reaching equilibrium fractionation. In order to accurately measure the temperature-dependent equilibrium fractionation equation, Sae et al. [18] applied extracellular pyrophosphatase to conduct experiments in different oxygen isotope-labeled PO$_4$ and water within the range of 3-37°C, and found that PO$_4$ reached equilibrium fractionation within 818 minutes.
3.3. Phosphate oxygen isotope signature of chemical degradation of organic phosphorus

Organophosphorus has a variety of chemical degradation pathways. Research on the oxygen isotope signature of chemical degradation of organic phosphorus has been gradually carried out in recent years.

Liang et al. [3] has studied the phosphate oxygen isotope signature of photo oxidation of organic phosphorus. 500W mercury lamp was applied to degrade α-D-glucose-1-phosphate, β-glycerophosphate and 5'-AMP in δ¹⁸Ow labeled water of -5.5‰ and 99.8‰, respectively. The difference in δ¹⁸O of produced phosphate is only about 1.8‰, which is much smaller than the δ¹⁸Ow span of water (greater than 100‰). It can be inferred that all four oxygen atoms of generated phosphate come from organic phosphorus rather than the ambient water. Therefore, δ¹⁸O values of -PO₄ groups in α-D-glucose-1-phosphate, β-glycerophosphate and 5'-AMP are calculated to be 20±1‰, 11±1‰ and 12±1‰, respectively.

Sandy et al. [19] has studied the phosphate oxygen isotope signature of UV oxidation of phosphonate (glyphosate and phosphonoacetic acid). 1200W mercury lamp was used to degrade phosphonate in δ¹⁸Ow labeled water. The linear fitting of the δ¹⁸Ow and δ¹⁸Op revealed that the δ¹⁸Op and δ¹⁸Ow positively correlated for glyphosate and phosphonoacetic acid, and the slopes are 0.17. The slopes mean that 17% oxygen of phosphate come from the surrounding water. According to the chemical structure of glyphosate and phosphonoacetic acid (C-PO₃), the exogenous bound oxygen of produced phosphate should account for 25% of the oxygen in PO₄. Therefore, it is speculated that the other 8% of the oxygen comes from O₂. As known that δ¹⁸O of oxygen in the air is about 23.5‰ [20], it can be calculated that the δ¹⁸O of the -PO₃ group in glyphosate and phosphorylacetic acid are 4.73‰ and -0.02‰, respectively. According to the phosphate oxygen isotope analysis, The C-P bond cleavage reaction mechanism can be gained as below, showed in figure 2.

Li [21] et al. has done research about the phosphate oxygen isotope signature of metal-catalysis UV degradation. Glyphosate and aminomethylphosphonic acid (AMPA) were degraded by UV irradiation under the catalysis of birnessite (MnO₂) minerals, in different δ¹⁸O labeled water MnO₂ (δ¹⁸O=−3.69±0.03‰, 55.31±0.02‰ and 94.79±0.02‰) and O₂ (δ¹⁸O=110.15±0.92‰, 2082.41±2.37‰). Through linear fitting analysis of δ¹⁸Op, δ¹⁸OMnO₂ and δ¹⁸OO₂, it was found that the exogenous oxygen does not come from MnO₂ or O₂. The δ¹⁸Op and δ¹⁸Ow were linearly fitted with the slope value 0.21, indicating that 21% of the oxygen in the generated phosphate comes from the surrounding water. The kinetic fractionation value of δ¹⁸Op produced by AMPA was calculated as -11.81 ± 4.14‰.

4. Conclusion

Nowadays, oxygen isotope as a new method is widely used in environmental science and gradually becomes a very useful tool for studying biogeochemical cycling of phosphorus in the natural
environment. This article provides a brief review about the progress of application of phosphate oxygen isotope in environment. It clarifies the principle for using phosphate oxygen isotope as a tracer and summarises its application in the organic compound degradation mechanism identification. Since last century, certain researchers have made a lot of experiments works which made great progress in this field, making it possible to trace the source and study the phosphorus cycle. However, in general, the research on phosphate oxygen isotope is still in its infancy. It needs more research and will become a very promising method for interpretation for phosphorus cycling in the future.

5. Acknowledgments

This work was financially supported by Youth Science and Technology Innovation Fund (2020) of BGRIMM Technology Group.

References

[1] A. Angert, T. Weiner, S. Mazeh, et al, Seasonal variability of soil phosphate stable oxygen isotopes in rainfall manipulation experiments, J. Geochimica et Cosmochimica Acta, 75(2011): 4216-4227.
[2] D.P. Jaisi, R.E. Blake, R.K. Kukkadapu, Fractionation of oxygen isotopes in phosphate during its interactions with iron oxides, J. Geochimica et Cosmochimica Acta, 74 (2010) 1309-1319.
[3] Y. Liang, R.E. Blake, Oxygen isotope signature of Pi regeneration from organic compounds by phosphomonoesterases and photooxidation, J. Geochimica et Cosmochimica Acta, 70(2006): 3957-3969.
[4] D.P. Jaisi, R.E. Blake, Y. Liang, et al, Investigation of Compound-Specific Organic-Inorganic Phosphorus Transformation Using Stable Isotope Ratios in Phosphate, J. (2014) 267-292.
[5] A.S. Colman, R.E. Blake, D.M. Karl, et al, Marine phosphate oxygen isotopes and organic matter remineralization in the oceans, J. P Natl Acad Sci USA, 102(2005) 13023-13028.
[6] R.E. Blake, J.R. O’Neil, G.A. Garcia, Oxygen isotope systematics of biologically mediated reactions of phosphate: I. Microbial degradation of organophosphorus compounds, J. Geochimica et Cosmochimica Acta, 61(1997) 4411-4422.
[7] I. Zohar, A. Shaviv, M. Young, et al, Phosphorus dynamics in soils irrigated with reclaimed waste water or fresh water - A study using oxygen isotopic composition of phosphate, J. Geoderma, 159(2010) 109-121.
[8] M.B. Young, K. McLaughlin, C. Kendall, et al, Characterizing the oxygen isotopic composition of phosphate sources to aquatic ecosystems, J. Environmental Science & Technology, 43(2009) 5190-5196.
[9] K. McLaughlin, F. Chavez, J.T. Pennington, et al, A time series investigation of the oxygen isotopic composition of dissolved inorganic phosphate in Monterey Bay, California, J. Limnology and Oceanography, 51(2006) 2370-2379.
[10] A.S. Colman, R.E. Blake, D.M. Karl, et al, Marine phosphate oxygen isotopes and organic matter remineralization in the oceans, J. P Natl Acad Sci USA, 102(2005): 13023-13028.
[11] H.L. Ehrlich, D.K. Newman, Geomicrobiology, M. 5th ed ed.: CRC Press (2009).
[12] R.E. Blake, Biogeochemical cycling of phosphorus: insights from oxygen isotope effects of phosphoenzymes, J. American Journal of Science (2005), 305.
[13] A. Longinelli, S. Nuti, Revised phosphate-water isotopic temperature scale, J. Earth & Planetary Science Letters, 19 (1973) 373-376.
[14] R.E. Blake, J.R. O’Neil, G.A. Garcia, Effects of microbial activity on the delta^{18}O of dissolved inorganic phosphate and textural features of synthetic apatites, J. American Mineralogist, 83 (1998) 1516-1531.
[15] Y. Liang, R.E. Blake, Compound- and enzyme-specific phosphodiester hydrolysis mechanisms revealed by delta^{18}O of dissolved inorganic phosphate: Implications for marine P cycling, J. Geochimica et Cosmochimica Acta, 73 (2009) 3782-3794.
[16] V-M. Leppänen, H. Nummelin, T. Hansen, et al, Sulfolobus acidocaldarius inorganic pyrophosphatase: structure, thermostability, and effect of metal ion in an archael pyrophosphatase, J. Protein Science, 8 (1999) 1218-1231.
[17] C. Von Sperber, F. Tamburini, B. Brunner, et al, The oxygen isotope composition of phosphate released from phytic acid by the activity of wheat and Aspergillus niger phytase, J. Biogeosciences, 12(2015) 4175-4184.
[18] S.J. Chang, R.E. Blake, Precise calibration of equilibrium oxygen isotope fractionations between dissolved phosphate and water from 3 to 37 °C, J. Geochemica et Cosmochimica Acta, 150 (2015) 314-329.
[19] E.H. Sandy, R.E. Blake, S.J. Chang, et al, Oxygen isotope signature of UV degradation of glyphosate and phosphonoacetate: Tracing sources and cycling of phosphonates, J. Journal of Hazardous Materials, 260 (2013) 947-954.
[20] R.F. Keeling, The atmospheric oxygen cycle: The oxygen isotopes of atmospheric CO₂ and O₂ and the O₂/N₂ ratio, J. Reviews of Geophysics, 33(1995) 2399-2409.
[21] H. Li, S.R. Joshi, D.P. Jaisi. Degradation and isotope source tracking of glyphosate and aminomethylphosphonic acid, J. Journal of Agricultural and Food Chemistry, 64 (2016) 529-538.
[22] A. Paytan, K. McLaughlin, The oceanic phosphorus cycle, J. Chemical Reviews, 107(2007) 563-576.