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Oxygen isotopes of phosphatic compounds—Application for marine particulate matter, sediments and soils

Karen McLaughlin [a,*], Adina Paytan [a,1], Carol Kendall [b,2], Steven Silva [b,3]

[a] Stanford University, Department of Geological and Environmental Sciences, Stanford, CA 94305-2115, United States
[b] United States Geological Survey, 345 Middlefield Rd, MS 434, Menlo Park, CA 94025, United States

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Abstract

The phosphate oxygen isotopic composition in naturally occurring particulate phosphatic compounds ($\delta^{18}O_p$) can be used as a tracer for phosphate sources and to evaluate the cycling of phosphorus (P) in the environment. However, phosphatic compounds must be converted to silver phosphate prior to isotopic analysis, a process that involves digestion of particulate matter in acid. This digestion will hydrolyze some of the phosphatic compounds such that oxygen from the acid solution will be incorporated into the sample as these phosphatic compounds are converted to orthophosphate (PO_4^{3-}). To determine the extent of incorporation of reagent oxygen into the sample, we digested various phosphatic compounds in both acid amended with $H_2^{18}O$ (spiked) and unspiked acid and then converted the samples to silver phosphate for $\delta^{18}O_p$ analysis. Our results indicate that there is no isotopic fractionation associated with acid digestion at 50 °C. Furthermore, we found that reagent oxygen incorporation is a function of the oxygen to phosphorus ratio (O:P) of the digested compound whereby the percentage of reagent oxygen incorporated into the sample is the same as that which is required to convert all of the P-compounds into orthophosphate. Based on these results, we developed a correction for reagent oxygen incorporation using simple mass balance, a procedure that allows for the determination of the $\delta^{18}O_p$ of samples containing a mixture of phosphatic compounds. We analyzed a variety of environmental samples for $\delta^{18}O_p$ to demonstrate the utility of this approach for understanding sources and cycling of P.

Keywords: Oxygen isotopes; Phosphate; Marine particulate matter; Marine sediment; Soils

1. Introduction

Phosphorus (P) is used by all living organisms for energy storage, metabolism and cell construction (Bridger and Henderson, 1983; Lehninger et al., 1993; Schlesinger, 1991). Upon uptake by a cell, phosphorus may be converted to a variety of different compounds from phospholipids for cell membranes to adenosine triphosphate (ATP) for metabolism. Phosphorus is also a principal component of deoxyribonucleic acid (DNA) and can be stored by cells as orthophosphate and polyphosphate (Lehninger et al., 1993).

Because P has only one stable isotope, P stable isotope ratios cannot be used as tracers to study sources and cycling of P in the environment as is the case for

* Corresponding author. Tel.: +1 949 502 8173; fax: +1 650 725 0979.
E-mail addresses: karenmcl@pangea.stanford.edu (K. McLaughlin), apaytan@pangea.stanford.edu (A. Paytan), ckendall@usgs.gov (C. Kendall), srsilva@usgs.gov (S. Silva).
[1] Tel.: +1 650 724 4073; fax: +1 650 725 0979.
[2] Tel.: +1 650 329 4576.
[3] Tel.: +1 650 329 4538.

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carbon, nitrogen and sulfur. However, most of the P found in nature is strongly bound to oxygen (O), which has three stable isotopes; hence, phosphate (PO_4^{3-}) can be analyzed for $\delta^{18}O$ (Blake et al., 1998; Longinelli et al., 1976; McLaughlin et al., 2004). Because the P–O bond in phosphate is resistant to inorganic hydrolysis and does not readily exchange oxygen with water without biological mediation (Blake et al., 1997; Longinelli et al., 1976), systematic isotopic variability of the oxygen isotopic composition of phosphate ($\delta^{18}O_p$) may provide information about environmental temperature and the $\delta^{18}O$ of water ($\delta^{18}O_w$), and can potentially be used to determine P sources and the extent of P cycling in the environment. Several studies have used the $\delta^{18}O_p$, particularly in apatite, to determine the $\delta^{18}O$ of environmental water (Longinelli et al., 2003; Luz et al., 1984), environmental temperatures (Kolodny et al., 1983; Kolodny and Raab, 1988), and the origin of phosphates in soil systems (Ayliffe et al., 1992; Mizota et al., 1992).

Few studies have been conducted to determine and utilize the oxygen isotopic composition of organic P compounds. The first of these (Longinelli et al., 1976) found that the $\delta^{18}O_p$ of soft tissue in marine organisms is approximately 20‰ higher than the $\delta^{18}O_w$ and is 3‰ higher compared to $\delta^{18}O_p$ of inorganic phosphate dissolved in the water column (DIP). These differences were attributed to kinetic fractionation effects during metabolism of phosphate (Longinelli et al., 1976). Paytan et al. (2002) found that oxygen isotope exchange between the surrounding water and intracellular P compounds is very rapid and occurs at all levels of the food chain without a significant temperature effect. Furthermore, Paytan et al. (2002) found that soft tissue $\delta^{18}O_p$ was 23–26‰ higher than $\delta^{18}O_w$, whereas DIP was ~20‰ higher than $\delta^{18}O_w$.

Investigation of the $\delta^{18}O_p$ of organic P compounds in the water column, in marine sediments, and in soils may provide information about the source and cycling of organic matter in the environment. For example, terrigenous organic matter is expected to have significantly different $\delta^{18}O_p$ as a result of exchange in fresh water systems which are isotopically low relative to seawater. Therefore, if some of the terrigenous organic matter containing phosphate is refractory and is not utilized by marine organisms, the source signal (i.e. fresh water imprint) will be preserved. If organic matter phosphate from terrestrial sources is biologically cycled in the oceanic water column, the extent of cycling could be determined by the amount of exchange between phosphate oxygen and seawater oxygen. As illustrated in the above example, the isotopic exchange of oxygen in phosphate attributable to biological processes can act as a tracer of phosphate cycling and the record of the $\delta^{18}O_p$ in the organic matter present in marine sediments could potentially provide a history of phosphate sources and cycling through time. Moreover, if different phosphatic organic compounds are remineralized at different rates, a variation in $\delta^{18}O_p$ of particulate organic phosphorus (POP) throughout the water column is expected. Such a variation may accompany changes in the composition of organo-P compounds, which has been observed with depth in the water column or in the sediment after burial (Clark et al., 1998, 1999; Paytan et al., 2003). In terrestrial environments, phosphate cycling in soils may be estimated by the degree of deviation of the $\delta^{18}O_p$ in the system from its source signature (bedrock, aerosols, fertilizer, etc.) (Ayliffe et al., 1992; Mizota et al., 1992).

Analyses of the oxygen isotopic composition of phosphate in organic matter involves the digestion of samples in nitric acid. Because some of the P in organic matter is in the form of polyphosphates, phosphonates, and other compounds not present in a 4:1 oxygen to phosphorus ratio (O:P), it is likely that there will be some incorporation of oxygen from the nitric acid solution when C–P and P–O–P bonds are cleaved and PO_4^{3-} is formed. Thus, it is crucial to determine the effect of this incorporation on the isotopic composition of the sample and to develop a protocol to systematically correct for this effect. It is also important to determine if this incorporation is related to a compound's molecular structure and if the acid digestion process involves isotopic fractionation in addition to simple oxygen incorporation.

Our primary objective was to quantify the incorporation of reagent oxygen during acid digestion of pure, reagent-grade phosphatic compounds and precipitation of silver phosphate from the digest. These results will determine if any isotopic exchange occurs during sample processing and allow for a mass balance approach to correct measured $\delta^{18}O_p$ of organic matter to that of the sample prior to any reagent incorporation. We have also included analyses of several natural samples to demonstrate the potential utility of this method for understanding sources and cycling of phosphate in various ecosystems.

2. Materials and methods

We used a four-step purification procedure to convert inorganic and organic phosphatic compounds to

silver phosphate for analysis of $\delta^{18}O_p$ via pyrolysis mass spectrometry. The method presented here was adapted from that described by McLaughlin et al. (2004) to accommodate digestion of solid compounds. We used reagent grade potassium phosphate (Mallinckrodt), sodium phosphate (Fisher Scientific), phytic acid (Sigma-Aldrich), DNA (Sigma-Aldrich), L-α-phosphatidylcholine (Sigma-Aldrich), calcium pyrophosphate (Sigma-Aldrich), tripolyphosphate (Sigma-Aldrich), ATP (Sigma-Aldrich), 2-aminoethylphosphonic acid (Sigma-Aldrich), and diethylcylcopropylmethyl phosphonate (Sigma-Aldrich) (Table 1) which represent a wide range of phosphatic compounds with various P bond configurations. Six replicates of each compound were weighed into separate 50-ml polyethylene depressed cap centrifuge tubes. Ten milliliters of 10 M nitric acid was added to three of these and ten milliliters of 10 M nitric acid that had been amended with $H_2^{18}O$ (Isotec T88-70022 batch # EQ0820) was added to the remaining three tubes of each compound. All samples were then heated in a water bath held at 50 °C for 24–36 h. This temperature was selected because it was sufficient to hasten the digestion whereby P would be hydrolyzed and converted to orthophosphate while insuring that no isotopic fractionation would result during the process. Indeed, O'Neil et al. (2003) found that at temperatures greater than 70 °C at pH 1, some exchange between phosphate and water will occur. Furthermore, they found that the longer the exposure of phosphate to acid, the greater the exchange. Thus, limiting both the temperature and digestion time is important to minimize fractionation. This nitric acid digestion procedure has been used to clean organic matter from bone material prior to preparation of apatite for $\delta^{18}O_p$ analysis and was determined to be sufficient to hydrolyze organic matter in bone samples (Luz et al., 1984). Hydrolysis was considered complete when compounds were completely dissolved. For sediment and soil samples, a residue remained after digestion which was separated from solution via centrifugation (20 min at 2000×g). For these samples, hydrolysis was assumed to be complete when there was no longer any visible sign of reaction between the acid and the sediment/soil. Incomplete hydrolysis of phosphatic compounds at this stage is a possible source of error in this method. Once hydrolysis is complete, the acid is neutralized with 8 M potassium hydroxide (Fisher Scientific, ACS grade), buffered with 1 M potassium acetate (Fisher Scientific, ACS grade), purified first to cerium phosphate and finally to silver phosphate and analyzed for $\delta^{18}O_p$ according to McLaughlin et al. (2004). Isotopic analyses were conducted on a Eurovector Elemental Analyzer coupled to a Micromass (now GVI) IsoPrime mass spectrometer at the U.S. Geological Survey in Menlo Park, CA. Results were calibrated and precision monitored using two internal silver phosphate standards, STDH ($\delta^{18}O_p$=20.0‰) and STDL ($\delta^{18}O_p$=11.3‰) which were analyzed throughout each mass spectrometer run. The isotopic values of these standards were determined previously by comparing to other standards which were also analyzed with the traditional fluorination method (McLaughlin et al., 2004). Oxygen isotopic measurements are reported in the standard delta notation in per mil units (‰) with respect to Vienna Standard Mean Ocean Water (VSMOW).

Table 1
$\delta^{18}O_p$ of reagent grade phosphatic compounds and the observed and expected percent oxygen incorporation based on their oxygen/phosphorus ratios (O:P)

Sample	Unspiked $\delta^{18}O_p$	Spiked $\delta^{18}O_p$	Corrected $\delta^{18}O_p$	Observed percent oxygen incorporation	Expected percent oxygen incorporation	O:P
Potassium phosphate	11.2 ± 0.1	11.4 ± 0.1	11.2 ± 0.1	0.2	0	4
Sodium phosphate	19.7 ± 0.2	19.8 ± 0.1	19.7 ± 0.4	0.1	0	4
Phytic acid	23.4 ± 0.1	23.5 ± 0.4	23.4 ± 0.5	0.1	0	4
DNA	24.0 ± 0.8	26.6 ± 0.1	24.9 ± 1.0	2.5	0	4
L-α-phosphatidylcholine	11.1 ± 0.4	20.1 ± 1.7	13.9 ± 2.0	8.2	0	4
Calcium pyrophosphate	15.1 ± 0.9	26.9 ± 0.5	18.8 ± 1.4	11.3	12.5	3.5
Tripolyphosphate	18.2 ± 0.4	35.9 ± 0.5	24.7 ± 0.9	17.1	16.75	3.33
ATP	17.2 ± 0.6	36.6 ± 1.1	24.3 ± 1.6	18.7	16.75	3.33
2-Aminoethylphosphonic acid	15.1 ± 1.8	39.9 ± 2.1	24.2 ± 3.9	23.9	25	3
Diethylcylcopropylmethyl phosphonate	27.3 ± 1.9	59.5 ± 2.5	48.8 ± 4.5	31.0	25	3

Results are reported with standard deviations (n=3).

3. Results and discussion

3.1. Determination of reagent oxygen incorporation into pure compounds

The difference between $\delta^{18}O_p$ of the spiked and unspiked samples for the suite of compounds varied from 0.1‰ to 32.2‰ (Fig. 1 and Table 1) and the extent of reagent oxygen incorporation is well correlated with the oxygen to phosphorus ratio of the compound (O:P) (Fig. 2). Potassium phosphate and sodium phosphate (O:P=4), both of which have phosphate groups ionically bonded to a cation, had no significant incorporation of reagent oxygen suggesting that the destruction of ionic bonds does not result in isotopic exchange and that no oxygen–phosphorus bond is broken during the acid digestion at 50 °C. The potassium phosphate and sodium phosphate used for these samples were the same as the potassium phosphate and sodium phosphate used as our internal phosphate standards (STDL and STDH), which have known isotopic compositions (of 11.3‰ and 20.0‰, respectively (McLaughlin et al., 2004)). Accordingly, these compounds can also be used to evaluate the extent of oxygen isotopic exchange resulting from thermodynamic or kinetic fractionation effects in addition to simple incorporation during digestion. Because no difference was observed between the spiked and unspiked potassium phosphate and sodium phosphate samples and because the measured isotopic composition of these compounds after treatment with hot acid (50 °C) was not different than the original $\delta^{18}O_p$ (previously determined without the digestion step), we conclude that there is no isotopic exchange resulting from fractionation between the reagent solution and phosphate during digestion with 10 M nitric acid at this temperature.

Phytic acid, in which phosphate groups are covalently bonded to a carbon ring (O:P=4), similarly did not incorporate reagent oxygen during digestion (e.g. no difference between the spiked and unspiked treatments) despite the fact that one of the oxygen atoms of each phosphate group is also bonded to a carbon atom. This can be explained by examining the relative bond strengths of the P–O and C–O bonds. Because phosphorus bonds much more strongly to oxygen than carbon bonds to oxygen (Corbridge, 1990), it is expected that when the phosphate group is cleaved from this molecule during digestion, the "sample" oxygen is retained by the phosphate group and the bond is broken between the carbon and oxygen atoms rather than between the phosphorus and oxygen atoms. Similarly, DNA experienced very little incorporation compared to the other P-compounds.

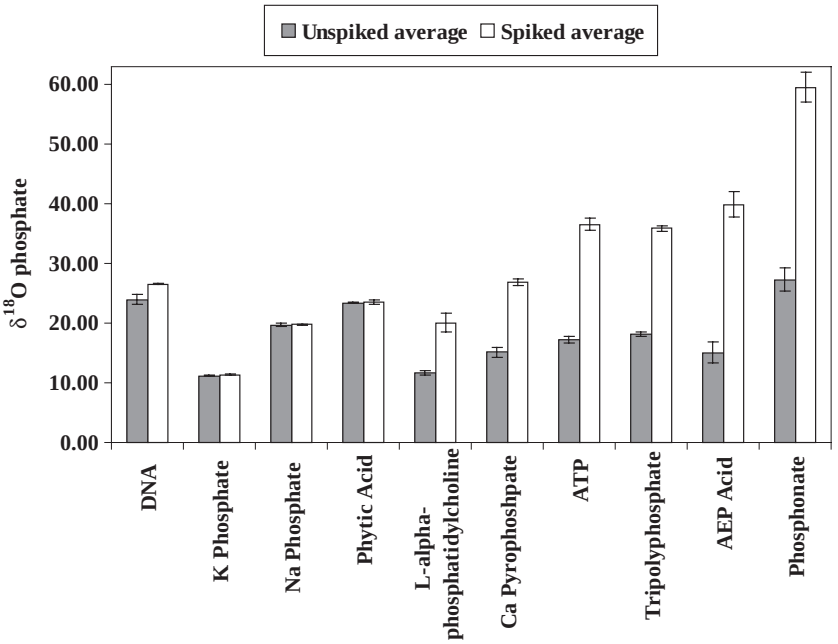

Fig. 1. Oxygen isotopic composition of phosphatic compounds digested in the spiked acid and the unspiked acid. Each compound was analyzed in triplicate for both the spiked digestion and the unspiked digestion. Error bars indicate the standard deviation between duplicates of each sample on the mass spectrometer.

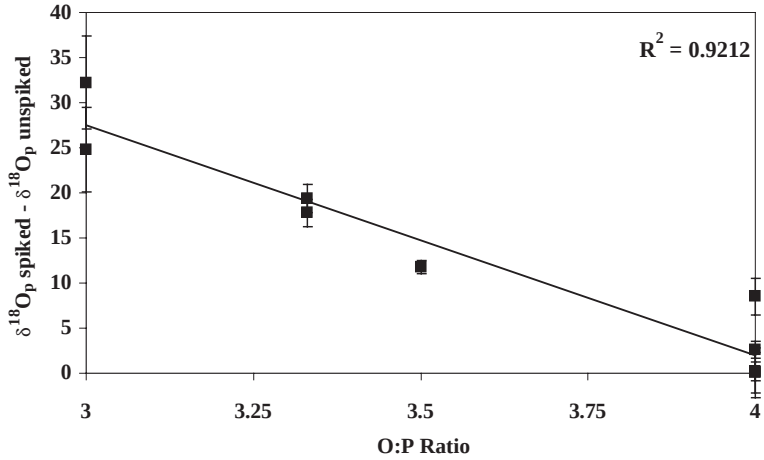

Fig. 2. The amount of incorporation of reagent oxygen is proportional to the oxygen/phosphorus ratio. During digestion, the phosphate group cleaves from the compound at a carbon–oxygen bond rather than a phosphorus–oxygen bond. Error bars represent one standard deviation.

Phosphate groups in DNA are covalently bonded to carbon atoms which link two nucleotides forming the framework for the "ladder" structure (O:P=4). Like DNA, the phosphate in L-α-phosphatidylcholine has two oxygen atoms bonded to two carbon atoms but upon digestion, incorporates a slightly greater amount of reagent oxygen. The incorporation of oxygen from the reagent during digestion of DNA and L-α-phosphatidylcholine suggests that those covalent bonds primarily break at the weaker oxygen–carbon bond site, but on rare occasions a phosphorus oxygen bond may also cleave. This discrepancy, however, could also be attributed to trace impurities within these chemical compounds.

The remaining P-compounds analyzed, each of which contain phosphate groups bonded together through at least one shared oxygen atom or contain a phosphate group with one P–C bond, experienced varying degrees of incorporation of reagent oxygen. ATP and Tripolyphosphate which have similar phosphate bond configurations, have similar reagent oxygen incorporation as would be expected. 2-Aminoethylphosphonic Acid and diethylcylcopropylmethyl phosphonate, which have similar P bond configurations (P is bonded to three oxygen atoms and one carbon), experienced the greatest incorporation of reagent oxygen of all the products analyzed but also showed the

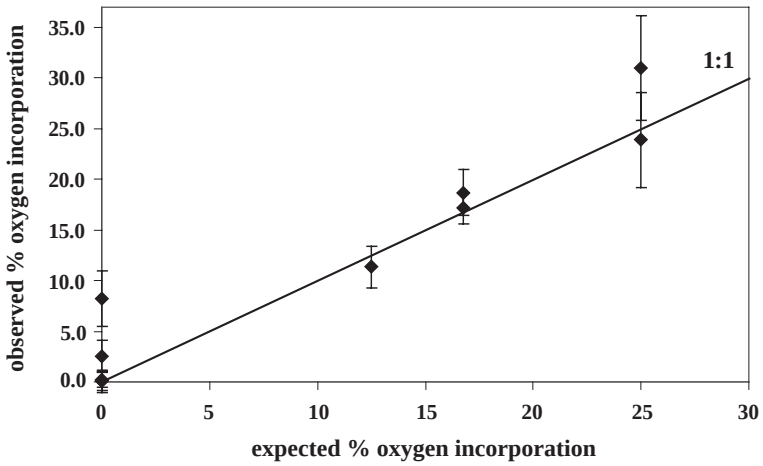

Fig. 3. The expected percent oxygen isotope incorporation calculated from the oxygen/phosphorus ratio plotted against the observed percent oxygen incorporation is nearly 1:1 within experimental error for all compounds. Expected percent oxygen is the amount of additional oxygen required such that each phosphorus atom is matched with four oxygen atoms and is determined from the oxygen/phosphorus ratio. Error bars represent one standard deviation.

Table 2
Oxygen isotopic composition and standard deviation of natural samples

Sample	Unspiked $\delta^{18}O_p$ (‰)	Spiked $\delta^{18}O_p$ (‰)	Spiked $\delta^{18}O_p$ – Unspiked $\delta^{18}O_p$ (‰)	Corrected $\delta^{18}O_p$ (‰)
Aidellia sp., Fish store	20.37 ± 0.44	38.48 ± 0.04	18.10	27.59 ± 0.48
Phytoplankton tow, Monterey Bay, CA, May 2000	18.03 ± 0.33	31.89 ± 0.13	13.87	22.93 ± 0.46
Zooplankton tow, Southern ocean, January 1997	20.20 ± 0.04	29.13 ± 0.29	8.93	23.40 ± 0.33
Ulva sp., Half Moon Bay, CA	19.79 ± 0.02	41.38 ± 0.54	21.61	28.61 ± 0.56
Santa Barbara Basin Site 4, Sediment	12.37 ± 0.95	38.14 + 0.24	38.14	21.00 ± 1.19
Portneuf silt–loam topsoil[a], Murtaugh, ID	9.45 ± 0.43	41.39	18.63	11.70 ± 0.43

[a] A full description of this topsoil is presented in Turner et al. (2003).

greatest standard deviations (variability) among sample replicates. These deviations may be attributable to incomplete digestion of the compounds.

3.2. Relationship between reagent oxygen incorporation and compound molecular structure

The amount of reagent oxygen incorporation is well correlated with the oxygen to phosphorus ratio of the compound ($R^2 = 0.921$) such that compounds with more shared oxygen atoms experienced greater incorporation of reagent oxygen (Fig. 2). Furthermore, the observed percent reagent oxygen incorporation is almost identical (1:1 slope, Fig. 3) to the expected percent reagent oxygen incorporation calculated from the O:P ratios and known isotopic composition of the spiked reagent. The expected incorporation is defined as the calculated amount of additional oxygen required to generate orthophosphate from each phosphorus atom present in the molecule (Fig. 3). This clearly suggests that the observed differences in isotopic composition between the spiked and the unspiked digestions are the result of reagent oxygen incorporation and are not the result of isotopic fractionation. Deviations from this 1:1 line are most likely explained by trace impurities in the phosphatic compound used (purity of these products ranged from 95% to 99 %).

The 1:1 relationship between the expected incorporation based on O:P ratio and the observed incorporation is evidence that the $\delta^{18}O_p$ measured is simply a combination of the oxygen isotopic composition of the sample before digestion and reagent oxygen incorporated during digestion. Thus it is possible to calculate the "true" isotopic composition of the sample (the measured isotopic composition minus the incorporated reagent oxygen) by mass balance. The isotopic composition of the sample can be calculated by solving a system of mass balance equations, one for the spiked reagent and one for the unspiked:

$$\delta^{18}O_{\text{spiked measured}=X} \quad \delta^{18}O_{\text{sample}} + (1 - X)\delta^{18}O_{\text{spiked reagent}}$$

$$\delta^{18}O_{\text{unspiked measured}=X} \quad \delta^{18}O_{\text{sample}} + (1 - X)\delta^{18}O_{\text{unspiked reagent}}$$

where X is the proportion of oxygen from the sample, $(1-X)$ is the proportion of oxygen from the reagent, $\delta^{18}O_{\text{spiked reagent}}$ is the isotopic composition of the ^{18}O amended acid (90‰), $\delta^{18}O_{\text{unspiked reagent}}$ is the isotopic composition of the unspiked acid solution (−13.74‰), $\delta^{18}O_{\text{sample}}$ is the "true" isotopic composition for the sample, $\delta^{18}O_{\text{spiked measured}}$ is the oxygen isotopic composition of the sample measured after digestion of the sample in the spiked nitric acid reagent, and $\delta^{18}O_{\text{unspiked measured}}$ is the isotopic composition of the sample after digestion in the unspiked nitric acid reagent. Solving these two equations for the $\delta^{18}O_{\text{sample}}$ yields:

$$\delta^{18}O_{\text{sample}} = \frac{\left(\delta^{18}O_{\text{spiked measured}} * \delta^{18}O_{\text{unspiked reagent}}\right) - \left(\delta^{18}O_{\text{spiked reagent}} * \delta^{18}O_{\text{unspiked measured}}\right)}{\left(\delta^{18}O_{\text{spiked measured}} - \delta^{18}O_{\text{unspiked measured}}\right) - \left(\delta^{18}O_{\text{spiked reagent}} + \delta^{18}O_{\text{unspiked reagent}}\right)}$$

The isotopic compositions for each of the compounds were calculated using the above equation and are given in Table 1.

3.3. Analysis of natural samples

In order to demonstrate the utility of this method for natural samples we analyzed a marine sediment, a silt–loam topsoil, a phytoplankton sample, a zooplankton sample, a saltwater macro-algae (*Ulva* sp.) and a freshwater macro-algae purchased at an aquarium store (*Aidellia* sp.) for $\delta^{18}O_p$. Results of spiked and unspiked samples as well as the corrected isotope values are listed in Table 2. A detailed interpretation of these data is beyond the scope of this paper; however, we have included the results here as a demonstration of the utility of this method for evaluating sources and cycling of phosphate in various ecosystems.

Several interesting observations can be made from this small data set. First, it appears that the $\delta^{18}O_p$ values of macro-algae (*Ulva* sp. and *Aidella* sp.) are significantly higher than marine phytoplankton and zooplankton (consistent with Paytan et al., 2002) and that zooplankton and phytoplankton are very similar in isotopic composition. This is despite collection of the *Ulva* and phytoplankton in Coastal California while the phytoplankton and zooplankton were collected in very different environments (Table 2). Fractionation of phosphate oxygen in organic matter has been attributed to kinetic biological effects in the metabolism of P (Blake et al., 1997; Longinelli et al., 1976) and it would appear that macro-algal P metabolism and associated oxygen isotopic exchange effects are different in both the phytoplankton and zooplankton, resulting in higher $\delta^{18}O_p$ values. The significant difference observed in the $\delta^{18}O_p$ of these samples suggests that this tracer could potentially be used to determine the sources and cycling of phosphate for various species of algae or other trophic levels in aquatic systems.

The phytoplankton sample collected in May 2002 had a $\delta^{18}O_p$ of 22.93‰ which is 3.45‰ higher than the oxygen isotopic composition of dissolved inorganic phosphate in seawater (19.48‰) and is 22.97‰ higher than seawater oxygen isotopic composition (−0.4‰) at the time of collection. This corroborates results from Longinelli et al. (1976) which found that $\delta^{18}O_p$ of soft tissue in marine organisms is approximately 3‰ higher than the isotopic composition of phosphate dissolved in the water column and about 20‰ higher than the $\delta^{18}O_w$. Moreover, the difference between the spiked and the unspiked digestions for the algae and the phytoplankton are greater than that for the zooplankton (Table 2). This is indicative of these organisms respective abilities to store phosphate as polyphosphates or of phosphonates being a constituent of cell organic matter (whereby the large differences between the spiked and unspiked $\delta^{18}O_p$ suggests an O:P ratio less than 4). Similarly, the Santa Barbara Basin sediment and the loam topsoil both show large differences between the digestions with spiked and unspiked acids which indicates that these samples have a substantial amount of phosphatic compounds with low O:P ratios, possibly phosphonates (Clark et al., 1999).

Another interesting finding was the relatively good agreement between organic matter in marine sediment in the Santa Barbara Basin and phytoplankton samples taken from Monterey Bay. Both sites are within the California Current and therefore, the seawater at these two sites should have similar temperatures, chemistry and biological communities. This suggests that the $\delta^{18}O_p$ signature (P cycling and source) incorporated into living organisms may be preserved in the sediment and could potentially be used for paleoceanographic studies.

4. Summary

Our results suggest that the magnitude of reagent oxygen incorporated into samples during digestion of solid compounds for purification and precipitation as silver phosphate for oxygen isotopic analyses is directly related to the molecular structure of the specific phosphatic compound. In addition, there is no apparent isotopic exchange or fractionation involved during the digestion step, simply incorporation of the reagent oxygen during hydrolysis of the compounds and conversion to orthophosphate. Because of the potential for reagent oxygen incorporation, natural samples that are not pure orthophosphate must be analyzed in replicate (once with an unspiked acid digestion and again with a spiked acid digestion). Using the results from these two digestions, the original isotopic composition of the material may be calculated by mass balance to correct for the incorporation of reagent oxygen during digestion. A preliminary investigation of the oxygen isotopic composition of phosphate in organic matter demonstrates the potential of this method for understanding sources and cycling of P in marine organic matter, sediments and soils and as a paleoceanographic tracer.

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