

Characterizing the Oxygen Isotopic Composition of Phosphate Sources to Aquatic Ecosystems

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The oxygen isotopic composition of dissolved inorganic phosphate ($\delta^{18}\text{O}_p$) in many aquatic ecosystems is not in isotopic equilibrium with ambient water and, therefore, may reflect the source $\delta^{18}\text{O}_p$. Identification of phosphate sources to water bodies is critical for designing best management practices for phosphate load reduction to control eutrophication. In order for $\delta^{18}\text{O}_p$ to be a useful tool for source tracking, the $\delta^{18}\text{O}_p$ of phosphate sources must be distinguishable from one another; however, the $\delta^{18}\text{O}_p$ of potential sources has not been well characterized. We measured the $\delta^{18}\text{O}_p$ of a variety of known phosphate sources, including fertilizers, semiprocessed phosphorite ore, particulate aerosols, detergents, leachates of vegetation, soil, animal feces, and wastewater treatment plant effluent. We found a considerable range of $\delta^{18}\text{O}_p$ values (from +8.4 to +24.9‰) for the various sources, and statistically significant differences were found between several of the source types. $\delta^{18}\text{O}_p$ measured in three different fresh water systems was generally not in equilibrium with ambient water. Although there is overlap in $\delta^{18}\text{O}_p$ values among the groups of samples, our results indicate that some sources are isotopically distinct and $\delta^{18}\text{O}_p$ can be used for identifying phosphate sources to aquatic systems.

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1. Introduction

Phosphorus (P) is an essential macronutrient in aquatic ecosystems, and eutrophication due to anthropogenic inputs of phosphorus has been observed in many places throughout the world (1, 2). Stable isotope analysis of carbon, nitrogen, and sulfur has been used successfully for source identification in numerous studies (3), but P has only one stable isotope, making this direct approach impossible. However, P in nature is strongly bound to oxygen (e.g., orthophosphate, polyphosphate, etc.), and oxygen has three stable isotopes. Advances in stable isotope mass spectrometry, dissolved inorganic phosphate (DIP) extraction, and purification techniques have made it possible to analyze the isotopic composition of the oxygen atoms bound to the phosphorus (P) in phosphate (PO_4) (4–7). The primary goal of this study is to characterize the oxygen isotopic composition of phosphate ($\delta^{18}\text{O}_p$) of a wide variety of sources that may contribute to the P load to aquatic ecosystems and to demonstrate that dissolved phosphate in fresh water systems is often not in isotopic equilibrium with ambient water, suggesting that $\delta^{18}\text{O}_p$ analysis may be useful for identifying and tracing phosphate sources.

Identifying point and nonpoint nutrient sources is critical to understanding ecosystem health and has important implications for ecosystem management practices, including industry regulation and allocation of funding for monitoring and restoration efforts (1, 8). P sources can be separated into point sources, such as sewage discharge sites, and nonpoint sources like atmospheric deposition and agricultural runoff. Orthophosphate (PO_4 , hereafter referred to as phosphate) is both the most common and most biologically available form of P and, thus, the most frequently regulated and monitored (9). Tracking phosphate as it travels and mixes in waterways is particularly important for identifying the relative importance of various phosphate sources to large aquatic systems. For example, it may be possible to trace phosphate input from a particular river into a lake, the coastal ocean, or another river if it has a distinct $\delta^{18}\text{O}_p$ from that of other local phosphate sources. This information can then be used to determine the importance of specific inputs within a drainage basin.

Although extensive data are available on the oxygen isotopic composition of phosphate found in mineral forms such as apatite in teeth, bones, and rocks (10–12), very little published information is available regarding the oxygen isotopic signatures of other sources of dissolved phosphate to water bodies. Recently, $\delta^{18}\text{O}_p$ has been used for identifying phosphate from wastewater treatment plant effluent and fertilizer inputs to aquatic ecosystems (7, 13, 14); however, these studies only measured a very small number of potential phosphate sources specific to the local areas.

$\delta^{18}\text{O}_p$ in any water sample is determined by both the $\delta^{18}\text{O}_p$ of the source phosphate and the amount of biological cycling that has occurred within the water (breaking the P–O bonds and resetting the isotopic signature primarily to a temperature-dependent equilibrium value (15)), although kinetic isotope effects have also been described (16–18). The P–O bond is highly resistant to inorganic hydrolysis and requires enzymatic biological mediation in order to exchange oxygen with water under standard environmental conditions (15–17). Biological cycling of P-compounds will work to erase the source signature and, if extensive intercellular cycling occurs, the $\delta^{18}\text{O}_p$ will reflect equilibrium rather than source values (14, 16, 19–21). Chemical processes (adsorption, dissolution) may also result in isotopic fractionation, and additional

research is needed to identify systems in which these processes produce measurable changes in $\delta^{18}\text{O}_p$.

The equilibrium $\delta^{18}\text{O}_p$ can be calculated using the empirically derived equation of Longinelli and Nuti (1973) (22):

$$T (^{\circ}\text{C}) = 111.4 - 4.3(\delta^{18}\text{O}_p - \delta^{18}\text{O}_w)$$

where the $\delta^{18}\text{O}_p$ is the oxygen isotope ratio of the dissolved phosphate, T is the water temperature, and $\delta^{18}\text{O}_w$ is the oxygen isotopic composition of the water in which the phosphate equilibrated.

Studies in open-ocean waters generally found that $\delta^{18}\text{O}_p$ values were near the expected temperature-dependent equilibrium, indicating almost complete biologically mediated equilibration with the surrounding water (20, 21). In contrast, recent studies in several rivers, lakes, and estuaries have found significant differences between the measured $\delta^{18}\text{O}_p$ and the expected equilibrium $\delta^{18}\text{O}_p$, indicating that at least some of the source signature is still retained in these waters (7, 13, 14, 23). However, so little data exist regarding the $\delta^{18}\text{O}_p$ of various sources that interpreting differences in $\delta^{18}\text{O}_p$ in receiving water bodies in relation to potential phosphate sources is currently difficult.

The objective of this study was to examine a set of known phosphate sources in order to determine whether measurable differences in the $\delta^{18}\text{O}_p$ of these sources exist. We also compiled existing published data on $\delta^{18}\text{O}_p$ of potential phosphate sources in order to present the most complete data set available. These data will provide a foundation for utilizing and interpreting $\delta^{18}\text{O}_p$ in aquatic systems where the $\delta^{18}\text{O}_p$ is not in isotopic equilibrium with the surrounding water and, thus, may reflect source signatures.

2. Experimental Procedures

A total of 65 samples representative of various potential phosphate sources were analyzed for this study, of which 12 were wastewater treatment plant (WWTP) water samples and 53 were solid or semisolid materials (fertilizers, detergents, etc). An additional 40 water samples from various fresh water environments consisting of rivers, tributaries, and groundwater were analyzed to assess their range of $\delta^{18}\text{O}_p$ and to determine if the $\delta^{18}\text{O}_p$ was in equilibrium with the environmental water. The WWTP samples were collected from facilities in California, Idaho, and Ohio. Surface water samples were collected from rivers and streams in the San Joaquin Valley and Lake Tahoe regions of California and from several tributaries entering Lake Erie from Michigan and Ohio. Groundwater samples were collected from two depths of a nested well group on the eastside of the San Joaquin River. Chemical fertilizers, household detergents, and toothpaste were all obtained from retail sources. Fertilizer ore from various processing stages was obtained from a processing plant in Israel. Aerosol samples were obtained from three separate collection deployments at Lake Kinneret, Israel, and a calcareous soil (for leachate analysis) was collected from Arka, Israel. Vegetation samples were collected from nine different plant species near Lake Tahoe, California. For each type of plant, both live and dead leaves (or needles) were collected directly from the plant, and a fallen plant litter sample was collected from beneath the plant. Fresh dog and goose feces samples were also collected near Lake Tahoe.

Phosphate in all samples was converted to silver phosphate and analyzed for $\delta^{18}\text{O}_p$ using thermal decomposition on an elemental analyzer coupled to a mass spectrometer at the Menlo Park U.S. Geological Survey Stable Isotope Laboratory. Water samples were processed and analyzed following the method of McLaughlin et al. (5). Briefly, between 2 and 40 L of water (depending on phosphate concentration)

was collected, and phosphate was quantitatively stripped from the water using magnesium-induced coprecipitation (MagIC) (24, 25). The samples were then purified using several precipitation steps and ion exchange and converted into silver phosphate (Ag_3PO_4). In order to calculate the predicted isotopic equilibrium value of $\delta^{18}\text{O}_p$ for each water sample, the temperature and $\delta^{18}\text{O}$ of water ($\delta^{18}\text{O}_w$) were measured for all WWTP, river, and groundwater samples. The temperature was measured in situ using a hand-held YSI probe, and $\delta^{18}\text{O}_w$ was measured at the Menlo Park U.S. Geological Survey Stable Isotope Laboratory by laser spectroscopy on a Los Gatos Research DLT-100 Liquid-Water Isotope analyzer, using a modification of the method described in Lis et al. (26).

$\delta^{18}\text{O}_p$ was measured on the water-soluble phosphate fraction in the aerosol, soil, vegetation, and feces samples. Aerosol samples were collected using a wet deposition method, in which open buckets of laboratory pure water containing sodium azide (to prevent biological activity) were deployed, and aerosol material was allowed to accumulate until the dissolved phosphate concentration was sufficient for $\delta^{18}\text{O}_p$ analysis. Vegetation, soil, and feces samples were dried and crushed, and 10 g of sample was added to about 200 mL of Milli-Q water and shaken for 3 h. The sample water was filtered to remove all particulate matter, and the phosphate in the filtered water was processed to silver phosphate following the method of McLaughlin et al. (5). Detergent and toothpaste samples were dissolved completely in distilled deionized water prior to sample processing. The fertilizers and semiprocessed fertilizer ores (including crushed phosphorite source rock) were dissolved with 8 N nitric acid. The semiprocessed fertilizer ores were processed to silver phosphate using a slightly different purification and precipitation technique following the method of Colman (7), modified from the method of O'Neil et al. (27). No differences in final $\delta^{18}\text{O}_p$ values were found between the two processing techniques using multiple samples and standards.

A subset of WWTP effluent and river water samples was also processed using the McLaughlin et al. (6) method which allows for the identification of reagent oxygen incorporation into the sample if significant amounts of certain organic phosphorus compounds are converted to orthophosphate during acid digestion. Although McLaughlin et al. (14) found no evidence of reagent oxygen incorporation during the processing of seawater samples, this method had not previously been tested on WWTP effluent or river water samples. No reagent incorporation was detected in these samples, which was expected, since the MagIC method preferentially concentrates inorganic phosphate while leaving behind most organic P (24, 25) and inorganic phosphate was the dominant form of P in the samples.

3. Results and Discussion

Comparison of Different Sources. $\delta^{18}\text{O}_p$ of potential phosphate sources and fresh water (river and groundwater) samples spanned a wide range of isotopic compositions, and statistically significant differences were found between the $\delta^{18}\text{O}_p$ distributions of several groups of samples. $\delta^{18}\text{O}_p$ ranged from +13.3 to +24.9‰ in the solid and semisolid samples, from +14.2 to +23.1‰ in the leachate samples (soil, vegetation, and feces), from +8.4 to +14.2‰ in the WWTP samples, and from +9.2 to +16.4‰ in the fresh water samples (Figure 1). $\delta^{18}\text{O}_p$ values for individual samples are provided in tabulated form in the Supporting Information.

Although there was overlap in the $\delta^{18}\text{O}_p$ among the various groups of samples, the broad range of $\delta^{18}\text{O}_p$ values measured in this study suggests that this technique may be useful for distinguishing between individual phosphate sources and between certain general types of phosphate sources. The $\delta^{18}\text{O}_p$ values of the different source types were compared

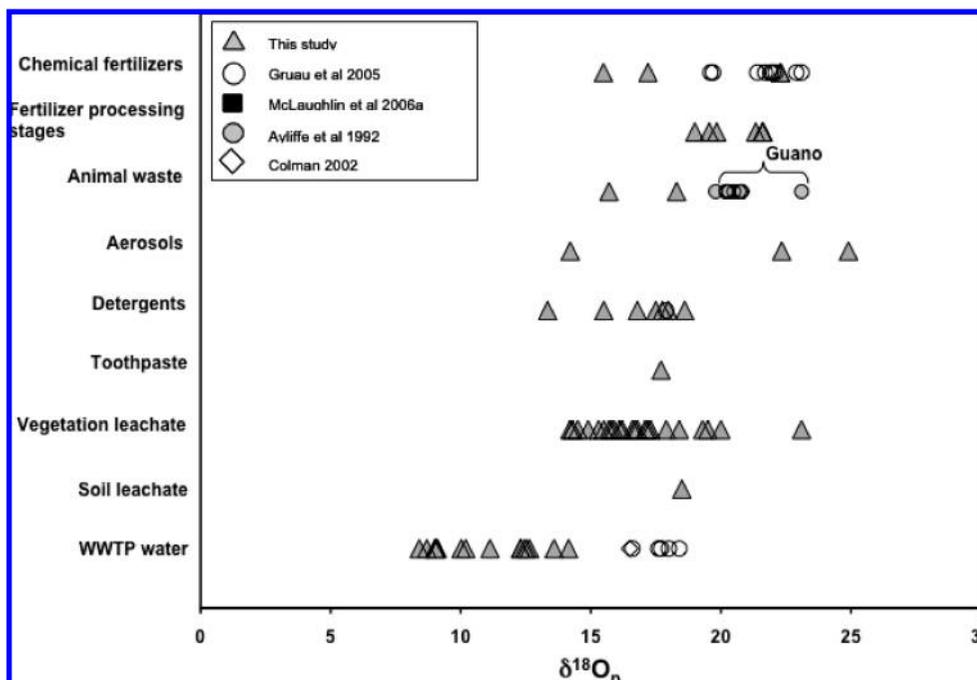


FIGURE 1. $\delta^{18}\text{O}_p$ for potential phosphate sources analyzed for this study compared to similar samples from other studies.

TABLE 1. Mean $\delta^{18}\text{O}_p$ Values for Types of Potential Phosphate Sources and Results of Statistical Comparisons Using Student's *t*-Test^a

sample group	<i>n</i>	mean $\delta^{18}\text{O}_p$ (this study only)		<i>n</i>	mean $\delta^{18}\text{O}_p$ (all studies)			
chemical fertilizers	4	+19.3		10	+20.8			
fertilizer ore	8	+20.1		8	+20.1 ^b			
all fertilizers	12	+19.8	A	18	+20.2	A		
aerosols	3	+20.5	A	3	+20.5 ^b	A	B	
detergents	7	+16.8		8	+16.9		B	C
vegetation	27	+16.9		27	+16.9 ^b			C
WWTP water ^c	11	+11.2		17	+13.4			D
WWTP water (other studies only)				6	+17.5		B	C
guano				10	+20.7	A		

^a Groups where no statistical difference between the means was found ($p > 0.05$) share the same letter designation, while groups that do not share a letter designation were found to have statistically different means ($p < 0.05$). All fertilizers were grouped together for statistical comparisons. ^b Indicates that no data from published studies were added to these categories; therefore, the number of samples and the mean value remain the same. ^c The primary effluent sample from the Bay Area WWTP was not included in the statistical analysis, because it represented incoming sewage rather than processed and/or outgoing water.

using student's *t* tests. No significant differences were found between the fertilizer and ore samples, so these samples were grouped together as fertilizers for comparison to other types of samples. Mean $\delta^{18}\text{O}_p$ for the sample types and statistical differences between types are presented in Table 1. The $\delta^{18}\text{O}_p$ of fertilizers was significantly higher than the $\delta^{18}\text{O}_p$ of all other sample types ($p < 0.05$), except for aerosols. The $\delta^{18}\text{O}_p$ of WWTP samples was significantly lower than the $\delta^{18}\text{O}_p$ of all other samples types ($p < 0.05$). We have combined the data from this study with previously published $\delta^{18}\text{O}_p$ values of potential phosphate sources in order to provide the most complete data set possible for $\delta^{18}\text{O}_p$ values for potential phosphate sources (Figure 1). Below, we discuss the $\delta^{18}\text{O}_p$ characteristics of different types of phosphate sources, compare the $\delta^{18}\text{O}_p$ values of different sources, and discuss the application of $\delta^{18}\text{O}_p$ as a phosphate source tracer in aquatic systems.

Fertilizers. A wide variety of fertilizers is available for both commercial and noncommercial applications, and fertilizer-derived phosphate may enter aquatic systems through either overland runoff or groundwater infiltration

(1, 8). Chemical fertilizers are derived from the mining and processing of phosphorites which are primarily from marine phosphorite deposits. In this study, the $\delta^{18}\text{O}_p$ of the chemical fertilizers ranged from +15.5 to +22.3‰. $\delta^{18}\text{O}_p$ of fertilizer ore from Israel in various stages of processing (from phosphorite ore to commercial fertilizer) clustered between +18.2 and +21.6‰, which is within the range of previously reported values for phosphorites in this region (10, 12). No consistent differences were found in $\delta^{18}\text{O}_p$ through the different degrees of processing, supporting the suggestion of Gruau et al. (28) that the $\delta^{18}\text{O}_p$ of chemical fertilizers is mainly controlled by the $\delta^{18}\text{O}_p$ of the source material and is not measurably altered during manufacturing processes. However, high temperatures were not used in the processing of the ores analyzed for this study and, as shown by Liang and Blake (29), solid inorganic phosphate may undergo significant isotopic exchange with atmospheric oxygen at high temperatures. Further research is needed to determine if certain fertilizer manufacturing processes significantly alter the $\delta^{18}\text{O}_p$ in relation to the source material.

The results from this study are similar to the small number

of $\delta^{18}\text{O}_p$ fertilizer values available in the published literature (Figure 1). Gruau et al. (28) found a range from +19.6 to +23.1‰ for three different types of chemical fertilizers used in an agricultural region in France. McLaughlin et al. (13) reported $\delta^{18}\text{O}_p$ values of +19.4 and +20.5‰ for two types of chemical fertilizers used in the Elkhorn Slough area, California. The inclusion of the published fertilizer $\delta^{18}\text{O}_p$ values in the statistical calculations only changed the mean fertilizer value slightly and did not change the distribution of statistically significant differences between the groups of samples (Table 1).

In this study, we report the $\delta^{18}\text{O}_p$ of water leachates for dog (+15.7‰) and goose (+18.3‰) feces; although they may not represent the original bulk $\delta^{18}\text{O}_p$ of the materials, they do represent the $\delta^{18}\text{O}_p$ of phosphate that would be released to the environment by water leaching. While the feces may include organic phosphorus compounds, treatment of the leachate with MagIC preferentially concentrated the phosphate in these samples (24, 25). No published $\delta^{18}\text{O}_p$ values exist for any type of pure animal waste other than seabird guano. Ayliffe et al. (31) reported a $\delta^{18}\text{O}_p$ range of +19.8 to +23.1‰ for modern guano from several different species of seabirds on six different islands. Seabird guano is mined as a source of fertilizer, and the mean of the $\delta^{18}\text{O}_p$ values reported by Ayliffe et al. (31) was not statistically different from the mean value of all fertilizers (this study and published data). Ayliffe et al. (31) concluded that the $\delta^{18}\text{O}_p$ of the guano was controlled primarily by sea surface temperature where the birds were feeding and the $\delta^{18}\text{O}$ of seawater.

Unprocessed animal waste and organic fertilizers may contribute phosphate to aquatic systems in areas where there are either large natural or managed animal populations or where organic fertilizers are applied. Unfortunately, determining the true bulk $\delta^{18}\text{O}_p$ value of organic compounds is difficult because exchange with reagent oxygen caused by acid hydrolysis during sample dissolution and possible concurrent water isotope exchange can alter the measured $\delta^{18}\text{O}_p$ (29, 30). Characterization of these potential sources awaits future method development for the analysis of organic phosphorus compounds.

Soil and Vegetation Leachates. Vegetation leachate samples ranged from +14.2 to +23.1‰, with an average $\delta^{18}\text{O}_p$ of $+16.9 \pm 2.0$ ‰, which was significantly different from the fertilizers and WWTP samples (this study only). There were measurable differences in $\delta^{18}\text{O}_p$ between at least two of the different stages for every species sampled, although there were no consistent trends between live, dead, and decaying vegetation. This indicates that there was variation in the $\delta^{18}\text{O}_p$ of the leaves throughout individual plants and/or that processes such as leaching and microbial decay were causing changes to the phosphate composition within the plant material after leaf death. Differences in $\delta^{18}\text{O}_p$ between stages ranged from 0.1‰ (live vs decayed cedar) to 7.6‰ (live vs decayed red fir). The soil leachate from Arka, Israel, had a value of +18.5‰, which matched the expected equilibrium value for phosphate in contact with irrigation water in that region. However, this value is also within the values found for fertilizers and those reported for phosphorites in the region, and research is currently underway to investigate the controls on soil leachate and soil water $\delta^{18}\text{O}_p$.

Detergents. Among the detergent samples measured in this study ($\delta^{18}\text{O}_p$ ranging from +13.3 to +18.6‰), no trends were found associated with either the brand of detergent or the form (liquid, gel, or solid). A $\delta^{18}\text{O}_p$ value of +17.7‰ was found for one toothpaste brand. Gruau et al. (28) reported a $\delta^{18}\text{O}_p$ value of +17.9‰ for a detergent phosphate builder, which falls within the range measured in this study. Based on the data we have so far, the detergent isotopic signature is slightly lower than that of fertilizer phosphate, although there is overlap between the two groups. The phosphate in

many products, including detergents and toothpastes, originates from the same worldwide deposits of phosphate rocks mined for fertilizers, although the total amount used in these products is small compared to the amount used for fertilizers. Differences in $\delta^{18}\text{O}_p$ between fertilizers and detergents most likely reflect differences in the source rocks used to produce the particular brands measured in this study and previous studies. Therefore, it is unlikely that there are significant differences between the $\delta^{18}\text{O}_p$ of fertilizers and detergents at the global scale but quite possible that there will be significant differences at the watershed or regional scales. In areas where detergents are a major source of phosphate to sewage, we would expect similar signatures in the solution entering a WWTP, although the signature might be reset prior to effluent discharge if extensive microbial cycling occurs during the treatment process (see discussion of WWTPs).

Waste Water Treatment Plants. The $\delta^{18}\text{O}_p$ of all WWTP samples (including treatment stages) ranged from +8.4 to +14.2‰. Some variation was found between the two different sampling events at the Bay Area WWTP. In October 2005, final effluent at the plant ranged from +8.4 to +11.1‰ ($n = 3$) during the course of a 48 h sampling period, while in January 2006, the final effluent $\delta^{18}\text{O}_p$ was +12.3‰. In January 2006, the $\delta^{18}\text{O}_p$ for five treatment stages (including final effluent) ranged from +12.3 to +12.6‰ and was indistinguishable from each other within sampling and analytical error. The water in incoming primary effluent at that time had a different $\delta^{18}\text{O}_p$ of +14.2‰. The sample from the Ohio WWTP and most of the Bay Area WWTP samples were close (<1‰ offset) to expected isotopic equilibrium with the treatment plant water (Figure 2 and Table S3 in the Supporting Information) with the exception of two Bay Area WWTP samples. Of these two samples, one final effluent sample from 10/29/2005 was 1.5‰ below expected equilibrium, while final effluent samples taken 12 h before and 12 h after were only 0.1‰ above and 0.6‰ below expected equilibrium, respectively. The primary effluent sample (1/11/06) was 1.9‰ below expected equilibrium, in contrast to samples from all other treatment stages collected on the same day, which were within 0.5‰ of expected equilibrium. The samples from the Idaho WWTP were both approximately 4‰ higher than expected equilibrium value. These results demonstrate that both measured $\delta^{18}\text{O}_p$ and offset from equilibrium can vary quite rapidly within a single treatment plant and are not consistent across WWTPs. This may be due to a combination of changes during treatment, or changes in source water, temperature, and residence time within the treatment plant.

WWTP effluent was the only sample group for which the range of $\delta^{18}\text{O}_p$ measured in this study were significantly different ($p < 0.05$) from the range of previously published values. The $\delta^{18}\text{O}_p$ of the WWTP effluent samples in this study ranged from +8.4 to +13.6‰ ($n = 7$, final effluent only), while Gruau et al. (28) reported a range of +16.6 to +18‰ for samples from three WWTPs in France ($n = 5$) and Colman (7) reported a value of +16.5‰ for effluent from a WWTP in Connecticut. Gruau et al. (28) reported that the WWTP samples from France were at or very near the estimated equilibrium values, while the Connecticut WWTP sample was not in isotopic equilibrium (7). The mean of all published WWTP effluent samples was statistically different ($p < 0.05$) from the mean values of the fertilizers (all values, this study and published data) and seabird guano but was not significantly different from the mean $\delta^{18}\text{O}_p$ of any other sample group. The variability in $\delta^{18}\text{O}_p$ values between different WWTPs is not surprising considering that different phosphate sources may be dominant in incoming sewage in different regions, and it is possible that different treatment processes may influence if and to what degree the original $\delta^{18}\text{O}_p$ values are reset during microbial processing within treatment plants.

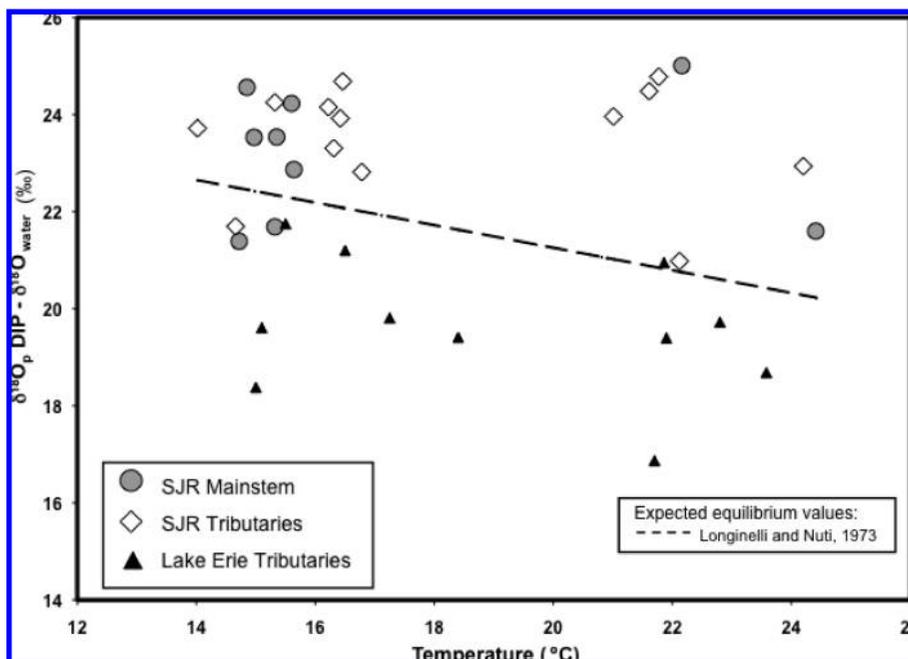


FIGURE 2. $\delta^{18}\text{O}_p - \delta^{18}\text{O}_w$ as a function of temperature for water samples collected from wastewater treatment plants. The use of $\delta^{18}\text{O}_p - \delta^{18}\text{O}_w$ for the y axis allows the equilibrium values to be plotted along a linear relationship with temperature.

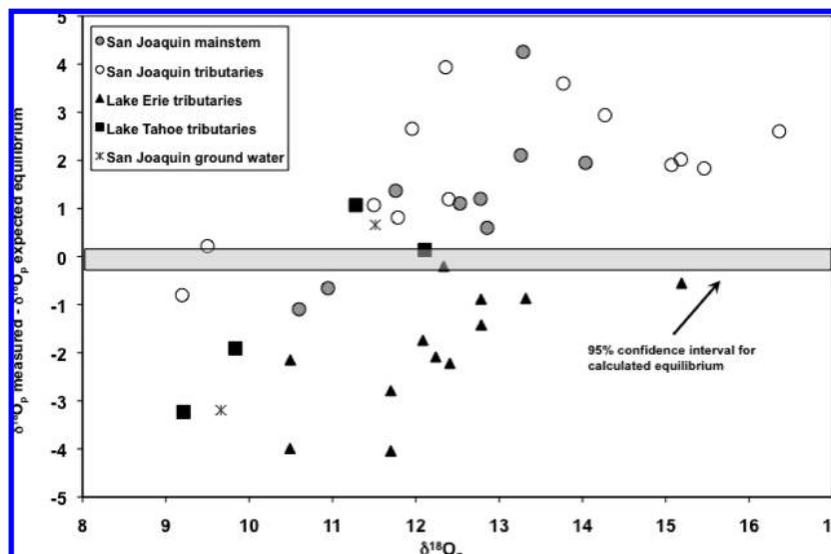


FIGURE 3. Offset from isotopic equilibrium of $\delta^{18}\text{O}_p$ for various river and groundwater samples.

If little microbial cycling of the phosphate takes place, then the $\delta^{18}\text{O}_p$ of the final effluent should directly reflect the mixed $\delta^{18}\text{O}_p$ values of the incoming phosphate sources to each WWTP. If, however, intracellular microbial cycling either partially or completely shifts the $\delta^{18}\text{O}_p$ values toward isotopic equilibrium, the $\delta^{18}\text{O}_p$ of the final effluent will be determined entirely by the water temperature and water isotopic composition in the treatment plant ponds, which may vary considerably depending upon the geographic region and season. In WWTPs with microbial reaction ponds that are not temperature controlled, there may also be seasonal shifts between source signatures and microbial processing as the dominant control on the $\delta^{18}\text{O}_p$ of the final effluent, since ambient temperature can have large effects on microbial processing rates and on water isotope values due to evaporation.

These results indicate that the $\delta^{18}\text{O}_p$ of WWTP effluent can vary considerably both within a single WWTP and between different WWTPs and sometimes may not be in isotopic equilibrium with the WWTP water. Therefore, the

$\delta^{18}\text{O}_p$ value of WWTP effluent must be directly measured for each study area where $\delta^{18}\text{O}_p$ measurements are being used to track source inputs. Additionally, it appears that the $\delta^{18}\text{O}_p$ of WWTP effluent may be distinct from the $\delta^{18}\text{O}_p$ of other phosphate sources but that these distinctions are dependent on the effluent $\delta^{18}\text{O}_p$ values from each specific WWTP.

River and Groundwater Samples. The $\delta^{18}\text{O}_p$ of all river water samples ranged from +9.2 to +16.4‰ (Figure 3). $\delta^{18}\text{O}_p$ ranged from +10.5 to +15.2‰ in the Lake Erie tributaries, from +9.2 to +16.4‰ in the San Joaquin River and tributaries, and from +9.2 to +12.2‰ in the Lake Tahoe tributaries. The mean $\delta^{18}\text{O}_p$ values of the Lake Erie tributary and the San Joaquin River and tributary samples were not statistically different, while the mean $\delta^{18}\text{O}_p$ value of the Lake Tahoe tributary samples was lower than the Lake Erie and San Joaquin River tributaries ($p < 0.05$). However, only four Lake Tahoe tributaries were sampled, and the apparent difference between the geographical locations may be due to the small sample set. Almost all of the fresh water samples collected were not within the expected equilibrium range for the

measured temperature and $\delta^{18}\text{O}_w$ (Figure 3). The $\delta^{18}\text{O}_p$ of the Lake Erie tributaries ranged from 4.0‰ lower than the expected isotopic equilibrium to 1.0‰ higher, with two samples falling within range of the expected equilibrium. San Joaquin River and tributary samples were between 1.0‰ lower and 4.3‰ higher than expected isotopic equilibrium, with only one sample within the expected equilibrium range. The Lake Tahoe tributary samples were between 3.2‰ lower and 1.1‰ higher, with one sample at the expected equilibrium value. Although the mean $\delta^{18}\text{O}_p$ values for the Lake Erie and San Joaquin sets of river samples were not statistically different, there was a significant ($p < 0.05$) difference in the offsets from expected isotopic equilibrium between the two sample sets. The majority of Lake Erie tributary samples had lower $\delta^{18}\text{O}_p$ than expected equilibrium (mean = 1.4‰ lower than expected equilibrium), while the majority of San Joaquin River and tributary samples had higher $\delta^{18}\text{O}_p$ than expected equilibrium (mean = 1.6‰ higher than expected equilibrium). Additional research is needed to better understand the mechanisms controlling $\delta^{18}\text{O}_p$ in fresh water systems, which may include a combination of source mixing, biological equilibrium and kinetic isotope effects, and biological processes such as precipitation and dissolution.

$\delta^{18}\text{O}_p$ in both San Joaquin groundwater samples was not in equilibrium with the ambient water. $\delta^{18}\text{O}_p$ was +11.5‰ in the shallow well and +9.7‰ in the deep well. The two well depths draw from chemically distinct water, and therefore, it is not surprising that the differences in $\delta^{18}\text{O}_p$ between the well depths did not go in the direction of expected equilibrium on the basis of differences in temperature and $\delta^{18}\text{O}_w$. In contrast, Blake et al. (32) found a positive correlation between $\delta^{18}\text{O}_p$ and $\delta^{18}\text{O}_w$ in four samples from the Cape Cod aquifer, demonstrating the influence of microbial activity on $\delta^{18}\text{O}_p$ within the aquifer, although the samples were also not at the expected equilibrium. Groundwater in both areas is known to have anthropogenic impacts to water quality, and the lack of complete equilibration suggests that $\delta^{18}\text{O}_p$ could be useful for studies of both microbial cycling of anthropogenic phosphate inputs within an aquifer and tracing groundwater phosphate as it discharges into surface waters.

Rivers can be an important source of phosphate into receiving water bodies such as larger rivers, lakes, and estuaries. Identifying and quantifying phosphate inputs from rivers to these receiving bodies are important for eutrophication research and for many regulatory activities. The results of our river studies in California and the Lake Erie area (Michigan and Ohio) demonstrate two important factors for using $\delta^{18}\text{O}_p$ as a source tracer in aquatic ecosystems. $\delta^{18}\text{O}_p$ in the majority of water samples was not in isotopic equilibrium, indicating that source signatures were not being rapidly overprinted by equilibrium signatures within the river. Also, in several instances, certain tributaries had $\delta^{18}\text{O}_p$ values that were distinct from those of other tributaries, indicating that the contribution of phosphate from specific tributaries to the receiving water body could be identified using this isotope tracing approach. The $\delta^{18}\text{O}_p$ in tributaries of the San Joaquin River spanned a relatively wide range of values, from +9.2 to +16.4‰, suggesting that phosphate inputs from the different tributaries to the mainstem river could be either qualitatively or quantitatively determined. $\delta^{18}\text{O}_p$ measurements alone could be used to determine whether or not phosphate from a specific tributary was a major source of phosphate to a receiving water body, or the $\delta^{18}\text{O}_p$ measurements could be combined with concentration, flow, and load calculations in order to construct a quantitative isotopic mass balance model for a chosen system.

This study greatly expands the amount of data available on $\delta^{18}\text{O}_p$ of various phosphate sources to aquatic ecosystems.

A broad range of $\delta^{18}\text{O}_p$ values was found in the phosphate samples, and statistically significant differences were found between the mean $\delta^{18}\text{O}_p$ values of certain sample groups, suggesting that in many systems this approach may be useful for distinguishing contributions from various phosphate sources. We also show that for the majority of rivers sampled in this study, phosphate was not in isotopic equilibrium with the surrounding water. Our results show that $\delta^{18}\text{O}_p$ has a strong potential for identifying and understanding phosphate source inputs in many different aquatic systems and that this tool could be used to construct isotope mass balance models and to constrain phosphate contribution of multiple water sources to a given system.

Additional research is necessary to increase the amount of $\delta^{18}\text{O}_p$ data available for different potential phosphate sources including groundwater and septic leakage inputs, the water leachable phosphate from various soil and sediment types, and phosphate from animal wastes and animal farming operations (including hatcheries and other aquaculture facilities). As the ranges of $\delta^{18}\text{O}_p$ values for various source groups become better characterized, it will be easier to apply this isotopic tool in many locations. The results of our study strongly suggest that $\delta^{18}\text{O}_p$ source signatures are not completely overprinted by biological cycling in many fresh water systems, and therefore, $\delta^{18}\text{O}_p$ measurements have great potential for tracing and partitioning phosphate inputs in a wide range of locations as long as these signatures are unique and well-characterized.

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Supporting Information Available

Four tables listing individual $\delta^{18}\text{O}_p$ results for all samples from this study. Table S1 lists $\delta^{18}\text{O}_p$ of solid, semisolid, and leachate potential phosphate sources. Table S2 lists $\delta^{18}\text{O}_p$ of water-soluble phosphate from vegetation leachates. Table S3 lists $\delta^{18}\text{O}_p$ of WWTP samples. Table S4 lists $\delta^{18}\text{O}_p$ values for various fresh water samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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