

# Oxygen Isotope Analysis of Phosphate by Electrospray Orbitrap Mass Spectrometry for Assessing the Microbial Metabolism in the Environment

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Understanding the impact of human activities on the metabolic state of soil and aquatic environments is of paramount importance to implement measures for maintaining ecosystem services and sustainable access to food, water, and energy. Variations of natural abundance oxygen isotope ratios in phosphate provide a new avenue for holistic assessments of metabolic activity in the environmental microbiome given the crucial importance of phosphoryl transfer reactions in fundamental biological processes. Here, we evaluated recently introduced instrumental approaches to oxygen isotope ratio measurements of oxyanions by electrospray ionisation (ESI) Orbitrap high-resolution mass spectrometry (Neubauer et al., *Anal. Chem.* 2020, 92, 4, 3077–3085). Specifically, we examined precision and accuracy of <sup>18</sup>O/<sup>16</sup>O ratio measurements in phosphate standards and tested different treatment protocols for quantifying  $\delta^{18}\text{O}$  of phosphate in environmental samples using a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, Germany). <sup>18</sup>O/<sup>16</sup>O ratios were obtained from quantifying signal intensities of two di-hydrogenphosphate isotopologue anions ( $\text{H}_2\text{P}^{16}\text{O}_4^-$  and  $\text{H}_2\text{P}^{16}\text{O}_3^{18}\text{O}^-$ ). Standards consisted of a series of eight diluted phosphoric acid solutions with known  $\delta^{18}\text{O}$  from -350‰ to +300‰ out of which, five covered the known environmental range between 0‰ and 60‰ (Tamburini et al., *Soil Sci. Soc. Am. J.* 2014, 78, 38–46).

Current results indicate that ESI-Orbitrap <sup>18</sup>O/<sup>16</sup>O ratio measurement matched those obtained by conventional elemental analysis/isotope ratio mass spectrometry within (99 ± 1)% over the studied range of  $\delta^{18}\text{O}$  values. Typical direct infusion measurements over a 15-min period of continuous signal acquisition yielded relative standard errors between ±1.2‰ to ±1.8‰ for  $\delta^{18}\text{O}$  of phosphate. However, variability between measurement campaigns distributed over several months revealed deviations of up to 10‰ for identical phosphate samples and identical instrumental settings. This observation points to yet unknown factors influencing the accuracy of <sup>18</sup>O/<sup>16</sup>O ratio measurements. Sample treatment protocols explored for quantitative transfer of phosphate ions from aqueous matrices to methanolic solutions used for isotopic analyses were based on

established workflows for phosphate purification and enrichment. The ESI-Orbitrap approach to  $\delta^{18}\text{O}$  in phosphate could potentially simplify sample preparation and thus increase sample throughput, thereby providing the circumstances for in-depth studies of changes in the microbial metabolism in the environment.