

## **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. In this project, we explore variations of natural abundance oxygen isotope ratios in phosphate as a new proxy for the holistic assessment of metabolic activity. Given the crucial importance of phosphoryl transfer reactions in fundamental biological processes, we hypothesise that changes in natural abundance  $^{18}\text{O}/^{16}\text{O}$  ratios in phosphate also reflect shifts in the metabolic state of the environmental microbiome as a response to anthropogenic impact.

To make such oxygen isotope ratio measurements available for metabolic studies, we evaluated the applicability of recently introduced instrumental approaches of oxyanion isotope analysis by electrospray ionisation (ESI) Orbitrap high-resolution mass spectrometry for phosphate. To that end, we characterised important ionisation and Orbitrap parameters for precise and accurate  $^{18}\text{O}/^{16}\text{O}$  ratio measurements of phosphate in solutions of variable pH and different sulfate and methanol concentrations through automated flow injection analyses. Based on optimised instrument parameters, we subsequently tested different protocols for the extraction of orthophosphate from aqueous and biological matrices into the methanolic solutions used for quantification of  $^{18}\text{O}/^{16}\text{O}$  ratio in phosphate from environmental samples.

Our data suggest that the ESI-Orbitrap approach to measure oxygen isotope ratios in phosphate could simplify sample preparation and thus increase sample throughput, thereby providing the procedures for in-depth studies of changes in the microbial phosphorus metabolism in the environment.