

INSPIRATION bulletin

CL:AIRE's INSPIRATION bulletins describe practical aspects of research which have direct application to the management of contaminated soil or groundwater in an agricultural context. This bulletin explores an isotopic approach to investigate the origin of phosphate pollution in agricultural catchments.

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Isotope techniques for the analysis of $\delta^{18}\text{O}$ of inorganic phosphate within aquatic ecosystems

1. Introduction

Phosphorus (P) is an essential nutrient for all living forms and is present in water ecosystems in a wide variety of chemical forms, both dissolved and particulate forms. The dissolved fraction includes inorganic P, generally orthophosphate (H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}), the most bioavailable form of P that is quickly taken up by microbial cells, plants and algae, and altered to organic phosphorus compounds. Inorganic P exists in very small concentrations in natural waters, limiting primary biological productivity in many freshwater systems and in oligotrophic regions of the ocean (Blake *et al.*, 2005). Recent increases in fluxes and concentrations of bioavailable P have induced undesirable ecosystem changes, such as increases in primary production, shifts in community composition, reduced biodiversity, algal blooms and hypoxia (Sondergaard & Jeppesen, 2007). Most scientists agree that these increases are largely caused by an inefficient treatment of human sewage and a high application of organic and synthetic fertilisers on agricultural lands (Schindler, 2012). In fact P is widely used and lost in agricultural landscapes and negatively affects water quality. Water pollution derived from agriculture is a global issue and the European Union has recently implemented agricultural and environmental policy to face the problem. Standards for "good status" of waterbodies have been set by the Water Framework Directive (WFD) but improvements seem to rise modestly (Smith *et al.*, 2017). In this context, the oxygen isotope ratio of dissolved inorganic phosphate ($\delta^{18}\text{O}_p$) represents a potentially powerful tracer, a useful apportionment tool to investigate the origin of phosphate pollution in agricultural catchments. Identifying point and nonpoint nutrient sources is critical to understanding ecosystem health and subsequently to developing management practices and mitigation measures in order to achieve the goals of the WFD and of Sustainable Intensification in agriculture.

2. Phosphorus Biogeochemistry

Phosphorus has only one stable isotope, ^{31}P , and stable isotope analysis requires an element to have at least two naturally-occurring stable isotopes, with changes in the ratio of individual isotopes of an element in a sample, against a known ratio in a reference (Davies *et al.*, 2014). P is often bound strongly to oxygen (O) in the dissolved inorganic phosphate ion (Blake *et al.*, 1997), P_i , and attention has recently focused on whether the stable isotope composition of O in P_i

($\delta^{18}\text{O}-\text{PO}_4$) can provide new insights into the P cycle. The value of the oxygen fractionation (^{16}O and ^{18}O) within a sample is expressed as $\delta^{18}\text{O}$, relative to Vienna Standard Mean Ocean Water (0‰ $_{\text{VSMOW}}$ on the $\delta^{18}\text{O}$ scale):

$$\delta^{18}\text{O}_{\text{sample}} = 1000 \left[\frac{\left(\frac{^{18}\text{O}}{^{16}\text{O}}\right)_{\text{sample}}}{\left(\frac{^{18}\text{O}}{^{16}\text{O}}\right)_{\text{standard}}} - 1 \right]$$

P-O bonds in P_i are resistant to inorganic hydrolysis under typical temperature, pressure and pH conditions in surface water and groundwater ecosystems, apparently negligible O exchange occurs within these ecosystems without biological mediation (Tudge, 1960; Blake *et al.*, 1997). However, biologically-mediated processes involving P_i cleave the P-O bonds and exchange O isotopes with ambient water, leading to a change in the O isotope composition of the P_i (Blake *et al.*, 2005). Hence, within environments in which biological activity is high, the initial $\delta^{18}\text{O}$ composition in the extracellular environment, reflecting sources of P, could be overprinted by equilibrium fractionation (Davies *et al.*, 2014). The $\delta^{18}\text{O}$ value of a P_i molecule in equilibrium with surrounding water can be predicted using the empirically-derived equation from Chang & Blake (2015):

$$\delta^{18}\text{O}_{\text{PO}_4} = (\delta^{18}\text{O}_{\text{H}_2\text{O}} + 1000) \times e^{[14.43 \times (1000/T) - 26.54]/1000} - 1000$$

Comparing the theoretical value for $\delta^{18}\text{O}_p$ at equilibrium with $\delta^{18}\text{O}_p$ observed in a sample can provide insight into the extent to which P_i has been recycled through intracellular metabolic reactions, assuming that the initial $\delta^{18}\text{O}_p$ value was at disequilibrium (Davies *et al.*, 2014).

3. Methods for the Determination of $\delta^{18}\text{O}_p$

Determining the oxygen isotope ratios of dissolved inorganic phosphate (DIP) in freshwater and in a seawater environment is a difficult task. The first method of isolating dissolved phosphate was published by Longinelli *et al.* (1976). Historically $\delta^{18}\text{O}_p$ has been determined through fluorination or bromination of bismuth(III) phosphate (BiPO_4), currently the pure phosphate salt Ag_3PO_4 is preferred (Lécuyer, 2004). Phosphate salt Ag_3PO_4 is analysed using a High Temperature Conversion Analyser (TC/EA) interfaced to a stable isotope ratio mass spectrometer (IRMS). TC/EA-IRMS requires a

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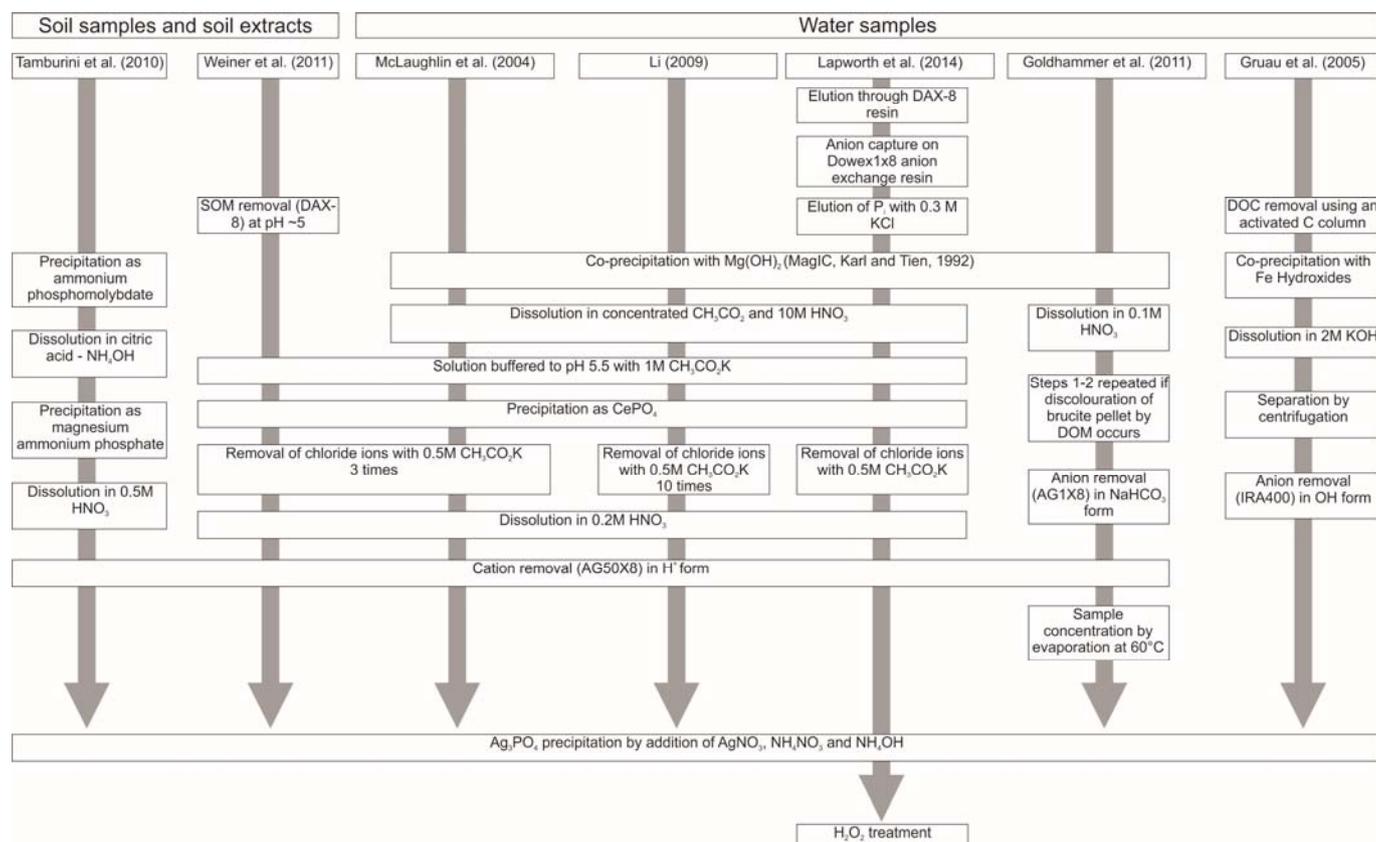


Figure 1: Categorisation of published protocols for the precipitation of Ag_3PO_4 . Modified from Davies *et al.* (2014).

relatively small sample mass for analysis (typically 400-500 μg Ag_3PO_4). The major protocol for extraction of P_i via precipitation of Ag_3PO_4 has been developed by McLaughlin *et al.* (2004). Although it has been successfully used to determine the $DIP-\delta^{18}O$ values of seawater and estuary waters, it is not directly applicable to freshwaters with high dissolved organic C (DOC). In fact Li (2009) and Davies (2016) demonstrated that using the method developed by McLaughlin *et al.* (2004), the Ag_3PO_4 precipitates contain a very high level of organic matter that contaminates the samples. Consequently all O-containing contaminants must be removed from the Ag_3PO_4 precipitate to ensure an accurate determination. Even though different attempts have been applied to develop a robust protocol for the extraction of inorganic P from water samples (Figure 1), there is no definitive approach for all aquatic matrices (Davies *et al.*, 2014).

4. $\delta^{18}O_p$ Data from Different Sources and Aquatic Ecosystems

A restricted dataset (Figure 2) is currently available across a range of aquatic ecosystems and sources of phosphate. Young *et al.* (2009) published the first collection of $\delta^{18}O_p$ source values reviewed from the literature and from their own study. Successively, Davies *et al.* (2014) expanded the global library of $\delta^{18}O_p$ data synthesising published values derived from both sources and aquatic ecosystems, while recently Granger *et al.* (2017), Tonderski *et al.* (2017) and Goody *et al.* (2018) increased the current available information on $\delta^{18}O_p$ in freshwaters. At present, Figure 1 suggests that there is an overlap of the $\delta^{18}O_p$ values among the various groups of samples. However, recent studies demonstrated that identifying local point sources and investigating specific geographical and temporal

variations of $\delta^{18}O_p$ would be of value in understanding phosphate biogeochemical cycling. In fact, site-specific parameters (i.e. DIP concentrations) and dynamics (i.e. seasonality, residence time) showed to influence the actual isotopic P_i -oxygen values (Goody *et al.*, 2016; Granger *et al.*, 2017; Tonderski *et al.*, 2017).

5. Conclusion

$\delta^{18}O_p - PO_4$ represents a novel stable isotope tracer that could be useful as a source apportionment tool. Its application in agricultural catchments could allow tracing the origin of water pollution, either

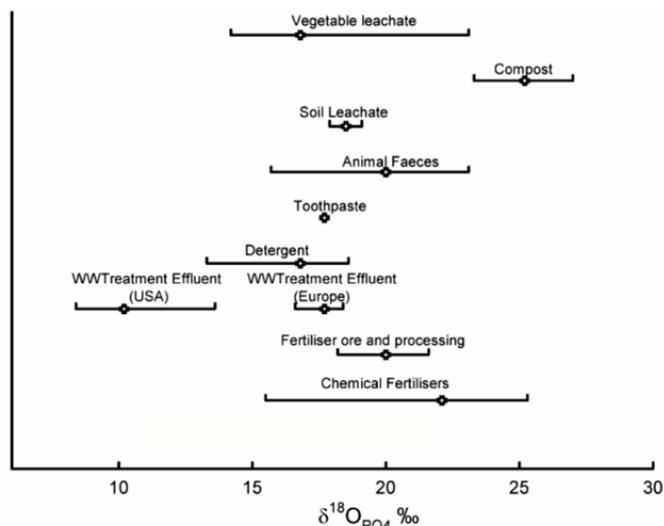


Figure 2: $\delta^{18}O_p$ for potential phosphate sources. Figure modified from Goody *et al.* (2015).

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from organic/inorganic fertilisers or other point sources, and consequently drawing up regulations to adopt mitigation practices to reach the goal of Sustainable Intensification. Nevertheless the research is still at an early stage and more study is needed to:

- Develop a robust protocol to analyse $\delta^{18}\text{O}_p - \text{PO}_4$;
- Expand the global dataset of $\delta^{18}\text{O}$ between sources of P and aquatic ecosystems;
- Investigate $\delta^{18}\text{O}_p$ together with the fluxes and dynamics of a catchment;
- Better understand the way and extent that biological activity controls P speciation.

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