

Identification of inositol phosphates (IP₆, IP₅ and IP₄) in hypobromite oxidised soil extracts as determined by solution ³¹P NMR spectroscopy

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Inositol phosphates (IP) are a major pool of identifiable organic P in soil and comprise on average one third of total organic P. Inositol hexakisphosphates (IP₆) are routinely determined in alkaline soil extracts using solution ³¹P nuclear magnetic resonance (NMR) spectroscopy. Before the advent of NMR as the method of choice to analyse organic P in soils, a greater diversity of IPs could be identified through the use of chromatography. This was possible by subjection of alkaline soil extracts to hypobromite oxidation and subsequent analysis by chromatography. This approach enabled the detection of IP₁ to IP₆ and their stereoisomers (myo, scyllo, D-chiro and neo) in soils. In contrast, there has been a lack of direct spectroscopic evidence for these IPs in soil extracts using NMR, and there is supporting evidence to suggest that the hypobromite oxidation technique may not completely isolate IPs from soil. The aim of this study was to identify the chemical nature of organic P, particularly the IPs, in hypobromite oxidised soil extracts using NMR spectroscopy. Four soils (Ferralsol, Vertisol, Cambisol and Gleysol; sampling depth 0-20 cm) with varying concentrations of organic P (93-1326 mg P/kg_{soil}) were analysed. The presence of lower order IPs was determined by spiking the hypobromite oxidised extracts with known standards from the collection of Dr Dennis Cosgrove and Dr Max Tate. For the first time, we identify many forms of IP in soil extracts using ${}^{31}P$ NMR spectroscopy. This included the four stereoisomers of IP₆ and IP₅ (myo, scyllo, neo and chiro), two different enantiomers of myo-IP₅, and also scyllo-IP₄. Concentrations of combined pools of IP₄ and IP₅ ranged from 0.5 to 30.3 mg P/kgsoil, which comprised 1 % to 10 % of the total organic P across all soils. Furthermore, a broad signal was present in the phosphomonoester region of hypobromite oxidised extracts, which comprised on average 46 % of the broad signal in untreated soil extracts. These results show that the organic P remaining in soil extracts following hypobromite oxidation is likely to include forms other than that of IPs. Many of the sharp peaks in NMR spectra of untreated soil extracts corresponded to the identified IPs of sharp peaks in soil extracts following hypobromite oxidation. Our results indicate that the majority of sharp peaks are due to IPs, which highlight their importance as constituents of the soil organic P.