Synthesis and initial characterization of metal-phytate compounds

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Recently, spectral techniques, such as, X-ray absorption near edge structure (XANES) spectroscopy, solid state ^{31}P nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), have been applied to investigate the metal-P species in animal manure and soil. But, it is hardly possible to resolve these spectral data on metal-organic P compounds partly due to the lack of model metal-organic P compounds for reference. Phytic acid (inositol hexa*kis*phosphate, IP6) has been identified as a major organic P form in soil, animal manure and other environmental samples. It contains a 6-carbon-ring with an H and a phosphate attached to each C moiety. Each of the six phosphate groups is attached in an ester linkage and retains two replaceable H atoms. This compound, thus, occurs in plants mainly in the forms of the Ca-Mg salt. Phytic acid can also exist in the salt forms with other metals (such as Fe and Al) in soil and manure. In this work, we synthesized phytate metal compounds in order to yield spectral information of these solid model compounds which would facilitate characterizing metal-organic P interactions in the environment.

Table 1. Composition of synthetic metal phytate compounds.

Metal-IP6	P (%)	C (%)	Metal (%)	P:C:Metal (mole)	Purity* (%)
Ca_6IP6	16.15	6.73	19.28	1:1.06:0.91	77.2
Mg_6IP6	17.07	7.33	12.95	1:1.11:0.96	72.9
Cu_6IP6	13.01	5.02	26.80	1:1.00:1.00	72.0
Mn_6IP6	15.53	6.63	27.40	1:1.10:1.10	81.6
Al_4IP6	17.7	6.54	10.95	1:0.96:0.72	72.0
Fe_4IP6	15.23	6.19	23.44	1:1.06:0.86	65.9
$Fe_{4-x}Al_x$IP6	15.59	6.42	17.3 (Fe) 2.96(Al)	1:1.08:0.84	70.2

*: Based on the difference in the P contents detected in the synthetic compounds and calculated from the theoretical formulas.

Synthesis of metal-IP6 compounds. Seven metal-phytate compounds were synthesized from Na_{12}IP6 and metal chlorides (Table 1). The theoretical mole ratio of P:C:metal is 1:1:1

for metal(II)-IP6, and 1:1:0.67 for metal(III)-IP6. Element analysis indicated that compounds with Ca, Mg, Cu, Mn, and Al were obtained near stoichiometrically (± 10%). The last two compounds containing Fe(III) were 25–28% higher in metal content, indicating some Fe hydroxide was present in the two compounds. The presence of Fe hydroxide might also partly explain the lowest purity of the two Fe-containing synthetic compounds.

FT-IR spectra. All the seven synthetic compounds and $Na_{12}IP6$ showed a weak absorption band around 1680 cm^{-1} (Figure 1) and a broad absorption band at 3400 cm^{-1} (data not shown). These two bands did not disappear when these samples were dried for 1 h at 105°C. These observations demonstrated the existence of O-H bonds in these compounds which could be due to the crystalline H_2O, impurity metal hydroxides, and unbounded P-O-H bonds although the last two should not be parts of these compounds theoretically. A sharp absorption band at 972 cm^{-1} and a broad band with trailing at 1106 cm^{-1}appeared in the spectrum of $Na_{12}IP6$. The band at 972 cm^{-1} shifted to 996 cm^{-1} with Ca_6IP6, and 1010 cm^{-1} with Mg_6IP6. Both compounds showed the broad band around 1135 cm^{-1} with less trailing character. In the spectra of Cu and Mn compounds, the broad band split to two overlapped but distinguishable bands at 1086 cm^{-1} and 1133 cm^{-1} or nearby with the sharp band at 997 cm^{-1}. The spectral characteristics of trivalent metal (Fe and Al) compounds were a shoulder band at 1007 cm^{-1} with a strong band at 1092 or 1166 cm^{-1}. Thus, the absorption bands from 900 to 1200 cm^{-1} in FT-IR could be used to classify metal phytate compounds to three groups: (1) light divalent metal (Ca and Mg) phytate compounds with a sharp band and a broad band, (2) heavy divalent metal (Cu and Mn) compounds with splitting broad bands, and (3) trivalent peaks with a shoulder band.

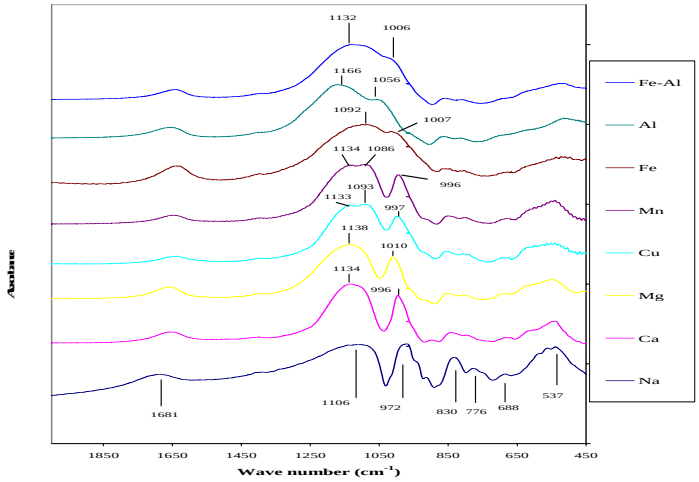

Figure 1. Fourier-transform infra-red spectra of metal-IP6 compounds.

Enzymatic hydrolysis. The metal-phytate compounds (2 mM P) were suspended in 100 mM glycine-HCl (pH 2.5) or 100 mM sodium acetate buffer (pH 5.0) with 3-phytase (0.1 U/ml). The reaction mixtures were incubated at an end-over-end shaker (40 rpm) at 22°C for 20 h. Whereas less than 0.1 mM of phosphate was detected at the end of incubation in the reaction in mixtures without 3-phytase, nearly all phytate P in Na, Ca, Mg, Mn, and Cu compounds were enzymatically hydrolyzed at either pH. No enzymatic release of P from Fe and Al phytates were detected.