Influence of phenolic acids on phosphorus mobilisation in acidic and calcareous soils

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Abstract

Phenolic acids and phenols are abundant in soils. However, little information is available on the role of these compounds in mobilisation of soil phosphorus (P). The present study examined the effects of three phenolics on P mobilisation in comparison with citric acid in three soils differing in chemical properties. The soils were incubated with organic compounds at concentrations of 0 to 100 μ mol g⁻¹ soil for 30 min. While the addition of phenolic acid anions and phenol decreased soil pH, citrate either increased or did not affect soil pH depending on the soil type. All the organic compounds increased the amounts of easily-mobilised P fractions in the order of catechol \geq citric acid \geq proto-catechuic acid \geq caffeic acid for the acid soils and citric acid > catechol = proto-catechuic acid > caffeic acid for the calcareous soil. Phosphorus mobilisation did not correspond to the amounts of Ca, Fe or Al ions released from the soils. These results suggest that organic acids and phenols altered the P fractions from stable, sparingly-soluble forms to easily dissolvable forms, and that chelation or precipitation of cations with organic ligands occur before biodegradation of the ligands.

Introduction

Phenolic acids contain phenolic hydroxyl and carboxyl groups, thus being aromatic organic acids. Phenolic acids are among the most widespread classes of secondary metabolites and are important in the plant-soil systems. Common phenolic acids in soil are caffeic, vanillic, and *p*-coumaric (Martens, 2002; Siqueria et al., 1991).

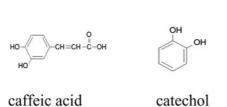
Phenols have been reported in some plants at the amounts up to 6 g kg⁻¹ fresh weight (Asami et al., 2003). When the plant residues are applied to a soil, the phenolic acids from the plant material may influence soil properties. In addition, phenolic acids are added to soil via root exudation and microbial activity (Blum, 1998; Dalton et al., 1989; Rice, 1984; Singh

et al., 2001). The roles of phenolic acids in allelopathy and microbial activity have been reported (Blum, 1996; Blum et al., 1999; Ohno, 2001; Singh et al., 2001), but the information on their role in nutrient mobilization is limited.

Phosphorus is one of the most limiting nutrients for plant growth in many soils. Its availability depends on soil characteristics and contents of mobilisable P fractions. The Ca-P is the main inorganic P fraction in calcareous soils, whereas Fe-P and Al-P are predominant forms in acid soils (Chang and Jackson, 1957). Organic P mineralisation contributes to the available P pool, but the degree of mineralisation is dependent on the stability of organic P fractions and the microbial activity (Bowman and Cole, 1978). Low-molecularweight aliphatic organic acids, such as citric and oxalic, have been reported to reduce P adsorption by soil and P-fixing minerals, and to desorb soil P, thus

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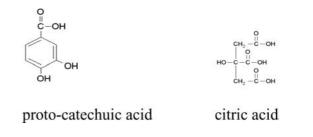
improving the availability of soil P to plants (Hu et al., 2001; 2002). In contrast, the role of phenolic acids in mobilisation of P has not been reported.

It is unclear what influence different species of phenolics may have on P availability in different soils. The objective of this study was to compare the capacity of phenolic acids and citric acid to extract P from three soils with different chemical properties.

Materials and methods

Soils

Three soils, representing Ca-, Fe- and Al-dominant soils, were sampled from the top 10-cm horizon on farms in Western Australia (Table 1). Samples were sieved (2 mm), air-dried and ground to pass through a 0.25-mm sieve for incubation and analysis. Amorphous Al and Fe were extracted by Tamm's solution (Rayment and Higginson, 1992) and determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) for Al, and atomic absorption spectrometry (AAS) for Fe. Exchangeable Ca, Mg, K and Na were determined using AAS after EDTA-NH₄OAc extraction (Nanjing Institute of Soil Science, CAS, 1978), and the total exchangeable bases were calculated by summation. The EDTA-NH₄OAc reagent contained 0.005 M EDTA and 1 M NH₄OAc adjusted to pH 7.0 (for acidic and neutral soils) or 8.5 (for alkaline soil). For extraction, 1.0 g soil was shaken with 50 mL EDTA-NH₄OAc reagent for 16 h, followed by centrifugation and cation determination using AAS (for measuring Ca 800 mg Sr kg⁻¹ was added). Clay content was determined by the particle analysis. Organic C was measured by a LECO CHN-1000 analyser (LECO Corporation, St. Joseph, MI, USA). Olsen P was extracted by shaking for 30 min in 0.5 M NaHCO₃ at the extractant-to-soil ratio of 50:1, and then determined colourimetrically (Page et al., 1995). The pH was measured by a pH meter (EA940) in water extract at the soil:water ratio of 1:1.



Organic ligands

Chemical reagents used in this study were citric acid (CIT), caffeic acid (CAF), catechol (CAT), and protocatechuic acid (PCA), representing different chemical compounds commonly found in root exudates. They were of analytical grade and purchased from Sigma Company. Their structures are listed above.

Experimental methods

Effect of shaking time on P extraction in the presence or absence of citric acid

Solution of citric acid was added to soil at 0 or 15 μ mol g⁻¹ soil. Soils were moistened to field capacity (150, 350 and 250 g kg⁻¹ for soils 1, 2 and 3, respectively) and then incubated at 20 °C for 30 min. Phosphorus was extracted in water or 0.5 M NaHCO₃ at the extractant-to-soil ratio of 50:1 by shaking on an end-over-end shaker for 10 to 480 min.

Soil incubation

Solutions of organic ligands were adjusted to pH 5.5 using dilute NaOH and were then added to soil at rates 0, 12.5, 50 or 100 μ mol g⁻¹ soil. The soils were watered to the field capacity, and then incubated at 20 °C for 30 min.

Phosphorus fractionation

Soil (0.5 g) was extracted sequentially in deionized water and 0.5 M NaHCO₃ at the extractant-to-soil ratio of 50:1. The extraction was facilitated by shaking at 20 °C for 30 min. Dowex 1X8-50 resin (HCO₃⁻ form) was used to separate resin P from each of the extracts. Resin P was exchanged with 0.5 M HCl by shaking for 2 hours. Total P in the extracts was determined after digesting in concentrated HNO₃ and H₂O₂ (Hedley et al., 1982; Tiessen and Moir, 1993).

Location	Soil no. 1 Kellerberrin	Soil no. 2 Harvey	Soil no. 3 Kojonup
Longitude	117° 46′00″E	115° 48′40″E	117° 04′42″E
Latitude	31° 23'00''S	33° 06′ 30″ S	33° 50′45″S
Amorphous Fe (g kg $^{-1}$)	1.65 ± 0.15	13.0 ± 0.0	1.18 ± 0.03
Amorphous Al (g kg $^{-1}$)	1.98 ± 0.03	3.08 ± 0.03	5.0 ± 0.0
Exch. Ca $(g kg^{-1})$	10.7 ± 0.1	2.59 ± 0.01	2.20 ± 0.04
Clay (%)	10	45	12
Organic C (g kg $^{-1}$)	7.17 ± 0.16	57.7 ± 0.7	51.5 ± 0.4
Olsen P ($\mu g \cdot g^{-1}$)	6.3 ± 0.1	9.7 ± 0.6	20.1 ± 1.5
pH (H ₂ O)	8.28 ± 0.06	5.02 ± 0.10	5.69 ± 0.01
pH (CaCl ₂)	7.83 ± 0.02	4.63 ± 0.03	5.02 ± 0.01
Total exchangeable bases (cmol kg^{-1})	32.5 ± 1.0	16.1 ± 0.2	6.7 ± 0.03

Table 1 The properties of the three soils from the farmland in Western Australia. Data represent means \pm s.e. (n = 2)

Measurement of P fractions and relevant chemical properties

Inorganic P was determined directly using the molybdate blue colourimetry (Murphy and Riley, 1962). The organic P was calculated as the difference between the contents of total extracted P and inorganic P. The pH of water extracts was measured immediately following extraction. Contents of Ca, Fe and Al in water extracts were measured using AAS (for Ca and Fe) and ICP-AES (for Al).

Statistics

All data were statistically analysed using Excel 2000. Separate models were created for each phosphorus fractions using two-factor analysis of variance (ANOVA), and differences between treatment means were analysed by LSD at $\alpha = 0.05$.

Results

Extraction efficiency as influenced by shaking time

Compared to water, bicarbonate extracted more P from the calcareous soil (no. 1) and Al-dominated acid soil (no. 3), but there was no significant difference between the two extractants in the acid Fe-dominated soil (no. 2) (Figure 1). The amount of P extracted by water or bicarbonate increased with the shaking time (Figure 1). Water extraction for 30 min released 60 to 95% of the amount of P extracted after 8 h for all three soils irrespective of citrate addition; hence, 30-min extraction was used in subsequent experiments. This extraction time was chosen because it was relatively short (thus minimising any potential effect of microbes on P extractability), yet long enough to extract a substantial amount of soil P under the conditions tested.

Influence of organic ligands on pH of water extracts

An addition of phenolic acids and catechol decreased pH of water extracts of all three soils (Figure 2). The pH decreased with increasing concentration of phenolic acids. In the calcareous soil (no. 1), the order of three phenolics in decreasing pH was CAT > CAF > PCA, whereas, in the acid soils, the order was PCA \geq CAF > CAT. In contrast, citric acid increased pH of the calcareous soil (no. 1) and the acid Al-dominated soil (no. 3), but did not influence pH of the acid Fe-dominated soil (no. 2).

The influence of organic ligands on the amount of P in various fractions

The content of resin-P was low (only $1-2 \ \mu g \ g^{-1}$) and not affected by organic ligands in any of the three soils (Table 2). The organic P fraction in the water extract was increased by all the organic ligands in the calcareous soil (CIT \ge CAT \ge PCA \approx CAF \approx Control) (no. 1), whereas no difference among the organic ligand treatments was noted in the acid Al-dominated soil (no. 3). Organic P in the acid Fe-dominated soil (no. 2) was increased by citric acid but not by phenolic acids.

P fraction	Calc	areous s	oil (no. 1	l)	Acid	Fe-dom	inated s	oil (no. 2)	Acid	Al-dom	inated s	oil (no. 3)
	P _{ri}	P_{wo}	P _{bi}	Sum	P _{ri}	P_{wo}	P _{bi}	Sum	P _{ri}	P_{wo}	P _{bi}	Sum
Control	1.5	29.1	8.8	39.4	1.2	27.8	10.7	39.7	2.1	29.9	13.6	45.5
CAF	1.3	31.5	12.8	44.6	1.4	30.6	14.4	46.4	1.3	27.0	17.4	45.7
CAT	1.5	35.3	11.9	48.7	1.4	29.8	17.2	48.3	1.5	32.2	18.4	52.2
PCA	1.2	32.8	10.6	44.6	1.9	31.1	15.5	48.4	1.9	29.5	18.1	49.4
CIT	1.3	36.8	7.9	45.9	0.9	61.9	13.8	76.5	1.9	32.8	16.3	50.9
LSD _{0.05}	ns	3.2	0.7	3.7	ns	5.0	2.4	5.9	ns	5.7	1.1	2.6

Control = without organic ligands addition; CAF = caffeic acid; CAT = catechol; PCA = proto-catechuic acid; CIT = citric acid.

ns, not significant.

The amount of NaHCO₃-extractable P in acid soils (no. 2 and 3) was increased by all organic ligands, with phenolics causing a greater increase than citric acid (CAT \geq PCA \geq CAF > CIT) (Table 2). The addition of citric acid to the calcareous soil (no. 1) decreased the NaHCO₃-extractable P (P_{bi}), whereas the addition of phenolics increased this P fraction.

Given that the P fractions extracted by water and NaHCO₃ could be directly utilised by plants, their sum was regarded as the total content of plant-available P (mobilised P). The addition of organic ligands increased the mobilised P in the following order: CAT \geq CIT \geq PCA \geq CAF for soils 1 and 3, and CIT > CAT = PCA > CAF for soil 2. Clearly, CAT and CIT were more effective in mobilising P than PCA and CAF in the present study.

The influence of organic ligand concentrations

Increasing addition rates of organic ligands increased the content of NaHCO₃-extractable inorganic P (Figure 3), except that addition of citrate to the calcareous soil (no. 1) and acid Fe-dominated soil (no. 2) at higher rates decreased this P fraction. Phenolics increased NaHCO₃-extractable inorganic P fraction more than citrate at the equivalent concentration in the three soils. Although there were different magnitudes of effects for various ligands and soils, caffeic acid displayed the strongest effect in the calcareous soil (no. 1), and catechol was the strongest in acid soils (no. 2 and 3). Most ligands had an effect even at 12.5 μ mol g⁻¹ soil.

Total mobilised P also increased with increasing concentration of organic ligands (Figure 4). In the calcareous soil (no. 1), the four organic ligands increased total mobilised P by 9 to 38% compared with the control. In the acid Fe-dominated soil (no. 2), citric acid increased the mobilised P by 77 to 116%, the effect being much greater than that of phenolics (an increase of 14 to 39%). In the acid Al-dominated soil (no. 3), citrate increased mobilisation of P to a greater extent than phenolics, particularly at the application rate of 100 μ mol g⁻¹ soil.

Extraction of Ca, Fe and Al from soils

Compared with the control, all the organic ligands substantially decreased the amount of Ca released from the calcareous soil (no. 1) (Table 3). With the increasing concentration of organic ligands, the amount of extracted Ca increased slightly. These observations suggest that the complexation between released Ca and organic ligands occurs before biodegradation of the ligands. Compared with citrate and catechol, proto-catechuic acid and caffeic acid inhibited Ca release to a lesser extent.

An addition of citric acid increased NaHCO₃extractable Fe in acid Fe-dominated soil (no. 2) by 6to 15-fold and the extractable Al in acid Al-dominated soil (no. 3) by 10- to 20-fold (Table 3). Increasing addition rates of caffeic acid decreased the amounts of the extractable Fe and Al. In contrast, increasing addition of catechol and proto-catechuic acid increased the extractable Fe from acid Fe-dominated soil (no. 2) and extractable Al from acid Al-dominated soil (no. 3), although the increases were lower than those observed after an addition of citric acid.

Organic ligands	Concentration $(\mu \mod g^{-1})$	Ca (µg g ⁻¹) Calcareous soil (no. 1)	Fe (µg g ⁻¹) Acid Fe-dominated soil (no. 2)	Al (μg g ⁻¹) Acid Al-dominated soil (no. 3)
Control	0	214	282	36
Caffeic acid	12.5	29	285	35
	50	44	206	33
	100	65	229	32
Catechol	12.5	26	346	42
	50	35	372	52
	100	43	404	84
Proto-catechuic acid	12.5	30	299	38
	50	50	311	45
	100	72	354	52
Citric acid	12.5	25	1662	376
	50	35	2684	545
	100	50	4248	722
	LSD _{0.05}	3.8	211	52

Table 3. Concentration of NaHCO₃-extractable Ca, Fe and Al in soils after 30-min incubation with organic ligands at various rates

Discussion

Role of phenolic acids in P mobilization

This study showed that phenolic acids can mobilise P from NaHCO3-insoluble forms in both acid and calcareous soils. In the Ca- and Al-dominant soils (no. 1 and 3), the extent of P mobilisation by phenolic acids was similar to that achieved by citric acid. However, in the Fe-dominant soil (no. 2), phenolic acids were less effective than citrate in mobilising P. These observations might be related to either the complexation of different acid ligands and metals (empirical observations indicate that citric acid is a stronger complexing agent for metals than the phenolics tested here), or transformation of P fractions. Furthermore, the effectiveness of phenolics decreased in the following order: $CAT \ge PCA \ge CAF$ for all three soils. It appears that the number of hydroxyl groups and their relative positions are more important than those of carboxyl groups. If the carboxyl group is positioned away from the benzol ring, its influence on the characteristics of the complex would be less.

With increasing concentration of organic ligands, NaHCO₃-extractable P and the total mobilised P increased in all three soils. For acid soils, citrate was more effective than phenolics in mobilising P at a given concentration. The reason might be that citrate was a stronger chelator for iron and aluminium than phenolics. For the calcareous soil, the effect of citrate and phenolics was similar in terms of an increase in mobilised P and the decrease in NaHCO₃-extractable inorganic P with an increase in ligand concentration.

When soil has high concentration of phenolics, the availability of soil P would be enhanced due to mobilisation. Phenolics could be derived from (i) decomposition of organic matter, (ii) through microbial activity, and/or (iii) by exudation from plant roots (Blum, 1996; Haider and Martin, 1975; Martens, 2000). Therefore, the P bioavailability in the rhizosphere soil as well as in a soil with high organic matter content could be higher than in a soil with low content of organic compounds.

Effects of citric acid and phenolics on soil pH

Organic acids could affect soil pH in at least two ways. Firstly, the microbial breakdown of organic acid anions causes an increase in soil pH. It has been shown that increases in soil pH following the addition of malate and citrate are highly correlated with CO_2 evolution during the decomposition of these two anions (Yan et al., 1996). Secondly, organic acids may contribute to soil pH change through their direct reactions

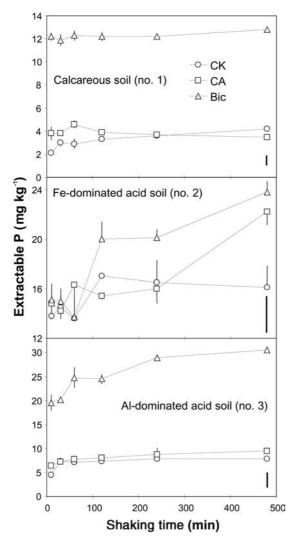


Figure 1. Phosphorus extracted by water and sodium bicarbonate during different shaking times. CK = water incubation (pH 5.5) and H₂O extract; CA = water + citrate incubation (pH 5.5) and H₂O extract; Bic = water incubation and bicarbonate extract. The bar at each data point represents the standard error of means of three replicates. The bar in the lower right corner of each graph represents LSD_{0.05}.

with soil surfaces. These reactions include ligand exchange between hydroxyl groups of soil aluminium and iron hydroxy oxides, and dissociation of organic acids. In the present study, addition of citric acid (mainly in an anion form at pH 6) generally increased soil pH even in the calcareous soil. As the initial pH values of the tested soils are above the pK values of citric acid ($pK_1 = 3.14$, $pK_2 = 4.77$ and $pK_3 = 6.39$), it appears that decomposition of citrate might have contributed to pH increase, even though the incubation and extraction times were kept as short as possible.

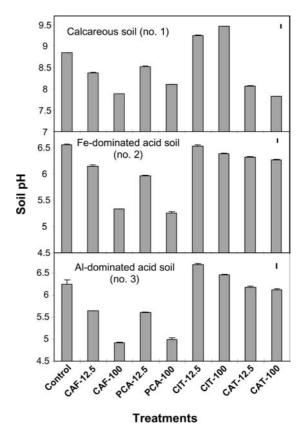


Figure 2. The pH (H₂O) of soils after incubation with organic ligands at field capacity for 30 min. The data on the X-axis are the amount of organic ligands addition in μ mol g⁻¹. Control = soil mixed with H₂O; CAF = caffeic acid; PCA = proto-catechuic acid; CIT = citric acid; CAT = catechol. The bar at each data point represents the standard error of means of three replicates. The bar in the top right corner of each graph represents LSD_{0.05}.

Adsorption of citric acid onto soil particles may result in pH changes as well. In addition, the dissociation constants of citric acid (as well as phenolics) may change in different soil conditions, thus affecting pH to a different degree. In the three soils tested, the difference in the magnitude of soil pH change upon the addition of the organic ligands might have also been influenced by the cations dominant in these soils.

The PCA and CAF decreased pH of all three soils tested, especially at the higher addition rate. The decreased pH by the phenolics could be explained by PCA and CAF having phenolic hydroxyls and carboxyl groups, thus the acidity of these ligands was relatively high, and an addition of these ligands could lead to the dissociation of acid function groups. The decreased pH by CAT only in the calcareous soil was probably corresponding to the dissociation of phenolic hydroxyls at higher soil pH.

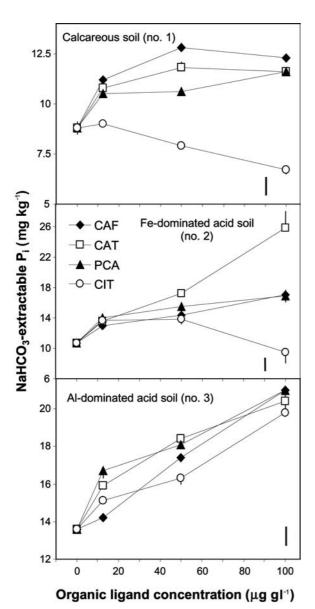


Figure 3. Amounts of NaHCO₃-extractable inorganic P in three soils after incubation with organic ligands at various rates for 30 min. CAF = caffeic acid; CAT = catechol; PCA = proto-catechuic acid; CIT = citric acid. The bar at each data point represents the standard error of means of three replicates. The bar in the lower right corner of each graph represents LSD_{0.05}.

Cation release from three soils incubated with organic ligands

The decrease of 6 to 8 times in the extractable Ca in the calcareous soil (no. 1) due to addition of organic acids suggested that chelation and/or precipitation occurred between calcium and organic ligands during the incubation period. It is also possible that some Ca-P

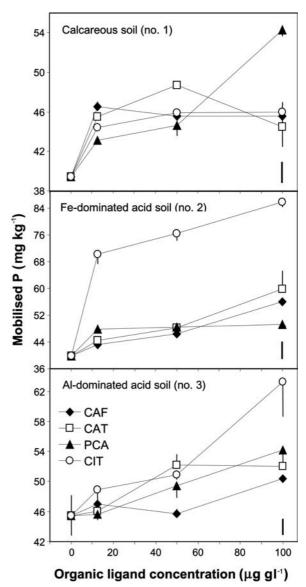


Figure 4. Amounts of the mobilised P (= resin P + water-extractable organic P + NaHCO₃-extractable inorganic P) in three soils after incubation with organic ligands at various rates for 30 min. CAF = caffeic acid; CAT = catechol; PCA = proto-catechuic acid; CIT = citric acid. The bar at each data point represents the standard error of means of three replicates. The bar in the lower right corner of each graph represents LSD_{0.05}.

complexes would be converted into other forms because of the low dissolution of complexes between Ca and organic ligands. In addition, the Fe and Al release from the acidic soils would result from chelation by the organic ligands tested (except caffeic acid, which could have formed insoluble compounds with Fe and Al). The amounts of cations extracted were not consistent with the amounts of P mobilised. This suggests that available P may be increased through the dissolution of insoluble inorganic fractions and mobilisation of organic P in the presence of organic ligands. Many studies have shown that organic ligands facilitate the dissolution of insoluble phosphates (eg. Buehler et al., 2002; Hedley et al., 1982). Without organic ligands, the newly mobilised inorganic P could be transformed back into insoluble fractions. Regarding mobilisation of organic P by the organic ligands tested (data not shown), the most effective ligand was catechol for the calcareous soil [increasing NaHCO3-extractable P by 25 to 46% (depending on concentration) in comparison with the control treatment], whereas no mobilisation of organic P by the ligands tested was noted in the other two soils. The apparent difference in the effectiveness of organic ligands in mobilising organic P in various soil types warrants further work.

Conclusion

Like aliphatic organic acids, phenolics could enhance soil P mobilisation in the three soils tested, although the magnitude depended on the soil type and the organic ligands used. The water- and NaHCO₃extractable P increased with an increase in concentration of organic ligands added. An addition of phenolic acids and catechol decreased pH of water extracts of all three soils, but addition of citric acid increased pH of the calcareous and the acid Al-dominated soil. Extraction of cations could not fully account for P mobilisation, indicating that chelation or precipitation of cations with organic ligands might have occurred.

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