

THE ROLE OF CITRIC ACID AS A PHOSPHORUS MOBILIZATION MECHANISM IN HIGHLY P-FIXING SOILS

PAPEL DEL ACIDO CITRICO COMO MECANISMO DE MOBILIZACION DE FÓSFORO EN SUELOS CON ALTA CAPACIDAD DE RETENCION DEL FOSFORO

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ABSTRACT

Exudation of low molecular weight carboxylates is an important phosphorus (P) acquisition strategy for plants in strongly P-fixing soils. The effect of citrate exudation on P availability was studied in five acid soils with a large organic matter content and a low available P pool. The amount of molybdate reactive P extracted by citric acid 10 mM (0.1–2 mg kg⁻¹) was larger than the molybdate reactive P (MRP) extracted by CaCl₂ 10⁻³ M (0.007–0.07 mg kg⁻¹). Both extractants were adjusted to soil pH prior to extraction. Also, the total P content of citric acid extracts (2–44 mg kg⁻¹) far exceeded the total P content of CaCl₂ (0.2–1.5 mg kg⁻¹). However, ratios of MRP to total dissolved P (TDP) remained constant. When citric acid was added to the CaCl₂-extracts (the pH of citric acid being adjusted to the pH of CaCl₂, and thus eliminating release of P through acidification) a significant pH increase was noted. Simultaneously, MRP increased significantly in all but one soil. This suggests that the P availability enhancing properties of citric acid are not only due to acidification of the plant rhizosphere, but also to its Al and Fe complexing capacity.

KEYWORDS: Andosols, citric acid, phosphorus availability.

RESUMEN

La exudación de ácidos orgánicos con bajo peso molecular, como el ácido cítrico, es una importante estrategia de adquisición de fósforo (P) para las plantas en suelos que fijan fuertemente el P. El objetivo de la presente investigación fue evaluar el efecto de la exudación de ácido cítrico sobre la disponibilidad de P. Dicho efecto fue estudiado en 5 suelos ácidos con un elevado contenido de materia orgánica y una baja disponibilidad de P. La cantidad de fosfato determinado con molibdato de amonio (MRP), extraído con ácido cítrico 10 mM (0,1–2 mg kg⁻¹) fue mayor que la cantidad de MRP extraído con CaCl₂ 10⁻³ M (0,007–0,07 mg kg⁻¹). Ambos extractantes fueron ajustados al pH del suelo antes de realizar la extracción. El contenido de P total extraído con ácido cítrico, superó en mucho el contenido de P total extraído con CaCl₂. Sin embargo, el ratio MRP:P total disuelto permaneció constante. Así mismo cuando el ácido cítrico fue añadido al CaCl₂ – el pH del ácido cítrico fué ajustado al pH del CaCl₂, eliminando así la liberación de P por acidificación– se observó un significativo aumento de pH. Simultáneamente, el MRP aumentó en todos los suelos menos en uno. Esto sugiere que las capacidades del ácido cítrico para aumentar la disponibilidad de P no es sólo debido a la acidificación de la rizosfera de la planta sino también a la capacidad de quelación del Al y Fe.

PALABRAS CLAVES: Acido cítrico, andosoles, disponibilidad de fósforo.

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INTRODUCTION

Carboxylate exudation is a major plant response to a number of well-defined stress situations, including

Al toxicity (Heim *et al.* 1999), deficiency of Fe (Marschner & Römheld 1994), K⁺ (Krafczyck *et al.* 1984), P (Ström *et al.* 2002) and O₂ (anoxia; Jones 1998). In the case of P deficiency, direct modification of the plant rhizosphere by carboxylate exudation is not the only possible P acquisition strategy. Also other mechanisms can be used such as excreting phosphatases to release organically bound P and the provision of extra C (López-Bucio *et al.* 2000) as a booster for micro-organisms, which in turn, also can produce carboxylates as well as phosphatases (Jones 1998).

The P availability enhancing effects of carboxylates can be grouped into direct and indirect effects. The direct effects generally result in immediate P release. They refer to the blocking of P adsorption sites (ligand exchange), oxide dissolution by complexing Al or Fe held in minerals or mobilization of P held in metal-humic substances (Staunton & Leprince 1996).

In contrast, far less is known about the indirect P mobilization effects of exudation. These indirect effects include both the stimulation of the microbial activity and a soil pH alteration, mostly a pH decrease. There is disagreement as to whether citric acid is exuded in its acid form, and only dissociates once it is outside the cell environment and consequently causing acidification (Geelhoed *et al.* 1999; Haynes & Mokolobate, 2001; Staunton & Leprince 1996) or in its conjugate form base form citrate³⁻, as cytosol pH is 7.1 – 7.3 (Hinsinger 2001; Jones 1998). In the latter case, the soil pH decrease is not caused by citric acid dissociation, but by the simultaneously excreted counterion H⁺ in order to maintain electrical neutrality. However, Sas *et al.* (2001) found that organic anion release was only partly accompanied by H⁺ extrusion. They suggest K⁺ as the alternative accompanying cation as does Hinsinger (2001). For most organic acid release situations, however, the counterion remains to be identified (Jones 1998). On the other hand, some authors report a pH increase (Jones & Darrah 1994, Jones and Kochian 1996). In this case, fewer possible mechanisms are proposed, except for the above mentioned citrate³⁻ equilibrating with citrate²⁻ and H⁺ not being the only counterion to adjust the anion – cation balance of the cell membrane. Haynes & Mokolobate (2001) name – next to the complexation of H⁺ by citrate – microbial decarboxylation as a proton consuming process. This effect is sometimes

referred to as the self liming effect in the context of organic residue amendment. A last possible mechanism resulting in a pH increase, is ligand exchange between the organic acid and soil solid phase hydroxyl groups (Yan 1996).

A third indirect exudation effect results from the fact that the complexation of Al and Fe bound to soil humic substances by organic anions renders them more soluble, smaller in size and more accessible to soil or root phosphatases. This facilitates enzymatic hydrolyzation of organically bound phosphorus which may be associated with these humic substances (Haynes & Mokolobate 2001; Jones 1998). Especially in soils containing a large amount of organic matter such as andosols, this mechanism may be crucial because the organic matter is often associated with Al and Fe (Borie & Zunino 1983). As long as organic P is bound with Al or Fe, it remains protected. Therefore, the limiting step in the utilization of the large organic P pool in andosols is not phosphatase activity but the release of Al and Fe from organic P (Otani & Ae 1999). However, the importance of the chelating ability of organic acids in comparison with their acidifying ability is still under debate (Subbarao *et al.* 1997).

Despite our limited knowledge of the mechanisms by which carboxylates improve P availability, empirical evidence indicates that exudation yields a competitive advantage to plants in natural ecosystems. Organic acid concentrations have been reported to be significantly lower in cultivated soils than in paired sites under native vegetation (Fox & Comerford 1990; Hue *et al.* 1986). Not only pristine forests (Schlesinger 1997), but also species rich ecosystems are reported to have plants that are capable of exudation. *Proteaceae* vegetations are known to use exudation to mobilize sparingly soluble phosphate in severely phosphate-impooverished soils (Lambers *et al.* 2002; López-Bucio *et al.* 2000; Sas *et al.* 2001). Exudation is also reported to play an important role in wetlands, as they are probably longer lived in O₂ poor conditions (Mitsch & Gosselink 2000).

In the present study, P mobilization by citrate was investigated in soils with a small available P pool and a high organic matter content. Citrate is usually considered as the most powerful organic anion, next to oxalate (Schlesinger 1997) and malate (Bolan *et al.* 1994). Firstly, the difference in P-fractions and Al and Fe contents extracted by citric

acid were compared with those extracted by CaCl_2 (= reference). Secondly, the importance of the Al- and Fe complexing power of citric acid is addressed by adding citric acid to CaCl_2 extracts at the same pH as the CaCl_2 extract. Changes in pH and molybdate reactive P will indicate whether or not complexation occurs without the presence of soil.

MATERIALS AND METHODS

A set of five acid soils from different countries was selected. The ferralsol originates from Đà Loan, located in the Vietnamese highlands (DL). The crop on the sampled field was maize. Both andosols were sampled in Chile. One site was located in Cordillera de los Andes in Puyehue National Park (PU). The second andosol was sampled in central valley near Paillaco (PA). Main tree species were respectively the evergreen *Nothofagus betuloides* (Mirb.) Oerst. and the deciduous *Nothofagus obliqua* (Mirb.) Blume. The Leiemeersen histosol was sampled in Oostkamp, Belgium (LM). The second histosol was sampled in Zegveld, The Netherlands (ZV). At all places topsoil was sampled (0–20 cm), except for PU where the 20–25 cm layer was analyzed, because previous experiments had shown that up to 97 % of the labile P pool of this layer was present under organic form (data not shown). The soils were air-dried and sieved over 2 mm. Only LM was stored wet after sieving over 1 cm to remove most plant remnants, root rhizomes and coarse roots.

After determination of soil pH (1:10, CaCl_2 10^{-3} M), soils were again extracted with CaCl_2 10^{-3} M. This time, the pH of CaCl_2 was adjusted to soil pH prior to extraction. Shaking time was 2 hours (s:l 1:4). Soils were also extracted using citric acid 10 mM, adjusted

to soil pH during 2 h (s:l 1:4). All extracts were filtered using a 0.45 μm membrane filter. Molybdate Reactive P (MRP) was determined according to Murphy and Riley (1962). When analyzing the citric acid extracts, ammonium molybdate and potassium antimony tartrate concentrations in the 'colour developing reagent' were increased to avoid interference of citric acid with colour formation. Total dissolved P (TDP) in both extracts was assessed by adding 1 mL of a digestion mixture (0.05 M H_2SO_4 , 16 mg L^{-1} $\text{K}_2\text{S}_2\text{O}_8$) to 1 mL extract in pyrex tubes. The tightly capped tubes were autoclaved at 121 °C for 30 min. The 10 mM citric acid extracts were diluted before destruction in order to minimize the C-load. Afterwards, P content was determined using malachite green (van Veldhoven & Mannaerts 1987). Molybdate Unreactive P (MUP) was calculated as the difference between TDP and MRP.

Al- and Fe-content of CaCl_2 and citric acid 10 mM extracts and acid ammonium oxalate extractable Al, Fe and P (Schwertmann 1973) were determined by ICP-OES (Perkin Elmer). Organic matter content was determined as percent loss on ignition on 550 °C (LOI).

In the next experiment, citric acid was added to the CaCl_2 extracts to make a final concentration of 10 mM. Before addition, the pH of citric acid was adjusted to the pH of the CaCl_2 extract, in order to eliminate the acidifying effect of citric acid. pH differences before and after addition were monitored (Metrohm 74). The change in MRP was assessed using the colour developing reagent with increased ammonium molybdate and potassium antimony tartrate content. All analyses were performed at the day of extraction. Citric acid had to be prepared daily, because of its susceptibility to microbial breakdown.

TABLE I. Selected soil properties. Mean values and standard deviations are based on 2 replicate measurements. Abbreviations: Đà Loan (DL), Leiemeersen (LM), Zegveld (ZV), Paillaco (PA) and Puyehue (PU).

Soil	Soil type	pH		LOI		Al_{ox}		Fe_{ox}		P_{ox}	
		s.d.		(%)	s.d.	g kg^{-1}	s.d.	g kg^{-1}	s.d.	g kg^{-1}	s.d.
DL	Ferralsol	4.2	0.1	18	1	3.86	0.1	1.78	0.05	0.15	0.01
LM	Histosol	5.8	0.1	54	0.2	0.27	0.0	1.58	0.23	0.08	0.01
ZV	Histosol	4.9	0.1	62	0.5	2.63	0.2	7.96	0.36	1.22	0.07
PA	Andosol	5.9	0.04	35	0.1	22.03	0.9	6.97	0.27	0.40	0.02
PU	Andosol	4.6	0.02	17	0.007	6.38	0.3	6.40	0.31	0.27	0.02

RESULTS

SOIL CHARACTERISTICS

LOI ranged from 17 (PU) to 88 % (LM) (Table I). Substantial amounts of organic matter were a prerequisite for the conducted experiments. The relatively low LOI of PU, bearing in mind that it is an andosol, is probably due to the fact that the 20–25 cm layer was analyzed and not the topsoil, where generally a more elevated LOI content is expected like in PA. The largest amount of oxalate extract-

able Al was found in the andosols (PA: 22 g kg⁻¹, PU: 6.4 g kg⁻¹). The same held for oxalate extractable Fe (PA: 7.0 g kg⁻¹, PU: 6.4 g kg⁻¹). The ZV histosol contained comparable amounts of oxalate extractable Fe (8.0 g kg⁻¹). Fe content of LM on the other hand, was significantly lower (1.6 g kg⁻¹). As for oxalate extractable P, the amount present in ZV (1.2 g kg⁻¹) was remarkably higher than in the other soils (0.15–0.40 g kg⁻¹). Probably, a substantial amount of P was released during the drying of the ZV histosol. This irreversibly alters the histosol soil matrix.

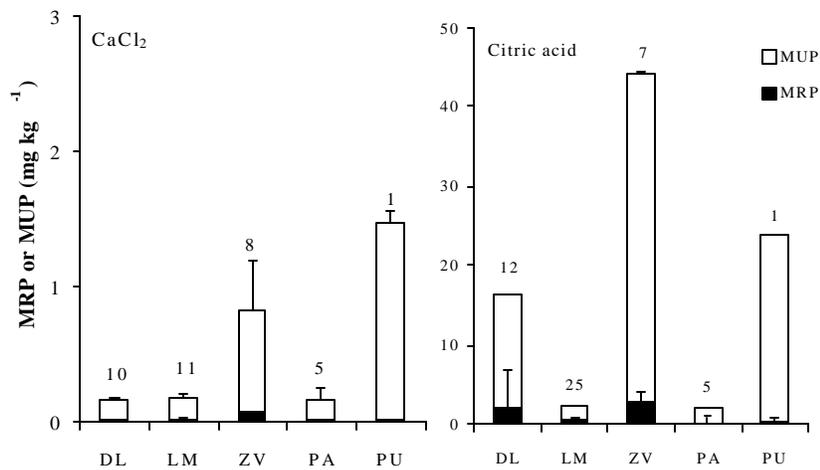


FIGURE 1. MRP and MUP content of CaCl₂ 10⁻³ M (left) and citric acid 10 mM extracts (right). Ratio of MRP to TDP, expressed as percentage, is indicated above the bars. Abbreviations: Dà Loan (DL), Leiemeersen (LM), Zegveld (ZV), Paillaco (PA) and Puyehue (PU). Error bars represent standard deviations of the means (n=2).

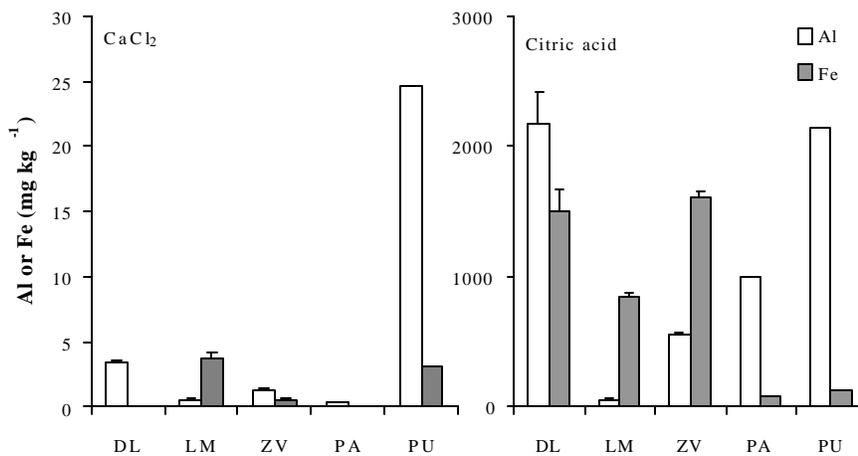


FIGURE 2. Al and Fe content of CaCl₂ 10⁻³ M (left) and citric acid 10 mM extracts (right). Site abbreviations as in Fig. 1. Error bars represent standard deviations of the means (n=2).

EFFECTS OF CITRIC ACID

The amount of MRP extracted by CaCl_2 adjusted to soil pH ranged between 0.01 (PU, PA) and 0.07 mg kg^{-1} (ZV). TDP in CaCl_2 ranged between 0.17 (DL) and 1.5 mg kg^{-1} (PU). The major part of the total P pool was obviously present in an organic form (Fig. 1). In the andosols, 95 to 99 % was present as MUP.

Citric acid clearly extracted more P than CaCl_2 did (Fig. 1). MRP increased at least tenfold (PA). In DL extracts, MRP (2 mg kg^{-1}) even mounted up to 116 times the amount present in CaCl_2 , which was the maximum increase observed. Also TDP amounts increased at least twelvefold. The largest TDP increase was noted in DL (*96) and in LM (*54). The ratio MRP:TDP remained constant, except for LM. In LM the MRP content

increased 30-fold when extracted with citric acid. However, TDP increased only 13-fold, hence the increase in MRP:TDP.

Similarly, the amounts of Al and Fe extracted by citric acid were huge in comparison to the amount extracted by CaCl_2 (Fig. 2). CaCl_2 extracted between 0.4 (PA) and 25 (PU) mg Al kg^{-1} . With citric acid, amounts up to 2177 (DL) and 2148 (PU) mg Al kg^{-1} were liberated. The highest relative increase was noted in PA (* 2200). The effect of citric acid was even more pronounced for Fe. The Fe amount liberated by citric acid increased by more than 3000-fold in comparison with the amount extracted by CaCl_2 . Maximum Fe amounts in citric acid were noted in DL (1506 mg kg^{-1}) and ZV (1619 mg kg^{-1}).

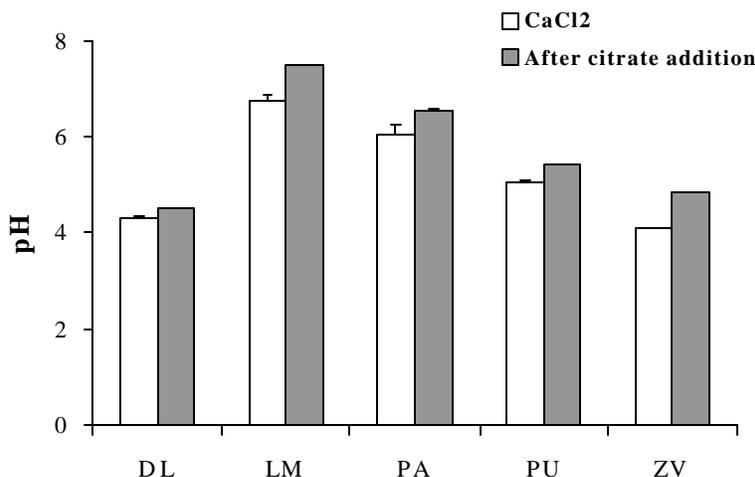


FIGURE 3. pH increase after citric acid addition to make a final 10 *mM* concentration. All differences significant at the 0.05 level. Site abbreviations as in Fig. 1. Error bars represent standard deviations of the means (n=2).

EFFECT OF ADDITION OF CITRIC ACID 10 *mM* AT SOIL pH

Citric acid was added to the same CaCl_2 extracts described above, to make a final 10 *mM* citric acid concentration. Before addition, citric acid was adjusted to the pH of the CaCl_2 extract. All soils showed a significant pH increase after the addition of citric acid ($\alpha = 0.05$) in the order of 0.5 (PA, PU) to 0.8 (LM, ZV). Results are presented in Figure 3. As was hypothesized, an increase in MRP was also detected (Fig. 4). Except for DL, the increase not statistically significant at $\alpha = 0.05$.

DISCUSSION

EFFECTS OF CITRIC ACID ON MOBILITY OF AL, FE, MRP AND MUP

Our results indicated that citric acid was more effective than CaCl_2 at mobilizing both MRP and MUP (Figs. 1, 2). Both P fractions were mobilized to the same extent, as the MRP:TDP ratios remain constant, except for LM. Why this is not the case for the LM soil remains unclear. LM citric acid-extracts contain the smallest amount of Al compared to the other soils. It is well established that the P

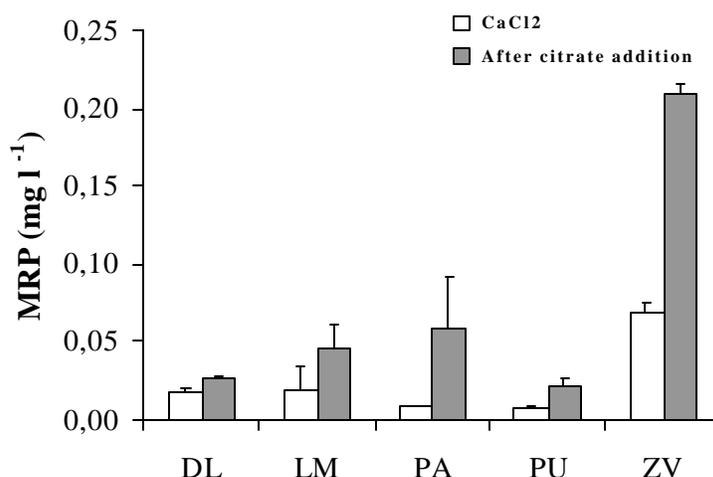


FIGURE 4. MRP increase after citric acid addition to make a final 10 mM concentration. All differences significant at the 0.05 level, except for Dà Loan. Site abbreviations as in Fig. 1. Error bars represent standard deviations of the means (n=2).

mobilizing capacities of citric acid are correlated with the high stability constant of the Al-citrate complex (Jones & Kochian 1996). In LM, citrate is mainly complexed with Fe. It is possible that the Fe-citrate complex was not stable up to MRP analysis, although always performed on the day of extraction (within 2 h). As comparable data are scarce, there are limited possibilities for comparison with other studies. Moreover, citric acid may interfere with the color reaction used to determine MRP (Galhardo & Masini 2000; Petterson & Karlberg 1999). Most authors do not mention whether they modified the color developing reagent to counteract this interference. Jones & Darrah (1994) state that 10 mM citrate solutions can be analyzed without problems with color formation. However, we corroborated observations by Helmke *et al.* (2003), that citrate concentrations above 5 mM impede color formation (results not shown). Thus, care must be taken when comparing results.

EFFECT OF CITRIC ACID 10 mM AT SOIL pH

Addition of citric acid to CaCl₂, in the absence of soil, resulted in a significant pH increase for all five soils. Because of the pH adjustment of citric acid to the pH of the CaCl₂ extract before addition, any pH change due to a change in the dissociation equilibrium is excluded. So, only microbial

decarboxylation and ligand exchange between citric acid and dissolved particles (< 0.45 μm) with hydroxyl groups are left as possible pH increasing mechanisms. Considering the fact that a simultaneous MRP increase is detected, our results point to ligand exchange with dissolved particles, being Al or Fe hydroxides or humic substances. The soils with the largest % LOI (ZV, LM) also exhibit the largest pH increase (0.8). PA and PU show a lower pH increase (0.5). Interestingly, Jones & Kochian (1996) report similar results. They performed column leachings with an artificial solution containing 60 μM citrate to assess the rate of dissolution of Al from soil. The solution pH was adjusted to soil pH before leaching, and prepared under sterile conditions. From the beginning onwards, they observed a leachate pH increase compared to control columns, which kept increasing steadily in the course of the experiment. On the other hand, MRP increase is apparently not directly related to LOI. PA and PU at least double MRP after citric acid addition, while this is the maximum increase observed for the histosols. However, it is important to bear in mind that P concentrations in CaCl₂ are close to detection limit.

Taken together, effects of citric acid are not attributable solely to rhizosphere pH modification, but also to the complexation capacities of citric acid. However, exact quantification of the effects of citrate

on P availability remains difficult. As yet, it is not clear what the typical concentrations are for bulk soil, rhizosphere soil and soil solution (Staunton & Leprince 1996), while the concentrations used during the experiment obviously are crucial. Furthermore, carboxylates are very susceptible to microbial breakdown. Thus, results tend to be very case specific. More research is needed to infer more general conclusions.

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BIBLIOGRAPHY

- BOLAN, N.S., R. NAIDU, S. MAHIMAIRAJA & S. BASKARAN. 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils* 18: 311-319.
- BORIE, F. & H. ZUNINO. 1983. Organic matter P associations as a sink in P-fixation processes in allophanic soils of Chile. *Soil Biology and Biochemistry* 15: 599-603.
- FOX, T.R. & N.B. COMERFORD. 1990. Low-molecular-weight organic acids in selected forest soils in the Southeastern USA. *Soil Science Society of America Journal* 54: 1139-1144.
- GALHARDO, C.X. & J.C. MASINI. 2000. Spectrophotometric determination of phosphate and silicate by sequential injection using molybdenum blue chemistry. *Analytica Chimica Acta* 417: 191-200.
- GEELHOED, J.S., W.H. VAN RIEMSDIJK & G.R. FINDENEGG. 1999. Simulation of the effect of citrate exudation from roots on the plant availability of phosphate sorbed on goethite. *European Journal of Soil Science* 50: 379-390.
- HAYNES, R.J. & M.S. MOKOLOBATE. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutrient Cycling in Agroecosystems* 59: 47-63.
- HEIM, A., J. LUSTER, I. BRUNNER, B. FREY & E. FROSSARD. 1999. Effect of Aluminium treatment on Norway spruce roots: Aluminium binding forms, element distribution, and release of organic substances. *Plant & Soil* 216: 103-116.
- HELMKE, P.A., T.J. DE BOERTH & X. HE. Bioavailability of organically-bound phosphorus. URL: <http://www.soils.wisc.edu/extension/FAPM/proceedings/1.Helmke.pdf>. Viewed: March 10, 2003.
- HINSINGER, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant & Soil* 237: 173-195.
- HUE, N.V., G.R. CRADDOCK & F. ADAMS. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Science Society of America Journal* 50: 28-34.
- IMAS, P., B. BAR-YOSEF, U. KAFKAFI & R. GANMORE-NEUMANN. 1997. Release of carboxylic anions and protons by tomato roots in response to ammonium nitrate ratio and pH in nutrient solution. *Plant Soil* 191: 27-34.
- JONES, D.L. & P.R. DARRAH. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant & Soil* 166:247-257.
- JONES, D.L. & L.V. KOCHIAN. 1996. Aluminium-organic acid interactions in acid soils. I Effect of root-derived organic acids on the kinetics of Al dissolution. *Plant & Soil*: 182: 221-228.
- JONES, D.L. 1998. Organic acids in the rhizosphere – a critical review. *Plant & Soil* 205: 25-44.
- KRAFCZYK, I., G. TOLLDENIER & H. BERINGER. 1984. Soluble root exudates of maize: Influence of potassium supply and rhizosphere micro-organisms. *Soil Biology and Biochemistry* 16: 315-322.
- LAMBERS, H., D. JUNIPER, G.R. CAWTHRAY, E.J. VENEKLAAS & E. MARTÍNEZ-FERRI. 2002. The pattern of carboxylate exudation in *Banksia grandis* (Proteaceae) is affected by the form of phosphate added to the soil. *Plant Soil* 238: 111-122.
- LÓPEZ-BUCIO, J., M.F. NIETO-JACOBO, V. RAMÍREZ-RODRÍGUEZ & L. HERRERA-ESTRELLA. 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Science* 160: 1-13.
- MARSCHNER, H. & V. RÖMHELD. 1994. Strategies of plants for acquisition of iron. *Plant Soil* 165: 261-274.
- MITSCHEW, W.J. & J.G. GOSSELINK. 2000. Wetlands. Third edition, Wiley, NYC. 920 pp.
- MURPHY, J. & J.P. RILEY. 1962. A modified single solution method for the determination of phosphorus in natural waters. *Analytica Chimica Acta* 27: 31-36.
- OTANI, T. & N. AE. 1999. Extraction of organic phosphorus in andosols by various methods. *Soil Science and Plant Nutrition* 45: 151-161.
- PETTERSON, A.K. & B. KARLBERG. 1999. Simultaneous determination of orthophosphate and silicate in brackish water. *Analytica Chimica Acta* 378: 183-189.
- SAS, L., Z. RENGEL & C. TANG. 2001. Excess cation uptake, and extrusion of protons and organic acid anions by *Lupinus albus* under phosphorus deficiency. *Plant Science* 160: 1191-1198.
- SCHLESINGER, W.H. 1997. Biogeochemistry. An analysis of global change. Second edition, Academic Press, USA. 588 pp.

- SCHWERTMANN, U. 1973. Use of oxalate for Fe extraction from soils. *Canadian Journal of Soil Science* 53: 244-246.
- STAUNTON, S. & F. LEPRINCE. 1996. Effect of pH and some organic anions on the solubility of soil phosphate: implications for P bioavailability. *European Journal of Soil Science* 47: 231-239.
- STRÖM, L., A.O. OWEN, D.L. GODBOLD & D.L. JONES. 2002. Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. *Soil Biology and Biochemistry* 34: 703-710.
- SUBBARAO, G.V., N.A.E. & T. OTANI. 1997. Genotypic variation in Iron-, and Aluminum-Phosphate solubilizing activity of Pigeonpea root exudates under P deficient conditions. *Soil Science and Plant Nutrition* 43: 295-305.
- VAN VELDHoven, P.P. & G.P. MANNAERTS. 1987. Inorganic and organic phosphate measurements in the nanomolar range. *Analytical Biochemistry* 161: 45-48.
- YAN, F., S. SCHUBERT & K. MENGEL. 1996. Soil pH increase due to biological decarboxylation of organic anions. *Soil Biology and Biochemistry* 28: 617-624.

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