



(RESEARCH ARTICLE)



Organic acid mediated phosphorus mobilization from phytin and glycerophosphate

Amita Gharu Dhariwal¹ and JC Tarafdar^{2,*}

¹ Department of Chemistry, Jai Narain Vyas University, Jodhpur 342001 Rajasthan, India.

² ICAR - Central Arid Zone Research Institute, Jodhpur-342008 Rajasthan, India.

GSC Advanced Research and Reviews, 2023, 15(01), 062–068

Publication history: Received on 22 February 2023; revised on 08 April 2023; accepted on 10 April 2023

Article DOI: <https://doi.org/10.30574/gscarr.2023.15.1.0103>

Abstract

More release of mineral-P from organic P compounds (phytin and glycerophosphate, 200 mg L⁻¹) was observed due to the action of 10 mg L⁻¹ organic acids (formic, citric, lactic, malic). Lactic acid was found to be most effective followed by formic and citric acid. In general, malic acid showed poor response as compared to the other organic acids tested for P mineralization. The inorganic P (Pi) release from glycerolphosphate, by different organic acids, was 62-66% higher than that release from phytin at the concentration of 10 mg L⁻¹. The maximum Pi release from glycerophosphate and phytin by the action of different organic acids varies between 7.50 mg L⁻¹ to 8.26 mg L⁻¹ and 4.64 mg L⁻¹ to 5.10 mg L⁻¹ respectively. The optimum time of incubation for more P mobilization from glycerophosphate varies between 90 and 135 minutes while phytin required from 60 to 105 minutes. The efficiency of different organic acids towards mineralization of P follows the order: lactic acid > formic acid > citric acid > malic acid.

Keywords: Glycerophosphate; Phytin; Organic acids; P mineralization

1. Introduction

Organic P must be hydrolyzed to inorganic P before it can be utilized by plants [1]. Low molecular weight organic acid (LMOA) could increase P solubility by releasing the bound organic P and by mineralization of organic P by increasing the rate of hydrolytic cleavage [2]. There are various forms of P with variable stabilities were noticed in the soil; the most resistant is inositol phosphate, which makes up more than half the organic phosphorus in majority of soils. Even some soils reported up to 83% of the total organic P as inositol phosphates [3]. The inositol phosphates in soils included esters myochiro-, seylo- and neo- inositol and known as phytic acid [C₆H₆(OHPO₃)₆]. Glycerophosphate is another important component of organic phosphorus present in the soil.

The organic acid normally present in the rhizosphere, were originate from microorganisms [4,5] or by exudation of root under P starvation condition [6] and by aluminum toxicity [7]. Typically the root contains formate, lactate, citrate, malate, oxalate, acetate, succinate, fumarate, isoutarate, aconitate etc. varying their chain structure and number of carboxyl groups. The low molecular weight organic anions function as organic ligands, which can increase P in solution by (i) replacing P sorbed at metal hydroxide surfaces through ligand – exchange reactions [8], (ii) dissolving metal oxide surfaces that sorb P [9] and (iii) complexing metals in solution and thus preventing precipitation of metal phosphates [10]. Low molecular weight organic acids release by plant roots increase soil availability by ligand exchange or dissolution [11]. Depending upon the dissociation properties and number of these carboxylic groups, organic acids can carry varying negative charges, thereby allowing the complexation of metal cations in solution and displacement of phosphate anions from the soil matrix [12]. Organic acids directly dissolve phosphate material by chelate cationic partners of the P ion [13]. For this reason, they have been implicated in many soil processes including the metabolisation and uptake of nutrients by plants and microorganisms. Therefore, they may also help for mobilization of organic P

*Corresponding author: JC Tarafdar

compounds. The aim of this study was to estimate the efficiency of different low molecular weight organic acids to mobilize P from two dominant soil organic P compounds, viz. phytin and glycerophosphate.

2. Material and methods

To find out the efficiency of organic acid towards the phosphorus mobilization, four organic acids reported to be secreted both by plants and microorganisms (formic, lactic, citric, malic) at a concentration of 10 mg L^{-1} each were tested on two most important soil organic P compounds phytin (200 mg L^{-1}) and glycerophosphate (200 mg L^{-1}). Both organic acid and solutions of organic P were prepared from extra-pure grade (E- merk) materials and deionized water was used for preparation of solution concentrations. Organic P compounds were incubated with organic acid at a constant shaking (60 rpm) at room temperature ($25 \pm 2^\circ\text{C}$). The release of inorganic P from organic P compounds due to action of organic acids was estimated with time (15 to 210 minutes). For control (blank), instead of organic acid solution same amount of deionized water was used with organic P compounds.

To measure the available phosphorus, five mL of the solution was taken in 25 mL volumetric flask and two to three drops of 2,4-dinitrophenyl indicator was added to it. The solution was discolor after using 0.5N HCl or $6\text{N NH}_4\text{OH}$ (as per requirement to adjust pH) solution drop by drop. Five mL of 15% ammonium molybdate solution was added to it and the color was developed by adding 1mL of 2.5% SnCl_2 . The blue color intensity was measured spectrophotometrically at 660 nm [14].

3. Results

The inorganic P (Pi) release with time from phytin and glycerophosphate organic P compounds was presented at Table 1. The result clearly demonstrated that release of Pi was more from glycerophosphate than phytin irrespective of different organic acid tested. In general, the pattern of Pi release with time was the same either with glycerophosphate (Fig. 1) or with phytin (Fig. 2). The release was more with lactic acid closely followed by formic acid. The least release of Pi was noted with malic acid. The Pi release from phytin by the action of different organic acids (10 mg L^{-1}) ranges from 2.46 to 5.10 mg L^{-1} (one liter contain 200 mg Po as phytin) or 2.70 to 8.26 mg L^{-1} glycerophosphate (one liter contain 200 mg Po as glycerophosphate). The maximum Pi release by the action of different organic acids varies between 7.50 mg L^{-1} (malic and citric acid) to 8.26 mg L^{-1} (lactic acid). The release by formic acid was found to be 8.15 mg L^{-1} .

The results (Table 2) clearly demonstrated that lactic acid was the most efficient organic acid to mobilize different organic P (Po) compounds, which may hydrolyze 4.13% of glycerophosphate Po within 90 minutes. On the other hand within the same period formic acid could hydrolyze only 4.08% of it. The citric acid took 105 minutes to hydrolyze 3.93% of the total glycerophosphate - Po while malic acid needed 135 minutes to hydrolyze only 3.75% of the total glycerophosphate - Po.

The Pi release from phytin by the action of different organic acids was presented (Table 3). The results demonstrated that the release was maximum (5.10 mg L^{-1}) due to the action of lactic acid followed by formic acid (4.92 mg L^{-1}), citric acid (4.82 mg L^{-1}) and malic acid (4.64 mg L^{-1}). The release of Pi from phytin P was 2.55% of total Po within 60 minutes by lactic acid and 2.46% by formic acid. The maximum release of Pi by the action of citric acid was noted after 75 minutes with 2.41%, which was further reduced when malic acid was used for hydrolysis.

Table 1 Release of available P (mg L⁻¹) with time from two organic P compounds (200 mg L⁻¹) by the organic acids

| Incubation time(min) | Lactic acid | | Formic acid | | Citric acid | | Malic acid | |
|----------------------|-------------|-------------------|-------------|-------------------|-------------|-------------------|------------|-------------------|
| | Phytin-P | Glycero phosphate | Phytin-P | Glycero phosphate | Phytin-P | Glycero phosphate | Phytin-P | Glycero phosphate |
| Initial | 2.46±0.08 | 2.70±0.06 | 2.46±0.08 | 2.70±0.06 | 2.46±0.08 | 2.70±0.06 | 2.46±0.08 | 2.70±0.06 |
| 15 | 4.05±0.12 | 4.98±0.10 | 3.94±0.09 | 4.96±0.11 | 3.88±0.17 | 4.88±0.29 | 3.75±0.13 | 4.69±0.09 |
| 30 | 4.65±0.20 | 5.73±0.23 | 4.23±0.15 | 5.62±0.22 | 4.15±0.19 | 5.50±0.31 | 3.79±0.36 | 4.99±0.15 |
| 45 | 4.80±0.24 | 6.73±0.25 | 4.55±0.16 | 6.64±0.21 | 4.32±0.40 | 6.56±0.24 | 3.85±0.28 | 5.49±0.11 |
| 60 | 5.10±0.18 | 7.53±0.31 | 4.92±0.18 | 7.56±0.25 | 4.50±0.33 | 7.18±0.22 | 4.03±0.31 | 6.16±0.31 |
| 75 | 5.04±0.17 | 7.94±0.24 | 4.82±0.20 | 7.72±0.24 | 4.82±0.35 | 7.59±0.19 | 4.25±0.22 | 6.43±0.34 |
| 90 | 4.90±0.22 | 8.26±0.36 | 4.77±0.21 | 8.15±0.20 | 4.74±0.22 | 7.70±0.16 | 4.40±0.40 | 6.83±0.22 |
| 105 | 4.78±0.28 | 8.20±0.40 | 4.62±0.19 | 8.06±0.18 | 4.59±0.18 | 7.86±0.15 | 4.64±0.33 | 6.97±0.29 |
| 120 | 4.66±0.21 | 8.18±0.45 | 4.55±0.18 | 8.02±0.14 | 4.52±0.17 | 7.72±0.10 | 4.57±0.30 | 7.37±0.35 |
| 135 | 4.63±0.14 | 8.12±0.41 | 4.48±0.24 | 7.99±0.13 | 4.45±0.13 | 7.70±0.09 | 4.45±0.25 | 7.50±0.39 |
| 150 | 4.56±0.16 | 8.10±0.40 | 4.40±0.21 | 7.97±0.26 | 4.37±0.10 | 7.70±0.30 | 4.35±0.22 | 7.48±0.18 |
| 165 | 4.46±0.18 | 8.10±0.47 | 4.38±0.25 | 7.97±0.27 | 4.30±0.21 | 7.67±0.31 | 4.32±0.18 | 7.42±0.19 |
| 180 | 4.41±0.18 | 8.02±0.35 | 4.34±0.22 | 7.91±0.29 | 4.25±0.25 | 7.64±0.25 | 4.25±0.11 | 7.40±0.31 |
| 195 | 4.41±0.14 | 7.89±0.38 | 4.31±0.27 | 7.77±0.40 | 4.21±0.29 | 7.52±0.26 | 4.20±0.29 | 7.34±0.29 |
| 210 | 4.35±0.13 | 7.67±0.33 | 4.25±0.22 | 7.54±0.31 | 4.15±0.31 | 7.44±0.22 | 4.08±0.41 | 7.21±0.20 |

Table 2 Time taken to release maximum inorganic P from glycerophosphate by different organic acids*

| Organic acids | Inorganic P release (mg L ⁻¹) | Time (min) |
|---------------|---|------------|
| Lactic | 8.26±0.36 (4.13)** | 90 |
| Formic | 8.15±0.20 (4.08) | 90 |
| Citric | 7.86±0.15 (3.93) | 105 |
| Mailc | 7.50±0.39 (3.75) | 135 |

* organic acid concentration: 10 mg L⁻¹; ** figure in parenthesis represent per cent of total mineral P release

Table 3 Time taken to release maximum inorganic P by different organic acids*

| Organic acids | Inorganic P release (mg L ⁻¹) | Time (min) |
|---------------|---|------------|
| Lactic | 5.10±0.17 (2.55)** | 60 |
| Formic | 4.92±0.18 (2.46) | 60 |
| Citric | 4.82±0.35 (2.41) | 75 |
| Mailc | 4.64±0.33 (2.32) | 105 |

* organic acid concentration: 10 mg L⁻¹; ** figure in parenthesis represent per cent of total mineral P release

Malic acid took 105 minutes to hydrolyze only 2.32% of phytin - P, the rate of release was lowest among the four organic acids tested. The total P release after optimum time of incubation (60 minutes for lactic and formic acid, 75 minutes for citric acid and 105 minutes for malic acid) was ranges from 2.46 to 5.10 mg L⁻¹ from phytin-P.

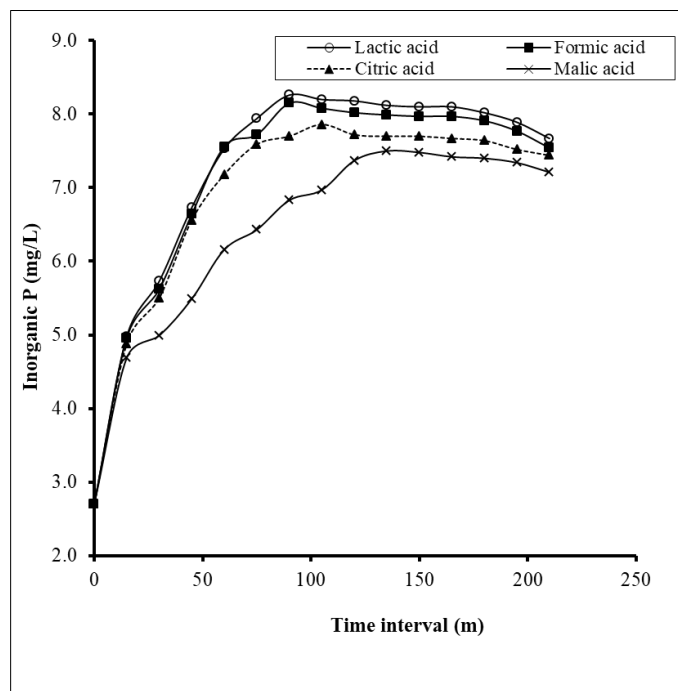


Figure 1 Periodical release of inorganic P from glycerophosphate by the action of different organic acids

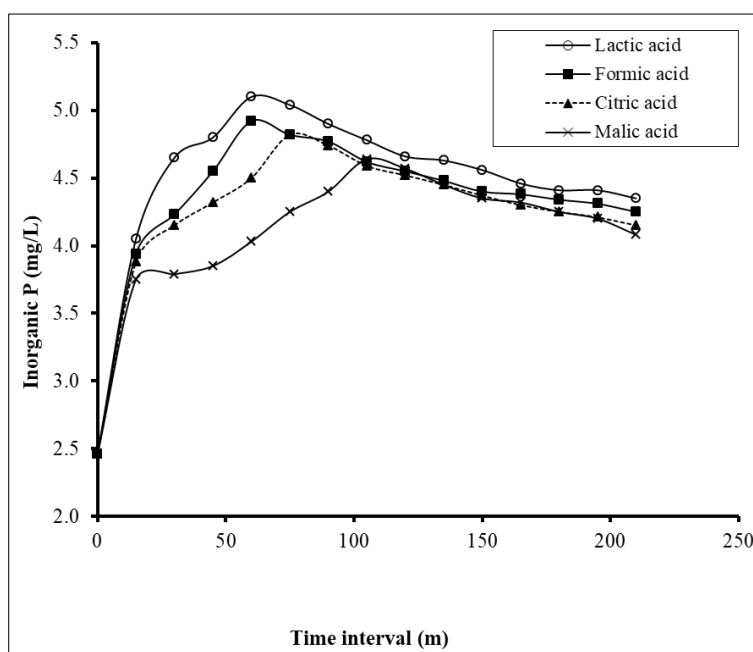


Figure 2 Periodical release of inorganic P from phytin by the action of different organic acids

4. Discussion

Organic acids are low-molecular weight -COOH containing compounds which are found in all organisms and which are characterized by the possession of one or more carboxyl groups. Depending on the dissociation properties and number of these carboxylic groups, organic acids can carry varying negative charge, thereby allowing the complexation of metal

cations in solution and the displacement of anions from the soil matrix [4]. Organic acids are a principal component of root exudates and have been hypothesized by many authors to be involved in the mobilization of nutrients within the rhizosphere [15, 16, 17]. The present study suggested that organic acids are capable of mobilizing significant quantities of soil P, elevating soil solution levels and consequently enhancing plant P acquisition. The sorption of organic acids to the mineral phase and mineralization by the soil's microbial biomass are critical to determining the effectiveness of organic acids in most rhizosphere processes. Organic forms of phosphorus constitute a large proportion of soil P. Although organic P is present in soil solution at higher concentration than inorganic phosphate [18], direct uptake of organic P compounds by plants is considered unlikely. Rather, plant roots acquire P as mineral phosphate ~~from~~ from the soil solution. Thus to contribute to plant P nutrition, soil P must be dephosphorylated by phosphatase or phytase [19, 20] and organic acids [21]. Mobilization of plant unavailable P by soil biota [22] mainly involved the solubilization of insoluble inorganic P and hydrolysis of organic P by the excretion of proton and organic acids.

The availability of organic P by plants depends on their cleavage of C-O-P bonds and rate of inorganic P release. Typically the root secrete many organic acid under P deficient condition [23, 24] and total concentration of organic acids in root is around 10-20 mM (1-4% of the total dry weight) [25]. The maximum concentration of organic acids present in soil will be 0.5-10 μ M. Jones and Darrah [25] concluded that organic acids can be expected to be of little consequence in nutrient mobilization from higher pH soils whilst in acid soil they may be involved both in a more general mechanism for micro nutrient uptake or as a potential detoxification mechanism. The release of Pi from Po sources was different with different organic acids, which was more with lactic acid and least with malic acid (Tables 1 to 3). The differential behavior of organic acids may be due to the differences in carboxyl groups present in their structure, which ultimately govern the release of proton. For example, lactic and formic acids have only one carboxyl group, malic acid has two and citric acid has three carboxyl groups.

A hydrogen bond (H-bond) is the bond between a functional group A-H and an atom or group of atoms in the same or a different molecule. Ionization of formic acid (i.e., release of cation) decrease due to the inter molecular H-bonding between two molecule of formic acid. While in case of lactic acid intermolecular H-bonding is less effective than formic acid, therefore, lactic acid is more efficient for P hydrolysis from phytin (Fig. 2) and glycerophosphate (Fig. 1). Malic acid has two and citric acid has three carboxyl groups. When more than one carboxyl groups are present the intra-molecular H-bonding will be more and due to dimer formation the affinity of organic acid decreases to react with metal, which ultimately helps to release the P. The higher release of P in citric than malic acid may be related to the fact that citric acid has three carboxyl groups while malic has only two. Therefore, intra-molecular H-bonding was more in malic acid while one carboxyl group was always free in case of citric acid to react and release P. Zhu et al. [26] reported significant P mobilization in subalpine forest soil due to citric acid through desorption and ligand exchange process rather than the acidification and lowering of the soil pH. Jones and Darrah [25] also reported that malic acid is poor comparison to citric acid for mobilizing micronutrients and P from all the test soils. Low molecular weight organic acids released Po rapidly in a short time, and then the release rate slowed down with the increase of time (Fig-1 and Fig-2). Zhu et al. [26] suggested that inorganic P and organic P were released by low molecular weight organic acids if the substrates of P and organic acid concentration were enough.

In low pH soil, organic acids mobilized much greater levels of phosphorus. In such environment, however, it is conceivable that the levels of free metals in solution may already be sufficient for plant growth. Organic acids released into high pH soils can be expected to have little effect on P mobilization, whilst at low pH they may be involved in a more general mechanism for the uptake of nutrients probably using a plasma lemma chelate-reductase. Fox et al. [27] suggested that it might be possible to estimate the relative effect of an organic acid on P availability based on its ability to complex metals. An interesting observation, we have, is the higher efficiency of lactic and formic acids for P mobilization, compared with citric acid. This is contradictory to previous reports of Traina et al. [28], where usually citric acid was found to be the most efficient. It seems that in the investigated soils with comparatively higher pH levels, a pH effect may also be responsible for organic acid induced mobilization of acid soluble P fractions. Steric effects which are related to the size or shape of the Po compounds, may affect the ability of the organic acids to displace the organic P. A definite explanation of these results will require a better understanding of the forms of Po present.

Phosphate dissolution rates can be greatly accelerated in soil in presence of organic acids such as malate, citrate and oxalate leading to 10-1000 fold higher soil solution P concentrations depending on soil type and speciation and concentration of organic acid [25, 27]. The extraction efficiency of inorganic P by organic acids from our experiments appears to follow the series: lactic acid > formic acid > citric acid > malic acid. Organic acid-induced P release depends on many factors including pH and soil mineralogy [25, 29]. There are at least two mechanisms by which P release can occur. The first involves direct ligand exchange, where organic acids, directly replace P on ligand exchange surfaces. The second could involve the complexation of metal exchange matrix holding the P. As metal ions are often released from soil concomitantly to P release it implies that the second pathway may be dominant although in reality both operate

simultaneously. However, the desorption/release of P is extremely soil dependent with generally high concentrations of organic acids [29] required to mobilize significant quantities of P into soil solution.

5. Conclusion

The results from our study suggest that organic acids play an important role in mineralization of organic P compound. To mobilize sufficient amount of organic P compounds for P nutrition of plants, it is necessary to increase organic acids concentration within the soil solution.

Compliance with ethical standards

Acknowledgments

The authors are thankful to the University Grants Commission (UGC) and Indian Council of Agricultural Research (ICAR) for providing the funds under which the present investigation was carried out.

Disclosure of conflict of interest

Both authors declare that there is no conflict of interest regarding the publication of this paper.

References

- [1] Tate K R. The biological transformation of P in soil. *Plant and Soil*. 1984; 76: 245-256.
- [2] George T S, Gregory P J, Wood M, Read D, Bresh R J. Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. *Soil Biology and Biochemistry*. 2002; 34: 1487-1494.
- [3] Islam A, Ahmed B. Distribution of inositol phosphate, phospholipids and nucleic acids and mineralization of inositol phosphate in some Bangladesh soils. *Journal of Soil Science*. 1973; 24: 193-198.
- [4] Jones D L. Organic acids in the rhizosphere – a critical review. *Plant and Soil*. 1998;205: 25-44.
- [5] Santi L P, Goenadi D H, Siswanto, Sailah I, Isroi. Solubilization of insoluble phosphates by *Aspergillus niger*. *Menara Perkebunan*. 2000; 68 : 37-47.
- [6] Feng-Guang L, Xiong-Liming. Exploring plant factors for increasing phosphorus utilization from rock phosphate and native soil phosphates in acidic soil. *International Atomic Energy Agency Technical Documents*. 2002; 1272: 211-223
- [7] Watanabe T, Osaki M, Yano H, Rao, I M. Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages. *Journal of Plant Nutrition*. 2006; 29(7): 1243-1255.
- [8] Stumm, W. Co-ordination interactions between soil solids and water. An aquatic chemist's point of view. *Geoderma*. 2006; 38: 19-30.
- [9] Martell A E, Motekaitis J J, Smith R M. Structure stability relationship of metal complexes and metal speciation in environmental aqueous solution. *Environmental Toxicology chem*. 1988; 7: 417-434.
- [10] Kee N G, Kwong K F, Huang P M. Influence of citric acid on the hydrolytic reaction of aluminium. *Soil Science Society of America Journal*. 1977; 41: 692-697.
- [11] Alberto A, George G, Sarmite K, Robert M, Leopold S, Jens B. Production of organic acids by arbuscular mycorrhizal fungi and their contribution in the mobilization of phosphorus bound to iron oxides. *Frontier in Plant Science*. 2021; 12 <https://doi.org/10.3389/fpls.2021.661842>
- [12] Lu W, Zhang F, Cao Y, Horst W J (ed), Schenk M K (ed.), Burkert A (ed), Classen N (ed.), Flessa H (ed.), Frommer W B (ed.), Goldbach H. (ed.), Olbs H W (ed.), Romheld V. Mobilization of soil phosphorus by low molecular weight organic acids. Fourteenth International Plant Nutrition Colloquium, Hannover Germany. 2001. 554-555.
- [13] Sperber J I. Solution of Apatite by Soil Microorganisms Producing Organic Acids. *Australian Journal of Agricultural Research*. 1958; 9: 778- 781.
- [14] Jackson M L. *Soil Chemical Analysis*. Delhi: Prentice-Hall of India; 1967. P. 498.
- [15] Marsehner H. *Mineral Nutrition of Higher Plants*. 2nd ed. London: Academic Press; 1995.

- [16] Ström L. Root exudation of organic acids : importance to nutrient availability and the calcifuge and calcicole behavior of plants. *Oikos*. 1997; 80: 459-466.
- [17] Farror J F, Jones D L. The control of carbon acquisition by roots. *New Phytologist*. 2000; 147: 187-199.
- [18] Seeling B, Jungk A. Utilization of organic phosphorus in calcium chloride extracts of soil by barley plants and hydrolysis of acid and alkaline phosphatases. *Plant and Soil*. 1996;178: 179-184.
- [19] Tarafdar J C, Jungk A. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biology and Fertility of Soils*. 1987;3: 199-204.
- [20] Tarafdar J C, Marschner H. Efficiency of VAM hyphae in utilization of organic phosphorus by wheat plants. *Soil Science and Plant Nutrition*. 1994; 40: 593-600.
- [21] Gharu A, Tarafdar J C. Influence of organic acids on mobilization of inorganic and organic phosphorus in soil. *Journal of the Indian Society of Soil Science*. 2004; 52 : 248-253.
- [22] Zhang B G, Li G T. Roles of soil organisms on the enhancement of plant availability of soil phosphorus. *Acta Pedologica Sinica*. 1998; 35: 104-111.
- [23] Zoysa A K N, Loganathan P, Hedley M J. Phosphate rock dissolution and transformation in the rhizosphere of tea (*Camellia sinensis* L.) compared with other plant species. *European Journal of Soil Science*. 1998; 49:477-486.
- [24] Krik, G J D. . A model of phosphate solubilization by organic anion excretion from plant roots. *European Journal of Soil Science*. 1999; 50: 369-378.
- [25] Jones D L, Darrah P R. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil*. 1994; 166: 247-257.
- [26] Zhu H, Bing, H. and Wu, Y. (2022). Citric Acid Promotes the Mobilization of Phosphorus under the Lower Concentration of Low Molecular Weight Organic Acids in Acidic Forest Soil. *Adsorption Science & Technology*. Volume 2022, Article ID 5071907
- [27] Fox R, Comerford N B, Mcfee W W. Phosphorous and Aluminium Release from a Spodic Horizon Mediated by Organic Acids. *Soil Science Society of America Journal*. 1990; 54: 1763-1767.
- [28] Traina S J, Sposito G, Bradford G R, Kafkafi V. Kinetic study of citrate effects on orthophosphate solubility in an acidic, montmorillonite soil. *Soil Science Society of America Journal*. 1987; 51: 1483-1487.
- [29] Lan M, Comerford N B, Fox T R. Organic anions effect on phosphorus release from spodic horizons. *Soil Science Society of America Journal*. 1995; 59: 1745-1749.