

# Phytate Exudation by the Roots of *Pteris Vittata* Can Dissolve Colloidal FePO<sub>4</sub>

**Sangar Khan**

Zhejiang University

**Paul J. Milham**

Western Sydney University - Hawkesbury Campus

**Kamel Mohamed Eltohamy**

Zhejiang University

**Yingbing Jin**

Zhejiang University

**Ziwan Wang**

Zhejiang University

**Xinqiang Liang** (✉ [liang410@zju.edu.cn](mailto:liang410@zju.edu.cn))

Zhejiang University <https://orcid.org/0000-0002-3521-9761>

---

## Research Article

**Keywords:** Phosphorus, iron phosphate, iron phosphate solubilizing activity, Phytate, *pteris vittata*

**Posted Date:** July 26th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-651377/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Environmental Science and Pollution Research on September 27th, 2021. See the published version at <https://doi.org/10.1007/s11356-021-16534-2>.

# Abstract

Phosphorus (P) is limiting nutrient in soil system. The P availability in soil strongly depend on Iron (Fe) speciation. Colloidal iron phosphate ( $\text{FePO}_{4\text{coll}}$ ) is an important phosphorus (P) fraction in soil solution that carry P from soil to water bodies. This study tested the hypothesis that phytate exudation by *Pteris vittata* (*P. vittata*) can dissolve  $\text{FePO}_{4\text{coll}}$  that leads to release of P and Fe. The phytate exudation in *P. vittata* increased from 434–2136  $\text{mg kg}^{-1}$  as the  $\text{FePO}_{4\text{coll}}$  concentration increased from 0–300 mM. The total P in *P. vittata* tissue increased from 2.88 to 8.28  $\text{g kg}^{-1}$ , the trichloroacetic acid P fractions (TCA fractions) were: inorganic P (0.86–5.10  $\text{mg g}^{-1}$ ), soluble organic P (0.25–0.87  $\text{mg g}^{-1}$ ), and insoluble organic P (0.16–2.03  $\text{mg g}^{-1}$ ) which leads to higher biomass as  $\text{FePO}_{4\text{coll}}$  increased from 0–300 mM. The linear regression analysis showed that  $\text{FePO}_4$  solubilizing activity has a positive correlation with TP, TCA P fractions in *P. vittata*, TP in growth media, and root exudates. This study shows that phytate exudation dissolved the  $\text{FePO}_{4\text{coll}}$  due to the chelation effect of phytic acid on Fe, and due to the high Fe–P solubilizing activity in root exudates of *P. vittata*.

## Introduction

Phosphorus (P) is an important nutrient for plant growth (Meyer et al. 2020) and world food production (Fresne et al., 2021), playing a major role in plant metabolic processes (He et al. 2020). Since the 1960s, the extensive use of P-fertilizers (Lin et al. 2019), and many other P products has caused excessive P levels to more frequently disturb water bodies and aquatic systems (Lei et al. 2020) and cause many environmental problems such as eutrophication (Liu et al. 2017a) and also threat to human health (Xu et al. 2020).

In soil solutions the colloids carrying P govern the mobility and availability of P in the aqueous phase of soil (Gottselig et al. 2017); however, the availability of P also depends on the P concentration and its speciation (Montalvo et al. 2015). The colloids (size 1–1000 nm in size) are rich in Al/Fe oxyhydroxides ((Montalvo et al., 2015; Wang et al., 2020; Eltohamy et al., 2021) and strongly sorb P in solution decreasing its availability (Montalvo et al. 2015), accelerate P mobility (Moradi et al. 2020), and transport P to the aquatic environment (Vendelboe et al. 2012). Also, the high amount of Fe/Al (hydr)oxides in Ah horizon promote the P binding in the colloidal phase via sorption and thereby promote P loss via leaching (Missong et al. 2018). The association of colloidal P with Fe oxyhydroxides showed the important role of Fe in catchment areas, the P mobility may be increased by the Fe rich colloids, but decreased the P availability (Baken et al. 2016a). Jiang et al.,(2015) claimed that the majority of P bind to Fe oxides, and after the dissolution of Fe oxides this P may be released and available for plants and microbes, while the previous study of Baken (et al., 2014) showed that Fe rich colloids can decrease the P bioavailability to algae and promote eutrophication of natural water. Thus, it is very important to reuse the  $\text{FePO}_{4\text{coll}}$  from soil solution before going to water bodies.

Plants release root exudates (Wang et al. 2013), which enhance the uptake of nutrients (Chen et al. 2017), however different plants have different root exudates which promote the uptake of nutrients (Han et al. 2017) and P utilization (Wang and Lambers 2019). In the rhizosphere, organic acids may act as metal chelators and affect the dissolution of mineral phases and the release of P from metals (Jones, 1998). P deficient conditions induce organic acid exudation in many plants (López-Arredondo et al. 2014), citric acid, malic acid, oxalic acid, and acetic acid are the most common organic acids in root exudates (Tao et al. 2020). For example, P deficiency induces citric acid exudations in white lupin (*Lupinus albus*) to manage P deficiency (Cheng et al. 2011), another study suggested that Oat (*Avena sativa*) roots also showed citric acid exudation in response to P deficiency (Wang et al. 2016) while one of a recent study suggested that Chinese brake fern (*Pteris vittata*) plant release phytic acid to cope with P deficiency (Fu et al. 2017). Chinese brake fern (*Pteris vittata*) is a widely distributed plant in Asia, Europe, Africa, Australia, can be grown in different environments (Wan et al. 2020), with a high yield of about 36 ton per hectare (Song et al. 2019). Besides, *Pteris vittata* (*P. vittata*) is also native to soils (tropical) of low nutrient status that contain mostly unavailable organic and inorganic P (Liu et al., 2017). This association suggests that *P. vittata* may access the P and Fe from insoluble phases such as those described in the preceding paragraph and/or mineralize organic P (Fu et al., 2017). Unlike the more typical organic acids (citrate and oxalate), phytic acid has been detected in root exudates of *P. vittata* (Tu et al., 2004) where it may be the main source of P storage as it is in cereal grains and their products (Thavarajah et al. 2009). Phytic acid forms a strong chelate with free Fe ions and consequently also adsorbs to Fe/Al minerals in the soil (Chen and Arai 2019). The twelve dissociable protons with six orthophosphate groups in phytic acid provide a strong chelation potential at different range pH (De Stefano et al. 2003). Its chelating ability increases as the valence cation increases, making it effective in chelating with Fe in soil (Trela 2010).

Colloidal P and Fe are frequently associated (Niyungeko et al., 2018; Fresne et al., 2021b; Jiang et al., 2015), and affect the uptake of P from colloids (Montalvo et al. 2015); Zhang et al., 2021). In addition, Segal et al. (2020) claimed that organic acid exudation may dissolve nano  $\text{FePO}_4$  and release P for plant uptake; however, they did not identify which organic acids may be involved. However, the above mentioned studies did not mention the potential role of phytate exudation in dissolution of  $\text{FePO}_{4\text{coll}}$ . There is however evidence that phytate exudation from *P. vittata* releases arsenic (an analog to P) from contaminated soil (Tu et al., (2004), but they were unable to explain the mechanism due to the complexities of working in a soil system. Therefore, in this study, we used a hydroponic system and investigated the effect of phytate exudation on  $\text{FePO}_{4\text{coll}}$  dissolution.

## Materials And Methods

### Plant conditioning

*P. vittata* (height 5–10 cm) was purchased from Guangdong province, and seedlings of pea (*Pisum sativum*) and lettuce (*Lactuca sativa*) from a local nursery in Hangzhou, Zhejiang province, China. The *P. vittata*, pea, and lettuce were first conditioned in 0.2-strength aerated Hoagland (Table S 1) solution at pH

6.5. The solution was buffered with 1 mM KOH-MES (2-(N-morpholino) ethane sulfonic acid) (Mathews et al. 2010). Water losses due to evapotranspiration were replaced daily with Milli-Q water and replaced every two weeks. The plants were raised in a growth chamber at ~70% relative humidity, 25 °C day/night temperature, and a 16 h light and 8 h darkness (Wan et al. 2010). When the new fronds and leaves were emerging, the conditioned plants were used in Experiments 2.2–2.4.

During conditioning, the plants were raised in a growth chamber at ~70% relative humidity, 25 °C day/night temperature, with 16 h of light per d (Wan et al. 2010).

### **Phosphorus accumulation experiment**

Six conditioned *P. vittata*, pea and lettuce plants were transferred to each pot containing 500 mL of half-strength aerated Hoagland nutrient solution containing 0, 50, 100, and 150 g P L<sup>-1</sup> as potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>). The modified half-strength aerated Hoagland nutrient solution was topped up daily with pure water to replace loss due to evapotranspiration, and solutions were replaced twice a week. Plants were harvested after 15 d, and the roots were separated from the leaves and dried at 80 °C.

Subsamples of dried leaves/fronds roots were ground and digested in concentrated nitric acid (HNO<sub>3</sub>). Diluted digests were analyzed for P using an inductively coupled plasma-atomic emission spectrometer (ICP-OES) according to the manufacturer's instructions (iCAP 6000 series, Thermo Fisher scientific, USA).

### **Organic acid excretion in *P. vittata*, pea and lettuce**

Six conditioned *P. vittata*, pea and lettuce plants (Section 2.1) were transferred to pots containing 500 mL of P and Fe free, half strength Hoagland nutrient solution, buffered at pH 6.0 with 0.5 M MES buffer for 3 d to measure the release of organic acids (Fayiga et al. 2008). The organic acid concentrations were determined as described in Section S 2.3.

### **Effect of phytate exudation on FePO<sub>4coll</sub> dissolution**

We conducted the experiment in Fe- and P-free 0.2-strength Hoagland media. Five conditioned *P. vittata* plants (Section 2.1) were grown in 500 mL per pot of sterile, Fe and P free, MES (0.5 M) buffered half strength Hoagland solution to investigate the role of phytic acid excretion on the dissolution of FePO<sub>4coll</sub>. The FePO<sub>4coll</sub> was prepared and characterized as described in the supplementary material (S 2.2, Fig. 1). The hydroponic solutions contained solid phase FePO<sub>4coll</sub> at mass/volume concentrations of: i) 0 mM FePO<sub>4coll</sub> (control), ii) 100 mM FePO<sub>4coll</sub>, iii) 200 mM FePO<sub>4coll</sub>, and iv) 300 mM FePO<sub>4coll</sub>. Chloramphenicol at 30 mg L<sup>-1</sup> was added to minimize microbial activity. Evapotranspiration losses were replaced with pure water daily and the solution was replaced every two weeks. Analysis for P and Fe in the growth media and *P. vittata* was conducted as in section S 2.4.

### **Organic acid reaction with FePO<sub>4coll</sub> in vitro**

Solid  $\text{FePO}_{4\text{coll}}$  was prepared and characterized after Liu et al., (2017) as detailed in supplementary material (S 2.2). The in vitro experiment was performed in 10 mM  $\text{NaClO}_4$ . The 100  $\mu\text{M}$   $\text{FePO}_{4\text{coll}}$  was mixed with phytic acid, oxalic and citric acid at organic acid:  $\text{FePO}_{4\text{coll}}$  molar ratio (1:1, 2:1, 3:1, 10:1, 30:1, and 60:1) in a centrifuge tube with final volume 50 mL. The pH was adjusted to 6.0 after the addition of organic acids using 0.1 L NaOH and HCl, and the redox potential of the solution were maintained at 67.2 mV. To minimize microbial activity, chloramphenicol at 30 mg  $\text{L}^{-1}$  was also added (Subbarao et al. 1997). Samples were shaken at 200 rpm for 24 h at 28 °C then centrifuged at 5000 rpm for 10 min. The supernatant was analyzed for P and Fe using ICP-MS calibrated and operated as per the manufacturer's instructions (NexION300X, PerkinElmer, USA).

## Statistical Analysis

Results were compared using analysis of variance and a 5% significance level with the least significant difference (Tukey). Linear regression modeling was used to predict the effect of the Fe–P solubilizing activity and TCA fractions in fern, TP in root exudates and growth media and effect of TP in *P. vittata*, growth media and root exudates and Fe in growth media on biomass of *P. vittata* by using the SPSS software (Version 20.0. SPSS Inc, USA), while the origin 2020 (Origin Lab Corporation, USA) was used for graphical illustration. Visual Minteq 3.1 was used to predict the phytate, and  $\text{FePO}_{4\text{coll}}$  speciation in hydroponic media.

## Results

### $\text{FePO}_{4\text{coll}}$ characterization

The SEM images of the white  $\text{FePO}_{4\text{coll}}$  powder showed that the particle had aggregates of 2  $\mu\text{m}$  and 500 nm size with particle diameter ranged from 273–435 nm (Fig 1 A, B, C, D). The XRD pattern identified  $\text{FePO}_{4\text{coll}}$  at 33° and DLS analysis showed the particle size ranged from 200–550 nm.

### P accumulation and organic acid exudation

When exposed to 0–150 mg  $\text{P L}^{-1}$ , the concentration of P in *P. vittata* was 2.2–9.6 g  $\text{P kg}^{-1}$ , while in lettuce and pea the concentrations were 3.2–7.6 and 3.3–6.5 g  $\text{P kg}^{-1}$  respectively (Fig 2). Limiting the supply of P and Fe induced *P. vittata* to excrete phytic acid (155 mg  $\text{kg}^{-1}$ g and citric acid 310 mg  $\text{kg}^{-1}$  of fresh weight) (Fig 2 A). Pea and lettuce excreted only citric acid, and the concentrations were about half that excreted by *P. vittata* (Fig 2 B). Increasing the  $\text{FePO}_{4\text{coll}}$  concentration caused progressive increases in the excreted concentration of phytic acid (Fig 2 C). The *P. vittata* exposed to 300 mM  $\text{FePO}_{4\text{coll}}$ , released significantly more phytic acid ( $P<0.05$ ) (2136 mg  $\text{kg}^{-1}$ ) than the plants exposed to 200 mM (749 mg  $\text{kg}^{-1}$ ), 100 mM (444 mg  $\text{kg}^{-1}$ ) and 0mM  $\text{FePO}_{4\text{coll}}$  (434 mg  $\text{kg}^{-1}$ ).

### Role of phytate exudation in $\text{FePO}_{4\text{coll}}$ dissolution

In *P. vittata*, TP, TCA fractions and Fe contents also increased with increasing  $\text{FePO}_{4\text{coll}}$  concentration. The total P concentration increased from 2.88 to 8.28  $\text{g kg}^{-1}$  and the TCA fractions were: inorganic P (0.86–5.10  $\text{mg g}^{-1}$ ), soluble organic P (0.25–0.87  $\text{mg g}^{-1}$ ), and insoluble organic P (0.16–2.03  $\text{mg g}^{-1}$ ) (Fig 3). There was also a dose response relationship between the external concentration of  $\text{FePO}_{4\text{coll}}$  and the internal concentrations of Fe, e.g., with 300 mM  $\text{FePO}_{4\text{coll}}$  the plants contained the highest Fe concentration ( $P < 0.05$ , 3.56  $\text{g kg}^{-1}$  fresh weight) (Fig 3). The total P in root exudates also increased as the treatments concentration increases (Fig 3).

### **Biomass Fe, P content in growth media, Phytase and Fe-P solubilizing activity**

The biomass of *P. vittata* also increased as the  $\text{FePO}_{4\text{coll}}$  concentration increased from 0–300 mM (Fig 4). The concentrations of P and Fe in the hydroponic media followed dose response relationship with the  $\text{FePO}_{4\text{coll}}$  addition. The P and Fe were higher (80.65 and 51.06 mM) ( $P < 0.05$ ) in 300 mM followed by 200 mM (72.32 and 36.1 mM) while 100 mM have less P and Fe concentration (Fig 4). In root exudates, the phytase and Fe-P solubilizing activity increased as the concentration of  $\text{FePO}_{4\text{coll}}$  increased from 0–300 mM. The phytase activity was greater in 300 mM  $\text{FePO}_{4\text{coll}}$  (8.3  $\text{h}^{-1}\text{g}^{-1}$  root FW), followed by 200 and 100 mM, while the control has the least phytase activity (3.2  $\text{h}^{-1}\text{g}^{-1}$  root FW). The same trend was observed in the Fe-P solubilizing activity which also increased from 0.86–6.85 ( $\mu\text{g P mL}^{-1}$ ) as  $\text{FePO}_{4\text{coll}}$  concentration increased from 0–300 mM (Fig 4).

### **Linear regression between Fe-P solubilizing activity and P fractions**

Linear regression analysis showed that  $\text{FePO}_4$  solubilizing activity have strong and significantly ( $P < 0.05$ ) correlate with *P. vittata* TP ( $R^2 = 0.93$ ), inorganic P ( $R^2 = 0.89$ ), soluble organic P ( $R^2 = 0.86$ ) but a weak correlation with insoluble organic P ( $R^2 = 0.21$ ) in *P. vittata* tissue (Fig 5). The analogous effects ( $P < 0.05$ ) in the growth media were on TP ( $R^2 = 0.86$ ), and on TP in root exudates ( $R^2 = 0.99$ ) (Figure 5). We also find a strong and significant correlation between biomass and P ( $R^2 = 0.80$ ), Fe ( $R^2 = 0.91$ ) in growth media, P in root exudates ( $R^2 = 0.80$ ) and TP in *P. vittata* tissue ( $R^2 = 0.91$ ) (Fig 5).

### **Solubilizing effects of organic acids on $\text{FePO}_{4\text{coll}}$ in vitro**

The dissolution of  $\text{FePO}_{4\text{coll}}$  increases as the organic acids:  $\text{FePO}_{4\text{coll}}$  molar ratio increases (Fig 6). In the case of phytic acid, at molar ratio 1:1 (phytic acid:  $\text{FePO}_{4\text{coll}}$ ), 32.5 and 3.12  $\mu\text{M}$  P and Fe released respectively, while the same molar ratios in citric and oxalic acids released P (14.65 and 16.3  $\mu\text{M}$ ) and Fe (4.5 and 5.4  $\mu\text{M}$ ). At a higher molar ratio of 60:1 (organic acids:  $\text{FePO}_{4\text{coll}}$ ) the phytic, citric acid, and oxalic acid released 69.8, 45.2 and 54.3  $\mu\text{M}$  P respectively. At the same molar ratio, the Fe concentrations were 69.8, 36.82 and 25.6  $\mu\text{M}$  in phytic acid, oxalic acid and citric acid respectively (Fig. 5). The results were confirmed by visual MINTEQ software, which showed that at lower phytic acid:  $\text{FePO}_{4\text{coll}}$  more P and less Fe released with high % of Fe-phytate, while at high molar ratio (60:1) the more P and Fe released with low % of Fe-phytate (Table 1).

# Discussion

## P accumulation by *P. vittata*

*Pteris vittata* accumulated a high amount of P (Fig 2 A) and released phytic acid (Fig. 2 B) while lettuce and pea plants accumulated less amount of P and did not release phytic acid. Our results are in agreement with Lessl and Ma (2013), who suggested *P. vittata* as a model plant for P uptake and Fu (et al., 2017) observed that under P limited condition, *P. vittata* can release phytic acid. On basis of high P uptake and phytic acid exudation, we only select *P. vittata* for  $\text{FePO}_{4\text{coll}}$  dissolution.

## Phytic acid exudation

*Pteris vittata* released phytic acid under P and Fe limited conditions ( $\text{FePO}_{4\text{coll}}$ , 0–300 mM) (Fig 2 C). Phytic acid is a major source of P in plants and most plants do not secrete it under environmental stress, e.g. *Arabidopsis thaliana*, *Triticum aestivum*, and *Brassica* species release malic and citric acids. Similarly, *Oryza sativa* releases only citric acid, and *P. esinoformis* releases oxalic and citric acids (Fu et al., 2017). Plants commonly release organic acids because they release P through the desorption of P from metal oxide via ligand exchange, and the release of  $\text{H}^+$  plays an important role in the dissolution of P from metal oxides (Jones 1998). The rhizosphere pH depends upon the  $\text{HCO}_3^-$ ,  $\text{OH}^-$  and  $\text{H}^+$  (secreted by roots) which is determined by the complement of ions taken up by roots (Hinsinger et al. 2003). There is also a possibility that *P. vittata* may release phytate due to Fe stress by analogy with the secretion of phytosiderophores (Ahmed and Holmström 2014) that complex Fe (Li et al., 2020), facilitating transport to the roots (Ahmed and Holmström 2014).

## Role of phytate in $\text{FePO}_{4\text{coll}}$ dissolution

The secreted phytate in root exudates may dissolve  $\text{FePO}_{4\text{coll}}$  in the following ways 1) lower down pH or forming the insoluble complex with metals at the mineral surface which further change the mineral electric surface potential negative and affect the P binding to mineral and 2) by the effect of phosphorylated inositol rings on Fe (Wang and Lambers, 2019; Lambers and Plaxton, 2015; Violante and Caporale, 2015). However, in our experiment the pH was not the main factor that dissolved the  $\text{FePO}_{4\text{coll}}$ , the organic acid exudation changes cause acidification, but the rhizosphere acidification and the organic acid exudations are the biochemically different process (Wang and Lambers 2019). There is also possibility that the negative charged phosphorylated inositol rings make chelate with Fe, more phosphorylated rings, stronger chelated and lower the solubility. The phytate from water soluble iron phytate with ferric ion but insoluble with tetra Fe (Nielsen et al. 2013). In our experiment, the uptake of P (Fig 3) shows that released phytic acid make chelated with Fe and dissolved the  $\text{FePO}_{4\text{coll}}$ . This result is consistent with the *in vitro* chelating experiments which show that at 1:1 molar ratio (phytic acid:  $\text{FePO}_{4\text{coll}}$ ) there was a strong chelating ability (Table 2 and Fig 6). The data showed that Fe was precipitated by phytic acid as Fe-phytate at a low concentration of phytic acid (Table 2). These data were confirmed by visual MINTEQ software (Table 1). At low concentration of phytic acid the strong chelation

may be due to the insoluble Fe-phytate, and as concentration increased the Fe-phytate became water soluble. The Fe uptake may be because of plant secreted siderophores phyto siderophores that make complex with Fe, then transported to plant across the root plasma membrane (Ahmed and Holmström 2014). The transported Fe-siderophore complex reduced to  $\text{Fe}^{2+}$  in the plant cell membrane and then uptake by cells. However, there is a need more investigation for *P. vittata* and phyto siderophores role in  $\text{FePO}_{4\text{coll}}$  dissolution. Our results agreed with Sega et al. (2020) who claimed that root exudation may dissolve the  $\text{FePO}_4$  nano particles which release P and Fe. However, they did not mention which organic acid can dissolve the  $\text{FePO}_4$ .

### **Role of phytase and Fe-P solubilizing activity in $\text{FePO}_{4\text{coll}}$ dissolution**

The phytase enzyme also plays the dominant role in P and Fe released in our experiment. Under P starvation, plant cells promote the activity of phytases (Tu et al. 2011). A previous study by Zhang et al., (2007) showed that Fe adsorption and bioavailability from Fe-phytate can be improved by phytase enzymes, added to oat-based beverages. The phytase secretion in our experiment is independent of microbial activity under limited P concentration, which further confirms that *P. vittata* can secrete phytase enzyme to promote plant growth by hydrolyzing phytic acid and Fe-phytate (Fig 4). This is consistent with our finding of high concentrations of Fe and P in growth media and also in *P. vittata* tissue (Fig 4). We also claimed that Fe-P solubilizing activity in root exudates of *P. vittata* showed an important role in  $\text{FePO}_{4\text{coll}}$  dissolution. The positive correlations showed (Fig 5) that with the increase in Fe-P solubilizing activity, the *P. vittata* uptake more Fe and P, which further confirmed by the linear regression of biomass with best predictive variable (Fig 5). These results extend those of Subbarao et al., (1997), by showing positive correlations between Fe-P solubilizing activity and the P fractions in *P. vittata*. This may explain the conflicted environmental adaptation of excreting a P-rich substance (phytate) in response to a P-limited environment. Several studies on *P. vittata* showed the exudation of phytic acid under stress caused by root exposure to minerals such as phosphate rock and  $\text{FeAsO}_4$  (Fu et al., 2017 and Liu et al., 2016).

## **Conclusion And Environmental Implications**

In conclusion, phytic acid released by *P. vittata* efficiently dissolved  $\text{FePO}_{4\text{coll}}$  releasing phosphate and iron. The phytase activity in root exudates may have facilitated the uptake of the dissolved Fe and P, since  $\text{FePO}_{4\text{coll}}$  increased both biomass and the internal concentrations of P and Fe. Our results indicate that Fe-P solubilizing activity is the unique property in root exudates of *P. vittata*, however the genetic mechanism should be investigated about the Fe-P solubilizing activity in root exudates.

Phosphorus play important role in non-point source pollution (Liu et al. 2016a). The colloidal P transported by Fe rice colloids from soil to water bodies which are mostly unavailable and leads to eutrophication (Baken et al. 2016b). In addition, many plants cannot dissolve iron phosphate in soil solution, however *P. vittata* is seems to be best solution to dissolve the  $\text{FePO}_{4\text{coll}}$  in soil before it reaching

to the water bodies. The molecular mechanisms and environmental adaptive significance of *P. vittata* and iron phosphate should be investigated.

## Declarations

**Ethics approval:** All ethical standards have been followed during this research.

**Consent to participate:** Not applicable.

**Consent to publish:** Not applicable.

**Availability of data and materials:** All data generated or analysed during this study are included in this article (and its supplementary information files).

**Competing interests:** The authors declare no competing interests

**Funding:** We are grateful for grants from the National Natural Science Foundation of China (22076163; 41522108) and the Natural Science Foundation of Zhejiang Province (LR16B070001).

## Authors contribution

Sangar Khan conducted the experiment, analysed the data and wrote the manuscript. Xinqiang Liang supervised and contributed to all aspects of the study. Paul J. Milham revised the manuscript, Kamel Mohamed Eltohamy revised the graphical work, Yingbing Jin contributed to data interpretation and Ziwan Wang helped in preparation of the manuscript.

## Acknowledgements

We acknowledge the National Natural Science Foundation of China, the Natural Science Foundation of Zhejiang Province and Key Laboratory of Water Pollution Control and Environmental Security Technology, Zhejiang Province, College of Environmental Resource Sciences, Zhejiang University.

## References

- Ahmed E, Holmström SJM (2014) Siderophores in environmental research: Roles and applications. *Microb Biotechnol* 7:196–208. <https://doi.org/10.1111/1751-7915.12117>
- Baken S, Moens C, van der Grift B, Smolders E (2016a) Phosphate binding by natural iron-rich colloids in streams. *Water Res* 98:326–333. <https://doi.org/10.1016/j.watres.2016.04.032>
- Baken S, Nawara S, Van Moorlegghem C, Smolders E (2014) Iron colloids reduce the bioavailability of phosphorus to the green alga *Raphidocelis subcapitata*. *Water Res* 59:198–206. <https://doi.org/10.1016/j.watres.2014.04.010>

- Baken S, Regelink IC, Comans RNJ, et al (2016b) Iron-rich colloids as carriers of phosphorus in streams: A field-flow fractionation study. *Water Res* 99:83–90. <https://doi.org/10.1016/j.watres.2016.04.060>
- Chen A, Arai Y (2019) Functional Group Specific Phytic Acid Adsorption at the Ferrihydrite-Water Interface. *Environ Sci Technol* 53:8205–8215. <https://doi.org/10.1021/acs.est.9b01511>
- Chen YT, Wang Y, Yeh KC (2017) Role of root exudates in metal acquisition and tolerance. *Curr Opin Plant Biol* 39:66–72. <https://doi.org/10.1016/j.pbi.2017.06.004>
- Cheng L, Bucciarelli B, Shen J, et al (2011) Update on white lupin cluster root acclimation to phosphorus deficiency. *Plant Physiol* 156:1025–1032. <https://doi.org/10.1104/pp.111.175174>
- De Stefano C, Milea D, Pettignano A, Sammartano S (2003) Speciation of phytate ion in aqueous solution. Alkali metal complex formation in different ionic media. *Anal Bioanal Chem* 376:1030–1040. <https://doi.org/10.1007/s00216-003-2056-1>
- Eltohamy KM, Liu C, Khan S, et al (2021) An internet-based smart irrigation approach for limiting phosphorus release from organic fertilizer-amended paddy soil. *J Clean Prod* 293:126254. <https://doi.org/10.1016/j.jclepro.2021.126254>
- Fayiga AO, Ma LQ, Rathinasabapathi B (2008) Effects of nutrients on arsenic accumulation by arsenic hyperaccumulator *Pteris vittata* L. *Environ Exp Bot* 62:231–237. <https://doi.org/10.1016/j.envexpbot.2007.09.001>
- Fresne M, Jordan P, Fenton O, et al (2021) Soil chemical and fertilizer influences on soluble and medium-sized colloidal phosphorus in agricultural soils. *Sci Total Environ* 754:. <https://doi.org/10.1016/j.scitotenv.2020.142112>
- Fu JW, Liu X, Han YH, et al (2017) Arsenic-hyperaccumulator *Pteris vittata* efficiently solubilized phosphate rock to sustain plant growth and As uptake. *J Hazard Mater* 330:68–75. <https://doi.org/10.1016/j.jhazmat.2017.01.049>
- Gottselig N, Nischwitz V, Meyn T, et al (2017) Phosphorus Binding to Nanoparticles and Colloids in Forest Stream Waters. *Vadose Zo J* 16:vzj2016.07.0064. <https://doi.org/10.2136/vzj2016.07.0064>
- Han YH, Liu X, Rathinasabapathi B, et al (2017) Mechanisms of efficient As solubilization in soils and As accumulation by As-hyperaccumulator *Pteris vittata*. *Environ Pollut* 227:569–577. <https://doi.org/10.1016/j.envpol.2017.05.001>
- He H, Wu M, Guo L, et al (2020) Release of tartrate as a major carboxylate by alfalfa (*Medicago sativa* L.) under phosphorus deficiency and the effect of soil nitrogen supply. *Plant Soil*. <https://doi.org/10.1007/s11104-020-04481-9>

- Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. *Plant Soil* 248:43–59.  
<https://doi.org/10.1023/A:1022371130939>
- Jiang X, Bol R, Willbold S, et al (2015) Speciation and distribution of P associated with Fe and Al oxides in aggregate-sized fraction of an arable soil. *Biogeosciences* 12:6443–6452. <https://doi.org/10.5194/bg-12-6443-2015>
- Jones DL (1998) Organic acids in the rhizosphere - A critical review. *Plant Soil* 205:25–44.  
<https://doi.org/10.1023/A:1004356007312>
- Lambers H, Plaxton WC (2015) Phosphorus: Back to The Roots. *Phosphorus Metab Plants* 48:3–22.  
<https://doi.org/10.1002/9781118958841.ch1>
- Lei Y, Geraets E, Saakes M, et al (2020) Electrochemical removal of phosphate in the presence of calcium at low current density: Precipitation or adsorption. *Water Res* 169:115207.  
<https://doi.org/10.1016/j.watres.2019.115207>
- Lessl JT, Ma LQ (2013) Sparingly-soluble phosphate rock induced significant plant growth and arsenic uptake by *Pteris vittata* from three contaminated soils. *Environ Sci Technol* 47:5311–5318.  
<https://doi.org/10.1021/es400892a>
- Li X, Peng P, Long J, et al (2020) Plant-induced insoluble Cd mobilization and Cd redistribution among different rice cultivars. *J Clean Prod* 256:120494. <https://doi.org/10.1016/j.jclepro.2020.120494>
- Lin Z, Wang Y, Huang W, et al (2019) Single-stage denitrifying phosphorus removal biofilter utilizing intracellular carbon source for advanced nutrient removal and phosphorus recovery. *Bioresour Technol* 277:27–36. <https://doi.org/10.1016/j.biortech.2019.01.025>
- Liu J, Luo X, Zhang N, Wu Y (2016a) Phosphorus released from sediment of Dianchi Lake and its effect on growth of *Microcystis aeruginosa*. *Environ Sci Pollut Res* 23:16321–16328.  
<https://doi.org/10.1007/s11356-016-6816-9>
- Liu X, Fu JW, Guan DX, et al (2016b) Arsenic induced phytate exudation, and promoted FeAsO<sub>4</sub> dissolution and plant growth in as-hyperaccumulator *pteris vittata*. *Environ Sci Technol* 50:9070–9077.  
<https://doi.org/10.1021/acs.est.6b00668>
- Liu Y, Cao X, Li H, et al (2017a) Distribution of phosphorus-solubilizing bacteria in relation to fractionation and sorption behaviors of phosphorus in sediment of the Three Gorges Reservoir. *Environ Sci Pollut Res* 24:17679–17687. <https://doi.org/10.1007/s11356-017-9339-0>
- Liu Y, Li Z, You Y, et al (2017b) Synthesis of different structured FePO<sub>4</sub> for the enhanced conversion of methyl cellulose to 5-hydroxymethylfurfural. *RSC Adv* 7:51281–51289.  
<https://doi.org/10.1039/c7ra09186a>

- López-Arredondo DL, Leyva-González MA, González-Morales SI, et al (2014) Phosphate nutrition: Improving low-phosphate tolerance in crops. *Annu Rev Plant Biol* 65:95–123. <https://doi.org/10.1146/annurev-arplant-050213-035949>
- Mathews S, Ma LQ, Rathinasabapathi B, et al (2010) Arsenic transformation in the growth media and biomass of hyperaccumulator *Pteris vittata* L. *Bioresour Technol* 101:8024–8030. <https://doi.org/10.1016/j.biortech.2010.05.042>
- Meyer G, Bell MJ, Doolette CL, et al (2020) Plant-Available Phosphorus in Highly Concentrated Fertilizer Bands: Effects of Soil Type, Phosphorus Form, and Coapplied Potassium. *J Agric Food Chem* 68:7571–7580. <https://doi.org/10.1021/acs.jafc.0c01287>
- Missong A, Holzmann S, Bol R, et al (2018) Leaching of natural colloids from forest topsoils and their relevance for phosphorus mobility. *Sci Total Environ* 634:305–315. <https://doi.org/10.1016/j.scitotenv.2018.03.265>
- Montalvo D, Degryse F, McLaughlin MJ (2015) Natural colloidal P and its contribution to plant P uptake. *Environ Sci Technol* 49:3427–3434. <https://doi.org/10.1021/es504643f>
- Moradi G, Bol R, Trbojevic L, et al (2020) Contrasting depth distribution of colloid-associated phosphorus in the active and abandoned sections of an alluvial fan in a hyper-arid region of the Atacama Desert. *Glob Planet Change* 185:. <https://doi.org/10.1016/j.gloplacha.2019.103090>
- Nielsen AVF, Tetens I, Meyer AS (2013) Potential of phytase-mediated iron release from cereal-based foods: A quantitative view. *Nutrients* 5:3074–3098. <https://doi.org/10.3390/nu5083074>
- Sega D, Baldan B, Zamboni A, Varanini Z (2020) FePO<sub>4</sub> NPs Are an Efficient Nutritional Source for Plants: Combination of Nano-Material Properties and Metabolic Responses to Nutritional Deficiencies. *Front Plant Sci* 11:. <https://doi.org/10.3389/fpls.2020.586470>
- Song Y, Liu J, Evrendilek F, et al (2019) Combustion behaviors of *Pteris vittata* using thermogravimetric, kinetic, emission and optimization analyses. *J Clean Prod* 237:117772. <https://doi.org/10.1016/j.jclepro.2019.117772>
- Subbarao GV, Ae N, Otani T (1997) Genotypic variation in iron-, and aluminum-phosphate solubilizing activity of pigeonpea root exudates under p deficient conditions. *Soil Sci Plant Nutr* 43:295–305. <https://doi.org/10.1080/00380768.1997.10414754>
- Tao Q, Zhao J, Li J, et al (2020) Unique root exudate tartaric acid enhanced cadmium mobilization and uptake in Cd-hyperaccumulator *Sedum alfredii*. *J Hazard Mater* 383:121177. <https://doi.org/10.1016/j.jhazmat.2019.121177>
- Thavarajah P, Thavarajah D, Vandenberg A (2009) Low phytic acid lentils (*Lens culinaris* L.): A Potential Solution for Increased Micronutrient Bioavailability. *J Agric Food Chem* 57:9044–9049.

<https://doi.org/10.1021/jf901636p>

Trela BC (2010) Iron stabilization with phytic acid in model wine and wine. *Am J Enol Vitic*

Tu S, Ma L, Luongo T (2004) Root exudates and arsenic accumulation in arsenic hyperaccumulating *Pteris vittata* and non-hyperaccumulating *Nephrolepis exaltata*. *Plant Soil* 258:9–19.

<https://doi.org/10.1023/B:PLSO.0000016499.95722.16>

Tu S, Ma L, Rathinasabapathi B (2011) Characterization of phytase from three ferns with differing arsenic tolerance. *Plant Physiol Biochem* 49:146–150. <https://doi.org/10.1016/j.plaphy.2010.11.004>

Vendelboe AL, Schjønning P, Moldrup P, et al (2012) Colloid Release from differently managed loess soil. *Soil Sci* 177:301–309. <https://doi.org/10.1097/SS.0b013e3182506dd3>

Violante A, Caporale AG (2015) Biogeochemical processes at soil-root interface. *J Soil Sci Plant Nutr* 15:422–448. <https://doi.org/10.4067/s0718-95162015005000038>

Wan X, Lei M, Chen T (2020) Review on remediation technologies for arsenic- contaminated soil. 14:

Wan XM, Lei M, Huang ZC, et al (2010) Sexual propagation of *Pteris vittata* L. influenced by pH, calcium, and temperature. *Int J Phytoremediation* 12:85–95. <https://doi.org/10.1080/15226510902767148>

Wang J, Lv J, Fu Y (2013) Effects of organic acids on Cd adsorption and desorption by two anthropic soils. *Front Environ Sci Eng China* 7:19–30. <https://doi.org/10.1007/s11783-012-0424-9>

Wang L, Missong A, Amelung W, et al (2020) Dissolved and colloidal phosphorus affect P cycling in calcareous forest soils. *Geoderma* 375:114507. <https://doi.org/10.1016/j.geoderma.2020.114507>

Wang Y, Krogstad T, Clarke JL, et al (2016) Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. *Front Plant Sci* 7:1–14. <https://doi.org/10.3389/fpls.2016.01664>

Wang Y, Lambers H (2019) Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. *Plant Soil* 2050:135–156. <https://doi.org/10.1007/s11104-019-03972-8>

Xu Q, Xiao K, Wang H, et al (2020) Insight into effects of organic and inorganic phosphorus speciations on phosphorus removal efficiency in secondary effluent. *Environ Sci Pollut Res* 27:11736–11748. <https://doi.org/10.1007/s11356-020-07774-9>

Zhang H, Önning G, Öste R, et al (2007) Improved iron bioavailability in an oat-based beverage: The combined effect of citric acid addition, dephytinization and iron supplementation. *Eur J Nutr* 46:95–102. <https://doi.org/10.1007/s00394-006-0637-4>

Zhang Q, Bol R, Amelung W, et al (2021) Geoderma Water dispersible colloids and related nutrient availability in Amazonian Terra Preta soils. *Geoderma* 397:115103.  
<https://doi.org/10.1016/j.geoderma.2021.115103>

## Tables

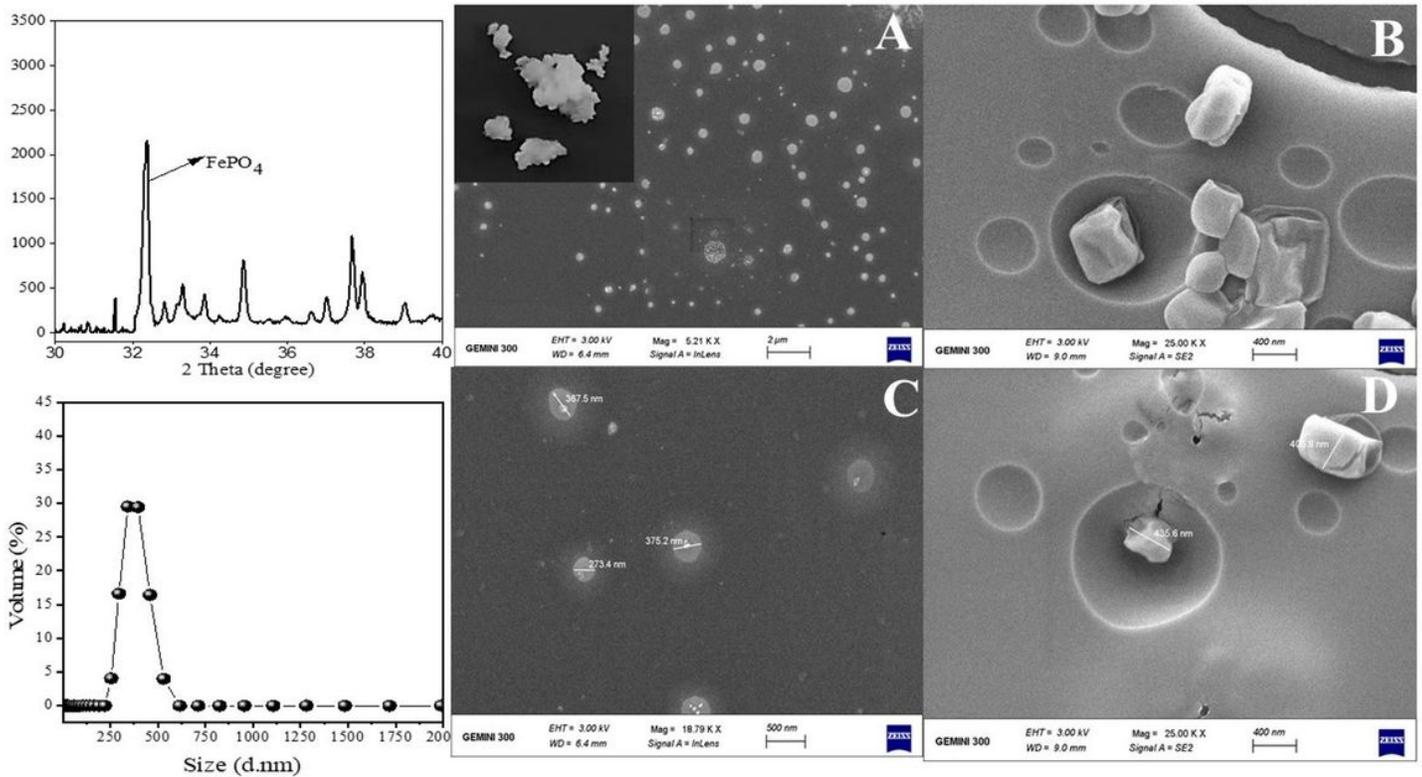
**Table 1. Prediction of P, Fe and Fe-phytate by using Visual MINTEQ**

Phytic acids: FePO <sub>4coll</sub>	Predicted P (µM)	Predicted Fe-phytate (µM)	Predicted Fe (µM)	Means with SE (n =3), values followed by the same letter are not significantly different (P<0.05).  <b>Table 2. Fe concentrations in solution released from FePO<sub>4coll</sub> by phytic acids at various organic acids: FePO<sub>4coll</sub> molar ratios <i>in vitro</i></b>
1:1	5.1±0.52 <sup>e</sup>	92±1.09 <sup>a</sup>	2.2±0.65 <sup>f</sup>	
2:1	8.0±0.12 <sup>d</sup>	83±0.68 <sup>b</sup>	3.2±0.88 <sup>e</sup>	
3:1	8.6±1.02 <sup>d</sup>	84±1.1 <sup>b</sup>	5.8±0.97 <sup>d</sup>	
10:1	22±1.09 <sup>c</sup>	69±0.68 <sup>c</sup>	19±1.0 <sup>c</sup>	
30:1	64±1.2 <sup>b</sup>	44±0.94 <sup>d</sup>	35±0.25 <sup>b</sup>	
60:1	95±0.78 <sup>a</sup>	2±0.25 <sup>e</sup>	55±0.68 <sup>a</sup>	

Phytic acids: FePO <sub>4coll</sub>	Organic acid (µM)	* Fe release (µM) in phytic acid	Fe in solution (µM)	Phytic acid: released Fe molar ratio	Fe precipitated by phytic acid (%)
1:1	100	32.5	3.12	3:1	90.4
2:1	200	37.7	7.13	5.3:1	81.0
3:1	300	41.57	7.76	7.2:1	81.3
10:1	1000	55.8	18.3	17.9:1	67.2
30:1	3000	63.23	35.4	47.6:1	43.9
60:1	6000	72.8	69.8	82.4:1	4.6

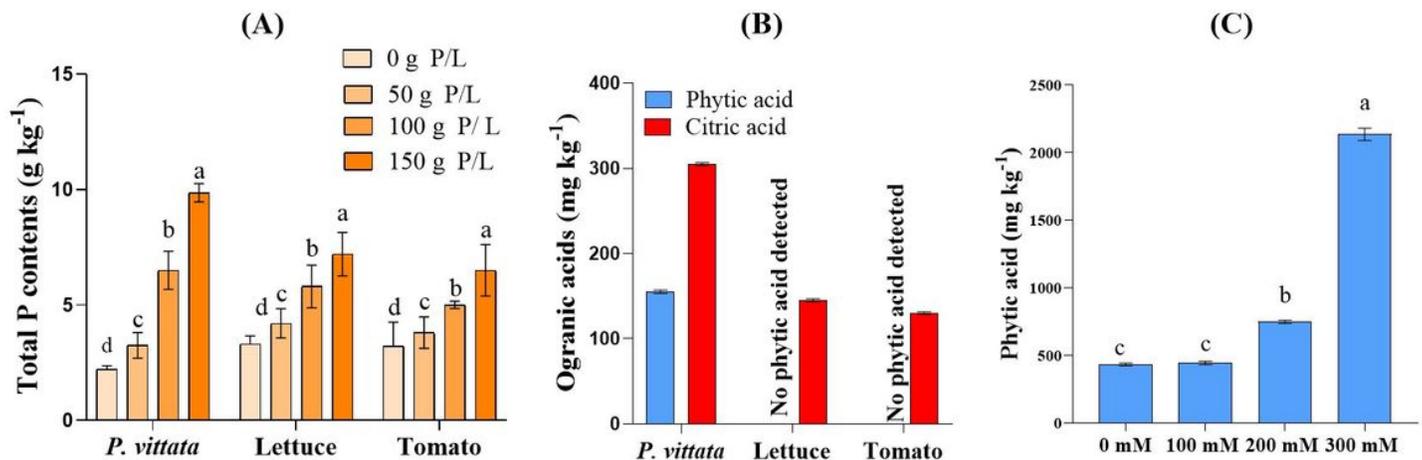
\* concentration of Fe released was calculated on the basis of the measured P concentration in the media.

## Figures



**Figure 1**

Characterization of FePO<sub>4</sub>coll by SEM, DLS and XRD pattern. Fig A represent the final product of FePO<sub>4</sub>coll and SEM characterization at 2μm, (B) 400nm, C and D represent the size diameter of FePO<sub>4</sub>coll at 500nm and 400 nm scale



**Figure 2**

Total phosphorus concentration (A) of *P. vittata*, lettuce and pea grown in half strength Hoagland solutions containing 0, 50, 100 and 150 g P L<sup>-1</sup> as KH<sub>2</sub>PO<sub>4</sub> and organic acid exudation (B) in *P. vittata*,

lettuce and pea grown in P and Fe free half strength Hoagland solutions containing and phytic acid exudation in *P. vittata* (C) grown in P and Fe half strength Hoagland solutions containing different concentration (0-100 mM) of  $\text{FePO}_4\text{coll}$ . Error bars indicate (A and C) the SE (n =3), values followed by the same letter are not significantly different (P<0.05).

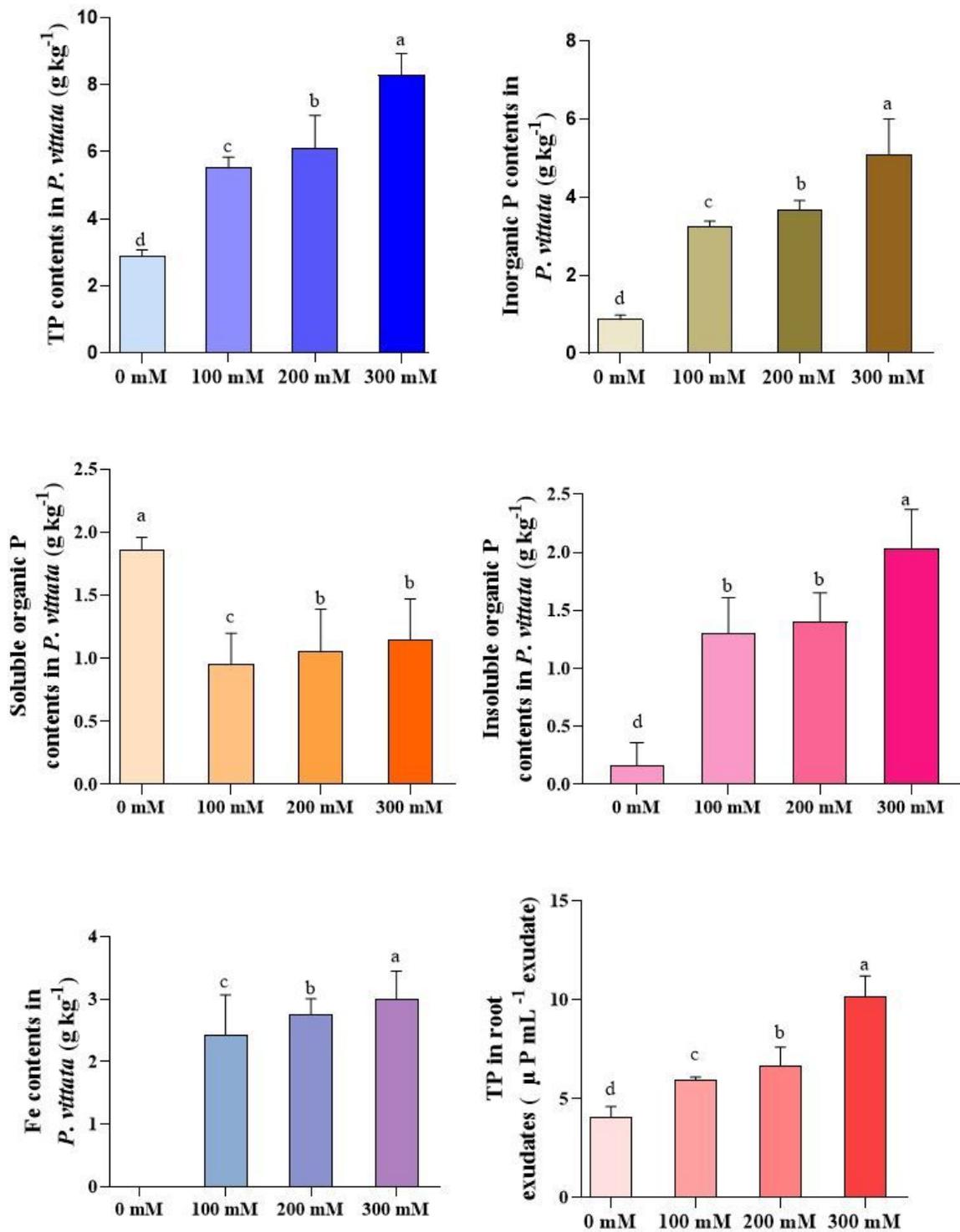
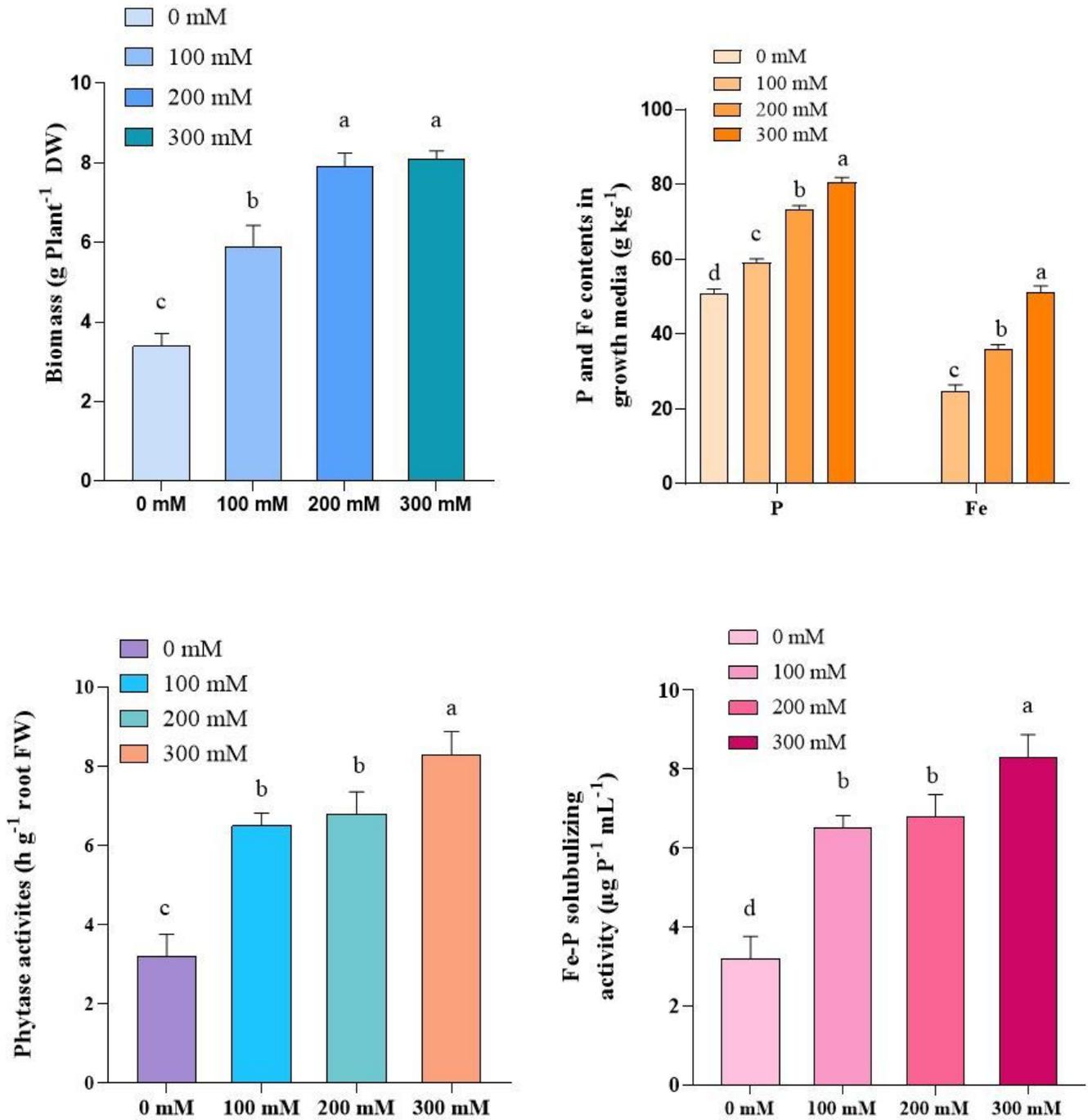


Figure 3

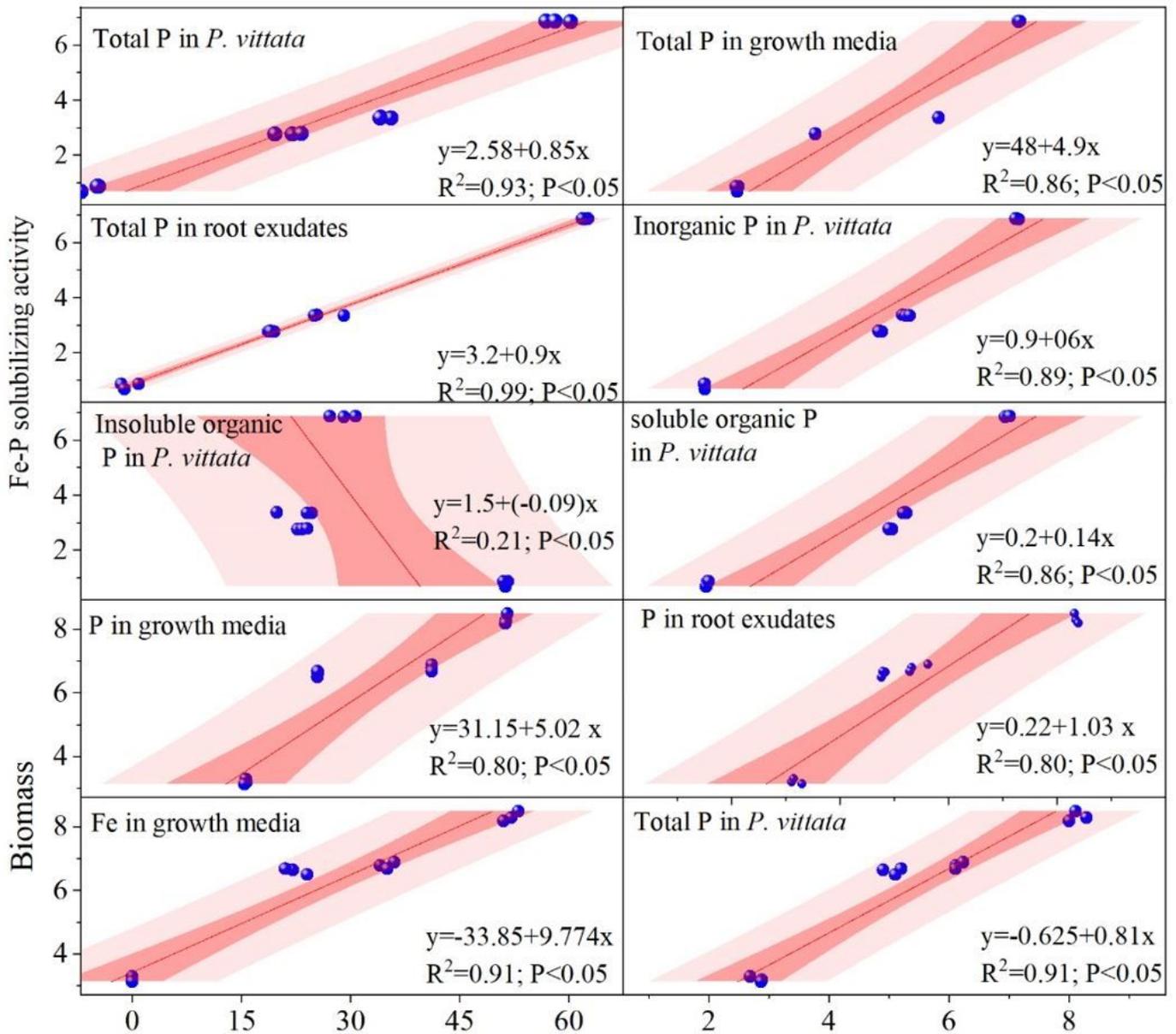
TCA P fractions in *P. vittata* and Linear regression between Fe-P solubilizing activity and TP, inorganic P, soluble organic P, insoluble organic P, in *P. vittata* tissue, TP in root exudates and growth media, Error bars indicate the SE (n =3), values followed by the same letter are not significantly different (P<0.05).



**Figure 4**

*Pteris vittata* biomass, P and Fe contents in growth media, phytase and Fe-P solubilizing activity in root exudates grown in P and Fe half strength Hoagland solutions containing different concentration (0-100

mM) of FePO<sub>4</sub>coll. Error bars indicate the SE (n =3), values followed by the same letter are not significantly different (P<0.05).



**Figure 5**

Simple linear regression between Fe-P solubilizing activity and TP in *P. vittata*, growth media, root exudates, insoluble, soluble and inorganic P in *P. vittata* and between biomass of *P. vittata* and TP in *P. vittata*, growth media, root exudates and Fe in growth media.

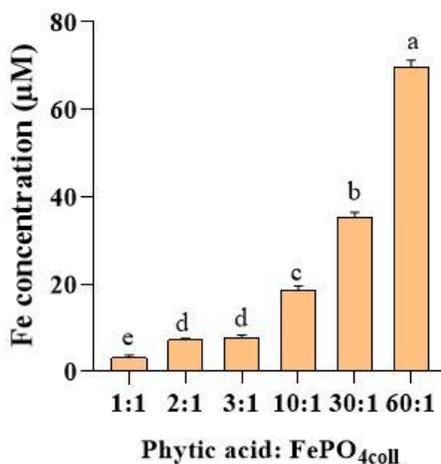
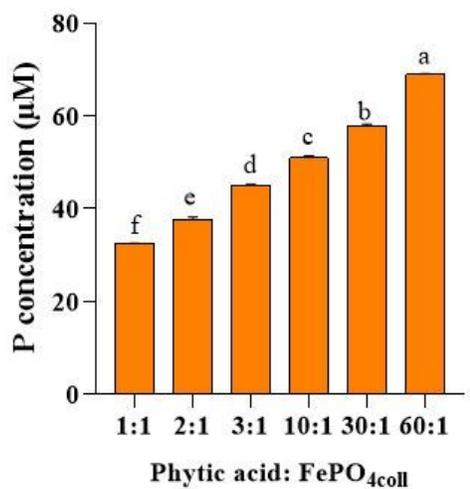
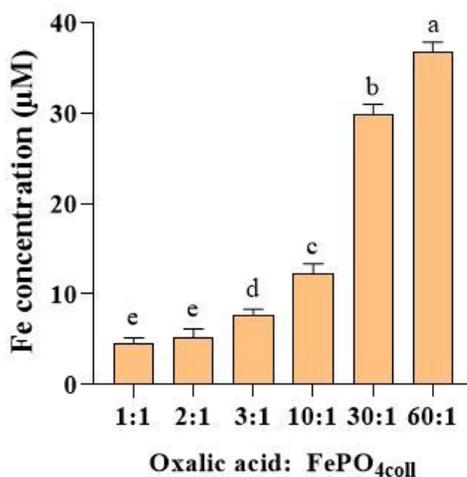
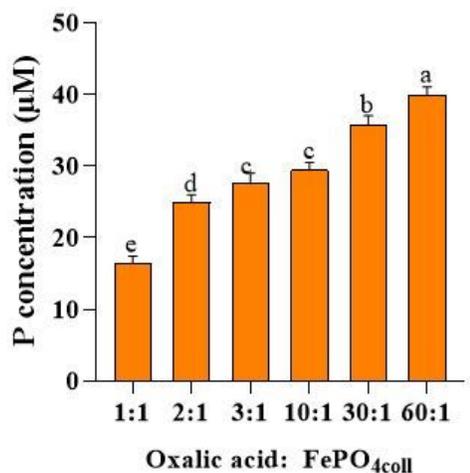
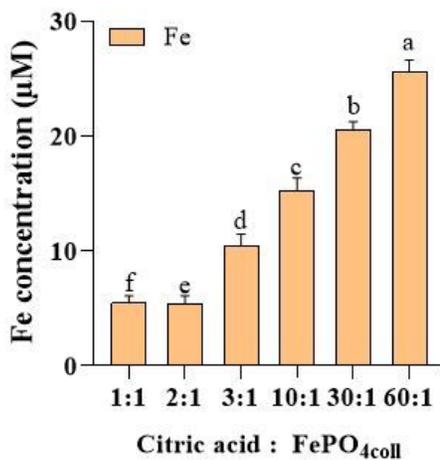
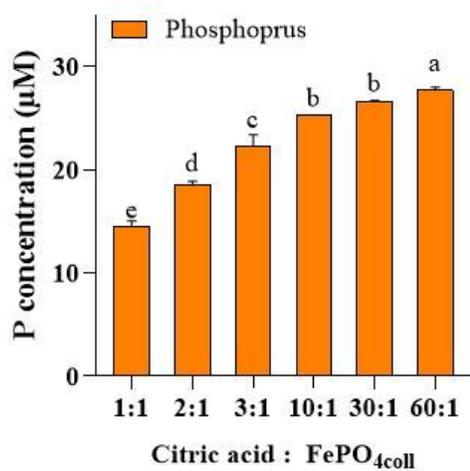


Figure 6

Effects of organic acids (phytic acid, oxalic acid, and citric acid) on P and Fe dissolution from FePO<sub>4</sub>coll (s) at a molar ratio of 1:1, 2:1, 3:1, 10:1, 30:1 and 60: 1. Error bars indicate the SE (n =3), values followed by the same letter are not significantly different (P<0.05).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supportinginformation.docx](#)