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Assessing microbially mediated vivianite as a novel phosphorus and iron fertilizer

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Abstract

Background Microorganisms can transform phosphorus (P)-enriched iron (Fe)-oxide sludge into products with higher P concentration or can directly promote the precipitation of P-rich compounds from water. However, there is no evidence of these products' efficiency as fertilizers. This study aimed to assess the effectiveness of microbially mediated vivianite (biovivianite) as P and Fe fertilizer for durum wheat and white lupin, respectively.

Results To this end, two completely randomized block experiments were conducted with wheat (phosphorus (P) experiment) and white lupin (iron (Fe) experiment). The P and Fe sources used included biovivianite produced by microbial reduction of P-containing ferrihydrite at pH 6.5 (VivInsol6.5) and pH 7.0 (VivInsol7.0), biovivianite produced with soluble Fe(III) citrate ($C_6H_5FeO_7$) in the presence of soluble phosphate at pH 7 (VivSol), and vivianite from a commercial company (ComViv). Potassium dihydrogen phosphate (KH_2PO_4) was used as a reference fertilizer in the P experiment, and Fe-EDDHA and Fe(II)-sulfate ($FeSO_4.7H_2O$) were used in the Fe experiment. Total P uptake by wheat plants from the product dominated by vivianite and phosphate-green rust (VivSol) was not significantly different from KH_2PO_4. The relative P use efficiency, i.e., the equivalence in terms of P recovery of VivSol was 74% of KH_2PO_4, making VivSol the effective P source for durum wheat among the products tested (aside from KH_2PO_4). For Fe uptake, product dominated by vivianite and metavivianite (VivInsol7.0), was the most effective Fe source for white lupin followed by Fe-EDDHA, ComViv, and VivSol with VivInsol6.5 as the least effective but without significant differences with Fe(II)-sulfate. The average crystallite sizes of the biovivianite were 59 nm, 63 nm, and 66 nm for VivSol, VivInsol7.0, and VivInsol6.5, respectively.

Conclusions The mineral constituents of the biovivianite coupled with their nano-crystallite sizes explained its effectiveness as P and Fe fertilizers. The results reveal that biovivianite production is a novel way of producing efficient P and Fe fertilizers from P-enriched Fe sludge or P-rich water. Thus, it can be used for producing fertilizers with high P and Fe concentrations from water purification, providing new tools for a circular economy approach in the use of a non-renewable resource such as P.

Highlights

- Vivianite is a sink for phosphorus (P), a scarce and non-renewable resource.
- Microbially mediated vivianite (biovivianite) was tested as P and Fe fertilizer on wheat and lupin.
- Biovivianite could replace soluble P (KH_2PO_4) by 74% as a P fertilizer for wheat.
- Biovivianite was a more efficient P source than chemically synthesized vivianite.
- The nano-crystallite size and mineral phases of biovivianite influenced its efficiency as P and Fe fertilizer.

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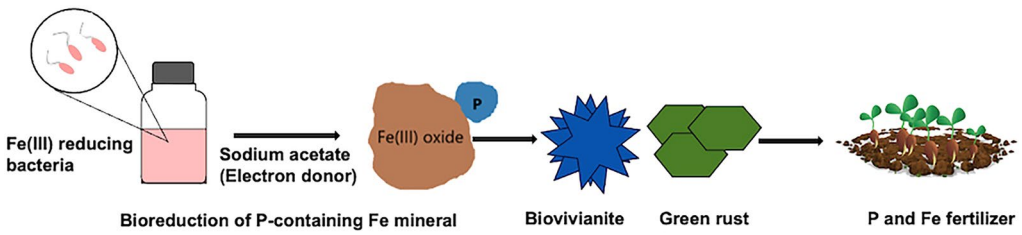
Keywords Bio-based fertilizer, Iron, Phosphorus, *Geobacter sulfurreducens*, Green rust, Ferrihydrite, Particle size, Durum wheat, White lupin

Graphical Abstract



Background

Aside from phosphorus (P) being essential for plants, it is a non-renewable resource with depleting reserves, and phosphate mineral deposits mined for phosphorus fertilizers are currently concentrated in only a few countries, such as Morocco and China [1]. Thus, phosphorus is a societal challenge as the continuous supply of this resource is critical for ensuring global food security [2]. Phosphorus recycling from wastewater is thus a crucial step for more efficient use of this non-renewable and strategic resource [3]. Chemical removal of P in water purification has been done by using sinks such as iron (Fe)-oxide sludge or precipitation as insoluble metal phosphates [4, 5]. In this regard, the precipitation of vivianite, a ferrous [Fe(II)] mineral rich in phosphate ($Fe_3^{2+}(PO_4^{3-})_2{\cdot}8H_2O$), is gaining attention due to the possibility of separating from digested sewage sludge by its magnetic properties [6]. The by-products from water purification could be used as fertilizers, however, the use of P-enriched Fe-oxide sludge is not practical due to its low P concentration, meanwhile, in the case of vivianite, a limitation could be ascribed to its low solubility. Constraints in the fertilizer use of water purification by-products pose a relevant problem for achieving a circular economy approach in the use of P.

Vivianite forms under reducing conditions in wastewater treatment facilities [7, 8], aquatic sediments and drained agricultural areas [9–11] and waterlogged soils [12, 13]. Vivianite can also be produced using dissimilatory Fe(III)-reducing bacteria such as *Geobacter sulfurreducens* and *Shewanella oneidensis* through the bioreduction of insoluble Fe(III) oxides and oxyhydroxides [14–18] or soluble ferric (Fe^{3+}) citrate [20, 21] in the presence of available phosphate. Here, the Fe(III)-reducing bacteria utilize organic carbon such as acetate or lactate as an electron donor with Fe(III) as the electron acceptor. This process results in the reduction of Fe(III) to Fe(II) which then can react with available phosphate to form vivianite (referred to henceforth as biovivianite, due to its microbially mediated nature). The high P and Fe content of vivianite make it a potential candidate as a fertilizer.

Phosphorus is one of the major nutrients required for plant growth and development. Natural soils have low levels of P available to plants; thus, low P fertilizer rates lead to the deficiency of this nutrient [22–24]. On the other hand, Fe is also essential for plants since it is responsible for, among other many physiological functions, chlorophyll synthesis in plants, and it underpins chloroplast development. Hence, Fe deficiency causes a typical symptom which is chlorosis of the leaves [25, 26]. Although Fe is an abundant element in the earth's crust and soils, it has been found to be less available to plants under both oxic conditions in soils, where Fe is mostly present as poorly soluble Fe^{3+}, and in calcareous soils, due to reduced mobility of Fe in the soil at alkaline pH [27]. Iron deficiency, the so-called Fe chlorosis, is thus frequent in oxic calcareous soils where, in addition, mechanisms for mobilizing Fe of plants sensitive to the problem (iron deficiency) are not effective [27, 28]. This is a relevant agronomic problem affecting sensitive crops in around 30% of the world's agricultural land [29]. The most common Fe fertilizers used to prevent Fe chlorosis are Fe chelates (the most usual Fe-EDDHA) and Fe(II)-sulfate. However, Fe-EDDHA is expensive, with reduced residual effect, and easily leaches out of the soil [30]. On the other hand, Fe(II)-sulfate is a cheap fertilizer which oxidizes quickly to ferric forms unavailable for plant uptake [31, 32].

Several studies have shown that synthetic vivianite can be an effective Fe fertilizer and can prevent Fe deficiency chlorosis in different crops [33–38]. However, there is little evidence on the effectiveness of vivianite as a P source

for plants [39–42]. The challenge is how easily the phosphate bound in vivianite can be dissolved and released, and how this release rate affects P adsorption and precipitation of poorly soluble metal phosphates in the soil and consequently its availability to plants. The microbially mediated nature of biovivianite could improve the P release rate, e.g., based on the typical smaller particle size associated with bioreduced Fe(II)-bearing minerals [43]; particle size has been shown to influence the dissolution of fertilizers in the soil and the uptake of such fertilizers by plants [44–47].

Therefore, this study's objective is to determine whether biovivianite can be an effective source of P and Fe for plant growth in durum wheat and white lupin. Microbial synthesis of biovivianite provides a potential low-cost and scalable route to obtaining a P-rich compound from wastewater or waste products, currently not of interest as a fertilizer due to their low P concentration, such as P-enriched Fe sludge resulting from water purification (Eshun et al. unpublished data). Therefore, the demonstration of effective fertilization using this novel biomineral phase (biovivianite) would open up new opportunities for the use of biotechnology to support a circular economy approach to fertilization and reduce overdependence on commercial fertilizers obtained from non-renewable and strategic resources.

Materials and methods

Preparation of biovivianite

Biovivianite was produced using 20 mmol l^{-1} of Fe(III) from either ferrihydrite (insoluble Fe) or Fe(III) citrate ($C_6H_5FeO_7$, soluble Fe). In a serum bottle, 30 mM sodium bicarbonate (buffer) solution containing 20 mM sodium acetate (electron donor), 20 mM sodium hydrogen phosphate (NaH_2PO_4) and 10 µM riboflavin (electron shuttle) was added to 20 mmol l^{-1} Fe(III) (either as ferrihydrite or Fe(III) citrate) [18]. The bioreduction medium was purged with a gas mix of N_2/CO_2 (80:20) to remove oxygen and two different pH values were used for the ferrihydrite experiments, pH 6.5 and 7.0. *Geobacter sulfurreducens* was cultured anaerobically using a modified freshwater medium [48] with 25 mM sodium acetate as the electron donor and 40 mM sodium fumarate as the electron acceptor in the dark at 30 °C. The grown cells were washed 3 times using a 30 mM sodium bicarbonate solution. Washed cells of *G. sulfurreducens* at an optical density (OD_{600}) of 0.4 were added to the bioreduction medium anaerobically and under sterile conditions and after that kept at 30 °C in an incubator in the dark. During bioreduction, ferrozine assay was used to determine the Fe(II) produced and Fe(total) [49, 50]. Briefly, 0.1 ml of a homogeneous aliquot of sample was added to 4.9 ml of 0.5 M HCl, left for 1 h, and the absorbance was measured at 562 nm. Thereafter, 0.2 ml of 6.25 M hydroxylamine hydrochloride was added to the digestate to reduce the Fe(III) to Fe(II) within 1 h and then absorbance was measured [known as Fe(total)]. The difference between Fe(total) and Fe(II) was calculated as the non-reduced Fe(III). After the bioreduction experiments, the reduced products were washed 3 times with degassed deionized water to remove any other salts that may be present, and the solids dried in the glove box.

Solid-phase characterization

Powder X-ray diffraction (XRD) and scanning electron microscopy with energy dispersive X-ray (SEM–EDX) were used to characterize the bioreduced products from the bioreduction experiments. For XRD analysis, samples were prepared anaerobically and analysed using a Bruker D8 advance diffractometer with Cu K α_1 radiation ($\lambda = 0.15406$ nm) at 5–70° 2-theta, with a step size of 0.02° and a count time of 0.5 s/step. Diffrac.Eva V14 software was used to match the peaks using standards from the International Centre for Diffraction Database (ICDD). The crystallite size of vivianite was calculated using the Scherer equation [51]. For SEM–EDX, the imaging was performed using an FEI Quanta 650 FEG SEM with a 15 kV beam in a high vacuum mode with EDX performed using the EDAX Gemini EDS system. ImageJ [52] was used to determine the particle size of the produced biovivianites.

Plant growth experimental design

Two completely randomized block experiments were performed at the same time with five replications each. The experiments were conducted using samples taken from the upper horizon (at 20 cm depth) of two soils, an Alfisol (Typic Haploxeralf) and a Vertisol (Chromic Haploxerert) according to Soil Taxonomy [53]. Soils were sampled in different locations in Spain (Alfisol: 37° 32′03″ N, 06° 13′22″ W, Vertisol: 37° 24′03″ N, 05° 35′15″ W), showing different soil properties (Additional file 1: Table S1). Soil particle size analyses were carried out using the densimeter method [54]. Soil organic carbon (SOC) was determined by dichromate oxidation [55] and the cation exchange capacity (CEC) by using 1 M NH_4OAc buffered at pH 7 [56]. The total $CaCO_3$ equivalent (CCE) was determined by the calcimeter method. pH was measured in water at a soil:extractant ratio of 1:2.5. Olsen P was used to determine the bioavailable P in the soils [57]. The experiments were performed to determine how effective biovivianite can be when used as a P and Fe source using durum wheat *(Triticum durum L.)* and white lupin (*Lupinus albus* L.), respectively. Wheat was used as a grain crop with significant P requirements [58], meanwhile, white lupin was selected as a Fe

chlorosis-sensitive plant [59, 60]. The Alfisol was used for wheat for the P experiment, whereas the Vertisol soil was used for lupin for the Fe experiment. The treatments used for each experiment were:

1. P source (6 treatments): Control without phosphate (non-fertilized with P), positive control (KH_2PO_4), biovivianite produced with insoluble Fe(III) oxyhydroxide (ferrihydrite) at pH 6.5, 7.0, and biovivianite produced with soluble Fe(III) citrate at pH 7.0 referred henceforth as VivInsol6.5, VivInsol7.0, and VivSol, respectively, and a synthesized vivianite from a commercial company (ComViv, from the company Fertiberia S.A., Madrid, Spain; it is produced from different P and Fe sources and used in commercial mixtures of NPK fertilizers).
2. Fe source (7 treatments): Control without Fe (non-fertilized with Fe), positive control (Fe(II)-sulfate), VivInsol6.5, VivInsol7.0, and VivSol, Fe chelate (as Fe-EDDHA), and ComViv were the Fe sources used for white lupin.

In both experiments, the biovivianite was applied as a suspension to the soil and mixed thoroughly. For the P uptake experiment, the P sources were applied at a rate of 15 mg P pot^{-1} (50 mg P kg^{-1} soil) for all treatments. ComViv was applied as a powder at the same rate as biovivianite (50 mg P kg^{-1}) and mixed with the soil before the experiment. KH_2PO_4 was applied in a crystalline presentation and mixed with the soil at a similar rate (50 mg P kg^{-1}). KH_2PO_4 was used as an efficient fertilizer which is a reference in terms of providing high P availability to plants in soils with basic pH. For the Fe chlorosis experiments, the Fe source was applied at a rate of 0.1 g Fe pot^{-1}(0.335 g Fe kg^{-1} soil). Fe–EDDHA at 0.02 mmol l^{-1} was applied together with the nutrient solution during irrigation while ComViv and Fe(II)-sulfate were applied as a powder at the same rate as biovivianite (0.335 g Fe kg^{-1} soil) and mixed with the soil before experiment.

Plant growth conditions

The seeds of white lupin and durum wheat were first germinated in perlite and irrigated with deionized water for 14 days until 4 true leaves appeared. Thereafter, one plant of white lupin and one plant of durum wheat were transplanted into a cylindrical pot (350 ml, 5.5 cm diameter, 15 cm high) with 0.3 kg of 2mm sieved soil in pots. Each pot was irrigated with 20 ml of Hoagland nutrient solution containing the following nutrients (all concentrations in mM): KH_2PO_4 (1)—only for the lupin experiment—$MgSO_4$ (2), $Ca(NO_3)_2$ (5), KNO_3 (5), KCl (0.05), Fe-EDDHA (0.02)—only for the wheat experiment—H_3BO_3 (0.024), $MnCl_2$ (0.0023), $CuSO_4$ (0.0005), and H_2MoO_4 (0.0005) every 2 days and 20 ml of deionised water was used on the third day to reduce the build-up of salinity from the nutrient solution. The pH of the nutrient solution was 6. The experiments were conducted in a growing chamber with a photoperiod of 14 h, a 25 °C/23 °C day/night temperature, 65% RH (relative humidity), and 22 W m^{-2} light intensity and harvested at 28 and 34 days after transplanting (DAT) for white lupin and wheat, respectively.

Plant analysis

The chlorophyll content of the plants was measured with a Minolta SPAD—502 chlorophyll meter (Soil plant analysis development) (Minolta Camera Co, Ltd., Osaka, Japan) at 10, 14, 18, 21, 28, and 34 days after transplanting (DAT). Correlation between SPAD units and leaf chlorophyll content was previously measured for wheat (chlorophyll = SPAD/136, $R^2 = 0.91$, $P < 0.001$, $n = 22$) [61] and for lupin (chlorophyll (mg [kg fresh weight]$^{-1}$) = 0.3 ln (SPAD)—0.48; $R^2 = 0.85$; $P < 0.001$, $n = 18$) [62]. The measurements were done in triplicate on the youngest fully expanded leaf. After harvest, the shoot and roots were separated, washed, dried in an air-forced oven at 65℃ and then weighed. The dried plant materials were ground to pass through a 1-mm sieve and then mineralized at 550℃ for 8 h in a furnace. The produced ash was analysed for its Fe and P content by dissolving it in 1 mol l^{-1} HCL and the solution was heated at 100℃ for 15 min for complete recovery of nutrients. The Fe content in the digestate was measured by atomic absorption spectrophotometry, whereas the P content was determined according to Murphy and Riley [63]. A certified material (tomato leaf) (standard reference material 1573a, National Institute of Standard and Technology, USA) was analysed in parallel to assess the total recovery of nutrients present in the plant material.

Soil analysis after harvest

After harvest, the soils were dried in an oven at 35–40℃ and weighed afterwards. After cropping, P availability to plants in the soil was assessed as Olsen P [57] with the colorimetric determination of P in the bicarbonate extracts [63]. Iron availability to plants was assessed using the diethylenetriamine pentaacetic acid (DTPA) method [64].

Fertilizer (P and Fe) use efficiency

The nutrient uptake was calculated as the product of the nutrient concentration in the plant and the dry matter in aerial parts. The relative use efficiency of P fertilizers (RPUE) was estimated according to Cabeza et al. [65] and using the formula,

$$\mathrm{RPUE\,(x)\,(\%)} = (\mathrm{PU_x} - \mathrm{PU_{Control}}) / (\mathrm{PU_{KH2PO4}} - \mathrm{PU_{Control}}),$$

Where PU_x is the phosphorus uptake of a fertilizer to be determined (mg P pot^{-1}), $PU_{Control}$ is the mean phosphorus uptake in the control without P fertilization (mg P pot^{-1}), and $PU_{KH_2PO_4}$ is the mean phosphorus uptake of the reference P fertilizer (KH_2PO_4). The same equation was used for the Fe experiments with Fe(II)-sulfate as the reference Fe fertilizer.

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the effect of the P and Fe sources on the chlorophyll content, dry matter (DM) yield, P and Fe uptake in the shoots and roots, the relative P or Fe use efficiency, available P and the available Fe extracted from the growing medium. Previously, normality and homoscedasticity were checked by using the Smirnoff–Kolmogorov and Levene tests, respectively [66], and data were transformed if one of both tests was not passed. Tukey's test at a probability level of 0.05 was also used to assess mean differences between treatments.

Results

Biovivianite was synthesized using *G. sulfurreducens* to test its effectiveness as a P and Fe fertilizer for wheat and white lupin, respectively. It was also used to determine whether biovivianite could be more effective than the chemically synthesized vivianite as a P source for wheat. For clarity, the biovivianite used for the study has been named based on the source of the starting Fe(III) material and the pH under which the bioreduction experiment was started, thus insoluble Fe(III) at pH 6.5 and 7.0 are known as VivInsol6.5 and VivInsol7.0, respectively, and soluble Fe(III) at pH 7.0 as VivSol. Chemically synthesized vivianite is referred to as ComViv. For the P experiment, KH_2PO_4 was used as the positive control. For Fe experiments, Fe(II)-sulfate was used as the positive control and Fe-EDDHA as another Fe source.

Solid characterization of the bioproduced fertilizers (biovivianite)

Vivianite ($Fe^{2+}{}_3(PO_4)_2{\cdot}8H_2O$) was the main mineral identified in all three biomineral products according to XRD results (Fig. 1a). Aside from vivianite, green rust II (GRII) was present in both VivSol and VivInsol6.5, however, VivSol showed a higher relative peak intensity for GRII signifying a greater abundance of GRII in VivSol compared to VivInsol6.5 (Fig. 1a). Metavivianite, a partially oxidized vivianite, ($Fe^{2+}Fe^{3+}{}_2(PO_4)_2(OH)_2{\cdot}6H_2O$) was identified together with vivianite in VivInsol7.0. The bioreduced products were digested using 37% HCl and digestate was analysed for phosphate and Fe content using the phosphomolybdate yellow assay and the ferrozine assay, respectively (Table 1). The Fe(II)/P ratios for the biovivianites were 1.29, 1.06, and 1.30 for VivInsol6.5, VivInsol7.0, and VivSol, respectively.

Compared with the other biovivianite, VivInsol7.0 had the lowest Fe(II)/Fe(total) (Table 1), depicting higher Fe(III) concentration in VivInsol7.0. This could explain the presence of the more oxidized form of vivianite (metavivianite) identified in VivInsol7.0. The Fe(III) content of VivInsol7.0 could also be attributed to any residual ferrihydrite, which can be assumed by the remaining darker colour at the endpoint of bioreduction. The visual structure of the precipitates differed among the 3 biovivianite samples (Fig. 1b). VivSol was less well structured (particle size 18 µm), VivInsol6.5 showed platy crystals (particle size 28 µm) and VivInsol7.0 was a mixture of both (particle size 16 µm) (Fig. 1b and c). Interestingly, the average crystallite sizes (measured using the Scherrer equation (Additional file 1: Eq. S1) [51]) of the biovivianites were all quite similar, 66 nm, 63 nm, and 59 nm for VivInsol6.5, VivInsol7.0, and VivSol, respectively. XRD analysis of ComViv, a synthetic vivianite tested, showed the presence of vivianite (Additional file 1: Fig. S1) with an average crystallite size of 55 nm.

Effect of the P source on durum wheat

The application of biovivianite and the other P sources on durum wheat influenced the SPAD readings (reflecting chlorophyll content), DM yield, shoot P concentrations, total P uptake, and relative P use efficiency. The SPAD readings for all treatments were significantly higher than the non-fertilized control at harvest (34 DAT) (Fig. 2a). The durum wheat dry matter yield was significantly higher for treatments with KH_2PO_4 and VivSol (Fig. 2b) than with other P sources; no significant difference was observed between KH_2PO_4 and VivSol. Among the vivianite-based treatments (referring to both synthetic vivianite (ComViv) and biovivianites), VivSol and ComViv led to significantly higher DM than VivInsol6.5, signifying the effectiveness of both vivianites (VivSol and ComViv) in contributing to plant development (specially to shoot development). For shoot P concentrations, significant differences were noted between KH_2PO_4, and all the other P sources tested (Fig. 2c). The total P uptake by wheat was significantly higher with KH_2PO_4 than with all vivianite-based treatments except for VivSol. Treatments with ComViv, VivInsol6.5 and VivInsol7.0 were not significantly different from the non-fertilized control (Fig. 2d). After the wheat crop was collected and analysed, treatment with VivSol resulted in the highest DTPA-extractable Fe in the soil, which was significantly different from

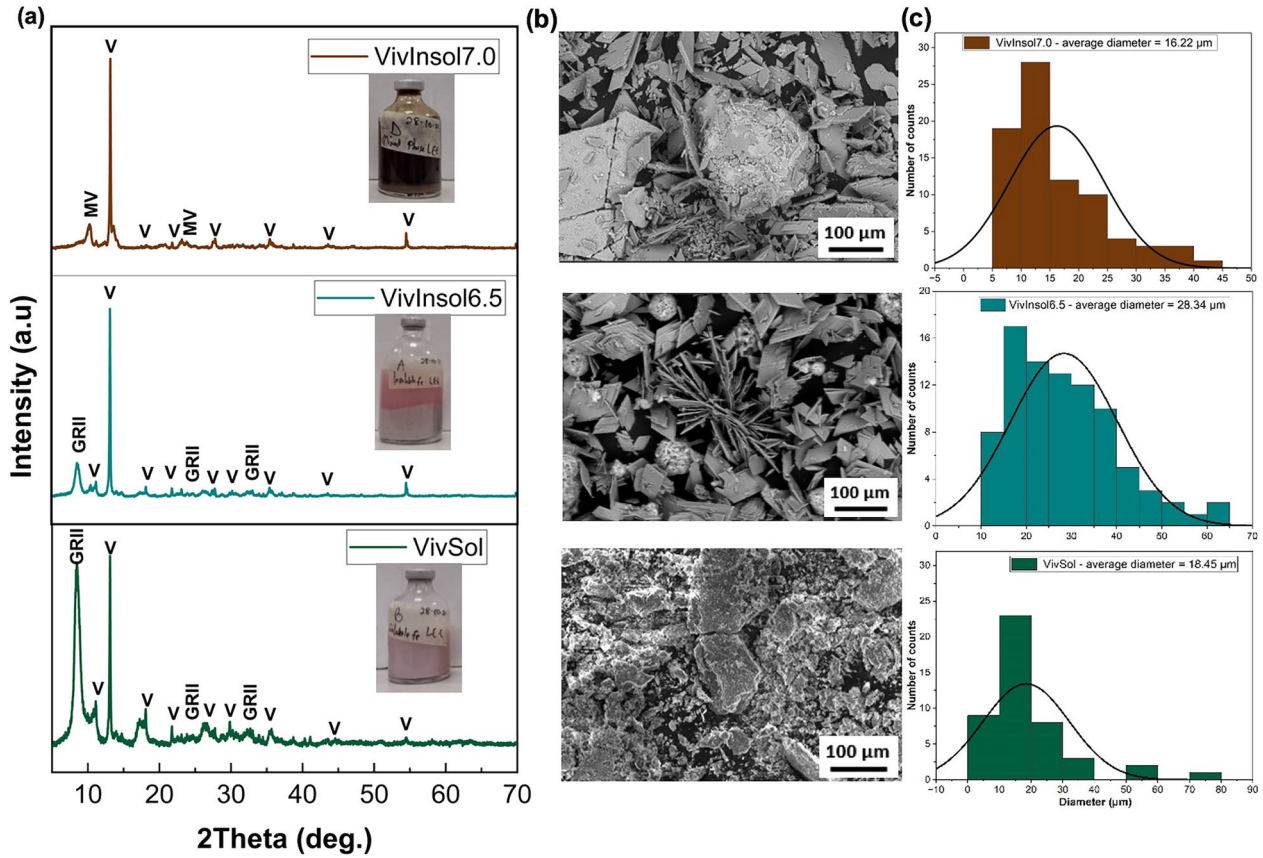


Fig. 1 XRD diffractogram (**a**) of the three different biovivianite with their respective SEM images (**b**) and particle size distribution (**c**). Top- VivInsol7.0—vivianite produced by the microbial reduction of insoluble ferrihydrite, at pH 7.0 and pH 6.5 (middle); bottom- VivSol—vivianite produced by the microbial reduction of soluble Fe(III) citrate. On the XRD diffractogram, GRII is green rust (PDF 13-0092), V is vivianite (PDF 30-0662), and MV is metavivianite (PDF 00-064-0286)

Table 1 Fe(II), Fe(III) and phosphate content of the biovivianite after acid digestion

Fertilizer products (Biovivianite)	Acid-extractable Fe (M)		Fe(II)/Fe(total)	Phosphate (M)
	Fe(II)	Fe(III)		
VivInsol6.5	1.13	0.03	0.97	0.875
VivInsol7.0	0.50	0.20	0.72	0.474
VivSol	0.19	0.04	0.86	0.143

all the other treatments (Additional file 1: Fig. S2). Olsen P values were not significantly different between the treatments (Additional file 1: Fig. S2). VivSol led to the highest relative phosphorus use efficiency (74%) followed by VivInsol7.0 (32%), ComViv (16.4%) and VivInsol6.5 (less than 0.5%) as the lowest (Fig. 2e). No significant difference was observed between VivSol and VivInsol7.0 but there was a difference between VivSol and ComViv.

Effect of the Fe source on white lupin

Biovivianites and other Fe sources tested influenced the parameters studied for white lupin (SPAD readings, DM yield, and total Fe uptake by plants). At 10 DAT, treatment with VivInsol6.5 recorded the lowest SPAD readings compared with all other treatments, including the control (no added Fe) (Fig. 3a). At 14 DAT, treatments with Fe-EDDHA and VivInsol7.0 had the highest SPAD readings followed by ComViv and Fe(II)-sulfate. However, at harvest (28 DAT), no significant difference in SPAD readings was observed between vivianite-based treatments, the negative and the positive controls ($P=0.188$), for white lupin. Dry matter yield for shoots and roots of white lupin was not significantly different between the Fe treatments. However, when VivInsol7.0 and ComViv were compared to the non-fertilized control, a significant difference was observed ($P=0.004$) (data not shown). Shoot concentrations of Fe in white lupin were not significantly different among all Fe treatments (Fig. 3c) whereas a significant difference was obtained among the root Fe concentrations (Additional file 1:

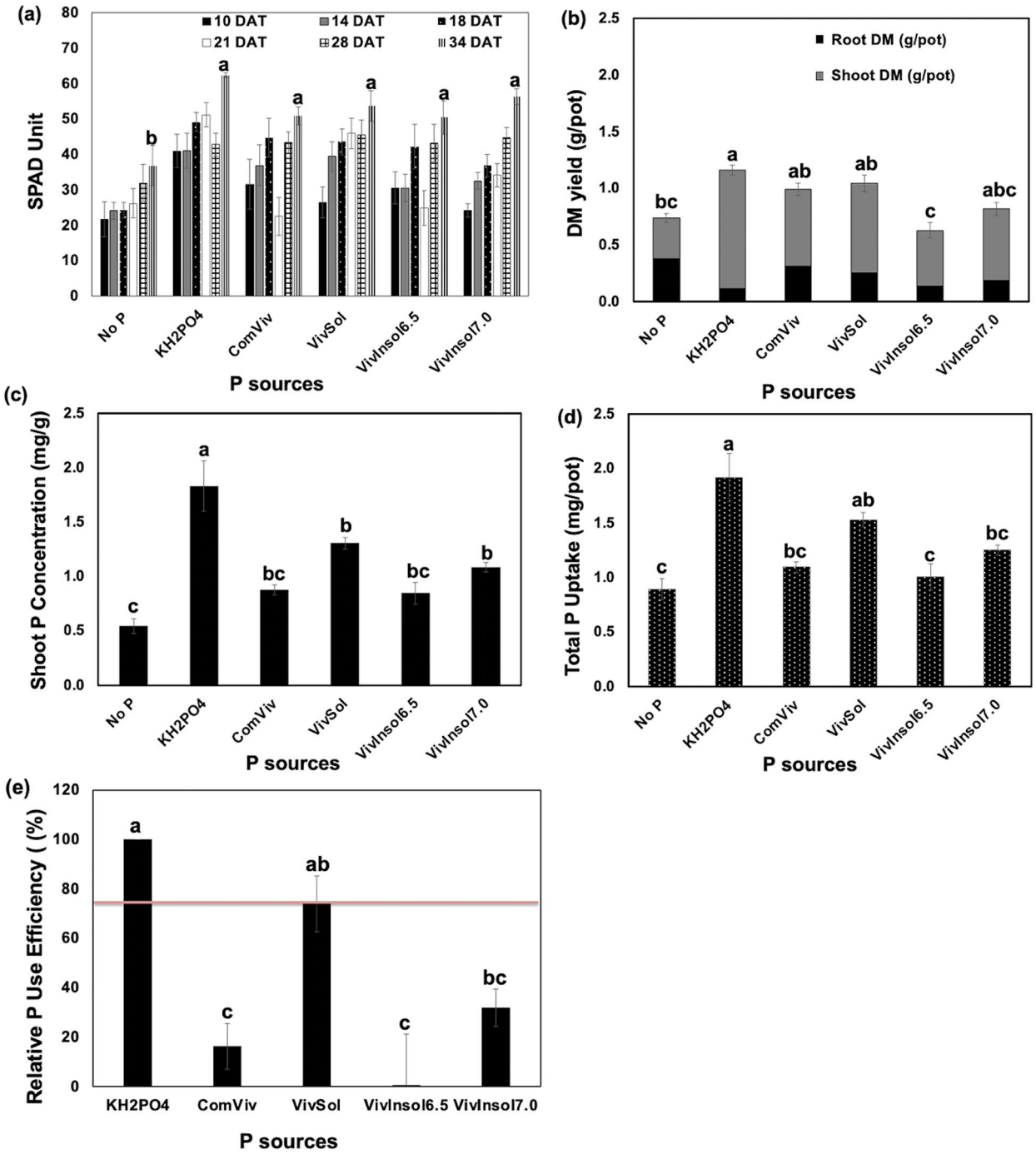


Fig. 2 Effect of the application of different P sources on (**a**) SPAD measurements for durum wheat, (**b**) dry matter (DM) yield, (**c**) shoot P concentration and (**d**) total P uptake for durum wheat harvested at 34 DAT. (**e**) Represents the relative P use efficiency (RPUE) (%) of the tested fertilizers using KH_2PO_4 as the reference P fertilizer. The data are means of 5 replicates and error bars indicate standard error. Means with the same letters were not significantly different according to the Tukey test at a probability level of 0.05

Table S2a). For instance, among the biovivianites tested, VivInsol7.0 promoted higher root Fe concentration than VivInsol6.5 and VivSol. VivInsol7.0 was the only treatment increasing total Fe uptake relative to the non-fertilized control, whereas results from VivInsol6.5, on the other hand, were not significantly different from the

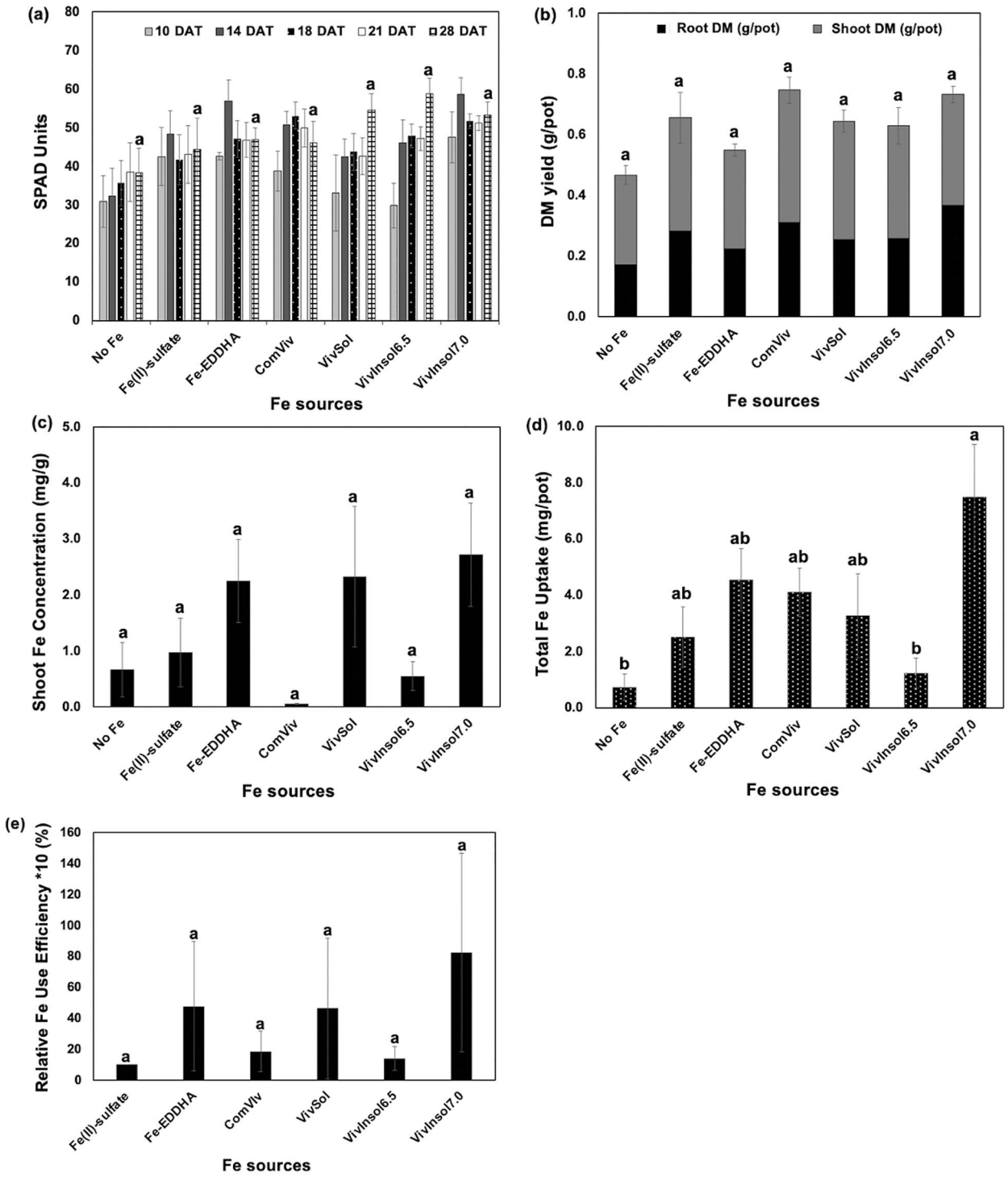


Fig. 3 Effect of the application of different Fe sources on the (**a**) SPAD measurements for white lupin, (**b**) dry matter (DM) yield, (**c**) shoot Fe concentration and (**d**) total Fe uptake for white lupin harvested at 28 DAT. (**e**) Represents the relative Fe use efficiency (RFeUE) (%) of the tested fertilizers using Fe(II)-sulfate as the reference fertilizer. The data are means of 5 replicates and error bars indicate standard error. Means with same letters were not significantly different according to Tukey test at a probability level of 0.05

non-fertilized control. Treatments with ComViv and Fe-EDDHA showed similar Fe uptake levels, which in turn were not significantly different from Fe(II)-sulfate treatments and the non-fertilized control (Fig. 3d). Treatment with ComViv was the least effective at increasing shoot Fe concentration but was the third highest in terms of total Fe uptake aside from VivInsol7.0 and Fe-EDDHA. Most of the Fe from ComViv was concentrated in the root. The Fe availability index in soil measured with the DTPA method was higher in Fe(II)-sulfate and was significantly different from all the treatments except treatment with VivSol in the soil after white lupin crop (Additional file 1: Fig. S2). Although this experiment focused on the effectiveness of the fertilizer products as a Fe source for white lupin, the shoot P concentration was the highest in ComViv and was significantly higher than the non-fertilized control (Additional file 1: Table S2a). Assuming P supplied from the nutrient solution contributed to P uptake in all the treatments, including the non-fertilized control, Fe(II)-sulfate, and Fe-EDDHA, then the highest P uptake noted in the treatments with ComViv could be the consequence of an additional P supply ascribed to vivianite. The relative Fe use efficiency (RFeUE) of the Fe sources was not significantly different compared with the fertilized control with Fe(II)-sulfate as the reference Fe source (Fig. 3e).

Discussion

Effect of pH on biovivianite production

During the bioreduction of phosphate-containing ferrihydrite into biovivianite, *G. sulfurreducens* couples the reduction of Fe(III) into biogenic Fe(II) through the oxidation of electron donors (sodium acetate) which generates HCO_3^- and OH^-. The production of OH^- increases the pH of the system [67]. A recent study by Eshun et al. [18] reported an increase in pH from 7 to approximately 8.5 and from 6.5 to 7.5 during biovivianite production experiments using sodium bicarbonate as the buffer solution. At pH > 8.5, the formation of Fe(II) hydroxide ($Fe(OH)_2$) is enhanced, which consumes Fe(II) needed for vivianite formation [68, 69]. The medium after biovivianite production at an initial pH of 6.5 (VivInsol6.5) in this study was pink in colour (residual from the cytochrome-containing *Geobacter* cells used during bioreduction) (Fig. 1a), whereas medium from the pH of 7.0 (VivInsol7.0) experiment was dark brown in colour, which is consistent with non-reduced ferrihydrite. Thus, pH indirectly influenced the mineralogical transformation by altering the rate and extent of Fe(II) production. This is critical for secondary mineral formation thereby explaining the higher Fe(III) concentration found in VivInsol7.0.

Effectiveness of biovivianite as a P source for wheat

Treatments with biovivianites (VivSol and VivInsol7.0) promoted higher P concentrations in plants than the non-P fertilized control, signifying that biovivianite was a P source for the wheat plant. Except for VivInsol6.5, all other vivianite-based treatments (both ComViv and biovivianite) led to no significant differences in DM yield when compared to the soluble P fertilizer, meanwhile, P concentrations in shoots were lower with vivianite-based treatments than with the soluble fertilizer. Thus, it seems that although less efficient in supplying P to plants than soluble fertilizer, most vivianite-based products provide enough P to ensure maximum plant development. This suggests that the soluble fertilizer promoted a luxury consumption of P, i.e., leading to P concentration in plant tissues well above the minimum required for optimal growth [39]. Overall, VivSol was an effective and efficient source of P since it did not lead to significantly lower DM nor P uptake than soluble fertilizer. In terms of relative P use efficiency for wheat, it was equivalent to the application of 74% of KH_2PO_4 at the same rate (Fig. 2e), thus, soluble KH_2PO_4 can be replaced by VivSol and still be equivalent to the application of KH_2PO_4 by 74% instead of ComViv which was 16%.

Unlike ComViv which was mainly vivianite according to XRD analysis and with an average crystallite size of 55 nm, the mineral composition of VivSol was vivianite and phosphate-green rust (GRII) with an average crystallite size of 59 nm. Green rusts are mixed valence Fe(II)/Fe(III) layered hydroxides, comprising a positively charged hydroxide layer $[Fe^{II}(1\text{-}x)\ Fe^{III}x(OH_2)]^{x+}$ which alternate with a negatively charged interlayer anions $[x/n\ A^{n-}\cdot(m\ x/n\ HO)]^{x-}$ where A can be SO_4^{2-}, PO_4^{3-}, Cl^-, or CO_3^{2-}, etc., and m is the amount of interlayer water [71, 72]. Green rust I (GRI) has either Cl^- or CO_3^{2-} as the interlayer anion, whereas green rust II (GRII) has SO_4^{2-} or PO_4^{3-} [73]. GRII(PO_4^{3-}) was identified in VivSol as SO_4^{2-} was absent in the bioreduction medium used in producing the biovivianite. Vivianite (PO_4^{3-}-rich Fe(II) mineral) and GRII(PO_4^{3-}) are both phosphate-rich, and their abundance in VivSol and absence in ComViv could explain why VivSol was an effective P source for wheat. The phosphate-rich nature of VivSol coupled with the smaller particle size of this product could have influenced its uptake as a P fertilizer to the wheat plant [44–47]. Although the crystallite size of ComViv was smaller than that of VivSol, the reason for the lower phosphorus use efficiency could be due to the well-structured nature of the precipitate as evidenced by the SEM image (Additional file 1: Fig. S1).

The residual effect of fertilizer, which can be estimated from Olsen P in the soil after crop, did not differ between the fertilizer treatments (Additional file 1: Fig.

S2). This shows that, neither soluble mineral fertilizer nor vivianite-based treatments increased bicarbonate-extractable P relative to the non-fertilized control, which was below the threshold value for fertilizer response [3]. This implies a loss of availability of the applied P since the amount of P extracted by crops was much lower than applied. This could be due to the soluble mineral fertilizer causing insoluble Ca phosphates to precipitate in this type of soils [74]. However, vivianite is a poorly soluble compound at basic pH like that in bicarbonate-extracted P. Bicarbonate extraction of P mostly promotes the desorption of adsorbed P and the dissolution of soluble metal phosphates [75], thus, it seems that vivianites-based products used in the study were not fully dissolved during the experiment, and therefore, could perform as a slow-release fertilizer [76]. This again, implies that the short-term experiment performed in the current study did not provide a full view of the effectiveness of the P fertilizer.

The release and phytoavailability of P from vivianite are enhanced in rhizospheric soil since organic anions exudated by roots such as citrate are capable of complexing Fe, which promotes the dissolution of vivianite [76, 77]. In a study by Fodoué et al. [42], bean plant *(Phaseolus vulgaris)* which can release organic acids (citric and formic acid) by the root was able to utilize phosphorus from natural vivianite (composed of iron, phosphorus, silica, and alumina) for growth of the plant. Additionally, Fe complexed by organic anions is assumed to increase the availability of P and Fe [78, 79] to plants, meaning P and Fe supply to plants from vivianite are related. This slow-release P fertilizer effect can minimize the precipitation of insoluble Ca phosphates which is expected around granules of soluble fertilizer. The precipitation of insoluble Ca phosphates is enhanced at high P concentrations in the soil solution and promoted by soluble fertilizers [80], thereby leading to a decreased P uptake by crops [73].

Effectiveness of biovivianite in preventing Fe chlorosis in white lupin

Biovivianite enhanced the chlorophyll content and the total Fe uptake by lupin compared with the non-fertilized control. Fe deficiency chlorosis causes the yellowing of young leaves [81]. These symptoms were observed in non-fertilized control, Fe(II)-sulfate, ComViv, and the biovivianite treatments except VivInsol7.0. The occurrence of chlorotic leaves, as revealed by the low SPAD measurements, particularly at the first growing steps, and the Fe concentration in the shoot were not always related in some of the treatments (Additional file 1: Table S3). For instance, ComViv had higher SPAD meter readings than the non-fertilized control but recorded the lowest shoot Fe concentrations. VivInsol6.5 also recorded the highest SPAD meter readings compared with the non-fertilized control at harvest, but had the lowest shoot Fe concentration among the biovivianites tested. For Fe(II)-sulfate, although it had the lowest SPAD meter readings, the Fe uptake was higher than VivInsol6.5. In contrast, the increase in SPAD meter reading by VivInsol7.0 and Fe-EDDHA was related to a significant increase in the total Fe uptake. These results confirm the assertion that higher chlorophyll content of leaves does not necessarily denote higher Fe concentration (evident in treatment with VivInsol6.5). Therefore, this concentration is not the most accurate measure of iron deficiency chlorosis [33, 62, 82], a phenomenon called the Fe paradox (i.e., inactivation of Fe in the leaf apoplast) [80, 83, 84]. This effect is well-known and has been usually ascribed to a decreased Fe transport through membranes leading to an accumulation of the nutrient in the organ, but not inside the cell where it performs its physiological functions [62].

Overall, treatment with VivInsol7.0 was the most effective Fe source increasing total Fe uptake by white lupin followed by Fe-EDDHA, ComViv, and VivSol, with VivInsol6.5 as the least effective. VivInsol7.0 was the only treatment, along with Fe-EDDHA, where Fe chlorosis symptoms were not observed. This is consistent with previous studies showing that vivianite was as effective as Fe-EDDHA in preventing Fe chlorosis in plants [34, 37, 85]. Fe(II)-sulfate oxidizes quickly to unavailable Fe(III) forms in the soil and that explains why it was ineffective as a Fe source for white lupin [31, 32].

VivInsol7.0 was mainly vivianite ($Fe^{2+}{}_3(PO_4)_2{\cdot}8H_2O$) and metavivianite ($Fe^{2+}Fe^{3+}{}_2(PO_4)_2(OH)_2{\cdot}6H_2O$) and it is assumed to contain non-reduced ferrihydrite. These Fe(III) minerals present in VivInsol7.0 explain why it had 28% of total Fe as Fe(III) compared with VivSol and VivInsol6.5 which had only 14% and 3%, respectively. The presence of Fe(III) compounds in VivInsol7.0 could be explained indirectly by the increase in pH observed during the Fe(III) bioreduction process as mentioned above. It, therefore, appears that the non-reduced ferrihydrite (poorly crystalline Fe(III) mineral) present in VivInsol7.0 did not limit, but rather increased, its efficiency as a source of Fe for plants. Consequently, the ratio of Fe(II) to Fe(III) in the fertilizer product is not a good index for predicting its efficiency as a Fe fertilizer product. This is because insoluble and poorly crystalline Fe(III) oxides, whose dissolution can be induced by organic anions released by roots [86] (forming Fe(III)–organic acid complexes), may also be sources of Fe for plants [25]. In fact, the effect of vivianite as a Fe source is ascribed to its oxidation to poorly crystalline Fe oxides in the soil [37, 87] and the subsequent reduction of the Fe(III)–organic acid complexes. The reduction of Fe(III) to Fe(II) via the use

of ferric chelate reductase [88] promotes the transport of Fe(II) by root cells using appropriate Fe(II) transporters [76, 89]. The secretion of exudates from the roots of lupin increases bacterial abundance in the rhizosphere [90], which influences the solubility and availability of nutrients (Fe, P, Zn, etc.) for plant use [91]. This is because the organic acids from the roots can be an effective energy source for bacterial growth and resulting metal (Fe) reduction [92]. Again, phosphate released by vivianite during its dissolution also promotes the formation of poorly crystalline Fe oxides, thus increasing the availability of Fe to plants [37, 86, 93]. This is another mechanism by which the availability of P and Fe in vivianite to plants is connected.

The effect of different Fe fertilizer treatments on DTPA extractable Fe (Additional file 1: Fig. S2) did not correspond with the efficiency in supplying this nutrient to plants (Fig. 3d). Oxidation products of Fe(II) sulfate and vivianite-based treatments are assumed to be essentially poorly crystalline Fe oxides [33], which are thought to be a source of Fe for plants. Although DTPA-extractable Fe has been usually related to poorly crystalline Fe oxides in soil [94], de Santiago and Delgado [62] concluded that in soils from Mediterranean areas such as those used in this study, Fe extracted with this method at 2 h was not related to poorly crystalline oxides. This is one of the reasons why these authors [62] explained that chemical extraction using DTPA was not an accurate availability index for predicting Fe deficiency chlorosis. Thus, although DTPA extraction can indicate the level of enrichment of the growing media by readily mobilizable Fe, it cannot be considered an accurate method for predicting the efficiency of Fe fertilizers. VivInsol7.0 had a particle size of 16 µm and an average crystallite size of 63 nm as compared with VivInsol6.5 which had 28 µm and an average crystallite size of 66 nm. Thus, the best results of VivInsol7.0 among vivianite-based treatments (including commercially synthesized one) could most likely be due to the smaller crystallite sizes and the mixed Fe phases identified in it. To achieve these characteristics, the control of pH in the precipitation process during phosphate-containing Fe(III) bioreduction is crucial for best results as Fe fertilizer.

Practical applications

The present results are promising with a view to using biovivianite as a P fertilizer. They also provide practical insight into the possible use of P-enriched waste Fe sludge produced during water purification or P-rich wastewater as cheap starting materials for biovivianite production. There were clear differences in the P use efficiency of vivianites produced via microbial reductions of soluble (VivSol) and insoluble Fe(III) oxyhydroxides (VivInsol6.5 and 7.0) and commercially synthesized vivianite (ComViv). The differences were mainly attributed to the mineralogical phases and the nano-crystallite sizes of the biomineral products identified via XRD analysis. Biomineral products from waste streams, although providing a cheap source of Fe(III) starting materials, would likely contain varying compounds, differing in crystallinity [95], particle sizes [96] and the incorporation of other elements such as magnesium and manganese [69]. These factors can affect the efficiency of these materials as P or Fe fertilizer and therefore require further investigation. Our results also showed that biovivianite performed better as a P fertilizer than the chemically synthesized vivianite and could be a suitable alternative to soluble mineral fertilizers in P-deficient soils. However, as a novel biomineral, further studies on the possible scale-up of biovivianite production, the effect of scale-up on the particle size of the products and how effective biovivianite can be used as a P fertilizer for a full growing season under field conditions are still needed.

Conclusion

Microbially mediated vivianite (biovivianite) can be used as an effective P and Fe source for plants. Biovivianite produced using soluble Fe(III) citrate (VivSol), which contained both vivianite and phosphate-green rust, was a more effective P source than the chemically synthesized vivianite (ComViv) in durum wheat. On the other hand, biovivianite produced using amorphous 2-line ferrihydrite at pH 7 (VivInsol7.0), which contained both vivianite and metavivianite, was the best Fe source, leading to higher Fe uptake than Fe-EDDHA in white lupin. The differences in the particle sizes of the bioreduced products (biovivianite), coupled with the mineralogical compositions, could explain why biovivianite was effective as both a P and Fe fertilizer for wheat and white lupin, respectively. The study, therefore, confirms that biovivianite can be used to correct Fe deficiency in plants, but it also provides evidence that P bound to biovivianite can be used as a P source for plants growing in P-deficient soils. Overall, the study gives insight into the possible use in agriculture of biotransformation products from other P and Fe sources such as P-enriched Fe waste sludge. This, will not only contribute to the reuse of waste materials, but will also help to reduce the overdependence on phosphate rock for P fertilizer production, thereby reinforcing a circular economy.

Abbreviations

VivInsol6.5	Biovivianite produced by microbial reduction of phosphorus-containing ferrihydrite at pH 6.5
VivInsol7.0	Biovivianite produced by microbial reduction of phosphorus-containing ferrihydrite at pH 7.0
VivSol	Biovivianite produced with soluble Fe(III) citrate ($C_6H_5FeO_7$) in the presence of soluble phosphate at pH 7.0

ComViv	Chemically synthesized vivianite from a commercial company
DAT	Days after transplanting
SPAD	Soil plant analysis development
RPUE	Relative phosphorus use efficiency
GRII	Green rust II
RFeUE	Relative iron (Fe) use efficiency

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00558-0.

Additional file 1: Table S1. Soil properties used in the growth experiments. **Fig. S1.** XRD diffractogram and SEM image of the chemically synthesized vivianite (ComViv) showing vivianite as the main mineral. **Table S2.** Amounts of P and Fe in the shoot and roots of white lupin (**a**) and wheat (**b**) respectively. **Fig. S2.** Soil Fe_{DTPA} extracted and Olsen P from white lupin (**a**) and durum wheat (**b**). Data points with different letters are significantly different according to the Tukey test at a probability of 0.05. Data points with * are not statistically significant at a probability level of 0.05. **Table S3.** Correlation coefficient between chlorophyll content of leaves and the Fe concentration in the shoot. **Table S4.** A summary of an analysis of the variance of several parameters of white lupin (**a**) and wheat (**b**) for all treatments studied.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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