A novel biological process for recycling phosphorus from wastewater as fertilizer

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Abstract

Background

For water purification, Fe-oxide sludge is frequently used as a P sink, generating P-enriched Fe sludge. Microorganisms can transform the P-enriched 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) production as Fe and P fertilizer for white lupin and durum wheat respectively.

Results

To this end, two completely randomized block experiments were conducted with white lupin (Fe experiment) and wheat (P experiment). The Fe and P sources used included biovivianite produced by microbial reduction of P-containing ferrihydrite (simulating saturated Fe-oxide sludge) 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) (simulating the direct removal from water) and vivianite from a commercial company (ComViv). Fe-EDDHA and Fe(II)-sulfate ($FeSO_4.7H_2O$) were used as reference fertilizers in the Fe experiment and potassium dihydrogen phosphate (KH_2PO_4) was used for the P experiment. Overall, products dominated by vivianite and metavivianite ($Fe^{2+}Fe^{3+}{}_2(PO_4)_2(OH)_2{\cdot}6H_2O$), 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. Total P uptake by wheat plants from VivSol (dominated by vivianite and phosphate-green rust) was not significantly different from KH_2PO_4 in supplying P to the wheat plant. The particle sizes of the biovivianites were 16 µm, 18 µm, and 28 µm for VivInsol7.0, VivSol and VivInsol6.5 respectively.

Conclusions

The mineral constituents of the biovivianite coupled with the smaller particle sizes contributed to its effective uptake by the plants. The results reveal that biovivianite production is a novel way of producing efficient P and Fe fertilizers from water purification, providing new tools for a circular economy approach in the use of a non-renewable resource.

Highlights

- Vivianite is a sink for phosphorus (P), a scarce and non-renewable resource.
- Microbially-mediated vivianite is tested as Fe and P fertilizer on lupin and wheat.
- 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 smaller particle size of biovivianite influenced its effective Fe and P uptake.

Background

Aside from 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 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-})$_2$.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 putrefaciens* through the bioreduction of insoluble Fe(III) oxides and oxyhydroxides [14–17] or soluble ferric (Fe^{3+}) citrate [18, 19] 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 phosphorus (P) and iron (Fe) content of vivianite make it a potential candidate as a fertilizer.

Phosphorus is one of the major nutrients for plant growth and development whose deficiency is ascribed to low P fertilizer rates since soils are in origin poor in available P to plants [20–23]. 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 [24, 25]. Iron is an abundant element in the earth's crust and soils. Its deficiency, the so-called Fe chlorosis, is ascribed to soil conditions restricting its solubility and absorption capability by plants such as calcareous soils leading to an increased pH [26, 27]. Iron has been found to be less available to plants under both oxic conditions in soils, as Fe is mostly present as poorly soluble Fe^{3+}, and in calcareous soils, due to reduced mobility of Fe in the soil with higher pH (Fe chlorosis) [27]. This is a relevant agronomic problem affecting sensitive crops in around 30% of the world's agricultural land [28]. 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 [29]. On the other hand, Fe(II)-sulfate is a cheap fertilizer which oxidizes quickly to ferric forms unavailable for plant uptake [30, 31].

Several studies have shown that synthetic vivianite can be an effective Fe fertilizer and can prevent Fe deficiency chlorosis in different crops [32–38]. However, there is little evidence on the effectiveness of vivianite as a P source for plants [39, 40]. 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 [41]; particle size has been shown to influence the dissolution of fertilizers in the soil and the uptake of such fertilizers by plants [42–45].

Therefore, this study's objective is whether biovivianite can be an effective source of Fe and P for plant growth in white lupin and durum wheat. 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) (Eshun et al. unpublished data). 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). 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 [46] 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) [47, 48]. Briefly, 0.1 ml of a homogeneous aliquot of sample was added to 4.9 ml of 0.5 M HCl, left for 1 hour, 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 hour 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 a week of bioreduction, the reduced products were washed 3 times with degassed deionized water to remove any other salts that may be present and 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 microcosm experiments. For XRD analysis, samples were prepared anaerobically and analysed using a Bruker D8 advance diffractometer with Cu K α_1 radiation (λ = 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 (Langford & Wilson, 1978). 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 [49] 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 [50]. Soils were sampled in different locations in Spain (alfisol: 37º 32´03´´ N, 06º 13´22´´ W, vertisol: 37º 24´03´´ N, 05º 35´15´´ W), showing different soil properties (Table S (supplementary data) 1). Soil particle size analyses were carried out using the densimeter method [51]. Soil organic carbon (SOC) was determined by dichromate oxidation [52] and the cation exchange capacity (CEC) by using 1 M NH_4OAc buffered at pH 7 [53]. 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 [54]. The experiments were performed to determine how effective biovivianite can be when used as a Fe and P source using white lupin (*Lupinus albus* L.) and durum wheat *(Triticum durum L.)* respectively. White lupin was selected as a Fe chlorosis-sensitive plant [55, 56], meanwhile, wheat was used as a grain crop with significant P requirements [57]. The vertisol soil was used for lupin for the Fe experiment whereas alfisol was used for wheat for the P experiment. The treatments used for each experiment were;

1. Fe source (7 treatments): Control without Fe (non-fertilized with Fe), positive control (Fe(II)-sulfate), 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, Fe chelate (as Fe-EDDHA), and a synthesized vivianite from a commercial company (as ComViv) were the Fe sources used for white lupin.
2. P source (6 treatments): Control without phosphate (non-fertilized with P), positive control (KH_2PO_4), VivInsol6.5, VivInsol7.0, VivSol, and ComViv were used as the P sources for wheat.

In both experiments, the biovivianite was applied as a suspension to the soil and mixed thoroughly. 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 Fe(II)-sulfate was applied as a powder at the same rate as biovivianite (0.335 g Fe kg^{-1} soil). For the P uptake experiment, the rate was 15 mg P pot^{-1} (50 mg P kg^{-1} soil) for all treatments. Fertilizers were applied similarly to the Fe experiment, with KH_2PO_4 applied as a powder at a similar rate (50 mg P kg^{-1}).

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 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, $R^2 = 0.91$, $P < 0.001$, $n = 22$) [58] and for lupin (Chlorophyll (mg [kg fresh weight]$^{-1}$) = 0.3 ln (SPAD) − 0.48; $R^2 = 0.85$; $P < 0.001$, $n = 18$) [59]. 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°C and then weighed. The dried plant materials were ground to pass through a 1mm sieve and then mineralized at 550°C 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 100º C 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 [60]. A certified material (tomato leaf) 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°C and weighed afterwards. Fe extractions were analysed using the diethylenetriamine pentaacetic acid (DTPA) method [61], which is considered an index

of Fe availability to plants. After cropping, P availability to plants in the soil was assessed as Olsen P [54] with the colorimetric determination of P in the bicarbonate extracts [60]. Acid and alkaline phosphatase activity in the growing media was determined by measuring the amount of p-nitrophenol produced after the addition of p-nitrophenyl phosphate as substrate according to Tabatabai and Bremner [62].

Fertilizer (Fe and P) 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, Steingrobe [63] and using the formula,

$$RPUE\ (x)\ (\%) = (PU_x - PU_{control})/(PU_{KH2PO4} - PU_{control})$$

Where PUx is the phosphorus uptake of a fertilizer (mg $Ppot^{-1}$), $PU_{control}$ is the mean phosphorus uptake in the control without P fertilization (mg $Ppot^{-1}$), and PU_{KH2PO4} 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 Fe and P sources on the chlorophyll content, dry matter (DM) yield, Fe and P uptake in the shoots and roots, the relative Fe or P 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 [64], 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 Fe and P fertilizer for white lupin and wheat, 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 Fe experiments, Fe(II)-sulfate was used as the positive control and Fe-EDDHA as another Fe source. For the P experiment, KH_2PO_4 was used as the positive control.

Solid characterization of the bioproduced fertilizers (biovivianite)

Vivianite ($Fe^{2+}_3(PO_4)_2.8H_2O$) was the main mineral identified in all three biomineral products according to XRD results (Fig. 1A). Aside from vivianite, green rust 2 (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. Acid digestion (0.5M HCl) of the final products of bioreduction with ferrozine assay showed that VivInsol6.5, VivSol, and VivInsol7.0 contained 3%, 14% and 28% Fe(III) compared to Fe(II). The relatively higher concentration of Fe(III) could explain the presence of the more oxidised form of vivianite, metavivianite, identified in VivInsol7.0, although this value could also be attributed to any residual ferrihydrite, which was eluded to 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) (Figs. 1B and 1C). Interestingly, the average crystallite size of the vivianites were all quite similar (measured using the Scherrer equation (Eq. S1) [65]) was 66 nm, 63 nm, and 59 nm for VivInsol6.5, VivInsol7.0, and VivSol, respectively. XRD analysis of ComViv, a synthetic Fe source tested, showed the presence of vivianite (Fig. S1) with a crystallite size of 55 nm.

Effect of the Fe source on white lupin

Vivianite and other Fe sources tested influenced the parameters studied for white lupin (Soil Plant Analysis Development (SPAD) readings, DM yield, total Fe uptake by plants). At 10 DAT, treatment with VivInsol6.5 recorded the lowest SPAD readings compared with treatment with no Fe (Fig. 2A). 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 treatments for white lupin. However, VivInsol6.5 led to higher SPAD readings at harvest (p = 0.030) than the non-fertilized control. DM yield for shoots and roots of white lupin were 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) (Fig. 2B). Shoot concentrations of Fe in white lupin were not significantly different among all Fe treatments (Fig. 2C) whereas a significant difference was obtained among the root Fe concentrations (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 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. 2D). 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 (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 (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 (Fig. 2E).

Effect of the P source on durum wheat

The durum wheat dry matter yield was significantly higher for treatments with KH_2PO_4 and VivSol (Fig. 3A) than with other P sources; no significant difference was observed between KH_2PO_4 and VivSol. Among the vivianite treatments, VivSol and ComViv led to significantly higher DM than VivInsol6.5, signifying the effectiveness of both vivianites 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. 3B). The total P uptake by wheat was significantly higher with KH_2PO_4 than with the vivianite treatments except for VivSol. Treatments with ComViv, VivInsol6.5 and VivInsol7.0 were not significantly different from the non-fertilized control (Fig. 3C). 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 all the other treatments (Fig. S2). Olsen P values were not significantly different between treatments (Fig. S2). This experiment focused on the effectiveness of the P sources but SPAD readings for all treatments were significantly higher than the non-fertilized control at harvest (34 DAT) (Fig. 3D). Regardless, vivianite treatments did not lead to higher Fe uptake in wheat when compared with soluble fertilized and non-fertilized controls. VivSol led to the highest relative phosphorus use efficiency among the vivianites tested (around 74%) followed by VivInsol7.0 (32%), ComViv (16.4%) and VivInsol6.5 (less than 0.5%) as the lowest (Fig. 3E). No significant difference was observed between VivSol and VivInsol7.0 but there was a difference between VivSol and ComViv.

Discussion

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 chlorosis causes the yellowing of young leaves [66]. These symptoms were observed in both treatments with non-fertilized control and Fe(II)-sulfate, and the biovivianite treatments except lupin treated with 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 (Table S4). 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 biovivianite 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) and therefore this concentration is not the most accurate measure of iron deficiency chlorosis [37, 59, 67], a phenomenon called the Fe paradox (i.e. inactivation of Fe in the leaf apoplast) [66, 68, 69]. 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 [59]. 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 the least effective. This is consistent with previous studies showing that vivianite was as effective as Fe-EDDHA in preventing Fe chlorosis in plants [32, 34, 70]. 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 [30, 31]. The best results of VivInsol7.0 among the biovivianites tested could most likely be due to the smaller particle sizes and the mixed Fe phases identified in it. It was mainly vivianite ($Fe^{2+}_3(PO_4)_2.8H_2O$) and metavivianite ($Fe^{2+}Fe^{3+}_2(PO_4)_2(OH)_2{\cdot}6H_2O$) (Fig. 1A) with a particle size of 16 µm as compared with 28 µm of VivInsol6.5. VivInsol7.0 had 28% of Fe(III) compared with VivSol and VivInsol6.5 which had only 14% and 3% respectively. Thus, the ratio of Fe(II) to Fe(III) in the fertilizer product is not a good index for predicting the efficiency of the fertilizer product, since poorly crystalline Fe(III) oxides may also be sources of Fe for plants [59]. Aside from the white crystalline precipitate formed in VivInsol7.0, the supernatant was dark brown in colour (Fig. 1A). This colour could be ascribed to residual Fe(III) oxyhydroxides (likely ferrihydrite and lepidocrocite) which are deemed effective Fe sources for plants [24, 70], in particular those formed after root uptake of phosphate from the vivianite mineral [34, 71, 72]. This contributes to explaining, in addition to the differences in particle sizes, why VivInsol7.0 was a better Fe source for white lupin among the biovivianite tested in the calcareous soil.

Effectiveness of biovivianite as a P source for wheat

Treatments with vivianite promoted higher P concentrations in plants than the non-P fertilized control. Therefore, vivianite was a P source for the wheat plant, however, it was less efficient than the soluble mineral fertilizer used ($KH_2PO_{4)}$ in terms of P recovery (RPUE). The residual effect of the fertilizer, which can be estimated from the Olsen P in the soil after crop, did not differ between the fertilizers. Thus, it seems that the vivianites performed as a slow-release fertilizer, and probably a short-term experiment did not provide a full view of its P fertilizer value in usual crop cycles. Vivianite can be beneficial as a slow-release P fertilizer in the soil since it can minimize the precipitation of insoluble Ca phosphates which is expected around granules of soluble fertilizer. This precipitation decreases the use of applied P by crops [73]. Except for VivInsol6.5, all other vivianite treatments led to no significant differences in DM yield, when compared to the soluble P fertilizer, meanwhile, P concentrations in shoots were lower with vivianite treatments than with the soluble fertilizer. Thus, it seems that although less efficient in supplying P to plants than soluble fertilizer, most vivianites provide enough P to ensure maximum plant development. This would suggest 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. 3E), thus, soluble KH_2PO_4 can be replaced by VivSol and still be equivalent to the application of KH_2PO_4 by 74%. The mineral composition of VivSol according to XRD analysis was vivianite and phosphate green rust (GRII) with a particle size of 18 µm, and a crystallite size of 59 nm. Green rusts are mixed valence Fe(II)/Fe(III) layered hydroxides, comprising of 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) [74, 75]. 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-} [76]. GRII(PO_4^{3-}) was identified in VivSol as SO_4^{2-} was absent in the bioreduction medium. Vivianite (PO_4^{3-} -rich Fe(II) mineral) and GRII(PO_4^{3-}) are both phosphate-rich, and their abundance in VivSol 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 [42–45]. Only vivianite was identified in ComViv with a crystallite size of 55 nm. Although the particle size of ComViv was smaller than that of VivSol, the reason for the lower phosphorus use efficiency could be due to the crystalline nature of ComViv as evidenced by the XRD pattern (Fig. S1).

Our results provide practical evidence that confirms the growing interest in the use of biovivianite obtained from the microbial reduction of P-containing waste Fe systems as fertilizers. However, biomineral products from such waste streams would contain varying compounds, differing in crystallinity [77], particle sizes [78] and the incorporation of other elements such as magnesium and manganese [79] (this can affect the morphology of the mineral). These factors can affect the efficiency of biovivianite obtained from such waste streams as Fe or P fertilizer. Although the present data is promising with a view to using biovivianite as fertilizer and for providing more practical use of P-enriched waste Fe sludge, further research is necessary for more consistent evidence of the effectiveness of biovivianite as both a Fe and P source for plants under different environmental conditions (e.g., under field conditions). Our results also showed that biovivianite performed better as a P fertilizer than the chemically synthesized vivianite and therefore could be a suitable alternative P source for plants grown 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 are still needed.

Conclusion

Microbially mediated vivianite (biovivianite) can be used as an effective Fe and P source for plants. Biovivianite produced using soluble Fe(III) citrate (VivSol), which contained both vivianite and phosphate green rust, was the most 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 most effective 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 Fe and P fertilizer for lupin and wheat 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 Fe and P sources such as P-enriched Fe waste sludge. This, not only will it contribute to the reuse of waste materials but will also help to reduce the overdependence on phosphate rock for P fertilizer production, thereby reinforcing 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 2

RFeUE – Relative iron (Fe) use efficiency

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, LE.E, J.L, A.D, V.C; **Methodology**, LE.E, A.M.G, R.R.G; **Formal analysis**, LE.E, A.M.G; **Visualization**, LE.E, A.M.G; **Writing – original draft preparation**, LE.E; **Writing – review & editing**, A.M.G, A.D, J.L, V.C, S.S, R.R.G; **Supervision**, A.D, J.L, V.C, S.S; **Funding acquisition**, J.L. All authors read and approved the final manuscript.

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References

1. Zapata F, Roy RN. Use of phosphate rocks for sustainable agriculture. Zapata F, Roy RN, editors. Rome, Italy: FAO Land and Water Development Division, The International Atomic Energy Agency; 2004. 126 p.
2. Cordell D, Drangert J-O, White S. The story of phosphorus: Global food security and food for thought. Global Environmental Change. 2009;19(2):292-305. doi:10.1016/j.gloenvcha.2008.10.009.
3. Recena R, García-López AM, Quintero JM, Skyttä A, Ylivainio K, Santner J, et al. Assessing the phosphorus demand in European agricultural soils based on the Olsen method. Journal of Cleaner Production. 2022;379:134749. doi:https://doi.org/10.1016/j.jclepro.2022.134749.
4. de-Bashan LE, Bashan Y. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003). Water Res. 2004;38(19):4222-46. doi:10.1016/j.watres.2004.07.014.
5. Xu H, He P, Gu W, Wang G, Shao L. Recovery of phosphorus as struvite from sewage sludge ash. J Environ Sci (China). 2012;24(8):1533-8. doi:10.1016/s1001-0742(11)60969-8.

6. Prot T, Nguyen VH, Wilfert P, Dugulan AI, Goubitz K, De Ridder DJ, et al. Magnetic separation and characterization of vivianite from digested sewage sludge. Separation and Purification Technology. 2019;224:564-79. doi:https://doi.org/10.1016/j.seppur.2019.05.057.
7. Wilfert P, Dugulan AI, Goubitz K, Korving L, Witkamp GJ, Van Loosdrecht MCM. Vivianite as the main phosphate mineral in digested sewage sludge and its role for phosphate recovery. Water Res. 2018;144:312-21. doi:10.1016/j.watres.2018.07.020.
8. Wilfert P, Mandalidis A, Dugulan AI, Goubitz K, Korving L, Temmink H, et al. Vivianite as an important iron phosphate precipitate in sewage treatment plants. Water Res. 2016;104:449-60. doi:10.1016/j.watres.2016.08.032.
9. Dijkstra N, Slomp CP, Behrends T. Vivianite is a key sink for phosphorus in sediments of the Landsort Deep, an intermittently anoxic deep basin in the Baltic Sea. Chemical Geology. 2016;438:58-72. doi:10.1016/j.chemgeo.2016.05.025.
10. Egger M, Jilbert T, Behrends T, Rivard C, Slomp CP. Vivianite is a major sink for phosphorus in methanogenic coastal surface sediments. Geochimica et Cosmochimica Acta. 2015;169:217-35. doi:10.1016/j.gca.2015.09.012.
11. Rothe M, Kleeberg A, Hupfer M. The occurrence, identification and environmental relevance of vivianite in waterlogged soils and aquatic sediments. Earth-Science Reviews. 2016;158:51-64. doi:10.1016/j.earscirev.2016.04.008.
12. Heiberg L, Koch CB, Kjaergaard C, Jensen HS, Hans Christian BH. Vivianite precipitation and phosphate sorption following iron reduction in anoxic soils. J Environ Qual. 2012;41(3):938-49. doi:10.2134/jeq2011.0067.
13. Nanzyo M, Onodera H, Hasegawa E, Ito K, Kanno H. Formation and Dissolution of Vivianite in Paddy Field Soil. Soil Science Society of America Journal. 2013;77(4):1452-9. doi:10.2136/sssaj2012.0437n.
14. Fredrickson JK, Zachara JM, Kennedy DW, Dong H, Onstott TC, LI S-M. Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. Geochimica et Cosmochimica Acta. 1998;62(19/20):3239–57.
15. Kukkadapu RK, Zachara JM, Fredrickson JK, Kennedy DW. Biotransformation of two-line silica-ferrihydrite by a dissimilatory Fe(III)-reducing bacterium: formation of carbonate green rust in the presence of phosphate. Geochimica et Cosmochimica Acta. 2004;68(13):2799-814. doi:10.1016/j.gca.2003.12.024.
16. Li X, Liu T, Li F, Zhang W, Zhou S, Li Y. Reduction of structural Fe(III) in oxyhydroxides by *Shewanella decolorationis S12* and characterization of the surface properties of iron minerals. Journal of Soils and Sediments. 2011;12(2):217-27. doi:10.1007/s11368-011-0433-5.
17. Zachara JM, Kukkadapu RK, Fredrickson JK, Gorby YA, Smith SC. Biomineralization of Poorly Crystalline Fe(III) Oxides by Dissimilatory Metal Reducing Bacteria (DMRB). Geomicrobiology Journal. 2002;19(2):179-207. doi:10.1080/01490450252864271.

18. Kooli WM, Comensoli L, Maillard J, Albini M, Gelb A, Junier P, et al. Bacterial iron reduction and biogenic mineral formation for the stabilisation of corroded iron objects. Sci Rep. 2018;8(1):764. doi:10.1038/s41598-017-19020-3.
19. Zhang G, Dong H, Jiang H, Kukkadapu RK, Kim J, Eberl D, et al. Biomineralization associated with microbial reduction of Fe3+ and oxidation of Fe2+ in solid minerals. American Mineralogist. 2009;94:1049–58. doi:10.2138/am.2009.3136.
20. Fredeen AL, Raab TK, Rao MI, Terry N. Effects of phosphorus nutrition on photosynthesis in *Glycine max (L.)* Merr. Planta. 1990;181:399 - 405.
21. Rao MI, Terry N. Leaf Phosphate Status, Photosynthesis, and Carbon Partitioning in Sugar Beet. Plant Physiol. 1995;107:1313 - 21.
22. Pieters AJ, Paul MJ, Lawlor DW. Low sink demand limits photosynthesis under Pi deficiency. Journal of Experimental Botany. 2001;52(358):1083 -91.
23. Meng X, Chen WW, Wang YY, Huang ZR, Ye X, Chen LS, et al. Effects of phosphorus deficiency on the absorption of mineral nutrients, photosynthetic system performance and antioxidant metabolism in Citrus grandis. PLoS One. 2021;16(2):e0246944. doi:10.1371/journal.pone.0246944.
24. Benítez ML, Pedrajas VM, del Campillo MC, Torrent J. Iron chlorosis in olive in relation to soil properties. Nutrient Cycling in Agroecosystems. 2002;62(1):47-52. doi:10.1023/a:1015116732580.
25. Li J, Cao X, Jia X, Liu L, Cao H, Qin W, et al. Iron Deficiency Leads to Chlorosis Through Impacting Chlorophyll Synthesis and Nitrogen Metabolism in *Areca catechuL*. Front Plant Sci. 2021;12:710093. doi:10.3389/fpls.2021.710093.
26. Lucena JJ. Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. Journal of Plant Nutrition. 2000;23(11-12):1591-606. doi:10.1080/01904160009382126.
27. Mengel K. Iron availability in plant tissues - iron chlorosis on calcareous soils. Plant and Soil. 1994;165:275-83.
28. de Santiago A, Quintero JM, Avilés M, Delgado A. Effect of *Trichoderma asperellum* strain T34 on iron nutrition in white lupin. Soil Biology and Biochemistry. 2009;41(12):2453-9. doi:https://doi.org/10.1016/j.soilbio.2009.07.033.
29. Rombola AD, Cremonini M, Lucchi A, Sorrenti G, Placucci G, Marangoni B. Leaching of soil-applied synthetic Fe chelates (Fe-EDDHA) in orchard ecosystems. Book of Abstracts of the XI International Symposium on Iron Nutrition and Interaction in Plants 2002:23-8.
30. Ahmad F, Maqsood MA, Aziz T, Cheema MA. Water soluble Iron (Fe) concentration in alkaline and calcareous soils influenced by various Fe sources. Pak J Agri Sci. 2014;51(2):407-11.
31. Lucena JJ. Fe Chelates for Remediation of Fe Chlorosis in Strategy I Plants. Journal of Plant Nutrition. 2003;26(10-11):1969-84. doi:10.1081/pln-120024257.
32. Eynard A, del Campillo MC, Barron V, Torrent J. Use of vivianite (Fe3(PO4)2.8H20) to prevent iron chlorosis in calcareous soils. Fertilizer Research. 1992;31:61 - 7.

33. Iglesias I, Dalmau R, Marcé X, Del Campillo MC, Barrón V, Torrent J. Fertlization with Iron(II)-phosphate effectively prevents iron chlorosis in pear trees (*Pyrus Communis L.Acta).* International Society for Horticultural Science (ISHS). 2000;511:65-72. doi:10.17660/ActaHortic.2000.511.7.
34. Rosado R, del Campillo MC, Martínez MA, Barrón V, Torrent J. Long-term effectiveness of vivianite in reducing iron chlorosis in olive trees. Plant and Soil. 2002;241:139–44.
35. Rombolà AD, Toselli M, Carpintero J, Ammari T, Quartieri M, Torrent J, et al. Prevention of Iron-Deficiency Induced Chlorosis in Kiwifruit (*Actinidia deliciosa*) Through Soil Application of Synthetic Vivianite in a Calcareous Soil. Journal of Plant Nutrition. 2003;26(10-11):2031-41. doi:10.1081/pln-120024262.
36. Díaz I, Barrón V, del Campillo MC, Torrent J. Testing the ability of vivianite to prevent iron deficiency in pot-grown grapevine. Scientia Horticulturae. 2010;123(4):464-8. doi:10.1016/j.scienta.2009.11.006.
37. de Santiago A, Delgado A. Interaction between beet vinasse and iron fertilisers in the prevention of iron deficiency in lupins. J Sci Food Agric. 2010;90(13):2188-94. doi:10.1002/jsfa.4068.
38. Fodoué Y, Nguetnkam JP, Tchameni R, Basga SD, Penaye J. Assessment of the Fertilizing effect of Vivianite on the Growth and yield of the Bean "*phaseolus vulgaris*" on Oxisoils from Ngaoundere (Central North Cameroon). International Research Journal of Earth Sciences. 2015;3(4):18-26.
39. Ayeyemi T, Recena R, García-López AM, Delgado A. Circular Economy Approach to Enhance Soil Fertility Based on Recovering Phosphorus from Wastewater. Agronomy. 2023;13(6). doi:10.3390/agronomy13061513.
40. Saracanlao RJ, Van Ryckel H, Verbeeck M, Everaert M, Smolders E. Increasing phosphorus fertilizer value of recycled iron phosphates by prolonged flooding and organic matter addition. Pedosphere. 2023. doi:10.1016/j.pedsph.2023.03.020.
41. Coker VS, Watts MP, Lloyd JR. Bioconversion of Fe(III) oxides into magnetic nanoparticles: Processes and applications. In: Ahmed IAM, Hudson-Edwards KA, editors. Redox-reactive Minerals: Properties, Reactions and Applications in Clean Technologies. EMU Notes. 17: Mineralogical Society of Great Britain and Ireland; 2017.
42. Degryse F, Baird R, da Silva RC, McLaughlin MJ. Dissolution rate and agronomic effectiveness of struvite fertilizers - effect of soil pH-granulation and base excess. Plant and Soil. 2017;410(1/2):139-52. doi:10.1007/sl.
43. Kanabo I, Gilkes R. The effect of particle size on North Carolina phosphate rock on its dissolution in soil and on levels of bicarbonate-soluble phosphorus. Fertilizer Research. 1988;15:137-45.
44. Saied HSH, Aboelenin SM, Kesba H, El-Sherbieny AEA, Helmy AM, Dahdouh SM, et al. Chemical evaluation of partially acidulated phosphate rocks and their impact on dry matter yield and phosphorus uptake of maize. Saudi J Biol Sci. 2022;29(5):3511-8. doi:10.1016/j.sjbs.2022.02.022.
45. Turgut N, Ozsert L, Kara M. Effect of particle size of fertilizers on the longitudinal distribution pattern of some delivery mechanisms. Int Agrophysics. 1994;8:147-54.

46. Lloyd JR, Leang C, Myerson ALH, Coppi MV, Stacey C, Methe B, et al. Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in *Geobacter sulfurreducens*. Biochemical Journal. 2003;369:153–61.
47. Stookey LL. Ferrozine-A New Spectrophotometric Reagent for Iron. Analytical Chemistry. 1970;42(7):779 - 81.
48. Viollier E, Inglett PW, Hunter K, Roychoudhury AN, Van Cappellen P. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Applied Geochemistry. 2000;15(6):785-90. doi:10.1016/s0883-2927(99)00097-9.
49. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nature Methods. 2012;9(7):676-82. doi:10.1038/nmeth.2019.
50. Soil Survey Staff. Keys to Soil Taxonomy. 12th ed. Washington DC: USDA-NAtural Resources Conservation Service; 2014.
51. Gee GW, Bauder JW. Particle-size Analysis. Methods of Soil Analysis. SSSA Book Series. 2 ed1986. p. 383-411.
52. Walkley A, Black IA. An Examination of the Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. Soil Science. 1934;37:29-38. doi:http://dx.doi.org/10.1097/00010694-193401000-00003.
53. Sumner ME, Miller WP. Cation Exchange Capacity and Exchange Coefficients. Methods of Soil Analysis. SSSA Book Series1996. p. 1201-29.
54. Olsen SR. Estimation of available phosphorus in soils by extraction with sodium bicarbonate: US Department of Agriculture; 1954.
55. Tang C, Zheng SJ, Qiao YF, Wang GH, Han XZ. Interactions Between High pH and Iron Supply on Nodulation and Iron Nutrition of *Lupinus albus L.* Genotypes Differing in Sensitivity to Iron Deficiency. Plant and Soil. 2006;279(1-2):153-62. doi:10.1007/s11104-005-0616-1.
56. White PF, Robson AD. Response of lupins (*Lupinus angustifolius L.*) and peas (*Pisum sativum L.*) to Fe deficiency induced by low concentrations of Fe in solution or by addition of HCO3^-1. Plant and Soil 1990;125(1):39-47.
57. Sandaña P, Pinochet D. Grain yield and phosphorus use efficiency of wheat and pea in a high yielding environment. Journal of Soil Science and Plant Nutrition. 2014;14(4):973-86.
58. de Santiago A, Quintero JM, Avilés M, Delgado A. Effect of *Trichoderma asperellum* strain T34 on iron, copper, manganese, and zinc uptake by wheat grown on a calcareous medium. Plant and Soil. 2011;342(1-2):97-104. doi:10.1007/s11104-010-0670-1.
59. de Santiago A, Delgado A. Predicting Iron Chlorosis of Lupin in Calcareous Spanish soils from Iron Extracts. Soil Science Society of America Journal. 2006;70(6):1945 - 50.
60. Murphy J, Riley JP. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Analytica Chimica Acta. 1962;27:31 - 6.

61. Lindsay WL, Norvell W. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil science society of America journal. 1978;42(3):421-8.
62. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem. 1969;1:301-7.
63. Cabeza R, Steingrobe B, Römer W, Claassen N. Effectiveness of recycled P products as P fertilizers, as evaluated in pot experiments. Nutrient Cycling in Agroecosystems. 2011;91(2):173-84. doi:10.1007/s10705-011-9454-0.
64. Acutis M, Scaglia B, Confalonieri R. Perfunctory analysis of variance in agronomy, and its consequences in experimental results interpretation. European Journal of Agronomy. 2012;43:129-35. doi:10.1016/j.eja.2012.06.006.
65. Langford JI, Wilson AJC. Scherrer after Sixty Years: A Survey and Some New Results in the Determination of Crystallite Size. Journal of Applied Crystallography. 1978;11:102-13. doi:http://dx.doi.org/10.1107/s0021889878012844.
66. Morales F, Grasa R, Abadía A, Abadía J. Iron chlorosis paradox in fruit trees. Journal of Plant Nutrition. 1998;21(4):815-25. doi:10.1080/01904169809365444.
67. de Santiago A, Quintero JM, Carmona E, Delgado A. Humic substances increase the effectiveness of iron sulfate and Vivianite preventing iron chlorosis in white lupin. Biology and Fertility of Soils. 2008;44(6):875-83. doi:10.1007/s00374-008-0272-8.
68. Römheld V. The chlorosis paradox: Fe inactivation as a secondary event in chlorotic leaves of grapevine. Journal of Plant Nutrition. 2000;23(11-12):1629-43. doi:10.1080/01904160009382129.
69. O'Toole MA. Differences in susceptibility to iron chlorosis of grass species grown on blanket peat. Nature. 1966;212:213. doi:doi:10.1038/212213a0, -213.
70. Ammari TG, Hattar B. Effectiveness of Vivianite to Prevent Lime-Induced Iron Deficiency in Lemon Trees Grown on Highly Calcareous Soil. Communications in Soil Science and Plant Analysis. 2011;42(21):2586-93. doi:10.1080/00103624.2011.614034.
71. Cumplido Js, Barro´n V, Torrent J. Effect of phosphate on the formation of nanophase lepidocrocite from Fe(II) sulfate. Clays and Clay Minerals. 2000;48(5):503–10.
72. de Santiago A, Carmona E, Quintero JM, Delgado A. Effectiveness of mixtures of vivianite and organic materials in preventing iron chlorosis in strawberry. Spanish Journal of Agricultural Research. 2013;11(1). doi:10.5424/sjar/2013111-2671.
73. Delgado A, Madrid A, Kassem S, Andreu L, del Campillo MıdC. Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant and Soil. 2002;245:277–86.
74. Benali O, Abdelmoula M, Refait P, Ge'nin J-MR. Effect of othophosphate on the oxidation product of green rust. Geochimica et Cosmochimica Acta. 2001;65(11).
75. Ona-Nguema G, Carteret C, Benali O, Abdelmoula M, Génin J-M, Jorand F. Competitive Formation of Hydroxycarbonate Green Rust 1 versus Hydroxysulphate Green Rust 2 in *Shewanella putrefaciens* Cultures. Geomicrobiology Journal. 2004;21(2):79-90. doi:10.1080/01490450490266316.

76. Zegeye A, Ona-Nguema G, Carteret C, Huguet L, Abdelmoula M, Génin J-M, et al. Formation of Hydroxysulphate Green Rust 2 as a Single Iron(II-III) Mineral in Microbial Culture. Geomicrobiology Journal. 2005;22:1-11. doi:10.1080/01490450500248960.
77. Joshi N, Filip J, Coker VS, Sadhukhan J, Safarik I, Bagshaw H, et al. Microbial Reduction of Natural Fe(III) Minerals; Toward the Sustainable Production of Functional Magnetic Nanoparticles. Frontiers in Environmental Science. 2018;6. doi:10.3389/fenvs.2018.00127.
78. Byrne JM, Telling ND, Coker VS, Pattrick RA, van der Laan G, Arenholz E, et al. Control of nanoparticle size, reactivity and magnetic properties during the bioproduction of magnetite by *Geobacter sulfurreducens*. Nanotechnology. 2011;22(45):455709. doi:10.1088/0957-4484/22/45/455709.
79. Joëlle Kubeneck L, ThomasArrigo LK, Rothwell KA, Kaegi R, Kretzschmar R. Competitive incorporation of Mn and Mg in vivianite at varying salinity and effects on crystal structure and morphology. Geochimica et Cosmochimica Acta. 2023;346:231-44. doi:10.1016/j.gca.2023.01.029.

Figures

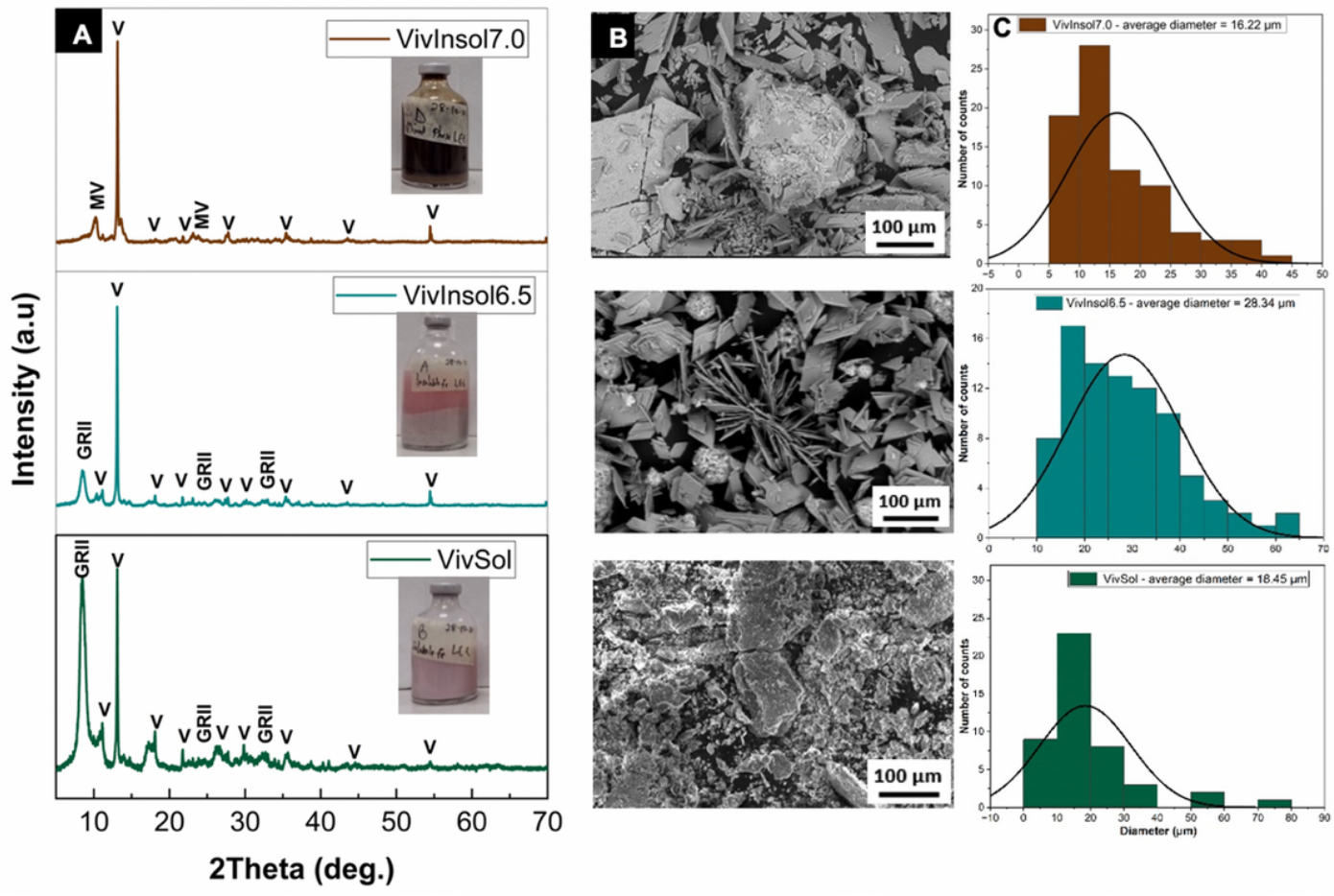

Figure 1

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.

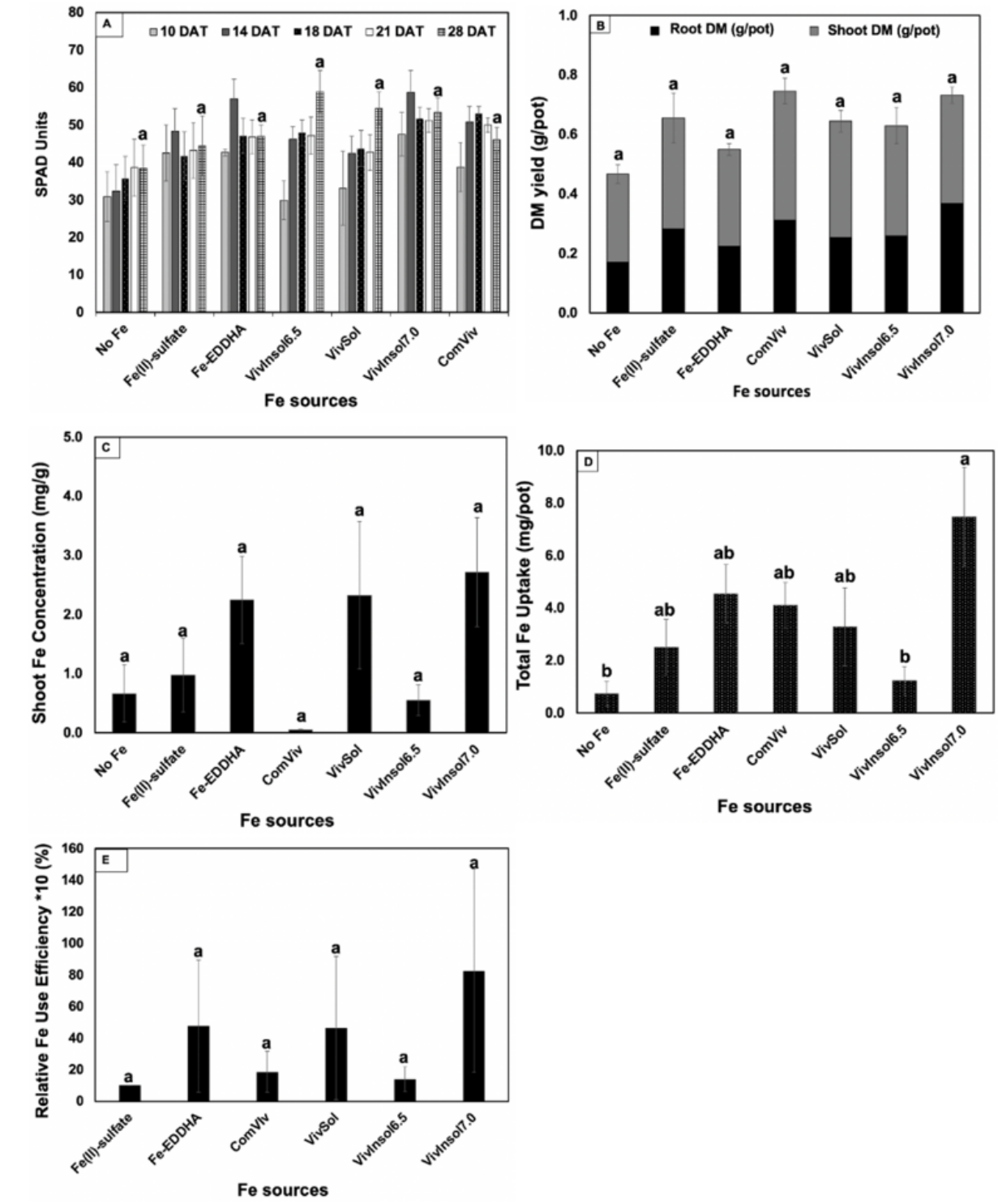

Figure 2

Effect of the application of different Fe sources on the SPAD measurements for lupin harvested at 28 DAT (A), dry matter (DM) yield (B), Shoot Fe concentration (C) and total Fe uptake (D) for white lupin. 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. E represents the Relative Fe use efficiency (%) of the tested fertilizers using Fe(II)-sulfate as the reference fertilizer.

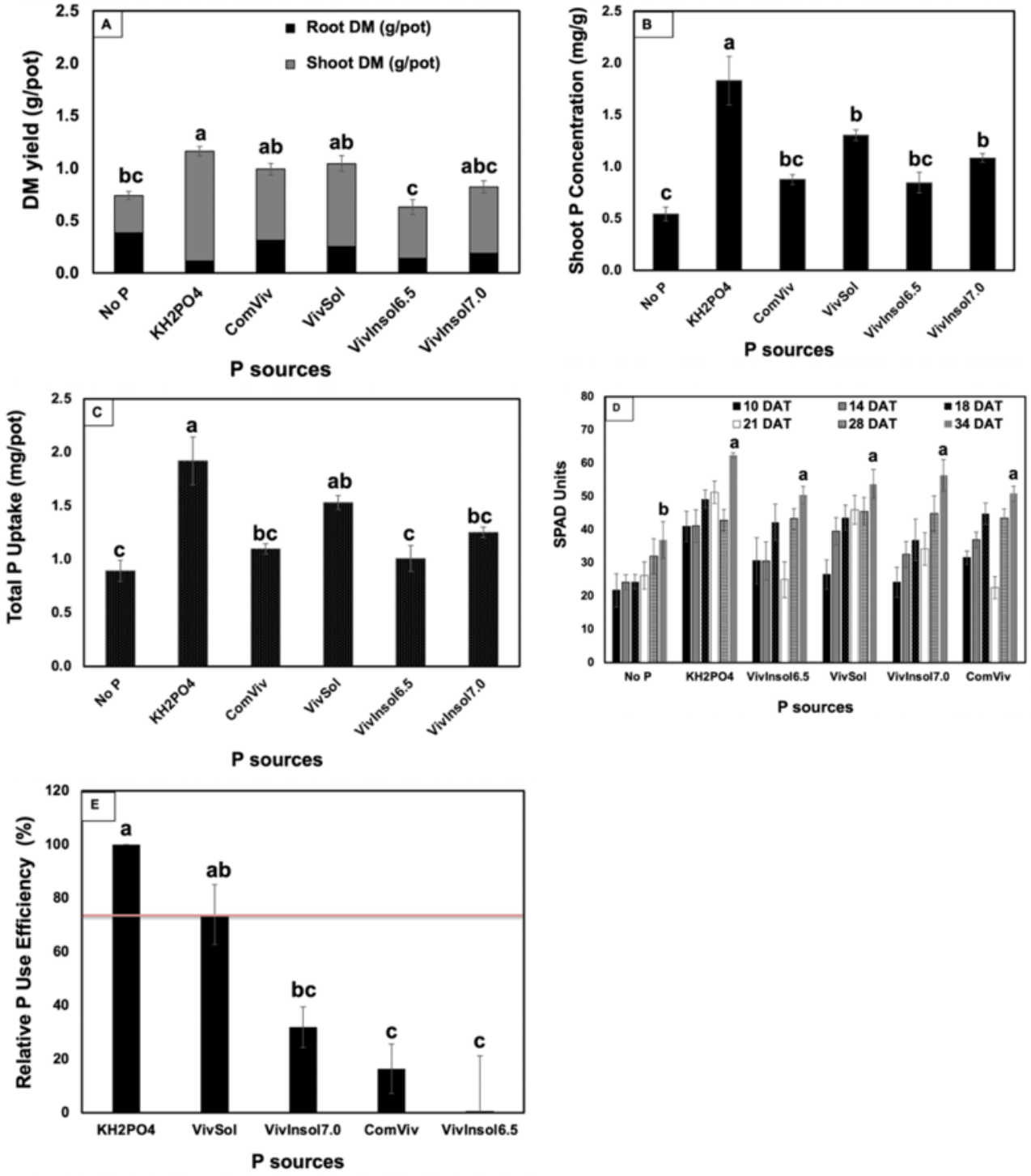

Figure 3

Effect of the application of different P sources on dry matter (DM) yield (A), Shoot P concentration (B) and total P uptake (C) for durum wheat. D is the SPAD measurements for durum wheat harvested at 34 DAT. 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. E represents the Relative P use efficiency (%) of the tested fertilizers using KH_2PO_4 as the reference fertilizer.

Supplementary Files

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- SupplementaryMaterial.docx
- GA.png