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**Microbial Volatile Organic Compound (VOC)-Driven Dissolution and Surface Modification of Phosphorus-Containing Soil Minerals for Plant Nutrition: An Indirect Route for VOC-Based Plant-Microbe Communications**

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(Article begins on next page)

1 **Bacterial VOC-driven surface engineering of phosphorus-containing soil minerals and its**  
2 **impact on plant growth: an indirect route for VOC-based plant-microbe communications**

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26

27 **Abstract**

28 Microbial volatile organic compounds (MVOCs) are believed to act as remote messengers in the interaction with  
29 plants, resulting in physiological changes in terms of plant growth and stress resistance. However, it remains unclear  
30 whether plants directly detect MVOCs using dedicated sensing machinery or whether other factors mediate this  
31 communication process. We investigated the ability of MVOCs secreted by *Bacillus megaterium* (a well-known  
32 MVOC producer) to modify the solid surface of hydroxyapatites, a widespread group of soil minerals. FT-IR spectral  
33 results indicated the spontaneous localization of MVOCs on the apatite surface based on variation in the COO<sup>-</sup> band  
34 with incubation time. The MVOCs facilitated the release of soluble phosphate from the minerals in a time-dependent

1 manner. The formation of calcium oxalate was revealed through SEM-EDS and XRD analyses, suggesting that the  
2 MVOC oxalic acid can block the recrystallization of phosphate into calcium phosphates. Gel- and soil-based plant  
3 cultivation tests employing *Arabidopsis thaliana* and solid calcium phosphates (*i.e.*, nano- and micro-sized  
4 hydroxyapatites and calcium phosphate dibasic) revealed that these MVOC mechanisms facilitated plant growth by  
5 ensuring the prolonged supply of plant-available phosphorus. The relationship between the enhancement of growth  
6 and the particle size of the calcium phosphates also substantiates the sorption of MVOCs onto soil minerals related to  
7 plant growth. Given that most previous studies have assumed that MVOCs are a molecular lexicon directly detected  
8 by plants, our approach provides a new mechanistic view of the presence of abiotic mediators in the interaction  
9 between plants and microbes via MVOCs.

10  
11  
12 **Keywords:** microbial volatile organic compounds, calcium phosphate, surface engineering, plant nutrition, plant-  
13 microbe interaction

## 14 15 **Introduction**

16 Microbial volatile organic compounds (MVOCs) are low-molecular-weight microbial metabolites derived from  
17 various synthetic pathways for aliphatic amino acids, fatty acids, terpenoids, pyrazines, and aromatic compounds  
18 (Schulz et al., 2020). In nature, MVOCs can move far from their site of production via the air, water, and pores in the  
19 soil, thus allowing microbes to interact with other biological species at a distance (Kanchiswamy et al., 2015). MVOCs  
20 appear to be particularly important in plant–microbe communications because, with the exception of epiphytes, the  
21 limited mobility and small size of microbes restrict physical contact with plants. In fact, scientific evidence supporting  
22 MVOC-driven plant–microbe interactions is abundant, with many microbial genera including *Arthrobacter*,  
23 *Pseudomonas*, *Bacillus*, *Trichoderma*, *Paraburkholderia*, and *Paenibacillus* shown to be capable of producing organic  
24 volatiles that modulate plant growth and stress resistance (Fincheira et al., 2018; Lee et al., 2012; Weisskopf et al.,  
25 2021). For example, several kinds of organics (*e.g.*, 2,3-butanediol, indole, dimethyl disulfide, and pentadecanol) in  
26 MVOCs are found to facilitate root, leaf, and shoot growth and chlorophyll production, which is accompanied by  
27 variation in a wide range of genetic expressions associated with photosynthesis, cell wall synthesis, and auxin hormone  
28 responsiveness (Fincheira et al., 2018). The resistance of plants to biotic (*e.g.*, pathogens) and abiotic (*e.g.*, drought  
29 and salt) stresses is promoted by MVOCs via the induction of systemic resistance, the synthesis of compatible solutes,  
30 and changes in sodium transporter expression (Liu et al., 2015; Weisskopf et al., 2021).

31  
32 Harnessing these effects of MVOCs on plants has been regarded as a promising strategy for sustainable agricultural  
33 practices, but detailed mechanistic explanations for how plants detect MVOCs still remain to be elucidated. It has

1 been hypothesized that plants inherently possess dedicated sensing machinery but, to the best of our knowledge, no  
2 molecular evidence has been reported to date. Moreover, MVOCs are structurally versatile, and a variety of MVOCs  
3 are produced simultaneously, meaning that it would be difficult for plants to directly detect them. Alternatively, other  
4 possible mechanisms could be non-specific interactions between organic moieties frequently found in MVOCs and  
5 plant components or that other factors controlled by MVOCs mediate plant–microbe interactions. It has been  
6 demonstrated that cellular membranes can be damaged by the penetration ability and non-specific binding of MVOCs  
7 to membrane components (Trombetta et al., 2015), indicating that initial non-specific stress- or toxicity-related  
8 responses can translate into MVOC-induced changes in plant physiology. Moreover, Jones et al. (2019) reported that  
9 trimethylamine (TMA), an MVOC produced by *Streptomyces*, alters iron availability by changing the environmental  
10 pH. Plant nutrition and associated plant physiology can be subsequently affected by changes such as this, thus other  
11 soil-related factors could be investigated as potential mediators to account for plant–microbe communications.

12  
13 MVOCs containing oxygen-based functional groups, which derive from the primary, fatty acid, and aromatic  
14 metabolism of microbes, are easily detected in the plant-microbe interactions (Weisskopf et al., 2021). Interestingly,  
15 these MVOCs are structural parallels to some organics associated with the surface engineering of metallic minerals  
16 found in soil environments. For example, stearic acid-modified apatite surfaces lead to dispersion in non-polar  
17 matrices (Li et al., 2008), and the spontaneous localization of citric acid on apatite surfaces is feasible when modulating  
18 the kinetics of apatite dissolution (Samavini et al., 2018; Schutze et al., 2020). Oxalic acid is also capable of modifying  
19 mineral surfaces, including silicate, gangue, and ilmenite, which consequently affects their floatation behavior (Nuri  
20 et al., 2019). It has been reported that gluconic acid has a role in phosphate solubilization (Lin et al., 2006; Rodriguez  
21 et al., 2004; Stella and Halimi., 2015). These non-specific actions associated with several minerals appear to exploit  
22 the ability of oxygen-based functional groups within MVOCs to facilitate coordination, hydrogen bonding, and  
23 dipole–dipole interactions. It is also important to note that these modifications coincide with changes in plant growth  
24 and physiology. Acidic oxygen-based functional groups can solubilize the surface of minerals, leading to the release  
25 of diffusible ions. In particular, phosphate, an important plant nutrient, is efficiently released in this manner. This  
26 surface engineering-based action can also be prolonged in a time-dependent manner (Samavini et al., 2018; Schutze  
27 et al., 2020; Wang et al., 2016), suggesting that soil quality evolves in terms of plant nutrition. Indeed, it has been  
28 demonstrated that plant nutrition directly affects plant growth, development, and stress resistance (Feller et al., 2018).  
29 Expression of diverse sets of genes and proteins is also modulated with plant nutrition (Brumbarova et al., 2019).

30  
31 Given the structural similarities between known MVOCs and organic compounds capable of mineral surface  
32 engineering, it is highly likely that a significant proportion of MVOCs play a crucial role in modifying mineral surfaces  
33 in soils. The mobility of MVOCs means that they can diffuse into the plant rhizosphere, subsequently modifying the  
34 surface of minerals required for plant nutrition. Because the effects of plant nutrition (*e.g.*, plant growth and stress  
35 resistance) are similar to the observations reported in previous studies that posit the direct sensing of MVOCs by plants

(Sharifi., 2018; Weisskopf et al., 2021), it is important to investigate the possibility that MVOCs are also involved in plant–microbe communications via the modification of minerals employed in plant nutrition. This study thus aimed to confirm whether the surface characteristics of solid ceramic particles are modified by MVOCs. Calcium phosphate and *Bacillus megaterium* were selected as the model ceramic and microorganism, respectively. After measuring phosphate release kinetics of calcium phosphate before and after MVOC treatment, Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD) were employed to characterize the effects of MVOCs on the mineral surface. Finally, the growth of *Arabidopsis thaliana* was assessed to evaluate the potential of MVOC-driven mineral surface modification as an abiotic mediator facilitating plant–microbe communications.

## Materials and methods

### *Chemicals and materials*

*Bacillus megaterium* GNU-01 was isolated from commercial humic acids and deposited at the Korean Collection for Type Cultures (No. 13087BP). Wild-type *A. thaliana* (Col-0 background) was kindly donated by Dr. Joon-Yung Cha (Gyeongsang National University, Jinju, Republic of Korea). Murashige and Skoog (MS) medium, calcium phosphate dibasic (purum p.a., ≥85%), micrometric hydroxyapatite (HA; Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>, reagent grade), vanadate-molybdate (p.a. for phosphate determination), calcium hydroxide (Ca(OH)<sub>2</sub>, ACS reagent, ≥95%), phosphoric acid (H<sub>3</sub>PO<sub>4</sub>, ACS reagent, ≥85%), potassium bromide (KBr, FT-IR grade, ≥99 trace metal basis), hydrochloric acid (HCl, extra pure grade), sodium bicarbonate (NaHCO<sub>3</sub>, ACS reagent, ≥99.7%), nutrient broth, and sodium hydroxide (NaOH, reagent grade, ≥99.7%), were purchased from Sigma-Aldrich. Plant agar and sucrose were purchased from Duchefa-Biochemie, and bed soil and potassium chloride (KCl) were purchased from Nongwoo Bio (Suwon, Republic of Korea) and Namhae Chemical (Yeosu, Republic of Korea), respectively. Unless otherwise noted, ultra-pure water (Milli-Q, Millipore) was used in the experiments and all reagents were used as received without any extra purification.

### *Plant and bacteria cultivation*

*Bacillus megaterium* was cultured in a 250 mL Erlenmeyer flask containing 100 mL of nutrient broth. The flask was kept in a shaking incubator (160 rpm) at 32 °C for 24 h before use. Plant cultures were created using MS medium containing 0.9% (w/v) agar and 2% (w/v) sucrose with the pH adjusted to 5.8 unless otherwise mentioned. The surface of the seeds was sterilized with 70% ethanol for 30 s and then 20% Clorox for 5 min followed by repeated washing with distilled water. After sowing, the plates were vernalized for 2 days at 4 °C in the absence of light and transferred to a conventional plant chamber (23 °C and 16/8 h light/dark cycle). In addition, MS medium that had been modified to remove phosphorus (P) was mechanically mixed with HA nanoparticles (HANPs) to ensure that the HANPs were the only source of P for plant nutrition.

1 ***Bacillus megaterium* VOCs profiling**

2 *Bacillus megaterium* was cultured in 20 ml glass vials containing MS agar. The vials were tightly sealed with a steel  
3 crimp cap fitted with a Teflon/silicon septum to minimize VOC leakage. Solid-phase microextraction (SPME) was  
4 then conducted using flexdivinylbenzene/carboxen/polydimethylsiloxane (50/30 lm) fibers (Supelco). The microbial  
5 colony-bearing agar was warmed to 80 °C and the SPME fibers were injected into the headspace for 20 min. The  
6 VOCs on the SPME fibers were then desorbed and profiled using gas chromatography–mass spectrometry (GC-MS,  
7 QP2020NX, Shimadzu) equipped with a DB5-WAX column (30 m, 0.25 mm id., 0.25 um film thickness, Agilent).  
8 The oven temperature started at 40 °C for 3 min and was increased to 80 °C at a rate of 7 °C/min and then to 240 °C  
9 at a rate of 25 °C/min, where it was held for 5 min. An electron impact method was used to obtain the mass-to-charge  
10 ratio ( $m/z$ ) from 40 to 500, and the mass fragment patterns for each significant peak were compared with those in the  
11 NIST database.

12  
13 ***Hydroxyapatite nanoparticle synthesis***

14 HANPs were synthesized using a modified version of the neutralization method described by Adamiano et al. (2017).  
15 Briefly, solution A was obtained by adding 10 g of Ca(OH)<sub>2</sub> to 100 mL of distilled water followed by stirring at 400  
16 rpm for 30 min to produce a homogenous solution. Solution B comprising 8.87 g of H<sub>3</sub>PO<sub>4</sub> dissolved in 30 mL of  
17 distilled water was added slowly to solution A under constant stirring at 400 rpm for 180 min followed by incubation  
18 overnight at room temperature. The Ca:P ratio was set at 1.67 mole/L for synthesis. The nanoparticle powder was  
19 washed thoroughly with distilled water and air-dried at 40 °C.

20  
21 ***Phosphate release analysis***

22 Biplates containing two physically separated spaces were used to assess the effects of MVOCs on the phosphate  
23 release of HANPs. One space contained HANPs (0.7 g) dispersed in 15 mL of distilled water, while the other space  
24 held MS agar that had been inoculated with 15 µL of *B. megaterium* (approximately 1×10<sup>9</sup> CFU) in a dropwise manner.  
25 The pH and phosphate concentration were examined weekly for up to 8 weeks and compared with those for a HANP  
26 dispersion without bacteria. The release of phosphate from the HANPs was measured using a vanadate/molybdate  
27 method. Briefly, the supernatant of the HANP suspension was harvested weekly using centrifugation (13,000 rpm and  
28 10 min), with 1 mL aliquots filtered using a syringe filter (0.2 µm) and then mixed with 250 µL of vanadate–molybdate  
29 reagent. After 10 min at room temperature, the absorption was recorded at 470 nm with a conventional UV-visible  
30 spectrophotometer. The separated HANP pellets were re-suspended in fresh distilled water.

31  
32 **Effect of *Bacillus megaterium* VOCs on HANP mineralogy**

1 For instrument-based analysis, the HANP solutions were treated with KCl to raise their ionic strength to 0.01 mole/L.  
2 The particles were then harvested using centrifugation (3,000 rpm and 20 min) followed by drying at room temperature.

3

#### 4 *Fourier-transform infrared spectroscopy analysis*

5 For all the FT-IR spectroscopic measurements, HANP powder, after exposure to the microbes for 7, 14, or 28 days,  
6 was pressed into self-supporting pellets and placed in quartz IR cells designed for spectroscopic measurements at  
7 room temperature (*i.e.*, cells equipped with KBr windows). The cells were connected to a conventional vacuum line  
8 (residual pressure =  $1 \times 10^{-3}$  mbar) to perform all *in situ* treatments and adsorption–desorption experiments. The  
9 spectra were collected at a resolution of  $4 \text{ cm}^{-1}$  with a Bruker Invenio R spectrometer equipped with a DTGS detector.  
10 The number of scans was adjusted to  $64 \text{ s}^{-1}$  to attain a suitable signal-to-noise ratio. The data were normalized to the  
11 optical density (*i.e.*, the weight of the pellet in mg/the area of the pellet in  $\text{cm}^2$ ) to account for differences in the  
12 thickness of the pellets. The HANPs were exposed to water ( $\text{H}_2\text{O}$ ) or heavy water ( $\text{D}_2\text{O}$ ) vapor pressure at the beam  
13 temperature (ca.  $50 \text{ }^\circ\text{C}$ ) and then outgassed for 3 h. To achieve the complete exchange of  $\text{H}_2\text{O}$  with  $\text{D}_2\text{O}$ , the sample  
14 underwent a series of heavy water admission/outgassing steps until the invariance of the spectra. For the adsorption  
15 of oxalic acid, the pellet was placed at the top of a closed chamber that had 200 mg of oxalic acid crystals at the bottom.  
16 The chamber was placed in an oven at  $80 \text{ }^\circ\text{C}$  for 4 h, saturating the chamber with oxalic acid vapor and allowing the  
17 adsorption of oxalic acid on the HANP surface.

18

#### 19 *X-ray diffraction analysis*

20 Each sample was analyzed using XRD with a D8 Advance diffractometer (Bruker, Karlsruhe, Germany) equipped  
21 with a Lynx-eye position-sensitive detector using  $\text{Cu K}\alpha$  radiation ( $\lambda = 1.54178 \text{ \AA}$ ) generated at 40 kV and 40 mA.  
22 The XRD profiles of the samples were acquired using a step size ( $2\theta$ ) of  $0.02^\circ$  and a counting time of 0.5 s in a  $2\theta$   
23 range of  $10\text{--}60^\circ$ . Phase identification was conducted with Highscore software (PANalytical) and powder diffraction  
24 files (ICDD PDF4+ 2013).

25 Equation (1) was used to calculate the crystallinity of each sample:

$$26 \text{ Crystallinity [\%]} = 100 \cdot C / (A + C) \quad (1)$$

27 where  $C$  is the sum of the peak areas and  $A$  is the area between the peaks and the background in the diffraction patterns  
28 (Adamiano et al, 2013).

29

#### 30 *Scanning electron microscopy*

31 The morphology of the samples was investigated using scanning electron microscopy (SEM) with a field-emission  
32 microscope (FE-SEM, SIGMA, ZEISS NTS GmbH, Oberkochen, Germany) coupled to an energy-dispersive X-ray

1 spectrometer (EDS; INCA Energy 300, Oxford Instruments, Abingdon-on-Thames, UK). A small quantity of the  
2 material was placed on carbon tape supported by an aluminum SEM stub for sputter-coating (Polaron E5100, Polaron  
3 Equipment, Watford, Hertfordshire, UK) with a 10 nm layer of gold for electrical conductance. A 3 kV accelerating  
4 voltage was used for sample observations in secondary electron imaging mode. Four random regions of the stub were  
5 imaged at magnifications of 5000×, 15,000×, 25,000×, and 50,000× for each sample. The elemental composition of  
6 the samples was analyzed using SEM-EDS with non-sputter-coated HANPs. An acceleration voltage of 10 kV was  
7 used to collect spectra from a minimum of four randomly selected regions of the samples at magnifications of 10,000×.

8

### 9 *Bioavailability analysis*

10 To assess whether the MVOC-mediated solubilization of HANPs is involved in plant nutrition, modified MS medium  
11 whose phosphate ions had been replaced with HANP particles (*i.e.*, 0, 2, 6, and 18 mg of HANPs in 15 mL of MS  
12 medium) was used to cultivate *A. thaliana*. Fourteen-day old *A. thaliana* seedlings were transferred to one half of a  
13 biplate containing the modified MS medium, with the other half containing normal MS agar inoculated with *B.*  
14 *megaterium* (approximately  $1 \times 10^9$  CFU) in a dropwise manner. The plates were incubated in a plant growth chamber  
15 (23 °C with a 16/8 h light/dark cycle). The fresh and dry weights (70 °C for 24 h) of the plants were compared with  
16 those of controls cultivated without the microbes and/or the HANPs. To measure pH and phosphate concentrations,  
17 agar slices from each plate were transferred to conical tubes after removing the plants and heated until the agar had  
18 completely melted. The pH was measured using a regular pH electrode, and phosphate was measured using the  
19 vanadate–molybdate method described above.

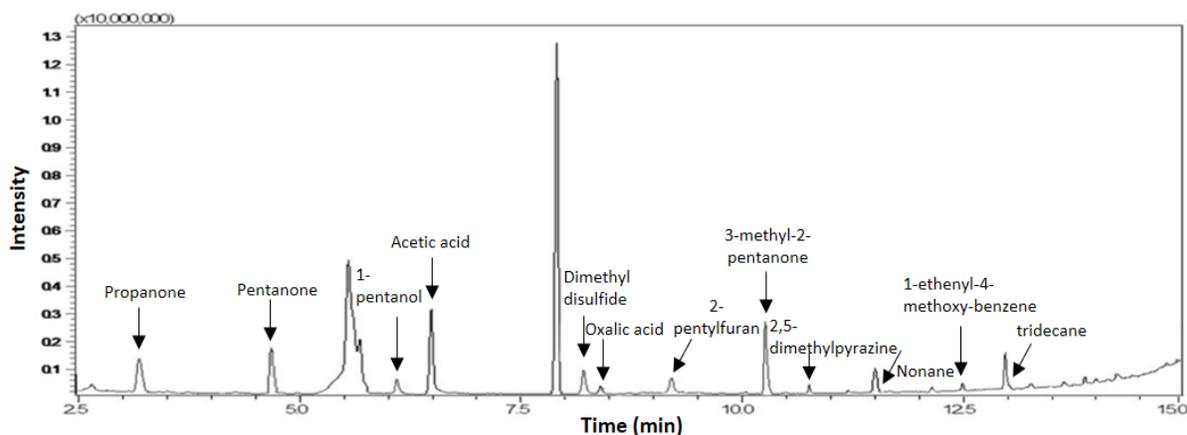
20 Soil systems were also created by mixing air-dried soil particles (50 g) with calcium phosphate powder (*i.e.*, 3 or 6  
21 mg of HANPs, calcium phosphate dibasic, or micrometric HA). A Petri dish (6 mm) containing normal MS agar  
22 inoculated with *B. megaterium* (approximately  $1 \times 10^9$  CFU) was placed at the bottom of the soil pot, thus allowing the  
23 microbes to emit MVOCs toward the soil. Sterilized *A. thaliana* seeds were sowed and then incubated in a plant  
24 chamber (23 °C with a 16/8 h light/dark cycle) for two months. Total P was obtained from 0.25 g of the soil from each  
25 pot, which was heated to 550 °C for 1 h followed by mixing the resulting dried soil with 25 mL of 1 M HCl. The  
26 mixture was incubated for 16 h at 60 °C. P content was estimated using inductively coupled plasma-optical emission  
27 spectrometry (ICP-OES, Thermo Scientific-United States) with 0.2 µm-filtered solutions. Available P was measured  
28 by adding 40 mL of 0.5 M NaHCO<sub>3</sub> to 0.5 g of soil. The pH was then adjusted to 8.5 with 1 N NaOH followed by  
29 gentle shaking for 16 h at room temperature. After centrifugation (3000 rpm and 5 min), the P in the supernatant was  
30 quantified using ICP-OES (Wuenschel et al., 2015; Zhao et al., 2018).

31

32

33

## 1 Results and Discussion



2

3 **Figure 1.** GC-MS profiling of VOCs emitted from *Bacillus megaterium*

4 MVOCs from *B. megaterium* were identified to evaluate whether the microbe has the potential to stimulate plant  
5 growth with airborne molecules. As shown in **Fig. 1 and Table S1**, six groups of compounds with 12 distinct organic  
6 structures were detected, including three ketones (propanone, 2-pentanone, and 3-methyl-2-pentanone), two organic  
7 acids (acetic acid and oxalic acid), one alcohol (1-pentanol), one sulfur-containing compound (dimethyl disulfide),  
8 three aromatics (2-pentylfuran, 2,5-dimethylpyrazine, and 1-ethyl-4-methoxy-benzene), and two alkanes (nonane and  
9 tridecane). Some of these, including dimethyl disulfide, pentanone, and pentanol, have been previously reported to  
10 promote plant growth and systemic resistance through airborne pathways (Choi et al., 2014; Kanchiswamy et al.,  
11 2015). It is also noticeable that small oxygen-containing organic compounds such as oxalic acid, acetic acid, and  
12 pentanol are produced as organic volatiles, as described previously for other microbes (Schulz et al., 2020; Weisskopf  
13 et al., 2021).

14

15 Previous studies have shown that the bioavailability of P from rock phosphate and P-containing ceramics is higher in  
16 the presence of oxygen-containing organic compounds (Wang et al., 2016; Jamal et al., 2018). The relatively low  $pK_a$   
17 of alcohols and carboxylic acid leads to a more acidic soil environment, thus facilitating the dissolution of P-containing  
18 solids. Another effect of small organic acids such as oxalic acid and citric acid is their spontaneous adsorption onto  
19 the surface of apatites (Samavini et al., 2018). Due to this surface engineering, the time-dependent release of phosphate  
20 ions has been observed, accompanied by enhanced plant growth (Samavini et al., 2018; Yoon et al., 2020; Yoon et al.,  
21 2021). This indicates that the MVOCs detected in the present study could be involved in the mobilization of inorganic  
22 nutrients in soil environments. To directly prove this, HANPs were tested because calcium phosphates are widespread  
23 in soils and act as a source of P for plant nutrition (Chang and Jackson, 1957).

24

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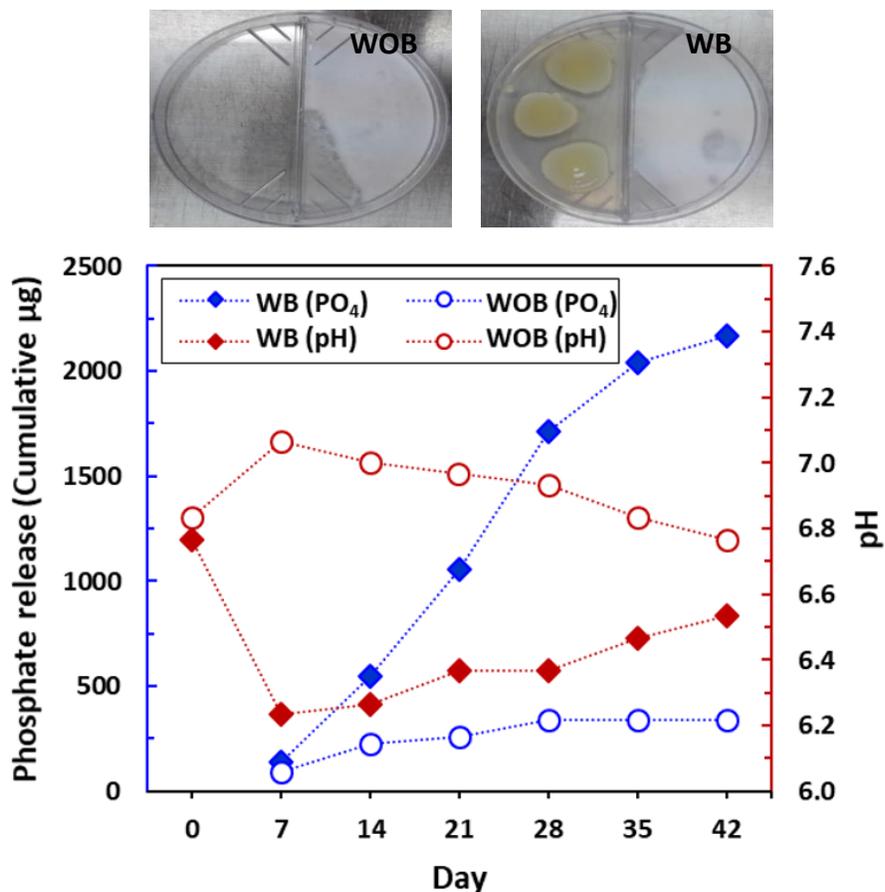
1 As shown in **Fig. 2**, the pH of aqueous solutions containing HANPs treated with MVOCs was significantly lower than  
2 that of the controls. Based on the identification of MVOCs containing alcohol and carboxylic acid (**Fig. 1**), this  
3 phenomenon could be attributable to MVOC-induced acidity. The release of phosphate from the HANPs was also  
4 improved by MVOC treatment, while the release without microbial treatment was very low (**Fig. 2**). The dependence  
5 of HANP dissolution on pH has been well-characterized (Wang et al., 2016), meaning that the enhanced release likely  
6 resulted from more rapid particle dissolution.

7  
8 To investigate MVOC binding on the surface of HANPs, FT-IR was used to identify the carboxylic groups before and  
9 after MVOC treatment. **Fig. 3** shows that the surface of the particles contained a significant quantity of  
10 carboxylic acids. FT-IR spectra were collected after outgassing the samples as such (**Fig. 3A**) and after the exchange  
11 with heavy water (**Fig. 3B**). In **Fig. 3A**, a broad band from ca. 3700 to ca. 2750  $\text{cm}^{-1}$  was observed for all spectra, due  
12 to the  $\nu(\text{OH})$  stretching modes of hydrogen-bonded water molecules adsorbed onto the surface of the HANPs (Mino  
13 et al., 2020). The narrow peak at 3569  $\text{cm}^{-1}$  was ascribed to the vibrations of columnar OH in the HA in the 4e position  
14 within the hexagonal lattice (Sakhno et al., 2015).

15  
16 In the 2200–1900  $\text{cm}^{-1}$  range, signals from the combination and overtones of the vibration modes of bulk phosphate  
17 groups were observed (Diallo-Garcia et al., 2014). A band associated with the  $\delta(\text{HOH})$  bending mode at 1645  $\text{cm}^{-1}$   
18 was also clearly observed in the control samples, and this signal overlapped with the antisymmetric stretching mode  
19  $\nu_{\text{asym}}(\text{COO}^-)$  from carboxylic species adsorbed onto the surface of the HANPs in the samples treated with MVOCs  
20 (Achelhi et al., 2010; Mino et al., 2016). This vibration ( $\nu_{\text{asym}}(\text{COO}^-)$ ) was coupled with the symmetric stretching mode  
21  $\nu_{\text{sym}}(\text{COO}^-)$  present as a narrow band centered at 1322  $\text{cm}^{-1}$  for 7B and 14B and as a doublet at 1322 and 1328  $\text{cm}^{-1}$   
22 for 28B. Additionally, an intense band due to carbonate species was present in the 1540–1350  $\text{cm}^{-1}$  range, originating  
23 from the  $\text{CO}_2$  dissolved introduced during the preparation of the materials (Fleet et al., 2009).

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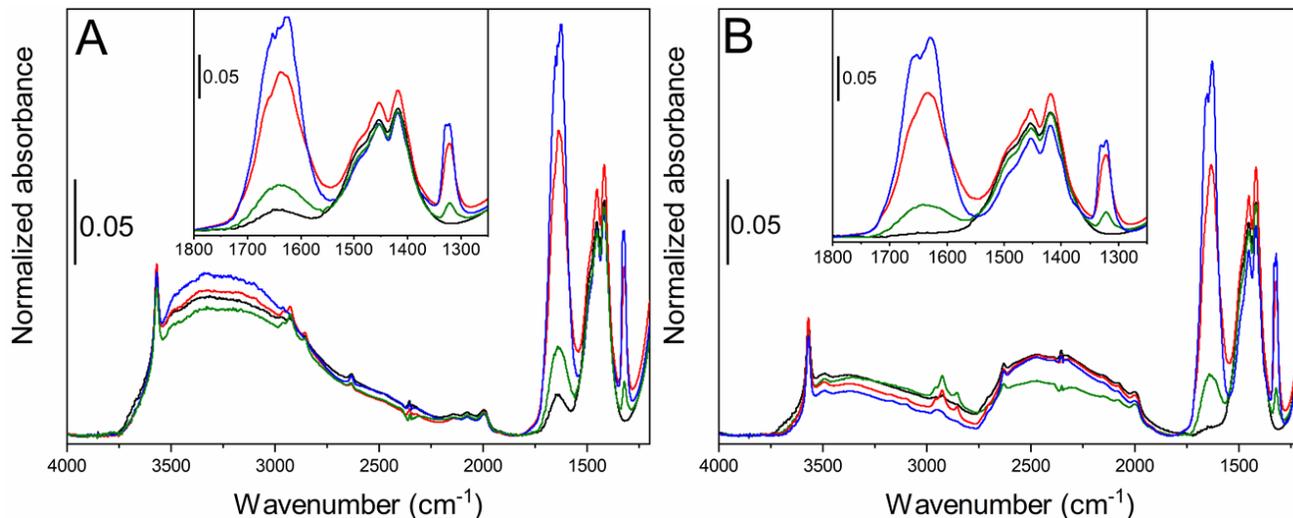
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**Figure 2.** Effect of *Bacillus megaterium* VOCs on (A) pH and (B) phosphate release of HANP-containing suspension with incubation times. Abbreviation: WOB, without bacteria; WB, with bacteria. Means and standard deviations (n = 3) are shown.

10 Because the band of interest related to  $\nu_{\text{asym}}(\text{COO}^-)$  overlapped with the bending mode of water, the HANPs were  
11 subjected to H/D exchange so that the surface-accessible OH species on the HANPs were replaced with OD, whose  
12 vibrations can be downshifted in frequency (Sakhno et al, 2015). The spectra of the materials recorded after this  
13 exchange are presented in **Fig. 3B**. Here, the OH stretching mode of the adsorbed water was replaced by the OD  
14 stretching mode within the 2760–1900  $\text{cm}^{-1}$  range, and the water bending mode shifted to 1200  $\text{cm}^{-1}$ , thus leaving the  
15 antisymmetric stretching mode of the carboxylate species unaffected. In particular, the spectrum of the sample at the  
16 earliest time point (7B) exhibited the Gaussian-like shape of either the  $\nu_{\text{asym}}$  or  $\nu_{\text{sym}}$  modes of  $\text{COO}^-$ . Further, an increase  
17 in time (14B) resulted not only in an increase in the intensity of the corresponding bands but also in changes to the  
18 shape of  $\nu_{\text{asym}}$ , which now consisted of a band centered at 1633  $\text{cm}^{-1}$  with a shoulder at higher wavenumbers (ca. 1663  
19  $\text{cm}^{-1}$ ). At the last time point (28B), the changes in the shape of  $\nu_{\text{asym}}$  could not be specifically quantified because the  
20 signal was out of scale. Interestingly, doubling the time of exposure of the HANPs to the MVOCs from one to two

1 weeks did not result in a noticeable increase in the intensity of the  $\nu_{\text{sym}}$  signal, but rather led to the formation of a  
2 doublet. A possible explanation for this is that almost full surface coverage was reached after 14 days, with further  
3 exposure resulting in the reorganization of the carboxylate species on the surface, as evidenced by the appearance of  
4 the additional signal at  $1330\text{ cm}^{-1}$ . Given the high sensitivity of these vibrations to the nature of the side groups of the  
5 acids and the types of acid–surface complexes (Arena et al., 2015), this is a strong indication of the presence of oxalic  
6 acid on the surface of the MVOC-treated samples.

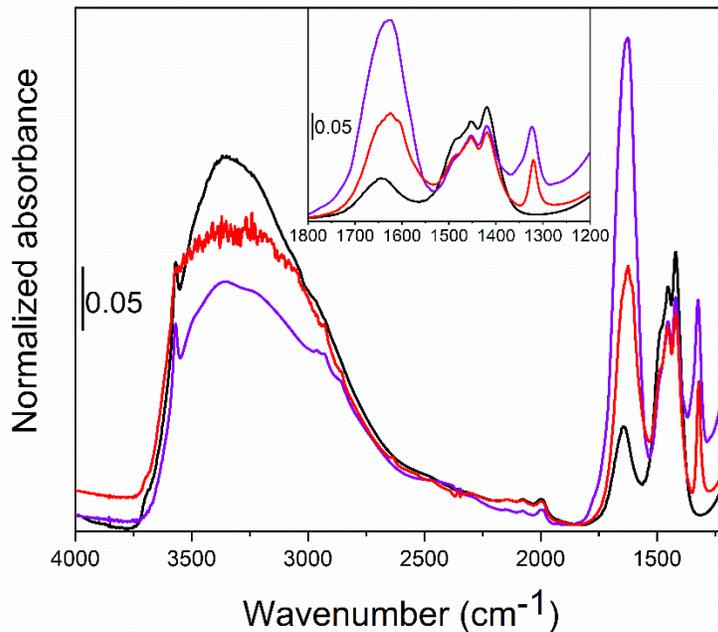
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9 **Figure 3.** FT-IR spectra of the WOB (black curve, without bacteria), 7B (green curve, with bacteria for 7 days), 14B  
10 (red curve, with bacteria for 14 days) and 28B (blue curve, with bacteria for 28 days) samples, (A) after outgassing at  
11 beam temperature for 3 hours and (B) after  $\text{D}_2\text{O}$  exchange and subsequent outgassing at beam temperature for 3 hours.  
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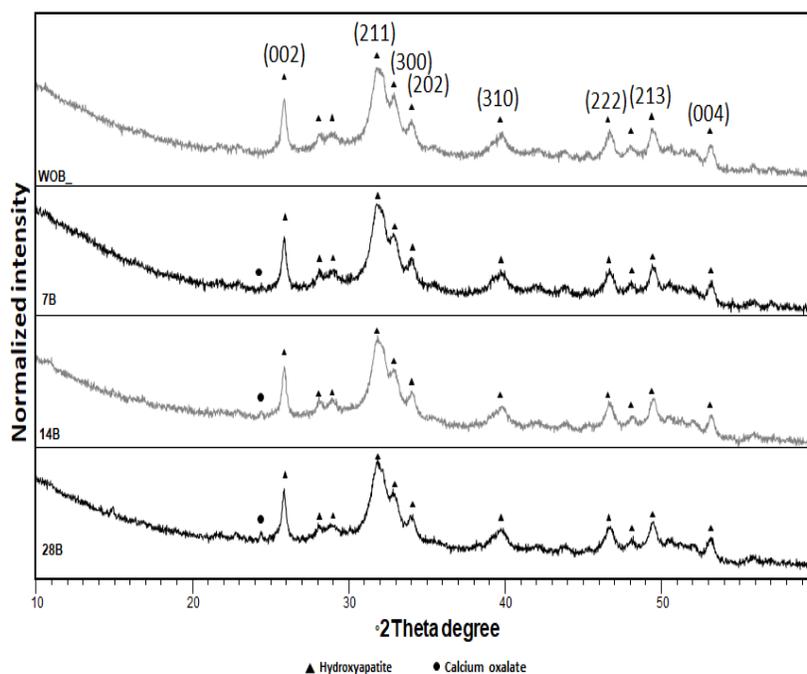
13 In order to more clearly understand if the observed signals were comparable with those of oxalate species on the  
14 surface of MVOC-treated HANPs, a model experiment was carried out. HANPs were exposed to pure oxalic acid  
15 vapor and their IR spectrum was compared to the 14B sample (**Fig. 4**). Interestingly, there was good correspondence  
16 between the bands due to the antisymmetric and symmetric stretching of  $\text{COO}^-$  in the two spectra.



1  
2 **Figure 4.** FT-IR spectra of the WOB (black curve, without bacteria), 14B (red curve, with bacteria for 14 days) and  
3 WOB with adsorbed oxalic acid (violet curve) samples. The spectra were acquired after outgassing at beam  
4 temperature for 2 hours

5  
6 We then analyzed the crystal structure of the HANPs treated with MVOCs to determine whether it was modified by  
7 the adsorbed oxalic acid. The XRD patterns collected for each sample are presented in **Fig. 5**. The spectra revealed  
8 that all of the samples consisted of poorly crystalline HA as the main phase, with a crystallinity index calculated  
9 according to Equation (1) of 13.0%. Peaks typical of HA diffraction patterns (PDF card file 00-009-0432) were  
10 observed at a  $2\theta$  of  $25.9^\circ$ ,  $31.7^\circ$ ,  $32.9^\circ$ ,  $34.0^\circ$ ,  $39.8^\circ$ ,  $46.7^\circ$ ,  $49.5^\circ$ , and  $53.1^\circ$  corresponding to the lattice planes with  
11 Miller indexes of (0 0 2), (2 1 1), (3 0 0), (2 0 2), (3 1 0), (2 2 2), (2 1 3), and (0 0 4), respectively. All of the samples  
12 had a similar domain size along the (002) and (310) directions ( $38 \pm 4$  and  $13 \pm 2$  nm, respectively). These results  
13 indicate that, similar to other examples of HA synthesized using wet precipitation reported in the literature (Adamiano  
14 et al., 2017; Stephanie et al., 2018; Marchiol et al., 2019), the crystallites are slightly elongated in the c-axis direction  
15 compared to the perpendicular a/b-axis.

16  
17 The XRD patterns of the HANPs treated with MVOCs featured a small peak around  $24.2^\circ$ , indicating the presence of  
18 low levels of secondary phase (e.g.,  $< 2.0$  wt.%) that could not be quantified using Rietveld refinement (because the  
19 signal was too weak). The intensity of this peak, which was assigned to calcium oxalate (PDF card file 00-016-0379),  
20 in particular the lattice planes with a Miller index of (0 2 0), increased with the exposure time (*i.e.*, from 7B to 28B).  
21 This indicates that the longer the HANPs are exposed to MVOCs, the higher the quantity of calcium oxalate, although  
22 the levels of this secondary phase remain low.



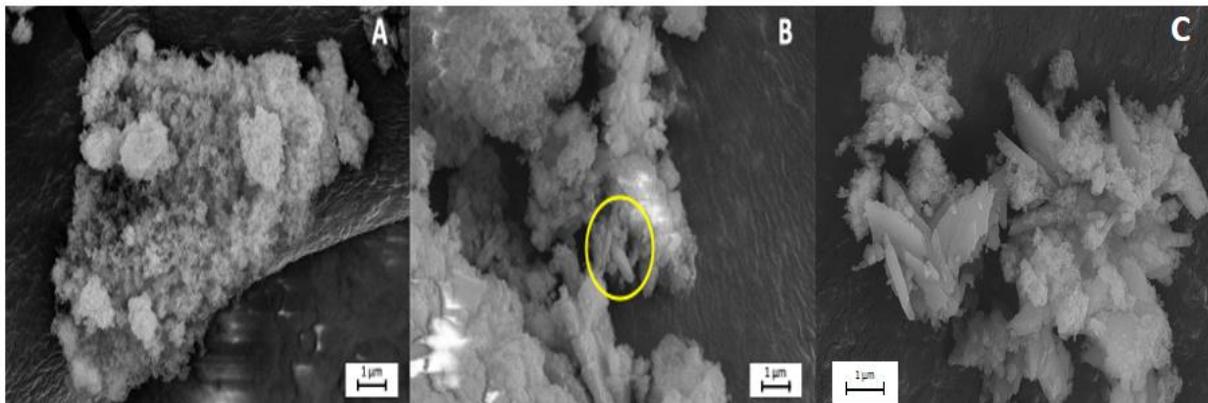
**Figure 5.** XRD pattern of the original HANPs (WOB, without bacteria) and that interacted with MVOCs (7B, with bacteria for 7 days; 14B, for 14 days; and 24B, for 24 days).

The results for the elemental analysis of the HANPs are summarized in **Table 1**. The HANPs had a similar composition before and after MVOC treatment, with the Ca/P ratio of all samples similar and slightly higher than that of stoichiometric HA (1.67). This lack of variation between the samples is primarily due to the low levels of calcium oxalate in the MVOC-treated HANPs; higher values are typical for partially carbonated HANPs in which the  $\text{CO}_3^{2-}$  anions present in the synthesis solution due to the dissolution of atmospheric  $\text{CO}_2$  partially substitute  $\text{PO}_4^{3-}$  in the HA structures during the reaction between the calcium and phosphorous precursors.

**Table 1.** Chemical composition of WOB (without bacteria), 7B (with bacteria for 7 days), 14B (for 14 days), and 28B (for 28 days) determined by ICP-OES

Sample	Ca		P		Ca/P	SD
	wt%	SD	wt%	SD		
WOB	35.4	1.7	16.2	0.7	0.01	1.69
7B	35.9	1.2	16.4	0.5	0.01	1.70
14B	35.1	1.6	15.9	0.7	0.01	1.70
28B	36.2	0.1	16.1	0.1	0.01	1.73

1 SEM micrographs of the WOB and 28B samples are displayed in **Fig. 6A and 6B**, respectively. For the MVOC-  
2 treated samples, we only present representative images for the 28B sample because all of the samples were found to  
3 have similar features. The WOB sample exhibited the morphology and size typical of nanocrystalline HA, with needle-  
4 like particles with a length of 75–125 nm and a width of 15–25 nm. The HANPs treated with MVOCs had a similar  
5 structure but with the occasional occurrence of a small number of larger particles (circled in yellow in **Fig. 6B**).



6  
7 **Figure 6.** SEM pictures at 25,000 x magnification of samples (A) WOB (without bacteria) and (B) 28B (with  
8 bacteria for 28 days), and (C) 35,000 x magnification of samples 28B collected on platy crystals. The circle in  
9 yellow highlights the presence of calcium oxalate crystals

10  
11 A micrograph of particles from the 28B sample at a higher magnification is presented in **Fig. 6C**. The crystals were  
12 characterized by a platy morphology with a prominent facet that was around 1.0 μm in width and length, with a  
13 thickness of around 0.15–0.25 μm. As reported in the literature, this morphology is typical of hydrated calcium oxalate  
14 crystals, also known as whewellite (Tazzoli et al., 1980). Thus, in accordance with the XRD data (**Fig. 5**), these  
15 particles were identified as hydrated calcium oxalate crystals.

16  
17 These findings were confirmed by EDS analysis of the nanometric needle-like HA crystals of the WOB and 28B  
18 samples and the micrometric platy-like crystals of the 28B sample (**Table 2**). The Ca/P ratio for the needle-like  
19 particles was close to that of stoichiometric HA for both samples. On the other hand, the Ca/P ratio for the platy  
20 crystals was significantly different from that for nanocrystalline HA, ranging between 1.50 and 1.75 for calcium  
21 deficient and carbonated HAs, respectively (Raynaud et al., 2002), while the C/Ca ratio was exactly the same as that  
22 for calcium oxalate. The formation of calcium oxalate in the presence of MVOCs would allow solubilized phosphate  
23 to be maintained because calcium ions are preferentially consumed to form calcium oxalate rather than calcium  
24 phosphates.

25

1 **Table 2.** EDS analysis of needle-like and platy crystals in (A) WOB (without bacteria) and (B) 28B (with bacteria  
2 for 28 days).

Sample	Ca/P	SD	C/Ca	SD
WOB	1.68	0.08	0.6	0.1
28B needle-like	1.65	0.05	0.8	0.2
28B platy-crystals	8	2	2.0	0.1

3  
4 The greater phosphate release from HANPs treated with MVOCs inspired us to test whether the surface engineering  
5 of soil minerals associated with plant nutrition would lead to enhancement in plant growth. This question is important  
6 because previous reports demonstrating the positive effects of MVOCs on plant growth have regarded MVOCs as a  
7 molecular lexicon that is directly detected by plants. If other factors found in the soil environment mediate plant–  
8 microbe interactions, these previous results could be re-interpreted from a new mechanistic perspective.

9  
10 The initial source of P in the MS agar was replaced with HANPs, and then the amount of available P was monitored  
11 as a function of incubation time with *B. megaterium*. As shown in **Fig. 7**, the pH fell and the levels of phosphate  
12 solubilized from the HANPs increased quantitatively. Given that the overall results were very similar to those obtained  
13 from HANPs in distilled water (**Fig. 2**), the release of P was not disrupted by the presence of other salts and plant  
14 nutrients. The percentage of HANPs that dissolved into phosphate ions by weight was found to be inversely  
15 proportional to the initial HANP levels, suggesting that an increase in the surface area of the HANPs in the MS agar  
16 was not directly proportional to the initial HANP levels.

17  
18 A similar decrease in the pH was observed when *A. thaliana* was treated with MVOCs, while their combination led  
19 to significantly lower phosphate concentrations (**Fig. 8**). This indicates that plants in the presence of MVOCs can  
20 more efficiently absorb phosphate in gel systems; this was further substantiated by an increase in the fresh and dry  
21 weight of the plants (47% to 65% and 26% to 47%, respectively) in the presence of MVOCs (**Fig. 9**). The results also  
22 showed that both the fresh and dry weight of the plants significantly increased with an increase in the phosphate  
23 concentration from 2 to 6 mg. However, no significant change was observed at phosphate concentrations above 6 mg  
24 (**Fig. 9**), which may be due to saturation of the available P necessary for plant growth in a gel system. Overall, these  
25 results indicate that the higher release of P due to the MOVC-driven surface engineering of HANPs can improve plant  
26 nutrition and thus plant growth.

27

1 We then tested whether these results were reproducible in a real soil system. Soils mixed with three types of P-  
2 containing mineral (*i.e.*, HANPs, calcium phosphate dibasic, and micro-HA) were evaluated to determine whether the  
3 levels of available P are higher in the presence of MVOCs. During the testing, we maintained a constant concentration  
4 of total P in our soils systems (**Fig. S1**). Although the levels of available P in the calcium phosphate-treated soils were  
5 similar to those of the untreated soil on the first day, they increased significantly with longer incubation times (**Figs.**  
6 **10 and S1**) both with and without MVOCs, though the phosphate release in the presence of MVOCs was significantly  
7 higher for all types of calcium phosphate. This indicates that MVOCs that are mobile and can diffuse into the soil  
8 matrix effectively modulate the P release kinetics of soil minerals. The concentration of the available P was also  
9 generally dependent on the quantity and size of the P-containing minerals, which suggests that the surface area of the  
10 minerals is critical to the P release kinetics. The sorption of MVOCs onto the mineral surfaces would activate the  
11 release of available P from the particles. The prolonged release effects with incubation times of up to 56 days were  
12 also typical of the surface engineering of P-containing ceramics (Samavini et al., 2018; Yoon et al., 2020; Yoon et al.,  
13 2021). It is important to note that the MVOC-induced increase in the available P in the soil occurred without adding  
14 calcium phosphate powder, indicating that the P-containing minerals within the soil are strongly affected by the  
15 MVOCs.

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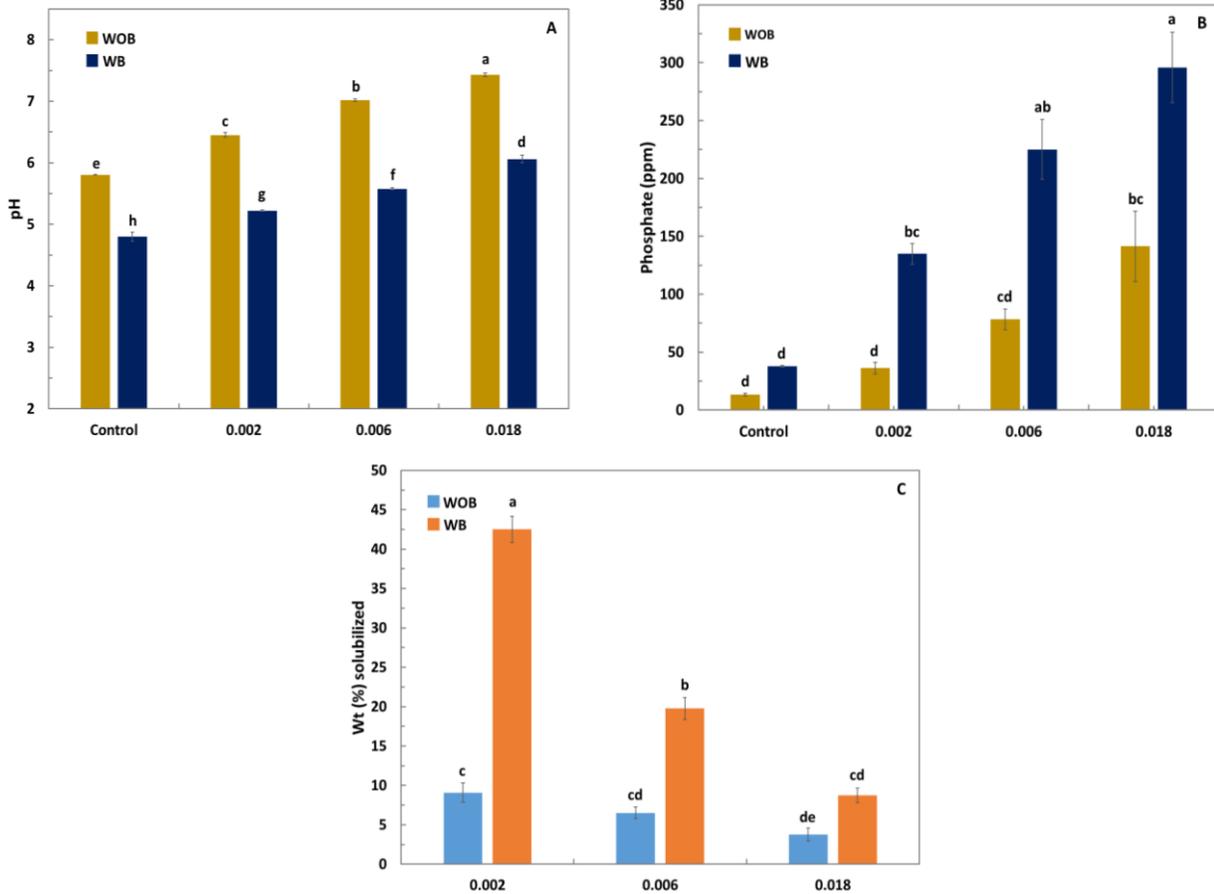
17 As shown in **Fig. 11**, the enhanced release of P increased plant growth, which was positively affected by the levels of  
18 P-containing minerals and the presence of MVOCs. The change in the phosphate concentration in soils with *A.*  
19 *thaliana* (**Fig. S2**) indicates that more phosphate was supplied to the plants via the MVOCs. In addition, the P content  
20 per unit mass of the plants was in accordance with the higher growth levels. Similarly, the concentration of the  
21 available P and total P in the soil was inversely proportional to the plant growth (**Fig. 12 and S3**). These data suggest  
22 that the MVOC-mediated increase in available P in the soil matrix is a direct influence on plant growth. Because plant  
23 nutrition is strongly associated with plant growth and stress resistance (Feller et al., 2018; Brumbarova et al., 2019),  
24 previous results demonstrating enhanced plant growth and stress resistance due to MVOCs should be re-evaluated to  
25 consider whether the soil minerals were modified. Experimental approaches to identify the molecular targets of  
26 MVOC-based plant stimulations should also be examined to determine whether plant nutrition is affected by soil  
27 mineral surface modification because plant nutrition coincides with changes in gene and protein expression  
28 (Brumbarova et al., 2019).

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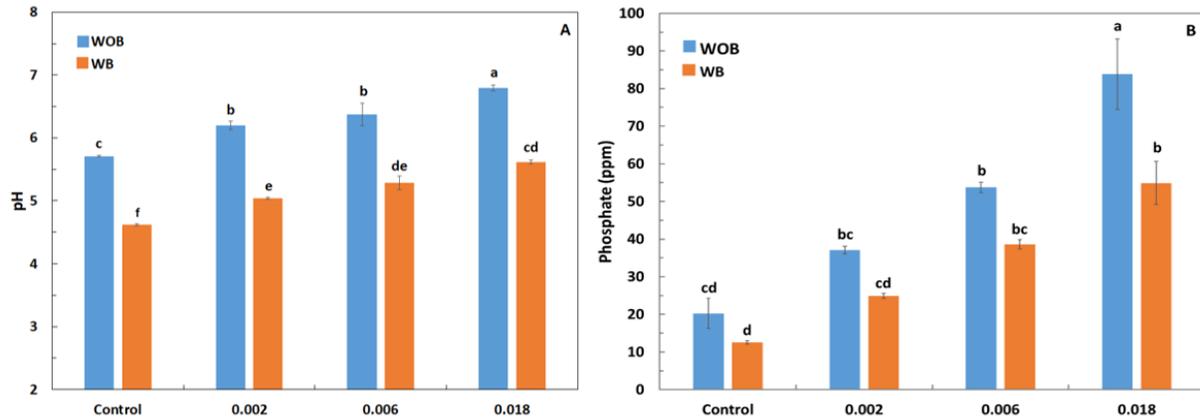
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**Figure 7.** Variations of (A) pH and (B) phosphate concentration in the MS agar (15 mL) containing 0, 2, 6 and 18 mg of HANPs with *Bacillus megaterium* VOCs for 2 weeks. The agars were maintained without plants during the cultivation. Abbreviation: WOB, without bacteria; WB, with bacteria. Means and standard deviations (n = 3) are shown. Statistical analyses are based on LSD ( $p < 0.05$ ).

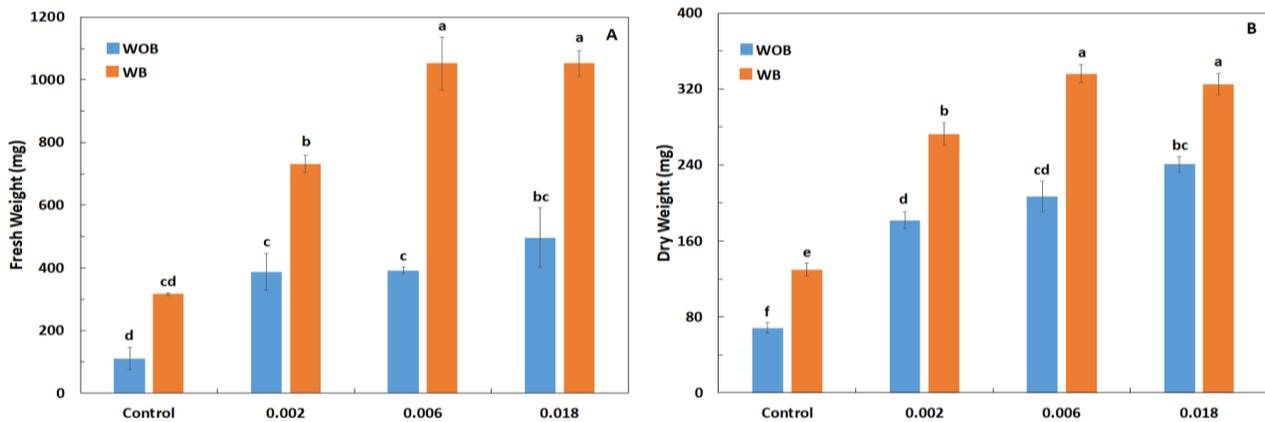


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2 **Figure 8.** Variations of (A) pH and (B) phosphate concentration in the MS agars containing 0, 2, 6 and 18 mg of  
 3 HANPs affected by VOCs of *Bacillus megaterium* and *Arabidopsis thaliana* seedlings for 2 weeks. Abbreviation:  
 4 WOB, without bacteria; WB, with bacteria. Means and standard deviations (n = 3) are shown. Statistical analyses are  
 5 based on LSD ( $p < 0.05$ ).

6

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8

9 **Figure 9.** Effect of *Bacillus megaterium* VOCs on *Arabidopsis thaliana* growth (i.e., (A) fresh weights and (B) dry  
 10 weight) on MS agars containing 0, 2, 6 and 18 mg of HANPs. Abbreviation: WOB, without bacteria; WB, with bacteria.  
 11 Means and standard deviations (n = 3, each n contains 12 plants) are shown. Statistical analyses are based on LSD ( $p$   
 12  $< 0.05$ ).

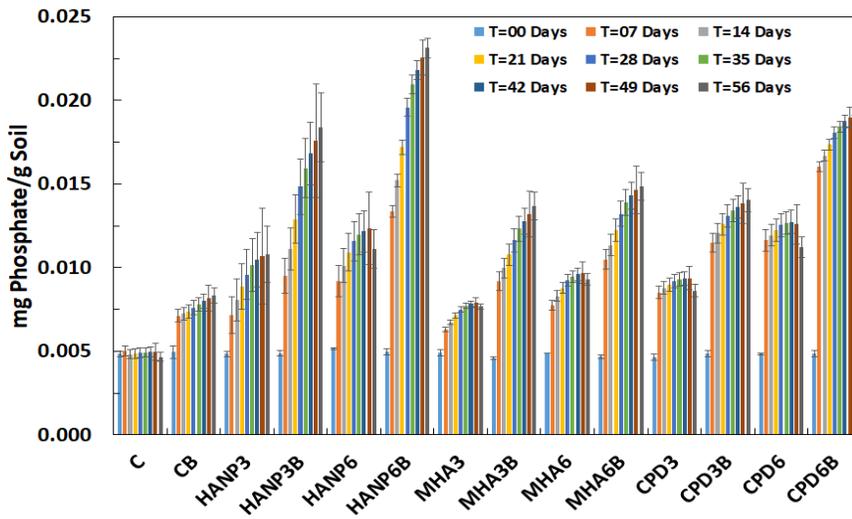
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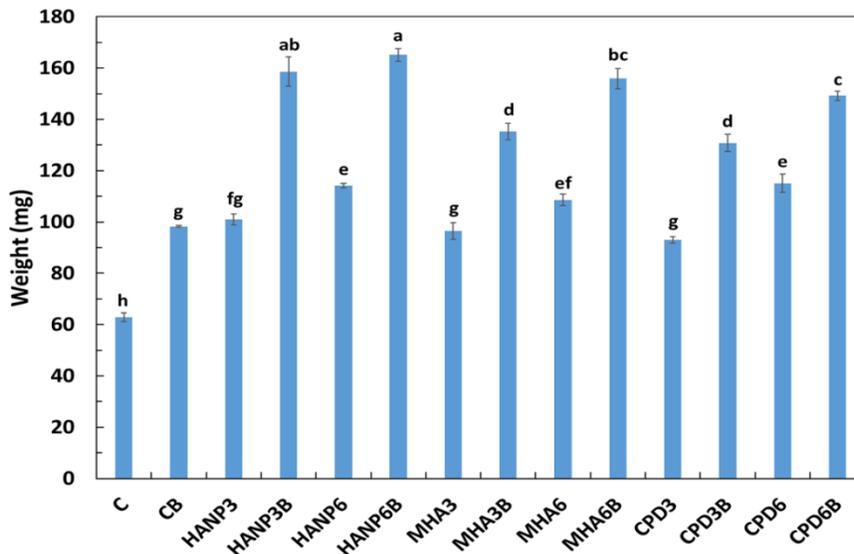
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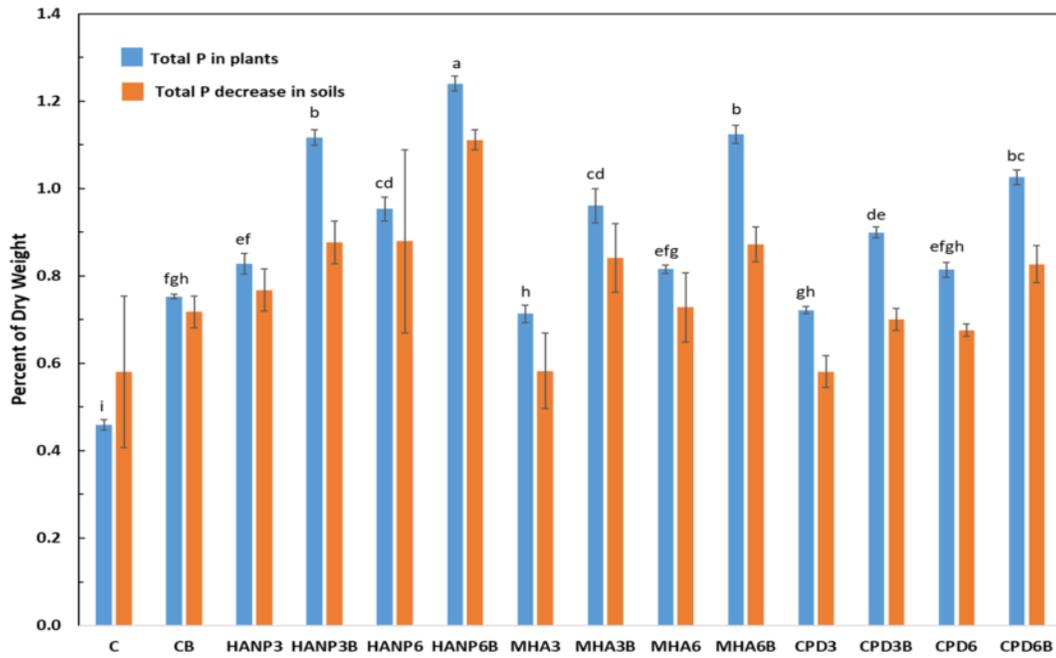
**Figure 10.** The effect of *Bacillus megaterium* VOCs on phosphate release from P-containing minerals (i.e., HANP, MHA, CPD) in soils for 56 days. Means and standard deviations ( $n = 3$ ) are shown. Abbreviations: **C**: Bulk soil; **CB**: without P source + Bacteria; **HANP3B**: 3mg HANP + Bacteria; **HANP6B**: 6mg HANP + Bacteria; **CPD3B**: 3mg CPD + Bacteria; **CPD6B**: 6mg CPD + Bacteria; **MHA3B**: 3mg MHA + bacteria; **MHA6B**: 6mg + Bacteria; **HANP**: hydroxyapatite nanoparticle; **CPD**: calcium phosphate dibasic; **MHA**: micro hydroxyapatite



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**Figure 11.** Biomass (dry weight) of *Arabidopsis thaliana* treated with HANP, MHA, and CPD in the presence and absence of *Bacillus megaterium* VOCs for 56 days. Means and standard deviations ( $n = 3$ , each  $n$  contains 6 plants) are shown. Statistical analyses are based on LSD ( $p < 0.05$ ).

1



2

3 **Figure 12.** P contents of *Arabidopsis thaliana* and corresponding P decrease in soils treated with HANP, MHA, and  
4 CPD in the presence and absence of *Bacillus megaterium* VOCs for 56 days. Means and standard deviations (n = 3,  
5 each n contains 6 plants) are shown. Statistical analyses are based on LSD ( $p < 0.05$ ).

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1 **Conclusion**

2 This research demonstrated the direct interaction between MOVCS and P-containing ceramics, which can then affect  
3 plant nutrition. It is evident that specific types of MOVCS containing oxygen-based functional groups are capable of  
4 modifying the surface of P-containing minerals including HANPs, calcium phosphate dibasic, and micro-HA, thus  
5 accelerating the release of soluble phosphate. By monitoring changes in pH and IR peak variations corresponding to  
6 COO<sup>-</sup>, it can be concluded that this release is due to the combined actions of a lower pH and surface engineering by  
7 carboxylic group-containing MVOCs. These actions cause the minerals to become more soluble for a longer period  
8 of time. XRD and SED-EDS analysis also revealed the formation of calcium oxalate during the solubilization process,  
9 suggesting that oxalate is solidified when calcium ions are released. As a result, phosphate is maintained within the  
10 soil by inhibiting its recrystallization into calcium phosphates. The higher concentration of phosphate for longer  
11 periods was also observed in soil and gel environments, and the growth of *A. thaliana* was also promoted. Given that  
12 the modulation of plant nutrition affects many components of plant physiology, including versatile gene expression,  
13 our findings raise the need to confirm whether the previously reported effects of MVOCs on plants are due to changes  
14 in soil minerals associated with plant nutrition.

15

16 **Acknowledgments**

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20 Korea Ministry of Environment (MOE) (2021003270006).

21

22 **Conflict of interest**

23 There is no conflict of interest.

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