Phosphorus Bioavailability and Migration of Hydroxyapatite in Different Sizes as Phosphorus Fertilizer in *Camellia Oleifera* Seedlings

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Abstract. Low mobility and solubility reduce the availability of traditional phosphorus (P) fertilizer in red acidic soil. Hydroxyapatite (HAP), especially nano-hydroxyapatite (n-HAP), may be more efficient than P fertilizer because of its nanoparticle characteristics. *Camellia oleifera* (*C. oleifera*) is an edible oil tree whose productivity is greatly affected by P fertilizer. During this study, we investigated the migration of different particle sizes of HAP (20 nm, 200 nm, and 80 μ m) and their effects on the seedling growth of *C. oleifera* cultivar Huashuo (HS) cuttings. A column experiment showed that the efflux ratio was negatively correlated with particle size in red acidic soil. The leaching results revealed that the contents of total P and available P in the 20-nm treatment were significantly higher than those in the 200-nm and 80- μ m treatments in the deep soil (10–15 cm or 15–20 cm), whereas the application of 20-nm n-HAP caused 13.43% wastage of available P. During the container experiments, 200-nm and 20-nm HAP significantly promoted the growth of the seedlings in terms of seedling height, stem diameter, and biomass. The available P contents in the rhizosphere and nonrhizosphere soils were negatively correlated with the HAP particle sizes. In conclusion, the migration of HAP is inversely correlated with particle size, and HAP improves the P bioavailability in red acidic soil. In summary, 200-nm HAP was the best P fertilizer for the seedlings of HS among the three particle sizes. This study offers preliminary results indicating that 200-nm HAP might be a better P fertilizer compared with other two HAP particle sizes for use in future *C. oleifera* orchards.

Phosphorus is an essential macronutrient that significantly influences crop growth and productivity (Qu et al., 2020). Emphasis has been placed on the efficient use of P fertilizer for sustainable plant yield and quality (Ryan 2002). Red soils with low pH cover an extensive area of southern China (He et al., 2011). Furthermore, P is easily fixed by clay minerals in red acidic soil, resulting in it being barely absorbed by the roots (Yuan et al., 2017), and it has been estimated that one-third to one-half

of the arable soils in China are P-deficient, especially red acidic soils (Li et al., 2020). Hence, serious environmental pollution is caused by increasing applications of P fertilizer. Greater environmental awareness and the demand for higher P availability have prompted researchers to focus on the effective utilization of P fertilizers in agriculture (Jiao et al., 2012). In addition, leaching is regarded as an important means of transporting P through the soil (Ukwattage et al., 2020).

Camellia oleifera is an important edible oil plant that is widely planted in red acidic soils (Yang et al., 2016). One of the main factors that restricts the growth of *C. oleifera* (He et al., 2011) is P deficiency, and P fertilizer has been reported to have an important role in the yield and quality of *C. oleifera* (Chen et al., 2007). Nanotechnology is an emerging technology that has been proposed to possess the potential to improve fertilizer formulations and augment plant nutrient uptake (Rai et al., 2015; Wang et al., 2016). Nanoparticles are defined as materials that have at least one dimension at the nano-level (Powers et al., 2006). In soil systems, nanomaterials have the capacity to be nanofertilizers. They can improve the yield of crops by increasing nutrient usage efficiency while

reducing the costs of production, thereby promoting the sustainable development of agriculture (Saleem and Zaidi, 2020).

Hydroxyapatite (HAP), especially nano-hydroxyapatite (n-HAP), is gradually receiving particular attention as a fertilizer (Koutsopoulos 2002; Szameitat et al., 2021). Research has shown that the zeolite NaP1/hydroxyapatite nanocomposite is useful as an inorganic fertilizer and can release nutrient ions for long periods (Watanabe et al., 2013). The positive effects of HAP on soybean plants are related to its longer permanence in soils compared with regular P fertilizer [$\text{Ca}(\text{H}_2\text{PO}_4)_2$] (Liu and Lal, 2014). Furthermore, triple superphosphate (20% P) fertilizer performed better than HAP for wheat (Montalvo et al., 2015). To date, the application of HAP as P fertilizers on *C. oleifera* has not been reported, and its P bioavailability in *C. oleifera* in red acidic soil is unknown.

During this study, the availability of three different particle sizes (20 nm, 200 nm, and 80 μ m) of HAP as P fertilizers in red soil was examined using container experiments involving cuttings of *C. oleifera* seedlings of the cultivar HS as indicator plants. Column experiments were also used to explore the migration of different HAP particle sizes. We hypothesized that 1) a small particle size would increase the migration intensity of HAP in red acidic soil, 2) smaller HAP particles would enhance the contents of total P and available P in the red soil because of their faster dissolution, and 3) n-HAP would have a significant positive effect on the growth of *C. oleifera* seedlings.

Materials and methods

Characterization of HAP and soil properties. Three HAP sizes (nominal particle sizes of 20 nm, 200 nm, and 80 μ m) were provided by Nanjing Emperor Nanomaterial, Co., Ltd. in China. The selection of the size of HAP was based on previous research (Montalvo et al., 2015). The HAP morphology was analyzed using a scanning electron microscope (Li et al., 2017).

The red acidic soils used in this study were collected at a depth of ≈ 30 cm from Wangcheng District, Changsha, China (lat. 28°23'44" N, long. 112°80'56" E). The samples of red soils were ground manually and air-dried for 48 h. Then, samples were passed through a mesh sieve (2 mm) for soil property determinations, column experiments, and container experiments. The pH values of the soils were determined using a pH electrode (e201-c) with the ratio of solid to liquid being 1:2.5 (Montalvo et al., 2015) and adding deionized water without CO_2 . The contents of soil organic matter were determined using the potassium dichromate method (Shi et al., 1996). The samples were digested with concentrated $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ to determine the total P and total nitrogen (Jiang et al., 2017), and the soils were extracted with 0.03 mol/L $\text{NH}_4\text{F} + 0.03$ mol/L HCl to determine the available P (Jin et al., 2018). The P contents (including total P and available P) and total nitrogen were determined using a discrete

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Table 1. Chemical properties of the soil used during this research.

Soil properties	pH (water)	Available P (mg/kg)	Total P (mg/kg)	Alox (g/kg)	Feox (g/kg)	SOM (g/kg)	Total N (g/kg)
Value	5.32 ± 0.02	0.15 ± 0.01	24.17 ± 0.83	25.79 ± 0.54	52.40 ± 0.59	3.24 ± 0.21	3.13 ± 0.10

N = nitrogen; P = phosphorus; SOM = soil organic matter.

auto analyzer (Smartchem 200; Westco Scientific Instruments, Rome, Italy). The elemental contents of the soil samples were directly measured using an FD-3022 γ spectrometer (Masayasu et al., 2000). The soil properties are shown in Table 1.

Column experiment. Three HAP suspensions (20 nm, 200 nm, 80 μ m) and a control were prepared with 0.1 g of HAP powder per mL of 0.1 M trisodium citrate solution (used to enhance the dispersion of nanoparticles), followed by mild sonication (1800 W, 3 min) (Montalvo et al., 2015).

With a grain size of 0.7 mm and a uniformity index of 1.25, quartz sand (purchased from Sinopharm Chemical Reagent Co., Ltd., China) was used as the packing material for the column experiments. Before use, the sand was soaked in 0.01 M NaOH solution for 24 h, rinsed with deionized water, and soaked again for an additional 24 h in 0.01 M HCl solution before being thoroughly rinsed with deionized water. Then, the sand was dried in an oven at 105 °C and stored for the experiments (Lv et al., 2012).

The porosity and pore volume (PV) of the columns were measured gravimetrically (Zhao et al., 2018); then, the columns (inner diameter, 10 cm; height, 30 cm) (Fig. 1) were oriented vertically and saturated with deionized water for 48 h to remove air pockets for subsequent tests (Du et al., 2010).

To prevent the loss of the soil during the experiment, the bottom of the column was covered with 2-cm-thick quartz sand under a 0.425-mm sieve. The air-dried soil samples were packed in the middle of the column. Placement of the quartz sand and sieve at the top of the column was contrary to that at the bottom. The soil columns were leached with 0.5 PV leachate (HAP suspensions) every 3 h, and the leachate was collected in a beaker. The absorbances of the obtained leachates and the initial suspension were measured by an ultraviolet spectrophotometer and recorded as C and C₀, respectively. The breakthrough curves (BTCs) of the HAP were drawn with the pore volume number as the abscissa and C/C₀ as the ordinate. All treatments were replicated six times.

After leaching, deionized water was added to half of the columns to elute the HAP in the red soil until there was no HAP measured in the obtained eluent. The soil columns were slowly pushed out after leaching and elution and cut into four parts every 5 cm; those parts were marked as 0 to 5 cm, 5 to 10 cm, 10 to 15 cm, and 15 to 20 cm from top to bottom and air-dried. The contents of total P and available P (including the treatments of leaching and elution) were determined as described.

Container experiments. The container experiments were performed with four treatments: 20 nm, 200 nm, 80 μ m, and control. The study used 1-year-old seedlings of *C. oleifera* cultivar HS that showed similar growth potential and had been managed similarly. Each seedling weighed an average of 3.80 g. First, 2 kg of air-dried soil samples were placed in each container (18 cm × 12 cm), and the cut seedlings were planted in the containers. The containers were covered with a layer of plastic film to minimize moisture loss by evaporation after being watered thoroughly. Five days after planting, Hoagland's nutrient solution without P was applied to each container (Ghanati et al., 2005). The P treatments were applied in the form of HAP suspensions at a ratio of 0 mg/kg (control) or 150 mg/kg (treatment) every 14 d. The containers were watered with 200 mL deionized water to maintain the moisture at field capacity every 7 d. All seedlings were grown in a glasshouse with 24 °C/17 °C day/night temperatures, and the containers were arranged in a completely randomized design. Six weeks after planting, the diameter and height of the seedlings were measured. The seedlings were then gently lifted to shake off the soil around the root system that adhered to the roots, which constituted rhizosphere soils, and the remaining soils were considered non-rhizosphere soils (Gremion et al., 2003). Then, soils were collected for drying in an oven at 65 °C. The treated seedlings were cut from the rhizome and divided into above-ground parts and belowground parts (root systems). The fresh and dry weights of the seedlings were measured, and the root to shoot ratios (root shoot ratio = root dry mass/aboveground dry mass) were calculated. The roots, stems, and leaves of the seedlings were ground. The methods used to determine the total P in the plants and available P in the soils were the same as those used for the column experiments.

Statistical analysis. All measurements were performed in triplicate, except the column experiments, and the data were recorded in Excel (version 2016; Microsoft Corp., Redmond, WA). The data were analyzed using a one-way analysis of variance at a significance level of $P < 0.05$. When significant, Duncan's multiple range tests were conducted to evaluate the outcomes of different HAP sizes. Data processing was performed using SPSS software (version 21.0; IBM Corp, Armonk, NY). All graphs were produced in Excel.

Fig. 1. Diagrammatic sketch of the soil column.

Fig. 2. Scanning electron microscopy images of hydroxyapatite (HAP) of different sizes: (A) 20 nm nano-hydroxyapatite (n-HAP); (B) 200 nm HAP; and (C) 80 μ m HAP.

Results

Characterization of HAP. The scanning electron microscopy results for HAP (Fig. 2) showed that the HAP morphology of the 20-nm and 200-nm HAP was needle-like, whereas that of the 80- μ m HAP was spherical. The P concentrations of the three HAP sizes were 19.40%, 20.01%, and 19.71%, respectively, which are close to the theoretical value of HAP (18.50%).

Column experiment. The BTCs of the column experiment (Fig. 3) characterized the change in HAP concentration with relative time (C/C_0) during the process of migration, thus reflecting the interaction between HAP and the soils (Zang 2011). Our results showed that the efflux ratios of HAP were negatively correlated with the particle sizes. The maximum C/C_0

value of the 20-nm n-HAP was 0.72. In contrast, when the particle size increased to 80 μ m, the C/C_0 values were close to 0, which confirmed that the migration of the 80- μ m HAP was negligible. With the increase in HAP particle size (20 nm to 200 nm), the observable efflux time of the HAP was also delayed from the initial position of the third PV (20 nm) to the four point five PV (200 nm). Compared with the other particle sizes, the 20-nm n-HAP significantly enhanced the efflux velocity and efflux ratio, which indicated that the migration intensity and solubility of the HAP were significantly increased by the n-HAP in the soil column.

The contents of available P were improved to varying degrees in all treatments after leaching, but not in the control groups (Fig. 4). The contents of available P in the shallow soils

(0–10 cm) increased significantly because of the accumulation of large HAP. The P contents were increased by n-HAP in the deep soil (10–20 cm), which can be explained by their better migration abilities. The available P contents of the 80- μ m and 200-nm HAP treatments in the 0- to 5-cm soil zone were increased by 26.50% and 20.93% compared with the control, respectively; however, this value was only 11.93% in the 20-nm n-HAP treatment. In the 5- to 10-cm soil column layer, the contents of soil-available P of the 200-nm HAP treatment increased most obviously by 14.21%, whereas increases of 7.77% and 13.92% were observed for 80- μ m HAP and 20-nm HAP, respectively. The available P contents of the 20 nm treatment in the 10- to 20-cm soil zone increased by 6.93% and 8.42%, respectively. There was no significant difference between the control and the 80- μ m treatment in the 10- to 20-cm soil column.

Adsorption of HAP by red acidic soil. As the soil depth increased, the total P contents in the 200-nm and 80- μ m treatments were reduced, and a significant difference was observed between the 20-nm and the control groups (Fig. 5). The total P content in the 0- to 5-cm layer of the 80- μ m treatment (38.94 mg/kg) was significantly higher than that of the other soil zones. In the 5- to 10-cm soil zone, the P contents of the 200 nm and 80 μ m treatments were 33.29 mg/kg and 33.16 mg/kg, respectively, which were significantly higher than that with the 20-nm treatment. However, the total P contents of the 20-nm treatment in the 10- to 15-cm and 15- to 20-cm soil layers were 30.74 mg/kg and 30.13 mg/kg, respectively, and remarkably higher than those of the others.

The changing trends of soil-available P contents were similar to those of total P, and the additions of HAP had significant effects on the available P contents of the soil (Fig. 6). The highest available P content of the 80- μ m treatment was 25.89-times that of the control, followed by those of the 200-nm and 20-nm treatments, which were 25.00-times and 9.28-times that of the control, respectively. In the 5- to 10-cm soil layer, the available P content of the 200-nm treatment was the highest at 1.92 mg/kg, although no significant difference was observed between those of the 20-nm and 80- μ m treatments, which were 1.25 mg/kg and 1.07 mg/kg, respectively. However, the contents of available P in the 20-nm treatment in the 10- to 15-cm and 15- to 20-cm soil zones were the highest at 1.25 mg/kg and 1.11 mg/kg, respectively; however, no significant difference was found between those of the 80- μ m and the control treatments, which were only 0.14 mg/kg and 0.18 mg/kg, respectively.

A comparison of the soil columns leached with the HAP suspension with the columns eluted with deionized water indicated the available P content of the 20-nm treatment was significantly increased by 19.36% in the deep soil (10–15 cm and 15–20 cm), indicating that 20-nm n-HAP demonstrated better migration ability in the red soil. However, the data also showed that 20-nm n-HAP had

Fig. 3. Effects of different particle sizes on the mobility of hydroxyapatite (HAP) during the soil column experiments.

Fig. 4. Effects of different particle sizes of hydroxyapatite (HAP) on the available phosphorus (P) content after leaching in soil columns.

Fig. 5. Changes in the total phosphorus (P) content in the red acidic soil after elution in different treatments. The results are the mean $\pm$ SE of three biological replicates. Different lowercase letters indicate significant differences at a 5% probability level according to Duncan's multiple range test.

13.43% wastage of available P in the soil after elution, and there were no significant differences between those of the control, 200-nm HAP, and 80- μ m HAP treatments.

Physiological indexes of *C. oleifera* cultivar HS seedlings. There were significant differences in the physiological indexes of the seedlings treated with different HAP particle sizes (Tab. 2). The height, stem diameter, and biomass of the seedlings were significantly improved by the 200-nm HAP, followed by the 20-nm n-HAP and 80- μ m HAP. The seedling heights of the 20-nm n-HAP and

200-nm HAP treatments were significantly higher than those of the 80- μ m HAP treatment and control. The stem diameter of the 200-nm treatment was the highest among all the treatments. The indexes of seedling fresh weight of the 200-nm treatment were 21.97% higher than the control. The root-to-shoot ratio of the 200-nm HAP was 0.59 and 1.13% higher than that of the 20-nm n-HAP.

Effects of different treatments on the P content of the seedlings of *C. oleifera* cultivar HS. The addition of different HAP particle sizes had significant effects on the contents of

total P in the roots, stems, and leaves of the seedlings (Fig. 7), with similar trends observed across the histograms. The total P contents of the seedlings treated with HAP were significantly higher than those of the control. The total P contents of the 20-nm treatment were the highest, followed by those of the 200-nm and 80- μ m treatments, whereas no significant differences were found between the 20-nm and 200-nm treatments, except in the leaf.

Available P contents in the rhizosphere and nonrhizosphere soil under different treatments. With the increase in HAP particle size, the available P contents in the rhizosphere and nonrhizosphere soil was reduced significantly (Fig. 8). The available P contents in the rhizosphere and nonrhizosphere soil of the 20-nm treatment were the highest at 1.79 mg/kg and 1.13 mg/kg, respectively, followed by those of the 200-nm treatment; the lowest contents were observed in the 80- μ m treatments, which were only 0.61 mg/kg and 0.41 mg/kg, respectively. The content of available P in the 20-nm treatment in the rhizosphere soil was significantly higher than that of the control. The paired *t* test showed that there were significant differences among the 20-nm, 80- μ m, and control treatments; however, there were no significant difference detected between the 20-nm and 200-nm treatments.

Discussion

Migration of HAP in red acidic soil. The migration of P in the soil is complex, and P can travel in different forms (e.g., dissolved, colloidal, and particulate) (Chen and Arai, 2020). Because of the long-term application of P fertilizer, a large amount of P has accumulated in the soil; however, the availability of traditional P fertilizer is limited by its low mobility and solubility (Yuan et al., 2013). Column experiments are a common way of testing the vertical transport of P in the soil. Our results showed that the mobility of the 20-nm n-HAP was the greatest, followed by that of the 200-nm HAP, whereas the 80- μ m HAP could hardly penetrate the soil column. This is probably because the large particles of HAP aggregates are easily adsorbed by active sites in the soil or blocked by the tension in the soil pores (Zhang and Wang, 2014). The column experiments indicated that in the 0- to 5-cm soil layer, the retention of 80- μ m HAP in the soil column led to an increase in the available P content. However, with the increase in depth, it ultimately did not differ from that of the control. A correlation analysis further confirmed that the adsorbed HAP of different particle sizes in the 0- to 5-cm column was positively correlated with the total P content of the soil after elution. We found that the distribution of available P with the 200-nm HAP was similar to that with 80- μ m HAP, but the content with the 200-nm HAP was higher, which was related to its stronger penetration ability. The uniform distribution of available P in the 20-nm HAP soil column indicated its better penetration ability in red soil, which was consistent with the results of the BTCs. In addition, it is believed that the

Fig. 6. Changes in available phosphorus (P) contents in red acidic soil after elution in different treatments. Mean values (n=3) followed by different lowercase letters in each column indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

Table 2. Effects of different treatments on the morphological indexes of the seedlings of *C. oleifera* cultivar Huashuo.

Treatment	Seedling ht (cm)	Stem diam (mm)	Fresh wt of seedlings (g)	Dry wt of seedlings (g)	Root-to-shoot ratio
20 nm	10.39 ± 0.26 a	2.25 ± 0.11 b	4.70 ± 0.06 a	1.69 ± 0.05 b	0.52 ± 0.02 c
200 nm	10.33 ± 0.18 a	2.48 ± 0.10 a	4.83 ± 0.11 a	1.97 ± 0.03 a	0.59 ± 0.07 c
80 μm	9.13 ± 0.06 b	2.27 ± 0.07 b	4.19 ± 0.08 b	1.30 ± 0.07 c	1.01 ± 0.03 b
Control	8.71 ± 0.09 c	2.06 ± 0.06 c	3.96 ± 0.08 c	1.14 ± 0.04 d	1.16 ± 0.04 a

Mean values (n = 3) followed by different lowercase letters in each column indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

Fig. 7. Effects of different treatments on total phosphorus (P) contents in the roots, stems, and leaves of the *C. oleifera* seedlings. Different letters in each column indicate significant differences at the 5% level according to Duncan's test.

main sorbents for P in acidic soil are iron (Fe) and aluminum (Al) oxides (Missong et al., 2016; Ilg et al., 2008). According to the soil elemental composition, the tested red acidic soil contained 25.79 g/kg Al and 52.40 g/kg Fe, and their contents were significantly higher than those of the other soil elements. Secondary phosphate minerals, which are relatively immobile and sparingly soluble, are formed by the complexation of n-HAP with high levels of Al^{3+} and Fe^{3+} cations (Zhao et al., 2006). This also partially explains the retention of some HAP in the soil. Conversely, one study found that the contents of available P in the 200-nm and 80-μm layers after elution by deionized water were similar to the contents before elution because of the large particle sizes and soil filtration effects (Liu and Lal, 2014). However, the total and available P

contents of the 20-nm treatment were significantly increased in the deep soil, which provided further evidence that 20-nm n-HAP exhibits strong mobility in red soil. This result is consistent with the effect of hydroxyapatite nanoparticles in the environment and oxides studied by Liu and Lal (2014). However, the rapid migration of 20-nm n-HAP results in greater loss of P. The present study showed that the 20-nm n-HAP had 13.43% wastage of available P in the soil after elution, which was far more than that of the 200-nm and 80-μm HAP treatments. Recent research found that coal fly ash and biochar showed synergistic effects in reducing the leaching loss of P (Ukwattage et al., 2020), which may offer a solution.

P bioavailability of HAP in red acidic soil. Exogenous P can increase the P content of

C. oleifera, thus significantly affecting the uptake of the contents of nitrogen and potassium (Yuan 2013). Research has found that the optimum P application for 5-year-old *C. oleifera* seedlings is 3.93 g/kg per month (Luo et al., 2016), and the growth of 1-year-old *C. oleifera* seedlings with a P content of 100 mg/kg was better than the growth with 50 mg/kg P (Qu et al., 2018). By combining sorption properties with a strong and large surface area, nanofertilizers have the potential advantages of sustained and slow release (Bindraban et al., 2015); therefore, n-HAP may be used as a more effective substitute for traditional phosphate fertilizers. With the 20-nm and 200-nm HAP particle treatments, the growth of the seedlings was significantly promoted because of the increased content of available P in the rhizosphere soil. As the rate of dissolution increases with decreasing particle size, migration and dissolution abilities improve (Borm et al., 2006). The low particle size also assists with uptake by plant roots. The level of available P in the rhizosphere soil was significantly higher than that in the nonrhizosphere soil because plants promote the transformation of HAP to available P by secreting small-molecule organic acids and adjusting the pH in the rhizosphere (Liao et al., 2006). The HAP particles were thus transported to the roots through the mass produced by plant transpiration. The results showed that the effects of 80-μm HAP on the seedlings were not as apparent as those of the 20-nm n-HAP and 200-nm HAP treatments. We found that most of the 80-μm HAP became trapped in the shallow soil and could not be effectively absorbed and used by plants, which may be related to its slow dissolution (Borm et al., 2006). Previous studies also indicated that reducing the particle size of slightly soluble P could improve agronomic efficiency (Alston and Chin, 1974) because increasing the dissolution rates can promote particle-to-root contact (Khasawneh and Doll, 1979; Watkinson 1994). There were no significant differences in the physiological indexes, total P content, and soil available P content between plants treated with HAP sizes of 20 nm and 200 nm. This showed that the practical application of 20-nm n-HAP is in line with that of 200-nm HAP. However, the results of the column experiment indicated that n-HAP of 20 nm migrated more easily in the red soil than HAP of 200 nm. We believe that the aggregation propensity of n-HAP has a significant effect on the dissolution rate of the particles, which affects the P bioavailability of HAP in *C. oleifera*. This is consistent with the earlier hypothesis of Montalvo et al. (2015).

Conclusions

The present study found that 20-nm n-HAP had better mobility than the 200-nm and 80-μm HAP. This was likely attributable to its smaller particle size. The 20-nm and 200-nm HAP treatments had similar effects on the growth of *C. oleifera* seedlings, but the higher adsorption of the 200 nm HAP

Fig. 8. Effects of different treatments on available phosphorus (P) contents in the rhizosphere and non-rhizosphere soil. Different letters in each column indicate significant differences at the 5% level according to Duncan's test.

indicated that it exhibited more efficient P fertilizer utilization. This research may help alleviate P deficiency in red acidic soil and provide a basis for the application of HAP as P fertilizer in *C. oleifera* orchards.

Literature Cited

Alston, A.M. and K.W. Chin. 1974. Response of subterranean clover to rock phosphates as affected by particle size and depth of mixing in the soil. *Austral. J. Expt. Agr.* 14(70):649–655, doi: 10.1071/EA9740649.

Bindraban, P.S., C. Dimkpa, L. Nagarajan, A. Roy, and R. Rabbinge. 2015. Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. *Biol. Fertil. Soils* 51:897–911, doi: 10.1007/s00374-015-1039-7.

Borm, P., F. Klaessig, T. Landry, B. Moudgil, J. Pauluhn, K. Thomas, R. Trotter, and S. Wood. 2006. Research strategies for safety evaluation of nanomaterials, Part V: Role of dissolution in biological fate and effects of nanoscale particles. *Toxicol. Sci.* 90(1):23–32, doi: 10.1093/toxsci/kfj084.

Chen, A. and Y. Arai. 2020. Current uncertainties in assessing the colloidal phosphorus loss from soil. *Adv. Agron.* 163: doi: 10.1016/bs.agron.2020.05.002.

Chen, Y., S. Peng, X. Wang, X. Yang, J. He, and D. Wang. 2007. Study of high yield cultivation technologies of oil-tea camellia (*Camellia oleifera*)—Formulate fertilization. *For. Res.* 20:650–655, doi: 10.3321/j.issn:1001-1498.2007.05.010.

Du, Z., J. Zhou, H. Wang, X. Chen, C. Du, and Q. Wang. 2010. Phosphorus movement and transformation in fertilizer microsites in red soil as affected by nitrogen and potassium application. *Acta Pedologica Sinica* 47(03):497–502, doi: 10.11766/trxb200812230315.

Ghanati, F., A. Morita, and H. Yokota. 2005. Effects of aluminum on the growth of tea plant and activation of antioxidant system. *Plant Soil* 276(1-2): doi: 10.1007/s11104-005-3697-y.

Gremion, F., A. Chatzinotas, and H. Harms. 2003. Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk

and rhizosphere soil. *Environ. Microbiol.* 5(10): doi: 10.1046/j.1462-2920.2003.00484.x.

He, G., J. Zhang, X. Hu, and J. Wu. 2011. Effect of aluminum toxicity and phosphorus deficiency on the growth and photosynthesis of oil tea (*Camellia oleifera* Abel.) seedlings in acidic red soils. *Acta Physiol. Plant.* 33(4):1285–1292, doi: 10.1007/s11738-010-0659-7.

Ilg, K., P. Dominik, M. Kaupenjohann, and J. Siemens. 2008. Phosphorus-induced mobilization of colloids: Model systems and soils. *Eur. J. Soil Sci.* 59(2):233–246, doi: 10.1111/j.1365-2389.2007.00982.x.

Jiang, H., J. Guo, X. Liu, S. Qiao, X. Zhang, K. Guo, J. Zhang, and J. Yang. 2017. Effects of combined application of nitrogen from different source on nitrogen utilization of spring maize and sustainability of soil fertility in Northeast China. *J. Plant Nutr. Fert.* 23(04):933–941, doi: 10.11674/zwyf.16249.

Jiao, W., W. Chen, A. Chang, and A. Page. 2012. Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. *Environ. Pollut.* 168:44–53, doi: 10.1016/j.envpol.2012.03.052.

Jin, X., X. Zeng, C. Qi, L. Yin, and Y. Deng. 2018. Influences of phosphorus application level on maize arbuscular mycorrhizal colonization and hyphal acquisition to heterogeneous phosphorus supply. *J. Plant Nutr. Fert.* 24(01):163–169, doi: 10.11674/zwyf.17239.

Khasawneh, F.E. and E.C. Doll. 1979. The use of phosphate rock for direct application to soils. *Adv. Agron.* 30(C):159–206, doi: 10.1016/S0065-2113(08)60706-3.

Koutsopoulos, S. 2002. Synthesis and characterization of hydroxyapatite crystals: A review study on the analytical methods. *J. Biomed. Mater. Res.* 62(4):600–612, doi: 10.1002/jbm.10280.

Li, D., J. Zhang, Y. He, Y. Qin, Y. Wei, P. Liu, L. Zhang, J. Wang, Q. Li, S. Fan, and K. Jiang. 2017. Scanning electron microscopy imaging of single-walled carbon nanotubes on substrates. *Nano Res.* 10(5):1804–1818, doi: 10.1007/s12274-017-1505-7.

Li, H., Z. Yang, M. Dai, X. Diao, S. Dai, T. Fang, and X. Dong. 2020. Input of Cd from agriculture phosphate fertilizer application in China

during 2006–2016. *Sci. Total Environ.* 2-3: doi: 10.1016/j.scitotenv.2019.134149.

Liao, H., H. Wan, J. Shaff, X. Wang, X. Yan, and L. Kochian. 2006. Phosphorus and aluminum interactions in soybean in relation to aluminum tolerance. exudation of specific organic acids from different regions of the intact root system. *Plant Physiol.* 141(2):674–684, doi: 10.1104/PP.105.076497.

Liu, R. and R. Lal. 2014. Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (*Glycine max*). *Sci. Rep.* 4:5686, doi: 10.1038/srep05686.

Luo, H., C. Zhu, L. Zhang, D. Hu, S. Tu, X. Guo, and D. Niu. 2016. Effects of phosphorus fertilization levels on vegetative growth in *Camellia oleifera*. *Non-wood For. Res.* 34(04):52–56, doi: 10.14067/j.cnki.1003-8981.2016.04.009.

Lv J., D. Xu, and F. Li. 2012. Effects of different environmental factors on the transportation of black soil colloid in saturated porous media. *Res. Environ. Sci.* 25(08):875–881, doi: CNKI: SUN:HJKX.0.2012-08-007.

Masayasu, N., K. Takashiet, and A. Masakazul. 2000. Correction methods of γ -ray self-absorption in bulk sample. *Radioisotopes* 49(4):189, doi: 10.3769/radioisotopes.49.189.

Missong, A., R. Bol, S. Willbold, J. Siemens, and E. Klumpp. 2016. Phosphorus forms in forest soil colloids as revealed by liquid-state ^{31}P -NMR. *J. Plant Nutr. Soil Sci.* 179(2): doi: 10.1002/jpln.201500119.

Montalvo, D., M. McLaughlin, and F. Degryse. 2015. Efficacy of hydroxyapatite nanoparticles as a P fertilizer in andisols and oxisols. *Soil Sci. Soc. Amer. J.* 60(5):14, doi: 10.2136/sssaj2014.09.0373.

Powers, K., S. Brown, V. Krishna, S. Wasdo, B. Moudgil, and S. Roberts. 2006. Research strategies for safety evaluation of nanomaterials, Part VI: Characterization of nanoscale particles for toxicological evaluation. *Toxicol. Sci.* 90:296–303, doi: 10.1093/toxsci/kfj099.

Qu X., H. Wang, X. Deng, Y. Yu, S. Dai, and J. Yuan. 2018. Effects of phosphorus and aluminum interaction on seedling growth of *Camellia vietnamensis* Huang. and phosphorus and aluminum contents in it. *J. Southern Agr.* 49(03):508–515, doi: CNKI:SUN:GXNY.0.2018-03-015.

Qu, X., J. Zhou, J. Masabni, and Y. Jun. 2020. Phosphorus relieves aluminum toxicity in oil tea seedlings by regulating the metabolic profiling in the roots. *Plant Physiol. Biochem.* 152:12–22, doi: 10.1016/j.plaphy.2020.04.030.

Rai, M., C. Ribeiro, L. Mattoso, and N. Duran. 2015. Nanotechnologies in food and agriculture. Springer, Cham, Switzerland.

Ryan, I. 2002. Efficient use of phosphate fertilizers for sustainable crop production in WANA. *Phosphate Newsletter.* 2-5.

Saleem, H. and S. Zaidi. 2020. Recent developments in the application of nanomaterials in agroecosystems. *Nanomaterials* 10(12):2411, doi: 10.3390/nano10122411.

Shi, R., S. Bao, and H. Qin. 1996. Soil agro-chemical analysis. Agriculture Press, Beijing.

Szameitat, A., A. Sharma, F. Minutello, A. Pinna, M. Er-Rafik, T. Hansen, and D. Persson. 2021. Unravelling the interactions between nano-hydroxyapatite and the roots of phosphorus deficient barley plants. *Environ. Sci. Nano* 8(2), doi: 10.1039/d0en00974a.

Ukwattage, N., Y. Li, Y. Gan, T. Li, and R. Gamage. 2020. Effect of biochar and coal fly ash soil amendments on the leaching loss of phosphorus in subtropical sandy ultisols. *Water Air Soil Pollut.* 231(2), doi: 10.1007/s11270-020-4393-5.

- Wang, D., Y. Xie, D. Jaisi, and Y. Jin. 2016. Effects of low-molecular-weight organic acids on the dissolution of hydroxyapatite nanoparticles in batch and column experiments: a perspective from phosphate oxygen isotope fractionation. *Environ. Sci. Nano* 3(4), doi: 10.1039/c6en00085a.
- Watanabe, Y., H. Yamada, T. Ikoma, J. Tanaka, G. Stevens, and Y. Komatsu. 2013. Preparation of a zeolite NaP1/hydroxyapatite nanocomposite and study of its behavior as inorganic fertilizer. *J. Chem. Eng. Data* 89(7):963–968, doi: 10.1002/jctb.4185.
- Watkinson, J.H. 1994. Dissolution rate of phosphate rock particles having a wide range of sizes. *Soil Res.* 32:1009–1014, doi: 10.1071/SR9941009.
- Yang, C., X. Liu, Z. Chen, Y. Lin, and S. Wang. 2016. Comparison of oil content and fatty acid profile of ten new *camellia oleifera* cultivars. *J. Lipids*, doi: 10.1155/2016/3982486.
- Yuan, J. 2013. Study on adaptive mechanism of *camellia oleifera* to low-phosphorous environment. Dissertation, Beijing Forestry University.
- Yuan, J., L. Huang, N. Zhou, H. Wang, and G. Niu. 2017. fractionation of inorganic phosphorus and aluminum in red acidic soil and the growth of *camellia oleifera*. *HortScience* 1293-1297: doi: 10.21273/hortsci12189-17.
- Yuan, J., X. Tan, S. Ye, N. Zhou, and B. Shi. 2013. The organic acids in root exudates of oil-tea and its role in mobilization of sparingly soluble phosphate in red soil. *J. Chem. Pharm. Res.* 5(11):572–577.
- Zang, L. 2011. Colloidal phosphorus transport and potential loss in paddy soil with different degree of phosphorus saturation. Dissertation, Zhejiang University.
- Zhang K., and M. Wang. 2014. Effect of ionic strength and fulvic acid on the transport of TiO₂ nanoparticles in soil. *Guangzhou Chem. Ind.* (23):110–111, doi: CNKI:SUN:GZHA.0.2014-23-044.
- Zhao, K., X. Zheng, C. Chen, and J. Shang. 2018. Effects of heterogeneity on migration of tracer and montmorillonite colloid in saturated porous medium. *J. Soil Water Conserv.* 32(3):140–145, doi: 10.13870/j.cnki.stbcxb.2018.03.022.
- Zhao, Q., D. Zeng, Z. Yu, B. Deng, and Z. Fang. 2006. Rhizosphere effects of *Pinus sylvestris* var. *mongolica* on soil phosphorus transformation. *Chinese J. Appl. Ecol.* 17(8):1377–1381, doi: 10.1360/yc-006-1280.

Supplemental Fig. 1. Soil energy spectrum (three replications).

Supplemental Fig. 2. Scanning electron microscopy images of soil.

Supplemental Table 1. Proportion of chemical compounds in the soil energy spectrum (three replications).

Element	Wt%	At%
NaK	00.05	00.07
MgK	00.80	01.03
AlK	19.90	23.09
SiK	54.57	60.82
KK	04.13	03.30
TiK	01.70	01.11
MnK	00.41	00.23
FeK	18.44	10.34
Matrix	Correction	ZAF
Element	Wt%	At%
NaK	00.00	00.00
MgK	00.56	00.72
AlK	18.88	21.86
SiK	56.33	62.67
KK	04.26	03.40
TiK	01.81	01.18
MnK	00.57	00.33
FeK	17.60	09.84
Matrix	Correction	ZAF
Element	Wt%	At%
NaK	00.00	00.00
MgK	00.79	01.01
AlK	19.67	22.70
SiK	55.86	61.91
KK	04.28	03.41
TiK	01.70	01.10
MnK	00.40	00.23
FeK	17.30	09.64
Matrix	Correction	ZAF