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ORIGINAL PAPER

Sewage Sludge Application Effects on Phosphorus Uptake by Cucumber and on Rhizosphere and Non-rhizosphere Soils Under Greenhouse Conditions

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Abstract
Within the growing greenhouse cultivation that we are facing these years, the use of phosphorus (P) fertilizers will inevitably increase. In this study, we used five different sewage sludges (SSs) as a P fertilizer, and studied the effect of these SSs on P uptake by cucumber plant (*Cucumis sativus* var. *Negin* L.), and specified their effects on availability and speciation of P in the rhizosphere and non-rhizosphere soils. A sandy clay loam texture soil was used and treated with 100 mg P kg^{-1} of five different SSs. Cucumber plants were grown under greenhouse conditions and after nearly 2 months, the plants were harvested. The plant's parts, root, shoot, and fruit were separated. Rhizosphere and non-rhizosphere soils for each treatment were also separated. The addition of different SSs increased shoot dry weight and also increased P content in root and shoot compared to control soil. The results showed that rhizosphere soil had a lower content of P extracted by water or Olsen compared to the non-rhizosphere soil. The mean proportion of HPO_4^{-2} in the SS-treated soils in the rhizosphere soil (74.3%) was significantly higher than non-rhizosphere soil (66.3%), while the proportion of $H_2PO_4^-$ in non-rhizosphere soil (29.8%) was significantly higher than rhizosphere soil (21.2%). Generally, the HPO_4^{-2} and $H_2PO_4^-$ were the dominant species of P in all treatments. The water-extractable P was better correlated to P content in shoot compared to Olsen-extractable P. This research offers additional insights into the effects of SS on soil solution characteristics and the availability of P in rhizosphere and non-rhizosphere soils which will be useful in understanding P uptake from soils treated with SS.

Keywords Phosphorus uptake · Greenhouse cultivation · Soil solution · Speciation

1 Introduction

Greenhouse crop production is increasing worldwide, and estimated to be 405 thousand hectares of greenhouses all over the world (FAO 2013). One of the greenhouse's most common crops is cucumber (*Cucumis sativus* L.). This crop is from the Cucurbitaceae family and its origin is from South Asia (Zhang et al. 2019). With a total production of 1.71 million tons in a year, Iran, along with China, Russia, and Turkey, is the top producing country of cucumber.

Sewage sludge (SS) is a semi solid-fluid waste material that is a by-product of municipal sewage treatment in urban areas. Studies show that the SS contains 1–8% (by dry weight) of phosphorus (P) (Liu et al. 2019), and after rocks, SS contains the highest amount of inorganic P (Falayi 2019). In Iran, the SS production in 2008 was about 650 thousand tons (dry) (Wichelns et al. 2015). In recent years, as a result of increasing agricultural activities, finding P sources is very critical. To deal with this issue, application of SS in agricultural soils in many countries as fertilizer is very common (e.g., Rittmann et al. 2011; Joo et al. 2015). There are some reports indicating that SS application to soil may have negative effects on soils and plant growth, mainly due to the presence of heavy metals, organic compounds, and pathogens in SS, which will contaminate the soils and groundwaters (Chu et al. 2017; Praspaliauskas et al. 2020). Taghipour and Jalali (2019) studied the effect of industrial solid wastes on heavy metal uptake by cucumber. They suggested that long-term application of industrial solid wastes increased the contents of heavy metals in cucumber and increased the risk of human health. On the

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other hand, there are a number of reports indicating that application of SS has positive effects on soil properties and plant growth (e.g., Antolín et al. 2005; Deeks et al. 2013; Eid et al. 2020). Eid et al. (2020) studied the application of SS on the growth of *Corchorus olitorius* L. plant. They suggested that the application of SS enhanced the content of organic matter (OM) and the plant growth, without accumulation of heavy metals to toxic levels for plants. Besides the positive effects of SS application on plant growth and available P in soil, it was reported that its application can increase P content in plants. Rehman and Qayyum (2020) studied the impact of co-composts of SS and farm manure as a source of P to rice in alkaline calcareous soils. They showed that the combined utilization of SS, farm manure, and rock phosphate has enhanced the effects of SS on P uptake and yield parameters and can be utilized visibly as a P fertilizer. Binder et al. (2002) indicated that due to the variability in soil, environment, SS composition, and management factors, various plants and soils may respond differently to the SS application, so further research is needed for each specific area, soil, and SS to evaluate the probability of substituting SS for P fertilization.

There are also variations between biological and chemical properties in rhizosphere and non-rhizosphere soils due to the various processes that exist in plant roots. Reddy et al. (1987) conducted a greenhouse analysis to evaluate enzyme activity in sludge-adjusted rhizosphere and non-rhizosphere soils. They indicated a higher activity for rhizosphere soils than non-rhizosphere soils. In the calcareous soils modified with SS, Raiesi et al. (2015) studied the influence of bean rhizosphere on the P fractionation. They suggested that organic P and calcium (Ca) phosphate fractions in rhizosphere soils may increase during the short-term application of SS to the calcareous soils, indicating that rhizosphere might induce some changes in P fractions in soil compared with non-rhizosphere soil. Wang et al. (2018) studied the effect of plant vegetation on P fractionation and nutrient stoichiometry in rhizosphere and non-rhizosphere of copper mine tailings. They indicated that the average pH values for the rhizosphere decreased relative to those in the non-rhizosphere, and that the alkaline phosphatase activities of the rhizosphere were substantially higher than those in the non-rhizosphere. In addition, the SS application can have different effects on soil solution characteristics for rhizosphere and non-rhizosphere soils. Phosphorus has various types in soil solution, and orthophosphate ion ($H_2PO_4^-$ and HPO_4^{2-}) can be taken up by plants from soil solution. The soil pH specifies the proportion in which these two types are taken up. In the soil solution phase, the P concentration is primarily controlled by the solubility of P minerals. The kinds of P compounds that occur in the soil are primarily determined by the soil's pH and mineral content. The most common P compounds are magnesium (Mg)-P, iron (Fe)-P, aluminum (Al)-P, and Ca-P. The recognition of P species and minerals controlling P concentration in soil solution in calcareous soils can be useful in managing crop production and in preventing groundwater degradation (Jalali and Jalali 2017). Sewage sludge application can affect the distribution of P types and P minerals controlling P availability.

As indicated, the previous studies were more about the effect of SS application on heavy metal uptake by plants and the potential risk for human consumption (e.g., Eid et al. 2017; Gomes et al. 2019), or the effect of the application of modified SS on soil and plant, such as SS ash (e.g., Lemming et al. 2017; Tsvetkov et al. 2020; Prabhakar et al. 2021), composting SS (e.g., Bożym and Siemiątkowski 2020; Rehman and Qayyum 2020), and vermicomposting of SS (e.g., Lv et al. 2020). In addition, there are some studies regarding the impacts of SS application on P uptake by plants (e.g., Petersen et al. 2003; Wang et al. 2016; Lemming et al. 2017; Wollmann et al. 2018). As mentioned above, soils and plants may react differently to different SSs and soil solution and P distribution may be affected differently in rhizosphere and non-rhizosphere soils, so it is important to determine P use efficiency of various SSs having different compositions for possibility of replacing P fertilization with SS. Additional insights into the effects of SS on the characteristics of soil solution and the availability of P in rhizosphere and non-rhizosphere soils will be helpful in understanding P uptake from SS-treated soils. In the current study, the hypothesis tested was that the P use efficiency of various SSs is different and that there is a separate effect of various SSs on soil P speciation and soil solution characteristics both in rhizosphere and non-rhizosphere soils. The main objectives of this study were (1) to examine P speciation and soil solution changes of the rhizosphere and non-rhizosphere soils treated by five various SSs and (2) to establish key factors that could possibly play significant roles in P uptake by different sections of the cucumber plants during the addition of SSs to soil.

2 Materials and Methods

2.1 Soil and Sewage Sludge

The soil was sampled from 0 to 30 cm of the Azandarian region in the Hamedan province in Iran (34° 29′ 35″ N, 48° 42′ 52″ E). The main cultivation of this region is vineyard. The soil was classified as Haplic Calcisols (Loamic, Ochric) according to word reference base (WRB). The physicochemical properties of this soil were previously analyzed by Jalali and Jalali (2020). The physicochemical properties of the studied soil include soil pH (1:5 soil to distilled water ratio, and 30-min shaking), cation exchange capacity (CEC) (sodium acetate and ammonium acetate method), OM (Walkley-Black method), $CaCO_3$ (back titration method), and soil texture (hydrometer method) that were determined according to the methods of Rowell (1994). The total P (0.2 g soil, 1 h, 550 °C, boiling with 25 ml 1 N HCl for 15 min) was determined according to the method of Andersen (1976). In this study, we used five SSs as a P source for greenhouse cultivation. These SSs come from Iranian cities of Rasht, Sanandaj, Saveh, Shiraz, and Tehran. The SSs are produced from municipal wastewater treatment plants, except for the SS of Saveh city, which is produced from municipal and industrial wastewaters. The processes applied to all SSs were settlement tank, waste stabilization pond, constructed wetland, percolating filter, and anaerobic digestion. Sampling and analysis of these SSs were done by Feizi et al. (2019). The chemical properties of SSs such as pH, OM, $CaCO_3$, and total P were measured according to the methods previously described for the soil. The electrical conductivity (EC) (1:5 soil to distilled water ratio and 30-min shaking) and nitrogen (N) (Kjeldahl digestion) were determined according to the methods of Rowell (1994).

2.2 Greenhouse Cultivation

The cultivation took place in the greenhouse of the Department of Soil Science, Faculty of Agriculture, Bu-Ali Sina University. Round plastic pots (12-cm top diameter and 11.5 cm in height) with a layer of gravel in the pots were prepared. To enrich the soil with 100 mg P kg^{-1} by five SSs, 6.4, 9.7, 13.3, 10.0, and 7.1 g of SS of Rasht, SS of Sanandaj, SS of Saveh, SS of Shiraz, and SS of Tehran respectively, were applied to 1-kg soil. The soil was thoroughly mixed with the SSs. The control soil which receives no P along with all other treatments had three replications.

Before sowing, the treatments were incubated for 40 days (the moisture content of each treatment was maintained at 60% field capacity using distilled water by daily weighing of each plot at 25 °C). Cucumber (*Cucumis sativus* var. *Negin* L.) seeds were sterilized with 10% H_2O_2 and rinsed with distilled water. Subsequently, seeds were planted in a seedling starter tray containing peat moss and perlite, and laid in the greenhouse. The greenhouse temperatures ranged from 25 to 35 °C at day and 15–18 °C at night and with 50–70% humidity, under 12 h of natural light. After about 10 days of sowing, on October 21, 2018, at the second leaf development, one plant was transferred to each pot.

Plants were watered every morning, and harvesting started after 55 days of plant transferring, on December 15, 2018. Roots, shoots, and fruits were separated. The fresh weight of plant shoots and fruits and the length of the shoots were measured. The roots were washed with tap water followed by distilled water to remove any substrate. Subsequently, fresh roots, shoots, and fruits were dried in an oven at 40 °C for 7 days. Then, the dry weight of roots, shoots, and fruits was measured. At the same time of separating roots, the rhizosphere soil was collected in a plastic bag by shaking roots vigorously. The soil outside the root zone was collected as the non-rhizosphere soil. In order to prepare the soils for analysis, the rhizosphere and non-rhizosphere soils were air-dried, crushed, and passed through a 2-mm sieve.

2.3 Elemental Analysis

The dry ashing method was used for digesting plant roots, shoots, and fruits. In total, 0.5 g of dry roots, shoots, or fruits was weighed in a crucible and placed in a furnace for 2 h at 500 °C. Five drops of deionized water were added when cooled, and then 2 ml of HNO_3 (1:1) was added as well. We led the solution on a hot plate to evaporate, after which the crucibles were again placed in a furnace at 500 °C for another 1 h. Five milliliters of HCl (1:1) was added after cooling and the solution was transferred to a volumetric flask with distilled water up to 50 ml (Williams 1984).

Soil solution from the rhizosphere and non-rhizosphere soils of each treatment was obtained using distilled water in a 1:5 soil to solution ratio and equilibrated for 30 min (Rowell 1994). The suspensions were centrifuged at 4000 rpm for 5 min, filtered through a filter paper.

Within the extractions obtained, EC and pH were measured by WTW- Cond 3110 and WTW- pH 7110, respectively. Calcium, Mg, chloride (Cl), and bicarbonate (HCO_3) were measured by the complexometric titration method (Rowell 1994). Sodium (Na) and potassium (K) were measured by a flame photometer (Jenway-PFP7). Phosphorus (determined using the colorimetrical method described by Murphy and Riley (1962)), sulfate (SO_4), and Al were measured by a UV-visible spectrophotometer (Analytik Jena-spekol 1500). Iron was measured by atomic absorption spectrophotometry (Varian, spectra 220). The water-extractable P in rhizosphere and non-rhizosphere soils, control soil, and different SSs were extracted by 1:5 soil to distilled water ratio and shaken for 30 min (Rowell 1994), and 0.5 M $NaHCO_3$ (Olsen-extractable P) adjusted pH 8.5 shaken for 30 min at the soil to solution ratio of 1:20 (Olsen and Sommers 1982). The P concentration in the extracts was also determined by the colorimetrical method (Murphy and Riley 1962), using a UV-visible spectrophotometer.

2.4 Data Analyses

The speciation of P in the water extract solution for rhizosphere and non-rhizosphere soils for all treatments was performed by Visual MINTEQ version 3.1 (Gustafsson 2019). The standard error of the mean and Pearson correlation (confidence level of 95%) were performed by Minitab version 19.1 (Minitab 2019). The Anderson-Darling test was used to check the normality of data. Analysis of variance (ANOVA) was used to evaluate the differences between the treatments and the significance differences were compared by the Duncan test at $p < 0.05$ using SAS version 9.4 (SAS 2013). All greenhouse cultivations and rhizosphere and non-rhizosphere soil measurements were conducted with three replicates.

The bioconcentration factor (BCF), bioaccumulation coefficient (BAC), and transfer factor (TF) were calculated in this study. The BCF was calculated as a ratio of P content in plant root to total P content in the soil (Pandey and Souza-Alonso 2019). The BAC was calculated as a ratio of P content in plant shoot to total P content in the soil (Koleli et al. 2015). The total P content in the sludge-treated soils was estimated from the total content of P in the control soil and SS mixed with the soil. The TF was calculated as a ratio of P content in the shoot to root ($TF_{S/R}$) and P content in the fruit to root ($TF_{F/R}$) (Taghipour and Jalali 2019).

Also, the P use efficiency (PUE), water-extractable efficiency (WEE), and Olsen-extractable efficiency (OEE) in the rhizosphere soil were calculated according to the following equations:

$$\text{PUE} = \frac{\text{Shoot P uptake (SS treatments)} - \text{Shoot P uptake (Control soil)}}{\text{P applied}} \times 100$$

$$\text{WEE} = \frac{\text{Water-extractable P (SS treatments)} - \text{Water-extractable P (Control soil)}}{\text{P applied}} \times 100$$

$$\text{OEE} = \frac{\text{Olsen-extractable P (SS treatments)} - \text{Olsen-extractable P (Control soil)}}{\text{P applied}} \times 100$$

3 Results

3.1 Soil and Sewage Sludge Properties

The soil used in this experiment had sandy clay loam texture, pH of 7.8, CEC of 4.7 $\text{cmol}_c\ \text{kg}^{-1}$, and low content of OM and $CaCO_3$, 0.46% and 1.57%, respectively. The water-extractable P, Olsen-extractable P, and total P content in the soil were also analyzed by Jalali and Jalali (2020) and are 11.08, 29.85, and 657.2 mg kg^{-1}, respectively.

The SS properties are presented in Table 1. The pH value was neutral, ranging from 6.7 to 7.5, except for Saveh, which had alkaline pH (8.9). Electrical conductivity values for SS of Rasht, SS of Sanandaj, SS of Saveh, SS of Shiraz, and SS of Tehran were 1.9, 2.3, 2.9, 1.9, and 1.5 dS m^{-1}, respectively. The highest water-extractable P and Olsen-extractable P belonged to SS of Tehran and the lowest was in SS of Shiraz. The total contents of P for SS of Rasht, SS of Sanandaj, SS of Saveh, SS of Shiraz, and SS of Tehran were 15,564, 10,299, 7479, 9972, and 14,109 mg kg^{-1}, respectively. Also, the highest carbon to P (C/P) and carbon to N (C/N) ratios have belonged to SS of Saveh (41.4 and 17.7%, respectively) and the lowest belonged to SS of Rasht (10.9 and 7.4%, respectively).

3.2 Soil Solution Properties in the Rhizosphere and Non-rhizosphere Soils

The chemical properties, soluble cations, and anions measured in the rhizosphere and non-rhizosphere soils with different treatments are presented in Table 2. The pH values in the control soil and SS of Saveh were significantly higher in the rhizosphere compared to non-rhizosphere soil. But in other treatments, no significant differences were observed. In the non-rhizosphere, the EC was lower than rhizosphere soil, with the exception of SS of Saveh, which in the non-rhizosphere soil was significantly higher than the rhizosphere soil. The content of Ca and Mg was not significantly different in most treatments in the rhizosphere and non-rhizosphere soils, but Ca in control and SS of Rasht was significantly higher in the rhizosphere than non-rhizosphere soils. In addition, the Mg content in SS of Shiraz was significantly lower in the rhizosphere soil than non-rhizosphere soil. The content of Na was significantly higher in the rhizosphere than non-rhizosphere soils (except in SS of Saveh which was not significant); however, the content of K in the non-rhizosphere was significantly higher than rhizosphere soil. The content of Cl, HCO_3, SO_4, Al, and Fe was not significantly different between treatments and rhizosphere and non-rhizosphere soils.

The P contents in water-extractable and Olsen-extractable are presented in Fig. 1a, b. Generally, the P content was lower in the rhizosphere compared to non-rhizosphere soils. However, the content of P in both extractants for SS of Rasht was significantly higher in the non-rhizosphere than rhizosphere soils.

The speciation of rhizosphere and non-rhizosphere soils in different treatments was also investigated in this study. The percentage of the total concentration of P species in the rhizosphere and non-rhizosphere soils is presented in Fig. 2a, b. In the rhizosphere soil, nearly 74% (average of all treatments including control) of total P species has belonged to HPO_4^{-2} and nearly 21% (average of all treatments including control) are for $H_2PO_4^{-}$. The $MgHPO_4$ (aq) and $CaHPO_4$ (aq) are between 1 and 2% (average of all treatments including control) of the total P species. Other P species were less than 0.5% of the total P species (Fig. 2a). In the non-rhizosphere soil, except for control soil which 51.7% of the total P species has belonged to $H_2PO_4^{-}$ and 45.7% was for HPO_4^{-2}, in other treatments, the HPO_4^{-2} had the higher percentage of the total P than $H_2PO_4^{-}$. The SS of Shiraz with 74.5% has the highest percentage for HPO_4^{-2} among treatments.

Table 1 The measured chemical properties of different sewage sludges along with standard errors of the means

Sewage sludges	pH[a]	EC[a,c] (dS m^{-1})	OM[a,d] (%)	Water-extractable P[b] (mg kg^{-1})	Olsen-extractable P[b] (mg kg^{-1})	Total P[b] (mg kg^{-1})	Total Fe[b] (mg kg^{-1})	Total Ca[b] (mg kg^{-1})	C/P[b,e] (%)	C/N[a,f] (%)
SS of Rasht	7.4 ± 0.09	1.9 ± 0.06	29.6 ± 0.8	215.2 ± 1.49	792.8 ± 53.8	15,564 ± 88	15,736 ± 8	6826 ± 100	10.9 ± 0.92	7.4 ± 0.6
SS of Sanandaj	6.7 ± 0.08	2.3 ± 0.09	44.7 ± 0.7	165.0 ± 1.13	699.2 ± 98.9	10,299 ± 72	14,785 ± 9	15,185 ± 120	24.9 ± 0.56	7.8 ± 0.5
SS of Saveh	8.9 ± 0.1	2.9 ± 0.08	54.0 ± 1.0	99.0 ± 6.05	703.8 ± 66.3	7479 ± 70	13,451 ± 3	9853 ± 110	41.4 ± 1.40	17.7 ± 0.7
SS of Shiraz	7.3 ± 0.09	1.9 ± 0.09	37.1 ± 0.7	17.9 ± 3.55	166.8 ± 17.1	9972 ± 77	10,874 ± 50	115,028 ± 310	21.3 ± 0.69	10.5 ± 0.3
SS of Tehran	7.5 ± 0.08	1.5 ± 0.08	48.4 ± 0.9	556.3 ± 5.78	1413.3 ± 50.4	14,109 ± 83	11,405 ± 56	10,214 ± 97	19.7 ± .77	8.3 ± 0.7

[a] Measured by Feizi et al. (2019), [b] measured by Jalali and Jalali (2020), [c] electrical conductivity, [d] organic matter, [e] carbon to phosphorus, [f] carbon to nitrogen

SS sewage sludge

Like the rhizosphere soil, the $MgHPO_4$ (aq) and $CaHPO_4$ (aq) were between 1 and 2% of the total P species (Fig. 2b). The mean proportion of HPO_4^{-2} in the SS-treated soils, except the control in the rhizosphere soil (74.3%), was significantly higher than that in the non-rhizosphere soil (66.3%), while the proportion of $H_2PO_4^{-}$ in the non-rhizosphere soil (29.8%) was significantly higher than that in the rhizosphere soil (21.2%). Jalali and Jalali (2017) studied the distributions of P species in 20 soil samples. They also found that HPO_4^{-2}, $H_2PO_4^{-}$, $CaHPO_4$, and $MgHPO_4$ had the highest percentage of P species among other species. The saturation index (SI) values are presented in Fig. 3a, b. The values of SI for different treatments and rhizosphere and non-rhizosphere soils were not very different from each other. The minerals ferrihydrite, gibbsite, goethite, hydroxyapatite, and strengite were supersaturated, and other minerals were undersaturated (Fig. 3a, b).

3.3 Effect of Sewage Sludges on Growth and P Uptake by Cucumber

The effect of different SSs on cucumber shoot length is shown in Fig. 4a. Shoot length in different SSs increased compared to control soil, but only SS of Rasht and SS of Tehran were significantly higher than control soil. The maximum length between SSs was observed at SS of Tehran with 41-cm length. Figure 4 also shows the effects of different SSs on biomass production of cucumber. Shoot dry weight in the control soil was lower than that in the soil treated with different SSs, but it is not significantly different from each other (Fig. 4b). The effects of different SSs on cucumber dry fruit weight did not vary significantly from each other (Fig. 4c). It may be because the replicates varied greatly, but the SS of Sanandaj, SS of Saveh, and SS of Tehran had a higher dry fruit weight than control, but the SS of Rasht and SS of Shiraz were lower than control soil.

The application of different SSs significantly increased the P content of root dry weight compared to the control soil (Fig. 5a). Although the P content of shoot dry weight in the control soil is lower than that in the other treatments, only a significant difference was observed in SS of Rasht (Fig. 5b). The contents of P in dry fruit weight in the control soil, SS of Rasht, and SS of Shiraz were significantly higher than those in the other treatments (Fig. 5c). Generally, the highest amount of shoot length, shoot weight, and fruit weight has belonged to the soils treated with SS of Rasht and SS of Tehran, which can be due to the highest amount of Ca, Mg, Na, K, and nitrate (NO_3) and the lower ratio of C/N in these SSs. On the other hand, SS of Shiraz had a relatively low amount of Mg, K, Na, and NO_3 and a higher ratio of C/N compared to other SSs.

Table 2 The soil solution properties in different treated soils in the rhizosphere and non-rhizosphere soils along with standard errors of the means

Parameters	Rhizosphere soil					
	Control	SS of Rasht	SS of Sanandaj	SS of Saveh	SS of Shiraz	SS of Tehran
pH	7.67 ± 0.13^{abc}	7.63 ± 0.03^{bcd}	7.65 ± 0.04^{abcd}	7.91 ± 0.04^{a}	7.77 ± 0.05^{ab}	7.54 ± 0.06^{bcd}
EC (dS m^{-1})	0.07 ± 1.07^{de}	0.08 ± 0.00^{cd}	0.08 ± 0.00^{c}	0.10 ± 0.00^{b}	0.07 ± 0.00^{de}	0.07 ± 0.00^{de}
Ca (mg kg^{-1})	18.78 ± 0.00^{a}	18.78 ± 0.00^{a}	18.78 ± 0.00^{a}	12.52 ± 0.00^{b}	18.78 ± 0.00^{a}	12.52 ± 0.00^{b}
Mg (mg kg^{-1})	5.70 ± 1.90^{bc}	5.70 ± 1.90^{bc}	9.49 ± 1.90^{a}	7.59 ± 0.00^{ab}	3.80 ± 0.00^{c}	7.59 ± 0.00^{ab}
Na (mg kg^{-1})	29.48 ± 0.74^{bc}	29.81 ± 0.27^{b}	28.03 ± 1.32^{bc}	57.13 ± 0.52^{a}	27.25 ± 1.02^{c}	30.12 ± 1.34^{b}
K (mg kg^{-1})	19.75 ± 2.05^{de}	15.53 ± 1.14^{fg}	14.76 ± 0.92^{fg}	17.35 ± 0.53^{ef}	16.89 ± 1.20^{ef}	12.81 ± 0.49^{g}
Cl (mg kg^{-1})	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}
HCO_3 (mg kg^{-1})	171.56 ± 19.1^{ab}	190.63 ± 0.00^{a}	171.56 ± 19.1^{ab}	190.63 ± 0.00^{a}	171.56 ± 19.1^{ab}	171.56 ± 19.1^{ab}
SO_4 (mg kg^{-1})	70.92 ± 2.97^{a}	50.76 ± 7.29^{b}	58.73 ± 4.78^{ab}	68.10 ± 6.90^{ab}	59.08 ± 5.33^{ab}	52.05 ± 1.10^{ab}
Al (mg kg^{-1})	0.97 ± 0.03^{a}	0.66 ± 0.10^{bc}	0.71 ± 0.18^{abc}	0.90 ± 0.04^{ab}	0.96 ± 0.10^{a}	0.90 ± 0.05^{ab}
Fe (mg kg^{-1})	18.62 ± 0.92^{b}	15.00 ± 0.09^{cde}	14.87 ± 1.38^{de}	17.65 ± 1.54^{bcd}	15.66 ± 0.40^{bcde}	13.17 ± 0.21^{e}

Parameters	Non-rhizosphere soil					
	Control	SS of Rasht	SS of Sanandaj	SS of Saveh	SS of Shiraz	SS of Tehran
pH	7.10 ± 0.11^{e}	7.50 ± 0.02^{bcd}	7.52 ± 0.08^{bcd}	7.41 ± 0.12^{cd}	7.71 ± 0.10^{ab}	7.39 ± 0.04^{d}
EC (dS m^{-1})	0.06 ± 0.00^{e}	0.06 ± 0.00^{e}	0.08 ± 0.00^{cd}	0.11 ± 0.00^{a}	0.07 ± 0.00^{de}	0.06 ± 0.00^{e}
Ca (mg kg^{-1})	12.52 ± 0.00^{b}	12.52 ± 0.00^{b}	18.78 ± 0.00^{a}	12.52 ± 0.00^{b}	18.78 ± 0.00^{a}	12.52 ± 0.00^{b}
Mg (mg kg^{-1})	7.59 ± 0.00^{ab}	7.59 ± 0.00^{ab}	7.59 ± 0.00^{ab}	7.59 ± 0.00^{ab}	7.59 ± 0.00^{ab}	7.59 ± 0.00^{ab}
Na (mg kg^{-1})	22.25 ± .20^{d}	21.25 ± 0.12^{de}	20.55 ± 1.02^{de}	55.22 ± 0.37^{a}	21.84 ± 0.07^{d}	19.24 ± 0.37^{e}
K (mg kg^{-1})	23.49 ± 0.43^{bc}	21.22 ± 0.65^{cd}	27.01 ± 0.88^{a}	22.87 ± 0.71^{bc}	25.14 ± 0.07^{ab}	22.30 ± 0.62^{bcd}
Cl (mg kg^{-1})	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}	27.70 ± 0.00^{a}
HCO_3 (mg kg^{-1})	152.50 ± 0.00^{b}	152.50 ± 0.00^{b}	152.50 ± 0.00^{b}	190.63 ± 0.00^{a}	152.50 ± 0.00^{b}	152.50 ± 0.00^{b}
SO_4 (mg kg^{-1})	70.37 ± 5.95^{a}	66.85 ± 7.27^{ab}	54.28 ± 3.49^{ab}	54.54 ± 5.15^{ab}	62.83 ± 1.71^{ab}	54.28 ± 7.78^{ab}
Al (mg kg^{-1})	0.72 ± 0.05^{abc}	0.61 ± 0.04^{bc}	0.60 ± 0.06^{c}	0.60 ± 0.04^{bc}	0.74 ± 0.13^{abc}	0.66 ± 0.05^{bc}
Fe (mg kg^{-1})	28.36 ± 0.79^{a}	18.01 ± 0.66^{bc}	17.92 ± 0.63^{bcd}	16.85 ± 0.54^{bcd}	18.26 ± 1.42^{b}	13.00 ± 0.52^{e}

Values followed by the same letter within each row in both rhizosphere and non-rhizosphere soils are not significantly different by the Duncan test at $p < 0.05$

SS sewage sludge

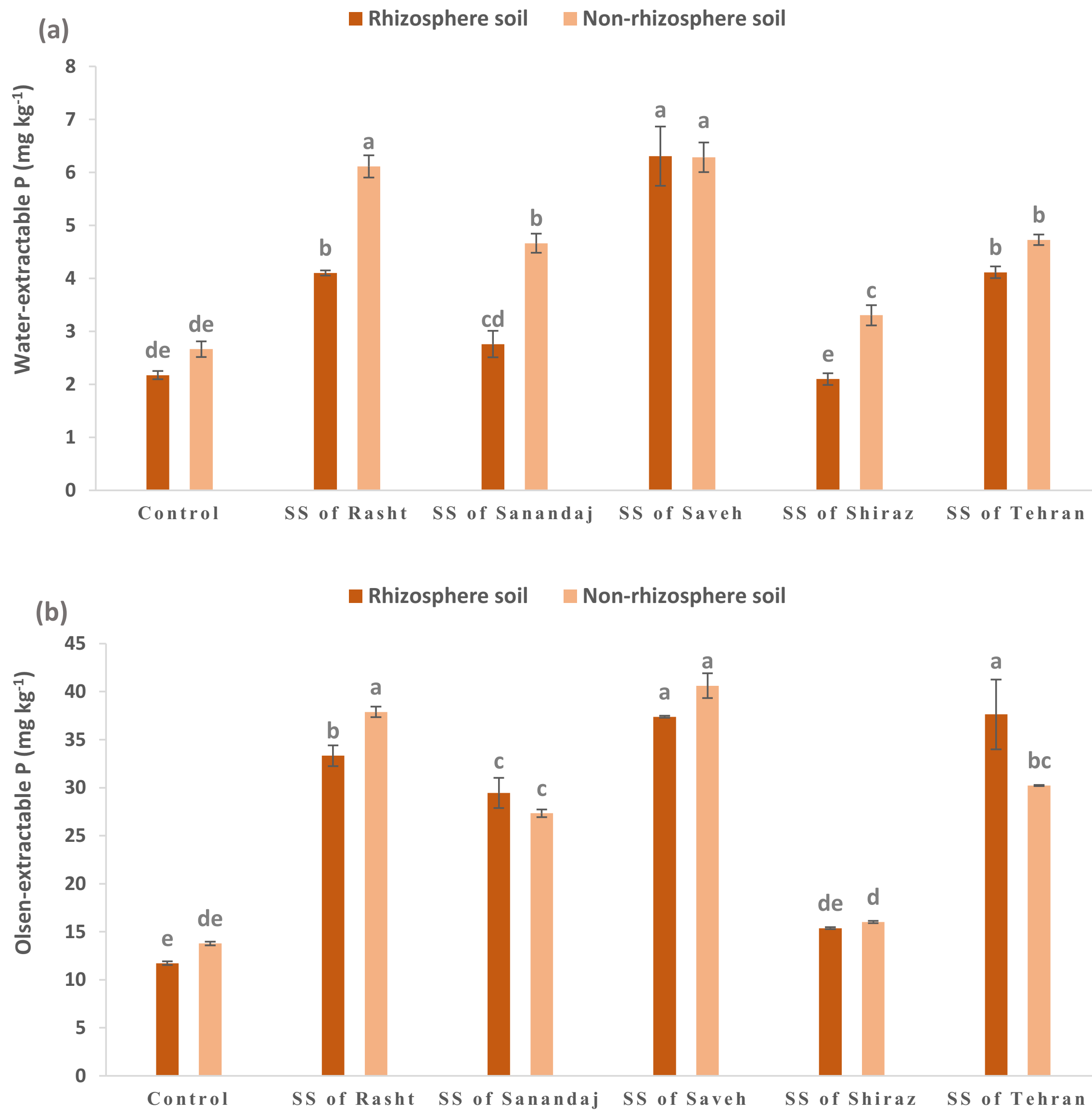

Fig. 1 The water-extractable P (a) and Olsen-extractable P (b) in the rhizosphere and non-rhizosphere soils in different treatments. Error bars show standard errors of the means. Two-way ANOVA was used to evaluate the differences between the treatments, and rhizosphere soil and non-rhizosphere soil as factors. Different letters show the difference significant at $p < 0.05$ by the Duncan test. SS, sewage sludge

3.4 Relationship Between P Uptake and P Extractants

The relationships between root and fruit P uptake with water-extractable P and Olsen-extractable P in the rhizosphere and non-rhizosphere soils were not significant, while significant relationships between shoot P uptake and extractable P were found. The regression equations between shoot P uptake and water-extractable P and Olsen-extractable P in the rhizosphere and non-rhizosphere soils are presented in Fig. 6. The results showed that the content of P in the plant was significantly related to the water-extractable P and Olsen-extractable P, except for the relation between shoot P uptake and Olsen-extractable P in the rhizosphere soil. Water-extractable P in both rhizosphere and non-rhizosphere soils was better described by second-order polynomial regression (Fig. 6a, b) and for Olsen-extractable P, the power regression best described these relations (Fig. 6c, d).

3.5 Phosphorus Uptake Parameters

Table 3 shows the values of BCF, BAC, $TF_{S/R}$, $TF_{F/R}$, PUE, WEE, and OEE calculated for cucumber in different treatments. The BCF value for SS of Tehran was higher than that for other treatments, with control soil being the lowest. Such values suggest that the amount of P in the soil is readily absorbed by the cucumber roots. The BAC value for SS of Rasht was higher than that for other treatments. The TF of shoot and fruit to root was also calculated. The values of

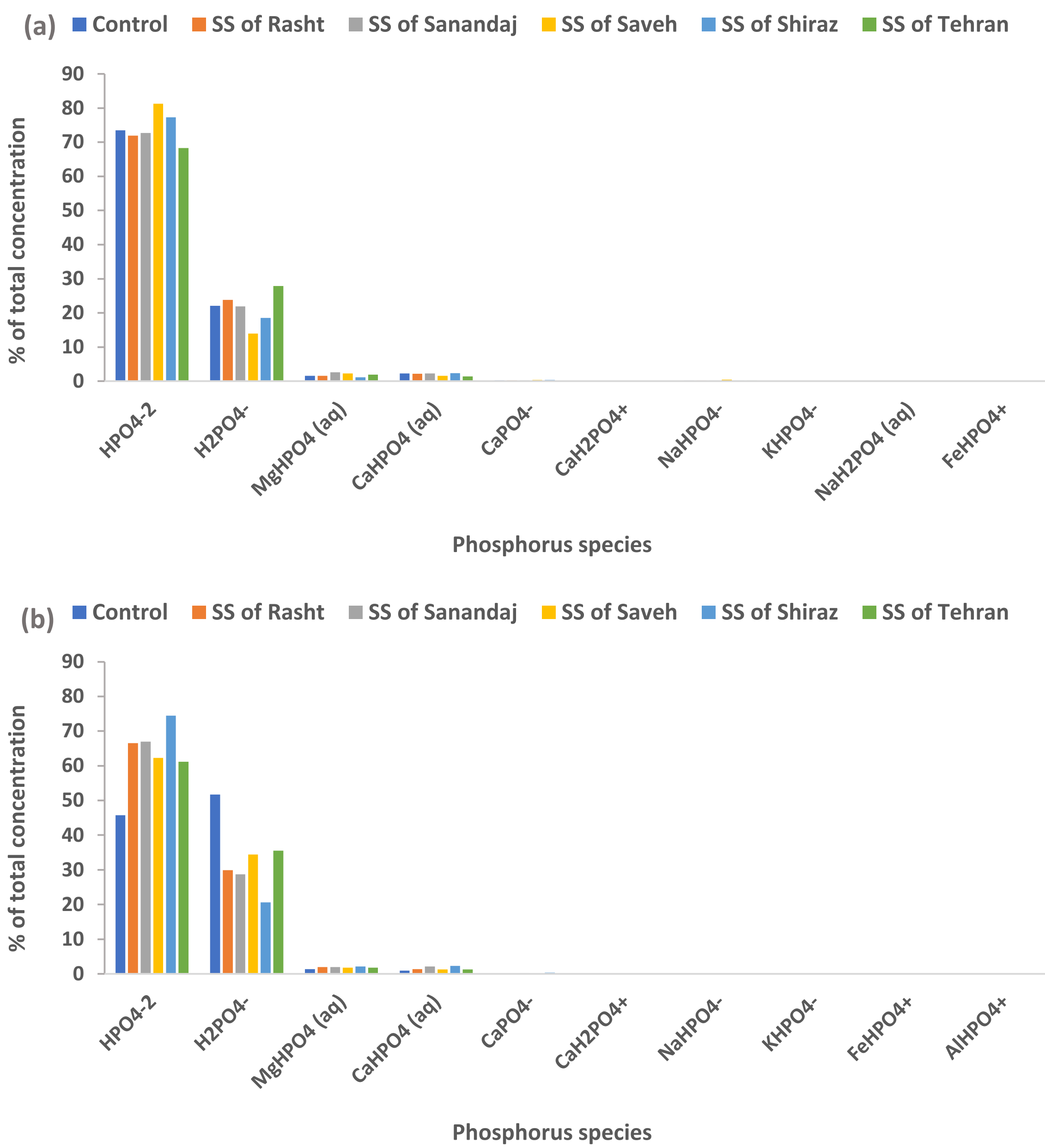

Fig. 2 The percentage of P species in (a) rhizosphere soil and (b) non-rhizosphere soil. SS, sewage sludge

$TF_{S/R}$ are much smaller than those of $TF_{F/R}$, indicating a higher amount of P transferred from root to fruit relative to root to shoot.

The PUE was calculated and presented in Table 3. The typical percentage for PUE is 10–15, and small values show that the applied P is inefficient (Roberts and Johnston 2015). The PUE percentage for SS of Rasht was higher than other treatments, and SS of Sanandaj was lower, but generally, the PUE was higher in all treatments and showed a good P efficiency.

The WEE and OEE were also measured (Table 3). These parameters show which one of SSs releases the P higher than the others. The SS of Rasht, SS of Saveh, and SS of Tehran release higher P than the other SSs in both parameters.

4 Discussion

The present study focuses on the effect of P present in various SSs and shows how the application of different SSs affects P uptake by cucumber and rhizosphere and non-rhizosphere soils. Five SS analyses showed variable total P, water-extractable P, and Olsen-extractable P contents depending on the different C/P and C/N ratios (Table 1). The highest C/N ratio has belonged to SSs of Saveh and Shiraz with low P contents, while the lowest C/N ratio belonged to SS of Rasht and SS of Sanandaj with relatively high P contents. In most treated soils, which are consistent with the results reported by Cao et al. (2018) and Wang et al. (2016), the pH value of the

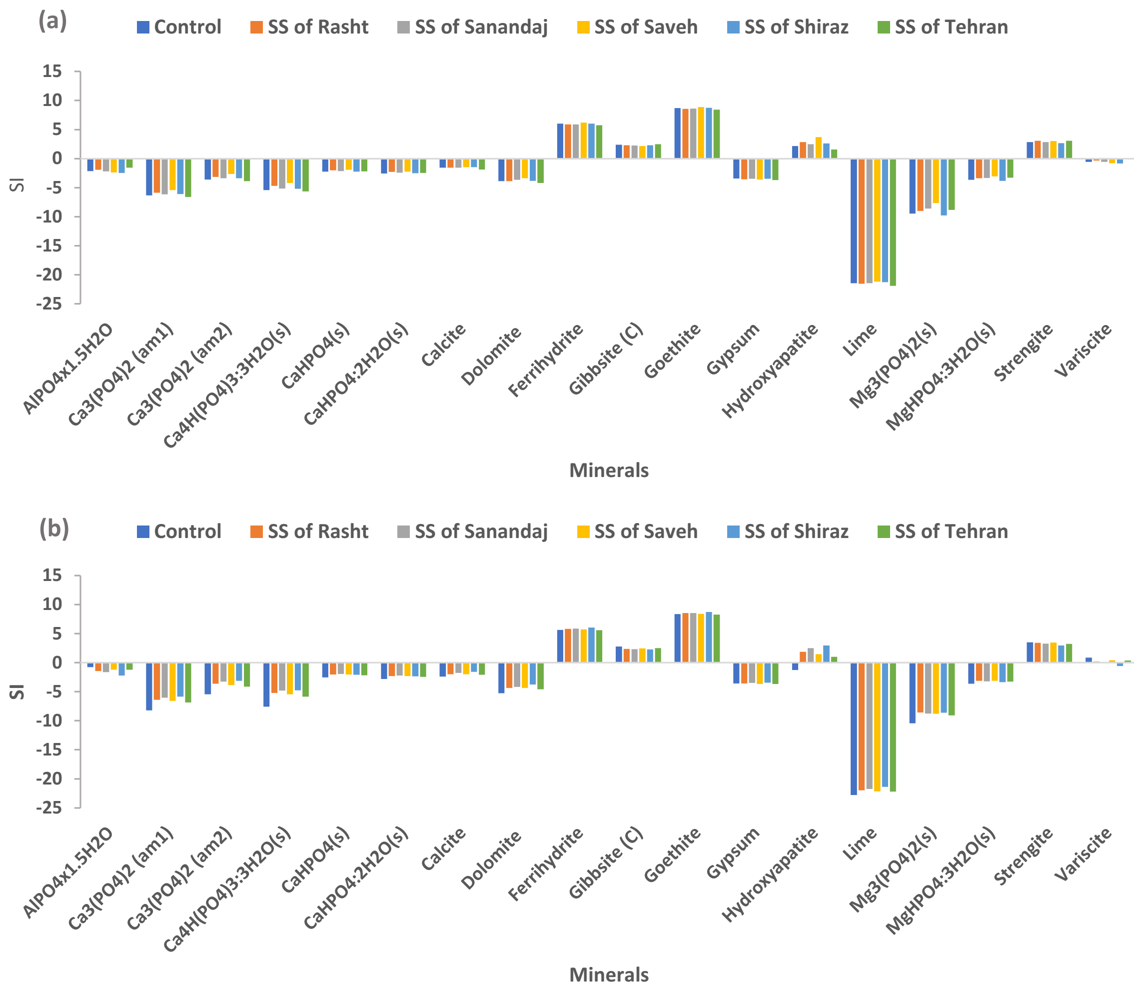

Fig. 3 Saturation indices of different minerals in (a) rhizosphere soil and (b) non-rhizosphere soil. SS, sewage sludge

rhizosphere and non-rhizosphere soils of cucumber plants was not different from each other. It has been shown that, as a result of deprotonation of organic matter decomposition and ammonium nitrification, SS appears to increase soil acidity (Britto and Kronzucker 2002). Due to low added SSs (0.64–1.34%) and relatively high calcium carbonate (1.57%), the pH value did not change in both the rhizosphere and non-rhizosphere soils in our experiment.

The addition of SS in both rhizosphere and non-rhizosphere soils of cucumber plant had different effects on cations and anions in soil solution (Table 2). In all treatments, the contents of Ca and Mg were not significantly different between rhizosphere and non-rhizosphere soils (except for both Ca in two treatment and Mg in one treatment). On the other hand, in soil solution, an opposite impact in Na and K was observed showing significantly higher Na in the rhizosphere than non-rhizosphere soils in all treatments (except SS of Saveh), although K was significantly higher in the non-rhizosphere soil. The lower K in rhizosphere soils may be due to plant uptake of K, which decreases its concentration in soil solution in rhizosphere soils relative to non-rhizosphere soils. Despite the addition of SS with elevated concentrations of Fe (Feizi et al. 2019), the water-extractable concentration of Fe in soil decreased with all SS amendments compared to the control in both rhizosphere and non-rhizosphere soils (Table 2). The same is true for Al, but total Al concentrations in SS were not measured in Feizi et al. (2019). This shows that the Fe (and Al) in the SS samples had very low availability and SS samples even are able to sorb or precipitate Fe (and Al) from the soil.

Application of all SSs increased P in both rhizosphere and non-rhizosphere soils of the cucumber plant (Fig. 1a, b) and was relatively lower in the rhizosphere compared with non-rhizosphere soils. Khalili-Rad and Mirseyed Hosseini (2017) studied the effect of rhizosphere and non-rhizosphere soils on P fractions. They found that the content of Olsen-extractable P in the rhizosphere soil was significantly lower than that in the non-rhizosphere soil. This could be due to the uptake of P by the plant. Microbial activity in the rhizosphere soil can be increased by releasing OM from the plant into the soil. The role of the microbial community should be taken into account in

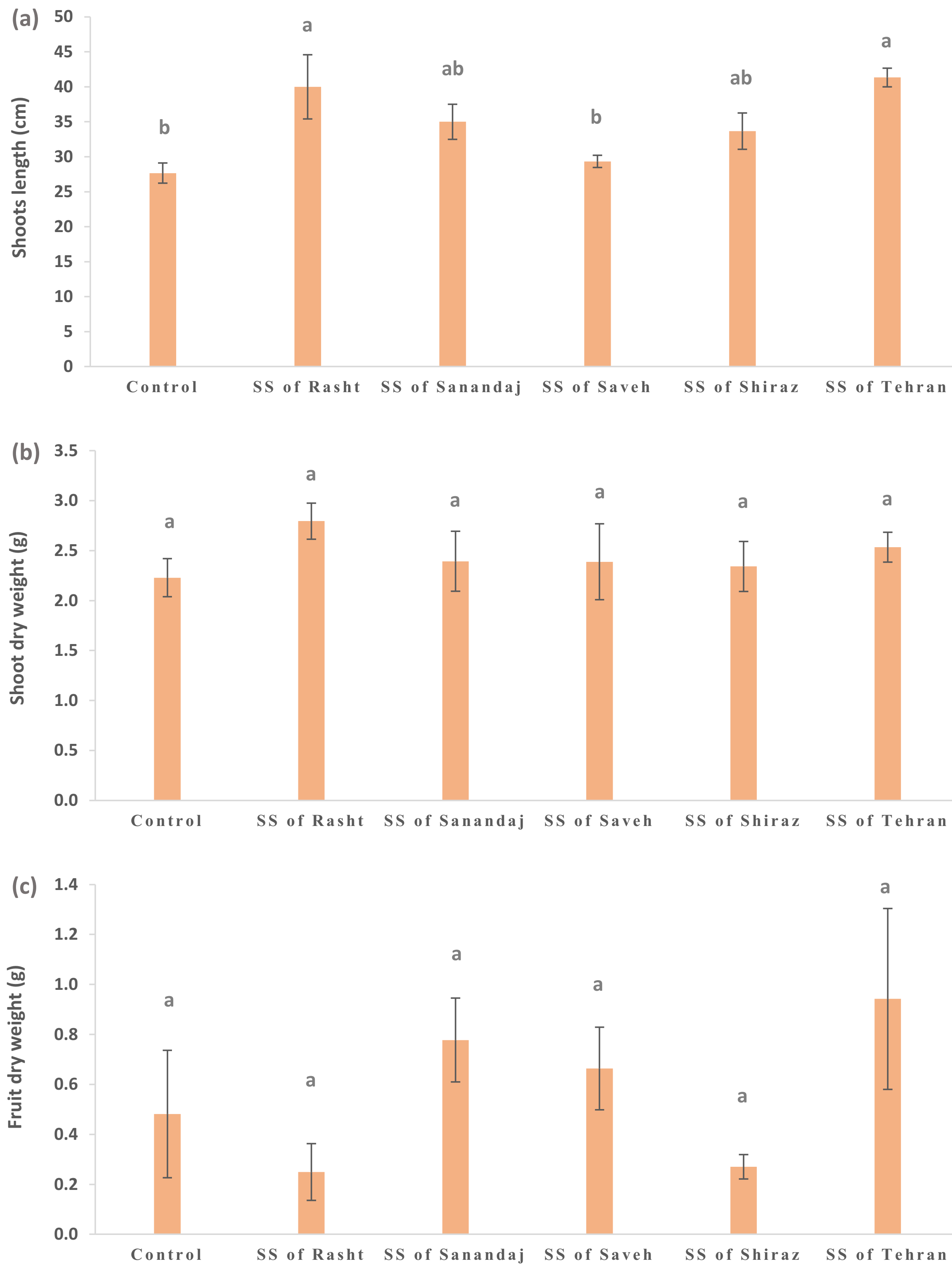

Fig. 4 Shoot length (a), shoot dry weight (b), and fruit dry weight (c) of cucumber as affected by soil treated with different SSs. Error bars show standard errors of the means. One-way ANOVA was used to evaluate the differences between the treatments. Different letters show the difference significant at $p < 0.05$ by the Duncan test. SS, sewage sludge

increasing the available P as it is very likely that SS application stimulated soil biological activity and, more specifically, the release of phosphatase, which is responsible for the hydrolysis of organic P. In the study conducted by Houben et al. (2019), they found that the application of SS stimulated the activity of phosphatase and results in increased release of available P. García-López et al. (2016) studied the addition of microorganisms to soil and

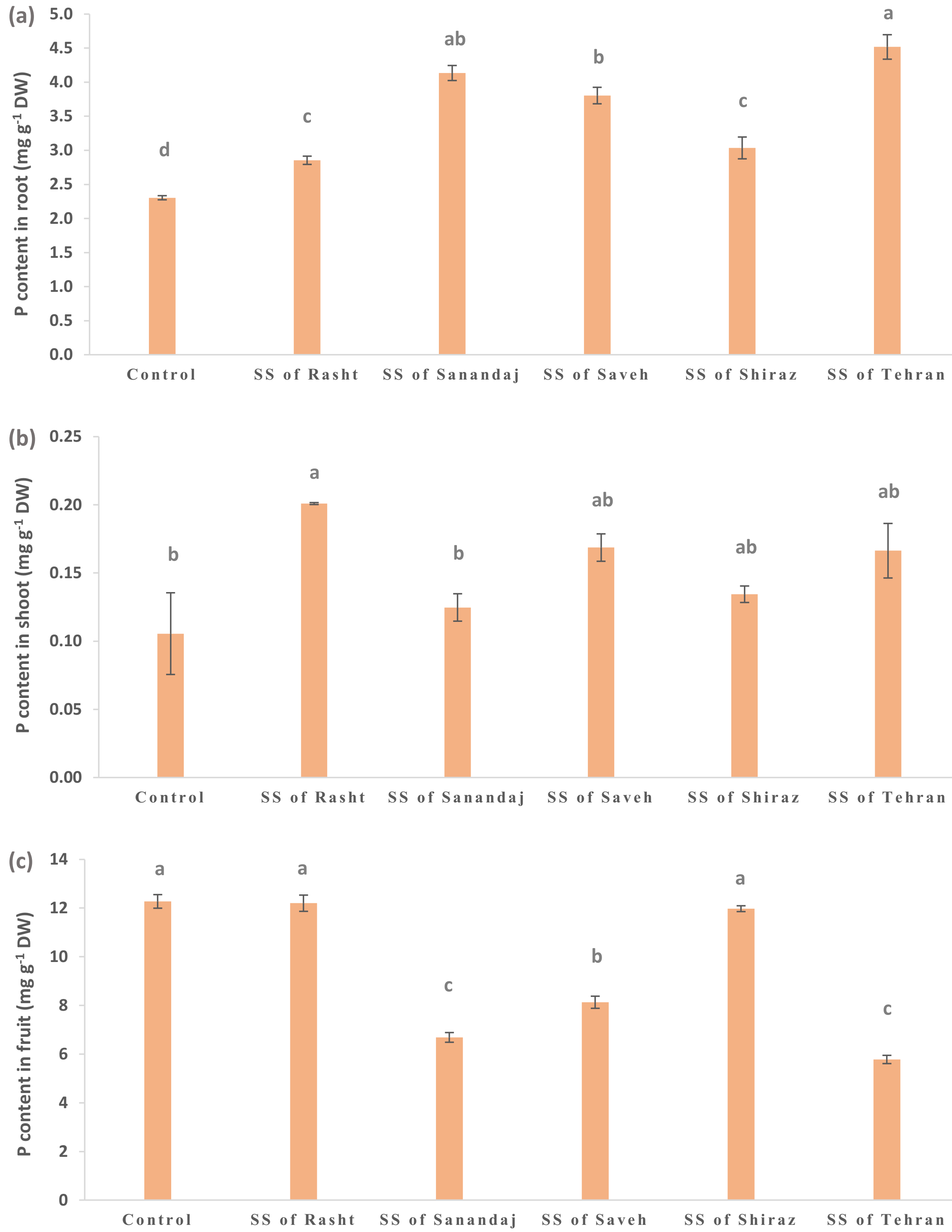

Fig. 5 The P content in (a) root dry weight (DW), (b) shoot dry weight, and (c) fruit dry weight of cucumber as affected by soil treated with different SSs. Error bars show standard errors of the means. One-way ANOVA was used to evaluate the differences between the treatments. Different letters show the difference significant at $p < 0.05$ by the Duncan test. SS, sewage sludge

its effect on P uptake by the cucumber plant. Generally, the results showed that the microorganisms increased P uptake by the cucumber. The structure and role of the microbial community in the rhizosphere differentiated considerably from those in the non-rhizosphere because of the influence of the rhizosphere (Chen et al. 2016). Carboxylate release from roots effectively increases the rhizosphere P concentration, some micronutrients, desorption of P from clay minerals, Fe and Al oxides, and hydroxides (Shane and Lambers 2005; Nobile et al. 2019).

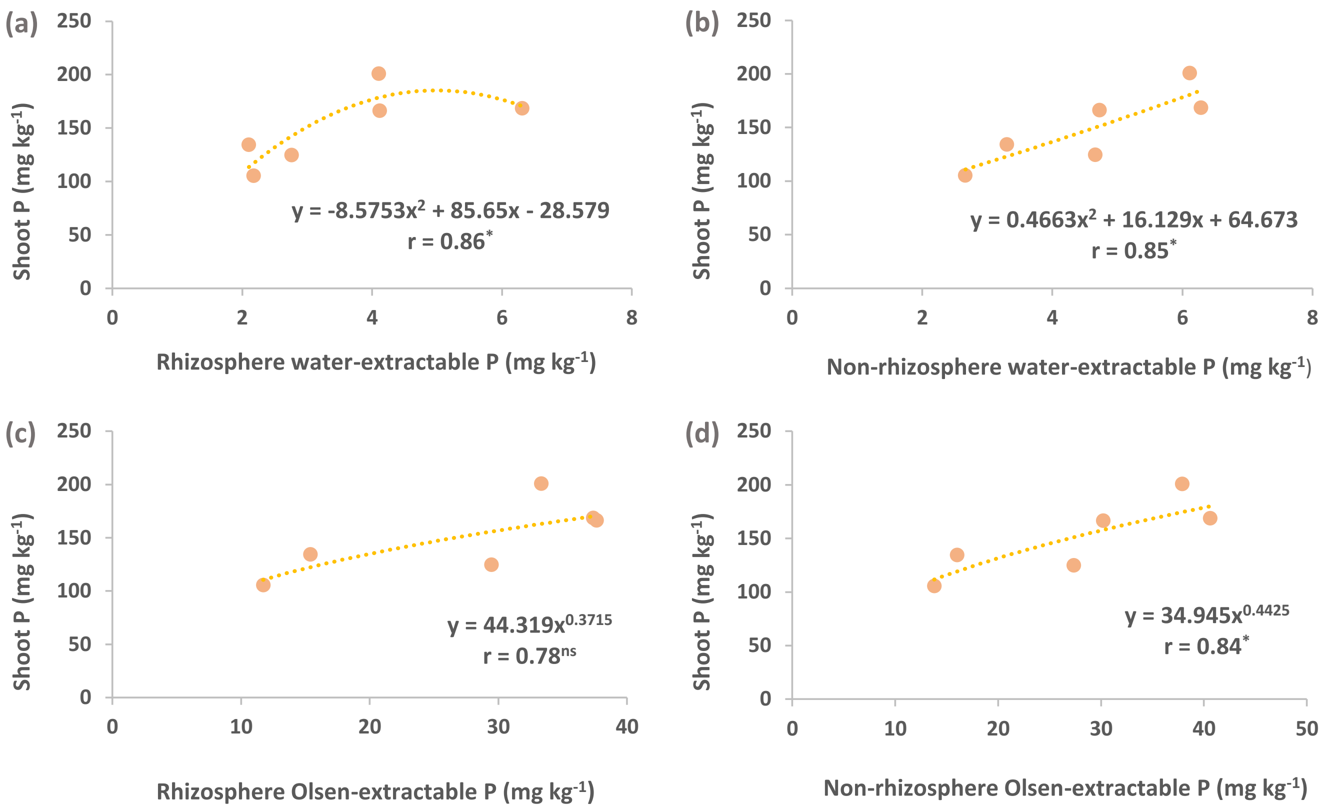

Fig. 6 Relationship between shoot P uptake and water-extractable P by second-order polynomial regression (a, b) and Olsen-extractable P by power regression (c, d) in the rhizosphere and non-rhizosphere soils

The concentration of P in the soil solution of calcareous soils is low and the added P is transformed into low-solubility P minerals and the availability of P to plants is largely dependent on the solid-phase solubility of P minerals, P in soil solution, and P sorbed on mineral surfaces (Zhang et al. 2014). Figures 2a, b and 3a, b show the impact of the SS application on the speciation of P in soil solution and minerals regulating the availability of P in cucumber plant rhizosphere and non-rhizosphere soils, respectively. Regardless of the rhizosphere and non-rhizosphere soils, the results indicated that the HPO_4^{-2} and $H_2PO_4^-$ were dominated in all treatments (Fig. 2a, b) consistent with other research (e.g., Zhang et al. 2014; Jalali and Jalali 2017). In the rhizosphere soil, the proportion of HPO_4^{-2} in all treatments was higher than $H_2PO_4^-$, while the dominant species is $H_2PO_4^-$ in the non-rhizosphere soil of control (Fig. 2a, b). The application of SSs has increased the proportion of HPO_4^{-2} in all treatments in the non-rhizosphere soil compared to control soils, but in

Table 3 The estimated BCF, BAC, $TF_{S/R}$, $TF_{F/R}$, PUE, WEE, and OEE for different treatments in cucumber plants along with standard errors of the means

Treatments	BCF[a]	BAC[b]	$TF_{S/R}$[c]	$TF_{F/R}$[d]	PUE[e] (%)	WEE[f] (%)	OEE[g] (%)
Control	3.5 ± 0.05[c]	0.16 ± 0.05[ab]	0.05 ± 0.00[ab]	5.3 ± 0.04[a]	-	-	-
SS of Rasht	3.8 ± 0.09[c]	0.27 ± 0.00[a]	0.07 ± 0.00[a]	4.3 ± 0.21[b]	95.4 ± 67[a]	1.93 ± 0.05[b]	21.61 ± 1.0[ab]
SS of Sanandaj	5.5 ± 0.15[ab]	0.16 ± 0.01[b]	0.03 ± 0.00[c]	1.6 ± 0.07[d]	19.2 ± 11[b]	0.59 ± 0.25[c]	17.73 ± 1.5[b]
SS of Saveh	5.0 ± 0.17[b]	0.22 ± 0.03[ab]	0.04 ± 0.00[bc]	2.1 ± 0.10[c]	63.2 ± 10[ab]	4.14 ± 0.56[a]	25.66 ± 0.1[a]
SS of Shiraz	4.0 ± 0.21[c]	0.18 ± 0.00[ab]	0.04 ± 0.00[bc]	3.9 ± 0.22[b]	28.9 ± 6[b]	0.00 ± 0.11[c]	3.66 ± 0.1[c]
SS of Tehran	6.0 ± 0.24[a]	0.22 ± 0.01[ab]	0.04 ± 0.00[bc]	1.3 ± 0.08[d]	60.9 ± 28[ab]	1.95 ± 0.11[b]	25.91 ± 3.6[a]

[a] Bioconcentration factor, [b] bioaccumulation coefficient, [c] transfer factor (shoot to root), [d] transfer factor (fruit to root), [e] phosphorus use efficiency, [f] water-extractable efficiency, [g] Olsen-extractable efficiency

Values followed by the same letter within each column are not significantly different by the Duncan test at $p < 0.05$

SS sewage sludge

rhizosphere soils, its proportion was not increased in all treatments and the increase was lower than that of the non-rhizosphere soil. Plant uptake P from soil solution is in the form of HPO_4^{-2} and $H_2PO_4^-$, but plant uptake of HPO_4^{-2} seems to be slower than $H_2PO_4^-$ uptake (Havlin et al. 2013). The introduction of SS to soil tends to increase the slower uptake of P species. Characterization of P minerals controlling P concentration in the rhizosphere and non-rhizosphere soils treated with various SSs is very important for managing P and protecting the quality of groundwater. It would appear that the solid-phase minerals controlling the availability of P are not different and that P minerals such as $CaHPO_4$ and $CaHPO_4$:$2H_2O$ control the P activity in SS-treated soils in both rhizosphere and non-rhizosphere soils (Fig. 3a, b).

Eid et al. (2017) observed that the application of SS up to 40 g kg^{-1} significantly increased cucumber shoot length. The abundant supply of macronutrients and micronutrients in SS (Arif et al. 2018; Guoqing et al. 2019) could be the reason for the increase observed in plant length (Fig. 4a). Figure 5a shows that compared with the control soil, the P content in cucumber plant roots has significantly increased with the application of different SSs. Application of SS to the soil, which contains a higher content of organic C, N, P, Na, K, Ca, and Mg, will improve plant growth (Singh and Agrawal 2010). In the present study, the rate of removal of P can be influenced by several factors. During the experiment, no additional nutrients were applied and it was assumed that nutrients in all SSs were sufficient to meet the demand for the cucumber plant. Nevertheless, if one of the SSs provides more N or other nutrients to the cucumber plant, the P uptake is likely to be increased and it may be impossible to differentiate the effect of P from the effect of other variables. Petersen et al. (2003) treated the soil with two different rates of SS and indicated that this results in different inputs of total P and particularly total N. Table 2 shows the content of nutrients in soil solution in the rhizosphere and non-rhizosphere soils. It can be seen that Ca in the rhizosphere soil did not significantly change except in SS of Saveh and SS of Tehran (Ca content was reduced). There were also no significant changes in the Mg content in all treated soils except in SS of Sanandaj and SS of Shiraz. Concerning K content, a decrease in K content was observed in all treated soils compared to control soil. Regarding N content in SSs (Table 1), the highest N content belonged to the SS of Rasht, but as shown in Fig. 5b, only P content in the shoot was the highest in the SS of Rasht, while P content in root was the highest in the SS of Sanandaj and SS of Tehran and, as indicated, the highest P content in the fruit belonged to the control, SS of Rasht, and SS of Shiraz (Fig. 5c). However, with the application of all SSs, as shown in Fig. 1a, b, both water-extractable P and Olsen-extractable P were increased. Therefore, it can be inferred that the nutrient content in different applied SSs may boost P uptake, but not generally, and that the improvement in P uptake by different parts of the cucumber plant may be mainly related to the P content in the applied SSs.

Lemming et al. (2017) showed that the P uptake by spring barley at SS-treated soil (50 mg P kg^{-1}) significantly increased compared to that of the control soil. Wang et al. (2016) proposed the application of SS as an alternative fertilizer. They found that SS application (40 mg P kg^{-1}) significantly increased shoot growth and N and P uptake by spring wheat. Wollmann et al. (2018) studied the effects of six P fertilizers recycled from SS on P uptake by maize. They observed that shoot P content in six fertilizers was significantly higher than unfertilized soil.

Regarding to the r values of regressions, it seems that water-extractable P (Fig. 6a, b) is a better indicator than Olsen-extractable P (Fig. 6c, d) for evaluating P availability in cucumber plants. Similar results were found by Matula (2011), which illustrates the relation between P content in barley shoots and water-extractable P best described by second-order polynomial regression ($r = 0.80$). Ayodele and Agboola (1981) reported that modified Olsen-extractable P significantly correlated to greenhouse and field experiment maize yield ($r = 0.90$ and $r = 0.70$, respectively). Over 8 years of study on Timothy grass in gravelly sandy loam soil, results showed that cumulative P budget linearly related to water-extractable P ($r = 0.92$) and Mehlich-3 ($r = 0.94$) (Messiga et al. 2014). Also, Kulhánek et al. (2007) observed that linear regression best described P uptake by barley and water-extractable P ($r = 0.90$), Mehlich-3-extractable P ($r = 0.60$), $CaCl_2$ ($r = 0.58$), and Olsen-extractable P ($r = 0.45$). The equations presented in Fig. 6 can be used to predict P uptake in soil treated with different SSs having various compositions.

It would appear that the regression equations that relate to P uptake in the shoot of cucumber with water-extractable P and Olsen-extractable P both in the rhizosphere and non-rhizosphere soils as the key soil variables were basically accurate in predicting the accumulation of P in the shoot of the cucumber plant. Olsen-extractable efficiency was different and was more than 17% (with the exception of Shiraz SS) for different SS-treated soils indicating high release of P from SS (Table 3). High Olsen-extractable P may also be clarified by the presence of soluble P types in SS (Jalali and Jalali, 2020). For some SSs, the low release of P may be due to the fact that SS contains a number of different proportions of both organic and inorganic forms of P which vary for plant availability (Kirchmann et al. 2017). The low-soluble P forms in SS such as Ca-P, Al-P, and Fe-P should be converted into readily available P, thereby delaying the supply of P for the plant.

The applications of different SSs resulted in an improvement in the available P in treated SS soils, thereby increasing BCF and BAC under different SSs. On the other hand, the highest BCF was obtained when plants of cucumber were grown in Tehran, Sanandaj, and Saveh SSs compared to those grown in other SSs (Table 3). This was due primarily to the highest rate of OEE for P in Tehran, Saveh, and to some extent in Sanandaj SSs compared with the other treated soils. In SS, availability of P to plants depends on the content of ions such as Al, Ca, Mg, and Fe that can produce an insoluble portion of P (Hedley and McLaughlin 2005). Table 1 displays the content of Fe and Ca in SSs. The results suggest a higher PUE (a greater supply of P) from the SS of Rasht, rather than from others. This SS had higher Fe (molar) and P and lower Ca among the SSs used, and it appears that there is a good relationship between Fe/Ca (molar ratio) in SSs and PUE ($r =$ 0.85), indicating that a higher PUE is related with a higher molar Fe/Ca ratio. This suggested that in the SSs studied, the role of Ca content in forming insoluble P species is more than Fe. To confirm this finding, further research is needed. It should be noted that the high PUE in all treated soils suggested that cucumber plants would uptake the released P from applied SSs. Hedley and McLaughlin (2005) concluded that waste products are less efficient in P uptake and increase first-season yields due to the lower available P relative to high-available P minerals. Our findings showed that due to high PUE values, most applied SSs with less water-soluble P may be comparable to mineral P fertilizers.

5 Conclusions

This study investigated the effect of SS application on P uptake by cucumber and its effects on rhizosphere and non-rhizosphere soils under greenhouse conditions. The findings suggested that the availability of P in both rhizosphere and non-rhizosphere soils was significantly affected by SS application, and that plant uptake of P was enhanced by SS application.

The application of various SSs to the soil not only affected soil P availability but also impacted soil solution characteristic and the proportion of HPO_4^{-2} and $H_2PO_4^{-}$ in the rhizosphere and non-rhizosphere soils, while minerals controlling the availability of P were not different in SS-treated soils in both rhizosphere and non-rhizosphere soils of cucumber plants. The results indicated that P uptake in the shoot of cucumber correlated with water-extractable P and Olsen-extractable P both in the rhizosphere and non-rhizosphere soils and may be used to predict P uptake in similar soils treated with various SSs. Due to their different composition, the P usage efficiency of SSs has varied, and most of them can be used to replace mineral P fertilization with SS.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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