Lettuce Response to Phosphorus Fertilization with Struvite Recovered from Municipal Wastewater

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Abstract. Struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) production is widely studied as a way to remove phosphorus (P) from wastewater and generate a potentially marketable P fertilizer, but its effects on crops have yet to be researched more thoroughly. This study was conducted to evaluate struvite recovered by the Spanish Research Council (CSIC) pilot process (STR) as a source of P for lettuce (*Lactuca sativa* L.) by comparing its effectiveness with that of single superphosphate (SUP), a common P fertilizer derived from phosphate rock. In a greenhouse pot experiment, a P-deficient loamy sand soil was amended with either SUP or STR at P rates of 0, 4, 8, 12, 16, and 20 $\text{mg} \cdot \text{kg}^{-1}$. Nitrogen and potassium were uniformly supplied to all treatments. The response of lettuce head fresh weight and P uptake to P rate exhibited statistically significant quadratic relationships for both SUP and STR. With respect to SUP, STR was significantly more effective in increasing lettuce yield and P uptake, probably because of the larger amount of magnesium (Mg) incorporated with this material and a synergistic effect on P uptake. This work supports previous findings based on other test crops in suggesting that STR can be a P source attractive to the fertilizer market with additional agronomic and environmental benefits such as providing available Mg and nitrogen, helping attenuate consumption of phosphate rock, and reducing release of P by discharge of treated wastewaters to surface and groundwater systems.

Phosphorus is one of the primary nutrients essential for plant growth and crop production (Mengel and Kirkby, 2004). At present, most commercial phosphorus (P) fertilizers on the market such as single superphosphate are derived from phosphate rock (Barker and Pilbeam, 2006). However, world reserves of currently exploitable phosphate rock are rather limited and increasingly dissipated (Abelson, 1999). For these reasons, alternative technologies to recover P from waste materials have received a great deal of interest in recent years (de-Bashan and Bashan, 2004; Shu et al., 2006).

Struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) is a potentially marketable product for the P fertilizer industry, which can be recovered at municipal wastewater treatment works from anaerobically digested sludge liquors (de-Bashan and Bashan, 2004; Gaterell et al., 2000). However, the production of struvite at full scale remains limited, partly because of the costs associated with chemical addition. In partic-

ular, anaerobically digested sludge liquors tend to be deficient in magnesium (Mg) (de-Bashan and Bashan, 2004; Nelson et al., 2003). Recently, scientists at the Spanish Research Council (CSIC) have developed a new, economically advantageous method for recovering struvite using a low-grade MgO-containing byproduct of the magnesite industry as a Mg source (Quintana et al., 2004, 2006, 2008). This technology is now being implemented at full scale in a municipal wastewater treatment plant in Madrid, Spain.

Several studies on struvite recovered from municipal wastewater have been focused on its production (e.g., de-Bashan and Bashan, 2004, and references therein), but its potential for use as a crop fertilizer requires additional research. In particular, positive agronomic results in terms of yields and P uptake have been reported for chickpea (*Cicer arietinum* L.) (Ghosh et al., 1996), lupin (*Lupinus albus* L.) (González-Ponce and García-López-de-Sá, 2008), and ryegrass (*Lolium perenne* L.) (González-Ponce and García-López-de-Sá, 2007; Johnston and Richards, 2003; Plaza et al., 2007). To date, however, no data are available in the literature for lettuce (*Lactuca sativa* L.), which is a leading vegetable crop in many parts of the world with a high demand for P (Kelling et al., 1998; Mou, 2008).

The objective of this work was to evaluate the potential of struvite recovered by the CSIC

pilot process (STR) as a source of P for the cultivation of lettuce under greenhouse conditions. Single superphosphate (SUP), a common P fertilizer derived from phosphate rock, which is considered to be fully plant-available (Johnston and Richards, 2003), was used as a reference for comparison.

Materials and Methods

Phosphorus sources and soil. The SUP used in this work was supplied by Fertiberia (Madrid, Spain). The STR sample was recovered by the CSIC pilot process from an anaerobically digested sludge liquor using a low-grade MgO byproduct from the calcination of natural magnesite as a Mg source. The anaerobically digested sludge liquor was collected from the municipal wastewater treatment plant located in Navalcarnero (Madrid, Spain), which is managed by Canal de Isabel II, whereas the low-grade MgO byproduct was supplied by Magnesitas Navarras, S.A. (Navarra, Spain). The mean composition of the SUP ($\pm$ SE of three replicates) was: total P content, $79.0 \pm 0.7 \text{ g} \cdot \text{kg}^{-1}$; total potassium (K) content, $1.41 \pm 0.07 \text{ g} \cdot \text{kg}^{-1}$; total calcium (Ca) content, $170.9 \pm 0.2 \text{ g} \cdot \text{kg}^{-1}$; total Mg content, $2.0 \pm 0.1 \text{ g} \cdot \text{kg}^{-1}$; and total iron (Fe) content, $864 \pm 105 \text{ mg} \cdot \text{kg}^{-1}$. The composition of the STR sample was: total nitrogen (N), $44.4 \pm 1.1 \text{ g} \cdot \text{kg}^{-1}$; total P content, $103.9 \pm 0.3 \text{ g} \cdot \text{kg}^{-1}$; total K content, $0.94 \pm 0.02 \text{ g} \cdot \text{kg}^{-1}$; total Ca content, $12.0 \pm 0.3 \text{ g} \cdot \text{kg}^{-1}$; total Mg content, $131.0 \pm 0.1 \text{ g} \cdot \text{kg}^{-1}$; and total Fe content, $1806 \pm 122 \text{ mg} \cdot \text{kg}^{-1}$. The principal chemical properties of the P sources were determined by conventional methods (Faithfull, 2002).

A nutrient-poor soil was sampled from the plow layer (Ap horizon, 0 to 20 cm depth) of a Typic Haploxeralf (Soil Survey Staff, 2006) in the experimental farm “La Higuera” located in Santa Olalla (Toledo, Spain). This soil is representative of the Spanish Mediterranean region, having a loamy sand texture (sand, $770 \pm 2 \text{ g} \cdot \text{kg}^{-1}$; silt, $190 \pm 2 \text{ g} \cdot \text{kg}^{-1}$; clay, $40 \pm 3 \text{ g} \cdot \text{kg}^{-1}$); pH measured in water suspension (soil-to-water ratio of 1:2.5), 5.9; electrical conductivity measured on a saturated paste extract, $2.01 \pm 0.09 \text{ dS} \cdot \text{m}^{-1}$; total organic carbon content, $2.5 \pm 0.1 \text{ g} \cdot \text{kg}^{-1}$; total N, $0.30 \pm 0.01 \text{ g} \cdot \text{kg}^{-1}$; available P, $19.6 \pm 0.7 \text{ mg} \cdot \text{kg}^{-1}$; available K, $150 \pm 2 \text{ mg} \cdot \text{kg}^{-1}$; available Ca, $204 \pm 3 \text{ mg} \cdot \text{kg}^{-1}$; available Mg, $5.9 \pm 2 \text{ mg} \cdot \text{kg}^{-1}$; and total Fe content, $4408 \pm 256 \text{ mg} \cdot \text{kg}^{-1}$. The properties of the soil were determined by conventional methods (Dane and Topp, 2002; Sparks, 1996) in a triplicate analysis.

Greenhouse pot experiment. The greenhouse pot experiment was set up with the following treatments applied in a randomized block design with three replicates: SUP at rates of 51, 101, 152, 203, and 253 $\text{mg} \cdot \text{kg}^{-1}$ (designated SUP4, SUP8, SUP12, SUP16, and SUP20, respectively) and STR at rates of 38, 77, 115, 154, and 192 $\text{mg} \cdot \text{kg}^{-1}$ (designated STR4, STR8, STR12, STR16, and STR20, respectively) equivalent to 4, 8, 12, 16, and 20 $\text{mg} \cdot \text{kg}^{-1}$ P. All treatments were

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Table 1. Analysis of variance for effects of time after phosphorus (P) fertilization, P source, P rate (P rate nested within P source), and block on available P, potassium (K), calcium (Ca), and magnesium (Mg) contents in soil.^z

Source of variation	df	Available P		Available K		Available Ca		Available Mg	
		MS	F-ratio	MS	F-ratio	MS	F-ratio	MS	F-ratio
Time	2	1,041.8	75.34***	65,016.9	176.58***	62,772.6	18.41**	3,323.3	37.35**
P source	1	148.6	7.78	1,074.7	3.16	8008.9	1.62	4,396.0	63.82*
P rate (P source)	4	133.9	6.08***	648.0	1.60	5291.4	2.30	153.9	3.24*
Block	2	0.5	0.02	294.3	0.67	64.6	0.01	52.9	0.48
P source × block	2	19.1	0.87	340.3	0.84	4,958.6	2.15	68.9	1.45
P rate × block	8	38.1	1.73	540.4	1.34	4,421.9	1.92	75.4	1.58
Time × block	4	13.8	0.63	368.2	0.91	3,409.2	1.48	89.0	1.87

^zdf = degrees of freedom; MS = mean square; *, **, and *** = significant at the 0.05, 0.01, and 0.001 level, respectively.

made 4 d before planting by thoroughly mixing either SUP or STR with 10 kg of air-dried, 2-mm sieved soil in plastic pots with no drainage hole. A control treatment that received no P was included (P0). To isolate P as the only growth-limiting nutrient, the amounts of N and K required to provide all treatments with 55 mg·kg⁻¹ N and 45 mg·kg⁻¹ K were supplied by variable additions of reagent-grade ammonium and potassium sulfates taking into account the N and K provided by the P sources.

Romaine-type lettuce (*Lactuca sativa* L. cv. Aitana) seedlings were germinated and grown in a 1:1 peat:sand mixture (v:v) under growth chamber conditions (24 °C for 15 d in a 16/8-h light/dark photoperiod). At the four-leaf stage, the lettuce seedlings were planted one per pot in mid-March. The pots were placed in a greenhouse with natural light and irrigated daily with deionized water. Growth temperatures ranged from 12 to 35 °C.

Soil samples were collected 1, 2, and 3 months after fertilization (mid-April, mid-May, and mid-June), coinciding with three distinct stages of lettuce development: rosette, early heading, and head maturity. After air-drying, crushing, and passing through a 2-mm sieve, each soil sample was analyzed for available P content by continuous-flow colorimetry after extraction with a calcium magnesium carbonate buffer (Burriel and Hernando, 1950) and for available K, Ca, and Mg contents by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after extraction with ammonium acetate (Sparks, 1996). Soon after the head marketable maturity was reached, the entire aerial lettuce biomass was collected from each pot, weighed quickly for yield determinations, dried at 60 °C in a forced-draft oven, ground to pass a 0.5-mm sieve, and then analyzed for total P, N, K, Ca, and Mg contents by ICP-AES after digestion in a nitric acid and perchloric acid mixture (Faithfull, 2002).

Data analysis. Data from dependent measures (i.e., soil available nutrient contents, lettuce head fresh weight, and nutrient uptake) were subjected to either three- or four-way analyses of variance. Time after fertilization, P source, and P rate nested within P source were treated as fixed independent variables and blocks as a random effect. Furthermore, the effects of time within the same P source and rate, P source within the same time and P rate, and P rate within the same time and P source on soil-available

nutrient contents were analyzed with repeated one-way analysis of variance. When variances were equal according to Levene statistics, the Bonferroni test at the 0.05 level

was performed for separation of means. Similarly, one-way analysis of variance followed by the Bonferroni test was performed to explore the effects of P source within the

Fig. 1. Available nutrient contents in soils amended with either single superphosphate (SUP) or struvite (STR) at phosphorus (P) rates of 0 (P0), 4, 8, 12, 16, and 20 mg·kg⁻¹ after 1, 2, and 3 months from fertilization. Error bars indicate SEs. Within the same P source and rate, different lowercase italic letters indicate statistically significant differences according to the Bonferroni test at the 0.05 level. Within the same time and P rate, different uppercase letters indicate statistically significant differences according to the Bonferroni test at the 0.05 level. Within the same time and P source, different lowercase letters indicate statistically significant differences according to the Bonferroni test at the 0.05 level.

Table 2. Analysis of variance for effects of phosphorus (P) source, P rate (P rate nested within P source), and block on lettuce head fresh weight and nutrient uptake.²

Source of variation	df	Head fresh wt		P uptake		N uptake		K uptake		Ca uptake		Mg uptake	
		MS	F-ratio	MS	F-ratio	MS	F-ratio	MS	F-ratio	MS	F-ratio	MS	F-ratio
P source	1	9,100.2	179.21**	496.87	121.87**	16,351.1	1.54	559.0	0.07	2,008.4	2.17	2,290.6	35.31*
P rate (P source)	4	227.0	0.42	37.51	1.15	4,188.4	0.76	8637.2	0.35	500.0	1.50	210.5	3.59
Block	2	865.3	1.36	17.43	1.12	4,231.7	0.35	10,239.1	0.71	282.4	0.26	24.5	0.30
P source × block	2	50.7	0.09	4.08	0.13	10,620.6	1.92	8,153.5	0.33	927.4	2.78	64.9	1.11
P rate × block	8	1,055.1	1.94	31.12	0.95	10,513.6	1.90	23,390.8	0.95	895.7	2.69	84.4	1.44

²df = degrees of freedom; MS = mean square; * and ** = significant at the 0.05 and 0.01 level, respectively.

same rate and P rate within the same source on lettuce head fresh weight and nutrient uptake.

The response of lettuce head fresh weight and P uptake to P rate was evaluated using three models (quadratic, exponential, and square root):

$$Y_i = a_i + b_iX + c_iX^2 \quad (1)$$

$$Y_i = a_i(1 - e^{-b(x+c)}) \quad (2)$$

$$Y_i = a_i + b_iX + c_iX^{1/2} \quad (3)$$

where Y_i is head fresh weight in grams or P uptake in mg/head obtained with source i ; X is the rate of P applied in $\text{mg}\cdot\text{kg}^{-1}$; and a_i , b_i , and c_i are constants obtained by fitting the models to the data for source i (Bélanger et al., 2000). By using a dummy variable, which took the value of 1 for the P source being considered and 0 for other source, a combined regression analysis resulted in a single value of coefficient of determination (R^2) and root mean square of error (RMSE) for the two regression equations (one for each P source) (Prochnow et al., 2004). The one representing the highest R^2 value and lowest RMSE was chosen to calculate the predicted maximum lettuce head fresh weight and P uptake and the corresponding optimum rates of fertilization. All statistical analyses were performed using SPSS 15 for Windows (Statistical Product and Service Solutions Inc., Chicago, IL).

Results and Discussion

Soil-available nutrient contents. The results of the analysis of main effects reveal that soil-available nutrient contents, especially P and K, are significantly influenced by the time after fertilization (Table 1). Furthermore, available P and, to a lesser extent, available Mg are significantly affected by the P rate applied, whereas only available Mg content is significantly affected by the P source (Table 1). The effects of block and the interactions between P source and block, P rate and block, and time and block are not significant for any parameter examined (Table 1).

Soil-available nutrient contents as affected by the time after fertilization, P source, and P rate are shown in Figure 1. In general, available P, K, Ca, and Mg contents decrease significantly with increasing time

after fertilization as a result of their absorption and use by lettuce plants. With very few exceptions, within the same time and rate of P applied, K and Ca available contents of soils fertilized with both SUP and STR are similar to each other. In contrast, with respect to SUP-fertilized soil, STR treatments exhibit slightly smaller available P content and larger available Mg content, especially at high rates of addition, which may be directly related to

the greater solubility of SUP (Bhuiyan et al., 2007; Johnston and Richards, 2003) and the larger amount of Mg incorporated with STR. With increasing the rates of SUP and STR, available K and Ca contents remain almost constant, whereas available P tends to increase significantly, especially in the case of SUP, and available Mg content increases significantly only with increasing the rates of STR after 1 and 2 months from its

Fig. 2. Lettuce head fresh weight and nutrient uptake as affected by phosphorus (P) sources (SUP, superphosphate; and STR, struvite) and rates of P applied. Error bars indicate SES. Within the same P rate, different lowercase letters indicate statistically significant differences according to the Bonferroni test at the 0.05 level. Within the same P source, different uppercase letters indicate statistically significant differences according to the Bonferroni test at the 0.05 level.

Table 3. Regression analysis for the quadratic, exponential, and square root models describing the relation between head fresh weight (HFW) or phosphorus (P) uptake by lettuce and rate of P applied.^z

	Quadratic		Exponential		Square root	
	HFW	P uptake	HFW	P uptake	HFW	P uptake
a _{SUP}	102.07	15.49	219.69	62.48	100.00	14.98
b _{SUP}	15.61	4.25	0.24	0.12	-4.07	0.04
c _{SUP}	-0.54	-0.12	3.70	2.55	42.77	9.22
a _{STR}	107.05	15.07	246.04	66.01	100.33	14.58
b _{STR}	21.27	5.56	0.25	3.62	-7.57	-0.07
c _{STR}	-0.76	-0.16	2.22	1.16	65.58	12.15
Observations	36	36	36	36	36	36
RMSE	20.26	4.36	21.43	6.79	22.26	5.11
R ²	0.864	0.940	0.848	0.853	0.836	0.917
MS	88,900	10,024	87,246	9,100	86,014	9,782
F-ratio	216.60***	528.50***	190.05***	197.22***	173.67***	374.67***

^za_{SUP}, b_{SUP}, and c_{SUP} and a_{STR}, b_{STR}, and c_{STR} = model coefficients for single superphosphate and struvite, respectively; RMSE = root mean square of error; R² = coefficient of determination. MS = mean square; *** = significant at the 0.001 level.

Fig. 3. Experimentally determined values (dots) and quadratic model derived regressions (lines) of head fresh weight (A) and phosphorus (P) uptake (B) by lettuce as a function of sources (SUP, superphosphate; and STR) and rates of P applied.

application. The effect of increasing the amount of STR on soil-available P content is not directly proportional to P rate as in the case of SUP, which may be attributed to its lower solubility.

Lettuce head fresh weight and nutrient uptake. The results of the analysis of main effects show that head fresh weight, P uptake, and, to a lesser extent, Mg uptake are significantly affected by the P source, whereas P rate induces no significant response on lettuce (Table 2). The effects of block and the interactions between P source and block and P rate and block are not significant either for lettuce head fresh weight or for nutrient uptake (Table 2).

Lettuce head fresh weight and nutrient uptake as affected by sources and rates of P applied are shown in Figure 2. With few exceptions, head fresh weight and nutrient uptake by lettuce tend to increase significantly with increasing P levels in the form of both SUP and STR, reaching maxima at P rates of 12 or 16 mg·kg⁻¹. Several studies have also reported yield responses to P fertilization of lettuce grown on diverse soils and under different environmental conditions (Mota et al., 2003; Nagata et al., 1992; Sanchez and El-Hout, 1995; Sanchez et al., 1990; Soundy et al., 2001). In general, within the same P rate applied, STR yields similar or slightly larger head fresh weight and P, N, K, and Ca uptake by lettuce as compared with SUP. Furthermore, at rates higher than 4 mg·kg⁻¹, STR enhances Mg uptake to a significantly greater extent than does SUP.

Especially noteworthy is the larger yield and P uptake by lettuce achieved with STR despite the similar amount and greater solubility of P added with SUP. These larger effects may be mostly attributed to the larger amount of Mg incorporated with STR and a synergistic effect on P uptake. Magnesium is part of the chlorophyll molecule, thereby essential for photosynthesis, helps activate many plant enzymes needed for growth, and plays a part in P transport within the plant (Mengel and Kirkby, 2004). Thus, yield increases as a result of Mg application have been recorded in a number of crops and soils (Choudhury and Khani, 2001; Hao and Papadopoulos, 2004; Lamarre and Payette, 1991; Vrataric et al., 2006). Furthermore, evidence in the literature suggests that increasing Mg level may result in increased absorption of P by plants and vice versa (Alloush et al., 2000; Li et al., 2004; Reinbott and Blevins, 1991, 1997, 1999; Skinner Saleque et al., 2001; and Matthews, 1990).

Response models, predicted maximum yield and phosphorus uptake, and optimum phosphorus rates. The best-fit parameters generated from the nonlinear regression of lettuce head fresh weight and P uptake as a function of P rate using the quadratic, exponential, and square root models are shown in Table 3. The three tested models explain a large proportion of the variability and fit well to the data reported in this study as indicated

by the large R² values and the small RMSEs (P < 0.001). Despite the little differences, the regression analysis indicates that the quadratic model best describes the experimental data sets for both head fresh weight and P uptake (Table 3; Fig. 3).

Previous studies have reported a significant quadratic response of lettuce yield and P uptake to common P fertilizers (Mota et al., 2003; Nagata et al., 1992; Sanchez and El-Hout, 1995; Soundy et al., 2001). The results obtained are also in general agreement with previous works indicating that the quadratic model is reliable in describing crop responses to fertilizers and, therefore, appropriate for predicting maximum yields and optimum rates (Bélanger et al., 2000). Such information is critical to maximize economic gains and minimize environmental risks of nutrient losses through runoff and leaching processes.

Predicted maximum lettuce head fresh weight and P uptake and the corresponding optimum rates of P for SUP and STR calculated by equating the first derivative of the quadratic response equation to 0 and solving for P rate are shown in Figure 4. With respect to SUP, STR results in significantly larger maximum lettuce yield and P uptake. However, maximum lettuce yield occurs at P rates of 14.7 ± 1.0 mg·kg⁻¹ for SUP and 14.0 ± 0.7 mg·kg⁻¹ for STR and maximum P uptake at 18.6 ± 1.2 mg·kg⁻¹ and 18.4 ± 1.8 mg·kg⁻¹, respectively. These results clearly indicate that STR is more efficient than SUP in increasing yield and supplying P to lettuce.

Conclusions

The results obtained indicate that STR recovered by the CSIC pilot process from anaerobically digested sludge liquors using a low-grade MgO byproduct from the calcination of natural magnesite is more effective than SUP in increasing yield and P uptake by lettuce. This better agronomic performance may be attributed to the larger amount of Mg incorporated with STR and its synergistic effect on P uptake. In conclusion, the present results, together with data reported previously based on other test crops (Ghosh et al., 1996; González-Ponce and García-López-de-Sá, 2007, 2008; Johnston and Richards, 2003;

Fig. 4. Predicted maximum lettuce head fresh weight (A) and phosphorus (P) uptake (B) and corresponding predicted optimum rates of P in the form of superphosphate (SUP) and struvite (STR). Error bars indicate SEs. Different lowercase letters indicate statistically significant differences in predicted maximum head fresh weight or P uptake according to the Bonferroni test at the 0.05 level. Different uppercase letters indicate statistically significant differences in predicted optimum P rate according to the Bonferroni test at the 0.05 level.

Plaza et al., 2007), demonstrate the potential value of STR as a marketable P fertilizer, especially for crops and soils having high P and Mg requirements. Struvite is comparable to other P fertilizers or even better as a P source, provides available Mg and N, and has additional environmental benefits such as helping attenuate consumption of phosphate rock and reducing release of P by discharge of treated wastewaters to surface and ground-water resources.

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