Phosphorus availability in recycled fertilizers: comparison of 11 chemical extraction methods with plant uptake during a 7-month growth experiment

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Abstract

Purpose

With the emerging production of recycled phosphorus (P) fertilizers, adequate measurements of their P availability are needed. Chemical extractions are commonly used to evaluate the P availability of commercial fertilizers. However, their appropriateness for the measurement of dynamic P availability from recycled P fertilizers is still not well understood.

Methods

Thus, this study examined the P availability of struvites (STRSL and STLQ), ashes (ASH1 and ASH2), and dry sludge iron phosphate (FeP) recycled from wastewater using eleven different chemical P extractions. Furthermore, perennial ryegrass was treated with these recycled P fertilizers during a seven-month pot trial to compare the shoots P uptake to the chemically extracted P.

Results

High P concentrations, comparable among the methods, were extracted from the STRLQ, STRSL and ASH1. In contrast, P concentrations extracted from less purified ASH2 and FeP were highly variable depending on the methods. The plant P uptake efficiency at the end of the pot trial indicated the same order among the products: STRSL(42%)≥STRLQ(36%)>ASH1(25%)>FeP(20%)>ASH2(14%). A correlation between the shoots P uptake and chemically extracted P by all the analyzed methods, except for Olsen's P, became the highest (r>0.8) only after four months of plant growth when the slow released P from the recycled fertilizers became available to plants.

Conclusions

The type of P recycling technology and wastewater used determined the P availability pattern of examined fertilizers. The P concentration in recycled P fertilizers extracted using ten chemical methods corresponded with a P uptake by plants grown for a minimum of four months.

Keywords: Chemical P extraction, Phosphorus availability, Struvite, Sewage sludge ash, Iron phosphate, Fertilizer Regulation

List of abbreviations and symbols

AAC	Alkaline ammonium citrate
AL AA	Ammonium lactate acetic acid buffer
AR	*Aqua regia*
ASH1	Chemically treated ash
ASH2	Chemically untreated ash
BR 2	Bray 2
CA	2% citric acid
CA CL	0.01M calcium chloride
CP_{uptake}	Cumulative shoots P uptake
FA	2 % formic acid
FeP	Dry sewage sludge iron phosphate
FR	Fertilizer Regulation
ICP-OES	inductively coupled plasma optical emission spectrometry
MA	Mineral acids
MEH 3	Mehlich 3
MW	Microwave digestion
NA	Nitric acid
NAC	Neutral ammonium citrate
OLS	Olsen's
PUE	Phosphorus uptake efficiency
r	Pearson’s correlation coefficient
SSA	Sewage sludge ash
STR	Struvites
STR LQ	Struvite from liquor
STR LQ	Struvite from sludge
TSP	Triple superphosphate
WAT	Water

1 Introduction

The current European Fertilizer Regulation (FR) (EC 2003; EC 2019), next to the national legislation (in each EU Member State), recommends the use of chemical extractions for the assessment of phosphorus (P) availability in fertilizers. This is because chemical extractions are quick, easily reproducible, and economic methods. According to the FR 2003/2003 (EC 2003), mineral acids (MA), neutral ammonium citrate (NAC), and water (WAT) chemical P extraction methods are defined as suitable for the majority of fertilizer types, while 2 % citric acid (CA) was defined as only applicable to basic slag, 2 % formic acid (FA) for soft ground rock phosphate and alkaline ammonium citrate (AAC) for dihydrated dicalcium phosphate. However, the new FR 1009/2019, which came into force recently, in July 2022, suggests using only two chemical P extraction methods, NAC and WAT, for the majority of fertilizer types. Still, the details regarding the chemical P extractions and fertilizing types are not yet fully specified in FR 1009/2019. In some countries, the soil P extraction method such as Bray 2 (BR 2), Mehlich 3 (MEH 3), Olsen's (OLS), and ammonium lactate acetic acid (AL AA) are used also as fertilizer P extraction methods (Kratz et al. 2019; Vogel et al. 2017; Wuenscher et al. 2015). Additionally, extraction using 0.01M calcium chloride (CA CL) is a method often used for the assessment of P availability of various types of materials (fertilizers, soils, plants, etc.) (Six et al. 2013).

These conventional methods, originally defined for commercial P fertilizers, have also been used in previous studies to determine the P concentration in recycled P fertilizers (Huygens et al. 2019; Kratz et al. 2019). However, those studies are scarce, performed on a limited number of chemical extraction methods and their findings are inconclusive. A few studies (Kratz et al. 2010; Krüger and Adam 2017) attested that MA is an optimal method for total P extraction of recycled P fertilizers. On the other hand, European Working Group (WG) STRUBIAS (Huygens et al. 2019), as well as Kratz et al. (2010), Duboc et al. (2017), and Krüger and Adam (2017) have recommended *aqua regia* (AR) as a standard P extraction methods for recycled P fertilizers. Furthermore, as it is a common practice to conduct digestion with nitric acid (MW NA) involving only one acid in comparison to AR (Christel et al. 2014; Delin 2016), this method has also been listed in the

STRUBIAS report (Huygens et al. 2019). In addition, for practical and economic reasons the main goal is to use fewer extractions to define the P availability of individual recycled P fertilizer categories. However, it was also noticed that the chemically extracted P concentration from recycled P fertilizers may differ within each fertilizer category depending on the technology used for P recovery (Huygens et al. 2019). Thus, the standardization of chemical methods for recycled P fertilizer appears to be complex and more investigation is needed to understand the patterns that impact the differences among the recycled P fertilizers.

Pot trials are typically performed to evaluate the fraction of P in fertilizer that is available to plants. Nevertheless, recycled P fertilizers can act as slow-release fertilizers, making it difficult to measure the P release at different plant growth stages (Duboc et al. 2017; Huygens and Saveyn 2018; Kratz et al. 2010; Vogel et al. 2017). Previous investigations using recycled P fertilizers in most cases did not show a good correlation between chemically extracted P using WAT, CA, FA, and NAC standard methods and the P taken by the plant (Duboc et al. 2017; Huygens et al. 2019; Ylivainio et al. 2021). Only a few studies in which CA (Brod et al. (2016); Meyer et al. (2018)), NAC (Kratz et al. 2010), and WAT (Lemming et al. 2017) were used, showed a good correlation. Moreover, the sequential fractionation conducted by Meyer et al. (2018) indicated a high correlation between the P extracted from recycled fertilizers using anion exchange resins and sodium bicarbonate ($NaHCO_3$), and the relative fertilizer efficiency. Nevertheless, as Kratz et al. (2019) emphasized, there is a discrepancy between a P solubility determined using chemical extraction methods at one point in time and changing equilibrium in plant growth trials. As STRUBIAS group (Huygens et al. 2019) pointed out there is still a lack of scientific publications that correlate plant P-availability with the fertilizer P-solubility for recycled P fertilizers.

Therefore, in this study, the P availability of two struvites (STR), two sewage sludge ashes (SSA), and one iron-phosphate (FeP) dry sludge were scrutinized using eleven chemical P extractions on fertilizers, of which two methods are commonly used for biosolids, five are commonly used methods for soils and four are official EU methods for fertilizers. Moreover, perennial ryegrass was grown on river sand supplied with the examined P fertilizers for seven months. The monthly measurements of shoots P uptake were compared to the chemically extracted P from recycled P fertilizers to create a proposal for standardized measurement of available P in slow-release recycled P fertilizers.

2 Materials and methods

2.1 Phosphorus fertilizers

The phosphorus availability of the following recycled P fertilizers was assessed: two STR (STRLQ and STRSL), two SSA (ASH1 and ASH2), and one FeP. The STRLQ was recycled from the liquid phase of sewage sludge and the STRSLfrom the solid phase of sewage sludge. The ASH1 was produced by thermal treatment of sewage sludge at 900–1000 ◦C and chemical treatment with sodium sulfate into the calcined ash with $CaNaPO_4$ phase. The ASH2 was produced from combined sewage and industrial sludge using pyrolysis at 650–750 ◦C and incineration at temperatures of 900 and 1100 ◦C without the addition of additives at this stage of technology development. The FeP was produced in the municipal wastewater treatment plant where the Fe-coagulants were used for P removal. Triple superphosphate (TSP), a well-studied commercial mineral (from P rock) phosphorus fertilizer, was used as a benchmark material (Bogdan et al. 2021). The complete chemical characterization of the examined fertilizers was previously described in Bogdan et al. (2021), and a reprint (with permission of Elsevier) is provided in the Supplementary material (Table S1).

2.2 Chemical extractions of P from fertilizers

A detailed list of chemical P extraction methods tested on each fertilizer is given in Table 1. Eleven chemical extractions were selected to assess the P concentration in recycled P fertilizers: two frequently named "total or pseudo-total" P extraction methods for biosolids, closed microwave (MW) AR and NA destruction methods, previously described in Bogdan et al. (2021), with a difference in addition of 10 mL extraction solution of AR (1 HNO_3, 65 % : 3 HCl, 37 %, v/v) or NA (HNO_3, 65 %) respectively; five soil/substrate P methods, AL AA (VLM 2010), MEH 3 (Pierzynski et al. 2005), BR 2, OLS, and CA CL (Van Ranst 1999); and four fertilizer P methods, W, MA, CA and NAC (EC 2003). Each extraction was performed in four replicates, except for CA CL method which was done in two replicates for FeP and ASH2 and three for STR and ASH1.

For most methods, protocols were followed according to the references, but with a reduction in sample weight (as presented in Table 1), while internal protocols were used for the two methods, MW AR and MW NA.

After P extraction, using BR 2, MEH 3, and OLS soil P methods, P concentration was determined using the colorimetric method of Scheel (Van Ranst, 1999) on Jenway 6400 spectrophotometer (Barloworld Scientific T/As Jenway, Felsted, UK) at a wavelength of 700 nm, as recommended in the protocols (Pierzynski et al. 2005; Van Ranst 1999). For the other eight P extraction methods, in addition to the colorimetric method, the determination of P was also done using inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista MPX CCD Simultaneous ICP-OES) at a wavelength of 213.618 nm. Detection limits of ICP-OES and spectrophotometer for P were 10 and 100 ppb, respectively.

2.3 Pot trial design

A pot trial, described in detail in Bogdan et al. (2021), was designed to measure the long-term shoots P uptake and compare it with the chemically extracted P from the fertilizers. Each pot was filled with 3 kg of river sand in four replicates. River sand substrate was supplied with the fertilizers at a P dose of 35 mg P pot^{-1}, and 46.9 mg of seeds, corresponding also to 3.5 g m^{-2} or 35 kg seeds ha^{-1} (Teagasc 2014), of novel variety (OAKPARK) perennial ryegrass (*Lolium perenne*) from Ireland. A modified Hoagland nutrient solution was added continuously in pots to fulfill plant demand for nutrients other than P through seven months of plant growth. The cutting of the perennial ryegrass was conducted every four weeks, except for the 1st cut which was done after five weeks.

2.4 Shoots P uptake and P uptake efficiency (PUE)

Shoots P uptake (mg P pot^{-1}) of each fertilizer at each plant cut and cumulative shoots P uptake (CP_{uptake}) after seven cuts were calculated based on previously reported (Bogdan et al. 2021) shoots dry matter (g pot^{-1}) and shoots P concentration (mg P g^{-1}) :

$$\text{Shoots P uptake (mg P pot}^{-1}\text{)} = \text{Shoots P concentration} * \text{Shoots dry matter} \quad (1)$$

$$CP_{uptake}\ \text{(mg P pot}^{-1}\text{)} = \sum_{1}^{i} \text{Shoots P uptake}_{i} \quad (2)$$

where, i: cut (1-7).

The P uptake efficiency (PUE, %) of the fertilizers at the end of the seven month pot trial was assessed as CP_{uptake} (mg P pot^{-1}) divided by the amount of P of fertilizer applied to the pots at the start of the experiment ($P_{applied}$ = 35 mg P pot^{-1}):

$$\text{PUE, \%} = \frac{CP_{uptake}}{P_{applied}} * 100 \quad (3)$$

2.5 Data analysis

Data were subjected to statistical analysis using the statistical program Minitab 16 software.

Statistical differences between the P concentration of six fertilizers (TSP, STRLQ, STRSL, ASH1, ASH2, and FeP) and within each of eleven chemical P extraction methods (MA, MW AR, MW NA, CA, AL AA, NAC, WAT, CA CL, MEH 3, BR 2 and OLS) were calculated according to the General linear model (two-way ANOVA) and Tukey test ($p < 0.05$). However, as the differences between the P concentrations extracted with various methods were large (strong acids undermined values and differences among fertilizers obtained with weak extractants) additional one-way ANOVA was conducted per each chemical extraction method.

The General linear model (two-way ANOVA) was used to investigate the effect of time (7 cuts) on shoots P uptake for all recycled fertilizers, while one-way ANOVA and Tukey test ($p < 0.05$) were used to compare the CP_{uptake} and the PUE at the end of seven month pot trial among the examined fertilizers. A post-hoc Tukey test at the 0.05 significance level was run to indicate the significant differences between the shoots P uptake, the CP_{uptake}, and the PUE among the fertilizer types, as well as cuts.

Standard statistical correlations (Pearson's *r*) were used to compare the results between:

i shoots P uptake at each plant cut and P concentrations in each chemical extraction method for all recycled P fertilizers combined to check which chemical P extraction method can be used for all recycled P fertilizers combined to determine the P that is available to plant at each plant cut;

ii and the CP_{uptake} and the fertilizer PUE at the end of the seven month pot trial with the chemically extracted P from all recycled P fertilizers combined for each method to check which chemical P extraction method can be used for all recycled P fertilizers combined to determine the P that is available to plant at the end of the pot trial (one growing season of the perennial ryegrass).

3 Results

3.1 Fertilizer P solubility according to chemical extraction methods

Overall, significantly higher ($p<0.05$) P concentration values for all recycled P fertilizers (Table 2) were obtained using strong acidic reagents (MA > MW AR ≥ CA ≥ MW NA > NAC > AL AA) than using weak extracting reagents (MEH 3 > BR 2 ≥ OLS ≥ WAT = CA CL) (Supplementary material, Table S2_A). The two STR, STRLQ and STRSL, had the highest P concentrations extracted ($p<0.05$) using all the chemical P extraction methods except the OLS method, which extracted the higher P concentrations from the ASH2. The second highest P concentrations were extracted from the ASH1, which was significantly higher ($p<0.05$) than the ASH2 and the FeP (Supplementary material, Table S2_B).

Moreover, the interaction among the fertilizer types and their P concentrations with the various chemical extraction methods was also significant ($p < 0.05$) (Supplementary material, Table S2_C). While the P concentrations in the STR remained comparable between all chemical extraction methods, the P concentrations from the two SSA significantly differed ($p < 0.05$), showing ASH1 a significantly higher P concentration than ASH2 when using the strong reagents MW NA, NAC, and AL AA. For both ash products, the P concentrations were equal using MA and MW AR extraction methods and comparable using the AL AA extraction method. The P concentrations of the two STR and ASH1 were comparable only when using the weaker soil extractions OLS and MEH 3, while the P concentrations of ASH2 and FeP were comparable using the MEH 3, but also AL AA, and NAC extractions. Moreover, the P concentrations using the BR 2, OLS, WAT, and CA CL soil extractions indicated no significant differences among all recycled fertilizers.

A comparison of the P concentrations in recycled fertilizers per each chemical extraction method can be found in the Supplementary material (Table S3).

The reference material, TSP, had equal P concentration with all ten extracts except in the OLS, which was lower (Supplementary material, Table S4). The relative fertilizer P concentration, a mean of comparing the concentration of P extracted by ten chemical extraction methods in relation to P concentration extracted by the strong " pseudo-total" MA method (%P-MA), was provided in Supplementary material (Fig.S5).

In addition, analyses of two instrumental methods for the determination of P, the ICP-OES and Scheel method, showed no significant differences ($p > 0.05$; average CV < 15 %) as confirmed by the Bland-Altman plot (Supplementary material, Fig.S6) and high *r* of 0.977 ($p < 0.001$).

3.2 Shoots P uptake over the course of seven cuts

The differences among the fertilizers in shoots P uptake measured at every cut are given in Table 3. All fertilizers had the lowest shoots P uptake at the first plant cut, with the highest P uptake was detected in the STRSL and the lowest in the ASH2 treatment. From the second cut onwards the plants had an equal shoots P uptake from the two STR. The shoots P uptake in the FeP and ASH1 treatments was comparable at the second cut, lower than in the treatments with two STR, but higher than in the ASH2 treatment. However, at the third cut, the shoots P uptake in the ASH1 treatment became comparable to treatments with two STR. At the fourth cut, the shoots P uptake was comparable among all recycled fertilizers except the ASH2 which was still significantly lower.

Furthermore, at the fifth and sixth cut, the shoots P uptake in FeP and ASH2 treatment became equal, as a sudden drop in the shoots P uptake occurred in the FeP and an increase in the ASH2 treatment. At the final harvest, the shoots P uptake in ASH1 was also comparable to the ones in the FeP and ASH2 treatment (statistical data can be found in Supplementary material, Table S7).

Overall, the shoots P uptake over the seven cuts differed among the recycled P fertilizers. For the treatments with FeP, ASH1, and STRSL the shoots P uptake was stable throughout the time. Contrary, the shoots P uptake in STRLQ treatment exhibited a slight reduction till the fourth cut, upon which it became stable. Moreover, the ASH2 was the only recycled P fertilizer that delivered significantly low ($p < 0.05$) P to the shoots during the first four cuts. However, its P release rapidly increased afterward.

There were no significant ($p > 0.05$) differences in shoots P uptake between the two STR, STRLQ and STRSL. A significantly ($p < 0.05$) lower shoots P uptake was achieved in the treatment with ASH1 compared to the two STR, but significantly ($p < 0.05$) higher than in the FeP treatment. The lowest ($p < 0.05$) shoots P uptake among the recycled P fertilized treatments was measured in the ASH2 treatment, but still significantly ($p < 0.05$) higher than in the unfertilized control that died after the three cuts. In summary, the $C_{Puptake}$, a sum of the seven monthly shoots P uptakes, indicated the following order among the fertilizers: STRSL = STRLQ > ASH1 ≥ FeP > ASH2 > Zero P (Table 3).

In addition, the positive control TSP had the highest ($p < 0.05$) shoot P uptake during four cuts. However, its shoot P uptake exhibited a continuous reduction with time until the fourth cut upon it got exhausted. On the contrary, the negative control Zero P had continuously the lowest ($p < 0.05$) shoot P uptake among all the treatments. Moreover, as the P from the substrate and seed got exhausted plant stopped the growth and no further measurements of shoot P uptake were possible in the Zero P treatment.

3.3 Phosphorus uptake efficiency (PUE) at the end of seven month pot trial

Phosphorus uptake efficiency (PUE), is often used to express a fertilizer P availability, calculated by the amount of P taken up by the plant divided by the amount of P supplied. Fig. 1 shows the differences in PUE (eq. 3) among the fertilizer at the end of seven month pot trial. In line with the shoots P uptake, both STR achieved the highest PUE (avg. 42% for the STRSL and 36% for the STRLQ). The PUE achieved in the ASH1 treatment (avg. 25%) was significantly lower ($p < 0.05$) than in the treatments with two STR, but slightly higher than the PUE in the treatment with FeP (avg. 20%) and significantly ($p < 0.05$) higher than the PUE in the ASH2 treatment (avg. 14%). In addition, the PUE of the positive control treatment TSP (avg. 37 %) was comparable to the ones of two STR.

3.4 Correlation between the chemically extracted P and the shoots P uptake during seven month trial

The correlation (*r*) between the shoots P uptake (mg P pot^{-1}) at each of seven plant cuts and P concentrations (mg P g^{-1}) in each of eleven chemical P extracts for all recycled P fertilizers combined is presented in Table 4 (and graphically in Supplementary material, Fig.S8_A, and Fig.S8_B). A low *r* was calculated between the fertilizer P concentrations extracted by the OLS method and the shoots P uptake at all cuts for all recycled P fertilizers combined. At the first plant cut, the highest *r* was achieved between the shoots P uptake and the fertilizers P concentrations in WAT extracts of all recycled P fertilizers combined. At the second plant cut, *r* was high between the shoots P uptake and almost all chemical extracts, except for the P extracted via the MA and OLS methods. At the third cut, *r* were similar to the ones at the second cut and slightly higher for the MW NA, NAC, MEH 3, and WAT extracts. At the fourth plant cut, the *r* with the shoots P uptake was still high in MW NA and WAT, but slightly lower for almost all other extracted P. Moreover, at this point, the *r* was no more significant in MW AR and MA extracts (besides OLS). Eventually, the highest *r* between the shoots P uptake and the chemically extracted P of all recycled P fertilizers combined using all extraction methods, except the OLS were observed at the last three cuts (fifth, sixth, and seventh) (Table 4). More precisely, the chemically extracted P of all recycled P fertilizers combined using seven extraction methods (except the MW AR, MA, and OLS) became comparable to the plant P uptake from the recycled P fertilizers (STR>ASH>FeP) from the fifth cut onwards.

In contrast, when the fast release fertilizer, TSP, was included in the correlation together with the recycled P fertilizers, the P concentrations measured in TSP were largely interfering with the correlation of all other recycled P fertilizers. The *r* between the shoots P uptake and the chemically extracted P concentrations from all fertilizers (recycled and TSP) exhibited a continuous reduction with time during four cuts, and no correlation from the fifth cut onwards as the P from TSP became exhausted from the pots . In a search for a common chemical P extraction method for all recycled fertilizers, the TSP proved to be an outlier. Thus, the correlation combining recycled fertilizers and TSP was excluded from the main findings and placed in the supplementary material (Table S9).

3.5 Correlation of chemically extracted P with shoots P uptake at the end of the seven month trial

The *r* between the CP_{uptake} (Fig.S10 in Supplementary material), as well as the fertilizer PUE at the end of the seven month pot trial (Fig.2), and the fertilizer P concentrations in eleven different extracts for all combined recycled P fertilizers, showed to be between 0.7 and 0.9 for all the extraction methods, except OLS (no correlation). The *r* was slightly lower (0.7) between the PUE and the P extracts in MW AR and MA than the *r* (of more than 0.8) between the PUE and the other eight P extracts. In contrast, no correlation was observed between the PUE and the fertilizer P concentrations in the BR 2, OLS, WAT, and CA CL extracts when the fast release fertilizer, TSP, was included in the correlation together with the recycled P fertilizers (Supplementary material, Table S11).

4 Discussion

The assessment of shoot P uptake during the seven month pot trial confirmed the slow P release from the recycled P fertilizers, in compliance with previous findings (Bogdan et al. 2021; Christiansen et al. 2020; Lemming et al. 2017; Nanzer et al. 2014; Romer and Steingrobe 2018; Thompson 2013). Consequently, the selection of optimal chemical P extraction method(s) for measuring available P concentration of the recycled P fertilizers proved to be significantly ($p<0.05$) dependent on the shoots P uptake at a specific period of plant growth (i.e. plant cut).

According to the present study, the P extracted in WAT corresponded to the “immediately” available P fraction and can be used to explain the percentage of P that is readily available from the slow release recycled P fertilizers during the first four months of ryegrass growth. The same was previously observed in the studies of Kratz et al. (2010) and Lemming et al. (2017). The P demand of perennial ryegrass (≥ 0.2% P) (Crush et al. 1989) was satisfied even by the low amounts of water-soluble P from the recycled P fertilizers while the large amounts of P delivered by water-soluble fertilizers such as TSP proved to be excessive.

Furthermore, since the shoots P uptake did not correlate as high for the two strongest chemical P extraction methods, MA and MW AR, as for the other methods, they should not be used for defining the plant P availability of recycled fertilizers when the plants are grown for less than seven months. The higher P concentrations observed for all tested recycled P fertilizers in the MA (the pseudo-total standard method for P fertilizers) and MW AR (the pseudo-total extraction method for biosolids) extracts are in agreement with the previous studies (Duboc et al. 2017; Huygens et al. 2019; Kratz et al. 2010; Krüger and Adam 2017). They are a consequence of these reagents' ability to dissolve P originating from apatite and Al- and Fe-bound P (Kuo 1996), as well as to break cation bridges and organic phosphate esters salts (Turner et al. 2005). Thus, MA and MW AR should be regarded as “pseudo-total” P extraction methods for all examined recycled P fertilizers. Moreover, as AR can be conducted with various modifications, and variations are even offered within a standard such as DIN EN 13346:2000, a more detailed specification should be considered, especially knowing that the hot plate (boiling under reflux) AR method can lead to significant losses of elements. Fast-drying, losses, and variations in results were observed in the pre-study using the AR method of Van Ranst (1999) similar to EN 13650 (Andersen and Kisser 2003) considered to become a standard in the new FR, and therefore we immediately switched to the AR digestion in closed MW. Possibly, further optimization of the hot plate AR method in terms of time and temperature could resolve some of the issues. Nevertheless, if the reaction occurs in the microwave oven, especially closed MW, the losses and the discrepancies in results are significantly reduced (Duboc et al. 2017). A variety in temperature, pressure, and, more frequently, time in the MW procedures is also a matter of necessary convention. While the EPA 3051a (EPA 3051a, SW846 methods, revision 2007) method is defined for 175 ± 5 °C, rise for 5.5 ± 0.25 min, hold for 4.5 min or the remainder of the 10-min digestion period, the protocol in the present study was conducted at a temperature of 180 °C and significantly longer holding time of 20 minutes, and a ramp time of 20-25 minutes to allow for the extraction of all elements and avoid the use of more potent and harmful chemicals such as hydrofluoric acid (HF). In terms of MA protocol, the official EU procedure (EC 2003) involves boiling in a Kjeldahl flask under reflux, allowing errors caused by the evaporation and overheating of the sensible novel P fertilizers. Therefore, we suggest that the method be optimized and further tested in closed MW.

Furthermore, the other seven chemical extraction methods (except OLS) proved to be suitable for measuring the P concentrations of recycled fertilizers that are available to plants from the fifth plant cut onwards. Still, their P solubility appeared to be somewhat influenced by the chemical composition of the recycled fertilizers. An equally high P solubility of both STR in the extracts of the four strong acid methods, MA, CA (standard fertilizer methods) and MW NA, MW AR (pseudo-total methods for biosolids), could be explained as a consequence of their high purity, characterizing them as a clean fertilizer that has a low interference of P with other elements present in these products (Huygens et al. 2019). The P concentrations of two STR obtained using the standardized NAC method followed in this study were slightly lower than the ones using the four other strong acid methods, and also lower than the concentration measured with the combined protocol of NAC with WAT used in other studies (Kratz et al. 2010; P-REX project 2012-2015). However, as the water addition next to NAC extracts only a small additional amount of P, the differences between the two protocols are not significant ($p > 0.05$), and the standardized protocol for NAC used in this study is considered a proper method. Although the NAC method from FR 2003/2003 is the most often recommended method for assessing available P in recycled P fertilizers (Huygens et al. 2019; Kratz et al. 2010), many deviations have been reported when the method was repeated on the same samples (Krüger and Adam 2017; Vogel et al. 2017). The P solubility of recycled P fertilizers in NAC was also found sensitive to changes in time and temperature used during this extraction (Krüger and Adam 2017). From the perspective of the present study, potential errors in preparing a neutral ammonium hydroxide (28 to 30 % of NH_3) can be avoided using the standardized ammonia solution of 28-30%. Alike for the STR, the comparable P concentrations using MW AR, MW NA, CA, as well as NAC for the ASH1, could be explained by the good quality of the product obtained using chemical additives during the thermal treatment of sewage sludge for its production, which reduced the concentration of P bonding elements (Herzel et al. 2016; Krüger and Adam 2017). In contrast, as the production of ASH2 did not involve the use of chemical additives in the thermal treatment of sewage sludge, the complexity of the matrix in ASH2 remained high. As a result, less P than in ASH1 was extracted from the ASH2 using the two strong acids extractions CA and MW NA. Therefore, although MW NA was considered a standard method for P extraction in recent studies (Christel et al. 2014; Delin 2016), it was shown inefficient in breaking the P bonds for these products (i.e. ASH2), and thus, it shouldn't be standardized for all the recovered P fertilizers or at least not for SSA. The P concentration extracted from ASH2 using the NAC method was very low but comparable to what was previously reported for similar German SSA (Krüger and Adam 2017). Conclusively, the higher sensitivity of the ASH2 to the chemical reagent used for P extraction can be explained by its interaction with the high organic carbon ($C_{org.}$ ≥ 7.5 %) and high iron concentrations (Fe:P >1; ~ 2.5 in MWAR) present in this product (Bogdan et al. 2021; Greb et al. 2016; Ylivainio et al. 2021). The presence of high C concentration in ASH2 was a consequence of using combined industrial and municipal sewage sludge for P recovery, and would not be expected if the typical municipal sewage sludge was used alone. In addition, the lower ratio of Fe:P concentration (~ 1.1) in the FeP (compared to the ASH2 of ~ 2.5) led to the higher P concentration of FeP in the extract of NAC than in CA, and comparable to the P concentration in MA extracts. This is in accordance with the previous observations for sewage sludge (Steckenmesser et al. 2017). Eventually, the ratio of P measurement in MW NA, CA, and NAC extracts of SSA to P concentration in MW AR or MA (P-MWNA, P-CA, P-NAC: P-MW AR, P-MA < 1) extracts could be used as an indicator for the strongly bonded iron phosphates (Steckenmesser et al. 2017). It appears that the type of sewage sludge is an essential parameter in defining the optimal procedure for recycling P from sewage sludge in the form of SSA, besides the type of thermal and chemical process used for their production.

Regarding the chemical extraction methods typically used for soils, the organic acids used in the AL AA extraction proved to be less efficient in mining P from products with complex matrixes (ASH1, ASH2, and FeP), compared to the previously discussed strong inorganic acid methods. Moreover, the preparation of the final AL AA extraction solution, consisting of three solutions, was very demanding in terms of precision and should be performed with high delicacy. Even minor errors in solution preparation mainly by the amount of acid-pH used were able to highly affect the results for the ASH1, ASH2, and FeP. A similar sensitivity of recycled P fertilizers to pH change in the AL AA extraction solution was observed in previous studies (Brod et al. 2016; P-REX project 2012-2015). Among the other tested soil extractions, the highest P concentration was extracted with the MEH 3 for most of the recycled P fertilizers, in agreement with reports by Jones (2001) and Dari et al. (2019). These results were expected knowing that the MEH 3 method is based on a complex extraction solution that can also dissolve several elements and release more P (Jones 2001; Wuenscher et al. 2015). However, for the FeP and ASH2, the use of hydrochloric acid (HCl) in the BR 2 extraction solution solubilized higher P concentrations than did the MEH 3 extraction. This is in agreement with the results from the sequential fractionation conducted by Meyer et al. (2018), indicating that SSA are highly soluble in HCl. This could be considered an "overestimation" of P concentration and has already limited the applicability of BR 2 for several commercial mineral P fertilizers (Zapata and Roy 2004). Finally, low P concentrations of the recycled P fertilizers in the extracts of CA CL were probably caused by the presence of Ca in the extracting solution of CA CL which led to P precipitations in form of calcium phosphates (Blombäck et al. 2021; Pierzynski et al. 2005). This interaction between the CA CL solution and the complex matrix of recycled P fertilizers has further resulted in a slightly lower correlation of the shoots P uptake with the P concentrations extracted using CA CL compared to one extracted using the WAT method.

Eventually, the comparison of the two strongest extraction methods, MW AR or MA, and other tested extraction methods, can be used to indicate the speed of the P release from the recycled P fertilizers. The ratio of P concentration between two extraction methods as a tool for assessing the P availability of the recycled fertilizers was also proposed by the STRUBIAS group (Huygens et al. 2019), more precisely labeling the ratio of water-extractable and acid-extractable P concentration (e.g. NAC, FA, CA) to the total P concentration. Considering that NAC and AR are already listed in Fertilizer Regulation 1009/2009 (EC 2019) the present study extends the recommendation by specifying that if the P concentrations measured in MW AR and NAC extracts of a recycled P product are equal then the recycled P fertilizer will be readily available to the plant already during the first four months of ryegrass growth. Similarly, if the fertilizer P concentration in NAC extract is significantly lower than in MWAR extract, the P availability of recycled P fertilizer to plant during the first four months of ryegrass growth will be restrained.

The present study showed no correlation between the shoots P uptake (and PUE) and P concentration extracted from recycled P fertilizers only when using the OLS method, similar to the work of Tandy et al. (2011) on soils. In contrast, the good correlation of plant P with the OLS-P in several other studies (Brod et al. 2016; Duboc et al. 2017) was a result of using different types of fertilizers. In those studies, the selected sewage sludge and recycled P fertilizers did not have high iron and carbon concentration as the ASH2 and FeP in the present study. The differences in P concentration among the examined recycled fertilizers using the OLS method were a consequence of the chemical composition of examined fertilizers and the capability of the OLS method to decrease the solution concentrations of soluble Ca^{2+} by precipitation of $CaCO_3$, as well as to reduce the concentration of soluble Al^{3+} and Fe^{+3} by forming Al and Fe oxyhydroxides. At high pHs, such as in the OLS method, many organic functional groups are ionized, and the increased charge density leads to increased solubility. Moreover, the stability of organic molecules also decreases as Na^{+} replaces polyvalent cations (Oades 1988; Soinne 2009; Swift 1996). Thus, for ASH2, containing high C, Ca, Fe, and Al concentrations, the OLS chemical P extraction overestimated P concentration (Jones 2001; Pierzynski et al. 2005). A similar, but less pronounced increase in P concentration was observed in the case of FeP, which is also a C- and Fe-rich fertilizer. It is most likely that the fertilizer interaction with the specific soil type used in the previous studies has led to a good correlation for P extracted by OLS. Additionally, many previous studies compared P concentration extracted from the soils by various chemical P methods (not extracted directly from the fertilizer as in this study) to the one in a plant. For example, Duboc et al. (2017) found a high correlation for the OLS P, but low for the CAL P. However, these tests may be suitable only if the tested P extraction method corresponds to the composition of the used soil. Nevertheless, since almost no P was detected in the present study after substrate extraction using a strong AR method on the sand (Bogdan et al. 2021), detecting any P using other weaker chemical extraction methods is doubtful, unless the amount of P fertilizer added to the pot is not increased above plant demand for P. Eventually, since the OLS method’s applicability strongly relies on differences in P chemistry, the method should neither be applied to all types of fertilizers nor all types of soils (Pierzynski et al. 2005). Besides, particular attention should be given to the variation in protocols for the OLS method. In this study "degassing" was done as the last step using sulfuric acid (not defined in the original method), to ease the determination, especially at ICP-OES.

Overall, the number of strong acid methods used (in fertilizer regulation) can be reduced to one method if the products are purified enough during production. The extractions typically used for commercial mineral fast release P fertilizers cannot be directly extended to the slow release recycled P products unless they have high purity and/or high P/other elements ratio. In the future, “slow-release chemical extraction tests” that involve the addition of extracting agents e.g. water, and collection of extracted samples at different points in time can be tested on recycled P fertilizers. These longer and more costly methods are currently in use for assessing controlled slow release fertilizers (Cancellier et al. 2018; EC 2002). **Moreover,** to provide a more holistic recommendation towards the use of chemical extractions for measuring P availability of recycled P fertilizers additional studies should be conducted with a larger number of recycled P fertilizers and under various environmental conditions (e.g. different soils, plant species). For example, according to a recent study of Bogdan et al. (2022), the ranking between the shoots P uptake of one P salt (same fertilizer category as STR) and one SSA (modification of ASH2) was the same on the alkali substrate as in the slightly acidic sand used in the present study. Thus, the findings of the present study regarding the chemical P extraction methods may remain valid for the application of these recycled P fertilizers on various substrates.

Regarding the methods used for the P determination, the spectrophotometric molybdenum-blue and optical emission spectroscopy with inductively coupled plasma (ICP-OES) are in common practice (Kratz et al. 2010; Krüger and Adam 2017; Vogel et al. 2017). Still, the Fertilizer Regulation 2003/2003 suggests a gravimetric method using quinoline phosphomolybdate as a standard method for P determination (EC 2003). The cost-effectiveness of the gravimetric method provides its obvious advantage, whereas disadvantages such as difficulty in purchasing the crucibles in pore size defined in Fertilizer Regulation 2003/2003 (EC 2003) and uncommon on the market, relevantly high risk of P losses during boiling and drying, lower sample throughput and higher labor compared to ICP-OES (but comparable to a spectrophotometric molybdenum-blue method), out-compete it. In regards to the spectrophotometric molybdenum-blue protocol, we suggest modification of the original method (Van Ranst 1999), as more applicable due to the long liveness (months) of its extraction solution compared to the one single day in the original method. Similar, more stable solutions, are most likely used for the "rapid P test" provided by MACHEREY-NAGEL GmbH & Co or Hanna Instruments. More important is that the colorimetric methods may interfere with some solutions or sample matrixes which limits their applicability in comparison to ICP-OES. In consequence, at least more procedures for P determination should be added to legislation if not fully replacing the old ones.

5 Conclusions

Eleven chemical P extraction methods were applied to measure the P concentration of different types of recycled P products and compare them with plant shoots P uptake. The type of fertilizer product played a determining role. In the case of struvites, a comparable P concentration was measured using the different strong acid chemical extraction methods. For the thermally treated sewage sludges, the P concentrations in sewage sludge ashes were strongly dependent on the technology used for P recovery. The ash produced using chemical additives in the thermal treatment of sludge led to better P extractability by several chemical methods compared to the other ash for which production additives were not applied. In contrast, the P concentrations extracted from the chemically untreated ash and the dry sludge iron phosphate varied strongly among the chemical extraction method used as their P was strongly bonded to high carbon and iron concentrations.

The shoots P uptake and its correlation with the P concentrations extracted using ten chemical methods were highly affected by the time and the interaction of time with the fertilizer type. During the first four months of plant growth, the strongest correlations with the shoots P uptake were measured for the water extraction method. Only after four plant cuts the correlation become high between the shoots P uptake and the P concentrations extracted using all methods, except the Olsen's. While Olsen's method is often recommended for the assessment of P in fertilizers, high limitations were observed in the present study. The comparison of the pseudo-total extraction methods, microwave digestion *aqua regia* or mineral acids, with the neutral ammonium citrate or other methods, can be used to indicate if P in recycled P fertilizers will be more or less readily available to plant during the first four months of ryegrass growth. In particular, the chemically untreated sewage sludge ashes that expressed delay in delivering P during four months of plant growth are prone to having a lower P concentration in neutral ammonium citrate, than the chemically treated sewage sludge ashes, and also lower than their P extracted in pseudo-total microwave digestion *aqua regia* extraction.

Overall, the P availability of recycled P fertilizers extracted using chemical methods should be associated with the longer plant growth experiments (> four months) due to their slow P release.

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Statements and Declarations

Competing Interests

The authors declare that they have no conflict of interest concerning the current research publication.

Author Contributions

Conceptualization: [Aleksandra Bogdan, Evi Michels, Erik Meers]; Methodology: [Aleksandra Bogdan, Erik Meers]; Software [Aleksandra Bogdan]; Validation: [Aleksandra Bogdan]; Formal analysis: [Aleksandra Bogdan, Olivier Nys]; Investigation, [Aleksandra Bogdan, Olivier Nys]; Resources: [Erik Meers, Evi Michels]; Data curation: [Aleksandra Bogdan, Olivier Nys]; Writing—original draft preparation: [Aleksandra Bogdan, Ana Alejandra Robles Aguilar, Evi Michels, Erik Meers]; Methodology: [Aleksandra Bogdan, Erik Meers]; Writing—review and editing: [Aleksandra Bogdan, Ana Alejandra Robles Aguilar, Erik Meers]; Visualization: [Aleksandra Bogdan]; Supervision, A.A.R.-A., and E.M.: [Ana Alejandra Robles Aguilar, Erik Meers]; Project administration: [Evi Michels, Erik Meers]; Funding acquisition: [Evi Michels, Erik Meers].

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Table 1 List of tested chemical P extraction methods

Extraction method	Extraction solution	Solid : Liquid ratio, g : mL
MA[b]	H_2SO_4 (1.84 g mL^{-1}), HNO_3 (1.40 g mL^{-1})	0.5 : (6 : 4)
MWAR[a]	HNO_3 (1.42 g mL^{-1}), HCl (1.18 g mL^{-1})	0.15 : (2.5 : 7.5)
MWNA[a]	HNO_3 (1.42 g mL^{-1})	0.15 : 10
NAC[b]	$C_6H_8O_7 \cdot H_2O$ (1.09 g mL^{-1}), NH_3 (28-29 %), pH 7.0	0.5 : 50
CA[b]	$C_6H_8O_7 \cdot H_2O$ (2 %)	0.5 : 50
AL AA[c]	$C_3H_6O_3$ (3 mol L^{1}), CH_3COOH (16 mol L^{-1}), NH_3 (13 mol L^{-1}), pH 3.75	0.5 : 10
MEH 3[e]	CH_3COOH (0.2 N), NH_4NO_3 (0.25 N), NH_4F (0.015 N), HNO_3 (0.13 N), EDTA (0.001 M)	0.5 : 10
BR 2[d]	NH_4F (1 N), HCl (0.5 N)	2.5 : 17.5
OLS[d]	$NaHCO_3$ (0.5 M), pH 8.5	0.5 : 10
WAT[b]	H_2O	0.5 : 50
CA CL[f]	$CaCl_2 \cdot 2H_2O$ (0.01 M)	5 : 25

MA-mineral acids, MW AR-microwave digestion *aqua regia*, MW NA-microwave digestion nitric acid, NAC-neutral ammonium citrate, CA-2% citric acid, AL AA-ammonium lactate acetic acid buffer, MEH 3-Mehlich 3, BR 2-Bray 2, OLS-Olsen's, WAT-water, CA CL-0.01M calcium chloride.

[a] Bogdan et al. (2021), [b] EC (2003), [c] VLM (2010), [d] Van Ranst (1999); [e] Pierzynski et al. (2005).

Table 2 Fertilizers P concentration (mg P g^{-1}) measured after eleven chemical extraction methods (mean ± stdv, n = 4 except for CA CL where n = 2 for FeP and ASH2 and n=3 for STR and ASH1).

Extraction method	FeP	STRLQ	STRSL	ASH1	ASH2
MA	22 ± 0	132 ± 8	140 ± 16	85 ± 8	78 ± 2
MW AR	18 ± 0	119 ± 17	114 ± 6	64 ± 2	66 ± 1
MW NA	19 ± 0	121 ± 3	121 ± 7	60 ± 2	26 ± 1
CA	16 ± 0	122 ± 2	128 ± 3	60 ± 4	46 ± 2
NAC	23 ± 1	87 ± 5	100 ± 9	67 ± 1	32 ± 2
AL AA	2.2 ± 0.1	95 ± 17	102 ± 18	25 ± 4	10 ± 1
MEH 3	0.59 ± 0.06	26 ± 1	27 ± 0	14 ± 1	1.6 ± 0.2
BR 2	1.1 ± 0.1	12 ± 0	13 ± 0	6.8 ± 0.9	5.0 ± 0.2
OLS	0.88 ± 0.11	3.3 ± 0.3	3.3 ± 0.8	2.9 ± 0.4	4.5 ± 0.2
WAT	0.11 ± 0.02	2.9 ± 0.1	4.7 ± 0.7	1.9 ± 0.0	0.26 ± 0.03
CA CL	0.044 ± 0.006	0.62 ± 0.02	1.1 ± 0.0	0.41 ± 0.03	0.019 ± 0.004

MA-mineral acids, MW AR-microwave digestion *aqua regia*, MW NA-microwave digestion nitric acid, NAC-neutral ammonium citrate, CA-2% citric acid, AL AA-ammonium lactate acetic acid buffer, MEH 3-Mehlich 3, BR 2-Bray 2, OLS-Olsen's, WAT-water, CA CL-0.01M calcium chloride.

FeP-iron phosphate dry sludge, STRLQ-struvite from liquor, STRSL-struvite from sludge, ASH1, and ASH2-sewage sludge ashes.

Table 3 Shoots P uptake (mg P pot^{-1}) during seven months of plant growth (mean ± stdv, n = 4)

Fertilizer type	Shoots P uptake, mg P pot^{-1}							
	1st Cut	2nd Cut	3rd Cut	4th Cut	5th Cut	6th Cut	7th Cut	CP_{uptake}
FeP	0.89 ± 0.18	1.3 ± 0.3	0.85 ± 0.09	1.2 ± 0.3	0.77 ± 0.10	0.95 ± 0.13	1.1 ± 0.1	7.0 ± 0.7
STRLQ	0.93 ± 0.10	2.2 ± 0.2	1.8 ± 0.6	1.3 ± 0.5	2.1 ± 0.5	1.9 ± 0.2	2.4 ± 0.2	13 ± 2
STRSL	1.8 ± 0.4	2.1 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	2.1 ± 0.3	2.2 ± 0.6	15 ± 2
ASH1	1.0 ± 0.4	1.1 ± 0.1	1.3 ± 0.4	1.1 ± 0.3	1.4 ± 0.2	1.8 ± 0.2	1.3 ± 0.3	8.8 ± 1
ASH2	0.19 ± 0.02	0.26 ± 0.06	0.50 ± 0.11	0.54 ± 0.13	1.1 ± 0.3	1.2 ± 0.1	1.2 ± 0.2	4.9 ± 0.4
TSP	1.9 ± 0.5	3.0 ± 0.3	3.0 ± 0.6	1.9 ± 0.3	1.2 ± 0.1	1.3 ± 0.4	0.85 ± 0.23	13 ± 1
Zero P	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	n.a.	n.a.	n.a.	n.a.	0.10 ± 0.03

FeP-iron phosphate dry sludge; STRLQ-struvite from liquor; STRSL-struvite from sludge; ASH1 and ASH2- sewage sludge ashes, TSP-triple superphosphate; Zero P-unfertilzed negative control; n.a.- sample weight was insufficient for the measurement of the P uptake (plant exhausted P from the substrate and seed and died after 3rd cut in Zero P treatment); CP_{uptake}-cumulative shoots P uptake

Statistical differences between the shoots P uptake of fertilizers in time calculated according to GLM ANOVA and post-hoc Tukey test at a significance level of 0.05 are provided in Supplementary material (Table S4)

Table 4 Correlation (Pearson, *r*) concerning shoots P uptake (mg P pot^{-1}) of recycled P fertilizer during the seven-month trial with chemically extracted P (mg P g^{-1}) from recycled P fertilizer

Extraction method, mg P g^{-1}	Shoots P uptake, mg P pot^{-1}						
	1st Cut	2nd Cut	3rd Cut	4th Cut	5th Cut	6th Cut	7th Cut
MA	0.457^{*}	0.589^{*}	0.691^{*}	0.381	0.797^{**}	0.846^{**}	0.819^{**}
MW AR	0.384	0.588^{*}	0.619^{*}	0.350	0.772^{**}	0.746^{**}	0.792^{**}
MW NA	0.620^{*}	0.841^{**}	0.835^{**}	0.865^{**}	0.844^{**}	0.548^{*}	0.871^{**}
CA	0.553^{*}	0.745^{*}	0.793^{**}	0.521^{*}	0.850^{**}	0.829^{**}	0.872^{**}
NAC	0.687^{*}	0.774^{**}	0.845^{**}	0.563^{*}	0.812^{**}	0.900^{**}	0.794^{**}
AL AA	0.610^{*}	0.781^{**}	0.771^{**}	0.534^{*}	0.760^{**}	0.749^{**}	0.865^{**}
MEH 3	0.653^{*}	0.835^{**}	0.835^{**}	0.581^{*}	0.832^{**}	0.864^{**}	0.828^{**}
BR 2	0.501^{*}	0.695^{*}	0.775^{**}	0.486^{*}	0.848^{**}	0.841^{**}	0.829^{**}
OLS	- 0.282	- 0.201	0.008	- 0.381	0.345	0.301	0.289
WAT	0.744^{**}	0.802^{**}	0.819^{**}	0.721^{**}	0.720^{**}	0.872^{**}	0.777^{**}
CA CL	0.730^{*}	0.785^{*}	0.780^{*}	0.684^{*}	0.699^{*}	0.795^{**}	0.691^{*}

* $p < 0.05$; ** $p < 0.001$

WAT-water, MA-mineral acids, CA-2% citric acid, NAC-neutral ammonium citrate, MW NA-microwave digestion nitric acid, MW AR-microwave digestion *aqua regia*, CA CL-0.01M calcium chloride, AL AA-ammonium lactate acetic acid buffer, BR 2-Bray 2, OLS-Olsen's, MEH 3-Mehlich 3

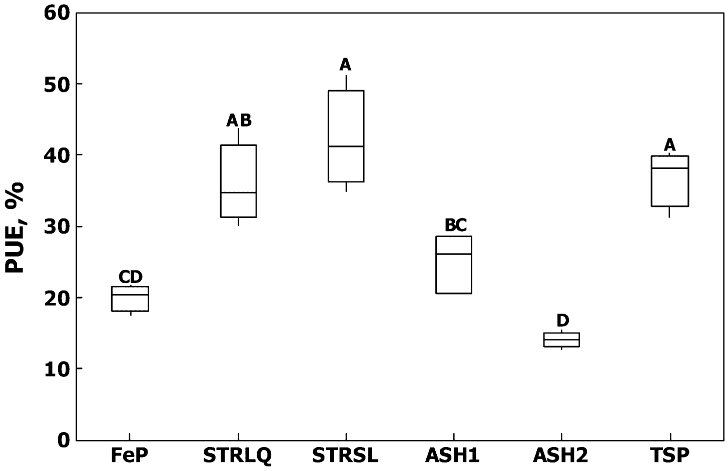

Fig. 1 Phosphorus uptake efficiency (PUE, %) of P fertilizers after seven months of plant growth in pots treated with fertilizers (STRLQ-struvite from liquor; STRSL-struvite from sludge; ASH1 and ASH2- sewage sludge ashes; FeP-iron phosphate dry sludge; TSP-triple superphosphate); The letters indicate statistical differences between the PUE of fertilizers according to 1-way ANOVA and post-hoc Tukey test at a significance level of 0.05

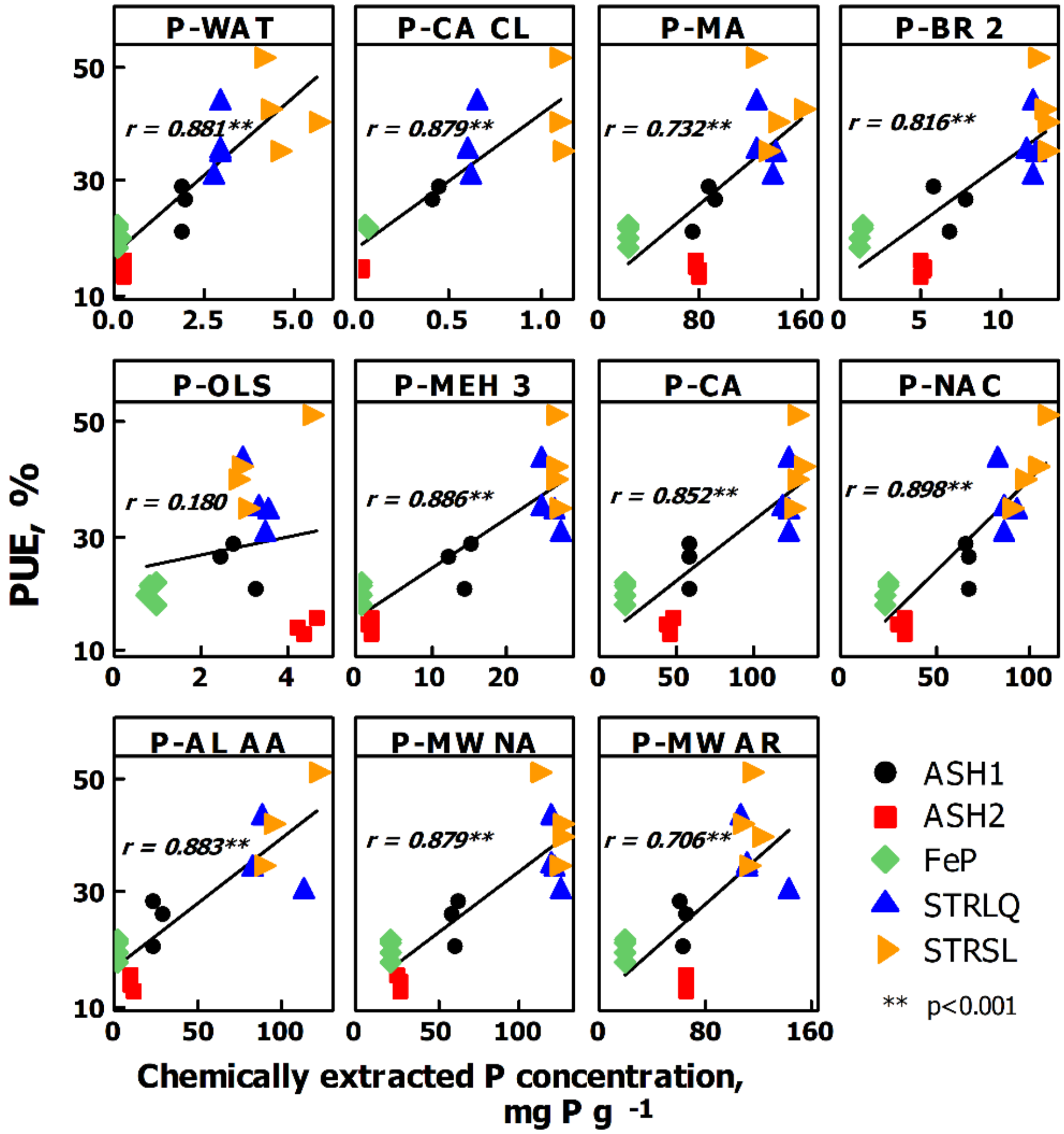

Fig. 2 Correlation (Pearson, *r*) concerning PUE (%) of recycled P fertilizer at the end of a seven-month trial with chemically extracted P concentration (mg P g^{-1}) from recycled P fertilizer. WAT-water, MA-mineral acids, CA-2% citric acid, NAC-neutral ammonium citrate, MW NA-microwave digestion nitric acid, MW AR-microwave digestion *aqua regia*, CA CL-0.01M calcium chloride, AL AA-ammonium lactate acetic acid buffer, BR 2-Bray 2, OLS-Olsen's, MEH 3-Mehlich 3. n = 4 for all chemical methods except for CA CL method where n = 2 for FeP and ASH2 and n=3 for STR and ASH1. *r* was given only if it was significant