Supplementary information

Uptake of pharmaceuticals by sorbent-amended struvite fertilisers recovered from human urine and their bioaccumulation in tomato fruit

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Supplementary information

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1 The physiochemical properties of pharmaceuticals, zeolite and biochar

Table S1. Physiochemical properties of pharmaceuticals selected for urine spiking

Micro-pollutant	RMM g/mol	pK_a	LogK_{ow}	Toxicity LD50: oral-rat mg/Kg	Medicinal function	References
Carbamazepine	236.3	13.9	2.45	1.957	epilepsy	Zheng et al., 2014
Propranolol HCl	295.8	9.24	3.48	466	blood pressure	Escher et al., 2006
Diclofenac sodium	318.1	3.99	4.51	53	anti-inflammatory	Escher et al., 2006
Sulfamethoxazole	253.3	5.7	0.89	6.200	anti-biotic	Zheng et al., 2014
Ibuprofen	206.3	4.91	3.97	636	pain relief	Zheng et al., 2014

Table S2. Physiochemical properties of zeolite and biochar

Property	Zeolite	Biochar
Type	Clinoptilolite	Wheat husk
pH	6.8 – 7.2	8.78 – 8.97
Surface area (m^2/g)	333-500	69.1
Porosity (%)	24 – 32	Not available
Water absorption (%)	34-36	40.78
NH_4^+ CEC (mol/Kg)	1.2 - 1.5	Not available
Chemical composition	70.0% SiO_2	63.2% C
	13.0% Al_2O_3	21.1% H
	1.5% Na_2O	1.5% N
	0.1% P_2O_5	1.8% P
	3.0% K_2O	1.5% K
	0.9% MgO	0.3% Mg
	7.0% CaO	7.0% Ca
	0.2% TiO_2	0.8% Al
	1.3% Fe_2O_3	0.1% Fe
	3.0% other	2.8% other

2 Determining the volume of $MgCl_2$ (32%) solution for struvite precipitation experiments

The desired volume of the $MgCl_2$ solution added to urine in order to precipitate struvite was based on the molar P concentration of the influent urine, and calculated using Equation S1.

Equation S1:

$$V_{\mathrm{MgCl_2}} = R_{\mathrm{Mg:P}} \cdot \frac{C_{\mathrm{P}}}{C_{\mathrm{Mg}}} \cdot \frac{V_{\mathrm{urine}} - V_{\mathrm{m\text{-}p}}}{1 + R_{\mathrm{Mg:P}} \cdot \frac{C_{\mathrm{P}}}{C_{\mathrm{Mg}}}}$$

Where:

$V_{\mathrm{MgCl_2}}$ = $MgCl_{2(aq)}$ solution volume

V_{urine} = total liquid volume

$V_{\text{m-p}}$ = micro-pollutant volume
C_{P} = P molar concentration
C_{Mg} = Mg molar concentration
$R_{\text{Mg:P}}$ = molar ratio Mg:P

3 Monitoring of pH and temperature during nutrient recovery

The variation in pH and temperature over the course of nutrient recovery were monitored in order to ensure similar conditions for each batch, and are shown in Figure S1 of the Supplementary Information. On average, the temperature was observed to vary between 9°C and 14°C, and pH was shown to remain fairly steady with the range of pH ~9.4 to 9.8. The variation in temperature was not large enough to affect the solubility of struvite significantly, which remains very low even at the highest temperature (16.3°C) in the pH range observed (Hanhoun et al., 2011). Furthermore, the steady pH indicated that the NH_4^+ concentration did not decrease significantly during nutrient recovery and demonstrated the high buffer capacity of urine, which was not influenced by the increase in temperature.

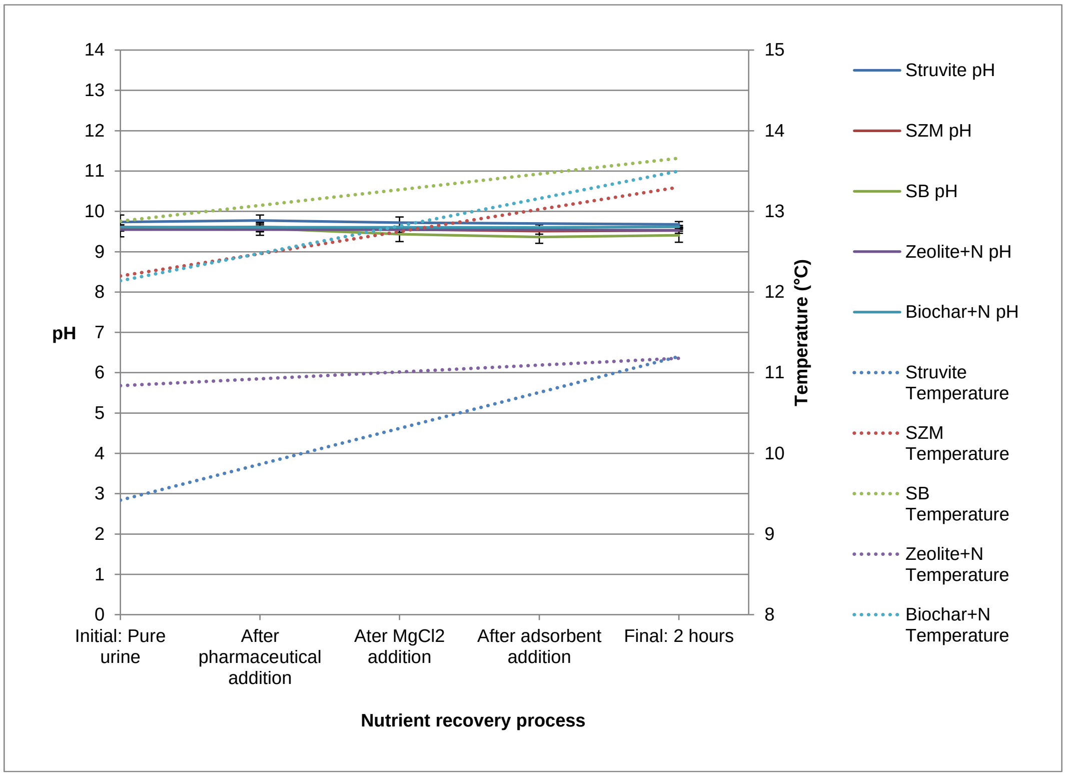

Figure S1. pH (solid line) and temperature (dotted line) monitoring of struvite crystallisation and N adsorption by zeolite and biochar during nutrient recovery experiments.

The addition of $MgCl_2$ (pH 6.5 aqueous solution) caused a slight decrease in pH in all struvite precipitation experiments, with the largest drop observed in the SB experiments. The ratio of Mg:P used was higher in the SB experiments because it was expected that biochar would adsorb some of the Mg^{2+} ions, reducing the availability for struvite precipitation. However, the difference in pH between the initial and final measurement, after struvite precipitation, was observed to be larger for SB (0.14) compared to Struvite (0.05) and Zeolite (0.07), indicating that some of the Mg^{2+} remained in solution, lowering the pH slightly. Experiments that did not include $MgCl_2$ addition exhibited negligible change in pH, indicating that neither zeolite nor biochar had a significant effect on the buffer capacity of urine.

4 Determining the number of batches

A single 25 L batch of urine was estimated to contain approximately 200 mg P/L, based on literature values, theoretically producing approximately 40 g struvite per batch and recovering 6.3 g P (Etter et al., 2011; Lui et al., 2014). The amount of struvite fertiliser required per plant was therefore calculated at 50 g, providing 6.5 g P. Five batches of each fertiliser stream were carried out in order to ensure a sufficient amount of struvite (150 g) would be produced to fertilize 3 tomato plants, taking variation in P concentration into consideration.

5 Balancing fertilisers by P

The P content of urine-derived fertilisers was measured in order to accurately balance the mass of fertilisers by P. The total-P content of urine-derived fertilisers was measured by first dissolving struvite (30 mg), SZM and SB (400 mg) in 500 mL deionised water and performing aqueous extraction of solid samples in triplicate. The total-P concentration of the resulting solutions was then determined colorimetrically. The struvite and struvite-sorbent P concentrations were calculated from the extract solution concentrations and are reported in Table S3.

Table S3. Crop trial experimental conditions, with fertiliser treatments balanced by P content. Fertiliser mass and resulting nutrient mass per plant used for each experiment is shown (if known) and the volume ratio of sand:soil:fertiliser treatment is given.

Fertiliser ID	Measured P Concentration (g/Kg)	Mass Fertiliser (g/plant)	N (g/plant)	P	K	Mg	Volume ratio of Sand:Soil:Fertiliser (L)
1. Struvite	106	50	10.7	5.3	16.3	4.1	11:1:Negligible
2. SZM	13	400	11.3	5.3	16.3	4.1	10.3:1:0.7
3. SB	13	400	10.6	5.3	16.3	4.1	8.8:1:2.2
4. Zeolite+N	-	350	7.3	0.3	16.3	-	10.2:1:0.8
5. Biochar+N	-	350	12	0.07	16.3	-	8.4:1:2.6

6 P analysis of potting materials

P levels of potting materials (sand, potting soil and biochar) were measured to determine approximate ratios to generate a potting mixture with low P. The P content of sand was low (<1 mg P/Kg sand), whereas the potting soil water soluble P was higher (~120 mg P/Kg soil), corresponding to the recommended P concentration for tomato plants (Warncke & Krauskopf, 1983). An 11:1 volume ratio of sand to potting soil was therefore used to produce nutrient poor soil (~5 mg P/Kg soil), likely to result in nutrient deficiencies in plants without addition of fertilisers. Measured biochar P was also high (~500 mg P/Kg biochar), but lower than the reported 1.8%$_{wt}$ biochar P content (Table S2), indicating that biochar P is largely insoluble at neutral pH and unavailable to plants. The 3% biochar P contribution to each plant was therefore disregarded as negligible.

7 Statistical analysis

Statistical analysis using the Kruskal-Wallis Rank Test non-parametric analysis for the One-way Anova was carried out for the total DW of plants grown in each fertiliser stream, as well as the final tomato fruit count and Fruit DW (Table 1). The null hypothesis was that each fertiliser stream resulted in plants that were statistically equal ($H<\chi^2$). Comparison of all fertiliser streams, using the whole data set, produced *H*-statistic values higher than the critical value (taken from Table S3 in the Supplementary Information) with 8 degrees of freedom, allowing the null hypothesis to be rejected. This indicated that there was statistically significant difference in terms of total DW, Fruit count and Fruit DW between the plants grown in the all the different fertiliser streams. The analysis of only Tier 1 plants produced *H* values lower than the critical value, indicating that statistically all Tier 1 fertilised plants were equal. The *H* values for Tier 2 plants were higher than the critical value, suggesting that there were statistically significant differences between Tier 2 plants. Exclusion of Zeolite+N plants from Tier 2 and inclusion in Tier 1 resulted in *H* values higher than the critical value in all cases, with the exception of Tier fa1 Fruit DW, where Zeolite+N was shown to produce statistically equal Fruit DW to the Tier 1 fertilisers.

Table 2. Kruskal-Wallis Rank Test *H*-statistic for non-parametric analysis of total DW, Fruit count and Fruit DW for all fertilisers, Tier 1 and Tier 2 fertilisers, including and excluding Zeolite+N plant data respectively at 95% probability. The null-hypothesis can be rejected if $H>\chi^2$.

Test group	df	Critical χ^2 value	Total DW		Fruit count		Fruit DW	
			H	$H<\chi^2$?	H	$H<\chi^2$?	H	$H<\chi^2$?
All Fertilisers	8	15.507	24.032	No	24.819	No	23.428	No
Tier 1 (Struvite, Struvite + zeolite, struvite + biochar, NPK benchmark)	3	7.815	5.821	Yes	6.692	Yes	3.410	Yes
Tier 2 (Zeolite + N, biochar + N, zeolite control, biochar control, soil control)	4	9.488	11.833	No	13.080	No	12.967	No
Tier 1 + Zeolite+N	4	9.488	9.867	No	11.120	No	6.833	Yes
Tier 2 – Zeolite+N	3	7.815	7.821	No	9.701	No	9.564	No

All Tier 1 fertilisers were all shown to have statistically equal nutrient availability, indicated by the Kruskal-Wallis rank test of Total DW, total fruit DW and number of fruit produced. The nutrient availability of Zeolite+N fertilisers was shown to be higher than that of other Tier 2 fertilisers, as the *H*-statistic decreased when Zeolite+N was excluded from the analysis. However, Zeolite+N nutrient availability was shown to also be statistically lower than Tier 1 in all categories but Fruit DW. This indicates that the nutrient availability of Zeolite+N fertilisers was possibly closer to Tier 1 than other Tier 2 fertilisers, including SB. The Kruskal-Wallis test is used where the conditions for a normal distribution are not met (ie. when the margin of error is > 5%) so a much larger crop trial (N>3), is necessary in order to carry out a one-way ANOVA *F* test and make more accurate distinctions between the different fertilisers.

8 Uncertainty calculations

Measurement uncertainty calculations were carried out using summation uncertainty Equation S2 and relative uncertainty Equation S3. The uncertainty of the initial micro-pollutant solutions concentration was calculated first, followed by the dilution uncertainty in the urine and finally the uncertainty of the measured pharmaceutical uptake.

Equation S2. Summation uncertainty

$$\sigma_C = \sqrt{\sigma_a{}^2 + \sigma_b{}^2}$$

Equation S3. Relative uncertainty:

$$\frac{\sigma_C}{C} = \sqrt{\frac{\sigma_a{}^2}{a^2} + \frac{\sigma_b{}^2}{b^2}}$$

Where:
a and *b* = measured parameters
C = calculated parameter
σ_a and σ_b = measurement uncertainty

9 Calculating the excess N recovered in Struvite experiments, relative to SZM and Zeolite+N

The excess N in Struvite experiments is calculated in Table 4 by subtracting the 1:1 molar ratio with P amount of N from the total recovered in Table S4. The Zeolite+N is shown to reach the CEC maximum for NH_4^+ exchange (shown in Table S2) and using the same zeolite N uptake, SZM is shown to have a much lower excess N uptake.

Table S4. Excess N calculated from a molar 1:1 ratio of N/recovered P and Zeolite+N recovered N for Struvite and SZM fertilisers

Fertiliser ID	Mode of N recovery	Molar N recovery mol_N	N in zeolite $mol_N/Kg_{zeolite}$	Percentage N recovery $\%_N$	Mass N g_N
Struvite:	Total	0.5	-	5	6.0
	Struvite*	0.18	-	4	4.9
	Excess	0.39	-	1	1.1

Zeolite+N:	Total	0.455	1.52	6.1	6.37
SZM:	Total	0.687	-	9.5	9.62
	Zeolite**	0.456	1.52	6.3	6.38
	Struvite*	0.186	-	2.6	2.6
	Excess	0.045	-	0.6	0.63

*Calculated from a 1:1 ratio with struvite P

**Based on Zeolite+N recovery of NH_4^+ by ion-exchange

10 Pharmaceutical micro-pollutant concentrations in tomato fruit biomass raw results

Table S5. Mean concentrations of micro-pollutants detected in fruit biomass

Micro-pollutant	Mean concentration measured in tomato fruit biomass				
	Struvite (μg/g)	SZM (μg/g)	SB (μg/g)	Zeolite+N (μg/g)	Biochar+N (μg/g)
Carbamazepine	nd	nd	nd	0.003* ±0.001	nd
Propranolol HCl	nd	nd	nd	nd	nd
Diclofenac sodium	nd	nd	nd	nd	nd
Sulfamethoxazole	nd	nd	nd	nd	nd
Ibuprofen	nd	nd	nd	nd	nd

*Under detection limits (0.001 μg/g biomass)

11 Literature values for bioaccumulation of pharmaceuticals in tomato plants

Table S6. Bioaccumulation factor (BAF) values of pharmaceuticals in lettuce and tomato plant tissues. BAF is the ratio of dry weigth of compounds in plant tissues compared to their initial conecentrations in the nutrient solutions. Source: Zheng et al. (2014)

Micro-pollutant	Tomato BAF			Lettuce BAF	
	Root	Leaf	Fruit	Root	Leaf
Carbamazepine	55.0	515	2.80	49.2	142
Ibuprofen	11.3	0.25	0.036	4.31	0.36
Sulfamethoxazole	418	1.86	0.22	580	17.8

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