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Antibiotic Residues in Struvite Fertilizers Precipitated by Different Processes in Municipal Wastewater Treatment Plants

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Abstract: Struvite ($MgNH_4PO_4 \cdot 6H_2O$) is a precipitation product that can be obtained in municipal wastewater treatment plants (WWTPs) and represents a promising fertilizer and technical solution for phosphorus recovery. Struvite can be recovered from the wastewater stream, during sludge digestion or by accelerated acid leaching from sludge followed by solid–liquid separation and struvite precipitation from the liquid phase. Moreover, struvite can be precipitated from industrial effluents or agricultural wastes. The resulting products are of different purity. Antibiotic residues are a relevant class of contaminants as already traces can induce or promote the development of antibiotic resistance in the environment. The aim of the current study was a screening of struvite raw materials precipitated by different processes in German WWTPs for their contamination by selected antibiotics out of the classes of sulfonamides (SAs), fluoroquinolones (FQs) and tetracyclines (TCs). Slightly higher antibiotic residues were detected when struvite was precipitated from the solid phase with a maximum of 133 µg TCs, 484 µg FQs and 8 µg SAs compared to 8 µg TCs, 86 µg FQs and 9 µg SAs per kg dry weight (DW) when struvite was obtained from the liquid phase. FQs were most frequently found in low but quantifiable concentrations in almost all struvite raw materials. Yet, the contamination level of struvite from WWTPs can generally be regarded as low compared to sewage sludge. Products received from effluents from the food industry were found to be almost free of antibiotic residues.

Keywords: antibiotic residues; fluoroquinolones; phosphorus removal; recycled fertilizers; sulfonamides; tetracyclines

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1. Introduction

Closing of nutrient cycles, with special regard to nitrogen (N) and phosphorus (P), becomes increasingly important for future sustainable agriculture, to prevent the environment from unwanted eutrophication and to maintain to some degree agricultural independence on a national or regional level [1]. This is of special relevance against the background of increasing and insecure energy and fertilizer prices and limited rock phosphate resources, which are often contaminated by heavy metals such as uranium and cadmium bearing the risk to contaminate agricultural soils in the long term [2]. The bulk of P fertilizers used in agriculture originates from nonrenewable phosphate rock, which is reported to last for only a few more centuries at current mining rates and in reasonable quality [3,4]. However, some phosphate deposits are highly underestimated, which was just recently observed in Norway [5].

P is an essential macronutrient for all living organisms and cannot be replaced by any other element with its central role in important metabolic processes such as photosynthesis and respiration, its unique function in genetic information (DNA), its important part in energy transfer, lipid metabolism and cell structure, to only mention some of the numerous functions in plant metabolism [6]. Currently, Europe is highly dependent on P imports as there is no significant European phosphate mining [7] apart from some activities in Finland and Norway. Accordingly, the EU [8] placed mineral phosphate rock on the list of critical raw materials in 2014. The largest sedimentary rock phosphate reserves that are currently mined are located in only a few countries, namely Morocco, China and the United States [3,9].

Closing of nutrient cycles by recycling of societal wastes and recovery of nutrients is an important possibility for sustainable fertilizer production, enabling also future generations to meet their food demand. It was calculated that approximately 50–60% of imported rock phosphates can be replaced in European agriculture when the most important societal wastes, in particular sewage sludge, which accounts for more than 50% of the off-farm P cycles, meat and bone meal, food wastes, by-products/wastes from the food industry and organic household wastes, are recycled for P [10–12]. Möller et al. [11] estimated the yearly P recycling potential in the EU, which accounted for 320,000 Mg P in biosolids from sewage sludge, 310,000 Mg P from slaughterhouse wastes and 215,000 Mg P from food wastes and by-products of food industries. Estimates for household wastes and urban wastes are hard to conduct as currently only 30% of these wastes are source separated across Europe. The P content of biosolids corresponded to 20% of the mineral fertilizer input in the EU-27 of 1487 Gg for imported fertilizer-P in 2005 [13–15].

There were two main recycling strategies for P from sewage sludge besides the direct use as sanitized biosolids or in composted or anaerobically digested form, by which P recovery rates close to 100% are achieved [11]. One strategy is thermal treatment yielding a P-rich ash almost free of organic pollutants that can be further processed to yield mineral fertilizers. Organic wastes can undergo traditional combustion or, alternatively, hydrothermal carbonization or pyrolysis resulting in different products. During or after incineration, chars or ashes can be treated to remove unwanted compounds and to increase the P availability of the product [11,16]. Most thermal approaches reveal high P recovery rates of 80 up to 100% [17] but result in the loss of organic matter and nutrients like nitrogen and sulfur. Moreover, the processes are highly energy demanding and costs for transportation and chemicals to increase P availability in the fertilizer product arise.

The second strategy is the precipitation of P out of anaerobically treated sludge or the anaerobic supernatant to produce struvite ($MgNH_4PO_4 \cdot 6H_2O$), water-soluble dicalcium phosphates or non-soluble Ca-phosphates [13,18,19]. Such chemical approaches have a lower P recovery (5–75%) because water soluble P and P attached to colloids are addressed in the first place [19,20]. There are different approaches to further increase P recovery, such as thermal hydrolysis of the sludge [13] or biological acidification of waste-activated sludge aiming to optimize P dissolution and recovery [21]. This paper deals with the second strategy, particularly with struvite as a precipitation product and its possible contamination by antibiotic residues. Struvite is regarded as a slow-release fertilizer with excellent plant availability [22]. Struvite is a fertilizer product of high relevance because of its potential to be used in organic farming systems.

Waste materials can contain different contaminants such as potentially toxic elements, persistent organic pollutants, pharmaceuticals, plastic particles, pathogens and resistance genes [23–26]. It is important to evaluate and to minimize the risks of recycled fertilizers to protect farm soils from contamination by heavy metals and organic contaminants. The concentration of potentially toxic elements is very low in struvite except for zinc (Zn) and chromium (Cr), while most element concentrations like Cd and Ni, but also Zn and Cr, are lower than in triple superphosphate or phosphate rock, which contain considerable amounts of potentially toxic elements [11].

High concentrations of organic pollutants are usually found in biosolids [27,28]. Only a few studies evaluated the contamination of struvite derived from municipal WWTPs by organic pollutants [29,30], while many publications are available reporting on the contamination of struvites derived from animal manures and piggery wastewater (e.g., [31–33]). It was already reported in 2007 that more than 98% of hormones and most pharmaceuticals remain in solution [30] during the precipitation process of struvite in WWTPs, and further clean-up can be achieved by rinsing the product. Generally, a low level of organic pollutants was detected in struvite [34–36]. But even low concentrations of antibiotic residues can be relevant as long-term and repeated exposure, even to non-lethal, sub-inhibitory concentrations of antibiotics, has the potential to function as signaling molecules and can accelerate the development and evolution of antibiotic resistance in exposed bacteria, which can be a serious threat for human and animal health [37,38]. Only little information is available on the occurrence and concentrations of antibiotic residues in struvite precipitated in municipal WWTPs.

The objective of this work was to evaluate the contamination of struvite raw materials recovered by different procedures in various German and one Dutch WWTP with residues of selected antibiotics out of the classes of tetracyclines (TCs), fluoroquinolones (FQs) and sulfonamides (SAs), representing common antibiotic classes frequently used in Germany. It was hypothesized that the contamination could be related to the organic carbon (C) content as higher C contents indicate a less purified product. Technical procedures that are commonly used in Germany for struvite precipitation are compared with respect to the contamination with antibiotic residues.

2. Materials and Methods

2.1. Struvite Samples

Thirty-four struvite raw materials were sampled from eight German and one Dutch WWTP using four different technical procedures for struvite precipitation (Table 1). German struvites were sampled from different batches that were delivered by truck to a German fertilizer producing company for fertilizer confection. From each truckload, a big bag of 800–1000 kg was filled as a retained sample from which the samples of the current study were taken. Samples were collected between 2019 and 2023, but most samples were taken in 2021. Process parameters are still under optimization in the WWTPs in Gifhorn and Braunschweig, which is why additional sampling took place in 2022 and 2023, respectively. A representative sample was always taken by mixing ten subsamples taken from the big bag by a shovel. Dutch samples were collected in May 2022, and one product sample was purchased in 2020.

Table 1. Origin of the struvite samples, precipitation technologies and year of sampling.

Country Code	Location of the WWTP	Precipitation Technique	No of Samples	Material	Comment
D	Berlin	AirPrex®	5	Raw material	Samples from 2019 (1), 2020 (1), 2021 (3)
D	Wolfsburg	AirPrex®	1	Raw material	Sample from 2021
D	Salzgitter	AirPrex®	4	Raw material	Samples from 2020 (3), 2021 (1)
D	Mönchen-gladbach	AirPrex®	3	Raw material	All samples from 2021
D	Braunschweig	NuReSys®	7	Raw material	System implemented in 2019 is still under improvement; samples from 2020 (1), 2021 (5), 2022 (1), 2023 (1)
NL	Apeldoorn	NuReSys®	1	Raw material	Sample collected in May 2022
D	Gifhorn	Modified Seaborne procedure	4	Raw material	Samples from 2021 (1), 2022 (3)
D	Hünfeld	PHOSPAQ™	8	Raw material	Samples from 2020 (1) 2021 (7)
NL	Amersfoort	Crystal Green Pearl®	2	Product	1st sample from 2020 and 2nd from May 2022
D	Hünxe	Mixture of struvite from different WWTPs	5	Pilot scale products	Products currently under development

Four different struvite precipitation approaches (Figure 1) differing in their precipitation method (1–3) or input material (4) were investigated. Struvite was precipitated in municipal WWTPs from sludge (1), centrate (2) or after forced P remobilization (3).

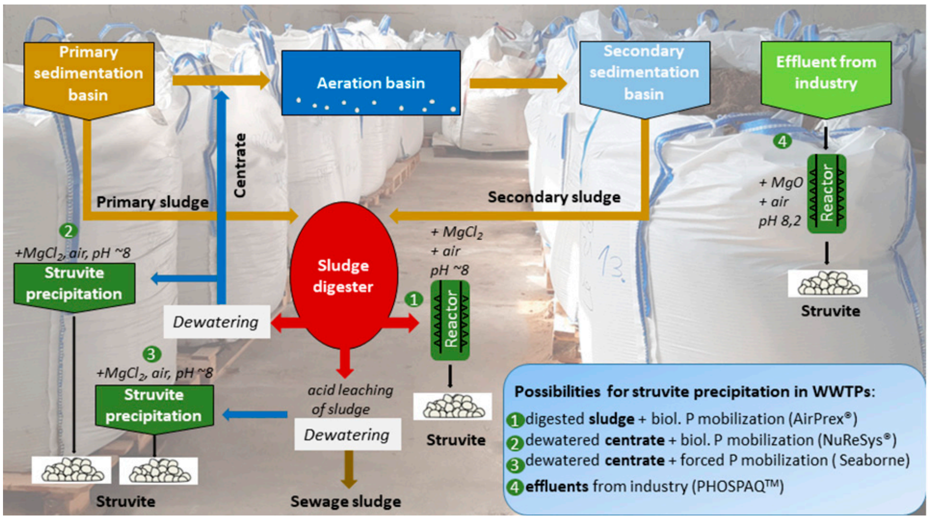

Figure 1. Possibilities for struvite precipitation in waste water treatment plants (WWTP) evaluated in the current paper.

The collected struvite samples differed in appearance, texture and color (Figure 2). Some struvite samples had the texture of sand while others were more stony and clotted.

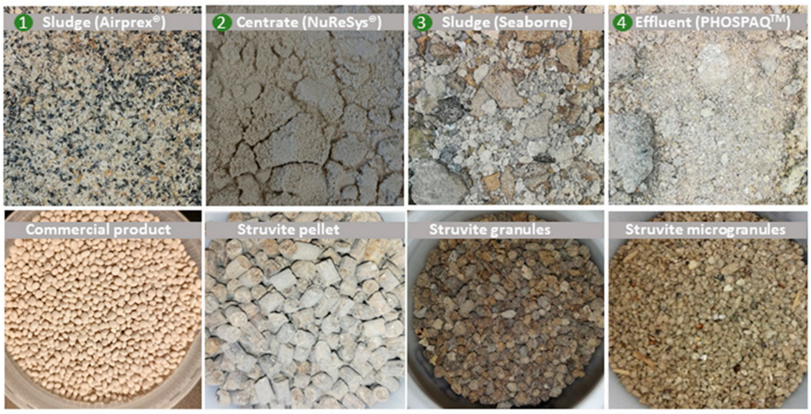

Figure 2. Struvite from different precipitation processes (see Figure 1) showing a different texture and appearance and products made from struvite.

Additionally, seven struvite products in pelletized or granulated form were analyzed (Figure 2). One product, which was measured twice from different batches, is already commercially available while the other five samples were pilot scale products (Table 1).

2.2. Pre-Treatment and Chemical Analysis of Struvite Raw Materials and Products

All samples were freeze-dried (Gamma 1-16 LSC plus, Martin Christ, Osterode am Harz, Germany) prior to grinding the samples with a vibratory disc mill at 700 rpm for 10 s (RS 200, Retsch, Haan, Germany) to a fine powder for further analysis. Total carbon (C) and nitrogen (N) were measured via dry combustion in a C/N analyzer (Vario Max Cube, Elementar, Langenselbold, Germany).

2.3. Determination of Antibiotic Residues

A method for the determination of antibiotic residues was used [39] that was formerly optimized for recycled organic fertilizers [40]. Three classes of antibiotics were detected by this method: tetracyclines (TCs), sulfonamides (SAs) and fluoroquinolones (FQs). In the current study, from the class of TCs, chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TC), doxycycline (DOX), demeclocycline (DMC) and metacycline (MC) were analyzed; from the SAs, sulfadiazine (SDZ), sulfamethazine (SMZ) and sulfamethoxazole (SMX); and from the FQs, enrofloxacin (ENR), ciprofloxacin (CIP), difloxacin (DF), norfloxacin (NOR) and ofloxacin (OF). We are aware that other antibiotics can be contained in struvite. However, it was the main target of this work to show exemplarily for commonly used antibiotics in which concentrations and frequencies they can be detected in struvite raw materials collected from a broad range of samples from different years and WWTPs.

2.3.1. Extraction Procedure

Each sample was extracted twice, one time the original sample and a second time the sample with the addition of internal standard to calculate the recovery rates for all antibiotics. Two times 0.75 g of freeze-dried material was weighed into falcon tubes and 50 µL of internal standard mix (containing 10 mg/L of each of the investigated antibiotics) was added to one sample. A three-stage extraction procedure was conducted using 20 mL of citric acid buffer (0.2 m, pH 3.7) in the first and second extraction steps. The samples were extracted for 20 min in an ultrasonic bath, centrifuged at 9000× *g* for 10 min and the supernatants were combined. In the third step the pellet was extracted with 10 mL of a solution containing 4.203 g of citric acid monohydrate solved in 100 mL of water filled up to 500 mL by methanol. Extraction was performed as before and the supernatant of this third extraction step was not mixed with the others. The pellet was mixed with 10 mL methanol containing 1% of formic acid, left for overnight reaction and was centrifuged the next day at 9000× *g* for 10 min. The supernatant was combined with that of the third extraction step and was vacuum concentrated (Eppendorf Concentrator plus, Hamburg, Germany) to a final volume of 5 mL. This concentrate and the supernatant of the first two extraction steps were subjected to a liquid-liquid extraction with 10–20 mL of heptane. The defatted extract was diluted with water to 100 mL, pH was adjusted to pH 3 with formic acid and vacuum-filtrated (85/90 BF from Macherey-Nagel, Düren, Germany) before it was loaded onto SPE cartridges (Strata-X 33u polymeric reversed phase, 200 mg/6 mL from Phenomenex, Torrance, CA, USA). SPE cartridges were pre-treated with 3 mL methanol first followed by 3 mL of citric acid buffer (pH 3). The sample was loaded onto the SPE at a flow rate of 5 mL/min, washed with 8 mL of distilled water and the SPEs were dried for 15 min under vacuum. The antibiotics were eluted from the cartridges in a two-step procedure starting with 4 mL of methanol followed by 4 mL of methanol containing 1% of formic acid. This extract was vacuum concentrated to 1 mL, filled up to 2 mL with methanol and was filtrated using a syringe filter (Perfect Flow, PVDF, 0.2 µm from Wicom, Heppenheim, Germany). Quantification was performed by standard addition.

2.3.2. Quantification of Antibiotics by HPLC with Electrospray Ionization Tandem Mass Spectrometry

For analysis of antibiotics, an Agilent 1200-HPLC (Agilent, Santa Clara, CA, USA) linked to an API 4000 triple quadrupole mass spectrometer (ABSciex, Framingham, MA, USA) was used in the positive electrospray ionization mode. The injection volume was 2 µL and the column temperature was 25 °C. A Poroshell 120 EC-C18 column (3 × 100 mm, 2.7 µm) from Agilent was used. A gradient made from water (containing 10% methanol, 77.08 mg/L ammonium acetate, 0.05% formic acid, eluent A) and methanol (containing 0.05% formic acid, eluent B) was used for elution and separation of antibiotics.

Quantification by standard addition was necessary to minimize matrix effects. More information about the extraction procedure, the ionization parameters of the LC/MS/MS and the transitions of the analytes used for determination and quantification can be found in Lehmann and Bloem [40].

2.3.3. Lower Limit of Quantification (LOQ)

The lower limit of detection (<LOD) and the lower limit of quantification (<LOQ) were calculated based on the signal-to-noise ratio (S/N). LOD is reached when this ratio is ≤ 3 and quantification is possible, at least theoretically, when this ratio is >10. The signal-to-noise ratio was calculated by the Analyst Software (Version 1.6.2). In practice, the LOQ varied depending on the sample and analyte and was reproducible when the signal-to-noise ratio was around 30. LOQ was determined exemplarily for one struvite sample that was almost free of antibiotics. The sample was spiked with increasing concentrations of antibiotics. When the signal-to-noise ratio was <10, the sample was below the limit for quantification even if there was a peak that could be integrated. LOQ can vary due to day-to-day variation, changing column characteristics and differences in the matrix, which is why the values in Table 2 have an indicative character.

Table 2. Range of the lower limit of quantification (LOQ) of antibiotic residues in struvite.

Target Antibiotic	Lower Limit of Quantification (LOQ) in Struvite [µg/kg DW]
Tetracycline (TC)	>1.0–2.02
Oxytetracycline (OTC)	1.33–6.67
Chlortetracycline (CTC)	8.91–17.86
Demeclocycline (DMC)	12.51–44.65
Metacycline (MC)	2.79–5.56
Doxycycline (DOX)	>1
Sulfadiazine (SDZ)	1.28–2.98
Sulfamethazine (SMZ)	0.99–1.55
Sulfamethoxazole (SMX)	0.45–1.59
Ciprofloxacin (CIP)	>1.0
Enrofloxacin (ENR)	0.27–1.24
Ofloxacin (OF)	0.73–1.50
Difloxacin (DF)	1.64–2.56
Norfloxacine (NOR)	>1.13

2.3.4. Recovery Rate of Antibiotic Residues in Struvite

Recovery rates of spiked antibiotics were determined for each sample and all antibiotics under determination as the matrix can differ in relation to the precipitation process and sampling date. Detection methods that determine antibiotic residues from different classes in one extraction procedure are always a compromise, and it is not possible to achieve very high recovery rates for all antibiotics in the same run.

The recovery rates can be used to correct the analyzed values, but this method is also risky as recovery rates vary from one measurement to the next. In the present paper, the original data are shown, but for the "worst case" scenario at the end of the discussion section, maximum contamination levels are calculated based on the recovery rates.

Each sample was measured twice, one time as the original sample and one time spiked with internal standard added at a concentration of 666 µg of each antibiotic/kg DW at the beginning of the extraction procedure. Recovery rates for most antibiotics were close to 50%, with the exception of the SAs with lower recoveries of about 36–43% (Table 3). The method was originally optimized for different organic materials such as digestates and sewage sludge. In digestates, recovery rates between 26 and 93% are reported [40]. The range of recovery rates for struvite raw materials shows that high recovery rates can also be reached by the method, which is why further optimization would not necessarily result in higher recovery rates (Table 3).

Table 3. Range of recovery rate (%) and medium recovery rate for different antibiotics added to struvite before extraction (n = 41).

Added Antibiotic	Range of the Recovery Rate [%]	Medium Recovery Rate [%] with Standard Deviation
Chlortetracycline (CTC)	35.7–93.1	53.4 ± 13.0
Doxycycline (DOX)	31.7–122.4	54.5 ± 16.5
Oxytetracycline (OTC)	32.9–96.7	53.6 ± 14.4
Tetracycline (TC)	35.2–104.1	55.3 ± 14.9
Demeclocycline (DMC)	29.1–98.7	52.9 ± 15.0
Metacycline (MC)	22.2–82.2	46.0 ± 14.0
Sulfadiazine (SDZ)	7.6–74.9	42.9 ± 17.5
Sulfamethazine (SMZ)	6.1–66.0	35.7 ± 15.7
Sulfamethoxazole (SMX)	12.8–66.2	41.4 ± 14.2
Ciprofloxacin (CIP)	24.8–79.2	48.6 ± 13.7
Difloxacin (DF)	26.4–91.9	46.7 ± 13.2
Enrofloxacin (ENR)	22.2–106.2	46.4 ± 16.0
Ofloxacin (OF)	29.9–91.1	48.7 ± 14.3
Norfloxacin (NOR)	20.5–76.7	46.2 ± 12.6

3. Results

3.1. Nitrogen and Organic Carbon in Struvite Raw Materials

The total carbon (C) and nitrogen (N) content was determined in different struvite raw materials (Table 4) to have an indication of possible contaminations with organic material and assess the homogeneity of the struvite samples yielded by the different processes (Figure 1). The data reveal that the variability in N was low compared to that of C. Clean magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) would contain a theoretical 5.7% of N. In reality, there are always some impurities causing a shift from this hypothetical value. The purest struvite was precipitated from centrate with a total N content of 5.3%. P recovery from sludge after forced P remobilization yielded a struvite containing 5.0% N, precipitation from effluent 4.7% N, and the lowest N content of 4.5% was found when struvite was precipitated from sludge (Table 4). It is important to keep in mind that these values refer to freeze-dried samples, which is why the values can differ from commonly reported values as commercial struvite contains residual moisture.

Table 4. Total carbon (C) and nitrogen (N) content in struvite raw materials originating from different P-recovery processes and WWTPs.

Medium/Process of P Recovery	Sludge/ AirPrex®	Centrate/ NuReSys®	Sludge after Forced P-Remobilization/ Modified Seaborne	Industrial Effluent/ PHOSPAQ™
No of samples	13	9	4	8
		Total carbon (C) content [%]		
Range	1.54–27.28	0.06–1.02	0.21–3.63	0.90–7.07
Mean ± SD	7.49 ± 7.74	0.48 ± 0.39	1.57 ± 1.54	2.32 ± 2.03
		Total nitrogen (N) content [%]		
Range	3.49–5.31	4.06–5.57	3.53–5.58	3.02–6.56
Mean ± SD	4.53 ± 0.54	5.33 ± 0.48	5.01 ± 0.99	4.73 ± 1.05

The total C content indicates a much higher variability in relation to process parameters. The lowest total C content of around 0.5% C was determined in products precipitated from centrate as expected, while the highest contents of about 7.5% C on average and up to 27.2% C at maximum were found when precipitation took place from sludge. It needs to be mentioned that the different processes are still under optimization in the WWTPs. For example, the NeReSys® facility sampled in this study was implemented in 2018/19 and the first struvite samples were received in 2020. The improvement of the system is still running. Moreover, most of the AirPrex® systems do not have the intention to produce a clean struvite but to protect the WWTP from unintended precipitation and choking of pipelines. Therefore, the data in Table 4 and the reported contamination levels of struvite raw materials by antibiotics (Table 5) have to be seen as "worst case" contents. The lower values indicate what can be easily achieved by further process optimization.

The seven products made from struvite that were analyzed in this study show partly increased nutrient or C contents due to their formulation, which is why their variability in C and N is not included in Table 4.

3.2. Contamination of Struvite by Antibiotic Residues

It was the aim of the current study to evaluate the contamination level of struvite raw materials obtained by different processes with antibiotic residues out of the classes of tetracyclines (TCs), sulfonamides (SAs) and fluoroquinolones (FQs). Antibiotic residues have the potential to be transferred from sewage sludge into precipitation products. Screening of the struvite raw materials derived from different processes for antibiotic residues will give an indication of the most promising technique to produce safe struvite fertilizers.

The data show that residues of SAs and TCs were rarely detected in struvite raw materials in concentrations that could be quantified; only in some outliers were SAs and TCs detected in higher concentrations (Figure 3). FQs were found with the highest frequency and often in concentrations that could be quantified (Table 5). For example, ciprofloxacin (CIP), enrofloxacin (ENR) and ofloxacin (OF) were detected in 33 out of 34 struvite raw materials (97%). Quantification was mainly possible in the case of FQs; for example, CIP could be quantified in 74% of all raw materials. The data in Figure 3 show that the concentrations of antibiotic residues are quite similar and in all cases low in the struvite raw materials, independent of the precipitation process. Struvites from food effluents revealed the lowest contamination. Therefore, all struvite precipitation procedures deliver products with a low contamination level with antibiotic residues.

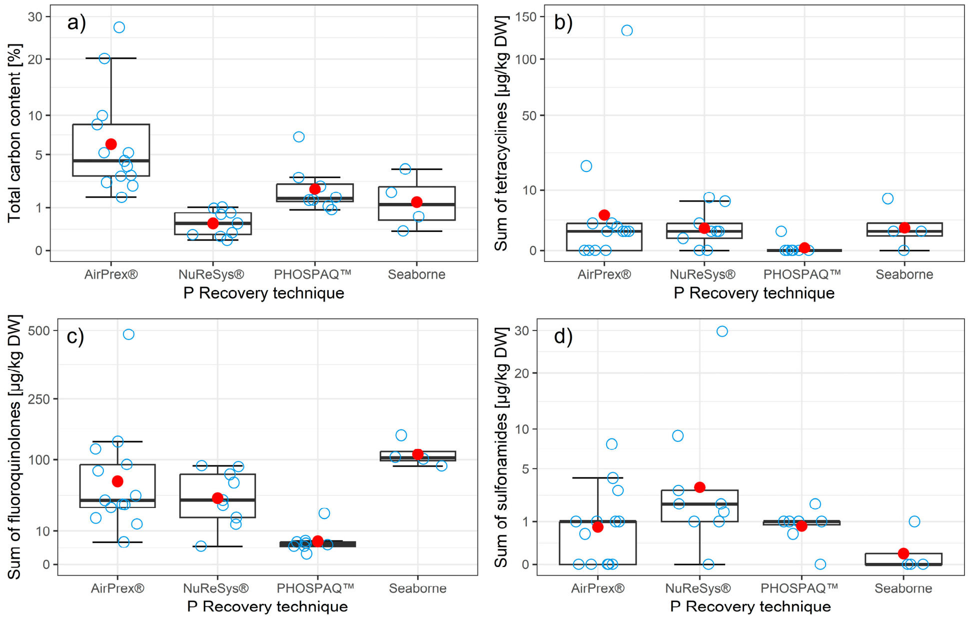

Figure 3. Boxplot of total carbon contents (**a**) and residues of antibiotic contaminations with tetracyclines (**b**), fluoroquinolones (**c**) and sulfonamides (**d**) in struvite raw materials in relation to P recovery technology. (Red dots indicate mean values, blue open circles are the single measurements from the WWTPs; scales of the *y*-axis were square root transformed for better visibility of low values).

Tetracyclines (DOX and TC) could be quantified in higher residual concentrations in some struvite samples precipitated from sludge (AirPrex®), in lower concentrations in precipitation products from centrate and in one sample from centrate after forced P remobilization. In precipitation products from effluents, TC was almost not detected (Figure 3).

Residues of FQs were found in most struvite raw materials independent of the precipitation technique (Figure 3c). Very low residues were detected when struvite was precipitated from industrial effluent, while maximum values were found in precipitates from centrate after forced P remobilization (Seaborne process). Municipal WWTPs always seem to be contaminated by residues of FQs that were often found in concentrations above the quantification limit (Table 5), indicating the regular widespread use of FQ-containing antibiotics in Germany. The high variation in residues of FQs in the struvites precipitated by AirPrex®-using plants (Figure 3c) reflects that the materials were precipitated in four different WWTPs. In one of the plants, the process was not always running perfectly according to the operators, and sometimes sewage sludge directly enters into the product, explaining the peak values. It needs to be stressed that struvite is precipitated in that plant to protect the system, not to produce a clean fertilizer raw material. Therefore, the higher concentrations should be treated as outliers that could be prevented when struvite becomes a valuable product.

The contamination of struvite by SA residues was lowest from the three antibiotic classes, with a maximum single concentration of 19.0 µg/kg DW of SMX (Table 4) and a maximum sum of SAs in one struvite of 29.8 µg/kg DW, which was detected in the Dutch sample (Figure 3d). Sulfonamides were quantified in slightly higher concentrations in some struvite samples precipitated from centrate, indicating their higher water solubility (Figure 3d). In accordance, the lowest contamination with SAs was observed when struvite was precipitated from centrate after forced P remobilization (Seaborne process), because the potentially SA-containing liquid phase is removed before the precipitation took place.

The rare contamination of struvite with residues of TCs indicates that this contamination can most likely be prevented by optimized process parameters, while especially the contamination by FQs was observed in most samples from all processes and even in the marketable products. FQs have a high tendency to bind to the sludge matrix, which is why these compounds show high persistence. Therefore, the highest attention should be paid to residues from FQs when struvite undergoes a further product optimization.

The seven products investigated in this study showed nearly no contamination by antibiotic residues with the exception of residues from CIP, where the median was above the LOQ (Table 5). These data indicate that under optimized process conditions with the intention to produce a fertilizer, very clean struvite raw materials can be produced, almost free of antibiotic residues.

Table 5. Contamination of struvite precipitated by different processes with antibiotic residues out of the classes of tetracyclines, sulfonamides and fluoroquinolones: mean values, median (Med.) and maximum (Max.) values and the detection frequency (DF-%) is shown (Values ≤ 1 indicate to a contamination at the limit of quantification; LOD = Limit of detection).

		Tetracyclines [µg/kg DW]					**Sulfonamides [µg/kg DW]**			**Fluoroquinolones [µg/kg DW]**				
Process		**OTC**	**CTC**	**DOX**	**TC**	**MC**	**SDZ**	**SMZ**	**SMX**	**CIP**	**DF**	**ENR**	**OF**	**NOR**
Air-Prex® (n = 13)	Mean	0.0	0.1	9.3	2.8	0.3	1.0	0.3	0.2	47.3	3.8	4.0	24.1	7.5
	Med.	0.0	0.0	0.0	1.0	0.0	0.5	0.0	0.0	25.2	1.0	1.0	11.1	1.0
	Max.	<LOD	1.0	103	29.9	1.0	7.9	3.1	1.0	251	16.1	26.2	131	59.9
	DF-%	0	8	15	54	31	46	15	15	100	92	100	100	100
NuRe-Sys® (n = 9)	Mean	0.1	0.0	0.0	0.6	1.4	2.3	0.6	2.6	9.8	4.7	4.0	14.7	13.1
	Med.	0.1	0.0	0.0	0.4	0.0	1.0	0.0	1.0	11.9	1.0	2.8	11.2	11.4
	Max.	1.0	<LOD	<LOD	2.3	7.6	9.0	3.4	19.0	16.2	17.2	11.0	33.7	35.9
	DF-%	11	0	0	56	44	67	33	67	100	89	100	89	89

Table 5. *Cont.*

		Tetracyclines [µg/kg DW]					Sulfonamides [µg/kg DW]			Fluoroquinolones [µg/kg DW]				
Process		**OTC**	**CTC**	**DOX**	**TC**	**MC**	**SDZ**	**SMZ**	**SMX**	**CIP**	**DF**	**ENR**	**OF**	**NOR**
Sea-borne (n = 4)	Mean	0.0	0.0	1.6	0.8	0.0	0.3	0.0	0.0	41.1	5.3	2.0	39.4	23.9
	Med.	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	42.2	4.1	1.3	40.9	20.6
	Max.	<LOD	<LOD	6.3	1.0	<LOD	1.0	<LOD	<LOD	67.0	12.4	4.4	51.8	46.0
	DF-%	0	0	25	75	0	25	0	0	100	100	100	100	100
PHOS-PAQ™ (n = 8)	Mean	0.0	0.0	0.0	0.0	0.1	0.8	0.1	0.0	2.1	0.4	0.8	2.0	0.6
	Med.	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.3	1.0	1.0	1.0
	Max.	<LOD	<LOD	<LOD	<LOD	1.0	1.0	1.0	<LOD	10.4	1.0	1.0	10.3	1.0
	DF-%	0	0	0	0	13	88	13	0	88	50	88	100	63
DF-% in all 34 raw materials		3	3	9	44	26	62	18	24	97	82	97	97	88
Products (n = 7)	Mean	0.1	0.1	0.5	0.4	0.0	0.1	0.0	0.0	8.8	0.6	2.3	6.2	0.5
	Med.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.0	1.0	1.0	1.0	0.0
	Max.	1.0	1.0	3.5	1.0	<LOD	1.0	<LOD	<LOD	17.2	1.0	6.7	17.3	1.4
	DF-%	14	14	14	43	0	14	0	0	100	57	86	86	43

OTC = oxytetracycline; CTC = chlortetracycline; DOX = doxycycline; TC = tetracycline; MC = metacycline; SDZ = sulfadiazine; SMZ = sulfamethazine; SMX = sulfamethoxazole; CIP = ciprofloxacin; DF = difloxacin; ENR = enrofloxacin; OF = ofloxacin; NOR = norfloxacin.

4. Discussion

Struvite recovered from WWTPs represents a renewable P-, N- and Mg-containing fertilizer with high potential to be used in agriculture [22,41–43]. A low contamination with organic contaminants is a prerequisite for safe fertilizer use. Antibiotic residues were excreted in high proportions unchanged with urine or feces due to poor adsorption in the gut [38,44,45]. The highest excretion rates of up to 96% were reported for the water-soluble SAs [44], while FQs and TCs are excreted at lower doses of 30–70% and 17–75%, respectively [46,47]. Due to these high excretion rates, substantial amounts of antibiotic residues can be detected in sewage sludge. Cheng et al. [48] reported that FQs and TCs were more abundant in sewage sludge in China than SAs, with contents of 1569–23,825 µg/kg DW ΣFQs (mean of 8274), 592–37,895 µg/kg DW ΣTCs (mean 8326) and 20.1–117 µg/kg DW ΣSAs (mean 55.4). Lehmann and Bloem [40] investigated 12 samples from European sewage sludge and found high residues of sulfadiazine (787 µg/kg DW), ciprofloxacin (1969 µg/kg DW) and tetracycline (2440 µg/kg DW).

In China, higher residues of quinolones were detected in sewage sludge compared to Europe [48,49]. FQs are strongly adsorbed and tend to accumulate in soils and sediments [50] and in sewage sludge. FQs and TCs have higher sludge-water partitioning coefficients (Kd) than SAs, explaining their higher occurrence in sewage sludge [51–53]. Precipitation of TCs with Mg, Ca and ferric iron and its accumulation in the solid fraction was observed [54,55].

4.1. Correlation between Antibiotic Residues and the C-Content of Struvite Raw Materials

The contamination with antibiotic residues was highest when struvite was precipitated from sludge (AirPrex®), but most struvite samples revealed a very low contamination close to the detection limit (Table 5). The data indicate that for some residues out of the class of FQs, the contamination increased slightly with increasing carbon content, but the relationship was proved to be not significant. Correlation analysis revealed that the contamination of struvite with residues of CIP could be explained by organic matter to less than 10%, that of OF to 5% and that of ENR to 2%. Ye et al. [33] found a linear relationship between TCs and the total organic C, which was not found in the present study due to the very low residual contamination by TCs. Ye et al. [33] also found a lower relationship for FQs. Other process factors seem to be more important with respect to the residual antibiotic contamination level.

In general, the contamination with antibiotic residues in sewage sludge is more than 95% higher than in struvite, indicating a very good exclusion of contamination in WWTPs.

The data also indicate a noticeable variability in the contamination even in the same WWTP. For example, all struvite samples from process 2 (NuReSys®) with the exception of one sample are from the same WWTP, and all samples from process 3 (Seaborne) and 4 (PHOSPAQ™) were from one WWTP, respectively. Only for the AirPrex® procedure, four different WWTPs could be sampled.

4.2. Predicted Environmental Application of Antibiotics via Struvite

For a reasonable risk assessment, it is necessary to calculate the amount of antibiotic residues applied to the field via struvite application in a worst-case scenario. For a targeted fertilization of 75 kg/ha P_2O_5 (≈32.7 kg/ha P), about 260 kg struvite are needed. Calculating with the highest ciprofloxacin (CIP) content detected in our study (251 µg/kg DW), this implies an input of 65 mg/ha CIP to the field. For comparison, this would be equal to 1/8 of a typically sold prescription drug containing 500 mg CIP/tablet. Even if the CIP content is corrected by the recovery rate, the CIP content would account for a maximum of 325 µg/kg DW, leading to an input of 84.5 mg/ha CIP (1/6 of a typical tablet). Data from Ryan et al. [56] indicate that struvite as a fertilizer has no negative effects on soil bacterial, fungal and nematode communities and can be safely applied as a recycled P fertilizer.

However, it should be noted that struvite could not only be recovered from municipal or industrial wastewater but also from agricultural wastes such as cow urine or waste water from pig farms [31,57]. Such wastes can contain considerably higher concentrations of antibiotic residues. Chen et al. [31] observed that struvite application to agricultural fields changed the antibiotic resistome in the soil, rhizosphere and phyllosphere. In that study, struvite was precipitated from swine wastewater in a large Chinese piggery. The struvite obtained in that study had much higher contamination levels by antibiotic residues than the struvite analyzed in the present study: doxycycline was found at 742.07 mg/kg DW, tetracycline at 415.21 mg/kg DW, sulfadiazine at 13.63 mg/kg DW, enrofloxacin at 59.10 mg/kg DW and ciprofloxacin at 5.10 mg/kg DW, to mention some of the results. The highest concentration observed in the present study was 251 µg/kg DW of CIP, which is 20 times lower than the values reported by Chen et al. [31]. The highest doxycycline content found in the present study was 6890 times lower than the value reported by Chen and coworkers. It can be assumed that this very high contamination by antibiotic residues in the struvite was responsible for the increased abundance and diversity of antibiotic resistance genes. Ye et al. [33] also reported a high antibiotic contamination of struvite precipitated from swine wastewater, especially with TCs (195.2–1995.0 µg/kg DW) and FQs (0.4–1104.0 µg/kg DW). It needs to be stressed that struvite produced from municipal WWTPs, which is currently the main source of struvite production in Europe, contains hardly any impurities [34].

Sub-inhibitory concentrations of antibiotic residues in the environment have the potential to increase the selection and development of antibiotic resistance by enrichment of pre-existing mutants and selection of de-novo resistance [37]. When the minimum inhibitory concentration (MIC) at which the antibiotic is controlling the bacteria is undercut but the minimum selective concentration (MSC) for the selection of resistant mutants is reached, there is a hazard for the development of antibiotic resistance. The MSC of a certain antibiotic is much lower than the MIC, which is why already low concentrations can cause serious problems: For TCs, the MSC was 100 times lower than the MIC, corresponding to 15 ng/mL TC [58]. For CIP, the MSC was 10-fold to 230-fold lower than the MIC, depending on the particular resistance mutation, corresponding to 2.5 ng/mL–100 pg/mL CIP [58]. Back to our worst-case scenario, a concentration of 2.5 ng/mL CIP is reached when 84.5 mg/ha CIP of the worst-case assumption were dissolved in 33,800 L of water, corresponding to 3.4 L/m^2. This calculation reveals that even low concentrations of antibiotic residues can be of relevance and it should always be the target to reduce the contamination level as much as possible.

It is important from which source struvite is precipitated. Struvite produced from animal manures can have a much higher contamination with antibiotic residues than struvite produced in WWTPs. Therefore, no general conclusions regarding the contamination and ecological relevance of struvite are possible, as the concentration can vary in relation to the raw material, the process of precipitation and several process parameters like pH, Mg application and granule size [33]. The class of antibiotic residue is also important: for example, Başakçilardan-Kabakci et al. [59] observed that TCs revealed a low adsorption efficiency to struvite of at most 22% of the wastewater concentration, as under alkaline precipitation conditions, struvite and TCs are both negatively charged. From the present study, it can be concluded that all struvite raw materials revealed a very low contamination with antibiotic residues. Special attention should be paid to the FQs because of their residual concentration and high detection frequency.

5. Conclusions

The tested struvite samples from municipal WWTPs and from industrial food effluents showed a very low contamination by antibiotic residues out of the classes of TCs, SAs and FQs. The data indicate that it is possible to produce struvite almost free of antibiotic residues with optimized process parameters. In struvite produced from industrial effluent from the food industry, hardly any antibiotic residues could be quantified. Struvite produced from sludge can contain a considerably higher contamination level. In relation to the process, special attention should be paid to residues from SAs when struvite is precipitated from centrate, while FQs can be detected in precipitates from all investigated procedures. The four technical solutions for struvite precipitation are proven to be all suitable to produce a fertilizer raw material. The variability in the carbon content indicates that the processes can be further optimized, and it should be the target of future optimization to produce precipitates low in carbon. Nevertheless, the residue data of antibiotics indicate that already without further optimization, the contaminations are low, showing only small variation over the years and between WWTPs.

The results are of high relevance for the decision process in German WWTPs regarding their P recovery strategy. Currently, the permission process for struvite as a P fertilizer for conventional and organic farming is under survey in Europe. The permission will promote the implementation of further struvite precipitation technologies in WWTPs when struvite becomes a valuable trade product.

Data from the literature reveal that struvite precipitated from animal wastes can contain multiple times the contamination found in the present study. It is therefore important to address from which input material struvite is precipitated when environmental aspects are discussed.

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