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Urine as a Biobased Fertilizer: The Netherlands as Case Study

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SIGNIFICANCE

- Activated carbon (AC) adsorbents can remove practically all detected contaminants from urine—notably pharmaceuticals—in a fast and facile way.
- Both target analysis and suspect screening were performed in this study to allow a thorough assessment of potentially present substances of concern.
- This study shows AC-treated urine adheres to all prerequisites listed in the Dutch fertilizer legislative framework for a material to be considered as fertilizer.

Keywords

Nutrient recycling, Biobased fertilizer, Activated carbon adsorbent, Pharmaceutical removal, Fertilizer legislative framework, Target analysis, Suspect screening

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Bibliography

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ABSTRACT

Humankind has grown increasingly dependent on mineral fertilizer to feed a growing world population. However, nutrients

are rarely recovered and recycled after consumption, leading to environmental waste issues now and potential supply issues in the future. Urine is rich in these nutrients and is therefore a prime candidate as a precursor for biobased fertilizers. This work presents a case study to remove potentially present contaminants from human urine using activated carbon adsorbents to adhere to Dutch fertilizer legislation. In the urine, three pharmaceuticals were identified by target analysis and 11 other contaminants by suspect screening using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). These were successfully removed by treatments with both granulated and powdered activated carbon, albeit with a loss of 16–17% and 2–4% of dissolved nutrients, respectively. Further screening of inorganic contaminants and persistent organic pollutants by inductively coupled plasma mass spectrometry (ICP-MS) and gas chromatography quadrupole-Orbitrap mass spectrometry (GC-Q-Orbitrap/MS) showed that all prerequisites for fertilizer status are met, paving the way for its future legal use as a biobased fertilizer in the Netherlands.

Introduction

Phosphorus (P), nitrogen (N), and potassium (K) are vital macronutrients for life [1]. Fertilization is essential for high crop yields, historically achieved by recycling nutrient-rich organic matter sources such as manure [2]. However, the 19th-century discovery of phosphate rock (PR) and the Haber–Bosch process for nitrogen fixation in the following century revolutionized agriculture [3, 4]. Today, mineral and chemical fertilizers are indispensable, supporting about 50% of food production [4–6]. However, PR is a finite resource, with depletion projected between 50 to 400 years from now [7, 8]. Deposits are unevenly distributed globally, with 75% located in Morocco and the Western Sahara [8]. Furthermore, PR often contains persistent heavy metal (HM) contaminants, such as cadmium (Cd) and uranium (U), which can pose serious health risks [9, 10]. As mined PR quality declines, this issue will worsen [11]. Although Haber–Bosch N fixation has no contamination problem, it is energy-intensive, consuming 1–2% of the world's energy production and responsible for about 1.8% of global CO_2 emissions [12, 13].

Both P and N are used inefficiently. In 2019, 89% of the 21 million tonnes (Mt) of mined P was intended for fertilizer production, yet only about 16% of mined P—or 14% of fertilizer P—was actually consumed by people due to agricultural runoff and losses along the agri-food chain [7, 14–17]. The efficiency of N is similar, with 16% of the 100 Mt of N fixed annually for fertilization being consumed by humans [12]. Following consumption, nutrients enter wastewater (WW) in areas with sanitary infrastructure. With increasing population growth, urbanization, and sewage system development, P and N flows in WW are expected to rise significantly [18]. As such, WW nutrient recovery and recycling are essential to move away from current linear practices [19–21]. Currently, however, less than 10% of P and generally even less N is recovered from WW—3% in the case of Paris, for example [7, 22]. This contributes to environmental issues like eutrophication, harming marine ecosystems, and perturbing biogeochemical cycles as underlined by the Planetary Boundaries framework [23–25].

Urban WW's primary nutrient contributor is urine, accounting for <1% of the volume but contributing 80% N, 70% K, and 50% P [26, 27]. Approximately 85% of N in urine is excreted as urea. This is rapidly hydrolyzed to ammonia due to the presence of urease, elevating the pH to about 9 [27–29]. Both ammonia and phosphate can be recovered, primarily using inorganic salt precipitation such as struvite ($MgNH_4PO_4{\cdot}6H_2O$), K-struvite ($MgKPO_4{\cdot}6H_2O$), or calcium phosphates ($CaHPO_4{\cdot}2H_2O/Ca_3(PO_4)_2$) [20, 30]. However, these products have low solubility, thus releasing nutrients slowly and often requiring further acid treatment to produce more soluble fertilizers [31]. Furthermore, precipitation usually involves chemical additives, such as magnesium (Mg) or calcium (Ca) sources [20, 29]. Alternatively, ammoniacal N can be recovered through ion exchange or air stripping and absorption via acid trapping [32–34]. The downsides are that ion exchangers are often quickly saturated and require frequent regeneration, and air stripping is generally time-consuming and inefficient for waste streams with high ammonia concentrations [35]. Furthermore, as with precipitation, acid trapping requires chemical additives to bind the ammonia [36].

Urine itself can also serve as a fertilizer but requires stabilization to prevent N loss as volatilized ammonia [29, 30]. This can be achieved through nitrification, converting ammonia to nitrate via intermediate nitrite formation using bacteria [37]. Other options include acidification or alkalinization to stabilize urea by inhibiting urease below pH 4 or above pH 10 [29, 30]. A urease inhibitor can also be added, encompassing a variety of compounds binding to the urease enzymatic pocket [30, 38] Post-treatment methods like evaporation, (membrane) distillation, forward/reverse osmosis, or freeze-thawing can reduce urine volume, increasing nutrient concentration or producing solid urine-based fertilizers while improving transportability [29]. Source-separation of urine thus presents an intriguing alternative to end-of-pipe solutions at wastewater treatment plants (WWTPs) [30, 39].

Importantly, approximately 64% of pharmaceuticals and/or their metabolites are excreted via urine [40]. If they end up in the environment, they may pose a significant environmental threat as pharmaceuticals are designed to be effective at low concentrations, potentially interfering with biochemical and physiological processes. [29, 30, 41] Hence, urine and its derived products must be free of pharmaceuticals for safe use as fertilizing materials [30]. Various methods for treating aqueous waste streams for pharmaceutical removal have been explored, including ozonation, UV/peroxidation, nanofiltration, and emerging technologies such as photoelectrochemical treatment [42–46]. Each has its advantages and challenges, requiring further research for practical implementation. Another promising method for removing pharmaceuticals is adsorption, which offers advantages such as low energy consumption, cost-effectiveness, ease of operation, and the potential for adsorbent regeneration [46–49]. Various materials have been proposed to this end, including

metal-organic frameworks, membranes, and activated carbon (AC) [50]. AC—divided into powdered (PAC) and granulated AC (GAC)—is well known for its effectiveness in pharmaceutical adsorption [51–55], with PAC boasting a larger surface area and GAC being easier to handle [48, 52–54]. Furthermore, AC does not induce the formation of pharmaceutical transformation products [56]. It is best used for apolar to moderately polar compound adsorption; highly polar compounds (log $K_{ow} \leq 0$) tend to adsorb less readily [57], although only few such strongly hydrophilic pharmaceuticals exist. Recent studies have explored the use of PAC and GAC for producing safe fertilizer from (nitrified) urine, yielding promising results [48–49, 55]

However, to penetrate the market and promote urine-based fertilizer recycling, adherence to relevant legislation is vital. In the Netherlands, companies GreenPee [58] and SEMiLLA Sanitation [59] have started a collaboration aiming to produce liquid fertilizer from source-separated urine [58, 59] Currently, urine is considered waste rather than a resource, hindering its utilization as a fertilizer [60]. European law permits materials to acquire End-of-Waste (EoW) status under specific conditions, namely having a defined purpose, demand, or market compliance with national technical and legislative requirements, and posing no environmental or public health risks [61]. Dutch legislative requirements for fertilizers include sufficient nutrient content and freedom from inorganic and organic contamination. Adhering to these requirements ensures compliance with environmental and health standards [62, 63].

This study focuses on a real-world case of urine recycling as a liquid biobased fertilizer, adhering to Dutch legislative standards. The primary goal was to remove pharmaceuticals and other micropollutants listed in Dutch fertilizer law from stabilized urine through AC treatment, enabling its legal use as fertilizer in the Netherlands. The removal of pharmaceutical contaminants guided treatment optimization. Subsequently, the treated urine was further investigated for persistent pollutants through target and suspect screening. To the best of our knowledge, no examples in the literature exist where a legislative framework is used to assess a urine-based fertilizer's market suitability, although an exception is found in Switzerland, where the urine-based fertilizer Aurin—produced by Vuna—has successfully entered the market [64]. Furthermore, there is only little precedence on urine suspect screening and none combined with contaminant removal [65].

Methods and Materials

Dutch Fertilizer Legislation

To facilitate the use of urine as a biobased fertilizer, this study uses the prerequisites for the application for fertilizer status in the Netherlands as a framework for conducted treatments and analyses. These can be divided into fertilizing components, inorganic contaminant concentrations, and organic contaminant concentrations, specified here for a liquid organic material [62]. Requirements for fertilizing components are 0.5% wt of N, P_2O_5, or K_2O in the respective dry matter of the liquid material. Should N be the only fertilizing component, at least 85% has to be bound organically. Alternatively, 20% of dry matter should be organic matter [62].

Inorganic contaminants refer to heavy metals (HMs) or metalloids. HMs subject to legal limits are cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn), as well as arsenic (As). Maximum allowable concentrations are given in ▶ **Table 1** and are expressed as mg HM per kg of the major fertilizing component, which is defined as the one that would reach either 80 kg P_2O_5, 100 kg N, 150 kg K_2O, or 3000 kg organic matter first with increasing application [62]. In the case of urine, this is N [28].

Organic contaminants are split into two different categories: contaminants with a defined concentration limit and those without. Contaminants falling in the first category are persistent organic pollutants (POPs), more specifically polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polychlorinated dibenzodioxins/furans (PCDF/PCDD). Maximum allowable concentrations are again expressed as mg POP per kg of the major fertilizing component and are listed in ▶ **Table 2**.

Contaminants for which no legal limits are set may, in principle, be present in fertilizer. However, their application to soil

▶**Table 1** Maximum inorganic contaminant concentrations allowed for a Dutch fertilizer [60].

Contaminant	Maximum concentration allowed per fertilizing component		
	(mg kg N^{-1})	(mg kg $P_2O_5^{-1}$)	(mg kg K_2O^{-1})
Cd	25	31.3	16.7
Cr	1500	1875	1000
Cu	1500	1875	1000
Hg	15	18.8	10
Ni	600	750	400
Pb	2000	2500	333
Zn	6000	7500	4000
As	300	375	200

▶**Table 2** Maximum organic contaminant concentrations allowed for a Dutch fertilizer [60].

Contaminant	Maximum concentration allowed per fertilizing component		
	(mg kg N^{-1})	(mg kg $P_2O_5^{-1}$)	(mg kg K_2O^{-1})
Σ PCDD/PCDF	0.015	0.019	0.010
α-HCH	248	310	165
β-HCH	9.6	12	6.4
γ-HCH (lindane)	0.96	1.2	0.64
HCB	31.2	31	20.8
Aldrin	5.6	7	3.7
Dieldrin	5.6	7	3.7
Σ Aldrin/Dieldrin	5.6	7	3.7
Endrin	5.6	7	3.7
Isodrin	5.6	7	3.7
Σ Endrin/Isodrin	5.6	7	3.7
Σ DDT + DDD + DDE	18.6	23	12.3
PCB-28	14.8	18.5	9.9
PCB-52	14.8	18.5	9.9
PCB-101	60	75	40
PCB-118	60	75	40
PCB-138	60	75	40
PCB-153	60	75	40
PCB-180	60	75	40
Σ 6-PCB (excl. PCB-118)	300	375	200
Naphtalene	480	600	320
Fenanthrene	600	750	400
Anthracene	480	600	320
Fluoranthene	148	185	98
Benzo(a)anthracene	184	230	123
Chrysene	184	230	123
Benzo(k)fluoranthene	216	270	144
Benzo(a)pyrene	232	290	155
Benzo(g,h,i)perylene	168	210	112
Indeno(1,2,3-c,d)pyrene	188	235	125
Σ 10-PAK	9200	11,500	6133
Mineral oil	748,000	935,000	498,668

can be limited by either legal soil concentration limits or "indicative environmental risk" (IER) values [62], the latter of which can be derived based on contaminant physicochemical characteristics.[66] In the absence of either parameter, the presence of the respective contaminant will result in the disqualification of the material as fertilizer [62]. For most pharmaceuticals, neither is available [67, 68]. In this study, no IER values were derived and the complete removal of pharmaceuticals was targeted as recommended in the literature [29, 30].

Analyte Selection

The selection of analytes assessed in this study was based on commonly used and frequently studied pharmaceuticals

(▶**Table 3**) [69, 70]. Analytical grade (>95% purity) reference- and isotope-labeled standards were purchased from Sigma Aldrich or Acros Organics and are listed in SI Table S1, as are other auxiliary chemicals.

Urine Sampling and Stabilization

Urine samples were collected in July 2021 at a Green Pee urinal situated in the city center of Amsterdam [74]. To avoid significant urea hydrolysis, the urease inhibitor solution "Fertiflow Add Green" was added to the collection tank of the urinal, consisting of 2,2-dimethyl-1,3-dioxolan-4-ylmethanol (60–64%) and n-butyl-thiophosphoric triamide (25–27.5%) in water [75]. The urease inhibitor solution was dosed at 5 mL per 100 L urine. Urine samples were stored at 4 °C prior to treatment and subsequent analysis.

AC Treatment

Collected urine samples were subjected to either PAC or GAC treatment. Treatment with PAC (Norit A SUPRA EUR, surface area 1700 m^2 g^{-1}, particle size D_{50} 20 µm, pH neutral, Norit, Amersfoort, The Netherlands) was performed batchwise in a 50 mL round bottom flask. PAC doses of 2 and 3 g L^{-1} were achieved by the addition of 50 and 75 mg PAC to 25 mL urine, respectively. Experiments were stirred at 400 rpm and room temperature. Samples were taken at regular time intervals by syringe, filtered over a 0.45 µm PTFE disposable syringe filter (Whatman), and stored at 4 °C in glass vials until sample preparation. Treatment with GAC (Norit GAC 1240 W, surface area 1100 m^2 g^{-1}, particle size D_{50} 1.7 mm, pH alkaline, Norit, Amersfoort, The Netherlands) was performed using a glass column (diameter 1 cm) fitted with a frit filter and tap. A volume of 300 mL of urine was used per experiment, with GAC loadings of 2, 10, and 30 g L^{-1} achieved by the addition of 0.6, 3, and 9 g GAC, respectively. Column lengths measured at 1.3, 6.7, and 20 cm, respectively. The percolation rate was controlled using the tap and calculated back after the column was finished, rounded to 15-minute increments. Samples were filtered over a 0.45 µm PTFE disposable syringe filter (Whatman) and stored at 4 °C in glass vials until sample preparation. See SI Table S2 for more information on the used ACs and SI Fig. S1 for a diagram of the cleaning process.

Sample Preparation and Analysis

Sample extraction was conducted based on the methods described in Kovalova et al. and Hernández et al., used for target analysis [76, 77].

Both treated and untreated urine matrices were filtered through a 0.45-µm PTFE disposable syringe filter (Whatman) and diluted 100-fold using a mixture of 0.1% formic acid (99%, ULC-MS, Biosolve) in Milli-Q water (Reference A+ System). An aliquot of 20 mL of the diluted matrix was taken, to which 5 µL of the isotope-labeled standard mix was added. SPE cartridges (Oasis HLB, 60 mg, 3 cc, Waters) were preconditioned with 3 mL of methanol (ULC-MS, Biosolve) followed by 3 mL Milli-Q water for LC-qToF analysis, whereas SPE cartridges (Sep-Pak C_{18}, 500 mg, 3 cc, Waters) were preconditioned with 5 mL of methanol followed by 5 mL Milli-Q water for GC-qOrbitrap analysis. Diluted samples were loaded on the SPE cartridges and percolated at a rate of 1 drop per 2 s. Upon completion, the cartridges were dried under vacuum for 15 min. Samples were eluted with 3 mL methanol and 5 mL cyclohexane (glass distilled grade, Rathburn) for LC and GC analysis, respectively at a rate of 1 drop per 2 s. Sample extracts for LC analysis were evaporated to dryness under a gentle nitrogen stream, reconstituted in 1 mL of 10:90 methanol:Milli-Q—0.1% formic acid, and filtered through a 0.22 µm polypropylene disposable syringe filter (FilterBio). Sample extracts for GC analysis were evaporated under a gentle nitrogen stream until approximately 0.5 mL was left, which were then increased to 1 mL by adding cyclohexane and filtered over a 0.22 µm PTFE disposable syringe filter (FilterBio) using a glass syringe. All samples were prepared in triplicate, and a sample blank was prepared for each set of urine samples.

Target and Suspect Screening Using LC-QToF-MS

A UHPLC system (Nexera, Shimadzu, Den Bosch, The Netherlands) coupled to a Bruker Daltonics maXis 4G high-resolution q-ToF/MS

▶**Table 3** Selected pharmaceutical analytes and relevant characteristics.

Analyte	Formula	pK_a	Log K_{ow}	Description
Atenolol	$C_{14}H_{22}N_2O_3$	9.54 [71]	0.22 [71]	Beta-blocker
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	9.96, 8.87 [71]	−0.03 [71]	Diuretic
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	3.99 [71]	4.51 [71]	NSAID
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	5.72 [72]	0.68 [72]	Antibiotic
N4-Acetylsulfamethoxazole	$C_{12}H_{13}N_3O_4S$	5.88[a]	0.86[a]	Metabolite of SMX
Ibuprofen	$C_{13}H_{18}O_2$	4.42 [71]	4.13 [71]	NSAID
Naproxen	$C_{14}H_{14}O_3$	4.18 [71]	3.24 [71]	NSAID
Trimethoprim	$C_{14}H_{18}N_4O_3$	7.13 [72]	0.91 [72]	Antibiotic

[a]Given values are predicted using Chemaxon [73].

upgraded with HD collision cell and equipped with an ESI source (Leiderdorp, The Netherlands) was used for analysis. The instrumental analysis method was based on previously published work by Narain-Ford et al. and Das et al [78, 79]. In short, a reversed-phase Kinetex 1.7 µm Biphenyl 100 Å, LC Column 150 × 2.1 mm (Phenomenex, Utrecht, The Netherlands) column was used to achieve separation. The mobile phases used for this study were ultrapure water with 0.05% acetic acid (mobile phase A) and MeOH (mobile phase B). The total flow rate was 0.3 mL/min. For the analysis, 20 µL of the sample was injected for positive and negative ESI mode analysis. The column oven and tray temperatures were 40 °C and 15 °C, respectively. Data-independent MS/MS scans were obtained for target analysis, while data-dependent MS/MS scans were acquired for suspect screening. TASQ software (version 2021, Bruker Daltonics) was used for data processing for the detection and quantification of target analytes. Details of the quantification method can be found elsewhere [78, 79], and the list of quantifier and qualifier ions for the target analytes can be found in SI Table S3.

The initial step in the suspect screening process involved manually *m/z* calibrating all raw HRMS data using Bruker DataAnalysis 4.4 software (Bruker Daltonics). The *m/z* calibration was conducted within a search range of 0.15 *m/z*, ensuring a calibration error of less than 0.5 ppm. Subsequently, centroiding and converting the data into mzML format were done in ProteoWizard v.3.0.22119. Further steps were performed in *R*, using the open-source platform *patRoon* (version 2.0.0) [80]. First, all features were found and grouped over multiple replicates using the *OpenMS* algorithm [81]. The amount of feature groups was then narrowed down using rule-based filtering. For example, a minimum intensity threshold of 10,000 was employed. Other settings can be found in SI Fig. S2. Componentization was performed using *RAMclustR* (version 1.2.2) [82], and features from positive and negative ionization data were grouped with a "sets workflow" [83]. The suspect screening itself was the next filter, where remaining feature groups were compared with a predetermined suspect list and kept if they match. For this, the NORMAN priority contaminants list was used, comprising 976 chemicals of concern including pharmaceuticals, pesticides, preservatives, and industrial chemicals [84]. Finally, feature annotation was performed by retrieving respective MS peak lists using *mzR* (version 2.28.0) [85], and matching them to generated formulae and *in silico*-predicted fragmentation data using *GenForm* and *MetFrag* (version 2.4.5) [86, 87], respectively. PubChemLite (January 2021) was used as a database [88]. Tentatively identified suspects were subsequently ranked according to their confidence level (1–5) based on the proposition by Schymanski et al. and explained in the supporting information (SI Fig. S2) [89]. Suspects ranked level 4 and lower were discarded and the remainder were manually inspected and compared to MS^2 data from the spectral libraries European MassBank and the MassBank of North America [90, 91].

Target Analysis Using GC-qOrbitrap/MS

Analyses were performed using a Thermo Trace 1300 gas chromatograph and a Thermo Exactive Orbitrap mass spectrometer. An Agilent J&W DB5-MS (30 m × 0.25 mm × 0.25 µm) column was used to achieve the separation. TriPlus RSH autosampler was used to inject 1 µL of extract, using a pulsed splitless injection with an injector temperature of 250 °C. Helium was used as the carrier gas and was set at a constant flow of 1.2 mL min^{-1}. The MS scan range was 50–650 *m/z*. Obtained chromatograms were interpreted with the Excalibur software (Version 3.1, Thermo Fischer Scientific Inc.) using the MS Search Program (Version 2.3, Thermo Fischer Scientific Inc.). Peaks in calibration sample chromatograms were matched to their respective analyte using a National Institute of Standards and Technology (NIST) user library containing detailed MS information of the target analytes. Calibration sample chromatograms were subsequently compared to treated urine sample chromatograms to assess the presence of screened analytes. All screened analytes are listed in SI Table S5.

Inorganic Measurements

The elemental compositions of two untreated urine samples were elucidated by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent ICP-MS 7800 according to NEN-EN-ISO 17294-2 and conducted by Groen Agro Control (Oss, The Netherlands). Values and standard deviations are listed in ▶ **Table 7** and are based on the average of the two datasets.

The N and P content of pre- and post-treated samples were measured using a Skalar San++ 5000 segmented flow analyzer equipped with an SA 1074 autosampler. Samples were diluted 1000-fold prior to analysis. For the determination of N concentration, the samples were mixed with a potassium peroxodisulfate/sodium hydroxide solution and heated to 70 °C. The solution was subsequently mixed with a borax buffer and brought into a UV digester. After dialysis, the nitrate content was determined by the Griess reaction after the reduction of the nitrate to nitrite using a cadmium-copper reductor. The color was measured at 540 nm. For the determination of P concentration, the diluted samples were reacted with ammonium heptamolybdate and potassium antimony(III) oxide tartrate in an acidic medium to form an antimony-phospho-molybdate complex. This complex was reduced to an intensely blue-colored complex by L(+)ascorbic acid and measured at 880 nm.

Quality Control

Method recovery experiments were performed in triplicate using the sample preparation method for LC-QToF-MS analysis described previously in *Sample Preparation and Analysis*, where samples were spiked with known concentrations of analytes pre-extraction. Recoveries were determined according to eq 1.

$$\text{Recovery}\ (\%) = \frac{C_{\text{Spiked Sample}} - C_{\text{Unspiked Sample}}}{C_{\text{Added}}} \times 100\% \quad (1)$$

Matrix effects (ME) were determined using the post-extraction addition method,[92] according to eq 2. Positive values indicate ion enhancement, whereas negative values indicate ion suppression.

$$\text{ME}\ (\%) = \left(\left(\frac{\text{Peak area in matrix}_{\text{post-extraction}}}{\text{Peak area without matrix}}\right) - 1\right) \times 100\% \quad (2)$$

Method performance was evaluated based on the linearity of the calibration curves, recoveries, the limit of detection (LOD), limit of quantification (LOQ), and the matrix effect.

Results and Discussion

Quality Control

Recoveries were within 70–130% for 75% of the target analytes and correlation coefficients were >0.99 in all cases. Both atenolol and trimethoprim showed low recoveries. The LOD ranged from 0.05 to 1.70 ng mL^{-1} and LOQ from 0.16 to 5.16 ng mL^{-1}. These numbers are further detailed in ▶**Table 4**. ME indicated signal suppression for all analytes except atenolol and trimethoprim, which were also the ones with low recoveries. Trimethoprim showed an unusually high matrix effect. ME were corrected during sample analysis by the addition of stable isotope-labeled internal standards pre-extraction, with the exception of hydrochlorothiazide (see SI Table S3 for standards used).

Pharmaceuticals in Untreated Urine

As pharmaceutical removal was leading in determining viable treatment conditions, pharmaceutical concentrations were first determined in untreated urine. Of the target pharmaceutical analytes, only ibuprofen, naproxen, and hydrochlorothiazide (HCT) were detected. Ibuprofen (465 ± 49.8 ng mL^{-1}) and naproxen (172 ± 48.4 ng mL^{-1}) showed high concentrations. Both are common non-steroidal anti-inflammatory drugs (NSAIDs), available for purchase without prescription at any drug store in the Netherlands. HCT—a commonly prescribed diuretic—was only detected in minor amounts (2.18 ± 0.24 ng mL^{-1}). Diuretics are used to a much lower degree than NSAIDs, explaining the lower concentration as compared to the detected NSAIDs. Furthermore, HCT is known to degrade over time in urine under anaerobic conditions at pH 9 via hydrolysis.[55] While these conditions differ from the ones employed in this work, degradation during storage remains a possibility.

Pharmaceutical Removal by PAC Treatment

A recent study by Özel Duygan et al. showed the efficacy of using PAC for pharmaceutical removal in spiked source-separated urine, reporting over 90% removal of 12 pharmaceuticals after 24 h using 200 mg L^{-1} PAC [55]. Concentrations of those pharmaceuticals were similar to those found in our samples, but they were different compounds. In a different study by Mailler et al., it is stated that ibuprofen adsorbs relatively poorly to PAC [93]. As this study aims for complete pharmaceutical removal—notably ibuprofen—using short treatment times, investigation of PAC treatment commenced using a loading of 2 g L^{-1}. Under these conditions, all three detected pharmaceuticals present in the untreated urine were almost completely removed after 1 h of mixing. Hydrochlorothiazide was not detected and naproxen was found to be below LOD. Naproxen was not detected anymore after 6 h of mixing. However, ibuprofen was consistently detected even after 24 h, albeit below LOQ at all time intervals (▶**Table 5**). Increasing the loading to 3 g L^{-1} showed detected analytes to be completely removed in 1 h (▶**Table 5**). The short contact time required can be attributed to PAC's high surface area and high dispersion by mixing. Yet, its physical appearance as a fine powder makes it a material difficult to handle, and post-treatment filtrations proved cumbersome. Attempts at using PAC in a column failed due to immediate frit clogging and consequent negligible flow rates, making PAC not ideal for larger-scale applications.

Pharmaceutical Removal by GAC Treatment

Next, we compared PAC with GAC and used initial column loadings for GAC treatment of 2 g L^{-1}. Two different percolation rates were investigated to assess the influence of residence time, corresponding to 6 and 18 h running time. Both ibuprofen and naproxen were still present in high concentrations of 304 ± 34.5 and 107 ± 31.6 ng mL^{-1}, respectively, using a 6 h running time (▶**Fig. 1**). Note that hydrochlorothiazide is not shown in ▶**Fig. 1** as it was not detected in any of the samples. Interestingly, a lower percolation rate appeared to have little

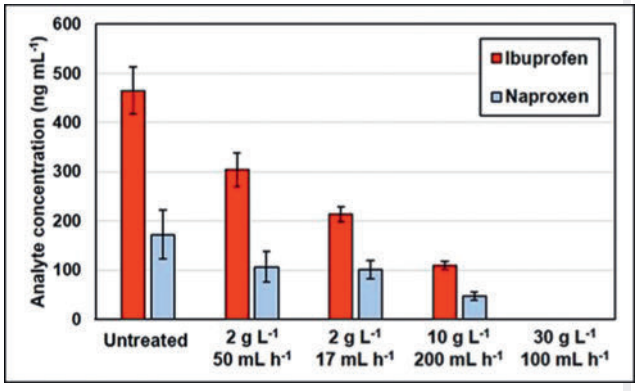
▶**Table 4** Analysis method performance (n = 3).

Analyte	Linearity (R^2)	Calibration range (ng mL^{-1})	LOQ (ng mL^{-1})	LOD (ng mL^{-1})	Recovery (% ± SD)	Matrix effect (%)
Atenolol	0.996	1–25	5.03	1.66	23 ± 1	+16
Hydrochlorothiazide	0.997	0.5–12.5	2.25	0.74	99 ± 3	−60
Diclofenac	0.997	0.2–5	0.92	0.30	90 ± 6	−25
Sulfamethoxazole	0.996	1–25	1.79	0.59	82 ± 7	−44
N4-Acetyl sulfamethoxazole	0.999	0.2–5	0.41	0.14	128 ± 2	−65
Ibuprofen	0.995	2–70	5.16	1.70	85 ± 5	−30
Naproxen	0.998	0.2–25	2.04	0.67	70 ± 16	−40
Trimethoprim	0.995	0.2–5	0.16	0.05	36 ± 4	+208

▶**Table 5** Results for PAC treatment experiments.[a]

Analyte	Initial concentration (ng mL^{-1})	2 g PAC L^{-1} (ng mL^{-1})			3 g PAC L^{-1} (ng mL^{-1})
		1 h	6 h	24 h	1 h
Ibuprofen	465 ± 48.4	<LOQ	<LOQ	<LOQ	nd
Naproxen	172 ± 49.8	nd	nd	nd	nd
Hydrochlorothiazide	2.18 ± 0.24	nd	nd	nd	nd

[a]nd, not detected.



▶**Figure 1** Ibuprofen and naproxen concentrations after GAC treatment, using different loadings and percolation rates.

effect on naproxen removal, whereas ibuprofen adsorption was significantly higher. Hydrophobicity—represented by log K_{ow}—is an important factor in assessing the adsorption tendency of micropollutants, influencing the adsorption capacity of micropollutants onto GAC [48, 56]. Hence, a possible explanation for the different responses between naproxen and ibuprofen could be the difference in log K_{ow}. Ibuprofen has a log K_{ow} of 4.13, whereas naproxen sits at 3.24, making ibuprofen the more lipophilic of the two. While others have found no correlation between log K_{ow} and pharmaceutical adsorption rates, those conclusions were drawn using PAC and varying AC loading instead of contact time [55, 70]. This result also points toward an interplay between advection and diffusion within the system, where diffusion can be regarded as analyte penetration into the porous structure of GAC. As aqueous diffusion coefficients of ibuprofen are in the order of 1–10^{-10} m^2 s^{-1}—many orders of magnitude smaller than accompanying advection rates in such systems—lower percolation rates will virtually always allow for more diffusion and thus adsorption, not taking into account possible saturation of the adsorbent surface.[94] This appears to hold especially true for more hydrophobic species, although a broader data set would be warranted to state this with more certainty.

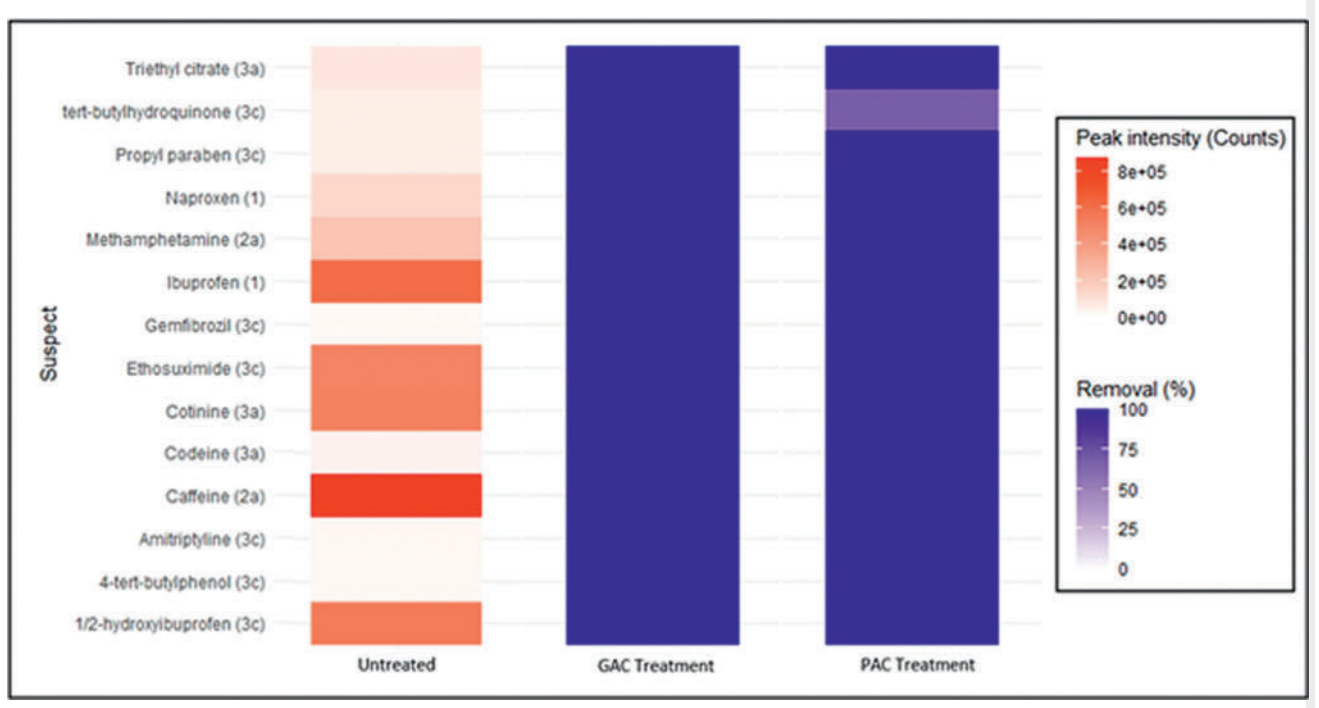
An adaptation to the setup, using a higher loading of 10 g L^{-1} GAC and 200 mL h^{-1} as percolation rate showed significantly improved removal at about 75% for both analytes (▶**Fig. 1**). This indicates that increased loading and column length are preferred over longer residence time for pharmaceutical removal—although both play an important role. Finally, complete removal of both analytes was achieved using 30 g L^{-1} at a percolation rate of 100 mL h^{-1}. To be able to truly make a predictive curve on pharmaceutical removal as a function of AC loading and percolation rate, detailed information on diffusion rate, advection rate, and adsorption tendency per analyte is needed. As these are also dependent on the matrix of the system, this would require significant resources to elucidate and was therefore deemed outside the scope of the current study.

Considerations on AC Treatment

GAC column treatment was preferred over PAC mixing due to ease of handling, implementation, and scale-up as well as the potential for continuous treatment. Important to consider is the regeneration of AC. Many studies have investigated this, and it is possible to extend AC lifetime by steam, microwave, thermal, and chemical means, among others [51, 95–97]. In some cases, adsorptive capacities were shown to be increased after regeneration [96]. Should the used AC become truly depleted, however, incineration will likely prove necessary as is the case for AC used in hazardous contexts [51]. An interesting alternative novel adsorbent is biochar, a porous carbonaceous material produced by the thermochemical treatment of biomass in an oxygen-deprived environment (pyrolysis) [98]. It is considered to be a more sustainable material than AC, especially when produced from lightly contaminated waste such as sewage sludge, and should thus be considered when investigating the adsorption of contaminants [99]. However, it does show significantly lower surface areas, which is why AC was chosen over biochar in this particular study.

Suspect Screening

To thoroughly assess the efficacy of AC treatment, urine samples both before and after complete pharmaceutical removal were subjected to suspect screening. For untreated urine, this yielded 95 unique hits with confidence level 4 and above (see SI Fig. S2 on confidence levels). This was narrowed down to 13 hits by manual investigation of MS/MS data for confidence levels 3 and above (▶**Fig. 2**), while hits with confidence level 4 were not taken into further consideration. Of the three pharmaceuticals detected using target screening, initially, only naproxen was tentatively identified during suspect screening.



▶ **Figure 2** Tentatively identified compounds in untreated, GAC- and PAC-treated urine, with identification levels in brackets.

An explanation for the absence of ibuprofen was found after inspecting the extracted chromatogram (*m/z* = 205.1234 ± 0.005) using *DataAnalysis* software. In the data-dependent acquisition method required for subsequent suspect screening, ibuprofen's qualifier ion (*m/z* 161.1330) cannot be detected. Lacking the MS/MS data required for a high enough confidence level, the feature was removed during the workflow. Ibuprofen's presence is certain as it was found during target analysis, therefore ibuprofen was incorporated in ▶ **Fig. 2**. Its metabolite 1/2-hydroxyibuprofen did show up in the suspect screening, corresponding to a significant feature intensity second only to caffeine and ibuprofen itself.

HCT was only found in low concentrations during target screening, so the possibility of it having been filtered out in the suspect screening workflow was checked manually. Indeed, extraction of the relevant chromatogram (*m/z* = 295.9572 ± 0.005) showed the intensity of the corresponding peak to be 3024, whereas an intensity threshold of 10,000 was used. For the sake of consistency with regard to other compounds which may also have been excluded due to this threshold, HCT was not included in ▶ **Fig. 2**.

The other tentatively identified suspects can be divided into food additives, pharmaceuticals, and—more roughly—stimulants. In the case of food additives, triethyl citrate (E1505), *tert*-butylhydroquinone (E319), and propylparaben (E216) were found. Identified pharmaceuticals—other than the ones discussed above—were codeine (opioid), ethosuximide (anticonvulsant), amitryptiline (antidepressant), and gemfibrozil (a cholesterol-lowering drug). Grouped as "stimulants", caffeine, cotinine (nicotine metabolite), and methamphetamine (drug of abuse) were tentatively identified. Perhaps unsurprisingly, caffeine showed the highest intensity count out of all suspects.

The only tentatively identified suspect that could not be readily placed in one of these categories is 4-*tert*-butylphenol. Most commonly used in the curing of epoxy resins, as a plasticizer and as a surfactant, its presence in urine is less easily explained as compared to the other suspects [100]. However, its presence in urine has been confirmed in a previous study, showing the plausibility of the suspect [101], ▶ **Fig. 2** lists all tentatively identified suspects and their respective peak intensity counts, as well as their removal rates by AC treatment. Identification levels for each suspect are given in brackets. After PAC and GAC treatment, suspect screening yielded 7 and 2 hits, respectively, brought back to only *tert*-butylhydroquinone being tentatively identified after manual inspection. As an isolated case, this sole suspect was not completely removed after PAC treatment with a removal rate of 69%.

Three other chemicals were identified but not incorporated into the final list of tentatively identified suspects. Firstly, diethyl phthalate was found in untreated urine as well as both PAC- and GAC-treated urine. However, it is a commonly used plasticizer and results indicated higher concentrations after AC treatment than before. Hence, it was concluded that the suspect was likely leached from plastics used in sample preparation and handling. Secondly and thirdly, (S)-mandelic acid and 4-hydroxyphenylacetic acid were tentatively identified but not part of the NORMAN suspect list. This is due to the nature of the suspect screening workflow: in these cases, the compounds

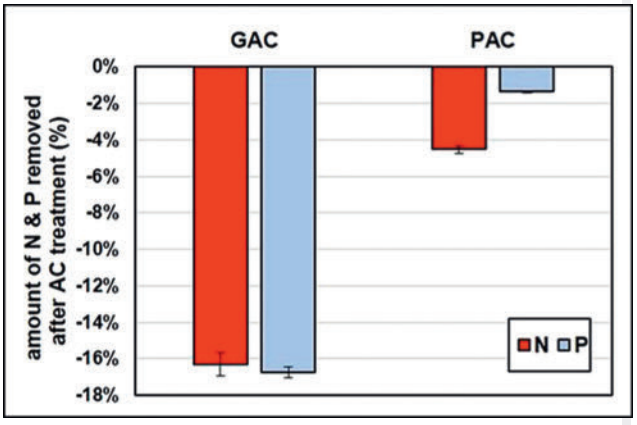
were part of the candidates obtained with MS/MS compound annotations, for which at least one candidate was part of the suspect list. Upon inspection, the MS/MS data pointed toward candidates 2nd and 5th in rank for their respective features, which were not part of the suspect list and therefore not further considered. See SI Part 2 S.1 and S.2 for detailed information on suspects found in negative and positive mode, respectively.

These results highlight the efficacy of both AC treatments; not only were all the tentatively identified suspects removed except one in the case of PAC (*tert*-butylhydroquinone), but the initial hits were reduced from 95 to 7 and 2 for PAC and GAC treatment, respectively. While these initial hits were not all tentatively identified, they do correspond with species in the urine matrix which were subsequently removed.

$$\text{Fertilizing component in dry matter (\%)} = \frac{\text{Nutrient content (\%)}}{\text{Dry matter content (\%)}} \times 100\% \quad (3)$$

Influence of AC Treatment on Nutrient Content

While recent work has shown that nutrient content is not significantly or only slightly affected by AC treatment [49, 102], the relatively high loadings used in this study could adversely affect nutrient concentrations to a higher degree. As such, selected urine samples were measured in triplicate for N and P content pre- and post-treatment. GAC treatment showed a similar reduction in both N and P content at 16.3 and 16.7%, respectively, where initial concentrations were 2426 ± 53 mg L^{-1} N and 817 ± 84 mg L^{-1} P_2O_5. PAC treatment had significantly less impact on nutrient concentrations, with only 4.5% N and 1.4% P removed (▶**Fig. 3**). This significant difference can be ascribed to the different AC loadings of 30 g L^{-1} GAC versus 3 g L^{-1} PAC, as well as shorter residence time in the case of the latter. Hence, while GAC offers much easier treatment in terms of handling, the removal of nutrients using this material with the chosen parameters is apparent and should be taken into account when aiming to produce fertilizer from urine at scale.



▶**Figure 3** P and N removal by AC treatment.

Closing the Loop: A Dutch Fertilizer

The removal of organic contaminants of concern using AC treatment has now been established. Yet, for a urine-based fertilizer—or any novel fertilizer for that matter—to qualify as legal in the Netherlands, it needs to adhere to legal limits regarding fertilizing components, inorganic contaminants, and POPs. Results verifying this adherence are discussed in the following paragraphs.

Fertilizing Components

As mentioned, adherence to fertilizing components requires the dry matter of the respective liquid material to contain 0.5% wt of either N, P_2O_5, or K_2O. In this study, N and P_2O_5 were focused on in particular. Dry matter content was determined according to EN 15934:2012 (see SI Table S4) and found to be 0.575% ± 0.003%. Based on eq 3, the fertilizing components N and P in dry matter after AC treatment were calculated and are given in ▶**Table 6**. Two datasets were used for this; values given are means and standard deviations are included. In both cases, the fertilizing component in dry matter adheres with ease to the legal minimum required for a material to be considered as fertilizer in the Netherlands: 11.9% ± 1.49 and 35.4% ± 0.81 for P_2O_5 and N, respectively.

Inorganic Contaminants

Inorganic contaminants (HMs) are defined as a function of the most abundant fertilizing component—in this case, N. HM contents per N (mg kg^{-1}) were determined based on the same datasets as above and offset against their legal limits in ▶**Table 7**. It should be noted that the given HM concentrations (second column) represent untreated urine, as HMs were not expected to be removed during AC treatment and therefore not measured post-treatment. However, the N concentration was corrected for N removal by GAC treatment (–16.3%) to establish a lower N limit. The HM content per N can thus be seen as an upper limit and worst-case scenario for these particular datasets. In all cases, HM content per N was far below the maximum allowed legal limit.

POP Screening

Adhering to maximum allowable POP concentrations is the final prerequisite for a fertilizer material to be legally eligible in the Netherlands. POPs in question include OCPs, PCBs, and PAHs, which were not included in the suspect screening. Due to the lipophilicity of these compounds, they tend to accumulate in body fat rather than being excreted [103]. As such, their presence in urine was not expected—especially following AC treatment. Screening for POPs was therefore done in a qualitative manner. Indeed, none of the screened POPs listed were detected in AC-treated urine (SI Table S5). Polychlorinated dibenzodioxins (PCDDs) and -furans (PCDFs) were not screened in this process due to budgetary constraints. They do, however, have the strictest limit with an allowable concentration of all POPs at 15 µg Σ PCDD/PCDF kg N^{-1}, which invites further discussion. In a recent study by Han et al., Σ_{27} PCDD/PCDF concentrations were determined

▶**Table 6** Determination of fertilizing components in dry matter (%) based on urine dry matter content and nutrient contents.

Fertilizing component	Required fertilizing component in dry matter (%)	Fertilizing component in liquid urine (%)	Fertilizing component in dry matter (%)
P_2O_5	0.5	0.068 ± 0.009	11.9 ± 1.49
N	0.5	0.20 ± 0.005	35.4 ± 0.81

▶**Table 7** HM concentrations in untreated urine, offset against N content and their legal limits.

Inorganic contaminant	Concentration (µg L^{-1})	HM content per N (mg HM kg N^{-1})	Max. HM allowed per N (mg HM kg N^{-1})[62]
Cd	<0.1	<0.05	25
Cr	36.6 ± 11.1	18.00 ± 5.46	1500
Cu	35.4 ± 12.5	17.38 ± 6.12	1500
Hg	<0.05	<0.02	15
Ni	3.3 ± 0.15	1.60 ± 0.07	600
Pb	0.21 ± 0.04	0.10 ± 0.02	2000
Zn	15 ± 0.02	7.38 ± 0.10	6000
As	0.99 ± 0.52	0.48 ± 0.25	300

at 1640–5415 pg L^{-1} in blood samples from people living in industrialized areas in China prone to dioxin emissions [104]. Considering a hypothetical scenario where a person at the upper limit of said PCDD/PCDF concentrations would excrete it all in one urinary session, this would result in about 14–27 µg Σ PCDD/PCDF kg N^{-1}; at or slightly above the legal limit (see SI Part 2 S.3 for assumptions made and data used). As this scenario is far from realistic, we can assume with reasonable confidence that dioxins will not pose a problem in adhering to legal fertilizer standards. Lastly, mineral oil was also not taken into account due to its very high permissible concentration of 748 g kg N^{-1}. Clearly, this limit was set for fertilizer materials originating from different sources and is not possible to reach in a urine-based fertilizer. As such, all prerequisites to apply for fertilizer- and EoW status are met for AC-treated urine, paving the way for its legal use as fertilizer in the Netherlands.

Conclusions

In this study, we set out to provide proof-of-principle for the possible production of a biobased fertilizer from source-separated urine using AC adsorbents, with the use of national (Dutch) fertilizer legislation as guiding framework and the application of additional suspect screening as notable novelties in this field. Three of the eight target pharmaceuticals were detected pre-treatment, of which common NSAIDs ibuprofen and naproxen were found in high concentrations. Furthermore, 11 contaminants of concern were tentatively identified using the NORMAN list of priority contaminants consisting of 976 substances. After 1 h of mixing with 3 g L^{-1} PAC, only one suspect was still detected. Treatment with 30 g L^{-1} GAC using a column with a running time of 3 h completely removed all identified substances. Screening for inorganic contaminants and POPs—in line with Dutch fertilizer legislation—showed all further requirements for fertilizer status to have been met. The high AC loadings employed did, however, affect the nutrient concentrations, especially using the GAC treatment with about 16% of both N and P removed. Still, GAC treatment is the preferred treatment method for further scale-up and implementation due to its significantly easier operation. Scaling up this method would be an interesting avenue for future investigation and should be explored to allow for the use of urine as biobased fertilizer in meaningful quantities. Subsequent investigations into the longevity and regeneration potential of the used GAC material could then shed further light on the large-scale potential of this method.

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Primary Data

Primary data for this article are available online at https://zenodo.org/record/10992983 and can be cited using the following DOI: 10.5281/zenodo.10992983

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*; Garland Science: New York **2008**.

[2] Garcia-Ruiz, R.; González de Molina, M.; Guzmán, G.; Soto, D.; Infante-Amate, J. *J. Sustain. Agric.* **2012**, *36*, 650–682.

[3] Corbridge, D. E. C. *Phosphorus - Chemistry, Biochemistry and Technology*; CRC Press: Boca Raton **2013**.

[4] Erisman, J. W.; Sutton, M. A.; Galloway, J.; Klimont, Z.; Winiwarter, W. *Nat. Geosci.* **2008**, *1*, 636–639.

[5] Scholz, R. W.; Roy, A. H.; Hellums, D. T. in *Sustain. Phosphorus Manag. - A Transdiscipl. Roadmap* (Eds.: Scholz, R.W.; Roy, H.; Brand, F.S.; Hellums, D.T.; Ulrich, A.E.); Springer: Dordrecht **2014**, 1–128.

[6] Galloway, J. N.; Townsend, A. R.; Erisman, J. W.; Bekunda, M.; Cai, Z.; Freney, J. R.; Martinelli, L. A.; Seitzinger, S. P.; Sutton, M. A. *Science* **2008**, *320*, 889–892.

[7] Cordell, D.; Drangert, J.; White, S. *Glob. Environ. Chang.* **2009**, *19*, 292–305.

[8] van Kauwenbergh, S. J.; Stewart, M.; Mikkelsen, R. *Better Crop.* **2013**, *97*, 18–20.

[9] Atafar, Z.; Mesdaghinia, A.; Nouri, J.; Homaee, M.; Yunesian, M.; Ahmadimoghaddam, M.; Mahvi, A. H. *Environ. Monit. Assess.* **2010**, *160*, 83–89.

[10] Cheraghi, M.; Lorestani, B.; Merrikhpour, H. *Biol. Trace Elem. Res.* **2012**, *145*, 87–92.

[11] Watson, I.; van Straaten, P.; Katz, T.; Botha, L. in *Sustain. Phosphorus Manag. - A Transdiscipl. Roadmap* (Eds.: Scholz, R.W.; Roy, H.; Brand, F.S.; Hellums, D.T.; Ulrich, A.E.) Springer: Dordrecht **2014**, 153–173.

[12] Matassa, S.; Batstone, D. J.; Hülsen, T.; Schnoor, J.; Verstraete, W. *Environ. Sci. Technol.* **2015**, *49*, 5247–5254.

[13] Mohamed, A. M. O.; Bicer, Y. *Comput. Aided Chem. Eng.* **2021**, *50*, 373–379.

[14] Rittmann, B. E.; Mayer, B.; Westerhoff, P.; Edwards, M. *Chemosphere* **2011**, *84*, 846–853.

[15] Gustavsson, J.; Cederberg, C.; Sonesson, U.; van Otterdijk, R.; Meybeck, A. *Global Food Losses and Food Wastes: Extent, Causes and Prevention*; Rome **2011**.

[16] Scherhaufer, S.; Moates, G.; Hartikainen, H.; Waldron, K.; Obersteiner, G. *Waste Manag.* **2018**, *77*, 98–113.

[17] *Summary of Joint European Commission—ESPP Webinar on P4 (Phosphorus) Critical Raw Material*; **2020**.

[18] Van Drecht, G.; Bouwman, A. F.; Harrison, J.; Knoop, J. M. *Glob. Biogeochem. Cycles* **2009**, *23*, 1–19.

[19] Beijer, S.; Slootweg, J. C. *Encycl. Inorg. Bioinorg. Chem.* **2021**, 1–26.

[20] Jupp, A. R.; Beijer, S.; Narain, G. C.; Schipper, W.; Slootweg, J. C. *Chem. Soc. Rev.* **2021**, *50*, 87–101.

[21] Ye, Y.; Ngo, H. H.; Guo, W.; Liu, Y.; Chang, S. W.; Nguyen, D. D.; Liang, H.; Wang, J. *Bioresour. Technol.* **2018**, *268*, 749–758.

[22] Esculier, F.; Le Noë, J.; Barles, S.; Billen, G.; Créno, B.; Garnier, J.; Lesavre, J.; Petit, L.; Tabuchi, J. P. *J. Hydrol.* **2019**, *573*, 1028–1045.

[23] Rockström, J.; Steffen, W.; Noone, K.; Persson, Å.; Chapin, F. S.; Lambin, E. F.; Lenton, T. M.; Scheffer, M.; Folke, C.; Schellnhuber, H. J.; Nykvist, B.; de Wit, C. A.; Hughes, T.; van der Leeuw, S.; Rodhe, H.; Sörlin, S.; Snyder, P. K.; Costanza, R.; Svedin, U.; Falkenmark, M.; Karlberg, L.; Corell, R. W.; Fabry, V. J.; Hansen, J.; Walker, B.; Liverman, D.; Richardson, K.; Crutzen, P.; Foley, J. A. *Nature* **2009**, *461*, 472–475.

[24] Steffen, W.; Richardson, K.; Rockström, J.; Cornell, S. E.; Fetzer, I.; Bennett, E. M.; Biggs, R.; Carpenter, S. R.; De Vries, W.; De Wit, C. A.; Folke, C.; Gerten, D.; Heinke, J.; Mace, G. M.; Persson, L. M.; Ramanathan, V.; Reyers, B.; Sörlin, S. *Science* **2015**, *347*, 1259855.

[25] Correll, D. L. *J. Environ. Qual.* **1998**, *27*, 261–266.

[26] Larsen, T. A.; Gujer, W. *Water Sci. Technol.* **1996**, *34*, 87–94.

[27] Friedler, E.; Butler, D.; Alfiya, Y. in *Source Sep. Decentralization Wastewater Manag.* (Eds.: Larsen, T.A.; Udert, K.M.; Lienert, J.); IWA Publishing, **2013**, 241–257.

[28] Udert, K. M.; Larsen, T. A.; Gujer, W. *Water Sci. Technol.* **2006**, *54*, 413–420.

[29] Martin, T. M. P.; Esculier, F.; Levavasseur, F.; Houot, S. *Crit. Rev. Environ. Sci. Technol.* **2022**, *52*, 890–936.

[30] Randall, D. G.; Naidoo, V. *J. Environ. Chem. Eng.* **2018**, *6*, 2627–2635.

[31] Sengupta, S.; Nawaz, T.; Beaudry, J. *Curr. Pollut. Reports* **2015**, *1*, 155–166.

[32] Tarpeh, W. A.; Barazesh, J. M.; Cath, T. Y.; Nelson, K. L. *Environ. Sci. Technol.* **2018**, *52*, 1453–1460.

[33] Christiaens, M. E. R.; Gildemyn, S.; Matassa, S.; Ysebaert, T.; De Vrieze, J.; Rabaey, K. *Environ. Sci. Technol.* **2017**, *51*, 13143–13150.

[34] Tarpeh, W. A.; Udert, K. M.; Nelson, K. L. *Environ. Sci. Technol.* **2017**, *51*, 2373–2381.

[35] Mutegoa, E.; Hilonga, A.; Njau, K. N. *Water Pract. Technol.* **2020**, *15*, 551–570.

[36] Basakcilardan-Kabakci, S.; Ipekoglu, A. N.; Talini, I. *Environ. Eng. Sci.* **2007**, *24*, 615–624.

[37] Etter, B.; Udert, K. Gounden, T. *Valorisation of Urine Nutrients - Vuna Final Project Report*; Dübendorf, Switzerland **2015**.

[38] Kafarski, P.; Talma, M. *J. Adv. Res.* **2018**, *13*, 101–112.

[39] Landry, K. A.; Boyer, T. H. *Water Res.* **2016**, *105*, 487–495.

[40] Lienert, J.; Bürki, T.; Escher, B. I. *Water Sci. Technol.* **2007**, *56*, 87–96.

[41] Mezzelani, M.; Gorbi, S.; Regoli, F. *Mar. Environ. Res.* **2018**, *140*, 41–60.

[42] Zhang, R.; Sun, P.; Boyer, T. H.; Zhao, L.; Huang, C. H. *Environ. Sci. Technol.* **2015**, *49*, 3056–3066.

[43] Dodd, M. C.; Zuleeg, S.; Von Gunten, U.; Pronk, W. *Environ. Sci. Technol.* **2008**, *42*, 9329–9337.

[44] Zimmermann, S. G.; Wittenwiler, M.; Hollender, J.; Krauss, M.; Ort, C.; Siegrist, H.; von Gunten, U. *Water Res.* **2011**, *45*, 605–617.

[45] Pronk, W.; Palmquist, H.; Biebow, M.; Boller, M. *Water Res.* **2006**, *40*, 1405–1412.

[46] Reis, R. Y. N.; Goulart, L. A.; Mascaro, L. H.; Alves, S. A. *J. Environ. Chem. Eng.* **2022**, *10*, 107859.

[47] Natarajan, R.; Saikia, K.; Ponnusamy, S. K.; Rathankumar, A. K.; Rajendran, D. S.; Venkataraman, S.; Tannani, D. B.; Arvind, V.; Somanna, T.; Banerjee, K.; Mohideen, N.; Vaidyanathan, V. K. *Chemosphere* **2022**, *287*, 131958.

[48] Almuntashiri, A.; Hosseinzadeh, A.; Volpin, F.; Ali, S. M.; Dorji, U.; Shon, H.; Phuntsho, S. *Chemosphere* **2021**, *280*, 130870.

[49] Köpping, I.; McArdell, C. S.; Borowska, E.; Böhler, M. A.; Udert, K. M. *Water Res. X* **2020**, *9*, 100057.

[50] Ilyasoglu, G.; Kose-mutlu, B.; Mutlu-salmanli, O.; Koyuncu, I. *Chemosphere* **2022**, *302*, 134775.

[51] Srivastava, A.; Gupta, B.; Majumder, A.; Gupta, A. K.; Nimbhorkar, S. K. *J. Environ. Chem. Eng.* **2021**, *9*, 106177.

[52] Delgado, N. Y.; Capparelli, A. L.; Marino, D. J.; Navarro, A. F.; Peñuela, G. A.; Ronco, A. E. *J. Surf. Eng. Mater. Adv. Technol.* **2016**, *06*, 183–200.

[53] Delgado, N.; Marino, D.; Capparelli, A.; Casas-Zapata, J. C.; Navarro, A. *J. Environ. Chem. Eng.* **2022**, *10*, 107706.

[54] Kim, S. H.; Shon, H. K.; Ngo, H. H. *J. Ind. Eng. Chem.* **2010**, *16*, 344–349.

[55] Özel Duygan, B. D.; Udert, K. M.; Remmele, A.; McArdell, C. S. *Resour. Conserv. Recycl.* **2021**, *166*, 105341.

[56] Delgado, L. F.; Charles, P.; Glucina, K.; Morlay, C. *Sci. Total Environ.* **2012**, *435–436*, 509–525.

[57] Kovalova, L.; Knappe, D. R. U.; Lehnberg, K.; Kazner, C.; Hollender, J. *Environ. Sci. Pollut. Res.* **2013**, *20*, 3607–3615.

[58] "GreenPee description webpage" can be found under https://greenpee.nl/en/the-process/.

[59] "Semilla Sanitation urine recycling webpage" can be found under https://www.nijhuisindustries.com/news/can-we-drink-tea-from-pee-yes-we-can-change-your-mindset.

[60] "Wettenbank overheid. Uitvoeringsregeling Meststoffen wet, Appendix Aa" can be found under https://wetten.overheid.nl/BWBR0018989/2021-02-18#BijlageAa

[61] European Commission. "DIRECTIVE 2008/98/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 19 November 2008 on waste and repealing certain Directives" can be found under https://eur-lex.europa.eu/legal-content/NL/TXT/?uri=CELEX:02008L0098-20180705**2008**.

[62] Commissie Deskundigen Meststoffenwet. *Protocol Beoordeling Stoffen Meststoffenwet. Versie 3.2*; Wettelijke Onderzoekstaken Natuur & Milieu: Wageningen **2016**.

[63] "Wettenbank overheid. Uitvoeringsregeling Meststoffen wet, Artikel 5" can be found under https://wetten.overheid.nl/BWBR0019031/2022-06-01#HoofdstukII_Paragraaf1_Artikel5.

[64] "Webpage on Aurin recycled fertilizer by Vuna" can be found under https://vuna.ch/en/aurin-recycling-dunger/.

[65] Caballero-Casero, N.; Castro, G.; Bastiaensen, M.; Gys, C.; van Larebeke, N.; Schoeters, G.; Covaci, A. *Chemosphere* **2021**, *280*, 130683.

[66] de Poorter, L. R. M.; van Herwijnen, R.; Janssen, P. J. C. M.; Smit, C. E. *RIVM Rapp. 2015-0057*; **2015**

[67] Hansler, R. J.; van Herwijnen, R.; Posthumus, R. *Indicatieve milieukwaliteitsnormen voor prioritaire stoffen 2004, RIVM Report 601782012/2008*; **2008**

[68] "Legal concentrations of substances" can be found under https://rvszoeksysteem.rivm.nl/Stoffen.

[69] Bischel, H. N.; Özel Duygan, B. D.; Strande, L.; McArdell, C. S.; Udert, K. M.; Kohn, T. *Water Res.* **2015**, *85*, 57–65.

[70] Kovalova, L.; Siegrist, H.; Von Gunten, U.; Eugster, J.; Hagenbuch, M.; Wittmer, A.; Moser, R.; McArdell, C. S. *Environ. Sci. Technol.* **2013**, *47*, 7899–7908.

[71] Avdeef, A.; Berger, C. M.; Brownell, C. *Pharm. Res.* **2000**, *17*, 85–89.

[72] Lee, S. H.; Kim, K. H.; Lee, M.; Lee, B. D. *J. Water Process Eng.* **2019**, *31*, 100828.

[73] "drugbank webpage on N-acetylsulfamethoxazole" can be found under https://go.drugbank.com/metabolites/DBMET00017.

[74] "GreenPee Webpage" can be found under https://greenpee.nl/.

[75] "Fertiflow Add Green Webpage" can be found under http://www.fertiflow.eu/nl/additieven/fertiflow-add-green.

[76] Kovalova, L.; Siegrist, H.; Singer, H.; Wittmer, A.; McArdell, C. S. *Environ. Sci. Technol.* **2012**, *46*, 1536–1545.

[77] Hernández, F.; Ibáñez, M.; Botero-Coy, A. M.; Bade, R.; Bustos-López, M. C.; Rincón, J.; Moncayo, A.; Bijlsma, L. *Anal. Bioanal. Chem.* **2015**, *407*, 6405–6416.

[78] Narain-Ford, D. M.; van Wezel, A. P.; Helmus, R.; Dekker, S. C.; Bartholomeus, R. P. *Water Res.* **2022**, *226*, 119303.

[79] Das, S.; Helmus, R.; Dong, Y.; Beijer, S.; Praetorius, A.; Parsons, J. R.; Jansen, B. *Chemosphere* **2023**, *337*, 139261.

[80] Helmus, R.; ter Laak, T. L.; van Wezel, A. P.; de Voogt, P.; Schymanski, E. L. *J. Cheminform.* **2021**, *13*, 1–25.

[81] Röst, H. L.; Sachsenberg, T.; Aiche, S.; Bielow, C.; Weisser, H.; Aicheler, F.; Andreotti, S.; Ehrlich, H. C.; Gutenbrunner, P.; Kenar, E.; Liang, X.; Nahnsen, S.; Nilse, L.; Pfeuffer, J.; Rosenberger, G.; Rurik, M.; Schmitt, U.; Veit, J.; Walzer, M.; Wojnar, D.; Wolski, W. E.; Schilling, O.; Choudhary, J. S.; Malmström, L.; Aebersold, R.; Reinert, K.; Kohlbacher, O. *Nat. Methods* **2016**, *13*, 741–748.

[82] Broeckling, C. D.; Afsar, F. A.; Neumann, S.; Ben-Hur, A.; Prenni, J. E. *Anal. Chem.* **2014**, *86*, 6812–6817.

[83] Helmus, R.; van de Velde, B.; Brunner, A. M.; ter Laak, T. L.; van Wezel, A. P.; Schymanski, E. L. *J. Open Source Softw.* **2022**, *7*, 4029.

[84] Dulio, V. "NORMAN priority list 2017" can be found under https://zenodo.org/record/2624273.

[85] Neumann, S. "mzR GitHub repository" can be found under https://github.com/sneumann/mzR.

[86] Meringer, M.; Reinker, S.; Zhang, J.; Muller, A. *MATCH Commun. Math. Comput. Chem.* **2011**, *65*, 259–290.

[87] Ruttkies, C.; Schymanski, E. L.; Wolf, S.; Hollender, J.; Neumann, S. *J. Cheminform.* **2016**, *8*, 1–16.

[88] Schymanski, E. L.; Kondić, T.; Neumann, S.; Thiessen, P. A.; Zhang, J.; Bolton, E. E. *J. Cheminform.* **2021**, *13*, 1–15.

[89] Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. *Environ. Sci. Technol.* **2014**, *48*, 2097–2098.

[90] "MassBank Europe Webpage" can be found under https://massbank.eu/MassBank/.

[91] "MassBank of North America Webpage" can be found under https://mona.fiehnlab.ucdavis.edu/.

[92] De Nicolò, A.; Cantù, M.; D'Avolio, A. *Bioanalysis* **2017**, *9*, 1093–1105.

[93] Mailler, R.; Gasperi, J.; Coquet, Y.; Deshayes, S.; Zedek, S.; Cren-Olivé, C.; Cartiser, N.; Eudes, V.; Bressy, A.; Caupos, E.; Moilleron, R.; Chebbo, G.; Rocher, V. *Water Res.* **2015**, *72*, 315–330.

[94] Cao, Y.; Nakhjiri, A. T.; Ghadiri, M. *Sci. Rep.* **2021**, *11*, 1–11.

[95] Larasati, A.; Fowler, G. D.; Graham, N. J. D. *Chemosphere* **2022**, *286*, 131888.

[96] Li, Q.; Qi, Y.; Gao, C. *J. Clean. Prod.* **2015**, *86*, 424–431.

[97] Marques, S. C. R.; Marcuzzo, J. M.; Baldan, M. R.; Mestre, A. S.; Carvalho, A. P. *Chem. Eng. J.* **2017**, *321*, 233–244.

[98] Xiang, W.; Zhang, X.; Chen, J.; Zou, W.; He, F.; Hu, X.; Tsang, D. C. W.; Ok, Y. S.; Gao, B. *Chemosphere* **2020**, *252*, 126539.

[99] Krahn, K. M.; Cornelissen, G.; Castro, G.; Arp, H. P. H.; Asimakopoulos, A. G.; Wolf, R.; Holmstad, R.; Zimmerman, A. R.; Sørmo, E. *J. Hazard. Mater.* **2023**, *445*, 130449.

[100] Wang, L.; Liu, J.; Liu, J.; Shi, L.; Wang, Z. *Chem. Ecol.* **2018**, *34*, 108–125.

[101] Kuklenyik, Z.; Ekong, J.; Cutchins, C. D.; Needham, L. L.; Calafat, A. M. *Anal. Chem.* **2003**, *75*, 6820–6825.

[102] Xu, X.; He, Z.; Tang, H.; Sun, Y.; Zhang, S.; Shi, D.; Ji, F. *Chemosphere* **2022**, *288*, 132317.

[103] Milbrath, M. O. G.; Wenger, Y.; Chang, C. W. C. W.; Emond, C.; Garabrant, D.; Gillespie, B. W.; Jolliet, O. *Environ. Health Perspect.* **2009**, *117*, 417–425.

[104] Han, Y.; Liu, W.; Lei, R.; Wang, M.; Xue, Y. *Chemosphere* **2022**, *303*, 134995.