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Organic micropollutants, heavy metals and pathogens in anaerobic digestate based on food waste

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ABSTRACT

Anaerobic digestate based on food waste is increasingly used as fertilizer in food production. This study examined the characteristics of anaerobic digestate based on food waste from three biogas plants in Sweden. The characterization included measurements of heavy metals ($n = 7$), chemicals of emerging concern (CECs), such as currently used drugs and pesticides ($n = 133$), and an extended range of food-borne pathogens, including two notable sporeformers and some widespread antibiotic-resistant bacteria. The amounts of *Escherichia coli*, enterococci, and *Salmonella* and the concentrations of the target heavy metals were all below the maximum accepted levels at all three locations studied. However, the spore-forming *Bacillus cereus* was found to be present at high levels in samples from all three biogas plants. Among the 133 CECs investigated, 48 were detected at least once, and the highest concentrations were found for pyroxidine, nicotine, caffeine, theobromine, and nicotine. The biofertilizers from the different biogas plants had similar CEC profiles, which indicate similarities in household waste composition and thorough mixing in the biogas plants. If this profile is found to be spatially and temporally consistent, it can help regulators to establish priority lists of CECs of top concern. Assuming increasing use of biofertilizers for food production in the future, it would be beneficial to have concentration limits for CECs Risk estimation based on risk quotients (RQs) indicated generally low environmental risks associated with application of biofertilizer to soils for food crop production. However, the toxicity of CEC mixtures needs to be considered when estimating the risks from application of biofertilizers on agricultural land or in other production systems.

CRediT author statement

Oksana Golovko Formal analysis, Conceptualization, Methodology, Writing- Original draft preparation. **Lutz Ahrens** Conceptualization, Writing- Reviewing and Editing. **Jenny Schelin** Formal analysis, Conceptualization, Methodology, Writing- Original draft preparation. **Mattias Sörengård** Methodology, Resources,. Formal analysis, Writing-Reviewing and Editing. **Karl-Johan Bergstrand** Writing- Reviewing and Editing. **Håkan Asp** Writing- Reviewing and Editing. **Malin Hultberg** Project administration, Conceptualization, Methodology, Resources, Writing- Reviewing and Editing. **Karin Wiberg** Conceptualization, Writing- Reviewing and Editing.

1. Introduction

In a circular economy, the negative effects of "take, make, and dispose" are avoided by using resources efficiently and in a circular manner (Ness, 2008; Merli et al., 2018). Global and national sustainable development goals call for responsible consumption and production in all sectors (https://www.un.org/sustainabledevelopment/sustainable-development-goals/), while EU regulations require all wastes to be treated to some degree (Directive, 1999/31/EC). Accordingly, there is a trend in waste management to transition from treatment of mixtures of residues towards sustainable solutions, including source separation, resource recovery, and overall waste reduction (Puyol et al., 2017; Mak et al., 2020; Ng et al., 2020). In the food sector, decreased generation of food waste is aimed for and has been achieved, e.g., in households and

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the food industry in Sweden (2012–2014) (Swedish EPA (Naturvårdsverket)) and elsewhere in the world (Lin et al., 2013). A commonly used treatment for food waste, often in combination with other waste fractions (Brändli et al., 2007a; Owamah et al., 2014; Suominen et al., 2014; Ali et al., 2019), is digestion under anaerobic conditions to produce biogas (biomethane) as a renewable energy source. This treatment also allows for nutrient recirculation, as the anaerobic digestate has a high content of organic matter and other nutritional components (Bergstrand et al., 2020; Pelayo Lind et al., 2020). The material is hygienized through the anaerobic process, and any aerobic treatments after the anaerobic digestion is performed mainly to oxidize ammonium to nitrate (Bergstrand et al., 2020). Today, anaerobic digestate is therefore considered an important resource for fertilization of arable land (Alburquerque et al., 2012a; Alburquerque et al., 2012b; de Groot et al., 2013).

Developing technical solutions for recirculation of nutrients from the biogas process is an important step towards a circular biobased society. Previous studies have focused mainly on basic parameters such as pH, conductivity, dry matter, organic carbon, nitrogen, phosphorus, ammonium, metals, and pathogenic bacteria (Alburquerque et al., 2012a). However, knowledge on the occurrence and levels of organic micropollutants in the residual material is very limited, particularly considering expected compositional differences due to short- and long-term variations in the type, mixtures, and quality of feedstock material. Some studies have reported the occurrence of highly hydrophobic organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and legacy persistent organic pollutants (POPs), in digestate from biogas plants processing food waste (Brändli et al., 2007a, 2007c; Govasmark et al., 2011; Alburquerque et al., 2012b; Owamah et al., 2014; Suominen et al., 2014; Barcauskaitė, 2019). Only a few studies have focused on pharmaceuticals (Ali et al., 2019), pesticides (Brändli et al., 2007c; Govasmark et al., 2011), and other contaminants of emerging concern (CECs) (Ali et al., 2019), and none has examined the full range of potentially deleterious components (organic pollutants, food-borne pathogens, and metals) in aerobic digestate based on food waste.

The digestion process substantially reduces the total mass of the easily degradable fraction of the starting material, while metals and organic micropollutants remain (Brändli et al., 2007a, 2007b, 2007c; Govasmark et al., 2011; Barcauskaitė, 2019), and may even be concentrated in relation to the feedstock material. Moreover, in batch experiments conducted under meso- and thermophilic conditions, anaerobic treatment of source-separated fecal sludge has been shown to achieve low removal rates of semi-persistent organic compounds such as pharmaceuticals (Gros et al., 2020). Application of anaerobic digestate as fertilizer on agricultural land may circulate these toxic elements back into the food production chain, raising concerns about potential negative effects after long-term use of recycled organic wastes (Govasmark et al., 2011; Suominen et al., 2014; Pivato et al., 2016; Ali et al., 2019).

In recent years, there has been increasing interest in food production in densely populated areas with low availability of arable soil. As a consequence of this, resource-optimised systems, such as hydroponics, where plants are grown directly in nutrient solution in soilless systems, have increased dramatically (Bergstrand, 2010). The anaerobic digestate from food waste has the potential to be used as plant nutrient source in the increasingly popular hydroponic systems (Bergstrand et al., 2020; Pelayo Lind et al., 2020). This proposed use is an additional argument to better define the occurrence and levels of the more water-soluble (hydrophilic), (semi-)persistent organic contaminants, especially residues from currently used chemicals, drugs, and pesticides. Thus, in-depth knowledge of the chemical and microbial characteristics of the anerobic digestate derived from organic waste is a necessity for safe urban food production. The aim of the this study was therefore to determine the characteristics of anaerobic digestate based on food waste in terms of risk factors. The main focus was on CECs, such as currently used drugs and pesticides, but also on heavy metals and an extended range of food-borne pathogens, including two notable sporeformers and some widespread antibiotic resistant bacteria. Grab samples of anaerobic digestates based on food waste and certified according to Swedish standards were collected at three different Swedish biogas plants and investigated in the study. To the best of our knowledge, this is the first study that shows the wide range of potentially deleterious components (organic pollutants, food-borne pathogens, and metals) in aerobic digestate based on food waste.

2. Materials and methods

2.1. Target analytes and chemicals

The biofertilizer samples were analyzed for a total of 133 CECs, comprising 60 pharmaceuticals, 58 pesticides, three industrial chemicals, one drug, three parabens, two stimulants, one food additive, two vitamins, one personal care product, one fatty acid, and one sweetener (Table S1 in Supporting Information (SI)). All analytical standards used for analysis were of high purity grade (>95%) and purchased from Sigma-Aldrich (Sweden). Isotopically labeled internal standards (ISs) ($n = 26$) were obtained from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), Sigma-Aldrich (Sweden), and Toronto Research Chemicals (Toronto, Canada). Detailed information about internal and native standards can be found in Table S1 in SI.

Ultrapure water was generated by a Milli-Q Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak Express membrane and LC-Pak polishing unit (Merck Millipore, Billercia, MA). Methanol, acetonitrile, ammonium acetate, formic acid, ammonia, and ethyl acetate of high analytical grade were acquired from Sigma-Aldrich (Sweden).

2.2. Sampling of residues from biogas production

Three biogas plants (BPs A-C) using various mixtures of source-separated waste fractions as feedstock and applying different conditions for anaerobic digestion were selected for sampling (Table 1). At each site, the residual product after digestion (digestate) was sampled on one occasion in June–July 2019. All sampled biogas plants were certified according to SPCR 120 (Avfall Sverige, 2020). Approximately 2 L of the final digestate product (biofertilizer) was collected from each of the BPs, in all cases in high-density polyethylene bottles that were pre-cleaned with methanol and distilled water. The samples were transported to the laboratory at the Swedish University of Agricultural Sciences (Uppsala, Sweden), freeze-dried within 7 days, and then stored at −20 °C before extraction.

Samples for microbial analysis were collected separately in 125-mL aseptic polypropylene straight sample containers with screw cap (VWR International AB, Stockholm), from two different locations in the anaerobic digestion process: immediately after completion of hygienization ("after hygienization") and in the final biofertilizer ("biofertilizer"). Duplicate samples were collected in all cases, and all samples were stored at +5 °C for transport to the respective analysis site.

Table 1
Composition of the feedstock used at the three biogas plants (BPs) sampled in this study.

Biogas plant (BP)	Feedstock[a]
BP-A	Food waste from households (37%), manure (29%), slaughterhouse waste (21%), fat from separators (industrial) 5%, other food waste (8%)
BP-B	Food waste (80%), animal byproducts (9%), industrial waste (11%)
BP-C	Food waste, animal byproducts, industrial waste

[a] Values provided by the BP operator, as annual averages.

2.3. Sample preparation for chemical analysis

The homogenized sample material was extracted in triplicate using ultrasonication, as described previously (Golovko et al., 2016). In brief, 2 g (dry weight (dw)) of sample were spiked with the IS mixture (20 ng absolute per compound and sample aliquot) and extracted by a two-step ultrasonic bath extraction procedure using: (i) 4 mL acetonitrile/MilliQ water (1/1, v/v) with 0.1% formic acid and (ii) 4 mL acetonitrile/2-propanol/MilliQ water mixture (3/3/4, v/v/v) with 0.1% formic acid, with an extraction duration of 15 min for each step. The two supernatants were combined, mixed, and filtered using a regenerated cellulose syringe filter (0.45 μm pores). For the instrumental analysis, an aliquot of 1 mL (out of 8 mL) of the extract was used.

2.4. Instrumental analysis of CECs

The extracts were analyzed using a DIONEX UltiMate 3000 ultra-high pressure liquid chromatography (UPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer (MS/MS) (TSQ Quantiva, Thermo Scientific, Waltham, MA, USA). A Kinetex® Biphenyl column (100 mm × 2.1 mm i. d., 2.6 μm particle size, Phenomenex) was used as separation column and ions were produced through heated electrospray ionization (H-ESI). The injection volume was 10 μL, and the mobile phase consisted of MilliQ water with 0.1% formic acid and methanol with 0.1% formic acid. The flow rate was 0.6 mL/min and the run time was 16 min, with switched positive and negative electrospray ionization modes. Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) was used for data acquisition.

2.5. Microbial analyses of food-borne pathogens and antibiotic resistant bacteria

The biofertilizer samples were analyzed for a total of eight different microorganisms, comprising five food-borne pathogens (*Escherichia coli*, enterococci, *Salmonella* spp, *Clostridium perfringens*, *Bacillus cereus*) and three antibiotic resistant bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactamase (ESBL)-resistant *E. coli*, and ESBL carbapenemase-producing (ESBL-CARBA) *Enterobacteriaceae*. *Escherichia coli*, enterococci, and *Salmonella* are included in regular monitoring of certified biofertilizer according to SPCR120 (Avfall Sverige, 2020), while *C. perfringens*, *B. cereus* MRSA, ESBL-resistant *E. coli*, and ESBL-CARBA resistant *Enterobacteriace* were included in the extended microbial analyses performed in this study.

The samples for the five food-borne pathogens were analyzed by an accredited laboratory (Eurofins Environment Testing Sweden AB, Linköping, Sweden, and Eurofins Food & Feed Testing, Uppsala, Sweden) according to the following NMKL procedures: *E. coli* NMKL 125, 4. Ed., 2005; *Enterococcus* NMKL 68, 5. Ed., 2011; *Salmonella* NMKL 71, 5. Ed., 1999; *C. perfringens* NMKL 95, 5. Ed., 2009 and *B. cereus* NMKL 67, 6. Ed., 2010. Levels of bacteria are expressed as colony forming units (CFU) per gram of wet digestate.

The analyses for MRSA, ESBL-resistant *E. coli*, and ESBL-CARBA resistant *Enterobacteriace* were performed by the accredited microbiology laboratory at the National Veterinary Institute (SVA), Uppsala, Sweden, according to the following procedures: SVA 23093 for MRSA, SVA 25505-6 for ESBL-resistant *E. coli*, and SVA 41275 for ESBL-CARBA resistant *Enterobacteriaceae*. In brief, SVA 23093 involves pre-enrichment in Mueller-Hinton broth at 37 °C for 16–20 h, followed by aerobic incubation in Tryptic Soy broth including 3.5 mg/L cefoxitin and 75 mg/L azetreonam at 37 °C for 16–20 h. Samples are then plated on chromogenic media (Oxoid Brilliance MRSA 2 agar) and blood (beef) agar plates, and incubated aerobically at 37 °C for 1–2 days. If needed, presumptive MRSA colonies are isolated and verified with PCR and MALDI-TOF-MS typing. SVA 25505-6 involves pre-enrichment in buffered peptone water at 37 °C for 18–24 h, followed by plating on McConkey agar with 1 mg cefotaxim/L and incubation at 37 °C for 18–24 h. If needed, presumptive ESBL-resistant *E. coli* colonies are isolated and re-streaked on blood (horse) agar plates, followed by verification using Spot Indole Reagent and PCR. SVA 41275 involves pre-enrichment in Buffered Peptone Water broth at 35 °C ± 2 °C for 18–24 h, followed by plating on ChromID Carba-agar and ChromIDOxa48-agar and incubation at 35 °C ± 2 °C for 18–24 h. Verification of presumptive ESBL-CARBA resistant *Enterobacteriaceae* is performed with blood (horse) agar plates, PCR, and MALDI-TOF-MS typing.

2.6. Heavy metal analysis

The heavy metals analyzed were cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn), which were selected based on the certification rules established by the Swedish Waste Management Association (Avfall Sverige, 2020). The heavy metals in the BP samples were measured according to standardized methods at an accredited laboratory (Eurofins, Linköping, Sweden). Cadmium, chromium, and lead were analyzed by inductively coupled plasma mass spectrometry (ICP)-MS, copper, nickel and zinc by ICP-atomic emission spectroscopy (AES), and mercury by atomic fluorescence spectroscopy (AFS). All analyses were performed according to ISO 11466/EN13346.

2.7. Environmental risk estimations for CECs

Environmental (ecotoxicological) risk quotient (RQ)-based risk estimation for the biofertilizer was applied, in accordance with similar studies performed on sludge and soil (EC, 2003; Magid et al., 2020). An RQ value > 1 is considered a risk and RQ values < 1 and >0.1 are considered a moderate risk. The RQs were calculated (Eq. (1)) considering application of the biofertilizer to agricultural land. All results from the following modeled parameters, measurements, and calculations can be found in Table S2 in SI.

$$RQ = \frac{c_{soil+biofertilizer}}{PNEC_{soil}} \quad (1)$$

where $c_{soil+biofertilizer}$ is predicted CEC concentration in soil [mg/kg] and $PNEC_{soil}$ is predicted no effect concentration in soil [mg/kg] for the individual CEC (used to reflect the ecotoxicological risk for soil organisms). $c_{soil+biofertilizer}$ was calculated as:

$$c_{soil+biofertilizer} = \frac{C_{biofertilizer,\ dw} \cdot APPL_{biofertilizer}}{DEPTH_{soil} \cdot RHO_{soil}} \quad (2)$$

where $c_{biofertilizer,\ dw}$ is measured biofertilizer concentration [mg/kg dw], $APPL_{biofertilizer}$ is amount of dry biofertilizer applied per unit area [kg/m^2], $DEPTH_{soil}$ is depth of soil used for application and mixing [m], and RHO_{soil} is bulk density of the soil [kg/m^3]. For $APPL_{biofertilizer}$, and bulk density ($DEPTH_{soil}$), standard EU assessment values were used (0.5 kg/m^2 for crops for human consumption, 0.2 m depth, and 1700 kg/m^3 for wet soil bulk density) (EC, 2003).

RHO_{soil} was calculated as:

$$PNEC_{soil} = \frac{K_{d,\ water-soil}}{RHO_{soil}} \cdot PNEC_{water} \cdot 1000 \quad (3)$$

where $PNEC_{water}$ [mg/L] was acquired from the QSAR model from the Norman Network database (https://www.norman-network.com/nds/susdat/), including an additional safety factor of 10 due to uncertainty in the data, and $K_{d,\ water-soil}$ [L/kg] was calculated as:

$$K_{d,\ water-soil} = K_{OC} \cdot f_{OC,\ soil} \quad (4)$$

where K_{OC} is organic carbon-water partition coefficient [L/kg] and $f_{OC,\ soil}$ is soil organic carbon content (0.05 (5%) was used). K_{OC} was estimated from an empirical model (Baker et al., 1997; Doucette, 2003):

$$log\ K_{OC} = 0.903\ log\ K_{OW} + 0.094 \quad (5)$$

where K_{OW} is the octanol-water partitioning coefficient (Golovko et al., 2021).

For calculation of the concentrations of individual CECs dissolved in the aqueous phase (*aq*) for hydroponic purposes, solid-water equilibrium partitioning (Eq. (2)) was used to assess the aqueous concentrations from 2.4 g dw added biofertilizer per liter of water. This amount of the test biofertilizer was chosen because it was the amount needed to reach adequate nutrient concentrations in hydroponic solution.

2.8. Quality control

Quality control of the CEC analyses included analysis of laboratory method blanks, limit of quantification (LOQ), matrix effect, and absolute recovery (Table S1 in SI). Calibration curves were prepared in the range 0.01–10 000 ng/mL and generally had R-values >0.99 (data not reported). The method blanks ($n = 3$) were prepared and extracted in the same way as the biofertilizer samples, but without sample. Matrix-matched standards were used to assess matrix effects and were prepared from biofertilizer sample extract spiked with ISs and native target CECs, at concentrations equivalent to 10 ng/g dw and 100 ng/g dw, respectively. All other analyses (microbial, metals) were performed using accredited methods.

3. Results and discussion

3.1. Microbial characterization of anaerobic digestate

The occurrence and levels of the eight microorganisms investigated in the anerobic digestate samples from the selected BPs are shown in Table 2. All investigated microorganisms showed similar occurrence and levels at the three BPs. In accordance with the certification requirements in SPCR120 (Avfall Sverige, 2020), the detected amounts of *E. coli*, enterococci, and *Salmonella* were below the specified maximum accepted level at all three study locations (Table 2). This is in agreement with previous findings that a combination of thermal pre-treatment followed by anaerobic digestion is sufficient for reducing *Salmonella*, enterococci, and *E. coli* to acceptable/non-detectable levels, as required by EU regulations (Bagge, 2009; Seruga et al., 2020). The antibiotic resistant bacteria (MRSA, ESBL-resistant *E. coli*, and ESBL-CARBA resistant *Enterobacteriaceae*) were not detected in any of the samples and *C. perfringens* was detected at levels <1.0 log CFU/g in all but one sample from the three BPs. The only species found to be present at high levels was the spore-forming food-borne pathogen *B. cereus*, which was detected in concentrations ranging from 3.3 to 4.8 log CFU/g at the three BPs. According to EFSA, food-borne diseases caused by *B. cereus* usually occur at 5–8 log CFU/spores per g of the food vehicle (EFSA, 2005). However, amounts as low as 3–4 log CFU per g food have been reported in some food-poisoning outbreaks (EFSA, 2005). Hence the high amount of *B. cereus* found in the biofertilizer samples from all biogas plants needs further attention and consideration if the biofertilizer is intended to be used in short nutrient loop systems, such as hydroponic food production systems (Tampio, 2016; Zhao and Liu, 2019). There is also a vast number of other possible pathogens that could occur in anerobic digestate, e.g., *Bacillus* spp. has been detected in a previous study which found that its occurrence was not affected by hygienization treatment or the following anaerobic digestion (Bagge, 2009).

3.2. Heavy metals

The concentrations of heavy metals (Cd, Cr, Cu, Pb, Hg, Ni, Zn) in the biofertilizer samples from the selected BPs are shown in Table 3. For all samples analyzed, the concentrations of heavy metals were lower than the maximum accepted level according to the certification (Avfall Sverige, 2020). Cadmium is a non-essential element that is a concern due to its toxicity. However, it is naturally present in varying concentrations in the environment, with different sources contributing to the total load, e.g., certain soils based on alum shale contain naturally high concentrations (Söderström and Eriksson, 2013). It is therefore of interest to note that all three biogas plants sampled had similar Cd concentrations (Table 3). For the other heavy metals analyzed, larger

Table 2
Detection of presence and levels of food-borne pathogens and antibiotic resistant bacteria in anerobic digestates collected from three selected biogas prodution plants (BP-A, BP-B and BP-C) in Sweden. Samples were collected immediately after completion of hygienization in the anaerobic digestion process and of the final biofertilizer. Two technical replicates (#1 and #2) were collected and analyzed for most samples. Levels of bacteria are given in colony forming units (CFU) per gram of wet digestate.

		BP-A		BP-B		BP-C	
Species Detection method	Technical replicates	After hygienization	Biofertilizer	After hygienization	Biofertilizer	After hygienization	Biofertilizer
E. coli NMKL 125, 4th ed, 2005	#1	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g
	#2	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g
Enterococcus NMKL 68, 5th ed, 2011	#1	<2.0 log cfu/g	<2.0 log cfu/g	<2.0 log cfu/g	<2.0 log cfu/g	3.0 log cfu/g	4.4 log cfu/g
	#2	<2.0 log cfu/g	<2.0 log cfu/g	<2.0 log cfu/g	<2.0 log cfu/g	3.5 log cfu/g	<2.0 log cfu/g
Salmonella NMKL 71, 5th ed, 1999	#1	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g
	#2	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g
C. perfringens NMKL 95, 5th ed, 2009	#1	2.7 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g
	#2	<1.0 log cfu/g	2.8 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g	<1.0 log cfu/g
B. cereus NMKL 67, 6th ed, 2010	#1	4.6 log cfu/g	4.3 log cfu/g	4.0 log cfu/g	3.3 log cfu/g	4.8 log cfu/g	4.7 log cfu/g
	#2	4.7 log cfu/g	4.1 log cfu/g	3.8 log cfu/g	3.6 log cfu/g	4.0 log cfu/g	4.7 log cfu/g
ESBL *E. coli* and ESBL-CARBA *Enterobacteriaceae* SVA 25505-6 and SVA 41275	#1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MRSA SVA 23093	#1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Note. n.d.: not detected.

Table 3
Dry matter content (%) and concentrations (mg/kg dry weight) of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) in biofertilizer samples from biogas production plants (BP) A-C.

	BP-A (mg/kg)	BP-B (mg/kg)	BP-C (mg/kg)	Maximum accepted level (mg/kg)[a]
Dry matter (%)	3.1	4.0	2.9	n.a.
Cd	0.35	0.34	0.37	1
Cr	8.2	13	20	100
Cu	100	41	67	600
Pb	2.0	2.7	7.4	100
Hg	<0.1	<0.1	<0.1	1
Ni	7.2	11	15	50
Zn	540	180	220	800

n.a. = not applicable.
[a] According to SPCR 120 (Avfall Sverige, 2020).

variations were observed. The highest concentrations of Cu and Zn were found in the biofertilizer from BP-A (100 and 540 mg/kg of dry matter, respectively), whereas Cr, Pb, and Ni concentrations were highest in biofertilizer from BP-C (20, 7.4, and 15 mg/kg, respectively). The fact that the concentration was lower than the maximum accepted level for all metals suggests that the levels of heavy metals in biofertilizer are well monitored and controlled by the producers, a possible explanation being the presence of clear statutory limits.

3.3. CECs in BPs

Of the 133 target CECs, 48 were detected at least once (Fig. 1A and Table S3 in SI). Different groups of CECs were found in the samples, including naturally formed food chemicals, food additives, stimulants, anti-fungal food preservatives, antiparasitic drugs, pesticides, and different types of pharmaceuticals. Stimulants detected included caffeine, nicotine, and theobromine; vitamins included pyridoxine (vitamin B6) and nicotinamide (vitamin B3); and antiparasitic drugs included levamisole, sulfaclozine, and fenbendazole. The highest detection frequencies and concentrations were found for biologically produced food chemicals, with five out of six compounds detected in average cumulative concentrations ranging from 200 (pyroxidine) to 8500 (nicotine) ng/g dw. Dominant detected stimulants were caffeine (up to 1500 ng/g, BP-C), theobromine (up to 2100 ng/g dw, BP-A), and nicotine (up to 12000 ng/g dw, BP-C). Theobromine and nicotine concentrations showed the highest variance in the detected CEC data, ranging from 13 to 2100 ng/g dw (coefficient of variance (CV) = 1.4) for theobromine and 6600–12000 ng/g dw (CV = 1.1) for nicotine. Stimulants are used in many food products, including drinks, and their occurrence in different environmental samples has been reported (Thompson and Darwish, 2019). The concentration of caffeine (240 ng/g dw) in the samples from BP-B was similar to that reported in food waste at a BP in Norway (Ali et al., 2019).

Among the 58 target pesticides, 13 were detected at least once, representing 10 fungicides, two insecticides, and one herbicide (chloridazon). Concentrations of detected pesticides at the study BPs ranged from 2.0 ng/g dw for chloridazon to 840 ng/g dw for imazalil. Imazalil showed the highest pesticide concentrations at all three BPs, with around eight-fold higher concentrations than the second most prevalent pesticide, prothioconazole (fungicide), with on average 93 ng/g dw. Imazalil is used post-harvest on citrus fruit to prevent rot, indicating the presence of citrus peel in the food waste used as feedstock at the BPs.

Antifungal food preservatives, such as ethylparaben, methylparaben, and propylparaben, were detected in average concentrations of 8.1, 11, and 23 ng/g dw, respectively (3 out of 3 target compounds detected). It is well known that antifungal preservatives are often used as food preservatives in e.g., processed vegetables, baked goods, fats and oils, seasonings, sugar substitutes, and frozen dairy products (Haman et al., 2015). To the best of our knowledge, this is the first time parabens have been measured in biofertilizer samples from BPs. Methylparaben is the paraben present in the highest concentrations in wastewater sludge in Sweden and in China (on average 68 and 97 ng/g dw, respectively) (Ma et al., 2018; Golovko et al., 2021).

The average concentrations of detected pharmaceuticals (26 out of 60 compounds detected) ranged from 0.7 (fenofibrate) to 99 (fenbendazole) ng/g dw. Other pharmaceuticals with high average concentrations were sertraline, losartan, furosemide, carbamazepine, and citalopram, with 82, 53, 45, and 25 ng/g dw, respectively. These compounds are frequently detected in sludge and soil (Golovko et al., 2016,

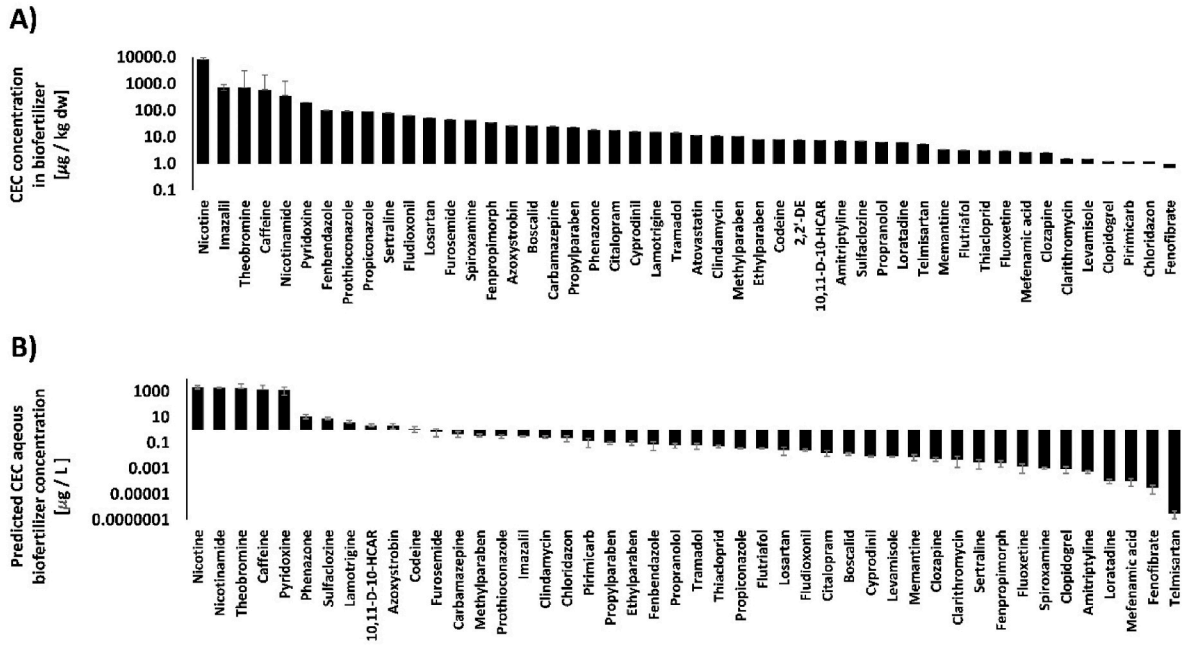

Fig. 1. Chemicals of emerging concern (CECs) detected in biofertilizer samples from biogas plants BP-A, BP-B, and BP-C. A) Measured log CEC concentrations (µg/kg dry weight (dw)) in the samples and B) calculated CEC concentrations in the aqeous phase, based on partitioning from 2.4 g dw biofertilizer per liter water (see Eq. (2)). Error bars represent standard deviation of concentrations in sludges from plants A, B, and C.

2021).

The log CEC concentration in the different waste feeds (BP-A, BP-B, BP-C) showed a significant correlation ($p < 0.001$, Spearman Correlation), with r^2 values of 0.63, 0.54, and 0.55 for BP-A *vs.* B, BP-A *vs.* C, and BP-B *vs.* C, respectively (Figure S1 in SI). The correlation was still significant after removing the six top concentrations of CECs from the data (Figure S1B in SI). A two-sided z-test (excluding nicotine, imazalil, theobromine, caffeine, nicotinamide, and pyridoxine for normality reasons) revealed no significant difference ($p = 0.86$) in average CEC concentrations for BP-A *vs.* B, while there were significant differences for BP-A *vs.* C and BP-B *vs.* C ($p < 0.05$ and $p < 0.001$, respectively). In conclusion, the different biofertilizers from the three BPs studied were similar in terms of CEC composition, which could be explained by similarities in household waste composition and well-mixed feedstock in the BPs. Overall, there are limited data available on CECs in foodwaste and biofertilizer, with previous studies mainly focusing on PCDD/Fs, PCBs, PBDEs, PFASs, and PAHs (Brändli et al., 2007a, 2007b, 2007c; Govasmark et al., 2011). Further studies on CECs in biofertilizer are urgently needed, since biofertilizer usage in food production is increasing rapidly (Lin et al., 2013).

Besides application to agricultural soil, biofertilizer can be used as a source of nutrients in hydroponic systems. The partitioning of CECs from the biofertilizer to the aqeous phase is therefore of high relevance (Eq. (4), Fig. 1B). Based on previous studies (Bergstrand et al., 2020), we assumed that up to 2.4 g dw biofertilizer would be mixed with 1 L of water, to be added to a hydroponic closed system as the nutrient solution. Similarly to the concentrations in the biofertilizer, caffeine, nicotine, theobromine, pyridoxine (vitamin B6), and nicotinamide (vitamin B3) showed the highest aqeous concentrations. Imazalil had relatively low concentrations in the aqueous phase, which can be attributed to its high log K_{OW} value (4.1). More water-soluble CECs are known to accumulate more easily in leafy vegetables (Kodešová et al., 2019), which are crops commonly grown in hydroponic systems. Bioaccumulation of water-soluble CECs and potential concerns associated with human consumption of crops farmed hydroponically need to be addressed in a future study.

3.4. Environmental risk estimation for CECs

According to the RQs-based risk estimates, soil fertilization using typical agricultural doses of biofertilizer from the study BPs was generally associated with low environmental risks (Fig. 2). Even the highest scoring CEC, theobromine, had a RQ almost three orders of magnitude lower than the moderate risk threshold. This is in alignment with previous risk estimation studies in sludge application for farming, where only two out of >150 CECs contributed to a moderate risk (Magid et al., 2020). Those two were phthalates and triclocarban, which were not included in the present study.

As shown by this study and elsewhere (Magid et al., 2020), CECs are not a major concern compared with metals, due to their low concentrations. However, individual CECs can have relatively high impact and occur at high concentrations, e.g., following consistent usage of wastewater sludge as fertilizer (Magid et al., 2020), and may contribute to the environmental risk. Furthermore, the toxicity of CEC mixtures needs to be considered when estimating the risks associated with application of biofertilizers on agricultural land or their use in hydroponic systems.

4. Conclusions

The occurrence and levels of food-borne pathogens, heavy metals, and CECs in anaerobic digestate produced from digestion of food waste were studied. Six out of eight microorganisms analyzed, *viz. E. coli*, enterococci, *Salmonella* spp., *C. perfringens,* MRSA, ESBL-resistant *E. coli,* and ESBL-CARBA resistant *Enterobacteriaceae*, showed similarly low levels or no occurrence in the anaerobic digestate (biofertilizer) from the three BPs studied. The detected amounts of *E. coli*, enterococci, and

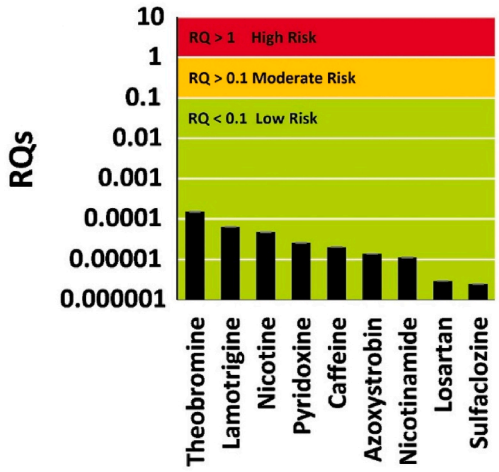

Fig. 2. Risk quotient (RQ) values based on the maximum detected concentrations of chemicals of emerging concern (CECs) in biofertilizer samples from the three biogas plants.

Salmonella were below the specified maximum accepted levels at all three study locations. However, the spore-forming *B. cereus* was found to be present at high levels in samples from all three BPs. For all samples analyzed, the concentrations of heavy metals were lower than the maximum accepted level according to the certification criteria.

For the CECs, 133 compounds were investigated and 48 were detected at least once in samples from the three BPs. The highest concentrations were found for pyroxidine and nicotine, with average cumulative concentrations of 200 and 8500 ng/g dw, respectively. Dominant detected stimulants were caffeine (up to 1500 ng/g), theobromine (up to 2100 ng/g dw), and nicotine (up to 12000 ng/g dw). Thirteen out of 58 pesticides investigated were detected, in concentrations ranging from 2.0 ng/g dw for chloridazon to 840 ng/g dw for imazalil.

The different biofertilizers from the three BPs had similar CEC profiles, which indicates similarities in household waste composition and good mixing in the biogas plants. It should, however, be noted that our study was based on grab samples and that variable occurrence and levels of the pollutants may occur. A long term-study could reveal if CEC profiles are spatially and temporally consistent. Such a study would help regulatory agencies to establish priority lists of CECs of top concern. Assuming increasing use of biofertilizers for food production in the future, it would be beneficial to have certification rules for CECs. There are currently limited data available on CECs in food waste and biofertilizer, and more studies, e.g., long-term investigations and foodwaste specific studies, are urgently needed.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.114997.

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