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Towards circular food production systems: Identification of chemical, microbial, and physical food safety hazards in municipal sludge and excess aerobic biomass of the food industry

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ABSTRACT

There is a need in Europe to re-use residual streams to move towards a more circular food production system in the future. Sludge from both municipal wastewater (sewage) treatment plants and from wastewater treatment plants of the food industry (i.e., aerobic biomass) are present in large volumes, contain nutrients, and are currently mostly incinerated in the Netherlands. This makes them an interesting source for re-use in food production systems. However, prior to re-use of these streams in a food production system, the presence of hazardous substances (chemicals, microbiological, and physical) needs to be evaluated to safeguard food safety of such systems. This study aims to evaluate this in sewage sludge collected from multiple locations and in excess aerobic biomass from food industry plants, sampled at 2 – 4 time points. Hazards were analyzed in all samples and included pathogenic bacteria, microbial composition, antimicrobial resistance (genes), viruses, heavy metals, pharmaceutical and pesticide residues, short-chain per- and polyfluoroalkyl substances, dioxins, and physical hazards. Generally, aerobic biomass from wastewater treatment plants of the food industry contained less of the above-mentioned hazards as compared to sludge from municipal wastewater treatment plants. When available, hazard concentrations were compared with maximum limits for fertilizer applications. For heavy metals, these limits were especially exceeded by found cadmium concentrations. Overall, the results of the present study will contribute to the design of safe circular food production systems and showcase the need for a fit-for-purpose and safe-by-design approach in the application of residual streams in food production systems.

1. Introduction

The current food production system is a major contributor to global greenhouse gas emissions and thus to climate change (Clark et al., 2020; Crippa et al., 2021; Vermeulen et al., 2012). It also adds to the depletion of important natural resources, with phosphorus (P) used for artificial fertilizer production being a well-known example. Thus, food production systems urgently need to become more sustainable to feed an

Abbreviations: AMPA, aminomethylphosphonic acid; AMR, antimicrobial resistance; dl, dioxin-like; DM, dry matter; ESBL, extended spectrum beta-lactamase; FTP, excess aerobic biomass from a food industry wastewater treatment plant; GC, gas chromatography; HR, high resolution; IE, inhabitant equivalents; K, potassium; LC, liquid chromatography; LOQ, limit of quantification; ML, maximum limit; MS, mass spectrometry; N, nitrogen; ndl, non-dioxin-like; NL, the Netherlands; P, phosphorus; PCDD/Fs, polychlorinated dibenzo-p-dioxins dibenzofurans; PCR, polymerase chain reaction; PFASs, per- and polyfluoroalkyl substances; PCBs, polychlorinated biphenyls; STP, sludge of a municipal wastewater (sewage) treatment plant; TEF, toxic equivalent factor; TEQ, toxic equivalence.

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increasing world population. The transition to more sustainable and resilient food production will require multiple extensive changes in our current food production system (Clark et al., 2020). A shift towards circularity that respects the limits of our planet is one of these (Muscat et al., 2021; van Zanten et al., 2023), and transitioning towards a circular economy is a key strategy of the European Union to achieve its sustainability goals (EC, 2020). In circular food production systems, residual streams are reduced, reused, and recycled in the most sustainable way (van Zanten et al., 2023). Highly abundant residual streams are sludge and excess aerobic biomass, which is the residue from wastewater treatment. These streams are typically rich in nitrogen (N), P, and potassium (K), and ideally these nutrients are re-used in food production, which increases the circularity of food production systems. Two major sources of these streams are sludge from municipal wastewater (sewage) treatment plants (abbreviated as STP), and excess aerobic biomass from food industry wastewater treatment plants (abbreviated as FTP) (CBS, 2023a).

In 2021, Dutch STPs disposed 1,263 ktonnes of sludge (~308 ktonnes dry matter), containing an estimated 14 ktonnes N, 10 ktonnes P and 1.3 ktonnes K (CBS, 2023a; Phyllis2, 2023). The application of artificial fertilizer in the Netherlands (NL) in 2021 was 207 ktonnes N, 5.3 ktonnes P, and 46 ktonnes K (FAOSTAT, 2023). In addition, industry produced 2,267 ktonnes sludge fresh matter (~540 ktonnes dry matter) in 2021, of which 519 ktonnes aerobic biomass fresh matter (~125 ktonnes dry matter) from FTPs, which may represent flows of N, P, and K in the same order of magnitude as those from STPs (CBS, 2023a). STP sludge alone, applied as fertilizer directly, could decrease the use of artificial fertilizer, as it more than covers the Dutch agricultural use of P. For N and K, approximately 7 % and 3 % could be covered by the application of STP sludge directly, respectively (CBS, 2023a; FAOSTAT, 2023). In addition, next to fertilizer value, sludge may be considered for other applications in (future) circular food production systems, such as insect feed (Raksasat et al., 2022) or possibly for seaweed cultivation.

When re-using STP and FTP streams in circular food production systems, attention needs to be paid to the presence of chemical, microbial, and physical hazards in these streams. The presence of these hazards can, besides a possible effect on the environment, result in the emergence of food safety issues. Returning STP and FTP streams into the food production system may introduce known and unknown food safety hazards that can subsequently possibly transfer through the food production system and/or may lead to accumulation in certain compartments of the food production system. For this reason, use of these streams as fertilizer is restricted in the Netherlands, where for example STP sludge is mainly incinerated (CBS, 2023b), while in some other European countries STP sludge is used as agricultural fertilizer (Eurostat, 2019). Recent reviews on food safety hazards in circular food production systems have, amongst others, identified pesticides, pharmaceuticals, heavy metals, microplastics, and organic contaminants as relevant (chemical) hazards (Egle, 2023; Focker et al., 2022; van Asselt et al., 2023). Compounds that may pose a risk particularly comprise of persistent, bio-accumulative, and toxic substances such as dioxins and certain per- and polyfluoroalkyl substances (PFASs) (Huygens, 2022). Moreover, the attention for persistent and mobile substances without necessarily bio-accumulative properties has increased over the past years. Examples of such substances are PFASs with a short or ultra-short chain length (Hale et al., 2022; Li et al., 2010; Liang et al., 2023). In addition, STP sludge is known to harbor pathogenic viruses, bacteria, and parasites (Bibby & Peccia, 2013; Hamilton et al., 2020; Jauregi et al., 2021; Pepper et al., 2006). In most cases, FTP streams are likely to be less polluted than STP sludges because of the more consistent and controlled input of the wastewater that originates from food production sites. However, data on the presence or absence of potential food safety hazards in FTP streams is currently lacking in scientific literature. This is needed as a first step to evaluate hazards and risks in the use of residual streams in circular food production systems (van Leeuwen et al., 2024). Therefore, the objective of this study is to assess the occurrence of a range of microbiological, chemical, and physical food safety hazards in STP sludge and aerobic biomass of FTPs (agri-food industry) potentially relevant for a circular food production system. This will provide input for possible mitigation measures and will contribute to the design of safe circular food production systems.

2. Materials and methods

2.1. Inventory, selection, and sampling of the residual streams

In this study, we considered different wastewater treatment plant streams with mainly low (economic) value application, produced in large quantities and with relatively high dry organic matter and nutrient content, focusing on municipal sewage sludge (STP) and excess aerobic biomass from food industry wastewater treatment plants (FTP).

Since the hazards in STP sludge were expected to vary between locations, four locations were sampled: STP sludge from two cities with a lot of industrial activity and at least one hospital, indicated as STP-city-1 (Den Bosch, NL) and STP-city-2 (Den Helder, NL), sludge from a STP in a mainly rural area, indicated as STP-rural (Cuijk, NL), and sludge from a Dutch Wadden island without a hospital or large industry, indicated as STP-island (Texel, NL). To give an impression of the size of the treatment plants from which the sludge was derived, in terms of Inhabitant Equivalents (IE): Den Bosch: 342,000 IE, Den Helder: 129,000 IE, Cuijk: 175,000 IE and Texel: less than 100,000 IE (Watersector, 2025). All these STPs receive storm water, e.g., run-off water from city roads, and consist of a pre-sedimentation tank, an aeration tank, a final clarifier, sludge return, thickeners to concentrate primary and secondary sludge, and systems to dewater the sludge. At three of the selected STPs the sludge is digested in an anaerobic digestion process in which the mixed primary and secondary sludge are partly converted into biogas. The original primary and secondary sludge are not subjected to aerobic stabilization before anaerobic digestion. The remainder of the digestion process, dewatered digested sludge, has the highest dry matter (DM) content, and is currently disposed, leaving room for further valorization. From the smallest STP (Texel, NL) only non-digested sludge was available.

In addition to the STP sludges we selected excess aerobic biomass originating from wastewater treatment plants of different food industry types, namely thickened aerobic biomass streams from a sugar factory (FTP-sugar), thickened activated biomass streams from the potato industry (FTP-potato), dewatered activated biomass streams from a dairy factory (FTP-dairy), and dewatered activated biomass streams from a processing plant of animal-based products (FTP-animal). An activated biomass stream is a mass with mainly bacteria and with an enhanced biological activity (to degrade pollutants). A third, but much smaller, source of excess biomass in the Netherlands is being produced in recirculation aquaculture systems. In most cases, this stream consists of suspended solids, primarily fish or shellfish feces and feed residues, directly collected from the water column without further processing. In this study we selected primary sludge produced by a freshwater recirculation aquaculture system (FTP-fish).

In Table 1, an overview of the collected streams and number and timing of sampling is provided. The samples were collected at multiple times throughout the year 2023, to consider differences in composition that may occur due to temporal (seasonal) variations in the type of wastewater that is treated. Samples were stored at 4 °C or −20 °C prior to further analyses, unless otherwise mentioned.

2.2. Nitrogen, phosphorus, potassium, and dry matter analyses

The dry matter content of the collected samples was determined by drying the samples overnight in an oven at 105 ± 5°C until a constant weight was reached. Furthermore, N, P, and K were quantified in all collected streams. Total N included organically bound ammonium nitrogen, urea nitrogen, nitrate, and nitrite. See for the detailed

Table 1
Overview and description of the collected sample types and the sampling in 2023 in the Netherlands (NL). DM: dry matter.

Abbreviation	Description	Type	Annual variation expected	Samples taken; number and timing
Municipal sewage sludge (STP)				
STP-city-1	Den Bosch, NL	Digested mixed sludge	Yes, in summer higher DM content than in winter	3; one in April, one in May, and one in July
STP-city −2	Den Helder, NL	Digested mixed sludge	No	4; one in March, two in May, and one in July
STP-rural	Cuijk, NL	Digested mixed sludge	Yes, in summer higher DM	3; one in April, one in May, and one in July
STP-island	Texel, NL	Mixed sludge	Yes, more wastewater during summer tourist season, but no extreme differences.	2; two in July
Excess aerobic biomass from the agri-food industry (FTP)				
FTP-sugar	Sugar factory	Thickened activated aerobic biomass	Yes, aerobic biomass is produced in campaign (Sept-Jan) and then stored	2; one in April, one in July
FTP-potato	Potato industry	Thickened activated aerobic biomass	Yes, aerobic biomass is produced in campaign (Aug-May) and then stored	2; one in May, one in July
FTP-dairy	Dairy factory	Dewatered activated aerobic biomass	No	2; one in April, one in July
FTP-animal	Processing plant of animal-based products	Dewatered activated aerobic biomass	No	2; one in May, one in June
FTP-fish	Fish farm	Primary sludge	No	2; one in May, one in September

methodology Appendix A1.

2.3. Microbiological analyses

Targeted and broad molecular approaches were used to detect microbes, bacterial pathogens, and antimicrobial resistance (AMR) and AMR genes. Targeted polymerase chain reaction (PCR) analyses were performed on *Campylobacter* spp., *Listeria monocytogenes,* and *Salmonella* spp., as these organisms are frequently found in sewage sludge and represent common gastro-intestinal infections (Focker et al., 2022; van Asselt et al., 2023). *Bacillus cereus* was included as a representative of spore forming bacteria with expected high environmental persistence (Jensen et al., 2003) and *Vibrio parahaemolyticus* as a possible emerging pathogen in aquatic systems. See Appendix A2 for the detailed methodology. To study AMR, extended-spectrum beta-lactamase (ESBL) producing or Carbapenem resistant *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. were cultivated from the samples (see Appendix A3 for the detailed methodology). Metagenomic approaches were applied to identify the presence of human viruses (see Appendix A4 for the detailed methodology) and the bacterial microbiome along with AMR genes in the collected streams (see Appendix A5 for the detailed methodology).

2.4. Chemical hazards

A wide range of chemical hazards were identified in the collected STP and FTP samples. These included heavy metals and (trace) elements, pharmaceutical and pesticide residues, short-chain PFASs, dioxins, and polychlorinated biphenyls (PCBs).

A selection of heavy metals and (trace) elements were quantified by inductively coupled plasma (ICP)-mass spectrometry (MS). All samples were analyzed for manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), mercury (Hg), thallium (Tl), lead (Pb), and uranium (U). See Appendix A6 for the detailed methodology.

For the detection of pharmaceutical residues as well as for pesticide residues, a screening analysis was performed first using liquid chromatography (LC)-high resolution (HR)-MS, followed by confirmatory analysis for samples with a positive screening result by LC-MS/MS, resulting in qualitative data on the presence or absence of the included substances. For the pharmaceutical residues, the screening analyses included 480 compounds of the following groups: antibiotics, anthelmintics, painkillers, blood pressure medication, antacids, antiepileptics, anti-depressants, lipid-lowering agents, anti-ADHD, pharmaceuticals for diabetes, diuretics, thyroid hormones, beta-agonists, hormones, beta-blockers, and benzodiazepines. See Appendix A7 for the detailed methodology of the pharmaceutical residues analyses. For the pesticide screening and confirmation, substances included fungicides, insecticides, and herbicides. Additionally, glyphosate and its degradation product AMPA (aminomethylphosphonic acid) were quantified in the samples by LC-MS/MS. See Appendix A8 for the detailed methodology.

Furthermore, a range of short-chain PFASs were quantified, because of their water-soluble nature that makes them more mobile, and thus potentially available for transfer and uptake by and through the food production system (e.g., crops, animals, humans). We focused on analyzing (ultra) short-chain PFASs, which included compounds with 1 to 6 fluorinated carbon atoms in the backbone. These included perfluorocarboxylic acids (PFCAs) (including TFA, PFPrA, PFBA, PFPeA, and PFHxA), in addition to perfluorosulfonic acids (PFSAs) (including TFMS, PFEtS, PFPrS, PFBS, PFPeS, and PFHxS), and perfluoroalkyl ether acids (PFEAs) (including PFMOAA, PFMOPrA, PFMOBA, PMPA, PEPA, and HFPO-DA). Besides quantification of these substances, we also screened for the presence of longer chain PFASs in the samples. These included PFCAs (including PFOA and PFNA), in addition to PFOS and n:2 fluorotelomer sulfonic acids (FTSs) (including 6:2 FTS and 8:2 FTS). See Appendix A9 for the detailed methodology, and a specified list of the included PFASs including their full name.

Finally, the lipophilic dioxins (polychlorinated dibenzo-p-dioxins, dibenzofurans; PCDD/Fs) and dioxin-like (dl) and non-dioxin-like (ndl) PCBs were detected and quantified in the collected samples by gas chromatography (GC)-HRMS. Concentrations of individual compounds were multiplied by their toxic equivalent factor (TEF) and summed to obtain a total toxic equivalence (TEQ). For the detailed methodology, see Appendix A10.

2.5. Physical hazards

The presence of physical hazards (metals, plastics, and plastic fibres) in the collected samples was qualitatively evaluated using an Olympus SZX 10 optical microscope (Olympus Corporation, Tokyo, Japan) with an attached Olympus SC50 CMOS camera (Olympus Corporation, Tokyo, Japan) at 4x-16x magnifications. This allowed for evaluation of particles $\geq$ 1 mm. See Appendix A11 for the detailed methodology.

2.6. Overview score of the collected data

To aid comparison between the different STP and FTP streams, an overview score was compiled per analyte group, for each STP or

FTP stream type. For qualitatively presented results (i.e., foodborne pathogenic bacteria, AMR presence, virology, pharmaceutical and pesticide residues, physical hazards), the overview score was compiled according to equation 1. For quantitatively presented results (i.e., concentrations of heavy metals and trace elements, short-chain PFASs, pesticide residues glyphosate and AMPA, dioxins and PCBs), the overview score was compiled using equations 2 and 3.

$$\text{Equation 1:. } Overview\ score(\%)_{jk} = \left(\frac{\sum_{i=1}^{n} sum\ of\ positives_{ijk}}{total\ possible\ positives_{k}}\right) x100$$

$$\text{Equation 2:. } Normalized\ concentration_{ij} = \frac{Concentration_{ij}}{Maximum\ concentration_{i}}$$

$$\text{Equation 3:. } Overview\ score(\%)_{j} = \left(\frac{\sum_{i=1}^{n} Normalized\ concentration_{ij}}{n}\right) x100$$

Each analyte group k consists of multiple individual analytes i, and the total number of analytes in that group is indicated with n. The STP or FTP stream type is indicated with j.

For the AMR genes, the average normalized total AMR gene count per sample type was expressed as percentage of the maximum amount of total AMR genes detected amongst all individual samples (set at 100 %). The overview scores of the STP and FTP streams per analyte group served as input for a heatmap, scaled 0 to 100 %. This was visualized using Excel (v2408).

3. Results

3.1. Dry matter contents and nitrogen, phosphorus, and potassium concentrations

Samples of 4 STPs and 5 FTPs were collected (as described in Table 1). DM concentrations of the samples ranged between 5.6 to 25.5 % (see Appendix B Table B.1) and were used for correction of the determined chemical concentrations. Besides, concentrations of three relevant elements of nutritional value were determined in the sludge samples (i.e., nitrogen, phosphorus, and potassium). Total nitrogen in the STP sludge ranged from 55.4 to 82.5 g/kg DM, while total nitrogen ranged from 14.0 to 72.4 g/kg DM in the FTP streams. The highest total nitrogen contents in STP- and in FTP streams were determined in STP-island (82.5 g/kg DM) and in FTP-potato (72.4 g/kg DM), respectively. Total phosphorus ranged from 33.5 to 43.7 g/kg DM in the STP sludge and ranged from 9.31 to 76.7 g/kg DM in the FTP streams. FTP-fish (76.7 g/kg DM) and STP-rural (43.7 g/kg DM) contained the highest total phosphorus contents. Total potassium content ranged from 1.12 to 12.9 g/kg DM in the STP sludge and from 0.42 to 12.2 g/kg DM in the FTP streams (Appendix B Table B.2).

3.2. Microbiological analyses

3.2.1. Pathogenic bacteria

To detect foodborne pathogenic bacteria, PCR analyses were performed on twelve STP samples and ten FTP samples, collected at two to four time points (Table 2). None of the samples was shown to be positive for *Salmonella* spp., *Campylobacter* spp., or *Vibrio parahaemolyticus*. *Listeria monocytogenes* positive samples were found amongst the STP samples, specifically for 2 out of 3 samples from STP-city-1 and for 2 out 3 samples from STP-rural. All samples except for FTP-fish were PCR positive for the *Bacillus cereus* group species.

3.2.2. Antimicrobial resistance

The presence of antimicrobial resistant bacteria in the STP and FTP samples was determined through studying the presence of ESBL bacteria and specific Carbapenem resistant bacteria (Table 2). In many of the samples ESBL bacteria were detected, with STP sludge samples, making up the majority of ESBL-positive samples, as nine out of 12 individual samples tested positive for *E. coli* ESBL. For all of these nine positive samples an *E. coli* ESBL was found, and for two samples an additional *Klebsiella pneumoniae* ESBL isolate was identified. *E. coli* ESBL positive samples were also observed in the FTP-animal samples, with two out of two positive samples. For the other FTP samples, one positive FTP-potato sample and one positive FTP-sugar sample was observed, being both an *E. coli* ESBL as well. In two samples, a carbapenem resistant isolate was obtained. Both cases concerned the STP-rural samples, and in both cases the isolate was identified as *E. coli*. In addition, the carbapenem resistant isolates showed a KPC (Klebsiella pneumoniae carbapenemase) gene to be present for one isolate and a NDM (New Delhi metallo-β-lactamase) gene for the second isolate, as the responsible carbapenemase gene.

3.2.3. Microbiome profiling, with a special focus on AMR genes

To profile the microbiome, long-read metagenomic sequencing was applied to twelve STP samples and ten FTP samples. Alpha and beta diversity parameters were calculated. Alpha diversity informs on how many different types of microorganisms are in each individual sample, and beta diversity describes the (dis)similarities in microbial communities between these different samples. Alpha diversity parameters showed a numerically higher richness for the STP samples (5774 ± 2374) compared to the FTP samples (3429 ± 1543). Whereas for the α-diversity Shannon index a significant effect was observed ($P = 0.03$), with STP samples 5.05 ± 0.27 compared to the FTP samples 4.92 ± 0.55. Lastly, α-diversity parameter Pielou's evenness was determined and showed no significant effect, with values of 0.58 ± 0.02 for STP and 0.61 ± 0.08 for FTP. When plotting each sample type separately, specific differences were observed for some sample types, where FTP-potato and FTP-fish showed a higher Shannon index compared to any of the STP sludge samples (see Appendix B Fig B.1). In addition to the differences in alpha diversity, beta diversity parameters showed a significant effect ($P < 0.001$) of source (i.e., STP or FTP) on the microbiota composition (Appendix B Fig B.2). Furthermore, within a source, samples showed high similarity towards their duplicate or triplicate samples from other timepoints, in each sample type.

A more detailed analysis was performed by zooming into the AMR genes. In general, more AMR genes were observed in the STP groups compared to the FTP groups (Fig. 1). For the FTP-sugar no AMR genes

Table 2
PCR results for the detection of foodborne pathogens and conventional plating results for the detection of antimicrobial resistant bacteria (ESBL and carbapenem-resistant) in sewage treatment plant (STP) samples and aerobic biomass samples from food industrial wastewater treatment plants (FTP). Numbers depict n positive samples/n total tested samples.

	Salmonella spp.	*Campylobacter* spp.	*Bacillus cereus* group	*Listeria monocytogenes*	*Vibrio parahaemolyticus*	ESBL	Carbapenem-resistant
STP-city-1	0/3	0/3	3/3	2/3	0/3	0/3	0/3
STP-city-2	0/4	0/4	4/4	0/4	0/4	4/4	0/4
STP-rural	0/3	0/3	3/3	2/3	0/3	3/3	2/3
STP-island	0/2	0/2	2/2	0/2	0/2	2/2	0/2
FTP-potato	0/2	0/2	2/2	0/2	0/2	1/2	0/2
FTP-sugar	0/2	0/2	2/2	0/2	0/2	1/2	0/2
FTP-animal	0/2	0/2	2/2	0/2	0/2	2/2	0/2
FTP-dairy	0/2	0/2	2/2	0/2	0/2	0/2	0/2
FTP-fish	0/2	0/2	0/2	0/2	0/2	0/2	0/2

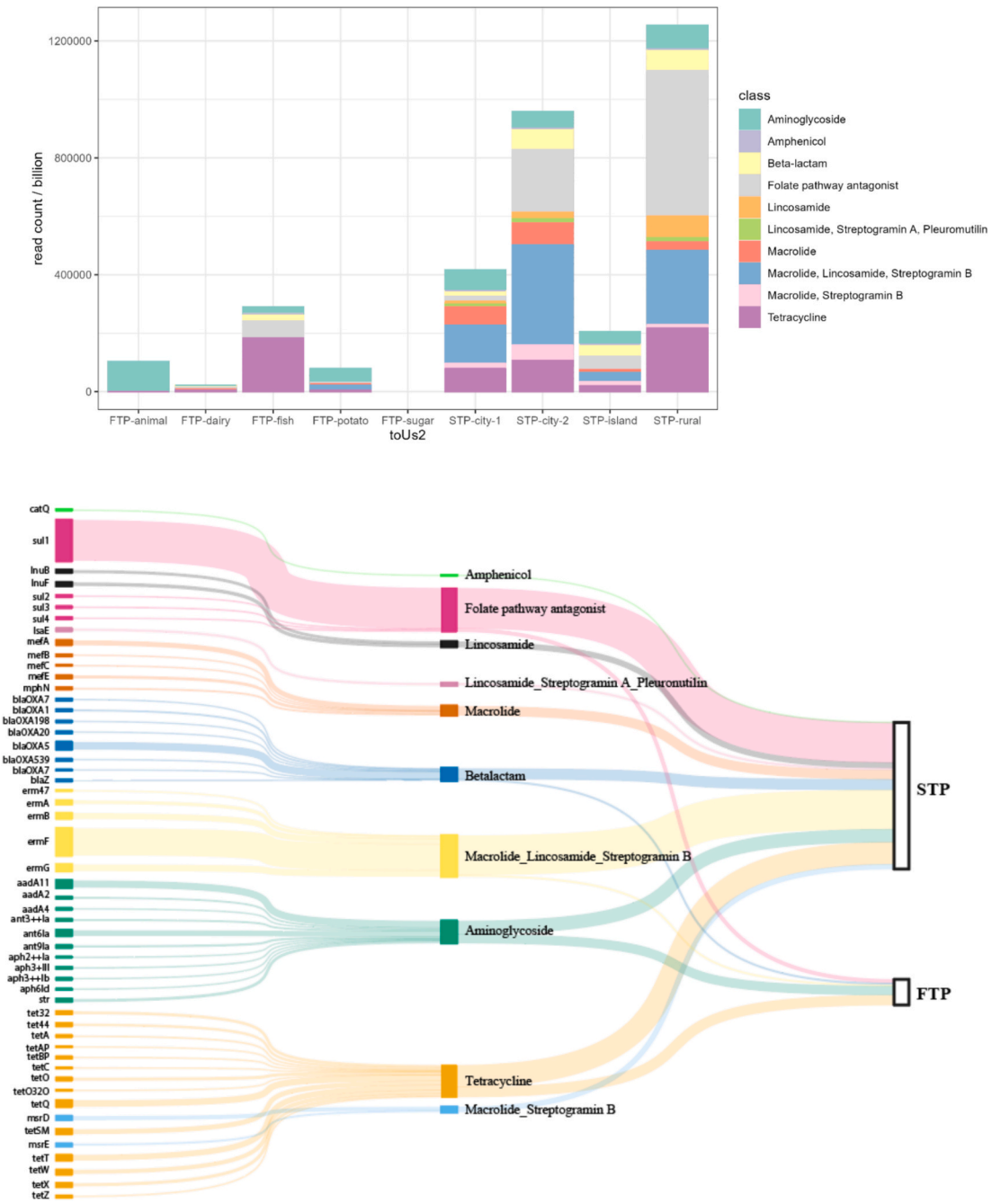

Fig. 1. Number of classes of antimicrobial resistance (AMR) genes per sample type. Streams from various origins were tested, i.e., aerobic biomass of wastewater treatment plants of the agri-food industry (FTP) or sewage treatment plants (STP). The top panel shows the read count per billion of the different AMR genes (x-axis) per individual FTP or STP stream. In the bottom panel, edges represent the read count per billion of the different AMR genes (minimum proportion for taxa above 0.001) and grouped per class of AMR, thereafter, grouping is based on STP or FTP.

were detected, and for FTP-animal, FTP-dairy, and FTP-potato, AMR genes were also detected at a low level. In contrast, STP-rural showed the highest amount of AMR genes, followed by STP-city-2, STP-city-1, and STP-island being the lowest of the STP sludge samples. The detected genes are grouped by the classes of antibiotics to which they confer resistance. The highest detection of individual genes was in the samples from STP-city-2 and STP-rural, including sulphonamide resistance genes (*sul*-genes), folate pathway antagonists which confer resistance to sulphonamides and *erm*-genes, conferring resistance to macrolides, lincosamides and streptogramin B. In FTP-fish, *tet*-genes, conferring resistance to tetracyclines, were detected notably high. Low-level resistance gene content was detected against medically important antimicrobials (MIA) including the critically important antimicrobials (CIA) macrolides and aminoglycosides, and highest-priority critically important antimicrobials (HP-CIA) in the form of the acquired carbapenemase gene *bla*OXA-198 in the STP (one STP-city-1, one STP-city-2, and two STP-rural) samples.

3.2.4. *Viromics*

Per FTP- and STP sample, sequencing reads for human infectious virus species were collected and assembled. The resulting number of positive samples per sample type including the mean genomic coverage ± standard deviation per species is depicted (Table 3).

Results for samples taken at the different time points were similar to

Table 3
Short list of genomic fragments from human virus species identified using the bait capture metagenome assay. Numbers depict n positive samples/n total tested samples, and the Mean ± SD of percentage genome recovery of virus fragments from the respective virus species. STP = municipal wastewater sewage treatment plant sludge; FTP = aerobic biomass of wastewater treatment plants of the agri-food industry.

Family	*Astroviridae*	*Reoviridae*	*Astroviridae*	*Astroviridae*	*Astroviridae*	*Astroviridae*	*Reoviridae*	*Astroviridae*	*Caliciviridae*
Species	Astro-virus MLB1	Rota-virus A	Human astro-virus	HMO astro-virus A	Mama-strovirus 1	Mama-strovirus 9	Rota-virus C	Astro-virus MLB2	Noro-virus
STP-city-1	1/3; 14.7 ± 0	3/3; 60.5 ± 19.4	0/3	2/3; 3.1 ± 0.8	0/3	0/3	3/3; 29.9 ± 1.8	0/3	1/3; 4 ± 0
STP-city-2	4/4; 87.1 ± 8	4/4; 57.9 ± 17.3	4/4; 68.4 ± 16.5	4/4; 57.4 ± 18.1	4/4; 58.5 ± 13.4	4/4; 20.4 ± 19.1	4/4; 32.3 ± 9.5	4/4; 10.2 ± 21.6	4/4; 11.7 ± 17.3
STP-rural	3/3; 90.4 ± 2.4	3/3; 81.5 ± 0.7	3/3; 64.6 ± 13.6	3/3; 96.5 ± 1.4	3/3; 50.1 ± 21	3/3; 37.9 ± 22.8	3/3; 34.8 ± 14.9	2/3; 3.6 ± 2	3/3; 25.7 ± 14.3
STP-island	2/2; 35.8 ± 8.4	2/2; 74.8 ± 4.6	2/2; 43.2 ± 14	2/2; 57.7 ± 7.7	2/2; 33.7 ± 13.4	2/2; 16.6 ± 13.5	1/2; 6.9 ± 0	2/2; 26 ± 7.1	2/2; 11.2 ± 5.6
FTP-potato	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
FTP-sugar	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
FTP-animal	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
FTP-dairy	0/2	1/2; 17.4 ± 0	0/2	0/2	0/2	0/2	0/2	0/2	0/2
FTP-fish	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2; 3.1 ± 0
Nega-tive control	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

each other. Major differences in virus species diversity and genomic coverage were observed between samples of different sampling locations. STP samples hosted a range of astrovirus, rotavirus and human norovirus genomic fragments. While the STP-city-2, STP-rural, and STP-island samples hosted the largest diversity of virus species, with nine different identified human virus species, the STP-city-1 samples were markedly cleaner with five different identified human virus species, with generally lower genomic coverage. FTP samples were mostly free of human infectious virus genomic fragments.

3.3. Chemical hazards

3.3.1. Heavy metals and trace elements

The concentrations of a selection of heavy metals and trace elements in the samples are presented in Table 4 (i.e., arsenic, cadmium, copper, lead, mercury, nickel, and zinc). In general, the highest concentrations were found in STP sludge samples, while the lowest concentrations were quantified in FTP-dairy. Arsenic concentrations ranged between 0.24 and 17.0 mg/kg DM with the lowest concentration found in the FTP-dairy, while highest arsenic concentrations within a similar range were detected in FTP-sugar (17.0 mg/kg DM), STP-city-2 (11.4 mg/kg DM), FTP-animal (11.0 mg/kg DM), and FTP-fish (9.38 mg/kg DM). Cadmium was not detected in FTP-dairy, while concentrations in the other samples ranged between 0.78 and 4.81 mg/kg DM, with the highest concentrations being detected in FTP-potato and FTP-animal with concentrations of 3.75 and 4.81 mg/kg DM, respectively. Mercury and lead concentrations were highest in the STP sludge samples, in which the concentrations ranged between 0.20 and 0.50 mg mercury/kg DM and 57.0 and 77.5 mg lead/kg DM. Copper concentrations in STP sludge exceeded those of the FTP samples, with the highest concentration measured in STP-city-1 (572 mg/kg DM) and the lowest in FTP-dairy (3.98 mg/kg DM). Nickel concentrations ranged between 3.20 mg/kg DM (FTP-dairy) and 25.6 mg/kg DM (STP-rural). Zinc concentrations were generally higher in the STP compared to the FTP samples, but highest concentrations were measured in FTP-fish (2078 mg/kg DM). The measured concentrations were compared to maximum limits (MLs) of the Dutch fertilizer law implementation decree for sludge and to limits for a potential application as co-material with manure in a digester (Rijksoverheid, 2024). The latter limits are dependent on the determined nutrient levels of the sample (sludge) and are thus expressed per amount of N, P and K per sample type accordingly. Comparisons are presented in Table 4 and are further elaborated on in the discussion.

Besides the above-mentioned heavy metals and elements, others were detected in the residual streams in the following range: manganese (<limit of quantification (LOQ) to 4.09 g/kg DM), iron (0.3 to 39.3 g/kg DM), cobalt (0.65 to 7.85 mg/kg DM), selenium (<LOQ to 5.30 mg/kg DM), molybdenum (1.25 to 9.30 mg/kg DM), thallium (<LOQ to 0.21 mg/kg DM), and uranium (<LOQ to 2.58 mg/kg DM) (Appendix B Table B.3).

Table 4
Concentrations of selected heavy metals and elements of 9 different sample types (4 sewage treatment plant (STP) sludge types, and 5 types of aerobic biomass from wastewater treatment plants of the agri-food industry (FTP)). Numbers depict mean ± SD in mg/kg dry matter (DM) and the number of samples (n) that were analysed per sample type. Cu = copper; Zn = zinc; Cd = cadmium; As = arsenic; P = phosphorus; N = nitrogen.

Sample types	n	Arsenic	Cadmium	Copper	Lead	Mercury	Nickel	Zinc	HM fold exceedance[b]
		mg/kg DM							
STP-city-1	3	4.26 ± 0.32	1.35[a] ± 0.44	572[a] ± 4.15	77.5 ± 5.85	0.28 ± 0.04	23.4 ± 1.16	1237[a] ± 67.0	Cu:3.4x; Zn:1.8x (P[d])
STP-city-2	4	11.4 ± 2.02	1.26[a] ± 0.11	292[a] ± 44.6	57.0 ± 15.8	0.35 ± 0.07	16.1 ± 1.20	986[a] ± 127	Cu:1.8x; Zn:1.5x (P[d])
STP-rural	3	6.61 ± 0.48	1.79[a] ± 0.12	406[a] ± 43.5	74.2 ± 2.94	0.50 ± 0.10	25.6 ± 1.57	1191[a] ± 38.6	Cu:2.2x; Zn:1.6x (P[d])
STP-island	2	4.07 ± 0.64	0.91 ± 0.26	226[a] ± 27.1	65.7 ± 53.9	0.20 ± 0.07	9.96 ± 1.65	606[a] ± 76.8	Cu:1.6x; Zn:1.1x (P[d])
FTP-potato	2	2.35 ± 0.35	3.75[a] ± 0.41	117[a] ± 4.48	15.9 ± 2.65	<0.12[c]	5.63 ± 2.25	298 ± 24.1	Cd:2.1x; Cu:1.1x (N=[d])
FTP-sugar	2	17.0[a] ± 1.14	0.78 ± 0.18	30.7 ± 4.41	22.2 ± 0.25	0.16 ± 0.03	7.56 ± 0.40	113 ± 6.99	Cd:1.2x; As:2.1x (P[d])
FTP-animal	2	11.0 ± 0.58	4.81[a] ± 2.73	35.3 ± 2.21	30.3 ± 18.61	0.10 ± 0.01	13.0 ± 2.63	240 ± 23.7	Cd:1.8x (P[d])
FTP-dairy	2	0.24 ± 0.03	<0.06[c]	3.98 ± 0.59	0.53 ± 0.22	<0.06[c]	3.20 ± 0.43	56.3 ± 6.66	– (P[d])
FTP-fish	2	9.38 ± 4.33	1.38[a] ± 0.50	113[a] ± 69.0	1.19 ± 0.20	0.18 ± 0.06	19.3 ± 5.96	2078[a] ± 488	Zn:1.6x (P[d])

[a] Concentration exceeded the limit of the Dutch fertilizer law implementation decree for sludge, which is 15 mg/kg DM for arsenic, 1.25 mg/kg DM for cadmium, 75 mg/kg DM for copper, 100 mg/kg DM for lead, 0.75 mg/kg DM for mercury, 30 mg/kg DM for nickel, and 300 mg/kg DM for zinc (Rijksoverheid, 2024).
[b] heavy metal (HM) fold-exceedance of the limit during incorporation as co-material in digester with manure for fertilizer application.
[c] Limit of Quantification (LOQ).
[d] Value-adding component for the sludge to which the comparison was done.

3.3.2. Pharmaceutical residues

A suspect screening and subsequently a confirmatory analysis approach was subjected to the samples to screen and confirm the presence of pharmaceutical residues. The STP sludge samples were found to be positive for a broad range of pharmaceuticals including painkillers, benzodiazepines, lipid-lowering agents, beta-agonists, blood-pressure medication, beta-blockers, anti-depressants, antibiotics, anthelmintics, and hormones. The number of confirmed pharmaceuticals was the lowest in STP-island sludge (13 confirmed compounds). The highest number of confirmed pharmaceuticals was found in STP-city-2 sludge (15–21 confirmed compounds). The confirmed pharmaceuticals per sample can be found in Appendix B Table B.4.

Most FTP samples contained considerably fewer pharmaceuticals compared to the STP sludge samples. None of the pharmaceuticals included were found in FTP-sugar, FTP-potato, and FTP-animal samples. In FTP-dairy, only albendazole and its metabolite albendazole sulfoxide were found. FTP-fish was positive for the antibiotics enrofloxacin and its metabolite ciprofloxacin, flumequine, levofloxacin, mebendazole, and its metabolites amino mebendazole and hydroxy mebendazole.

3.3.3. Pesticides

Glyphosate and its primary degradation product AMPA were quantitatively determined in the samples (Table 5). Glyphosate was only detected in the four STP samples, of which concentrations were in a similar range. AMPA was detected in all STP sludge samples, as well as in FTP-potato and FTP-sugar. STP-city-1 contained the highest AMPA concentration. In FTP-animal, FTP-dairy, and FTP-fish, neither glyphosate nor AMPA were quantified.

Moreover, a screening approach was applied and included a wide range of pesticides. Subsequently, a confirmatory analysis was performed only on positive screened samples. This included all STP sludge types, one collected sample of FTP-potato, and two FTP-sugar samples. The confirmatory analysis included 38 water-soluble pesticidal compounds, which can be classified as 22 fungicides, 9 insecticides, and 7 herbicides (Appendix B Table B.5). This analysis showed that of the STP sludge types, STP-island contained the lowest number of confirmed pesticidal compounds (1–3 confirmed compounds). The total number of confirmed pesticidal compounds in the other STP samples ranged between 4 and 17. In all STP sludge samples, the insecticidal compound permethrin was detected, while four other insecticides (chlorpyrifos, cyhalothrin, diazinon, and pirimiphos-methyl) were detected in only some of the STP sludge samples. In one of the FTP-potato samples, 9 pesticidal compounds were confirmed, and for the FTP-sugar the confirmed number of compounds ranged between 4 and 6.

Table 5
Concentrations of glyphosate and its primary degradation product aminomethylphosphonic acid (AMPA) in the sewage sludge (STP), and in the aerobic biomass of the wastewater treatment plants of the agri-food industry (FTP), in mg/kg dried material. SD refers to the standard deviation and n refers to the number of collected samples analyzed.

Sludge types	n	AMPA (mg/kg DM) Mean ± SD	Glyphosate (mg/kg DM) Mean ± SD
STP-city-1	3	6.76 ± 0.069	0.11 ± 0.026**
STP-city-2	4	1.77 ± 0.851	0.20 ± 0.082
STP-rural	3	2.11 ± 1.061	0.05 ± 0.081*
STP-island	2	1.10 ± 0.163	0.21 ± 0.124
FTP-potato	2	0.655 ± 0.636**	<LOQ
FTP-sugar	2	0.16 ± 0.057	<LOQ
FTP-animal	2	<LOQ	<LOQ
FTP-dairy	2	<LOQ	<LOQ
FTP-fish	2	<LOQ	<LOQ

Limit of quantification (LOQ) was 0.1 mg/kg; *Two samples were < LOQ and values were set at 0; **Contains sample(s) with an indicative quantitative result, and thus the LOQ was used for calculations.

3.3.4. Short-chain PFASs

Seven of the 17 short-chain-PFASs analysed in this study were frequently detected in the samples and concentrations are presented in Fig. 2. Results of the other compounds (PFHxA, PFEtS, PFPrS, PFPeS, PFMOAA, PFMOBA, PMPA, PEPA, and HFPO-DA) were mostly < LOQ, or only scarcely detected, and presented in Appendix B Table B.6 for concentrations per kg wet weight and Appendix B Table B.7 for the concentrations per kg DM. While short-chain PFASs are very water soluble, it may seem arbitrary to express it on DM content. However, considering that water contents of sludges were variable, we decided to express on DM basis, also to align better with other chemical hazard classes in this paper. The results showed that levels of TFA were substantially higher than for the other included short-chain PFASs (Fig. 2B). TFA is present in nearly all samples at average levels up to roughly 170 μg/kg DM (STP-island). TFMS was also detected in nearly all samples, except FTP-fish. PFPrA, PFHxA, PFBS, and PFHxS were detected in six out of the nine sample types. FTP-potato and FTP-fish contained only detectable amounts of respectively two and one studied PFASs. In general, the FTP samples were contaminated to a lesser degree than the STP samples.

Besides quantification of these short-chain PFASs, the samples were screened qualitatively for presence of PFOA, PFNA, PFOS, 6:2 FTS, 8:2 FTS. The methodology did not allow accurate quantification, but presence/absence of the substances could be indicated. These PFASs were shown present in most samples, with highest detection frequency for PFOA, PFNA, and PFOS, and lower frequency for 6:2 FTS and 8:2 FTS. These results are presented in Appendix B Table B.8.

3.3.5. Dioxins and PCBs

TEQ levels of dioxins, dl-PCBs, and concentrations of ndl-PCBs were highest in the four STP-sludge types and in the FTP-animal and FTP-fish (Fig. 3 for upper bound; Appendix B Table B.9 for lower bound values). Total dioxins and dl-PCBs (WHO2005-PCDD/F-PCB) TEQ, ranged between 2.31 and 3.87 ng TEQ/kg DM in the STP sludge types, and was lower for the FTP samples in which concentrations ranged between 0.25 and 1.56 ng TEQ/kg DM. Total ndl-PCBs ranged between 4.42 and 19.24 μg/kg DM in the STP samples, and were generally lower in FTP samples with concentrations ranging between 0.71 and 5.06 μg/kg DM. The lowest levels of dioxins, dl-PCBs, and ndl-PCBs were detected in FTP-dairy, FTP-potato, and FTP-sugar (Fig. 3). Dl-PCBs are comprised of non-ortho PCBs (WHO2005-NO-PCB), of which upper bound values varied between 0.03 and 1.31 ng TEQ/kg DM, and mono-ortho PCBs (WHO2005-MO-PCB), which varied between 0.003 and 0.11 ng TEQ/kg DM (Appendix B Table B.10).

3.4. Physical hazards

The samples were qualitatively evaluated for the presence of physical hazards (see also Appendix B Table B.11). In the STP sludge samples, contamination with plastic fibres was observed, except for STP-island. For the FTP samples, plastic fibres were only found in the FTP-animal sample. Microscopic observations of the FTP-potato, FTP-sugar, FTP-dairy, and FTP-fish revealed contamination with plastics only in one of the two samples analysed per sample type. Moreover, metal contaminations were observed in the FTP-sugar, FTP-animal, and FTP-fish. No metal contaminations were determined in the FTP-potato, FTP-dairy, and STP sludge samples.

3.5. Overview score of the collected data

Based on the findings of the present study, we aimed to provide a comprehensive overview of the results per included hazard group, and per sample type included in the present study. An overview score for each sample type was compiled per food safety hazard group, and aids comparison amongst the different sample types, but cannot be used for comparison between the groups. The food safety hazard groups were

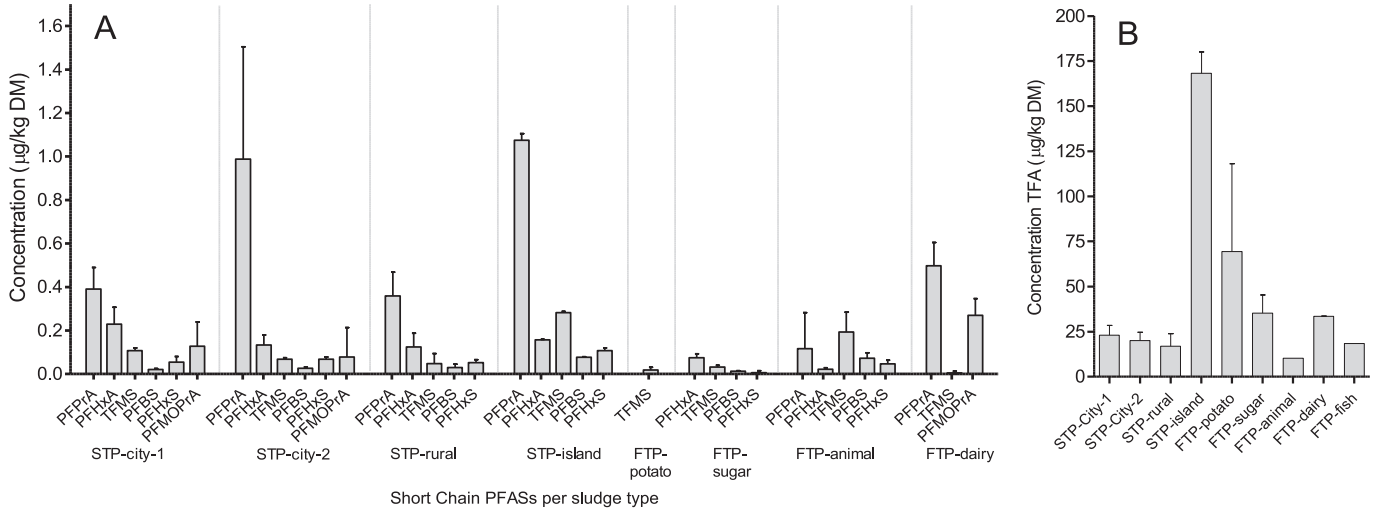

Fig. 2. Concentrations of the short-chain PFASs PFPrA, PFHxA, TFMS, PFBS, PFHxS, PFMOPrA (panel A) and TFA (panel B) in sludge from municipal wastewater treatment plants (STP) and aerobic biomass from wastewater treatment plants of the agri-food industry (FTP), in µg/kg DM. Data points represent the average ± SD of the different collected sludge samples. FTP-fish was not included in panel A as all data points were < LOQ. PFASs values < LOQ are not shown. DM: dry matter.

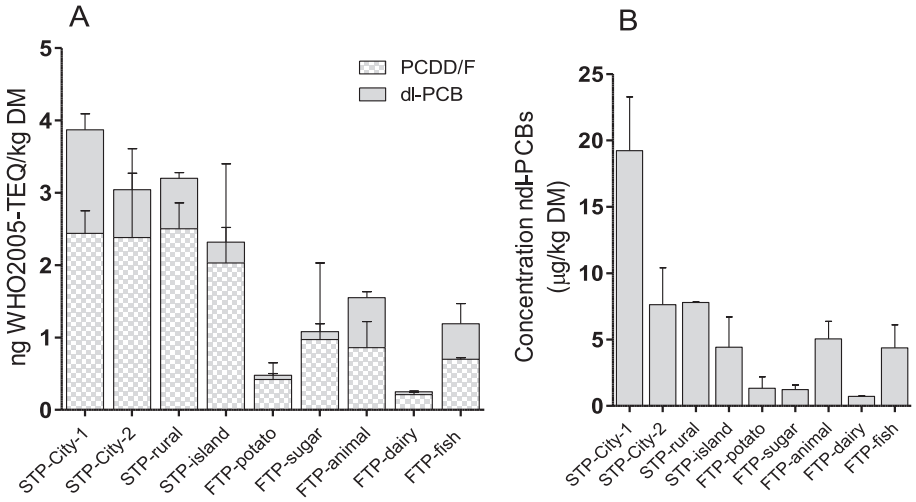

Fig. 3. Total toxic equivalent (TEQ) of dioxins (WHO2005-PCDD/F) and dioxin-like PCBs (WHO2005-dl-PCB) levels are presented in panel A and together represent WHO2005-PCDD/F-PCB-TEQ. Non-dioxin like PCBs (Total ndl-PCBs) are presented in panel B. STP: sludge from municipal wastewater treatment plants; FTP: aerobic biomass from food industry wastewater treatment plants. Upper bound (ub) values are presented, which incorporated the LOQ for analytes determined in case of < LOQ. Average ± SD values are presented, of the number of samples taken per sludge type (3 for STP-city-1, 4 for STP-city-2, 3 for STP-rural and 2 for all other sludge types). DM: dry matter.

evaluated qualitatively (i.e., foodborne pathogenic bacteria, AMR, viromics, pesticide residues, pharmaceutical residues, physical hazards), or quantitively (AMR genes, heavy metals and trace elements, pesticide residues glyphosate and AMPA, short-chain PFASs, dioxins). For the qualitative obtained data, a score of 100 % indicates that all analytes included in the analysis of that food safety hazard group were present in the sample type. For the quantitative data obtained, a score of 100 % indicates that for each individual analyte part of the analyte group, the concentration was highest in that sample type. A visual representation of the scores can be found in Fig. 4. In general, FTP samples cluster together within their group, and similarly, STP samples also form clusters within their own group for most analyte groups.

4. Discussion

In the present study, we evaluated the presence and occurrence of a range of food safety hazards in STP and FTP streams. Generally, the presence and quantity of chemical, microbiological, and physical hazards in FTP streams were found to be lower compared to their presence in the STP streams.

4.1. Microbiological analyses

Microbiological analyses included untargeted and targeted approaches to evaluate the presence of pathogenic bacteria, viruses, the microbiome, AMR, and AMR genes in the samples. This is relevant for safe, circular food production systems since pathogens were reported to persist in soils amended with STP sludge (Roberts et al., 2016) and might contaminate produce by uptake via the roots or direct contact of contaminated soil with produce (Black et al., 2021). Studies addressing the transfer of biological contaminants to food crops are still limited, however, it has been a recent topic of interest (McKenzie-Reynolds et al., 2025; Xiong et al., 2025). Their presence in the STP and FTP samples was expected but their relative contribution had not previously been compared. For example, *Bacillus* spp. were reported in literature to predominate in aerobic spore-forming communities in activated sludge

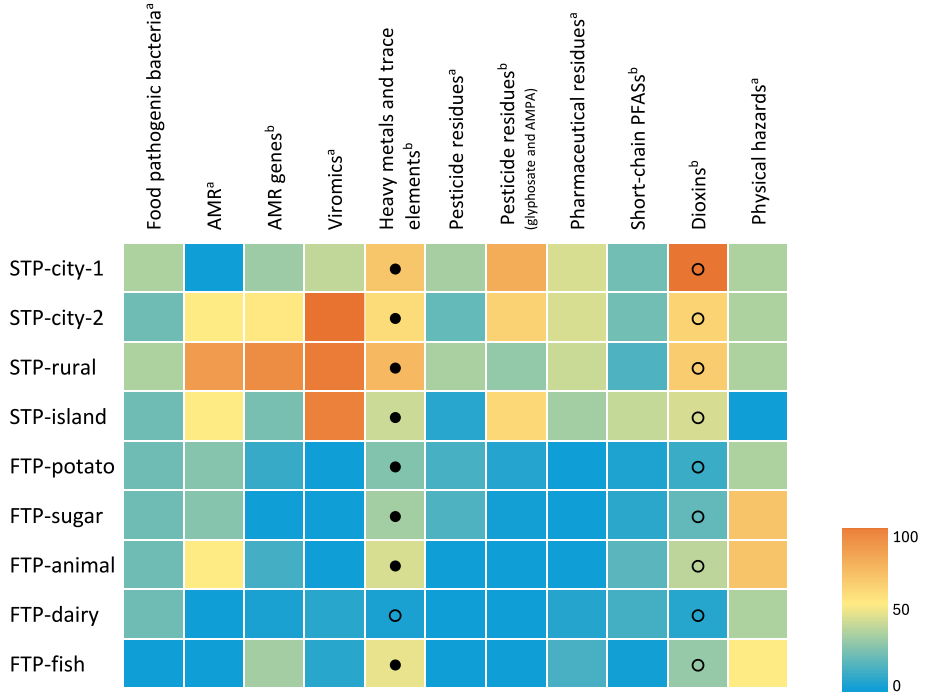

Fig. 4. Overview score of each sample type on the presence of analytes per included group of hazards, expressed on a scale of 0 (low) to 100% (high). [a] Qualitative evaluation; [b] quantitative evaluation. One of the included analytes exceeding the available maximum limit (ML) when available is indicated with full circles; no analyte exceeding available MLs is indicated with open circles. AMR: antimicrobial resistance; STP: sludge of municipal wastewater treatment plants; FTP: excess aerobic biomass of food industry wastewater treatment plants.

of STPs (Mizuki et al., 2001). It is therefore not surprising that *Bacillus cereus* was found in almost all analyzed samples, except FTP-fish. *Listeria monocytogenes*, however, was only found positive for some of the STP samples and for none of the FTP samples. There is no clear reason why *L. monocytogenes* was only reported in some STP-sludge types, since *L. monocytogenes* is known to be present in all environments and is also a well-known bacterium that can reside in process environments (Osek et al., 2022). For the other included pathogenic bacteria, no positive samples were observed. In general, this indicates that the amount of foodborne pathogens was low. However, not all known foodborne pathogens were tested for, and it is unclear what kind of bacteria can be transferred to food products when the sludge is applied in circular food production systems. Also recent studies have focused on the biological risks associated with the use of STP streams and suggest to monitor risks carefully by a combination of conventional and molecular techniques (Peruzzo et al., 2024). Furthermore, it is important to note that the PCR-obtained results as such must be regarded as an indication, since positive results are based on the presence of DNA, and thus viability of the pathogenic bacteria present in the sludge remains to be determined.

Besides the presence of pathogenic bacteria, for many of the samples ESBL isolates were obtained through culturing. No clear difference was observed in the number of *E. coli* ESBL positive samples between the STP sludge and FTP samples. The fact that *E. coli* ESBLs were found in all these different sludge samples is not very surprising as ESBL bacteria are found in many environments (Husna et al., 2023) and *E. coli* is a commensal for humans and ruminants (Ramos et al., 2020). *Klebsiella pneumonia* ESBLs were found in only two STP sludge samples. *Klebsiella* are bacteria that originally occur in the human intestines (Bray & Zafar, 2024) and are thus expected to be more abundantly present in STP sludge as compared to FTP streams. In addition, for two STP sludge samples a carbapenem resistant *E. coli* isolate was obtained. Carbapenem resistance is unwanted as this class of antibiotics is usually reserved as a last resort to treat multidrug-resistant (MDR) bacterial infections in humans (Husna et al., 2023). However, since carbapenems are only allowed to be used in humans and not in animals (Bonardi & Pitino, 2019), it is not surprising that carbapenem resistance was only found in STP sludge samples. MDR foodborne pathogenic bacteria, such as *E. coli* (EHEC), *Salmonella*, or *Campylobacter* can be hard to treat in case of infection. In general antibiotics are not recommended for treatment of EHEC infections, as antibiotics could trigger the production of Shiga toxins. However, antibiotics-based treatments are still being investigated (Ramstad et al., 2021). Other problems in which resistant bacteria can be involved are for instance opportunistic infections on the respiratory tract, the skin, or the urinary tract (Bray & Zafar, 2024). In addition to the detection of ESBL-isolates and carbapenem resistance through culturing, long-read metagenomic sequencing was used for microbiome profiling and for detection of a wider group of AMR genes. STP samples showed greater microbial diversity compared to FTP samples, likely due to the narrow range of nutrients and distinct microbial composition of input materials within FTP streams. Similarly, there was a greater abundance and diversity of AMR genes detected in the STP samples. The World Health Organization has recently reclassified AMR genes according to their importance in medical treatment and potential to spread amongst microbial pathogens (WHO, 2024). Resistance against the critically important classes aminoglycosides and macrolides were detected mostly in STP samples in the present study. Similarly, carbapenems are considered highest-priority critical antimicrobials, and resistance against them was detected with gene $bla_{\text{OXA-198}}$ in samples from STP sample types (STP-city-1, STP-city-2, and STP-city-rural), although not at all timepoints. It is remarkable that the carbapenemase gene bla_{KPC} and the ESBL-genes detected via culturing as described above, could not be detected through metagenomic sequencing. These differences are likely caused by the relatively low number of *E. coli* compared to other bacterial species identified through metagenomic sequencing, while through culturing carbapenem resistant *E. coli* are isolated, specifically resulting in a lower detection limit. This highlights the need for deeper sequencing to detect the full spectrum of AMR genes in sludge and aerobic biomass samples. Overall, if transmission of AMR genes from bacteria present in food or food production systems to opportunistic infectious bacteria can occur is unclear. Also,

no regulation applies for AMR parameters in food, although monitoring is mandatory for EU countries. We believe that, from a precautionary principal point of view, if the role of food production systems in the spread of AMR is not resolved, it is crucial to remain vigilant about the accumulation or introduction of AMR bacteria in the food chain.

The virome analyses applied in the present study allowed for a broad characterization of the genetic diversity of human pathogenic viruses in the STP and FTP samples. All STP samples were found to be positive for multiple virus species, while for most FTP samples no virus species were identified, except for one FTP-dairy sample (rotavirus A) and one FTP-fish sample (norovirus). Only species of the viral families *astroviridae*, *reoviridae,* and *caliciviridae* were detected in the STP samples. Indeed, these families of viruses have been previously reported in STP sludge (Bibby & Peccia, 2013; Gholipour et al., 2022; Schaeffer et al., 2023). All three virus families are an important cause of acute gastroenteritis (Bishop RF, 2008), with noroviruses being the leading cause of acute gastroenteritis worldwide, and frequent transmission and infection via food is reported (Bosch et al., 2018). Specifically, high coverage of the species mamastrovirus 1, mamastrovirus 9, astrovirus MLB1 and MLB2, rotavirus A, rotavirus C, and norovirus were reported in the STP sludge in the present study. In the Netherlands in 2022, norovirus and rotavirus caused an estimated health burden of $2*10^3$ and $1.5*10^3$ disability-adjusted life years (DALYs)/year, respectively (Bos et al., 2023). Although it is important to note that we cannot conclude on the viability of the detected viral genomic fragments, all virus species detected in the present study are small, non-enveloped enteric viruses and as such are generally characterized by high environmental stability and high probability of infection per ingested virus particle (Bishop RF, 2008; Gholipour et al., 2022). Therefore, we cannot rule out the possibility that re-use of digested STP sludge in circular food production systems may result in the transmission of viable virus particles and thus poses a food safety risk. Next to a direct food safety risk, the reuse of human excreta, being a relevant inflow of the STP sludge, in circular food production systems may result in an increased introduction of human viruses into the environment. We reason that this could increase the likelihood of recombination of human and animal viruses in hosts, contributing to the risk of new emerging viruses. Treatment of the STP sludge (e.g., heating or acidification) could be an option to mitigate the presence of pathogens and viruses when these materials are used in a circular food production system, as is for example to some extent in place in Sweden (Ekane et al., 2021). Human virus genomic material was hardly detected in any of the FTP streams analyzed, indicating that re-use of FTP streams is less likely to result in transmission compared to re-use of STP sludge.

4.2. Chemical hazards

Besides microbiological analyses, a range of chemical hazards were identified in the samples, including heavy metals and trace elements. In general, the highest heavy metal and trace elements concentrations were found in the STP samples, while some were present in high levels in the FTP samples as well. Concentrations were compared to available MLs in sludge with fertilizer application according to the Dutch fertilizer law implementation decree (Rijksoverheid, 2024). Generally, a higher number of heavy metal or trace element concentrations exceeded a ML in the STP streams compared to the FTP streams.

In FTP-dairy, concentrations were all below the available MLs. Additionally, MLs exist when the sludge serves as co-material with manure in a digester for fertilizer application. Dependent on the nutrient levels of the sludge, limits for this application are expressed in heavy metal content per kg N, P, or K (Egle, 2023). One or two heavy metals or trace element concentrations exceeded these Dutch MLs in the FTP and STP streams. Only FTP-dairy concentrations did not exceed these limits. Based on our results, the FTP-dairy stream thus complies with these available Dutch MLs for heavy metals and trace elements, showing the potential to use this stream in circular food systems. However, for all other streams (both FTP and STP streams), at least one heavy metal or trace element does not comply with these available Dutch MLs.

A range of pharmaceutical residues were qualitatively identified in the samples. Especially in the STP samples a high number of pharmaceutical substances were identified. This ranged from 13 to 21 confirmed compounds, belonging to the pharmaceutical class antibiotics, non-steroidal anti-inflammatory drugs, painkillers, benzodiazepines, anti-hypertensive agents, β-blockers, antidepressants, anthelmintics, and hormones. These pharmaceutical classes are mostly used in humans, but some detected pharmaceuticals such as anthelmintics are probably originating from their applications for pets. STP sludge has been previously reported to contain a variety of pharmaceutical residues, such as the pharmaceutical classes detected in the present study (Mejías et al., 2021)(Mejías, Martín et al. 2021). Of the FTP streams, only in FTP-dairy and FTP-fish samples there were some pharmaceutical residues identified (2 to 6 confirmed compounds). The number of different substances and pharmaceutical classes identified was much lower than for the STP sludge types. In FTP-fish, some residues of pharmaceuticals that are not allowed for production animals in the Netherlands were detected, such as ciprofloxacin, mebendazole, and corresponding metabolites (EU, 2009). It is important to note that the source of contamination was not further investigated in the present study, and it is not known if for example reclaimed or fresh water was used. Reclaimed water can be a potential source of e.g., pharmaceutical residues (Garcia-Valverde et al., 2023). Nevertheless, the permitted use of enrofloxacin probably causes the presence of ciprofloxacin, since ciprofloxacin is a known metabolite of enrofloxacin (Idowu et al., 2010). Moreover, it must be noted that human wastewater was connected to the wastewater treatment plant of FTP-fish, which might explain the detection of mebendazole and ciprofloxacin in this stream.

With a similar screening approach as for the pharmaceutical residues, pesticide residues were identified in the sludge. Especially in the STP sludge, multiple pesticides were identified, ranging between 1 and 17 substances per sludge sample. This included multiple substances that are allowed in the Netherlands, such as permethrin, which was detected in all STP sludge samples. Permethrin is, besides being a widely applied biocide against for example ants, also registered as a veterinary drug, for instance to treat pets against fleas and ticks (Buijs et al., 2022). This may result in exposure to humans and subsequently inputs into the STP. Our findings correspond to a human biomonitoring exposure study which showed that permethrin was the most widely detected substance included in their analyses (Wacławik et al., 2024). Besides allowed substances, also some pesticides were detected that were not allowed as a plant protection product in the Netherlands at the moment of sampling. For example, diazinon was detected in one STP-city-1 sample, while diazinon has been banned for use as a plant protection product in the EU since 2007 (EU, 2007). However, it is a dual use substance and is still allowed in flea collars for pets (EU, 2016), which might explain this finding. Also, the insecticide chlorpyrifos, banned for use as a plant protection product in the EU since 2020, was detected in one STP-rural sample. It has been reported that chlorpyrifos is moderately persistent at least in some environments (Bose et al., 2021), which might partially explain this finding. Of the FTP streams, pesticides were only identified in the crop-associated FTP sample types, which is as expected because of their relation to the terrestrial food production system. In these samples (i.e., FTP-potato and FTP-sugar), the number of confirmed substances ranged from 4 to 9. These were all fungicides, and one additional herbicide in FTP-potato, prosulfocarb, which was indeed allowed for application in potato cultivation at the moment of sampling in the Netherlands (ctgb, 2024). In the FTP-sugar samples, the azole fungicide cyproconazole was detected. Cyproconazole was intended to control a wide range of fungi on crops, including sugar beets. Although the approval of its application in the EU has been discontinued since 2021, cyproconazole has been reported to possibly be persistent in soil and water systems (Wightwick et al., 2012). The herbicide glyphosate and its degradation product AMPA were detected with a separate method,

allowing proper quantification. Of the FTP samples, only AMPA was detected in FTP-potato and FTP-sugar, while both glyphosate and AMPA were detected in all STP sludge types. The widespread detection of glyphosate and AMPA is not surprising, since it is allowed for agricultural and household applications in the Netherlands. The European Commission recently prolonged its approval in 2023 for ten years (Alvarez et al., 2023). Higher concentrations of glyphosate and AMPA were detected in STP sludge as compared to FTP-potato and FTP-sugar streams. It is important to note that detected concentrations in the samples cannot be directly extrapolated to their use in agricultural or household applications.

Some of the short-chain PFASs analyzed in this study were present in most sample types. The highest detected concentrations were for TFA, and these levels (on DM basis) varied substantially amongst the several sample types. The concentrations in STP-island stood out with 2.5 times higher levels than the next highest sample (FTP-potato). PFPrA, PFHxA, TFMS, PFBS, and PFHxS were frequently detected (in $\geq$ 6 out of 9 sample types) and at lower levels compared to TFA (<LOQ to 1075 ng/kg DM). Several other short-chain PFASs were not detected in any of the samples. The sources of the short-chain PFASs identified in the samples are yet to be elucidated. For example, TFA is detected in high levels in surface water samples from, for example, deposition (Freeling et al., 2020), degradation of industrial chemicals (Holland et al., 2021) and CF_3 containing pesticides (Joerss et al., 2024). Moreover, TFA is reported in Dutch tap waters (Inspectie Leefomgeving en Transport, 2023). Various sources of water are used in the agri-food industry, and it is therefore difficult to pinpoint the actual source of TFA (or other short-chain PFASs) for every sludge type. The concentrations of the short-chain PFASs PFHxA, PFBS, and PFHxS in the STP sludge samples can be compared to a global overview provided by Zhou et al. (2024), who compared PFASs in STP sludge between a collection of European (6), North American (2), Asian (3), Oceanian (1), and African countries (2). Within this overview, mean concentrations of PFHxA, PFBS, and PFHxS were higher than our findings in STP and FTP samples (Zhou et al., 2024). Moreover, Yan et al. (2012) investigated sludge from 25 different STPs in Shanghai (China) and detected concentrations of PFPrA and PFBS that were much higher as compared to the STP and FTP samples analyzed in the present study. In the Netherlands, STOWA (i.e. foundation for applied water research in the Netherlands) has investigated STP sludge from Dutch municipal wastewater treatment plants with high, moderate, and low PFASs load (Derksen & Baltussen, 2021). Although their selection of analyzed PFASs was different than in the current study, they found sum-PFASs amounts of approximately 40 – 170 μg/kg DM for the high and moderate load PFASs STPs, and approximately 26 – 41 μg/kg DM for the low-load STPs. Their observed levels for PFHxA, PFBS, and PFHxS were higher than observed in the present study. It is important to note that in this study we aimed for detection of the more mobile short-chain PFASs and thus did not focus on quantitative analysis of long-chain PFASs. However, the screening approach applied allowed confirmation of the presence of PFOS, PFOA, PFNA, 6:2 FTS, and 8:2 FTS in several of the samples, which is in line with previous findings in the Netherlands (Derksen & Baltussen, 2021). Future work may be directed towards the inclusion of longer-chain PFASs, as well as a more diverse set of PFASs. There are no Dutch MLs for any of the PFASs in sludge or excess aerobic biomass, neither there are MLs at an EU level. Application of these streams in food production systems requires a thorough assessment of the fates of these contaminants in such systems. This is important since, for example, it has been shown that short-chain PFASs and long-chain PFASs may be assimilated from a soil–water system by agricultural crops (Adu et al., 2023; Lesmeister et al., 2021).

Dioxins and ndl- and dl-PCBs were detected in all sample types. Dioxins can be unintentionally formed during chemical and combustion processes, while PCBs were intentionally produced and manufactured until their use and marketing has been very heavily restricted in Europe since 1985. They are persistent, lipophilic, and toxic chemicals, which can accumulate in the food chain (Smulders et al., 2019) and are detected widely in the environment (Srogi, 2007). In the present study, the TEQ of the concentrations of dioxins and dl-PCBs was generally lower in the FTP samples as compared to the STP samples. Regarding the ndl-PCBs, lowest concentrations were observed in the FTP-potato, FTP-sugar, and FTP-dairy. TEQ concentrations of dioxins and dl-PCBs were in the same order of magnitude as in some STP samples collected in Spain in a recently published study (Gomez-Rico et al., 2024). These concentrations seem to be lower as compared to concentrations analysed in STP samples collected in 2012 in Switzerland (Zennegg et al., 2013). Concentrations of ndl-PCBs were in a similar range of STP sludge collected and analysed in the Netherlands in 2019 (Laan, 2020). For a potential fertilizer application of the sludge streams as co-material with manure in a digester, limits exist for dioxins (WHO2005-PCDD/F) and ndl-PCBs, which depend on the nutrient levels within the specific sludge. These fertilizer limits for dioxins and for non-dioxin like PCBs were not exceeded in any sample type included in the present study.

4.3. Physical hazards

Besides microbiological and chemical analyses, some physical hazards such as metal contaminations and plastics were detected qualitatively as well. Some plastic and plastic fibres were found in most sample types, while metal contaminations were only detected in some FTP samples. There can be multiple reasons for the identified presence and absence of particles in the STP and FTP streams, such as source and effect of particle size on homogeneity (Sarkar & Wassgren, 2014). However, further investigating this was out of the scope of the present study. Previous studies reported on the presence of small plastic particles and fibres in STP sludge (Carr et al., 2016; Edo et al., 2020), and there are concerns about their release in the environment via sludge applications (Hatinoglu & Sanin, 2021). This might be similar for metal particles. Additionally, microplastics were labelled as emerging pollutants of concern, specifically in STP sludge (Egle, 2023). Besides, microplastics have shown to migrate and accumulate within plants, and seem to affect plant health (Gao et al., 2025). Although microplastics smaller than 1 mm were not included in the present study, their presence in the analysed samples is plausible, especially in cases where larger plastics and plastic fibres were detected.

4.4. STP and FTP streams in circular food production systems

Besides the presence of chemical, microbial and physical hazards, both the STP sludge as well as the FTP samples contained relevant agricultural nutrients like nitrogen, phosphorus, and potassium. This makes them of interest for potential applications in circular food production systems, for example as fertilizer. Available MLs in the Dutch fertilizer law implementation decree and MLs during incorporation as co-material in a digester with manure for fertilizer application were thus compared to detected concentrations in the present study. These limits were only available for some heavy metals and trace elements and for dioxins and PCBs, and as earlier described, especially arsenic, cadmium, copper, and zinc concentrations in some of the sample types exceeded (one of) these MLs. When comparing the detected concentrations to MLs of other EU member states, often having less stringent limits as compared to the Netherlands, we observed that the concentrations of the respective analytes in the samples still fall within the MLs of other national legislations (see Appendix B Table B.12) (Egle, 2023). This variability in MLs means that whether a residual stream can be applied as fertilizer is also highly dependent on the regulations that apply in a specific country or region. Furthermore, in some countries, certain certification schemes and treatment processes are in place to ensure a certain quality of STP streams applied as fertilizer (Ekane et al., 2021). Besides Dutch and other EU member states' available MLs, the current EU legal framework on specifically sewage (STP) sludge management (Directive 86/278/EEC) contains guidelines on maximum heavy metal

concentrations in STP sludge as well. In this EU directive, limits are absent for some heavy metals (e.g., arsenic), while for other heavy metals limits are much more relaxed as compared to the available relevant Dutch and other EU member state MLs. It needs to be stressed that evaluation of the MLs of this Directive (86/278/EEC) were deemed insufficient, as potentially associated health risks and environmental risks on the (repeated) application of STP sludge as fertilizer were insufficiently considered (Egle, 2023). This was further supported by experimental evidence that confirmed increasing concentrations of metals in soils and edible crops at levels that are considered environmentally risky when soils were amended with STP sludge (Charlton et al., 2016; Zaragüeta et al., 2021). Furthermore, the EU Regulation 2019/1009 (Fertilising Products Regulation) has been recently in place and includes organic fertilizers in its scope. Currently (STP) sludge is not included in its component material categories (Cucina et al., 2021) and thus falls outside the scope of this regulation. Nevertheless, multiple quality criteria related to contaminations were formulated for organic fertilizers to be allowed on the European market that go beyond heavy metals and trace elements. These include for example plastic impurities (>2 mm, maximum 2.5 g/kg DM), the absence of *Salmonella* spp. in 25 g of total fertilizing product, and a maximum of 1000 colony-forming unit (CFU) Enterococcaceae per gram product. In the present study, other pathogenic bacteria such as *B. cereus* and *L. monocytogenes* were detected in all or some of the sample types. Depending on their (dis)similarities in the possibility to transfer to food products, the absence of *Salmonella* spp. and a ML of Enterococcaceae may thus serve insufficiently as an indicator for microbiological safety of sludge when used as a fertilizer product.

Besides hazards of which currently MLs exist, a variety of other hazards were found to be present in the samples as well. Some of them were less expected. For example, the detection of (low concentrations of) some unauthorized pesticide and pharmaceutical residues, of which their source is unknown. It is important to further understand and evaluate the impact on food safety of the hazards without available MLs, when these are being introduced in a circular food production system. Their toxic or adverse properties relevant for human, animal, and environmental health as well as their fate through such a system need to be investigated to be able to perform such a risk analysis (van Leeuwen et al., 2024). That information can also aid further prioritization of the hazards, for example to control or mitigate these hazards. As such, fate studies with STP and FTP streams and circular food production systems to further understand and evaluate the possible impact of the identified hazards on food safety would be needed, for both chemical hazards and for microbiological hazards including AMR genes. For the chemical hazards, this would probably especially be relevant for substances that are persistent (i.e., having a high half-life), mobile (defined by e.g., the organic carbon–water partition coefficient log K_{oc}) and toxic (PMT substances), very persistent and very mobile (vPvM) or persistent, and bio-accumulative and toxic (PBT). These groups of substances have been suggested to have an equivalent level of concern as they can cause long-term harm to humans and the environment (Chirsir et al., 2024). Additionally, a mixture of hazards was present in all samples of the present study. Thus, in case STP or FTP streams are applied in circular food production systems, a mixture of these hazards can probably be expected in the derived food products as well. The potential combined toxicity of these diverse chemicals and other hazards warrants further investigation (Egle, 2023). Additionally, a range of chemical and microbial pollutants not included in the analytical scope of the present study can be present in the sludge as well. This is expected especially in the STP sludge samples given their widespread variety and accompanied differences in quality of the input flows. Further and improved usage of untargeted analytical approaches could be helpful to identify these (Nanusha et al., 2024). This can also aid identification of substances that might have a less obvious relevance for food safety, such as surfactants or flocculants, which are often being used in the sludge or aerobic biomass creation processes. Hazard identification of currently known and unknown substances in these streams thus remains relevant to investigate further to safeguard the safety of its application in circular food production systems.

In the present study, the occurrence and quantity of the hazards identified in the sludge were generally lower for the FTP samples as compared to the STP samples. Furthermore, the input flows of the FTP samples are expected to be more homogeneous as compared to these of STP samples, resulting in a more stable produced stream. Besides, FTP streams are expected to be more easily controllable than STP streams in terms of wastewater inflows. This would make it easier to possibly implement critical control points or mitigation measures during the sludge or aerobic biomass creation process, and aids producing stable, controllable, and safe(r) residual streams for application in circular food production systems. Thus, STP and FTP streams have different valorization potentials in circular food production systems and probably require separate evaluation for their impact on food safety as well. Given the results of the present study, only FTP-dairy would be deemed to be safely applicable based on the Dutch ML for heavy metals and dioxins and PCBs. The other FTP sample types were generally less contaminated as compared to the STP samples, which makes them more suitable for direct application as well. Besides direct application of STP and FTP streams as fertilizer, derivate compost and digestate could be applied as well, of which the processes might affect the quantity of some of the investigated hazards (such as some drug residues (Ezzariai et al., 2018)). Besides STP and FTP streams as a fertilizer application, other applications in a more closed food production system warrant further investigation, for example as substrate for insect rearing (Bohm et al., 2022) or seaweed cultivation for feed or food applications. The presence and possible transfer of food safety hazards should however also be studied to ensure the safety of the derived food or feed products, as some hazards can transfer and bio-accumulate in these systems as well (Meyer et al., 2021). In case certain streams cannot be directly applied in circular food production systems, other valorization options such as the extracting of valuable nutrients such as P could be valuable (Zheng et al., 2022).

5. Conclusions

To conclude, the results of the present study can serve as a first step to further evaluate what the possible application of the STP sludge and FTP aerobic biomass in circular food production systems would entail, when looking at involved hazards. The results can help policy makers to provide guidance on which hazards need to be prioritized for monitoring of future circular food production systems and control of new circular food production systems. Also, it will help in identifying critical points where mitigation strategies need to be developed and implemented. Overall, the results of the present study will contribute to the design of safe circular food production systems and showcase the need for a fit-for-purpose and safe-by-design approach in the application of residual streams in food production systems. This can allow us to move towards a more sustainable food production system without compromising human, animal, and environmental health.

CRediT authorship contribution statement

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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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Appendices A and B. Supplementary data

Supplementary data (Appendix A and Appendix B) to this article can be found online at https://doi.org/10.1016/j.envint.2025.109624.

Data availability

Data will be made available on request.

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