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Large-scale assessment of organic contaminant emissions from chemical and pharmaceutical manufacturing into Swiss surface waters

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ABSTRACT

This study presents a nation-wide assessment of the influence of chemical and pharmaceutical manufacturing (CPM) wastewaters on synthetic organic contaminant (SOC) emissions to Swiss surface waters. Geographic Information System (GIS) based analysis of the presence of CPM in wastewater treatment plant (WWTP) catchments revealed wide distribution of this industrial sector across Switzerland, suggesting that one-third of the 718 Swiss WWTPs may be influenced by CPM wastewaters. To reflect the diversity of this type of wastewaters, we investigated the effluents of 11 WWTPs of diverse sizes and technologies, which treated 0-100% wastewater from a variety of CPM activities. In an extensive sampling campaign, we collected temporally high resolved (*i.e.,* daily) samples for 2-3 months to capture the dynamics of CPM discharges. The > 850 samples were then measured with liquid chromatography high-resolution mass spectrometry (LC-HRMS). Non-target characterization of the LC-HRMS time series datasets revealed that CPM wastewaters left a highly variable and site-specific signature in the effluents of the WWTPs. Particularly, compared to WWTPs with purely domestic input, a larger variety of substances (up to 15 times more compounds) with higher maximum concentrations (1-2 orders of magnitude) and more uncommon substances were found in CPM-influenced effluents. Moreover, in the latter, highly fluctuating discharges often contributed to a substantial fraction of the overall emissions. The largely varying characteristics of CPM discharges between different facilities were primarily related to the type of activities at the industries (*i.e.*, production *versus* processing of chemicals) as well as to the pre-treatment and storage of CPM wastewaters. Eventually, for one WWTP, LC-HRMS time series were correlated with ecotoxicity time series obtained from bioassays and major toxic components could be identified. Overall, in view of their potential relevance to water quality, a strong focus on SOC discharges from CPM is essential, including the design of situation-specific monitoring, as well as risk assessment and mitigation strategies that consider the variability of industrial emissions.

1. Introduction

Wastewater treatment plant (WWTP) effluents are a major source of synthetic organic contaminants (SOCs) to the aquatic environment. There is evidence that, in addition to domestic wastewater, wastewaters from chemical and pharmaceutical manufacturing (CPM) can contribute substantially to the loads of SOCs emitted to surface waters (Anliker et al. 2020a, Anliker et al. 2020b, Cardoso et al. 2014, Kleywegt et al. 2019, Larsson 2014, Schlusener et al. 2015, Scott et al. 2018). However, to date, most studies on CPM emissions in WWTP effluents, including our own (Anliker et al. 2020a, Anliker et al. 2020b), investigated the wastewaters of individual industries or industrial sites. Hence, it is currently unclear whether SOC emissions from CPM are isolated events or widespread.

To the best of our knowledge there are only two studies, both focusing on pharmaceutical manufacturing facilities in North America (Kleywegt et al. 2019, Scott et al. 2018), that have assessed CPM discharges from different locations. Based on large sets of > 100 target

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compounds, it was clearly shown that the wastewaters of every company emitted substantially higher pharmaceutical loads than those detected in domestic wastewaters. However, these studies focused on target compounds in individual grab samples and therefore do not fully reflect the complexity and dynamics of CPM wastewaters. Indeed, the temporal and compositional dynamics of CPM discharges overall have been rarely investigated to date. The available studies have shown that CPM discharges strongly vary over time and that long-term and high temporal resolution sampling is essential to capture production cycle dependent CPM emissions and related peak concentrations (Anliker et al. 2020a, Anliker et al. 2020b, Ruppe et al. 2018, Schlusener et al. 2015). For a thorough chemical characterization of such complex samples, high-resolution mass spectrometry (HRMS) has been established as the method of choice, as it provides comprehensive information on both known and unknown compounds, and hence, does not rely on prior knowledge of the sample composition, *e.g.*, comprehensive production data, which is commonly very difficult to obtain for CPM (Cardoso et al. 2014, Phillips et al. 2010). Moreover, recently, the analysis of entire datasets of unidentified LC-HRMS signals (chemical "fingerprints") has increasingly been applied for the characterization and differentiation of various emissions sources of SOCs, often in combination with time trend or spatial analysis (Anliker et al. 2020a, Beckers et al. 2020, Chiaia--Hernandez et al. 2017, Du et al. 2020, Schollée et al. 2021). Without identifying specific compounds, these non-target investigations of HRMS datasets are extremely valuable for a better understanding of SOC sources in general. However, chemical characterization alone does not provide information about the ecotoxicological relevance of the detected HRMS signals. To evaluate the potential impacts of the WWTP effluents on aquatic communities, LC-HRMS data eventually need to be related to toxicological effects (Brack et al. 2018). Yet, time resolved toxicological evaluations are time-consuming, costly and therefore rare.

The goal of this study was to characterize SOC discharges from CPM via WWTPs on a national scale and to evaluate their relevance as SOC source affecting surface water quality in comparison to domestic wastewaters. Specifically, we were interested in (i) investigating the occurrence and spatial distribution of CPM discharges in Switzerland, (ii) describing their influence on effluent chemical composition and dynamics, and (iii) providing a first assessment of potential factors that influence the extent and the characteristics of SOC emissions from CPM in WWTP effluents. Finally, we aimed to exemplarily link comprehensive temporal HRMS data with temporal toxicity data to identify the cause of adverse effects observed in CPM wastewater.

To achieve these goals, we extended our previous investigations of SOC discharges from pharmaceutical manufacturing (Anliker et al. 2020a, Anliker et al. 2020b) to a much larger set of WWTPs, including ones that receive substantial shares of wastewater from chemical facilities. In a first step, Swiss WWTP catchments were analyzed systematically for the presence of CPM using Geographic Information System (GIS) analysis and structural business statistics data. Based on this analysis seven WWTPs were selected as sampling sites, each receiving various shares of wastewater from diverse CPM facilities. For 2-3 months, daily effluent samples were taken at each WWTP and analyzed with LC-HRMS. We then compared the time series datasets of the seven WWTPs measured for the current study, along with previously acquired data of four additional WWTPs (Anliker et al. 2020a, Anliker et al. 2020b), using a non-target approach. Rather than on structure elucidation, the analysis focused on general characteristics of the HRMS time series inventories, such as the number of compounds that persisted during wastewater treatment, intensity variations over time and maximal intensities. Eventually, due to the commitment of one of the connected industries, comprehensive bioassays were performed at one WWTP in parallel to our LC-HRMS-based monitoring. For this WWTP LC-HRMS time series were correlated with ecotoxicity time series to find toxicity causing components based on matching time patterns. As far as we are aware, the current study is the first attempt to assess the relevance of SOC discharges from CPM on a national level using comprehensive temporal LC-HRMS data.

2. Materials and methods

2.1. Geospatial data analysis

To assess the CPM industrial density per WWTP catchment, geospatial data analysis was performed with the open source Geographic Information System software QGIS (version 3.10) (QGIS Development Team 2020). The Swiss business statistics dataset (Swiss Federal Statistical Office 2016) was filtered for entries of chemical and pharmaceutical industries, *i.e.,* for General Classification of Economic Activities (NOGA) (Swiss Federal Office 2021b) divisions 20 (manufacture of chemicals and chemical products) and 21 (manufacture of basic pharmaceutical products and pharmaceutical preparations). The spatial resolution of the business statistics data was one hectare and values ≤ 3 were set to 3 in the original dataset to prevent the identification of individual industries. The number of full-time equivalent employees and the number of workplaces (*i.e.*, facilities) within the chemical and pharmaceutical sector, extracted from the business statistics, was then intersected with the WWTP catchment areas (Eawag 2014). Additionally, for the visualization (Fig. 1), the following basic Swiss geospatial data were used: national boundaries (swisstopo 2015), lake shorelines (swisstopo 2007) and the water network (Swiss Federal Office for the Environment 2015)

2.2. Sampling site description

This study analyzed the effluents of 11 WWTPs of different sizes and technologies across Switzerland, which treated differing shares of CPM wastewater (0-100%, see Supplementary Information (SI) 1 for the sampling site selection criteria). The effluents of seven WWTPs were sampled for the present study and samples from four WWTPs have been taken previously (Anliker et al. 2020a, Anliker et al. 2020b). Eight of the investigated WWTPs receive both domestic and industrial wastewater, one exclusively treats CPM wastewater and, for comparison, two only treat domestic wastewater. This selection is representative of the situation in Switzerland, where the vast majority of CPM sites discharge their wastewater to municipal WWTPs. Summary information on the 11 WWTPs considered in this study are given in Table 1.

Because the sampling at all WWTPs was conducted under non-disclosure agreements, the locations of the WWTPs and the names of the companies have been anonymized. Similarly, the names of the compounds reported to be processed by industry have been removed. Hereafter, the WWTPs receiving CPM wastewater are referred to as CP## and CPF##, with CPF referring specifically to WWTPs that receive wastewater only from formulating (and not producing) CPM activities, ## indicating the volume (in percent) industrial wastewater, and the purely domestic WWTPs are referred to as D01 and D02.

The absolute volume of industrial wastewater treated varied between 50 and 9'600 m^3/d for the WWTPs with CPM in their catchments. In this context, it is important to notice that a small amount of industrial wastewater can already contribute substantially to the total influent DOC (see Table 1). The purely industrial WWTP CP100 represents an exception insofar as it has much lower capacity than all other WWTPs and is equipped with an advanced treatment specifically for SOC removal (*i.e.*, activated carbon adsorption). The other WWTPs receive mixed influents and have only mechanical and conventional biological treatment steps, which are, in some cases, complemented with phosphorous removal, nitrification and denitrification. These mostly biology-based treatments will remain standard at most municipal Swiss WWTPs also in the foreseeable future, as only selected (100 out of 718) WWTPs will be upgraded with an additional treatment for SOC removal (Swiss Federal Office 2021a). Biological treatment options are also the standard in other counties worldwide that have central wastewater treatment.

In terms of connected industries, it should be noted that at four

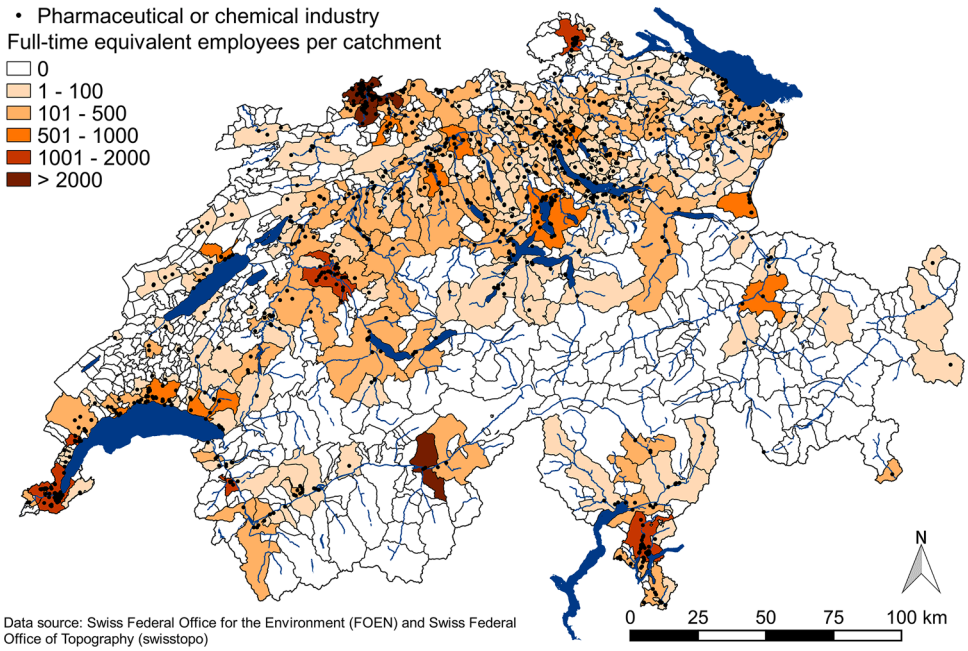

Fig. 1. Map visualizing the density of chemical and pharmaceutical industry employees per WWTP catchment in Switzerland. The catchment areas of the 718 Swiss WWTPs are colored according to the number of full-time equivalent employees in the chemical and pharmaceutical industry. Darker colors mark WWTP catchments with a large number of employees in the respective industrial sectors (legend in the figure's upper left corner). Black dots mark the sites of chemical or pharmaceutical industries. Blue areas indicate surface waters, while black lines indicate the WWTP catchment boundaries.

Table 1
Characteristics of the investigated WWTPs.

WWTP	CPM Full-time equivalent employees	Volume[a] [m³/day]	vol% CPM[b]	DOC% CPM[b]	Effluent DOC mean[c] [mg/L] (n)	Type of CPM	CPM Cluster[d]	Industrial pretreatment (other than incineration)	wastewater treatment (in additional to mechanical and conventional biological treatment)	Production information
D01*	501-1000	60'000	0	0	5.3 (3)	Production of diagnostic systems	-	n.a.	Phosphorous removal, nitrification, denitrification	n.a.
D02	101-500	16'000	n.a.	n.a.	20 (26)	Pharmaceutical R&D	-	n.a.	Phosphorous removal, nitrification	n.a.
CPF0.1*	>1001	50'000	0.1	1	5 (45)	Pharmaceutical formulation	+	Flocculation or neutralization, in specific cases inactivation or chemical oxidation	Phosphorous removal, nitrification, denitrification	Confidential substance list Current production plan during sampling period
CP1.0	101-500	8'000	1	12	7.7 (17)	Pharmaceutical and pharmaceutical intermediates production	-	Neutralization, activated carbon adsorption	Phosphorous removal	n.a.
CP1.7	>1001	18'000	1.7	n.a.	12 (12)	Pharmaceutical production and formulation	-	Neutralization	Phosphorous removal	Confidential substance list
CPF2.3*	101-500	4'000	2.3	2	9.1 (21)	Pharmaceutical formulation	+	Filtration 1µm	Phosphorous removal	Confidential substance list Current production plan during sampling period
CP3.0*	501-1000	18'000	3	60	7.1 (87)	Pharmaceutical production and formulation	-	n.a.	Phosphorous removal, nitrification, denitrification	Publicly available product list
CP25	>1001	18'000	25	85	55 (56)	Chemical and pharmaceutical production	+	Precipitation, chemical Oxidation, neutralization		Confidential substance list
CP50	>1001	13'000	50	90	26 (91)	Chemical production	+	n.a.	Phosphorous removal	Confidential substance list
CP60	>1001	16'000	60	95	59 (39)	Chemical and pharmaceutical production	+	Specific for each wastewater stream (oxidation, adsorption, distillation)	Phosphorous removal, nitrification	Confidential substance list
CP100	101-500	260	100	100	55 (36)	Pharmaceutical and pharmaceutical intermediates production and R&D	-	n.a.	Activated carbon adsorption	Publicly available product list

n.a.: Data not available.
n: Number of available measurements.
*: WWTPs discussed in our previous studies, *i.e.*, D01 and CP3.0 in Anliker et al. (2020a) and CPF0.1 and CPF2.3 in Anliker et al. (2020b)
[a] Average WWTP discharge during the sampling period.
[b] Calculated based on the influent values at the respective WWTP.
[c] Average DOC concentration during sampling period, except for CPF0.1 and CPF2.3 where the value corresponds to the annual average of measurements performed in 2017.
[d] CPM Cluster commonly describes the presence of several facilities on one industrial site, here also "pseudo-cluster" are included, i.e., facilities at different locations connected to the same WWTP. Cases where more than one CPM facility is connected to the WWTP are marked with "+" in the table, whereas "-" indicates that the respective WWTP only treats wastewater of a single facility

WWTPs the CPM wastewater originates from a single company, whereas at five it stems from several companies (so-called CPM clusters). The in total 23 facilities covered by the study include producing chemical industries that synthesize plant protection products and base chemicals, as well as producing and formulating (*i.e.*, facilities that process active pharmaceutical ingredients into medical end products) pharmaceutical industries. Several companies are involved in research and development activities, and for the majority of the companies on-demand, custom synthesis is an important part of their business activities. There are WWTP catchments with a relatively low number of 101-500 employees in CPM, as well as two catchments with > 2'000 employees.

While this might not be the case everywhere (Kleywegt et al. 2019),

in Switzerland, CPM wastewater is generally pretreated on-site before its collection in large buffer tanks, from where it is then directed to a WWTP (Straub et al. 2020). All the CPM companies covered in this study incinerate their most contaminated wastewaters. Based on information provided by companies connected to CP60 and CP100, the volume of incinerated wastewater amounts to 20'000 m^3 and 10'000 m^3 per year, respectively. Apart from incinerating the most problematic wastewaters, the level of on-site wastewater pretreatment differed substantially among the companies. It ranged from simple neutralization to highly specific treatment of individual wastewater streams, using *e.g.*, distillation, chemical oxidation or activated carbon adsorption. In some cases, detailed information on pretreatment and on-site wastewater handling was difficult to obtain and is therefore missing in **Table 1**. While each constituting a specific case, we think that together the sampling sites included in this study do reflect the variety of CPM wastewater in Switzerland, and are therefore suitable to describe SOC discharges from this industrial sector on a national scale.

2.3. Chemicals and solutions

Information on the chemicals and solutions used for chemical analysis is provided in the SI 2.

2.4. Sample collection and storage

WWTP effluents were sampled to determine the SOCs released to surface waters. To take into account the dynamics of CPM emissions and to increase the probability of capturing short-term discharges, highly resolved temporal sampling was performed for several months. At CP1.0 and CP25 sampling was conducted for 2 months, from early September until end of October 2017 (56 samples per sampling site). At D02, CP1.7, CP50, CP60 and CP100 the sampling campaign lasted for 3 months, from end of October 2017 until end of January 2018 (91 samples per sampling site). Daily 24-h composite samples were collected by the staff of the WWTPs using the available on-site infrastructure. Each day, a sample aliquot of 50 mL was filled in a 100 mL glass bottle and stored at 4°C. At the end of each week, samples were shipped on ice to the laboratory and stored at -20°C until chemical analysis (maximum six months after the end of the sampling campaign).

2.5. Sample preparation

Samples were prepared and analyzed in one batch per WWTP. After thawing, 5 mL of each sample were centrifuged (Megafuge 1.0 R, Heraeus Sepatech) at a relative centrifugal force of 3 g at 25°C for 15 min in glass vials to remove suspended particles. Then 1.5 mL supernatant were transferred to 2 mL glass measurement vials and spiked with 15 µL of a solution containing 235 isotope-labeled internal standards (ISTDs, SI 3 Table S1). The concentration of each ISTD was 1 µg/L for the CP1.0 and CP25 samples and 2 µg/L for all other WWTPs (see SI 5 for details). For the CP1.0 and CP25 samples, a ten-point calibration ranging from 0.005 to 10 µg/L with a mix of 659 compounds (SI 4 Table S2, including 114 compounds known to be manufactured at one of the CPM sites considered in this study) was prepared in ultrapure water. For all other WWTPs, a seven-point calibration from 0.025 to 10 µg/L was used. In the following, compounds for which an authentic reference standard was available and analyzed are referred to as "target compounds". During sample preparation, for each sampling site, four samples were spiked with the target compounds (two samples each at a concentration of 0.1 and 1 µg/L) to assess recoveries. Additionally, duplicates were prepared for three samples of each WWTP and measured for quality control. Finally, laboratory method blank samples were included in the measurement to determine contamination and carry-over

2.6. LC-HRMS measurements

The WWTP effluent samples were analyzed by large volume direct injection LC-HRMS. The samples of the different WWTPs were measured in separate sequences. To ensure the quality of the measurements, the analytical instrument was cleaned and calibrated before each sequence. In each sequence, after every tenth WWTP sample a blank (ultrapure water) without and a blank with ISTDs was analyzed. The calibration curve was measured at the beginning and at the end of each sequence. A sample volume of 50 µL was injected and separated with a mobile phase gradient (water-methanol, both acidified with 0.1% formic acid) on a reversed-phase C18 column (Atlantis T3, 3 µm particle size, 3.0 × 150 mm inner diameter, Waters) at a flow rate of 300 µL/min. HRMS data was acquired on a hybrid quadrupole-orbitrap mass spectrometer (QExactive Plus, Thermo Scientific) in positive and negative electrospray ionization (ESI) mode in separate runs. Full scan spectra of two mass ranges, *i.e.*, 50-105 *m/z* and 100-1000 *m/z*, were recorded at a resolution (R) of 35'000 and 140'000 (at *m/z* 200), respectively. These full scans were followed by six data independent MS/MS experiments (R=17'500 at *m/z* 200). Because of the large differences in sample matrix, slight adaptions to the method between sample measurements of different WWTPs were necessary; these are detailed in SI 5. Further details on the analytical instrumentation and the method are provided elsewhere (Anliker et al. 2020b). Based on the ISTDs and the quantified target compounds measurements generally resulted in mass errors < 2 ppm and retention time (RT) deviations < 30 sec. Measurement quality control data is presented in SI 6. Although the focus of this work is on SOCs, the generic analytical method used may also have captured natural organic compounds and a few inorganics.

2.7. LC-HRMS data processing and data analysis

The LC-HRMS non-target data processing closely followed the procedure described in Anliker et al. (2020a). Here, the enviMass workflow (version 4.3) (Loos 2021) was used for the automatic generation of intensity time series of all LC-HRMS full scan features (*i.e.*, chromatographic peaks, defined by *m/z* and RT) detected in the mass range of 100-1000 *m/z*. The data of each WWTP were processed in a separate batch. The processing steps and settings of the enviMass workflow are given in SI 7 and SI 8, respectively, and details on the extraction of time series and subsequent filtering for relevant entries are described in Anliker et al. (2020a). Importantly, only signals of an intensity $\geq$ 1E5 and time series with an average sample/blank ratio > 10 were considered in the analysis. Since componentization was performed (*i.e.*, features of adduct and isotopologues belonging to the same component were grouped and only the most intense entry was retained) it was assumed that each feature in the final datasets corresponds to one compound. Selected target compounds were quantified with the software TraceFinder 4.1 (Thermo Scientific). Quantification was based on the area ratio of the reference standard and the ISTD of the analyte. Compounds were considered quantifiable if the relative recovery (based on spiked samples) and the reproducibility (based on replicate samples) was in a range of 75% to 125%.

Overall, the LC-HRMS data of 853 effluent samples were analyzed in this study, between 56 and 91 for each of the 11 WWTPs. As mentioned above, the data of the daily WWTP effluents samples from D01 and CP3.0 (3 months observation period) and CPF0.1 and CPF2.3 (2 months observation period) were acquired in the framework of previous studies. These data were acquired and processed using a very similar approach to the one described here, details are given in Anliker et al. (2020a) (for D01 and CP3.0) and in Anliker et al. (2020b) (for CPF0.1 and CPF2.3).

Data analysis was performed using the statistical software R (version 3.6.1) (R Core Team 2017) with the basic functions, if not stated otherwise. For quantification of the overall intensity variation, the fold change observed in HRMS time series was calculated as the ratio of the 0.95-quantile to the 0.05-quantile of the intensity values according to

Anliker et al. (2020a). As proposed previously (Anliker et al. 2020a) a threshold for overall time series variation > 10-fold was used to detect potential CPM emissions, assuming that large fluctuations were linked to production cycles. A mass window tolerance of ±2ppm and a RT tolerance of ±30s was applied to define matching feature pairs between different WWTPs. For the correlation analysis, non-detects in the LC-HRMS data were set to zero. Spearman's rank order correlation coefficients (ρ) between HRMS time series and toxicity time series were calculated using the R *cor.test* function.

2.8. Bioassays

A comprehensive ecotoxicological study with the effluents of CP60 was performed by a private laboratory (Soluval Santiago, Couvet, Switzerland) on behalf of one of the companies connected to the WWTP. For this, simultaneously to the LC-HRMS sample collection, daily 24-h composite effluent samples were taken. *In vivo* bioassays were then conducted for the 91 samples according to ISO standards in screening mode, *i.e.*, at a reduced number of concentrations and replicates. Acute toxicity tests were performed for microcrustaceans (*Daphnia magna*, endpoint: immobilization and mortality, duration: 48 h, ISO 6341), bacteria (Microtox^{TM}, *Vibrio fischeri,* bioluminescence, 0.5 h, ISO 11348-3) as well as chronic toxicity tests with green algae (*Raphidocelis subcapitata*, growth, 72 h, ISO 8692) and macrophytes (*Lemna minor*, population growth, 7 d, ISO 20079). The results of the ecotoxicological assessment were expressed in toxic units (see SI 9 for details). However, as requested by the company that provided the data, all toxicity data are given as normalized values only. A fixed value was used for results below the sensitivity limit of the bioassays (see SI 9).

In this work, the toxicity time series were correlated with the LC-HRMS time series of CP60, thereby obtaining a "virtual fractionation" to identify features that potentially cause the observed ecotoxicological effects (see section 2.7). Similar approaches, based on multivariate variate data analysis instead of time series, have successfully been applied previously (Eide et al. 2004, Eide et al. 2002, Hug et al. 2015).

3. Results and discussion

3.1. Presence of chemical and pharmaceutical manufacturing facilities in Swiss WWTP catchments

According to the statistical records (Swiss Federal Statistical Office 2016), there is – relative to the country's small area of 41'300 km^2 – a considerable number of > 1'000 industrial premises in the chemical (727) and pharmaceutical (279) sector registered. The GIS based analysis showed that chemical and pharmaceutical industries are located in 271 (38%) of the 718 Swiss WWTP catchments. The number of full-time equivalent employees per catchment as a proxy for CPM industrial density is illustrated in the map in **Fig. 1**. Discussions with the respective cantonal authorities revealed that a high number of full-time employees in CPM industries was, for most catchments, a good indicator for identifying WWTPs that treat substantial amounts of CPM wastewater. In a few cases only, it was found that CPM locations are purely administrative or the respective companies do not discharge any manufacturing wastewater (as this was the case for WWTP D01, for instance, **Table 1**). In the majority of the WWTP catchments with CPM (172 of 271), there are < 100 employees in the respective industry branch; the analysis identified only four catchments with more than > 2000 full-time employees in the CPM sector. These findings indicate that many WWTPs in the Swiss Plateau potentially receive wastewater from CPM, mostly from small to middle sized facilities. Hence, contamination from CPM is potentially widespread nationwide and focusing on the well-known CPM hotspots might miss important contributions. It can be assumed that the situation is similar in other regions of the world, where CPM locations are far more numerous than in Switzerland, *e.g.*, > 30'000 all over Europe (European Chemical Industry Council 2020), > 25'000 in the US (The International Trade Administration 2021) and > 20'000 in China (Chen and Reniers 2020). To the best of our knowledge, systematic assessments of CPM emissions in these countries are missing and an analysis as the one presented here could provide valuable first insights into the prevalence of potential CPM contamination.

3.2. Characteristics of temporal LC-HRMS signatures in WWTP effluents

Next, we were interested if the presence of CPM in the wastewater WWTP catchment has an actual impact on the effluents. To this end, we analyzed the effluent data of the 11 WWTPs. Data processing resulted in a time series for each of the several thousand features detected in the LC-HRMS measurements of the effluent samples, ranging from 4'542 (CPF0.1) to 69'480 (CP50) per WWTP (**Fig. 2a**).

First, we characterized the time series datasets with a non-target approach and then compared them, considering the different contributions of CPM wastewater at each WWTP. In the interest of clarity, the data of the ESI positive and negative mode measurements are discussed together.

3.2.1. Comparison of purely domestic and CPM wastewater receiving WWTP effluents

Starting with a general comparison of purely domestic and CPM-influenced WWTP effluents, clear differences were already apparent at the level of DOC concentrations, which were generally higher and fluctuated more at WWTPs receiving CPM wastewater (SI 10). However, DOC measurements do not provide information on the composition of the effluents. The LC-HRMS time series datasets revealed that, compared to purely domestic WWTP effluents, up to 15 times more features were detected in CPM wastewater receiving WWTP effluents (**Fig. 2a**), suggesting that a larger substance spectrum was emitted in the latter. Furthermore, the share of features with very high intensities (*i.e.*, > 1E8) was larger at the WWTPs that receive CPM wastewater, ranging up to > 7‰, compared to D01 and D02, with a share < 1‰ (SI 11 Figure S3). Even though feature intensities cannot be directly transferred into concentrations (because of different ionization efficiencies of the underlying compounds), high intensities still often correspond to high concentrations. Hence, the results imply that more compounds with very high concentrations were discharged in the effluents of WWTPs receiving CPM wastewater compared to purely domestic ones.

We then focused on features with intensity variations > 10-fold in their time series (in the following referred to as highly fluctuating features), *i.e.*, potential industrial emissions (see section 2.7). Here, the percentage of highly fluctuating features varied between 2.6% (D01) and 16.7% (CP50) (**Fig. 2a**, black bars). The number of highly fluctuating features was generally larger at WWTPs that treat CPM wastewater and features with extreme variations (*i.e.*, > 1'000-fold) were only detected at these WWTPs (SI 11). Overall, these general differences between purely domestic and WWTPs receiving CPM wastewater were consistent with the findings of our previous work (Anliker et al. 2020a), which was based on comparing D01 and CP3.0 only.

Given the large share of CPM wastewater in some of the WWTPs, the percentages of highly fluctuating features might seem relatively small. However, highly fluctuating features accounted for a much larger proportion (> 40%) of the total measured intensity, at the majority of the WWTPs receiving CPM wastewater compared to purely domestic WWTPs (< 20%, **Fig. 2b**). This implies that highly fluctuating features, most likely indicative of industrial discharges, contributed considerably to total substance emissions. The temporal patterns of the share of highly fluctuating features relative to the total intensity is provided in SI 12.

Overall, the comparison of the non-target time series datasets of purely domestic and CPM-influenced WWTP effluents indicated that substantial emissions (in terms of both substance diversity and magnitude) from CPM are rather the rule than the exception. This conclusion is in accordance with the two other large-scale studies on SOC discharges from CPM, both of which concluded that pharmaceutical manufacturing

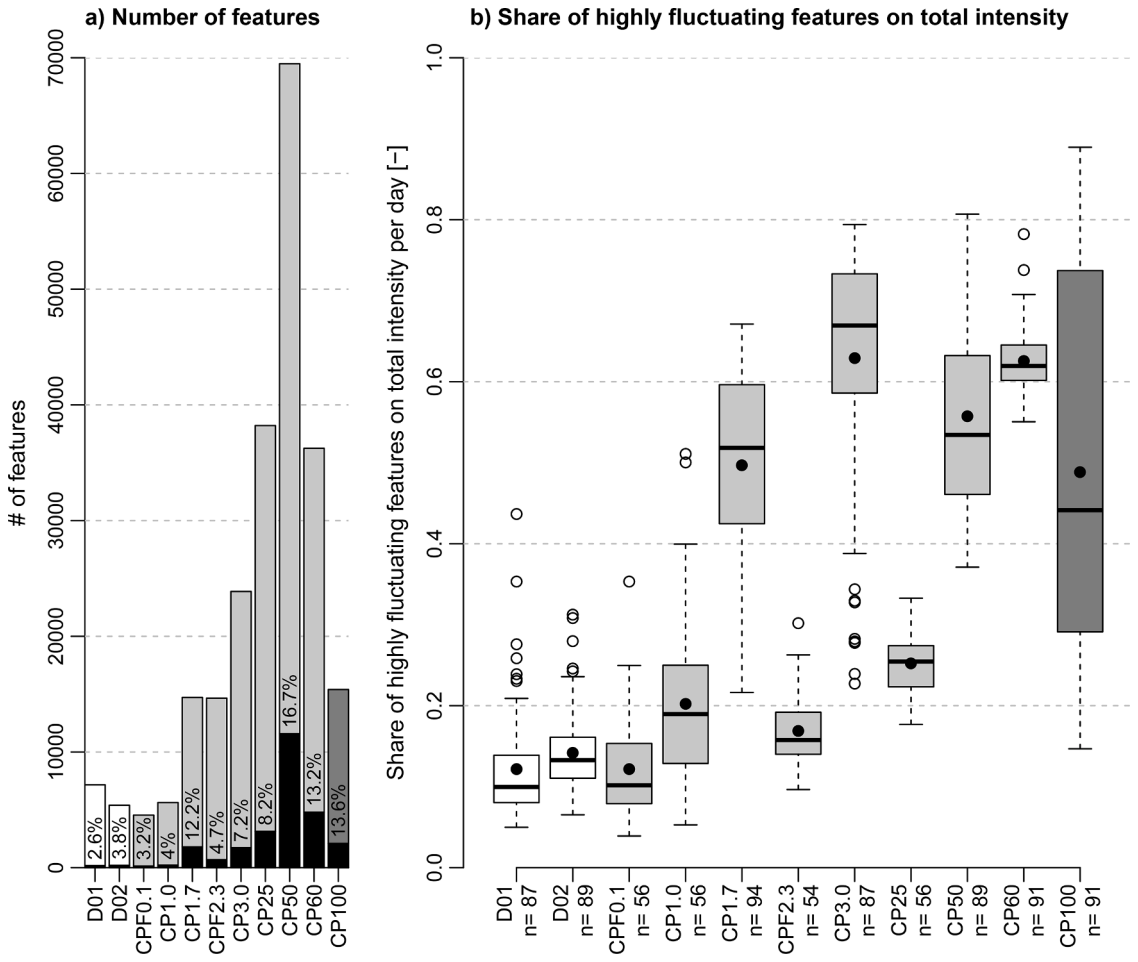

Fig. 2. **a)** Bar plot displaying the number of features detected at each WWTP (sum of positive and negative ESI and only most intense feature per compound). Black bars show the number of highly fluctuating features (*i.e.*, overall variation > 10-fold) and are labelled with the respective percentage of highly fluctuating features on total features. **b)** Box-whisker plots showing the distribution of the share of the intensity of highly fluctuating features on the daily total intensity (*i.e.*, for each sample, the sum of the intensities of highly fluctuating features divided by the sum of the intensities of all features detected) for the 11 WWTPs. On the x-axis, the WWTP code and the number of data points (n, *i.e.*, days) are indicated. The upper and the lower limit of the box indicates the third quartile and first quartile, respectively; the line represents the median, the black dots mark the mean, the whiskers extend to 1.5 times the interquartile range from the bottom and the top of the box, and any data beyond that range are represented as empty circles. In **a)** and **b)** empty bars and boxes indicate purely domestic WWTPs, light grey mark mixed WWTPs and the data of the purely industrial CP100 are shown in dark grey.

wastewaters were generally a relevant source of pharmaceuticals to the aquatic environment (Kleywegt et al. 2019, Scott et al. 2018). In the future, as a consequence of the current COVID pandemic, CPM activities are expected to increase in Switzerland and the EU, due to recent initiatives to bring back pharmaceutical production to Europe to reduce the risk of medicine shortages (European Commission 2020). At the international level, based on available literature (Larsson et al. 2007, Li et al. 2008), it can be assumed that contamination from CPM is even more relevant in regions of the world with much higher production volumes and less focus on surface water quality. Therefore, in many parts or the world, CPM is likely a currently underestimated source of SOCs influencing water quality.

3.2.2. *Differences between the effluents of CPM wastewater receiving WWTPs*

As described in the section above, CPM-influenced WWTPs were generally observed to have a large number of total features, a large portion of intense features and a large proportion of highly fluctuating features. However, these characteristics did not correlate well to other factors, such as the percentage of industrial wastewater, the number of employees, or the DOC concentration. A comprehensive analysis of the factors affecting CPM emissions was not possible due to missing information on industrial processes, wastewater handling and pretreatment. Nevertheless, a closer inspection of specific cases did reveal potential explanations for the differences observed among the CPM-influenced WWTPs.

First, there seems to be a difference between producing and formulating facilities. This difference might originate from the fact that formulating industries commonly emit fewer compounds. For example, during our sampling campaign at WWTP CPF0.1 and CPF2.3, only 77 different compounds were processed by the five companies involved in the study (Anliker et al. 2020b), compared to hundreds of compounds expected from synthesis activities (including starting materials, end products, catalysts, solvents, intermediates and byproducts) (Howard and Muir 2013). Apart from their small shares of CPM wastewater, this might explain why, at CPF0.1 and CPF2.3, highly fluctuating emissions contributed comparatively little (< 20%) to the overall intensity (**Fig. 2b**). Still, active pharmaceutical ingredients processed by formulating industries were responsible for the highest peak concentrations measured in the effluents of both WWTPs (Anliker et al. 2020b).

Second, as expected, the extent to which CPM discharges are detected in WWTPs effluents seems to largely depend on the wastewater (pre-)treatment. On the one hand, CP1.7 and CP3.0, where highly fluctuating features contributed disproportionately (> 50%, despite

industrial wastewater shares < 5 vol%) to the total intensities (Fig. 2**b**), are known to have insufficient pretreatment and requirements for mitigation measures have been imposed on the respective companies. On the other hand, activated carbon adsorption is in place at two WWTPs, as an on-site advanced pretreatment prior to release to CP1.0 and as final WWTP treatment step at CP100. At both WWTPs, relatively few features and smaller proportions of highly fluctuating emissions were detected (Fig. 2**a**). However, at CP100, the daily share of highly fluctuating features on the total emissions varied substantially (Fig. 2**b**). When considering the time pattern of the total intensities at CP100 (SI 12 Figure S4 k), it becomes apparent that this variation was caused by one event of very high cumulative intensities (also reflected in increased DOC concentrations). This event was attributable to a malfunction of the WWTP, highlighting that a correctly operating treatment is fundamental to prevent emissions, and that the fluctuating emissions observed at CP100 were, in contrast to our initial assumption, not *per se* caused by production cycles.

Third, industrial wastewater storage and discharge practices appear to strongly influence the dynamics of CPM discharges observed in WWTP effluents. In particular, at CP25, CPM wastewater is collected in large stacking tanks and released little by little into the WWTP. This controlled discharge likely averages out production dependent fluctuations and might explain why relatively few highly fluctuating features were detected in the effluents of CP25. Hence, as has been highlighted previously (Anliker et al. 2020b, Kleywegt et al. 2019), for data interpretation, knowledge on the production cycles and the companies' wastewater handling is essential. In some cases, focusing on highly fluctuating features might be misleading and will miss relevant CPM discharges. Therefore, considering additional indicators for industrial emissions might be necessary, such as site-specificity of features or much higher intensities of relatively constant features compared to other sites (Krauss et al. 2019).

3.3. Comparison of the composition of WWTP effluents

So far, the focus was on the overall feature characteristics of the investigated WWTP effluents. Next, we compared the composition of the effluents with a focus on the top most intense features, assuming that those are the most relevant from a concentration point of view. First, we investigated the overlap of features between the different WWTPs. Specifically, we extracted the 100 most intense features at each WWTP and analyzed the percentage of matching features (*i.e.*, same *m/z* and RT). The results are displayed as overlap matrices in Fig. 3. Generally, more overlapping features were found between the effluents of WWTPs that are less influenced by CPM wastewater, *i.e.*, up to 35% (D02 and CPF2.3) in positive and up to 49% (CPF0.1 and CP1.0) in negative ESI mode. In contrast, the WWTPs that receive ≥ 25 vol% CPM wastewater had maximally 7% overlapping features with any other WWTP in ESI positive mode and 18% in ESI negative mode, respectively. This result indicates that compounds with highest concentrations in domestic wastewater are rather similar between different WWTPs, while they are site-specific in the CPM wastewater influenced effluents.

We then looked for target compounds (*i.e.*, known substances for which reference standards were available and analyzed) among the overlapping features. It was found that compounds detected at more than two WWTPs were substances known to be present at high concentrations in domestic wastewater effluents: benzotriazole (detected among the most intense features at 8 WWTPs), 4-/5-methylbenzotriazole (6), metformin (5), 4-acetamidoantipyrine (5), tramadol and o-desvenlafaxine (isobaric & co-eluting, 5), gabapentin (5), N-N-diethyl-3-methylbenzamide (DEET, 4) and irbesartan (3) in positive ESI, and the artificial sweeteners acesulfame (7) and saccharine (4) in negative ESI mode. However, these well-known domestic wastewater contaminants only rarely appeared among the 100 most intense features at WWTPs strongly influenced by CPM wastewater. Generally, the 100 most intense features of these WWTPs only contained few target compounds anyhow and the ones found were typically reported *(i.e.*, either confidentially by the company or on a publicly available product list) to be manufactured by a CPM company in the catchment of the respective WWTP. These "CPM target compounds" were usually not measured in domestic wastewater, or at much lower concentrations (*i.e.*, 1-2 orders of magnitude, SI 13 Table S4), thus supporting our previous results from a smaller set of WWTPs (Anliker et al. 2020a, Anliker et al. 2020b).

In summary, each CPM site displays its very own substance spectrum and relevant compounds are hence site-specific. Searching for features of high intensity variations in LC-HRMS time series (Anliker et al. 2020a) proved efficient in most cases to detect potential CPM discharges and thus can be applied in other regions of the world to obtain an initial estimate of the extent of such emissions. However, compound-specific monitoring and sound environmental risk assessments of CPM discharges remain very challenging since each situation has to be assessed individually.

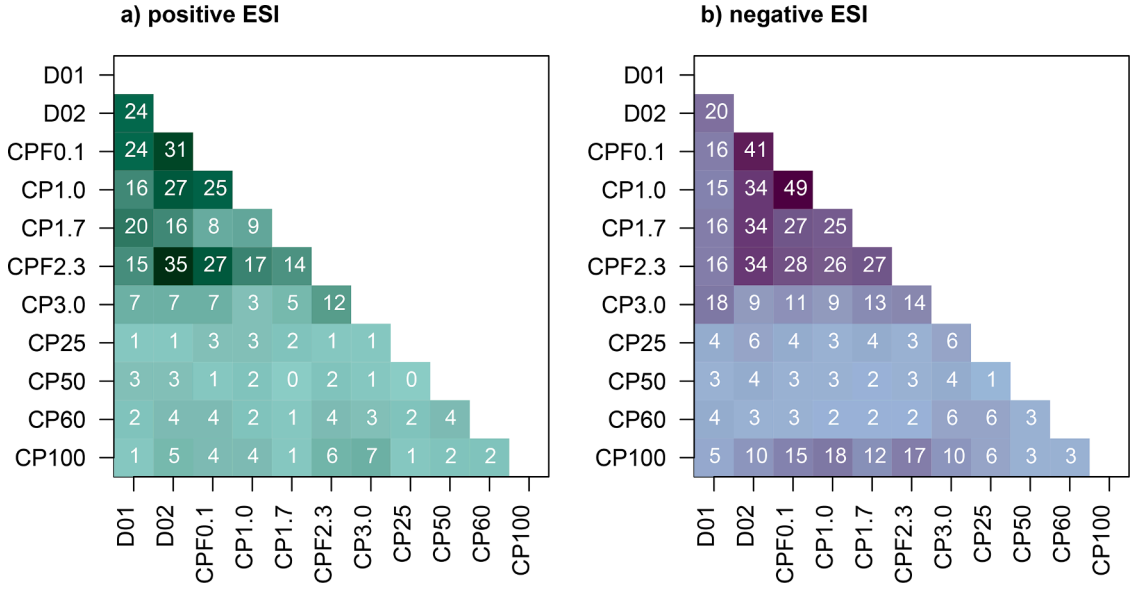

Fig. 3. Matrices visualizing the number of overlapping features between WWTPs of the 100 most intense features, **a)** in positive and **b)** in negative ESI mode, respectively, at each WWTP. The number in each box is the percent of overlapping features between the respective WWTPs.

3.4. Identifying LC-HRMS features causing toxicity

In the following, we discuss the results of correlating the toxicity data with the LC-HRMS chemical data. While no significant toxicity was observed in the acute toxicity assays with microcrustaceans and luminescent bacteria, distinct time periods of substantial effects occurred in the chronic tests with green algae and macrophytes.

For the green algae, significant toxicity was observed in the effluent samples during the last 20 days of the sampling campaign. Correlation of the HRMS data with the algae toxicity data resulted in only one feature with a highly correlated time series (*i.e.*, Spearman's $\rho \geq 0.9$) (**Fig. 4a**). The chemical structure of the measured *m/z* 112.9857 (deprotonated molecular ion) was identified and confirmed with a reference standard as trifluoracetic acid (TFA, level 1 identification (Schymanski et al. 2014)). The maximum effluent concentration was estimated retrospectively based on a one-point calibration and amounted to approximately 10 mg/L. Although TFA is widely used in organic synthesis, intensive follow-up investigations of the concerned company showed that, in this case, TFA was formed during a specific waste stream pre-treatment. Changing the pre-treatment process substantially reduced the effluent toxicity. Importantly, this example demonstrates that unidentified compounds in CPM wastewater might be responsible for considerable toxicity and as a consequence, that risk assessments focusing only on active ingredients are insufficient.

For the macrophytes (duckweed), increased toxicity was observed during two short periods of 3 and 1 days. The time series of three HRMS features that were very well correlated with the macrophytes toxicity could be associated (through different adducts, indicating that feature componentization was not always successful) to a herbicide (herb_A) produced by a company connected to CP60 (in **Fig. 4b** the data of the most intense adduct $[M+H]^+$ is shown, the other time series are given in SI 14 Figures S5 m and n). Indeed, duckweed is well known to be sensitive to herb_A (EPA 2020). While TFA corresponded to the 10th most intense feature (*i.e.*, according to maximum intensity during the sampling period in negative ESI), the most intense adduct of herb_A was on rank 1204 only (in positive ESI), demonstrating that also less intense features might be ecotoxicologically relevant. Herb_A was on the confidential product list provided by the company and was included in our measurements as CPM target compound (*i.e.*, an authentic reference standard was measured, leading to level 1 identification (Schymanski et al. 2014)). Quantification resulted in a maximal effluent concentration of 9.3 µg/L.

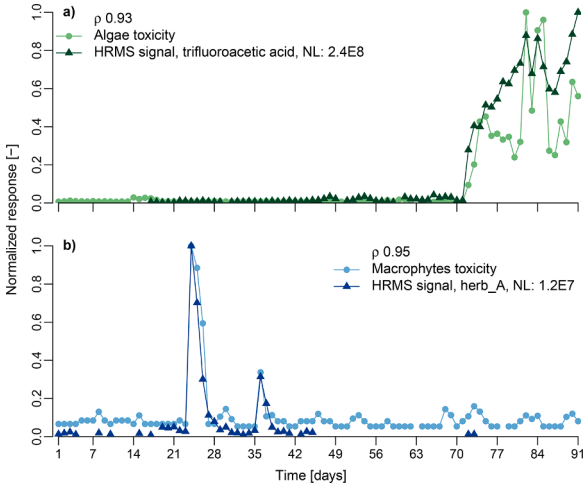

Fig. 4. Time profiles showing the highly correlated time patterns between toxicity data and HRMS chemical data: **a)** Time series of the algae toxicity and **b)** the time series of the macrophytes toxicity, along with the HRMS intensity time series of the features that highly correlate with the respective toxicity pattern. The Spearman's rank-order correlation coefficient (ρ) and the normalization levels (NL) of the HRMS time series are indicated in the figure legends. Missing data in HRMS time series (triangles), correspond to non-detects (signals < LOD). In toxicity time series, a fixed value was used for results below the sensitivity limit of the bioassays (see SI 9), and missing values correspond to missing samples.

Considering the complex composition of the effluents, it is surprising that in both algae and macrophytes toxicity only one single compound was found to be responsible for the observed effects. Only for the macrophytes toxicity, additional HRMS features resulted in very high correlation coefficients (SI 14 Figure S5); however, none of them explained both periods of increased toxicity. Moreover, some of the observed high correlations were rather uncertain because intensities of the corresponding HRMS features were close to the limit of detection (LOD) and/or few data points were detected. Yet, experiments with the individual substances showed increased toxicity (by 170% and 140% for herb_A and TFA, respectively) in non-toxic CP60 effluent (7-day composite sample) compared to standard test medium (EPA-AAP (EPA 1996)). This observation suggests that the sample matrix can influence observed compound toxicity. This might be due to synergistic effects with matrix components or enhanced bioavailability in the effluent samples .

In summary, combining temporal HRMS chemical data with temporal toxicity data proved highly successful in identifying compounds in CPM wastewaters that were toxic in *in vivo* bioassays. Fundamental to the success of time series correlation was the availability of high temporally resolved data reflecting daily fluctuations and short-term peaks, as well as the presence of distinct time patterns in these data.

4. Conclusions

Long-term, daily LC-HRMS effluent signatures of 11 Swiss WWTPs receiving 0-100 vol% of CPM wastewater were acquired and analyzed in this study. Using mainly a non-target approach, this unique dataset enabled assessing the complexity, diversity, and contaminant dynamics of CPM discharges via WWTP effluents for the first time on a national level. The results showed that:

- CPM is surprisingly widely distributed across Switzerland and > 30% of the municipal WWTPs potentially receive CPM wastewater, suggesting that, also in other countries, CPM discharges might be a more widespread source of SOC contamination than anticipated.
- Substantial SOC emissions from CPM have to be expected, unless (i) the share of industrial wastewater on the total WWTP flow is very low (< 5%), (ii) a highly advanced wastewater pre-treatment is in place, and (iii) the number of compounds handled on site is small.
- Effluents mainly dominated by households exhibited relatively constant temporal dynamics and their composition was similar among different WWTPs, whereas CPM-influenced effluents generally showed higher complexity, variability, and site specificity. In some cases, even small amounts of CPM wastewater (< 5%) caused significant changes in the effluent composition.
- Compared to emissions in domestic wastewaters, amounts of SOCs discharged in CPM wastewater may be very high, *i.e.*, up to 15 times more compounds and 1-2 orders of magnitude higher signal intensities (indicating higher concentrations) were observed.
- Because of the diversity of this industrial branch, there are large differences in the dynamics and compositions between the effluents of CPM-influenced WWTPs. Compounds relevant for monitoring differ from facility to facility and often include non-registered chemicals. Hence, the strategies currently applied to monitor SOC emissions in domestic WWTP effluents, by performing sporadic measurements of a predefined set of target compounds, are not suitable to assess CPM discharges. Rather, the site-specificity of CPM discharges requires considering the individual situation to achieve sound risk assessments and to define suitable monitoring programs and mitigation measures.

- Combining temporal HRMS data and temporal toxicity data was highly successful to identify toxicity causing compounds in CPM wastewater and indicated that unknown, unexpected and low abundant compounds may be ecotoxicologically relevant.

Overall, we conclude that, in countries where CPM is present, SOC contamination from this industrial source is likely an underestimated factor influencing the quality of surface waters. Applying multiple mitigation strategies is essential to reduce the potential environmental impact of CPM wastewaters, including reducing SOC load and complexity, as well as removing the most toxic compounds. In the future, innovative technical solutions are needed as an alternative to wastewater incineration, which is very energy-inefficient. Finally, further studies are required to contribute to a better awareness of SOC discharges from CPM and to increase the companies' commitment to put their principles of environmental sustainability more rigorously into action. In fact, at CPM sites where mitigation measures have been implemented, the impact of SOC discharges on water quality could be substantially reduced.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118221.

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