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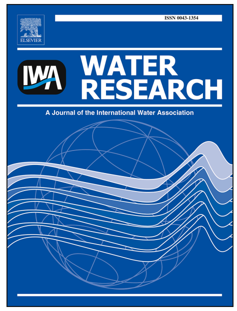

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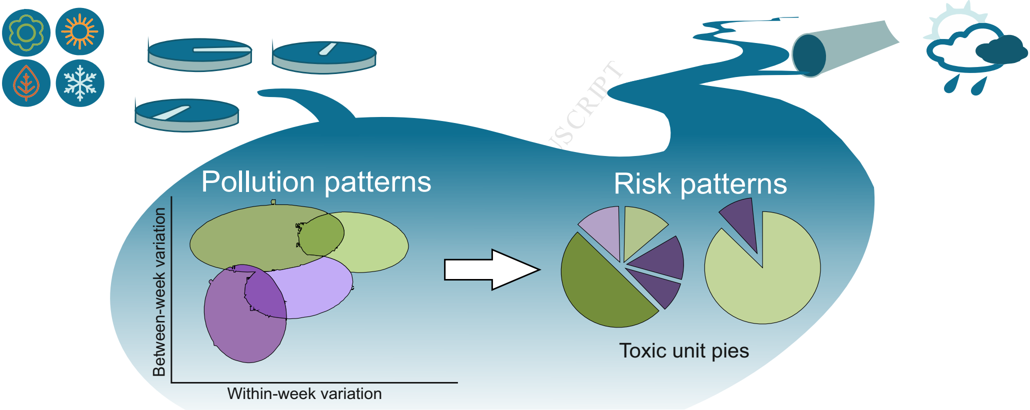
Pollution patterns
Between-week variation
Within-week variation
Risk patterns
Toxic unit pies

Title

Characterization and risk assessment of seasonal and weather dynamics in organic pollutant mixtures from discharge of a separate sewer system

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Abstract

Sites of wastewater discharge are hotspots for pollution of freshwaters with organic micropollutants and are often associated with adverse effects to aquatic organisms. The assessment, monitoring and managment of these hotspots is challenged by variations in the pollutant mixture composition due to season, weather conditions and random spills. In this study, we unraveled temporal exposure patterns in organic micropollutant mixtures from wastewater discharge and analyzed respective acute and sublethal risks for aquatic organisms. Samples were taken from two components of a separate sewer system i) a wastewater treatment plant (WWTP) and ii) a rain sewer of a medium size town as well as from the receiving river in different seasons. Rain sewer samples were separately collected for rain and dry - weather conditions. We analyzed 149 compounds by liquid chromatography-tandem mass spectrometry (LC-MS/MS). By considering the pollution dynamics in the point sources, we reduced the complexity of pollutant mixtures by k-means clustering to a few emission groups representing temporal and weather-related pollution patterns. From these groups, we derived biological quality element (BQE) - specific risk patterns. In most cases, one main risk driving emission group and a few individual risk driving compounds were identified for each BQE. While acute risk for fish was quite low, algae were exposed to seasonally emitted herbicides (terbuthylazine, spiroxamine) and crustaceans to randomly spilled insecticides (diazinon, dimethoate). Sublethal risks for all BQE were strongly influenced by constantly emitted pollutants, above all, pharmaceuticals. Variability of risks in the river was mainly driven by water discharge of the river rather than by season or peak events. Overall, the studied WWTP represented the major pollution source with a specific emission of agricultural compounds. However, the investigated rain sewer showed to be a constant pollution source due to illicit connections and was an important entry route for high loads of insecticides and biocides due to spills or incorrect disposal. By considering these pollution and risk dynamics, monitoring strategies may be

optimized with a special focus on times of low flow conditions in the river, rain events and seasonally emitted risk drivers.

Keywords

Organic micropollutants; wastewater treatment plant; rain sewer; pollutant patterns; acute and sublethal risks; seasonal and weather dynamics

1. Introduction

Synthetic organic chemicals are essential for our modern lifestyle. They are used as pharmaceuticals, pesticides, and biocides, as well as in all types of industrial production and as components of everyday consumer products. As organic micropollutants they are ubiquitous in the aquatic environment and may be detected in great numbers and in complex and variable mixture compositions (Busch et al., 2016, Loos et al., 2009). Wastewater including urban stormwater is an important source for these pollutants potentially affecting aquatic organisms (Inostroza et al., 2016, König et al., 2017, Munz et al., 2017, Münze et al., 2017). Consequently, a comprehensive characterization, assessment, monitoring and management of wastewater effluents and receiving waters is crucial. This requires considering variations in the mixture composition due to season, weather conditions and random spills (Petrie et al., 2015, Wittmer et al., 2010).

In small rivers, wastewater treatment plant (WWTP) effluents but also rain sewers may significantly contribute to the overall discharge and pollution load (Munz et al., 2017, Wittmer et al., 2010). In separate sewer systems, stormwater is discharged directly to rain storage reservoirs and receiving water bodies with less or no treatment. Consequently, poly aromatic compounds from atmospheric deposition or traffic as well as pesticides and biocides used for urban pest control in private or public settings may be washed off from surfaces at rain events and enter surface waters via rain sewers (Gasperi et al., 2014, Wittmer et al., 2010).

While for rain sewers only limited data are available, there have been extensive studies on pollutants and pollutant mixtures emitted from WWTPs (e.g. Kostich et al., 2014, Loos et al., 2013, Munz et al., 2017). Loos et al. (2013) found 80% of their 156 target compounds in the effluents of 90 WWTPs across Europe. Munz et al. (2017) detected 50% of 57 compounds at eight time points in the effluents of 24 Swiss WWTPs. These studies suggest the occurrence of common baseline pollution patterns. In addition, the review by Petrie et al. (2015) summarizes knowledge on diurnal, weekly, seasonal and precipitation-related patterns of individual compounds or compound classes entering or emitted from WWTPs due to changing WWTP performance or consumption patterns. Season-specific emission has been observed for the insect repellent DEET (Nelson et al., 2011), pharmaceuticals such as antibiotics and nonsteroidal anti-inflammatory drugs (e.g. Castiglioni et al., 2006, Golovko et al., 2014, Vieno et al., 2005) and pesticides (Neumann et al., 2002).

It may be hypothesized that the complex contamination in receiving rivers reflects an overlay of constant baseline emission of municipal wastewater components via WWTPs and seasonal and event-driven pollution patterns from WWTPs, rain sewers, agricultural runoff and other sources. Temporal pollution patterns and seasonal concentration peaks may result in temporal risk and survival patterns of aquatic organisms (Ashauer et al., 2007). In order to enhance the efficiency and explanatory power of chemical monitoring, priority mixtures and compounds may be identified on the basis of pollution and effect patterns (Altenburger et al., 2015). Simple indicators for risk by toxic chemicals are toxic units (TU), which are the ratio between a measured environmental concentration of a compound and its respective effect or lethal concentration. Sum TUs characterize mixture effects. To get a quick, but holistic overview on the risk, the Water Framework Directive defined biological quality elements (BQE) as representative organisms groups for the aquatic community for monitoring and status assessment (EEA, 2008).

The objective of the present study was to unravel temporal exposure and risk patterns of chemical mixtures in wastewater discharge from a separate sewer system including a municipal WWTP and an associated rain sewer. In detail four major questions were addressed: 1) How can pollutants from wastewater discharge be grouped? 2) Can these groups be used to derive and discriminate risk patterns for different BQE? 3) Are there individual dominating drivers of risk or are the risks driven by the complex mixtures? 4) To what extent do WWTP and rain sewer effluent contribute to BQE-specific risks in the river? Finally, this study should contribute to the discussion on future monitoring strategies and management of pollution hotspots.

2. Methods

2.1 Study site

The study was performed at the River Holtemme (Saxony Anhalt, Germany) at a conventional WWTP (80,000 population equivalents), which receives municipal and industrial wastewater from the municipality of Wernigerode and nine villages in an area of intensive agriculture. This catchment is connected by 180 km of sewers. For biological wastewater treatment, the WWTP uses activated sludge technology in its aeration tank with the addition of ferric salt for phosphorus removal. The WWTP operates in a separate sewer system. Stormwater is drained via rain sewers. Consequently, the WWTP is not strongly affected by rainfalls and has a hydraulic retention time of 72 hours. Discharge and temperature data of the effluent during the sampling periods are shown in the supporting information (Table S1). The studied rain sewer discharges into the river approximately 6 km upstream of the WWTP effluent. It serves the old town and a residential area of Wernigerode with private houses, gardens, roads and commercial areas. The discharge data of the rain sewer and precipitation data are presented in Table S2. Discharge data at the nearest river gauge was retrieved from the public database of the State Office for Flood Protection and Water Management Saxony-Anhalt (LHW) (2017).

2.2 Sampling

From the WWTP effluent, eight daily composite samples were taken during one week in May, July/August and October 2015, and February 2016, respectively. Each composite sample was compiled of sub-samples taken every two minutes. In total, 32 samples (250 ml each) were provided by the WWTP operators.

Monthly composite samples from a rain sewer from April 2015 to April 2016 were provided by the Environmental Agency for Saxony-Anhalt (LAU). Under dry conditions, volume-proportional samples were collected by an auto-sampler equipped with an ultrasonic probe. Samples of 150 ml were collected after 16 m³ of water passed the sampling point. Rain samples were taken discharge-proportionally every two minutes if discharge rates exceeded 30 L s^{-1}. The samples were taken continuously over one month and immediately stored in separate five-liter containers placed in an onsite freezer connected to the auto-sampler. For each weather condition and month, one-liter samples were sent to our laboratory. The samples from May and June 2015 could not be analyzed due to transport damage.

Monthly composite samples were also taken from the river approximately 1.2 km downstream of the WWTP effluent. In hourly intervals, sub-samples of 500 ml were automatically collected using an on-site large volume solid phase extraction (LVSPE) device described by Schulze et al. (2017). Since the monitoring station was not operable in the winter months due to possible frost damage, LVSPE samples could only be taken from April to November 2015.

All samples were stored at -20℃ until analysis.

2.3 Chemical analysis of samples

In total, 149 compounds were selected for target analysis of water samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The selection was based on previous knowledge of the occurrence of compounds in river water, general consumption habits as well as specific industrial activities in the study area. Finally, the list included pharmaceuticals, pesticides, biocides, household and industrial compounds, artificial sweeteners and important transformation products (TP) (Table S3).

Analysis of WWTP and rain sewer samples

Chemical analysis was performed on a 1260 Infinity LC system (Agilent) coupled to a QTrap 6500 MS/MS (ABSciex) with an IonDrive Turbo V electrospray ion source system. From each sample an aliquot of 1 mL was taken for chemical analysis. For the WWTP samples, a 1:10

dilution was additionally prepared. Details on sample preparation can be found in the supplementary information (SI 3). The target compounds were addressed with three LC-MS methods according to ionization behavior (Table 1). Details on LC-MS/MS settings are presented in Tables S4 and S5. Individual compound settings are provided in Table S3. Details on the preparation and analysis of LVSPE samples are provided in the supplementary information (SI 4).

2.4 Statistical analysis

In order to account for different discharge across the sampling periods, the concentration data were converted into loads using discharge data provided by the operators of the WWTP and the LAU.

Statistical analysis was performed in R (R Core Team, 2016). Data from the WWTP and the rain sewer were analyzed separately due to different system and sampling settings. Prior to statistical analysis the data was transformed by the natural logarithm, scaled and centered to reduce skewness and ensure equal variance of all variables. Values below MDL were treated as zeroes. Due to several non-detects in the data sets, the 'glog' function from the package 'FitAR' was used (McLeod and Zhang, 2008). Temporal patterns of all detected compounds and sampling days or months were displayed by a heatmap (function 'heatmap.2', R package 'gplots' (Warnes et al., 2016)). K-means clustering was applied as an exploratory data analysis tool to identify emission groups among all chemicals in the WWTP and rain sewer effluent. The factoextra package was used for k-means clustering and the preparation of graphs (Kassambara and Mundt, 2016). The number of clusters was determined by the elbow method, which calculates the within sum of squares for different numbers of k clusters ("wss" method, 'fviz_nbclust' function). The clustering of WWTP compounds was based on the between-week (BV) and within-week (WV) variation of a compound's load calculated according to equation (1) and (2). From the WV values of each week, an average WV was calculated for each compound. The R package FactoMineR was used for analysis of mixed data (FAMD) (Le et al., 2008).

Eq. (1) Calculation of between-week variation (BV):

$$BV = \frac{SD(Means\ per\ week\ )}{X(Means\ per\ week\ )} * 100\%$$

Eq. (2) Calculation of within-week variation (WV):

SD = standard deviation

$$WV = \frac{(\sum_{4\ weeks} \frac{SD\ (Loads\ all\ samples\ per\ week)}{X(Loads\ all\ samples\ per\ week)})}{4} * 100\%$$

X = mean value

2.5 Calculation of toxic units

Toxic units were calculated for acute toxicity (TU_{acute}) and sublethal risk (TU_{sub}) such as effects on growth, reproduction and behavior. The 95th percentile of the measured environmental concentration (MEC_{95}) [mg L^{-1}] of each compound was calculated for a) each sampling week of the WWTP effluent, b) for each emission group of the WWTP effluent and the rain sewer effluent and c) for each weather condition in the rain sewer samples. The MEC_{95} was divided by acute effect concentrations (ECs) and lowest-observable-effect-concentrations (LOEC), respectively, for fish, green algae and daphnia as a representative for crustaceans. The ECs were either based on the 5th percentile of the measured acute ECs retrieved from the US Environmental Protection Agency's ECOTOX database (according to Busch et al. (2016)) or on predicted ECs (read-across or ECOSAR) in case of missing effect data (Table S8). Details on selected LOECs retrieved from the ECOTOX database are provided in Table S9. Risk driving compounds were determined by calculating the contribution of the individual chemical's toxicity (TU_i) to the sum of all individual toxicity values of a sampling week (TU_w) or an emission group (TU_e) (Eq.3).

Furthermore, the contribution of an emission group to sum toxicity of all emission groups (Eq.4) was assessed.

Eq. 3: Contribution of individual compound (i)

$$\%\mathrm{i} = \frac{TUi}{TUw/e} * 100\%$$

Eq. 4: Contribution of emission group (e)

$$\%\mathrm{e} = \frac{TUe}{\sum TUe} * 100\%$$

3. Results and Discussion

3.1 Target compounds emitted from WWTP and rain sewer

In total, 89 out of 149 target compounds were detected in the WWTP effluent (Table S10). In general, concentrations of common target compounds (e.g. carbamazepine, tramadol, benzotriazole, terbuthylazine) were in the same range as concentrations detected in Europe-wide surveys of WWTP effluents (Loos et al., 2013, Munz et al., 2017). Since the WWTP is part of a separate sewer system, we observed slightly lower concentrations for insecticides that are used as outdoor biocides and for the turf herbicide mecoprop than Munz et al. (2017). Those compounds were likely emitted via rain sewers during runoff and did not pass the WWTP. On the other hand, high concentrations of agricultural pesticides in May, for example boscalid and MCPA (max = 962 ng L^{-1} and 17,836 ng L^{-1}, respectively) were in contrast to previous studies and suggested a strong influence of the surrounding agricultural activities on the WWTP emissions. Here, stormwater runoff could not explain this input but rather incorrect disposal and cleaning practices. Even though seasonal pesticide peaks have been reported before in WWTP effluent (Munz et al., 2017, Neumann et al., 2002, Wittmer et al., 2010), it was not expected that this WWTP contributed directly and to a great extent to the pesticide emissions. In our case, the WWTP was also an important entry route for fungicides such as propiconazole and epoxiconazole. Pharmaceutical industry in the investigated catchment might have been the source for high concentrations of amitriyptyline and pipamperone. The concentrations of pipamperone were similar to those in a WWTP investigated by Van De Steene et al. (2010), which received wastewater from a chemo pharmaceutical plant. In other WWTPs not connected to pharmaceutical industry, the concentrations of pipamperone were at least two magnitudes lower (Van De Steene et al., 2010).

In the rain sewer samples, 67 of 149 target compounds were detected (Table S11). The samples shared 55 compounds with the WWTP effluent, but at lower concentrations. The presence of pharmaceuticals and - most importantly - the artificial sweetener cyclamate indicated the discharge of untreated wastewater from the rain sewer. Cyclamate is almost completely removed in WWTPs, thus it is a suitable tracer for raw wastewater (Buerge et al., 2009). According to the operators of the local wastewater system, the connection rate was about 98%, but some households were still erroneously connected to the rain sewer system. The rain sewer was also characterized by the emission of biocides and urban pesticides as described by Wittmer et al. (2010).

By including both point sources in the study, we were able to identify use and emission patterns of common wastewater pollutants from households as well as source-specific pollutants from agriculture and private and urban pest control.

3.2 Temporal patterns in pollutant mixtures

In the following, temporal patterns in pollutant mixtures are discussed. Detailed concentration data for each compound and sample can be found in Tables S10 and S11.

3.2.1 WWTP effluent

Temporal emission patterns

The discharge volume of the WWTP fluctuated only by 14% over the whole sampling period. The temperature in the biological treatment compartment (i.e. aeration tank) showed a minimum temperature of 11.1℃ in February and a maximum temperature of 19.4℃ in July/August, with mean temperatures of 15.9℃ in May and of 17.7℃ in October (Table S1). However, we detected clear seasonal differences in the pollution load and composition (Fig. 1). In general, the lowest loads were emitted in July. Since we did not analyze the respective influent samples, we cannot conclude on the removal efficiency of the WWTP. Rather we determined the overall patterns resulting from variations in treatment efficiencies and input. However, low pollutant loads in summer months were also detected in previous studies and were explained by increased biodegradation in the WWTP (Castiglioni et al., 2006, Munz et al., 2017, Vieno et al., 2005). In contrast to other studies, we detected highest loads in May, especially for pharmaceuticals. Usually higher pharmaceutical concentrations and loads were observed in winter due to decreased biodegradation and increased consumption (Castiglioni et al., 2006, Golovko et al., 2014, Vieno et al., 2005). As we also detected the most diverse pollutant mixture in May, the higher loads might have resulted from a higher burden on the microbial community thus reducing the efficiency of biodegradation in the system (Onesios et al., 2009).

Seasonal differences were driven by month-specific clusters of chemicals (Fig. 1). For example, high loads of herbicides were detected in May, fungicide loads increased in October and February showed a specific emission of a few pharmaceuticals.

Emission groups in the pollutant mixture

The target chemicals were separated into four emission groups (Fig. 2) based on the variation of each compound load within a week (Eq. 2) and among the weeks (Eq. 1). In general, the four groups were distinguished into two "constant" (i.e., group 2 and group 4), and two "seasonal" emission groups (i.e., group 1 and 3).

Compounds observed at constant levels were assigned to group 2. The lowest WV and BV was 17% and 14%, respectively, for lidocaine. Considering these minimum variations, this group was neither affected by seasonal nor by great weekly fluctuations and thus reflected baseline emission. Most members of this group were pharmaceuticals for long-term treatments (e.g. carbamazepine, beta-blockers) or daily used drugs (e.g. diclofenac, lidocaine). For these substances, our findings were in agreement with previous studies (Castiglioni et al., 2006, Golovko et al., 2014, Munz et al., 2017, Vieno et al., 2005). For antibiotics like sulfamethoxazole and trimethoprim, we also observed a rather constant emission in agreement with a study by Marx et al. (2015), indicating a joint prescription of these antibiotics in Germany throughout the year. In contrast, seasonal peaks for sulfamethoxazole have been detected by Castiglioni et al. (2006) and Golovko et al. (2014). Finally, steady emission was observed for industrial compounds with wide and constant areas of application, e.g. benzotriazole, and for the legacy herbicide fenuron and the biocides carbendazim and fipronil. Those biocides are applied as a fungicide in outdoor-paints and as an insecticide in fly traps, respectively (BAuA, 2017).

In contrast to group 2, group 4 compounds were characterized by a higher WV. This variation was observed for high-consumption compounds (e.g propiconazole and saccharin) or might have resulted from variations in the production process, for example, of the pharmaceuticals pipamerone and melperone, from which medicines are formulated batch-like in a local plant. Furthermore, low concentrations around the MDL led to higher variation for the herbicide metabolites and low-use fungicides.

Seasonal compounds were characterized by a high BV and were covered in groups 1 and 3. Group 3 included chemicals, which were i) constantly emitted within each sampling week, however at significantly different loads among the weeks or ii) were only found in one of the weeks. Most agricultural pesticides were among the seasonal compounds with peak application in May. Thus, group 3 compounds were considered as “season-specific”. Five pharmaceuticals were assigned to this group. They had seasonal peaks in February (i.e. sertraline and nitrendipin) and in October (i.e. loperamide), respectively. The occurrence of antihistamine cetirizine (peak in May) and the anti-coughing agent ambroxol (peak in February) indicated season-dependent consumption. Interestingly, the artificial sweetener acesulfame was assigned to this group with a clear peak in the May week. Since acesulfame is rather persistent during wastewater treatment processes (Buerge et al., 2009), an increased influent load was the only explanation. However, to our knowledge season-dependent removal efficiencies of acesulfame and other sweeteners have not been studied yet.

Group 1 contained seasonal compounds with high WV resulting from four phenomena. 1) In case of the agricultural pesticide pethoxamide, this was due to a short application peak in May indicating that the effluent directly reflected the agricultural activities of the surrounding area. 2) For other compounds, apparent seasonal emission resulted from non-systematic consumption of privately used chemicals or accidental spills (e.g. dimethoate and diazinon) or 3) from a few individual data points around the MDL (e.g., the legacy pesticide TP deisopropylatrazine). 4) Additionally, compounds such as 2-napthtalenesulfonic acid showed a large general variation most likely because of its wide area of application. Due to these diverse reasons for the higher WV, this group was called “seasonal-random”.

Overlaps of the clusters’ confidence intervals may be reduced by repeated and extended sampling to clarify group membership. Furthermore, the intervals indicated outliers, e.g. *p*-toluenesulfonamide (TSA) and mecoprop (MCP), which did not fit to any cluster.

3.2.2 Weather-related emission patterns and emission groups in rain sewer effluent

In the rain sewer samples, the most prominent pattern was driven by rain- and dry-weather conditions (Fig. 3). Based on the chemical load profile, dry weather samples were clearly separated from rain weather samples. These dynamics were in agreement with Wittmer et al. (2010).

The chemicals were assigned to two emission groups representing chemicals either related to wastewater (i.e. illicit connections) or surface runoff (Fig. 4). The validity of cluster outcome was confirmed by FAMD and k-means clustering with a reduced dataset (Fig. S1, S2).

In general, pharmaceuticals and legacy pesticides dominated the chemical profile under dry weather conditions indicating wastewater as a source (group 1). Pesticides and biocides correlated with wet weather conditions (group 2). The legacy pesticide atrazine (banned since 1991) and pesticide metabolites were detected only in dry-weather samples due to their continuous low concentrations, which were diluted below the MDL during rain events. The input of these compounds occurs likely via infiltrating groundwater as atrazine is still often present after these years in aquifers and the vadose zone in Germany and released in low concentrations into surface waters (Vonberg et al., 2014).

Surface runoff (group 2) was characterized by biocides, fungicides, insecticides and urban pesticides. Pollution with these compounds from urban areas is considerable due to comparably larger amounts of herbicides applied by urban users than by farmers (Blanchoud et al., 2004). Furthermore, larger fractions of precipitation enter receiving waters as runoff from paved areas with impermeable materials than from areas with permeable soil. Biocides and fungicides are often applied on facades and other outdoor surfaces. Herbicides like MCPA and mecoprop are commonly applied on turf for weed control (Phillips and Bode, 2004, Wittmer et al., 2010). The runoff of terbuthylazine was unexpected as there is no agricultural area connected to the rain sewer.

Outliers of both groups showed a random and precipitation-independent emission behavior (e.g. propiconazole) or had concentrations close or below the MDL (e.g. 2-octyl-4-isothiazolin). Deisopropylatrazine and N-acetyl-4-aminoantipyrine showed correlation with dry-weather discharge but were outliers due to many non-detects.

In contrast to patterns in the WWTP effluent, clear seasonal dynamics could not be confirmed in rain sewer discharge. In general, the pharmaceuticals found were associated with long-term treatment and were therefore constantly emitted. Distinct high loads were only observed in October (Fig. 3), which may have resulted from maintenance works in the sewer system. For rain-related emissions, clear seasonal patterns were missing due to the lack of strong rain events (discharge > 30 L s^{-1}) in most fall and winter months. Still, privately used pesticides are not as systematically and efficiently applied as agricultural pesticides (Templeton et al., 1998). Inter-seasonal presence of these compounds was observed in streams due to an extended application period and continuous runoff from urban areas (Phillips and Bode, 2004). Moreover, some non-agricultural pesticides have a broad field of application as biocides e.g. in facade protection. Single peaks might be further due to spills and incorrect disposal (Phillips and Bode, 2004, Wittmer et al., 2010). This probably holds true for the high peak of dimethoate in the November dry-weather sample. The rain sewer system is quite sensitive to discharge variations and immediately reflects the activities in its catchment.

3.3 Risk estimation for temporal emissions and emission groups

In order to understand temporal dynamics of risks for fish, crustaceans and algae, acute (TU_{acute}) and sublethal risks (TU_{sub}) were associated with emission groups of chemicals as identified in chapter 3.2. The pie charts describe the contribution of each emission group (Eq. 4) and the main drivers of each group (Eq. 3) for each biological quality element (BQE) (Fig. 5, Fig. 6).

3.3.1 Risk patterns and risk driving compounds in WWTP effluent

Acute and sublethal risk from wastewater discharge of the WWTP was assessed per season and emission group. Concerning the sum toxicity of each sampling week, similar seasonal patterns for acute and for sublethal risk were observed for all BQE (Fig. 5a). Only sublethal risk for fish did not show any strong temporal dependency. In general, acute toxic risk for fish was lower compared to risks for other BQE and was driven by the seasonally emitted chemicals; metolachlor and MCPA (Fig. 5b). The potential toxic effect of these two herbicides was mainly explained by their high maximum effluent concentrations (i.e. 849 ng L^{-1} and 17,836 ng L^{-1}, respectively). The exceedence of TU_{acute} by TU_{sub} by up to three orders of magnitude indicated the underestimation of risk for fish if only acute toxicity is considered (Fig. 5a). Due to physiological similarities to mammals, fish are potentially more susceptible to pharmaceuticals, which are not designed to exhibit acute toxicity but to be biologically active substances (Corcoran et al., 2010). In fact, constantly emitted pharmaceuticals explained 90% of the TU_{sub} for fish (Fig. 5e). The main driver was citalopram (65%), which was demonstrated to alter the swimming behavior of fish (Olsén et al., 2014). This might be important for predator-prey relations. Antidepressants have been reported to affect a number of physiological functions (Corcoran et al., 2010). For example, the second driver, amitriptyline (19%), decreased the body length of fish embryos (Yang et al., 2014). Yet, long-term consequences of exposure to these substances in wildlife and on population level are largely unknown.

For daphnia, TU_{acute} and TU_{sub} were driven by a diazinon peak in October (Fig. 5c,f). However, in agreement with Munz et al. (2017), diazinon also dominated the risk pattern in the absence of peak concentrations (Fig. S3, S4). Diazinon is no longer approved for plant protection, but is still registered as an insecticide in pet collars against fleas. The application in private households might explain the rather random emissions (Wittmer et al., 2010). Due to the long hydraulic retention time of 72 hours, the diazinon concentrations detected during a few days in October likely resulted from a single spill. The second driver for TU_{acute} was fipronil (10%), which was constantly emitted at low concentrations (Fig. 5c). Like diazinon, fipronil is registered for veterinary and indoor pest control. Both are neuroactive compounds (Busch et al., 2016). Due to their high acute toxicity to non-target insects, they have been banned as plant protection products. However, their continuous application in private households still poses an issue for aquatic ecosystems.

The temporal risk for algae was explained by the seasonal emission of agricultural pesticides (Fig. 5d,g). The herbicide terbuthylazine (51%) and the fungicide spiroxamine (21%) explained most of the TU_{acute} (Fig. 5d). Both drivers were season-specific for May, while spiroxamine showed a short peak application in the May week and was thus assigned to group 1. A similar emission pattern was observed for MCPA, which was the main driver for TU_{sub} (Fig. 5g).

In addition to seasonal sublethal risk drivers, daphnia and algae were constantly exposed to sublethal concentrations of diclofenac (Fig. 5f,g). Diclofenac may affect growth in daphnia and cell multiplication in algae (Dietrich et al., 2010, Lawrence et al., 2012). The anti-inflammatory agent was previously identified as a main risk driver in environmental mixtures (Busch et al., 2016, Munz et al., 2017) and has been associated with risk to fish and mammals (Corcoran et al., 2010).

3.3.2 Risk patterns and risk driving compounds in rain sewer effluent

The chemical profiles in the rain sewer were mainly driven by the weather conditions but most compounds were present in both conditions. Therefore, TUs were calculated for dry and rain conditions based on the MEC_{95} of each detected compound. Likewise, the risk contribution of the two emission groups was evaluated for both weather conditions (Fig. 6). Higher TUs were observed for fish during dry weather. The TU_{acute} in the rain sewer was similar to TU_{acute} in May in the WWTP effluent. The TU_{acute} for algae was slightly higher during rain events. For daphnia, again an extraordinary peak was observed (TU_{acute} of 2.3). This peak was due to a high concentration (5161 ng L^{-1}) of the neuroactive insecticide dimethoate in the November sample.

Dimethoate is approved for private use as an insecticide in gardens. The extraordinary peak in the dry sample in November likely resulted from incorrect disposal after the application period leading to acute risk at the effluent. This peak strongly influenced the TU_{acute} patterns for fish and daphnia. Dimethoate always dominated the acute toxicity for daphnia (Fig. 6c), while fipronil strongly contributed to TU_{acute} in rain events and in the absence of the spill event (Fig. S5c). For fish, dimethoate explained 59% of the TU_{acute} in dry weather. In the absence of this spill, the risk pattern became more complex with carbendazim as the main driver also under dry weather conditions (Fig. S5b). Carbendazim is classified as hazardous for human and environmental health (ECHA, 2017).

For algae, the herbicides diuron and terbuthlyazine were important acute risk drivers under both weather conditions (Fig. 6d). Elevated concentrations of diuron and terbuthlyazine were observed during runoff events. However, they were still present in low concentrations during dry discharge (Fig. 3). Similarly to carbendazim, diuron is used in biocides in outdoor paints (BAuA, 2017). Often both compounds occur as constituents of the same product and are consequently washed off together.

Overall, TU_{sub} were lower than in WWTP effluent but were dominated by the same pharmaceuticals (Fig. 5 and 6). Additionally, TU_{sub} for daphnia was driven by an exceptionally high metoprolol concentration in the September rain sample (3837 ng L^{-1}). For algae, TU_{sub} was similar in rain and dry conditions with diuron as main driver (Fig. 6g).

Four out of seven risk drivers in the rain sewer effluent were biocides. Similar to the patterns in the WWTP effluent, randomly used and discarded insecticides posed high risk to aquatic organisms. The assessment of both point sources implied that acute and sublethal risk was driven by mainly one emission group. In all cases, one or two dominating risk driving compounds could be identified. Lower total risk was often accompanied by a decreasing contribution of the main risk drivers and increasing importance of other compounds leading to a more complex risk pattern and highlighting the need for further investigations of mixture effects (Fig. S3, S4).

3.4 Exposure in the receiving river

In order to assess the contribution of the studied WWTP and rain sewer to the risk in the receiving river, we analyzed monthly composite (LVSPE) samples from the river downstream of these two point sources. Here, we focused on the main risk driving compounds identified in chapter 3.3 (Table S 12). In general, the TUs increased from May with a peak in July and August (Fig. 7). The discrepancy between the expected risk pattern based on the identified emission groups (Chapter 3.2.1) and the high summer risk can be explained by the low discharge volume in the river and thus lower dilution from June onwards (Fig. 7) (Ankley et al., 2007). This effect can be clearly seen for TU_{sub} for fish, which was mostly driven by constantly emitted pharmaceuticals, i.e. similar river discharge resulted in similar TU_{sub} (Fig. 7b). A season-specific risk pattern due to seasonal application could still be deduced for algae. Higher dilution in spring and input from diffuse sources during summer rains contributed to elevated risk even after the main application period (Neumann et al., 2002). Thus, these seasonally emitted pollutants require monitoring in higher temporal resolution during the respective peak times and rain events in the following months. Generally, rain discharge calls for event-based monitoring. Short peak emissions via the WWTP effluent and the rain sewer were averaged out in monthly composite samples and the rain sewer effluent was strongly diluted in the river. For crustaceans, average risk in the river was dominated by constantly emitted fipronil. Only, the dimethoate spill in November was intense enough to be observed in the monthly composite river samples and contributed to the monthly average TU_{acute}. Concerning the contribution of point sources to the discharge of the river, the rain sewer played a minor role (0.01 - 2.3%). Still, adverse acute and sublethal effects to organisms might be observed at the vicinity of the effluent. Moreover, similar patterns and contributions may be assumed for other rain sewers discharging into the river and contributing to the pollution and risk patterns upstream of the WWTP. The studied WWTP contributed up to 40% to the river discharge under low flow conditions and was the main contributor to the pollution with organic micropollutants in the river. The final pollution and risk pattern in the river was mainly driven by the total river discharge (Ankley et al., 2007).

4. Conclusions

In this study,

- Clustering WWTP effluents according to temporal contamination patterns revealed a clear seasonality in the emission reflected by compounds such as agricultural pesticides in spring or fungicides in fall. The concentrations of other compounds such as many pharmaceuticals were quite constant. In the rain sewer, the discrimination between rain and dry discharge dominated contamination patterns, which were driven by surface runoff and illicit connections. In addition to constantly and seasonally emitted pollutants, there were randomly emitted non-agricultural pesticide and biocide peaks in both WWTP effluent and rain sewer calling for management. These patterns may be used for hypothesis testing in other catchments with similar and different wastewater systems (e.g. concerning CSO structures, land use and hydraulic retention time characteristics).
- Chemical patterns could be directly translated to BQE-specific risk patterns. While fish were potentially most affected by sublethal effects of constantly emitted chemicals (i.e. baseline emission), algae were exposed to seasonal risks. Random emission of insecticides and biocides from private and urban sources were a strong potential threat for crustaceans.
- High risks were typically related to one or few risk drivers, while there may be more contributing chemicals at lower risks. This particularly applied for algae and crustaceans, which were under high risk during pesticide applications and spills. Variability of acute and sublethal risks in the river was mainly driven by water discharge of the river rather than by season or peak events. Due to the lack of sublethal effect data for several compounds and endpoints, the assessment provided only a rough estimate on the potential risks to aquatic organisms. However, this estimate already highlighted the importance of sublethal effects on growth, behavior and reproduction in risk assessments and especially underlined the major role of constantly emitted pharmaceuticals in these

effects for all BQE. Furthermore, identified risk drivers and risk patterns should be tested in bioassays to confirm their effects and investigate mixture effects on the BQE.

- Despite the occurrence of occasionally high loads of insecticides and biocides in the rain sewer, the WWTP was still the more important pollution source with a considerable emission of agricultural pesticides and compounds used and produced by local industries. The risk posed by non-agricultural pesticides and biocides may require rethinking of approval and stormwater management, while input of agricultural pesticides may be reduced by awareness-rising of professional users.
- By considering pollution dynamics due to temporal and weather influences, water authorities may optimize monitoring strategies suggesting high frequency monitoring during main emission times of pesticides and focusing on low water discharge seasons. Furthermore, event-based monitoring will support the identification of peak contamination triggered by rain events but will fail to capture random spills. Event sampling might be especially important for catchments with WWTPs strongly affected by rainfall, e.g. combined sewer systems.

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Figures

Fig. 1: Temporal patterns in WWTP effluent based on compound loads [mg d^{-1}] in relation to the sampling days (data log-transformed, scaled and centered

Fig. 2: Emission groups of chemicals in WWTP effluent based on within-week variation and between-week variation. Ellipses represent 95% confidence interval. Full compound names and details are given in Table S10.

Fig. 3: Temporal and weather patterns in rain sewer samples (D = dry; R = rain). Chemical loads were log-transformed, scaled and centered prior to clustering.

Fig. 4: Emission groups of chemicals in rain sewer effluent based on loads [mg/d] detected in each sample. Data was standardized prior to PCA and clustering; zeros treated by glog transformation. Ellipses represent 95% confidence interval. Full compound names and details are given in Table S11.

Fig. 5: a) Sum TU_{acute} and TU_{sub} for seasonal emission from WWTP effluent (based on weekly MEC_{95} of each compound). Contributions of WWTP emission groups and individual risk drivers to sum TU_{acute} and TU_{sub} are shown for a)/e) fish, c)/f) daphnia and d)/g) algae (based on MEC_{95} of each compound in respective emission group). (NFA= N-Formyl-4-aminoantipyrine; FIP = Fipronil; DFC = Diclofenac)

Fig. 6: a) Sum TU_{acute} and TU_{sub} for dry and rain emission from rain sewer effluent (based on MEC_{95} of each compound in each weather condition). Contributions of weather conditions and individual risk drivers to sum TU_{acute} and TU_{sub} are shown for b)/e) fish, c)/f) daphnia and d)/g) algae (based on MEC_{95} of each compound in each weather condition). (ATP = Amitriptyline; CBZ = Carbamazepine; CP = Citalopram; DCF = Diclofenac; DIU = Diuron; DMT= Dimethoate; DNP = 2,4-Dinitrophenol; FIP = Fipronil; FPP= Fenpropimorph; TBA= Terbuthylazine, TBY= Terbutryn)

Fig. 7: TUs based on concentrations of main risk driving compounds in river samples in relation discharge volume in the river [m^3]

Tables

Table 1: Overview LC-MS/MS methods used for target screening

Method	LC mobile phase	Flow rate [µL min^{-1}]	Ionization mode	Number of compounds
1	A: Water + 0.1% formic acid B: MeOH + 0.1% formic acid	0.40	ESI positive	52
2	A: Water + 2 mM NH_4 formate B: MeOH + 2 mM NH4 formate	0.40	ESI positive	62
3	A: Water + 1 mM NH_4F + 1 mM NH_4 formate B: MeOH	0.35	ESI negative	35

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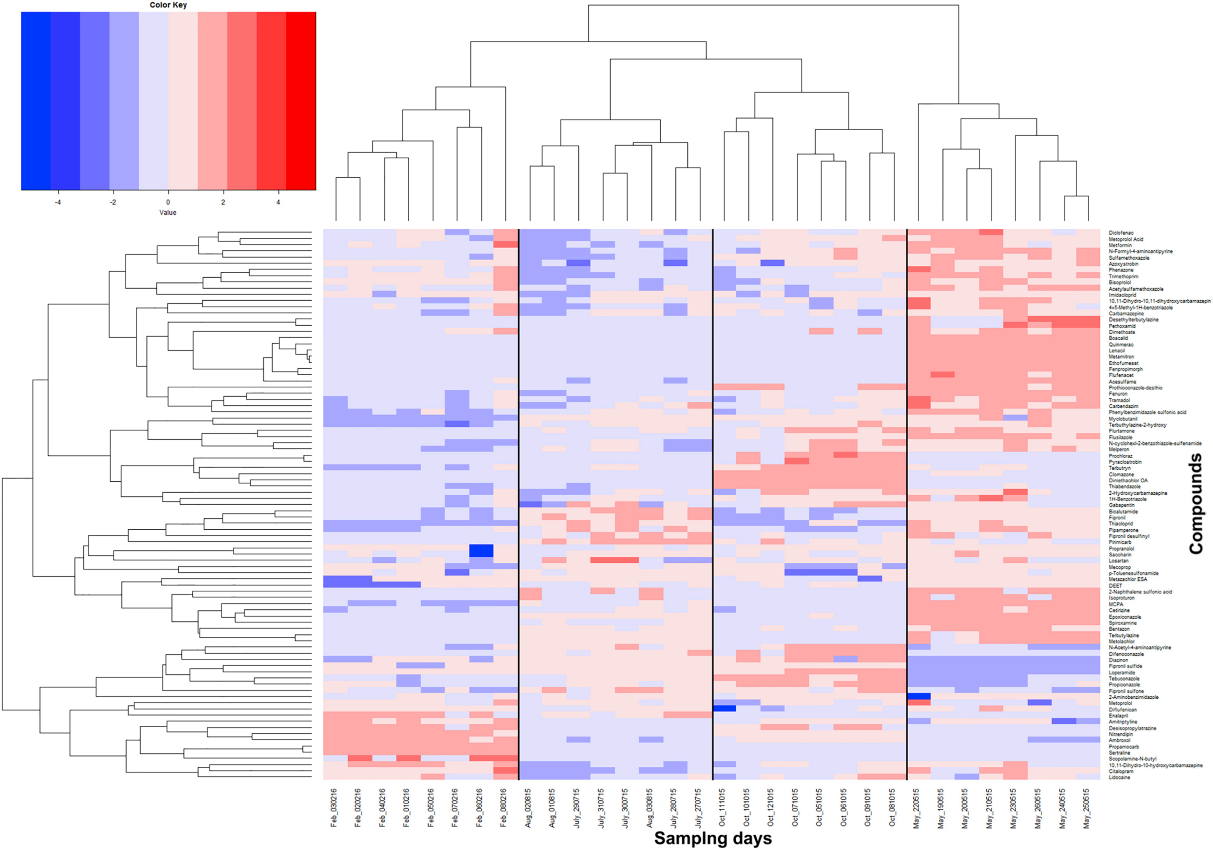
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Clomazone
Dimethachlor OA
Thiabendazole
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1H-Benzotriazole
Gabapentin
Bicalutamide
Fipronil
Thiacloprid
Pipamperone
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Pirimicarb
Propranolol
Saccharin
Losartan
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DEET
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MCPA
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Bentazon
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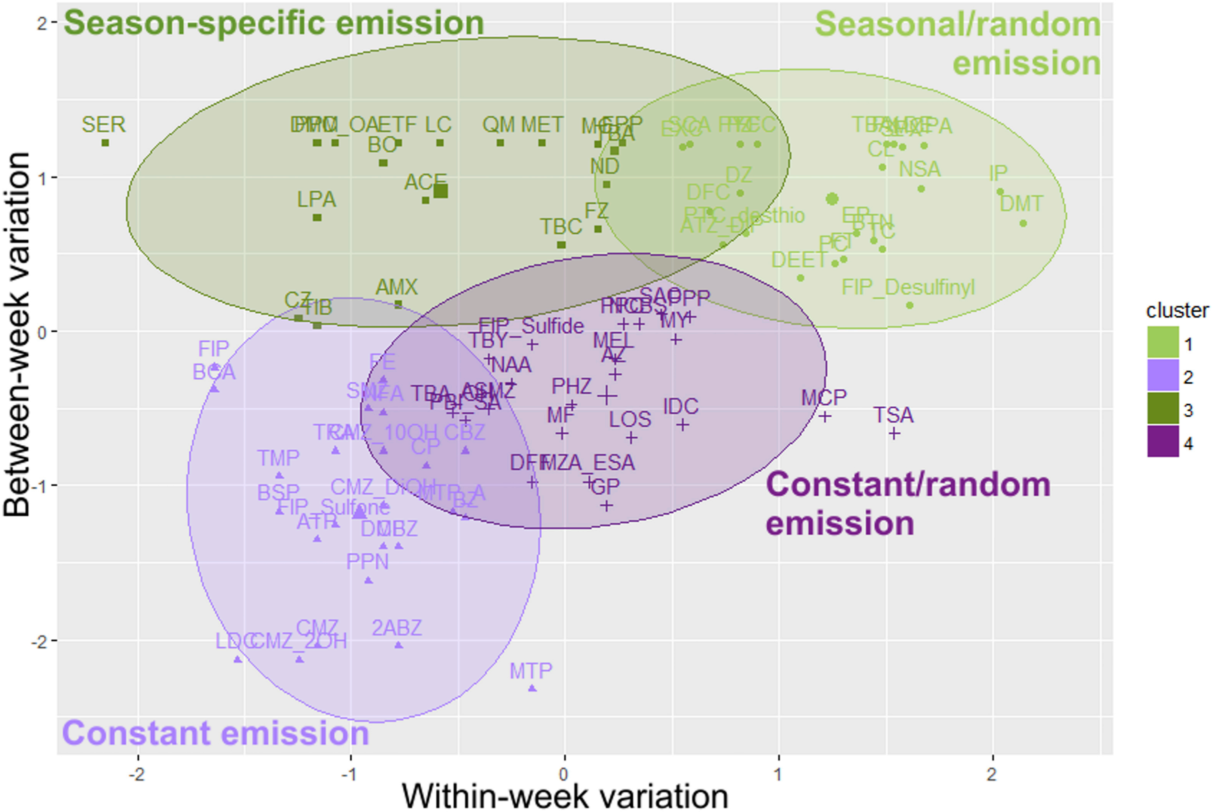

Season-specific emission
Seasonal/random emission
Constant/random emission
Constant emission
Between-week variation
Within-week variation
cluster
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4
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LC
QM
MET
BO
ACE
LPA
ND
FZ
TBC
AMX
CZ
TIB
DZ
DFC
DEET
NSA
IP
DMT
CL
FIP_Desulfinyl
FIP_Sulfide
TBY
NAA
MEL
AZ
PHZ
MF
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IDC
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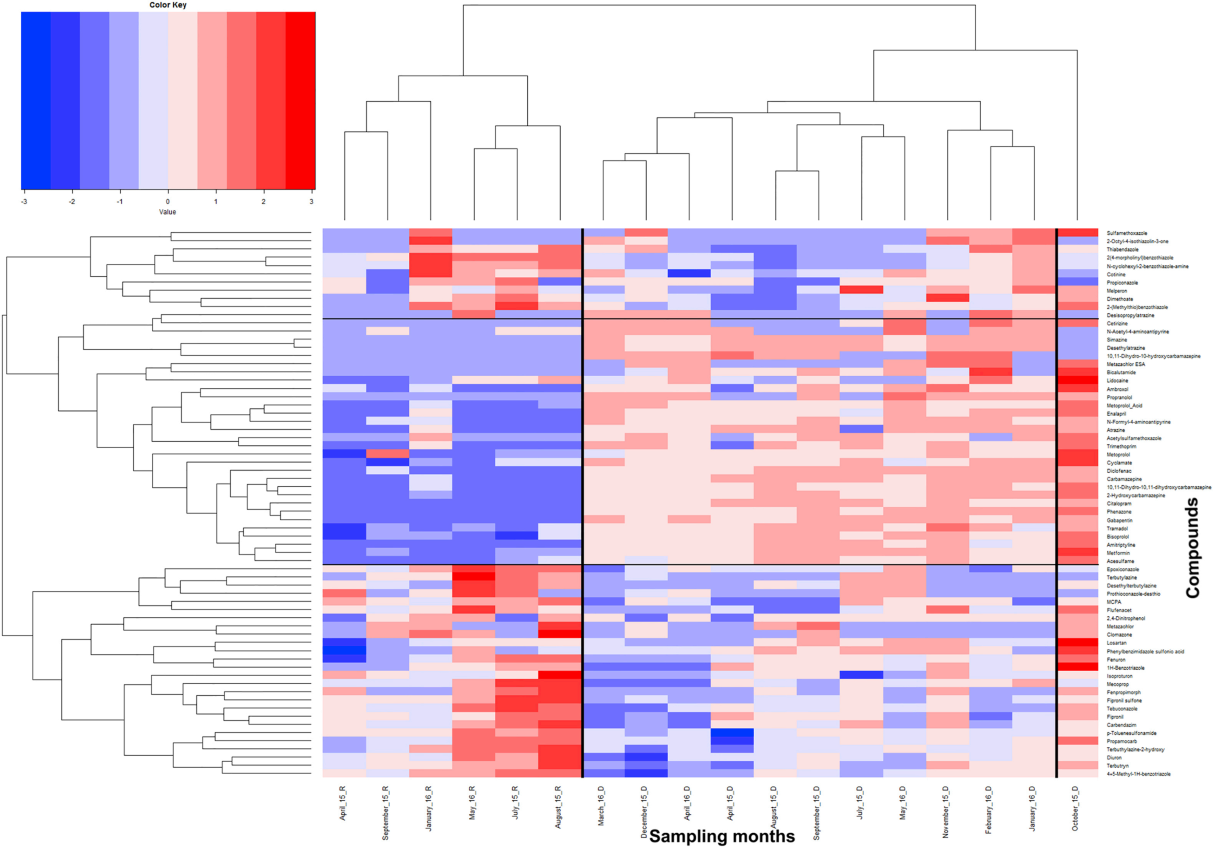

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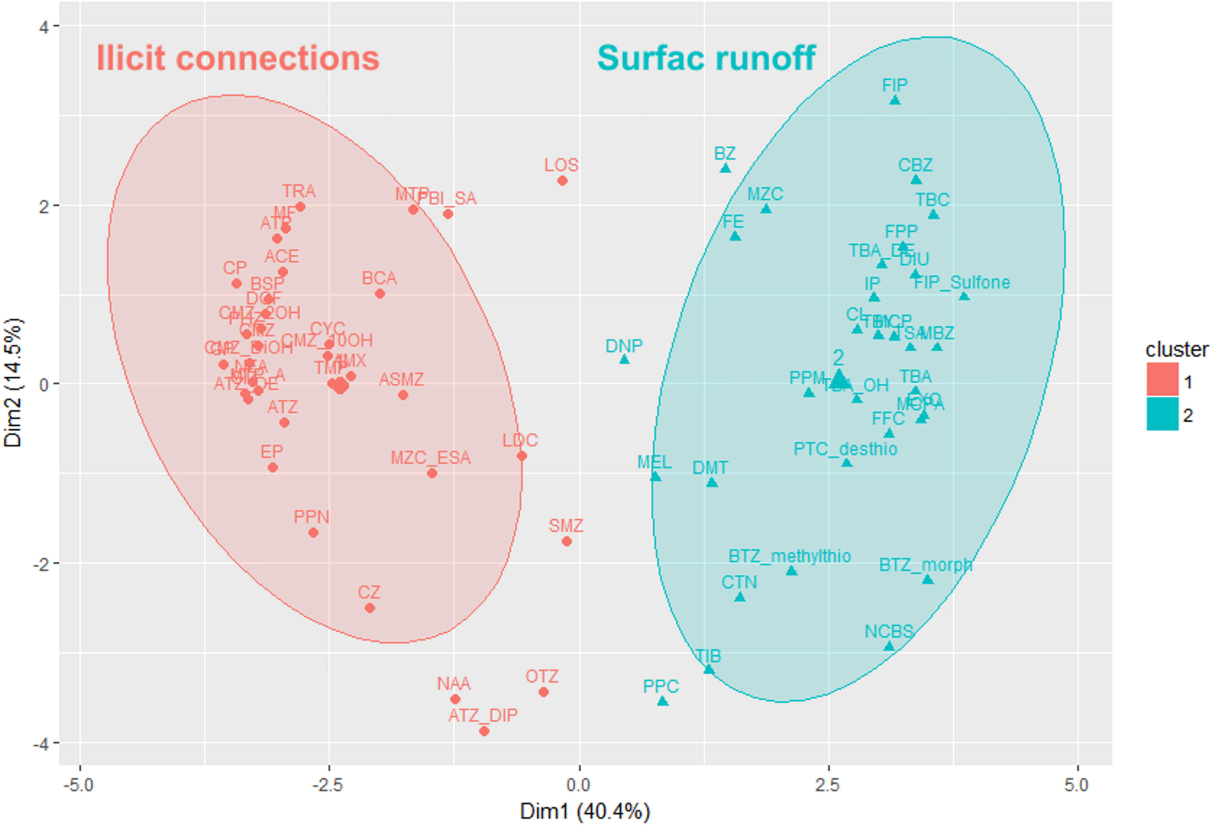
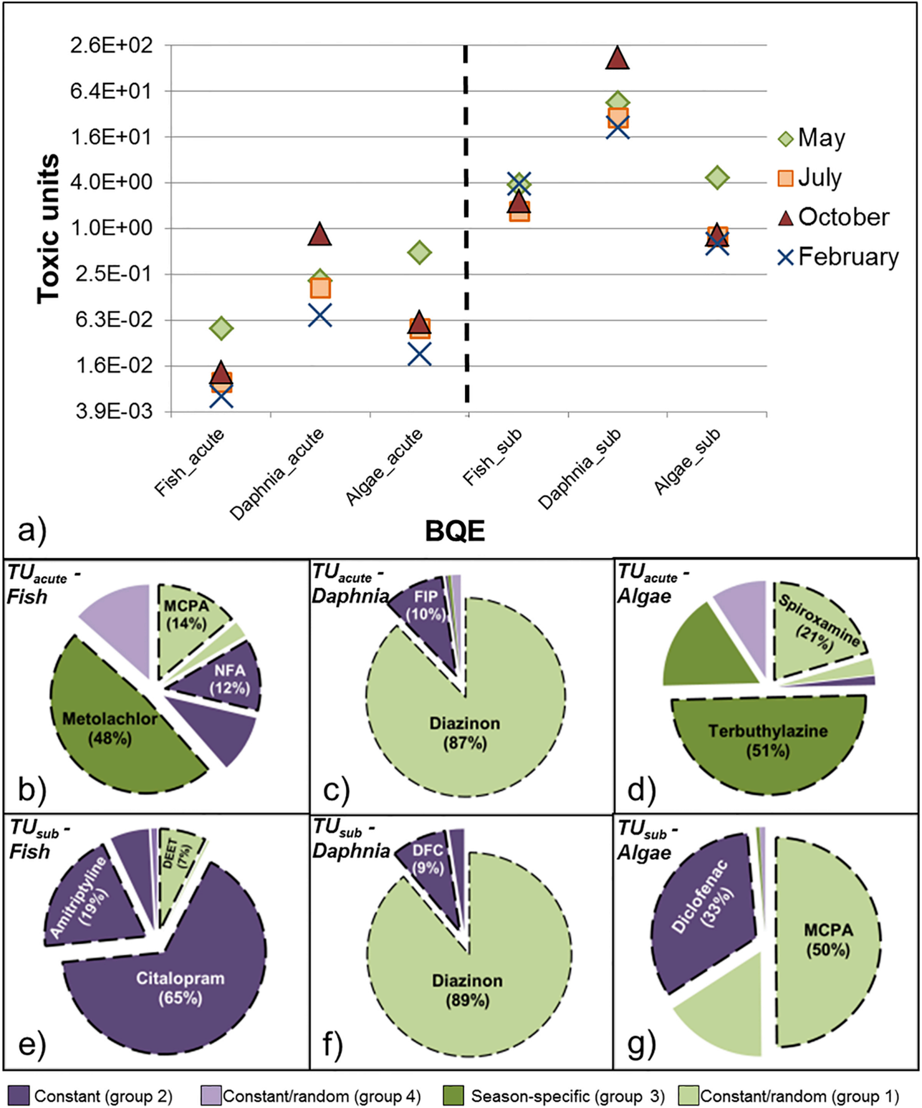
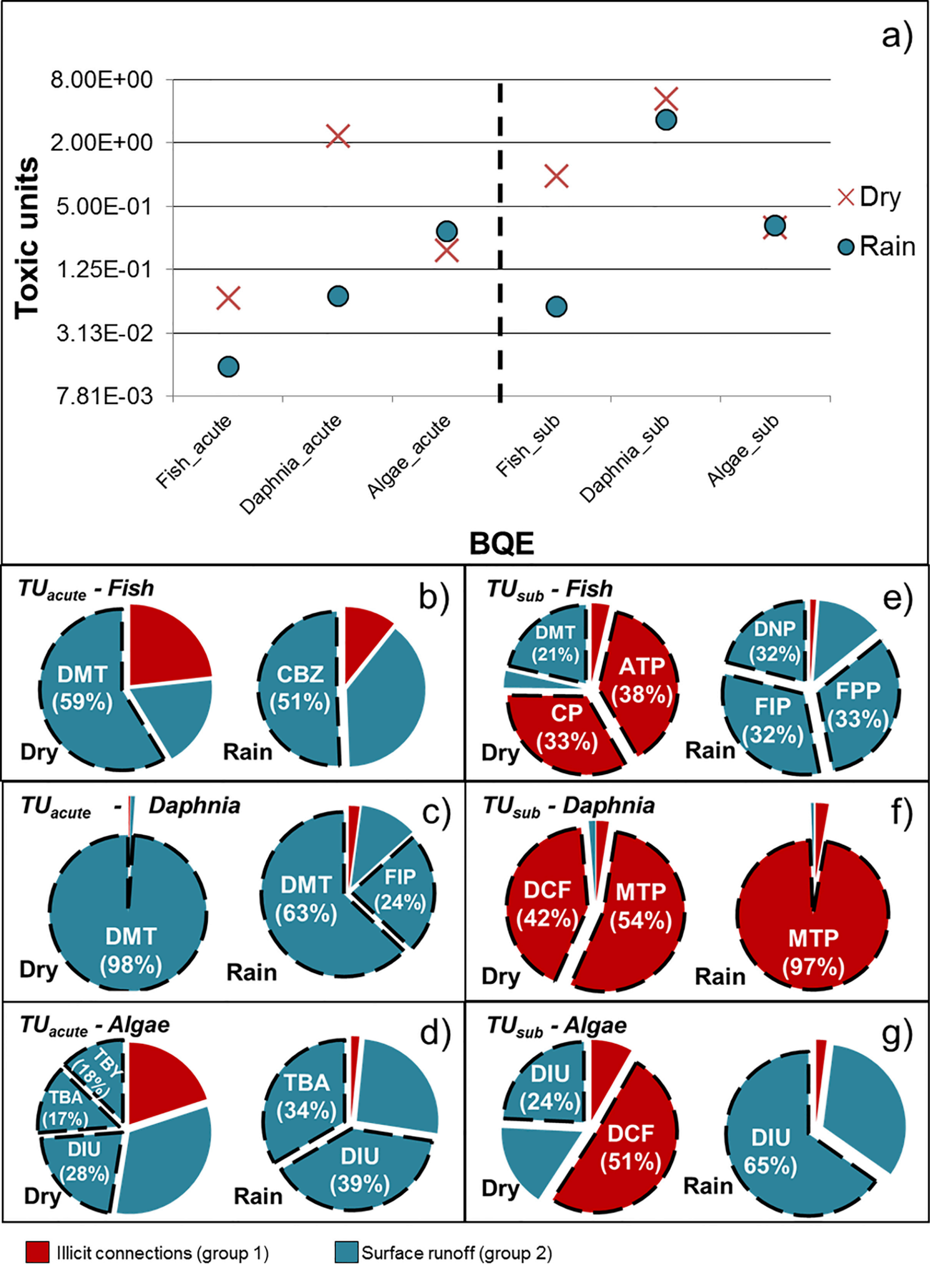
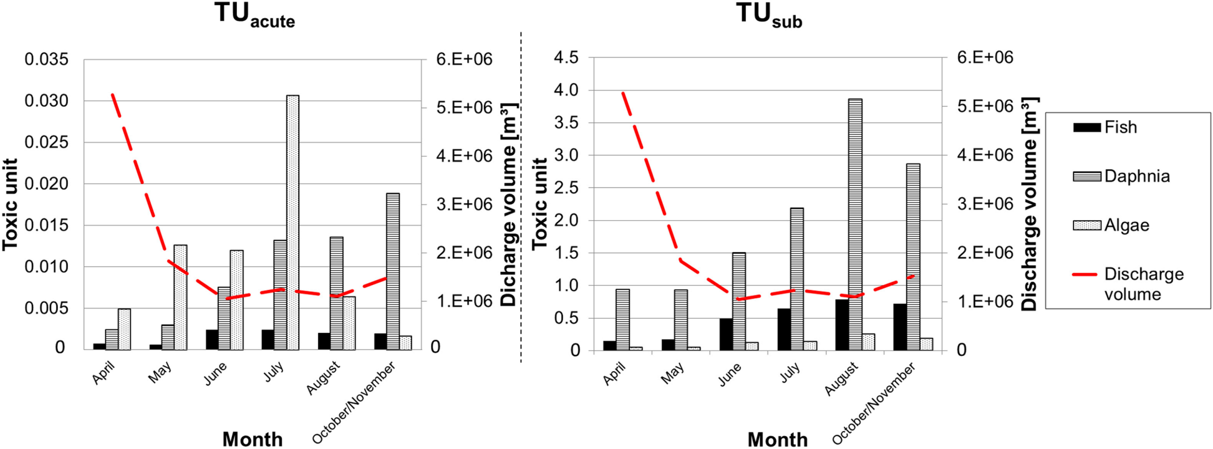
Cotinine
Propiconazole
Melperon
Dimethoate
2-(Methylthio)benzothiazole
Desisopropylatrazine
Cetirizine
N-Acetyl-4-aminoantipyrine
Simazine
Desethylatrazine
10,11-Dihydro-10-hydroxycarbamazepine
Metazachlor ESA
Bicalutamide
Lidocaine
Ambroxol
Propranolol
Metoprolol_Acid
Enalapril
N-Formyl-4-aminoantipyrine
Atrazine
Acetylsulfamethoxazole
Trimethoprim
Metoprolol
Cyclamate
Diclofenac
Carbamazepine
10,11-Dihydro-10,11-dihydroxycarbamazepine
2-Hydroxycarbamazepine
Citalopram
Phenazone
Gabapentin
Tramadol
Bisoprolol
Amitriptyline
Metformin
Acesulfame
Epoxiconazole
Terbutylazine
Desethylterbutylazine
Prothioconazole-desthio
MCPA
Flufenacet
2,4-Dinitrophenol
Metazachlor
Clomazone
Losartan
Phenylbenzimidazole sulfonic acid
Fenuron
1H-Benzotriazole
Isoproturon
Mecoprop
Fenpropimorph
Fipronil sulfone
Tebuconazole
Fipronil
Carbendazim
p-Toluenesulfonamide
Propamocarb
Terbuthylazine-2-hydroxy
Diuron
Terbutryn
4+5-Methyl-1H-benzotriazole
Compounds
April_15_R
September_15_R
January_16_R
May_16_R
July_15_R
August_15_R
March_16_D
December_15_D
April_16_D
April_15_D
August_15_D
September_15_D
July_15_D
May_16_D
November_15_D
February_16_D
January_16_D
October_15_D
Sampling months


Ilicit connections
Surfac runoff
cluster
1
2
Dim1 (40.4%)
Dim2 (14.5%)
-5.0
-2.5
0.0
2.5
5.0
4
2
0
-2
-4
LOS
TRA
MTP
PBI_SA
ACE
CP
BSP
BCA
CYC
CMZ_10OH
ASMZ
ATZ
EP
LDC
MZC_ESA
PPN
SMZ
CZ
NAA
OTZ
ATZ_DIP
FIP
BZ
CBZ
MZC
TBC
FE
FPP
DIU
IP
FIP_Sulfone
MBZ
DNP
TBA
PTC_desthio
MEL
DMT
BTZ_methylthio
BTZ_morph
CTN
NCBS
TIB
PPC



2.6E+02
6.4E+01
1.6E+01
4.0E+00
1.0E+00
2.5E-01
6.3E-02
1.6E-02
3.9E-03
Toxic units
May
July
October
February
Fish_acute
Daphnia_acute
Algae_acute
Fish_sub
Daphnia_sub
Algae_sub
a)
BQE
TUacute - Fish
MCPA (14%)
NFA (12%)
Metolachlor (48%)
b)
TUacute - Daphnia
FIP (10%)
Diazinon (87%)
c)
TUacute - Algae
Spiroxamine (21%)
Terbuthylazine (51%)
d)
TUsub - Fish
Amitriptyline (19%)
DEET (7%)
Citalopram (65%)
e)
TUsub - Daphnia
DFC (9%)
Diazinon (89%)
f)
TUsub - Algae
Diclofenac (33%)
MCPA (50%)
g)
Constant (group 2)
Constant/random (group 4)
Season-specific (group 3)
Constant/random (group 1)


a)
8.00E+00
2.00E+00
5.00E-01
1.25E-01
3.13E-02
7.81E-03
Toxic units
Dry
Rain
Fish_acute
Daphnia_acute
Algae_acute
Fish_sub
Daphnia_sub
Algae_sub
BQE
TUacute - Fish
b)
DMT (59%)
Dry
CBZ (51%)
Rain
TUsub - Fish
e)
DMT (21%)
ATP (38%)
CP (33%)
Dry
DNP (32%)
FIP (32%)
FPP (33%)
Rain
TUacute - Daphnia
c)
DMT (98%)
Dry
DMT (63%)
FIP (24%)
Rain
TUsub - Daphnia
f)
DCF (42%)
MTP (54%)
Dry
MTP (97%)
Rain
TUacute - Algae
d)
TBY (18%)
TBA (17%)
DIU (28%)
Dry
TBA (34%)
DIU (39%)
Rain
TUsub - Algae
g)
DIU (24%)
DCF (51%)
Dry
DIU 65%)
Rain
Illicit connections (group 1)
Surface runoff (group 2)



TU$_{acute}$
Toxic unit
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0
Dicharge volume [m³]
April
May
June
July
August
October/November
Month
TU$_{sub}$
Toxic unit
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0
Discharge volume [m³]
April
May
June
July
August
October/November
Month
Fish
Daphnia
Algae
Discharge volume

Highlights

- 149 compounds analyzed in wastewater discharge in different seasons and weather.
- Pollutant mixtures reduced to few emission groups by k-means clustering.
- Emission groups translate into organism-specific risk patterns.
- Sublethal risk driven by constantly emitted compounds (mainly pharmaceuticals).
- Risk in river depends on river discharge and WWTP emissions (main contributor).