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Identification of contaminants of emerging concern through temporal trend analysis of suspended particulate matter in the Rhine River catchments (2005–2022): A case study using LC-HRMS to support early-warning systems

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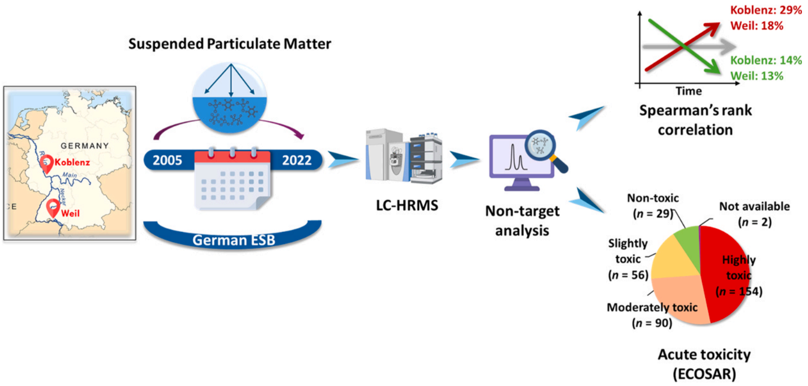
HIGHLIGHTS

- SS/NTS applied to archived SPM samples using LC-HRMS and computational tools
- SPM matrices from two Rhine sites (2005 and 2022) analyzed for temporal trends
- 332 compounds identified; 29 % showed increasing time trends at Koblenz site
- 47 % of compounds were highly acutely toxic using ECOSAR models
- SS/NTS of SPM archives supports early-warning-oriented risk management

GRAPHICAL ABSTRACT



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ABSTRACT

The continuous release of synthetic chemicals into aquatic systems underscores the need for long-term assessments of contaminants of emerging concern (CECs). Archived suspended particulate matter (SPM) samples from the German Environmental Specimen Bank (ESB) (2005–2022) at two Rhine sites (Weil am Rhein and Koblenz) were analyzed using liquid chromatography–high-resolution mass spectrometry (LC-HRMS) suspect/non-target screening (SS/NTS) to evaluate temporal trends. Using retention-time indices and orthogonal MS/MS evidence (mzCloud, FISh, CFM-ID), 332 compounds were identified at varying Schymanski confidence levels (2.1: 3.0 %; 2.2: 5.7 %; 3.1: 53 %; 3.2: 38 %). Temporal analysis of LC-HRMS peak areas revealed that 25 % of contaminants

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increased over time, with a higher proportion at Koblenz (29 %) than Weil (18 %). Conversely, several compounds exhibited statistically significant decreasing trends at both Koblenz (14 %) and Weil sites (13 %), consistent with regulatory measures, improved wastewater treatment, and shifts in industrial practices. Aquatic toxicity prediction (ECOSAR) indicated that 47 % (154 of 332) of annotated structures were highly acutely toxic ($LC_{50}/EC_{50} \leq 1$ mg/L) to at least one test group (fish, Daphnia, or green algae). This study provides the first 18-year, site-specific non-target time series from a national archive and integrating orthogonal identification with hazard prediction to support chemical prioritization. Archived SPM enables retrospective and comparable assessment of particle-associated contaminants, complementing dissolved-phase monitoring, and supporting the identification of unmonitored emerging CECs. Crucially, long-term NTS of these SPM samples provides screening-level early-warning signals and a watch list for targeted confirmation and risk management.

1. Introduction

Suspended particulate matter (SPM) comprises fine particles suspended in rivers, lakes, reservoirs, and coastal/seawater systems and plays a vital role in aquatic ecosystems. It influences biogeochemical cycles, water-sediment interactions, and facilitates the transport and transformation of trace organic micropollutants [1,2]. SPM originates from both natural and anthropogenic sources, including terrigenous and autochthonous particles as well as human-made materials [1]. The composition of SPM, both inorganic and organic, is strongly influenced by climatic conditions and hydrology, which in turn affect biogeochemical cycles [3]. In addition to its ecological effects, SPM significantly impacts water quality by transporting contaminants, both elemental and organic micropollutants, depending on its source and composition [4]. Contaminants with higher organic carbon normalized partition coefficients (K_{OC}) increasingly partition to SPM under typical suspended solids and organic carbon conditions in large rivers, whereas more polar compounds remain predominantly in the dissolved phase [5]. Consequently, SPM reflects the particle-bound fraction of the contaminant load and complements dissolved-phase monitoring rather than capturing the full chemical mixture on its own [5,6]. Recent research on the Yellow River showed that SPM plays a critical role in the distribution and transport of heavy metals, underscoring its function as a major pathway for contaminants in large river systems [7]. Similarly, common detected organic micropollutants including per- and polyfluoroalkyl substances (PFASs) [8], pharmaceuticals, surfactants, biocides, and industrial intermediates in rivers downstream of urban and industrial sources can contribute substantially to particle-associated contaminant mixtures [9,10].

SPM particles are coated with a bio-organic film rich in carbon and hydroxyl functional groups, promoting adsorption and transformation of pollutants, making SPM a major driver of contaminant transport in aquatic systems [1,11]. However, its heterogeneity (particle type/size/surface) and hydrological variability require careful interpretation and cross-year comparability checks [5]. Analyzing contaminants of emerging concern (CECs) in SPM remains challenging due to diverse composition, variable particle sizes, matrix effects, and complex chemical interactions [12–15]. Addressing these challenges requires integrated temporal and spatial monitoring with robust statistical trend analysis to support sustainable river and sediment management strategies [16].

Non-target screening (NTS), recognized for its ability to detect unknown chemicals, alongside suspect screening (SS), has been extensively utilized in environmental sciences [16]. However, its use in the analysis of SPM and sediments remains comparatively limited [12–15]. When coupled with gas chromatography (GC) or liquid chromatography (LC) and high-resolution mass spectrometry (HRMS), SS/NTS plays a pivotal role in full-scan analysis of aquatic environments, enabling the detection of emerging contaminants and providing crucial insights for risk assessment and regulatory action [17]. Although NTS is increasingly used in monitoring of CECs, studies investigating temporal trends (especially for particle-phase matrices) remain scarce [17]. Environmental specimen banks (ESBs) provide archived samples that enable retrospective NTS, supporting the creation of multi-year datasets, characterization of complex contaminant mixtures, evaluation of management effectiveness, and strengthening early-warning systems (EWS) [18,19]. This approach complements EU Watch List frameworks for regulatory monitoring, including the Water Framework Directive (WFD) surface water Watch List (Commission Implementing Decision (EU) 2025/439) [20], and the German Drinking Water Ordinance Watch List (Commission Implementing Decision (EU) 2022/679) [21], alongside associated analytical guidance for Watch List substances [22]. However, since NTS signals are prone to ionization and matrix effects, and identification confidence relies on MS/MS library coverage and *in-silico* predictions, Schymanski levels are reported [23]. Retention-time indices (RTI), orthogonal evidence (MS/MS library/annotation tools), and quality assurance/quality control (QA/QC) measures, are applied to minimize false positives and interpret trends cautiously [17]. This approach is consistent with recent interlaboratory findings, showing that experimental RT projection and RTI provides robust cross-system retention constraints compared with purely predictive models [24]. Combining these strategies enables early detection of emerging contaminant signals and supports timely preventive measures [17]. A recent bibliometric analysis highlights the rapid growth of NTS in water-pollution research and identifies key hotspots, underscoring current gaps that long-term time series can help address [25]. Archived materials therefore provide a practical approach to obtaining long-term chemical trend information without the need for new field campaigns.

A recent study demonstrated the feasibility of applying SS/NTS to archived SPM from large river systems. LC-HRMS-based NTS of SPM samples from Rhine and Saar revealed increasing trends of previously overlooked cationic contaminants and enabled an initial ecotoxicological prioritization of quaternary ammonium compounds and dyes [12, 26]. In the present study, archived SPM samples from the German ESB, collected between 2005 and 2022 at two contrasting Rhine sites (Weil am Rhein and Koblenz), were analyzed using LC-HRMS with SS/NTS to evaluate temporal trends and screen for emerging contaminants across a much broader range of contaminant classes. While previous investigations of these archives, such as analyses of heavy metals and PFAS profiles (https://www.umweltprobenbank.de) [27], have provided valuable insights, our work extends this scope by characterizing a wider suite of CECs and assessing long-term pollution dynamics to support trend-based prioritization. Supported by *in-silico* predictions and orthogonal evidence, these findings advance long-term environmental monitoring and provide screening-level signals that support early warning and prioritization of emerging contaminants. This site-specific, non-target time series analysis of 36 archived annual composites from a national archive integrates retention-time indices and MS/MS library-based annotation tools with predicted acute toxicity information. The results demonstrate how archived SPM can be used to prioritize chemicals for targeted confirmation and source control, while complementing dissolved-phase monitoring strategies.

2. Materials and methods

2.1. Sampling

SPM samples were obtained from the German ESB, a long-term national program dedicated to the systematic collection, analysis, and archiving of samples from human populations as well as marine, freshwater, and terrestrial ecosystems. The ESB supports chemical and environmental policy and regulation by providing high-quality data for long-term monitoring and research [18]. The SPM samples were collected monthly between 2005 and 2022 using sedimentation boxes at two Rhine River sites: Weil and Koblenz (Figure S1 in Supplementary Information, SI1). Weil (Upper Rhine, just downstream of Basel) and Koblenz (Middle Rhine, directly upstream of the Moselle confluence) were selected to span an upstream–downstream pressure gradient, well-treated/closely monitored upstream *vs* cumulative industrial–municipal inputs downstream, enabling site-resolved trend analysis and early-warning assessment [27–29].

Monthly samples were collected using sedimentation traps installed at about 1 m depth. Samples were pre-sieved at 2 mm and immediately cryo-archived on site using liquid nitrogen to preserve its biological and chemical integrity, in line with ESB standard operating procedures [30]. The bulk < 2 mm fraction was analyzed without further < 63 µm size fractionation. At the end of each year, the twelve-monthly samples were freeze-dried and pooled to an equal-mass (dry-weight) basis into a single annual composite sample per site (18 per site; $n = 36$ total). No flow-weighting or additional normalization was applied during compositing. Aliquots of the samples were stored above liquid nitrogen at below −130 °C in the German ESB archive [30]. For this study, cryo-archived aliquots were dispatched in insulated shippers packed with dry ice to maintain sub-zero temperature during transport. Shipments were received cold and upon arrival, aliquots were immediately transferred to −20 °C storage and handled on ice to avoid thaw–freeze cycles prior to extraction.

The detailed sampling locations are described in Göckener et al. [27] and on the website of the German ESB (https://www.umweltprobenbank.de/en/documents/profiles/sampling_areas). Physicochemical characterization of the annual SPM composites including pH ($CaCl_2$), total carbon (TC), total organic carbon (TOC), total inorganic carbon (TIC) (% dry weight), as well as grain size (sand/silt/clay, %) is provided for Weil (Table S1, SI1) and Koblenz (Table S2, SI1) in SI1.

2.2. Chemicals

All solvents and chemicals utilized in the study were of analytical grade. Dichloromethane (DCM) was purchased from Honeywell (34411–2.5 L), *n*-hexane from Supelco (1.04371.2500), and methanol (HPLC grade, ≥99.8 %) from Merck (M/4056/17X). Filtration was performed using Whatman GF/F filters (1825–047, Cytiva). VWR centrifuge tubes (525–1588) with PTFE screw caps (10132422, Fisherbrand) were used for sample processing.

2.3. Extraction and pre-treatment of the SPM Samples

Before proceeding with the extraction procedure, all glassware and laboratory tools were pre-cleaned and heat-treated at 450 °C for 3 h. The SPM samples were extracted using sequential ultrasonic extraction with *n*-hexane:dichloromethane (Hx:DCM) and methanol (MeOH). Briefly, 5 g of each SPM sample was weighed and extracted twice by adding 25 mL of Hx:DCM (1:1, v/v) each time, followed by ultrasonication for 30 min per extraction. The supernatant was then transferred to a round flask. Subsequently, the remaining samples were extracted twice each with 25 mL of MeOH using ultrasonication. The obtained extracts were then concentrated to approximately 1 mL using rotary evaporation and filtered (Whatman GF/F filters) into 8 mL vials. To recover analytes retained on the filter, it was rinsed with approximately 7 mL of the respective extraction solvent (Hx:DCM or MeOH) into the 8 mL vial. The volume was adjusted to 5 mL under a gentle nitrogen flow, and the extracts were stored at −20°C until analysis. For each annual sample, extracts were prepared in duplicate ($n = 2$), except for one sample (Koblenz 2005), where limited material permitted only a single extraction.

An aliquot corresponding to approximately 0.4 g dry weight of each sample was used for LC-HRMS analysis, with injections performed in triplicate. For this study, only the methanolic extracts were analyzed, selected after a preliminary screening showing higher LC-HRMS feature yield; while the Hx:DCM extracts are being analyzed by GC-HRMS in a separate study. Procedural blanks, consisting of the extraction solvents, were included with each extraction and processed identically to the samples to monitor potential contamination.

Unspiked sediment QC samples were included in each batch. These QC samples were prepared by pooling different sediment samples that had previously been shown to induce AhR activity and exert antagonistic AR effects, making them particularly relevant for the accompanying bioassay testing. Prior to analysis, the QC samples were freeze-dried and extracted using the same procedure as the SPM samples, ensuring consistency in processing. QC samples and procedural blanks were analyzed in parallel to identify potential background signals or laboratory-introduced contaminants. Moreover, procedural blanks were included specifically to monitor potential contamination. While the QC samples provided qualitative information on procedural cleanliness and matrix-derived background, they were not used for quantitative correction.

2.4. Chemical analysis using LC-HESI-HRMS

LC separation was carried out using a Vanquish Horizon ultra-performance liquid chromatography (UPLC) system (Thermo Fisher Scientific, Bremen, Germany). MilliQ water and MeOH were used as mobile phases, modified both with 0.1 % formic acid for positive ionization mode (+ESI) and with 5 mM ammonium acetate for negative ionization mode (−ESI). A CORTECS C18+ column (90 Å, 2.7 µm, 2.1 mm × 100 mm) was used at a flow rate of 0.3 mL/min, applying a gradient elution from 10 % to 90 % of organic solvent over 18 min, followed by an equilibrating step, while 10 µL of each sample was injected in triplicate (triplicate responses were averaged; standard deviation (SD) retained; duplicate-extract handling is described in 2.3).

HRMS detection was carried out on a QExactive Focus Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated electrospray ionization (HESI-II) source. Both +ESI and −ESI ionization modes were utilized to maximize the detection of diverse compounds with different ionization behaviours. The full MS scan range was 80–1000 *m/z* with a resolution of 70,000 (at *m/z* 200). To collect MS/MS spectra for the most intense ions, the data-dependent acquisition (DDA) mode was employed, using stepped collision energies of 10, 20, and 40 eV. These MS/MS spectra were acquired at a resolution of 17,500. Instrument settings included sheath and auxiliary gas flow rates of 35 arbitrary units (a.u.) and 10 a.u., respectively, a spray voltage of 3.0 kV, capillary temperature of 350 °C, and auxiliary gas heater temperature of 300 °C. To ensure and monitor mass accuracy throughout the analytical sequence, the LC-HRMS system was calibrated prior to each batch using Cal-Mix and Thermo Scientific™ Pierce™ FlexMix™ calibration solutions. In addition, an in-house system suitability test (SST) standard was injected throughout the sequence to monitor mass accuracy and detect drifts [31]. Each batch was bracketed by MeOH and control blanks, an unspiked QC sediment, and MQ blanks. Calibration and SST checks were performed before and after each batch. Quality control focused on carry-over and background signals in blanks, mass accuracy (±5 ppm), and retention time consistency for both RTI and SST mixtures. Reproducibility of RTI calibrants (mean retention time and %RSD) is reported in Tables S3–S5 (SI1), along with calibration curve of calibrants presented in Figure S2 (SI1). Features that failed blank evaluation, mass accuracy, or RT/RTI criteria were excluded from further analysis. Temporal trends were inferred from unnormalized peak areas under controlled conditions. At the screening stage, *p*-values were not adjusted for multiple comparisons, and trends were interpreted as directional indicators rather than definitive statistical outcomes.

Targeted quantification was beyond the scope of this study. All 36 annual composites were extracted and analyzed using identical protocols and LC–HRMS settings within a single analytical campaign for each ionization mode, incorporating bracketed QC samples and RTI calibrants to ensure maximum comparability across years.

3. Results and discussion

3.1. Identification of compounds

After data preprocessing using Compound Discoverer 3.3 (Thermo Fisher Scientific), following the workflow outlined in Table S3 (SI1), a total of 24,691 and 27,143 features, defined as distinct signals characterized by mass-to-charge ratio, retention time, and intensity, representing potential chemical compounds, were initially detected in the negative (–ESI) (NEG) and positive (+ESI) ionization modes (POS), respectively (Fig. 1). Stepwise feature numbers per sample and per ionization mode are summarized in Table S4a and S4b (SI1) and text in SI1. Background removal reduced the number of features to 15,612 (NEG; 63 % of initial) and 19,120 (POS; 70 % of initial). Further refinement, including multiple filtering steps, was applied to retain only features with MS^2 spectra, ensuring robust feature selection. This resulted in 2634 (NEG; 11 % of initial) and 2512 (POS; 9.3 % of initial) features. Subsequent time-trend prioritization retained only features consistently detected for at least five years at each sampling site, narrowed the dataset to 215 (NEG; 0.87 % of initial) and 186 (POS; 0.69 % of initial) features. Finally, eliminating false positives manually ($n = 65$, Tables S5–7), and considering that some compounds were detected in both POS and NEG modes, reduced the number of features to 332 compounds (173 in NEG; 0.70 % of initial and 159 in POS; 0.59 % of initial).

The 332 unique compounds identified in the Rhine-Koblenz ($n = 18$) and Rhine-Weil ($n = 18$) catchments were classified into seven main categories (Fig. 2). The most prevalent group consisted of non-anthropogenic compounds (39 %), followed by industrial chemicals (33 %) (including per- and polyfluoroalkyl substances (PFASs) (1.0 %)), pharmaceuticals (12 %), other chemicals (9.0 %), personal care products (4.5 %), and pesticides (1.5 %).

Identified compounds were assigned Schymanski confidence levels and reported as two tiers: probable/putative (levels 2.1 and 2.2) and tentative (levels 3.1 and 3.2) [23]. A detailed list of compounds identified at each confidence level, along with their corresponding molecular formula, *m/z*, calculated molecular weight, SMILES, neutral loss, mass error, retention time, category, applications, and related peak area across samples, is provided in SI2. Overall, among the 332 compounds identified, 29 (8.7 %) were robustly assigned to Schymanski Level 2 (2.1, $n = 10$, 3.0 % of total compounds identified; 2.2, $n = 19$, 5.7 %), while 303 (91 %) were tentatively identified at Level 3 (3.1, $n = 175$, 53 %; 3.2, $n = 128$, 38 %) (Figure S3 SI1). Confidence levels were assigned based on MS^2 similarity against mass spectral libraries (mzCloud, mzVault, and ChemSpider), MS^2 fragmentation libraries (MassBank), and *in silico* fragmentation tools (CFM-ID). Detailed levelling criteria and spectral-validation steps are provided in SI1. Additionally, Figure S4 (SI1) illustrates MS^2 spectra matching for several compounds identified at level 2, showing spectral similarity comparisons with the mzCloud mass spectral library. It should be noted that this NTS analysis provides screening-level early warning signals based on annual SPM composite peak areas, whereas quantification concentrations measurements are beyond the scope of this study.

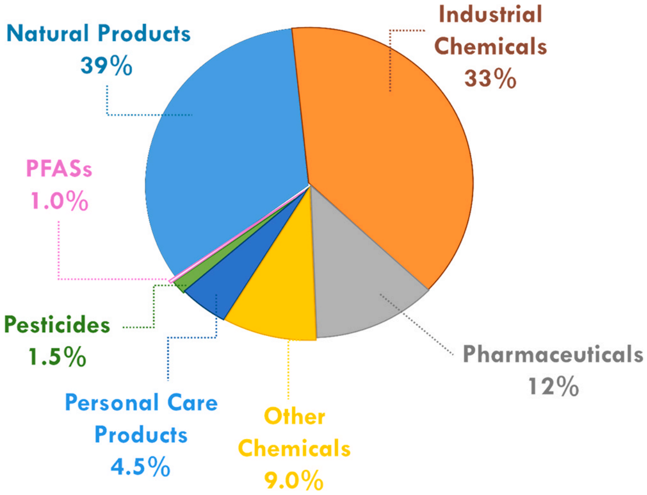


Fig. 2. Classification of identified compounds in SPM samples collected from Rhine-Koblenz and Rhine-Weil ($n = 332$).

Pharmaceuticals were among the most prominent compound groups identified in this study. Due to the substantial variability in removal efficiencies across conventional wastewater treatment plants (WWTPs), readily biodegradable pharmaceuticals—such as paracetamol—are typically well eliminated from the aqueous phase during treatment [32]. Consequently, they are often not detected in effluent samples, which is consistent with our observations. In contrast, β-blockers like metoprolol exhibit moderate and variable removal, primarily through biotransformation [33], consistent with our detection of this compound at both sites. Moreover, persistent pharmaceuticals, such as carbamazepine, tend to exhibit low removal efficiencies in conventional WWTPs [34], as confirmed by our detection of this compound at both sites. The predominance of pharmaceuticals in Rhine SPM is consistent with recent LC-HRMS studies of rural surface waters, where pharmaceuticals also dominated the CEC mixture despite limited local wastewater infrastructure [35].

Recent research on non-steroidal anti-inflammatory drugs (NSAIDs)

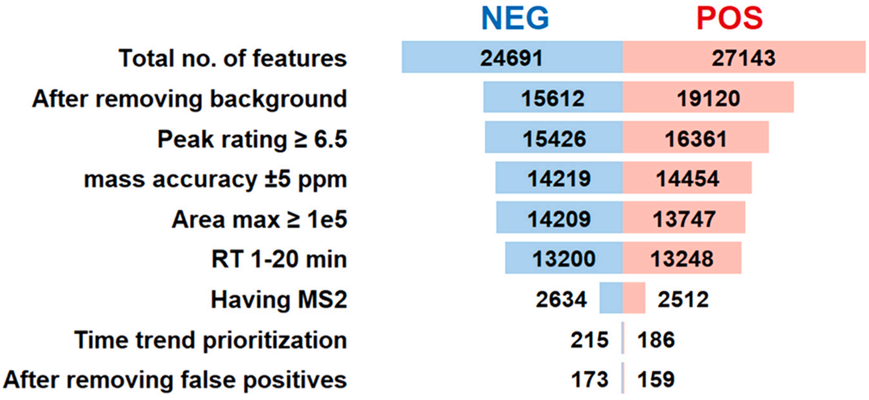


Fig. 1. Number of features at each processing step in positive (POS) and negative (NEG) ionization modes based on the 36 SPM samples.

indicates that pharmaceutical residues can still spread across various environmental compartments, even when WWTPs effectively remove them from the dissolved phase. In a comprehensive case study, Dolu and Nas (2023) monitored four widely used NSAIDs and six metabolites from an advanced WWTP into its discharge channel, sewage sludge, irrigated soils, and crops. They observed that, despite achieving over 93 % removal from the aqueous phase (except for diclofenac), several NSAIDs and metabolites accumulated in these downstream compartments [36]. These findings show that WWTPs and discharge channels act as redistribution hubs between aquatic and terrestrial systems. This is relevant to our case study, as NSAID signals in Rhine SPM likely stem from combined inputs of WWTP effluents, irrigation channels, and contaminated soils.

The fate of surfactants and industrial additives is more variable and depends on both their chemical class and the specific configuration of the treatment process [37]. Among the industrial chemicals identified in this study, surfactant classes such as alkyl (ether) sulfates (AES) and alkyl sulfates (AS) were detected. Although AES (e.g., myreth sulfate, laureth-2 sulfate) and AS (e.g., undecyl hydrogen sulfate, myristyl sulfate) are generally well removed in activated sludge-based WWTPs—with removal efficiencies typically ranging from $\sim$98 % to $>$ 99 %—residual concentrations can still persist in effluents and be continuously discharged into receiving waters. In contrast, WWTPs employing trickling filters exhibit lower and more variable removal efficiencies, with AES removal averaging around 83 % [38]. A national German campaign across 33 WWTPs reported an average effluent concentration of 0.57 μg/L for total AES, despite their high removal efficiencies. This finding highlights that basin-scale loads can remain detectable downstream [39]. The detection of AS and AES compounds at both Rhine sites reflects consistent usage of these substances. However, the absence of significant temporal trends at either location suggests stable performance of WWTPs throughout the study period, rather than an increase in environmental inputs [40].

3.2. *Temporal analysis of identified contaminants*

A time trend analysis was conducted to prioritize features exhibiting increasing trends over the study period (R scripts in Table S8, SI1). In this study, “increasing” indicates a significant monotonic association between year and unnormalized LC–HRMS peak area ($p \leq 0.05$) for features present in ≥ 5 annual composites per site. This $\geq$ 5-year presence requirement makes the workflow a conservative, confirmatory workflow focused on repeatedly observed signals. These counts are therefore screening-level directionality indicators rather than quantified changes in concentration.

Spearman’s rank correlation was selected as the primary method for trend analysis due to its sensitivity to directional changes and its ability to identify more statistically significant monotonic trends under the sampling cadence ($n = 142$ in Rhine-Koblenz and $n = 103$ in Rhine-Weil), compared to the more conservative Mann–Kendall test ($n = 42$ in Koblenz and $n = 94$ in Weil), which is more robust in the presence of tied values (Fig. 3). Annual SPM composites vary between years, therefore linear regressions in figures (Figures S4, S5, S8, S9, and S11 in SI1) are shown only as visual aids. Since p-values were not adjusted during this screening level, trends should be interpreted primarily as directional rather than definitive. Peak-area patterns may also be influenced by hydrological variability, SPM heterogeneity, and temporal shifts in chemical usage or production; therefore, they represent preliminary signals that warrant targeted follow-up for risk assessment. Using temporal trends as a prioritization layer is consistent with recent NTS case studies, where time profiles helped distinguish persistent or increasing signals from transient background variability [41]; however a recent study has shown that the apparent temporal variability in LC-HRMS NTS time series can depend strongly on the chosen data-processing approach and emphasize the need for conservative trend interpretation [42,43].

In our study, site-specific differences are interpreted qualitatively due to the lack of quantitative data on domestic *vs* industrial wastewater contributions. Reliable source attribution would require flow-normalized loads and verified shares of municipal and industrial effluents. Complete cross-tabulations and agreement rates are provided in SI2.

At the Rhine-Koblenz site, 97 compounds showed statistically significant increasing trends (29 %), 45 compounds exhibited decreasing trends (14 %), and 190 compounds showed no significant trend (57 %) (Figure S5, for details see SI2). At the Rhine-Weil site, 59 increased (18 %), 44 decreased (13 %), and 229 compounds exhibited no significant change over time (69 %).

Interpreted as screening-level directionality, the larger number of increasing compounds at Koblenz suggests a more dynamic contaminant profile, plausibly reflecting its role as a downstream confluence integrating cumulative municipal/industrial inputs and tributaries with episodes of reduced dilution, whereas Weil (immediately downstream of Basel) benefits from advanced wastewater treatment and intensive surveillance that may constrain SPM accumulation (Fig. 3); however, without flow-normalized loads, hydrological covariates, and verified effluent shares, this remains a qualitative interpretation [27–29].

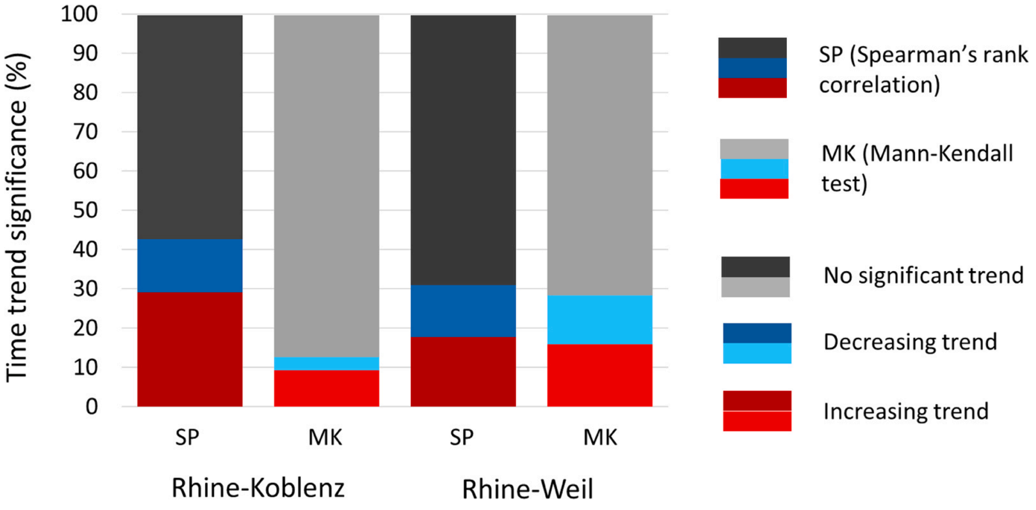


Fig. 3. Percentage of the chemicals identified showing significant trend using Spearman’s rank correlation (SP) and Mann-Kendall test (MK) at Koblenz and Weil ($n = 332$). Colors denote trend direction (red = increasing, blue = decreasing, grey = no significant trend).

3.2.1. *Increasing time trends*

A greater number of contaminants exhibited statistically significant upward trends (Spearman's Rs ≥ 0.8) over time at the Rhine-Koblenz site ($n = 20$) compared to the Rhine-Weil site ($n = 9$) (for details see Tables S9 and S10 in SI1 and SI2). Despite its proximity to the industrial Basel region, Rhine-Weil benefits from Switzerland's advanced wastewater treatment and a long-standing monitoring station that analyzes over 680 substances, enabling early pollution detection and limiting long-term contaminant buildup in SPM samples [27,28]. In contrast, industrial pollution remains a pressing concern for the Rhine near Rhine-Koblenz, originating from upstream hubs including Mannheim or Ludwigshafen [27]. For example, Ludwigshafen is home to BASF, the world's largest chemical producer, while Mannheim accommodates a wide range of chemical and industrial facilities [29].

Within the highest confidence set (level 2; $n = 29$), industrial chemicals comprised the largest group ($n = 11$), followed by natural substances ($n = 9$) and pharmaceuticals ($n = 5$). Notably, most features flagged for acute toxicity (see SI2) were associated with industrial chemicals, underscoring the need for heightened attention to this subset. The persistent detection of industrial chemicals corroborates recent findings, highlighting the widespread occurrence of industrial CECs across European aquatic environments [44]. For instance, a recent study monitoring WWTPs and transnational river basins in Spain and Portugal reported incomplete removal of several industrial chemicals, including xylenesulfonate, TBEP (tris(2-butoxyethyl) phosphate), and TCPP (tris(1-chloro-2-propyl) phosphate). Despite treatment, elevated concentrations of these substances were still observed in effluents and surface waters, underscoring the limitations of conventional wastewater treatment technologies in effectively eliminating industrial contaminants [45].

Pharmaceuticals, plant-protection compounds, and personal-care products significantly contribute to pollution in the Rhine River. These contaminants originate primarily from municipal wastewater and catchment inputs *via* runoff and tributaries. For most micropollutants, particularly pharmaceuticals, effluents from WWTPs represent the dominant source. In contrast, plant-protection compounds mainly enter the river through diffuse agricultural runoff and drainage [46].

Numerous studies underscore the need for targeted monitoring and regulation of CECs, particularly due to their presence and potential risks in drinking water, highlighting the importance of effective water quality management [47]. Furthermore, special attention should be given to the application of diverse analytical tools to capture a wide range of chemicals with varying physicochemical properties, for instance, PMT/vPvM substances (persistent, mobile, and toxic/very persistent and very mobile), which can potentially evade conventional treatment and migrate through aquatic systems into drinking water sources [48]. This highlights the importance of incorporating hydrophilic interaction liquid chromatography (HILIC) as a complementary separation technique to broaden the detection scope for such contaminants [49].

Rhine-Koblenz

The chemical profile at the Rhine-Koblenz site reflects a complex mixture of industrial chemicals, natural substances, pharmaceuticals, and personal care products (Figure S6 and SI2). This composition reflects the site's downstream location along the Rhine, where it is influenced by cumulative discharges from several upstream industrialized regions. Among the 20 substances showing statistically significant upward trends, several industrial chemicals ($n = 6$) are identified as likely originating from upstream industrial hubs such as Mannheim and Ludwigshafen [27]. The fitted trend lines are included solely to visualize the direction of change, acknowledging the substantial year-to-year variability observed in the SPM composite samples. For example, 3′,4′-(dioctyloxy)acetophenone (Fig. 4A), commonly used as a plastic additive and UV stabilizer, reflects the expansion of plastic production and may enter aquatic environments *via* leaching and degradation. Its environmental behavior is similar to that of other UV filters, such as benzotriazoles and benzophenones, which have been shown to hinder algal growth and disrupt photosynthesis [50], regulated under the EU cosmetics legislation (Regulation (EC) No 1223/2009, Annex VI) [51].

Octyl sulfate, a surfactant found in personal care and cleaning products, is being detected more frequently due to its widespread usage and poor removal efficiency in WWTPs (Fig. 4B) [52]. Its persistence in aquatic environments highlights the challenges associated with treating

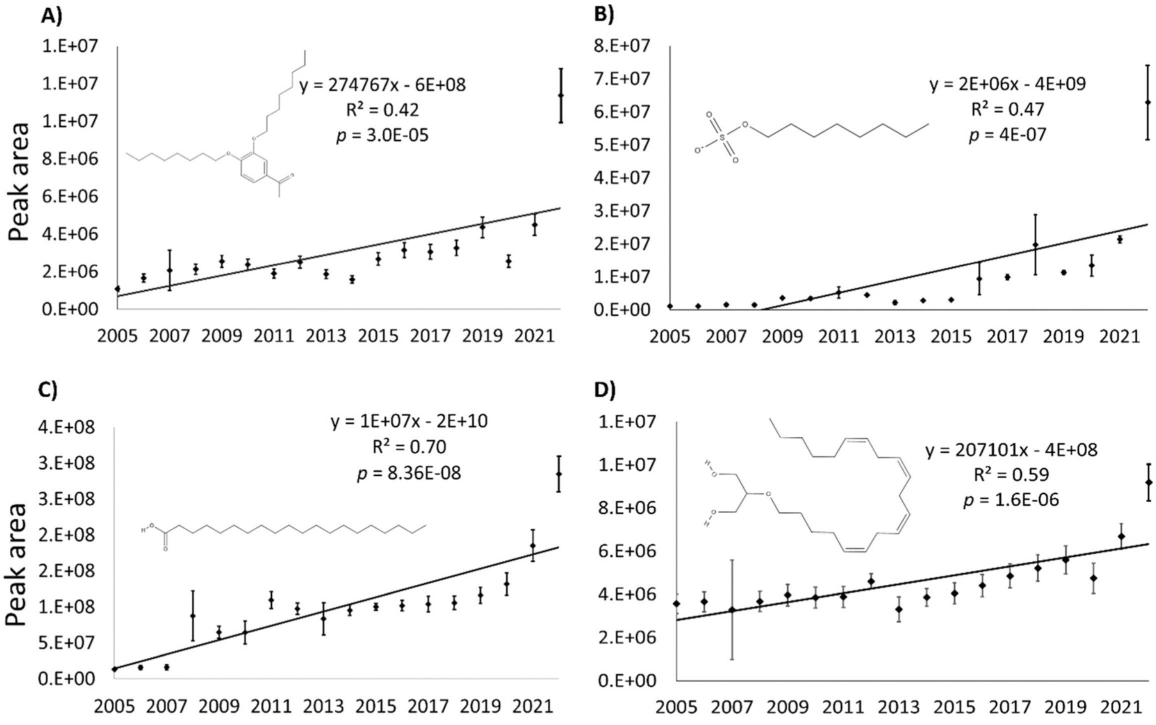


Fig. 4. Time trend analysis of selected compounds at the Rhine-Koblenz site ($n = 18$), showing statistically significant increasing trends (Spearman's rank correlation): (A) 3′,4′-(Dioctyloxy)acetophenone, (B) octyl sulfate, (C) arachidic acid, and (D) 2-arachidonyl glycerol ether.

surfactant-rich effluents, especially in densely populated or industrialized catchments [53].

Pharmaceuticals are also prominent in the chemical profile. These compounds often resist conventional treatment methods, leading to a lasting presence in surface waters [54]. The environmental fate of many pharmaceuticals in WWTPs and receiving rivers is largely influenced by their ionization state and hydrophobicity, which govern their sorption to SPM and affect removal efficiency. Moreover, in large lake–river systems, mass-flow based assessments reveal that hydrology and pollutant loads, not just concentrations, play a critical role in shaping downstream occurrence [55,56]. As an example, metoprolol, an antihypertensive drug, was tentatively identified at level 3.1 and exhibited increasing trends at both sites. This finding aligns with its high prescription rates and frequent detection in European surface waters, reflecting its persistence and incomplete removal during conventional wastewater treatment [57].

In addition to anthropogenic chemicals, a large number of naturally occurring compounds ($n = 129$) were characterized (SI2). At Koblenz, increasing examples included arachidic acid, chrysin, and 2-arachidonyl glycerol ether (2-AGE). Since each feature was assigned to a single primary category, compounds with potential mixed provenance (e.g., arachidic acid, 2-AGE) were classified as natural, although their signals likely reflect mixed origins, biogenic organic matter cycling and anthropogenic wastewater inputs, commonly observed in urban rivers [28]. Arachidic acid has mixed origins—biogenic (plant waxes/organic matter) and anthropogenic (domestic/industrial; e.g., food processing, cosmetics) (Fig. 4C) [58]. 2-Arachidonyl glycerol ether (noladin ether, 2-AGE) was originally reported as an endogenous CB1 agonist isolated from porcine brain, but its endogenous occurrence remains debated. In this study, it is treated putative endogenous, with environmental detections likely reflecting wastewater-influenced inputs (Fig. 4D) [59]. Furthermore, cholestane derivatives (fecal sterol biomarkers) indicate human or animal fecal inputs [60].

The diversity and persistence of these compounds, along with their increasing temporal trends, emphasize the relevance of the Rhine catchments, particularly Koblenz, as a receptor site for both legacy and emerging contaminants. This pattern is likely intensified by reduced dilution capacity during low-flow conditions and the potential for sediment remobilization, as highlighted in recent ICPR assessments of Rhine water quality [61]. These factors emphasize the need for systematic monitoring and implementation of effective source control strategies.

Rhine-Weil

The chemical profile at the Rhine-Weil site reveals a complex mixture of industrial chemicals, pharmaceuticals, personal care products, and synthetic intermediates, reflecting its location downstream of Basel, as a major industrial and pharmaceutical center [61] (Table S10 in SI and SI2). This composition is consistent with localized discharges from urban and manufacturing sources in the region. Although fewer substances at Rhine-Weil showed statistically significant upward trends ($n = 59$) compared to Rhine-Koblenz ($n = 97$), the detected compounds are noteworthy for their emerging use, limited regulatory oversight, and potential environmental persistence (Figure S7 in SI). Several of the detected industrial compounds ($n = 17$) reflect the increasing use of industrial and consumer products. For example, N-2-ethylhexyl bicycloheptenedicarboximide (Fig. 5A), used for pesticide production, and octhilinone (Fig. 5B), commonly found in paints and coatings, point to rising applications in the construction industry and highlight gaps in regulatory oversight, emphasizing their potential environmental significance [62].

Among the features showing upward trends at Weil, two quinone-type compounds, (2*Z*,6 R)-5,6-dihydroxy-2-[(2E,4E)-1-hydroxy-2,4-hexadien-1-ylidene]-4,6-dimethyl-4-cyclohexene-1,3-dione and phenanthrenequinone, were particularly notable. These increases are interpreted as consistent with *in-situ* oxidative transformation of aromatic precursors (e.g., polycyclic aromatic hydrocarbons (PAHs) and phenolic/*p*-phenylenediamine (PPD) antioxidants) within WWTPs and stormwater systems under photochemical aging, rather than a single point source [63]. Downstream mixing and attenuation processes (e.g. redox reactions, biodegradation, sorption, and settling) along the Weil–Koblenz reach likely diminish these signals by the time they reach Koblenz [27–29].

The detection of pharmaceutical-related substances, such as peptide-based drug intermediates, and synthetic research chemicals like pyrrolidinedione derivatives reflects ongoing advancements in chemical synthesis and therapeutic development [64]. For instance, Boc-LALALW was tentatively identified at level 3.1., possessing its alternating hydrophobic alanine and leucine residues together with two tryptophan

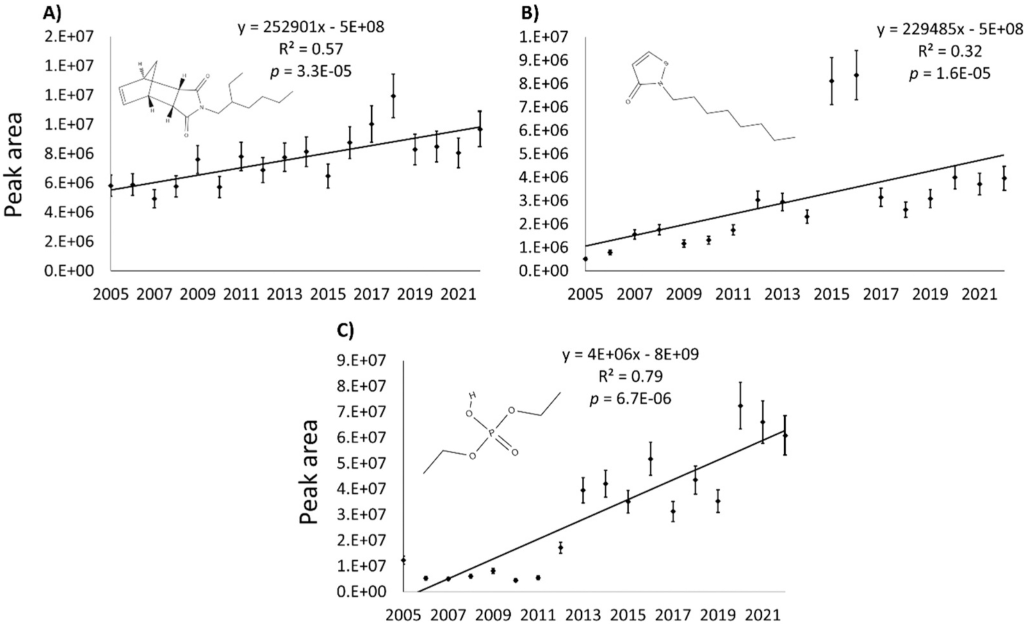


Fig. 5. Time trend analysis of selected compounds at the Rhine-Weil site ($n = 18$), showing statistically significant increasing trends (Spearman's rank correlation): (A) N-2-ethylhexyl bicycloheptenedicarboximide, (B) octhilinone, (C) diethyl phosphate.

residues, this synthetic peptide has been widely used as a model system for studying conformation, sidechain dynamics, and peptide–membrane interactions [65].

These compounds often fall outside current regulatory frameworks and environmental monitoring efforts. Although increasingly used in medicine, antimicrobial peptides and peptide-based drugs have poorly understood environmental fates, with limited data on their degradation and ecological effects [66].

Octyl sulfate, a surfactant commonly found in shampoos and soaps, is a typical personal care product contaminant whose persistence in surface waters is primarily due to incomplete removal during wastewater treatment processes [67]. Beyond its chemical persistence, recent studies have shown that sodium octyl sulfate can indirectly affect aquatic ecosystems by inducing colony formation in green algae, leading to the inhibition of grazing by key zooplankton species such as *Daphnia* and *Bosmina* [68]. These surfactants can enter aquatic environments through wastewater discharges from municipal sources or industrial facilities, including those located near pharmaceutical production sites. Such interactions suggest that surfactants can disrupt food web dynamics, further emphasizing the importance of long-term ecological monitoring and comprehensive risk assessment [69].

Diethyl phosphate, a degradation product of organophosphate pesticides, serves as a recognized indicator of agricultural runoff. It is known for its chemical stability in the environment and its potential to add to long-term pollution levels in surface waters (Fig. 5C) [58].

Although excluding 2022 slightly reduced the magnitude of the slopes, the direction of temporal trends for all plotted compounds at both sites remained unchanged (Figures S8 and S9 in SI1). Pettitt tests and Sen's slope estimates for the selected compounds consistently confirmed positive trends across both segments (Figures S8 and S9; Table S11 in SI1). Given the consistency in sampling protocols, extraction procedures, and quality control diagnostics, with no analytical or batch-related anomalies observed, the detected trends are likely driven by environmental factors, such as hydrological dynamics or variations in SPM loads.

These findings highlight the importance of upstream monitoring at sites like Rhine-Weil, where localized industrial activity and urban wastewater inputs contribute to a chemically diverse and evolving contaminant profile. While the number of increasing trends is lower than at Rhine-Koblenz, the nature of the compounds detected at Rhine-Weil suggests a need for proactive monitoring of emerging contaminants and their transformation products [70]. These findings underscore the strong influence of upstream sources and highlight the transboundary nature of chemical pollution in this part of the Rhine [70].

3.2.2. *Decreasing time trend compounds identified at both sites*

Several compounds showed statistically significant declines at Rhine-Koblenz ($n = 45$) and Rhine-Weil ($n = 44$), indicating the positive impact of regulatory actions, improved wastewater treatment systems, and changes in industrial practices (Table S12, Figures S10 and S11 in SI1; SI2). Among them, 11 and 13 compounds demonstrated a pronounced downward trend (Spearman's Rs ≤ -0.8) at the Koblenz and Weil sites, respectively.

Perfluorooctanesulfonic acid (PFOS), a persistent and bioaccumulative PFAS, has significantly declined in usage since the voluntary phase out by producers in the early 2000s and its inclusion in the Stockholm Convention in 2009, leading to a global phase-out of most of its applications [71] and declining trends in human populations and environment, including Germany, where PFAS levels show clear declining trends across human plasma and environmental matrices [72].

Climbazole, an antifungal agent frequently applied in anti-dandruff shampoos, has shown a decreasing trend, likely due to regulatory limits introduced by the EU Cosmetics Regulation (EC) No 1223/2009 [73]. Similarly, a tentatively identified anticonvulsant, carbamazepine (level 3.2), showed a downward trend at both sites. Given its poor removal in conventional WWTPs [57], this decline likely reflects improved catchment-scale attenuations such as the implementation or optimization of advanced treatment, rather than reduced usage. Notably, carbamazepine is designated by the EU Urban Wastewater Treatment Directive as an indicator pharmaceutical for verifying quaternary treatment, with a benchmark of $\geq 80\ \%$ removal [74]. The observed decrease thus suggests enhanced treatment efficiency over time downstream of both catchments. However, interpretation is limited by the lower identification confidence and the screening-level nature of the SPM data.

Within the pesticide group, diazinon, an organophosphate insecticide, has dropped in usage, primarily due to its removal from residential use and regulatory restrictions imposed by European Commission Directive 2007/6/EC, reflecting concerns about its neurotoxic effects and long-term environmental persistence [75]. Additionally, diuron, a well-known herbicide, has also shown a downward trend in Rhine-Koblenz, likely influenced by regulatory measures by the European-Commission in 2007 [75], followed by the European Commission in 2023, along with improved runoff management and wastewater treatment [76].

The decline in key pollutants at both sites highlights the success of regulatory efforts and improvements in treatment technologies. These findings also emphasize the role of long-term monitoring in supporting early-warning–oriented assessment, enabling the timely detection of environmental changes and supporting informed policy-making.

3.3. *Acute toxicity assessment of the identified chemicals using the QSAR model*

Ecological Structure Activity Relationships (ECOSAR) predictions of the 332 identified compounds covering acute toxicity in aquatic organisms including fish, daphnia (*Daphnia magna*), and green algae, are detailed in SI2. In this study, ECOSAR outputs were used as screening-level indicators of intrinsic hazard for the 332 identified compounds, covering acute toxicity in aquatic organisms including fish, *Daphnia magna*, and green algae (SI2). These predictions are not intended for quantitative risk assessment or mixture prioritization and have limited applicability to ionizable or surface-active compounds, transformation products outside the model domain, and complex chemical mixtures. All analytes were derived from the SPM-bound fraction. Sorption to SPM typically reduces freely dissolved concentrations, thereby limiting direct exposure. Nevertheless, SPM-bound compounds can still pose ecological risks through trophic transfer (e.g., ingestion of particles) and processes such as sediment deposition or resuspension [5]. Accordingly, ECOSAR predictions, based on freely dissolved chemical structures, are interpreted here as indicators of intrinsic hazard rather than exposure-adjusted risk for SPM-associated compounds [77,78]. To illustrate sorption, we used the measured TOC of Rhine SPM (2.4–5.4 %; Tables S1 and S2 in SI1). The resulting SPM-bound fraction was consistently high across all years ($F_{SPM} \approx 0.83$–0.92), supporting our interpretation that SPM can retain the majority of a moderately sorbing compound while still enabling trophic uptake through particle ingestion.

Based on the highest predicted toxicity for each compound, 47 % ($n = 154$) were classified as highly acutely toxic, 27 % ($n = 90$) demonstrated moderate toxicity, and 17 % ($n = 56$) showed slight toxicity. The remaining 8.7 % ($n = 29$) showed no significant toxicity. Among the highly toxic responses, daphnia indicated the highest frequency ($n = 69$), underscoring its pronounced sensitivity to the tested compounds. This supports its function as a sentinel species in aquatic ecotoxicology, as daphnia is widely recognized for its responsiveness to a broad spectrum of environmental contaminants, making it a reliable indicator for evaluating ecological risks in aquatic environments [79].

Several compounds predicted as highly toxic by ECOSAR have also been reported in the literature to exhibit significant aquatic toxicity. For instance, TCC (triclocarban), a widely used antimicrobial agent in personal care products, has been detected in surface waters and sediments.

It is highly toxic to aquatic invertebrates and other organisms, with effects observed at environmentally relevant concentrations [80]. TCC is also bioaccumulative and persistent in sediments, raising concerns about long-term ecological impacts [81]. Furthermore, it acts as an endocrine disruptor, impairing thyroid homeostasis and affecting reproduction and development in aquatic species [82]. Irganox 1098, a hindered amine antioxidant used as a stabilizer in polymers [83], is considered potentially toxic to aquatic life due to its persistence, low biodegradability, and bioaccumulative potential, which align with ECOSAR-based screening criteria [84].

Several compounds identified at level 2 of confidence exhibited increasing trends over time at the Rhine-Koblenz ($n = 8$) and Rhine-Weil ($n = 1$), suggesting growing inputs of certain emerging contaminants into the Rhine River. Table 1 integrates ECOSAR (most sensitive species/endpoint) with temporal trends derived from LC–HRMS peak areas (presence ≥5 years per site; Spearman $p \leq 0.05$; Rs reported with Rs ≥ 0.80 flagged) to prioritize Level-2 identifications. This approach yields a screening-level watch list of contaminants with the highest potential for environmental concern.

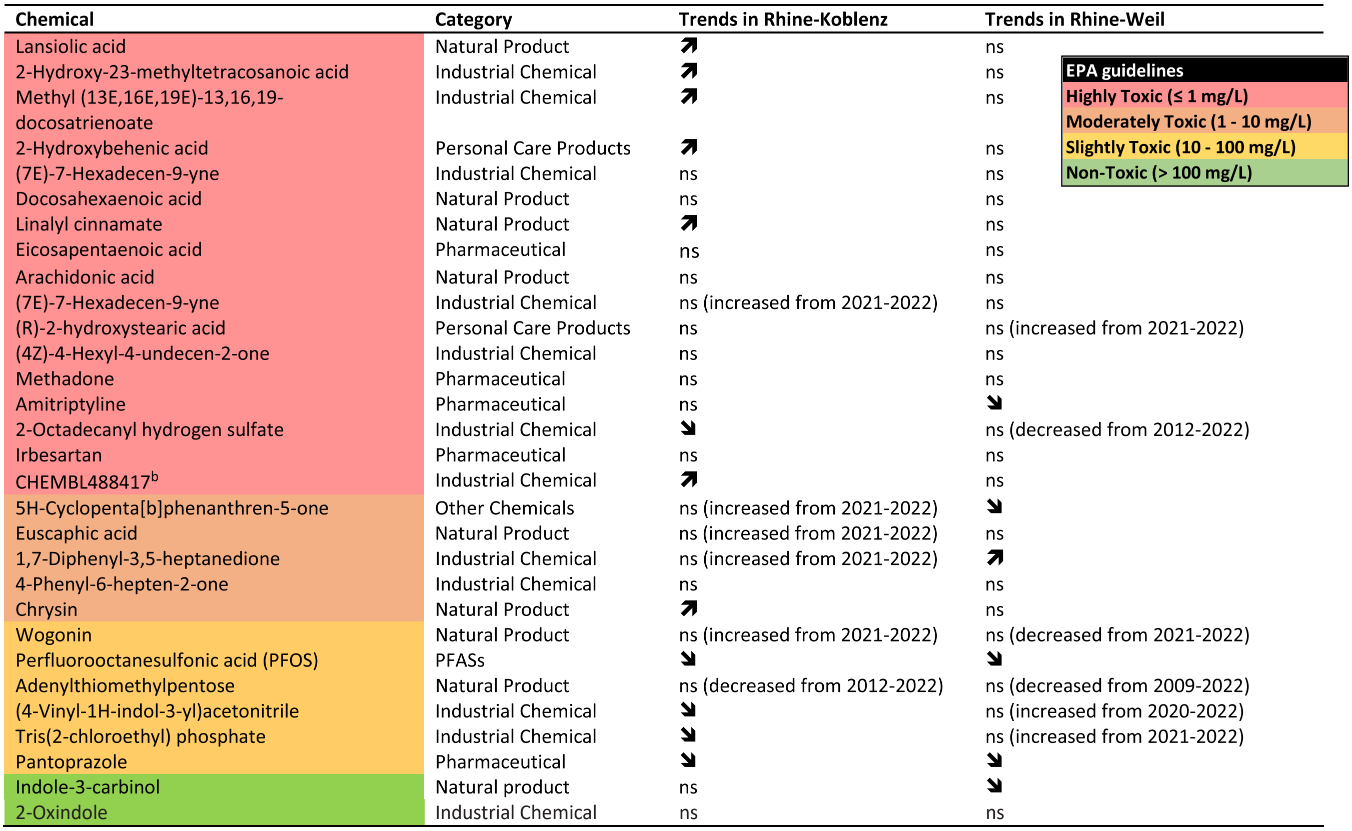
Among them, linalyl cinnamate, a fragrance compound commonly used in cosmetics and personal care products, was identified for the first time in the SPM samples at both sites. Despite its widespread use, existing safety assessments focus solely on human exposure, with no documented environmental occurrence or aquatic toxicity data [85]. Its predicted high aquatic toxicity based on the QSAR model (EC_{50}: 2.90×10^{-2} mg/L for green algae over 96 h), combined with the absence of prior environmental monitoring, highlights its potential as a novel ecological risk. Consistent with these observations, another study on the SPM samples reported declining concentrations of two synthetic fragrances used in personal care products and household cleaners, Galaxolide® and Tonalide®, supporting the trends observed in the present findings [86].

Moreover, chrysin, a flavonoid naturally present in honey and propolis, exhibited an upward trend at Rhine-Koblenz. Although it has moderate predicted aquatic toxicity (LC_{50}: 8.66 mg/L for daphnids over 48 h), its detection may be linked to plant runoff or increased use in dietary supplements. While recent studies support its biological safety [87], its environmental behavior remains poorly understood. The present study is a relative, peak-area–based trend screening, while trend directionality was interpreted from unnormalized LC-HRMS peak areas under documented QA/QC (calibration/SST, bracketed blanks, unspiked QC sediment, RTI checks; see 2.4 and SI1), noting that SPM heterogeneity and hydrology can affect signal magnitude. Accordingly, ECOSAR-based hazard flags should be interpreted cautiously and corroborated with targeted confirmation before any risk characterization.

At the Rhine-Koblenz site, long-chain fatty acids and esters like 2-hydroxybehenic acid and methyl (13E,16E,19E)-13,16,19-docosatrienoate suggest inputs from biological activity, cosmetics, and industrial use. Both compounds pose notable ecological risks, showing high aquatic toxicity (Fish LC_{50}: 2.90×10^{-3} mg/L; Algae EC_{50}: 1.90×10^{-4} mg/L). Other compounds with increasing trends at Rhine-Koblenz include 2-hydroxy-23-methyltetracosanoic acid and CHEMBL488417 ((2E,4E,6E,8E)-9-(2-Acetyl-5,5-dimethyl-1-cyclopenten-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid), while 1,7-diphenyl-3,5-heptanedione showed a significant rise at Rhine-Weil.

Table 1

Schymanski Level 2 identifications with modeled acute aquatic toxicity (most sensitive species/endpoint) and site-specific trends from Spearman's tests on annual LC-HRMS peak areas ($p \leq 0.05$)[a]



Chemical	Category	Trends in Rhine-Koblenz	Trends in Rhine-Weil
🟥 Lansiolic acid	Natural Product	↗	ns
🟥 2-Hydroxy-23-methyltetracosanoic acid	Industrial Chemical	↗	ns
🟥 Methyl (13E,16E,19E)-13,16,19-docosatrienoate	Industrial Chemical	↗	ns
🟥 2-Hydroxybehenic acid	Personal Care Products	↗	ns
🟥 (7E)-7-Hexadecen-9-yne	Industrial Chemical	ns	ns
🟥 Docosahexaenoic acid	Natural Product	ns	ns
🟥 Linalyl cinnamate	Natural Product	↗	ns
🟥 Eicosapentaenoic acid	Pharmaceutical	ns	ns
🟥 Arachidonic acid	Natural Product	ns	ns
🟥 (7E)-7-Hexadecen-9-yne	Industrial Chemical	ns (increased from 2021-2022)	ns
🟥 (R)-2-hydroxystearic acid	Personal Care Products	ns	ns (increased from 2021-2022)
🟥 (4Z)-4-Hexyl-4-undecen-2-one	Industrial Chemical	ns	ns
🟥 Methadone	Pharmaceutical	ns	ns
🟥 Amitriptyline	Pharmaceutical	ns	↘
🟥 2-Octadecanyl hydrogen sulfate	Industrial Chemical	↘	ns (decreased from 2012-2022)
🟥 Irbesartan	Pharmaceutical	ns	ns
🟥 CHEMBL488417[b]	Industrial Chemical	↗	ns
🟧 5H-Cyclopenta[b]phenanthren-5-one	Other Chemicals	ns (increased from 2021-2022)	↘
🟧 Euscaphic acid	Natural Product	ns (increased from 2021-2022)	ns
🟧 1,7-Diphenyl-3,5-heptanedione	Industrial Chemical	ns (increased from 2021-2022)	↗
🟧 4-Phenyl-6-hepten-2-one	Industrial Chemical	ns	ns
🟧 Chrysin	Natural Product	↗	ns
🟨 Wogonin	Natural Product	ns (increased from 2021-2022)	ns (decreased from 2021-2022)
🟨 Perfluorooctanesulfonic acid (PFOS)	PFASs	↘	ns
🟨 Adenylthiomethylpentose	Natural Product	ns (decreased from 2012-2022)	ns (decreased from 2009-2022)
🟨 (4-Vinyl-1H-indol-3-yl)acetonitrile	Industrial Chemical	↘	ns (increased from 2020-2022)
🟨 Tris(2-chloroethyl) phosphate	Industrial Chemical	↘	ns (increased from 2021-2022)
🟨 Pantoprazole	Pharmaceutical	↘	↘
🟩 Indole-3-carbinol	Natural product	ns	↘
🟩 2-Oxindole	Industrial Chemical	ns	ns

EPA guidelines
🟥 Highly Toxic (≤ 1 mg/L)
🟧 Moderately Toxic (1 - 10 mg/L)
🟨 Slightly Toxic (10 - 100 mg/L)
🟩 Non-Toxic (> 100 mg/L)

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[a] ↗: significant increasing trend ($p < 0.05$); ↘: significant decreasing trend ($p < 0.05$); ns: no significant trend ($p > 0.05$); more details on temporal behavior is provided in parentheses. [b] (2E,4E,6E,8E)-9-(2-Acetyl-5,5-dimethyl-1-cyclopenten-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid.

As demonstrated in Table S13 (SI1), 55 % of level 2 compounds ($n = 29$) were classified as highly acutely toxic, while 17 % were moderately toxic, 21 % slightly toxic, and 7 % showed no toxicity. Among the source categories, personal care products had the highest proportion of highly toxic predictions (100 %), followed by pharmaceuticals (80 %), industrial chemicals (55 %), and natural products (44 %). PFAS exhibited only slight toxicity, and the single compound in the "other chemicals" category was moderately toxic. These results are intended solely for prioritization purposes, given the uncertainties associated with compound identification and predictive modeling.

These SPM-based trends and the resulting watch list complement previous LC–HRMS NTS studies on Rhine and Saar SPM that focused on permanent cations [12] by extending the chemical space to PFAS, pharmaceuticals, surfactants, biocides and industrial additives and integrating acute-toxicity–based prioritization. Together, these datasets illustrate the value of archived SPM for reconstructing long-term contaminant dynamics and supporting across-class substance prioritization. However, given the limited data on their environmental occurrence, referring to the detection of these compounds in environmental samples without corresponding concentration data, these upward trends warrant further investigation into their ecological relevance. While the actual impact of these findings depends on concentration levels, their consistent detection and increasing trends highlight the need for additional studies to support future risk assessment efforts. Ultimately, this work contributes to the development of early-warning–oriented monitoring frameworks that (i) flag potential watch list chemicals, (ii) trigger targeted confirmation, quantification, and risk quotient screening, and (iii) inform source control and surveillance priorities in aquatic ecosystems.

4. Conclusion

This case study highlights the value of long-term SPM archives in conjunction with suspect screening and NTS for the identification of emerging contaminants in aquatic environments. The identification of various compounds with increasing trends, particularly at the Rhine-Koblenz site, indicates continued chemical exposure from diverse sources, including industrial chemicals, pharmaceuticals, and biocides. Across both Rhine sites, a total of 332 compounds were identified with varying levels of confidence. Spearman trend analysis revealed 97 increasing (29 %) and 45 decreasing (14 %) compounds at Koblenz, and 59 increasing (18 %) and 44 decreasing (13 %) compounds at the Weil catchment.

At the acute screening level, ECOSAR-based toxicity predictions were used to further prioritize these substances for ecological risk assessment. The results indicated that 47 % of the compounds exhibited high acute toxicity, 27 % moderate, 17 % slight, and 8.7 % low or no acute toxicity. Daphnia frequently emerged as the most sensitive species across the dataset.

In contrast, the observed declining trends in regulated organic micropollutants such as PFOS and diazinon reflect the effectiveness of environmental regulations and improved wastewater treatment. The combined application of LC-HRMS and SS/NTS provides a powerful analytical framework for high-resolution detection, trend analysis, and prioritization of emerging contaminants, thereby supporting early-warning–oriented monitoring and risk-based prioritization. Future studies focusing on targeted analysis of prioritized chemicals, supported by Level 1 confirmation using reference standards, validated extraction protocols and matrix-effect assessment, combined with integration of hydrological covariates, effect-based approaches, and complementary GC-HRMS data, can further strengthen the regulatory relevance of this work. This study provides a robust framework for extending similar investigations on sediments, soils, and biota by integrating temporal trend analysis with LC-HRMS–based SS/NTS. Such an approach enables early detection of emerging contaminants and supports timely management and policy decisions in coordination with regulators for source tracing and mitigation.

Environmental implications

Long-term non-target screening (NTS) of suspended particulate matter (SPM) from two Rhine sites (2005–2022) identified 332 contaminants, with about 25 % showing significant upward trends, indicating emerging particle-associated exposure risks. Combining temporal trends (Spearman $p \leq 0.05$), persistence markers and ECOSAR-based hazard predictions, we derive an SPM-based watch list including PFAS, pharmaceuticals, anionic surfactants, UV stabilizers, biocides, organophosphate indicators, and fragrance ingredients as candidates for targeted confirmation, load-based monitoring and source control measures. The approach provides screening-level early-warning signals that complement dissolved-phase monitoring and can support regulators and utilities prioritize mitigation efforts, while recognizing that quantitative risk assessment still requires targeted concentration data and hydrological context.

CRediT authorship contribution statement

Javad Mottaghipisheh: Writing – original draft, Validation, Software, Investigation, Formal analysis, Data curation. **Erica Selin:** Writing – review & editing, Investigation, Formal analysis. **Anna Kärrman:** Writing – review & editing, Methodology, Conceptualization. **Maria Larsson:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Fredric Södergren-Seilitz:** Investigation, Formal analysis. **Jan Koschorreck:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Bernd Göckener:** Writing – review & editing, Resources. **Lutz Ahrens:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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Supporting Information

All data supporting the findings of this study are available within the paper and its Supplementary Information (SI), SI1 (the word file) and SI2 (spreadsheet excel file).

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.140993.

Data Availability

Data will be made available on request.

References

[1] Fan, J., Yang, J., Cheng, F., Zhang, S., 2023. The Source, distribution, and environmental effects of suspended particulate matter in the yangtze river system. Water 15. https://doi.org/10.3390/w15193429.

[2] He, Y., Song, K., Yang, C., Li, Y., He, W., Xu, F., 2021. Suspended particulate matter (SPM)-bound polycyclic aromatic hydrocarbons (PAHs) in lakes and reservoirs across a large geographical scale. Sci Total Environ 752, 142863. https://doi.org/10.1016/j.scitotenv.2020.142863.

[3] Huynh, T.T., Kim, J., Lee, S.D., Fettweis, M., Bi, Q., Kim, S., Lee, S., Choi, Y.Y., Nguyen, H.S., Bui, T.V., et al., 2024. Spatiotemporal Dynamics of Suspended Particulate Matter in Water Environments: A Review. Water 16. https://doi.org/10.3390/w16243613.

[4] Amalfitano, S., Corno, G., Eckert, E., Fazi, S., Ninio, S., Callieri, C., Grossart, H.-P., Eckert, W., 2017. Tracing particulate matter and associated microorganisms in freshwaters. Hydrobiologia 800, 145–154. https://doi.org/10.1007/s10750-017-3260-x.

[5] Niu, L., Gartner, A.A.E., Konig, M., Krauss, M., Spahr, S., Escher, B.I., 2025. Role of suspended particulate matter for the transport and risks of organic micropollutant mixtures in rivers: a comparison between baseflow and high discharge conditions. Environ Sci Technol 59, 4857–4867. https://doi.org/10.1021/acs.est.4c13378.

[6] Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2017. Spatial distribution of organic contaminants in three rivers of Southern England bound to suspended particulate material and dissolved in water. Sci Total Environ 593-594, 487–497. https://doi.org/10.1016/j.scitotenv.2017.03.167.

[7] Liu, X., Sheng, Y., Liu, Q., Li, Z., 2024. Suspended particulate matter affects the distribution and migration of heavy metals in the Yellow River. Sci Total Environ 912, 169537. https://doi.org/10.1016/j.scitotenv.2023.169537.

[8] Ahrens, L., Taniyasu, S., Yeung, L.W., Yamashita, N., Lam, P.K., Ebinghaus, R., 2010. Distribution of polyfluoroalkyl compounds in water, suspended particulate matter and sediment from Tokyo Bay, Japan. Chemosphere 79, 266–272. https://doi.org/10.1016/j.chemosphere.2010.01.045.

[9] Finckh, S., Carmona, E., Borchardt, D., Buttner, O., Krauss, M., Schulze, T., Yang, S., Brack, W., 2024. Mapping chemical footprints of organic micropollutants in European streams. Environ Int 183, 108371. https://doi.org/10.1016/j.envint.2023.108371.

[10] Soriano, Y., Carmona, E., Renovell, J., Pico, Y., Brack, W., Krauss, M., Backhaus, T., Inostroza, P.A., 2024. Co-occurrence and spatial distribution of organic micropollutants in surface waters of the River Aconcagua and Maipo basins in Central Chile. Sci Total Environ 954, 176314. https://doi.org/10.1016/j.scitotenv.2024.176314.

[11] Jiang, Z.-P., Tong, Y., Tong, M., Yuan, J., Cao, Q., Pan, Y., 2021. The Effects of suspended particulate matter, nutrient, and salinity on the growth of amphidinium carterae under estuary environmental conditions. Front Mar Sci 8. https://doi.org/10.3389/fmars.2021.690764.

[12] Koppe, T., Jewell, K.S., Ehlig, B., Wick, A., Koschorreck, J., Ternes, T.A., 2023. Identification and trend analysis of organic cationic contaminants via non-target screening in suspended particulate matter of the German rivers Rhine and Saar. Water Res 229, 119304. https://doi.org/10.1016/j.watres.2022.119304.

[13] Schwarzbauer, J., Littke, R., Weigelt, V., 2000. Identification of specific organic contaminants for estimating the contribution of the Elbe river to the pollution of the German Bight. Org Geochem 31, 1713–1731. https://doi.org/10.1016/s0146-6380(00)00076-0.

[14] Sibiya, I., Poma, G., Cuykx, M., Covaci, A., Daso Adegbenro, P., Okonkwo, J., 2019. Targeted and non-target screening of persistent organic pollutants and organophosphorus flame retardants in leachate and sediment from landfill sites in Gauteng Province, South Africa. Sci Total Environ 653, 1231–1239. https://doi.org/10.1016/j.scitotenv.2018.10.356.

[15] Terzic, S., Ahel, M., 2011. Nontarget analysis of polar contaminants in freshwater sediments influenced by pharmaceutical industry using ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry. Environ Pollut 159, 557–566. https://doi.org/10.1016/j.envpol.2010.10.009.

[16] Durig, W., Alygizakis, N.A., Wiberg, K., Ahrens, L., 2022. Application of a novel prioritisation strategy using non-target screening for evaluation of temporal trends (1969-2017) of contaminants of emerging concern (CECs) in archived lynx muscle tissue samples. Sci Total Environ 817, 153035. https://doi.org/10.1016/j.scitotenv.2022.153035.

[17] Hollender, J., Schymanski, E.L., Ahrens, L., Alygizakis, N., Béen, F., Bijlsma, L., Brunner, A.M., Celma, A., Fildier, A., Fu, Q., et al., 2023. NORMAN guidance on suspect and non-target screening in environmental monitoring. Environ Sci Eur 35. https://doi.org/10.1186/s12302-023-00779-4.

[18] Fliedner, A., Rudel, H., Gockener, B., Krehenwinkel, H., Paulus, M., Koschorreck, J., 2022. Environmental specimen banks and the European Green Deal. Sci Total Environ 852, 158430. https://doi.org/10.1016/j.scitotenv.2022.158430.

[19] Brack, W., Hollender, J., de Alda, M.L., Müller, C., Schulze, T., Schymanski, E., Slobodnik, J., Krauss, M., 2019. High-resolution mass spectrometry to complement monitoring and track emerging chemicals and pollution trends in European water resources. Environ Sci Eur 31. https://doi.org/10.1186/s12302-019-0230-0.

[20] Commission, E. Commission Implementing Decision (EU) 2025/439 of 28 February 2025 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C(2025) 1244). 2025.

[21] Commission, E. Commission Implementing Decision (EU) 2022/679 of 19 January 2022 establishing a watch list of substances and compounds of concern for water intended for human consumption as provided for in Directive (EU) 2020/2184 of the European Parliament and of the Council (notified under document C(2022) 142). 2022, OJ L 124, 41–43.

[22] Loos, R., Daouk, S., Marinov, D., Gomez, L., Porcel-Rodriguez, E., Sanseverino, I., Amalric, L., Potalivo, M., Calabretta, E., Ferencik, M., et al., 2024. Summary recommendations on "Analytical methods for substances in the Watch List under the Water Framework Directive". Sci Total Environ 912, 168707. https://doi.org/10.1016/j.scitotenv.2023.168707.

[23] Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol 48, 2097–2098. https://doi.org/10.1021/es5002105.

[24] Malm, L., Kruve, A., 2025. Do experimental projection methods outcompete retention time prediction models in non-target screening? A case study on LC/HRMS interlaboratory comparison data. Analyst 150, 3567–3577. https://doi.org/10.1039/d5an00323g.

[25] Wu, Y., Shi, Y., Gu, T., Du, X., Du, Z., Zhang, C., Sun, K., Zhang, Y., Guo, X., Wang, S., et al., 2024. Global trends and hotspots in non-targeted screening of water pollution research: bibliometric and visual analysis. Toxics 12. https://doi.org/10.3390/toxics12120844.

[26] Simon, F., Gehrenkemper, L., Becher, S., Dierkes, G., Langhammer, N., Cossmer, A., von der Au, M., Gockener, B., Fliedner, A., Rudel, H., et al., 2023. Quantification and characterization of PFASs in suspended particulate matter (SPM) of German rivers using EOF, dTOPA, (non-)target HRMS. Sci Total Environ 885, 163753. https://doi.org/10.1016/j.scitotenv.2023.163753.

[27] Gockener, B., Fliedner, A., Rudel, H., Badry, A., Koschorreck, J., 2022. Long-Term Trends of Per- and Polyfluoroalkyl Substances (PFAS) in Suspended Particular Matter from German Rivers Using the Direct Total Oxidizable Precursor (dTOP) Assay. Environ Sci Technol 56, 208–217. https://doi.org/10.1021/acs.est.1c04165.

[28] ICPR, I.CftPotR.. Water Quality of the Rhine. Available online: ⟨https://www.iksr.org/en/topics/water-quality/⟩ (accessed on 2025-06-11).

[29] MacErlean, F. Industrial pollution of the Rhine not improving. Available online: ⟨https://www.waternewseurope.com/industrial-pollution-of-the-rhine-not-improving/⟩ (accessed on 23 June 2025).

[30] Ricking, M., Keller, M., Heininger, P., Körner, A., 2017. *Richtlinie zur Probenahme und Probenbearbeitung Schwebstoffe*; Bundesanstalt für Gewässerkunde (BfG). Umweltbundesamt. UBA.

[31] Loffler, P., Rehnstam, S., Ahrens, L., Lai, F.Y., Celma, A., 2025. Long-Term System Suitability Evaluation for Mass Accuracy in the Analysis of Small Molecules by High-Resolution Mass Spectrometry. J Am Soc Mass Spectrom 36, 2005–2012. https://doi.org/10.1021/jasms.5c00128.

[32] Khalidi-Idrissi, A., Madinzi, A., Anouzla, A., Pala, A., Mouhir, L., Kadmi, Y., Souabi, S., 2023. Recent advances in the biological treatment of wastewater rich in emerging pollutants produced by pharmaceutical industrial discharges. Int J Environ Sci Technol (Tehran) 1–22. https://doi.org/10.1007/s13762-023-04867-z.

[33] Rubirola, A., Llorca, M., Rodriguez-Mozaz, S., Casas, N., Rodriguez-Roda, I., Barcelo, D., Buttiglieri, G., 2014. Characterization of metoprolol biodegradation and its transformation products generated in activated sludge batch experiments and in full scale WWTPs. Water Res 63, 21–32. https://doi.org/10.1016/j.watres.2014.05.031.

[34] Chaber-Jarlachowicz, P., Gworek, B., Kalinowski, R., 2025. Removal efficiency of pharmaceuticals during the wastewater treatment process: Emission and environmental risk assessment. PLoS One 20, e0331211. https://doi.org/10.1371/journal.pone.0331211.

[35] Fernandez-Garcia, A., Martinez-Piernas, A.B., Moreno-Gonzalez, D., Gilbert-Lopez, B., Garcia-Reyes, J.F., 2024. Chemical profiling of organic contaminants in rural surface waters combining target and non-target LC-HRMS/MS analysis. Sci Total Environ 954, 176587. https://doi.org/10.1016/j.scitotenv.2024.176587.

[36] Dolu, T., Nas, B., 2023. Dissemination of nonsteroidal anti-inflammatory drugs (NSAIDs) and metabolites from wastewater treatment plant to soils and agricultural crops via real-scale different agronomic practices. Environ Res 227, 115731. https://doi.org/10.1016/j.envres.2023.115731.

[37] Arora, U., Khuntia, H.K., Chanakya, H.N., Kapley, A., 2022. Surfactants: combating the fate, impact, and aftermath of their release in the environment. Int J Environ Sci Technol 20, 11551–11574. https://doi.org/10.1007/s13762-022-04549-2.

[38] Sanderson, H., Price, B.B., Dyer, S.D., DeCarvalho, A.J., Robaugh, D., Waite, S.W., Morrall, S.W., Nielsen, A.M., Cano, M.L., Evans, K.A., 2006. Occurrence and hazard screening of alkyl sulfates and alkyl ethoxysulfates in river sediments. Sci Total Environ 367, 312–323. https://doi.org/10.1016/j.scitotenv.2005.11.021.

[39] Scheurer, M.F.., Finnian; Alygizakis, Nikiforos A.; Slobodnik, Jaroslav; Oswald, Peter; Thomaidis, Nikolaos S. *Development of an analytical method for the quantification of surfactants and its application to wastewater treatment plant effluents*; TEXTE 108/2019; Dessau-Roßlau, Germany, 2019; p. 101.

[40] Ying, G.G.Fate, 2006. behavior and effects of surfactants and their degradation products in the environment. Environ Int 32, 417–431. https://doi.org/10.1016/j.envint.2005.07.004.

[41] Aurich, D., Diderich, P., Helmus, R., Schymanski, E.L., 2023. Non-target screening of surface water samples to identify exposome-related pollutants: a case study from Luxembourg. Environ Sci Eur 35. https://doi.org/10.1186/s12302-023-00805-5.

[42] Drees, F., Vosough, M., Schmidt, T.C., 2025. Unraveling non-target screening variability for LC-HRMS data: a chemometric comparative analysis of river water samples impacted by treated wastewater. Anal Bioanal Chem 417, 6075–6088. https://doi.org/10.1007/s00216-025-05966-1.

[43] Armin, R., Vosough, M., Schmidt, T.C., 2025. Exploring non-target screening variability in unsupervised multivariate time trend analysis of LC-HRMS data. Anal Bioanal Chem. https://doi.org/10.1007/s00216-025-06225-z.

[44] Schulze, S., Zahn, D., Montes, R., Rodil, R., Quintana, J.B., Knepper, T.P., Reemtsma, T., Berger, U., 2019. Occurrence of emerging persistent and mobile organic contaminants in European water samples. Water Res 153, 80–90. https://doi.org/10.1016/j.watres.2019.01.008.

[45] Montes, R., Mendez, S., Cobas, J., Carro, N., Neuparth, T., Alves, N., Santos, M.M., Quintana, J.B., Rodil, R., 2023. Occurrence of persistent and mobile chemicals and other contaminants of emerging concern in Spanish and Portuguese wastewater treatment plants, transnational river basins and coastal water. Sci Total Environ 885, 163737. https://doi.org/10.1016/j.scitotenv.2023.163737.

[46] ICPR), I.C.f.t.P.o.t.R. *Micropollutants in the Rhine Catchment – Evaluation of Long-Term Monitoring Data in Suspended Particulate Matter*; ICPR Report No. 246; Koblenz, Germany, 2023.

[47] Wu, X., Ren, J., Xu, Q., Xiao, Y., Li, X., Peng, Y., 2023. Priority screening of contaminant of emerging concern (CECs) in surface water from drinking water sources in the lower reaches of the Yangtze River based on exposure-activity ratios (EARs). Sci Total Environ 856, 159016. https://doi.org/10.1016/j.scitotenv.2022.159016.

[48] Hale, S.E., Arp, H.P.H., Schliebner, I., Neumann, M., 2020. Persistent, mobile and toxic (PMT) and very persistent and very mobile (vPvM) substances pose an equivalent level of concern to persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) substances under REACH. Environ Sci Eur 32. https://doi.org/10.1186/s12302-020-00440-4.

[49] Neuwald, I., Muschket, M., Zahn, D., Berger, U., Seiwert, B., Meier, T., Kuckelkorn, J., Strobel, C., Knepper, T.P., Reemtsma, T., 2021. Filling the knowledge gap: A suspect screening study for 1310 potentially persistent and mobile chemicals with SFC- and HILIC-HRMS in two German river systems. Water Res 204, 117645. https://doi.org/10.1016/j.watres.2021.117645.

[50] Rummel, C.D., Schafer, H., Jahnke, A., Arp, H.P.H., Schmitt-Jansen, M., 2022. Effects of leachates from UV-weathered microplastic on the microalgae Scenedesmus vacuolatus. Anal Bioanal Chem 414, 1469–1479. https://doi.org/10.1007/s00216-021-03798-3.

[51] Narloch, I., Wejnerowska, G., 2021. An overview of the analytical methods for the determination of organic ultraviolet filters in cosmetic products and human samples. Molecules 26. https://doi.org/10.3390/molecules26164780.

[52] Badmus, S.O., Amusa, H.K., Oyehan, T.A., Saleh, T.A., 2021. Environmental risks and toxicity of surfactants: overview of analysis, assessment, and remediation techniques. Environ Sci Pollut Res Int 28, 62085–62104. https://doi.org/10.1007/s11356-021-16483-w.

[53] Jardak, K., Drogui, P., Daghrir, R., 2016. Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes. Environ Sci Pollut Res Int 23, 3195–3216. https://doi.org/10.1007/s11356-015-5803-x.

[54] Kummerer, K., 2009. The presence of pharmaceuticals in the environment due to human use–present knowledge and future challenges. J Environ Manag 90, 2354–2366. https://doi.org/10.1016/j.jenvman.2009.01.023.

[55] Huangfu, Y., Li, Q., Yang, W., Bu, Q., Yang, L., Tang, J., Gan, J., 2024. Occurrence, Source Apportionment, and Ecological Risk of Typical Pharmaceuticals in Surface Waters of Beijing, China. Toxics 12. https://doi.org/10.3390/toxics12030171.

[56] Malnes, D., Ahrens, L., Kohler, S., Forsberg, M., Golovko, O., 2022. Occurrence and mass flows of contaminants of emerging concern (CECs) in Sweden's three largest lakes and associated rivers. Chemosphere 294, 133825. https://doi.org/10.1016/j.chemosphere.2022.133825.

[57] Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment–a review. Sci Total Environ 429, 123–155. https://doi.org/10.1016/j.scitotenv.2012.04.028.

[58] Turrio-Baldassarri, L., Battistelli, C.L., Conti, L., Crebelli, R., De Berardis, B., Iamiceli, A.L., Gambino, M., Iannaccone, S., 2006. Evaluation of emission toxicity of urban bus engines: compressed natural gas and comparison with liquid fuels. Sci Total Environ 355, 64–77. https://doi.org/10.1016/j.scitotenv.2005.02.037.

[59] Hanus, L., Abu-Lafi, S., Fride, E., Breuer, A., Vogel, Z., Shalev, D.E., Kustanovich, I., Mechoulam, R., 2001. 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc Natl Acad Sci USA 98, 3662–3665. https://doi.org/10.1073/pnas.061029898.

[60] Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res 30, 2893–2900. https://doi.org/10.1016/s0043-1354(96)00011-5.

[61] ICPR, I.CftPotR.. How is the Rhine today? ICPR publishes technical reports on the ecology and water quality of the Rhine. Available online: ⟨https://www.iksr.org/en/press/press-releases/press-releases-individual-presentation/how-is-the-rhine-today-icpr-publishes-technical-reports-on-the-ecology-and-water-quality-of-the-rhine⟩ (accessed on 2025-06-11).

[62] Weidemann, E., Niinipuu, M., Fick, J., Jansson, S., 2018. Using carbonized low-cost materials for removal of chemicals of environmental concern from water. Environ Sci Pollut Res Int 25, 15793–15801. https://doi.org/10.1007/s11356-018-1781-0.

[63] Cao, G., Wang, W., Zhang, J., Wu, P., Zhao, X., Yang, Z., Hu, D., Cai, Z., 2022. New Evidence of Rubber-Derived Quinones in Water, Air, and Soil. Environ Sci Technol 56, 4142–4150. https://doi.org/10.1021/acs.est.1c07376.

[64] Fu, C., Yu, L., Miao, Y., Liu, X., Yu, Z., Wei, M., 2023. Peptide-drug conjugates (PDCs): a novel trend of research and development on targeted therapy, hype or hope? Acta Pharm Sin B 13, 498–516. https://doi.org/10.1016/j.apsb.2022.07.020.

[65] Killian, J.A., Nyholm, T.K., 2006. Peptides in lipid bilayers: the power of simple models. Curr Opin Struct Biol 16, 473–479. https://doi.org/10.1016/j.sbi.2006.06.007.

[66] Daniel, O., Thiebault, T., Guigon, E., 2025. Medicinal applications and environmental fate of antimicrobial peptides: a review. Environ Chem Lett. https://doi.org/10.1007/s10311-025-01854-3.

[67] Pham, H.D., Dang, T.H.M., Nguyen, T.T.N., Nguyen, T.A.H., Pham, T.N.M., Pham, T.D., 2021. Separation and determination of alkyl sulfate surfactants in wastewater by capillary electrophoresis coupled with contactless conductivity detection after preconcentration by simultaneous adsorption using alumina beads. Electrophoresis 42, 191–199. https://doi.org/10.1002/elps.202000086.

[68] Oda, Y., Miyabara, Y., 2023. Grazing inhibition in Daphnia and Bosmina by colony formation of Desmodesmus subspicatus triggered by sodium octyl sulfate. Ecotoxicology 32, 884–894. https://doi.org/10.1007/s10646-023-02694-z.

[69] Salatin, S., Farjami, A., Siahi-Shadbad, M., Hamidi, S., 2023. Biological Stability of Peptides/Proteins Therapeutic Agents. Int J Pept Res Ther 29. https://doi.org/10.1007/s10989-023-10549-8.

[70] Eggen, R.I., Hollender, J., Joss, A., Scharer, M., Stamm, C., 2014. Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ Sci Technol 48, 7683–7689. https://doi.org/10.1021/es500907n.

[71] Stockholm-Convention. Governments unite to step-up reduction on global DDT reliance and add nine new chemicals under international treaty. Available online: ⟨https://chm.pops.int/Convention/Pressrelease/COP4Geneva9May2009/tabid/542/language/en-US/Default.aspx⟩ (accessed on June 11).

[72] Badry, A., Lämmer, R., Göckener, B., Koschorreck, J., 2025. Pre-regulatory actions as a driver for reduced PFAS emissions? long-term trends and change points for human and environmental samples from Germany. Environ Sci Eur 37. https://doi.org/10.1186/s12302-025-01124-7.

[73] European-Commission-Regulation, 2019. Comm Regul (EU) 2019/698 30 April 2019 Amend Annex III V Regul (EC) No 1223/2009 Cosmet Prod (Text EEA Relev) 66–69.

[74] European-Commission. Proposal for a Directive concerning urban wastewater treatment (recast). 2022.

[75] European-Commission. Commission Decision of 6 June 2007 concerning the non-inclusion of diazinon in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance (2007/393/EC). 2007, L 148, 149–110.

[76] European-Commission-Regulation. Commission Regulation (EU) 2023/1783 of 15 September 2023 amending Annexes II and V to Regulation (EC) No 396/2005 as regards maximum residue levels for denatonium benzoate, diuron, etoxazole, methomyl and teflubenzuron in or on certain products. 2023, L 229, 263–288.

[77] U.S.EPA. Ecological Structure Activity Relationships (ECOSAR) Predictive Model. Available online: ⟨https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model⟩ (accessed on 2025.08.06).

[78] EPA, U.S., 2004. Quantitative Risk Assessment. Calculations.

[79] Salmani, M.H., Garzegar, S., Ehrampoush, M.H., Askarishahi, M., 2021. Predicting anionic surfactant toxicity to Daphnia magna in aquatic environment: a green approach for evaluation of EC(50) values. Environ Sci Pollut Res Int 28, 50731–50746. https://doi.org/10.1007/s11356-021-14107-x.

[80] Chalew, T.E., Halden, R.U., 2009. Environmental exposure of aquatic and terrestrial biota to triclosan and triclocarban. J Am Water Works Assoc 45, 4–13. https://doi.org/10.1111/j.1752-1688.2008.00284.x.

[81] Pycke, B.F., Roll, I.B., Brownawell, B.J., Kinney, C.A., Furlong, E.T., Kolpin, D.W., Halden, R.U., 2014. Transformation products and human metabolites of triclocarban and triclosan in sewage sludge across the United States. Environ Sci Technol 48, 7881–7890. https://doi.org/10.1021/es5006362.

[82] Milanovic, M., Duric, L., Milosevic, N., Milic, N., 2023. Comprehensive insight into triclosan-from widespread occurrence to health outcomes. Environ Sci Pollut Res Int 30, 25119–25140. https://doi.org/10.1007/s11356-021-17273-0.

[83] Okamba-Diogo, O., Richaud, E., Verdu, J., Fernagut, F., Guilment, J., Pery, F., Fayolle, B., 2016. Quantification of hindered phenols in polyamide 11 during thermal aging. Polym Test 52, 63–70. https://doi.org/10.1016/j.polymertesting.2016.03.023.

[84] Holmquist, H., Lexén, J., Rahmberg, M., Sahlin, U., Palm, J.G., Rydberg, T., 2018. The potential to use QSAR to populate ecotoxicity characterisation factors for

simplified LCIA and chemical prioritisation. Int J Life Cycle Assess 23, 2208–2216. https://doi.org/10.1007/s11367-018-1452-x.

[85] Najam, L., Alam, T., 2023. Occurrence, distribution, and fate of emerging persistent organic pollutants (POPs) in the Environment. In: Aftab, T. (Ed.), Emerging Contaminants and Plants. Emerging Contaminants and Associated Treatment Technologies; Springer, Cham.

[86] Rudel, H., Bohmer, W., Schroter-Kermani, C., 2006. Retrospective monitoring of synthetic musk compounds in aquatic biota from German rivers and coastal areas. J Environ Monit 8, 812–823. https://doi.org/10.1039/b602389b.

[87] Pitchakarn, P., Ting, P., Buacheen, P., Karinchai, J., Inthachat, W., Chantong, B., Suttisansanee, U., Nuchuchua, O., Temviriyanukul, P., 2024. Multi-endpoint toxicological assessment of chrysin loaded oil-in-water emulsion system in different biological models. Nanomater (Basel) 14. https://doi.org/10.3390/nano14121001.