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Övervakning för bevakningslistan 2022-2023
Monitoring for the WFD watch list 2022-2023

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Sammanfattning

Huvudsyftet med projektet var att undersöka förekomsten av utvalda ämnen från EU Vattendirektivs bevakningslista (n=21), inklusive sulfametoxazol, trimetoprim, venlafaxin, O-desmetylvenlafaxin, klindamycin, metformin, guanylurea, flukonazol, klotrimazol, imazalil, ipkonazol, metkonazol, mikonazol, prokloraz, tebukonazol, penkonazol, fipronil, tetrakonazol, butyl metoxydibensoylmetan (avobenzon), oktokrylen och bensofenon-3 i sju svenska avrinningsområden påverkade av reningsverk (WWTPs). Andra organiska mikroföreningar (OMPs) inkluderades också, såsom läkemedel, personliga vårdprodukter, industrikemikalier och PFAS, på grund av deras potentiellt skadliga effekter på miljön även vid låga doser. Ämnen som sulfametoxazol, trimetoprim, venlafaxin och flukonazol upptäcktes konsekvent nedströms reningsverk, vilket indikerar ofullständig rening i avloppsvattenbehandling. Säsongsfaktorer, som minskat vattenflöde under sommaren, verkar öka förekomsten av dessa föroreningar. Metformin och dess nedbrytningsprodukt guanylurea hittades i höga koncentrationer på grund av dess omfattande användning och miljöbeständighet. De högsta PFAS-nivåerna återfanns i Sagån, där PFHxA, PFHxS, PFHpA och PFBS var de dominerande föreningarna. Variationen i PFAS-koncentrationer kopplades till faktorer som snösmältning och begränsningarna med provtagning med stickprov, som kan överskatta eller underskatta kortvariga föroreningstoppar. Höga nivåer av OMPs, särskilt tolytriazol, upptäcktes i avloppsvatten från reningsverk, tillsammans med vanliga läkemedel som tramadol och metoprolol. 1,4-Dioxan fanns på alla undersökta platser, vilket indikerar dess beständighet i vattenmiljöer. Upptäckten av 6PPD och 6PPD-kinon, särskilt i ytvatten från vägavrinning, väcker oro på grund av deras toxicitet för

vattenlevande organismer. Studien understryker behovet av mer frekvent och omfattande övervakning för att bättre förstå beteendet och miljöriskerna med beständiga föroreningar som 1,4-Dioxan och 6PPD-kinon.

Summary

The main aim of the project was to investigate the occurrence of selected EU Water Framework Watch List substances (n=21) which include sulfametoxazol, trimetoprim, venlafaxin, O-desmetylvenlafaxin, clindamycin, metformin, guanylurea, fluconazole, clotrimazole, imazalil, ipconazole, metconazole, miconazole, procloraz, tebuconazole, penconazole, fipronil, tetraconazole, butyl methoxydibenzoyl-methane (avobenzone), octocrylene and benzophenone-3 in seven Swedish river catchments impacted by WWTPs. Other OMPs were also included, such as pharmaceuticals, personal care products, industrial chemicals, and PFASs, due to potential of their adverse effects at low doses to the environment. Compounds such as sulfamethoxazole, trimethoprim, venlafaxine, and fluconazole were consistently detected downstream, indicating incomplete removal during wastewater treatment. Seasonal factors, such as reduced water flow in summer, appear to increase contaminant persistence. Metformin and its byproduct, guanyl urea, were found in high concentrations due to widespread use and environmental persistence. The highest PFAS levels were found in Sagån, with PFHxA, PFHxS, PFHpA, and PFBS being the dominant compounds. PFAS concentration variability was linked to factors like snowmelt dilution and the limitations of grab sampling. High levels of OMPs, particularly tolytriazole, were detected in WWTP effluents, along with common pharmaceuticals such as tramadol and metoprolol. 1,4-Dioxane was present at all sites, indicating its persistence in aquatic environments. The detection of 6PPD and 6PPD-quinone, particularly in surface waters from road runoff, raises concerns due to their toxicity to aquatic organisms. The study highlights the need for more frequent, comprehensive monitoring to better understand the behavior and environmental risks of persistent contaminants like 1,4-Dioxane and 6PPD-quinone.

Sammanfattning

Huvudsyftet med projektet var att undersöka förekomsten av utvalda ämnen från EU Vattendirektivs bevakningslista (n=21), inklusive sulfametoxazol, trimetoprim, venlafaxin, O-desmetylvenlafaxin, klindamycin, metformin, guanylurea, flukonazol, klotrimazol, imazalil, ipkonazol, metkonazol, mikonazol, prokloraz, tebukonazol, penkonazol, fipronil, tetrakonazol, butyl metoxydibensoylmetan (avobenzon), oktokrylen och bensofenon-3 i sju svenska avrinningsområden påverkade av reningsverk (WWTPs). Andra organiska mikroföreningar (OMPs) inkluderades också, såsom läkemedel, personliga vårdprodukter, industrikemikalier och PFAS, på grund av deras potentiellt skadliga effekter på miljön även vid låga doser. Ämnen som sulfametoxazol, trimetoprim, venlafaxin och flukonazol upptäcktes konsekvent nedströms, vilket indikerar ofullständig rening i avloppsvattenbehandling. Säsongsfaktorer, som minskat vattenflöde under sommaren, verkar öka beständigheten av dessa föroreningar. Metformin och dess nedbrytningsprodukt guanylurea hittades i höga koncentrationer på grund av dess omfattande användning och miljöbeständighet. De högsta PFAS-nivåerna återfanns i Sagån, där PFHxA, PFHxS, PFHpA och PFBS var de dominerande föreningarna. Variationen i PFAS-koncentrationer kopplades till faktorer som snösmältning och begränsningarna med provtagning med ögonblicksbilder, som kan överskatta eller underskatta kortvariga föroreningstoppar. Höga nivåer av OMPs, särskilt tolytriazol, upptäcktes i avloppsvatten från reningsverk, tillsammans med vanliga läkemedel som tramadol och metoprolol. 1,4-Dioxan fanns på alla undersökta platser, vilket indikerar dess beständighet i vattenmiljöer. Upptäckten av 6PPD och 6PPD-kinon, särskilt i ytvatten från vägavrinning, väcker oro på grund av deras toxicitet för vattenlevande organismer. Studien understryker behovet av mer frekvent och omfattande övervakning för att bättre förstå beteendet och miljöriskerna med beständiga föroreningar som 1,4-Dioxan och 6PPD-kinon.

1. Introduction

Currently, urban wastewater is recognized as a primary source of organic micropollutants (OMPs) in aquatic environments (1, 2). These pollutants include pharmaceuticals, personal care products, stimulants, per- and polyfluoroalkyl substances (PFASs), and hormones. The potential human and ecological adverse effects resulting from the presence of these compounds in aquatic environments remain largely unknown (3, 4). Many OMPs are not removed in conventional treatment processes and/or are accumulated in quantities that pose challenges to wastewater treatment plants (WWTPs). During wastewater treatment, some OMPs undergo degradation or transformation via chemical or biological processes, while others are got removed from the aqueous phase through adsorption onto solid matrices (5, 6). Nevertheless, a substantial fraction of OMPs persist through treatment processes and are subsequently discharged into the environment (6, 7). The Water Framework Directive aims to assess Member States for environmental pollutants to identify chemicals potentially hazardous to human health or the environment. The EU Watch List includes 22 substances (8).

The primary aim of this project, as a continuation of previous monitoring efforts (9), was to investigate the occurrence of selected EU Watch List substances (n=21) which include pharmaceuticals (sulfametoxazol, trimetoprim, venlafaxin, O-desmetylvenlafaxin, clindamycin, metformin, guanylurea), pesticides (fluconazole, clotrimazole, imazalil, ipconazole, metconazole, miconazole, procloraz, tebuconazole, penconazole, fipronil and tetraconazole) and three UV filters butyl methoxydibenzoyl-methane (avobenzone), octocrylene, benzophenone-3 in seven Swedish river catchments impacted by WWTPs, including other OMPs (pharmaceuticals, personal care products, industrial chemicals, PFASs).

Table 1. Target compounds from the watch list (2022/1307/EU)

Compound	Class	Highest accepted LOD or LOQ* (ng/L)
Sulfametoxazol	Antibiotic	100
Trimetoprim	Antibiotic	100
Venlafaxin	Antidepressant	6
O-desmetylvenlafaxin	Metabolite of Antidepressant	6
Klotrimazol	Antifungal	20
Flukonazol	Antifungal	250
Imazalil	Fungicide	800
Ipkonazol	Fungicide	44
Metkonazol	Fungicide	29
Mikonazol	Antifungal	200

Penkonazol	Fungicide	1 700
Prokloraz	Fungicide	161
Tebukonazol	Fungicide	240
Tetrakonazol	Fungicide	1 900
Fipronil	Veterinary pharmaceutical (insecticide)	0,77*
Clindamycin	Antibiotic	44*
Ofloxacin	Antibiotic	26*
Metformin	Pharmaceutical	156 000*
Guanylurea	Metabolite of metformin	100 000*
Butyl methoxydibenzoyl-methane	UV-filter	3 000*
Octocrylene	UV-filter	266*
Benzophenone-3	UV-filter	670*

2. Material and methods

2.1 Target OMPs and other chemicals

All analytical standards used for analysis were of high purity grade (>95%). Native standards (*n*=96) were acquired from Sigma-Aldrich (Sweden). Isotopically labeled standards (IS) (*n*=30) for the target compounds were obtained from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), Sigma-Aldrich, and Toronto Research Chemicals (Toronto, Canada). Detailed information about internal standards (IS) and native standards can be found elsewhere (10). The target OMPs were selected for analysis, based on occurrence and distribution in the aquatic environment, and production and consumption patterns (5, 11), Table S1 in the Appendix. Ultrapure water was generated by a Milli-Q (MQ) Advantage Ultrapure Water purification system and filtered through a 0.22 µm Millipak Express membrane and an LC-Pak polishing unit (Merk Millipore, Billercia, MA). Methanol, acetonitrile, formic acid and ammonia of high analytical grade were acquired from Sigma-Aldrich (Sweden).

The 1,4-dioxane analysis was conducted by a commercial lab in Karlsruhe, Germany (https://tzw.de/) whereby headspace-GC-MS (standard method DIN 38407-43:2014) was used.

2.2 Sampling

In this study, seven river catchments were monitored. The WWTPs were selected based on previous screening campaign which were performed by SLU from 2018 till 2020 (11, 12). The sites were selected based on different scenarios including population density, waste source, WWTPs with potentially large impacts on the receiving water body, and flow conditions (Table 2). Sampling was performed for four sampling events for rivers and the wastewater effluent (November 2022, April 2023, July 2023 and November 2023) (in total *n*=84 samples). Surface water samples (0.1 m below the water surface) were collected in the recipient of WWTPs (both downstream and upstream of the WWTP effluent entry point). All samples were collected as grab samples in polypropylene bottles.
After collection the samples were stored frozen (-20^{o}C) in darkness until extraction.

Table 2. Sampling sites for the EU Watch List compounds monitoring.

Sampling sites	Reason for sampling	Person equivalent	Distance, sampling point to WWTP [km]
Fyrisån Flottsund	Recipient of WWTPs	180 000	6.6
Örsundaån	Agricultural run-offs, many unconnected wastewater effluents, recipient of many minor WWTPs	>2 000	0.3
Enköpingsån	Possibly recipient of WWTP	18 000	0.5
Lövstaån (Knivsta)	Recipient of WWTP and close to raw water intake for drinking WTP.	12 000	7.6
Ösan, bron vid Asketorp	Recipient of WWTP containing hospital's wastewater.		2.7
Kolbäcksån Strömsholm	Recipient of two WWTPs	21 500	3.4
Sagån	Small WWTP	>2 000	0.07

2.3 Sample preparation, instrumental analysis of OMPs and quality control

Water samples from rivers (500 mL) and wastewater effluents (200 mL) were extracted by solid-phase extraction (SPE) using Oasis HLB-cartridges (6 mL, 200 mg, 30 μm) following the procedure described by Sörengård et al. (2019). Samples were analysed using a DIONEX UltiMate 3000 ultraperformance liquid chromatograph (UPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer (MS/MS) (TSQ Quantiva, Thermo Fischer Scientific, Waltham, MA, USA). The data were evaluated with Tracefinder 4.1 (Thermo Fischer Scientific, MA, USA). Detailed information regarding instrument configuration and analysis is described elsewhere (5, 12). Table S2 in the Appendix presents results for individual concentrations of selected compounds in wastewater effluent and in recipient waters. Information about average limit of quantification (LOQ, ng/L), average absolute recovery and matrix effect of studied OMPs can be found in Table S3 in the Appendix.

3. Results and discussion

3.1 Occurrence of OMPs in wastewater effluent and in recipient waters (upstream and downstream)

Table S2 in the Appendix presents results for individual concentrations of selected compounds in wastewater effluent and in recipient waters. The compounds were divided into three groups:

1) EU Watch List compounds (n=21) which include pharmaceuticals (sulfamethoxazole, trimethoprim, venlafaxine, O-desmetylvenlafaxine, clindamycin, metformin, guanylurea), pesticides (fluconazole, clotrimazole, imazalil, ipconazole, metconazole, miconazole, procloraz, tebuconazole, penconazole, fipronil and tetraconazole) and three UV filters butyl methoxydibenzoyl-methane, octocrylene and benzophenone-3). Tetraconazole, penconazole and ipconazole were below their respective limit of quantification (LOQ) in all analyzed samples, Table S2 in the Appendix.

Metconazole was found in only one upstream sample at 3 ng/L (sampling location Örsundaån, November 2022). Prochloraz was found in two upstream samples, at 2.4 ng/L (sampling location Örsundaån, November 2023) and 2.3 ng/L (sampling location Sagån, November 2023), respectively. Benzophenone-3 was found in only one downstream sample, at 5 ng/L, (sampling location Sagån, November 2022).

2) PFASs (*n*=14) (Table S2 in the Appendix), PFBA were not analyzed due to analytical challenges. PFTeDA was found in one upstream sample only at 1.1 ng/L (sampling location Knivstaån, April 2023). PFUnDA was found in two samples:

- effluent sample from Örsundsbro WWTP, November 2022, 2.1 ng/L and

- upstream surface water from sample Knivstaån, April 2023, 3.7 ng/L.

3) Other OMPs (*n*=62) including pharmaceuticals, personal care products, stimulants etc. (Table S2 in the Appendix). Chloramphenicol, simvastatin and ranitidine were below LOQ in all analyzed samples, Table S2 in the Appendix. Erythromycin was found in two wastewater effluent samples which were collected in July 2023, at concentration 77 ng/L (sampling location Kungsängen) and concentration 95 ng/L (sampling location Knivsta), respectively.

Throughout the sampling campaign, the upstream locations consistently exhibited the lowest concentrations of all studied compounds. The presence of OMPs upstream of the WWTP can be attributed to contamination originating from other sources further upstream in the river (11, 13).

In general, the highest concentrations of OMPs were observed in the effluent, followed by the downstream locations. Seasonal variations were observed for most substances, with higher concentrations detected in June and November compared to other sampling months. This pattern may be explained by annual fluctuations in river discharge, suggesting that prevailing weather conditions significantly influence the levels of OMPs in the rivers (9).

It is important to note that OMPs were analyzed using a multi-residue analytical method, which includes a large number of compounds (n=96 native compounds) in a single run. While this approach is efficient in terms of sample usage, solvent consumption, time, and analytical costs, it may not provide the sensitivity or specificity required for some of compounds. Therefore, there is a need to develop more targeted, reliable, and accurate analytical methods specifically optimized for the detection and quantification of specific compounds across various environmental compartments (14, 15).

3.1.1 Occurrence of EU Watch List compounds in wastewater effluent and in recipient waters (upstream and downstream)

EU Watch List compounds (*n*=21) pharmaceuticals (sulfamethoxazole, trimethoprim, venlafaxine, O-desmetylvenlafaxine, clindamycin, metformin, guanylurea), pesticides (fluconazole, clotrimazole, imazalil, ipconazole, metconazole, miconazole, procloraz, tebuconazole, penconazole, fipronil and tetraconazole) and three UV filters (avobenzone - butyl methoxydibenzoyl-methane, octocrylene and benzophenone-3) were studied in wastewater effluent and in recipient waters, Table 3.

Table 3. Detection frequency (DF, %), average (AV), maximum (MAX) and minimum (MIN) concentrations (ng/L) of EU Watch List compounds in wastewater effluent (WW) and in recipient waters (upstream - U and downstream - D).

Compounds	Wastewater effluent				Upstream				Downstream			
	DF_WW	AV_WW	MAX_WW	Min_WW	DF_U	AV_U	MAX_U	Min_U	DF_D	AV_D	MAX_D	Min_D
Sulfamethoxazole	100%	251	1300	41	21%	4	5	2	71%	44	230	2
Trimethoprim	100%	261	1300	12	32%	15	23	2	79%	35	130	3
Venlafaxine	100%	410	850	55	39%	4	9	2	93%	45	290	2
Desvenlafaxine	100%	229	530	23	21%	4	6	2	86%	32	250	3
Clindamycin	100%	37	190	4	0%	-	-	-	36%	11	36	2
Fluconazole	96%	82	210	7	0%	-	-	-	25%	24	69	7
Tebuconazole	82%	8	18	3	21%	8	32	2	36%	7	20	2
Imazalil	75%	5	14	2	0%	-	-	-	0%	-	-	0
Fipronil	86%	6	19	2	0%	-	-	-	7%	3	4	2
Clotrimazole	25%	10	24	2	25%	8	13	5	25%	7	11	3
Miconazole	25%	18	40	3	25%	12	24	6	25%	11	20	4
Octocrylene	18%	6	12	3	4%	3	3	3	11%	3	3	3
Avobenzone (butyl methoxydibenzoylmethane)	25%	5	6	3	25%	3	7	2	25%	4	6	1
Metformin	100%	171	1000	7	61%	4	9	2	86%	8	24	2
Tetraconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Penconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Ipconazole	0%	-	-	-	0%	-	-	-	0%	-	-	-
Guanyl urea	100%	2788	6500	22	32%	14	59	3	68%	214	1200	3
Metconazole	0%	-	-	-	4%	3	3	3	0%	-	-	-
Procloraz	0%	-	-	-	7%	2	2	2	0%	-	-	-
Benzophenone-3	0%	-	-	-	0%	-	-	-	7%	5.35	5.7	5

Combined concentrations of the Watch list compounds in WWTP effluent samples is shown in Figure 1A and the highest concentration levels were detected in WWTP effluents emitted to Ösan in July 2023. This location showed the highest concentrations in the sample collected in July 2023, dominated by venlafaxine, sulfamethoxazole and trimethoprim. In Ösan, the total concentration in WWTP effluents reached up to 5 µg/l with the highest contributions from antibiotics (sulfamethoxazole and trimethoprim), venlafaxine and its metabolite desvenlafaxine and

fluconazole, Figure 1A. Fyrisån showed a consistent pattern with moderate concentrations across different sampling events (except of sampling in July 2023), with significant amounts of sulfamethoxazole, trimethoprim, and venlafaxine. Enköpingsån was similar to Fyrisån, with moderate concentrations and a noticeable presence of venlafaxine. Örsundån showed lower concentrations compared to other locations. Kolbäcksån showed low to moderate concentrations, similar to Sagån, with a significant presence of venlafaxine, desvenlafaxine and sulfamethoxazole.

Variations in reported concentrations of Watch list compounds in wastewater effluents across different locations can be attributed to several factors, including differences in medicine consumption patterns or applications, the type of influent received (it was not studied in this project), the efficiency of the treatment technologies employed, the size of the WWTP, and the population served by the plant (16-20).

Combined concentrations of the Watch list compounds in surface water samples is shown in Figure 1B and it highlights variability in contamination levels, with notable spikes at Enköpingsån, Knivstaån and Sagån in their respective downstream samples collected in November 2022 and July 2023. The data indicates that certain chemicals, such as sulfamethoxazole and venlafaxine, were consistently present, but their concentrations can vary significantly depending on the location and sampling time. For the downstream samples most of the compounds showed higher concentrations in November and July compared to the other sampling periods.

Fyrisån showed low concentrations across different sampling events, with higher amounts of sulfamethoxazole, trimethoprim, and venlafaxine in July 2023. Örsundaån and Kolbäcksån showed similar low concentrations in downstream samples. Enköpingsån showed a significant spike in concentration for downstream sample collected in November 2022 and July 2023, dominated by venlafaxine, sulfamethoxazole, and desvenlafaxine. Knivstaån, Ösan and Sagån showed similar pater of the watch list compounds with pick concentrations in downstream samples collected in November and July.

The most frequently detected compounds were sulfamethoxazole, venlafaxine, desvenlafaxine and fluconazole. These compounds are often detected in municipal waste water and surface waters globally (21, 22). Venlafaxine and desvenlafaxine were detected in 77% and 69% of all analyzed samples, in the range of <LOQ up to 850 ng/L and 530 ng/L, respectively. Their concentrations in the downstream river samples were remarkably higher than in the upstream sampling locations, suggesting that their presence is related to wastewaters and their incomplete removal in WWTPs (5, 11, 23). These results are similar to our previously published report where the same monitoring program was performed (9). These results are consistent with findings from a recently published study that investigated watch list compounds in surface waters in Italy (14)

Tebuconazole and imazalil were found only in wastewater effluent samples with maximum concentrations 32 ng/L and 14 ng/L respectively. It is interesting to mention that clotrimazole and miconazole were detected in only November 2023 samples (wastewater effluent, upstream and downstream samples) in all studied locations, Figure 1. Further investigation is required to

determine whether this observation represents a genuine pattern or is attributable to analytical artifacts, such as sample contamination or other methodological issues.

Fluconazole was mostly detected in wastewater effluent samples in most of studied locations (Figure 1A), likely reflecting that fluconazole is intended for systemic use and the many products available for human use on the Swedish market (24). The azole substances have previously been reported to have seasonally high concentrations in surface waters during spring or summer in Switzerland (25). Clotrimazole and miconazole are currently available on the Swedish market for treatment of fungus (26) or humans and animals. Both are used for foot fungus in humans, and clotrimazole is also used for vaginal fungal infections. Whether clotrimazole and miconazole have a reoccurring trend for their detection during winter months in Sweden remains to be more closely studied.

The mode of action of azole compounds suggests a potential to disrupt endocrine systems in various organisms, raising significant environmental concerns (27-30). The presence of azole antifungals, including ketoconazole, clotrimazole, miconazole, itraconazole, and econazole, has been documented in multiple environmental matrices, particularly in wastewater and surface water (27-30). Although the concentrations of azole antifungals are generally low (ranging from ng/L to µg/L), the continuous discharge of trace amounts into the environment may lead to cumulative, unforeseen ecological impacts. Conducting risk assessments for azole antifungal drugs is therefore crucial to evaluate the likelihood of harmful effects resulting from their toxicity.

Fipronil was found in most of wastewater effluent samples collected in all studied locations and in surface water samples it was found only in downstream river samples collected in November 2023 from Enköpingsån location.

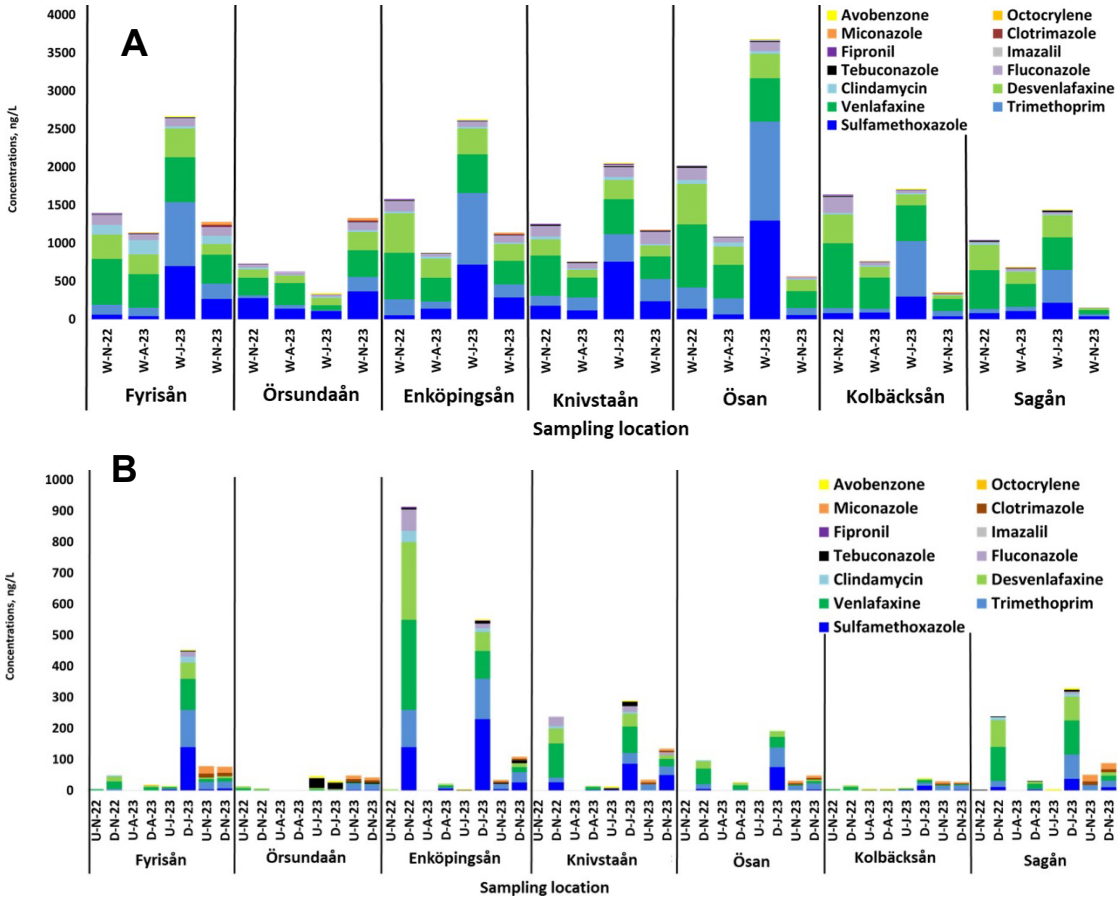
The antidiabetic drug metformin and its metabolite guanyl urea exhibited similar distribution patterns in both wastewater and receiving water samples, consistent with other compounds from the watch list. However, their concentration levels were significantly higher, Table 3. The maximum concentrations detected in wastewater effluent were 1000 ng/L for metformin and 6500 ng/L for guanyl urea. In the downstream receiving waters, the maximum concentrations were 24 ng/L for metformin and 1200 ng/L for guanyl urea, respectively (Table 3, Figure 1C and 1D). Similar findings were reported in Germany, where surface water monitoring showed higher concentrations of guanyl urea compared to its parent compound, metformin (31).

The extensive prescription and global use of metformin are well-documented in the literature and are reflected in its frequent detection at elevated concentrations in wastewater and receiving waters (31-33). Metformin has higher solubility (300 g/L) and lower Kow (-4.9) values which explain its greater distribution in the aqueous phase, contributing to the presence of metformin in water bodies and aqueous mobility is expected to be high.

The presence of metformin in surface waters is largely influenced by its consumption volume, removal efficiency in WWTPs, and environmental persistence. However, there are significant

analytical challenges in the detection of metformin and its transformation product, guanyl urea, particularly in complex wastewater and surface water matrices (15). Chromatographic separation of these highly polar compounds, especially in the presence of other contaminants, remains a considerable obstacle for analytical chemists. While several analytical methods exist for detecting these compounds in environmental samples, their extreme polarity complicates detection limits and elution times. Additionally, reproducibility and recovery rates pose further challenges, underscoring the need for careful method optimization when analyzing samples from surface waters and wastewater.

The results show that the occurrence of EU Watch List compounds varies with season (Figure 1). For the wastewater samples most of the compounds showed higher concentrations in July compared to the other sampling periods. It could be explained by the fact that water levels decrease during the summer due to lower rainfall or drought conditions. As a result, wastewater effluent may be less diluted, leading to relatively higher concentrations of some of compounds compared to wetter seasons (12, 34). Also, high temperatures in summer can impact wastewater treatment processes, such as biological degradation in treatment plants. While some microbial activity might increase, others may be less effective at removing pharmaceuticals, leading to higher residual concentrations (35).



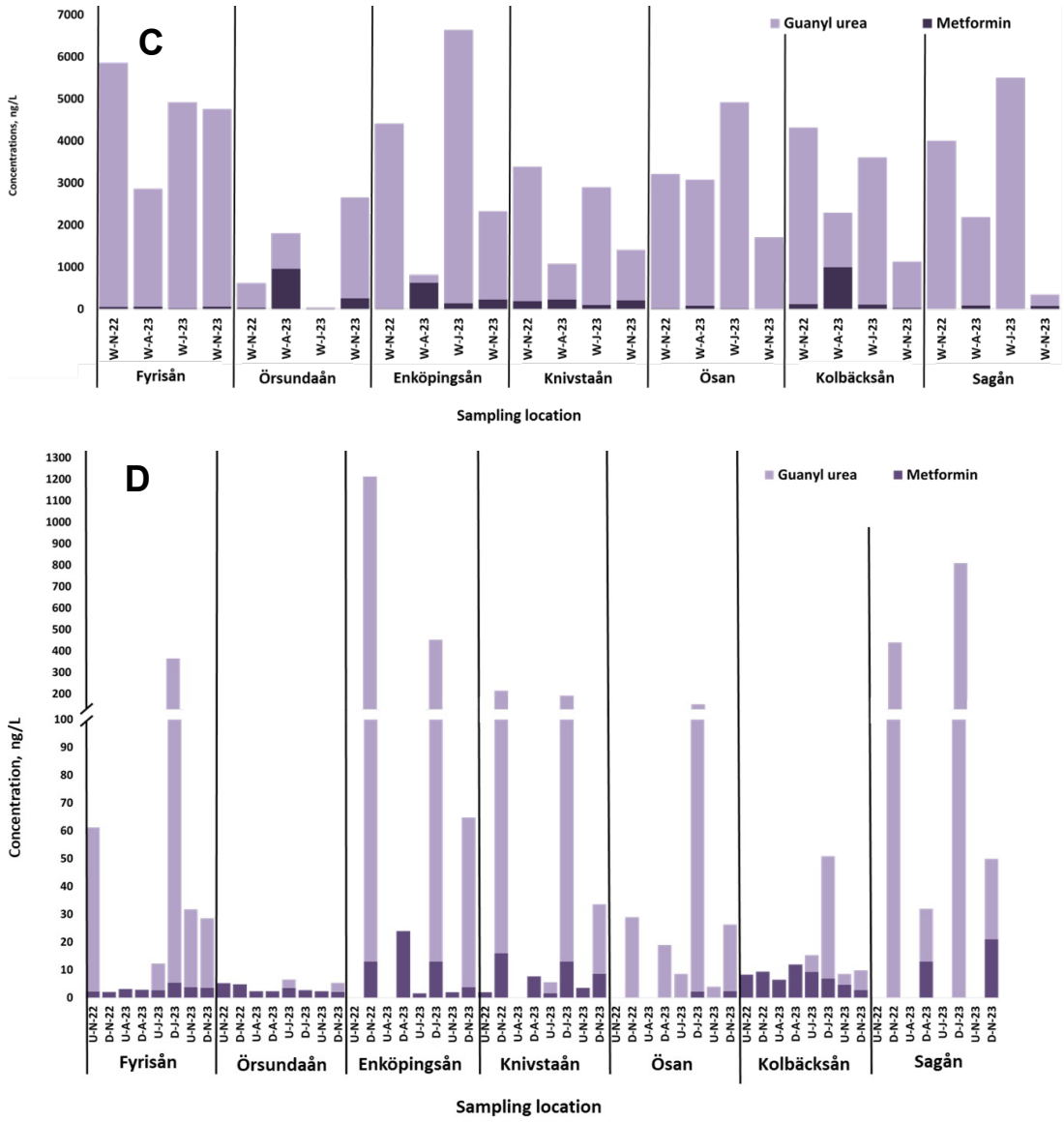


Figure 1. Cumulative concentrations of targeted EU Watch List compounds in studied locations in A) wastewater effluent, B) surface water samples, C) wastewater effluent for guanil urea and metformin, D) surface water samples guanil urea and metformin. Sampling locations: W - wastewater effluent; U - recipient upstream; D - recipient downstream. Sampling time: N-22 – November 2022; A-23– April 2023, J-23 – July 2023; N-23 – November 2023.

3.1.2 Occurrence of PFAS in wastewater effluent and in recipient waters (upstream and downstream)

The concentration of ∑PFAS in water samples (WWTP effluent and recipients) was generally in the range from 1 ng/L up to 300 ng/L, concentrations probably correlating with the level of industrial pressure of the site and the dilution capacity of the recipient, Figure 2.

Table 4. Detection frequency (DF, %), average (AV), maximum (MAX) and minimum (MIN) concentrations (ng/L) of PFASs in wastewater effluent (WW) and in recipient waters (upstream - U and downstream - D).

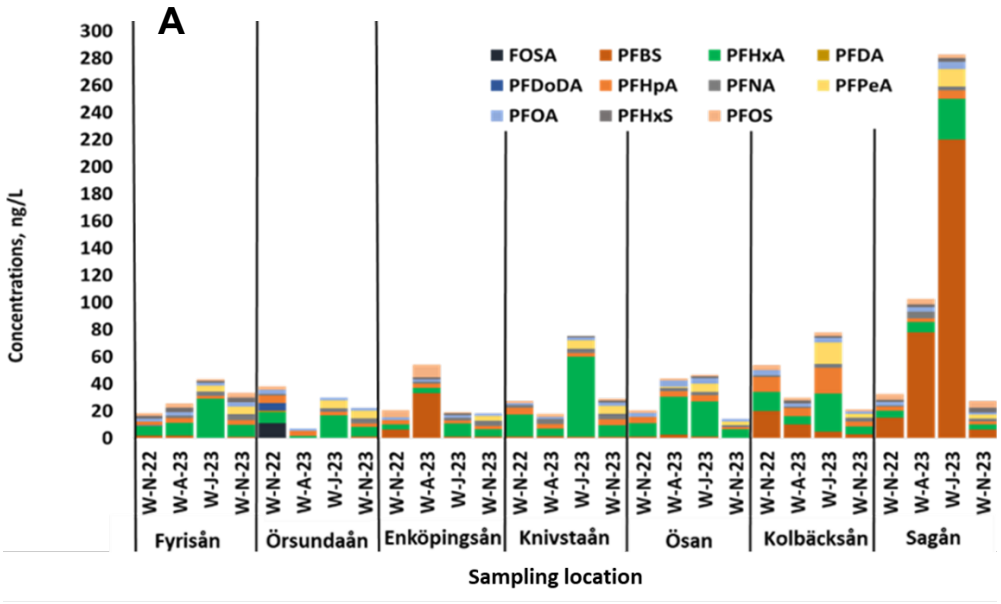
Compounds	Wastewater effluent				Upstream				Downstream			
	DF_WW	AV_WW	MAX_WW	Min_WW	DF_U	AV_U	MAX_U	Min_U	DF_D	AV_D	MAX_D	Min_D
FOSA	4%	11	11	11	0%	-	-	-	4%	1.4	1.4	1.4
PFBS	82%	18	220	0.85	54%	2	2.7	1.1	68%	10	79	0.93
PFHxA	100%	13	59	1.8	75%	2	4.5	0.85	75%	5	39	1.2
PFDA	4%	1	1	1	4%	1	1.1	1.1	0%	-	-	-
PFDoDA	4%	6	5.7	5.7	4%	9	9.1	9.1	7%	1	1.5	1.3
PFHpA	100%	4	19	1.7	39%	1	1.7	0.78	57%	2	6.5	0.98
PFNA	86%	3	4.7	0.77	36%	1	1.8	0.78	32%	2	6.7	0.78
PFPeA	50%	6	16	1.1	0%	-	-	-	0%	-	-	-
PFOA	100%	3	5.2	1.2	25%	1	3.1	0.73	39%	2	12	0.71
PFHxS	54%	2	3.7	0.97	29%	2	4.4	1.4	32%	11	88	0.83
PFOS	79%	3	9.4	0.84	43%	3	8.9	0.8	50%	3	8.8	0.71

The most contaminated sites in all sampling campaigns were Sagån (WWTP effluent and downstream samples). The highest concentrations were found in effluent and downstream samples, with a ∑PFAS concentration up to 250 ng/L. PFHxA, PFHxS, PFHpA and PFBS, were the most abundant pollutants in wastewater and surface water samples, Figure 2. These results are similar to our previously published report where the same monitoring program was performed (9).

The highest concentration of ∑PFAS was detected in WWTP effluent emitted to Sagån, particularly in samples collected in April and July 2023, with a considerable presence of PFBS. The data indicates that certain PFAS, such as PFHxA and PFBS, were consistently present across most locations and sampling events, with their concentrations varying significantly depending on the location and time of sampling. Fyrisån showed low concentrations across different sampling events, with minor amounts of various PFAS. Örsundaån showed similar low concentrations, with some presence of PFHxA and PFPeA. Enköpingsån showed slightly higher concentrations compared to Fyrisån and Örsundån, with notable amounts of PFHxA and PFPeA. Knivstaån showed moderate concentrations, particularly in July, with significant amounts of PFHxA and PFPeA. Ösan showed moderate concentrations with a slight increase in the sample collected in

April 2023 and July 2023, dominated by PFHxA and PFPeA. Kolbäcksån showed moderate concentrations.

∑PFASs in surface water samples is shown in Figure 2B. The highest concentration levels of PFAS were detected in Sagån with a significant spike in concentration for sample collected downstream recipient in July 2023. PFBS showed the highest concentration levels compared to the rest of the PFAS, followed by PFHxA and PFOS. These results are similar to our previously published report where the same monitoring program was performed (9). Örsundaån and Ösan showed similar low combined concentrations, although the PFAS profile is different: PFBS was the main contaminant in Örsundån, and in Ösan PFHxA and PFBS made up the majority of the PFAS profile. Enköpingsån showed a significant spike in concentration for the sample collected in November 2022 and 2023, largely constituted by PFBS and PFHxA. It is noteworthy that PFAS were not detected in the samples collected from locations Knivstaån, Ösan and Kolbäcksån in the downstream recipient in April 2023. The observed variation in concentration levels could be attributed to dilution caused by snowmelt. Another possible explanation is the limitation inherent in grab sampling. Grab sampling captures water quality at a single moment in time, providing only a snapshot of conditions. While it is useful for detecting short-term pollution events, it may not accurately represent fluctuations in concentration levels over time, potentially either capturing or missing spikes/ impulses in contaminant concentrations.



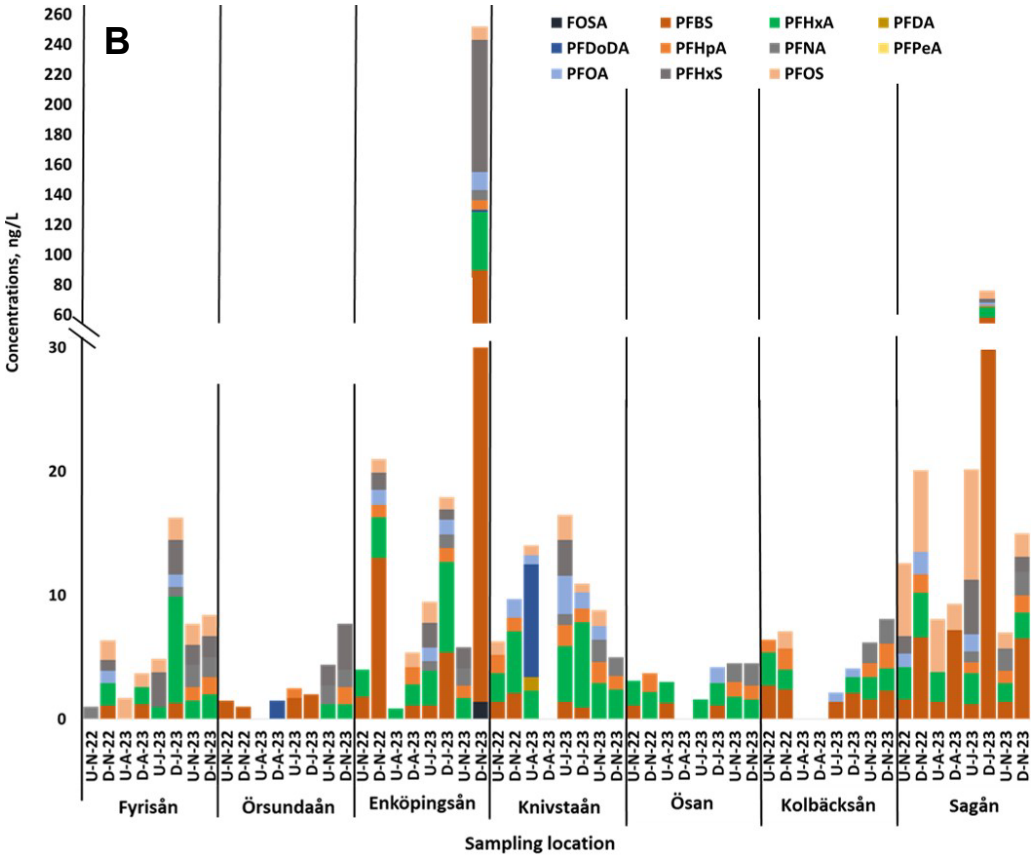


Figure 2. Combined concentrations of PFASs in studied locations in A) wastewater effluent and B) surface water samples. Sampling locations: W - wastewater effluent; U - recipient upstream; D - recipient downstream. Sampling time: N-22 – November 2022; A-23– April 2023, J-23 – July 2023; N-23 – November 2023.

3.1.3 Occurrence of other OMPs in wastewater effluent and in recipient waters (upstream and downstream)

Other OMPs were also included for monitoring study and Table S2 in the Appendix presents results for individual concentrations of selected compounds in wastewater effluent and in recipient waters, Table 5. The compounds were divided into following groups:

- Beta blockers (albuterol, atenolol, sotalol, metoprolol, propranolol, terbutaline)
- Antidepressants (citalopram, mirtazapine, oxazepam, amitriptyline, sertraline, fluoxetine, diazepam)
- Antihypertensives (bisoprolol, valsartan, diltiazem, irbesartan, losartan, ramipril)
- Antiepileptics (carbamazepine, lamotrigine)
- Opiates (oxycodone, tramadol, codeine, lidocaine)
- Pesticides (DEET and BAM)

- Stimulants (nicotine, caffeine)
- Antibiotics (erythromycin, amoxicillin, ciprofloxacin, clarithromycin, azithromycin, metronidazole, roxithromycin)
- Antifungal preservatives (ethylparaben, propylparaben, methylparaben)
- Antilipidemic agents (atovastatin, bezafibrate, gemfibrozil)
- Diuretics (hydrochlorothiazide (HCTZ), furosemide)
- Antihistamines (cetirizine, fexofenadine)
- Others (bicalutamide, climbazole, clozapine, loperamide, memantine, primidone, tolytriazole, ifosfamide, chlorzoxazone, panthenol, salicylic acid, thiabendazole, diclofenac, melamine, 6-PPD, 6-PPD quinone)

Table 5. Detection frequency (DF, %), average (AV), maximum (MAX) and minimum (MIN) concentrations (ng/L) of other OMPs compounds in wastewater effluent (WW) and in recipient waters (upstream - U and downstream - D).

Compounds	Wastewater effluent				Upstream				Downstream			
	DF_WW	AV_WW	MAX_WW	MIN_WW	DF_U	AV_U	MAX_U	MIN_U	DF_D	AV_D	MAX_D	MIN_D
Albuterol (Salbutamol)	96%	9	19	2	0%	-	-	-	18%	4	7	2
Atenolol	100%	475	830	96	29%	4	7	2	89%	47	250	3
Sotalol	86%	41	100	5	0%	-	-	-	39%	13	56	2
Metoprolol	100%	702	1400	84	46%	5	11	2	100 %	76	880	2
Propranolol	100%	91	360	2	18%	3	5	2	75%	11	53	2
Terbutaline	75%	5	13	2	0%	-	-	-	0%	-	-	-
Citalopram	100%	151	360	3	21%	3	4	2	75%	14	95	2
Mirtazapine	100%	152	340	7	21%	2	4	2	79%	17	100	2
Oxazepam	100%	260	620	21	21%	4	6	2	86%	34	270	4
Amitriptyline	96%	52	120	5	25%	8	12	6	61%	11	50	3
Sertraline	100%	55	130	3	46%	11	22	2	50%	12	25	3
Fluoxetine	96%	17	33	2	25%	4	7	3	36%	4	7	2
Diazepam	14%	3	4	2	0%	-	-	-	0%	-	-	-
Bisoprolol	100%	247	710	40	18%	3	6	2	82%	31	200	2
Valsartan	100%	586	1300	110	14%	9	13	7	79%	57	550	8
Diltiazem	93%	9	19	3	7%	2	3	2	14%	2	3	2
Irbesartan	93%	75	200	8	0%	-	-	-	54%	14	79	3
Losartan	100%	1716	3800	290	75%	15	41	2	100 %	159	1800	10
Ramipril	75%	4	12	2	0%	-	-	-	0%	-	-	-
Carbamazepine	100%	311	890	33	46%	5	9	2	93%	39	250	3

Lamotrigine	100%	744	1700	90	64%	8	34	2	100 %	71	270	2
Oxycodone	96%	27	53	7	0%	-	-	-	29%	7	17	2
Tramadol	100%	870	1700	76	25%	9	16	5	89%	83	590	4
Codeine	100%	178	680	9	4%	5	5	5	57%	30	230	4
Lidocaine	100%	214	440	23	0%	-	-	-	54%	37	230	7
Nicotine	100%	734	10000	10	96%	9	31	2	100 %	44	790	2
Caffeine	100%	2250	15000	8	100 %	108	640	16	100 %	298	4300	26
Erythromycin	7%	86	95	77	0%	-	-	-	0%	-	-	-
Amoxicillin	0%	-	-	-	0%	-	-	-	0%	-	-	-
Ciprofloxacin	0%	-	-	-	0%	-	-	-	0%	-	-	-
Clarithromycin	93%	66	300	3	0%	-	-	-	39%	9	19	2
Azithromycin	86%	97	260	4	18%	8	9	5	39%	19	62	4
Metronidazole	89%	37	280	2	0%	-	-	-	25%	12	37	2
Roxithromycin	14%	8	18	3	0%	-	-	-	0%	-	-	-
Ethylparaben	50%	4	7	2	29%	2	3	2	46%	3	9	2
Propylparaben	39%	4	10	2	14%	3	4	2	11%	4	6	2
Methylparaben	79%	4	10	2	32%	3	5	2	32%	3	6	2
Atovastatin (Lipitor)	100%	42	120	5	0%	-	-	-	29%	8	41	2
Bezafibrate	100%	88	570	2	11%	3	3	2	64%	8	46	2
Gemfibrozil	86%	131	530	8	0%	-	-	-	29%	43	270	7
Hydrochlorothiazide (HCTZ)	100%	1988	5000	410	79%	9	45	3	100 %	192	1900	3
Furosemide	100%	787	2800	27	25%	8	13	3	89%	63	360	5
Cetirizine	100%	989	1800	180	71%	8	23	1	100 %	114	760	4
Fexofenadine	100%	566	2600	25	57%	7	22	2	100 %	63	310	2
DEET	100%	207	980	27	68%	9	31	2	96%	31	250	2
BAM (Dichlorobenzamide)	93%	509	1200	280	0%	-	-	-	0%	-	-	-
Bicalutamide	100%	576	2600	63	50%	5	14	2	96%	53	240	3
Climbazole	100%	34	190	2	0%	-	-	-	29%	8	30	2
Clozapine	100%	39	140	2	21%	5	7	2	50%	7	21	2
Loperamide	75%	5	17	2	14%	4	5	3	11%	4	5	3
Memantine	100%	54	150	10	0%	-	-	-	46%	11	31	2
Primidone	14%	151	250	62	4%	34	34	34	7%	124	210	37
Tolytriazole	100%	2043	27000	52	79%	136	2500	2	93%	259	2500	2
Ifosfamide	14%	9	18	3	0%	-	-	-	4%	4	4	4
Chlorzoxazone	96%	56	240	4	0%	-	-	-	29%	13	27	3
Panthenol	61%	21	97	3	0%	-	-	-	0%	-	-	-
Salicylic acid	96%	22	65	5	93%	5	13	2	93%	6	15	3
Thiabendazole	25%	2	3	2	0%	-	-	-	0%	-	-	-

Diclofenac	100%	590	1500	73	64%	5	13	2	86%	63	510	2
Melamine	25%	17	52	2	0%	-	-	-	7%	6	10	2
6-PPD	14%	31	34	27	18%	40	72	22	14%	29	43	21
6-PPD quinone	54%	19	34	7.8	54%	49	150	2	54%	23	73	3

The concentrations of each detected compound were generally lower in the water samples collected upstream of the WWTP effluents than those collected downstream the treated wastewater discharges (Figure 3). This exhibited the impact the treated wastewater had on the recipients. ΣOMPs in WWTP effluent samples is shown in Figure 3A. The highest concentration levels were detected in wastewater effluent from Ösan. A significant spike in ΣOMPs concentration for the sample collected in July 2023 from Ösan was observed. These results can be explained by the fact that Ösan is a recipient of WWTP containing hospital's wastewater which contributes to the high level of different pharmaceuticals. The concentration spike for Ösan was largely attributed to the highly elevated concentration of tolytriazole, 27000 ng/L compared to 4400-5900 ng/L for the other samples collected at the same site. Across all sampling sites, tramadol, lidocaine, metoprolol and caffeine were the most abundant compounds (Figure 3 and Table S2). These compounds are often detected in WWTP influent in many countries (5, 22, 36). The occurrence of studied pharmaceuticals was previously monitored in the selected locations by Malnes et al. (2022b). These results may be attributed to day-to-day variability of influent flow, dependent on OMPs consumption patterns and WWTP operational performance.

ΣOMPs in surface water samples was characterized by significant variability in contamination levels, with notable spikes at Enköpingsån and Sagån in downstream recipient samples collected in November 2022 and July 2023. Fyrisån showed an elevated concentration of tolytriazole in the upstream recipient sample in July 2023. Knivstaån showed similar patterns to Ösan, with a spike in concentration for downstream recipient samples collected in November 2022 and July 2023. Örsundaån and Kolbäcksån showed similar low concentrations over the sampling events.

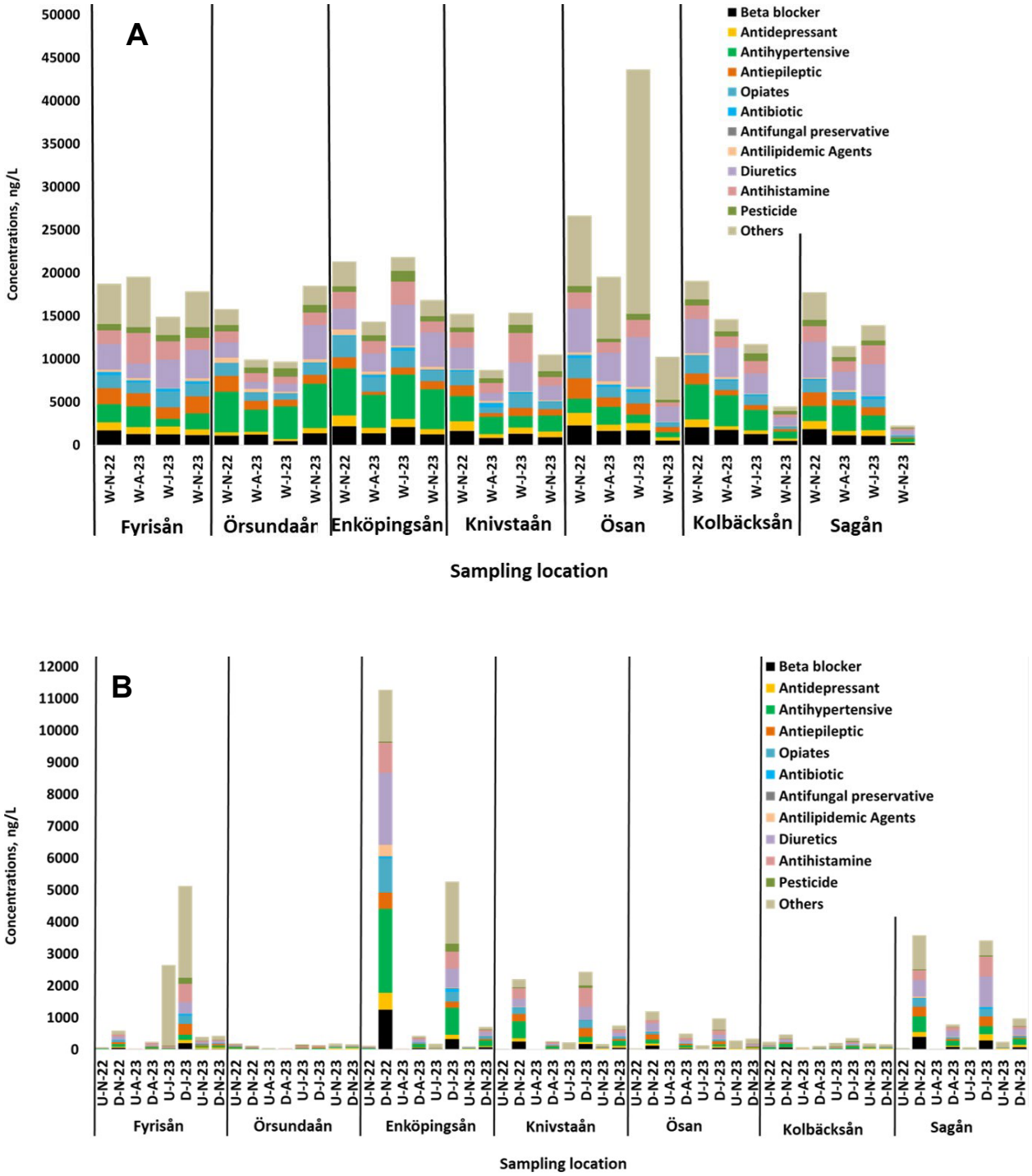


Figure 3. Combined concentrations of OMPs in studied locations in A) wastewater effluent and B) surface water samples. Sampling locations: W - wastewater effluent; U - recipient upstream; D - recipient downstream. Sampling time: N-22 – November 2022; A-23– April 2023, J-23 – July 2023; N-23 – November 2023.

Σstimulants (nicotine, caffeine) in WWTP effluent samples is shown in Figure 4A and the highest contamination was detected in Örsundån and Enköpingsån WWTP effluents. These locations showed the highest concentrations among all locations, particularly in samples collected in April 2023 and November 2023 for Örsundån and November 2023 for Enköpingsån (more than 20 µg/L). These concentrations were close to three-fold higher compared to any of the other sites in this study. Fyrisån showed low concentrations with increase in sample collected in November 2023. Knivstaån and Sagån showed high concentration of stimulants in samples collected in April and November 2023. Kolbäcksån showed moderate concentrations, with a significant presence of stimulants in July 2023 samples. Ösan showed high concentration levels in samples collected in November 2022 and April 2023 compared to the samples collected in July and November 2023.

Combined concentrations of stimulants in surface water samples is shown in Figure 4B and the highest contamination was detected in Enköpingsån and Knivstaån. Ösan showed low to moderate concentrations, similar to Kolbäcksån and Fyrisån, with a significant spike in concentration for upstream recipient samples collected in July and November 2023. Sagån showed low concentrations with a significant increase in downstream recipient samples collected in April and November 2023.

Caffeine and nicotine are among the most widely used legal stimulants in modern societies (37, 38). Caffeine, the main psychoactive component in coffee, is also found in tea, soft drinks, and energy beverages, while nicotine is the primary addictive agent in cigarettes and tobacco products (37). In general, the concentrations of caffeine and other pharmaceutical were found to be higher in water samples collected during the dry season, typically occurring in winter in many regions worldwide (38). Furthermore, seasonal variations in human social activities appear to influence anthropogenic inputs of caffeine into the environment, potentially contributing to the observed fluctuations in caffeine concentrations across different seasons (38). The presence of caffeine and nicotine in aquatic environments is characterized by significant complexity where different factors should be considered.

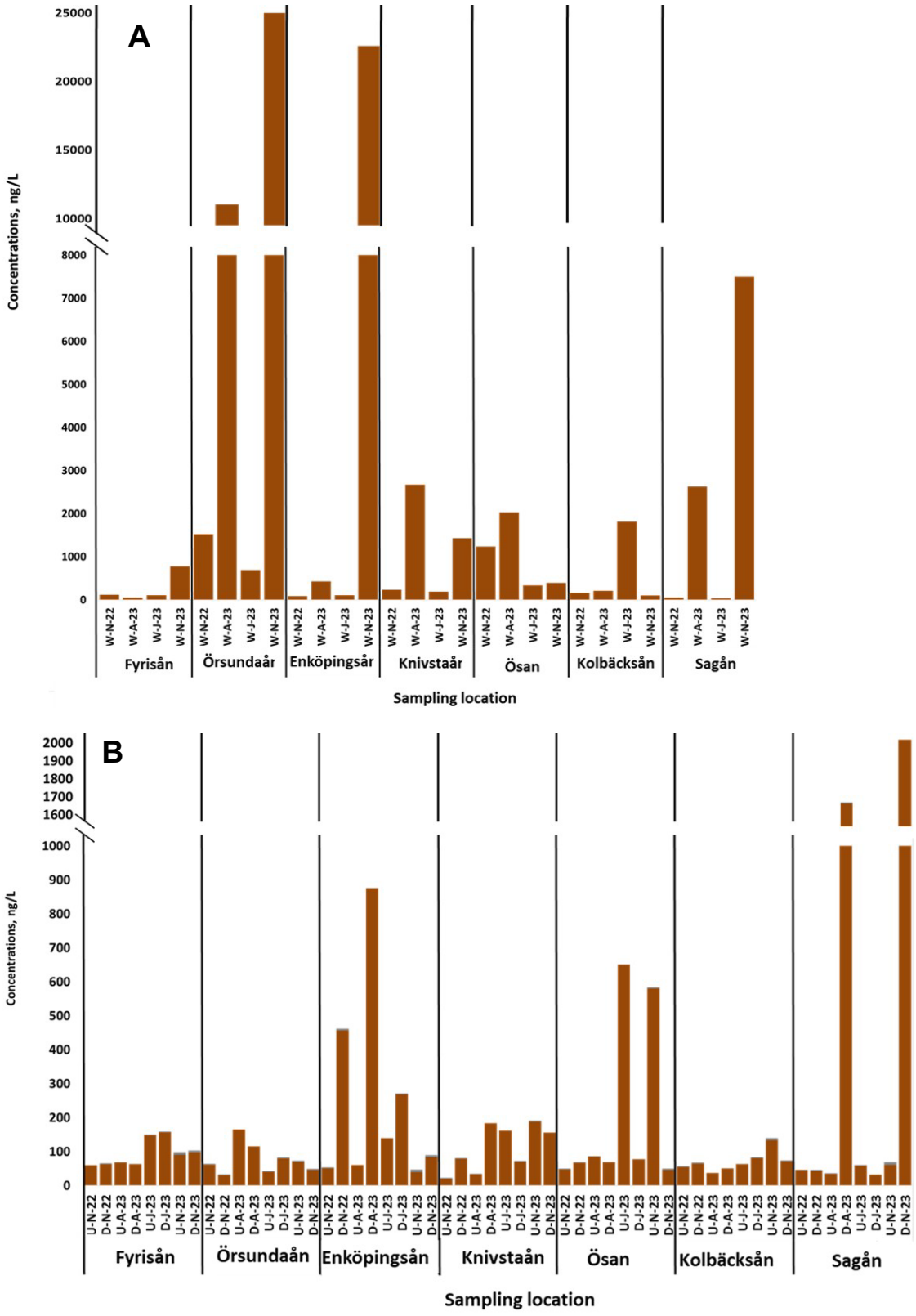


Figure 4. Combined concentrations of stimulants in studied locations in A) wastewater effluent and B) surface water samples. Sampling locations: W - wastewater effluent; U - recipient

upstream; D - recipient downstream. Sampling time: N-22 – November 2022; A-23– April 2023, J-23 – July 2023; N-23 – November 2023.

In this study 1,4-Dioxane was analyzed (Figure 5). This compound is highly persistent and it was found in all studied locations at concentration levels 44 – 430 ng/L. This substance has been highlighted as a contaminant of drinking water resources which are difficult to remove (39-41). It is important to keep monitoring the occurrence of 1,4-dioxane in water because of its adverse health effects. Concentrations of 1,4-Dioxane in WWTP effluent samples is shown in Figure 5A and the highest contamination was detected in WWTP effluents emitted to Ösan.

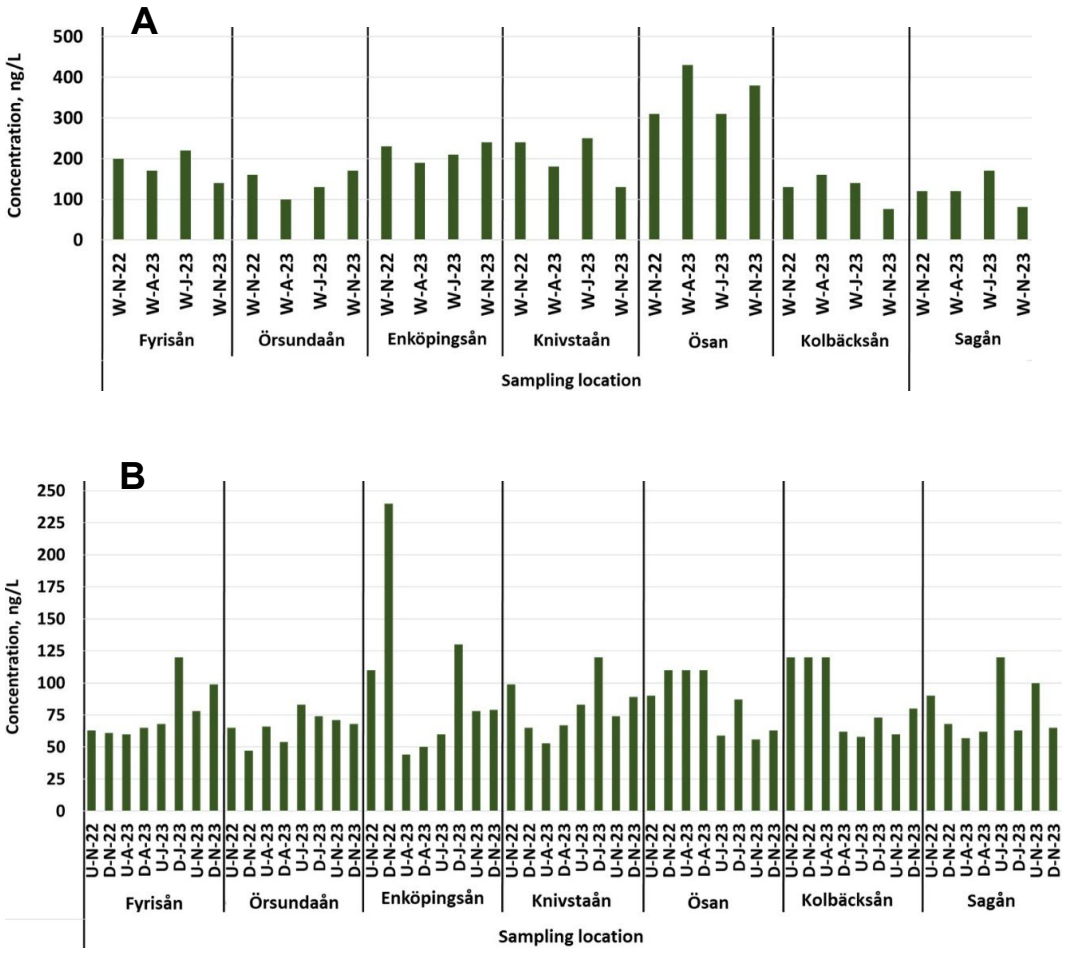


Figure 5. Concentrations of 1,4-Dioxane in studied locations in A) wastewater effluent and B) surface water samples. Sampling locations: W - wastewater effluent; U - recipient upstream; D - recipient downstream. Sampling time: N-22 – November 2022; A-23– April 2023, J-23 – July 2023; N-23 – November 2023.

Concentrations of 1,4-Dioxane in surface water samples is shown in Figure 5B with a low variability in contamination levels, with notable spikes at Enköpingsån in downstream recipient samples collected in November 2022 and July 2023. Similar results with higher spike in concentration for downstream recipient samples collected in July 2023 were identified for Fyrisån, Knivstaån and Sagån.

In this study, the presence of 6PPD (N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine) and its transformation product, 6PPD-quinone, was investigated (Figure 6 and Table 5). 6PPD is widely utilized in the rubber industry and is recognized as an environmental contaminant frequently detected in various environmental matrices, including surface waters (42).

6PPD was detected in only a subset of wastewater effluent samples, specifically from Fyrisån, Örsundån, Enköpingsån, and Kolbäcksån in November 2023, with concentrations ranging between 27 and 34 ng/L. At other sampling locations and times, 6PPD was below the limit of quantification (LOQ). In contrast, 6PPD-quinone was consistently found in all studied locations during the July 2023 and November 2023 sampling campaigns, with concentrations ranging from 7.8 to 34 ng/L.

The concentrations of both 6PPD and 6PPD-quinone in surface water samples are illustrated in Figure 6B. 6PPD exhibited a similar distribution pattern to that observed in wastewater effluents. It was detected in both upstream and downstream surface water samples from Fyrisån, Örsundån, Ösan, and Sagån in November 2023. Notably, there was no significant difference in the concentration levels of 6PPD between upstream and downstream samples from Fyrisån, Örsundån, and Sagån. However, at the Ösan sampling location, the concentration of 6PPD was higher in upstream samples compared to downstream.

6PPD-quinone was detected at all recipient water sampling locations in both July and November 2023, exhibiting a similar spatial distribution to that found in wastewater effluent samples. Particularly elevated concentrations of 6PPD-quinone were observed in upstream samples from Knivstaån (July 2023), Ösan (July and November 2023), and Sagån (November 2023), with values reaching up to 150 ng/L. These observed spikes may be attributed to various factors, such as the duration of rainfall and fluctuations in river water levels. However, further investigation is required to reach definitive conclusions regarding these results. In contrast, contamination levels of 6PPD-quinone at Fyrisån showed low variability between upstream and downstream samples during sampling events in July 2023 and November 2023.

In general, the concentrations of 6PPD and 6PPD-quinone were higher in recipient water samples compared to those in wastewater effluent, suggesting that WWTPs are not the primary source of these contaminants. Instead, road runoff, particularly from particle emissions, likely plays a more significant role, as highlighted in several studies (42-44).

6PPD-quinone has been demonstrated to induce species-specific toxicity in aquatic organisms (42, 45). As a result, it is crucial to continue monitoring both the occurrence and concentration levels of this contaminant in aquatic environments.

Based on our findings, drawing definitive conclusions regarding the seasonality of 6PPD and its transformation product is challenging, as they were not detected in the samples collected in November 2022. Increasing the sampling frequency would be advantageous for further investigation into the seasonal dynamics of these contaminants in surface waters. Furthermore, it would be beneficial to analyze both the aqueous and particulate phases of discharges containing suspended particles to accurately quantify the transfer of 6PPD and 6PPD-quinone to surface waters.

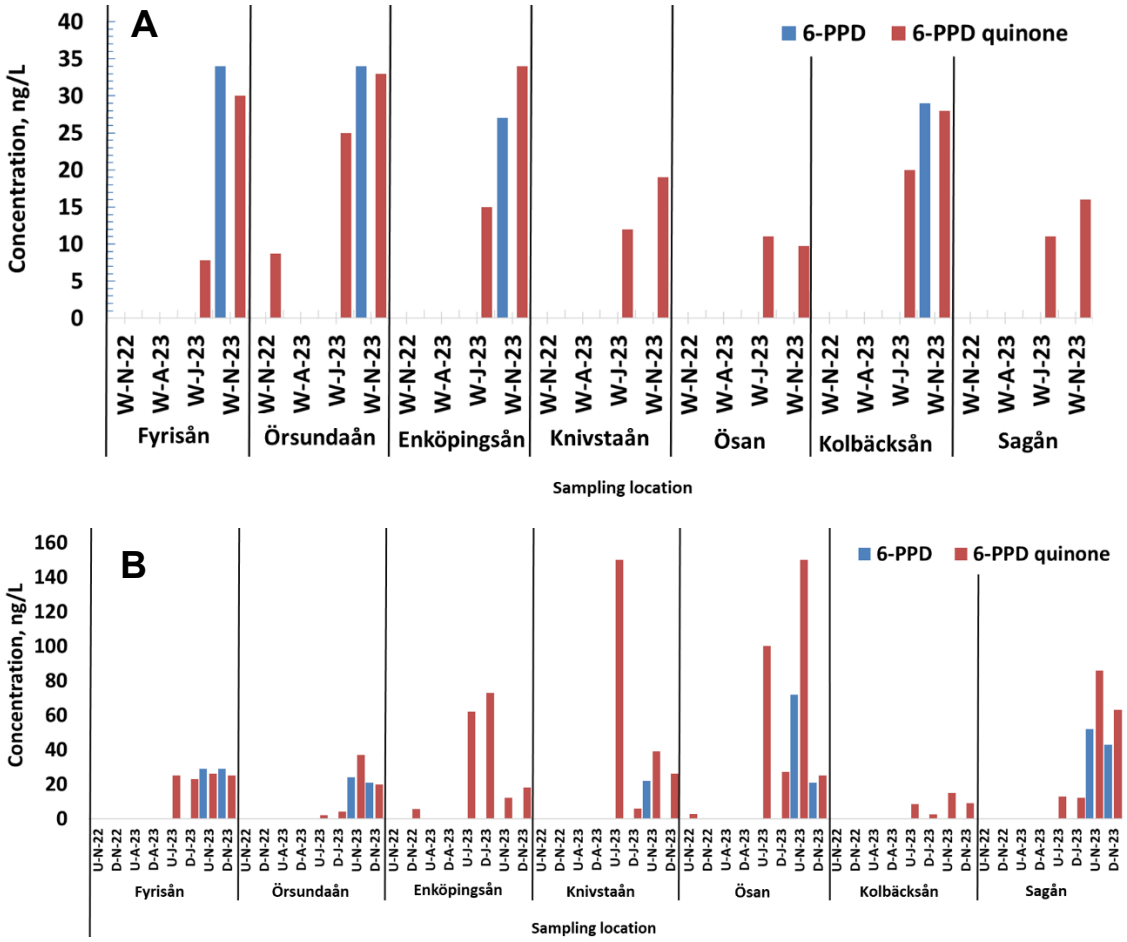


Figure 6. Concentrations of 6PPD and 6PPD-quinone in studied locations in A) wastewater effluent and B) surface water samples. Sampling locations: W - wastewater effluent; U - recipient upstream; D - recipient downstream. Sampling time: N-22 – November 2022; A-23– April 2023, J-23 – July 2023; N-23 – November 2023.

4. Conclusions.

The occurrence of selected EU Water Framework Watch List substances, including PFAS and other OMPs was investigated in seven Swedish river catchments impacted by WWTPs.

The consistent detection of compounds such as sulfamethoxazole, trimethoprim, venlafaxine, and fluconazole, particularly in downstream water samples, shows their incomplete removal by conventional wastewater treatment processes. The elevated concentrations observed during summer months suggest that seasonal factors, including reduced water flow and potential alterations in microbial degradation efficiency, may enhance the persistence of these contaminants. Metformin and its transformation product, guanyl urea, were detected at notably high concentrations, reflecting their widespread usage, high solubility, and environmental persistence, which complicates their removal in WWTPs.

The highest ∑PFAS concentrations, particularly in WWTP effluent and downstream samples was detected in Sagån. PFHxA, PFHxS, PFHpA, and PFBS were the dominant PFAS. The variability in PFAS concentrations across sites and seasons can be attributed to several factors, including potential dilution from snowmelt and the limitations of grab sampling, which may either overestimate or underestimate transient contaminant levels by capturing short-term pollution events. Differences in PFAS profiles between sites, highlight the spatial heterogeneity of PFAS contamination.

The highest concentrations of OMPs were observed in WWTP effluents, with notable peaks primarily driven by elevated tolytriazole concentrations. Pharmaceuticals such as tramadol, lidocaine, metoprolol, and two stimulants (caffeine and nicotine) were the predominant compounds detected, consistent with global monitoring trends of wastewater effluents.

The detection of 1,4-Dioxane across all studied locations, highlights the persistence and potential risk posed by this compound in aquatic environments.

The presence of 6PPD and 6PPD-quinone in both WWTP effluents and surface waters indicates that these pollutants are of emerging concern. The elevated concentrations of 6PPD-quinone, particularly in surface waters, suggest that non-point sources such as road runoff, rather than WWTP effluents, are the primary contributors to its environmental loading. The consistent detection of 6PPD-quinone across sampling locations is concerning given its known toxicity to aquatic organisms, reinforcing the necessity for targeted monitoring and further investigation into its environmental fate and impact.

The results underscore the need for more frequent and spatially comprehensive monitoring to capture fluctuations in pollutant concentrations and better understand the environmental behavior of persistent contaminants such as 1,4-Dioxane and 6PPD-quinone.

Recommendations:

- These findings highlight the variability and seasonal dependence of EU Watch List compounds in wastewater and surface waters, underscoring the need for effective monitoring and treatment strategies.
- Continued monitoring and research on OMPs are essential steps for gaining deeper insights into their origins, environmental levels and ecological impact.
- Continued development of targeted, reliable, and highly accurate analytical methods specifically optimized for the detection and quantification of individual compounds across various environmental compartments.

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CRediT authorship contribution statement

Daniel Malnes: Methodology, sampling, writing – review & editing.

Maximilian Tyka: Sampling, writing – review & editing.

Oksana Golovko: Methodology, formal analysis and data handling; Writing – original draft, Project administration.

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Table S1. Physico-chemical properties for studied OMPs

Table S2. The concentrations (ng/L) of OMPs in waste effluent and surface water.

Table S3. Average limit of quantification (LOQ, ng/L), average absolute recovery and matrix effect of studied OMPs.