Supplementary Information

Estrogen occurrence and removal at a municipal wastewater treatment plant in Greater Poland Voivodeship, Poland

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LC-MS/MS conditions

Chromatographic separation was performed using an UltiMate 3000 RSLC liquid chromatograph (Dionex) equipped with a Hypersil Gold C18 reversed-phase column (100 mm × 2.1 mm, 1.9 μm). Mobile phase components: A consisted of water containing 0.1% (v/v) formic acid, and mobile phase B consisted of acetonitrile. The chromatographic gradient programme was as follows: 0.0 min (30% A, 70% B), 4.0 min (10% A, 90% B), 4.5 min (0% A, 100% B), and 5.5 min (0% A, 100% B). The flow rate was 0.20 mL min^{-1}, the column temperature was maintained at 35 °C and the injection volume was 10 μL.

Mass spectrometric detection was performed on an API 4000 QTRAP tandem mass spectrometer (Biosystems/MDS Sciex) with an electrospray ionization (ESI) source operated in positive-ion mode. The ion source temperature was set to 500 °C; curtain gas was 10 psi, nebulizer gas 40 psi and drying gas 40 psi; the ion spray voltage was 5500 V. Data were acquired in multiple reaction monitoring (MRM). For each analyte, two transitions were monitored: MRM1 (quantifier) and MRM2 (qualifier). Optimized declustering potentials (DP) and collision energies (CE) are provided in Table S1.

Identification criteria

Analyte identification was based on agreement of retention time (t_R) with the corresponding reference standard and the presence of two MRM transitions (quantifier and qualifier) acquired under the same chromatographic conditions. In addition, the qualifier-to-quantifier ion ratio was checked for consistency with the reference standard.

Table S1. MRM transitions and optimized MS/MS parameters for dansylated estrogens

Analyte	Precursor ion $[M+H]^+$ (m/z)	DP (V)	MRM1* (quantifier)	CE (V)	MRM2* (qualifier)	CE (V)
E1	504	136	504→171	49	504→156	77
E2α	506	136	506→171	51	506→156	75
E2β	506	126	506→171	53	506→156	73
E3	522	126	522→171	51	522→156	81
EE2	530	156	530→171	51	530→156	79

*MRM denotes the transition of the protonated molecular ion (precursor ion) to the respective product ion.

Table S2. Influent and effluent pH, TSS, COD and BOD_5 concentrations and calculated removal efficiencies for each monitoring date.

Removal (%) was calculated as $(C_{\text{in}} - C_{\text{out}})/C_{\text{in}} \times 100$using paired concentrations for each date.

Date	pH_{in}	pH_{out}	TSS_{in} (mg/L)	TSS_{out} (mg/L)	TSS removal (%)	COD_{in} (mg O_2/L)	COD_{out} (mg O_2/L)	COD removal (%)	BOD_{5in}(mg O_2/L)	BOD_{5out} (mg O_2/L)	BOD_5 removal (%)
02.10.2024	6.7	8.2	590	25.0	95.8	1868	88.5	95.3	870	15.0	98.3
17.10.2024	7.4	8.1	262	20.0	92.4	882	65.5	92.6	359	7.6	97.9
06.11.2024	7.0	8.0	480	23.0	95.2	1556	69.2	95.6	703	10.0	98.6
20.11.2024	6.8	8.1	633	11.0	98.3	2064	58.5	97.2	1228	8.8	99.3
04.12.2024	7.0	7.9	514	27.0	94.7	1607	74.9	95.3	855	6.8	99.2
19.12.2024	7.0	7.5	662	4.6	99.3	1454	44.9	96.9	886	5.9	99.3
08.01.2025	7.0	7.5	376	4.1	98.9	1769	52.7	97.0	882	5.3	99.4

Date	pH_{in}	pH_{out}	TSS_{in} (mg/L)	TSS_{out} (mg/L)	TSS removal (%)	COD_{in} (mg O_2/L)	COD_{out} (mg O_2/L)	COD removal (%)	BOD_{5in}(mg O_2/L)	BOD_{5out} (mg O_2/L)	BOD_5 removal (%)
22.01.2025	7.1	8.0	530	7.1	98.7	1544	61.0	96.0	1069	7.2	99.3
05.02.2025	7.3	8.1	730	5.1	99.3	1544	62.7	95.9	704	6.5	99.1
20.02.2025	7.3	7.9	654	15.0	97.7	1701	46.4	97.3	982	7.1	99.3
05.03.2025	7.5	8.0	268	5.0	98.1	961	63.1	93.4	490	6.2	98.7
19.03.2025	7.7	8.0	384	9.5	97.5	1153	58.7	94.9	509	6.6	98.7
02.04.2025	7.7	8.1	418	10.0	97.6	1407	61.3	95.6	545	6.5	98.8
17.04.2025	7.7	8.1	206	7.0	96.6	831	61.0	92.7	341	6.8	98.0
Mean	7.2	8.0	479	12.4	97.2	1453	62.0	95.4	745	7.6	98.9

Table S3. Influent and effluent P_{tot}, N_{tot} and Fe concentrations and calculated removal efficiencies for each monitoring date.

Removal (%) was calculated as $(C_{in} - C_{out})/C_{in} \times 100$using paired concentrations for each date.

Date	$P_{tot,in}$ (mg P/L)	$P_{tot,out}$ (mg P/L)	P_{tot} removal (%)	$N_{tot,in}$ (mg N/L)	$N_{tot,out}$ (mg N/L)	N_{tot} removal (%)	Fe_{in} (mg Fe/L)	Fe_{out} (mg Fe/L)	Fe removal (%)
02.10.2024	6.10	1.95	68.0	87.90	7.68	91.3	-	-	-
17.10.2024	10.10	1.96	80.6	74.00	7.48	89.9	2.780	0.851	69.4
06.11.2024	17.10	2.00	88.3	96.40	6.05	93.7	-	-	-
20.11.2024	12.80	1.42	88.9	88.60	4.05	95.4	-	-	-
04.12.2024	11.60	1.81	84.4	75.80	6.57	91.3	-	-	-
19.12.2024	15.10	1.45	90.4	110.00	5.29	95.2	3.150	0.892	71.7
08.01.2025	16.20	1.37	91.5	99.50	6.21	93.8	-	-	-
22.01.2025	14.20	1.75	87.7	102.00	7.13	93.0	-	-	-

Date	$P_{tot,in}$ (mg P/L)	$P_{tot,out}$ (mg P/L)	P_{tot} removal (%)	$N_{tot,in}$ (mg N/L)	$N_{tot,out}$ (mg N/L)	N_{tot} removal (%)	Fe_{in} (mg Fe/L)	Fe_{out} (mg Fe/L)	Fe removal (%)
05.02.2025	17.20	1.71	90.1	90.00	7.47	91.7	-	-	-
20.02.2025	13.20	1.05	92.0	107.00	11.10	89.6	3.310	0.749	77.4
05.03.2025	12.20	1.60	86.9	94.40	7.32	92.2	-	-	-
19.03.2025	11.60	1.00	91.4	89.90	7.71	91.4	-	-	-
02.04.2025	13.40	1.66	87.6	97.00	6.27	93.5	-	-	-
17.04.2025	9.70	1.73	82.2	92.10	4.35	95.3	2.490	0.557	77.6
Mean	12.89	1.60	86.4	93.19	6.76	92.7	2.933	0.762	74.0

Note: Fe was available only for selected dates.

Supplementary Materials - Estrogens (Tables S4a-S4e)

Supplementary note

Concentrations are reported as mean ± SD (µg/L) of three independent extractions of the same wastewater sample. Samples were analyzed without prior filtration; therefore, the reported variability reflects both analytical variation and heterogeneity of the wastewater matrix, including the contribution of suspended solids. Values below the method quantification limit (MQL = 0.025 µg/L) are reported as "<MQL".

Table S4a. Estrone (E1) concentrations (µg/L; mean ± SD)

Date	Influent	Effluent
23 Oct 2024	0.19 ± 0.01	0.17 ± 0.03
13 Nov 2024	0.19 ± 0.02	0.14 ± 0.06
11 Dec 2024	0.17 ± 0.01	0.047 ± 0.003
05 Feb 2025	0.35 ± 0.07	0.050 ± 0.004
26 Feb 2025	0.47 ± 0.05	0.12 ± 0.06
26 Mar 2025	0.19 ± 0.03	0.072 ± 0.023
09 Apr 2025	0.11 ± 0.03	0.027 ± 0.004

Table S4b. 17α-estradiol (E2α) concentrations (µg/L; mean ± SD)

Date	Influent	Effluent
23 Oct 2024	0.077 ± 0.007	0.083 ± 0.045
13 Nov 2024	0.056 ± 0.014	0.12 ± 0.10
11 Dec 2024	0.12 ± 0.06	0.14 ± 0.03
05 Feb 2025	1.21 ± 0.75	0.64 ± 0.43
26 Feb 2025	0.22 ± 0.03	0.38 ± 0.14
26 Mar 2025	1.40 ± 0.65	1.05 ± 0.24
09 Apr 2025	1.72 ± 1.07	0.95 ± 0.30

Table S4c. 17β-estradiol (E2β) concentrations (µg/L; mean ± SD)

Date	Influent	Effluent
23 Oct 2024	0.17 ± 0.06	0.16 ± 0.05
13 Nov 2024	0.11 ± 0.04	0.045 ± 0.001
11 Dec 2024	0.091 ± 0.006	<MQL
05 Feb 2025	0.039 ± 0.002	0.347 ± 0.004
26 Feb 2025	0.16 ± 0.03	<MQL
26 Mar 2025	9.52 ± 5.20	8.39 ± 3.71
09 Apr 2025	8.16 ± 2.51	4.85 ± 1.57

Table S4d. Estriol (E3) concentrations (µg/L; mean ± SD)

Date	Influent	Effluent
23 Oct 2024	0.23 ± 0.07	0.10 ± 0.11
13 Nov 2024	0.60 ± 0.05	0.16 ± 0.09
11 Dec 2024	0.85 ± 0.06	0.051 ± 0.007
05 Feb 2025	2.51 ± 0.38	0.24 ± 0.42
26 Feb 2025	2.48 ± 0.33	0.22 ± 0.19
26 Mar 2025	3.10 ± 1.28	4.77 ± 1.76
09 Apr 2025	1.44 ± 0.56	0.21 ± 0.09

Table S4e. 17α-ethinylestradiol (EE2) concentrations (µg/L; mean ± SD)

Date	Influent	Effluent
23 Oct 2024	<MQL	<MQL
13 Nov 2024	<MQL	0.057 ± 0.017
11 Dec 2024	0.073 ± 0.007	0.062 ± 0.005
05 Feb 2025	<MQL	0.066 ± 0.008
26 Feb 2025	0.033 ± 0.017	<MQL
26 Mar 2025	<MQL	<MQL
09 Apr 2025	<MQL	<MQL