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

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Abstract

Steroidal estrogens are endocrine-disrupting compounds of concern due to their continuous release from municipal wastewater treatment plants (WWTPs). This study investigated the occurrence and removal of estrone (E1), 17α-estradiol (E2α), 17β-estradiol (E2β), estriol (E3), and 17α-ethinylestradiol (EE2) in a full-scale municipal WWTP in the Greater Poland Voivodeship, Poland. Influent and effluent samples collected between October 2024 and April 2025 were analyzed by LC-MS/MS following QuEChERS extraction and dansyl chloride derivatization. The plant showed stable and efficient removal of conventional pollutants, whereas estrogen concentrations exhibited marked temporal variability. This variability may reflect temporal changes in influent composition and operational conditions of the treatment process. The summed estrogen concentration (ΣE) varied substantially between sampling campaigns, indicating inconsistent estrogen removal despite stable overall treatment performance. The reduction in ΣE ranged from 0% to 79% across sampling campaigns. The results suggest that sorption, biodegradation, and transformation processes may influence the final estrogen profile in treated effluent particularly through deconjugation and interconversion between estrogen species. The study provides baseline data relevant to future WWTP modernization and micropollutant monitoring in Europe.

1. Introduction

Endocrine-disrupting compounds (EDCs) comprise a broad group of chemicals capable of interfering with hormonal signalling and inducing adverse biological effects in exposed organisms, in some cases even at very low concentrations (Pironti et al. 2021). Among them, steroidal estrogens including estrone (E1), 17β-estradiol (E2β), estriol (E3), and the synthetic 17α-ethinylestradiol (EE2) - are of particular concern because of their high endocrine potency and their continuous release into the aquatic environment. These compounds are excreted by humans and animals in both free and conjugated forms, and they may enter surface waters through municipal wastewater discharges as well as diffuse agricultural sources.

Exposure to estrogenic compounds has been linked to a wide range of adverse effects in aquatic organisms, particularly reproductive and developmental disturbances such as feminisation, altered gonadal development, and impaired fertility (Wojnarowski et al. 2021). For this reason, steroidal estrogens have remained under regulatory scrutiny in Europe and were included in EU-wide monitoring initiatives in 2018–2020, reflecting persistent concern regarding their occurrence and ecological significance (European Commission 2018). Available European datasets indicate that estrogens are usually detected in surface waters at ng/L levels, commonly in the range of approximately 0.1–10 ng/L, although exceedances of proposed or existing benchmark values have also been reported (Ciślak et al. 2023). However, monitoring coverage remains spatially uneven, particularly in parts of Eastern Europe, which limits robust continent-wide assessment of estrogen occurrence and trends (Ciślak et al. 2023).

Municipal wastewater treatment plants (WWTPs) represent key control points for limiting estrogen emissions to receiving waters. Nevertheless, reported removal efficiencies vary widely among facilities

and depend on treatment configuration, operational conditions, and the physicochemical properties of individual compounds. Many published datasets are based on short monitoring windows and a limited number of target analytes, which may obscure temporal variability and changes in estrogen profiles caused by biological transformation processes during treatment (Kumar et al. 2011; Martín et al. 2012; Margot et al. 2013). Previous studies have shown that residual estrogens in WWTP effluents, especially E2 and EE2, may still contribute substantially to ecological risk, as reflected by elevated risk quotient (RQ) and estradiol equivalent (EEQ) values in treated wastewater (Wu et al. 2023). Moreover, effective removal of conventional wastewater quality parameters does not necessarily imply elimination of endocrine or toxic activity, which highlights the limitations of relying exclusively on targeted chemical analysis of selected compounds (Bertanza et al. 2022).

The interpretation of estrogen occurrence in wastewater is further complicated by the presence of conjugated forms and by transformation processes occurring during biological treatment. Deconjugation, oxidation, and interconversion between estrogen species may alter the profile of free estrogens detected in influent and effluent wastewater, making concentration changes difficult to interpret solely in terms of direct removal. Previous studies have shown that secondary biological treatment is a key stage for estrogen removal, with natural estrogens generally being removed more efficiently than EE2 (Islam et al. 2021). Estrogens discharged to aquatic systems may also partition between water and sediments, so their environmental burden may not be fully reflected by aqueous concentrations alone (Pizzini et al. 2024).

From an analytical perspective, reliable determination of estrogens in wastewater remains challenging because these compounds occur at trace levels in complex matrices containing a wide range of interfering substances. Recent analytical studies have shown that alternative sample-preparation strategies, including QuEChERS-based protocols, may improve the quantitation of estrogens in difficult wastewater matrices (Sweeney et al. 2021). Owing to their relatively hydrophobic character and strong affinity for particulate matter, estrogens may partition between dissolved and suspended phases in wastewater; therefore, QuEChERS-based extraction, which does not require prior filtration, may improve recovery by enabling extraction of both aqueous and particle-bound fractions (Piosik et al. 2025).

The regulatory relevance of such datasets is increasing. The recast Urban Wastewater Treatment Directive (Directive (EU) 2024/3019) introduces stricter requirements for collection, treatment, monitoring, and reporting, and foresees the implementation or assessment of quaternary treatment for micropollutant removal (European Parliament and Council of the European Union 2024). These changes are expected to drive upgrades in many European WWTPs and highlight the need for robust baseline data on endocrine-active substances before modernization. In this context, studies from full-scale plants are needed not only to document current occurrence levels, but also to support future assessment of treatment upgrades and micropollutant control strategies.

Against this background, the present study investigated the occurrence and removal of selected steroidal estrogens in a full-scale municipal WWTP located in the Greater Poland Voivodeship, Poland.

The study combined routine operational monitoring with targeted determination of E1, E2α, E2β, E3, and EE2 in influent and effluent wastewater. LC-MS/MS quantified estrogens following QuEChERS extraction and dansyl chloride derivatization. The work provides date-resolved concentration data for individual estrogens and for summed estrogen concentration (ΣE) as an integrated concentration metric, and offers an empirical basis for interpreting estrogen removal patterns in the context of biological treatment processes and emerging regulatory drivers for WWTP modernization in Europe. This is important because there is still a notable lack of data from full-scale wastewater treatment plants in Central and Eastern Europe, particularly concerning temporal variability and transformation-driven alterations in estrogen profiles.

2. Materials and Methods

2.1. Reagents

Estrone (E1, ≥ 99%), 17α-estradiol (E2α, ≥ 98%), 17β-estradiol (E2β, ≥ 98%), estriol (E3, ≥ 97%) and 17α-ethinylestradiol (EE2, ≥ 98%), as well as acetonitrile (ACN; LC-MS grade), methanol (MeOH; LC-MS grade) and dansyl chloride, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (99%), anhydrous magnesium sulfate (98%), sodium carbonate (99%) and acetone (99.9%) were obtained from Chempur (Piekary Śląskie, Poland). Formic acid (90%), disodium citrate dihydrate (99%) and trisodium citrate dihydrate (99%) were purchased from Merck (Darmstadt, Germany). C18 ODS SPE bulk sorbent and PSA SPE bulk sorbent were purchased from Agilent Technologies (USA). Methyl tert-butyl ether (MTBE) was obtained from Honeywell Riedel-de Haën (Seelze, Germany). Ultrapure water obtained by reverse osmosis in a Watek-Demiwa 5 Rosa system (Czech Republic) was used throughout.

2.2. Characterization of wastewater treatment plant

The study was conducted at a municipal mechanical-biological wastewater treatment plant (WWTP) located in Chwałkowo (Środa Wielkopolska municipality, western Poland). The facility operates an activated-sludge process with nutrient removal (N and P). The average daily flow is approximately 5,000 $m^3\ d^{-1}$, with a maximum wet-weather hydraulic capacity of up to ~ 12,000 $m^3\ d^{-1}$. Septage delivered by vacuum trucks accounts for approximately 8% of the total inflow. Treated effluent is discharged to a local drainage ditch (D-4), which flows into the Drgań stream, a tributary of the Moskawa River.

The treatment line includes preliminary treatment (screening and grit removal), biological treatment in activated-sludge tanks with zones supporting nitrogen and phosphorus removal, and secondary clarification. Excess sludge is thickened and mechanically dewatered, and side streams from sludge processing are returned to the headworks.

Influent and effluent wastewater samples were collected on seven dates: 23 October, 13 November and 11 December 2024, and 5 February, 26 February, 26 March and 9 April 2025. On each sampling date, 24-h composite samples were collected automatically at both the influent and effluent sampling points using automatic samplers. Aliquots of 200 mL were transferred into sterile glass bottles, transported to the

laboratory in cooled conditions and stored at 4°C until analysis. Wastewater sampling was performed according to PN-ISO 5667-10:2021-11.

2.3. Analytical methods

Conventional wastewater quality parameters, including temperature, pH, total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total phosphorus (P_{tot}), ammonium nitrogen (NH_4^+-N), total nitrogen (N_{tot}) and total iron (Fe), were obtained from routine operational monitoring performed by the WWTP laboratory (accredited laboratory, certificate AB 1193). For each sampling campaign, removal efficiencies were calculated from paired influent and effluent concentrations. Target estrogens were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following QuEChERS-based extraction and dansyl chloride derivatization. The applied method enabled the extraction of both dissolved and particle-bound fractions; therefore, the reported concentrations represent the total-phase concentrations of the target estrogens.

2.4. Extraction procedure

A modified QuEChERS procedure was used for estrogen extraction. Briefly, 10 mL of wastewater was transferred to a 50 mL polypropylene centrifuge tube and 15 mL of acetonitrile (ACN) was added. Next, a pre-mixed salting-out mixture (“mix 1”) consisting of 1.0 g NaCl, 0.5 g disodium citrate dihydrate, 1.0 g trisodium citrate dihydrate and 4 g $MgSO_4$ was added. The samples were shaken manually for 1 min, placed in a HulaMixer™ rotator (10 min, 100 rpm, 360°) and centrifuged (5 min, 4000 rpm).

An aliquot (5 mL) of the ACN layer was transferred to a 15 mL QuEChERS dispersive clean-up tube containing 900 mg $MgSO_4$, 150 mg PSA and 150 mg C18, shaken manually for 1 min, placed in a HulaMixer™ rotator (10 min, 100 rpm, 360°) and centrifuged (5 min, 4000 rpm). 2 mL of the cleaned extract was evaporated to dryness at 60°C. The residue was reconstituted in 200 µL of 0.1 M aqueous sodium carbonate (Na_2CO_3) solution prior to derivatization.

2.5. Derivatization procedure

Derivatization with dansyl chloride (Dns-Cl) was performed to enable the identification of E2 isomers and improve detection sensitivity. To 200 µL of the reconstituted extract, 180 µL of Dns-Cl solution (4 mg Dns-Cl dissolved in 10 mL acetone) was added. The reaction mixture was incubated at 40°C for 10 min in a thermoblock.

After derivatization, 1 mL of methyl tert-butyl ether (MTBE) was added, and the mixture was shaken for 1 min and centrifuged (5 min, 12,000 rpm). The organic phase (800 µL) was collected and evaporated to dryness at 60°C. The residue was reconstituted in 200 µL of ACN/0.1% aqueous formic acid (70:30, v/v), transferred to autosampler vials and analysed by LC-MS/MS.

2.6. LC-MS/MS analysis

Chromatographic separation was performed on an UltiMate 3000 RSLC system (Dionex) coupled to an API 4000 QTRAP tandem mass spectrometer (AB Sciex) with electrospray ionization operated in positive-ion mode. Data were acquired in multiple reaction monitoring (MRM). Detailed chromatographic conditions and MRM transitions are provided in the Supplementary Material (Table S1).

2.7. Method validation and quality assurance/quality control (QA/QC)

The LC-MS/MS method was validated for the determination of E1, E2α, E2β, E3, and EE2 in wastewater. Validation included assessment of linearity, method detection limit (MDL), method quantification limit (MQL), recovery, and repeatability. Matrix-matched calibration was linear over 0.025-10 µg/L for all analytes, with coefficients of determination (R^2) of 0.978–0.997. The method quantification limit (MQL) was defined as the lowest concentration on the calibration curve providing acceptable precision and accuracy and was established at 0.025 µg/L for all analytes. The method detection limit (MDL) was estimated as one-third of the MQL and was therefore 0.008 µg/L.

Method accuracy, evaluated as recovery in spiked wastewater samples processed through the full extraction-derivatization-analysis workflow, ranged from 78% to 96% (E1: 96 ± 5%; E2α: 78 ± 3%; E2β: 78 ± 7%; E3: 78 ± 8%; EE2: 93 ± 6%). Method repeatability was evaluated separately for raw and treated wastewater at a spiking level of 5 µg/L using five independent extractions of each matrix. This relatively high fortification level was selected to minimize the contribution of endogenous estrogens present in the wastewater matrix to the repeatability assessment. Relative standard deviation (RSD) values ranged from 3% to 16% across analytes and matrices. Quality assurance measures included the analysis of blanks and replicate measurements to control for contamination and analytical repeatability.

3. Results and Discussion

3.1. Removal of conventional parameters

Operational monitoring data indicated stable WWTP performance over the study period (October 2024-April 2025). Influent pH was near-neutral, while effluent pH was slightly alkaline. Based on paired influent-effluent data, high removal efficiencies were achieved for solids and organic matter, with mean reductions of approximately 97.2% for total suspended solids (TSS), 95.4% for COD, and 98.9% for BOD_5 (Table 1). Nutrient-related parameters also decreased substantially; total phosphorus (P_{tot}) and total nitrogen (N_{tot}) were reduced by ~ 86.4% and ~ 92.7%, respectively. Date-by-date concentrations and calculated removal efficiencies are provided in Supplementary Tables S2-S3.

Table 1
Conventional wastewater quality parameters in influent and effluent during the monitoring period (October 2024-April 2025) and overall removal efficiencies (n = 14; Fe reported for n = 4). Removal (%) was calculated as .

Parameter	Unit	Influent (mean)	Effluent (mean)	Removal (%)
pH	-	7.3	8.0	-
Total suspended solids (TSS)	mg/L	479	12.4	97.2
Chemical oxygen demand (COD)	mg O_2/L	1453	62.0	95.4
Biochemical oxygen demand (BOD_5)	mg O_2/L	745	7.6	98.9
Total phosphorus (P_{tot})	mg P/L	12.89	1.60	86.4
Total nitrogen (N_{tot})	mg N/L	93.19	6.76	92.7
Total iron (Fe)*	mg Fe/L	2.933	0.762	74.0

*Fe values were available only for selected dates (n = 4; see Supplementary Table S3).

3.2. Estrogen removal

Five estrogens: E1, E2α, E2β, E3 and EE2 were detected in influent and/or effluent wastewater during the monitoring period (October 2024-April 2025). Concentrations were reported in µg/L and exhibited pronounced temporal variability. Values below the method quantification limit (MQL = 0.025 µg/L) were reported as < MQL.

In influent wastewater, concentrations (µg/L) ranged from 0.11 to 0.47 for E1, 0.06 to 1.72 for E2α, 0.04 to 9.52 for E2β, 0.23 to 3.10 for E3, and from < MQL to 0.07 for EE2. In effluent wastewater, the corresponding ranges (µg/L) were 0.03–0.17, 0.08–1.05, <MQL-8.39, 0.05–4.77, and < MQL-0.07, respectively (Table 2). Detailed concentrations for each sampling date are presented in Supplementary Tables S4a-S4e.

To assess the overall estrogen burden, summed estrogen concentration (ΣE) was calculated as E1 + E2α + E2β + E3 + EE2. Values below the MQL were assigned a value of zero for the purposes of ΣE calculation. ΣE varied markedly across sampling campaigns, ranging from 0.67 to 14.21 µg/L in influent and from 0.30 to 14.28 µg/L in effluent wastewater (Table 3). The reduction in ΣE ranged from 0% to 79%, demonstrating considerable fluctuations in treatment performance over time. Overall, these results indicate that estrogen removal at the studied wastewater treatment plant was inconsistent across sampling campaigns.

Table 2
Concentration ranges of individual estrogens in influent and effluent wastewater during the monitoring period.

Analyte	Influent (min-max, µg/L)	Effluent (min-max, µg/L)
E1	0.11–0.47	0.03–0.17
E2α	0.06–1.72	0.08–1.05
E2β	0.04–9.52	<MQL-8.39
E3	0.23–3.10	0.05–4.77
EE2	<MQL-0.07	<MQL-0.07

Notes: Detailed date-resolved concentrations reported as mean ± SD from replicate measurements are provided in Supplementary Tables S4a-S4e. All concentrations are reported in µg/L.

Table 3
Summed estrogen concentration (ΣE) in influent and effluent wastewater and reduction by sampling date.

Date	ΣE_in (µg/L)	ΣE_out (µg/L)	Reduction of ΣE (%)
23 Oct 2024	0.67	0.51	23
13 Nov 2024	0.96	0.52	45
11 Dec 2024	1.30	0.30	77
05 Feb 2025	4.11	1.34	67
26 Feb 2025	3.36	0.72	79
26 Mar 2025	14.21	14.28	0*
09 Apr 2025	11.43	6.04	47

Notes: ΣE = E1 + E2α + E2β + E3 + EE2. Values below the method quantification limit (MQL = 0.025 µg/L) were assigned a value of zero for the purposes of ΣE calculation. *For the 26 March 2025 campaign, the calculated change in ΣE was − 0.5%. Given the small magnitude of this difference relative to the observed method repeatability, it was interpreted as no measurable reduction (0%) rather than as evidence of an actual increase in estrogen concentration.

4. Discussion

4.1. Occurrence and removal of estrogens in the studied WWTP

Operational monitoring confirmed stable WWTP performance throughout the study period, with high removal efficiencies for total suspended solids (TSS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD_5), indicating effective treatment of bulk pollutants. Such performance is generally

consistent with efficient activated-sludge treatment, in which steroidal estrogens may be attenuated through sorption onto sludge solids and biodegradation mediated by microbial communities. However, the fate of micropollutants such as estrogens cannot be inferred directly from conventional treatment performance alone. In the present study, conventional removal remained consistently high, whereas estrogen concentrations and overall estrogen reduction varied markedly between sampling campaigns. This distinction is consistent with previous reports showing that WWTP effluents may comply with conventional treatment criteria while still retaining residual endocrine activity, and that targeted chemical measurements alone may not fully capture the biological relevance of treated effluents (Wolf et al. 2022; Bertanza et al. 2022).

All five target estrogens - E1, E2α, E2β, E3, and EE2 - were detected during the monitoring period, although individual results below the method quantification limit occurred for E2β and EE2. Concentrations showed pronounced temporal variability. In influent wastewater, concentrations ranged from 0.11 to 0.47 µg/L for E1, 0.06 to 1.72 µg/L for E2α, 0.04 to 9.52 µg/L for E2β, 0.23 to 3.10 µg/L for E3, and from < MQL to 0.07 µg/L for EE2. In effluent wastewater, the corresponding ranges were 0.03–0.17, 0.08–1.05, <MQL-8.39, 0.05–4.77, and < MQL-0.07 µg/L, respectively. These data indicate that although the plant reduced the concentrations of several estrogens on many sampling dates, removal was not consistent, and residual concentrations remained measurable in treated effluent.

This pattern agrees with the broader literature showing that estrogen removal efficiencies in municipal WWTPs vary markedly between facilities and over time, depending on treatment configuration, operational conditions, and the physicochemical and biological behavior of individual compounds. Reported removal efficiencies in municipal WWTPs may differ substantially even among activated-sludge systems, confirming that treatment design and operation strongly influence estrogen fate (Yien Fang et al. 2019; Abusam et al. 2022). Previous studies have shown that secondary treatment based on nitrifying activated sludge can play a major role in estrogen removal, although treatment performance may depend on wastewater matrix, season, and transformation or desorption processes affecting individual analytes (Wu et al. 2023). In this context, the present study provides full-scale evidence from a Polish municipal WWTP and demonstrates marked temporal variability in estrogen occurrence and removal across sampling campaigns. Previous studies indicate that secondary biological treatment is a key stage for estrogen removal, while natural estrogens are often removed more efficiently than EE2 (Islam et al. 2021).

To support integrated interpretation of treatment performance, summed estrogen concentration (ΣE), calculated as E1 + E2α + E2β + E3 + EE2, was used as an integrated metric representing the combined mass concentration of the five estrogens quantified in this study. Influent ΣE ranged from 0.67 to 14.21 µg/L, while effluent ΣE ranged from 0.30 to 14.28 µg/L. The reduction in ΣE ranged from 0% to 79%, confirming marked temporal variability in treatment performance. These results indicate that estrogen removal in the studied WWTP was highly variable over time and that campaign-specific interpretation is more informative than a single average removal value. From a practical perspective, ΣE provides a useful integrated descriptor of the estrogen burden in wastewater while retaining the possibility of compound-

specific interpretation through the underlying analytical dataset. However, ΣE should not be interpreted as a measure of total estrogenic activity, as the individual compounds differ in their biological potency.

4.2. Temporal variability and possible transformation pathways

The pronounced variability observed in both individual estrogens and ΣE suggests that the behavior of estrogens in the studied WWTP may have been influenced not only by net removal, but also by biological and physicochemical transformation processes occurring during treatment. The conceptual scheme presented in Fig. 1 provides a useful framework for interpreting these shifts (Gomes et al. 2022). Natural estrogens are biochemically related, and transformations between them may occur both before excretion and within the treatment system itself. In particular, estradiol may be oxidized to estrone, while estriol may represent a downstream metabolite contributing substantially to the estrogen signal in wastewater. These transformation pathways may partly explain the high relative contribution of E3 observed in several sampling campaigns.

An additional interpretative factor is the occurrence of conjugated estrogen forms in municipal wastewater. These conjugates, including sulfates and glucuronides, may be hydrolyzed during biological treatment, thereby releasing free parent compounds and altering the concentrations measured in influent and effluent samples. As a result, the free estrogen profile observed after treatment may reflect not only direct removal, but also ongoing deconjugation and interconversion processes. Previous studies have shown that conjugated estrogens can undergo extensive transformation in activated sludge, with deconjugation, oxidation, sulfation, and hydroxylation contributing to complex interconversion pathways and altering the profile of free estrogens during wastewater treatment; free estrogens and hydroxylated transformation products may therefore act as central nodes linking multiple pathways in wastewater systems (Yu et al. 2026). Likewise, the free estrogen profile observed after biological treatment may reflect not only net removal, but also ongoing deconjugation and interconversion processes, including the formation of E1 as a transformation product (Gomes et al. 2022).

The persistence of E1 is particularly relevant in this context. In line with other European monitoring studies, estrone has been identified as one of the most recurrently detected estrogens in environmental waters, which may partly reflect its formation as a degradation product of E2 (Merlo et al. 2024). Previous studies have also shown that changes in the concentration of parent steroid compounds across treatment may reflect deconjugation and hydrolysis processes occurring during wastewater treatment (Tanui et al. 2025). This is consistent with the present dataset, in which decreases in one analyte were not always accompanied by proportional decreases in others, and in which estrogen reduction based on ΣE remained limited in some campaigns despite effective conventional treatment. The observed variability in estrogen profiles may also reflect the formation of transformation products that retain or even increase estrogenic activity relative to parent compounds (Bertanza et al. 2022).

Besides biotransformation, process-related factors may also have contributed to the observed variability. Published evidence indicates that estrogen removal in conventional WWTPs is influenced by hydraulic

retention time, sludge age, organic loading, solids retention, redox conditions, and microbial community structure. The present results are compatible with this view. Although routine performance indicators suggested stable plant operation, the concentrations of individual estrogens and corresponding ΣE reductions varied substantially between campaigns. This may reflect the combined influence of sorption, biodegradation, deconjugation, and interconversion processes acting simultaneously in the treatment train. Reported mainly for livestock and municipal systems, previous evidence suggests that estrogen removal is strongly affected by process conditions, including redox environment, hydraulic retention time, and sludge retention time, and that aerobic treatment generally favors higher removal than anaerobic treatment (Gomes et al. 2022). Likewise, previous studies have shown that the removal of E1, E2 and EE2 may improve with increasing sludge retention time and hydraulic retention time, likely due to the development of nitrifying biomass (Amin et al. 2018).

Taken together, the present results are consistent with a treatment scenario in which attenuation of estrogens may occur through sorption and biodegradation, while deconjugation and interconversion processes may simultaneously reshape the composition of the estrogen mixture. Such a scenario could help explain why the estrogen profile of the treated effluent did not simply mirror the influent composition and why treatment outcomes differed considerably between sampling campaigns. Figure 1 should therefore be regarded as a mechanistic framework for interpreting profile shifts rather than as a definitive process model.

4.3. Environmental implications, limitations and future perspectives

The present findings have important implications for both environmental monitoring and wastewater treatment evaluation. First, they show clearly that treatment performance for endocrine-active micropollutants should not be inferred solely from bulk removal of conventional wastewater parameters. In the studied WWTP, high and stable removal of TSS, COD, and BOD_5 coexisted with marked variability in estrogen concentrations and in the reduction of ΣE. This means that conventional compliance indicators and targeted micropollutant measurements provide complementary rather than interchangeable information.

Second, the results support the need for temporally resolved monitoring. In systems showing marked temporal variability, date-specific concentration profiles and integrated metrics such as ΣE are likely to provide a more realistic basis for treatment assessment than aggregated mean values alone. This is particularly relevant in light of emerging European regulatory requirements. The recast Urban Wastewater Treatment Directive places increasing emphasis on micropollutant control and is expected to drive upgrades toward advanced or quaternary treatment in many WWTPs. Although the estrogens investigated in the present study are not among the indicator substances used to assess compliance with the micropollutant removal requirements of Directive (EU) 2024/3019, the Directive provides an important broader regulatory context for improving micropollutant monitoring and treatment. In this

context, robust baseline datasets from full-scale plants are needed to characterize concentration ranges, identify temporal variability, and provide a reference for evaluating future modernization efforts.

At the same time, several limitations of the present study should be acknowledged. First, the study was based on seven 24-h composite sampling campaigns conducted between October 2024 and April 2025. Although composite sampling provides a more representative estimate of daily wastewater composition than grab sampling, the limited number of campaigns may not fully capture longer-term, seasonal, or event-driven variability in estrogen occurrence and removal. Second, the analytical scope was limited to free estrogens; direct measurement of conjugated forms would allow more explicit evaluation of the deconjugation mechanism proposed in Fig. 1. Third, inclusion of more detailed process variables, such as sludge age, reactor temperature, or biomass characteristics, would strengthen mechanistic interpretation and improve comparison with literature. These limitations are important because previous studies have shown that considering only free estrogens may substantially underestimate estrogen-associated risks, since conjugated estrogens and their transformation products can persist through treatment and many retain estrogenic activity in the final effluent (Yu et al. 2026). In addition, monitoring limited to E1, E2 and E3 may underestimate the overall burden of natural estrogens in municipal wastewater, because less commonly studied metabolites can also occur at appreciable concentrations and contribute substantially to total estrogenic equivalents (Tang et al. 2020). This study demonstrated that estrogen removal in the investigated WWTP was highly variable despite stable conventional treatment performance. The results highlight the importance of transformation processes and the need for improved monitoring strategies.

Future work should therefore include higher-frequency 24-h composite sampling campaigns, broader analyte lists including conjugated estrogens and transformation products, and integration of targeted chemical analysis with bioanalytical tools. Such an approach would improve understanding of treatment-driven variability and provide a stronger basis for evaluating the effectiveness of future WWTP upgrades. From a practical perspective, optimization of hydraulic and sludge retention times, together with implementation of advanced treatment technologies such as adsorption or advanced oxidation, may become increasingly relevant for reducing estrogenic micropollutants under future regulatory frameworks (Wang et al. 2025).

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval

This is not applicable.

Consent to Participate

This is not applicable.

Consent to Publish

This is not applicable.

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Authors' Contributions

Marianna Piosik, Wiktoria Skotarczak, Anastazja Borisławska, Joanna Zembrzuska and Izabela Kruszelnicka contributed to the study conception and design, material preparation, data collection and data analysis. Marianna Piosik wrote the first draft of the manuscript. Joanna Zembrzuska and Izabela Kruszelnicka reviewed and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

The data supporting the findings of this study are provided within the article and its Supplementary Information. Additional data are available from the corresponding author upon reasonable request.

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Figures

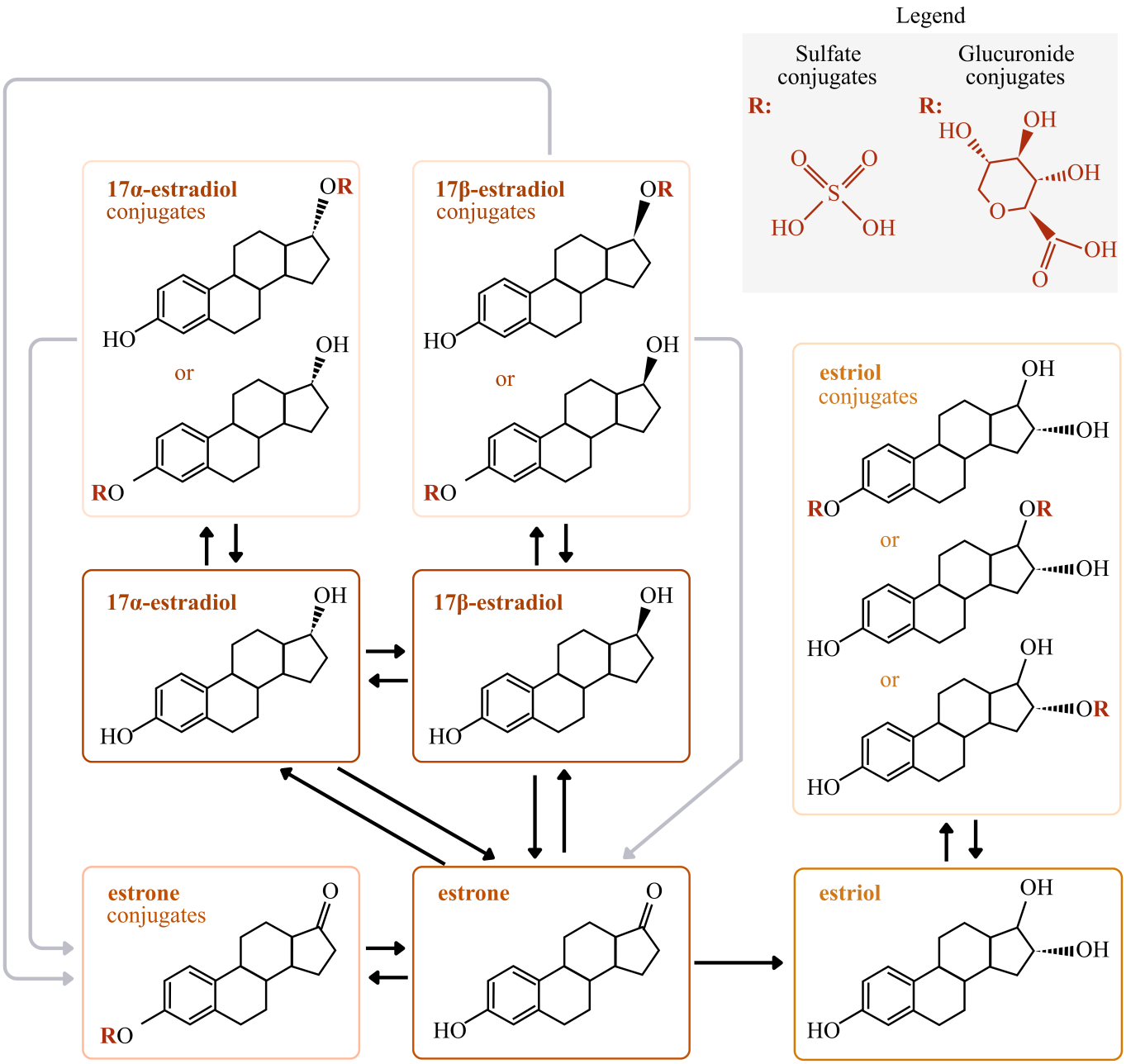

Figure 1

Conceptual scheme of biotransformation pathways among natural estrogens (E1, E2α/E2β and E3) and their sulfate/glucuronide conjugates in wastewater systems.

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