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Analysis of (Anti-)Oestrogenic and (Anti-)Androgenic Activities in Wastewater from the Lodz Sewer System

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Abstract: This article presents the results of a study on the oestrogenicity and androgenicity of urban wastewater in Lodz, and the possibility of their removal by the Group Wastewater Treatment Plant (GWWTP). Wastewater samples were taken at five points of the sewer system in the city and at the inlet and outlet of the GWWTP. The study was conducted using Yeast Oestrogen Screen (YES)/Yeast Androgen Screen (YAS) tests, which allow a general assessment of the content of compounds with (anti-)oestrogenic and (anti-)androgenic effects in wastewater, without identifying specific substances. Wastewater samples taken from the sewage network did not show (anti-)oestrogenic activity, while oestrogenic and antagonistic properties to androgens were detected in most of them. In the influent of the treatment plant, oestrogen agonistic activity was detected only in one sample (oestrogen equivalent—EEQ equal to 1.31×10^5 ng 17 β–oestradiol/L) and was 100% removed. The purification efficiencies in GWWTP for oestrogen and androgen antagonistic activity were 51.5–99.2% and 39.4–47.1%, respectively. Although no oestrogenic activity was detected in general wastewater in Lodz, observed high-antagonistic–androgenic activities may adversely affect the water body and cause, among others, the feminization of fish, especially in the case of discharge of untreated wastewater by combined sewer overflows.

Keywords: YES/YAS assay; (anti-)oestrogenicity; (anti-)androgenicity; urban wastewater; wastewater treatment plant

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Citation: Brzezinska, A.; Sakson, G.; Olejnik, D. Analysis of (Anti-)Oestrogenic and (Anti-)Androgenic Activities in Wastewater from the Lodz Sewer System. *Water* **2023**, *15*, 2454. https://doi.org/10.3390/w15132454

Academic Editor: Laura Bulgariu

Received: 24 May 2023
Revised: 29 June 2023
Accepted: 30 June 2023
Published: 4 July 2023

1. Introduction

Currently, the water crisis has become a global problem, and the reuse of water is one of the elements of its mitigation. In addition, the development of various branches industries, including the pharmaceutical industry, are a source of surface water pollution with toxic and dangerous substances not only for human health but also for wildlife (fishes, mammals, birds, reptiles, amphibians, and invertebrates). Micropollutants are continuously entering into the environment and can be highly recalcitrant causing acute and/or long-term harmful effects to inter alia water organisms [1–5]. Medications containing oestrogens are used for various health reasons, including contraception, treatment for menopausal symptoms and osteoporosis prevention [6], and its concentration in surface waters still increases [7]. Sex hormones cause extremely unfavourable effects on living organisms, including fish at an exceptionally low concentration of 0.1 ng/L [8–11]. These compounds can cause various adverse biological effects on organisms, such as its feminisation, dysregulation of natural processes related to reproduction, lowering the physiological condition of the organisms [12], and even the occurrence of neoplastic processes, thus drastically decreasing animal welfare [13–15]. Oestrogens contained in food and water can cause premature menopause in women and affect the decline of fertility and feminisation of men [16,17]. The feminisation of fish can also be caused by anti-androgenic substances that may be present in treated sewage discharged into surface waters [18].

The analysed literature shows that oestrone (E1), oestradiol (E2), oestriol (E3), and synthetic 17α-*ethinylestradiol* (EE2) are the most significant in terms of environmental impact [19–21]. Studies of these hormones conducted in China, in the rivers of southern Jiangsu, showed significant differences in their concentrations (ranging from 1.96 to 143.29 ng/L), together with the observation of their seasonality. Higher concentrations in waters were observed in winter than in spring and autumn periods [22,23]. These results indicate that different anthropogenic activities and hydrological regimes will lead to differences in the distributions of oestrogenic compounds. Research conducted by Barel–Cohen et al. [24] suggest that hormones in readily measured quantities can be transported considerable distances from the source of pollution. In recent years, increasing efforts have been made to investigate the presence of natural hormones and drug-derived synthetic hormones in the aquatic environment [25].

Nowadays, wastewater has been recognised by scientists around the world as a major source of oestrogenic contaminants because humans excrete oestrogen daily [16,26], and the ability of wastewater treatment processes to mitigate current and future environmental risks from those compounds is being investigated [4,27,28]. Currently, there is no standard for safe levels of oestrogen to be discharged into surface waters from wastewater treatment plant (WWTP) effluents. The quality of the effluent discharges depends on the quality of the raw wastewater received and the treatment process performed by the WWTP. Study on oestrogen load directed to wastewater treatment plants conducted by [29] indicated that its level depends primarily on population data, demographic profiles, consumption rates (only for EE2), and excretion rates. Pregnant women excrete between 260–790 μg/day of oestrone, 280–600 17β-oestradiol, and form 6000 to nearly 10,000 μg/day of oestriol [30]. In addition, there is the consumption of hormonal drugs, which additionally increases the oestrogenicity of wastewater. WWTPs do not completely remove oestrogens in the effluent and, therefore, biosolids and wastewater effluents containing significant concentrations of oestrogens are sometimes discharged into the natural environment [31,32]. This is due to the fact that most conventional treatment plants do not have advanced treatment technologies, such as ozonation [33], advanced oxidation [34–36], activated carbon adsorption [37] or membrane filtration [38,39]. Therefore, residues of micropollutants are still found in effluents, and it is necessary to modernise the existing treatment plants by implementing additional advanced wastewater treatment technologies to reduce or eliminate them [40]. In addition to WWTPs, hospitals have also been identified as another major source of steroidal oestrogen contamination, and several studies have shown that steroidal oestrogens, especially high levels of oestriol, have been found in all hospital wastewater samples [41]. Livestock farms, slaughterhouses, and large urban agglomerations are also important sources of oestrogen. [30,42,43]. According to [44] the world's human population, about seven billion people approximately discharge 30,000 kg/yr. of natural steroidal oestrogens (E1, E2, and E3) and an additional 700 kg/yr. of synthetic oestrogens (EE2) solely from birth control pill practices. It should be noted that higher concentrations of oestrogen are discharged into the environment by livestock. In the United States and countries under the European Union, the annual oestrogen discharge via this way, at 83,000 kg/yr., is more than twice the rate of human discharge. According to a National Sewage Sludge Survey of the US EPA, 76 tons of oestrogen was released into the environment form solid waste, especially animal manures [19].

Much of the literature focuses primarily on detecting agonist activity in water and wastewater, but some environmental contaminants can act as antagonists. If they are present in the sample, they can reduce the agonist response in vitro [45], highlighting the importance of assessing both agonism and antagonism in wastewater samples. (Anti-)androgenic activities have been reported in aquatic environments in many countries [46–49], but compounds responsible for those activities often remain unidentified [50–52].

Despite more research conducted around the world on the occurrence and concentration of oestrogens in surface waters and wastewater, the number of these data is still limited. Some oestrogenic hormones (including, for example, 17β-estradiol, EE2) have been included in the updated watch list of 10 other substances [53], but according to Article 8b (2) [54], the Commission is to update the watch list every two years. When updating the list, the Commission is to remove any substance for which a risk-based assessment, as referred to in Article 16 (2) [55], can be concluded without additional monitoring data. Currently, according to the last Commission Implementing Decision [56], these compounds are no longer observed, but they still remain unregulated pollutants.

The aim of this paper was to determine both the oestrogenic and androgenic properties of wastewater in the Lodz sewage system, and the possibility of reducing those properties at the wastewater treatment plant. The research was carried out using YES/YAS assay, which enable, at the same time, a general assessment of the content of compounds with (anti-)oestrogenic and (anti-)androgenic activity in wastewater, without the need to identify specific substances. Studies using both (anti-)oestrogenic and (anti-)androgenic assays are necessary for the assessment of threats to receiving water, but the results of such studies are still lacking. They are usually carried out in wastewater after the treatment process, possibly flowing to the wastewater treatment plant. However, there are no studies of this type carried out in the combined sewer system, where untreated wastewater is periodically discharged into surface waters through combined sewer overflows (CSOs). In this case, a significant amount of pollutants, including hormonal substances, enters directly into surface waters.

2. Materials and Methods

2.1. Characteristics of the Studied Catchment—Lodz City

Lodz is the fourth city in Poland in terms of population (about 690,000 inhabitants in 2021) and the fourth in terms of area 293.25 km^2 (Statistical Office in Lodz, 2022 [57]. The city is located in the centre of the country (Poland) and due to the presence of larger or smaller rivers and streams (18 rivers) was, in the past, a large centre of the textile industry. Currently, these are small watercourses in the city centre, mostly hidden in underground channels. Unfortunately, since the nineties of the twentieth century, mainly due to the collapse of the textile industry, a gradual decrease in the number of people in the city has been observed. In addition, with the increase in age, there is a change in the proportions between women and men in Lodz. In the total number of inhabitants over thirty, women start to dominate (Figure 1, Table 1). Such differences in reproductive age may affect the concentration of these hormones in the wastewater. The percentages shown in Figure 1 refer to the proportions of women and men, respectively, in a given age group.

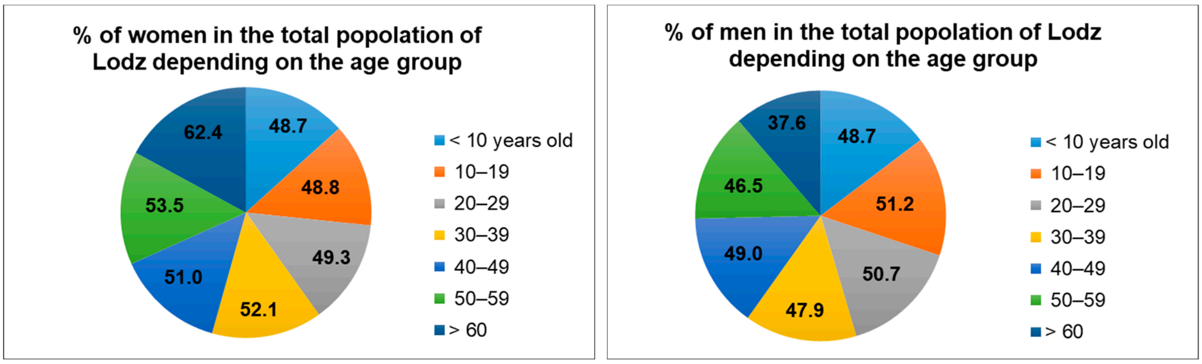

Figure 1. Breakdown of population in Lodz city depending on the age group, based on [57].

Table 1. Feminisation and masculinisation coefficient for the population of Lodz city based on [57].

Parameter	Value (%)
feminisation coefficient	119
masculinisation coefficient	84

2.2. Wastewater System of Lodz

Lodz is equipped with a hybrid sewage system (Figure 2). A combined sewer system exists in the central part of the city, whereas in the remaining districts, there is a separate sewer system. The first of them is equipped with 18 CSOs operating during heavy rainfall and discharging excess wastewater into four urban rivers. The Ner River is the main receiver of all wastewaters generated in Lodz (discharge form wastewater treatment plant, CSOs and drainage system).

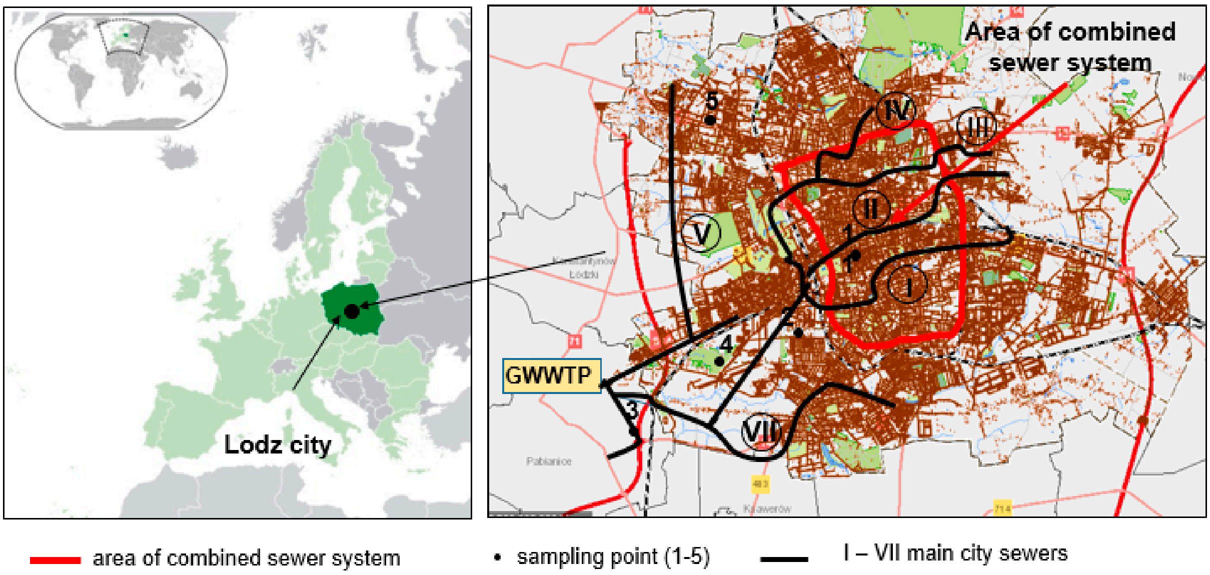

Figure 2. Lodz city location and sewage system in the city with sampling points and a marked area of combined sewer system.

2.3. Group Wastewater Treatment Plant of Lodz

The Group Wastewater Treatment Plant (GWWTP) in Lodz serve a population of about 700,000 inhabitants and treats on average about 170,000 m^3/day of combined wastewater coming from the city. Additionally, wastewater from three nearby small towns flows into the treatment plant. The biological stage of the GWWTP works in the MUCT (Modified University of Cape Town) system (Figure 3).

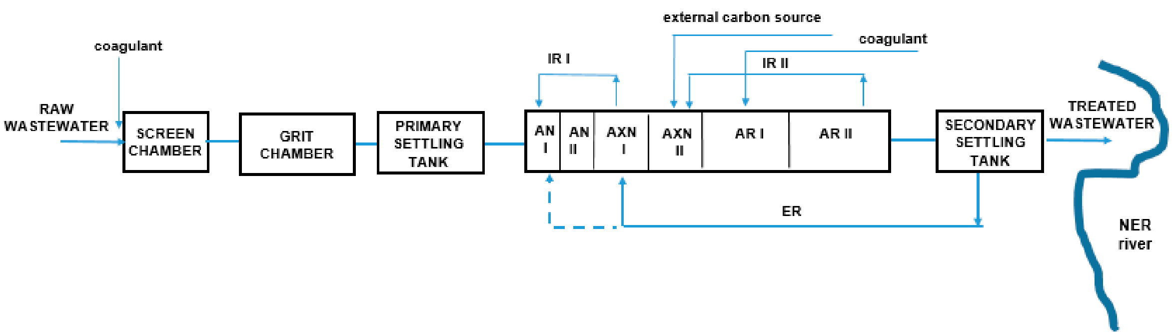

Figure 3. Scheme of the GWWTP technological system (for one process line). AN—anaerobic chamber, AR—aerobic chamber, AXN—anoxic chamber, IR—internal recirculation, ER—external recirculation.

2.4. Sample Collection

Wastewater sampling points were located in several parts of the city, both on a sanitary and combined sewer system. Wastewater was also collected at the inflow and outflow of the treatment plant. Determination of oestrogenicity is part of a large 3-year measurement campaign on the extensive urban sewage system of Lodz. Samples for testing were collected at each point of sewerage during dry weather (D), and in two cases (sampling point 2 and 5) during wet weather (W), using an automatic sampler Teledyne ISCO 6712 every hour around the clock. Samples were prepared for analysis as cumulative samples from four subsamples, thus obtaining six test samples in 24 h.

2.5. Methodology of Wastewater Tests

During the 2018–2021 monitoring campaign, the oestrogenic and androgenic activity, as well as a wide range of physicochemical parameters [58] and the toxicity of the wastewater, was tested. Oestrogenic and androgenic activities were determined using YES/YAS method (Xenometrix AG, Swiss Commitment for Bioassays) [59]. In the assay, the genetically modified yeast (*Saccharomyces cerevisiae*) to identify compounds that can interact with the human oestrogen and androgen receptors hERα and hAR were used. Oestrogens and androgens, as well as substances with a similar effect, can form complex compounds with receptors of modified yeast. *Saccharomyces cerevisiae* produce β-galactosidase, which converts the yellow substrate chlorophenol red-β-D-galactopyranoside (CPRG) into a red product, can be quantified colourimetrically at 570 nm.

The system can identify both activating (agonistic) and inhibitory (antagonistic) activities of tests compounds. For the determination of antagonist activities, the samples were incubated in the presence of a fixed concentration of a reference agonist (17 β -oestradiol for YES and 5α-dihydrotestosterone for YAS). Inhibition of the response relative to the fixed agonist concentration is a sign of antagonist activity [59].

Results were evaluated for oestrogenic and androgenic properties, agonistic and antagonistic, and yeast growth inhibition or possible cytotoxicity. The assay consists of four main steps: (1) Yeast cultivation (usually 5–10 days + 1 day); (2) Proper assay, including sample and control preparation, as well as dilution plate preparation (17β-oestradiol (E2) for YES agonists, 4-hydroxytamoxylene. (TH) for YES antagonists, and 5α-dihydrotestosterone (DHT) for YAS agonists and flutamide (FL). After preparation, the plates with yeast cells are incubated for 48 h at 31 °C in the presence of a substrate for β-galactosidase. (3) Reading the plates with a spectrophotometer at 570 and 690 nm to determine the colour of the CPRG breakdown product and yeast growth, respectively. (4) Calculations and interpretation of results. Outcomes are evaluated for oestrogenic and androgenic properties, agonistic and antagonistic, and yeast growth inhibition or possible cytotoxicity.

The growth factor (G) and Induction Ratio (IR) were calculated as follows:

$$G = \frac{A_{690,S}}{A_{690,N}}$$

where $A_{570,S}$ is the net absorbance of the sample S at 570–690 nm

$A_{570,N}$ is the net absorbance of the solvent control at 570–690 nm.

$$IR = \frac{1}{G} \times \frac{A_{570,S}}{A_{570,N}}$$

where $A_{570,S}$ is the net absorbance of the sample S at 570–690 nm

$A_{570,N}$ is the net absorbance of the solvent control at 570–690 nm.

For all samples, the oestrogen equivalent (EEQ) was calculated. EEQ corresponds to the concentration of 17 β–oestradiol (E2), which would provide the same activity as a sample. Equivalents for antagonistic oestrogenic activity (aEEQ), agonistic androgenic activity (AEQ), antagonistic androgenic activity (aAEQ) were calculated analogously. For wastewater samples taken from the wastewater system, a higher toxicity was observed in the tested oestrogenicity. Toxicity was determined using the ToxTrak method in accordance with the guidelines (Toxicity ToxTrakTM Method 10017, HACH LANGE Manual). ToxTrak tests results—wastewater toxicity was expressed as the degree of inhibition—DI (%), which was calculated according to the formula below:

$$DI = 1 - (\Delta A\ sample/\Delta A\ control) \times 100\ (\%)$$

where ΔA = Initial absorbance value–Final absorbance value.

The absorbance of samples and controls was measured using a spectrophotometer DR 6000 at a wavelength of 603 nm.

The methodology of laboratory tests of physicochemical wastewater parameters was presented in the previous article [58].

3. Results and Discussion

Results of wastewater physicochemical characteristics at sampling points of the sewage system are presented in Table 2. Contaminant concentrations in these wastewater samples flowing through the sewage system in Lodz were similar to those observed in other European countries [60,61].

Table 2. Wastewater characteristic in sampling point.

SP	pH	BOD_5 mgO_2/L	COD mgO_2/L	TSS mg/L	PC mg/L	TOX Degree of Inhibition—DI (%)
GWWTP	7.80	575	1093	586	0.03	40.5
1.	8.53	70	316	158	-	65.8
2.	7.50	472	790	440	0.08	31.5
3.	7.62	575	1014	438	0.038	-
4.	7.46–7.79	435–1250	937–2416	236–3264	0.055–0.125	20.7–51.8
5.	7.27–7.65	380–1000	728–1636	236–584	0.039–0.087	40.6–52.7
Limit values for sewage discharged into sewer system *	6.5–9.5	600	1200	600	15	-

Notes: * Permissible values of pollutants in industrial wastewater discharged into the Lodz sewer system.

Oestrogenicity was tested in wastewater samples of higher toxicity and its results are presented in Table 3. Wastewater toxicity, expressed as a degree of inhibition (DI), was variable and ranged from 2.3 to 72.4%, depending on the tested point, but in the tested samples collected for oestrogenicity analyses, it ranged from 20.7 to 65.8%. Most of these samples showed toxicity >40% DI, which means that the effluent may have toxic properties.

However, the research conducted during the YES/YAS procedure showed that none of the wastewater samples had a cytotoxic effect on cells of any of the genetically modified yeast strains YES or YAS. The growth of yeast cells measured spectrophotometrically was normal with a growth factor of G greater than 0.5. This means that the obtained results are reliable and undisturbed by additional effects.

The oestrogenic activity via the yeast screening assay of the wastewater sample from sewer system, can be expressed as an equivalent relative oestrogenicity and androgenicity, as summarized in Table 3.

In most samples, the test results showed the absence of oestrogenic agonistic properties. Only at point No. 4 were oestrogenic agonistic effects detected in two out of the three tested wastewater samples (Table 3). The first sample was collected on 05.06.2019 (EEQ = 0.9 ng/L), and the second one on 09.01.2020 (EEQ = 13.70 ng/L). In addition, it should be noted that the first result concerns dry weather and the second wet weather, which indicates the possibility of higher agonistic oestrogenic activity in wastewater during wet weather. The increased values of the activity in the wastewater during wet weather may be associated with sewer sediments washing out. The study conducted by [62,63] showed a significant contribution of sewer deposits to the pollutant load in combined wastewater during wet weather. The effect of sewer sediments on the concentration of endocrine compounds in wastewater requires verification and further research. In collected wastewater samples, no agonistic properties for androgenic activity were found (Table 3). Oestrogenic antagonistic properties (aEEQ ranged from 3.01×10^2–2.2×10^6 ng HT/L) were found in all wastewater samples collected from the sewer system, as well as in most samples where androgenic antagonistic properties (aAEQ = from 1.88×10^4–2.13×10^7 ng FLU/L) were detected. The wastewater characteristic—basic physicochemical parameters and toxicity level—at the inlet of GWWTP is shown in Table 4.

Table 3. Evaluation of (anti-)oestrogenic and (anti-)androgenic activities of wastewater from the sewage system of Lodz, based upon the IR criterion.

Sampling Point	Data	Oestrogenic Activity				Androgenic Activity			
		Agonistic Oestrogenic Activity		Antagonistic Oestrogenic Activity		Agonistic Androgenic Activity		Antagonistic Androgenic Activity	
		EEQ, ng E2/L	IR	aEEQ, ng HT/L	IR	AEQ, ng DHT/L	IR	aAEQ, ng FLU/L	IR
1.	09.01.2019 W	ND	−	7.59×10^4	+	ND	−	1.36×10^7	+
2.	29.01.2019 D	ND	−	1.29×10^4	+	ND	−	1.57×10^7	+
3.	28.11.2019 D	ND	−	3.52×10^5	+	ND	−	9.45×10^6	+
4.	05.06.2019 D	0.90	+	3.03×10^2	+	ND	−	ND	−
4.	05.12.2019 D	ND	−	3.50×10^5	+	ND	−	1.70×10^7	+
4.	09.01.2020 W	13.70	+	8.49×10^5	+	ND	−	ND	−
5.	10.12.2020 D	ND	−	3.01×10^2	+	ND	−	ND	−
5.	17.12.2020 D	ND	−	2.20×10^6	+	ND	−	ND	−
5.	13.01.2021 D	ND	−	1.04×10^5	+	ND	−	1.88×10^4	+

Notes: D—dry weather; W—wet weather. E2—17 β-estradiol (reference agonist YES). HT—4-hydroxytamoxifene (reference antagonist YES). DHT—5α-dihydrotestosterone (reference agonist YAS). FLU—flutamide (reference antagonist YAS). IR "+"—means that the sample shows the given property; for agonists, the induction factor (IR) of the assay is greater than or equal to IR10, where IR10 is defined as an IR that is 10% (IR max − solvent IR) above the solvent IR.; for antagonists, the IR of the sample is less than or equal to the IR50 and the IR50 is defined as 50% (Control IR − Solvent IR), where the Control IR is the corresponding fixed concentration of the agonist used. IR "−"—means that the sample does not show the given property; for agonists, the induction factor (IR) of the assay is less than IR10; for antagonists, the IR of the sample is greater than the IR50. ND—means that the concentration of oestrogenic or androgenic substances was below 10^{-10} M for oestrogens (YES) or below 10^{-9} M for androgens (YAS) in terms of the standard.

Table 4. Wastewater characteristic at the inlet of GWWTP (samples used for the YES/YAS assay).

Parameter	pH	BOD_5 mgO_2/L	COD mgO_2/L	TSS mg/L	PC mg/L	TOX Degree of Inhibition—DI (%)
Inflow	7.41–7.66	186–700	442–1742	212–828	0.01–0.051	5.4–40.5
Limit values for treated sewage **	-	15	125	35	-	-

Notes: ** Regulation of the Minister of Maritime Economy and Inland Navigation of 12 July 2019 on substances particularly harmful to the aquatic environment and the conditions to be met when introducing sewage into waters or into the ground, as well as when discharging rainwater or meltwater into waters or to devices water.

The range of oestrogenic and androgenic activities in the inlet to GWWTP is shown in Table 5. Comparing the results from the sampling points located on the sewer system (Table 3) and on the inflow to the GWWTP (Table 5), one can notice a slightly greater variation in the first case. This may be due to, among others, the differentiation of land use. Higher local values of oestrogenic and androgenic activity are important in the case of the CSOs operation, when untreated wastewater are discharged into surface waters without treatment.

Table 5. (Anti)oestrogenic and (anti)androgenic activities of wastewater at the inlet of GWWTP.

	Oestrogenic Activity				Androgenic Activity			
	Agonistic Oestrogenic Activity		Antagonistic Oestrogenic Activity		Agonistic Androgenic Activity		Antagonistic Androgenic Activity	
	EEQ, ng E2/L	**IR**	**aEEQ, ng HT/L**	**IR**	**AEQ, ng DHT/L**	**IR**	**aAEQ, ng FLU/L**	**IR**
Min.	ND	–	2.20×10^2	+	ND	–	7.53×10^5	+
Max.	1.31	+	8.49×10^5		ND	–	2.32×10^7	+

Conducted research indicated that the oestrogenic and androgenic properties of wastewater at the inlet to GWWTP may undergo some changes even during the day (Figure 4). For example, on 11.09.2019, samples from 9 a.m. and 1 p.m. showed agonistic oestrogenicity of 1.31 ng E2/L and 0.9 ng E2/L (this value, due to the logarithmic scale, is not visible on the figure), respectively (Figure 4). In samples from 5 p.m. and 9 p.m., wastewater did not show these properties. Antagonistic oestrogenic activity was significantly higher in the 9 a.m. sample (8.49×10^5 ng HT/L) than in the other samples 2.20–3.01 $\times$ 10^2 ng/L). No AEQ property was detected in any sample and the aAEQ was relatively constant.

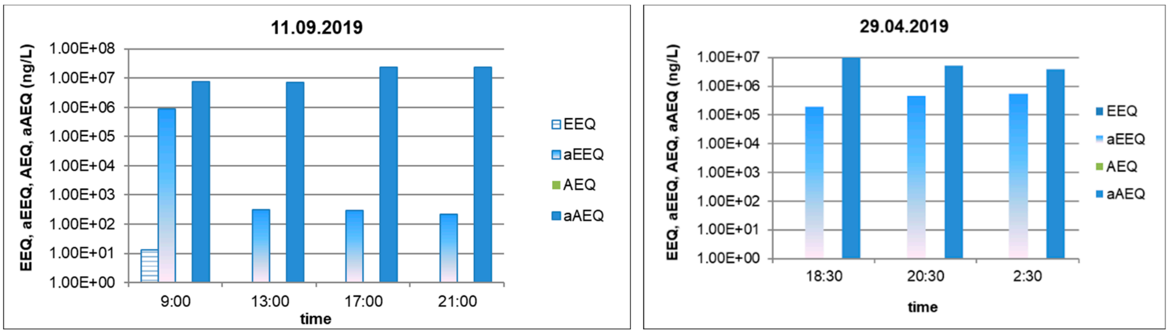

Figure 4. Daily variability of EEQ, aEEQ, AEQ, and aAEQ in influent to GWWTP.

The results of oestrogenicity and androgenicity tests of raw and treated wastewater are presented in Table 6. The tests were carried out twice, collecting wastewater at the inlet and outlet with the time delay resulting from the wastewater treatment processes.

Table 6. Evaluation of (anti)oestrogenic and (anti)androgenic activities of wastewater at the inlet and outlet of WWTP, based upon the IR criterion.

Sample/Date	Oestrogenic Activity				Androgenic Activity			
	Agonistic Oestrogenic Activity		Antagonistic Oestrogenic Activity		Agonistic Androgenic Activity		Antagonistic Androgenic Activity	
	EEQ ng E2/L	**IR**	**aEEQ, ng HT/L**	**IR**	**AEQ ng DHT/L**	**IR**	**aAEQ, ng FLU/L**	**IR**
Inlet 11.09.2019	1.31	+	8.49×10^5	+	ND	–	7.33×10^6	+
Outlet 11.09.2019	ND	–	6.73×10^4	+	ND	–	3.88×10^6	+
Reduction (%)	100		99.2				47.1	
Inlet 10.03.2021	ND	–	4.66×10^5	+	ND	–	2.03×10^7	+
Outlet 10.03.2021	ND	–	2.26×10^5	+	ND	–	1.23×10^7	+
Reduction (%)			51.5				39.4	

Wastewater treatment processes have an impact on reducing the oestrogenic and androgenic activities. In the case of GWWTP on 11.09.2019, oestrogenic agonistic properties were identified in the inflow but not in the outflow, which corresponds to 100% reductions. In the sample dated 10.03.2021, no agonistic oestrogenic activity was identified either in the inflow or outflow from the treatment plant.

In treated wastewater samples, the agonistic properties for oestrogenicity and androgenicity were not detected during the study (Table 6). Similar results were obtained by [64] in the determination of these properties in wastewater discharged from several treatment plants into the Gdansk bay (Poland). Oestrogenic and androgenic antagonistic properties were identified in each sample of wastewater from GWWTP with higher values observed at the treatment plant inlet. The reduction was 99.2 and 51.5% for the antagonistic oestrogenic properties, and 47.1 and 39.4% for the antagonistic androgenic properties, respectively. The obtained results of antagonistic oestrogenic and androgenic activity in treated wastewater were similar to the values provided by [65]—for aEEQ between 10^4–10^5 ng/L and for aAEQ between 10^6–10^7 ng/L, respectively. According to [12], in the influent to the four tested WWTPs, agonistic androgenic activity (AEQ) ranged from 47 to 59 ng DHT/L, and the treated wastewater was between 0.34–0.79 ng DHT/L. Anti-androgenic activity in the influent to WWTP and effluent from WWTP showed a level between 5.2×10^3 to 2.6×10^4 ng FLU/L and 3.5–8.9×10^3 ng FLU/L, respectively. Research conducted during 3 years by [66] showed that the average EEQ concentrations of treated wastewater discharged by investigated treatment plants were estimated as 23, 33.9 and 24.2 ng/L for 2005, 2006 and 2007, respectively, and were significantly lower than in raw wastewater coming to those treatment plants. This indicated that treatment processes remarkably reduced the oestrogenic activity of oestrogenic compounds before being discharged into river waters. Usually, reduction rates of oestrogenicity at conventional WWTPs with tertiary treatment are high—from about 80% to more than 90% [67,68]. Although, high reductions during the treatment process residual concentrations occurring in WWTP discharges may still pose a risk to aquatic fauna and flora [69]. Especially in crowded and development urban areas, the wastewater treatment systems might be insufficient to eliminate all oestrogenic activity from municipal wastewaters [70].

During the study in Lodz, the correlations between the hormonal activity of wastewater and their main physicochemical parameters, listed in Table 2, were also analysed. Strong correlations between hormonal activity and easily measurable wastewater indicators would facilitate the identification of potential hazards resulting from the presence of substances, which caused possible endocrine disruption. Unfortunately, no correlation was found with pH, BOD_5, COD, TSS and PC. There was only a negative medium correlation between toxicity and antagonistic activity for oestrogenicity (r = −0.31) and a strong negative correlation between toxicity and antagonistic activity for androgenicity (r = −0.53) (Figure 5).

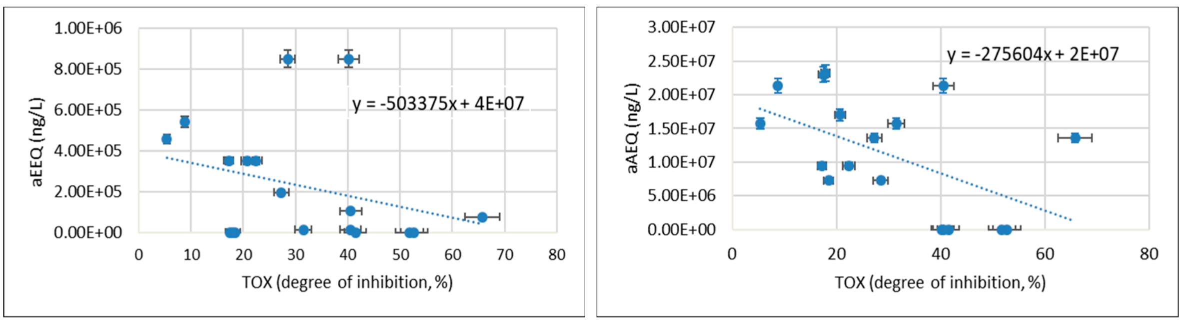

Figure 5. Correlation between aEEQ, aAEQ and toxicity in wastewater from the Lodz sewer system.

The studies of the wastewater hormonal activity, with the use of YES/YAS tests carried out in Lodz, allowed estimated values only. According to studies conducted by other researchers [71,72], the use of yeast-based assays can be effective in the first assessment of oestrogenic activity of waters. It should be noted, however, that the data of methods depends mainly on the matrix; that effect is very important in the real samples. According to [73] and previous studies [74–77], bioassays are promising tools for environmental monitoring and risk assessment of chemical substances, allowing the evaluation of biological responses to a given complex sample, including possible interactions of different pollutants. Nevertheless, for example, cytotoxicity and anti-oestrogenic activity found in the sample tested might mask the oestrogenic activity of dissolved and particulate fractions of samples, leading in this case to possibly underestimated results. Bioassays are less affected by the sample matrix and can detect the oestrogenic effects of water samples at ng or pg level, therefore they could be used as screening methods to complement chemical analysis [78]. The observed difference in responsiveness among bioassays—based on mixture composition—is probably due to biological differences between them, suggesting that panels of bioassays with different characteristics should be applied according to specific environmental pollution conditions. According to [79], the YES assay was less sensitive than other tests, which may result in a higher number of undetected results. Perhaps for this reason, with the low hormonal activity occurring in the tested wastewater in Lodz, the tests provided a negative response.

Current in vitro bioassays are sufficiently sensitive to detect the presence of endocrine disrupting chemicals, which have a potential risk on human and ecosystem health [65]; however, due to the high purchase price, they are unlikely to be included in monitoring wastewater in sewer systems. Therefore, other simplified methods are being sought to identify potential threats. In the case of oestrogens, it is possible to estimate their concentrations on the basis of excretion rates of demographic groups, flows and of the wastewater treatment plant's operation [29,80]. However, due to the negative impact of these compounds on living organisms [81], even at very low concentrations, possible predictions should be verified by testing samples in a laboratory environment.

It should be noted that inhibitory effects may also be caused by non-specific effects, which can lead to a reduction of the response inducted by the fixed concentrations of E2 and DHT. Parallel inhibition in the YES and YAS assay may be indicative of such non-specific inhibition, which is not a true oestrogen or androgen antagonistic effect.

The use of YES/YAS assays to assess the occurrence of oestrogenicity and androgenicity allows, among others, to identify areas in the city where endocrine substances may appear in wastewater transported through the sewer system. In case of a combined sewer system, those substances can be discharged with raw wastewater to the receiving water through combined sewer overflows. Therefore, it is necessary to identify the sources of their emission in the catchment area, such as hospitals, pharmaceutical manufactories, etc., and then modernise the wastewater management. The dynamically developing industry and the pharmaceutical market may cause an intensification of problems related to the increased emission of hormonal compounds into the aquatic environment. In Lodz, the risk of polluting water bodies with these compounds is additionally increasing due to the population aging and the increasing women percentage in the population. A separate issue is the modernisation of old, combined sewer systems in cities, so as to eliminate or limit the combined sewer overflow activity. The new proposal for amendments to the Wastewater Directive on urban wastewater treatment form [82], emphasizes the need to reduce the pollutant load discharged through combined sewer overflows to collect a 1% load of urban wastewater, calculated in dry weather conditions, and to introduce the fourth stage of treatment in wastewater treatment plants, thus enabling the removal of micropollutants, including pharmaceuticals.

4. Conclusions

- YES/YAS bioassays may be used to assess general levels of endocrine disrupting chemicals and, therefore, could be included, either as an alternative method or complementary to the chemical analysis of wastewater, surface water, as well as for assessing the removal those substances in WWTP.
- The samples of wastewater from the sewer system in Lodz did not exhibit agonistic oestrogenic activity with two exceptions (EEQ = 0.9 and 13.7 ng E2/L). No agonistic androgenic activity was found in any of the tested samples. Oestrogenic antagonistic

properties (aEEQ) were found in all wastewater samples collected from the sewer system (max = 8.49×10^5 ng HT/L), as well as in most samples, where androgenic antagonistic properties (aAEQ) were detected (max = 1.70×10^7 ng/L).

- Oestrogen and androgen agonist activity was not found in wastewater from the treatment plant. Both oestrogenic and androgenic antagonistic properties were identified in the inflow and outflow from the treatment plant. In the treatment process, they were reduced from 39.4 to 99.2%, depending on the type of activity. Those results confirm that in the case of conventional wastewater treatment plants, a constant high reduction of hormonal pollutants is not guaranteed.
- YES/YAS bioassays used to analyse wastewater containing mixtures of both agonists and antagonists provided general information about the presence of all active chemical compounds with oestrogenic and androgenic effects. No correlation was found between the hormonal activity of wastewater and their basic physicochemical parameters, which could facilitate the identification of oestrogenic and androgenic contaminates that pose a threat to the aquatic environment.
- The results of the YES/YAS assay indicate that due to the CSOs activity in Lodz, with discharge of untreated wastewater into small urban rivers, there is the possibility of threats to the aquatic ecosystem, resulting from the presence of endocrine disrupting chemicals. Reducing the risk to the receiving waters from endocrine disruptors contained in wastewater can be achieved both by eliminating/reducing significant point sources of pollution in the catchment, and by reducing CSO activities and the modernisation of existing wastewater treatment plants to increase their micropollutant removal efficiency.

Author Contributions: Conceptualization, A.B. and G.S.; Methodology, A.B. and G.S.; Formal Analysis, A.B. and G.S.; Investigation, D.O.; Resources, D.O.; Data Curation, A.B. and G.S.; Writing—Original Draft Preparation, A.B. and G.S.; Writing—Review & Editing, G.S. and D.O.; Visualization, A.B. and G.S.; Supervision, A.B., Funding Acquisition, A.B. and G.S. All authors have read and agreed to the published version of the manuscript.

Funding: The research results presented in the article were created as part of Project entitled "Development of a monitoring, early warning and sustainable management system for wastewater treatment plant minimizing pollutant emission to aquatic environment from urbanized area" co-financed by the European Union financed from the European Regional Development Fund under the Smart Growth Operational Programme. The project is implemented as a part of the National Centre for Research and Development: Regional Science and Research Agendas competition. Co-financing contract number: POIR.04.01.02-00-0060/17.

Data Availability Statement: The data sets generated and analysed during the current study are not publicly available due to the duration of the NCRD Project but are available from the corresponding author on reasonable request.

Acknowledgments: The Authors would like to acknowledge Ewa Liwarska-Bizukojć for her technical support in the determinations of wastewater toxicity.

Conflicts of Interest: The authors declare no conflict of interest.

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