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STEROIDAL ESTROGENS OCCURRENCE AND REMOVAL IN TWO STPs AND COASTAL MARINE WATER FROM JEDDAH, SAUDI ARABIA

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ABSTRACT

In the present study, the concentration of four steroidal estrogens (estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2)) was determined in two sewage treatment plants (STPs) and in surface marine water from Jeddah's coast. The average removal efficiencies (REs) of steroidal estrogens ranged 46.7-89.5%, in King Abdulaziz University (KAU) STP. In the Al-Khumrah (AK) STP wastewater, the RE was 77.7-88.5%. In the marine water samples, E1 was the most frequently detected estrogen with concentrations ranging from not detected (ND) to 50 ng/L. E2 was detected in seven samples with variable concentrations up to 3.2 ng/L. E3 was detected in two samples from one site (0.8 and 1 ng/L), whereas EE2 was detected above limits of quantification (LOQ) in three samples with a maximum concentration of 1.9 ng/L in three different sites. Estrogen levels on the Red Sea coast were generally low compared to some published reports elsewhere. This study provides baseline data for steroidal estrogens occurrence and removal in STP wastewater and their concentrations in the coastal water likely impacted by STP effluents for the first time in Middle East which lacks such data.

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1.Introduction

A wide range of chemicals is continuously released to the aquatic environments. Domestic and industrial sewage effluents are the major sources of environmental pollutants, such as pharmaceuticals, personal care products, hormones, metals, and pesticides [1-3]. Most of these substances are known also as endocrine disrupting chemicals (EDCs) because they interfere with the function of the organismal endocrine system and adversely affect major biological events including reproduction, growth, and development [4]. Priority EDCs include steroidal estrogens, estrone (E1), β (E2), estriol (E3), and 17α-ethinylestradiol (EE2) [1]. They are classified as the major contributors to the estrogenic activity and potency associated with STP effluents hindering the reproductive fitness in various aquatic species [5, 6, 3]. Residues of estrogens even at low concentrations (i.e. nanograms per litter) in sewage effluents can mimic or modulate the action of endogenous hormones in exposed biota [7, 8]. Thus, an efficient removal of steroidal estrogens from domestic wastewater prior to discharge is critical for the protection of aquatic organisms and human health. A complete removal of EDCs from sewage effluents during sewage treatment works is a challenging task [9]. Several operational and technical factors may affect performance quality and removal rates in a STP, such as the modernity of the facility, the type of technology utilized, and the hydraulic load [10]. Therefore, the REs may vary significantly between different treatment facilities and even vary within the same STP [11, 12].

Large scale field and laboratory surveys have provided strong evidence that chronic exposure to substances released from STPs was associated with the abnormalities seen in wild fish [13]. Shortly after the first publication documented wild fish feminization in U.K. rivers [14], scientific reports from different parts of the world supported the "cause and effect" findings by Jobling and colleagues [13]. However, there is a clear gap of knowledge on the occurrence of steroidal estrogens in wastewater and surface water and any associated endocrine disruption effects in the aquatic ecosystems of the Middle East in general and in Saudi Arabia in particular. A literature search revealed only a few papers reporting endocrine disruption related

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work in the area. Mohagheghian et al [15] reported the distribution of estrogenic steroids in various STPs in Tehran. Other studies, such as [16, 17], linked water quality and chemistry data, including biological and chemical oxygen demand, phosphorus and nitrogen, to point and non-point sources of coastal sewage discharge without in depth characterization of EDCs constituent in the effluents or the receiving waters, except for a few heavy metal determination studies [18-22]. To the best of our knowledge, there are no available published reports to date that documented the occurrence of steroidal estrogens in any Middle Eastern water body or their removal rates from STPs. It is crucial to establish such field of research in the region to ensure that the reproduction fitness of our marine life is not adversely affected by EDCs. The unique environment and weather in Saudi Arabia as a semi-arid land country makes it critical to study the fate of EDCs in general and estrogenic chemicals in particular in aquatic systems; and evaluating the threats that they may impose on the magnificent Red Sea biodiversity. The broad objective of this study was to start the first step toward establishing the endocrine disruption research in the Kingdom, specifically by investigating the occurrence of E1, E2, E3, and EE2 in the Red Sea water and to test the removal capacity of these EDCs from two selected STPs. The findings would allow us to put the data into the global context to know where we fit and what future direction ought to be considered.

2.Materials and methods

2.1. Chemicals and reagents

The reference standards of E1, E2, E3, and EE2 were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The deuterated standard of E2 (E2-d4) was purchased from the CDN Isotopes (Quebec, Canada). High-performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Scientific (Hanover Park, Illinois, USA).

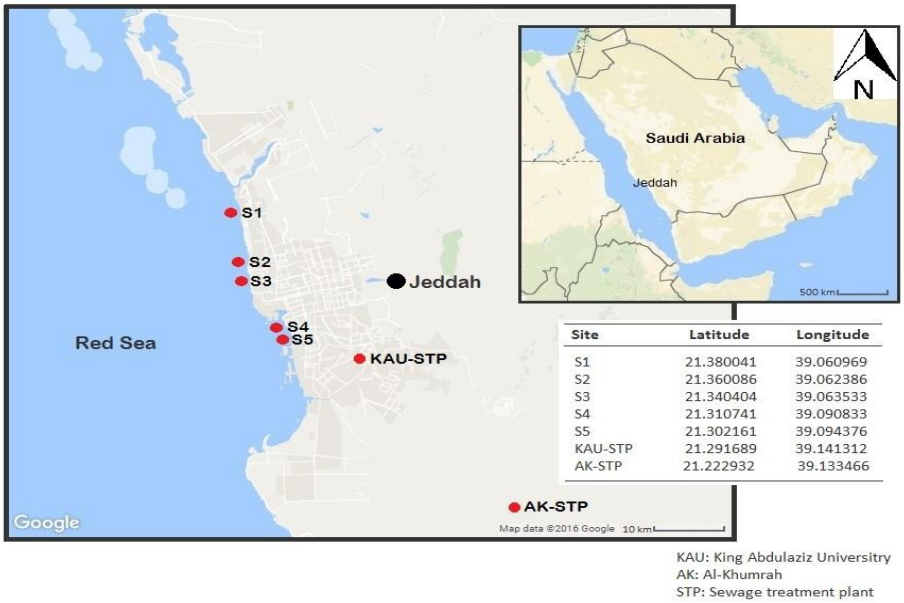
2.2. Sample collection

2.2.1. Municipal wastewater samples

Municipal wastewater samples (n = 36, 1 liter each) were collected into polyethylene bottles from the Al Khumrah District (AK) STP and the King Abdulaziz University (KAU) STP. Samples were collected in triplicates per matrix (influent and effluent) every other day giving a total of 18 samples per investigated STP. The field visits for the KAU-STP were conducted in the early mornings of the 16th, 18th and 20th of March 2014. Similarly, the field visits for the AK-STP were conducted on the mornings of the 23rd, 25th and 27th of March 2014. The KAU-STP is a small capacity (6000 m^3/day) membrane bioreactor secondary treatment facility that serves the campus community and uses its treated effluent for irrigation within KAU campus [23]. The AK-STP is the largest wastewater treatment facility located in the industrial region south of Jeddah. It consists of four phases with a collective capacity of 250.000 m^3/day, and employs tertiary treatment with ultraviolet disinfection process [24] and sells the treated water to different factories in the industrial region for cooling and manufacturing purposes.

2.2.2. Costal water collection

A total of 24 surface water samples were collected into 1-L polyethylene bottles from five different sites along 17 km of the Red Sea coastline (Figure 1). Four sites (S1, S2, S4, S5) were characterized by visible sewage effluent and underground water discharge points in shallow areas along Jeddah's coast [23], whereas S3 was a deep coastal area (approximately greater than 30 m in depth) without any noticed point or non-point sources of effluent. Signs of pollution such as odour, effluent blooms, and mass algal growth can be easily observed in S1, S2, S4, and S5. Furthermore, the recreational fishing activity of consumable species was also seen in a number of sites like S4 and S5 (Figure 2).



Site	Latitude	Longitude
S1	21.380041	39.060969
S2	21.360086	39.062386
S3	21.340404	39.063533
S4	21.310741	39.090833
S5	21.302161	39.094376
KAU-STP	21.291689	39.141312
AK-STP	21.222932	39.133466

KAU: King Abdulaziz Universitry
AK: Al-Khumrah
STP: Sewage treatment plant

Figure 1. Study sites map and sample collection coordinates (latitude, longitude) data for the investigated STPs and coastal surface water.

Figure 2. Photos **a** and **b** show field observations of visible pollution impacting Jeddah coast from site 2 **(S2)**. A clear point source of effluents as indicated by the red arrows in **a** and the pathway of wastewater as it is entering the sea **b**. Photos **d** and **e** are field observations from site 4 **(S4)**. A clear pipe draining polluted water to the sea **d** and mass algal growth from excess phosphorus and nitrogen as shown in **e**. Fishing activities taking place in S4 as shown by photo **c** and in site 5 **(S5)** as shown by photo **f**. Photos were taken by Al-Ansari A.

2.2.3. Sample pre-treatment and storage

Immediately after collection, all samples were transported to the laboratory on ice and were preserved by pH adjustment to approximately 2.4 and kept refrigerated at 4°C. Subsequently, they were shipped to the analytical laboratory at the Southern Illinois University (USA) in thick styrofoam coolers supplemented with sufficient dry ice.

2.3. Sample extraction

A volume of 500 mL of water sample was filtered through a pre-rinsed GF/F filter (0.7 µm pore size, Whatman, GE Healthcare Life Sciences, Pittsburg, MA, USA). A procedural blank (HPLC water) was processed along with every five authentic samples. The filtered sample or blank was spiked with 50 ng E2-d4 and then pulled through a J.T. Baker Bakerbond C_{18} Speedisk (Avantor Performance Materials, Center Valley, PA, USA) under vacuum until the Speedisk was completely dry. The Speedisk was pre-rinsed with 10 mL acetonitrile, 10 mL methanol, and 10 mL HPLC water. Estrogen analytes were eluted from the Speedisk by 15 mL methanol under vacuum. The collected extracts were concentrated, filtered through a 0.2 µm Nylon membrane (VWR, Radnor, PA, USA), and then transferred to LC vials. The deuterated 2-methoxy estrone (2-MeO-E1-d4) was used as an internal standard and added to each extract prior to instrumental analysis.

2.4. Instrumental analysis

Separation of steroidal estrogens was achieved using an Agilent HPLC (Agilent Technologies, fPalo Alto, CA, USA) equipped with a Waters Xterra® phenyl column (150 mm, 2.1 mm i.d., 1.7 µm particle diameter; Waters, Milford, MA, USA) and a 10 µL sample loop. The column temperature was kept at 40 °C. The mobile phase consisted of water (A) and methanol (B), both spiked with 0.1% formic acid (v/v). The mobile phase flow rate was 200 µL/min and the following gradient was employed: 5% B ramped to 62% B in 3 min (linear) and then ramped to 82% B in 30 min (linear), followed by a linear increase to 100% B in 1 min (held for 3 min) and then a change to 5% B in 1 min (held for 7 min). The HPLC was interfaced with a 3200 Q Trap® triple quadrupole/linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex; Toronto, Canada) equipped with a TurboIonSpray® electrospray ionization (ESI) probe operated in positive mode and in multiple reaction monitoring (MRM) mode for quantitative determination. The ion pairs monitored were *m/z* 269.0 → 145.0 (143.0) for E1, 271.1 → 145.0 (183.1) for E2, 287.1 → 171.0 (145.0) for E3, 295.0 → 145.0 (143.0) for EE2, 275.1 → 147.0 (187.0) for E2-d4, 303.1 → 162.0 (175.2) for 2-MeO-E1-d4, respectively. Table 1 shows Steroidal estrogen spike recovery data.

Table 1. Steroidal estrogen spike recovery data, limits of quantification (LOQ), and limits of detection (LOD)

Estrogen	Recovery (%)	SD*	LOQ	LOD (ng/L)
E2	82.7	5.2	0.3	0.1
E1	83.3	3.1	0.2	0.1
E3	78.5	6.3	0.3	0.1
EE2	80.3	4.6	0.5	0.2
*n = 5				

2.5. Quality assurance and quality control

Procedures to ensure analytical precision and accuracy included matrix effect tests, matrix spiking recovery tests, and the examination of procedural blank contamination and surrogate recoveries in authentic samples. Matrix effect tests followed the

standard addition approach similar to that described in [25] and were introduced in detail in Figure 2. The mean (± standard deviation) matrix effect value (ME%) ranged from 91% (±5%) to 102% (±5%) for E1, E2, E3, EE2, and E2-d4, revealing minimal matrix effects and demonstrating satisfactory sample extraction efficiencies as well as reliable instrumental performances. Matrix spiking recovery tests were conducted by adding 20 ng each of target estrogen analyses and E2-d4 to a composite of water samples produced from 10 randomly selected wastewater samples, following the same analytical procedures described above. After subtracting original concentrations in the composite, recoveries of estrogens and E2-d4 added to the three replicate composite samples ranged from 78.5% (±6.3%) to 83.3% (±3.1%). No estrogen analytes were detectable or quantifiable in any of procedural blanks. Recoveries of E2-d4 ranged from 75% to 96% in authentic samples. The concentration of each analyte was corrected based on the recovery of E2-d4. The limit of quantification (LOQ) of estrogens, estimated as an analyte response 10 times the standard deviation of the noise during instrumental analyse, ranged from 0.2 to 0.5 ng/L. The limit of detection (LOD), defined as an analyte reponse three times the standard deviation of the noise, ranged from 0.1 to 0.2 ng/mL. An analyte with detection below LOD is considered non-detectable (ND).

3. Results and discussion

3.1. Occurrence of estrogens and removal in STPs

The concentrations of hormones in influent, effluent, and removal efficiencies (RE, %) are listed in table 2. All four steroidal estrogens were detected in the influent samples of both KAU-STP and AK-STP. In both STPs, E1 had a detection frequency (DF, %) of 100% being detected in all influent samples whereas each of E2, E3, and EE2 had a DF of 85.7%. The total number of the analyzed and documented wastewater samples was 31 samples due to the loss of five samples during shipment.

The highest reported concentrations of all four estrogens in the influent samples from both STPs were 121, 41.9, 38.2, and 8 ng/L for E1, E2, E3, and EE2 respectively. These concentrations fall within similar maximum concentrations in a number of studies from various countries. A recent study by [12] reported comparable data to our findings, i.e. 182.1, 45.7, 65.7, and 19.9 ng/L as the highest concentrations of E1, E2, E3, and EE2 in the effluent of six STPs released into Dianchi lake in China. Another study in Canada also reported 104, 66.9, and 5.7 ng/L for E1, E2, and EE2 with no measurements aimed for E3 [26]. [15] reported maximum levels of 18.76, 8, and 11.76 ng/L for E1, E2, and EE2, respectively, in influents from seven STPs in Tehran, whereas E3 was below their detection limits (0.5 ng/L). In [12] and [26] studies, E1 was always the highest hormone in the influent and EE2 was the lowest in concentration. This observation was consistent with our data. The worst-case scenario illustrated above as the maximum detectable levels of estrogens in raw sewage collected from KAU-STP and AK-STP in Jeddah did not reveal any surprising findings in comparison to documented concentrations in STP influent from various parts of the world. In the effluent samples, the mean concentrations of E1, E2, E3 and EE2 were 5.3, 0.5, 0.2 and 0.2 ng/L in KAU-STP and 2.8, 0.1, 1.1 and 0.2 ng/L in AK-STP respectively. Daily estrogen concentrations of both influent and effluent are shown in table 2. Generally, E1 appeared to be the most abundant estrogen in the effluents with a DF of 100%. The DF of E2 was 62.5% in KAU-STP and no detection in AK-STP effluents.

Table 2. Occurrence and removal efficiencies of steroidal estrogens in two STPs in Jeddah, Saudi Arabia.

		KAU-STP						
Estrogen	**Day**	**influent**	**[Range]**	***n***	**effluent**	**[Range]**	***n***	**RE**
		[conc.], ng/L			**[conc.],ng/L**			**(%)**
E1	1	15.1	8.5 - 21.6	2	2.5	1.9 - 2.8	3	83.5
	2	97.8	63.6 - 121	3	4.7	2 - 7.4	2	95.2
	3	56.1	20.6 - 80	3	8.6	1.3 - 12.5	3	84.7
	Mean	56.3			5.3			87.8
	SD	41.4			3.1			6.4
E2	1	0.4	ND - 0.8	2	0.6	0.4 - 0.8	3	-50.1
	2	4.2	2.9 - 6.1	3	0.3	ND - 0.5	2	92.8
	3	16.6	2.6 - 41.9	3	0.4	ND - 1.1	3	97.4
	Mean	7.1			0.5			46.7
	SD	8.5			0.2			83.9
E3	1	0.5	<LOQ - 0.8	2	0.4	ND - 0.9	3	17.9
	2	9.0	7.8 - 11.3	3	0.1	ND	2	99.0
	3	8.7	5.3 - 10.6	3	0.2	ND - <LOQ	3	98.2
	Mean	6.1			0.2			71.7
	SD	4.8			0.2			46.6
EE2	1	1.2	<LOQ - 1.9	2	0.3	ND - <LOQ	3	77.7
	2	2.9	1.6 - 5	3	0.2	ND	2	94.7
	3	3.7	1.2 - 8	3	0.2	ND	3	95.9
	Mean	2.6			0.2			89.5
	SD	1.3			0.1			10.2

Table 2 (continued)

		AK-STP						
Estrogen	**Day**	**influent**	**[Range]**	**n**	**effluent**	**[Range]**	**n**	**RE**
		[conc.], ng/L			**[conc.], ng/L**			**(%)**
E1	1	9.8	2.3 - 23.5	3	4.0	1.4 - 6.1	3	59.1
	2	21.0	2.9 - 4.6	2	3.7	14.1 - 27.7	2	82.2
	3	7.4	6.9 - 7.9	2	0.6	<LOQ- 1.1	3	91.6
	Mean	12.7			2.8			77.7
	SD	7.3			1.9			16.8
E2	1	0.8	<LOQ - 1.3	3	0.1	ND	3	88.3
	2	0.4	ND - 0.8	2	0.1	ND	2	79.8
	3	1.0	0.8 - 1.2	2	0.1	ND	3	91.0
	Mean	0.7			0.1			86.3
	SD	0.3			0.0			5.9
E3	1	17.7	14.1 -24.4	3	0.1	ND	3	99.5
	2	9.1	ND - 18.1	2	3.0	3 - 3.1	2	66.6
	3	31.0	23.7 - 38.2	2	0.2	ND - <LOQ	3	99.5
	Mean	19.3			1.1			88.5
	SD	11.0			1.7			19.0
EE2	1	1.2	0.9 - 1.5	3	0.2	ND	3	87.3
	2	0.7	ND - 1.3	2	0.3	ND - <LOQ	2	55.2
	3	1.9	2.3 - 1.5	2	0.2	ND	3	92.1
	Mean	1.3			0.2			78.2
	SD	0.6			0.1			20.1

KAU: King Abdulaziz University, AK: Al-Khomra, nd: not detected, LOQ: limits of quantification, Std: standard deviation, RE: Removal efficiency

The DF of E3 was 37.5% in both STPs. [12] reported 16.2, 4.4, 2.6, and 3.3 as their mean concentrations of E1, E2, E3, and EE2. Among 30 different EDCs surveyed in four different STPs, [27] reported steroidal estrogens in effluents reaching ~5 ng/L for EE2, the most frequently detected estrogen, whereas E1, E2, and E3 were all below 20 ng/L. An earlier survey of 18 STPs across Canada showed lower average concentrations of 17 and 1.8 ng/L of E1 and E2 [28]. E1 and E2 were detected at mean concentrations of 12 and 4 ng/L respectively at five of the eight surveyed STPs effluents in the U.S. [6].

A recent meta-analysis study conducted by [29] investigated global trends of multiple EDCs mostly reported in STP effluents from ten countries in the last decade. Data shown in Figure 3 compares median concentrations of natural estrogens from the present study to those reported in [29]. The concentration of estrogens in Saudi Arabian STP effluents was generally in the lower end of the range of levels reported elsewhere. For instance, the median concentrations of E1 were 2.8, 3.15, 5.56, 6.25, and 7.2 ng/L in Saudi Arabia, Germany, Korea, China, and Spain, respectively.

The median concentrations of E2 in increasing order were 0.7, 1.4, 2.3, 2.85, 3.44 ng/L in Saudi Arabia, Italy, U.K, Germany, and China, respectively. The only exception was for E3, which indicated an increasing order of 1.2, 1.4, 2, 2.5, and 3 ng/L in Italy, U.K, U.S.A, Korea, and Saudi Arabia, respectively. The relatively low concentrations of estrogens in the Saudi Arabian STP effluents may be attributed to a number of reasons. Firstly, the starting concentrations in the influent are considered low which might be related to high microbial degradation or photo degradation activates [30, 31]. Secondly, the continuous upgrade and development of the studied STP facilities could have improved their treatment efficacies. Indeed, the KAU-STP is a well-maintained membrane bio-reactor facility working under its operational capacity (6000 m^3/day) [23]. On the other hand, the AK-STP was recently upgraded to a tertiary treatment facility with an operational capacity reaching 250.000 m^3/day [24] which is equal to 25% of the whole city STPs treatment capacity.

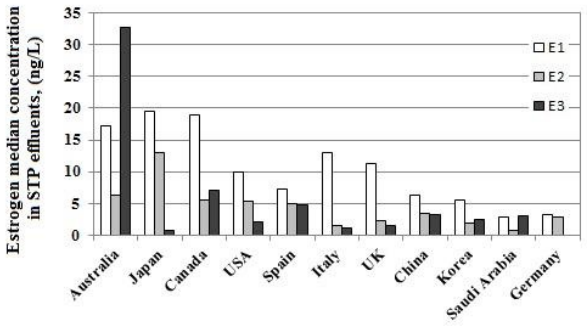


Figure 3. Steroidal estrogen median concentrations in STP effluents in ten countries as adopted from Sun et al. (2014) in comparison to median concentrations of two STPs effluent from Jeddah, Saudi Arabia as reported in this study

The mean REs calculated from three days sampling revealed that the KAU-STP recorded 87.8%, 46.7%, 71.7%, and 89.5% for E1, E2, E3, and EE2, respectively. Similar REs were also determined in the AK STP, 77.7%, 86.3%, 88.5 and 78.2% for E1, E2, E3, and EE2, respectively. As shown in table 2, the best REs achieved per day for KAU STP reached 95.2% (day 2), 97.4% (day 3), 99% (day 2) and 95.9% (day 3) for E1, E2, E3, and EE2, respectively. Comparably, the best REs achieved per day for AK STP reached 91.6% (day 3), 91% (day 3), 99.5% (days 1 & 3), and 92.1% (day 3) for E1, E2, E3, and EE2, respectively. The low average removal rate for E2 in KAU STP was due to the higher concentration of the estrogen measured in the effluent (0.6 ng/L) than that of the influent (0.4 ng/L) in day 1 assessment. The limited number of samples in our study could be the main drawback, which led to this low removal rate since day 2 & 3 assessments reached greater than 90%. Excluding the samples with low removal rates for some estrogens, our data is comparable to other recent studies. For instance, [12] reported an average of 75 – 92% REs for the steroidal estrogens in six Chinese STPs with operational Characteristics. In another study, [32] reported 72, 78, 100, 90% mean REs for E1, E2, E3, and EE2 respectively in Darvill STP in South Africa. [11] compared the REs of steroidal estrogens in two nitrifying/denitrifying activated sludge STPs. Their data showed high average removal rates 89-91%, 94-96%, 98-99%, 92-93% for E1, E2, E3, and EE2.

3.2. Occurrence of estrogens in coastal marine water

All four estrogens were detectable in coastal marine water samples with variable detection rates (table 3). The most abundant estrogen was E1 with a high DF of 83.3%, followed by 37.5, 29.2, and 20.8% for E3, E2, and EE2, respectively. The highest measured concentrations of estrogens were found per site as the following: E2 (3.2 ng/L in S1), E1 (11.6 ng/L in S2), E3 (46.5 ng/L in S4), and EE2 (1.9 ng/L in S2). The detection of steroidal estrogens from the sampled sites was highly anticipated. All sampling locations were impacted by either STP effluents and/or groundwater drainage outfalls. In particular, as the following: S1 and S5 were receiving STP effluents and groundwater discharges, S2 and S4 were receiving only groundwater effluents. The site used as a reference site (S3) was a deep coastal site without any visual point source of effluents. The sampled sites fall within an area of Jeddah's coast that received a great deal of attention from many research groups as polluted areas due to anthropogenic activates [16, 17, 19, 23]. However, since no STP in Jeddah is located near the shoreline, it was hard to confirm the exact origin of the STP effluents. The effluent releasing point for the AK-STP is located within a government sector with restricted access.

Table 3. Red Sea surface water samples steroidal estrogens concentrations

Estrogen	Site	*N*	*n**	[conc.], ng/L	SD	[Range]
E2	S1	8	2	3.2	n/a	ND-3.2
	S2	6	3	1.5	0.8	ND-2.3
	S3	3	0	ND	n/a	n/a
	S4	4	1	0.8	n/a	n/a
	S5	3	1	1.5	n/a	n/a
E2, DF (%)	all	24	7			29.17
E1	S1	8	6	10.5	22.09	ND-50
	S2	6	6	11.6	15.92	0.9-43.2
	S3	3	1	1.6	n/a	n/a
	S4	4	4	9.3	13.59	1.1-29.5
	S5	3	3	1.9	1.05	0.9-3
E1, DF (%)	all	24	20			83.33
E3	S1	8	1	14.9	n/a	n/a
	S2	6	4	4.6	5.34	0.8-12.2
	S3	3	1	<LOQ	n/a	n/a
	S4	4	2	46.5	n/a	<LOQ-46.5
	S5	3	1	<LOQ	n/a	n/a
E3, DF (%)	all	24	9			37.5
EE2	S1	8	1	0.8	n/a	n/a
	S2	6	3	1.9	n/a	<LOQ-1.9
	S3	3	0	ND	n/a	n/a
	S4	4	1	1.3	n/a	n/a
	S5	3	0	ND	n/a	n/a
EE2, DF (%)	all	24	5			20.83

n*: number of samples detected including signals below LOQ,
ND: Not detected, n/a: Not applicable, DF: Detection frequency (%)

The surface water levels of the steroidal estrogens from this study was compared to various studies worldwide. The data is expressed as median values and shown in table 4. The data demonstrates that the surface water levels of steroidal estrogens from polluted sites in Jeddah coast were relatively low. For instance, E1 levels in surface water were in the following order: 228.5, 36.2, 21, 19.7, 17.7, 4.8, 4.7, 1.8, 1.5, and 1.1 ng/L in Taiwan, Japan, China, China*, South Korea, Australia, Italy, France, Saudi Arabia, and China*** respectively. Furthermore, the concentrations of EE2 were also much lower in Saudi surface waters than others. 27.7, 9.5, 7.2, 2.4, 1.6, 1.5, 1.1, 0.8, 0.7, and 0.1 ng/L in China, China*, Italy, Czech, Republic, China**, France, South Korea, Saudi Arabia, Germany, and Australia respectively [33-43]. Besides the environmental factors that could reduce the bioavailability of steroidal estrogens in the receiving waters, such low surface concentrations of the studied hormones in Jeddah coast can be attributed mainly to two reasons. Firstly, as shown in this study the treated sewage effluent contained very low levels of steroidal estrogens compared to other countries. Secondly, most of the groundwater in Jeddah is originally raw sewage which travels through the soil unlit it gets pumped to the sea [23, 44]. Such journey would defiantly reduce the concentration of the steroidal estrogens in the runoff water [45, 3].

3.3. Implications of Jeddah coast pollution

Although AK-STP is the largest sewage treatment facility in Jeddah, there are four other smaller STPs serving the city with a population size of approximately 3.4 million [46]. The other STPs claim to employ either secondary or tertiary treatment technologies, which results in different effluent quality [28]. In fact, the performance of the two investigated STPs in Jeddah was better than expected and their effluent quality was comparable to the effluent quality in other countries (Figure 3). However, potential influences of various point and non-point sources of pollution to Jeddah's coastline worth continuous and in-depth research and regulatory monitoring.

Country (Reference)	**E1 (ng/L)**	**E2**	**E3**	**EE2**
Taiwan [33]	228.5	18.5	43.1	n.a
China [35]	21.0	2.1	40.6	27.7
China* [36]	19.7	8.6	10.3	9.5
Japan [34]	36.2	4.5	n.a	n.a
South Korea [37]	17.7	6.8	n.a	1.1
Saudi Arabia (this study)	**1.5**	**9.3**	**4.6**	**0.8**
Italy [39]	4.7	2.1	n.a	7.2
France [40]	1.8	2.1	2.1	1.5
China** [41]	1.1	0.6	3.0	1.6
Australia [38]	4.8	1.2	n.a	0.1
Germany [42]	n.a	4.0	n.a	0.7
Czech Republic [43]	n.a	2.6	n.a	2.4

n.a: means not available because either the sited study did not measure or the concentration or it was below LOD or LOQ

* or **: used to distinguish studies from the same country

Table 4: Steroidal estrogen median concentrations (ng/L) in surface waters in compiled from different countries in comparison to median concentrations of seawater from Jeddah, Saudi Arabia as reported in this study

A number of critical factors may contribute to the observed pollution in Jeddah's coast (see Figure 2). The most important were the obvious weakness in the environmental protection regulations and law enforcement that resulted in constant violations in the release of domestic and industrial sewage effluents, the drainage of untreated groundwater, and the illegal dumbs ships waste. Furthermore, the violations may include the release of raw sewage from the transportation tankers in the nearest rainwater drainages and the illegal dumping of sewage to the sea from recreational places like restaurants and hotels may likely occur [16, 23, 17, 19]. Approximately 66% of Jeddah's residential areas lack proper underground sewage networks [23]. Thus,

storing residential sewage in permeable septic tanks is a common practice that contaminates and increases the levels of underground water [23, 44].

The potencies of steroidal estrogen at environmentally relevant concentrations were investigated by [47] and [48]. Vitellogenin (VTG), widely known as the estrogenic activity biomarker, was induced in Juvenile rainbow trout. The single hormone exposure experiments showed that EE2 was 11 to 27 times more potent than E2 whereas E2 was 2.3 to 3.2 times more potent than E1. The lowest observable effect concentrations (LOEC) for single hormone were 3, 14, 1 ng/L for E1, E2, and EE2 respectively. The lowest level tested mixture (5.3 ng/L, E2 plus 0.2 ng/L, EE2) was enough to induce VTG in the exposed species [47]. Changing the laboratory conditions such as the duration of the experiment, the assessed biological endpoint, and the tested species gender and age would affect the acquired data. For instance, [48] observed alterations in gonad development, conditions known as intersex, in Japanese medaka after hatch to 100 days post§§1 hatch. The LOECs were 10, 10, 1000, 0.1 ng/L for E1, E2, E3, and EE2 respectively. The data showed that EE2 was 100 times more estrogenic than E1 and E2 and 10000 times more potent than E3. In our study, the total concentration of E1, E2, and EE2 in each surface water of each site was 14.5, 15.5, 2.1, 11.4, 3.6 ng/L in S1, S2, S3, S4, and S5 respectively. Assuming that the steroidal estrogens would act in an additive manner as per [48] findings we hypothesize that the coastal water in Jeddah might be estrogenic to the exposed biota. Even when we at EE2 as the most potent estrogens we found that its concentrations (table 3) were close to or above than the LOECs determined in [47] and [48] studies. Furthermore, environmental regulatory agencies like the ministry of environment in Canada have recommended that EE2 in surface water should not exceed 0.5 ng/L in an unfiltered water sample since EE2 tends to bind organic matter [49]. Thus it should be noted that the measured EE2 levels in this study were determined from filtered samples and we did not measure EE2 in the water suspended particulates. Low level effects of EE2 in fish (< 5 ng/L) were extensively studied in the last three decays. As low as 0.47, 1.54 and 3.92 ng/L EE2 exposure to fathead minnows for 21 days resulted in reduced egg production in females while only the highest two levels induced VTG production in males [50]. Aquatic exposure to EE2 at 1.1 ng/L for 75 days adversely affected zebra fish sexual maturity and fecundity [51]. Reduced testicular growth was observed in rainbow trout after a 21-day exposure to 2 ng/L EE2 [52]. Complete population collapse of fathead minnows was documented in a whole exposure to EE2 at 5 ng/L in a multi-year exposure [53]. All these examples showing that low level EE2 or steroidal estrogens mixtures could impose a threat to the exposed biota to STP effluents in Jeddah just like other parts of the world. Furthermore, in addition to the known adverse effects of EE2, there is a growing body of literature supporting its bioaccumulation in fish tissues as well [3, 54-57].

4. Conclusions

The data presented here determined environmental levels of steroidal estrogens, E1, E2, E3, and EE2 in two STPs and marine coastal water near point sources in Jeddah. All estrogens were detected with variable detection frequencies in the tested environmental mediums. The REs of the investigated STPs ranged from 46.7 to 89.5% for studied analytes. Low levels of estrogens detected in marine surface water supported previous studies [16, 17, 19] relating the clear pollution seen in Jeddah coast to treated and untreated sewage discharges. This study reported the occurrence of such important endocrine disrupters in the Saudi aquatic environment for the first time. However, future research should evaluate the degree by which coastal biota are affected by estrogens as well as their environmental uptake.

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