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Occurrence and removal of steroidal estrogens in Centre Eastern Tunisia municipal sewage treatment plant

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ABSTRACT

Occurrence and removal efficiencies of both natural estrogens, estrone (E1), 17β-estradiol (E2) and estriol (E3), and a synthetic estrogen, 17α-ethinylestradiol (EE2), were investigated in sewage treatment plant in Centre Eastern Tunisia employing simple activated sludge process. Concentrations of target estrogens were determined in both wastewater and sludge phases by gas chromatography coupled with mass spectrometer. Among the estrogens studied, E3 was found as the dominant compound detected in wastewater samples with average concentration up to 300 ± 4 ng/L in influent and up to 36 ± 2 in effluent. High aqueous phase removals (>85%) were achieved for E3, while only low to moderate removals for E1, E2, and EE2 (<75%). Based on the mass balance analysis, sorption onto sludge played a dominant role in the removal of estrogens in warm season, especially for E1 and E2 (69.5 and 66.3%, respectively), while biological degradation played a significant role in hot season (⩾61%).

Keywords: Activated sludge; Estrogens; Gas chromatography–mass spectrometry (GC–MS); Sewage treatment plant

1. Introduction

During the last decade, the main focus when trying to improve the quality of water has gradually shifted from conventional pollutants (organic matter, solids, and nutrients) to more specific endocrine disrupting compounds (EDCs). As some of these substances are detected at the ng/L level, they are described as micropollutants. These include aromatic hydrocarbons, heavy metals, and more recently, steroidal estrogens, whose occurrence in urban wastewaters from all over the world is demonstrated nowadays [1–4]. Estrogens have both natural and synthetic sources [5]. Natural estrogens include 17β-estradiol (E2), estrone (E1), and estriol (E3) are present in human urine, and synthetic compounds (e.g. 17α-ethinylestradiol (EE2)) are commonly used as a major ingredient in many oral

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contraceptives. Estrogens can potentially lead to a host of adverse effects on wildlife, such as the feminization of fish, the lack of reproductive in some species, birth defects and the development of physical abnormalities [6–10]. Previous studies have clearly shown that the municipal sewage treatment plants (STPs) are an important pollution source of EDCs released into the environment [4,11,12]. Their relative concentrations vary with the type of STP-urban or industrial [13]. Continued research efforts dealing with this subject have been made to investigate the occurrence and fate of these EDCs in the STPs [3,4,14]. Liu et al. [15] reported that the concentrations of estrogens were commonly detected at ng/L level. Zhou et al. [16] investigated the change of concentrations of eight EDCs in wastewater along the treatment processes of three STPs in Beijing.

As wastewater effluents are the most likely sources of estrogens in surface waters [17–19], removal of these compounds in wastewater treatment plant have been the focus of much attention [4,14]. Significant concentrations of estrogens in effluents have been attributed to their incomplete removal during the wastewater treatment process [20]. Biodegradation has been reported as the primary removal means for estrogens in wastewater [21].

To the best of our knowledge, data concerning estrogens occurrence are lacking in Tunisia. Therefore, the purpose of this study was to investigate the occurrence, fate, and removal of most potent natural and synthetic estrogens (E1, E2, E3, and EE2), during the treatment processes in a STP located in North Eastern Tunisia (Sousse), which constitutes one of the most significant touristic poles of the country. The levels of these compounds were investigated in wastewater and sludge of different stages of treatment by using gas chromatography–mass spectrometry (GC–MS). A mass balance analysis was applied to establish mass flux in the plant and removal mechanisms of these compounds inside the treatment units.

2. Experimental

2.1. Chemical and standard solutions

All solvents used in this work, including methanol, ethyl acetate, dichloromethane, pyridine, and hexane, were of distilled-in glass grade and were purchased from Rathburn Chemicals Ltd., Walkerburn, Scotland. Standards (E1, E2, E3, and EE2), internal standards (E2-d4), and the derivatization (N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA)) were all purchased from Sigma and Qmx laboratories Ltd, UK, with an isotopic purity > 98%.

Separate stock solutions of individual standards were made up at a concentration of 1,000 mg/L in methanol and kept at −18 °C. The stock solutions were used to regularly prepare working standards solutions for calibration and spiking experiments. Ultrapure water was supplied by a Maxima Unit from USF Elga, UK.

2.2. Sample collections

Samples were obtained from Sousse-STP located in Centre Eastern Tunisia (Fig. 1), which serves a population of 300,000 and has an average flow rate 17,430 m^3/d, hydraulic retention time (HRT) 4 h. Domestic source accounts for approximately 45% of influent, while touristic sources account for 52% of influent. Effluent reuse is an integral part of the treatment strategy at this STP. The STP consists of primary sedimentation and secondary activated sludge treatment. The mechanical treatment comprises a screen, aerated grit chamber, degreaser and a primary clarifier. The primary effluent flows through the biological treatment, consists of three parallel aeration tanks,

Fig. 1. Localization of the study site.

that separates and breaks down organic contaminants, for example, nitrogen and BOD/COD, with the aid of microorganisms. During periods of high flow (e.g. during rainfall events), a portion of the flow from the primary sedimentation is being diverted to bypass the municipal secondary clarifier. 65% of the secondary effluent was directly discharged to a close aquifer, the rest to a nearby touristic zone ≪El- Kantaoui≫ in order to reuse for the golf irrigation. Part of the settled sludge was returned to the aeration tanks, and the remaining part was pumped into a storage tank as excess sludge. After being dewatered in natural drying beds, the excess sludge was carried away for final disposal of discharges. The scheme of the STP and sampling locations are shown in Fig. 2.

Sampling along different treatment processes in the STP were taken in two seasons throughout 2010; 1 April (spring) and 3 July (August), corresponding to a mixed liquor suspended solids (MLSS) concentration in the return sludge of 5.2 and 5.9 g/L, respectively. Fig. 2 shows the sampling sites with three points (W_1–W_3) for wastewater samples and two points (S_1 and S_2) for sludge samples. Totally, 30 samples were collected between 2:00 and 4:00 pm, because the hourly fluctuation of the effluent estrogenicity was found to be insignificant on one sampling day except the morning period when a urine peak load usually appeared in the STP [22]. The samples were collected in precleaned amber glass bottles into which 1% formaldehyde (v/v) was preadded to restrain the microbial activity. The samples kept cool after sampling and during transport to the laboratory.

In the laboratory, the wastewater samples were acidified to pH 2.5–3.0 with 40% H_2SO_4 (v/v) and stored at 4°C in refrigerator for analysis within 24 h. Sludge samples were first centrifuged at 6,000 × g for 20 min (Sigma, 2 K-1S, China) and divided into the liquid (i.e. interstitial wastewater in sludge) and solid phases. Afterwards, the liquid phase was pretreated in the same way as the bulk wastewater sample, while the solid phase was freeze-dried, stored at −18°C in refrigerator, and analyzed within one week.

Information of the STP in the two operational monitoring periods coinciding with sampling time, including flow rates, HRT, solid retention time (SRT), mixed liquor suspending solid of the return sludge, Information in particular temperatures and rainfall levels in study area were recorded (Table 1).

2.3. Sample preparation

The sample processing and analysis is a modification method of the previously described method of Liu and Nie [23,24]. Briefly, wastewater and the liquid phase of activated sludge samples (1 L each) were filtered using precombusted Whatman GF/F filter paper

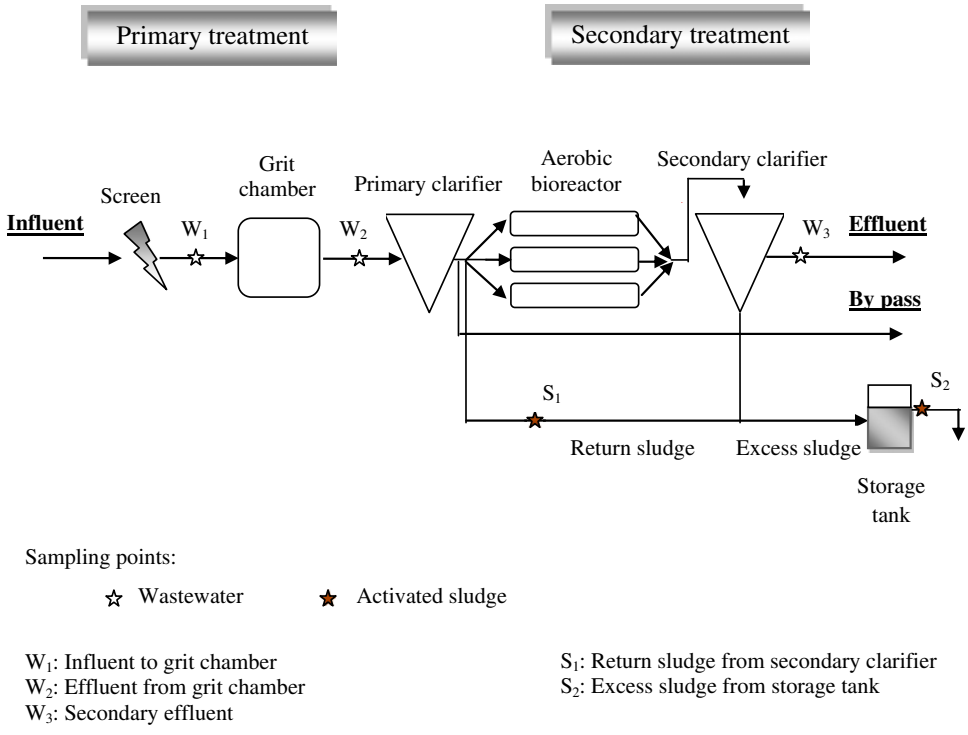

Fig. 2. Schematic wastewater treatment configuration in Sousse STP and sampling locations.

Table 1
Information of the STP during the first (April) and second (July) sampling campaign

Parameters	Flow rates (m^3/d)	MLSS (g/L)	HRT (h)	SRT (d)	Temperature (°C)	Rainfall (ml)
April	28.360	5.2	4	3	16	27
July	36.960	5.9	6	4	39	0.001

Note: MLSS: mixed liquor suspending solids; HRT: hydraulic retention time; SRT: solid retention time.

(0.7 μm) to remove particulate matter and spiked with 100 ng of E2-d_2. Estrogens from filtrates were recovered after solid-phase extraction (SPE) through Oasis HLB cartridges. The cartridges were conditioned sequentially with 5 ml of ethyl acetate, 5 mL of methanol, and 3 × 5 ml of ultrapure water to remove residual bonding agents. Then, the filtrates were percolated through the cartridges at a flow rate of 5 ml/mn, which were subsequently eluted with 10 ml of ethyl acetate solvent. The eluates were reduced to 0.5 ml with rotary evaporation (45°C) and until dryness under a gentle stream of nitrogen. The dried samples were submitted to the derivatization procedure.

Estrogens from freeze-dried solid phase of sludge sample (1.0 g) were recovered after sonication with 10 mL of ethyl acetate/methanol (1:1, v/v) under continuous ultrasonication for 10 min, in triplicate. The supernatant was collected after centrifugation (1,600 × g for 8 min), spiked with 100 ng of E2-d_2, acidified to pH 3 with 40% H_2SO_4 and diluted to a final volume of 300 mL using ultrapure water, after which, was filtered using precombusted Whatman GF/F filter paper (0.7 μm) to remove particulate matter and extracted, according to the aforementioned SPE method using Oasis HLB cartridges. The eluates were reduced to about 0.5 mL with rotary evaporator (BUCHI, RE5310/1, China) and further dried with a gentle stream of nitrogen. A clean-up step through a neutral Al_2O_3/silica gel column [24] was necessary to purify the extract post-SPE. The eluates were reduced to about 1 mL with rotary evaporation (25°C) and until dryness under a gentle stream of nitrogen. The dried samples were submitted to the derivatization procedure.

The above refined extracts from the wastewater and sludge samples were derivatized by 50 μL of each pyridine and BSTFA (to produce nonpolar derivatives) and heated at 65 ± 5°C for 30 min. The derivatives were cooled to room temperature, evaporated under a gentle stream of nitrogen to dryness and reconstituted in 100 μL of hexane vial for GC–MS analysis.

2.4. Analytical determination

The analysis of silylated derivatives was performed using a gas chromatograph (Trace GC 2000, Thermoquest CE Instruments, TX, USA) coupled with an ion trap mass spectrometer (Polaris Q, Thermoquest CE Instruments, Texas, USA) and an autosampler (AS 2000, Thermoquest). A ZB5 (5% diphenyl–95% dimethylpolysiloxane) capillary column of 30 m × 0.25 mm i.d. (0.25 m film thickness) is used. Helium carrier gas was maintained at a constant flow rate of 1.5 mL/min. The GC column temperature was programmed from 100°C (initial equilibrium time 1 min) to 200°C via a ramp of 10°C/min, 200–260°C via a ramp of 15°C/min, 260–300°C via a ramp of 3°C/min and maintained at 300°C for 2 min. The MS operates with electron impact ionization in full-scan mode from 50 m/z to 600 for qualitative analysis. The inlet and MS transfer line temperatures were maintained at 280°C, and the ion source temperature is 250°C. Sample injection (1 μL) was in splitless mode.

2.5. Performance method application

An internal instrument calibration, for both liquid and solid phase, was carried out with E2-d_2 as internal standard for concentrations ranged from 10 to 10,000 ng/L for each analyte with three replicates per concentration. E2-d_2 was present at a concentration of 100 ng/L in every standard solution. A linear fit with a high correlation coefficient was obtained for the studied compounds ($R^2 > 0.99$).

For the determination of the limit of detection (LOD), 1 L of wastewater and 1 g of particulate matter were extracted and then spiked with 100 ng of E2-d_2. The LOD of each compound for the two types of samples was determined as three times the standard deviation of ten independent replicate analyses. For wastewater samples, the obtained LODs ranged from 0.8 (EE2) to 3.4 ng/L (E2), whereas for solid samples, the LODs varied between 0.3 (E1 and E2) and 0.4 ng/g (E3 and EE2). The limit of quantification (LOQ) was determined as the analyte concentration corresponding to a signal/noise ratio of 10. As shown in Table 2, the LOQs varied from 2.6 (EE2) to 11.2 ng/L (E2) for wastewater samples and from 0.9 (E1and E2) to 1.4 ng/g (E3 and EE2) for solid samples.

For both types of samples, precision was assessed by performing repeatability and reproducibility

Table 2
Performance parameters of application method

Compounds	Wastewater	Sludge						
	Linear range (ng/L)	R^2	LOQ (ng/L)	LOD (ng/L)	Linear range (ng/g)	R^2	LOQ (ng/g)	LOD (ng/g)
E1	3–500	0.9969	5.6	1.7	7–200	0.9981	0.9	0.
E2	2.5–500	0.9975	11.2	3.4	8–200	0.9962	0.9	0.3
E3	6–500	0.9987	5.5	2.7	17–200	0.9996	1.4	0.4
EE2	5–500	0.9998	2.6	0.8	19–200	0.9973	1.4	0.4

experiments. For repeatability experiments, six replicates of a sample (either wastewater or sewage sludge) were spiked at a level of 100 ng of the target compounds and analyzed during 1 day ($n = 6$, intraday precision). For reproducibility experiments, three replicates ($n = 3$) of wastewater or sludge samples spiked at the same level as above were analyzed at three different days ($k = 3$) over a period of 1 week (interday precision). Precision data of the extraction procedure for the two types of the samples are given in Table 3. The results had shown satisfactory intra - and interday precision of the analytical procedure, both for wastewater and sludge samples. RSDs were less than 20% for all the compounds in both samples, indicating the good precision.

In order to evaluate the trueness of the method, recovery experiments were performed. To accomplish this, wastewater (1 L) and biomass samples (1 g) were spiked at three fortification levels (50, 100 and 200 ng) for each compound. The recoveries ranged between 88 and 99% and between 87 and 101%, for wastewater (Table 4) and sludge samples (Table 5), respectively.

2.6. Determination of physico-chemical parameters

Physico-chemical characteristics of wastewater (Table 6) were validated according to French standard NF XPT 90–210 [25]. Biochemical oxygen demand (BOD5) was determined by the manometric method with a respirometer (BSB-Controlled Model OxiTop (WTW)) and the chemical oxygen demand (COD) was estimated using the method described by Knechtel [26]. Total nitrogen contents (TN) were measured by the Kjeldhal method using an automated apparatus (Buchi, Switzerland). Phosphorus was determined colorimetrically at 430 nm using a Shimadzu U 1000 spectrophotometer [27]. The phosphorus content (TP) was measured calorimetrically by atomic absorption (HITACHI, Z-6100 model). The volatile solids content was deduced after weighing the incinerated dry sludge at 550˚C for 6 h [27]. The MLSS was determined by wastewater settling for 2 h, using an Imhoff cone.

Table 3
Precision data of the extraction procedures for the two types of samples

Compounds	Wastewater	Sludge		
	Intra-day precision RSD (%), $n = 6$	Inter-day precision RSD (%), $n = 3$, $k = 3$	Intra-day precision RSD (%), $n = 6$	Intra-day precision RSD (%), $n = 3$, $k = 3$
E1	8.1	15.2	12.8	18.2
E2	9.7	20.0	11.0	17.0
E3	8.0	17.5	13.0	20.0
EE2	8.6	19.4	11.6	17.5

Table 4
Mean recoveries (%) and standards deviation ($n = 6$) of the target compounds in spiked wastewater samples

Compounds	Concentration level		
	50 ng/L Recovery (%)	100 ng/L Recovery (%)	200 ng/L Recovery (%)
E1	85.1 ± 5.1	88.0 ± 6.2	86.4 ± 5.1
E2	89.0 ± 6.4	92.0 ± 7	91.0 ± 19
E3	90.0 ± 10.1	93.0 ± 5.2	93.2 ± 13
EE2	93.6 ± 5.4	99.0 ± 12	97.8 ± 10.1

Table 5
Mean recoveries (%) and standards deviation ($n = 6$) of the target compounds in spiked sludge samples

Compounds	Concentration level		
	50 ng/g Recovery (%)	100 ng/g Recovery (%)	200 ng/g Recovery (%)
E1	84.0 ± 8	87.0 ± 9.2	87.0 ± 9.2
E2	90.0 ± 10.2	92.0 ± 12.1	91.3 ± 14.6
E3	94.2 ± 9.5	95.0 ± 20	93.1 ± 20
EE2	98.6 ± 12.3	101.0 ± 12.5	99.2 ± 14.1

Table 6
General water quality parameters for influent and effluent during the first (April) and second (July) sampling campaign

Sampling point	COD (mg/L)		BOD_5 (mg/L)		TP (mg/L)		TN (mg/L)		VSS (mg/L)	
	Campaign									
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Influent	1,016	1,010	400	398	75	70	10.2	8	618	469
Effluent	118	101	38	30	66	61	5.6	5.6	32	31

Note: COD: chemical oxygen demand; BOD_5: biochemical oxygen demand; TP: total phosphorus; TN: total nitrogen; VSS: volatile suspending solids.

3. Results and discussion

3.1. Estrogens concentration and removal in the water line

Concentrations of target estrogens in the influent, primary effluent, and secondary effluent are shown in Fig. 3. In general, a large portion of the estrogenic materials excreted by humans are originally present in the conjugated forms (i.e. glucuronides and sulfates) which have a less estrogenic activity than their unconjugated (or free) forms. However, they may get deconjugated by microorganisms and thus converted into free estrogens during transport in the sewers. D'Ascenzo et al. [28] reported that the daily amounts of conjugated E1, E2, and E3 excreted by women were approximately 32, 14, and 106 μg, respectively; so the mass ratio of conjugated E2 is about 9.2% in the three natural estrogens. However, concentrations of target estrogens in the influent (Fig. 3) during April and July show that the specific concentration ratio of E2, if only the three natural estrogens are considered in the raw influent was 1.2 and 0.9%, respectively, and obviously lower than that in the urine. It is inferred that the deconjugated E2 could be partially metabolized to E1 and E3 [29] in the sewers due to its high biodegradability [28], thus leading to an increase in the specific concentration ratios of E1 and E3 in raw influent. Overall, E2 concentrations detected in Sousse STP (4 ng/L) were similar to those found in previous surveys in Japan in the range of 4.0–25 ng/L [18].

E3 was the highest concentration detected in the influent during the studied period (April and July), 300 and 360 ng/L, respectively. Such a high concentration detected in wastewater is probably due to its excretion in the largest amount by humans [30]. Those values are generally lower than those reported by Nie et al. [24] with a concentration of 459 ng/L and similar to the range (100–376 ng/L) reported by Hashimoto et al. [31] in Japan.

The influent of the studied STP was also found to contain the synthetic estrogen EE2 (50–105 ng/L). The results can be ascribed to the contraceptives released in STP influent. The results are consistent with those reported by Miege et al. [32].

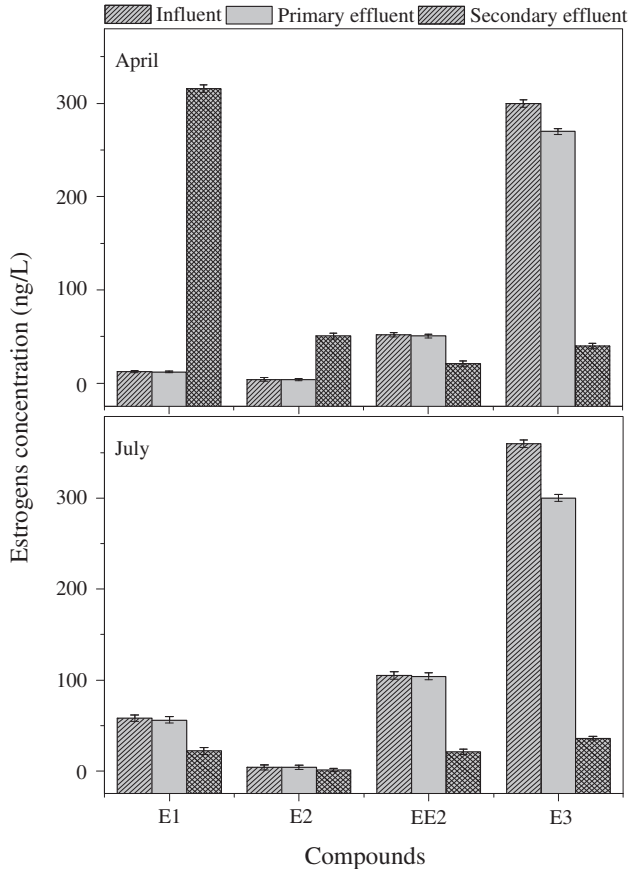

Fig. 3. Concentrations (ng/L) of the investigated estrogens detected in influent, primary effluent and secondary effluent of the STP.

In terms of aqueous phase removal, the comparison between primary effluent and raw influent indicates that the concentrations of estrogens (except E3) remained quite stable after the wastewater passed through the aerated grit chamber (Fig. 4). It is known that the decrease in estrogens concentration through

the activated sludge treatment is primarily due to biodegradation [33]. Removal due to sorption onto excess activated sludge was found to be insignificant, less than a few percentages [34–36]. Thus, the amount of estrogens biodegraded might offset that desorbed from the grits or partitioned with grease in the aerated grit chamber. The degree of estrogenic compound removed is largely determined by physiochemical properties of compound, for example, hydrophobicity, suspended solid content of the wastewater and their settling characteristics, and retention time in the settling tank etc. [37]. E3 is the least hydrophobic among all target estrogens since the log K_{ow} values of E1, E2, EE2, and E3 are 3.43, 3.94, 4.15, and 2.81, respectively [38]. Hence, E3 is more biodegradable than E1 and EE2 [39]. The two reasons tended to account for a notable primary removal of E3, 10.3 and 9% in April and July sampling campaigns, respectively. Andersen et al. [34] reported that the concentrations of estrogens did not significantly decrease in the primary effluent. Neither the aerated grit chamber nor the primary sedimentation tank could notably remove any target estrogens.

All target estrogens were detected in STP effluent. A few other studies have also investigated the occurrence of these endocrine disruptors in the effluent of the STP [2,40,41,17]. In some of these studies, estrone concentration increased over the course of the treatment as in our study during April sampling compaign (Fig. 4), illustrating both degradation of estradiol plus probable deconjugation of estrone [17,18,40]. High concentration of estradiol was also observed at the secondary effluent with respect to the influent, during April sampling campaign, which explains the high negative secondary removal efficiencies (Fig. 4). However, this is not a large dataset and the methods (grab sampling) would probably be inadequate to pick out subtle differences at these concentration levels. Further, although the reason behind the consistent difference in the effluent concentrations is still unclear due to the limited access to the STPs, the similarity in the specific bacterial population (nitrifying), possible adsorption into the suspended solids, operational conditions, drop of sewage temperature, influent characteristics and persistent of conjugate form during transport in the sewers [42] were probably responsible factors.

The secondary treatment contributed 59.6 and 86.6% in the removal of EE2 and E3, respectively (Fig. 4).

During July sampling campaigns, E1 was the lowest compound removal (62%), while the other compounds were decreased by more than 75% in their concentrations (Fig. 4). The results also showed that

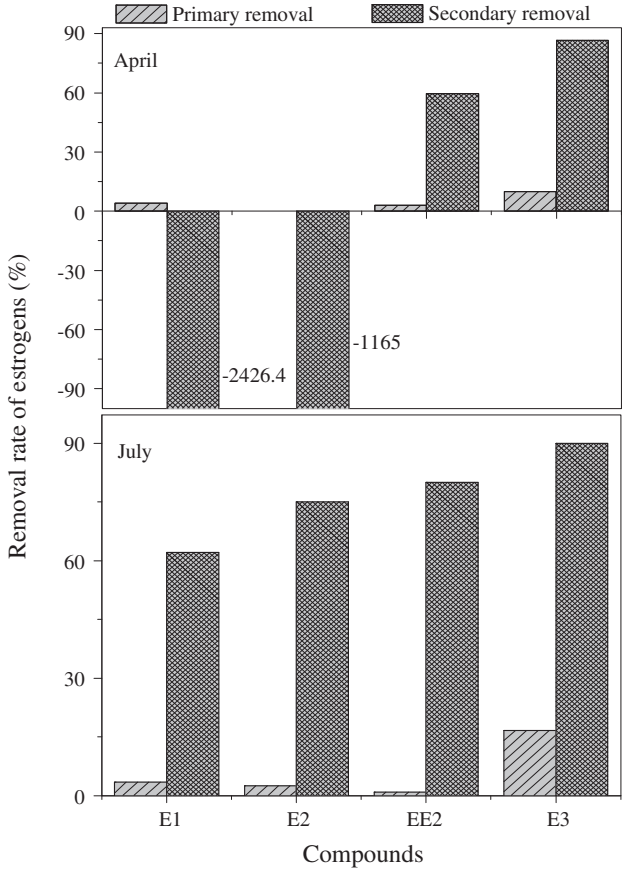

Fig. 4. The water phase removal rates of the target estrogens in the primary and secondary treatments.

the secondary treatment is the most useful process for removing natural as well as synthetic estrogens in water phase line of studied STP (Fig. 4).

3.2. Estrogens concentration in the sludge line

As shown in Fig. 5, the four target compounds were all detected in solid phase of both return and excess sludge samples. The profiles of detected estrogens in the sludge of two stages were similar.

In solid phase of return sludge, E1, E2, EE2, and E3 reached a maximal concentration of 72, 22, 30, and 61 ng/g SS in April, respectively. Nie et al. [24] reported that the concentrations of E2, EE2, and E3 in the secondary sludge samples, which were collected from an STP, located in Beijing, ranged from 22.0, 28.5, and 12.5 ng/g SS in spring, respectively. This STP adopted an anaerobic/anoxic/oxic process with a comparatively longer SRT, which probably accounted for the more removal of estrogens than the simple process examined in this study.

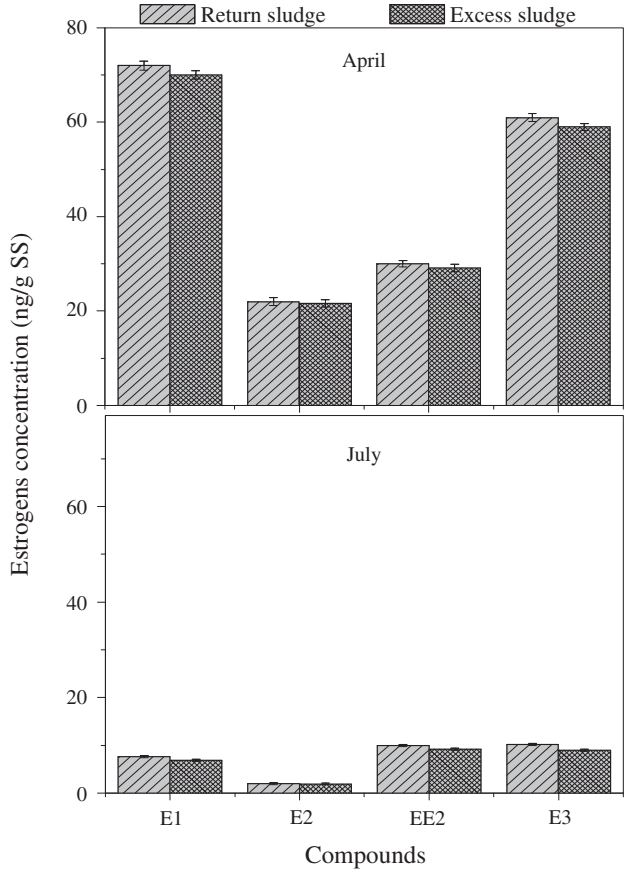

Fig. 5. Concentrations (ng/g SS) of selected estrogens in the solid phase of return sludge and excess sludge of the STP in April and July sampling campaigns.

3.3. Fate of estrogens in STP

There are three pathways for the removal/transport of estrogens in the STP: (1) biodegradation; (2) discharge into the aquatic environment with the secondary effluent; and (3) discharge with the excess sludge. Based on the mass balance of target estrogens and some important operational conditions including the flux of wastewater (28,360 and 36,960 m^3/d in April and July, respectively) and the discharge rate of excess sludge (3,420 and 3,496 kg SS/d in April and July, respectively) in the STP, the mass flux ratios of studied estrogens through biodegradation, secondary effluent and excess sludge could be estimated, as shown in Fig. 6. Results indicate that sorption to the excess sludge was the most important pathway of E1 and E2 (69.4 and 66.3%, respectively), while EE2 and E3 showed substantial losses during secondary treatment (biodegradation): 52.7 and 84.2%, respectively, in April sampling campaigns. This contradicts some previous results where biological treatment played a key role in the removal of these natural estrogens [16]. During July sampling campaigns, biodegradation was the most important pathway of all target estrogens, which agrees with that reported by Pothitou and Voutsa [43]. Among all target estrogens, E1 showed the lowest mass flux ratio via biodegradation (60.8%) but the highest mass flux ratio via the secondary effluent (38%).

Variations in secondary effluent concentrations of E1 and E2 between the two sampling campaigns might be one of the factors responsible for these differences in mass flux ratio. The difference may also be due to environmental factors such as temperature and rainfall, the water quality of influents, plant configurations, HRT, and SRT [5]. Therefore, more sampling events in the future research can provide better information in seasonal variation of these compounds.

Andersen et al. [35] measured the solid/water distribution coefficients (K_d) of several steroid estrogens between water and activated sludge particles and pointed out that the mass flux ratio of estrogens discharged with the excess sludge was below 1.8% if equilibrium conditions could be met in the STP.

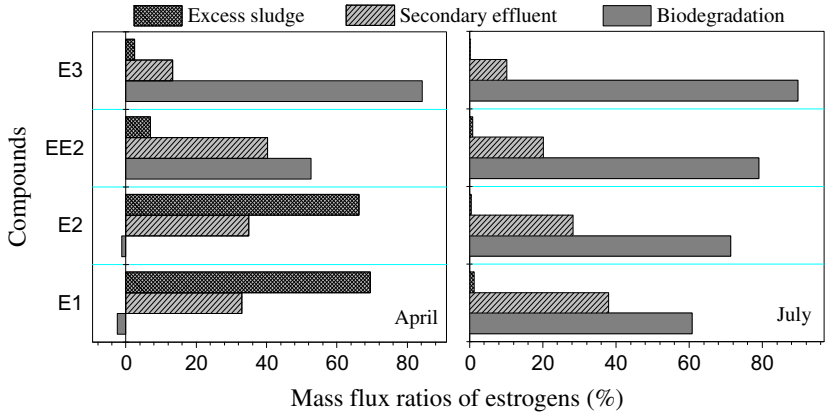

Fig. 6. Mass flux ratios of estrogens through three different pathways (%).

4. Conclusions

Both natural and synthetic estrogens were detected in sewage influent of an STP located in Centre Eastern Tunisia employing simple activated sludge process. All estrogens found in the wastewater were also detected in the sludge phase. Among investigated estrogens, E3 was found in the raw influent at high concentration (300–360 ng/L). It is noted that in April, the conjugated E1 and E2 could not be effectively deconjugated during transport in sewers. Therefore, the concentrations of E1 and E2 in the effluent largely exceeded those in the influent. High aqueous phase removals (>85%) were achieved for E3, while only low to moderate removals for E1, E2, and EE2 (<75%). Sorption onto sludge played a dominant role in the removal of estrogens in cold season (April), especially for E1 and E2 with a percentage of 69.5 and 66.3%, while biological degradation played a significant role in hot season (July).

References

[1] T.A. Ternes, Occurrence of drugs in German sewage treatment plants and rivers, Water Res. 32 (1998) 3245–3260.

[2] M. Carballa, F. Omil, J.M. Lema, M. Llompart, C. Garcia-Jares, I. Rodriguez, M. Gomez, T. Ternes, Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant, Water Res. 38 (2004) 2918–2926.

[3] N. Nacada, T. Tanishima, H. Shinohara, K. Kiri, H. Takada, Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment, Water Res. 40 (2006) 3297–3303.

[4] A.S. Stasinakis, G. Gatidou, D. Mamais, N.S. Thomaidis, T.D. Lekkas, Occurrence and fate of endocrine disrupters in Greek sewage treatment plants, Water Res. 42 (2008) 1796–1804.

[5] M.R. Servos, D.T. Bennie, B.K. Burnison, A. Jurkovic, R. McInnis, T. Neheli, A. Schnell, P. Seto, S.A. Smyth, T.A. Ternes, Distribution of estrogens, 17 b-estradiol and estrone, in Canadian municipal wastewater treatment plants, Sci. Total Environ. 336 (2005) 155–160.

[6] G. Levy, I. Lutz, A. Kruger, W. Kloas, Bisphenol A induces feminization in Xenopus laevis tadpoles, Environ. Res. 94 (2004) 102–111.

[7] S.K. Khanal, B. Xie, M.L. Thompson, S.W. Sung, S.K. Ong, J. Van Leeuwen, Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems, Environ. Sci. Technol. 40 (2006) 6537–6546.

[8] L.J. Mills, C. Chichester, Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? Sci. Total Environ. 343 (2005) 1–34.

[9] S. Jobling, D. Casey, T. Rodgers-Gray, J. Oehlmann, U. Schulte- Oehlmann, S. Pawlowski, T. Baunbeck, A.P. Turner, C.R. Tyler, Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent, Aquat. Toxico. 65 (2003) 205–220.

[10] S.K. Lee, D.N. Veeramachaneni, Subchronic exposure to low concentrations of di-n-Butyl Phthalate disrupts spermatogenesis in Xenopus laevis frogs, Toxicol. Sci. 84 (2005) 394–407.

[11] Y. Zhang, J.L. Zhou, Occurrence and removal of endocrine disrupting chemicals in wastewater, Chemosphere 73 (2008) 848–853.

[12] J.L. Zhou, Z.L. Zhang, E. Banks, D. Grover, J.Q. Jiang, Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water, J. Hazard. Mat. 166 (2009) 655–661.

[13] G. Fent, W.J. Hein, M. Moendel, R. Kubiak, Fate of 14C-bisphenol A in soils, Chemosphere 51 (2003) 735–746.

[14] W. Zheng, S.R. Yates, S.A. Bradford, Analysis of steroid hormones in a typical dairy waste disposal system, Environ. Sci. Technol. 42 (2008) 530–535.

[15] Z.H. Liu, Y. Kanjo, S. Mizutani, Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment—physical means, biodegradation, and chemical advanced oxidation: A review, Sci. Total Environ. 407 (2009) 731–748.

[16] H.D. Zhou, X. Huang, X.L. Wang, X.H. Zhi, C.D. Yang, X.H. Wen, Q.H. Wang, H. Tsuno, H. Tanaka, Behaviour of selected endocrine-disrupting chemicals in three sewage treatment plants of Beijing, China, Environ. Monit. Assess. 161 (2010) 107–121.

[17] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, M. Servos, Behavior and occurrence of estrogens in municipal sewage treatment plants-I. Investigations in Germany, Canada and Brazil, Sci. Total Environ. 225 (1999) 81–90.

[18] C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Samperi, Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water, Environ. Sci. Technol. 34 (2000) 5059–5066.

[19] D. Grover, J.L. Zhou, P.E. Frickers, J.W. Readman, Improved removal of estrogenic and pharmaceutical compounds in sewage effluent by full scale granular activated carbon: Impact on receiving river water, J. Hazard. Mater. 185 (2011) 1005–1011.

[20] K.K. Samir, B. Xie, M.L. Thompson, S. Sung, S.K. Ong, J. Hans Van Leeuwen, Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems, Environ. Sci. Technol. 40 (2006) 6537–6546.

[21] G.G. Ying, R.S. Kookana, Y.J. Ru, Occurrence and fate of hormone steroids in the environment, Environ. Inter. 28 (2002) 545–551.

[22] D. Martinovic, J.S. Denny, P.K. Schmieder, G.T. Ankley, P.W. Sorensen, Temporal variation in the estrogenicity of a sewage treatment plant effluent and its biological significance, Environ. Sci. Technol. 42 (2008) 3421–3427.

[23] R. Liu, A. Wilding, J. Zhou, Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction- gas chromatography–mass spectrometry, J. Chromatogr. A 2004 (1022) 179–189.

[24] Y. Nie, Z. Qiang, H. Zhang, C. Adams, Determination of endocrine-disrupting chemicals in the liquid and solid phases of activated sludge by solid phase extraction and gas chromatography–mass spectrometry, J. Chromatogr. 1216 (2009) 7071–7080.

[25] AFNOR Protocole d'évaluation d'une méthode alternative d'analyse physico-chimique par rapport a une méthode de référence [Protocol evaluation of an alternative physico-chemical methode analysis report to a referring methode], Norme NF XPT [Standard NF XPT] 58 (1999) 90–210.

[26] R.J. Knetchel, A more economical method for the determination of chemical oxygen demand, Water Pollut. Control 71 (1978) 25–29.

[27] Japanese Standards Association, JIS handbook, in: Y. Ohama (Ed.), Environmental Technology. Japanese Standards Association, Tokyo (1995) 553–638.

[28] G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, R. Samperi, Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities, Sci. Total Environ. 302 (2003) 199–209.

[29] H.B. Lee, D. Liu, Degradation of 17 beta-estradiol and its metabolites by sewage bacteria, Water Air Soil Pollut. 134 (2002) 353–368.

[30] T.A. Ternes, A. Joss, Human Pharmaceuticals, Hormones and Fragrances: The Challenge of Micropollutants in Urban Water Management, IWA, London, 2006.

[31] T. Hashimoto, K. Onda, Y. Nakamura, K. Tada, A. Miya, T. Murakami, Comparison of natural estrogen removal efficiency in the conventional activated sludge process and the oxidation ditch process, Water Res. 41 (2007) 2117–2126.

[32] C. Miege, J.M. Choubert, L. Ribeiro, M. Eusebe, M. Coquery, Fate of pharmaceuticals and personal care products in wastewater treatment plants—conception of a database and first results, Environ. Pollut. 157 (2009) 1721–1726.
[33] X. Nan, X. Yi-Feng, X. Shuo, L. Jing, T. Hu-Chun, Removal of estrogens in municipal wastewater treatment plants: A Chinese perspective, Environ. Pollut. 165 (2012) 215–224.
[34] H. Andersen, H. Siegrist, B. Halling-Sorensen, T.A. Ternes, Fate of estrogens in a municipal sewage treatment plant, Environ. Sci. Technol. 37 (2003) 4021–4026.
[35] H. Andersen, M. Hansen, J. Kjolholt, F. Stuer-Lauridsen, T. Ternes, B. Halling- Sorensen, Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment, Chemosphere 61 (2005) 139–146.
[36] F.S. Li, A. Yuasa, A. Obara, A.P. Mathews, Aerobic batch degradation of 17-beta estradiol (E2) by activated sludge: Effects of spiking E2 concentrations, MLVSS, and temperatures, Water Res. 39 (2005) 2065–2075.
[37] Y.K.K. Koh, T.Y. Chiu, A. Boobis, E. Cartmell, M.D. Scrimshaw, J.N. Lester, Treatment and removal strategies for estrogens from wastewater, Environ. Technol. 29 (2008) 245–267.
[38] G.G. Ying, R.S. Kookana, Y.J. Ru, Occurrence and fate of hormone steroids in the environment, Environ. Int. 28 (2002) 545–551.
[39] A.C. Johnson, J.P. Sumpter, Removal of endocrine-disrupting chemicals in activated sludge treatment works, Environ. Sci. Technol. 35 (2001) 4697–4703.
[40] S. Castiglioni, R. Bagnati, R. Fanelli, F. Pomati, D. Calamari, E. Zuccato, Removal of pharmaceuticals in sewage treatment plants in Italy, Environ. Sci. Technol. 40 (2006) 357–363.
[41] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters, Water Res. 41 (2007) 1013–1021.
[42] K. Vimal, N. Norihide, Y. Makoto, Y. Naoyuki, C.J. Andrew, T. Hiroaki, The arrival and discharge of conjugated estrogens from a range of different sewage treatment plants in the UK, Chemosphere 82 (2011) 1124–1128.
[43] P. Pothitou, D. Voutsa, Endocrine disrupting compounds in municipal and industrial wastewater treatment plants in Northern Greece, Chemosphere 73 (2008) 1716–1723.