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REMOVAL EFFICIENCY AND POTENTIAL RISKS OF STEROID ESTROGENS IN SELECTED WASTEWATER TREATMENT PLANTS IN THE KLANG VALLEY, MALAYSIA

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By

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Wastewater treatment plant (WWTP) is the major source for steroid estrogens loaded into aquatic environment because wastewater treatment systems are generally designed to remove basic wastewater parameters. Amongst steroid estrogens, natural 17β-estradiol (E_2) and synthetic 17α-ethinylestradiol (EE_2) are potent in estrogenic potency and have been classified as "Watch List" in Directive 2013/39/EU. The general objective of the study is to analyze the occurrence, removal, and potential risks of natural E_2 and synthetic EE_2 in six selected WWTPs located in Klang Valley. The specific objectives of the study are: (1) to determine natural E_2 and synthetic EE_2 concentrations and wastewater physicochemical parameters in influent and effluent, (2) to identify the influence of socio-demographic profile on natural E_2 and synthetic EE_2 concentration in influent, (3) to evaluate the removal efficiency of natural E_2 and synthetic EE_2 in WWTP, (4) to analyze relationship between wastewater physicochemical parameters associated with natural E_2 and synthetic EE_2 removal in WWTP, and (5) to assess the potential risks of natural E_2 and synthetic EE_2 to human and aquatic environment. Wastewater samples were collected bimonthly for a period of six months (from March to July 2016) from influent and effluent of six selected WWTPs located in Klang Valley. The wastewater samples were analyzed using 17β-estradiol and 17α-ethynylestradiol ELISA kit. Kruskal-Wallis test and principal component analysis (PCA) were used for statistical analysis. Risk quotient (RQ) was applied for human risk while mathematical models such as estradiol equivalent concentration (EEQ) and concentration addition (CA) models were applied for aquatic environment risk. Quantitative results showed natural E_2 concentration was 88.17±7.03 ng/L to 93.93±6.91 ng/L in influent and 35.10±17.33 ng/L to 85.20±7.54 ng/L in effluent. Synthetic EE_2 concentration was 0.22±0.21 ng/L to 4.94±6.32 ng/L in influent and 0.02±0.02 ng/L to 1.04±0.77 ng/L in effluent. For wastewater physicochemical parameters, total suspended solid was 95.17±3.96 mg/L to 129.80±4.95 mg/L in influent and 2.84±0.95 mg/L to 14.67±0.57 mg/L in effluent, pH was 6.73±0.01 to 7.04±0.03 in influent and 6.79±0.06 to 6.95±0.02 in effluent, oxidation reduction potential was -29.20±3.20 mV to -18.07±1.93 mV in influent and 5.37±3.25 mV to

16.77±3.93 mV in effluent, and temperature was 32.20±0.1°C to 35.60±2.31°C in influent and 32.47±0.12°C to 34.63±0.67°C in effluent. Kruskal-Wallis test indicated significant difference between natural E_2, synthetic EE_2 concentrations in influent and socio-demographic profile (gender, marital status, education, and household). The removal efficiency was 6.4% to 63.0% for natural E_2 and 28.3% to 99.3% for synthetic EE_2. The PCA indicated wastewater physicochemical parameters were associated significantly with natural E_2 and synthetic EE_2 removal efficiency in WWTP. For human risk, cumulative RQ value was below the allowable limit, except WWTP 1. For aquatic environment risk, EEQ model predicted estrogenic activity of 35.1 EEQ-ng/L to 85.3 EEQ-ng/L, while CA model predicted estrogenic activity of 105.4 ng/L. However, these estrogenic activity in aquatic environment could be lower due to dilution effect through rainfall. This study output is useful as baseline quantitative data of E_2 and EE_2 in Klang Valley WWTPs and its potential risks to human and aquatic environment.

Keywords: Wastewater treatment plants, Steroid estrogens, Removal efficiency, Potential Risks

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KECEKAPAN PENYINGKIRAN DAN RISIKO POTENSI ESTROGEN STEROID DI LOJI RAWATAN KUMBAHAN TERPILIH DI LEMBAH KLANG, MALAYSIA

Oleh

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Loji rawatan kumbahan (WWTP) merupakan punca utama estrogen steroid masuk ke dalam lingkungan akuatik kerana sistem rawatan kumbahan biasanya direka untuk menyingkirkan parameter air kumbahan asas. Antara estrogen steroid, 17β-estradiol (E_2) semulajadi dan 17α-ethinylestradiol (EE_2) sintetik berpotensi dalam potensi estrogenik dan telah dikelaskan sebagai "Watch List" dalam Directive 2013/39/EU. Objektif umum kajian ini adalah untuk menganalisis kepekatan, kecekapan penyingkiran, dan potensi risiko E_2 semulajadi dan EE_2 sintetik di enam buah WWTP terpilih yang terletak di Lembah Klang. Objektif kajian ini adalah: (1) untuk menentukan kepekatan E_2 semulajadi dan sintetik EE_2, dan parameter fizikokimia kumbahan di influen dan efluen, (2) untuk mengenal pasti pengaruh profil sosio-demografi terhadap kepekatan E_2 semulajadi dan EE_2 sintetik di influen, (3) untuk menilai kecekapan penyingkiran E_2 semulajadi dan EE_2 sintetik dalam WWTP, (4) untuk menganalisis hubungan antara parameter fizikokimia kumbahan dikaitkanan dengan kecekapan penyingkiran E_2 semulajadi dan EE_2 sintetik dalam WWTP, dan (5) untuk menilai potensi risiko E_2 semulajadi dan EE_2 sintetik terhadap manusia dan lingkungan akuatik. Sampel air kumbahan dikumpulkan dua bulan sekali dalam tempoh enam bulan (dari Mac hingga Julai 2016) dari influen dan effluen enam buah WWTP terpilih yang terletak di Lembah Klang. Sampel air kumbahan dianalisis dengan menggunakan kit ELISA 17β-estradiol dan 17α-ethynylestradiol. Ujian Kruskal-Wallis dan analisis komponen utama (PCA) telah digunakan untuk analisis statistik. Risk quotient (RQ) digunakan untuk menilai risiko manusia manakala model matematik seperti model kepekatan estradiol (EEQ) dan model penambahan kepekatan (CA) digunakan untuk menilai risiko lingkungan akuatik. Hasil kuantitatif menunjukkan kepekatan E_2 semulajadi adalah 88.17±7.03 ng/L hingga 93.93±6.91 ng/L bagi influen dan 35.10±17.33 ng/L hingga 85.20±7.54 ng/L bagi efluen. Kepekatan EE_2 sintetik adalah 0.22±0.21 ng/L hingga 4.94±6.32 ng/L bagi influen dan 0.02±0.02 ng/L hingga 1.04±0.77 ng/L bagi efluen. Untuk parameter fizikokimia kumbahan, jumlah pepejal terampai adalah 95.17±3.96 mg/L hingga 129.80±4.95 mg/L bagi influen dan 2.84±0.95 mg/L hingga 14.67±0.57 mg/L bagi efluen, pH adalah 6.73±0.01 hingga 7.04±0.03 bagi influen dan 6.79±0.06

hingga 6.95±0.02 bagi efluen, potensi penurunan oksidasi adalah -29.20±3.20 mV hingga -18.07±1.93 mV bagi influen dan 5.37±3.25 mV hingga 16.77±3.93 mV bagi efluen, dan suhu adalah 32.20±0.1°C hingga 35.60±2.31°C bagi influen dan 32.47±0.12°C hingga 34.63±0.67°C bagi efluen. Ujian Kruskal-Wallis menunjukkan perbezaan bererti antara kepekatan E_2 semulajadi, EE_2 sintetik di influen dan profil sosio-demografi (jantina, status perkahwinan, pendidikan, dan isi rumah). Kecekapan penyingkiran adalah 6.4% hingga 63.0% bagi E_2 semula jadi dan 28.3% hingga 99.3% bagi EE_2 sintetik. Analisis PCA menunjukkan parameter fizikokimia kumbahan dikaitkan dengan kecekapan penyingkiran E_2 semulajadi dan EE_2 sintetik dalam WWTP. Untuk risiko manusia, nilai kumulatif RQ adalah di bawah had yang dibenarkan, kecuali WWTP 1. Untuk risiko lingkungan akuatik, model EEQ meramalkan aktiviti estrogenik dari julat 35.1 EEQ-ng/L hingga 85.3 EEQ-ng/L, manakala model CA meramalkan aktiviti estrogenik 105.4 ng/L. Walau bagaimanapun, aktiviti estrogenik dalam lingkungan akuatik boleh lebih rendah disebabkan kesan pencairan melalui hujan. Output kajian ini berguna sebagai data kuantitatif asas bagi E_2 semulajadi dan EE_2 sintetik di WWTP Lembah Klang dan potensi risiko terhadap manusia dan lingkungan akuatik.

Kata kunci: Loji rawatan kumbahan, Estrogen steroid, Kecekapan penyingkiran, Risiko potensi

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LIST OF ABBREVIATIONS

AA-EQS	Annual average value of environmental quality standards
AL	Aerated lagoon
BSA	Bovine Serum Albumin
CA	Concentration addition
CAS	Conventional activated sludge
CCL_3	Contaminant Candidate List
CDER	Center for Drug Evaluation and Research
CI	Combination index
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
E_1	Estrone
E_2	17β-estradiol
E_3	Estriol
E. coli	*Escherichia coli*
EA	Extended aeration
EC_{50}	Half maximal effective concentration
EDC	Endocrine disrupting compound
EDSP	Endocrine Disruptor Screening Program
EDTA	Endocrine Disrupters Testing and Assessment
EE_2	17α-ethynylestradiol
EEF	Estrogen equivalency factor
EEQ	Estradiol equivalent concentration
EEQ_t	Total estrogenic activity
ELISA	Enzyme-linked immune sorbent assay
EQS	Environmental quality standards
ER	Estrogen receptor
ETV	Exposure threshold value
E-screen	Cell proliferation
FDA	Food and Drug Administration
GC-MS	Gas chromatography mass spectrometry
H	Henry's law constant
h	Hill coefficient
HOCL	Hypochlorous acid
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
HRT	Hydraulic retention time
K_{ow}	Log octanol–water partition
K_d	Distribution coefficient
LC-MS	Liquid chromatography mass spectrometry
LOD	Limit of detection
LOEC	Lowest observable effect concentration
LOQ	Limit of quantitation
MBR	Membrane bio-reactor
MCF-7	Proliferation of breast cancer cell
MDL	Method detection limit
mV	millivolt
NABC	Need, Approach, Benefit, and Competition
OD	Oxidation ditch

ORP	Oxidation reduction potential
PCA	Principal component analysis
PE	Population equivalent
QA	Quality assurance
QC	Quality control
r^2	Correlation coefficient
RBCs	Rotating biological contactors
RIA	Radio immune assay
RQ	Risk quotient
SBR	Sequencing batch reactor
SPE	Solid phase extraction
SPEED	Strategic Program on Endocrine Disruptors
SRT	Solid retention time
TCA	Tricarboxylic acid
TF	Trickling filter
TMD	Tetramethylbenzidine
TSS	Total suspended solid
USEPA	United States Environmental Protection Agency
USNEPA	United States National Environmental Policy Act
UV	Ultraviolet
WFD	Water Framework Directive
WHO	World Health Organization
WWTP	Wastewater treatment plant
YES	Yeast estrogen screen

CHAPTER 1

INTRODUCTION

1.1 Background of study

Endocrine disrupting compounds (EDCs) comprise a wide variety of contaminants, involving surfactants, plasticizers, pesticides, as well as natural and synthetic estrogens (Esperanza et al., 2007). Endocrine disrupting compounds have received considerable attention worldwide because of their potential as environmental contaminants that are capable of interfering with the normal functions of the endocrine system (Yang et al., 2011). These EDCs could either mimic or prevent the endocrine system in human and aquatic organisms by disrupting the normal function of these glands thus potentially elicit negative effects on endocrine systems (Chang et al., 2009). The lowest observable effect concentration (LOEC), particularly for estrogens, was at the range of 0.2 ng/L to 1.0 ng/L, which could cause estrogenic effects in fish at laboratory (Zha et al., 2008). Based on Adeel et al. (2017), human health could be affected by consuming contaminated fish or drinking water.

Amongst this large group of EDCs, steroid estrogens are of particular interest for their relatively high estrogenic potency (Ternes et al., 1999), which found to be 10^2-10^7 times more potent than other EDCs (Legler et al., 1999, 2002a, 2002b). Farre et al. (2007) stated that steroid estrogens have high estrogenic potency with physiological active concentrations ranging from picograms per liter (pg/L) to nanograms per liter (ng/L). Based on Water Framework Directive (WFD), the proposed annual average environmental quality standards (AA-EQS) value for priority substances were as low as 3600 pg/L, 400 pg/L and 35 pg/L for estrone (E_1), 17β-estradiol (E_2), and 17α-ethynylestradiol (EE_2), respectively (Kase et al., 2018).

Natural estrogens (E_1, E_2, and E_3) naturally secreted by the adrenal cortex, placenta, testis, and ovary in human, while synthetic EE_2 is a major component in oral contraceptive and hormone therapy pills (Zhang et al., 2011; Ye et al., 2012). Amongst these steroid estrogens, natural E_2 and synthetic EE_2 are the most potent in estrogenic potency (Desbrow et al., 1998; Racs and Goel, 2009). Natural E_2 and synthetic EE_2 have been classified as “Watch List” in Directive 2013/39/EU, because they are used as active ingredients in oral contraceptive and hormone therapy pills (Manickum and John, 2015; Cunha et al., 2016), in order to collect more monitoring data to support the priority substances list under the WFD since there is no maximum tolerable concentrations were determined so far (Cunha et al., 2016).

Municipal wastewater is the main disposal pathway for human waste born estrogenic compounds providing synthetic estrogen ingested will excrete together with natural estrogens as urine and feces that eventually enter the wastewater stream (Hamid and Eskicioglu, 2012). Wastewater treatment plant (WWTP) is regarded as the major source for steroid estrogens release to aquatic environment because the wastewater treatment systems are generally designed to remove basic physical, chemical, and biological wastewater parameters (Aerni et al., 2004). Instead, estrogen are organic compounds (Sparkman et al., 2011), which involve basic wastewater parameters for removal, such as sorp to suspended solid (physically), or biodegrade by organism where biological oxygen demand is measured (biologically).

1.2 Problem statement

Wastewater treatment plant has been a major point source of emerging pollutants into water bodies, provided that estrogenic compounds are not a design criterion for conventional wastewater treatment system (Hamid and Eskicioglu, 2012). Globally, the WWTPs were estimated to eliminate about 50.0% of the total estrogens in wastewater (Cargouet et al., 2004). To date, quantification of steroid estrogens in WWTPs has been conducted in several countries. Mohagheghian et al. (2014) study in Iran reported natural E_1 (71.8% removal), natural E_2 (68.2% removal) and synthetic EE_2 (80.4% removal) were partially removed in activated sludge treatment systems, with concentration of 3.2±0.8 ng/L, 0.9±0.5 ng/L, and 1.2±0.4 ng/L respectively detected in effluent, and indicated adverse effects to environment. Besides, Manickum and John (2014) study in South Africa observed contamination of downstream river due to partial estrogens removal in wastewater treatment systems, at estrogenic activity of 3.5 ng-EEQ/L (natural E_2) and 15.2 ng-EEQ/L (synthetic EE_2), which eventually served as raw source to drinking water treatment plants. In Malaysia, limited studies were conducted on occurrence and removal of steroid estrogens in WWTPs. Study done by Al-Odaini et al. (2013) determined occurrence of synthetic hormones (levonorgestrel, norethindrone, cyproterone acetate, and EE_2) in WWTP effluent, with concentration detected less than method detection limit (<MDL), <MDL–11336 ng/L, <MDL-262 ng/L, and <MDL-1898 ng/L respectively, and indicated potential risks to aquatic environment and drinking water. However, understanding on the occurrence and removal of steroid estrogens (natural E_2 and synthetic EE_2) in both influent and effluent of Klang Valley WWTPs together with its risks to the receiving rivers is very limited.

Steroid estrogens quantification involves high cost and challenging due to complexity of wastewater matrix, low concentration (pg/L to ng/L) in wastewater, and laborious instrumental analysis protocols (Johnson and Williams, 2004; Umali et al., 2012). Previous studies had shown wide application of instrumental analytical techniques such as gas chromatography mass spectrometry (GCMS) (Atkinson et al., 2012; Pessoa et al., 2014) and liquid chromatography mass spectrometry (LCMS) (Sim et al., 2011; Muz et al., 2012) to quantify steroid estrogens in wastewater samples. However, application of instrumental GCMS often required derivatization step for better sensitivity, attribute to low volatility of steroid estrogens (Briciu et al., 2009). The application of LCMS is expensive because high consumption of solvents is required, and qualified personnel is needed for complex instrumental operation (Mauricio et al., 2006; Pacakova et al., 2009). Therefore, there is a necessity for rapid, simple, and cost-effective analytical technique such as non-instrumental enzyme-linked immunosorbent assay (ELISA) in steroid estrogens quantification (Farre et al., 2007). To date, ELISA has been used by Dorabawila and Gupta (2005) for determination of natural E_2 in environmental samples, because of its good sensitivity that can detect as low as 50 pg/L. The ELISA was also used by Swart and Pool (2007) to quantify steroid hormones in environmental samples, which is simple because large number of samples can be analysed simultaneously, and cheap because the kit provides manual and all needed chemical for amalysis. In Malaysia, ELISA was used to diagnose infectious disease, such as avian influenza (Ong et al., 2010), human immunodeficiency virus (Paranthaman et al., 2015), and foot-and-mouse disease (Zubaidah et al., 2017). Yet, application of ELISA for steroid estrogens quantification in wastewater samples is very limited studied.

The European Commission had highlighted the estrogenic activity of estrogens mixture in wastewater effluent, and acknowledged the need to implement monitoring and risk assessments methods (European Commission, 2012). However, the potential risks of estrogenic activity to human is rarely evaluated since the exposure threshold value (ETV) is unavailable. In perspectives of potential risks to aquatic environment, direct measuring of estrogenic activity by analytical monitoring is an expensive and time-consuming approach, which may pose challenges during study design, data analysis and interpretation (Sumpter et al., 2006). The measuring techniques could combine with mathematical models for greatest confidence in risk assessment. Several studies have employed mathematical model to predict the toxicity of chemicals mixture. Zhang et al. (2011) used estradiol equivalent concentration (EEQ) model to predict total estrogenic activity (EEQ_t) of natural E_1, E_2, E_3, synthetic EE_2, diethylstilbestrol, OP and NP in WWTP effluent (3.5-29.6 ng-EEQ/L), and indicated the potential to pose ecological risk to the receiving river. Avbersek et al. (2011) also used EEQ model to determine EEQ_t of natural E_2, E_3, and synthetic EE_2 in WWTP effluent (1.7 ng-EEQ/L) and highlighted low potency natural E_3 could contribute to EEQ_t. On the other hand, Rider and LeBlanc (2005) used concentration addition (CA) model to assess the toxicity of pesticides chemical in ternary mixture. Hadrup et al. (2013) also used CA model to predict the toxicity of pesticide chemical mixture, and proven the presence of multiple chemicals contribute to mixture effects. To date, only Bermudez et al. (2012) employed both EEQ and CA models to evaluate the estrogenic activity of environmental estrogens mixtures (equilin, mestranol, natural E_1, E_2, and synthetic EE_2) in animal waste. However, the quality of Klang Valley WWTPs effluent in terms of estrogenic pollution remains unclear.

1.3 Study justification

Steroid estrogens, as emerging organic compounds in wastewater are limited studied because there is currently no legislation for maximum allowable levels in water matrix (Umali et al., 2012). By understanding the occurrence and removal of natural E_2 and synthetic EE_2 in Klang Valley WWTPs, the adverse effects of estrogenic pollutants to human and aquatic environment could be determined. There is a great concern regarding the threats of steroid estrogens presence in WWTP effluent, as the treated wastewater is eventually released to the receiving river (Muz et al., 2012). Their eventual presence in the receiving river has the potential to bio-accumulate and enter the food chain as drinking water (Magi et al., 2010), thus affect reproduction and development of human (Manickuma and John, 2014). Besides, studies reported concentration as low as 1.0 ng/L of natural E_2 (Routledge et al., 1998) and 0.5 ng/L of synthetic EE_2 (Hansen et al., 1998) could induce vitellogenin production in fish.

Non-instrumental ELISA is indispensable for rapid, simple, and cost-effective method for steroid estrogens quantification in environmental samples (Farre et al., 2007; Swart and Pool, 2007). This is because, ELISA analysis is performed using 96 well plates, where large numbers of samples involving blank and spike samples can be analyzed simultaneously (Pacakova et al., 2009). The assay involved in ELISA is relatively cheap compared to the cost of the instrumental analytical techniques because ELISA kit includes all chemicals needed as well as manual for the analysis (Mispagel et al., 2009). Besides, ELISA has good sensitivity with detection limit as low as picogram, which is more sensitive than instrumental analytical techniques that detect up to nanogram (Mauricio et al., 2006). Based on Auckland Regional Council, ELISA was validated to be adequate for steroid estrogens analysis with good recovery rates (Singhal et al., 2009).

The potential risks of estrogenic activity to human is assessed because Aerni et al. (2004) observed estrogenic activity as low as 0.1 ng/L are sufficient to cause estrogenic effects to human. The potential risks of estrogenic activity to aquatic environment is assessed because exposure studies reported fish exposed to 1.0 ng/L of natural E_2 (Routledge et al., 1998) and 0.1 ng/L of synthetic EE_2 (Desbrow et al., 1998) could exhibit changes in biomarkers due to estrogenic activity. Application of both EEQ and CA models to predict the estrogenic activity in WWTP effluent is for the comparison of two predictive models. This is because, CA model is extensively used in chemical mixture toxicity research, while EEQ model which is a derived approach of CA model required less data for prediction due to assumptions of parallel concentration curve is made (Bermudez et al., 2012). Despite the fact complex chemical matrices in a real-world mixture could weaken both CA and EEQ models in estrogenic activity prediction (Kortenkamp et al., 2009), these models had the strength that allow for easier and quick interpretation of effect-based mixture quality monitoring data (Bermudez et al., 2012). Besides, application of EEQ and CA models in estrogenic activity predictions require less time and low cost (Ting et al., 2017), because they involved mathematical formulae, where the raw data could be obtained from measuring technique.

1.4 Significance of study

This study is a pioneer work to understand the occurrence and removal of natural E_2 and synthetic EE_2 in Klang Valley WWTPs. This is the first attempt that involved non-instrumental ELISA to quantify steroid estrogens in wastewater samples, which subsequently assessed the potential risks to aquatic environment by using mathematical modelings. Hence, the outputs from present study are applicable as baseline data in Malaysia. The data obtained from present study will be useful and enables alert relevant practitioners regarding this issue. By understanding fate of steroid estrogens in six different WWTPs, will provide insights into which wastewater treatment systems can remove estrogenic compounds more effectively. Besides, understanding the fate of steroid estrogens within the WWTPs will yield better removal technology based on minor modifications of current wastewater treatment systems, so the effluent discharged to the receiving river will be more environmental friendly and safe. Moreover, the outputs from mathematical modelings (CA and EEQ models) can be used to address the shortage of information on environmental toxicity caused by WWTPs.

1.5 Research objectives

The general objective of the study is to analyze the occurrence, removal, and potential risks of natural E_2 and synthetic EE_2 in six selected WWTPs located in Klang Valley.

The specific objectives of the study are:

i. To determine natural E_2 and synthetic EE_2 concentrations and wastewater physicochemical parameters (TSS, pH, temperature, and ORP) in influent and effluent.
ii. To identify the influence of socio-demographic profile on natural E_2 and synthetic EE_2 concentration in influent.
iii. To evaluate the removal efficiency of natural E_2 and synthetic EE_2 in WWTP.
iv. To analyze relationship between wastewater physicochemical parameters associated with natural E_2 and synthetic EE_2 removal in WWTP.
v. To assess the potential risks of natural E_2 and synthetic EE_2 to human and aquatic environment.

1.6 Research hypotheses

The research hypotheses of the study are:

i. There is a significant difference between socio-demographic profile based on natural E_2 and synthetic EE_2 concentration in influent.
ii. There is a significant association between wastewater physicochemical parameters with natural E_2 and synthetic EE_2 removal in WWTP.

1.7 Scope of study

This study focused on the occurrence, removal, and potential risks of natural E_2 and synthetic EE_2 (estrogenic pollutants) in six selected WWTPs in Klang Valley. The quantification was carried out by using simple, rapid, and cost-effective non-instrumental ELISA, which was appeared to be the most reliable analytical techniques to quantify ultra-trace concentration of steroid estrogens in wastewater samples. Socio-demographic profile for population living nearby six selected WWTPs was obtained to describe the potential sources of estrogens in WWTPs influent. The influence of socio-demographic profile on natural E_2 and synthetic EE_2 concentration in influent was determined. Besides, the removal efficiency of natural E_2 and synthetic EE_2 in WWTP was evaluated. The wastewater physicochemical parameters (TSS, pH, temperature, and ORP) associated with natural E_2 and synthetic EE_2 removal in WWTP was identified. Finally, the potential risks of estrogenic activity (estrogenic pollutants mimic action of natural E_2, binds to normal estrogen receptor and interfere normal estrogen signalling process) to human and aquatic environment were assessed.

1.8 Study limitation

The quantification and assessment of estrogenic activity in WWTPs effluent would be more ideal if the study was conducted continuously over long term; daily, weekly or monthly. Burt et al. (2008) emphasize that generating long term data sets will provide more reliable outputs and allow observations on the gradual change of estrogenic activity in WWTPs effluent. The long-term monitoring is essential to further understand data stability and its reasons, as well as observe gradual change and its causes. On the other hand, prediction of estrogenic activity in a real-world WWTP effluent mixture is more challenging by application of EEQ and CA models, due to the complex chemical matrices involved. There are other industrial chemicals (Bisphenol A, phathalates) and estrogenic compounds (estrone, estriol, other steroidal estrogens, conjugates of steroidal estrogens, phyoestrogens) that are capable of invoking the estrogenic response present in WWTPs effluent (Tanaka et al., 2001; Pawlowski et al., 2003; Aerni et al., 2004; Liney et al., 2005). Likewise, the occurrence of antagonists in WWTP effluent may inhibit the estrogen responsive pathway and alter the estrogenic potency of the mixture, which could lead to reduction in estrogenic activity (Thorpe et al., 2006).

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LIST OF PUBLICATIONS

Published

Fang, T.Y., Praveena, S.M., Aris, A.Z., Ismail, S.N.S. and Rasdi, I. (2019). Quantification of selected steroid hormones (17β-estradiol and 17α-ethynylestradiol) in wastewater treatment plants in Klang Valley (Malaysia). *Chemosphere* 215: 153-162. (IF: 4.208, Q1)

Fang, T.Y., Praveena, S.M., deBurbure, C., Aris, A.Z., Ismail, S.N.S. and Rasdi, I. (2016). Analytical techniques for steroid estrogens in wastewater samples-A review. *Chemosphere* 165:358-368. (IF: 4.208, Q1)

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