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Occurrence of selected estrogenic compounds and estrogenic activity in surface water and sediment of Langat River (Malaysia)

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Abstract The occurrence and estrogenic activities of steroid estrogens, such as the natural estrone (E1), 17β estradiol (E2), and estriol (E3), as well as the synthetic 17α-ethynylestradiol (EE2), were investigated in eight sampling points along the Langat River (Malaysia). Surface water samples were collected at 0.5 m and surface sediment 0–5 cm from the river surface. Instrument analysis of steroid estrogens was determined by UPLC-ESI-MS with an ultra-performance liquid chromatograph (Perkin Elmer FX15) coupled to a Q Trap function mass spectrophotometer (model 3200: AB Sciex). Steroid estrogen concentrations were higher in the Langat River sediments than those in its surface water. In surface water, E1 was not detected in any sampling point, E2 was only detected in two midstream sampling points (range 0–0.004 ng/L), E3 in three sampling points (range 0–0.002 ng/L), and EE2 in four sampling points (range 0–0.02 ng/L). E1 and E2 were detected in sediments from all sampling points, E3 in five sampling points, while EE2 only in one midstream sample (3.29E−4 ng/g). Sewage treatment plants, farming waste, and agricultural activities particularly present midstream and downstream were identified as potential sources of estrogens. Estrogenic activity expressed as estradiol equivalents (EEQs) was below 1 ng/L in all samples for both surface water and sediment, indicating therefore a low potential estrogenic risk to the aquatic environment. Although the health risks are still uncertain for drinking water consumers exposed to low levels of steroid estrogen concentrations, Langat River water is unacceptable for direct drinking purposes without treatment. Further studies of endocrine disruptors in Malaysian waters are highly recommended.

Keywords Occurrence · Steroid estrogen · Estrogenic activity · Langat River

Introduction

Environmental contaminants that can disrupt the endocrine system in humans and wildlife are known as endocrine-disrupting compounds (Esteban et al. 2014; Shi et al. 2012). Much research has focused on environmental contaminants, such as pesticidesals, which may interfere with the endocrine system (Colborn et al. 1993;

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Wang et al. 2009). By contrast, less information is available on estrogenic compounds with hormone-disrupting activities. Steroid estrogens are estrogenic compounds and are among important contributors to endocrine-disrupting activity in the environment (Huang et al. 2013). In medicine, steroid estrogens are used for menopausal syndrome management and for treating prostate and breast cancers. In animal farming, steroid estrogens are applied to promote single sex populations, especially in aquaculture, and to function as growth promoters (Kuster et al. 2004). Steroid estrogens are also constantly released into the environment through direct discharge, sewage treatment plants, and sewage sludge in agriculture (Baronti et al. 2000). The effects of estrogenic activity include imposex of aquatic organisms, feminization, and interference in the normal function of the endocrine system; estrogens affect wildlife production and development, and their effects possibly appear in the subsequent generations, thereby endangering the sustainable development of humans and ecosystems (Aris et al. 2014; Peng et al. 2006).

Even low concentrations of steroid estrogens in rivers could alter normal hormone function in humans and wildlife, as observed in the Tiber River in Italy (Laganà et al. 2004), Songhua river, China (Zhang et al. 2014), and the Iberian River (Gorga et al. 2014). Steroid hormones have lipophilic characteristics with high octanol-water partition coefficient values (high log Kow 2.81–5.67). These characteristics give steroid hormones a high tendency to accumulate from water into solid matrices, such as sediment. In anaerobic and dark environments, such as rivers and lakes, steroid estrogens are transported and diffused across the sediment water column interface or removed by scouring during storm events (Lai et al. 2000). Steroid estrogen in the subsurface layers of river sediments may undergo low biodegradation and photodecomposition to be adsorbed in sediment (Jürgens et al. 1999). Sediments can act as sinks for pollutants, including steroid estrogens, which may persist for a long time and pose potential threats to the environment, especially to aquatic biota (Praveena et al. 2012). Jürgens et al. (1999) showed that approximately 53 % of the steroid estrogens that enter the river system would end up in the bed-sediment compartment, with maximum adsorption within the first 24 h of contact (Aris et al. 2014; Kuster et al. 2004; Ternes et al. 2002). However, interactions between steroid estrogens in water and sediment have rarely been directly analyzed; quantitative data are also scarce and restricted to a few locations (Peck et al. 2004; Zhang et al. 2011; Zhao et al. 2011).

The Langat River is one of the four major river systems in the Selangor state, which provides approximately 50 % of the population of Selangor with water for drinking, agriculture, and manufacturing. The land area from the middle to lower stream of the Langat River has been exploited to support the expanding population. In addition, poor land management practices along the Langat River have increased pollution loading into the river water and sediment. According to the Department of Environment of Malaysia (2009), Langat River is one of the most polluted rivers in the state of Selangor because of rapid industrialization and urbanization arising from infrastructural development and the expansion of the population. Chemical pollution has recently caused public concern regarding drinking water safety because the Langat River is the primary source of drinking water (Al-Odaini et al. 2013; Azrina et al. 2006; Lim et al. 2013b; Sarmani 1989; Yusoff et al. 2008). Continuous exposure to large amounts of sewage effluent from private and public sewage treatment plants, individual septic tanks, and domestic sewage can be one of the steroid hormone sources in the river water and sediment of the Langat River (Al-Odaini et al. 2013). Sewage effluent is directly released into the river water, which is used as a source of raw water for the production of potable supplies for communities living downstream. Thus, the continuous release and exposure to these steroid estrogens can have various effects on aquatic species and pose as human health risks associated with the consumption of contaminated drinking water over a lifetime. The detailed quantification of these pollutants in water and sediment is essential in the assessment of endocrine-disrupting effects on the aquatic environment and human health risks.

This study aims to investigate the concentrations of natural (estrone, estriol, and 17β-estradiol) and synthetic (17α-ethynyl estradiol) steroid estrogens in the Langat River and its sediment. This study was also conducted to assess the estrogenic activity and indicate potential risks to the aquatic environment. Thus, this study is a pioneer work on the steroid estrogen concentration and the estrogenic activity in river water and sediment in Malaysia. The output of this study will provide a baseline value to broaden our current knowledge of steroid estrogen contamination in Malaysia.

Materials and methods

Chemicals and materials

Steroid estrogen standards of estrone (E1), 17ß-estradiol (E2), estriol (E3), and 17ß ethynylestradiol (EE2) were purchased from Sigma-Aldrich Canada, Ltd. (Oakville, Ontario, Canada). All HPLC-grade reagents (methanol, ethyl acetate, and methanol-dichloromethane) were obtained from J. T. Baker, Inc. The cartridges for solid-phase extraction (SPE) were C_{18} SPE cartridges (500 mg/6 cc) from Thermo Fisher Scientific (Sunnyvale, USA). Deionized water was prepared with a Milli-Q water purification system.

Study area and sampling

The Langat River basin is located between 101° 25’ and 101° 54’ of the Greenwich meridian with an area of approximately 2423 m^2. Water from the upstream area of the Langat River (Hulu Langat; ~182 km from the main range of Banjaran Titiwangsa) flows to the downstream area in Kuala Selangor and drains into the Straits of Malacca. The Strait of Malacca is one of the world’s busiest sea lanes; this strait connects the Indian Ocean, South China Sea, and Pacific Ocean (Law et al., 2002). The sediment of the Langat River is composed of granite and sandstone. The climate of the Langat River is characterized by uniform and high rainfall and humidity, with an average annual temperature of 26.7 °C. The climate is influenced by the South West monsoon, which blows across the Strait of Malacca. A significant increase in human population along the Langat River was recorded; the number of people increased by 94.66 % from 598,661 people in 1990 to 1,165,267 people in 2000 (Juahir et al. 2011; Lim et al. 2013a).

Surface water and river sediment samples were collected from the Langat River in April 2015 (Fig. 1). The Langat River dam (S1) and Hulu Langat (S2) sampling locations were selected because these areas have relatively low river pollution. The sampling locations of Cheras Batu 11 (S3), Bangi (S4), Semenyih River (S5), and Salak Tinggi (S6) were located in the middle stream region with moderate river pollution. The sampling locations of Bukit Tampoi (S7) and Banting (S8) are areas of high pollution because a number of wastewater treatment plants are located along the river (Juahir et al. 2011). Surface water samples were collected in triplicate with precleaned 1-L amber glass bottles. The amber glass bottles were rinsed twice with river water before collection. Surface water samples were collected at a depth of 0.5 m below the water surface. Before surface water sampling, amber glass bottles were rinsed with acid wash and dried in the oven to reduce the possibility of interferences (Wang et al. 2012; Zhang et al. 2014). River sediment was sampled from 0 cm to 5 cm of the river surface. The river sediment samples were freeze-dried, homogenized, and stored in the dark at −20 °C until further analysis.

Sample analysis

Surface water samples were filtered through a 0.45 μm Whatman membrane filter to remove impurities. Subsequently, 50 mL of methanol was added as a preservative. The amber glass bottles were wrapped with aluminum foil and kept in refrigerator until SPE and analysis (Chang et al. 2009). River sediment samples were extracted by ultrasonic extraction based on Zhang et al. (2014). Sediment sample (10 g; oven-dried and homogenized) was mixed with methanol–dichloromethane (1:1 *v*/*v*; 25 mL), ultrasonically extracted for 20 min, and centrifuged at 1100*g* for 5 min. The extraction was repeated twice, and the sediment extracts were then combined into a flask for SPE. The river water samples and sediment extracts were loaded into C_{18} SPE cartridges (500 mg/6 cc; Thermo Fisher Scientific, Sunnyvale, USA) preconditioned with 5 mL of ethyl acetate and 5 mL of methanol, followed by 5 mL of ultrapure water. After sample loading, the cartridges were washed with 3 mL of ultrapure water at a flow rate of 1 mL/min. The cartridges were dried under a gentle stream of nitrogen gas for 20 min and eluted with 10 mL of methanol in ethyl acetate (15 %, *v*/*v*) at a flow rate of 1 mL/

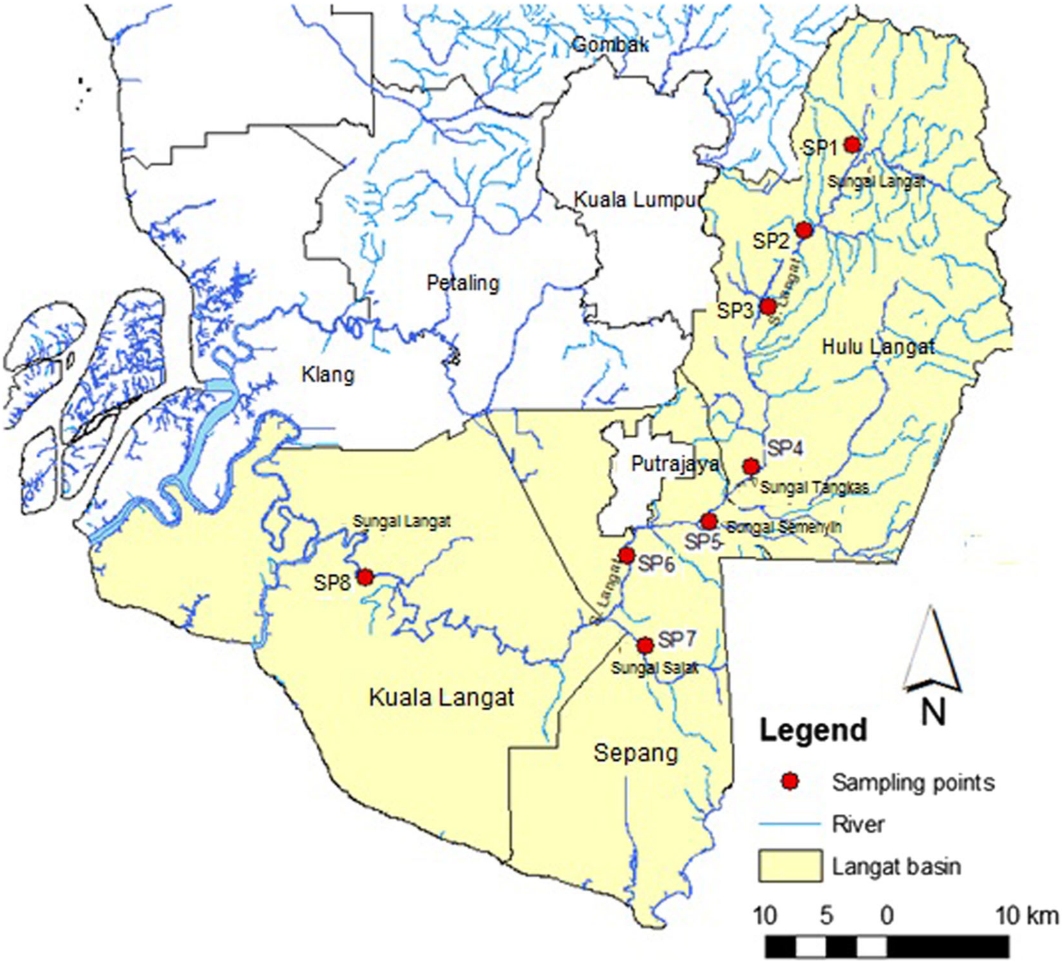

Fig. 1 Sampling locations along the Langat River

min. The extracts were evaporated into complete dryness under a gentle flow of nitrogen gas, reconstituted with 0.2 mL of methanol–ultrapure water (10:90, *v*/*v*), and transferred into autosampler vials prior to instrument analysis.

Instrument analysis of steroid estrogens was performed by ultra-performance liquid chromatography (Perkin Elmer FX15) electrospray ionization-mass spectrometry coupled to a QTrap function mass spectrometer (model 3200; AB Sciex). Ultra-high performance liquid chromatography shortens analysis time and sample volume, enhancement in speed and solvent consumption, can operate at high system back-pressures with mobile phase running at upper velocities as compared to high performance liquid chromatography (Nováková et al. 2006; Hamdy and Belal 2014). Ultra-performance liquid chromatography electrospray ionization-mass spectrometry also offers more advantages in quantifying steroid estrogen at low concentrations, as most of the detections of steroid estrogens in sediment is mainly performed using gas chromatography–mass spectrometry, as indicated by the reviews conducted by Aris et al. (2014), Kuster et al. (2004), and Ternes et al. (2002).

Approximately 20 μL of each sample was injected into a Zorbax C18 column (5 μm particle size; 150 mm × 4.6 mm i.d.; Phenomenex, Macclesfield, UK). Gradient separation was achieved with two solvents, namely, a mixture of water and 0.2 % formic acid (A) and a mixture of methanol with 0.2 % formic acid (B). The total flow rate for the mobile phases was 0.8 mL/min. Methanol was used as the organic mobile phase because it provides superior chromatographic separation and increased sensitivity (Koh et al. 2007).

The gradient condition started at 60 % A and 40 % B, linearly increased to 80 % B in 8 min, maintained for 2 min, and linearly decreased to 40 % B in 1 min. The total chromatographic run time was 12 min for a sample volume of 20 μL. The mass spectrophotometer was operated in the negative electrospray ionization mode by multiple-reaction monitoring (MRM). Detection by the mass spectrophotometer was performed under the following conditions: ion source gas 1 and 2, 40 psi; ion spray voltage, −4500 V; source temperature, 250 °C; and curtain gas (N_2), 10 psi.

Six-point calibration curves were constructed for each steroid estrogen within the linear range of the instrument (20–200 ng/L). Standard addition was selected as the calibration method of choice because steroid estrogen concentrations were present in ultra-trace concentrations (ng/L). Method of standard addition was adapted because of several factors, namely, the signal of the analyte is affected by very low concentrations of steroid estrogens present in the sample and matrix interference should be prevented from appearing (Abbasi Tarighat 2014; Danzer and Currie 1998). A comprehensive list of MRM transitions was generated based on the product ion analysis. Data analysis was performed with the Analyst™ v1.5.2 software. Optimization of mass spectrometry was carried out in negative mode with electrospray ionization. Single standard solutions were used to identify the negative ions and peak retention times (Table 1). Optimization of mass spectrometry, namely, the retention time, MRM, dwell time, and collision energy of each steroid estrogen, was performed by direct infusion of 200 ng/L of the standard steroid estrogen at a flow rate of 20 μL/min. The highest intensity was achieved for each steroid estrogen when optimum conditions were reached. The intensity of E1 was obtained at 9.41 min, E2 at 9.60 min, E3 at 6.03 min, and EE2 at 9.32 min (Table 1).

All the analytical procedures were done with certain quality assurance and control measures. All the glassware were cleaned by soaking in nitric acid overnight, washing with distilled water, and drying in the oven. For each sample, reagent blanks and procedural blanks were similarly processed and handled with actual river surface water and sediment samples. All the river water and sediment samples were analyzed in triplicate. The calibration curves for determining steroid estrogens were obtained by linear regression analysis of standard solutions. Linear regression analysis on standard solutions was performed with the ratio of standard to internal standard areas with coefficient correlation values of more than 0.996 (Table 2). For each steroid estrogen, the estimated instrument limit of quantification and limit of detection were based on the concentration with minimal signal-to-noise (S/N) ratios of 10 and 3, respectively (Table 2). The recoveries were evaluated at low (20 ng/mL) and high (200 ng/mL) concentrations of river sediment. Recoveries were calculated by subtracting the concentrations observed in the unspiked samples. Recoveries for three replicate river sediment samples ranged from 97 to 107 %, with relative standard deviations in the range 0.8–17 % (Table 2). The concentrations of steroid estrogens were reported as ng/L for surface water samples and ng/g dry weight (dw) for surface sediment.

Data analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences, version 15.0. Descriptive statistics was performed to obtain mean and range values of natural and synthetic steroid estrogen. Estrogenic activities in river water and sediment samples (EEQs) were calculated by comparing activity of natural and synthetic steroid estrogen concentrations (C_i) with relative estrogenicity values (E2) as shown in

Table 1 LC-MS/MS conditions for MRM transitions of each steroid estrogen

Steroid estrogen	Retention time (min)	MRM *m/z*	Dwell time (ms)	Collision energy (eV)
E1	9.41	269 > 145	150	70
E2	9.60	270.9 > 145	150	56
E3	6.03	287.1 > 171	150	52
EE2	9.32	295.05 > 145	150	50

Table 2 Analytical characteristics of the method performance in this study

Steroid estrogens	LOD (ng/mL)	LOQ (ng/mL)	Correlation coefficient (r^2)	Linear range (ng/mL)	Recovery rate (%)		Relative standard deviation (%)
					High	Low	
E1	0.003	0.010	0.9999	0.02–200	102	99	10
E2	0.003	0.010	0.9996	0.02–200	104	103	17
E3	0.007	0.020	0.9997	0.02–200	97	98	6
EE2	0.007	0.020	0.9998	0.02–200	107	106	0.8

Eq. 1. The relative estrogenicity values (E2) were obtained from a study done by Céspedes et al. (2004).

$$\mathrm{EEQ} = \mathrm{C_i} \times \mathrm{E2} \tag{1}$$

where

EEQ	Estradiol equivalents
C_i	Concentration of each steroid estrogen detected in water and sediment
E2	Relative estrogenicity values

Results and discussion

Steroid estrogen concentrations in surface water and sediment of Langat River

This study evaluated the presence of steroid estrogen in river water and sediment at eight sampling points along the Langat River in Selangor. In river water, E1 concentrations were lower than LOD and were not detected in all the sampling points. E2 concentrations were only detected in SP5 and SP7. These river samples were obtained from areas located near agricultural activities. The strong influence of agricultural activities on the occurrence of steroid hormones is also reported by Lee et al. (2007) and Rao et al. (2013). Moreover, Kolodziej et al. (2003) demonstrated that farming waste could contribute to occurrence of steroid hormones in surface water. According to (Lee et al. 2007), E2 can transform to E1 and then degrade to E3 in the aquatic environment. EE2 concentrations were also found in SP2, SP3, SP5, and SP6. EE2 had lower solubility than the other steroid estrogens (E1, E2, and E3). Therefore, EE2 remains stable despite oxidation in the environment (Aris et al. 2014).

The concentrations of steroid estrogen in river surface sediment are summarized in Table 3. The results showed that the concentration of E1 in SP2 was 0.035 ng/g, which is the highest among all sampling locations. The concentration of E1 was probably high because the river sediments were obtained from wastewater outflows. The concentration of E2 was highest in SP8, whereas E3 (0.024 ng/g) was highest in SP7. These areas are locations where the river receives waste from agriculture and residential areas (Zhang et al. 2014), and Ternes et al. (1999) stated that the sources of natural E1, E2, and E3 are human and livestock excretions. Meanwhile, synthetic EE2 was not detected in nearly all the samples and was only detected in one sediment sample (SP6) at a very low concentration (in the range of ND to 3.29E–4 ng/g). This sampling location is near a sewage treatment plant. According to Aris et al. (2014), one of the contributors to the presence of steroid estrogen hormones in the environment is the sewage treatment plant. Barel-Cohen et al. (2006) observed that the concentration of EE2 may be reduced by half at 25 km away from the sewage effluent source but can still be detected up to 100 km along the river.

Steroid estrogens concentrations in surface water and sediment in this study were compared to those reported in previous studies throughout the world (Table 4). Steroid estrogen concentrations in surface water of the Langat River were found to be relatively low as compared with the surface waters of China, Turkey, Spain, Italy, Brazil, and Japan. In river sediment from the present study, steroid estrogen was relatively low compared with the river sediments of China, Japan, Australia, and Spain. The present levels of steroid estrogens in surface water and sediment of the Songhua River (China) originated from municipal wastewater; these steroid estrogens were supersaturated in sediments related to the river (Zhang et al. 2014). Similarly, steroid estrogen

Table 3 Mean concentration of steroid estrogens in the Langat River sediment

Compound	E1	E2	E3	EE2
Water (ng/L)				
SP1	ND	ND	0.003	ND
SP2	<LOD	ND	<LOD	0.003
SP3	ND	ND	ND	0.004
SP4	ND	ND	ND	ND
SP5	<LOD	0.004	0.003	0.002
SP6	ND	ND	ND	0.005
SP7	<LOD	0.003	0.002	ND
SP8	ND	ND	ND	ND
Range	ND–<LOD	ND–0.004	ND–0.003	ND–0.005
Sediment (ng/g)				
SP1	0.029	0.0189	0.011	ND
SP2	0.035	0.0251	0.010	ND
SP3	0.002	0.0053	0.014	ND
SP4	0.0160	0.0159	0.014	ND
SP5	0.0061	0.0096	ND	ND
SP6	0.0072	0.0178	ND	3.29E−4
SP7	0.0035	0.0113	0.024	ND
SP8	0.0091	0.0281	ND	ND
Range	0.002–0.035	0.0053–0.0281	ND–0.024	ND–3.29E−4

concentrations were higher in the northern part of Tokyo Bay, which is related to huge quantities of pollutants from adjacent rivers that contain a large amount of municipal and industrial wastewater (Isobe et al. 2006). Although the steroid estrogen concentrations in the Langat River surface water

Table 4 Comparison of steroid estrogens concentrations in various rivers in the world

Location	E1	E2	E3	EE2	Reference
Water (ng/L)					
Langat River	ND–<0.002	ND–0.004	ND–0.003	ND–0.005	This study
Songhua river (China)	0.84–17.8	ND	ND–8.08	ND–24.4	Zhang et al. (2014)
Gediz river (Turkey)	7.53	0.10	7.33	ND	Aftafa et al., (2014)
Tiber river (Italy)	5–12	2–6	2–5	ND–1	Laganà et al. (2004)
Iberian river (Spain)	ND–7.3	ND–7.8	ND–5.7	ND–2.2	Gorga et al. (2014)
Rivers in Brazil	BD–39	ND–7.3	ND–2.3	ND–25	Sodré et al., (2010)
Tokyo Bay, Japan	1.6	ND	ND	38	Isobe et al. (2006)
Sediment (ng/g)					
Langat River	0.002–0.035	0.0053–0.0281	ND–0.024	ND–3.29E−4	This study
Songhua river (China)	0.5	0.11	ND	ND	Zhang et al. (2014)
Tokyo Bay, Japan	0.05–3.6	ND–0.59	ND	ND	Isobe et al. (2006)
Malabar ocean, Australia	0.16–1.17	0.22–2.48	–	ND–0.5	Braga et al., (2005)
Anoia and Cardener rivers (Spain)	ND–11.9	ND	ND–3.37	ND–22.8	López de Alda et al. (2002)

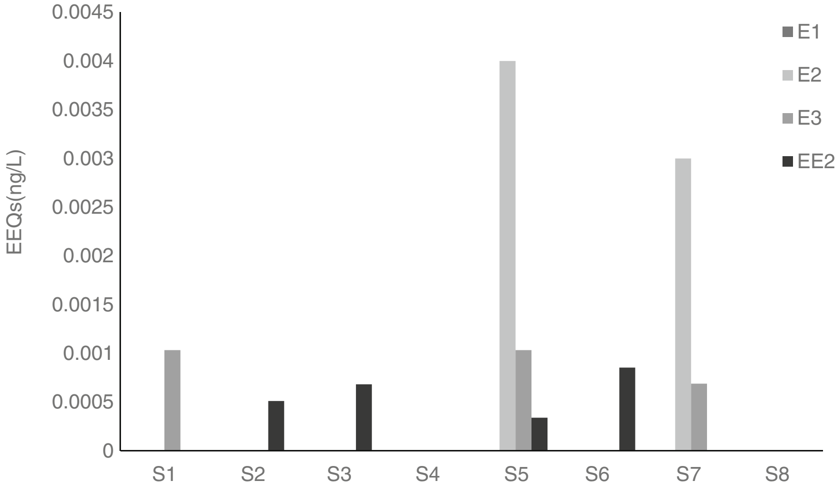

Fig. 2 Potential estrogenic activity (EEQ) for surface water of the Langat River

and sediment were relatively low compared with the results of other studies elsewhere, the estrogenic activities should be analyzed.

Knowledge on estrogenic activities will allow the proper interpretation of potential threats to aquatic environments. The Langat River is continuously exposed to micropollutants from sewage effluent of public and private sewage treatment plants, individual septic tanks, and sewage from squatters' areas (Al-Odaini et al. 2013). Steroid estrogen does not have established drinking water standards or guidelines to date. Thus, the risks associated with drinking water intake remain unclear.

Estrogenic activities

In addition to chemical analysis, the estrogenic activity in surface water and sediment of Langat River were also analyzed. Information on steroid estrogen concentrations based on chemical analysis is limited. Thus, we cannot provide a clear picture of the potential risks to the aquatic environment. According to Gorga et al. (2014), each steroid estrogen possesses different estrogenic potencies in an aquatic environment. The effects of mixtures and the persistence of these estrogenically active substances should be considered (Esteban et al. 2014). The EEQ level is set at 1. Potential risks to the aquatic environment are present if the EEQ values exceed 1. Conversely, EEQ values lower than 1 indicate the absence of any potential risks to the aquatic environment. From Figs. 2 and 3, the EEQ values for surface water and sediment were below 1, thereby indicating the absence of potential risks to the aquatic environment.

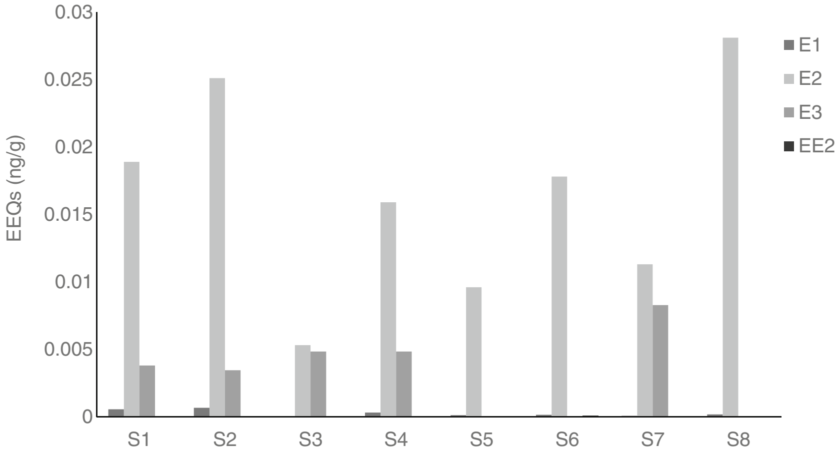

Fig. 3 Potential estrogenic activity (EEQ) for surface sediment of the Langat River

Therefore, none of the sampling locations of the river represent a potential estrogenic risk to the aquatic environment.

Conclusion

This study is the first to analyze the steroid estrogen concentrations and estrogenic activities in the surface water and sediment of the Langat River (Malaysia). Natural and synthetic estrogens, namely, estrone (E1), 17ß-estradiol (E2), estriol (E3), and 17α-ethynyl estradiol (EE2), were found in the surface water and sediment. These findings indicate that the continuous output of estrogens from sewage treatment plants, farming waste, and agricultural activities are possible sources of steroid estrogens. However, given their low concentrations observed in this study, their potential estrogenic risk to aquatic organisms is low in the Langat River. Yet, the present detection of steroid estrogens in the Langat River strongly suggests that it may be of interest to proceed to representative sampling of other potential endocrine-disrupting environmental pollutants. In particular, the presence of alkylphenolic compounds of category 1 in the endocrine disrupter priority list, which have clear endocrine-disrupting effects in humans and wildlife, ought to be monitored. Since these only represent a small proportion of the 194 listed category 1 endocrine disrupters, which are given highest priorities, further similar studies along the Langat River are strongly recommended.

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