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AN OPTIMIZED ANALYTICAL METHOD TO STUDY THE OCCURRENCE AND DISTRIBUTION OF BISPHENOL A (BPA) AND 17ß-ESTRADIOL (E2) IN THE SURFACE WATER OF IBAI RIVER, TERENGGANU, MALAYSIA

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Abstract: Endocrine disrupting compounds (EDCs) are currently a global issue and have been ubiquitously found in various environmental compartment particularly in surface water. An analytical method employing a solid phase extraction in combination with HPLC-DAD was optimized for analysis of EDCs in surface river water. The optimized method showed a high mean extraction recovery for BPA at 73.84% while satisfactory recovery was achieved for E2 at 35.78%. It was then applied to analyse the level of BPA and E2 in the surface water of Ibai River, Terengganu. BPA and E2 were detected in this river with the concentration for both compounds ranging from 0.54 µg/L to 50.10 µg/L and 1.14 µg/L to 8.64 µg/L, respectively. Domestic waste discharge and animal manure might contribute to the presence of BPA and E2 in this river system as both compounds exhibited higher concentrations at sampling locations near those sources.

Keywords: Endocrine Disrupting Compounds (EDCs), 17ß-estradiol (E2), bisphenol A, Solid Phase Extraction, HPLC–DAD.

Introduction

The World Health Organization (WHO) defines endocrine disrupting compound (EDC) as an exogenous agent or a combination that changes the function of the endocrine system and subsequently triggers harmful health effects in an entire organism or subpopulation (Wooding *et al.,* 2017). EDCs such as phenolic EDCs have attracted substantial attention for their ability to disrupt the endocrine systems of living organisms, including humans. EDCs have been found in various environmental substances such as surface and underground water, soil and sediment as well as biota (Santhi *et al.,* 2012; Omar *et al.,* 2013; 2016).

The presence of EDCs in the aquatic environment has drawn considerable interest from environmental researchers due to their harmful effects on the reproductive function of aquatic and human population by disrupting the endogenous substances (Wee & Aris, 2017; Luo *et al.,* 2019). In addition, due to the various uses of EDCs in livestock farming, aquaculture and agriculture, as well as modern medical applications, it is increasingly possible for EDCs to enter the aquatic environment through various routes such as release during manufacturing, leaching from landfills, direct discharge through pesticide application and wastewater treatment plants (WWTP) (Liu *et al.,* 2017; Bertoldi *et al.,* 2019).

Bisphenol A (BPA) is one of the essential organic chemicals commonly used in plastic products as an additive agent and another form of EDCs is food contact materials. It can significantly interfere with the endocrine and fertility of humans and other species. BPA is widely found at significant levels in the environment, which can present potential risks of adverse ecological effects. The quantitative identification of BPA in water samples is therefore, important to ensure that water for human use is safe from pollution (Zhao *et al.,* 2019).

Several reproductive EDCs that affect humans and environment are derived from natural estrogens such as estrone (E1), 17ß-estradiol (E2), estriol (E3) and synthetic estrogenic compounds such as 17α-ethynylestrodiol (EE2). 17β-estradiol (E2) for instance is one of the primary estrogens found in animal discharge and is associated with the preservation of sexual characteristics and animal reproductive systems. E2 is excreted primarily by urine and faeces, with the excretions varying in age, sex, fitness as well as physiological and developmental status (Liu *et al.,* 2018).

Concerns about phenolic EDCs in aquatic ecosystems have resulted in an increasing number of worldwide studies (Omar *et al.,* 2016; Saeed *et al.,* 2017; Jusoh *et al.,* 2019). Such compounds can be adsorbed by aquatic organisms upon entering the water system and create a serious threat to the ecosystem. In addition, a lot of studies on the nature, fate and distribution of these environmental pollutants in the aquatic environment have been carried out in recent years (Liu *et al.,* 2017).

In this study, an optimized analytical method was applied to determine the level of EDCs in the surface water of Ibai River, Terengganu. This river flows through numerous areas of socio-economic activity, such as rubber and oil palm plantations, forestry, commercial manufacturing, aquaculture, urban and rural communities, and forest reserves. The selected EDCs were characterized by Solid Phase Extraction accompanied by High Performance Liquid Chromatography (HPLC), attached with a Diode Array Detector (SPE – HPLC – DAD). This method allowed the selected compounds to be determined and quantified at trace levels in the collected surface river water.

Materials and Methods

Sampling Location

Ibai River is one of the river basins in the Kuala Terengganu district. This river basin occupies approximately 94 km^2 of catchment areas. The basin consists of the Ibai River as the main river and its tributaries such as the Pak Su Man river, the Serai river, the Udang river, the Laca river and the Crocodile river. Previously, most of the economic activity in the basin consisted of agricultural operations situated

Table 1: Coordinate and description of sampling location

No.	Abbreviation	Coordinate	Description
1	S1	5˚17’05” N 103˚10’22” E	Near lagoon Kuala Ibai
2	S2	5˚16’57” N 103˚10’21” E	Near lagoon Kuala Ibai
3	S3	5˚16’57” N 103˚10’15” E	Across Tengku Zaharah Mosque
4	S4	5˚17’04” N 103˚10’15” E	Waste from Tengku Zaharah Mosque
5	S5	5˚16’46” N 103˚10’08” E	Under bridge
6	S6	5˚16’38” N 103˚10’00” E	Near Noor Arfa Batik Craft
7	S7	5˚16’39” N 103˚09’52” E	Fish aquaculture area
8	S8	5˚16’40” N 103˚09’53” E	Fish aquaculture area
9	S9	5˚16’37” N 103˚09’20” E	Under bridge, unpleasant odour
10	S10	5˚16’25” N 103˚08’47” E	Domestic waste
11	S11	5˚16’50” N 103˚08’27” E	Near Pusat Sains Kuala Terengganu
12	S12	5˚16’26” N 103˚07’46” E	Near construction area
13	S13	5˚16’11” N 103˚07’35” E	Domestic waste
14	S14	5˚15’29” N 103˚07’07” E	Fishing area
15	S15	5˚15’55” N 103˚07’28” E	Cattle breeding area

in the upper reaches of the river system, other operations are municipal and settlements that are more concentrated in the region upstream to downstream (Laila *et al.,* 2018). Figure 1 shows a map of Ibai River, Terengganu, Malaysia and sampling locations denoted by S1 to S15 while Table 1 shows the description of the sampling site.

Chemicals, Standards and Sample Collection

Methanol (MeOH) and acetonitrile (ACN) were acquired from Fisher Scientific (Loughborough, UK). The ultrapure water was generated from Milli-Q water purification system (Millipore, USA). Standard solutions were prepared in MeOH under exact volume dilution from a stock solution. All standard solutions were prepared fresh and stored in glass-stoppered volumetric flasks at 4°C.

Sampling was carried out on 9 October 2019, covering fifteen sampling points along the Ibai River system. The van Dorn sampler was employed to collect surface water samples. Samples were then transferred into 500 mL ambient glass bottles which were previously cleaned with MeOH and placed in a cool box at ± 4 - 6°C. Physico-chemical parameters of water samples such as salinity, dissolved oxygen (DO), temperature and pH were determined onsite using YSI multiparameter probe while turbidity was measured using portable turbidimeter. The samples were instantly filtered through a 47 mm (1.7 µm pore size) glass microfibre filter upon arrival at the laboratory. Preservation of samples was accomplished by modifying the filtrates to pH 2 using 1 M of hydrochloric acid and stored in a freezer before extraction and clean-up.

Extraction and Sample Analysis

Solid phase extraction was carried out in a series of vacuum manifolds procured from Phenomenex (USA). The extraction procedure was carried out according to Omar *et al.* (2019). Two hours before extraction, approximately 100 mg of tetrasodium-ethylenediaminetetraacetate-dehydrate (Na^2-EDTA) was added into samples. The cartridges were preconditioned with 5 mL of methanol followed by 5 mL of ultrapure water and 5 mL of ultrapure water (pH=2). Elution was completed by adding 10 mL of methanol, then, 5 mL of methanol/acetone (50:50, v/v). The extracts were concentrated using a rotary evaporator and then, further concentrated with a gentle stream of nitrogen (N_2) blow until near dryness. The extracts were reconstituted with ultrapure water/ACN (70:30, v/v) to 1 mL and

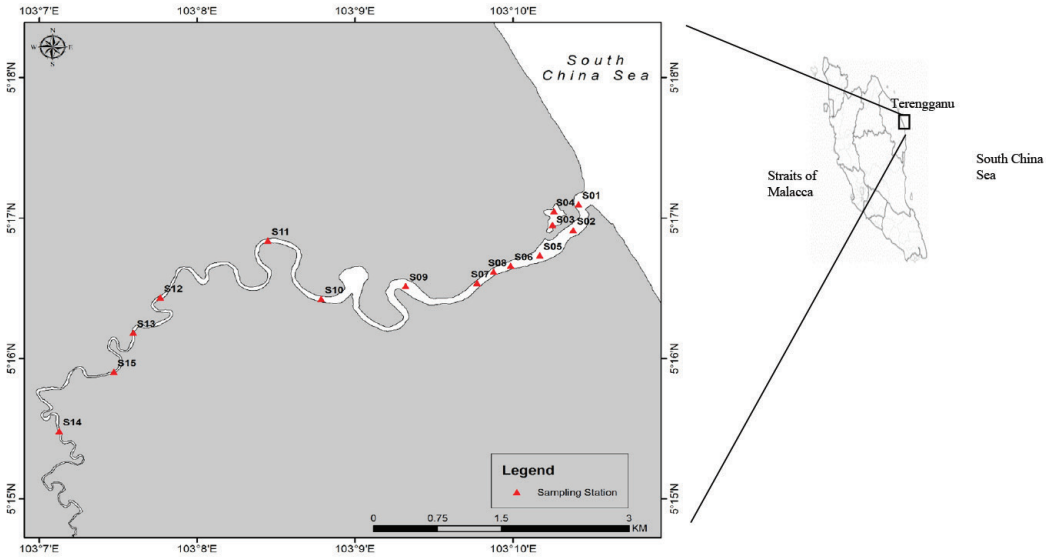

Figure 1: Map of Ibai River, Terengganu and sampling location denoted by S1-S15

filtered through 0.2 µm PTFE membrane filter before HPLC analysis.

The BPA and E2 were identified by peak area and respective retention time by different concentrations were 0.1 mg/L, 0.5 mg/L, 2.0 mg/L, 4.0 mg/L, 6.0 mg/L and 8.0 mg/L. For optimum chromatographic condition, a mixed standard was tested under mobile phase manipulation, flow rate, wavelength, elution, volume of injection and column temperature. The compounds were quantified and detected using HPLC (Agilent Series 1200) equipped with a Diode Array Detector (DAD). Reversed phase C18 analytical column (Supelco C18, 15 cm x 4.6 mm, 5 µm) was chosen with the chromatographic condition developed and optimized. Flow rate of mobile phase was set at 0.8 mL/min with an injection volume of 20 µL and a total run time of 10 minutes. BPA and E2 were confirmed by the respective retention time of the individual standard and quantified by the external standard calibration.

The percentage of extraction recoveries for BPA and E2 were evaluated to assess the extraction and clean-up efficiency. In this study, the artificial seawater was prepared for the purpose of evaluating the extraction recovery of analytical methods by dissolving Instant Ocean Sea salt into ultrapure water with a salinity adjusted to 25 parts per trillion. The artificial seawater was used to spike 20 µg/L of mix standard of selected EDCs for recovery analysis and quality control spike during the analysis of samples.

Results and Discussion

The optimization of several parameters was carried out to develop the analytical method for evaluating BPA and E2 in the surface water samples. The composition of mobile phase, which was 40% deionized water (A) and 60% MeOH: ACN (B) was evaluated for isocratic elution. Isocratic elution was used for the better separation of both BPA and E2. Different wavelengths of 210, 230, 254 and 280 nanometres (nm) were subsequently evaluated. Through consideration of the higher signal intensities and symmetries as illustrated in Figure 2, a wavelength of 210 nm was selected as optimal to separate multi-residue EDCs. Identification of peaks was done by using a chromatogram of the standard solution. The respective retention time was identified for BPA and E2.

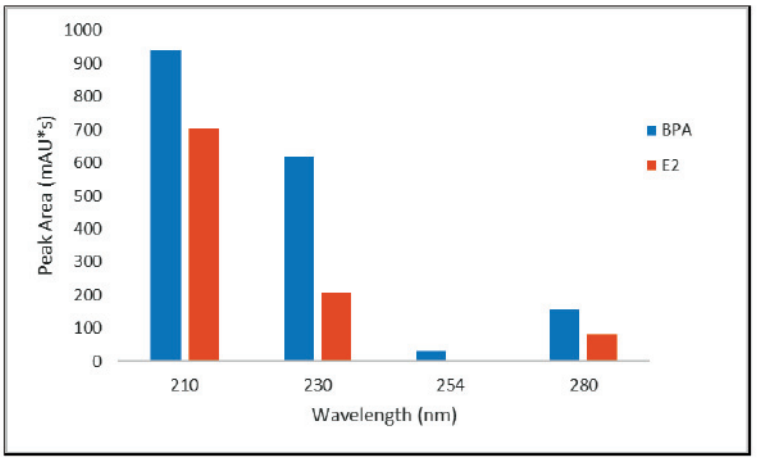

Figure 2: Peak areas of Bisphenol A (BPA) and 17β-estradiol (E2) at different wavelength

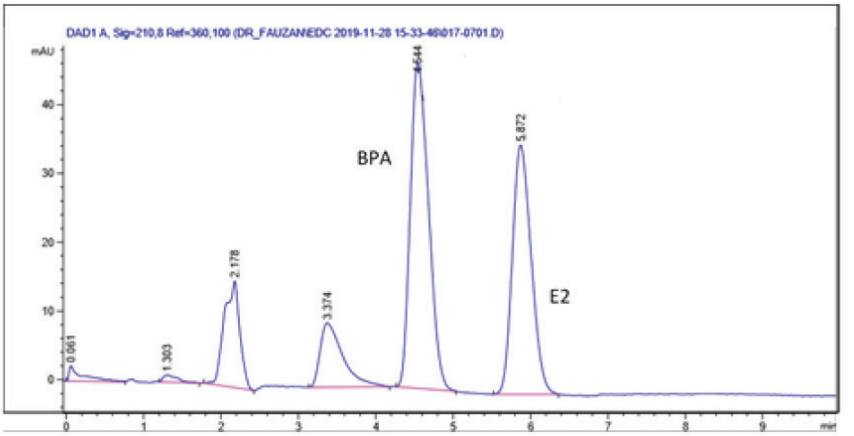

Figure 3: Chromatographic separation for Bisphenol A and 17β-estradiol in a single analytical run

The rate of solvent elution has also been measured. In the first attempt, the extracts were considered elucidating in a lower amount of elution of 3 mL, followed by 10 mL elution volume. A comparison of the recovery rates of 10 mL and 3 mL elution of the SPE method reveals that MeOH has shown improved recovery by higher elution volume. Consequently, the optimal amount of solvent elution was chosen as 10 ml of methanol elution. Instead of achieving an optimal extraction owing to sorbent retention and a comparatively low elution rate, the larger volume can be subject to major problems, which can lead to a substantial loss of sample. To avoid overloading and clogging of cartridges, the sample size (100 mL) was chosen to ensure that the cartridges are not overloaded and thus, performance is robust.

In order to validate the method of recovery, a mixed BPA and E2 standard was spiked into artificial seawater before SPE extraction. The percentage of recoveries was calculated using Equation 1.

$$\% \text{ Recovery} = \frac{Area\ of\ analyte\ in\ spike\ matrix}{Area\ of\ analyte\ in\ reference\ matrix} \times 100 \quad (1)$$

This study achieved recovery rates of 73.84% and 35.78% of BPA and E2 in 100 mL artificial seawater, respectively. E2 exhibited a satisfactory method recovery. The lower recovery of E2 is due to the limitations in multi-residue analysis in a single analytical run, optimization of chromatographic conditions, as well as recovery for each of the compounds. In the case of determining several residues, there is also a limitation in calculating the recovery rate in a single analytical run (Wee *et al.,* 2016, Ismail *et al.,* 2019). The calibration curve for each residue was constructed for HPLC optimization at six concentrations of mixed standards, comprising 0.1 mg/L, 0.5 mg/L, 2.0 mg/L, 4.0 mg/L, 6.0 mg/L and 8.0 mg/L. These curves demonstrated excellent linearity for BPA and E2 with r values at 0.998 and 0.9982, respectively.

Malaysian Marine Water Quality Criteria and Standards (MMWQCS) and the Malaysia National Water Quality Standards (NWQS) were used to compare the physico-chemical parameters of water determined in this study.

Based on in-situ determination, the pH value (6.4 – 8.1) was still in the range set by NWQS (pH range = 5-9) and MMWCQS (n.a). Dissolved oxygen (0.22 – 5.48 mg/L) of the surface water of Ibai River was found to be slightly higher than the MWQCS' (4 mg/L) and NWQS (3-5 mg/L). The present monitoring results showed that the industrial and domestic wastes as well as human activities might influence the physicochemical parameters of water samples within this sampling area. Physicochemical parameters are a good indicator of water quality, as well as an excellent attribute of human impact on environments. Based on NWQS, the level of most of the parameters measured remained at Class III, which means extensive treatment required for common economic use and is tolerant of species but suitable for livestock drinking.

Table 2 shows the concentration of BPA and E2 in the surface water of Ibai River while Figure 4 shows the distribution pattern of BPA and E2 along this river ecosystem. Both compounds were detected in the surface water collected with the concentration of BPA ranging from 0.54 µg/L to 50.10 µg/L while E2 was found in the range of < 0.75 µg/L to 8.64 µg/L. The level of BPA was found at higher concentration than E2 at most of the sampling stations and it was observed that BPA was not present in the upper reaches of the river (S14 and S15). The highest concentration of BPA was detected at S10 while the highest concentration of E2 was observed at S4. Based on site description, domestic waste discharge contributed to the presence of BPA and E2 in this river ecosystem, as both compounds exhibited high concentration at those sites. E2 was also found at a higher concentration at the sampling site near the cattle breeding area, suggesting that animal manure might also contribute to the presence of this compound in this river ecosystem. The presence of BPA and E2 in this river system also indicated similar trend of concentration as described by Santhi *et al.* (2012) in Selangor River, Omar *et al.* (2019) in Klang River and Shehab *et al.* (2020). On the other hand, comparison with other EDCs showed that pharmaceutical compounds, namely caffeine were also detected in the surface water of Ibai River. A study by Khalik *et al.* (2020)

Table 2: Concentration of BPA and E2 in surface water of Ibai River

	Concentration, µg/L ± %RSD					
Sampling Point	**BPA**			**E2**		
S1	10.5	±	2.60	7.60	±	1.16
S2	12.80	±	5.10	6.18	±	3.15
S3	6.42	±	3.10	7.12	±	1.65
S4	7.05	±	3.40	8.64	±	4.04
S5	5.70	±	2.20	1.45	±	2.46
S6	30.45	±	6.30	1.14	±	0.56
S7	8.30	±	4.32	6.14	±	0.45
S8	7.76	±	1.63	5.50	±	0.86
S9	11.30	±	1.55		< 0.75	
S10	50.10	±	5.50	7.12	±	3.46
S11	1.54	±	3.82		< 0.75	
S12	0.54	±	4.46		< 0.75	
S13	47.25	±	1.12	5.32	±	2.13
S14		< 0.28			< 0.75	
S15		< 0.28		8.44	±	1.65

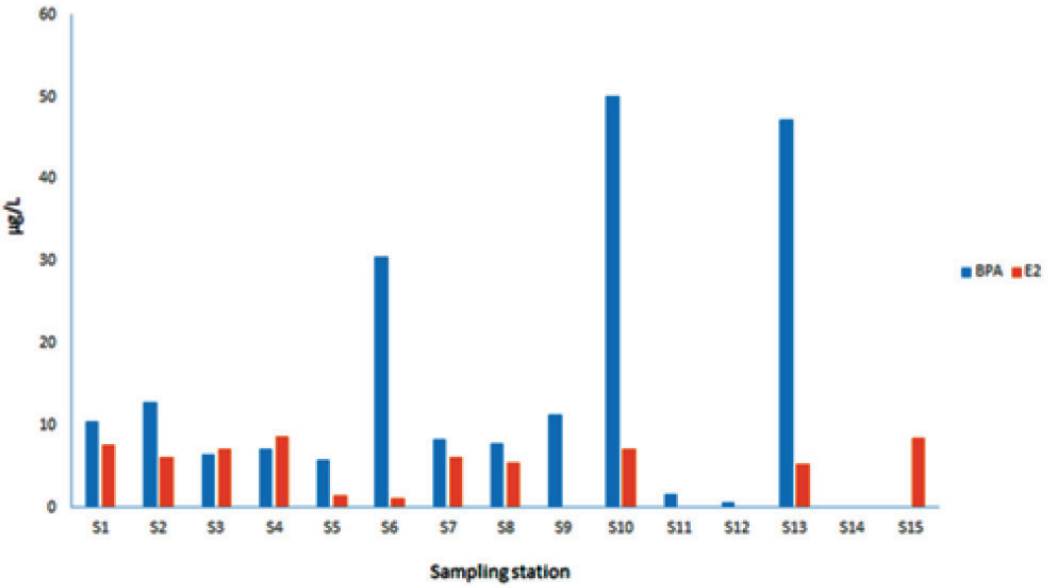

Figure 4: Distribution pattern of BPA and E2 along Ibai River

reported concentration of caffeine in the range of 391 – 415 ng/L at five sampling points along this river.

Conclusion

This study was conducted to assess the occurrence and distribution of EDCs, specifically BPA and E2 contamination, in the Ibai River ecosystem. The results from the optimized analytical method can be used to determine the level of multiclass EDCs in the surface water of this river. The optimized method showed a high mean extraction recovery for Bisphenol A (BPA) at 73.84% and satisfactory recovery for 17ß-estradiol (E2) at 35.78%. The lower recovery of E2 is due to limitations on multi-residue analysis in a single analytical run, optimization of chromatographic conditions, and recoveries for each of the compounds. Sampling was conducted along this river with various physicochemical parameters of water samples such as pH, temperature, salinity, dissolved oxygen and turbidity were determined on site. BPA and E2 were detected in this river with the concentration for both of compounds ranging from 0.54 µg/L to 50.10 µg/L and 1.14 µg/L to 8.64 µg/L, respectively. Domestic waste discharge and animal manure might contribute to the presence of BPA and E2 in this river ecosystem, as both compounds exhibited higher concentration at sampling locations near these sources. Therefore, a comprehensive study should be carried out to evaluate further the potential sources of BPA and E2 in this river. At this point in time, the present study provides a baseline assessment for understanding the pollution profile and distribution of EDCs in the surface river water of Ibai River.

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