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# Sex Steroid Hormone in Recreational Beach Case Study

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Birth control pill is one of synthetic steroid hormones are believed to cause higher transgender case in the environment today, thus to affect more cancer risk to human populations. Beaches are the last extent for steroid hormones residue that can be reached by waste or surface water. The research is significant to develop a database and standard allowable limits for the non-traditional contaminants. Sex steroid hormones are known as endocrine disruptor compounds (EDC) a substance that can interrupt the reproductive system of human and normal hormonal signaling pathways that capable of affecting both the environment and human health even at a very low concentration. Hence, this research was focused on the determination of sex steroid hormone particularly on estradiol in Tanjung Aru Recreational Beach, Sabah. For this study, the steroid sex hormone was extracted using dispersive liquid-liquid micro extraction (DLLME-SFO) methodology with solidification of floating organic drop and injected into High-Performance Liquid Chromatography (HPLC) for detection. Based on the result obtained it is found that there were few detections of steroid sex hormone occurrences and this is alarming although in nanogram per milliliter in environment as it will bring and posed a life threat towards the living organism in the environment.

**Keywords:** Endocrine disrupting compounds (EDCs), estradiol, sex hormone, steroid, recreational beach

## I. INTRODUCTION

Beach is the medium where the freshwater blends with the ocean water in that coastal zone. Coastal area provides various recreational opportunities such as snorkeling, diving, swimming, kayaking and boating. However, coastal waters could be polluted through direct and indirect harmful pollutants discharging from inshore or upstream activities (Makin & Gower 2010). Recently, discharge of anthropogenic chemicals into the environment mostly is from endocrine disruptor compounds (EDCs) (Vandenberg *et al.*, 2010) which has been a major concern for the last decade (Hohenblum *et al.*, 2004). EDC is known as a substance that can interrupt the reproductive system of human and normal hormonal

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signaling pathways in animals (Lee *et al.*, 2013) which causes abnormal reproduction, stimulation of cancer growth, dysfunction of neuronal and immune system as it interrupts the actions of endogenous hormones (Hohenblum *et al.*, 2004). Deformation of sex organs is one of the significant harmful effects of EDCs which have been discovered in marine gastropods, fish and birds (An *et al.*, 2006). Nevertheless, it can affect both the environment and human health at very low concentration (Melnick *et al.*, 2002).

In Malaysia, there is no research done in determining steroid hormones in coastal waters. Incomplete elimination of the steroid hormones in wastewater treatment plants also could lead to surface waters pollution (Liu *et al.*, 2009) especially coastal waters as it is the end of where the pollutants accumulated. This was proven by a research on seawater samples in coastal area of Singapore reported that there was presence of sex hormone activities which was then further the research on the sex hormone activities in the green mussel, *Perna viridis*, in coastal waters of Singapore (Barel-Cohen *et al.*, 2006). It indicates that green mussel is in danger condition because estrogenic chemicals can disrupt the marine wildlife endocrine systems in which domestic seafood has high possibility to be affected (Koh *et al.*, 2005; Aoki *et al.*, 2010). This explain why EDC has become a big concern to human as human is the final consumer in the trophic level in the process of biomagnifications. The accumulation of estrogenic toxicity starting from lower trophic levels to the highest trophic levels may cause more poisoning effect to larger species (Cailleaud *et al.*, 2011). Hence, the aim of this research is to determine the occurrences of the sex steroid hormone in Tanjung Aru Recreational Beach, Sabah.

## II. MATERIALS AND METHODS

The research area chosen is along Tanjung Aru Recreational Beach, Kota Kinabalu, Sabah. The beach is located at the North Borneo and at the East of Malaysia. Figure 1 shows the chosen area of the research area while Table 1 lists the coordinate of each sampling station. The water sample was obtained through grab sample or spot samples and taken along the coastline of Tanjung Aru Beach with standardized depth of 20 to 30 cm from the water surface. The recreational beach coastline has been seen under an intensive development over the last few decades where the recreation park, hotel, hawker stalls, public toilets and beachside cafes are located. As there are a number of people particularly tourists located in this area, public toilets are often in use (Hamzah et al. 2011). Based on the Environmental Impact Assessment (EIA) of The Proposed Kota Kinabalu Beach Resort Sdn. Bhd. at Tanjung Aru, Kota Kinabalu (Forever Sabah 2014), there were three major drains discharging wastewater effluent directly into the South China Sea especially when there is high tide. This indicates that wastes are flowed into the sea from public toilets and from other sources. There were 5 locations chosen along the study

area as shown in Table 1. In-situ parameters involved in this research were pH, temperature, dissolved oxygen and turbidity. The equipment used was pH meter (Digimed DM-2; pH range 0 to 14 with 0.01 pH resolution on lowest range) and turbidimeter (HACH 2100P; 0 to 1000 NTU with 0.01 NTU resolution on lowest range) for pH and turbidity analysis respectively. For both the measurement of temperature and dissolved oxygen, dissolved oxygen (DO) meter was used (Model 1056; temperature range 0.0 to 100°C; dissolved oxygen range 0 to 50 mg/L with 0.01 mg/L resolution).

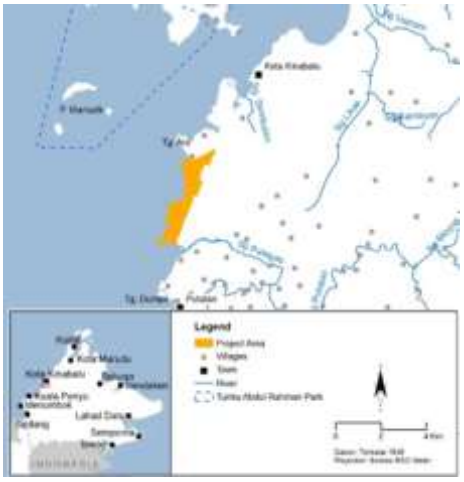


Figure 1. Danish Hydraulic Institute 2014

Table 1. Details of sampling locations

| Location | Site Description | Latitude | Longitude |
|---|---|---|---|
| 1 | Major drain was seen discharging effluent into the South China Sea | N 05° 56" 872' | E 116° 02" 562' |
| 2 | Dead fishes were seen floating on the shore | N 05° 56" 897' | E 116° 02" 550' |
| 3 | Dead fish sank on the sediment of the shore | N 05° 56" 921' | E 116° 02" 545' |
| 4 | Surroundings had rubbish | N 05° 56" 950' | E 116° 02" 538' |
| 5 | Situated near the public toilets of the beach | N 05° 56" 996' | E 116° 02" 517' |

Before the analysis of steroid sex hormone, standard stock solutions will be prepared at concentration of 2,000,000 ng/mL in acetonitrile. The solutions are to be diluted to the suitable concentration by using acetonitrile and maintained at temperature of 4 °C. Extraction of steroid hormone was carried out using the dispersive liquid-liquid micro extraction based on solidification of floating organic drop (DLLME-SFO) (Payus *et al.*, 2016). Prior to extraction of steroid hormones, a mixture which contains dispersive solvent and extraction solvent will be injected into 5 mL of water samples spiked with 50 µg/L of analytes. The dispersive solvent is methanol whereas the extraction solvent is 1-Undecanol.

All water samples with analytes undergo centrifugation at 4500 rpm for 3 minutes. Liquid organic drop will be floated on the surface of the water sample. The water sample is left to cool in ice bath for few minutes until frozen liquid organic drop is formed. The solid liquid organic drop can now be scooped from the water sample easily by using a customized scoop. The solid liquid organic drop will melt rapidly at room temperature. To separate the water and organic solvent of the scooped melted solid organic drop, the whole drop of organic solvent will be transferred to a micro tube by using a syringe. Next, the organic solvent transferred into an auto sampler vial and mixed with dimethyl sulfoxide. The mixture injected into a High-Performance Liquid Chromatography (HPLC) system for detection of steroid hormone.

The HPLC system used is Agilent Technologies 1200 series equipped with VWD (UV-DAD) detector for detection of steroid hormones. HPLC Phenyl Column (1.7 µm, 2.1 mm × 100 mm) was chosen for separation of analytes. Column temperature was maintained at 30 °C whereas the wavelength was adjusted to 280 nm which is the UV detector with fixed wavelength. The separation gradient is the acetonitrile-water mobile phase in which it is start with 5% of acetonitrile and then increase slowly to 40% after 2 minutes, followed up by a slightly increase to 50% after 4 minutes, then finally increased to 60% after 1 minutes. The flow rate was kept at 0.4 mL/min. However, flow rate was adjusted to 1.5 mL/min so that the amount of samples was obtained in short time. All the mobile phases were pre-treated by filtering through a 0.22 µm membrane of nylon filter media. Vial was used to hold the sample whereas the injection volume was set at 25 µL for each sample. All the Pyrex glassware, petri plates and forceps are sterilized by autoclaving for 15 minutes at 121°C. Extraction is to be carried out as early as possible to avoid addition of chemical preservatives. For steroid hormone analysis, standard stock solutions will be prepared in 2,000,000 ng/mL of acetonitrile for all the steroid hormones. The standard solutions will be run through the HPLC system to identify the peak and retention time.

## III. RESULTS AND DISCUSSIONS

Based on Table 2 at the above, it shows the occurrences of sex steroid hormone on 17α-ethynylestradiol in five locations along the coastline study area within the range of detection concentration, however the rest of the hormones type show no occurrences. For 17α-ethynylestradiol, the total average concentration is recorded at 301.570 ± 122.330 ng/mL. Based on the result obtained it is found that there were often detections and occurrences of 17α-ethynylestradiol in the study with detection range from lowest concentration of 2.709 ± 0.001 ng/mL and the highest concentration recorded at 265.705 ± 10.515 ng/mL. The 17α-ethynylestradiol is a synthetic estrogen that is widely used as active ingredient in birth control pills and other menopause treatment (Jones-Lepp & Stevens,

2007). Every consumption of this steroid hormone in women will resulted in excretion of 17α-ethynylestradiol into the water body such as wastewater through the renal and bowel pathway. In order to be transported out from the human and animal bodies, 17α-ethynylestradiol exists in the form of metabolites as water-soluble estrogen glucuronides so that they can be excreted in the form of urine while some in feces which exists in the form of glucuronide and sulfate conjugates (Khanal *et al.*, 2006). As a result, these glucuronides will eventually discharge and deposit in the sewage system. However, in the sewage treatment plant, stability of 17α-ethynylestradiol maintained relatively strong when undergoing activated sludge process, hence, preventing itself from breaking down and eliminated from sewage treatment plant resulting incomplete treatment (Zuo *et al.*, 2006; Forrez *et al.*, 2009). On the other hand, prior to bacterial modification, 17α-ethynylestradiol may also be activated to its free form in the sewage treatment plant (Al-Ansari *et al.*, 2010; Atkinson *et al.*, 2012) after been digested by bacterial as food and then it will bond again to other organic or colloids to form flocculation.

Table 2.Concentration of 17α-ethynylestradiol for each of the stations

| Sampling Location | 17α-ethynylestradiol (ng/mL) Concentration Mean | Standard Deviation |
|---|---|---|
| 1 | 2.714 | ±0.005 |
| 2 | 2.709 | ±0.001 |
| 3 | 161.213 | ±274.527 |
| 4 | No Detection | No Detection |
| 5 | 265.705 | ±10.515 |
| Total Average Concentration | 301.570 | ± 122.330 |

Previous studies showed that there was presence of 17α-ethynylestradiol not only in wastewater treatment plant but also in surface water (Desbrow *et al.*, 1998; Kuch & Ballschmiter, 2001). In this study, sewage outfall could be the most probable factor that caused the occurrence of 17α-ethynylestradiol in coastal water as industrial sites were not present in the area. Hence, from the sewage system, the 17α-ethynylestradiol glucuronides were carried from the domestic wastewater along to the wastewater treatment plant. Based on the location of Tanjung Aru Beach, there were many residential houses, primary school and even public toilet near the beach. It was found that human urine is most probably the major source of 17α-ethynylestradiol (Vethaak *et al.*, 2005; Pauwels 2008).

Thus, 17α-ethynylestradiol detection in surface water is a significant sign of contamination by domestic sewage discharges, since coastal water is the end point of pollutants where most of the pollution occurs at the inlands and flow out to the sea. In addition, even 100 km away from the sewage effluent source, the 17α-ethynylestradiol occurrence can still be detected in this study which can be alarming for human and environment safety.

For the no detection of 17α-ethynylestradiol at Location 4, it might be the cause of photodegradation factor in promoting

elimination of 17α-ethynylestradiol in the water body (Crisp *et al.*, 1998). The rate of degradation of 17α-ethynylestradiol was faster in summer than in winter, where summer receives most of the solar radiation (Jurgens *et al.*, 2002). In addition, estrogens tend to exhibit behavior of sensitive to light with the presence of phenolic structures which can be easily degraded by the process of photolysis (Mazellier *et al.*, 2008). Other than that, based on its physical chemical properties, 17α-ethynylestradiol has the ability to sorbs organic matter and accumulates in sediments. The 17α-ethynylestradiol is more likely to be sorbed, on contact to fine bed sediments or suspended solids than the ones with high organic carbon content (Holthaus *et al.*, 2002). Hence, stations with no detection, the sediment instead might have the detection of 17α-ethynylestradiol as it tends to sorbs and accumulate in the sediments.

Table 3. Summary of the in-situ measurements of pH, temperature (°C), Turbidity (NTU), Conductivity (mS/cm) and Salinity (ppt)

| pH | Temperature (°C) | Dissolved oxygen (mg/L) | Turbidity (NTU) | Conductivity (mS/cm) | Salinity (ppt) |
|---|---|---|---|---|---|
| 6.73 ±0.26 | 28.70 ±0.29 | 4.22 ±0.21 | 14.28 ±0.45 | 11.97 ±0.03 | 10.90 ±0.00 |

The 17α-ethynylestradiol is said to be more recalcitrant in the water body because it has an ethinyl group at C-17 atom (Moschet & Hollender, 2009) as shown in Figure 2. This feature prevents the oxidation of this C-atom; hence, 17α-ethynylestradiol has greater influence on the environment for the reason of its stability in the water body (Zhang *et al.*, 2011; Li *et al.*, 2013). Unlike synthetic estrogen, natural female sex hormone is not as potent and persistent compare to synthetic (Zhang *et al.*, 2011; Li *et al.*, 2013). According to the physicochemical properties of the synthetic estrogens, it can be said that 17α-ethynylestradiol is a type of non-polar and hydrophobic organic compound with low volatility and is more biodegradation resistance (Feng *et al.*, 2010; Tanaka & Kodama, 2007). This can explain why the synthetic shows more occurrences and existence compare to natural sex hormone. Ethinyl group which is triple bond attached at C-17 atom and the strength of bond in a chemical structure increases with the increase number of bonds. Single bond < double bond < triple bond.

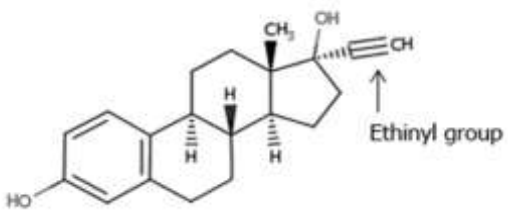


Figure 2. 17α-ethynylestradiol

(Source: Canadian Institutes of Health Research 2013)

F or total average of water quality in-situ analysis, pH, temperature, dissolved oxygen (DO), turbidity, conductivity and salinity were conducted, as shown in Table 3. -ethynylestradiol showed moderate negative relationship between pH and temperature which were r=-0.689 and r=-0.660. pH and temperature were inversely proportional to 17α-ethynylestradiol. When temperature is higher such as 37 °C, the faecal-derived enzymes existed together with steroid hormone when excreted out via the faeces were at the optimum temperature to undergo degradation on steroid hormone (Yang, 2010). The steroid hormone degraded faster at temperature 37 °C than 22 °C which is the room temperature (Yang, 2010). Based on this relationship, 17α-ethynylestradiol may encounter die-off behavior at higher temperature.

Moreover, 17α-ethynylestradiol cannot sustain in high pH condition due to the fact that steroid hormone is acidic based. A treatment was conducted using ursodeoxycholic acid on 17α-ethynylestradiol, the outcome showed that the acid does not affect the bioavailability in women consuming oral contraceptives (Lorensen *et al.*, 2005). In meantime, DO is the only parameter that had positive strong relationship with 17α-ethynylestradiol at r=0.841. However, degradation rate of increased sharply when DO is increasing (Ren *et al.*, 2016). This is due to the high yields of excited 17α-ethynylestradiol and reactive species configuration that contribute direct photo degradation to 17α-ethynylestradiol. In meantime, turbidity, conductivity and salinity were found as weak relationship with 17α-ethynylestradiol, at r==440; r=-130; and r=-0.032.

For conductivity, logically, it shows weak relationship as 17α-ethynylestradiol exists in a form of organic compound, which is steroid hormone. Hence, organic compound does not exhibit electrical charge. One of the applications of the study is to provide an indicator for hormone level detection in the water as it has been proven that water activities such as swimming and waddling at the coastal water might cause the public to consume these exogenous steroid hormones indirectly. Consequently, not only marine species will encounter the adverse effects of steroid hormones but the same goes to human as they could alter and interrupt the reproductive system of human which will cause abnormal reproduction, stimulation of cancer growth, dysfunction of neuronal and immune system as it interrupts the actions of endogenous hormones.

## IV. SUMMARY

Determination of 17α-ethynylestradiol in Tanjung Aru Beach was recorded at mean concentration of 301.570 ± 122.330 ng/mL, with detection range from lowest concentration of 2.709 ± 0.001 ng/mL and the highest concentration recorded at 265.705 ± 10.515 ng/mL. In conclusion from the result

obtained for the beach water is alarming and worrying as the steroid sex hormone 17α-ethynylestradiol is still presence and detected although in coastal water at the very end point of surface water. Future study in monitoring the occurrence of synthetic estrogen should be done more often in the future. Monitoring for estrogenic concentration or other steroid sex hormones in the environment is very much in need due to the compound itself can undergo sorption and conversion that its concentration even in trace level could affect badly towards the water ecosystem.

## V. ACKNOWLEDGMENT

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