STEROID HORMONES ASSAY ON ESTROGEN AND PROGESTERONE GROUP OCCURRENCES FATE AND PATHWAY DISTRIBUTIONS IN RIVER AND COASTAL ENVIRONMENT

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Abstract: Birth control pills are one of the synthetic steroid hormones used as pharmaceutical drugs that are believed to cause higher transgender cases in the environment today, thus causing more cancer risk, such as vaginal and breast cancer, to human populations. This research aims to detect the occurrence and quantify the concentration of three types of steroid hormones (progesterone, 17∝-ethynylestradiol and estrone) in the riverine and coastal environment. A method called dispersive liquid-liquid microextraction with solidification of floating organic drop followed by HPLC was used for hormone determination. Water samples were obtained through grab sampling along coastal and river courses. The results show a trend of hormone concentration, where progesterone had the highest occurrence, with total average of 0.11679 ± 0.08796 ng/mL, followed by 17∝-ethynylestradiol at 0.53329 ± 0.30904 ng/mL, while estrone showed the least frequency of concentration with total average of 0.02406 ± 0.00481 ng/mL.

Keywords: Steroid hormone, estrogen, progesterone, estrone, 17∝-ethynylestradiol, river, coastal.

Introduction

The occurrence of natural and synthetic steroids, especially in parts per trillion concentrations has worried researchers because these chemicals have shown to cause adverse impacts on aquatic organisms even in the very trace concentrations. The natural and synthetic discharge of sex steroids from menstruation and excretion will end up in domestic wastewater effluents, which flow into treatment plants and eventually into the lakes and rivers. The gradual buildup of these sex steroids drew wide concerns on their endocrine-disrupting effects and how they will affect the drinking water supply, since various studies have shown that they remain in wastewater even after treatment due to incomplete removal from the old conservative wastewater treatment system or from direct discharge into the watercourse (Weigel *et al.*, 2012; Seiler, 2008). Previous studies on steroid hormones and their effects on the aquatic environment were found to be lacking compared to other more common water

quality parameters studied, such as heavy metal contamination, fecal coliform contamination and nutrient contamination. On the other hand, there has been no environmental legislation that covers hormones and antibiotics in the context of environmental law up to this date, especially on developing a permissible limit or standard for this emerging endocrine disrupting compounds (EDCs) residues in the environment. Hence, the objective of this research is to determine the occurrence of steroid hormones and their pathway from waste and surface water environment and moving on to beaches and coastal environment.

The presence and occurrence of the steroid hormones are low in the environment, however, it is very unsafe and risky for humans and the ecosystem health, as the micro-constituent pollutants are capable of altering the functioning of the endocrine system in humans and wildlife by long term accumulation (Fuhrman *et al.*, 2015). It is very toxic despite in very low

concentration (ng/L), and will interfere with the production, release, transportation, metabolism process, binding, action or elimination of the natural hormones in the body (Kavlok, 2009). Steroids typically possess more strong physiological effects at very low concentrations via binding to nuclear receptors at nano- or picomolar levels and will affect the toxicity level (Segner *et al.*, 2013). In addition, the excretion of steroids and their metabolites into the environment are due to their incomplete removal in wastewater treatment plants or direct discharge into watercourses (Weigel *et al.*, 2012; Seiler, 2008). The most significant entry route for these compounds into aquatic environments are their release from wastewater treatment plants due to their conservative legacy systems (Lindqvist *et al.*, 2005). Substantial amounts of medication are usually excreted unmodified and travel via urine and feces into wastewater (Jones *et al.*, 2011), and as a result these compounds are normally detected at elevated levels in wastewater influents (Kolpin *et al.*, 2012; Sacher *et al.*, 2011). Therefore, it is necessary to develop a database that will document the occurrence of EDCs, especially for steroid hormones, in the environment and to understand the extent of this micro-pollutant transport of steroid hormones in the ecosystem, as it contributes long-term negative impacts on public health and nature.

The occurrence of steroid hormones in surface water has increased because of the release of treated or untreated sewage along the river and coastal ecosystems. Beaches or the coastal environment is the last extent point for steroid hormones discharge that can be reached by wastewater and surface water flow (Lagana *et al.*, 2008;). Steroid hormones are expected to be detected in the river and coastal areas due to sewage effluents and leakage that will bring continuous accumulation in the ecosystems (Kolpin *et al.*, 2012). Hence, this unique group of hazardous organic micro-pollutants is gradually becoming prominent as they could pose a new threat to the decline in water quality with their endocrine-disrupting effects on both humans and wildlife. Human excretion and

menstrual flow are major sources of natural steroidal estrogens found in surface waters due to the lack of proper piping and sewage that causes waste to leak from housing areas, even from illegal homes and slums. Most of this domestic waste, which contains high amounts of steroidal estrogens, flow into the rivers and seas. This could be compounded by the fact that global consumption of estrogens had increased exponentially over the last 50 years because of contraceptives, menopausal therapies, as a treatment for some human cancers and as veterinary growth promoters. Estrogens are now considered to be the most prescribed drugs in the world (Guedes-Alonso *et al.*, 2017). Steroids are among the major groups of chemicals that are categorized as endocrine disruptors (Zhang *et al*., 2013).

Natural and synthetic steroid hormones, such as estrone, estradiol and ethinyl estradiol are capable of acting as EDC in the environment. Metabolites of steroid hormones form glucuronides with specific enzymes in order to be excreted out of the human body in the form of water-soluble conjugates (Makin & Gower, 2010). Glucuronides, which have a higher solubility in water, are excreted via the kidney while some is excreted in feces. In this modern medical science era, both natural and synthetic steroid hormones are widely used as contraceptive pills, infertility treatment, menstrual and menopausal hormonal treatment, hormone replacement therapy (HRT), to treat particular cancers (Zarzycki *et al.*, 2009), growth enhancement for men and testosterone for male infertility prevention. Hence, these steroid hormones are excreted without control by humans and end up in the sewage system, and is listed as the main source of steroid hormone contamination in the environment (Liu *et al*., 2013). The research is significant in viewing analysis on the biosynthetic pathway of steroid hormone in the water system, specifically for riverine and coastal environments.

From the hypothesis of this study, natural and synthetic discharge of sex steroids from menstruation and excretion will end up in

domestic wastewater effluents, which flow into treatment plants and eventually into the lakes and rivers. Therefore, this research is significant for water security studies to develop a database for the androgens and progestogens concentrations in the biosynthetic pathway in the water system. Specifically, for riverine and coastal environments that deal with non-traditional human waste contamination markers on steroid hormones. In addition, from the baseline data obtained, it is hoped to stimulate government action on the need for a new set of laws to limit concentration of hormones and antibiotics and combat this emerging threat to the health of aquatic environment.

Materials and Methods

A method called dispersive liquid-liquid micro extraction with solidification of floating organic drop followed by HPLC was performed for hormone determination. The HPLC system used is Agilent Technologies 1200 series equipped with VWD (UV-DAD) detector for detection of the steroid hormones. The water sample obtained through grab sampling along coastal and river courses. The river and coastal waterways chosen for sampling involved the Inanam River, Papar River, Sepanggar River, Moyog River, Sembulan River and to the end point of Tanjung Aru Beach. The study areas were selected based on their topographic location that is near to the main city, with heavily dense populated areas, surrounded by residential areas as well as hotels and a large number of leaky sewage collectors, as most the properties nearby are served by old and aging septic tanks. Each sampling area includes six sites along every drainage basin, where it meets at the Likas Bay and Tanjung Aru Beach. The sites have much-restricted water flows since the discharge is too shallow along the coastline. The chosen locations span from upstream to the downstream at which the river basins converge to flow out to the sea. Apart from that, the chosen rivers also receive water from the catchment point of resources for drinking water and consumption and are also the main locations where the wastewater treatment plants discharge

their effluents. Steroid hormones were expected to be detected in the river and coastal area due to the sewerage effluent and leakage that will bring continuous accumulations in the ecosystem (Kolpin *et al.*, 2012).

For the hormone steroid analysis, Dispersive Liquid-Liquid Micro Extraction based on Solidification Floating Organic Drop (DLLME-SFO) methodology was carried out for the steroid hormone extraction (Payus *et al.*, 2016; Chang & Huang, 2010). Before the extraction of steroid hormones, a mixture that contains a dispersive solvent and extraction solvent was injected into 5 mL of water sample spiked with 50 µg/L of analytes (Standard Stock solution of 17α-ethynylestradiol). The dispersive solvent is methanol whereas the extraction solvent is 1-Undecanol. All water samples with analytes (Standard stock solution of 17α-ethynylestradiol) undergo centrifugation at 4500 rpm for 3 minutes. Liquid organic drop was floated on the surface of the water sample. The water sample was cooled in an ice bath for a few minutes until the frozen liquid organic drop was formed. The solid-liquid organic drop was then removed from the water sample using a customized scoop. The solid-liquid organic drop was melted rapidly at room temperature. In order to separate the water and organic solvent of the scooped melted solid organic drop, the whole drop of organic solvent was transferred to a microtube by using a syringe. Next, the organic solvent was transferred into an autosampler vial and mixed with dimethyl sulfoxide. The mixture was then injected into a High-Performance Liquid Chromatography (HPLC) system for the detection of steroid hormone (APHA, 2018).

Three replications for each water sample from each sampling location were involved in this study. Before the analysis of steroid hormone, standard stock solutions were prepared at a concentration of 2,000,000 ng/mL in acetonitrile. The solutions were diluted to the suitable concentration by using acetonitrile and maintained at a temperature of 4°C. HPLC Phenyl Column (1.7 µm, 2.1 mm x 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 started with 5% of acetonitrile and then increased slowly to 40% after 2 minutes, followed by a slight increase to 50% after 4 minutes, then finally increased to 60% after 1 minute. The flow rate was kept at 0.4 mL/min. all the mobile phases were pre-treated by filtering through a 0.22 μm membrane of nylon filter media. Samples were held in vials, 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 was carried out as early as possible to avoid addition of chemical preservatives. The water samples and standard solutions were run through the HPLC system to identify the peak and retention time of the steroid hormone concentrations (Payus *et al*., 2016).

Results and Discussion

The steroid hormones concentrations that managed to be detected in this research, are progesterone, 17∝-ethynylestradiol and estrone. These were measured from the samples taken from the Inanam River, Moyog River, Sepanggar River, Sembulan River, Papar River to the end point of Likas Bay and Tanjung Aru Beach. The findings of the research for the occurrences and concentrations of the steroid hormones can be summarized in Table 1, Table 2 and Table 3, according to each river basin at the studied area, with each of the steroid hormone involved in the analysis.

Table 1: Minimum value, maximum value, mean and standard deviation of the trend detection of progesterone (ng/mL) in each of the river basins at the studied area

Location	Min Value	Max value	Mean	Standard Deviation
Inanam River	0.24660	0.41054	0.32300	0.04120
Moyog River	0.08828	0.19387	0.13048	0.02085
Sepanggar River	0.02350	0.05095	0.03815	0.02384
Sembulan River	0.01262	0.04128	0.01561	0.01508
Papar River	0.02668	0.14977	0.07671	0.02606
Likas Bay		No Detection		
Tanjung Aru Beach		No Detection		

Table 2: Minimum value, maximum value, mean and standard deviation of the trend detection of 17∝-ethynylestradiol (ng/mL) in each of the river basins at the studied area

Location	Min Value	Max value	Mean	Standard Deviation
Inanam River	0.12880	0.16190	0.14694	0.01305
Moyog River	11.62375	13.19611	0.77497	0.12013
Sepanggar River		No Detection		
Sembulan River	0.22681	2.19559	0.90968	0.84139
Papar River		No Detection		
Likas Bay		No Detection		
Tanjung Aru Beach	0.25827	0.47821	0.30157	0.12233

The findings show that the average concentration of the steroid hormone progesterone at all studied river basins and coastal sites are recorded at 0.11679 ± 0.08796 ng/mL, with a range of 0.01262 ng/mL at the lowest to 0.41054 ng/mL at the highest concentration detected. The trend of detections showed the hormone progesterone had the highest occurrences compared to 17∝-ethynylestradiol and estrone.

For the hormone steroid 17∝-ethynylestradiol, total average concentrations recorded at 0.53329 ± 0.30904 ng/mL, with a range of concentration detections from the lowest of 0.12880 ng/mL to the highest concentration of 13.19611 ng/mL. But 17∝-ethynylestradiol steroid hormone was not detected in all river basins. The same situation was found for estrone: the hormone was not found in most rivers but only in Inanam River and Tanjung Aru Beach, with a total average concentration of 0.02406 ± 0.00481 ng/mL, with the lowest concentration recorded at 0.00014 ng/mL to the highest at 0.11510 ng/mL. On the other hand, the concentration of steroid hormones saw an increasing pattern from upstream to downstream of the river and shows the least concentration of trend concentration at coastal waters. This might be due to the higher or lower occurrence of steroid concentration that the analyte in a sample could not be detected by the limit detection value (LOD) and limit of quantification (LOQ) in this analysis, with a probability that the concentration of the component in the material analyzed is greater than the blank sample (Baross *et al.*, 2015). The research also showed that estrone has the least frequency of non-detection, followed by 17∝-ethynylestradiol, whereas the progesterone reported having the greatest frequency of occurrences.

Comparisons of the steroid hormone concentrations and detections with previous studies, for progesterone, 17-α ethynylestradiol and estrone found that the level of detection in this study was lower, as shown in Table 4. Liu *et al.* (2011) detected progesterone at concentrations 0.5 to 2.5 ng/L in the river which receives heavy load of effluents from a nearby WWTP, and domestic wastewater forms the majority of the effluent. Machado *et al.* (2014) also detected higher progesterone level at 60 and 450 ng/L and argued that the different profiles could be due to the discharge of a mixture of treated wastewater and naturally attenuated untreated wastewater during the study period. This has also been expected since it is a menstrual cycle-related hormone and released throughout the ovarian cycle, causing higher concentrations of this hormone to be released in wastewater (Machado *et al.*, 2014; Liu *et al.*, 2011; Chang *et al.*, 2011). For 17-α ethynylestradiol in this research, it is of utmost concern to assess its impact on to the environment, especially when significant amounts were detected in a better quality upstream water. When compared with previous studies shown in Table 4 done by De

Table 3: Minimum value, maximum value, mean and standard deviation of the trend detection of estrone (ng/mL) in each of the river basins at the studied area

Location	Min Value	Max value	Mean	Standard Deviation
Inanam River	0.06680	0.11510	0.04977	0.01123
Moyog River		No Detection		
Sepanggar River		No Detection		
Sembulan River		No Detection		
Papar River		No Detection		
Likas Bay		No Detection		
Tanjung Aru Beach	0.00014	0.00014	0.00014	0.00000

Table 4: Comparison of progesterone, 17-α ethynylestradiol and estrone concentrations (ng/mL) in this
research compared with previous studies

Reference (Literatures)	Detection Level (ng/L)	Study Location (Country)
Progesterone		
In this Research	0.01 – 0.41	Malaysia
Machado *et al.* (2014)	60 – 450	Brazil
Liu *et al.* (2012)	2.5	China
Chang *et al.* (2011)	River A 0.3 – 1.2 River B 0.1 – 0.9	China
17-α ethynylestradiol		
In this Research	0.13 – 13.20	Malaysia
De Mes *et al.* (2005)	River (Meuse) 0.2 River (Rhine) 1.2 – 4.3	Netherlands
Rao *et al.* (2013)	1.55 – 24.4	China
Lei *et al.* (2009)	River (Beitang) 1.64 – 24.4 River (Dagu) No detection – 35.6 River (Yongding New) No detection – 12.0	China
Estrone		
In this Research	0.0001 – 0.1151	Malaysia
Liu *et al.* (2011)	River (Upstream) 6.0 River (Downstream) 13.3	China
Xin Zhou *et al.* (2011)	14.0 – 180.0	China
Chen *et al.* (2010)	Creek (Wulo) Upstream 376.1 Downstream 87.7	China

Mes *et al.* 2005; Rao *et al.* 2013; Lei *et al.* 2009, the loading of 17-α ethynylestradiol pollution in the water system should spark concern and mitigation measures should be taken in order to prevent the aquatic systems and organisms from endocrine-disrupting effects caused by these estrogen mimics, as the 17-α ethynylestradiol is a synthetic steroid hormone and it is more potent and stable compared to the others that can cause more severe endocrine disrupting effects. For estrone, in this study it shows least occurrence as compared to the previous studies by Liu *et*

al. 2011; Xin Zhou *et al.* 2011; and Chen *et al.* 2010.

According to Kabir *et al.* (2015), progesterone and synthetic progestins were mostly detected when analyzed and occurred in the low ng/mL range in wastewater and surface water. It is important to note that many of the used progestins have never been analyzed and their fate in wastewater treatment and the aquatic environment is poorly known (Karl, 2015). The ovaries produce progesterone even during the follicular phase of the cycle, and is also synthetically produced for birth control pills (Peng *et al.*, 2014). According to Karl (2015), progesterone excreted from our body through urine and feces. The analysis by Payus *et al.* (2016) found that if there was a higher amount of fecal coliform then there will be fewer progesterone concentrations detected because fecal coliforms can degrade progesterone. In conclusion, the presence of progesterone is from women using contraceptive pills or through the menstrual cycle and estrous.

The non-detection concentration of progesterone could be due to the degradation of the steroid hormone. Photodegradation is an abiotic degradation pathway that takes place in natural water. Indirect photodegradation occurs when another compound absorbs light, becomes excited and reacts with the organic contaminant, while direct photodegradation occurs when organic contaminants absorb light, become excited and undergo chemical changes (Khetan & Collins, 2007). Unsaturated molecules can absorb light in the 200-700 nm range, which includes all visible light and much of the low energy UV spectrum (Vialaton & Richard, 2002). Natural water usually contains many unsaturated molecules for example, dissolved organic matter and other reactive intermediates, such as nitrate and nitrite, that absorb light in this range (Lin & Reinhard, 2005). As a result, natural water bodies have been described as "large photochemical reactor systems". Because progesterone is an unsaturated molecule, it has the potential to undergo direct and indirect photodegradation. Progesterone's biological

damage starts in transit to the sewage treatment plant (WWTP) in the sewer, as this channel is known to become an active bioreactor that contains natural biofilms with varying metabolic abilities (Jelic *et al.*, 2015). Generally, the transformation of progesterone may be caused by the process of biotic or abiotic degeneration of steroids and associated toxicity (Jasper *et al.*, 2017). The removal of organic pollutants at WWTPs is a complex process with many plausible mechanisms (Evgenidou *et al.*, 2015). The main transport route for this compound to the environment is through WWTP, where it may be only partially removed.

Detection of 17-α ethynylestradiol has always been the major concern as it has high resistance towards degradation and has high tendency to absorb organic matter, accumulate in sediment and concentrate in biota (Ahmad *et al.*, 2014). The 17α-ethynylestradiol is also known as a synthetic estrogen compound that can be found in drugs and contraceptive pills. Wastewater effluent containing estrogen compounds are discharged into the river, while the sludge is used as fertilizer. Steroid hormone 17-α ethynylestradiol is insoluble in water but will dissolve in ethanol, and can resist the biodegradation process due to its physicochemical properties as a nonpolar and hydrophobic organic compound with low volatility. It has a higher possibility to bind with the estrogen receptor too in the human body compared to estradiol (Zhuo *et al.*, 2019; Sanaz *et al.*, 2016; Ahmad *et al.*, 2014). From these findings, it shows that 17-α ethynylestradiol is a more powerful estrogenic compound in stimulating the estrogenic response compared to estradiol that can be naturally produced (Lima *et al.*, 2012). It was widely used as contraceptive pills because 17-α ethynylestradiol has a high resistance to the biodegradation process, as well as greater oral bioavailability in the human body. Therefore, it is not surprising to find 17-α ethynylestradiol detection in the river ecosystem as it was allowed to flow into the water body through domestic wastewater. A study also found that 17-α ethynylestradiol was highly

stable towards oxidation, which means it is more resistant against degradation due to the presence of the ethynyl group in the 17th position in the chemical structure (Li *et al.*, 2013). When the hormones are excreted from the human body or undergo surface runoff from agricultural activity such as from fertilizer, it will flow into the water body and some might penetrate through the groundwater system. Human waste such as urine is considered one of the main sources of 17-α ethynylestradiol. However, the hormone excretions from the human body have been metabolized to form biologically inactive and water-soluble sulfate (Andrew *et al.*, 2010).

The quantity of hormone secretion depends on the sex, hormonal status, stage of menstruation, consumption of contraceptive pills and pregnancy. Besides, waste discharged from factories producing these synthetic hormones is also the main contributor to the high hormone concentration in water sources especially estrogen compound. As a result, water bodies can contain large loads of estrogen compounds, where a small amount may be absorbed by sediments and stay for a long period. Besides, other factors could contribute to the high concentration of the hormone in the water body which includes hydrologic flow-paths such as infiltration and vertical drainage. The wide consumption of both natural and synthetic estrogens by humans has resulted in an increase of 17-α ethynylestradiol presence and availability in the environment. The increase of population in an area has led to an irregular distribution of these pollutants (Sengul, 2016). Ismail *et al.* (2014) found that even 100km away from the sewage effluent source, steroid hormones such as 17-α ethynylestradiol can still be detected. Pollutants may have been diluted when they reach coastal areas, as waters there are well mixed, which might cause scattering of 17-α ethynylestradiol glucuronides (Emnet *et al.*, 2015). Nonetheless, a low concentration of steroid hormone occurrence is still capable of affecting both the environment and human health even in trace concentrations (Barber *et al.*, 2000).

In this study, estrone was detected only in Inanam River and Tanjung Aru Beach. Generally, estrone is one of the estrogens groups that can be found widely in the aquatic environment and the most abundant natural estrogen in aquatic systems. However, it seemed that in this research estrone had the opposite fate. One of the reasons is because coastal waters are well mixed and causes scattering of estrone glucuronides (Emnet *et al.*, 2015). This can explain the low concentration of estrone that was detected and it was mostly below LOD. Humans are not the only ones that excrete large amounts of estrone in glucuronide- and sulfate-conjugated forms via urine, but also animals (Sarmah *et al.*, 2006), which normally could be discharged from the major storm drains (Ahmad *et al.*, 2014). Non-detection of estrone could also be due to the photodegradation that degraded estrone rapidly under simulated sunlight (Combalbert & Hernandez-Raquet, 2010). This is due to the fact that there is an absence of no ethinyl group in estrone which makes it appear less potent as a natural estrogen as compared to synthetic estrogen. Therefore, estrone tends to degrade faster and does not appear in the environment, which explains why there were so many non-detected concentrations of estrone in the study areas. Estrone has high photon absorption capability and will be degraded by UVC and UVA direct photolysis, in which the photon energy is absorbed by the estrogen molecule, undergoes excitation and irreversible reactions that will break down molecular bonds, thus reduced the estrone (Ma *et al.*, 2015).

In comparison of estrone and 17-α ethynylestradiol, degradation of estrone does not have strong estrogenic activity observed but 17-α ethynylestradiol is said to be more recalcitrant in the water body (Moschet & Hollender, 2009). This is because 17-α ethynylestradiol has an ethinyl group at C-17 atom as shown in Figure 1 whereas estrone does not have. This feature prevents the oxidation of this C-atom, hence, 17-α ethynylestradiol has a greater influence on the environment for the reason of its stability in the water body (Li *et al*., 2013). Unlike synthetic estrogen 17-α ethynylestradiol, the estrone

that acts as the natural female sex hormone is not as potent as the 17-α ethynylestradiol. In other words, 17-α ethynylestradiol is more persistent compared to estrone. According to the physicochemical properties of the synthetic estrogens, 17-α ethynylestradiol is a nonpolar and hydrophobic organic compound with low volatility and is more biodegradation resistant (Feng *et al.*, 2010). This can explain why 17-α ethynylestradiol in this study were recorded more than the estrone. Most of the study areas show higher concentrations of 17-α ethynylestradiol, whereas estrone was detected at only two sites. Previous studies reported similar results. Emnet *et al.* (2015), did not detect estrone in all the water samples, even in the sewage effluent, whereas 17-α ethynylestradiol was detected in all the samples. Therefore, detection of synthetic 17-α ethynylestradiol is much easier than the natural and non-synthetic steroid hormone, estrone.

Whidbey *et al.* (2012) reported that estrone photodegraded faster than 17-α ethynylestradiol, which means estrone has a shorter half-life. This is since 17-α ethynylestradiol has an ethinyl group, which is a triple-bond attached at the C-17 atom. Generally, the strength of the bond in a chemical structure increases with the increasing number of bonds. Single bond < double bond < triple bond. This also explains why 17-α ethynylestradiol is more difficult to be broken down and has a slower rate of degradation. Ma *et al.* (2015) found that the conversion sequence of estrogens was estrone > 17-α ethynylestradiol under the direct radiation of mainly UVA and UVC. It also found that estrone has a higher photon absorption capability at 0.042 cm^{-1}, which is 0.026 cm^{-1} stronger than 17-α ethynylestradiol. Moreover, natural sunlight has been shown to degrade estrogens to some extent, particularly in seawater (Xiaolin *et al.*, 2013). Based on a study done by Kabir *et al* (2015), wastewater treatment plants removed estrone and 17-α ethynylestradiol at efficiencies of 93.7% and 71.2% respectively. Hence, the occurrence of estrone in the environment will be lower as compared to 17-α ethynylestradiol. Higher distribution of 17-α ethynylestradiol in the environment than estrone can most probably be explained by the rapid use of contraceptive pills in the modern era of medical technology (Elsa *et al.*, 2014). Overall, one of the main characteristics that are associated with the use of contraceptives is the high 17-α ethynylestradiol degradation resistance and higher oral bioavailability in the human body (Gallo *et al.*, 2016). However, the drawback of this characteristic is 17-α ethynylestradiol tends to remain longer in the environment and thus cause adverse effects to the human health and ecosystem. The 17-α ethynylestradiol ranked highest in terms of estrogenic potency during in vitro tests as compared to other natural estrogens, such as E1 (Gorga *et al.*, 2012).
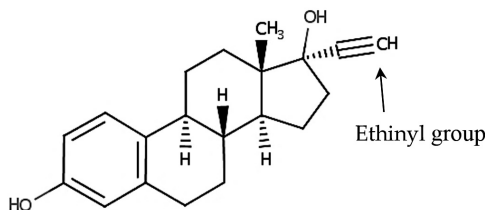

Figure 1: Chemical structure of 17α-ethynylestradiol (Source: Canadian Institutes of Health Research, 2013)

Conclusion

The average concentration of the steroid hormone progesterone at all studied riverine and coastal environments are recorded at 116.79 ± 87.96 ng/mL, with concentrations ranging from 12.62 ng/mL to 410.54 ng/mL; 17∝-ethynylestradiol at 533.29 ± 309.04 ng/mL, range of concentrations from 128.80 ng/mL to 2195.59 ng/mL; and estrone 24.06 ± 4.81 ng/mL, range from 0.14 ng/mL to 115.10 ng/mL of concentrations. These results are alarming as the steroid hormones are still present despite going through wastewater treatment and reaching the coast, which is at the very end point of surface water. Future studies in monitoring the occurrence and emergence of these hormones should be done more often in the future. Monitoring for estrogenic concentration or other steroid hormones and hazardous organic micro-pollutants in the environment is needed because these compounds can undergo sorption and conversion, and even in trace-level concentrations could badly affect the water ecosystem.

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