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QUANTIFICATION OF ESTROGENIC ENDOCRINE DISRUPTING CHEMICALS IN RIVER ESTUARY AND LAKE WATER FROM SELECT SITES IN THE WINAM GULF OF LAKE VICTORIA, KENYA

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ABSTRACT

This study investigates the presence of estrogenic endocrine disrupting chemicals (eEDCs) in waters from the Winam Gulf of Lake Victoria in Kisumu. The detection of these compounds, including natural estrogens (Estrone - E1 and 17-β estradiol - E2) and synthetic estradiol (ethynyl estradiol - EE2), highlights their significance as neglected pollutants in East Africa. The concentration levels of these eEDCs in water were measured from select river estuaries and fish landing sites competitive Enzyme-linked Immuno-Sorbent Assay. Estrone exhibited the highest concentration, while 17-β estradiol had the lowest. The concentration levels of estrone ranged 0.59-90.74 pg./ml, 17β estradiol (2.47-7.76 pg/ml) and ethinyl estradiol (0.93-15.62 pg./ml). Estrone was observed to be the highest at 115.75 pg/ml. Although the concentrations varied, no significant statistical difference was observed between river and land sites. Notably, rivers passing through densely populated areas showed higher estrogen concentrations. Seasonal variations indicated lower concentrations during wet seasons and higher concentrations during dry seasons. The study suggests that dilution effects may influence concentration levels depending on the distance from shore or river estuary. Overall, the study highlights the potential risk of estrogenic pollutants to aquatic and terrestrial life, emphasizing the need for urgent mitigation strategies to prevent adverse impacts as populations and pollution levels increase.

KEYWORDS: Estrone, 17β Estradiol, Ethynyl Estradiol, Aquatic Pollution, Lake Victoria, Neglected Pollutants.

INTRODUCTION

Estrogenic endocrine disrupting chemicals are a subset of the wide range of chemicals known as endocrine disrupting chemicals comprising natural and synthetic estrogenic steroids. Examples are estrogens (for instance, estrone, ethynylestradiol), industrial chemicals (such as bisphenol A, alkylphenol) and organochlorine pesticides such as Aldrin, Bendiocarb, Carbofuran**).**[1] Some endocrine-disrupting chemicals that have been detected in Lake Victoria waters are polychlorinated biphenyls (PCBs)[2], chlorinated pesticides[3] and polycyclic aromatic hydrocarbons.[4] However, no studies have quantified the estrogenic endocrine disrupting chemicals in Lake Victoria.

Estrogenic endocrine disrupting chemicals have been defined as exogenous chemicals or agents that interfere with the production, release, transport, metabolism, binding action or removal of endogenous hormones that are produced naturally by the body for the maintenance of homeostasis and regulation of developmental processes.[5] Adverse effects on aquatic animals, wildlife, livestock and humans have been reported to physiological alterations to the endocrine system's normal functioning.[6] Aquatic and terrestrial animals are exposed to estrogenic endocrine disrupting chemicals through dietary and water intake from a contaminated supply.[7]

It is a vital source of drinking water and food such as fish. The increasing human populations have seen rapid urbanisation, expansion of agriculture and industrialisation around the Winam Gulf of Lake Victoria. The population increase has had unintended environmental pollution of the lake through increased wastewater and sewage, finding its way into the lake.[8] Some of the chemicals are endocrine disrupting chemicals. Of interest in this study is a subclass of endocrine disrupting chemicals known as the estrogenic endocrine disrupting chemicals (eEDC) that are steroids classified as either as natural and synthetic estrogens and are excreted in the urine and faeces of humans and animals.[9]

The public health aspects of endocrine disrupting chemicals have raised global concern for the last few decades because they polluted the surface water, groundwater, soil) and food and drinking water.[10] The estrogenic endocrine disrupting chemicals have been reported as risk factors for a variety of health problems such as immune deficiencies, cancer (breast and prostate cancer in women and men, respectively), diabetes, obesity, induction of premature menopause, infertility in humans, and cardiovascular diseases[11] and this makes them a subject of interest regarding human health. However, this study's interest was a smaller class of EDCs known as the estrogenic endocrine disrupting chemicals.

Estrogenic endocrine disrupting chemicals that are produced naturally by humans and animals include estrone (E1), 17β estradiol (E2) and estriol (E3). Examples of synthetic estrogens include ethynylestradiol (EE2). The chemical structure of the three estrogens is shown in Figure 1. Estradiol is the primary estrogen produced by mammals; it is higher in females than the males.[12] Synthetic estrogens are mainly used for medical interventions such as a cure for osteoporosis and oral contraceptives.[13] These estrogens find their way into the aquatic ecosystem through treated and untreated sewage discharges and agricultural runoffs. The fate of the various estrogens differs depending on the estrogen, some persisting for longer than others. Some estrogen types are converted to other estrogen types, and others become conjugated with other metabolites.[14] For instance, 17-β estradiol is converted to estrone through the biodegradation process, and the ethinyl estradiol is the most difficult to degrade.[15]

Globally, estrogens are identified as the major contributors to the endocrine-disrupting activity observed in environmental waters.[16] The effects of estrogens on fauna have been widely reported in human, wildlife, and aquatic animals. Outcomes reported in aquatic animals include intersex, increase vitellogenin levels in male fish, and altered sexual behavior.[17] This study's objective was to determine the presence and quantify the estrogens' concentration levels in the water and determine their distribution in the Winam Gulf sites.

MATERIALS AND METHODS

Study Area and sample collection

Lake Victoria is the largest tropical freshwater lake in Africa, and second-largest globally with a surface area of about 68,800 km^2 and a maximum depth of about 70 m. The lake is shared by Kenya, Uganda and Tanzania. Seven rivers and two landing sites were selected from the Kenya side of the lake were selected for this study. The rivers include; River Kisat (1), River Auji (4), River Luanda (5), River Nyamasaria (7), River Oriatiko (8), River Tako (9), River Wigwa (10), one effluent stream - Railways point (3) and two lakeshores (Dunga beach (6) and Tilapia beach (2) of the Winam Gulf of Lake Victoria (Figure 2). All the sites are around Kisumu city, located in Kisumu County in Kenya's western region. In reference to his study, river refers to river estuary and landing site to fish landing site or beach. The lake is fed by rivers whose catchment area consists of agricultural, industrial and residential zones. Therefore, the lake serves as a sink for agricultural runoff, domestic effluent and industrial waste, treated and untreated sewage.

A total of fifty-nine water samples were collected: forty-one from the rivers and eighteen from the fish landing sites. Water samples were collected from the river mouths and the fish landing sites in Kisumu, and labelled. These sites were points of enhanced confluence pollution points of agricultural runoff and beach activities, respectively. Three water samples were collected at each collection point in three sampling events spread through the wet and dry seasons. Three river water samples were collected at each site: 100 meters upstream, 0m (at the mouth) and 100 meters offshore; for the fish landing sites samples, they were collected at 0 meters at the shore, 100 meters offshore and 200 meters offshore.

The samples were collected in amber 2.5-litre bottles pre-rinsed with distilled water and kept at 4°C during transportation for processing in the laboratory.

Solid Phase Extraction

All water samples were filtered using 47-millimetre 1-micrometre pore Telkroma filter paper to remove the suspended debris. The estrogens were extracted using Carbon-18 solid-phase extraction (SPE) columns (Abaxis, USA). The SPE columns were preconditioned using 4 ml of a solvent mixture (40% hexane, 45% methanol and 15% 2-propanol), followed by another wash with 4 ml of ethanol. The solid phase extraction (SPE) columns were again washed with one column volume of High-performance liquid chromatograph (HPLC) grade water. Two litres of the filtered water were passed through the preconditioned C18 SPE columns and then air dried with a vacuum pump. The hydrophobic substances bound to the SPE columns matrix were eluted into glass vials using 10 ml dichloromethane.

Enzyme-linked Immuno-Sorbent Assay (ELISA)

The estrogen levels were detected and quantified using the competitive Enzyme-linked Immuno-Sorbent Assay (ELISA) based on the competitive reaction between the sampled estrogen and the enzyme-labelled standard competing for binding to the specific monoclonal antibody on the surface of the microtiter plate. The assay was performed according to the manufacturers' protocols for three estrogens: Estrone (E1), 17-β estradiol (E2) and Ethinyl Estradiol (EE2). Dried samples were reconstituted in dimethyl sulphoxide (DMSO) to a final volume of 0.1% of the original sample volume extracted. The reconstituted conjugate solution was stored at 4 °C and used within 48 hours. The colour's absorbance was analysed using a microplate spectrophotometer (THERMO MULTISKAN EX) at 450 nm. The steroid hormone levels measured by the microplate spectrophotometer were calibrated using curves obtained from the respective standard solutions for Estrone (E1), 17-β estradiol (E2) and Ethinyl Estradiol (EE2) provided by the manufacturer (ECOLOGIENA®, Japan Enviro-Chemicals, Ltd).

Statistical analysis

Descriptive statistics were used to represent. Kruskal Wallis test was used to determine the statistical difference between the mean concentration levels from the surveys. Minitab 17 Statistical Software (2010). State College, PA: Minitab, Inc. (www.minitab.com). A significance level of $\alpha = 0.05$ was used.

RESULTS AND DISCUSSION

This study confirms estrogenic endocrine disrupting chemicals in waters from the study sites in Kisumu's Winam Gulf of Lake Victoria. The confirmation of estrogens' presence in Lake Victoria waters is essential for managing the steroidal pollutants by planning the region's estrogens' management. The estrogenic endocrine disrupting chemicals can be classified as a class of neglected pollutants in East Africa, whose importance will increase as the lake basin population grows. Their disruptive effect on human and animal endocrine systems should present a point of concern.

The two natural estrogenic compounds (Estrone - E1 and 17-β estradiol - E2) and synthetic estradiol (ethynyl estradiol - EE2) were detected in water samples obtained at the various river and landing sites. Table 1 shows the mean of general study, river and landing sites mean concentration levels for the three estrogens tested: the general mean concentration levels show that estrone (E1) had the highest concentration and 17-β estradiol (E2) was the lowest. The river water concentrations were also higher than the landing sites concentrations for E, E2 and EE2, and the mean concentration levels were not statistically significantly different between the river and land sites ($p<0.05$). This study's concentration levels for 17-βestradiol were congruent with the other studies conducted in Morogoro, where the quantities registered were 0.34-9.53.[18] However, the concentration levels for the estrone and ethynyl estradiol in the same study were much lower, ranging between 0.17-11.49 pg/ml and 0-0.92 pg/ml, respectively.

The mean concentrations of the quantified estrogenic endocrine disrupting chemicals in the waters obtained from each of the river estuaries and fish landing sites in the Winam Gulf of Lake Victoria are shown in Figure 3. The fish landing sites, Dunga and Tilapia beaches, recorded higher 17β estradiol concentrations than the river estuaries. The estrone and ethynyl estradiol concentration levels in the rivers and fish landing sites were comparable, however the estrone levels in the river was generally elevated than the fish landing site. The River Kisat, River Auji and the effluent stream Railways point having the highest concentrations. The highest concentration levels for estrone (E1) were recorded in the River Auji, River Kisat and Railway point. While 17-β estradiol was highest in the fish landing sites, Dunga and Tilapia fish landing sites. River Auji and River Kisat recorded the highest concentrations of ethynylestradiol. These rivers showing a high concentration of estrogens were passing through areas of high human populations.

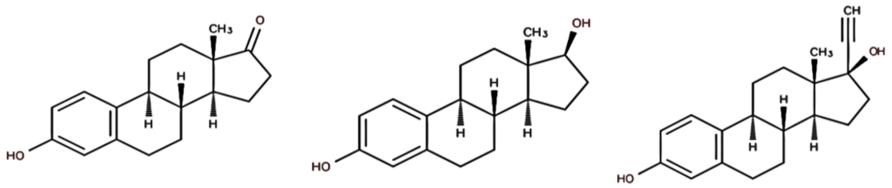
The 17-β estradiol (E2) and Ethinyl Estradiol (EE2) concentrations were lowest in the wet seasons and higher in the dry season (Figure 4). This finding was in concurrence with a study in Huangpu River, China which indicated the estrogen concentrations were lower in the wet season and higher in the dry season, and this was attributed to the dilution effect of more waters from the rain.[19] However, the estrone concentration in the June wet season was lowest, however the Sept wet season turned out to be the highest, and the cause for this variation was not presently clear.

The estrogens concentration was compared among the three collection points for both the river and fish landing sites, as shown in Figure 5. The river concentration levels indicate that estrone and 17-β estradiol levels were highest at the 100-meter upstream, and lowest at the 100m offshore, however the ethynyl estradiol was highest at the 0m collection point. Estrone and 17-β estradiol at the fish landings sites was also highest at the 100 m offshore collection point and lowest at the 200 m offshore points, however the ethynyl estradiol levels were almost the same at the 100m and 200m offshore. The that estrone and 17-β estradiol had relatively similar trends, with the 100 m offshore collection point having the highest concentration levels and the 100m offshore (river) and 200 m offshore (landing site) having the lowest concentration levels. The difference in eEDC concentrations at the river estuary and the landing sites was consistent with the dilution effect as the distance towards open water increases.[21] The lower concentration levels at the 0m at the river and om at the land sites for the estrone and 17-β estradiol could be due to the effect of suspended solids and sediments in the water absorbing some of the estrogens since the water is slowed down.[22]

The landing site concentrations were highest at the 100m offshore collection point for E1 and E2, followed by the 0m and lowest in the 200m offshore collection point. The low concentration levels at the 0m collection point were attributed to estrogens being absorbed by sediments due to the slow movement of the water at the shore. The study established that the estrone and ethinyl estradiol mean concentrations were higher in the river estuaries than in the fish landing sites. Whereas, the fish landing sites' 17β estradiol concentrations were higher than the river estuaries. Compared to the river for estrone and ethinyl estradiol, the lower concentrations at the fish landing sites could be attributed to the dilution effect after the water from the river's mixes with lake waters. The ethinyl estradiol is usually aerobically hydrolysed by bacteria to estrone[19], and that could probably explain why it is low in concentrations in the study. However, it should be noted that ethinyl estradiol is more persistent in the environment and more potent due to its hydrophobic property, making it quickly absorbed into sediments in the aquatic ecosystem.[20]

The concentration levels of the three estrogens in Lake Victoria were relatively moderate compared to global levels. However, these levels are expected to rise due to the increasing human population and expanding livestock farming in the region. The presence of estrogens in the water continues to pose a public health risk to both people and animals relying on or inhabiting the lake water. Although no immediate effects of Endocrine Disrupting Chemicals (EDCs) on Lake Victoria's fauna have been reported, prolonged exposure could lead to estrogen-driven changes, a phenomenon observed globally even at low concentrations.[18] Notably, among the three studied hormones, EE2 stands out as the most potent. Its Predicted No Effect Concentration (PNEC) is as low as 0.1 ng L-1, rendering it toxic to aquatic organisms like fish, thereby posing a substantial threat.[23, 24]

These estrogens are likely to exert negative effects on aquatic and terrestrial animals consuming the water over time. Their presence in the water raises significant public health concerns regarding potential exposure risks for aquatic and terrestrial creatures, including humans, who may come into contact with these estrogens through their diets and water consumption. Consequently, it is imperative to urgently address the issue by reducing estrogen levels in the water that enters the lake. This preventive action is crucial to curbing any further increase in concentrations, thus averting potential detrimental consequences of these estrogens on aquatic ecosystems.



Estrone (E1) 17-β estradiol (E2) Ethynylestradiol (EE2)

Figure 1: Chemical structures of estrogens found in the environment.

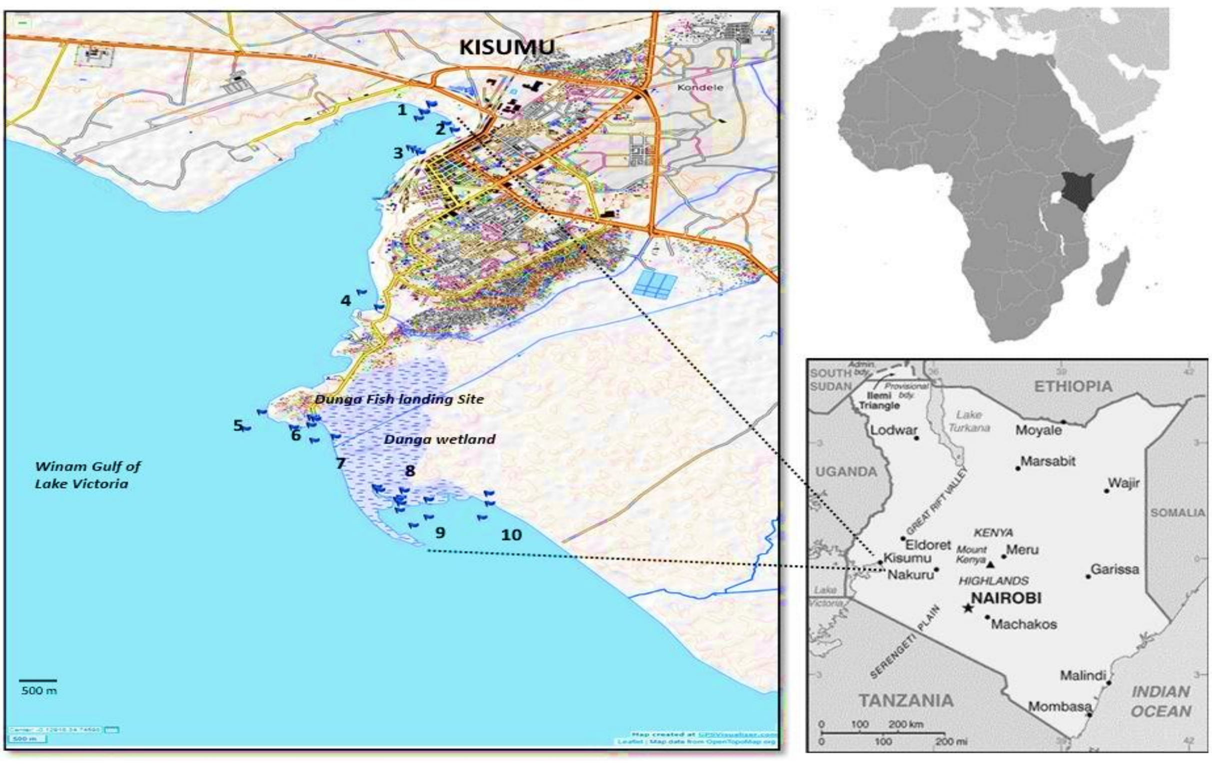


Figure 2: Map of Lake Victoria basin, Kenya showing the drainage area and main town.

Table 1: mean concentrations (pg/L) pooled for all samples for the overall study river and landing sites' water samples.

	n	Pooled study concentrations	n_R	River water concentrations	n_{fls}	Fish landing sites concentrations	P value
Estrone (E1)	59	101.68 ± 5.84	41	112.59± 6.08	18	76.8 ± 11.4	0.383
17-β estradiol (E2)	59	19.57 ± 2.53	41	22.53 ± 3.43	18	12.84 ± 2.15	0.189
Ethynyl estradiol (EE2)	59	40.14 ± 4.5	41	40.48 ± 5.52	18	39.38 ± 7.92	0.985

n-pooled ssample size, nR – River water sample size, nfls – Landing sites' water sample size

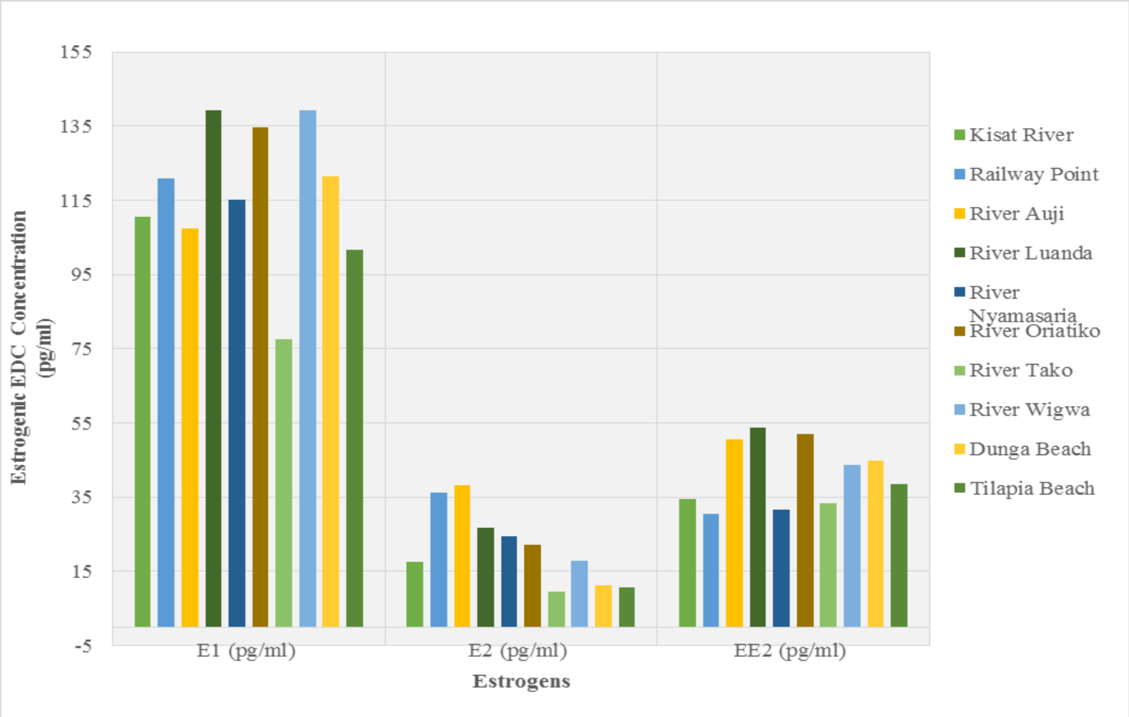


Figure 3: Mean estrogen concentration levels from sampled sites.

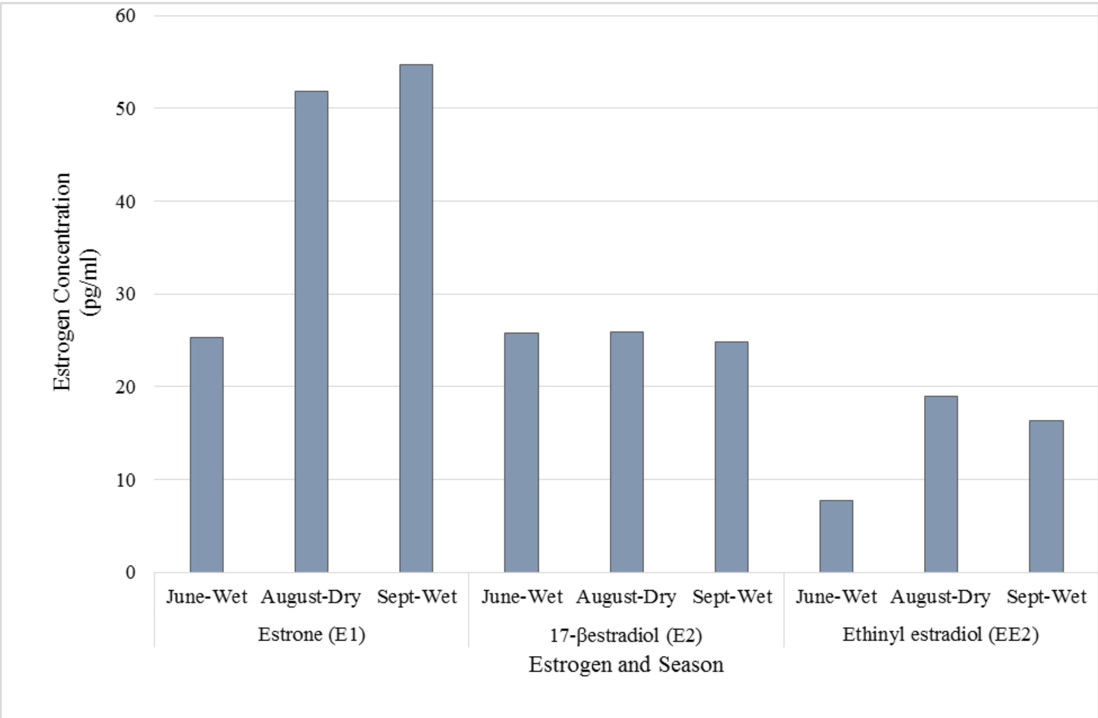


Figure 4: Variation in mean concentration levels of the steroidal estrogens by season.

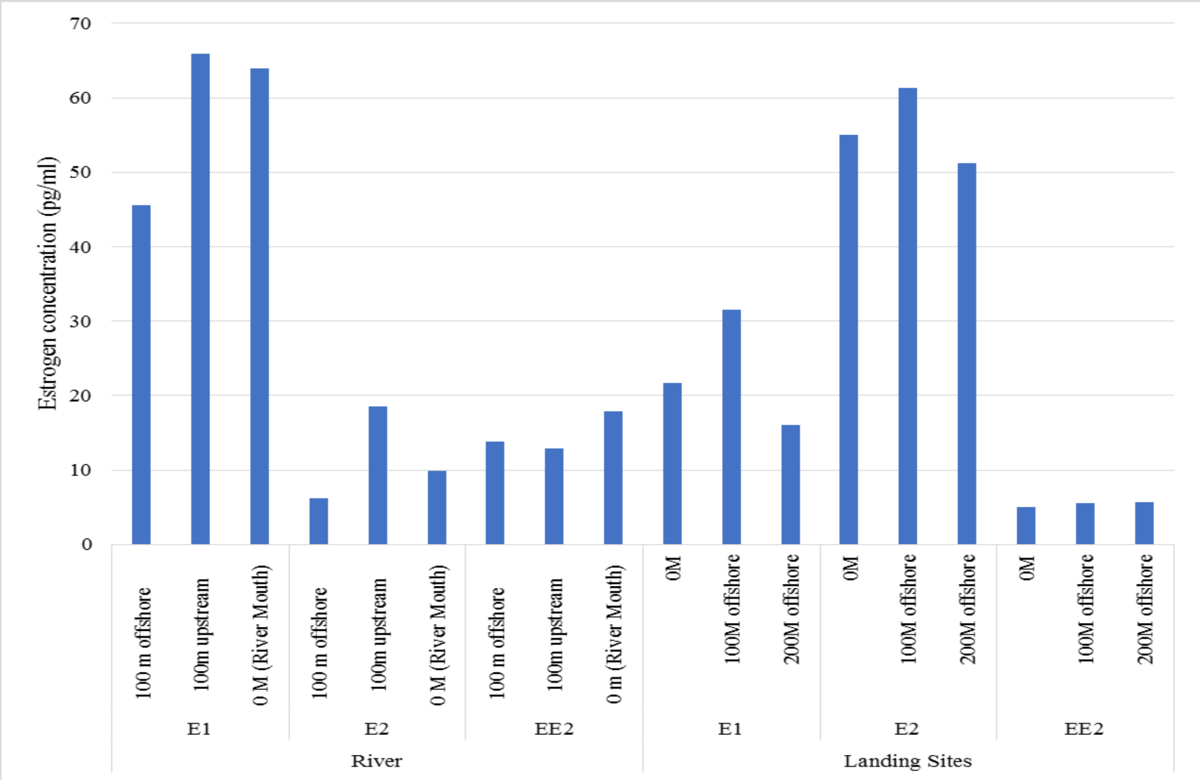


Figure 5: Concentration levels of various oestrogens at different collection points.

Conflict of Interest

The authors affirm that they do not possess any identifiable financial conflicts or personal affiliations that could have potentially influenced the outcomes presented in this research article.

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