

**ESTIMATE OF BIOCONCENTRATION OF ENDOCRINE  
DISRUPTING CHEMICALS IN EUPHRATES RIVER IN  
AL-NASSIRYIA CITY OF IRAQ**

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**ABSTRACT:**

Endocrine disrupting chemicals (EDCs) in water have been of overworld concern due to their potential adverse effects in organisms . The objective of this study was to examine endocrine-disrupting factors in Euphrates river , in Al-Nassiryia city/Iraq.

Samples from all locations were collected during autumn2016 and winter2016, extracted by solid phase method and analyzed by HPLC-UV in the wave length of 210 nm for ethinyl estradiol and 280 nm for other compounds. The results showed that presence of bisphenol A with concentrations ranging 0.001 to 0.98 µg/L and 0.09 – 5.7 µg/L nonylphenol and 2.05 – 19.9 ng/L for 17-beta estradiol. Estron was 0.1 to 54 ng/L and 0.91-6.2 ng/L for ethinyl estradiol.

The concentration levels of pollutants in st.2 were higher than the st.1 and st.3. Analysis of water showed that concentration levels of pollutants were higher during autumn comparing with winter in st.2 (Pvalue  $\leq 0.005$ ), which may be due to the changes of environmental conditions.

The widespread use of birth control pills formulated with these content of potent estrogenic chemicals appear to be the more important path for the freeing of estrogenic compounds into the aquatic environment.

**Keywords:** Endocrine disrupters, Bisphenol A, 17-beta estradiol, Ethinyl, Nonylphenol, Euphrates River, al-Nassirya city, Iraq

## **INTRODUCTION:**

Recently, many types of chemical substances realise in large quantities to the environment, which can load unavoidable costs to be paid for technological remediation processes (Hirano *et al.*, 2001). Evidences have indicated the humans, domestic and wildlife species have pained pernicious health consequences from exposure to environmental chemicals that react with the endocrine system and because that they are called "endocrine disrupting chemicals", EDCs (Moreno *et al.*, 2001).The existence in our environment of compounds with estrogenic properties has become very important of world-wide growing apprehension , because these compounds may intervene with the reproduction of human, livestock and wild-living animals.

Endocrine disrupting chemicals (EDCs) enter aquatic environment through discharged effluents, mainly through wastewater treatment works and diffuse run off from land, and affect a wide range of aquatic biota (Soffker and Tyler, 2012).

The identity of the estrogenic substance is unknown. It is suggested that the two most likely possibilities are ethynylestradiol, originating from pharmaceutical use, or alkylphenol-ethoxylates (APE), coming from the biodegradation of surfactants and detergents during sewage treatment.(Purdom, et al., 2006).

Chemicals endocrine disrupters that is Man-made affectation the potential to modulate endocrine function and thus adversely affect human reproductive development (Foster *et al.*, 2000). A great deal of research attempt has been achieved to the identification, occurrence, fate and effects of organic

contaminants found in municipal wastewaters two decades ago. Some contaminants that have the possible to disrupt the normal functions of the endocrine system in wildlife, fish, and humans have drawn specific attention. (Lee *et al.*, 2005).

One particular synthetic estrogen has been distinguished for purportedly detrimental effects on the environment: ethynyl-estradiol, or EE2, a synthetic estrogen used in birth control pills, patches, rings and injectable. (Moore, *et al.*, 2011)

Bisphenol A is an estrogenic endocrine-disrupting chemical with two unsaturated phenol rings which, which are used in dentistry, food packaging, and as lacquers to coated food cans, bottle tops, and water pipes (Tsutsumi, 2005).

Furthermore, woman using control are not the only ones flushing estrogen down the drain. Pregnant woman excrete high levels of natural estrogens and nearly everyone (both women and man) produce some amount of natural estrogen also released into wastewater. (Wise *et al.*, 2011)

Estrogens included in this work were the female hormones  $17\beta$ -estradiol (E2), estrone (E1) and the synthetic contraceptive additive  $17\alpha$ -ethynylestradiol (EE2) that has potency similar to natural hormones.  $17\beta$ -estradiol (E2) is the one that display the highest estrogenic capacities (Smeets *et al.*, 1999).

The objective of this study was to obtain a first impression of the lack of hormones information in the Euphrate river, including station near selected waste water effluents.

## **MATERIALS AND METHODS**

-Study area:

The study area included 3 stations on Euphrates river in Al-Nassirya city, the first station located at 5 km before the second station located at convergence zone stream discharge waste water, while the third station located before the river leaving the city of Al-Nassirya and far from the second station by 10 km. Fig.(1)



**Fig.(1) Map of the study stations**

### **Sample collection**

Subsurface water samples were collected from the middle and two banks of the Euphrates river during autumn 2016 to winter 2016. Samples were collected in brown glass bottles and stored at 4°C in the dark box until analysis from each station.

## **Extraction and HPLC analysis**

The current study followed a chemical method approved by the Jafari *et.al.*,( 2009).First of all we extracted by solid phase method and after that analyzed by HPLC-UV in the wave length of 210 nm for ethinyl estradiol and 280 nm for other compounds. Applied SPSS program for the statistical analysis to show the significant difference between seasons and stations at ( $P \leq 0.05$ )

<b>Compound</b>	<b>Autumn2016</b>	<b>Winter2016</b>
Esteron (ng/L)	0.3	0.1
bisphenol A ( $\mu\text{g/L}$ )	0.01	0.06
17-beta estradiol(ng/L)	12.9	9
ethinyl estradiol(ng/L)	2.09	3.9
Nonylphenol ( $\mu\text{g/L}$ )	0.09	0.32

Table 1: Concentration of estrogenic compounds in St.1

## **RESULTS :**

The results showed that EDCs concentration levels in st.1 were from 0.1 ng/L for Esteron in winter 2016 to 0.32  $\mu\text{g/L}$  for Nonylphenol in the same season while in autumn2016 the results ranged between 0.3 ng/L for Esteron to 0.09  $\mu\text{g/L}$  for Nonylphenol . (Table 1)

The statistical analysis did not show a significant difference between seasons and between st.1 and st.3 at ( $P \leq 0.05$ ) , the concentration levels of Nonylphenol A and 17 beta estradiol were 0.32  $\mu\text{g/L}$  in winter and 12.9 ng/L in autumn , respectively, which are more than the others.

The results in st.2 were from 6.2 ng/L for ethinyl estradiol in Autumn2016 to 4.09 µg/L for Nonylphenol in the same season while in Winter2016 the results ranged between 6.03 ng/L for ethinyl estradiol to 5.07 µg/L for Nonylphenol (Table 2).

Table 2: Concentration of estrogenic compounds in St.2

<b>Compound</b>	<b>Autumn2016</b>	<b>Winter2016</b>
Esteron (ng/L)	54	23
bisphenol A (µg/L)	0.98	0.32
17-beta estradiol(ng/L)	19.9	11.7
ethinyl estradiol(ng/L)	6.2	6.03
Nonylphenol (µg/L)	4.09	5.7

The statistical analysis showed a significant difference between seasons and between st.2 compared with st.1 and st.3 at ( $P \leq 0.05$ ), and concentration levels of Nonylphenol A and 17 beta estradiol and Esteron were 5.7 µg/L in winter and 19.9 ng/L in autumn, 54 ng/L in autumn respectively, which are more than the others

Values obtained for water samples in st.3 showed that contaminates ranged between 1.12 ng/L for ethinyl estradiol to 0.51µg/L for nonylphenol in Autumn2016 and in Winter2016 the results ranged between 0.91ng/L for ethinyl estradiol to 0.4 µg/L for Nonylphenol (Table 3).

Table 3 : Concentration of estrogenic compounds in St.3

<b>Compound</b>	<b>autumn</b>	<b>winter</b>
Esteron (ng/L)	3.2	1.01

bisphenol A ( $\mu\text{g/L}$ )	0.078	0.001
17-beta estradiol( $\text{ng/L}$ )	2.05	3
ethinyl estradiol( $\text{ng/L}$ )	1.12	0.91
Nonylphenol ( $\mu\text{g/L}$ )	0.51	0.4

The statistical analysis showed a significant difference between seasons and between st.2 compared with st.1 and st.3 at ( $P \leq 0.05$ ) . and concentration levels of Nonylphenol A and 17 beta estradiol and Esteron were 0.51 in autumn  $\mu\text{g/L}$  and 3  $\text{ng/L}$  in winter, 3.2  $\text{ng/L}$  in autumn respectively, which are more than the others.

## **DISCUSSION:**

The effect estrogenic compounds in the water suooly from industry, agriculture and other sources raises concerns about humun health and deserves scrutiny. Estrogenic compounds are part of a large category of chemicals known as endocrine disruptors, chemicals that can alter the hormonal and homeostatic systems enabling for an organism- to communicate with and respond to its environment.(Diamanti-Kandarakis *et al.*, 2009).

A study performed on station near samples from sewage treatment plants has evidenced the presence of Nonylphenol, bisphenol A, estrone and 17 $\beta$ -estradiol in the Euphrates . Much research has been conducted on the source and fate of estrogens in wastewater treatment plants. Sorption and biodegradation are the initial removal techniques for estrogens in activated sludge systems, which are vastly used biological treatment techniques for municipal wastewater treatment. However, when removal of estrogens in a wastewater treatment plant is incomplete.(Racz & Goel.,2010)

The concentration of estrogenic compound in samples showed that all five studied compounds found in station 2, Nonylphenol had largest amounts in both seasons. The swage treatment plant efficiency in removing BPA is better (90%) than for NP (75%), probably because of the major resistance of NP to microbial decay occurring during the treatment operation. In addition, NP is generated during the break down of products containing alkyl phenol poly ethoxylates which is one of the world's largest groups of surfactants. (Lagana *et al.*, 2004). It was found that the concentrations did not change obviously during the period of study, suggesting that the NP contamination is still prevalent, possibly due to their continuous use in car washes and other service industries (Pettersson *et al.*, 2006).

These results indicate that environmental endocrine-disrupting estrogens are found larger at st.2 on the river since there was not enough removed in the process of sewage treatment although the treatment are not enough and it can be carried over into the aquatic environment. After land passage, they can finally be found in drinking water (Kuch and Ballschmiter, 2001). The concentration levels of pollutants were higher during autumn comparing with winter in st.2 ( $P \leq 0.005$ ) and also in other sites, which may be due to the changes of environmental conditions.

Effluent from towns and industrial wastewater treatment plants repeatedly contain bisphenols, because of the incomplete removal of these contaminants during treatment (Moral *et al.*, 2005). Estrogens are in fact fundamentally secreted as conjugates of sulfuric acid and glucuronic acids. In this form they would not possess a direct biological activity, but they can act as precursor hormone containers able to be deconjugated into the major compounds during the sewage treatment.



This event is especially clear for E1, the most abundant estrogen excreted by cycling women, that is even believe to be the by output of biodegradation of E2 in the sewer. And so on, Jafari *et.al*(2009) show that tiny level of the most potent estrogen is discharged into extraditing waters.

However, this low concentration could be significant with assessment to endocrine activity at sites where sewage treatment plant effluents can make up a considerable concentrations of the river flow, even because estrogens may cause dangerous effects on organisms even at such low concentrations (Lagana *et al.*, 2004).

## **CONCLUSION:**

Application of the procedure revealed that in Euphrates river the estrogenic hormones can be detected at some locations and at certain time points in surface water and thier concentrations that near of effluents of waste water treatment plants was higher than other locations.

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تقدير التركيز الحيوي لكيميائيات خلل الغدد الصم في نهر الفرات في مدينة الناصرية

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### **الخلاصة:**

العديد من المركبات الكيميائية في البيئة المائية اقلقت انحاء العالم بسبب تاثيرها السلبي للانسان والحياة البرية. هذه المركبات وصفت بالمركبات الكيميائية المعطلة للغدد الصم . ان هدف الدراسة هو قياس عوامل تعطيل الغدد الصم في نهر الفرات في مدينة الناصرية /العراق . جمعت العينات من المحطات خلال فصلي الخريف والشتاء لعام 2016 حيث استخلصت بطريقة الطور الصلب وحللت بجهاز HPLC وعلى طول موجي 210 نانوميتر لاثيناييل استيرودايل و280 نانوميتر للمركبات الاخرى . وضحت النتائج بان تركيز الفينول الثنائي أي تراوح بين 0.001 الى 0.98 مايكروغرام/لتر وكانت النونيل فينول بحدود 0.09 الى 5.7 مايكروغرام /لتر , وكان التركيز 2.05 الى 19.9 نانوغرام /لتر للمركب 17-بيتا استراديول . الاسترون كان بين 0.1 الى 54 نانوغرام/لتر وتراوحت تراكيز الاثيناييل استرودايل بين 0.91 الى 6.2 نانوغرام/لتر.

ان مستوى تركيز الملوثات في المحطة الثانية كان اعلى من التراكيز المسجلة في المحطتين الاولى والثالثة . ان تحليل عينات المياه وضحت مستويات التركيز كانت اعلى تقريبا خلال فصل الخريف مقارنة مع الشتاء خاصة في المحطة الثانية عند مستوى معنوي (> 0.05) , وقد يعود لتغيرات في الظروف البيئية . ان الانتشار الواسع باستخدام حبوب منع الحمل شكلت مع المركبات الكيميائية الاستروجينية الكامنة لتكون طريق الاكثر اهمية بتحرير المركبات الاستروجينية للبيئة المائية .

**الكلمات المفتاحية :** خلل الغدد الصم , الفينول الثنائي أي , 17-بيتا استرودايل , الاثيناييل

استرودايل , نونيل فينول , نهر الفرات , مدينة الناصرية , العراق .