Monitoring of 17 β-Estradiol Residues in the Suez Canal Region

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ABSTRACT

This study was initiated to provide the first record of monitoring of 17 β-estradiol (E2) residues in some Egyptian aquatic ecosystems. Samples of water were collected from three water bodies located in the Suez Canal region. Samples were extracted, filtered and examined by HPLC on a C_{18} column using Florescence detectors.

Results provided evidence of the presence of estradiol in the studied area. Interestingly, marine lakes contained significant levels of 17 β-estradiol ($P < 0.05$). Lower levels were also detected in the rivulet streams supplied by River Nile.

Detection of estradiol in the aquatic ecosystems of the Suez Canal region grabs the attention towards the heavy reliance on some esterogenic medicinal products in the area, and the eventual effect on the aquatic systems including biodiversity of a variety of organisms. Therefore, it is recommended to enlarge the detection scanning of estradiol in other Egyptian areas.

Key words: Estradiol, Temsah Lake, Suez Canal region

INTRODUCTION

There is a global concern about the presence of the estrogenic residues in the aquatic ecosystems as one of the dangerous pharmaceutical residues; this is due to its impact on marine life ecosystems and on human health (Seifert *et al*., 2003). The source of these estrogenic residues is industrial wastes and medicines (Nolan *et al*, 2001), and as additives in animal feed (Bruce, 2005; Hailing *et al*., 2002). Even though that these compounds are found in small amounts in the environment (Hensen *et al*., 2002), but the effect of these traces is remarkable on marine animals and consequently on humans.

It was evidenced that estrogenic residues were detected in some lakes, rivers and ground waters in many places around Europe (Burton *et al*., 2002). It was also reported that it caused severe damage to the marine system balance and on human welfare.

17 β estradiol (E2) is one of these compounds which belong to (EDCs) and have been of important concern since the 1930s (Cook *et al.*, 1934; Tawfic, 2006). 17 β Estradiol, as well as other steroids, are excreted from the human body and live

stocks in high quantities (Narender and Cindy, 2009); so when they are discharged into marine environments as waste waters or sewage, they occur with concentration levels in the range of nanograms /L (Koh *et al.*, 2008). Concentrations less than 1 ng/L in the aquatic environment can cause infertility and weaken estrogenic activity, decrease reproductivity of female fish (Burton and Wells, 2002), change behavioral habits which may change reproductive physiology of fish (Denslow and Sepúlveda, 2007), and sex reverse in some fish turning males to females (Hansen *et al*., 1998).

Since contraceptives have been widely used in the last 50 years in many countries, 17 β estradiol, and its derivatives, were detected in aquatic environments in different industrial countries, even where high precautions of water and environmental safety are considered. They were detected in British raw domestic sewage discharged into rivers (Desbrow *et al.*, 1998; Rujiralai *et al.*, 2011) and waste water in South Korea (Ra *et al.*, 2011), China (Liu *et al.*, 2011; Lu *et al.*, 2011; Ra *et al.*, 2011; Zhou *et al.*, 2011), The Netherlands (Belfroid *et al.*, 2006), Italy (Pojana *et al.*, 2007), and Germany (Körner *et al.*, 2001; Hintemann *et al.*, 2006). In addition, it was detected in prawn and fish (Jiang *et al.*, 2009), Japanese Spanish mackerel (*Scomberomorus niphonius)* and mollusks (Zou *et al.*, 2007), Iin France, it was also detected in shellfish (Lagadic *et al.*, 2007). Therefore, the aim of this project was to investigate and locate 17 β estradiol residues in Suez canal region as a first step to scan other Egyptian aquatic ecosystems.

MATERIALS AND METHODS

Study areas:

The study was carried out in the Suez Canal region (Fig. 1). Samples were collected form Bahr Elbakar drainage, Ismailia stream, Abo shehata., and from two parts of Temsah Lake which consists of two different ecosystems together, a fresh water part supplied from the River Nile, and a marine water part supplied by the Suez canal. In addition to Temsah Lake, samples were collected from Manzala lake. A volume of 100 ml were collected at 50 cm depth, filtered, then stored frozen until they were thawed as a pre-step to analysis.

Quantitative verification of target Residues:

Extraction of estradiol

A volume of 100 ml of each sample was freeze-dried. (Hetovac). The remaining dried material was re-dissolved in 6 ml of n-hexane (Merck, Lichrosolv) and then shaked vigorously for 30 minutes on a shaker.

Clean up of the samples:

Molecular Imprinted Polymer (MIP) was prepared according to Le Noir *et al.*, (2007), and packed in a glass columns. Just before clean up, the MIP columns were conditioned with 5 ml of methanol: acetic acid (2:1), at a flow rate of 1ml/ 10 min. Hexane extract from each sample was applied to MIP column at a flow rate of 1ml/ min. Finally, the column was eluted with 10 ml of a mixture of methanol: acetic acid (2:1) at a flow rate of 0.5 ml /minuet (Le Noir *et al.*, 2007).

The collected samples were concentrated to 500µl by evaporation under nitrogen gas stream. The final samples were filtered through 0.45 □m filters and transferred to 2 ml vials and estradiol concentrations were determined by HPLC system using C_{18} column and and florescence detectors.

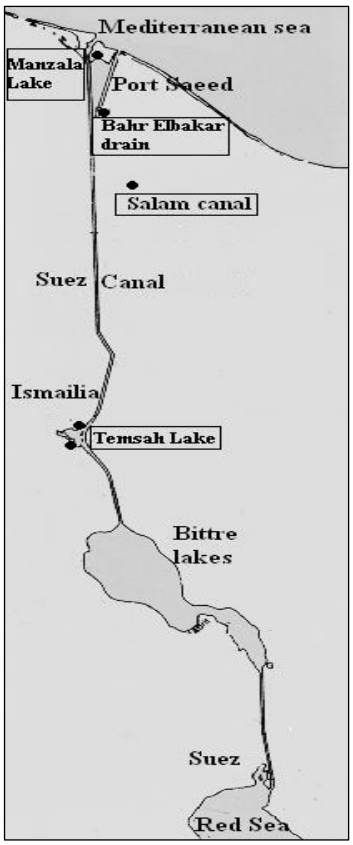

Fig. 1: location of sampling sites in Suez Canal region

Detction by HPLC- fluorescence analysis

A series of 17β-estradiol (98% purity, Sigma Aldrich) dilutions (2×10^6 ng/ml-0.0001 ng/ ml) were analyzed to construct a standard curve, and the cleaned up samples were subjected to HPLC- fluorescence for detection under the same conditions. Results were evaluated using the standard curve to determine the concentrations in the tested samples.

The analysis was made using an Auto-sampler G13129, and the separation was carried out on Supelcosil C_{18} column (150 x 4.60 mm, 5 µm). Elution was performed with gradient elution using acetontrile 10% in ultra pure water up to 100% acetontrile (Merck, Lichrosolv-for chromatography) and water at flow rate of 0.8 ml /min (Lopez de Alda *et al.*, 2002). Estradiol was detected using fluorescence detector G 1321A at excitation at 420 nm after emission at 330 nm. Detection was possible in the range 1.0 mg/ml -0.01 ng/ml.

Statistical analysis:

The statistical analysis was performed by applying ANOVA test to the results to compare differences between sites. The correlation between samples was performed using IBM SPSS Version19, 2010.

RESULTS

Estradiol was spiked up at minute 23.68 in the standard curve as well as in the contaminated samples as shown in Figure 2. Estradiol was detected in all tested samples as summarized in Table (1) and illustrated in Fig (3). The highest level of estradiol (1029 ±0.1µg/L) was found in Bahr Elbakar samples. Also, it was detected in both marine and fresh water parts of Temsah Lake; (925 ±0.1 and 462 ±0.1µg/L

respectively). Lower concentrations were detected in Ismailia stream, El Salam canal, and Abo Shehata (556 ±0.1, 552±0.1, and 284 ±0.1 µg/L) respectively.

Table 1: Detected concentrations of 17 β-estradiol (± St. Div.)

Location	Sample	µg/L	St. Div.
Bahr El Bakar	w9	1029	0.1
Manzala Lake	w10	684	0.1
Salam canal	w12	552	0.1
Temsah lake "Marine"	w13	925	0.1
Temsah lake "fresh"	w14	462	0.1
Ismailia stream	w15	556	0.1
Abo Shehata	w16	284	0.1

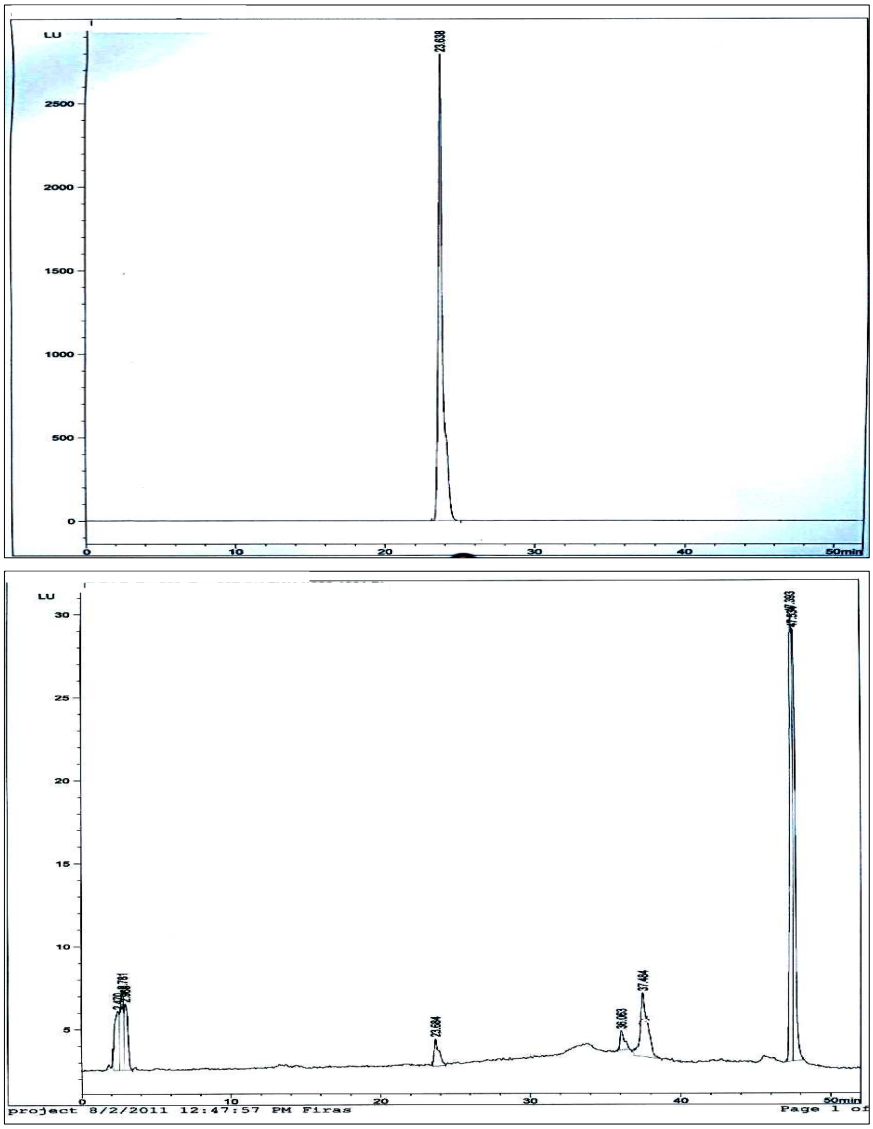

Fig. 2: HPLC analysis showing the standard of 17 β estradiol (a) and its detection in Bahr elbakar sample (b) at retention time 23.684.

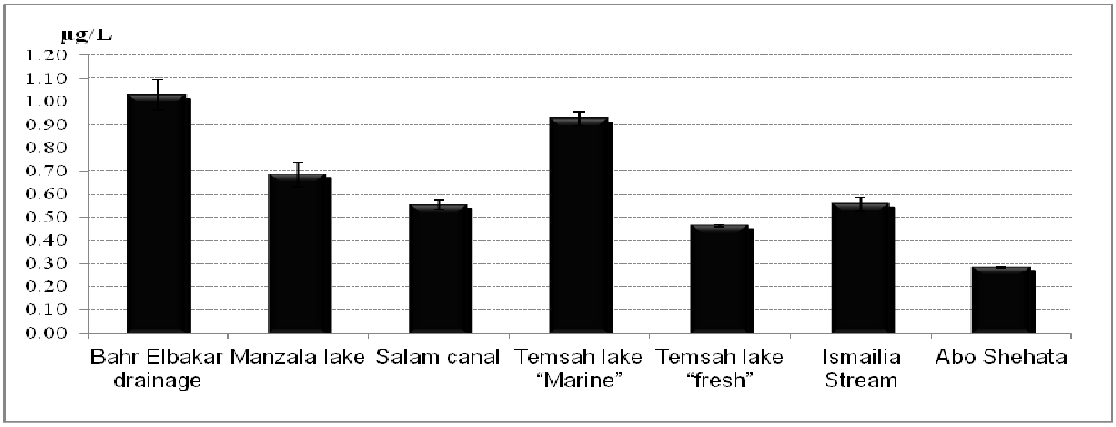

Fig. 3: Detected concentration in of 17 β estradiol in the tested samples

DISCUSSION

Bahr Elbakar drain had the highest level of estradiol (1029 ±0.1µg/L) as this drain is the end point stream of Cairo and Delta discharges. This high detected concentration must be because the drain receives untreated and somehow primarily treated waste water from east Cairo moving east to Manzala lake (Taha *et al*., 2004). In addition, the industrial activities, waste water discharged from Belbeis drain to Bahr El-Bakar drain, as well as domestic discharge from the country side around Bahr El-Bakar drain, all these sources may have resulted in having such high concentrations of estradiol.

E2 detection in the marine and the fresh water parts of Temsah Lake; (925 ±0.1 and 462 ±0.1 respectively) is interesting because that lake is unique as it receives high saline water supply from the Suez Canal moving from south (Red sea) to north (Mediterranean sea), and receives freshwater supply as a mixture coming from the Nile and other sub-Nile streams (El-Serehy and Sleigh, 1992). The lake is an important source of feeding for many living species; most of the aquatic migrating birds stop around Temsah lake in their migratory trip from Europe to Africa in winter and vise versa in summer. Local birds also live around the lake and its surrounding areas, where they find their feeding resources of fish and crustaceans. People in this area depend on fish as their main source of food. The annual fish production from Temsah lake and the bitter lakes is 4557 tons of the total fish production in Egypt which is estimated with 387398 tons according to GAFRD (2009). However, Temsah lake seems to be in need of proper attention and conservation. The lake has been suffering from different sources of pollution for decades endangering the environment, marine life and human welfare (El-Sherif *et al.* 2009).

The concentration of estradiol detected in Manzala lake was 684 ±0.1 µg/L. This lake is also known to be the end point of different discharges of the surrounding area though it contributes with 48023 tons to the total Egyptian fish production (GAFRD, 2009). In addition, the lake is the final destination of 3 billion m^3 of waste water discharge coming from Bahr Elbakar drain every year (EU-SMAP, 2007). Thus, the water quality in Manzala Lake is affected by the water quality received from Bahr Elbakar. However, this concentration is in one way or another below the expected concentrations, taking into consideration the huge amount of polluted discharges in the lake. But this may be due to the dilution effect by the water flow from Suez Canal towards the Mediterranean sea, or most likely due to the absence of medical industrial discharges or animal farms' drainage which usually contains high amounts of

esterogens incorporated in the animal feed as additives (Bruce, 2005; Hailing *et al*. 2002), uunlike the case in Temsah lake which is surrounded by agricultural farms, and few animal production activities by the farmers.

Furthermore, the lower concentrations of estradiol detected in El Salam canal, Ismailia stream and Abo Shehata (552±0.1, 556 ±0.1 and 284 ±0.1 µg/L) respectively is most likely because they are bronchial streams and canals where the dilution factor through hundreds of kilometers of water flow away from the main sources would have a considerable effect. For instance, El Salam canal, which is an artificial canal established to supply eastern parts of the Suez canal region with irrigation water, is 262 Km^2 long. It is supplied by a mixture of 2.11 billion m^3/year of the Nile fresh water from the Domietta branch, 1.905 billion m3/year of the drainage water from Bahr Hadous and 0.435 billion m^3/year of El Serw drainage water (Hafez,2005). Thus, the ratio of Nile water to drainage water is approximately 1:1. However, the water is used in fish farming and animal feeding as additional farm activities (Elnwishy *et. al*, 2008) besides being used in irrigation for reclamation of east Suez canal which is the major purpose for establishing the canal (Hafez, 2005). The mixture of the sewage water and the farm's discharges may have resulted in some harmful biological effects on fish and animal reproduction in the area.

Also, the concentrations in Ismailia stream (556 ±0.1 µg/L) which is supplied by Nile fresh water from north Cairo to Ismailia were higher than that detected in the fresh water part of Temsah lake (462 ±0.1 µg/L) though they are supplied by the Nile and closely located branches. But this may be due to a higher load of discharges in the lake, while on the other hand, the dilution factor to the discharges in the stream from Cairo to Ismailia slightly reduce the concentration.

It was observed that the tested samples from the marine water contained higher concentrations of estradiol than the samples from fresh and brackish waters. The marine part of Temsah lake had an approximately 50 % higher contamination level of estradiol than the fresh water part of the same lake, and both Temsah and Manzala lakes showed higher concentrations of estradiol than other samples. It is worth to mention that the salinity in both lakes are ~40 ‰ (El-Serehy and Sleigh, 1992). Different factors may also be involved in such higher level in marine water than fresh water; such as faster degradation of estradiol in the anaerobic freshwater sediments than in the anaerobic marine sediments (Lopez de Alda *et al.*, 2002; Tyler *et al.*, 2005; Czajka and Londry, 2006; Christoph and Juliane, 2009), salinity assistance to estradiol to become more resistant to degradation, or the inability and sometimes total failing of marine microorganisms to be involved in biodegradation of estradiol are all possible factors open for upcoming investigations.

CONCLUSION

It was concluded that the Suez canal region is contaminated with 17 β-estradiol residues. Temsah and Manzala lakes showed relatively high concentrations. Though these levels are not extremely high but, they may result in unhealthy effects to human consumption of sea food due to accumulation factor.

However, this research provides the first data about the presence and the levels of estradiol in Egypt. Thus, further investigations are recommended in the future in other regions in Egypt as well as to adopting new techniques for the removal of 17 β-estradiol in order to maintain marine life ecosystem in the tested locations.

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ARABIC SUMMARY

رصد بقايا مركب 17 - بيتا استراديول في منطقة قناة السويس

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هذه الدراسة تسجل اول رصد لمتبقيات مركب 17 بيتا استراديول في بعض النظم الايكولوجية المائية المصريه.حيث تم جمع عينات من المسطحات المائية في منطقة قناة السويس. وتم استخلاص العينات وفحصها بأستخدام جهاز HPLCعلى عمود C18 باستخدام أجهزة الكشف عن التنوير.

وقد قدمت النتائج أدلة على وجود الاستراديول في المنطقة التي شملتها الدراسة. ومن المثير للاهتمام، وجود مستويات كبيرة من β-17 استراديول (P. 0.05>) في البحيرات المالحه عنها في المياة العذبه. ورصدت أدنى المستويات في تيارات المصارف المائية المتفرعة من نهر النيل.

وتبين وجود الاستراديول في النظم الايكولوجية المائية في منطقة قناة السويس يجذب الانتباه نحو خطورة الاعتماد الكبير على بعض المنتجات الطبية الاستيروجينية في المنطقة، والتأثيرالنهائي لوجودها على النظم المائية بما في ذلك التنوع البيولوجي من مجموعة متنوعة من الكائنات الحية. ولذلك فمن المستحسن توسيع نطاق عملية الكشف عن الاستراديول في مسطحات مائية اخرى في جمهورية مصر العربية.