Original Article

Environmental Monitoring of 17β- estradiol and Estrone in Ardabil's Drinking Water Source as Endocrine Disrupting Chemicals

Khodavirdi Abbaszadeh Maroufan[1], Hamid Mirzaei[1*], Amir Abbas Matin[2], Afshin Javadi[1], Alireza Amani-gadim[2]

[1] Department of Food Hygiene, Tabriz Branch, Islamic Azad University, Tabriz, Iran. [2]Department of Chemistry, Faculty of Basic Sciences, Azerbaijan Shahid Madani University, Tabriz, Iran.

Abstract

The aim of this study was the monitoring of estrogens along the Baliqlychay River as a drinking water source of Ardabil City in Iran, annually. Eight sites were selected and the effect of Nir City crude sewage and wastewater of two fish farms on the amount of hormones in Baliqlychay River and drinking water distribution network of Ardabil City were studied. Samples were tested for Estrone and 17β- estradiol using HPLC. It was found that there was no Estrone in the studied stations but all of them were polluted by 17β- estradiol. During the grazing season and entrance of contaminated crude sewage of Nir City, animal feces are the main source of 17β- estradiol pollution. The efficiency of the Ardabil wastewater treatment plant depends on the water consumption rate. In normal conditions, the wastewater treatment plant showed an acceptable performance and removed 58.82- 63.25% of E2. But, at high consumption seasons, hormone removal is negligible.

Keywords: 17β- estradiol, Estrone, Ardabil, drinking water

INTRODUCTION

Water is the most abundant compound worldwide and the main source of life. The water availability affects the life and health of all living beings, including humans, plants, and animals [1]. The availability of clean water is of tremendous importance. As water consumption increases, especially in industries; water resources are being contaminated by pollutants in different ways and these issues will result in problems in the future. Roughly, three decades ago, the World Health Organization (WHO) and the United Nations Global Environment Monitoring System (GEMS) have recognized environmental quality regarding air and water quality, food contamination etc. Moreover, the biological indicators have been monitored [2]. The presence of endocrine-disrupting compounds (EDCs) in the environment and their adverse effects on human and animal health has been favored recently [3]. Estrogens, androgens and thyroid hormones are the most common endocrine disrupters [4]. Estrogens include natural and artificial estrogens and industrial compounds that have estrogenic activity. Estrogens are steroid hormones that are mainly synthesized from cholesterol [5].17β- estradiol (E2) is the strongest natural primary estrogen among the numerous classes of natural and synthetic estrogen hormones. Estrone (E1) is a metabolite of E2 and slightly weaker (Fig.1). Estriol (E3) is the ultimate metabolite and the weakest natural estrogen, which has only 10% E2 activity. 17α- Ethynyl estradiol (EE2) is one of the synthetic estrogens applied in contraception [6].

The reproductive performance of mammals [7], fish [8], amphibian [9], reptiles [10], birds [11], aquatic invertebrates [12] and plants [13, 14] can be influenced by estrogens and estrogenic endocrine-disrupting compounds. Estrogen has an exasperating influence on breast tumor development and about 36% of breast cancers depend on this hormone. These hormones also contribute to cancers of the uterus, ovaries, and other cancers [15, 16]. 17β- estradiol is at the highest level in women, particularly pregnant women; it is involved in breast growth, the proliferation of epithelial cells and sexual development [17]. Recently, researchers have been trying to shed light on the fate of these compounds entering the environment with large volumes and thousands of sources of release [18]. The EDCs are released into the environment chiefly through sewages. Sludge and treated water are the

Address for correspondence: Hamid Mirzaei, Department of Food Hygiene, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
E-mail: mirzaeih51 @ gmail.com

How to cite this article: Abbaszadeh Maroufan, Kh., Mirzaei, H., Matin, A. A., Javadi, A., Amani-gadim, A. Environmental Monitoring of 17β- estradiol and Estrone in Ardabil's Drinking Water Source as Endocrine Disrupting Chemicals. Arch Pharma Pract 2019;10(3):98-106.

results of sewage treatment in treatment plants. Sludge is frequently applied as a fertilizer in farms and treated water for irrigation [19]. Possible problems of EDCs discharge to the environment are influencing soil microorganisms, pollution of ground and surface waters and affecting all living creatures. Day-to-day excretion of estrogens from women, men and pregnant women are 2.4, 1.5, and 259 µg, in the respective order [20-22]. It is believed that estrogen in the sheep is excreted more often through feces and in the pigs through urine [23]. Most of the estrogen excreted from animals consists of naturally occurring estrogen, E1 and E2 [24, 25]. Following excretion, the first estrogen is exposed to the stool and urine, enters the soil, and then enters the flow of natural water. Estrogens are found in current waters around worldwide [26, 27]. It is necessary to manage these compounds because of the high diversity and high environmental sustainability and impacts on cellular and molecular levels, the physical and destructive effects of EDCs [28]. The negative effects of these compounds include changes in cycles and sexual behaviors, negative effects on the immune system, abnormal sexual differentiation, tumors, and cancer. The main negative effect is there in involvement in the development of cancer in the body's hormonal regions, such as the breast, testicles and prostate as well as damaging the uterus during the postpartum period [20]. Such effects are dependent on the level of contact, life span and exposure time of the population. It has been reported that the maximum impact happens during fetal growth and childhood development [29]. The release of these compounds into water resources affects the aquatic and fish ecosystems negatively. Damage to fertility and mistakes during spawning are two of the negative effects of the hormone in aquatic animals [30]. In 1995, Palmer reported that 1 µg of hormone E2 per gram of frog and tortoise is capable of exerting carcinogenic effects [13, 14, 31].

The increased presence of EDCs in the surface, groundwater, and even drinking water have raised concerns in terms of preventing and removing these compounds using conventional treatment processes [32]. Humans and animals excrete large quantities of medicine and they enter the soil or nature by sewage, fertilizer, or sludge and most importantly, contraceptives including estrogen hormones that are excreted at high levels in humans. Upon the consumption of a drug, roughly 50-90 percent of it is excreted unchanged, and the rest is excreted from the body interactions in the form of chemical metabolites, such as byproducts, [33, 34]. The level of estrogen hormone in urban sewage is higher in many countries ranging from 12.5 to 23.7 ng L^{-1} [35]. The steroid hormones in the British internal wastewater are released into the rivers [36, 37], Italy [38, 39], China [40-42], the Netherlands [43], sewage in South Korea [40] and Germany [44, 45] and in water and wastewater in the cities of Tehran, Hamedan and Shiraz in Iran [16]. It is also found in drinking water in some parts of the United States [46]. Moreover, it has been observed in the shellfish and fish of Japan and Spain [47], China [48], river sediments in Spain [47, 49, 50] and French molluscs [51], as well as in crustaceans [52]. 17β- estradiol might amass in the bedding and plants of the sea and enter the human body through the food chain threatening the safety of the environment and marine life and human health in the end [53, 54]. As specified by the Australian Recycling Organization, the highest concentration of estrogen hormone is 74ngL^{-1} in water. According to WHO and FAO, estrogen hormone levels in drinking water is 0.03ng L^{-1} and the daily human intake is at a maximum of 0.0008 Micrograms per kilogram of weight [55, 56]. The European Watch list of emerging pollutants has been updated in 2013 by adding E2 and EE2. Contaminated food (milk, meat, etc.) and water expose human to estrogenic hormones [57].

The current study was carried out to assess drinking water sources in the city of Ardabil, Ardabil Province, Iran (Baliqlychay River and Yamchi dam). This catchment area is entirely inland and is one of the natural and virgin areas of Iran. Recently, this area has been experiencing developed touristic industry (hot water springs), fish farming, and urbanization. Thus, monitoring the target pollutants could be a good indicator of water quality in this region.

Experimental

Materials and Reagents

Estrone and 17β- estradiol; both were of analytical grade and were purchased from Sigma-Aldrich (USA). HPLC grade solvents including acetonitrile and water, KOH and n-Octanol were purchased from Merck (Germany). Polypropylene hollow fibers were purchased from Membrana (Germany).

Instruments

A Rigol high-performance liquid chromatograph (HPLC) model L3000 (China) equipped with UV/Vis detector was used for separation and quantification of Estrone and 17β-estradiol. The separation was carried out on Knauer ODS column, 250×4.6, 5 µm (Germany) using acetonitrile: water mixture (80:20) with a 1 mL min^{-1} flow rate as the mobile phase.

The Study Area

Ardabil is the capital of Ardabil Province in the north-west of Iran (N 38° latitude and E 48° 02' longitude). It is in the west south of the Caspian Sea and between two mountains of "Sabalan and Baghro" (Fig.2) [58]. Ardabil is 70km far away from the Caspian Sea and 210km away from the city of Tabriz. It is located in the basin of the Baliqlychay River. It has an average altitude of 1263 meters (4144 ft.) and a total area of 18011 km^2 (6954 sq. mi). Neighboring the Caspian Sea and the Republic of Azerbaijan, Ardabil has been of great political and economic significance throughout history, especially within the Caucasus region. It is also located in the East of Mount Sabalan (4811 m), where its peak is covered with snow throughout the year. According to the reports of the statistics center of Iran, the population of this city in 2011 was about 485,153 persons and the density of the city was 74 persons in a hectare and in 2016 was about 605,992 persons.

Yamchi dam in Ardabil Province, with the aim of providing safe water for the needs of agriculture and drinking water in Ardabil, was constructed on the Baliqlychay River. This dam was designed to provide 65 million m^3 of water for agriculture and 20 million m^3 per year for urban use in Ardabil [59]

Climate

Due to its cool climate during hot summer months, many tourists come from regions with a semi-arid climate. The winters are long and cold, with a record of a low temperature of −33 °C. The annual rainfall is around 380mm per year. The temperature of the Ardebil is between 35°C to -33°C during a year.

Sampling

This study was conducted over a 12-month period including four sampling periods (four seasons) from March 2018 to February 2019 and 23 samples were collected from the Ardabil drinking water supply river (Baliqlychay River). Samples were collected from 8 stations in the specified areas along the Baliqlychay River, the sewage outlet of Nir fish farms, crude sewage of Nir City, sewage treatment plant of Ardabil City and Ardabil drinking water distribution network, according to the standard sampling procedure of water and sewage using 250ml glass containers. The samples were transferred to the lab in an ice-cold box at 4 °C. Sampling areas (Fig. 3) were selected based on the research objectives as follows, using the GPS device and the geographic coordinates were presented in Table 1.

Extraction and Determination of Estrogenic Hormones in Water Samples

N-octanol loaded HF with length 2.5cm was immersed directly in the 50ml of aqueous sample (pH= 10.6). After 25 min of laboratory temperature, the extraction equilibrium was reached. The fiber was transferred into 2ml methanol as a desorption solvent under sonication. After 15 minutes, the analytes were desorbed. Finally, methanol was evaporated to dryness under the nitrogen stream; then, the residue was re-dissolved in the 40μL mobile phase and directly injected into the HPLC system for analysis.

Results and Discussion

The Concentration of 17β- estradiol (E2) and Estrone (E1) in Water Samples of the Ilianjug Station (Station No. 1)

The 17β- estradiol (E2) is the primary natural estrogen released from natural sources. The Estrone (E1) is a metabolite of (E2) and is slightly weaker in terms of estrogenic activity. The results show that in all samples for one year, the amount of E1 was less than the detection limit of the method. However, E2 concentration changed during four seasons. At this station, the concentration levels of E2 in the summer and autumn were under the detection limit but in winter, they reached the maximum level. The source of water in Ilianjug station melted snow and ice on Mount Sabalan peak which is covered by snow and glaciers throughout the year. During summer and autumn, the source of river water springs which are uncontaminated. During the summer, the slopes of the Sabalan Mountains, which are used as nomadic litter and the sheep grazing fields, soil contamination occurred due to sheep feces and urine. In recent years, the area has been favored by tourists and climbers. Regarding the dryness of the air and lack of proper rainfall during the summer and early spring, E2 accumulates on the surface of the soil. During autumn and winter with the beginning of the rainfall and flowing the surface waters, the entry of hormones into the water stream reaches its maximum level, with the highest concentration of E2 in winter estimated at 6.82μgL^{-1}.

Soil surface and its pollution were washed by rainfall during the autumn and winter; on the other hand, the accumulation of the hormone contamination was decreased due to nomadic litter and tourists leaving the region. In addition, frost on the slopes of Mount Sabalan decreases the concentration of hormones in the spring significantly. The amount of E2 was reported as 0.86 μgL^{-1} (Fig. 4).

Monitoring of the E2 and E1 in Reserved Water of Yamchi dam at the Jorab Area

At station No. 2 (Jorab area, the entrance of the dam), the lowest, and highest levels of E2 hormone were measured in the spring and summer, respectively, at 0.59 and 5.53 μgL^{-1}. In autumn and winter, the concentrations of E2 were found at 1.42 and 4.89μgL^{-1}, respectively. Various factors affect the amount of E2 in the reserved water of the dam including the volume of inputs to the dam by the rivers (including sewage of the city of Nir and wastewater of fish farms). So, the difference between the amount of E2 in stations No.1 and No.2 in summer was mainly related to the sewage of Nir and wastewater of fish farms. Thus, the amount of E2 in Nir crude sewage and fish farms wastewater were studied too. In addition, the sedimentation of E2 in sedimentary layers, its release, and re-entry from sediments, plants, and animals in the dam can affect the E2 amount in this station. In all seasons, E1 was not detected in this station just like station No.1.

The Concentration of E2 and E1 in Yamchi dam Outlet

Water drainage from the dam, which is used as drinking water in Ardabil, and for agricultural use, is without lining system and after about one km; it is entered into Ardabil Drinking Water Treatment Plant. Sampling was performed exactly at the exit point of the dam. E2 was detected in all samples during four seasons. The results show that in spring and winter, the lowest amount was 1.53 and the highest was 6.15μgL^{-1}, respectively. In addition, the reported E2 for summer and autumn levels were 5.30 and 1.74 μgL^{-1}, respectively. Due to sunlight and composition of entry flows to the dam, the water drainage layer, and evaporation affect

E2 level in station No. 3. E1 was not detected in this station for a year.

The Monitoring of the E2 and E1 in Ardabil Drinking Water Treatment Plant

Similar to previously studied stations, E1 was not detected in the Ardabil drinking water treatment plant outlet. However, E2 was detected in all samples. The results show that the lowest level of E2 was 0.63 μgL^{-1}in spring and its highest level was 5.23μgL^{-1} in summer. In addition, in autumn and winter, it was 1.98 and 2.26 μgL^{-1}, respectively. These results show that during spring and winter, the water treatment plant has acceptable efficiency and removes about 58.82% and 63.25% of E2, respectively. Nevertheless, during summer and autumn, there are no significant differences between input and outlet of the plant, which could be related to the increased water consumption during these seasons affecting the performance of the water treatment process negatively.

The Concentration of E2 and E1 in Ardabil Drinking Water Distribution Network

The drinking water distribution network of the Ardabil is 26km long, and during this period of study, it was monitored for E1 and E2. The E2 hormone was detected in all samples during four seasons. According to the results, the lowest level of E2 was recorded in the summer at 0.06μgL^{-1} and the highest level of E2 was measured in the autumn at 1.68μgL^{-1}. The concentration of E2 was measured at 1.57 and 0.87μgL^{-1} in spring and winter, respectively. According to section 3.4, the highest amount of E2 was recorded in the outlet of the water treatment plant (5.23μgL^{-1}). So, a significant decrease was observed from the water treatment plant to the water distribution network (0.06μgL^{-1}). Due to the weather conditions, it is obvious that water consumption in the urban area is high during summer. So, this excess consumed water was supplied from wells in the city. Therefore, the most logical reason for the decreasing concentration of the target compound in this study is dilution by adding well waters to treated water from the water treatment plant.

The Concentration of E2 and E1 in Wastewater of Fish Farms (Bolaghlar area of Nir)

The Bolaghlar area is located in the northwest of the city of Nir. In recent years, the salmon waterfowl farms were established in this region. Estrogenic hormones are widely used as growth agents and mono sexuality factors in fish production. The maximum feeding of the fish and their reproduction happens in spring. So, it seems that the amount of estrogenic hormones in the wastewater of the fish farms reaches the maximum level in this season. The wastewater of these farms will eventually enter the Yam Dam reservoir without any treatment. For this purpose, the outlet of two fish farms were sampled and analyzed. According to the results during the four seasons of the year, E1 was not detected in the samples, but E2 contaminated the Baliqlychay River through fish farms. The amount of E2 in the first fish farm station 6 (S6) during the study period was estimated to be 3.60, 3.52, 3.30 and 2.98 μgL^{-1} and in the second fish farm station 7 (S7), it was 3.54 and 3.49 and 3.22 and 2.25 μgL^{-1} respectively. The S7 fish farm was at a higher elevation than the S6 and part of the water needed was supplied from the water outlet S7. Therefore, it is only natural that the level of E2 contamination in the fish farm S6 was above S7.

The Concentration of E2 and E1 in Crude Sewage Discharges of Nir

The city of Nir is the closest city to the Yamchi dam and in the southwest area, has a population of roughly 20,000 people. The city lacks a wastewater treatment plant and its crude sewage containing urban, hospital, industrial and agricultural wastewater enters the Yamchi dam reservoir without treatment. For evaluation of its effect on the amount of estrogenic hormones on the quality of reserved water in the dam, sampling was carried out from crude sewage of the Nir during four seasons. Results show that E1 was not detected in the samples, but the E2 level in sewage cannot be ignored. Results show that the concentration of E2 in these samples was 4.12, 6.20, 3.89, and 3.98 μgL^{-1}during spring until winter. The results of hormones residues annual variation in 8 stations are presented in (Fig. 4).

Conclusions

In this study, the annual variation of E1 and E2 concentrations in Ardabil city water sources were investigated. According to the results, E1 was not reported at any of the stations, but E2 contamination was found in all seasons, with the main source being urban sewage and fish farms wastewater and surface wastewater from livestock and sheep grazing pastures.

References

1. Eisenberg D, Kauzmann W. The structure and properties of water. Oxford University Press on Demand; 2005 Oct 20.
2. Fischer K, Fries E, Körner W, Schmalz C, Zwiener C. New developments in the trace analysis of organic water pollutants. Applied microbiology and biotechnology. 2012 Apr 1;94(1):11-28.
3. Baldigo BP, George SD, Phillips PJ, Hemming JD, Denslow ND, Kroll KJ. Potential estrogenic effects of wastewaters on gene expression in Pimephales promelas and fish assemblages in streams of southeastern New York. Environmental toxicology and chemistry. 2015 Dec;34(12):2803-15.
4. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs Jr DR, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine reviews. 2012 Mar 14;33(3):378-455.
5. Pazoki M, Takdastan A, Jaafarzadeh N. Investigation of minimization of excess sludge production in sequencing batch reactor by heating some sludge. Asian Journal of Chemistry. 2010;22(3):1751-9.
6. Spencer AL, Bonnema R, McNamara MC. Helping women choose appropriate hormonal contraception: update on risks, benefits, and indications. The American journal of medicine. 2009 Jun 1;122(6):497-506.
7. Doshi T, Mehta SS, Dighe V, Balasinor N, Vanage G. Hypermethylation of estrogen receptor promoter region in adult testis of rats exposed neonatally to bisphenol A. Toxicology. 2011 Nov 18;289(2-3):74-82.
8. Runnalls TJ, Beresford N, Losty E, Scott AP, Sumpter JP. Several synthetic progestins with different potencies adversely affect reproduction of fish. Environmental science & technology. 2013 Feb 8;47(4):2077-84.

9. Kvarnryd M, Grabic R, Brandt I, Berg C. Early life progestin exposure causes arrested oocyte development, oviductal agenesis and sterility in adult Xenopus tropicalis frogs. Aquatic Toxicology. 2011 May 1;103(1-2):18-24.
10. Verderame M, Limatola E, Scudiero R. Estrogenic contamination by manure fertilizer in organic farming: a case study with the lizard Podarcis sicula. Ecotoxicology. 2016 Jan 1;25(1):105-14.
11. Tubbs CW. California condors and DDT: Examining the effects of endocrine disrupting chemicals in a critically endangered species. Endocrine Disruptors. 2016 Apr 1;4(1):e1173766.
12. Ciocan CM, Cubero-Leon E, Puinean AM, Hill EM, Minier C, Osada M, Fenlon K, Rotchell JM. Effects of estrogen exposure in mussels, Mytilus edulis, at different stages of gametogenesis. Environmental Pollution. 2010 Sep 1;158(9):2977-84.
13. Liu ZH, Kanjo Y, Mizutani S. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment—physical means, biodegradation, and chemical advanced oxidation: a review. Science of the total environment. 2009 Jan 1;407(2):731-48.
14. Mohagheghian A, Nabizadeh R, Mesdghinia A, Rastkari N, Mahvi AH, Alimohammadi M, Yunesian M, Ahmadkhaniha R, Nazmara S. Distribution of estrogenic steroids in municipal wastewater treatment plants in Tehran, Iran. Journal of Environmental Health Science and Engineering. 2014 Dec;12(1):97.
15. Zitnick KK, Shappell NW, Hakk H, DeSutter TM, Khan E, Casey FX. Effects of liquid swine manure on dissipation of 17β-estradiol in soil. Journal of hazardous materials. 2011 Feb 28;186(2-3):1111-7.
16. Mohebzadeh T, Taghizadeh MM, Takdastan A, Dehghani M. Comparing the performance of wastewater treatment using activated sludge and aerated lagoons processes in the removal efficiency of estradiol hormones. Jundishapur J Health Sci. 2013 Jun;5(3):149-56.
17. Havlíková L, Nováková L, Matysová L, Šícha J, Solich P. Determination of estradiol and its degradation products by liquid chromatography. Journal of Chromatography A. 2006 Jun 30;1119(1-2):216-23.
18. Thomas KV, Langford K. .1 Occurrence of pharmaceuticals in the aqueous environment. Comprehensive Analytical Chemistry. 2007 Jan 1;50:337-59.
19. Dębska B, Długosz J, Piotrowska-Długosz A, Banach-Szott M. The impact of a bio-fertilizer on the soil organic matter status and carbon sequestration—results from a field-scale study. Journal of Soils and Sediments. 2016 Oct 1;16(10):2335-43.
20. Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G. Global assessment of the state-of-the-science of endocrine disruptors. Geneva: World Health Organization. 2002 Feb:11-32.
21. Johnson AC, Belfroid A, Di Corcia A. Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Science of the Total Environment. 2000 Jul 10;256(2-3):163-73.
22. Sun W, Zhou K. Adsorption of 17β-estradiol by multi-walled carbon nanotubes in natural waters with or without aquatic colloids. Chemical Engineering Journal. 2014 Dec 15;258:185-93.
23. Schwarzenberger F, Möstl E, Palme R, Bamberg E. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Animal Reproduction Science. 1996 Apr 1;42(1-4):515-26.
24. Feng X, Ding S, Tu J, Wu F, Deng N. Degradation of estrone in aqueous solution by photo-Fenton system. Science of the Total Environment. 2005 Jun 1;345(1-3):229-37.
25. Combalbert S, Bellet V, Dabert P, Bernet N, Balaguer P, Hernandez-Raquet G. Fate of steroid hormones and endocrine activities in swine manure disposal and treatment facilities. Water research. 2012 Mar 1;46(3):895-906.
26. Lange IG, Daxenberger A, Schiffer B, Witters H, Ibarreta D, Meyer HH. Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. Analytica Chimica Acta. 2002 Nov 25;473(1-2):27-37.
27. Lorenzen A, Hendel JG, Conn KL, Bittman S, Kwabiah AB, Lazarovitz G, Masse D, McAllister TA, Topp E. Survey of hormone activities in municipal biosolids and animal manures. Environmental Toxicology: An International Journal. 2004 Jun;19(3):216-25.
28. Hamid H, Eskicioglu C. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. Water Research. 2012 Nov 15;46(18):5813-33.
29. Krein A, Pailler JY, Guignard C, Gutleb AC, Hoffmann L, Meyer B, Keßler S, Berckmans P, Witters HE. Determination of estrogen activity in river waters and wastewater in Luxembourg by chemical analysis and the yeast estrogen screen assay. Environment and Pollution. 2012 Jul 1;1(2):86.
30. Esplugas S, Bila DM, Krause LG, Dezotti M. Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. Journal of hazardous materials. 2007 Nov 19;149(3):631-42.
31. Palme R, Fischer P, Schildorfer H, Ismail MN. Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. Animal Reproduction Science. 1996 May 1;43(1):43-63.
32. Yıldırım SÖ, Akkurt M, Safari N, Amani V, McKee V. 2-[3-(2-Pyridyl) pyrazin-2-yl] pyridinium tetrachloridoaurate (III). Acta Crystallographica Section E: Structure Reports Online. 2009 May 1;65(5):m491-2.
33. Koh YK, Chiu TY, Boobis A, Cartmell E, Scrimshaw MD, Lester JN. Treatment and removal strategies for estrogens from wastewater. Environmental Technology. 2008 Mar 1;29(3):245-67.
34. Nejad MH, Takdastan A, Jaafarzadeh N, Mogadam MA, Mengelizadeh N. Removal of orthophosphate from municipal wastewater using chemical precipitation process in Ahvaz wastewater treatment plant, Iran. Asian Journal of Chemistry. 2013 Mar 21;25(5):2565.
35. Chang H, Wan Y, Hu J. Determination and source apportionment of five classes of steroid hormones in urban rivers. Environmental science & technology. 2009 Sep 17;43(20):7691-8.
36. Desbrow CE, Routledge EJ, Brighty GC, Sumpter JP, Waldock M. Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. Environmental science & technology. 1998 Jun 1;32(11):1549-58.
37. Rujiralai T, Bull ID, Llewellyn N, Evershed RP. In situ polar organic chemical integrative sampling (POCIS) of steroidal estrogens in sewage treatment works discharge and river water. Journal of Environmental Monitoring. 2011;13(5):1427-34.
38. Pojana G, Gomiero A, Jonkers N, Marcomini A. Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environment International. 2007 Oct 1;33(7):929-36.
39. Pojana G, Bonfà A, Busetti F, Collarin A, Marcomini A. Estrogenic potential of the Venice, Italy, lagoon waters. Environmental Toxicology and Chemistry: An International Journal. 2004 Aug;23(8):1874-80.
40. Ra JS, Lee SH, Lee J, Kim HY, Lim BJ, Kim SH, Kim SD. Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters. Journal of Environmental Monitoring. 2011;13(1):101-9.
41. Lu G, Yan Z, Wang Y, Chen W. Assessment of estrogenic contamination and biological effects in Lake Taihu. Ecotoxicology. 2011 Jul 1;20(5):974.
42. Zhou M, Li L, Dunson D, Carin L. Lognormal and gamma mixed negative binomial regression. InProceedings of the... International Conference on Machine Learning. International Conference on Machine Learning 2012 (Vol. 2012, p. 1343). NIH Public Access.
43. Belfroid, A.C., Schrap, S.M., Voogt, P. The occurrence of estrogenic hormones, bisphenol-A and phthalates in the aquatic environment of The Netherlands, In Vethaak AD (Ed.) Estrogens and xenoestrogens in the aquatic environment: an integrated approach for field monitoring and effect assessment, 2006; 53-75.
44. Körner W, Bolz U, Triebskorn R, Schwaiger J, Negele RD, Marx A, Hagnemaier H. Steroid analysis and xenosteroid potentials in two small streams in southwest Germany. Journal of Aquatic Ecosystem Stress and Recovery. 2001 Sep 1;8(3-4):215-29.
45. Hintemann T, Schneider C, Schöler HF, Schneider RJ. Field study using two immunoassays for the determination of estradiol and ethinylestradiol in the aquatic environment. Water research. 2006 Jul 1;40(12):2287-94.
46. Caldwell DJ, Mastrocco F, Nowak E, Johnston J, Yekel H, Pfeiffer D, Hoyt M, DuPlessie BM, Anderson PD. An assessment of potential

exposure and risk from estrogens in drinking water. Environmental health perspectives. 2009 Oct 13;118(3):338-44.
47. Jiang T, Zhao L, Chu B, Feng Q, Yan W, Lin JM. Molecularly imprinted solid-phase extraction for the selective determination of 17β-estradiol in fishery samples with high performance liquid chromatography. Talanta. 2009 Apr 30;78(2):442-7.
48. Gong J, Ran Y, Chen DY, Yang Y. Occurrence of endocrine-disrupting chemicals in riverine sediments from the Pearl River Delta, China. Marine pollution bulletin. 2011 Jan 1;63(5-12):556-63.
49. Petrović M, Eljarrat E, de Alda MJ, Barceló D. Analysis and environmental levels of endocrine-disrupting compounds in freshwater sediments. TrAC Trends in Analytical Chemistry. 2001 Nov 1;20(11):637-48.
50. de Alda MJ, Gil A, Paz E, Barceló D. Occurrence and analysis of estrogens and progestogens in river sediments by liquid chromatography-electrospray-mass spectrometry. Analyst. 2002;127(10):1299-304.
51. Zou L, Lin H, Jiang J. Determination of β-estradiol residues in fish/shellfish muscle by gas chromatography-mass spectrometry. Chinese Journal of Analytical Chemistry. 2007 Jul 1;35(7):983-7.
52. Lagadic L, Coutellec MA, Caquet T. Endocrine disruption in aquatic pulmonate molluscs: few evidences, many challenges. Ecotoxicology. 2007 Feb 1;16(1):45-59.
53. Lai KM, Scrimshaw MD, Lester JN. Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems. Science of the Total Environment. 2002 Apr 22;289(1-3):159-68.
54. Gomes RL, Deacon HE, Lai KM, Birkett JW, Scrimshaw MD, Lester JN. An assessment of the bioaccumulation of estrone in Daphnia magna. Environmental Toxicology and Chemistry: An International Journal. 2004 Jan;23(1):105-8.
55. Stumpe B, Marschner B. Long-term sewage sludge application and wastewater irrigation on the mineralization and sorption of 17β-estradiol and testosterone in soils. Science of the total environment. 2007 Mar 15;374(2-3):282-91.
56. Kakavandi B, Takdastan A, Jaafarzadeh N, Azizi M, Mirzaei A, Azari A. Application of Fe3O4@ C catalyzing heterogeneous UV-Fenton system for tetracycline removal with a focus on optimization by a response surface method. Journal of Photochemistry and Photobiology A: Chemistry. 2016 Jan 1;314:178-88.
57. Keresztes S, Tatár E, Czégény Z, Záray G, Mihucz VG. Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water. Science of the total environment. 2013 Aug 1;458:451-8.
58. Asmin HE. An Analysis on Studies of Population Developments of Ardabil City, 2014.
59. Torabi Jafroudi H, Rahimibashar MR, Taghavi H. The effect of environmental factors on the distribution pattern of Mytilaster lineatus (Gmelin, 1789) bivalves in the rocky shores of the southern Caspian Sea basin. Journal of Marine Science and Technology. 2017;16(2):93-102.

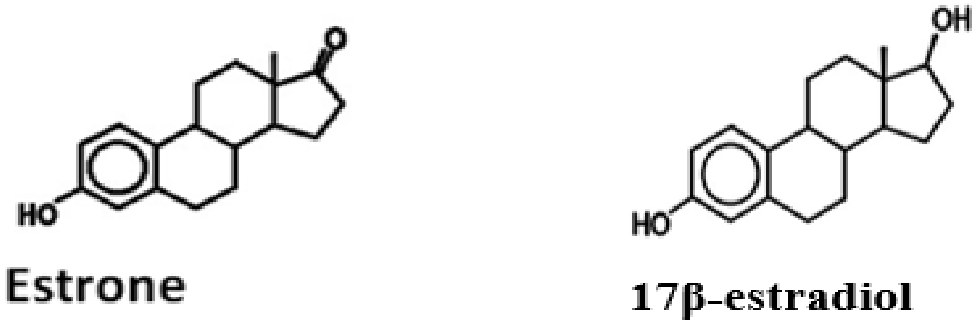

Figure 1. Structure of the Estrone, 17β- estradiol

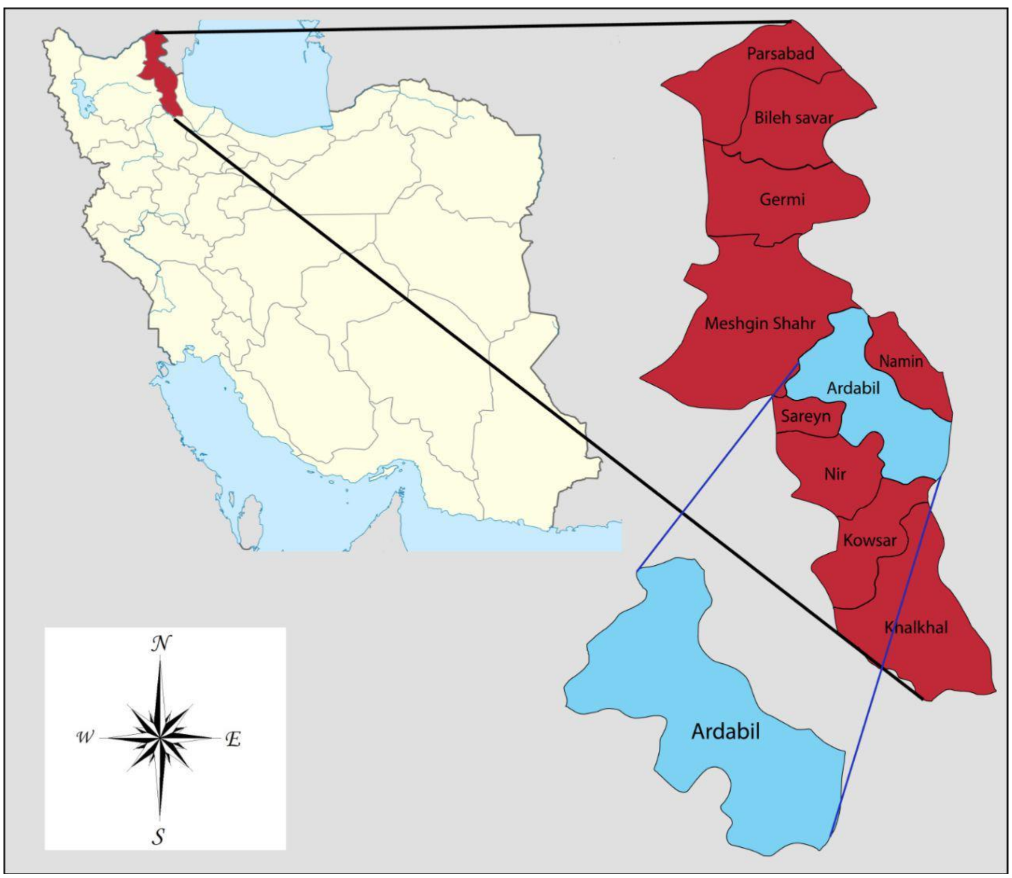

Figure 2. The geographic location of Ardabil City

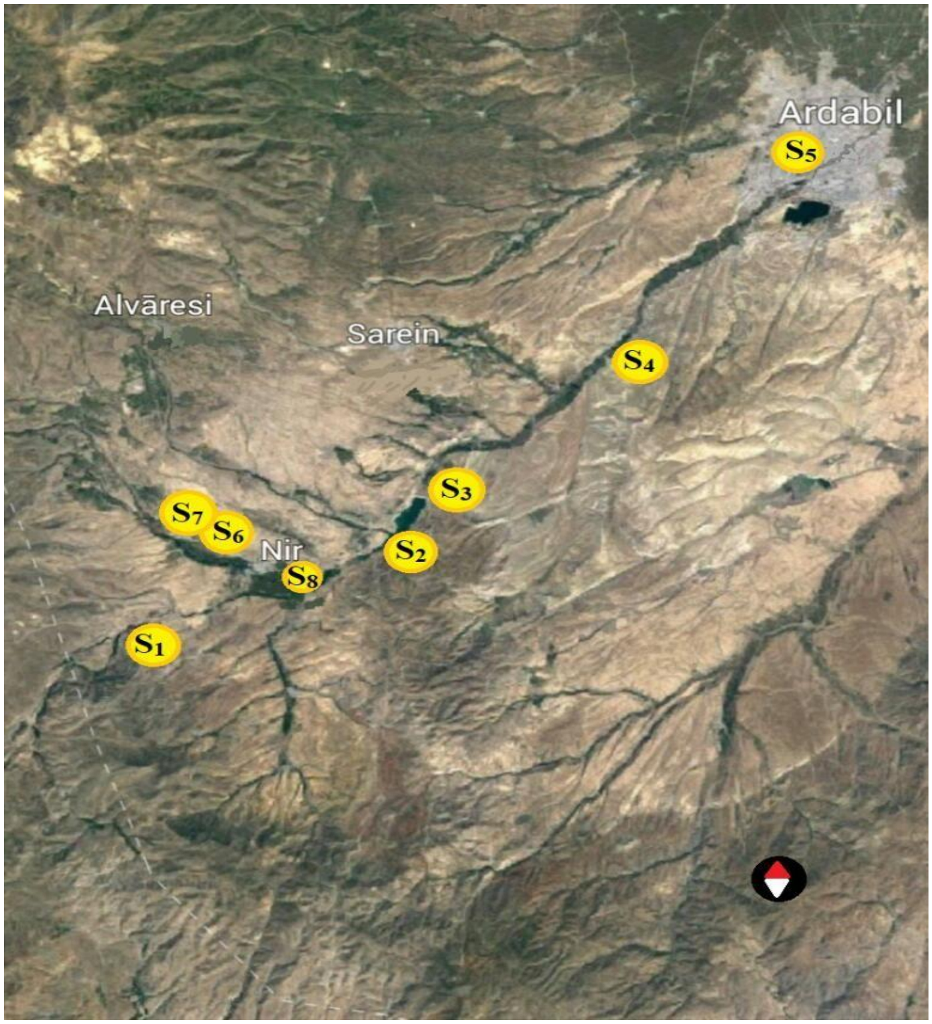

Figure 3. Location of sampling stations

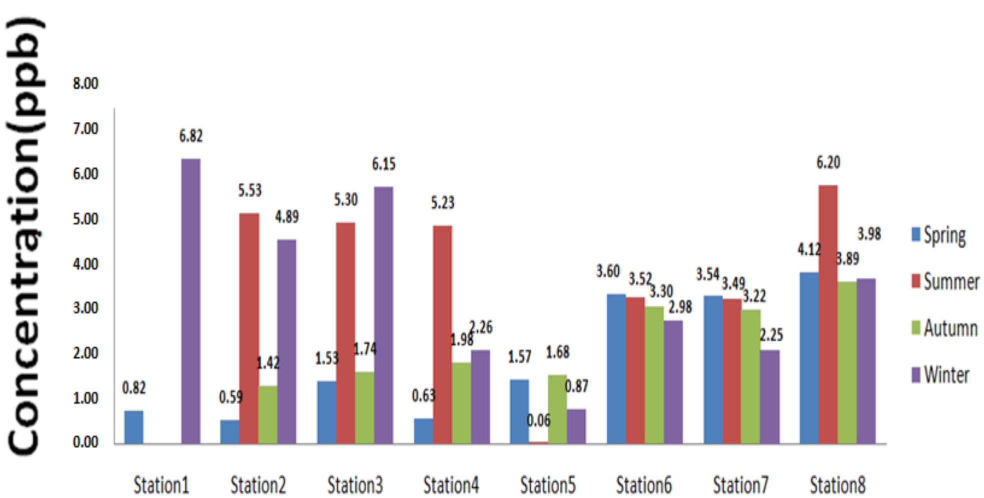

Figure 4. E2 concentrations in 8 stations during four seasons

Table 1: Coordinates of sampling sites Extraction and determination of estrogenic Hormones in water samples

Station. no	X	Y
Station 1	380048	475924
Station 2	380305	480356
Station 3	380426	480502
Station 4	380509	480502
Station 5	380509	480522
Station 6	380209	475902
Station 7	38008	475800
Station 8	380141	480017