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"Study of the Measurement of Environmental Estrogen Level from Drinking Water in Dhaka City" - A Cross Sectional Study

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Abstract: Environmental pollutants appearing in waste water, bottled mineral water, tap water, and bottled drinking water are potential, but yet poorly characterized, sources of human exposure to endocrine disrupting chemicals (EDCs) globally. Here, we have investigated the level of environmental oestrogen in drinking water (filter/bottle and jar drinking water) in the most densely populated region in Dhaka city. We draw attention in drinking water to the potential risk of intensive modern agriculture and waste disposal systems on oestrogen release levels and their effects on human health. Influent and effluent bimonthly samples were taken at two distinct times throughout the previous and current years (2023 and 2024) from a major water treatment plant (WASA) in Dhaka city. In addition to tap water (direct supply from WASA) from households, different brands of bottled (mineral water), filter drinking water, and jar drinking water were also examined, as were equivalent samples from a household water purification facility situated in the same region. Samples were collected in sterile, one-litre containers, and an enzyme-linked immunosorbent assay (ELISA) was used to determine the samples' oestrogenic potential. To address this knowledge gap, this study measured the environmental oestrogen level in tap drinking water (jar-container) and bottle drinking water (mineral). Quantitative enzyme-linked immunosorbent assays (ELISA) were used to determine concentrations of 17α-ethinyl estradiol (EE2). The highest concentrations were measured in samples taken from the Jar drinking water at 20.0 ng/L, and the lowest were 3.02 ng/L, respectively. The concentrations of 17α-ethinyl estradiol (3.0–20.0 ng/L) varied somewhat between locations and sampling periods ($p < 0.00$); however, patterns were not consistent. The EE2 concentrations measured in the filter water and bottle water were all undefined values (mostly below 0.01 ng/L), which created difficulties in interpretation due to problems associated with trying to measure such low concentrations with confidence. In this study, we found the higher level of environmental oestrogen in Jar drinking water in this region.

Keywords: *Endocrine-disrupting chemicals, environmental estrogen level, drinking water, tap water, mineral water (bottle water), Enzyme-Linked Immunosorbent Assay (ELISA)*

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1. Introduction

Endocrine disruptors, also referred to as environmental hormones, are environmental substances capable of inducing abnormal effects on the biological endocrine system and other related pathways [1]. Among these, environmental oestrogens stand out as the most prevalent hazard category currently, garnering significant attention due to their extensive sources, substantial risks, and prolonged latency periods [2,3].

EEs are a class of compounds primarily composed of benzene rings, with molecular weights ranging from 150 to 400. Their chemical structures bear resemblance to endogenous estrogens. EEs can be broadly categorised into two groups based on their sources: natural and synthetic estrogens [4]. In addition to these, various synthetic chemicals, including pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), alkyl phenolic compounds in nonionic surfactants, plasticisers, and antioxidants, exhibit weak EE activity [5]. Studies have shown that EEs are predominantly detected in the environment at concentrations in the ng/L range, with heavily polluted areas reaching levels as high as μg/L. Although the concentrations may be relatively low, EEs present in natural water bodies can imitate the effects of endogenous oestrogens or disrupt their normal functioning upon entering organisms [6]. This interference can have adverse effects on animal behaviour, disrupt endocrine systems, impede reproductive development, and ultimately lead to increased rates of biological lethality, carcinogenicity, and teratogenicity. Consequently, the water environment, ecosystem, and human health can be significantly impacted by the presence of EEs [7,8].

In recent decades, there has been a significant focus on the study of endocrine-disrupting compounds (EDCs) due to their potential negative impacts on both human and wildlife endocrine systems [9]. Among the various EDCs that have been identified, 17α-ethynyl-oestradiol (EE2) has garnered considerable attention as a synthetic oestrogen with a particularly potent ooestrogenic effect. The global consumption of EE2 was estimated to be approximately 1000 kg in 1998, highlighting its widespread use and potential environmental implications [9,10].

Initially discovered in Germany in January 1938, EE2 was primarily utilised for medical purposes such as treating prostate and breast cancers, chronic hemispheric, and inducing labour [11]. However, it was not until the 1990s that EE2 began to be recognised as a significant environmental contaminant. Since then, research on the occurrence, fate, and toxicological effects of EE2 in wastewater and other environmental settings has become a prominent area of study worldwide, reflecting the growing concern over its potential impact [12,13].

Given the potential risks posed by EE2 to human health, efforts have been made to address its presence in municipal wastewater treatment plants (WWTPs) and other environments [14]. The inclusion of EE2 in the Japanese Drinking Water Quality Standard and the consideration of regulating it as a priority micropollutant in the Drinking Water Quality Standards of European countries underscore the importance of monitoring and managing EE2 contamination [15]. As surface water serves as a crucial source of drinking water for many nations, the global contamination status of EE2 in surface waters has emerged as a significant area of interest and concern [16].

The global human population, estimated at around 7 billion individuals, contributes to the discharge of approximately 30,000 kg/yr. of natural steroidal ooestrogens (E1, E2, and E3) and an additional 700 kg/yr [17]. of synthetic ooestrogens (EE2) through the widespread use of birth control pills. Despite these significant figures, the discharge of oestrogens from livestock activities surpasses that from human sources by a considerable margin. In regions such as the United States and the European Union, the annual discharge of oestrogens by livestock stands at 83,000 kg/yr, more than double the rate of human discharge. This disparity underscores the substantial impact of livestock-related activities on environmental oestrogen levels [18,19].

The presence of oestrogens in the environment, originating from both human and animal waste, poses a significant risk to ecological systems and human health. Studies have linked the detection of ooestrogens in aquatic environments to concentrated animal feeding operations (CAFOs), highlighting the potential role of livestock activities in oestrogen lution [20]. Furthermore, the application of animal manure or sludge biosolids to agricultural lands, commonly used as organic fertilisers, has been identified as a key contributor to environmental oestrogen evels [21]. This practice, prevalent in modern agriculture, underscores the need for a comprehensive understanding of the sources and impacts of oestrogen pollutants [22].

Given the serious implications of oestrogen pollution on environmental and human health, it is crucial to address the issue from a holistic perspective. By examining the sources, pathways, and impacts of oestrogens in the environment, we can develop effective strategies to mitigate their harmful ffects [23]. Understanding the role of livestock activities, agricultural practices, and human behaviours in oestrogen pollution is essential for implementing sustainable solutions that safeguard both ecological integrity and public health [24]. Through comprehensive research and informed decision-making, we can work towards minimising the environmental impact of oestrogens and promoting a healthier, more sustainable future for all [25].

2. Materials and Methods

Sampling and description of study site

Water samples, treated and untreated, were collected bimonthly from various cities and districts in Bangladesh over a 20-month period in 2022 and 2024. Dhaka WASA, the largest water plant in the country, caters to over ten million residents by processing both household (71%) and industrial (29%) water from five districts. The plant receives around 20,00,000 m^3 of wastewater daily and treats an average of 2550 million m^3 annually. Influent samples were taken before treatment, while effluent samples were collected post-purification before discharge into the distribution system.

In addition to the samples from Dhaka WASA, water

samples were also obtained from a household purification plant in the same region in March, April, and June of 2024. The household plant sources its raw water from Dhaka WASA and Jar Drinking Water. This allowed for a comparison between the quality of water at the centralised treatment plant and the household purification facility, providing valuable insights into the overall water quality in the area.

Furthermore, tap water samples (both hot and cold) were gathered twice a month from various locations in Bangladesh, including residential buildings in Dhaka, during a 3-month period in 2024. Additionally, ten different brands of bottled still and mineral waters were purchased from a local grocery store in Dhaka for further analysis. This comprehensive sampling approach aimed to assess the quality of water from different sources and distribution systems to ensure the safety and reliability of the drinking water supply in the region.

Chemicals and methods

The research paper's method and materials section employed a variety of equipment and substances to carry out the experiment. Initially, a pipette was utilised to precisely measure and transfer small volumes of liquids. Following that, an ELISA microtiter plate was used as the experimental platform. To ensure the cleanliness of the plate, a specialised device designed for plate washing was employed, effectively eliminating any residual substances and guaranteeing reliable results.

Additionally, an ELISA reader and computer were incorporated into the experiment. The ELISA reader was responsible for measuring the optical density of the samples, while the computer was utilised to analyse and record the acquired data. Alongside the apparatus, various reagents were employed throughout the experiment. An incubator, specifically a test kit, was used to create the ideal conditions for the experiment.

To coat the ELISA microtiter plate, a coating buffer was employed, providing the necessary conditions for the immobilization of the target molecules onto the plate surface. A washing buffer was also utilized to eliminate any unbound substances and ensure the experiment's specificity. Furthermore, an HRP conjugate served as a detection reagent, enabling the visualization and quantification of the target molecules. Lastly, a stop solution was employed to halt the reaction and stabilize the results, preventing any further alterations in the samples and allowing for accurate measurements to be obtained.

Sample preparation

The initial step in preparing the sample involves adding 0.5 ml of water into a centrifuge tube. This critical step is necessary to ensure that the sample is adequately diluted and primed for subsequent analysis. The addition of water creates an optimal environment for the sample to be effectively processed, setting the stage for accurate results to be obtained.

Following the addition of water, the next step is to introduce 0.5 ml of sample diluent into the mixture within the centrifuge tube. The sample diluent serves a pivotal role in the preparation process by facilitating the proper conditioning of the sample for analysis. Through the incorporation of the diluent, the conditions are optimised to yield precise and dependable results from the sample.

Once the sample and diluent have been thoroughly mixed, it is imperative to extract 50 microlitres of the supernatant for analysis. This final step is crucial in guaranteeing that the sample is suitably processed and ready for further examination. By meticulously measuring the supernatant, the analysis can be conducted with precision and efficiency, ensuring the reliability of the results obtained.

Procedure

The initial step in the ELISA procedure involves the systematic numbering of both the samples and standard samples in the wells of the microtiter plate. This numbering system is crucial for maintaining a clear record of the various components added to each well during the experiment. Proper labelling of each well is essential to guaranteeing the precision and accuracy of the results obtained from the ELISA assay.

Subsequently, a volume of 50 microlitres of either the standard or sample is carefully added to each well. This step is fundamental as it introduces the substances that will undergo analysis in the ELISA assay. The precise measurement of 50 microlitres is vital to ensuring the consistency and reliability of the results obtained from the experiment.

After the samples have been added, 50 microlitres of the antibody working solution should be added to each well. Following this, the plate should be sealed with a plate sealer to prevent any potential contamination or evaporation during the incubation process. This particular step is critical in facilitating the effective binding of antibodies to the target molecules present in the samples. Subsequently, the plate should be gently shaken for 10 seconds and then placed in an incubator for 30 minutes at a temperature of 26 degrees Celsius. This incubation period allows for the formation of antigen-antibody complexes, while the shaking ensures the uniform distribution of components in the wells, ultimately leading to more precise results in the ELISA assay [26].

Data analysis

The data analysis section of the research paper employed a combination of statistical software tools, namely SPSS, MS Excel, and R, to ensure a comprehensive and robust analysis. SPSS, a widely recognized statistical software, was utilized to conduct various statistical tests and analyses on the collected data. This software offered a user-friendly interface and a wide range of statistical techniques, allowing for the exploration of relationships and patterns within the dataset. MS Excel, a versatile spreadsheet programmed, played a crucial role in organizing and manipulating the data, facilitating efficient calculations and dualizations. Lastly, R, a powerful programming language, was employed for advanced statistical modelling and data visualization, enabling the researchers to delve deeper into the research findings and uncover additional insights. The utilization of these software tools ensured a rigorous and comprehensive analysis of the data, contributing to the overall quality and validity of the research findings.

3. Results and Discussion

Table 1 and Figure 1 show that filtered/bottled drinking water (mineral) has significantly different oestrogen contents than jar drinking water. In particular, compared to Jar drinking water, where it is found in a significant portion of the samples, oestrogen is absent from most Filtered/Bottled drinking water (mineral) samples. There were 45 examples where oestrogen was absent from iltered/bottled drinking water (mineral) and none where it was present. The statistical significance of the difference is indicated by the P-value of 0.000. Consequently, it implies that oestrogen is much less present in this kind of water than it is in others. Additionally, there were 21 situations where oestrogen was identified in Jar drinking water and 34 cases where it was not. Here, too, the p-value (0.000) suggests a statistically significant result. This implies that the amount of oestrogen present in this kind of water differs significantly from that which is absent.

Table 1. Presence of Estrogen level in different types of drinking water samples

Water Type	Estrogen (Number of Samples=100)		P-Value
Filtered/Bottled drinking water (Mineral)	Absent	45	0.000*
	Present	0	
Jar drinking Water	Absent	34	0.000*
	Present	21	
Total		100	

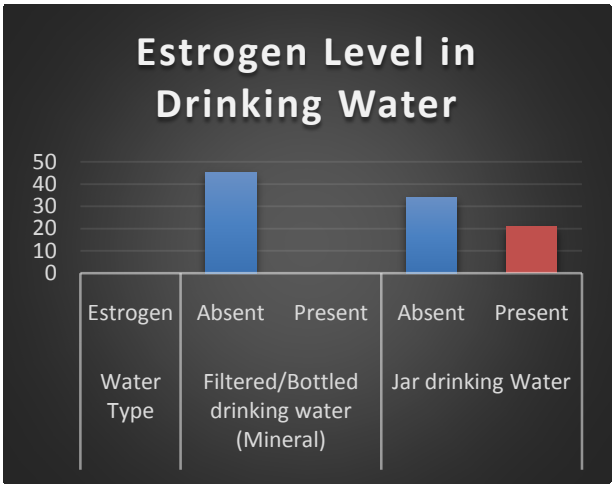

Figure 1. Presence of Estrogen level in different types of drinking water

According to the Table 1 data, compared to filtered/bottled drinking water (mineral)—where ooestrogen is mostly absent—Jar drinking water tends to have larger measurable levels of ooestrogen across different concentration ranges. Statistical significance is indicated by the supplied p-value for this difference. Understanding these distinctions is essential for evaluating the possible negative health effects of oestrogen exposure from different types of drinking water, and it can guide decisions regarding policy and public health recommendations. Especially oestrogen is mostly absent (45 cases) in this type of water, particularly for filtered or bottled drinking water (mineral); no detectable quantities were found in any other concentration category. Furthermore, oestrogen levels in Jar drinking water vary more than in other types of water; three samples had levels below 5 ng/L, while the percentages of samples in higher concentration categories (6–10 ng/L, 11–15 ng/L, and 16–20 ng/L) increased (Table 2 and Figure 2).

Table 2. Distribution of Estrogen levels in Filtered / Bottled and Jar Drinking water samples

	Water Type		
Estrogen Level (ng/L)	Filtered/Bottled drinking water (Mineral)	Jar drinking Water	P-Value
Absent	45	34	0.000*
Less than 5	0	3	
6 - 10	0	4	
11 - 15	0	6	
16 - 20	0	8	
Total	45	55	

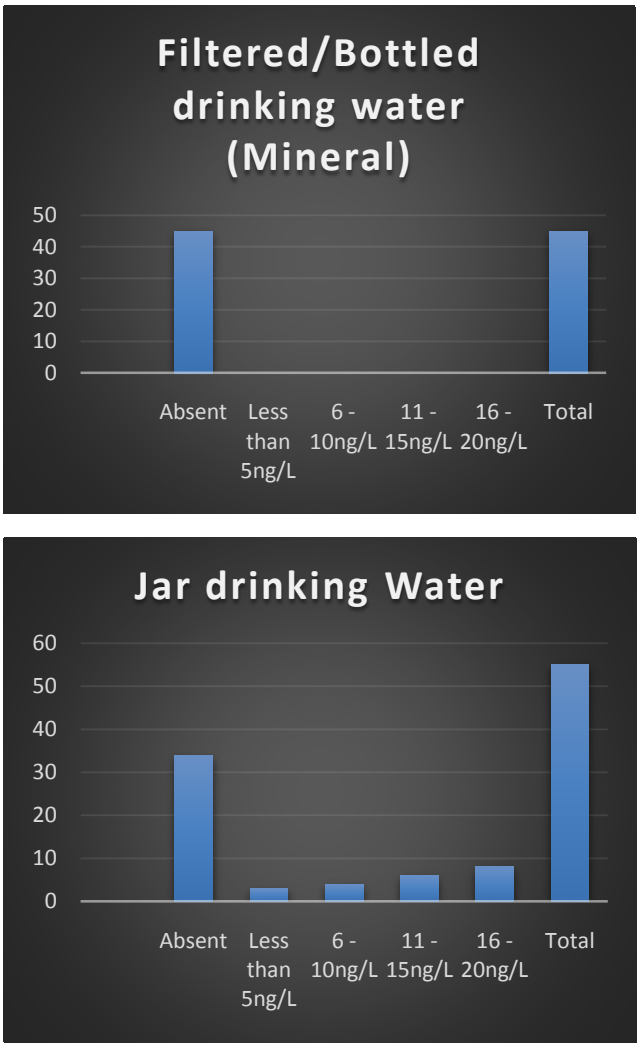

Figure 2. Quantity of estrogen level in different types of drinking water samples

4. Conclusion

These findings indicate that the purification method applied in Dhaka city with mechanical, chemical, and biological purification steps is effective in reducing oestrogenic activity. On the other hand, jar drinking water that was collected from different suppliers is not free of oestrogen compounds for the population in this region.

Recommendations

Future studies can examine the oestrogen level from tube well water in the rural areas of Bangladesh. Also, we can investigate the river and pond water in the different areas of Bangladesh where a large number of populations are using this water frequently to prepare their food. Already some reference articles reported that boiling drinking water could not destroy the environmental oestrogen in drinking water. In our study, we found that only a good-quality water filter machine can exclude the environmental oestrogen from Jar water.

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Conflict of interest: None

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