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Determination of Bisphenol A and Phthalate Levels in Wastewater Samples

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ABSTRACT

Objective: The use of endocrine-disrupting chemicals (EDCs) such as bisphenol A (BPA) in plastics manufacturing, agriculture, livestock, and paint manufacturing increas daily. The water treated in wastewater treatment plants is used in many areas such as irrigation of parks and gardens, and reinforcement of underground water resources. However, whether the treatment process eliminates EDCs in wastewater is not exactly known, and determining this as well as the amounts of these chemicals in treated water are important in terms of protecting the environment and human health. The aim of the study was to determine BPA and phthalate concentrations in the influent and effluent flow samples obtained from wastewater treatment plants.

Materials and Methods: BPA and phthalate concentrations were measured in influent and effluent flow samples using the enzyme-linked immunosorbent assay (ELISA) method. BPA and phthalate measurements were performed as competitive measurements of BPA and total phthalates in samples using specific monoclonal antibodies.

Results: BPA and phthalate levels were measured respectively as 7.69 µg/L and 78.27 µg/L in the influent water samples and 3.17 µg/L and 25.56 µg/L in the effluent water samples. The concentration of BPA and phthalates in the effluent samples decreased significantly compared to the influent water samples.

Conclusion: This study is believed to shed light on the importance of monitoring BPA and phthalate concentrations in wastewater treatment plants and inspections for detecting other EDCs in wastewater.

Keywords: Wastewater, bisphenol A, phthalate, endocrine-disrupting chemical

INTRODUCTION

Although water is the most abundant molecule on Earth, humans are able to use only 0.3% of this water for drinking purposes. In addition, the need for potable water and utility water have increased daily due to rapid population growth, environmental pollution, global warming, and limited fresh water resources. Therefore, urgent measures should be taken against environmental and water pollution, and new water sources should be sought. In this regard, being able to effectively remove toxic chemicals from wastewater is important for reuse in homes, agriculture, and industry (1-3). Around the globe, domestic water and safe water resources are rapidly diminishing and heading toward a

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point of depletion. Wastewater needs to be treated in order to minimize the possibility of contaminating drinking and utility water and using water resources more efficiently. Wastewater treatment is an applied physical, chemical, and biological recycling process for regaining some or all of the physical, chemical, and bacteriological properties of water that have been lost as a result of various uses (4, 5). After being subjected to appropriate treatment processes in wastewater treatment plants, water that registers non-toxic values by considering its biological characteristics can be used for irrigating agricultural areas, recreation areas, urban green spaces, car washes, toilets, heating pipes, concrete construction works, firefighting, and drinking water and can be assessed as an alternative to domestic water supplies (3, 6).

Endocrine-disrupting chemicals (EDCs) are exogenous agents that can mimic hormones and are responsible for maintaining homeostasis and regulating developmental processes; EDCs can also cause the production, release, transport, binding, and elimination of natural hormones in the body (7).

Agriculture, animal husbandry, industrial production, and technological developments have increased alongside the rapid human population growth around the world. These increases have caused rising for the use of EDCs such as bisphenol A (BPA), phthalates, atrazine, dioxins, diethylstilbesterol, and genistein in pesticides, herbicides, the manufacture of plastics, pharmaceuticals, the textile industry, livestock, the dye industry, and sewage systems.

EDCs cannot be completely removed through conventional wastewater treatment methods in treatment plants and thus spread to the environment. The literature has reported these EDCs to largely be fully non-degradable in treatment plants, to be detected in effluent flows, and to contaminate groundwater, potable water, and utility water supplies (7).

The aim of this study was to evaluate BPA and phthalate concentrations in the influent and effluent water samples from wastewater treatment plants. For this aim, BPA and total phthalate concentrations were measured using the enzyme-linked immunosorbent assay (ELISA) method in the influent and effluent water samples obtained from wastewater treatment plants. The BPA and phthalate levels were measured competitively with regard to BPA and total phthalate levels in specimens using specific monoclonal antibodies.

MATERIALS AND METHODS

The study has been carried out over seven influent and seven effluent water samples obtained from the Ataköy Advanced Biological Wastewater Treatment Plant at various times over a six-month period during the spring and summer. Influent water samples were obtained from the post-screen, pre-sand and grease trap sections by means of a sample collection device, while the effluent samples were obtained from a part of the facility close to the discharge point using a composite sampling device and then transferred to sterile 250 mL plastic containers. These influent and effluent water samples were then delivered to the laboratory through a light-proof black bag. The samples were transferred to 50 mL centrifuge tubes and centrifuged at 3,000 rpm for 2 minutes to separate the supernatants. Next, the wastewater and treated wastewater samples were transferred to prewashed glass containers that had been autoclaved and rinsed with methanol. The wastewater samples were filtered through a 0.45 micrometer Sartorius Minisart® NML injector filter containing cellulose acetate. Subsequently, the glass containers were wrapped with aluminum foil and stored in a deep freezer at -80°C. One day prior to the day of the study, the samples were transferred to a refrigerator at +4°C (8).

BPA Measurement

The assay is based on the principle that BPA is directly recognized and bound by monoclonal antibodies (Ecologiena Supersensitive BPA ELISA Kit; Tokiwa Chemical Industries, Tokyo, Japan). Samples containing BPA and the BPA-enzyme mixture were added to each well of the microplate and competed for the binding sites of specific antibodies fixed on the surface of these wells.

BPA and the excess BPA-enzyme mixtures that didn't bind after the first treatment were washed away. The presence of BPA is detected by addition of a color solution. Enzyme-labeled BPA bound to the BPA antibody on the plate triggers the substrate to turn a specific color. After a certain incubation time, the reaction is stopped by addition of a diluted acid. The absorbance measured by the intensity of the color that formed varied inversely with the amount of BPA present in the samples. In this way, the BPA concentrations in the examined samples were determined.

The standard curve was obtained according to the absorbance value at 450 nm based on the BPA standards of known concentrations. The BPA concentration for each sample has been calculated using the absorbance values obtained from the standard curve.

Di-(2-ethylhexyl) Phthalate (DEHP) Measurement

Direct competitive ELISA (Abraxis Phthalates ELISA, Railroad Drive, Warminster, PA) method was used based on the detection of test phthalates with specific antibodies. Samples containing DEHP and a phthalate-enzyme mixture were added to the wells of the microplate. The phthalates present in the samples and the phthalate-enzyme mixture competed for the binding sites of the anti-phthalate antibodies in solution. The phthalate antibodies then bind with a second antibody (goat anti-rabbit) fixed to the surface of the wells.

After the washing step and the addition of the substrate solution, a blue color developed. The color reaction was stopped after 30 minutes by addition of a diluted acid. The intensity of the blue color was inversely proportional to the phthalate concentration in the sample.

A standard curve was obtained with respect to absorbance at 450 nm based on DEHP standards of known concentrations.

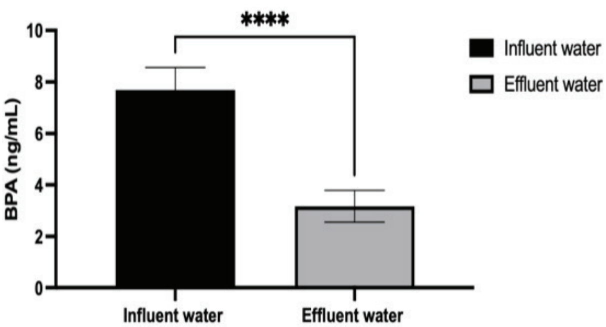
The DEHP concentration in each sample was calculated using the absorbance values obtained from the standard curve.

Statistical Analyses

GraphPad Prism 9.0 (GraphPad Software, San Diego, USA) was used for all statistical analyses. All data are expressed as a mean ± the standard deviation (SD). Unpaired t-test was used to evaluate the differences between the groups. The correlation between BPA and DEHP levels in the water samples were determined using the Pearson correlation analysis, with a value of $p < 0.05$ being considered statistically significant.

RESULTS

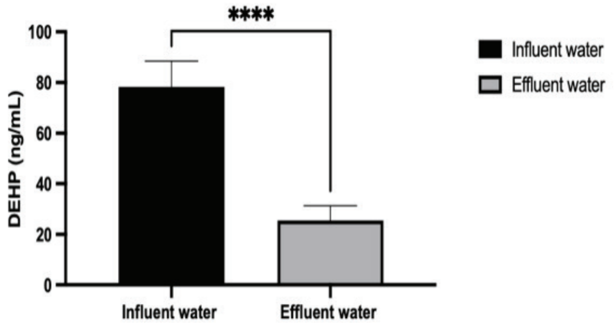
Figure 1 provides the BPA levels (ng/mL) in the influent and effluent water samples from the wastewater treatment plant. BPA levels decreased significantly in the effluent water samples when compared to the influent water samples ($p < 0.0001$). Figure 2 provides the DEHP levels in the influent and effluent water samples of the wastewater treatment plant. Significantly decreased DEHP levels were found in the effluent water samples when compared to the influent water samples ($p < 0.0001$).



	Influent Water	Effluent Water
BPA (ng/mL)	7.69±0.87	3.17±0.62****

Figure 1. BPA (ng/mL) levels in the influent and effluent water samples of the wastewater treatment plant.

Table 1 presents the findings from the correlation analysis of the BPA and DEHP levels in the influent and effluent water samples from the wastewater treatment plant. Accordingly, BPA levels in the influent water and effluent water samples were found to be positively correlated.



	Influent Water	Effluent Water
DEHP (ng/mL)	78.27±10.15	25.56±5.75****

Figure 2. DEHP levels in the influent and effluent water samples of the wastewater treatment plant.

DISCUSSION

Endocrine disruptors such as BPA and phthalates that come to wastewater treatment plants through plastics cannot be completely eliminated during treatment processes. Such chemicals cause environmental pollution by being discharged to environments such as rivers, lakes, and seas through the effluent flows of the treatment plant. However, some studies have reported BFA to be degradable within 2 days after being discharged into a receiving environment (9, 10). The concentrations of BPA and similar chemicals in rivers and streams under hydrological and climatic conditions have been shown to occur in the range of 0.5-16 ng/L in Germany and 90-12.000 ng/L in the United States (11, 12). Another study conducted in Germany determined BPA levels to be 1927 ng/L at the point where the wastewater treatment plant discharged into a river (13). The presence of BPA has been detected even in wastewater sediments in China, Korea, and Japan (14-16).

Phthalates are distributed over a wide range of concentrations in streams where wastewater is discharged based on their physicochemical characteristics. Among these phthalates, DEHP is the most common, with a concentration range of 0.05-4.95 µg/L (17). Phthalates are found in wastewater sediments as high molecular-weight compounds such as di-n-butyl phthalate, butyl benzyl phthalate, di-isobutyl phthalate, and diethyl-hexyl phthalate (18, 19).

Table 1. Correlation of BPA and DEHP levels in the influent and effluent water samples of the wastewater treatment plant.

	BPA in influent water samples	BPA in effluent water samples	DEHP in influent water samples	DEHP in effluent water samples
BPA in influent water samples		r=0.921 p=0.026*	r= 0.109 p=0.861	r= -0.547 p=0.340
BPA in effluent water samples	r= 0.921 p=0.026*		r= 0.091 p=0.884	r= -0.382 p=0.526
DEHP in influent water samples	r= 0.109 p=0.861	r= 0.091 p=0,884		r=0.670 p=0.216
DEHP in effluent water samples	r= -0.547 p=0.340	r= -0.382 p=0.526	r= 0.670 p=0.216	

*: p< 0.05

At the beginning of the 2000s, the European Water Framework Directive identified DEHP as one of 33 hazardous materials. According to the Environmental Quality Standards (EQS), the recommended annual average concentration value for surface waters is 1.3 µg/L, with a maximum of 4.720 µg/kg dry weight for sediments. The discharge values for DEHP in receiving waters such as the lakes, rivers, and streams where wastewater is discharged in Europe are 3.2 µg/L, with a 30 mg/kg dry weight for sediments (20). Wastewaters host quite a few chemicals that cause various pathologies in humans. Due to the reusable properties of these waters, determining the presence and amount of EDCs in treated wastewater in terms of protecting public health and preventing environmental pollution has gained importance. In accordance with this argument, the current study aimed to determine the concentrations of BPA and phthalates in the inlet and outlet waters of wastewater treatment plants, with both phthalate and BPA being found in all the wastewater samples that were taken. According to the obtained data, the overall BPA and phthalate levels in the influent flow to the facility that underwent advanced biological wastewater treatment were found to be 7.69 µg/L and 78.27 µg/L, respectively, with the respective levels in the effluent water being 3.17 µg/L and 25.56 µg/L. The study found the concentrations of BPA and phthalates in the effluent water samples to have significantly decreased compared to the influent water samples.

Because some endocrine disruptors are not fully biologically degradable due to their physicochemical structures, they spread rapidly from one environment to another. For example, the percentage of BPA purified from wastewater treatment plants varies between 30-90%. As can be seen, not all of these chemicals can be eliminated. On the other hand, the removal efficiency for phthalates is higher than for BPA at a range of 68-95% (21, 22). Most of the research in the literature has been based on the removal efficiency of EDCs in wastewater treatment plants. Tran et al. found the BPA and DEHP levels in the influent wastewater coming to the wastewater treatment plants near the Seine River to be 4 µg/L and 33 µg/L, respectively, and to reduce respectively to 0.4 µg/L and 2µg/L in the effluent water samples. According to the treatment techniques used in their study, they reported a BPA removal in the range of 30-90% and phthalate removal in the range of 68-95% (20).

Significant numerical differences are found worldwide in studies determining the phthalate concentrations in untreated wastewater. The minimum and maximum concentrations of DEHP (13-101 µg/L) as measured by Vethaak et al. (23) for the inlet water of a treatment plant in the Netherlands in 2005 prior to the implementation of the European Wastewater Regulation Decisions (DCE2008) resemble the numerical values found in the current study. Similarly, Mattinen et al. (24) measured the minimum and maximum value ranges of DEHP concentrations to be 28-122 µg/L in Finland in 2003, which is also consistent with the current study. As a result of the research, this study has observed the BPA data regarding the treatment plant effluent flow to average 3.17 µg/L, which matches the values of 0.47-12 µg/L as found by Höhne and Püttmann (25) in Germany. Sousa et al.'s study (26) in Portugal stated effluent BPA levels of 3-316 ng/L after decantation and biological treatment applications. Considering the physicochemical properties of endocrine disruptors, the most effective treatment methods are suggested as decantation, biodegradation, ultrafiltration, volatilization, advanced oxidation, and ozonation applications.

In our study we found the BPA and phthalate concentrations in the effluent flow of the treatment plant to be significantly lower compared to the influent water samples. By using advanced biological wastewater treatment methods on untreated wastewater, BPA was seen to be removed at a rate of 58.8% and phthalates at a rate of 67.4%. These data also reveal the efficiency with which EDCs are purified from the wastewater of the treatment plant. These data also overlap with the data from the study of Sanchez-Avila (21) in Spain, in which the percentage of phthalates removed was reported as 68%. When analyzing the wastewater characteristics, the inlet waters of the treatment plant where the study was conducted mainly pertain to industrial areas and domestic wastewater.

According to the data this study obtained, BPA and phthalate levels in wastewater treatment plants were observed to be significantly lower in the effluent samples compared to the influent water samples. While these data reveal the treatment performance of the facility, they also reveal the treatment performance, which can be further improved by using advanced treatment technologies.

In order to protect public health, the environment, and natural life, BPA and phthalate concentrations in the influent and effluent water samples of the wastewater treatment plants need to be constantly monitored. Further research on this area would shed light on the enactment of new laws and regulations, the inclusion of heavy penal sanctions on businesses that discharge illegal wastewater into the receiving aquatic environment, the intensification and dissemination of inspections, and the application of proper treatment technologies.

Ethics Committee Approval: Ethics committee approval is not required because of no material or experimental animal that would require permission.

Peer-review: Externally peer-reviewed.

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Conflicts of Interest: The authors declare no conflict of interest.

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