RESEARCH ARTICLE

BISPHENOL A DETECTION IN PACKAGED DRINKING WATER AND BEVERAGES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Dr. Neha P. Sangai and Dr. Himanshu A. Pandya

Department of Botany, University School of Sciences, Gujarat University, Navrangpura, Ahmedabad-380 009, Gujarat, India.

……………………………………………………………………………………………………....

Manuscript Info

Abstract

………………………….
………………………………………………………………

Background: Bisphenol A is characterized as an endocrine disruptor as it interferes with the synthesis of hormones and metabolism resulting in abnormality in the homeostatis of exposed persons. It is used in the production of polycarbonate and epoxy resins which are utilized in the preparation of almost all plastic packaging materials like plastic bottles, cans, food containers, and coating on food containers.

Objective: To detect leaching of Bisphenol A in 15 samples of Bottled water and Beverages using High Performance Liquid Chromatography.

Methods: Liquid-liquid extraction technique was used for analytical detection of BPA from bottled drinking water and beverages.

Results: BPA contamination in Bottled drinking water was calculated through mean concentration for a time period of 30 days as (0.38 ng/ml - 0 day), 8.86 ng/ml (5th day), 17.85 ng/ml (10th day), 30.35 ng/ml (20th day) and 44.48 ng/ml (30th day)). The mean concentration of BPA was observed to be 0.25 to 2.25 ng/ml. Also, the mean concentration of BPA at different temperatures was observed to be 5.96 ng/ml (at 4^{0}C), 5.62 ng/ml (at 20^{0}C) and 8.80 ng/ml (at 55^{0}C). The above results revealed presence of high concentration of BPA in all the samples of bottled drinking water and beverages.

Conclusion: The results obtained in the above study depicted considerable amount of BPA leaching from bottled containers into drinking water and beverages. Prolong usage of bottled water and beverages should be avoided to reduce the risk of human exposure to BPA through leaching. Also, it was found that high temperatures resulted in increased BPA leaching.

……………………………………………………………………………………………………....

Introduction:-

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) is a high-volume industrial chemical obtained through condensation of acetone and phenol (Adeyi et al., 2019). The chemical structure of BPA is composed of two phenol molecules bonded by a methyl bridge and two methyl groups (Almeida et al., 2018). BPA is an imperative component in the binding, plasticizing, and hardening of plastics, paints/lacquers, binding materials, and filling materials. Additionally, it is used as an additive for flame-retardants, brake fluids, and thermal papers (Omer et al., 2016, Khan et al., 2020). BPA depicts the tendency to migrate into food materials stored in BPA containing

Corresponding Author:- Dr. Neha P. Sangai
Address:- Department of Botany, University School of Sciences, Gujarat University, Navrangpura, Ahmedabad-380 009, Gujarat, India.

111

packaging material (Adeyi et al., 2019). Huang *et al.,* (2012) reported that 95% of BPA produced in the industry is used to make plastics, in particular polycarbonate resins (71 %) and epoxy resins (29%).

BPA is a synthetic high production volume chemical compound and enlisted as endocrine disrupter (Khan et al., 2020). It belongs to category 1 of Endocrine Disruptive Chemicals (EDC) that is severely toxic to living organisms (Adeyi et al., 2019). BPA depicts estrogenic activity even at concentrations below 1 μg/m^3, still it is used widely in the production of consumer goods, polycarbonate plastics, and epoxy resins, the coating used to line metallic food and beverage cans, and other products (Rykowska et al., 2006). This results in BPA leaching into foods and beverages through packaging or storage containers. Therefore, there is great concern regarding the harmful effects of its exposure. Le et al., (2008) explained that BPA migrates into the water from polycarbonate drinking bottles, an effect that was stimulated by exposure of plastic to increased temperatures. This led to extensive exposure of BPA to human population. Repeated BPA exposure is reported to cause type 2 diabetes, deformity in male reproductive system, thyroid dysfunction, hypertension, obesity and cardiovascular diseases (Adeyi et al., 2019).

The migration of BPA to food products is determined by performing direct analysis of food samples. Predominantly for BPA detection a pre-validated High Performance Liquid Chromatography (HPLC) technique is preferred for quantitative determination (Park et al., 2008, Suryadi et al., 2018, Rodriguez et al.,2019). The main aim of the present study is to detect the presence of Bisphenol A in 15 samples of Bottled drinking water and beverages purchased from the local market in Ahmedabad, Gujrat.

Material and Method:-
Reagents:
BPA (minimum purity 97%) was purchased from Sigma Aldrich. HPLC grade methanol and acetonitrile were obtained from Merck. A standard solution containing 100 ppm of BPA and a sub stock containing 10 ppm of BPA were prepared in methanol and kept refrigerated. Solutions of the required concentrations were prepared daily by dilution.

Sampling plan:
In this study, a total of 15 samples of commercial brands of drinking water bottles and beverages (Non-carbonated and Milk) were purchased from the local market of Navarangpura, Ahemadabad, Gujrat. Samples used in the study were tested as consumed. All samples were extracted under the same conditions.

HPLC analysis parameters:
The HPLC system water's Acquity UPLC, BSM, PDA detector consisted of SM auto-injector and a C18 column (Sunfire C18 150*4.6mm, 5μm) with water/acetonitrile (35:65) at a flow-rate of 0.4 ml min^{-1}. The injection volume was 10 µl and elute was monitored at 210 nm. Methanol is used as diluent.

Quality Control and Quality Assurance:
Calibration of instrument:
The instrument was calibrated using calibration standards. Calibration Standards were prepared from the stock solution of BPA to obtain a standard calibration curve.

Quantitation and Quality Control:
Confirmation of BPA identity was based on the retention time and the ion ratios. The calculation of BPA concentration in samples was based on the calibration curves of peak area ratios of BPA (ion m/z 228) over the internal standard peak area plotted with the ratios of native BPA concentration over the internal standard concentration.

BPA contents in bottled drinking water samples :
BPA is used ubiquitously in almost all plastic bottles and it has been remarked as toxicant. US FDA has reported that BPA leaches into liquids from plastic bottles or containers when kept for longer time period. This is the first study in the Gujrat (West India). Water samples from the local market place were collected and analyzed for BPA contamination.

Results and Discussions:-

In the present study, BPA analysis was carried out using HPLC method **(Figure 1).** The time based leaching of BPA in drinking bottled water was studied for a time span of 0 to 30 days and readings were taken at a 10 day time interval. The readings were noted and the average values were calculated of the Bottled water samples. The mean concentration of BPA was observed to be 0.38 ng/ml on 0 day, 17.85 ng/ml on 10th day, 30.35 ng/ml on 20th day and 44.48ng/ml on the 30th day. The effect of different temperatures on BPA leaching was also evaluated at three different temperatures (4^0C, 25^0C and 55^0C) (**Graph 1 to 4, Table 1 to 3**).

The average concentration of BPA was found to be 5.96 ng/ml at 4^0C, 5.62 ng/ml at 25^0C and 8.80 ng/ml at 55^0C. BPA leaching in canned beverages was also studied. For the study seven samples of non-carbonated and milk beverages were analyzed. The average concentration of BPA in tested beverages ranged from 0.25 ng/ml to 2.25 ng/ml.

The results obtained in the temperature dependent study were in corroboration with Zaki and Shoeib (2018). The authors reported temperature dependent BPA leaching at 4^0C, 25^0C and 40^0C and found a positive correlation between the effect of time and temperature on leaching of phthalates in bottled drinking water. The results of the above study also evidence similar finding.

Concentration of BPA leached from bottles increased with storage time and under elevated temperature (Amiridou et al., 2011). It has been described that during the photolysis of BPA in deionized water under natural sun radiation, the concentration of BPA remained about 80% of the initial concentration during the first 5 days of exposure monitored with a progressive decrease. Conversely, another study reported that BPA offerings show direct photolysis in neutral pure water under simulated solar irradiation, but the process is rapid in the presence of humic substances (Mezcua et al., 2006). Additionally, a study determined endocrine-disrupting chemicals (EDCs) such as BPA in water samples from PET and polyethylene (PE) bottles after outdoor exposure for 10 weeks at temperatures up to 30^0C (Zhan et al., 2006).

The concentration of BPA in drinking water samples varied from 0.213 ng/ml (Table1, sample 8) to 0.726 ng/ml (Table1, sample 6) at day 0. However, when the samples were kept for longer duration BPA concentration increased in all the analyzed samples. Also, the rise in temperature fueled the concentration of BPA. Bae and Hong (2015) have reported that BPA present in the epoxy lining of cans could leak into the beverages and its repeated exposure could lead to increased blood pressure and heart rate. Regular change of columns in branded water filters should be done as regular practice and also storage for long periods of branded mineral water in plastic bottles or containers must be avoided (Honeycutt et al., 2017). The awareness about the ill-effects of BPA is highly recommended to ensure the safety of public health. Wastes and leachates from landfills and paper mills are suspected to be the most important source that may result in BPA discharge and contaminate natural waters bodies (Taskeen et al., 2012). Similarly, other studies reported that significant amounts of BPA leached from bottle containers into the water. Long storage of bottled water under direct sunlight should be avoided to reduce the risk of human exposure to BPA (Biello et al., 2008, Elobeid et al., 2012). This is a preliminary report on the analysis of BPA in drinking water samples. More research needs to be done on the analysis and bio-monitoring of BPA in India in for brighter future.

Conclusions:-

In the present study, BPA contamination was observed in all bottled drinking water and beverages sampled for analysis. Bottled drinking water and canned beverages is an important avenue for BPA exposure to human population. The concentration of BPA was high in the analyzed samples and increased with time and temperature speculating high risk to public health. Thus, prolonged use of BPA as packaged drinking water bottles as well as beverages bottle drastically affects human health if they are used for a longer time period. So, use and storage of BPA products for longer period of time should be avoided.

Figure 1:- BPA analysis using HPLC method.
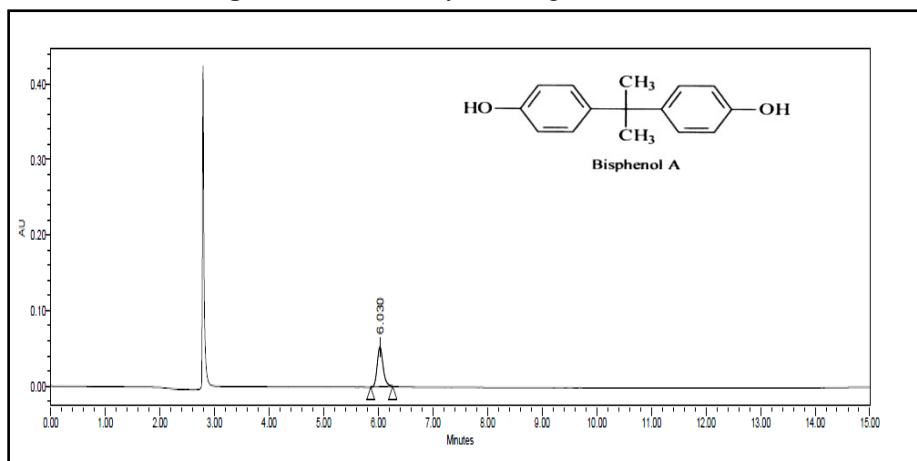
Graph 1: Time dependent leaching of BPA
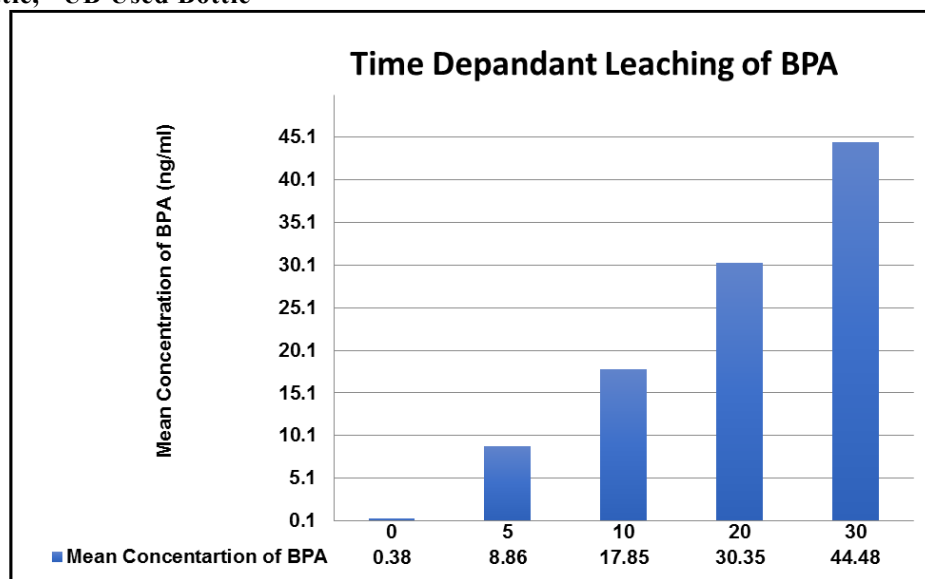**NB New Bottle, *UB Used Bottle**

Table 1:- Time dependent leaching of BPA in 0, 10, 20 and 30 days.

| Sr. No.	Beverages	BPA concentration (ng/ml)
1	B-1	0.634
2	B-2	0.257
3	B-3	1.722
4	B-4	0.335
5	B-5	2.256
6	B-6	0.537
7	B-7	0.442

Table 2:- BPA leaching in beverages.

| Sr. No.	Brand Codes (Drinking Water Bottles)	Bottle	BPA concentration (ng/mL)				
			0 Day	5 Days	10 Days	20 Days	30 Days
1	DWB-1	NB	0.452	3.912	5.231	12.912	16.213
2	DWB-2	NB	0.293	4.931	7.023	18.012	29.023
3	DWB-3	NB	0.372	5.875	10.916	24.076	32.934
4	DWB-4	NB	0.231	9.014	13.054	23.914	35.627
5	DWB-5	NB	0.329	7.234	14.913	26.067	40.156
6	DWB-6	UB	0.726	13.456	25.234	44.324	60.453
7	DWB-7	UB	0.531	15.234	32.756	46.256	65.321
8	DWB-8	UB	0.213	8.674	16.321	26.432	40.213
9	DWB-9	UB	0.278	11.423	35.236	51.236	80.456

Graph 2:- Mean concentration of BPA in beverages
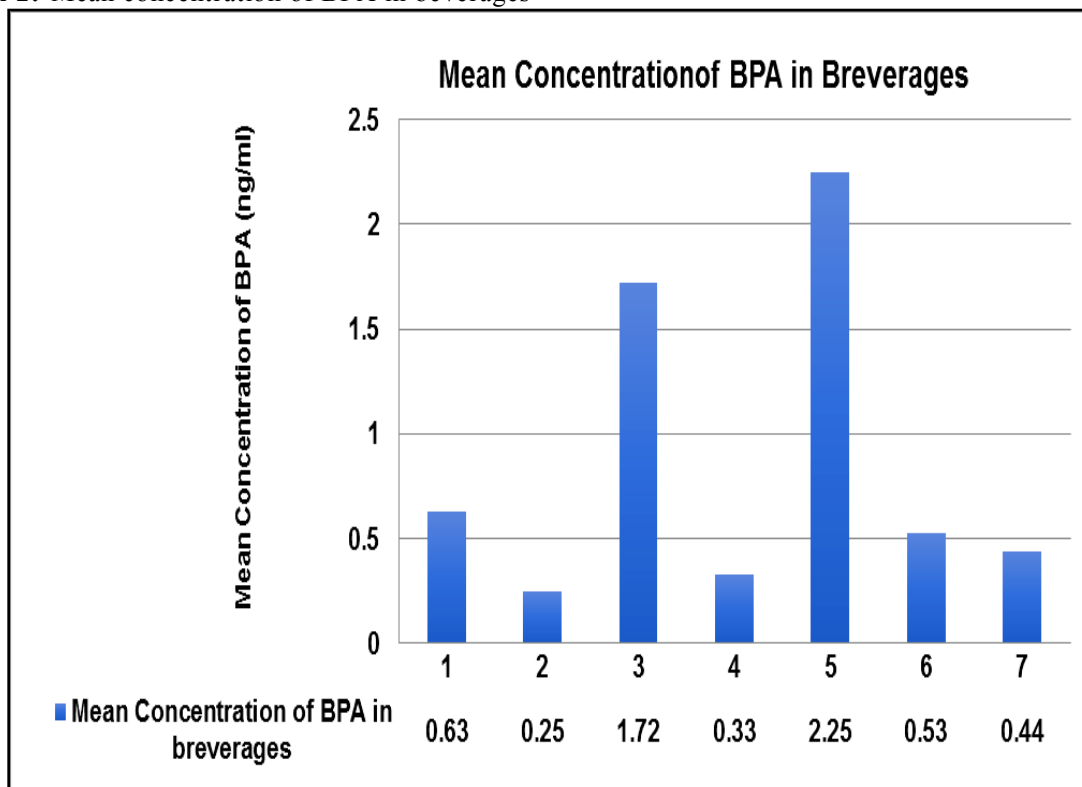
Table 3:- Temperature dependent leaching of BPA at 4^0C, 25^0 C and 55^0C.

Sr. No.	Brand Codes (Drinking Water Bottles)		Temperature		
		Bottle	4°C	25°C	55°C
1	DWB-1	NB	6.567	4.931	8.965
2	DWB-2	NB	4.385	3.456	7.234
3	DWB-6	UB	7.912	7.234	10.254
4	DWB-7	UB	5.012	6.897	8.756

Graph 3:- Time and temperature dependent leaching of BPA at 4^0C, 25^0 C, 55^0C.
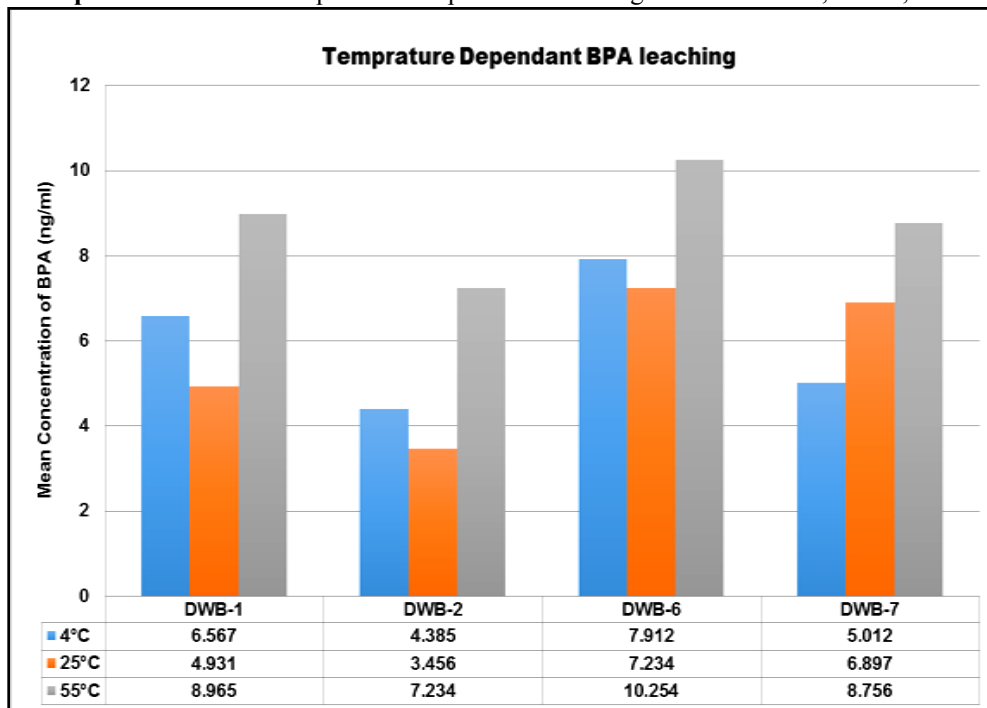
Graph 4:- Mean concentration of BPA at temperature 4^0C, 25^0 C, 55^0C.
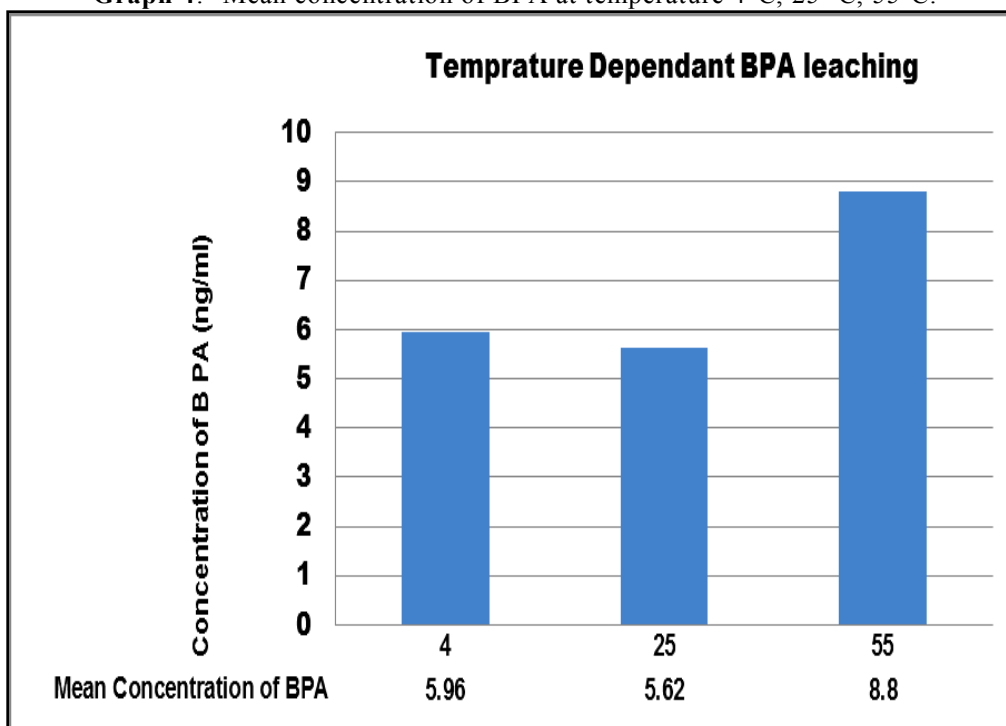
References:-

1] Adeyi, A. A. and Babalola, B. A. (2019). Bisphenol-A (BPA) in foods commonly consumed in Southwest Nigeria and its human health risk. Scientific Reports. 9, 17458.

2] Almeida, S., Raposo, A., Almeida-Gonzalez, M., Carrascosa, C. (2018).Bisphenol A: Food Exposure and Impact on Human Health. Comprehensive Reviews in Food Science and Food Safety, 17, 1-10.

3] Omer, L., Ahmed, H., Elbashir, A. (2016). Determination of bisphenol A in exposed bottled water samples to direct sun light using multi walled carbon nanotubes as solid phase extraction sorbent. J. Environ. Chem. Ecotoxicol., 8 (7) 51-57.

4] Khan, M. R., Alammari, A. M., Aqel, A., Azam, M. (2020). Trace analysis of environmental endocrine disrupting contaminant Bisphenol-A in canned, glass and polyethylene terephthalate plastic carbonated beverages of diverse flavors and origin. Food Science and Technology. 1-10.

5] Huang, Y.Q.,Wong, C.K.C., Zheng, J.S., Bouwman, H., Barra, R.,Walhlstrom, B., Neretin, L.,Wong M H (2012). Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. *Environ Int.,* 42, 91–99.

6] Rykowska, I., Wasiak, A. (2006). Properties, threats, and methods of analysis of bisphenol A and its derivates. Acta *Chromatography* 16, 7–27.

7] Le, H. H., Carlson, E.M., Chua, J.P., Belcher, S.M. (2008). Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. Toxicology Letters. 176, 149–156.

8] Park, H. J., Lee, Y., Kim, M.R., Kim, K. (2008). Safety of polyethylene terephthalate food containers evaluated by HPLC, migration test, and estimated daily intake. J. Food Sci. 73, T83–T89.

9] Suryadi, H., Naja, F., Harahap, Y. (2018). Detection of Bisphenol A contamination in canned carbonated beverages by Gas Chromatography. International Journal of Applied Pharmaceutics. 10, 1, 116-119.

10] Rodriguez, R.,Castillo, E., Sinuco, D. (2019). Validation of an HPLC method for determination of Bisphenol-A migration from baby feeding bottles. Journal of Analytical Methods in Chemistry. 1989042.

11] Zaki, G. and Shoeib, T. (2018). Concentrations of several phthalates contaminants in Egyptian bottled water: Effects of storage conditions and estimate of human exposure. Science of The Total Environment. 618: 142-150.

12] Amiridou, D, Voutsa, D. (2011). Alkylphenols and phthalates in bottled waters. Journal of *Hazardous Materials*, 185: 281–286.

13] Mezcua, M, Ferrer, I, Hernando, MD, Fernandez-Alba, AR. (2006). Photolysis and photocatalysis of bisphenol A: identification of degradation products by liquid chromatography with electrospray ionization/time-of-flight/mass spectrometry (LC/ESI/ToF/MS). Food Addit Contam., 23: 1242–1251

14] Zhan, M, Yang X, Xian Q, Kong, L. (2006). Photosensitized degradation of bisphenol A involving reactive oxygen species in the presence of humic substances. Chemosphere, 3: 378–386.

15] Bae, S and Hong, YC. Exposure to Bisphenol A from drinking canned beverages increases blood pressure randomized crossover trial. Hypertension 2015; 313-319.

16] Honeycutt, J., Nguyen, J., Kentner, A., Brenhouse, H. (2017).Effects of Water Bottle Materials and Filtration on Bisphenol A Content in Laboratory Animal Drinking Water. *J* Am Assoc Lab Anim Sci. 56(3): 269–272.

17] Taskeen, A, Hameed, R, Naeem, I. (2012).Sources of Bisphenol A Contamination in Drinking Water in Pakistan and Determination of Migration Rates. *Biomed* Pharmacol J. 5(2).

18] Biello, D. (2008). Plastic (not) fantastic: Food containers leach a potentially harmful chemical. Scientific American.1-6.

19] Elobeid, M., Almarhoon, Z., Virk, P., Hassan, Z. et al., (2012).Bisphenol A Detection in Various Brands of Drinking Bottled Water in Riyadh, Saudi Arabia Using Gas Chromatography/Mass Spectrometer. Tropical Journal of Pharmaceutical Research. 11 (3) 455-459.