UNVEILING THE SILENT THREAT - ANALYZING THE PRESENCE OF BPA IN DIFFERENT SOURCES AND ITS IMPACT

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Abstract:
The study aimed to establish the baseline data of Bisphenol A (BPA) presence in the unexplored, and the largest district named Kachchh. BPA is a monomer and endocrine disruptor that is utilized in plastic and epoxy resin manufacture. The fact that it is widely used, and its possible health threats call for the urgent monitoring of BPA in environmental as well as human samples. The study adopted analytical method to detect baseline level of BPA leaching, due to temperature variations in mineral water bottles and pouches, as well as urine samples from gynecological patients. A Waters HPLC device, equipped with a 515 pump and an ultraviolet detector at 278 nm, was used to detect BPA in a methanol-water mobile phase using the 2489 detector. Findings suggested that BPA concentration in stored pouches were significantly higher in comparison to the samples taken directly from the refrigerator. Also, the BPA level was almost (0.25-2.1 µg/L) in the urine sample of the Gynec patient compared with the standard peaks. The results prove the urgent need for more awareness about BPA contamination across this region. Such contamination may have the effect of environmental pollution and thus affects public health. The determinations of high BPA levels in the stored water containers for drinking purposes aroused the issues of long-term exposure to this hormone disruptor chemical. Also, sensitive method of detection to be performed in the future as extended part of this study. Consequently, it is essential that regulations and consumer awareness education are implemented to minimize the BPA exposure risks and protect human health as well as the environment.
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Keywords: Bisphenol-A (BPA), environmental pollution, health risks, HPLC analysis, drinking water, urine samples, temperature variability.

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1. INTRODUCTION

Bisphenol A (BPA) is a man-made chemical often applied in the manufacture of polycarbonate plastics and epoxy resins. Most of them are typically used in food and drink packaging and in other consumer products (Karalius et al., 2014). BPA from the packaging materials is believed to enter the food and beverages via variation in temperature, pH and storage period. Several studies have reported that BPA is an endocrine disruptor, a chemical that can interfere with normal hormone regulation (Li and Franke 2015; Nachman et al., 2015). Accurate assessments of BPA levels in environmental and biological samples aid in the evaluation of the associated human health risks (Vandenberg et al., 2014; Teeguarden et al., 2015; Shankar and Teppala 2015; Thayer et al., 2016).

The European Union's (EFSA) experts have established a tolerable daily intake (TDI) of 0.2 nanograms (0.2ng) of Bisphenol A (BPA) per kilogram of body weight per day (kg/bw/day). This is 20,000 times lower than the previous TDI of 4 micrograms (4μg) per kilogram of body weight per day, which EFSA published in 2015.

The aim of the present study was to identify and to quantify BPA leaching from plastic water bottles, pouches and urine samples in the city of Bhuj, using analytical method by high performance liquid chromatography (HPLC). Pouches and bottles were observed after different storage periods to determine if the duration of storage affects the leaching of BPA. For comparison, samples from nearby water bodies were also collected. Furthermore, urine samples of gynec patients in Bhuj city were tested to evaluate BPA concentrations which would be a result of environmental exposure (Syed, 2011).

HPLC is a sensitive and accurate technique, that is often used for separation, identification, and quantification of chemical compounds in samples of unknown origin (Wolfgang et al., 2005; Ballesteros-Gómez et al., 2009; Manuela et al., 2011; Liu et al., 2014). For the present study mobile phase is methanol and water where methanol is in the maximum concentration for best BPA elution and detection. With careful calibration HPLC gives an opportunity for the quantification of BPA to a level of 1ppt which makes this method relevant for both environmental and human sample studies. Previous research studies showed the alarming leaching of BPA from polycarbonate drinking water bottles and pouches (Azevedo et al., 2001; Rodriguez-Mozaz et al., 2004; Wolfgang et al., 2005; Manuela et al., 2011; Battal et al., 2014). Most of the people in the rural area are not aware about the long term consequences of using water from plastic pouches. BPA has different leaching rate depending on the factors i.e. storage period, temperature, technique of cleaning, as well as pH. Nevertheless, the base line data of BPA contamination in Indian water sources, and its levels in local people is still lacking. This study focuses to reveal some information of BPA exposure in the Bhuj region by using HPLC method.

The data obtained in this study will supplement the risk assessment procedures associated with the water bottles/pouches and environmental exposures in the city of Bhuj. A considerable amount of BPA from human serum and urine is detected (Vandenberg et al., 2010; Taskeen and Naeem 2010; Tsukioka et al., 2014; Thayer et al., 2016 serum Teeguarden et al., 2011; 2015; Nachman et al., 2015) it could be taken seriously by regulatory authorities for the purpose of reducing the exposure to BPA, for evidence-based decision-making, concerning pollution of BPA and health risks.

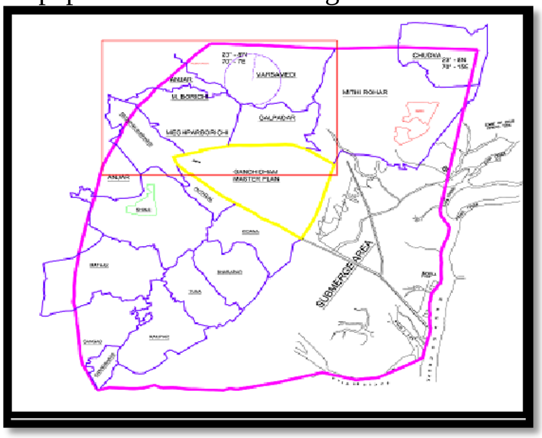
2. MATERIAL AND METHODS

HPLC grade methanol, water, acetonitrile Merck make and BPA (99%) from Hi-media, were purchased from Krishna Scientific, Gandhidham. Throughout the experiment only glass materials were used except the samples. All the glassware used for the experiments were cleaned with HPLC grade methanol & water and dried in the oven at 100ºC to avoid additional contamination/noise in baseline/peak while injecting in the instrument/column. Each standard solution of 0.01 ppm to 10.0 ppm was prepared in methanol.

2.1 Sample Collection

Surface Water: Water samples collected from the surface of many water bodies in the Kachchh region such areas as Bhid, GMDC, Hamirsar, Khatri, Kukma, and Mavji which are vital water sources/bodies. Six sampling points were selected and collected before monsoon season.

Samples were gathered in 50 ml clean and thoroughly cleaned glass bottles, those bottles were filled with sample water and washed right before closing to prevent contamination. The samples were stored in cool boxes during their transport to the laboratories, where they were analyzed within 48 hours of collection. Filtration was the first step in the analysis process. Samples were filtered with Whatman filter paper and GF/F glass fiber filter.



Bottled Mineral Water

The bottles of mineral water, from 7 different brands, were procured from local small stores and hawkers around Kachchh city.

-made pouches for mineral water

A number of water bottles of the same or different brands were bought whenever we sighted the local hawkers or roadside vendors. Duplex or triplex samples were taken, to have comparative samples stored in different situations, against the effort of some suppliers who had their pouches stored in neither cool boxes nor air-conditioning.

Urine samples from thirty women of first second and third trimester belong to lower economical class from GK General Hospital was collected from Department of Gynecology, Gujarat Adani Institute of Medical Science. Urine samples were collected in polypropylene container and kept at 4°C until the analysis.

Analysis of BPA from Urine sample

Urine samples from thirty women of first second and third trimester belong to lower economical class from GK General Hospital was collected. (Required ethical committee permission was obtained from Gujarat Adani Institute of Medical Science and consent form from women was also signed). Urine samples were collected in polypropylene container and kept at 4°C until the analysis.

According to modified method of Matsumoto et al., 2003 Urine (500µl) was buffered with 30µl of 2.0 M sodium acetate buffer (pH 5.0) and hydrolyzed enzymatically with β-glucuronidase (*E. coli*, 5 µl, 200U ml^{-1}) followed by incubation at 37°C for 90 min, (Brock et al., 2001). After hydrolysis, the hydrolysate was extracted once with 5 ml of ethyl acetate. After centrifugation, 4 ml of supernatant was transferred to a new tube and evaporated with N_2 gas. The residue was dissolved with 200 µl of 60 % acetonitrile in water (HPLC grade) and 20 µl of the solution was injected onto the WATERS high performance liquid chromatography (HPLC-UV) with 515 pump and 2489 detector. HPLC conditions is same as mentioned above.

Quantitative analysis

Calibration curves were obtained under the optimized condition with linear dynamic range of 0.01ppm to 10ppm from stock solution of 100 ppm BPA and correlation of determination (r^2) of 0.997.

Findings and Results

The HPLC analysis revealed varying levels of Bisphenol A (BPA) in different water sources and bottled mineral water samples collected from the Kachchh region. The retention time and peak area/Rf values for each sample are summarized in Table 1. Among the various samples collected from various water bodies, water bottles, water pouches and urine samples, the mean BPA concentrations ranged from approx. 0.3 to 30 µg/L compared with the standard peaks of HPLC. Notably, the highest BPA concentration was observed in samples collected from the water pouch after 96 hours. However, higher concentrations were detected in certain brands depending on the temperature variation and time duration. Temporal variations in BPA concentrations were also observed in bottled mineral water samples stored for different durations. For example, the BPA concentration in one of the brands of water bottle was increased from 0.22 µg/L at the 0th hour to 4.0 µg/L at the 96th hour, indicating a potential leaching of BPA over time.

Additionally, substantial differences were observed between different brands of bottled mineral water, with some brands exhibiting significantly higher BPA concentrations compared to others. For instance, one of the brands of water pouch showed a remarkable increase in BPA concentration from 0.28 µg/L at the 0th hour to 14.77 µg/L at the 48th hour, highlighting the importance of brand-specific monitoring and regulation.

Equipment

The analytical HPLC system employed was WATERS Series HPLC equipped with 515 pump and 2489 detector a reversed-phase analytical column (C18, 250 x 4.6 mm, 5µm) was used. Mobile phase of Methanol, water 80:20 was prepared and degassed by magnetic stirrer for 45 minutes before use, to reduce the noise. 20µL of sample injection from C18 column at elution time 1min/ml, detection response time 0.1 sec at 278 nm with run time of 8 minutes and eluted peaks were detected at 278 nm.

Table 1: Retention Time and BPA Peak in Different Sources

	Retention Time	Area	% Area	Height
GMDC	4.306	1100	7.18	153
Kukma	4.315	10754	24.76	1536
Hamirsar	4.301	651	3.82	95
Bhid	4.302	1161	19.18	156
Khatri	4.298	1134	12.55	149
Mavji	4.303	2609	14.6	362
(WP 1) 0th hour	4.067	1413	31.35	209
(WP 2) 0th hour	4.074	2536	29.19	352
(WB 1) 0th hour	4.072	10280	43.75	646
(WB 2) 0th hour	4.084	114404	79.48	16342
(WP 1) 96th hour	4.266	13726	67.19	1812
(WB 2) 96th hour	4.291	18311	24.23	2410
(WP 1) 48th hour	4.257	3628	85.2	473
(WP 2) 48th hour	4.26	6444	86.33	856
(WB 1) 48th hour	4.299	520222	85.81	69085
(WP 1) 24th hour	4.083	3206	46.48	449
(WB 2) 24th hour	4.087	31439	88.04	4312
(WP 2) 24th hour	4.093	59907	82.82	8185
(WP 1) 0th hour	4.115	28101	83.66	3920
(WP 2) 0th hour	4.139	9742	19.9	975
(WP 3) 0th hour	4.125	30619	62.9	4172
Urine Sample 1	4.064	74466	2.35	5742
Urine Sample 2	3.705	45782	3.34	4690
Urine Sample 3	3.386	1384631	9.69	101516
Urine Sample 4	3.394	35250	1.06	4573
Urine Sample 5	3.033	179281	4.85	19188
Urine Sample 6	3.712	52296	2.85	4479
Urine Sample 7	3.717	72298	6.1	7801

The application of HPLC led to the revelation of the heterogeneous distribution of BPA in water samples taken from different sources. Some samples had slightly detectable BPA contamination, while some had high content, especially in some specific mineral water pouches. Such outcomes emphasize the significance of constant monitoring of BPA concentration in water sources to avert possible health complications related to BPA intake. Nevertheless, additional research is needed to determine factors that contribute to BPA leaching and to understand better the impact on human health and sustainable environment.

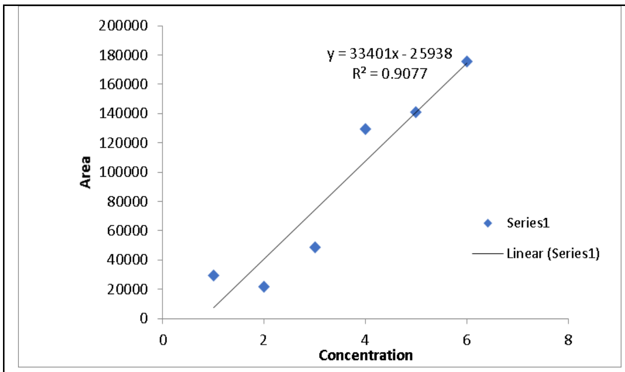


Figure 1. Calibration curve were obtained under the optimized condition with linear dynamic range of 0.01ppm to 10ppm from stock solution of 100 ppm BPA and correlation of determination (r^2) of 0.997.

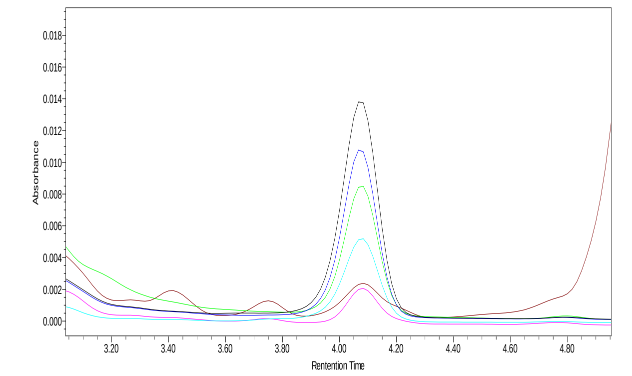


Figure 2. Standards (0.01 ppm, 0.1 ppm, 0.5 ppm, 1.0 ppm, 10.0 ppm)

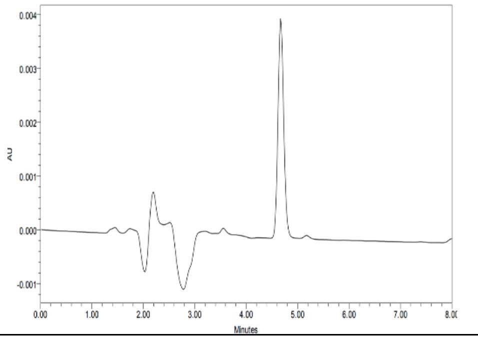


Figure 3. Spectrum of packed water sample old less than 15 days.

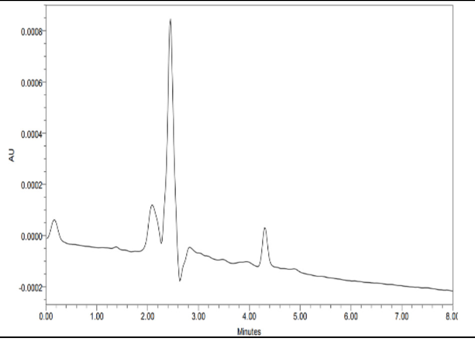


Figure 4. Spectrum of (water body) sample

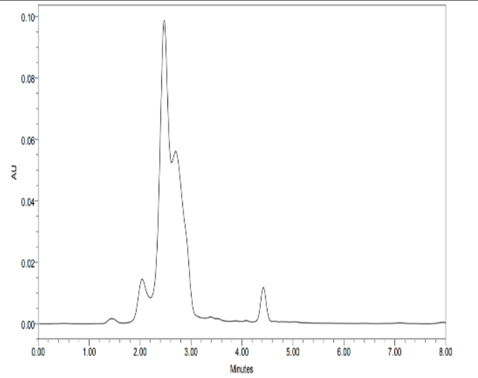


Figure 5. Spectrum of urine sample no. 1a

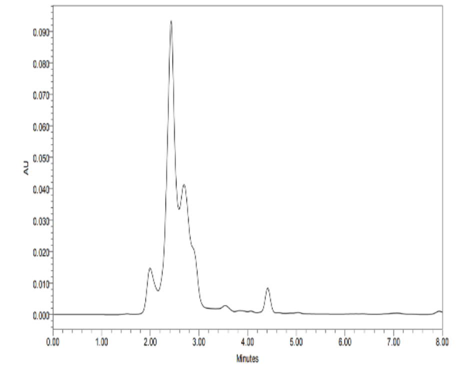


Figure 6. Spectrum of urine sample no. 2a

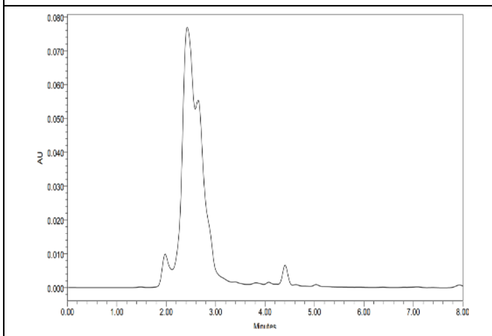


Figure 7. Spectrum of urine sample no. 3a

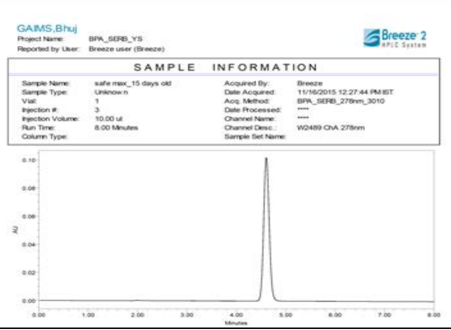


Figure 8. Lab report generated from Breeze software for standard

Figure 1-8) Retention Times and Chromatograms obtained by HPLC in different matrices

The analysis of urine samples belonging to the gynecological patients performed to test for Bisphenol-A (BPA) and its relevance to reproductive health. The table below captures the urinary BPA levels that have been recorded in women going through various reproductive health disorders, as well as in different pregnancy stages. The findings are summarized in the table 2 below.

Table 2: Number of samples collected based on disorder / history of gynaecology patients.

DISORDER	NO OF SAMPLES
Polycystic	0
Irregular menstrual	7
Infertility	8
Recurrent miscarriage	3
1st trimester	9
2nd Trimester	6
3rd Trimester	3
Obesity	1

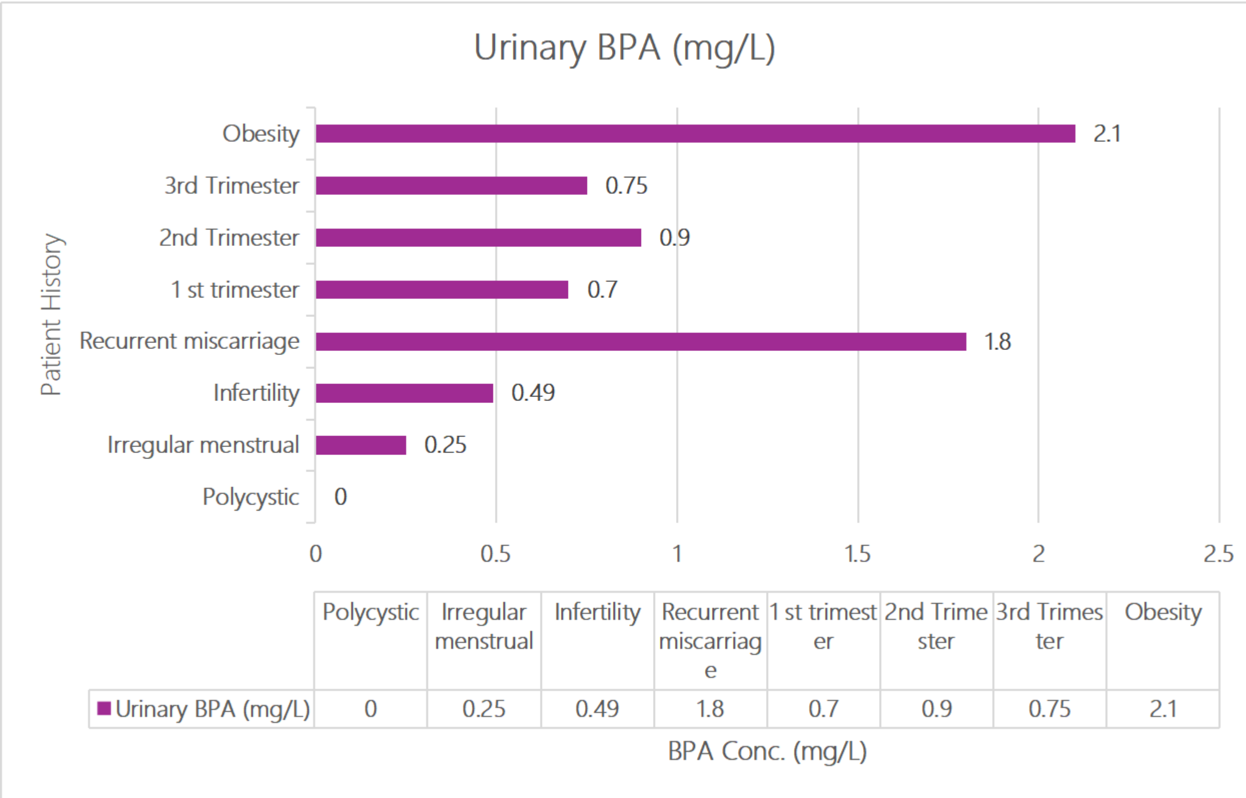


Figure 9: BPA concentration observed in urine samples

1. Irregular Menstrual Cycles and Infertility: Women with irregular menstrual cycle had an average urinary concentration of BPA in 0.25 mg/L, but those who were infertile had a slightly higher mean BPA concentration of 0.49 mg/L. These results can be considered as evidence of a probable role of exposure to BPA in menstrual irregularities or/and infertility, which requires further research to identify the mechanisms that are at the base of this relationship.

2. Recurrent Miscarriages: In the study, individuals with past experiences of multiple miscarriages were found to demonstrate the highest urinary BPA levels, with the mean concentration of 1.8 mg/L. This acknowledgment brings in the idea that such may be a cause of recurrent miscarriages and highlights the need to research the issue further in high-risk populations.

3. Pregnancy Trimesters: The results also indicated clinically variable changes in urinary BPA levels during different trimesters of pregnancy. During the first trimester, 0.7 mg/L of

BPA were detected on average. This value rose to 0.9 mg/L during the second trimester before slightly decreasing in the third trimester to 0.75 mg/L. These fluctuations emphasize the requirement for integrative assessment of BPA exposure during pregnancy, being able to express the possible impact on motherhood and newborn health.

4. Obesity: Obesity becomes a major risk factor in the body BPA levels, for the obese individual the mean concentration of BPA in the body is 2.1mg/L. This finding highlights the complexity of the metabolic health and BPA levels interaction, which implies that obesity may influence BPA levels through body accumulation.

The findings of this study suggest the existence of BPA in many of the samples including urine of gynecological patients. These patients had heterogeneous reproductive health histories. The results of these studies point to possible adverse consequences of BPA on reproductive health and give rise to a necessity of implementing precautionary measures towards exposure of high-risk populations.

1. Reproductive Health Disorders: The above-mentioned correlations between urinary levels of BPA (Vom Saal & Vandenberg, 2021) and reproductive health disorders like irregular menstrual cycles, infertility and repeatedly miscarriages hint at a possible role of BPA in reproductive hormonal imbalance and functions. These studies demonstrated that exposure to BPA can lead to reproductive toxicity and that should be further explored to expand the knowledge about the involved mechanisms and the development of preventive strategies to avoid adverse outcomes.

2. Pregnancy Monitoring: The changing nature of urinary BPA levels during pregnancy demonstrates the necessity of ongoing monitoring to evaluate the extent of fetal exposure to BPA. With a strong focus on pre-natal development as an important foundation of lifelong health outcomes, it is imperative to put in place measures to mitigate prenatal BPA exposure since it is the key to protecting the health of mothers and fetuses.

3. Obesity and BPA Exposure: A striking correlation between obesity and increased urinary BPA concentration indicates the requirement for a synergetic strategy which applies to individuals with metabolic disturbance. Strategies directed to cut BPA exposure, either through diet modification or consumer product regulation, are the most likely to reduce adverse health outcomes linked to BPA and obesity (Rivas et al., 2009).

Generalized, these facts suggest a lack of joint effort to minimize BPA exposure and preserve reproductive and metabolic health throughout the population. Researchers address the complicated interplay between environmental exposures and reproductive outcomes in this study, thus providing a better understanding of the potential health risks associated with BPA and help evidence-based interventions to be developed to enhance public health and welfare.

BPA is used ubiquitously in almost all plastic bottles in spite of “BPA-free” claims, the substitute of BPA is not surely safe for the human health, and it has been remarked as toxicant. The US FDA has reported that BPA leaches into liquids from plastic bottles or containers when kept for a longer period. This is the first study of its kind in the largest district of the Gujrat state. Water samples from pouches and bottles from the local marketplace as well as urine samples were collected and analyzed for BPA contamination.

Conclusion

The proposed extraction and determination of BPA demonstrated to be able to do the extraction and determination using a mobile phase (15.0% (v/v) Methanol, 3.0% (m/v) water, and using C18 cartridge and acetonitrile as eluent. The method was selective, robust, simple, fast, and adopted concept of green chemistry by using enzyme from live *E. coli* for the hydrolysis process. We assume that this is the first ever study to elucidate the presence of BPA from different water sources and urine sample from this specific region. The present study can be an important tool for laboratories dedicated to the analysis of emerging pollutants that do not have MS detector or limitation with the advanced instruments. (Geissen et al., 2015).

Bisphenol A (BPA) has possibly emerged as an environmental contaminant with its still unnoticed detrimental effects on the human health. The findings of this research enable the creation of the method of BPA extraction and its identification at different sources using high-performance liquid chromatography. The suggested green chemistry technique show that BPA contamination does not only occur in water bottles but is also present in urine samples. The findings clearly identify BPA leaching from a wide range of plastic materials already in circulation to present in the surrounding environment and human bodies. Biological communication across the entire population no matter how small exposures to BPA may face a great consequence, especially for particular groups like pregnant women and young children. This observation in water bottles and urine sample made during the research demonstrated the invisible danger that plastic materials our substitute with no concern about health safety. Hence, the burning issue of today is to search for alternative, eco-friendly materials and place stricter controls around BPA production and consumption in order to fight the invisible disaster of this specific pollution. In the wake of the extended exposure of BPA in the environment and the bio-accumulation of this chemical, we call for evidence-based public policy in order to prevent further damage to ecological and human health. This requires multi-sectored cooperation between scientists, industries, governments, and people in community organization to unfold the hidden nature including excessive BPA pollution. Further detailed study is required to monitor lower dose of BPA using more sensitive techniques.

References:

1. Azevedo, D. D. A., Lacorte, S., Viana, P., & Barceló, D. (2001). Occurrence of Nonylphenol and Bisphenol-A in Surface Waters from Portugal. *Journal of the Brazilian Chemical Society*, *12*(4), 532–537. https://doi.org/10.1590/S0103-50532001000400015
2. Ballesteros-Gómez, A., Rubio, S., & Pérez-Bendito, D. (2009). Analytical methods for the determination of bisphenol A in food. *Journal of Chromatography A*, *1216*(3), 449–469. https://doi.org/10.1016/j.chroma.2008.06.037
3. Battal, D., Cok, I., Unlusayin, I., & Tunctan, B. (2014). Development and validation of an LC-MS/MS method for simultaneous quantitative analysis of free and conjugated bisphenol A in human urine. *Biomedical Chromatography*, *28*(5), 686–693. https://doi.org/10.1002/bmc.3090
4. Blount, B. C., Milgram, K. E., Silva, M. J., Malek, N. A., Reidy, J. A., Needham, L. L., & Brock, J. W. (2000). Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. *Analytical Chemistry*, *72*(17), 4127–4134. https://doi.org/10.1021/ac000422r
5. Bosch, M. E., Sánchez, A. J. R., Rojas, F. S., & Ojeda, C. B. (2008). Analytical methodologies for the determination of cisplatin. *Journal of Pharmaceutical and Biomedical Analysis*, *47*(3), 451–459. https://doi.org/10.1016/j.jpba.2008.01.047
6. Breemen, R. B. van, Li, G., Yuan, Y., & Huang, K. (n.d.). *Shimadzu_Journal_2-1_P32-34*. 13–15.
7. Brock, J. W., Yoshimura, Y., Barr, J. R., Maggio, V. L., Graiser, S. R., Nakazawa, H., & Needham, L. L. (2001). Measurement of bisphenol A levels in human urine. *Journal of Exposure Analysis and Environmental Epidemiology*, *11*(4), 323–328. https://doi.org/10.1038/sj.jea.7500174
8. Cantonwine, D., Meeker, J. D., Hu, H., Sánchez, B. N., Lamadrid-Figueroa, H., Mercado-García, A., Fortenberry, G. Z., Calafat, A. M., & Téllez-Rojo, M. M. (2010). Bisphenol a exposure in Mexico City and risk of prematurity: A pilot nested case control study. *Environmental Health: A Global Access Science Source*, *9*(1). https://doi.org/10.1186/1476-069X-9-62
9. Carwile, J. L., Luu, H. T., Bassett, L. S., Driscoll, D. A., Yuan, C., Chang, J. Y., Ye, X., Calafat, A. M., & Michels, K. B. (2009). Polycarbonate bottle use and urinary bisphenol A concentration. *Environmental Health Perspectives*, *117*(9), 1368–1372. https://doi.org/10.1289/ehp.0900604
10. Chang, H. S., Choo, K. H., Lee, B., & Choi, S. J. (2009). The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water. *Journal of Hazardous Materials*, *172*(1), 1–12. https://doi.org/10.1016/j.jhazmat.2009.06.135
11. Chen, M., Edlow, A. G., Lin, T., Smith, N. A., McElrath, T. F., & Lu, C. (2011). Determination of bisphenol-A levels in human amniotic fluid samples by liquid chromatography coupled with mass spectrometry. *Journal of Separation Science*, *34*(14), 1648–1655. https://doi.org/10.1002/jssc.201100152
12. Erdem, Y. K., & Acar, F. (2012). Migration of Bisphenol-A into the Natural Spring Water Packaged in Polycarbonate Carboys. *International Journal of Applied Science and Technology*, *2*(1), 152–156.
13. Geissen V, Mol H, Klumpp E, Umlauf G et al. Emerging pollutants in the environment: A challenge for water resource management. *International Soil and Water Conservation Research*, 2015:*3*(1), 57–65. https://doi.org/10.1016/j.iswcr.2015.03.002
14. Gerona, R. R., Pan, J., Zota, A. R., Schwartz, J. M., Friesen, M., Taylor, J. A., Hunt, P. A., & Woodruff, T. J. (2016). Direct

measurement of Bisphenol A (BPA), BPA glucuronide and BPA sulfate in a diverse and low-income population of pregnant women reveals high exposure, with potential implications for previous exposure estimates: A cross-sectional study. *Environmental Health: A Global Access Science Source*, *15*(1), 1–14. https://doi.org/10.1186/s12940-016-0131-2

15. Karalius, V. P., Harbison, J. E., Plange-rhule, J., Breemen, R. B. van, Li, G., Huang, K., Durazo-arvizu, R. A., Mora, N., Dugas, L. R., Vail, L., Tuchman, N. C., Forrester, T., & Luke, A. (2014). Environmental Health Insights with Distinctly Different Levels of Economic Development. *Environmental Health Insights*, *8*, 1–3. https://doi.org/10.4137/EHI.S13130.TYPE
16. Khateb, F., Khatib, N., & Kubánek, D. (2012). Low-voltage ultra-low-power current conveyor based on quasi-floating gate transistors. *Radioengineering*, *21*(2), 725–735. https://doi.org/10.4103/2229-5186.79345
17. Li, X., & Franke, A. A. (2015). Improvement of bisphenol A quantitation from urine by LCMS. *Analytical and Bioanalytical Chemistry*, *407*(13), 3869–3874. https://doi.org/10.1007/s00216-015-8563-z
18. Manuela DDi Giovanni, Andreas Esposito, Barbara Romagnoli, Maria Ferrari, Rino Calori, Cecilia Bergamini, A. C. (2011). Determination of Bisphenol A in water bottles by solid phase extraction by high performance liquid chromatography and tandem mass spectrometry. *Agenzia Regionale Prevenzione e Ambiente Dell'emilia-Romagna*, 1. https://doi.org/10.1242/dmm.019414
19. Matsumoto, A., Kunugita, N., Kitagawa, K., Isse, T., Oyama, T., Foureman, G. L., Morita, M., & Kawamoto, T. (2003). Bisphenol A levels in human urine. *Environmental Health Perspectives*, *111*(1), 101–104. https://doi.org/10.1289/ehp.5512
20. Moors, S., Blaszkewicz, M., Bolt, H. M., & Degen, G. H. (2007). Simultaneous determination of daidzein, equol, genistein and bisphenol A in human urine by a fast and simple method using SPE and GC-MS. *Molecular Nutrition and Food Research*, *51*(7), 787–798. https://doi.org/10.1002/mnfr.200600289
21. Nachman, R. M., Fox, S. D., Golden, W. C., Sibinga, E., Groopman, J. D., & Lees, P. S. J. (2015). Serial free bisphenol A and bisphenol A glucuronide concentrations in neonates. *Journal of Pediatrics*, *167*(1), 64–69. https://doi.org/10.1016/j.jpeds.2015.03.036
22. Rodriguez-Mozaz, S., López De Alda, M. J., & Barceló, D. (2004). Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry. *Journal of Chromatography A*, *1045*(1–2), 85–92. https://doi.org/10.1016/j.chroma.2004.06.040
23. Shankar, A., & Teppala, S. (2011). Relationship between urinary bisphenol A levels and diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, *96*(12), 3822–3826. https://doi.org/10.1210/jc.2011-1682
24. Taskeen, A., & Naeem, I. (2010). Analysis of bisphenol A in blood and urine samples: A mini review. *Asian Journal of Chemistry*, *22*(5), 4136–4140.
25. Teeguarden, J. G., Calafat, A. M., Ye, X., Doerge, D. R., Churchwell, M. I., Gunawan, R., & Graham, M. K. (2011). Twenty-four-hour human urine and serum profiles of bisphenol A during high-dietary exposure. *Toxicological Sciences*, *123*(1), 48–57. https://doi.org/10.1093/toxsci/kfr160
26. Teeguarden, J. G., Twaddle, N. C., Churchwell, M. I., Yang, X., Fisher, J. W., Seryak, L. M., & Doerge, D. R. (2015). 24-hour human urine and serum profiles of bisphenol A following ingestion in soup: Individual pharmacokinetic data and emographics. *Data in Brief*, *4*, 83–86. https://doi.org/10.1016/j.dib.2015.03.002
27. Thayer, K. A., Taylor, K. W., Garantziotis, S., Schurman, S. H., Kissling, G. E., Hunt, D., Herbert, B., Church, R., Jankowich, R., Churchwell, M. I., Scheri, R. C., Birnbaum, L. S., & Bucher, J. R. (2016). Bisphenol a, bisphenol s, and 4-hydro xyphenyl 4-isopro oxyphenyl sulfone (bpsip) in urine and blood of cashiers. *Environmental Health Perspectives*, *124*(4), 437–444. https://doi.org/10.1289/ehp.1409427
28. Tsukioka, T., Terasawa, J., Sato, S., Hatayama, Y., MAKINO, T., & NAKAZAWA, H. (2014). Development of analytical method for determining trace amounts of BPA in urine samples and estimation of exposure to BPA. *Journal of Environmental Chemistry*, *14*(1), 57–63. https://www.jstage.jst.go.jp/article/jec1991/14/1/14_1_57.
29. Vandenberg, L. N., Chahoud, I., Heindel, J. J., Padmanabhan, V., Paumgartten, F. J. R., & Schoenfelder, G. (2010). Urinary,

circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environmental Health Perspectives*, *118*(8), 1055–1070. https://doi.org/10.1289/ehp.0901716.

30. Vandenberg, L. N., Chahoud, I., Heindel, J. J., Padmanabhan, V., Paumgartten, F. J. R., & Schoenfelder, G. (2010). Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environmental Health Perspectives*, *118*(8), 1055–1070. https://doi.org/10.1289/ehp.0901716
31. Vandenberg, L. N., Gerona, R. R., Kannan, K., Taylor, J. A., van Breemen, R. B., Dickenson, C. A., Liao, C., Yuan, Y., Newbold, R. R., Padmanabhan, V., vom Saal, F. S., & Woodruff, T. J. (2014). A round robin approach to the analysis of bisphenol a (BPA) in human blood samples. *Environmental Health: A Global Access Science Source*, *13*(1). https://doi.org/10.1186/1476-069X-13-25
32. Vom Saal FS, Vandenberg LN. Update on the Health Effects of Bisphenol A: Overwhelming Evidence of Harm. Endocrinology. 2021 Mar 1;162(3):bqaa171. doi: 10.1210/endocr/bqaa171.
33. Wolfgang, V., Bittner, N., & Dekant, W. (2005). Quantitation of Bisphenol a and Bisphenol a Glucuronide in Biological Samples By High Performance Liquid Chromatography-Tandem Mass Abstract: *Pharmacology*, *33*(11), 1748–1757. https://doi.org/10.1124/dmd.105.005454.unintentionally
34. Yokota, H., Kato, S., Physiology, V., Ku, S., Ka, S., & Iw, V. B. H. (2004). *BISPHENOL A GLUCURONIDATION AND EXCRETION IN LIVER OF PREGNANT AND NONPREGNANT FEMALE RATS Hiroki Inoue, Akio Tsuruta , Satoko Kudo , Takako Ishii , Yusuke Fukushima , Hidetomo Iwano , ABSTRACT :* *33*(1), 55–59. https://doi.org/10.1124/dmd.104.001537.is
35. Liu X, Yu Y, Zhao M, Zhang H, Li Y, Duan G. Solid phase extraction using magnetic core mesoporous shell microspheres with C18-modified interior pore-walls for residue analysis of cephalosporins in milk by LC-MS/MS. Food Chem. 2014;150:206-212.
36. Rivas MA, Granada A, Monteagudo C, Murcia MA, Olea-Serrano MF, Mariscal-Arcas M. Dietary exposure assessment of pregnant women to bisphenol-A form cans and microwave containers in Southern Spain. Food Chem Toxicol. 2009;47(2):506-510.
37. Syed SL. Analysis of bisphenol A leaching from baby feeding bottles. Agilent Technologies, Inc. Bangalore, India. 2011.
38. Schecter A, Malik N, Haffner D, Smith S, Harris TR, Paepke O, Birnbaum L. Bisphenol A (BPA) in U.S. food. 2010;44(24):9425-30.