BISPHENOL-A CONCENTRATIONS IN WASTEWATER AND RIVER WATER SAMPLES FROM WESTERN KENTUCKY, USA

Subhadra Vemu, Bommanna G. Loganathan

Department of Chemistry and Watershed Studies Institute, 1201 Jesse D. Jones Hall, Murray State University, Murray, KY 42071-3300, USA.

Abstract

Bisphenol A (BPA) concentration was measured in Murray Wastewater Treatment Plant (MWWTP) samples including influent, influent composite, effluent and effluent composite. Upstream Bee Creek and downstream Bee Creek water samples were also analyzed to study input of BPA from the WWTP. Natural waters such as Clarks River and Kentucky Lake water samples were also analyzed to understand the levels contamination of BPA by non-point source. The results showed that measurable levels of BPA were found in all samples analyzed. Among the samples analyzed, the concentration of BPA ranged from 103 ng/L in Upstream Bee Creek) to 153 ng/L in influent. Removal efficiencies of Bisphenol A from this WWTP varied from 6.62 % – 15.7 %. Bisphenol A is resistant to the waste water treatment process and hence a significant level of BPA reaches the receiving waters such as Bee Creek and Clarks River.

Introduction

In recent years, there exists a serious concern about the man-made chemicals that can mimic the natural estrogen known as "Endocrine disrupting chemicals" [1]. Some pesticides and industrial chemicals can affect animal physiology by mimicking the effect of endogenous hormones. Bisphenol-A (2, 2-bis (hydroxyphenyl) propane) is a well known endocrine disruptor. Bisphenol A (BPA), is proved to be the cause of various 'developmental and reproductive toxicities' posing a serious threat to human and wild life species[2,3]. Every year, over six billion pounds of BPA are used in the manufacturing of epoxy resins and poly-carbonate plastics used in a wide variety of domestic products [4]. BPA has lipophilic property, therefore, it is easily absorbed into small intestine and binds to intracellular endocrine receptors to bring about the estrogenic activity [5].

Because of BPA's high volume production and extensive use in plastics, there is a widespread environmental contamination and well documented human exposure to BPA. To our knowledge, there exist no studies conducted on BPA contamination levels in western Kentucky regional waters. Therefore, this study was designed to assess the concentrations of BPA in our waste water treatment plant samples. To assess the levels of BPA in natural waters, water samples from Clarks River and Kentucky Lake were collected and analyzed. Enzyme linked Immunosorbent Assay is the most flexible and accurate method developed till date and produces parallel data when estimated through GC, HPLC or other analytical techniques.

Murray is a small university town located in the Calloway County, in the western Kentucky. MWWTP serves the residence to a population of approximately 15,100 people as per the 2002 U.S. census. In addition, MWWTP also serves about 10,000 students from surrounding states during an academic year. The Murray waste water treatment plant receives sewage and other waste water from the residential houses, hospitals, university and commercial and industrial plants. It has the processing capacity of 20 megalitres. day^{-6}. Based on the sample types and the possible sources of BPA, we hypothesize that detectable levels of BPA may be found in MWWTP samples, natural waters such as Clarks River, Kentucky Lake. Influent and Effluent BPA concentrations would reveal the removal efficiencies and loading estimates of BPA into Clarks River from MWWTP.

Materials and Methods

Five individual sampling events were carried out from the Murray Waste Water Treatment Plant, Clarks River, Kentucky Lake sampling locations during December, 2008 through April, 2009. Four sampling points: Influent, Effluent from MWWTP, Upstream Bee creek, Downstream Bee creek were also chosen to study the input of BPA into the receiving water (Figure 1).

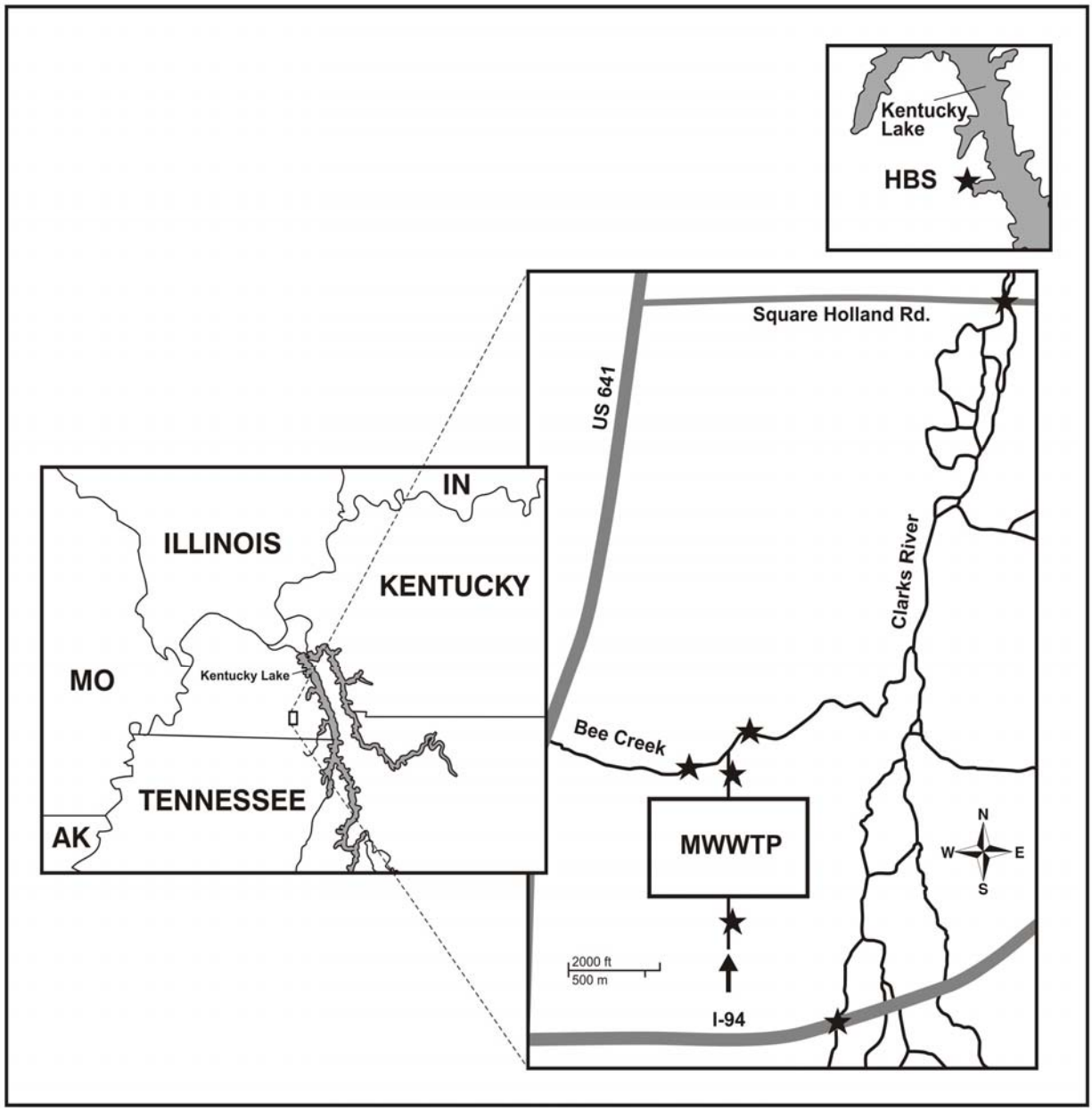

Figure 1. Map showing sampling locations in Murray Wastewater Treatment Plant, Bee Creek, Clarks River and Kentucky Lake, USA.

Influent (coordinates: 36° 37.685' N & 88° 17.640' W) consists of incoming raw sewage and waste water from the residents and business complexes of Murray and neighboring communities. This influent after treatment process it is discharged as effluent at 36° 37.770 N & 88°.17.597' W). Two samples were taken from the Bee Creek, Upstream Bee Creek (located at 36°.37.762' W & 88°.17.759' N) is at the front end of MWWTP while

downstream Bee Creek (located at 36°.37.831' N & 88° 17.759' W) receives the processed water. The sample details are shown in Table 1.

Table 1. Details of samples collected at Murray Wastewater Treatment Plant, Clarks River and Kentucky Lake.

Survey	Sampling Date	Sample Type	Precipitation	Temperature
1	12/11/2008	Influent Influent Composite Effluent Effluent Composite Upstream Bee Creek Downstream Bee Creek	0.7"	28°F
2	12/19/2008	Influent Influent Composite Effluent Effluent Composite Upstream Bee Creek Downstream Bee Creek	0.21	37°F
3	1/21/2009	Influent Influent Composite Effluent Effluent Composite Upstream Bee Creek Downstream Bee Creek Clarks River Kentucky Lake	none	33°F
4	2/14/2009	Influent Influent Composite Effluent Effluent Composite Upstream Bee Creek Downstream Bee Creek Clarks River Kentucky Lake	trace	38°F
5	2/22/2009	Influent Influent Composite Effluent Effluent Composite Upstream Bee Creek Downstream Bee Creek Clarks River Kentucky Lake	0.0	40°F

Waste water, river water samples were collected using adjustable length sampling pole with sample bottle strapped. After rinsing several times with sample, water samples were collected and transferred to pre-cleaned amber colored glass bottles with Teflon lined caps. The sample bottles were transported in ice in a cooler to the laboratory for analysis. The samples were immediately filtered using preashed glass fiber filters (0.45 µm pore diameter) and stored at -20^0 C.

Solid phase extraction of the samples was performed for all samples with OASIS Hydrophilic Lipophilic Balanced – Solis Phase Extraction cartridges (HLB-SPE), 3.0mL, and 0.2 g, 30 µm, obtained from Waters Limited. All cartridges were pretreated with 2 * 3.5 mL portions of Methanol followed by 2 x 3 mL portions of 18 ΩM deionized water. 100mL of each sample was passed through the cartridges with a flow rate of 3-5

mL/min by applying vacuum. The samples were eluted with approximately 15 mL portions of Dichloromethane – Hexane (4:1) ratio (99.9 % OPTIMA grade obtained from Fischer Scientific). The eluent volumes were further concentrated to 1 mL portions using a gentle stream of ultra high purity nitrogen gas to a residue. 10 % v/v methanol was added to extract the residue through efficient mixing of the sample. Final samples were assayed with BPA specific supersensitive kit provided by Japan Envirochemicals Limited. The measurement of fluorescence was determined using precalibrated M6+ Mini spectrophotometer set at single wavelength of 450 nm.

Results and Discussion

Five point calibration curve was constructed with the standard analyte Standard solutions provided with the BPA specific ELISA kit was used for calibration curve. All the standard values gave highly reproducible data with $R^2 > O.99$. Measured BPA concentrations (ng/L) are shown in Table 2.

Table 2. Concentration (ng/L) of Bisphenol A in waste water samples collected from Murray Wastewater Treatment Plant.

Sample	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	**Mean**
Influent	134	135	152	151	139	142.6
Influent Composite	126	139	156	NA	149	137.3
Effluent	113	118	140	141	119	128.6
Effluent Composite	103	119	155	NA	139	129.0
Upstream Bee Creek	79	118	70	129	118	101.2

Detectable concentrations of BPA was found in all of the samples collected from MWWTP. Influent samples showed the highest mean concentration of 142.60 ng/L (Range: 132-152 ng/L). The 24 h influent composite samples had a mean concentration of 137.25 ng/L (Range 118-156 ng/L), showed a similar in magnitude to that of influent samples. The effluent samples had relatively lower levels of BPA than that of influent and contained a mean concentration of 128.6 ng/L (Range: 109-141 ng/L), and 24 h effluent composite samples had a mean concentration of 129 ng/L (Range: 103-155 ng/L). The concentration of BPA in the upstream Bee Creek was assessed to have a mean concentration of 101.2 ng /L (Range: 72-13 ng/L). The downstream Bee Creek samples that received the treated waters from MWWTP, (effluent) had interestingly BPA concentrations of mean 133.60 ng/L a little higher than that of upstream Bee Creek indicating the loading of BPA from the treated effluent from MWWTP. Duplicate analysis was performed for a few of the sample types, for which the above figures represent the mean. In order to compare the grab versus composite sample concentration, 24-h composite samples were analyzed. A slight difference in concentration was noticed in influent samples, but, the effluents contained very similar mean values (Table 2). 1. In general, influent samples recorded highest concentration of BPA when compared to the rest of the samples.

BPA concentrations found in the natural water bodies such as Kentucky Lake and Clarks River were given in Table 3. The Kentucky Lake showed a BPA mean concentration of 132 ng/L and Clarks River 117 ng/L. These mean concentrations clearly lie in the range of effluent and downstream Bee Creek samples as observed from Table 3.

Table 3. Bisphenol A concentrations (ng/L) in Kentucky Lake and Clarks River, Kentucky, USA.

Sampling Location	Survey 1	Survey 2	Survey 3	Mean
Kentucky Lake (HBS)	135	159	103	132.3
Clarks River Up Stream	124	116	111	117.3
Clarks River Downstream	119	114	120	117.6

The amount of BPA eliminated during the waste water treatment process, removal efficiency was calculated from differences in the magnitude of influent and effluent (Table 4). The highest removal efficiency as calculated to be 15.67 % occurred in the survey number one, least being in survey number four of the magnitude of 6.62 %. BPA removal efficiency of the MWWTP was in the following order: Removal efficiency of survey #1 > survey # 5 > survey # 2 > survey # 3 > survey # 4. Loading estimates of BPA from the MWWTP to the downstream Bee Creek site were calculated by taking concentration of BPA and multiplied to the total volume of processed water through the waste water treatment plant. The final loading estimates ranged from 1440 mg/day to a maximum of 2580 mg/day (Table 4).

Table 4. Bisphenol A removal efficiency and loading estimate of Murray Wastewater Treatment Plant during different sampling surveys.

Bisphenol A	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5	Mean
Removal Efficiency (%)	15.7	12.6	7.89	6.62	14.4	9.82
Loading Estimate (mg/day)	1580	2360	1440	2580	2360	2064

To our knowledge, this is the first study on bisphenol A in Murray Wastewater Treatment Plant samples, Clarks River and Kentucky Lake. Our results provide evidence that detectable concentrations of BPA were found in all samples analyzed. Influent contained highest concentrations of BPA than effluent. Upstream Bee Creek had relatively lower concentrations than downstream, indicating the input of BPA from WWTP to the receiving waters, Bee Creek and Clarks River (Table 4).

Acknowledgements

The authors are thankful to Mr. Jason Henderson, Murray Wastewater Treatment Plant for his help in sampling at the Plant. The authors also thankful to Mr. Vidyasagar Kummarikunta, Miss. Nanditha Billa, Mr. Sudan Loganathan, Ms. Annette Fowler and Mr. Dylan Benningfield for their help during sampling and analysis.

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