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Determination of bisphenol A traces in water samples from the Vrbas River and its tributaries, Bosnia and Herzegovina

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Abstract: The bisphenol A (BPA) concentration was determined in 12 surface water samples of the Vrbas River and its five tributaries. The samples were taken in the area that belongs to the city of Banja Luka (Bosnia and Herzegovina). BPA was isolated using micro liquid–liquid extraction followed by derivatization and gas chromatography–mass spectrometry analysis (GC–MS). Silylation was used as a derivatization method to increase volatility and allow the GC–MS determination of BPA. The limits of detection (*LOD*) and quantification (*LOQ*), obtained by validating the procedure, were determined at 4 and 10 ng L⁻¹, respectively. The concentrations of BPA were ranged between 33 and 354 ng L⁻¹, and all were above the *LOQ* value. The lowest amount of BPA was found in the sample collected in the river Vrbas, near Švrakava estuary upstream from the city of Banja Luka. The highest concentration of BPA was recorded at the confluence of the Crkvena and Vrbas rivers, which is located in the city center. This study shows that population and human activity could affect the level of BPA in the environment.

Keywords: bisphenol A; GC–MS; microextraction; derivatization; river water.

INTRODUCTION

It is well known that the presence of certain chemicals in the environment adversely affects human health by damaging the endocrine system.¹ These compounds can interact with specific receptors and therefore interfere with the hormonal action and function of human cells.²

Bisphenol A (BPA; 4,4'-dihydroxy-2,2-diphenyl propane) is a chemical synthesized worldwide with over 5.5 million tons per year and is defined as one of the endocrine-disrupting compounds (EDCs).³ BPA is known as a monomer – for the synthesis of polycarbonate plastics, epoxy, and polyester resins, and it is

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also used in the production of thermal paper.^{4,5} Consequently, it can be found in materials which are used to produce CDs and DVDs, baby bottles, containers for beverages and foods, toys, and medical devices.^{6,7} Traces of BPA can leak from these materials leading to releases into the environment and causing pollution. Some studies have shown that the BPA is mainly found in water, soil, and sediments.⁸ The primary sources of BPA in the aquatic environment are domestic and industrial wastewater and runoff from agriculture.⁹ It has been found that the presence of BPA in the aquatic ecosystem may have a potentially harmful influence on the reproductive system and metabolic processes of organisms such as fish and mussels.¹⁰ In addition, BPA has been detected in human serum, urine, saliva, and breast milk.¹¹ Some studies indicate that BPA could be effective as estradiol in activating specific receptor responses and may act as an androgen receptor antagonist.^{12,13} Furthermore, it has been reported that human exposure to BPA could cause several health issues, including various types of cancers, obesity, cardiovascular and neurodegenerative disorders.^{14–17}

Canada is the first country to consider BPA regulation more strictly and ban its presence in infant feeding bottles.¹⁸ Moreover, BPA is listed among compounds considered hazardous to human health and the environment.¹⁹ BPA-contained products are forbidden in some American states. At the same time, in the EU, France was the first state that banned the production, trade, and marketing of food cans coated with epoxy-resins containing BPA in January 2015.²⁰ Realizing the seriousness of the negative impact on human health, the European Food Safety Authority (EFSA) has recently set a new specific migration limit (*SML*) of BPA that can migrate from the plastic food contact material into the food at $50 \mu\text{g kg}^{-1}$.²¹ The new *SML* is based on a temporary tolerable daily intake (*t-TDI*) of BPA, which from 2015 onwards amounts to $4 \mu\text{g (kg BW)}^{-1} \text{ day}^{-1}$.²² Further, according to the European Water Framework Directive and the Environmental Quality Standards Directive, BPA has recently been listed as a priority hazardous pollutant.^{23,24}

Due to the toxicity and widespread human exposure to BPA, it is necessary to monitor the presence of this compound in the environment. The examinations of wastewater, surface water, and drinking water samples collected in Germany showed that the BPA concentration in drinking water samples ranged from 0.5 to 2 ng L^{-1} , while the mean BPA concentration in river water samples and the wastewater was 4.7 and 16 ng L^{-1} , respectively.²⁵ A study conducted by measuring BPA in the Danube River, near Novi Sad in Serbia, showed that the concentration of BPA varies significantly depending on the seasons and is highest in summer, probably due to increased human activities and weather conditions.²⁶

Accordingly, our study aimed to determine the amount of bisphenol A in real water samples collected in the Vrbas River and its tributaries using the GC–MS technique. The samples were collected in early July 2021, during the low water

levels. BPA was isolated from water samples by a very simple and accessible micro-liquid-liquid extraction technique, followed by a derivatization step by silylation with BSTFA reagent (*N,O*-bis(trimethylsilyl)-trifluoroacetamide). Silylated BPA was quantified by GC-MS due to increased volatility.

EXPERIMENTAL

Chemicals

BPA was purchased from Sigma-Aldrich with a minimum of 98% purity. Deuterated bisphenol A- d_{16} (BPA- d_{16}), purchased from Sigma-Aldrich, was used as an internal standard (IS). The stock solution was prepared by dissolving a certain amount of BPA in acetone (HPLC purity, Fisher Scientific, UK). Working solutions for recovery check and calibration were prepared by diluting the stock solution at the required concentrations in acetone. All solutions were stored in the refrigerator before analysis. During the experiment, only equipment made of glass was used. Sodium chloride and dichloromethane (both from Lach-Ner, Czech Republic) were used for the microextraction. The derivatization process was performed using the following derivatization reagent: BSTFA (*N,O*-bis(trimethylsilyl)trifluoroacetamide) + 1% TMCS (trimethylchlorosilane) reagent (Supelco, Sigma-Aldrich). The silylated derivative of BPA was dissolved in hexane (HPLC purity, Fisher Scientific, UK) before GC-MS analysis.

Description of the sampling sites

Details related to the sampling sites are given in Supplementary material to this paper.

Pretreatment and micro liquid-liquid extraction of the samples

150 g of NaCl was dissolved in a 500 mL water sample. Afterward, the pH value was adjusted between 3 and 5 by adding diluted HCl (1:1).^{27,28} The sample was transferred to a separatory funnel connected to a mechanical stirrer. Then 3.5 mL of dichloromethane and 20 μ L of BPA- d_{16} ($c = 0.5 \text{ mg mL}^{-1}$) were added to the funnel. The content of the funnel was stirred for 1 min at 900 rpm.²⁹ After mixing, the organic phase (bottom layer) was discharged directly through a separatory funnel into a vial, via a layer of anhydrous Na_2SO_4 and cotton wool, to remove residual water due to sensitivity of the derivatizing agent to moisture.

Derivatization and GC-MS (SIM) analysis

The dichloromethane phase obtained after microextraction was exposed to a gentle stream of nitrogen to remove the solvent. Then derivatization of the residue (extracted BPA) was performed by adding 100 μ L of derivatization reagent (BSTFA + 1 % TMCS). The content was stirred on vortex and heated to 80 °C for 30 min. After derivatization, the solution was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 100 μ L of hexane.³⁰

The samples were analyzed using the GC-MS technique (Varian 450-GC gas chromatograph with 220-MS IT mass spectrometer, USA). The separation of compounds was performed on FactorFour capillary column VF-5ms 30 m \times 0.25 mm with 0.25 μ m film thickness (Varian). The injector temperature was 270 °C, and the injection volume was 1 μ L in splitless mode. The carrier gas was helium at a constant flow rate of 1 mL min⁻¹. The oven program started at 50 °C. Afterward, the temperature was incremented at 50 °C per min to reach 150 °C, and then immediately at 30 °C per minute until it reached 270 °C, where it was held for 5 min. The parameters for MS analysis were as follows: EI (70 eV) temperature 200 °C, solvent cut time 6 min, scan 45–650 m/z . The identification was based on comparing a particular compound's mass spectra and retention time with the reference materials using the "Wiley regis-

tryTM of mass spectral data” library. The quantitative analysis was performed using the internal standard (BPA-*d*₁₆, IS), which was added to each sample in the same amount and considered the relative response of the analyte to the IS. The calibration curve, which was used for quantification, is described in the “linearity” section.

The validation of the method

The validation was performed by determining several important parameters, such as linearity, precision and trueness, limit of detection (*LOD*), and quantification (*LOQ*). Deionised water without adding BPA was analyzed as a blank sample before each analysis in order to track the residual amounts of BPA in solvents and the used reagent.

Linearity. The linearity of the method was determined by calibration curve construction. A series of dilutions (50, 100, 200, 400, 600 and 800 ng mL⁻¹) were made from the working solution (2000 ng mL⁻¹) to obtain a calibration curve with six different concentration levels in hexane: 0.1, 0.2, 0.4, 0.8, 1.2 and 1.6 ng µL⁻¹. Each concentration level was analyzed in duplicate. In addition, lower concentrations were also analyzed (0.02 and 0.05 ng µL⁻¹), in order to determine the *LOD* and *LOQ*. The calibration curve was formed based on the peak area ratio of the BPA and the BPA-*d*₁₆. The internal standard was always added at the same concentration of 0.2 ng µL⁻¹ hexane. The linearity of the calibration curve was determined by the coefficient of determination (Table I). The obtained calibration curve was used for the quantification of BPA in water samples.

LOD and LOQ. The quantification limit is presented as the lowest concentration that can be quantified with a precision lower than 5 %. The sample with the minimum concentration of 0.02 ng in 1 µL hexane was analyzed in six replicates and the standard deviation (*SD*) was calculated.

The detection limit was calculated according to the following equation:

$$LOD = X + \frac{3SD}{m} \quad (1)$$

where *X* is the average value of concentrations found in analyzed samples and *m* is the slope of a calibration curve.

The detection limit was calculated according to the following equation:

$$LOQ = X + \frac{10SD}{m} \quad (2)$$

The *LOQ* and *LOD* values are shown in Table I.

Precision and trueness. Trueness was proved by the spiking/recovery method. Precision was calculated as the relative standard deviation (*RSD*, %) of concentrations found after the repetition of spiking six times per day. The validation was performed in duplicate by spiking the deionised water samples (test portion 500 mL) with the BPA to obtain the following concentrations: 80, 120 and 160 ng L⁻¹. The IS was added to each sample in the same concentration of 40 ng L⁻¹. The amount of recovered BPA ranged between 72.5 and 82.5 wt. %, with a mean value of 77.5 wt. %, calculated based on the initial weight of BPA. The obtained results are given in Table I.

TABLE I. The validation parameters of the microextraction – GC–MS method for the analysis of BPA in water samples

| <i>m/z</i> | <i>R</i> ² | Method trueness – recovery ± <i>RSD</i> , wt. % | <i>LOQ</i> / ng L ⁻¹ | <i>LOD</i> / ng L ⁻¹ |
|------------|-----------------------|---|---------------------------------|---------------------------------|
| 357/372 | 0.9982 | 77.50±5.42 | 10 | 4 |

RESULTS AND DISCUSSION

The real water samples collected at the locations, as shown in Fig. S-1 of the Supplementary material, were tested to determine the content of BPA. After microextraction and derivatization processes, the prepared samples were analyzed by GC-MS technique. The qualitative analysis was performed using a characteristic retention time and at least two m/z values. The first, always required, was the basic ion. The second was the molecular ion or characteristic/reference ion of a given compound. The characteristic total ion chromatogram and mass spectra of identified silylated BPA and silylated BPA- d_{16} for the sample T4 are presented in Fig. 1.

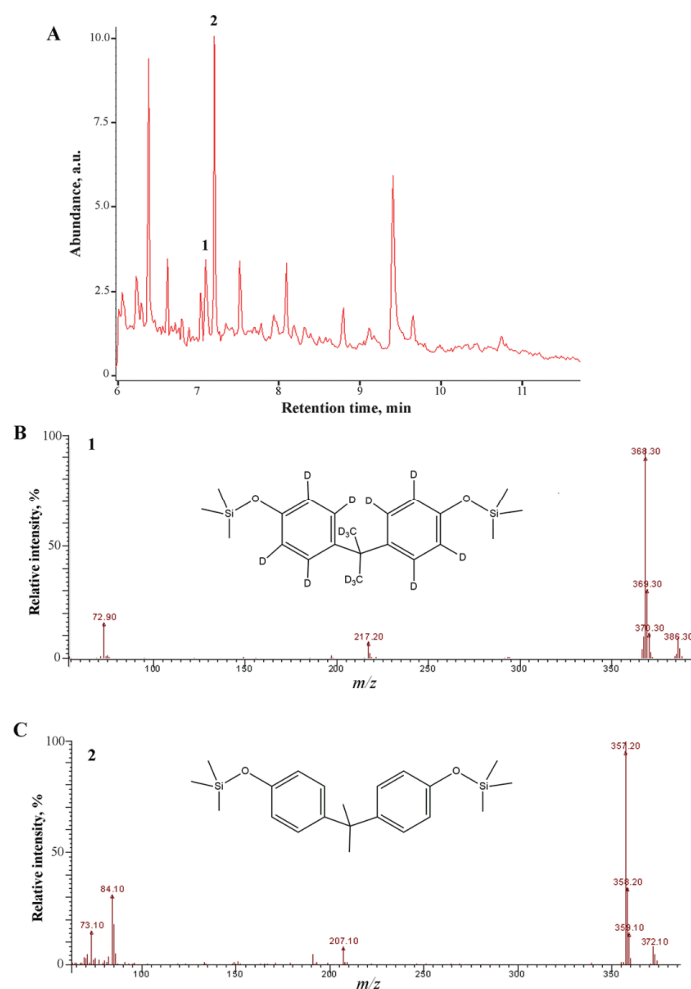


Fig. 1. The total ion chromatogram (A) and mass spectra of identified silylated BPA- d_{16} (B) and silylated BPA (C) for the sample T4.

The main required ions used for the identification were 357 and 368 for silylated BPA and silylated BPA, respectively. As a second characteristic ion, the molecular ion of a particular compound is generally sought, which was 372 (silylated BPA) and 386 (silylated BPA- d_{16}).

The obtained results are shown in Table II. The mass of BPA (ng) in 1 μL hexane, which was injected in the GC column, was calculated using the calibration curve. The mass of BPA in 100 μL hexane was the same found in 500 mL of the initial sample before any treatment. It can be seen that the concentration of BPA in the investigated river samples ranged from 33 to 354 ng L^{-1} . The highest concentration was measured in the samples collected at the mouth of the Crkvena into the Vrbas River (MT2). The lowest value of BPA was recorded in the sample taken at the location where the Švrakava River flows into the Vrbas upstream from the city (V1).

TABLE II. Results of GC–MS analysis of the water samples

| Sample | BPA concentration | |
|--------|-------------------------------|--------------------|
| | $\text{ng } \mu\text{L}^{-1}$ | ng L^{-1} |
| T1 | 0.32 | 65 |
| T2 | 0.34 | 69 |
| T3 | 0.77 | 153 |
| T4 | 0.49 | 99 |
| T5 | 0.96 | 193 |
| MT1 | 0.60 | 121 |
| MT2 | 1.77 | 354 |
| MT3 | 0.67 | 133 |
| V1 | 0.16 | 33 |
| V2 | 0.42 | 84 |
| V3 | 0.80 | 159 |
| V4 | 0.52 | 104 |

Additionally, it can be observed that in all analyzed samples, the BPA concentration was higher than the *LOQ* value, which was 0.06 $\text{ng } \mu\text{L}^{-1}$. The obtained results indicated the contamination of the Vrbas River and its tributaries at all sampling sites.

The river Švrakava is a right tributary of the Vrbas, with the mouth around 15 km upstream from the city center, positioned in a relatively clean but populated area called Karanovac. It was not unexpected that the lowest BPA values were determined for the samples T1 and V1, although agricultural land and partly construction facilities are located immediately before the mouth along the riverbank. Both samples were clear, with no visible contaminants.

The course and the catchment area of the river Suturlija, which is about 17 km long, is located in the area southwest of Banja Luka. The river flows through a relatively uninhabited area, however, at the confluence into the Vrbas River in

Srpske Toplice, at the altitude of 159 m, it is under a certain anthropogenic factor. The mouth of the river Suturlija in the Vrbas is located just before the beginning of the inner city zone. The results show that the concentration of BPA in the sample taken at the mouth of the river Suturlija in the Vrbas (MT1) is two-fold higher than the concentration found in the sample taken in the river itself (T2). All samples taken in this area were clear, with no visible contaminants.

The Crkvena River, with its course of 11.5 km, flows through a very populated and active area. It finally flows into the Vrbas directly into the city centre and near the Kastel fortress. The last part of the stream, about 1.5 km long, is located in an underground tunnel under the road. This tunnel ends 100 m before the mouth of the Vrbas at the sewer in the central zone of Banja Luka. This could explain the high concentration of BPA recorded in the sample taken at the mouth of this river in the Vrbas (MT2), which is the highest value obtained in this study. The water samples taken from the Crkvena River were visibly polluted, turbid, and had an unpleasant odour.

The river Vrbanja is more than 90 km long and the largest tributary of the Vrbas. The river Vrbas upstream from the mouth of the Vrbanja is burdened with discharges. This could be why the higher measured concentration of BPA at the location V3, compared to the results obtained at locations T4 and MT3 (Table II). Furthermore, the Vrbanja samples were clear, with no visible contaminants. However, the sample of the Vrbas (V3) in this area was somewhat stained.

The source of the river Dragočajska is located on the hill Ruštevaca. Its confluence into the Vrbas is near the settlement Zalužani. The length of the stream is about 21 km. In the middle and lower course, this river is of a flat character, with many tributaries. A significant part of the flow of this watercourse passes through a densely populated area and is under significant anthropogenic influence. The sample of the Dragočajska River was visibly polluted and turbid, while the sample of the Vrbas River (V4) was slightly stained. Moreover, at the time of sampling on the banks and in the river itself, the last few hundred meters before the mouth, plastic waste (bottles and bags) could be seen. The concentration of BPA in the Dragočajska sample was the highest (T5), which can be attributed to its position. In addition, it should be noted that one of the tributaries of the Dragočajska river (Ivaštanka) is located near the municipal landfill in Ramići, whose leachate could contribute to higher BPA value. It flows into the Vrbas about 9 km downstream from the central zone of the city of Banja Luka, which may explain the lower concentration of BPA in the sample of the Vrbas River in the zone downstream from the mouth (V4).

A study similar to ours, conducted in southern India, has shown that the BPA concentration ranged from 2.8 to 136 ng L⁻¹, in the samples collected from three rivers (Kaveri, Vellar and Tamiraparani).³¹ The values of BPA are comparable to those found in the Vrbas River area. In addition, in the study performed in the

Danube River (Serbia) and its tributaries the Tisa and the Sava, the BPA concentration varies from the area where the samples were taken.³² Moreover, high amounts of BPA were detected near industrial zones (up to 338 ng L⁻¹), which agrees with the result obtained in our study (sample MT2). On the other side, a much lower concentration was measured in the samples taken in the municipal areas ranging from 0.6 to 31.2 ng L⁻¹. This finding confirms the observation obtained in our study that waste such as plastics and other contaminants could significantly impact the level of BPA in water resources. Furthermore, similar observation can be found in the study where sampling was performed from three wastewater treatment plants in Poland and municipal surface waters.³³ The obtained results have demonstrated extremely high concentration of BPA in wastewater (up to 1465 ng L⁻¹), while this parameter was even higher in surface water (up to 3113 ng L⁻¹), which is significantly higher than the concentrations found in surface water analyzed in this study.

CONCLUSION

The concentration of bisphenol A in the Vrbas River and its tributaries was examined by GC–MS technique. For that purpose, 15 samples were taken from three locations around 5 tributaries of the Vrbas River. The presence of BPA was detected in all analyzed samples, with the concentrations higher than the *LOQ* value. The highest BPA concentration was recorded at the mouth of the river Crkvena in the Vrbas River, where the Crkvena flows into the Vrbas direct to the city centre. On the other side, the lowest amount of BPA was found in the sample collected in the river Vrbas near Švrakava estuary upstream from the city of Banja Luka. This study indicates that population and human activity could influence the level of BPA in the environment and therefore the monitoring this compound is required. According to the legislation of the Western Balkan countries, the maximum permitted concentration of BPA is not defined. Thus, it is important to calculate the exposure rates of this pollutant as an endocrine-disrupting compound. Compared to the available literature data, the concentrations of BPA found in this study are not considered extremely hazardous to human health and the environment.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/11238>, or from the corresponding author on request.

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ИЗВОД

ОДРЕЂИВАЊЕ ТРАГОВА БИСФЕНОЛА А У УЗОРЦИМА ВОДЕ ИЗ РЕКЕ ВРБАС И ЊЕНИХ ПРИТОКА, БОСНА И ХЕРЦЕГОВИНА

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Концентрација бисфенола А (ВРА) одређена је у 12 узорака површинских вода реке Врбас и њених пет притока. Узорци су узети на подручју које припада граду Бања Лука (Босна и Херцеговина). ВРА је изолован микро течно-течном екстракцијом, након чега је уследила дериватизација и гасно хроматографска-масено спектрометријска анализа (GC-MS). Силиловање је коришћено као метода дериватизације, како би се повећала испарљивост и омогућило GC-MS одређивање ВРА. Границе детекције (LOD) и квантификације (LOQ), су утврђене валидацијом коришћене процедуре, и износиле су 4 и 10 ng L⁻¹, редом. Концентрације ВРА су се кретале између 33 и 354 ng L⁻¹, и све су биле изнад вредности LOQ. Најнижа количина ВРА пронађена је у узорку узетом из реке Врбас код ушћа Швракаве, узводно од града Бања Лука. Највећа концентрација ВРА забележена је на ушћу реке Црквене у Врбас, које се налази у центру града. Ова студија показује да би број становника и људска активност могли да утичу на ниво ВРА у животној средини, па је стога потребно стално праћење концентрације овог једињења.

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