

Temporal bisphenol A occurrence in the Danube surface water during different seasons

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Abstract:

This research describes for the first time the identification and quantification of bisphenol A (BPA) in the Danube surface water in Novi Sad (Serbia).

A gas chromatographic mass spectrometric (GC-MS) method with a solid-phase extraction (SPE) procedure was used to investigate the seasonal variations of the BPA concentrations in the water samples. These variations were significant, showing higher concentrations in summer comparing with those in winter. Although it has a short half-life in surface water (0.5-5 days), BPA was frequently detected at relevant concentration levels of up to 693 ng/L.

The risk quotients, expressed as the ratio of the 95th percentile of the measured environmental concentrations for each sampling site and the predicted no-effect concentration (PNEC) were higher than one (> 1) at seven sampling locations, indicating a significant ecotoxicological risk to the human health and the environment, which confirmed a considerable contamination of the Danube surface water along the Novi Sad bank, Serbia.

Keywords: bisphenol A; the Danube river; endocrine disrupter; GC-MS; surface water.

RUNNING TITLE: BISPHENOL A IN THE DANUBE

Bisphenol A (BPA) is one of the most investigated and discussed endocrine disrupting compounds (EDCs), and has received large attention as an emerging chemical of concern in order to protect wildlife and human health.¹ Endocrine disruptors (EDs) can interfere with hormonal action and with the human cell functions mainly through the interaction with specific receptors and can induce numerous adverse health effects. Therefore, the occurrence of BPA in the environment could have potential harmful effects on the reproductive system, growth, digestion and metabolic processes of aquatic organisms like fish or mussels.² Moreover, effects on the human endocrine system and its function have been discussed by direct exposure via drinking water (BPA was used in polycarbonate plastic bottles).³ BPA is also believed to pose a potential risk of developing various types of cancers.^{4,5}

BPA, 2,2-bis(4-hydroxyphenyl) propane, is an organic compound extensively used as an intermediate in the production of polycarbonate plastics and epoxy resins, unsaturated polyester-styrene resins and flame retardants. It is used in a broad range of the products of modern consumer society, including reusable baby bottles, toys, food and drink storage containers, protective coatings for metal cans, adhesives and paints, electronic components, personal computers, CDs and DVDs, sports equipment, medical devices, eyeglass lenses, dental sealants and dental bonding agents, etc.^{6,7} More than 3.6 million tons of BPA is synthesized worldwide annually and its production is constantly increasing owing to a growing demand for plastic products.⁸

The first country that has recognized the importance and necessity to legislate and regulate the environmental issue of BPA was Canada.¹ Although, the European Commission and the US government have banned the BPA use in baby bottles and packaging items for children aged 0-3, BPA is still under discussion to be prioritized for regulation by the European Union and the United States because of its possible adverse impact on human health and environment. Various declarations and statements have been issued [The Berlaymont declaration, the Ramazzini statement, European Environmental Bureau, World Health Organization, etc.] on this challenging topic, and the debate is still going on how to regulate and reduce the BPA concentrations effectively in consumer products and aquatic systems in order to protect humans and wildlife.¹ Recent studies confirmed the presence of BPA in different human biological matrices such as urine⁹, breast milk¹⁰ and hair¹¹.

The main source of the BPA occurrence in the aquatic environment is municipal and industrial wastewater. BPA is mostly found in water and food (including drinking water) associated with the manufacture of various polycarbonate plastic products (packages or food contact materials) or it is leached from the final products as a result of the conducted hydrolysis of polymers at high temperature, or under acid/basic conditions.^{3,12,13} Despite its rapid degradation, favoured by aerobic conditions, with a half-life of about 0.5-5 days in the aquatic environment, the constant release into the surface water makes BPA a pseudo-persistent pollutant in the environment.¹⁴⁻¹⁶

Although, European legislation set environmental quality standards for priority and certain other pollutants, BPA permitted levels, together with pharmaceuticals, personal care products, algal toxins and various endocrine disrupters have not been regulated by any legislative bodies yet because of the insufficient reliable data on their safety or risks.^{14,17}

In the Western Balkan Region, particularly in Serbia, only limited monitoring data on water status are available. A screening analysis of the wastewater discharged into the Danube along the Novi Sad municipality was carried out during the period 2011-2012, and more than 130 chemical contaminants were detected.¹⁸ Furthermore, non-target screening analysis of the Danube water samples in the area of Belgrade confirmed the presence of the wide range of organic contaminants.¹⁹

The primary goal of this study, conducted for the first time, was to examine the occurrence of BPA along the Danube River in the area of Novi Sad (Serbia). The obtained results were also used to evaluate the seasonal variations of this endocrine disrupter and to assess the potential ecological risks.

EXPERIMENTAL

Chemicals

The standard of bisphenol A, (2,3-dihydroxypropyl) glycid was obtained from Sigma Aldrich (Deisenhofen, Germany). Methanol, dichloromethane and ethyl acetate were of HPLC grade (LGC Promochem, Wesel, Germany). The silylation agent N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Fluka (Sigma Aldrich, Deisenhofen, Germany) and ultra pure water was used. The regenerated cellulose filters (0.45 µm) and cartridges were obtained from Agilent Technologies. All glassware was rinsed with methanol and dried in an oven at 100 °C for 1 h before use.

Collection of water samples

The surface water samples (1 L) were collected in dark amber glass bottles at eight representative locations along the Danube River (Fig. 1) of the Novi Sad municipality, Serbia, during autumn (November 2012), winter (March 2013), spring (May 2013) and summer (September 2013) under comparable meteorological conditions at all sites for each sample at the depth of 50 cm. The sampling locations, presented in Fig. 1, are in downstream order of the Danube River through the specified section.

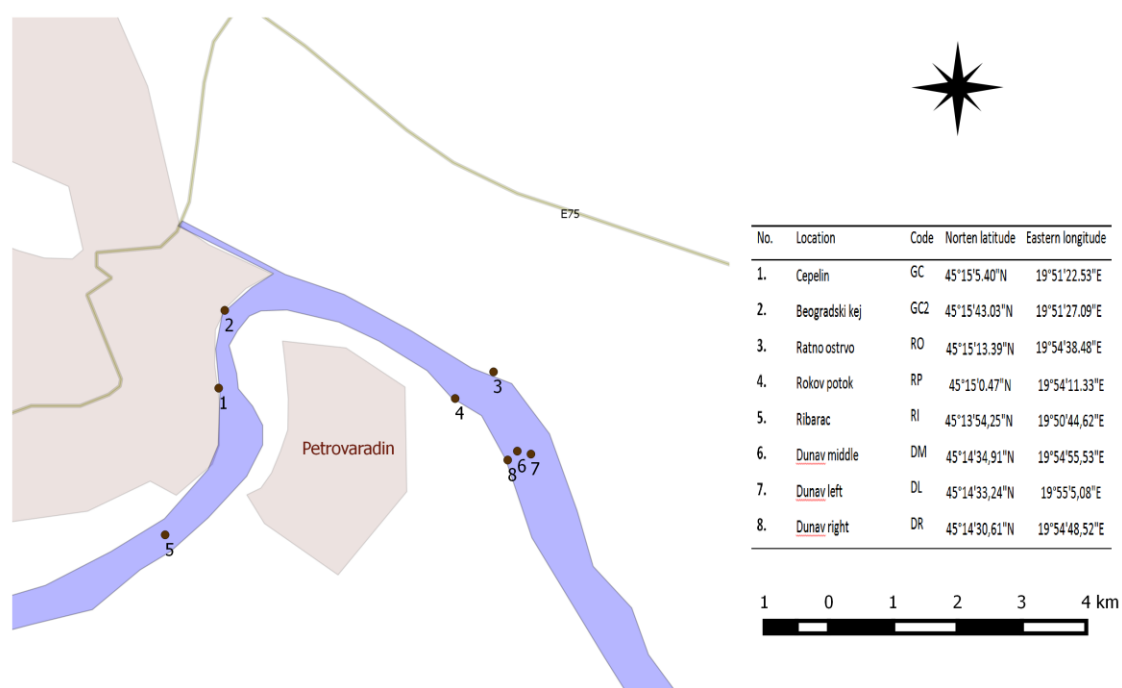


Fig. 1. Map of the Danube River sampling sites.

The locations *GC*, *GC2*, *RO* and *RP* were positioned 100 meters downstream of untreated sewage discharge. The sampling sites *DM*, *DL* and *DR* were collected few hundred meters from the urban area to determine the eco-toxicological status of the river after the effect of dilution and filtration by the river bank layers. The sampling site *RI* was included in order to overview the influence of the incoming pollution to the Novi Sad section of the river. All the samples were filtered through the regenerated cellulose 0.45 µm membrane filter (Agilent Technologies, Germany) and extracted within 24 h in the laboratory.

Sample preparation

The sample preparation was done by using the previously developed method by Ballesteros et al. (2006).²⁰ A solid-phase extraction procedure (SPE) with Agilent Bond Elut Plexa (200 mg/6 mL) type of cartridges was used for the sample preconcentration and removal of the existing interferents. SPE was carried out on an Agilent vacuum manifold for 12 columns connected to a water vacuum pump. The pH values of filtered water samples were adjusted to pH 3 with 1M hydrochloric acid to increase the extraction efficiency.^{20,21} The cartridges were activated with 5 mL mixture of dichloromethane and methanol (50:50, v/v) followed with 5 mL of pure methanol and 15 mL of ultra pure water (pH 3). The final extraction volume of each sample was 500 mL. The BPA was eluted with 5 mL of methanol. After isolation, the eluate was gently evaporated to dryness under nitrogen stream at 40 °C (5.0 purity) and the residue of all samples was reconstituted in 50 µL of ethyl acetate. A silylation step was carried out using 50 µL of BSTFA prior to the gas chromatography-mass spectrometry (GC-MS) analysis (Fig. 2).

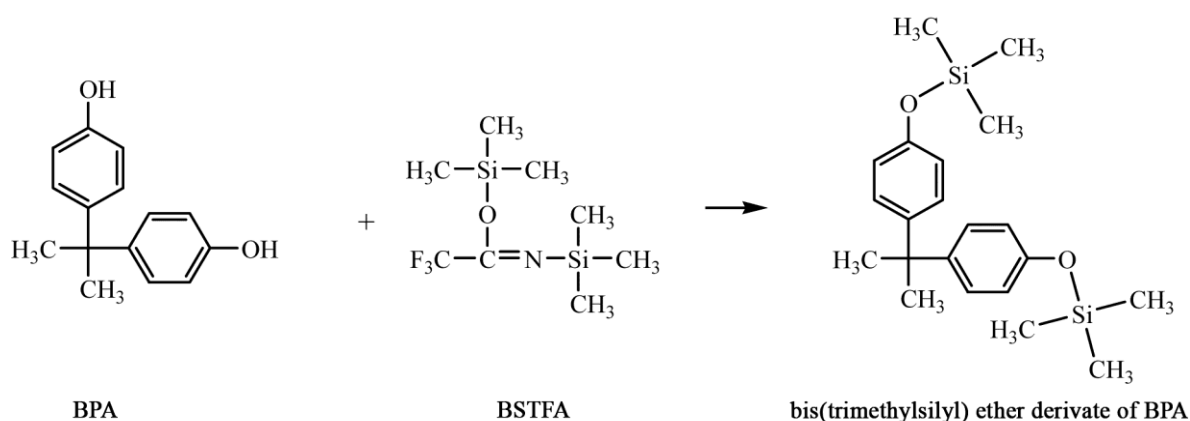


Fig. 2. Derivatization of bisphenol A.

GC-MS analysis

The analyses were done with an Agilent GC 7890A coupled with 5975C VL mass detector using the method developed by Zafra et al. (2003).²² The system was used in SCAN (complete spectrum) and in selected ion monitoring (SIM) modes. The separation was done using a fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). The MS quad temperature was set at 150 °C and the electron impact (EI) ion source temperature of the MS was 230 °C. A 2 µL aliquot of the silylated samples were automatically injected using the splitless injection mode at 250 °C. The GC oven temperature program was applied. The initial oven temperature was set at 50 °C and held

for 1 min. Then, the temperature was increased to 150 °C (held 1 min) and 270 °C via ramp of 25 and 10 °C/min respectively and maintained at 270 °C for 2 min. High purity helium (99.99 %) was used as carrier gas with a flow rate of 1.0916 mL/min. The characteristic ions listed in Table I for SIM mode operation were used for the BPA quantification in the water samples.

TABLE I. SIM mode characterization

Analyte	Monitored ions (<i>m/z</i>)		Retention time (min)
	Quantifier	Qualifier	
BPA	357	373 and 207	15.114

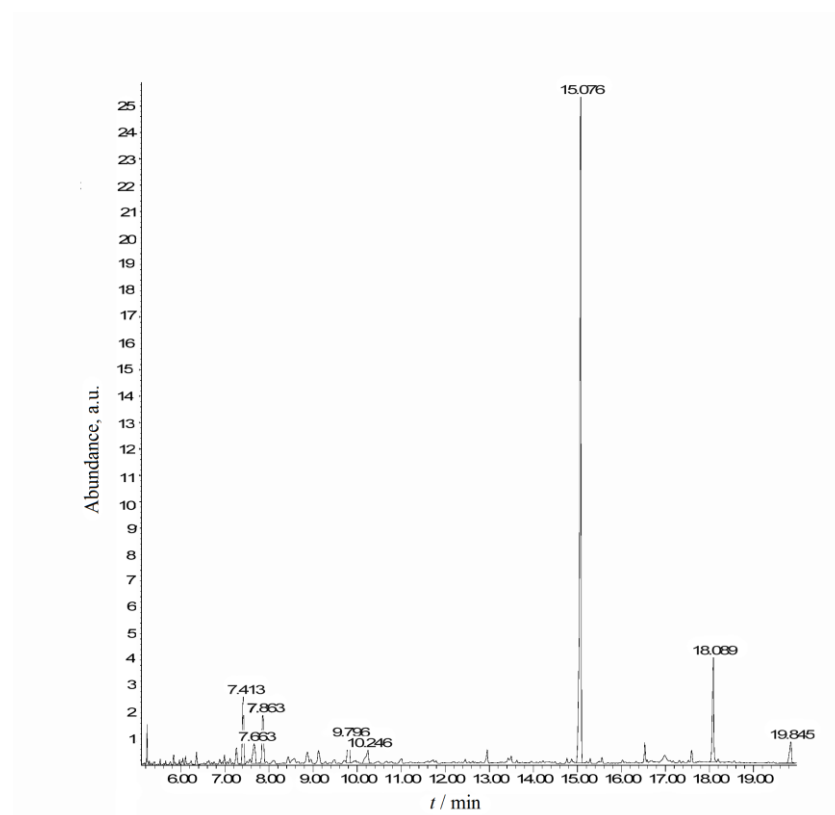


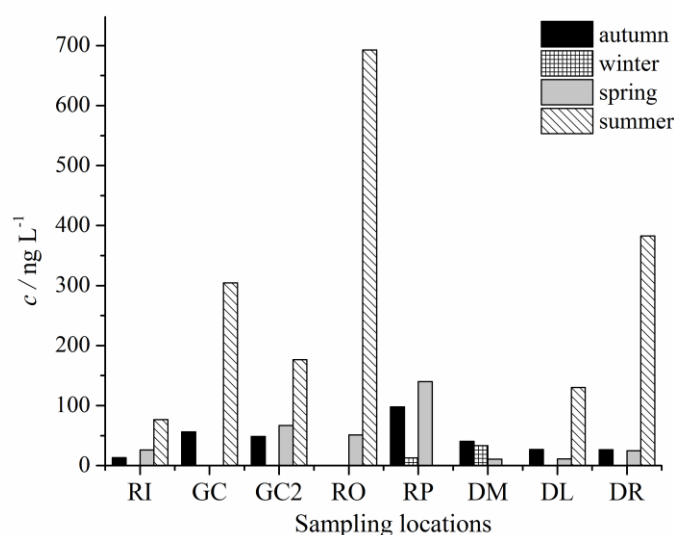
Fig. 3. Representative GC-MS chromatogram of the Danube water sample (SIM mode).

The BPA in the water samples was quantified with external standard bis(trimethylsilyl)bisphenol A. The calibration curve was linear for the concentration range 35-100 ng/mL ($y=40197x+10266$, $R^2=0.997$) and the limit of quantification (LOQ) was set at S/N ratios ≥ 10 . All instrumental and procedural blanks were far below LOQ, 6 ng/L. The identification of BPA was confirmed using Fiehn and NIST08 mass spectral libraries. The characteristic chromatogram of the examined water samples containing BPA is presented in Fig. 3.

149 *Occurrence of BPA in surface water*

150 Seasonal occurrence of bisphenol A in the surface water at the chosen sites along the
 151 Danube of the Novi Sad section is shown in Fig. 4.

152 BPA was detected above the limit of quantification (LOQ), 6 ng/L, in 7 out of 8
 153 collected samples in autumn. High frequency of BPA detection at concentration levels up to
 154 98 ng/L confirmed the presence of the Danube surface water in this area. The maximum
 155 detected concentration was in the *RP* sample, which is near the numerous illegal settlements
 156 with septic tanks and waste disposal sites, unauthorized traffic and an old industrial complex.



157

158 Fig. 4. Concentrations of BPA during the year.

159 In winter, BPA was only detected in two samples, *RP* and *DM*, at concentrations of 13
 160 and 33 ng/L respectively, when the level of the Danube was increased as a result of the
 161 weather conditions. These concentrations are assumed to be due to the dilution effects. In
 162 winter the biodegradation by microorganisms should be slower. Two strains of *Pseudomonas*
 163 (*Pseudomonas sp.* and *Pseudomonas putida*) and *Streptomyces sp.* strains were found to have
 164 BPA biodegradability capacity about 90% or more. The study conducted in China suggests
 165 that these bacteria are widely distributed in the rivers.¹⁴

166 However, high frequency of BPA detection was observed in the spring samples. Again,
 167 the maximum detected concentration of BPA was in the *RP* sample (140 ng/L), while the
 168 lowest concentration was in the *DM* sample (10.8 ng/L), the site which is far away from any

direct anthropological influence. The *DM*, *DL* and *DR* sites were chosen to determine if the Novi Sad area had an impact on the eco-toxicological status of the Danube.

The *RO* sampling site is located 100 m downstream of the discharge site of the sewage collector for collecting the wastewater from the local big oil refinery complex and the rain and urban run-off water from the industrial and underdeveloped suburban area. The possible pollution of the main water supplying source of the Novi Sad drinking water waterworks by the Oil Refinery and a Thermal Power Plant, situated only a few hundred meters upstream, presents the major danger for the river.¹⁸ The detected BPA levels were above the LOQ in six summer samples at significantly high concentrations, 77 ng/L in the *RI* sample and 693 ng/L in the *RO* sample.

Although BPA has a short half-life in surface water due to the rapid degradation, this chemical was often detected in our study. The *RI* sampling point was included in order to overview the influence of the possible high incoming pollution as the Croatian biggest river port, Vukovar, is located 80 km upstream and presents the most important source of anthropological pollution of the Danube before Novi Sad. The BPA measured levels were above the LOQ (Table II) in autumn, spring and summer at this site.

The BPA concentrations in the surface water samples varied significantly depending on the chosen sites, possible sources and routes of pollution and the seasons (Table II).

TABLE II. Seasonal variation of BPA concentrations in the Danube surface water samples

Sample	Range (ng/L)	Mean (ng/L)	Median (ng/L)	95th percentile
<i>RI</i>	n.d.-77	29	20	77
<i>GC</i>	n.d.-304	90	28	304
<i>GC2</i>	n.d.-177	73	58	177
<i>RO</i>	n.d.-693	186	26	693
<i>RP</i>	n.d.-140	63	55	140
<i>DM</i>	n.d.-41	21	22	41
<i>DL</i>	n.d.-130	42	19	130
<i>DR</i>	n.d.-383	108	26	383

n.d.: <LOQ

The obtained BPA results in our study were compared with the available data on some European rivers. BPA was quantified in the Dutch surface waters at the levels up to 330 ng/L, but it was below the LOQ in 60-80% of water samples.²³ In the Seine River, BPA was found in the range of 11-154 ng/L²⁴, while in the Rhine surface water the concentration of BPA

193 ranged from 42 to 229 ng/L.²¹ However, in freshwater streams of Germany, the BPA was
194 detected in the concentrations up to 1927 ng/L, but only in 13% of the samples.²⁵ The
195 maximum detected BPA concentration in our study (693 ng/L) was significantly higher than
196 the obtained concentration in the recent publication analysing the Danube samples for this
197 endocrine disrupter (68 ng/L).²⁶ The relatively high BPA concentrations found in the
198 investigated Danube River stretch in Serbia can be explained by the fact that Novi Sad has
199 still no urban waste water treatment plant and that most of the industries around Novi Sad
200 municipality are without wastewater treatment facilities.

201 *Risk evaluation*

202 The presence of the BPA endocrine disrupter might have adverse effects on aquatic
203 organisms²⁷ and the consumption of BPA contaminated freshwater fish²³ is probably one of
204 the main routes of the contamination of humans.¹⁴ The environmental risk assessment is
205 evaluated by comparing the predicted environmental concentration (PEC) or by measuring the
206 environmental concentration (MEC) with the sensitivity of the aquatic ecosystem for BPA
207 (predicted no-effect concentration, PNEC). The ratio PEC/PNEC (or MEC/PNEC), known as
208 the risk quotient (RQ), should not exceed 1. Otherwise, a risk to the aquatic environment is
209 assumed.²⁸

210 The PNEC values are based on eco-toxicity data from single species laboratory tests
211 combined with the safety factors or even on the derived provisional PNEC.²⁹ Based on the
212 literature data, the PNEC value of the BPA for fresh surface water varies from 64 µg/L³⁰ to
213 1.5 µg/L according to the European Union risk assessment report.³¹ However, the latest
214 research of Wright-Walters et al. (2011)¹⁶ estimated an aquatic PNEC of 0.06 µg/L for BPA
215 using a non-parametric methodology.

216 In order to evaluate the BPA adverse effects on the Danube surface water, the 95th
217 percentile of MEC was used together with PNEC to calculate the RQ for each sampling site
218 using Wright-Walters and co-workers methodology.¹⁶

219 The calculated ratio ranged from 0.68 to 11.54 and the RQ was above 1 at seven
220 locations out of eight (Fig. 5). Consequently, the frequency of exceedance was significantly
221 high, 0.87. It is evident that a potential risk exists in this part of the Danube, especially near
222 the RO sampling site.

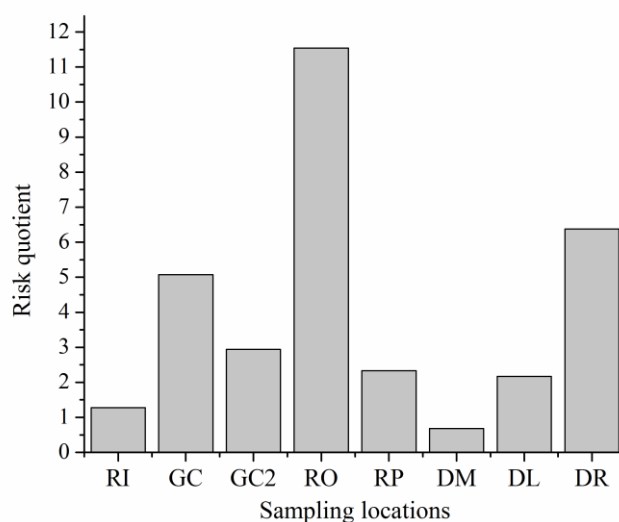


Fig. 5. Distribution of the eco-toxicological risk of BPA.

This risk assessment approach was based on an evaluation of the toxic potential of a single plasticizer to the aquatic environment without taking into account the possibility and effects of BPA acting additively with other EDs.

CONCLUSIONS

This research is a pioneering study on the eco-toxicological impact of BPA as an endocrine disrupter on the surface water in the Western Balkan Region. The obtained results suggest that the aquatic environment along the Danube River of the Novi Sad section, Serbia, is not sufficiently protected from adverse effects to BPA exposure at the established water concentrations of < 6 ng/L to 693 ng/L. The lack of the EDs legislation indicates that the aquatic environment is not sufficiently protected from the detrimental effects of BPA toxicant. Constant and extensive monitoring of the high-risk sites, as well as the biological laboratory experiments, are indispensable to get better insight into the potential toxicological risks of BPA and understand the full scope of effects of the BPA concentration influence on aquatic organisms along this part of the Danube. More reliable research on endocrine disruptive substances such as BPA is necessary in the field of ecotoxicology (derivation of reliable PNECs) and analytical chemistry (monitoring in the environment) in order to regulate these xenoestrogens in the field of water policy.

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The authors have declared no conflict of interest.

ИЗВОД

СЕЗОНСКО ОДРЕЂИВАЊЕ ПРИСУСТВА БИСФЕНОЛА А У ПОВРШИНСКОЈ
ВОДИ ДУНАВА

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Сезонске промене концентрација бисфенола А у површинској води Дунава, на територији Новог Сада, анализирани су, по први пут на овом подручју, применом гасне хроматографије са масеном спектрометријом. И поред кратког полуживота у површинској води (0,5-5 дана), бисфенол А често је детектован дуж тока Дунава у релевантним концентрационим нивоима (до 693 ng/l). Регистроване вредности бисфенола А током четири годишња доба значајно се разликују и указују на већу контаминацију у летњем периоду.

Процењен ризик по животну средину, изражен као однос 95. перцентила измерених концентрација бисфенола А у површинској води и концентрације за коју је предвиђено да нема биолошке ефекте, био је већи од јединице на чак седам локација узорковања.

Добијени резултати у оквиру овог истраживања потврдили су загађење површинске воде Дунава бисфенолом А, дуж обала Новог Сада, и указују на постојање значајног ризика по људско здравље и животну средину у овом подручју.

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