Preliminary investigation of bisphenol A in sediments and suspended matter in Kaštela Bay (Adriatic Sea, Croatia)

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The occurrence, seasonal and vertical distribution of bisphenol A (BPA) was investigated in the sediments and suspended matter sampled in Kaštela Bay, Adriatic Sea, Croatia. Granulometric composition of sediment and organic matter (OM) in sediments and suspended matter were also examined. BPA was detected in all samples of sediments and suspended matter. Concentration of BPA in sediment ranged from 5.32 µg kg¹ to 15.52 µg kg¹, while in suspended matter BPA ranged from 4.66 µg kg¹ to 103.06 µg kg¹. Seasonality in distribution of BPA concentrations in sediment and suspended matter was not found. The most abundant grain size fractions in sediment were silt (64.40±8.63%) and sand (26.97±9.11%) with accordingly determined sediment type as sandy silt. Average content of OM was 12.01±1.85% and 12.51±1.30%, for sediment and suspended matter, respectively. Monthly distribution of OM content showed higher values during October-March period and lower values during April-September period, while for the suspended matter situation was opposite. Statistical analysis of obtained data showed no existing significant correlation between concentration of BPA in sediments and suspended matter, or between BPA concentration and OM in sediments and suspended matter.

Key words: BPA, marine environment, sediment, suspended matter, Adriatic Sea

INTRODUCTION

Kaštela Bay as the largest bay in the coastal area of middle Adriatic includes city of Split, Kaštela and Trogir with approximately 350.000 inhabitants. Due to the rapid urbanization and industrialization in the 1970s, Kaštela Bay has been receiving large quantities of untreated municipal and industrial effluents for decades, and became one of the most industrialized and contaminated areas in the eastern Adriatic, Croatia (KUŠPILIĆ et al., 2009). The situation has improved due to the fact that most chemical industries were closed down during last 20

years, and especially after the activation of the modern sewage system "Eko Kaštela Bay" in 2004 (VIDJAK et al. 2012; UJEVIĆ et al., 2000; BOGNER et al., 1998). Through monitoring programs conducted by the Institute of Oceanography and Fisheries (IOF-Split) on behalf of Croatian water management company (Croatian Waters), United Nations Environment Program - Mediterranean Action Plan (UNEP-MAP), as well as for the implementation of EU Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD), physical and chemical properties of the water column and sediment have been monitored including the heavy met-

als and persistent organic pollutants in Kaštela Bay area (UJEVIĆ *et al.*, 1998; BOGNER *et al.*, 2004; MILUN *et al.*, 2006). There were no data obtained for the bisphenol A (BPA) in sediments or in suspended matter.

BPA is an organic compound composed of two phenol molecules bonded by a methyl bridge and two methyl groups (2,2-bis(4hydroxyphenyl)propane). BPA is one of many anthropogenic chemicals currently released into the environment which belongs to a group of endocrine-disrupting compounds (VANDEN-BERG et al., 2009; FALCONER et al., 2006). BPA has become ubiquitous in the marine environment within the past decades because of its presence in plastics, paints/lacquers, binding materials, and filling materials (HUANG et al. 2012). It is a pseudo-persistent chemical, which despite its short half-life can be found in the environment because of its continuous release (OEHLMANN et al., 2009). Releases are primarily via effluent discharge from municipal wastewater treatment plants, leaching from landfills, combustion of domestic waste, and the natural breakdown of plastics in the environment (CRAIN et al., 2007; KANG et al., 2007; SIDHU et al., 2005). Investigations of KRISHNAN et al. (1993) and TAKAO et al. (2002) confirmed that BPA can leach from polycarbonates and epoxy resins. Various authors confirmed detection of BPA in marine surface waters, treatment plant influents and effluents, groundwater, sediments and soils (CAREGHINI et al., 2015).

BPA has low (HEINONEN *et al.*, 2002) or moderate (COUSINS *et al.*, 2002) hydrophobicity and thus a modest capacity for bioaccumulation. Based on its chemical characteristics, it is estimated that the BPA in the environment is mostly present in abiotic components: in water and suspended solids (53%), soil (25%), or in sediments (23%) (COUSINS *et al.*, 2002; STAPLES *et al.*, 1998).

BPA leaching could be a concern at marine sites where plastic waste has accumulated, as BPA leaches more rapidly in marine than in freshwater systems (CRAIN *et al.*, 2007; SAJIKI & YONEKUBO, 2003) and microbial degradation may occur more slowly in marine waters (KANG & KONDO, 2005). It was determined that the bioa-

vailable fraction of dissolved BPA may increase with salinity (HU *et al.*, 2006).

Information with sufficient geographical and temporal coverage of BPA to enable a pan-European assessment is limited (EEA, 2011; DAVID *et al.*, 2010). Considering characteristics of BPA and its potential impact as an endocrine disruptive compound, BPA is included in the MSFD (Marine Strategy Framework Directive), OSPAR commission and WFD (Water Framework Directive) list of priority substances.

Since there is no information about BPA in the marine environment of Croatian part of the Adriatic, in this paper are presented preliminary data of BPA investigations obtained in sediments and suspended matter from the water column at Kaštela Bay area, Adriatic Sea.

MATERIAL AND METHODS

Study area

Kaštela Bay is semienclosed bay with a surface area of 61 km² and an average depth of 23 m, and communicates with the adjacent island Brač channel through the 1.8 km wide and 40 m deep strait (Fig. 1).

The most important fresh-water source is River Jadro with an average annual inflow of 10 m³ s⁻¹. The eastern part of the bay also receives large quantities of untreated municipal, agricultural wastes and industrial effluents (from food processing, cement and metallurgic industry, shipyard, recreational boating capacities) (UJEVIĆ et al., 2010). Water circulation in the bay is generated mostly by the local wind, which is related to the passage of mid-latitude cyclones over the area (GAČIĆ et al., 1987; BEG PAKLAR et al., 2002). Anthropogenic influence on the chemical and biological properties was demonstrated through phytoplankton blooms and small nonloricate ciliate proliferation during the warmer part of the year (MARASOVIĆ et al., 1991; 2005; BOJANIĆ et al., 2006).

Possible sources of the suspended matter in the bay are marine organisms production, river input (Jadro river), coastal erosion, and particles of anthropogenic origin. Prevailing

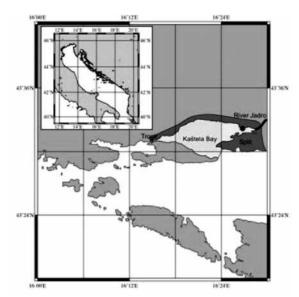


Fig. 1. Location of experimental site S1 in the Kaštela Bay (Adriatic Sea, Croatia)

circulation, which is mostly induced by local winds, is controlling transport of sediment and suspended particles in the bay (BEG PAKLAR et al., 2002). Typical estuarine circulation with incoming flow in the surface layer and the outgoing flow in the deeper layers with turbulent vertical exchanges is induced by SE wind. Anticyclonic gyre in the eastern part of the basin and cyclonic circulation in the western part of basin is associated with NE wind while water outflow in the surface layer is compensated by an upwelling process. Bay behaves as a typical dilution basin during calm weather with an outgoing current in the surface layer and an incoming current in the deeper layers (ZORE-ARMANDA et al., 1974). Vertical stratification is present from April to September, while in winter (from November to March) the water column is well mixed (ZORE ARMANDA, 1980).

In order to determine preliminary spatial and seasonal distribution of BPA in the Kaštela Bay one sampling site in eastern part of bay was selected (S1; 43°32'34"N; 16°24'39"E). The water depth at the S1 site was 10 m while distance from the coastline was 20 m, nearby the previously settled factory that produced polyvinyl chloride (PVC) and was closed during 1990's (Fig. 1).

Sampling of sediments and suspended matter

For investigation of vertical BPA distribution, sediment cores 10 cm long were sampled in March 2000 and July 2014. Collection of sediments was obtained by SCUBA-divers using plastic tubes which were inserted into the sediment to preserve an undisturbed core. After the sampling, sediment cores were sliced horizontally into 2 cm long sections and frozen until analysis.

For determination of BPA temporal distribution, sediments were sampled monthly (April 2001 to March 2002), using a gravity corer and frozen until analysis.

Samples of suspended matter were also collected monthly from April 2000 to March 2002 using sediment traps placed at three different depths of the water column. Sediments traps were made from PVC tubes (height 50 cm, 10 cm in diameter). Samples of suspended matter were frozen until analysis. Sampling months were classified into warm and cold season (April - September and October - March).

Sample preparation, determination of granulometric content and organic matter

Frozen samples of sediments and suspended matter were lyophilized and homogenized for BPA and organic matter content determination.

Organic matter (OM) content was determined gravimetrically by H2O2 treatment of sediment and suspended matter samples at 450 °C for 6 hours. The loss of weight of the samples as the result of this treatment was assumed to be due to OM content. Granulometric composition in non-homogenized sediment samples was determined by sieving (>63 µm) and hydrometric method (<63 μm). Sediment type was determined according to Shepard classification (SHEPARD, 1954).

Extraction of BPA in sediment and suspended matter

For extraction of BPA in sediment and suspended matter adapted method was used (GUERRA et al., 2010; POJANA et al., 2007). Aliquots (5.00±0.10 g) of each sediment samples were weighted into 50 mL polypropylene centrifuge tubes, extracted in 5 ml solution mixture (1:9, v/v) of dichloromethane (DCM) and methanol (MeOH). Extraction of BPA was enhanced by sonication for 60 min at 25°C and then by shaking for 3 h at 25°C in a rotary shaker. Samples were centrifuged for 40 min at 4000 rpm, (Hettich Rotina 380, Germany) and filtered through a 0.45 μm polytetrafluoroethylene filter (PTFE) prior to HPLC analysis.

Suspended matter samples were collected from three depths and pulled in one sample because weights of subsamples at separate depth were insufficient for HPLC analysis. Aliquots (5.00±0.10 g) of each suspended matter samples were weighted into 50 mL polypropylene centrifuge tubes, extracted in 10 mL extraction solution, DCM: MeOH (1:9, v/v) and following the extraction procedure for sediment samples.

Analysis

Sediment and suspended matter extracts were analyzed using a HPLC Varian ProSTAR 230, analytical solvent system coupled with a ProStar 410 autosampler, ProStar 500 column valve module and ProSTAR 363 fluorescence detector, excitation wavelength was set to 227 nm and emission wavelength to 313 nm. Separation of BPA was carried on reversed phase C18 column (Restek, Pinnacle II 5 μ m, 250 x 4.6 mm column) protected by a guard cartridge Pinnacle II C18, 20 x 4.0 mm (Varian). Column temperature was kept at 30°C, run time was 12 min and partial loopfill volume was set to the 10 μ L.

Data collection and result treatment were performed with the Varian Star Chromatography software. The BPA of sediment and suspended matter samples was identified by comparing with chromatograms of standard solutions.

Reagents

Analytical standard of bisphenol A (4,4-iso-propylidenediphenol) was obtained from the Acros Organics (Morris Plains, NJ). Methanol (MeOH) was obtained from J. T Baker (USA/

Netherlands), and dichloromethane (DCM) from Sigma Aldrich (St. Louis, MO), both HPLC grade. Water for chromatographic purposes was purified by a Milli-Q system (Millipore, Billerica, MS, USA). Standard stock solution was prepared for BPA at 1 μ M by dissolving solid standard in methanol. All working solutions (0.05-0.5 μ M) were prepared by diluting stock solutions in 2 mL glass vials for HPLC (Varian ProSTAR 230).

RESULTS AND DISCUSSION

The contribution of grain size fractions (gravel-particles >2 mm, sand (0.063-2 mm), silt (0.004-0.063 mm) and clay-particles <0.004 mm) in the sediments at investigated site is presented in Figure 2.

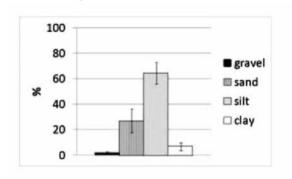


Fig. 2. Granulometric composition of grain size fractions (gravel, sand, silt and clay) in sediment at site S1

Predominant grain size fraction was silt with average content of (64.40±8.63%). Average sand content was 26.97±9.11%, while clay and gravel were minor fractions with 6.83±3.12% and 1.80±1.41%, respectively, (Fig. 2). According to Shepard's classification (SHEPARD, 1954) the sediment type was sandy silt. Station S1 is influenced by the river Jadro that brings great amounts of fine sized fraction to the Kaštela Bay area. This is in accordance with the previous results of the terrigenous sedimentation at near shore sites in the middle Adriatic (MATIJEVIĆ *et. al.* 2008).

Organic matter content in surface sediment layer (0-4cm depth) ranged from 8.94% (August) to 14.37% (April) with the average content of 12.01±1.85% (Fig 3).

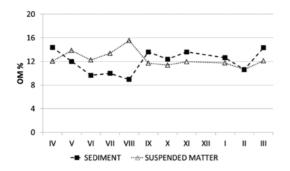


Fig. 3. Monthly distribution of OM content in sediment surface layer (0-4 cm depth) and suspended matter at site S1 sampled during 2001/2002

Extremely high OM content was found in sediments at S1 in relation to OM previously determined for the middle Adriatic sediments (range: 0.55-8.72% with the average content of 3.84±2.40%). High OM content for sediment at site S1 was in good accordance with highest silt content in sediment of the investigated area (Fig. 2). Affinity of the organic matter particles to fine-grained sediments is well documented for the near-shore stations along the eastern Adriatic coast (UJEVIĆ et al., 1998; BOGNER et al., 2004; BOGNER et al., 2005). S1 is located in the eastern part of the bay, very close to the coastline where large amount of industrial and domestic waste waters are discharged that certainly contributes to organic matter increase in this area.

From monthly distribution of OM content in surface sediments at S1 site (Fig. 3) higher values were noticed during the cold season (October-March) 13.13±1.32%, while lower values were obtained during warm period (April-September) 11.56±0.54%. Vertical profiles of OM indicated relatively uniform distribution with elevated values in deeper sediment layer (8-10 cm depth) that can be attributed to local organic matter agglomeration in sediment (Fig. 4).

In suspended matter OM content ranged from 10.60% (February) to 15.54% (August). Average content of OM in suspended matter $(12.51\pm1.30\%)$ was very close to the average OM content in sediment (Fig. 3). Higher OM content in suspended matter could be expected due to OM affinity to fine grained particles that prevail in the water column (UJEVIĆ et al., 2010).

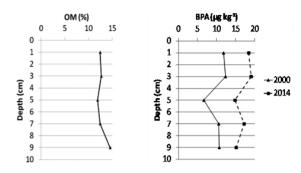


Fig. 4. Vertical profiles of OM content (sampled in March 2000) and BPA content in sediment cores (sampled in March 2000 and July 2014)

Monthly distribution of OM content in suspended matter indicated lower average value (11.4±4.7%) during worm part of the year (April-September), than in cold part of the year (October-March) (12.7±1.3%). This was the opposite of the seasonal pattern obtained for the OM in sediments at S1 site.

Concentrations of BPA in sediment surface layer ranged from 5.32 µg kg⁻¹ to 15.52 µg kg⁻¹ with the average content of 11.82±2.82 µg kg⁻¹ (Fig. 5). Concentrations of BPA in Kaštela Bay were lower than those found in sediments of the Venice Lagoon (0-118 µg kg⁻¹), Italy (POJANA et al., 2007) that presents only available data related to BPA in Adriatic Sea. BPA results presented in this paper were also lower than those reported for Mediterranean, Thermaikos Bay (7.2-39 μg kg⁻¹) in Greece (ARDITSOGLOU et al., 2012). BPA concentration range in Kaštela Bay is more similar with data reported for river sediments (<0.5-15 µg kg⁻¹) in Germany (BOLZ et al., 2001), Pearl River estuary and adjacent South Chinese Sea sediments (1-12 µg kg⁻¹) in China (PENG et al., 2007).

From monthly distribution of BPA concentrations in surface sediments relatively high variability during the investigated years was obvious, and no significant seasonal changes were established (Fig. 5).

Linear regression analysis of BPA concentration was applied for period of maximum samplings obtained (April 2001-March 2002), divided into warm and cold season. This analysis did not point to trend of increase or decrease in BPA concentrations.

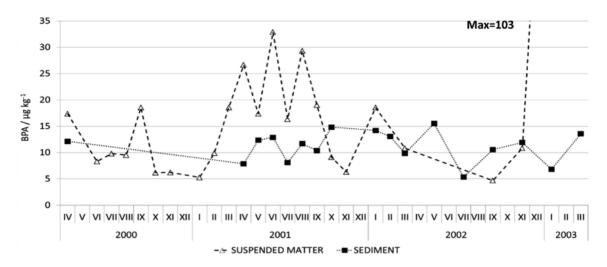


Fig. 5. BPA concentration in sediment surface layer and suspended matter at site S1 during investigated period

Relatively high variability during the years, and non-existing trends in BPA concentrations indicate that BPA is not impacted with seasonal input but rather with BPA's degradation processes in the water column and sediment as was suggested by YING & KOOKANA (2003).

BPA was quantifiable in vertical profiles throughout all layers of sediment cores, with average concentration 10.44±2.00 μg kg⁻¹ in year 2000 (Fig. 4). During 2014 BPA average value was higher 17.00±1.71 μg kg⁻¹. Maximum BPA concentrations were detected in 2-4cm layer of sediment cores.

Investigations of BPA vertical profiles by using ²¹⁰Pb dating method of sediment layers, PENG *et al.* (2007) have estimated age of sediment layers, calculated average sedimentation rates (0.34-0.97 cm y⁻¹) and estimated loadings of BPA in each decade since 1940's. Due to relatively small number of data presented for Kaštela Bay area, as well as to lack of sediment dating, similar estimation of age or origin of BPA was not possible.

Concentrations of BPA in suspended matter ranged from 4.66 µg kg⁻¹ to 103.06 µg kg⁻¹ (18.01±19.69 µg kg⁻¹) (Fig. 5). Due to relatively scarce literature data for BPA in suspended matter there was no enough data for comparison of BPA concentration range in similar marine environments such as anthropogenic impacted bay. BPA concentrations obtained in this inves-

tigation were in range of data reported for BPA In suspended matter of Thermaikos Bay (26-160 µg kg⁻¹) in Greece (ARDITSOGLOU *et al.*, 2012), higher than those reported for superficial waters in Netherlands (5.56-56 µg kg⁻¹) (VETHAAK *et al.*, 2005), but lower than data published for suspended matter from Tiber River (360-660 µg kg⁻¹) in Italy (PATROLECCO *et al.*, 2006).

According to obtained results for Kaštela Bay, higher concentrations of BPA were generally measured in suspended matter than in sediments at site S1 (Fig. 5). Monthly distribution of BPA concentrations in suspended matter showed very high variability through investigated years. Linear regression analysis of BPA concentra-

Table 1. Linear correlation coefficients between organic matter in suspended matter (OM / s.m.), organic matter in sediment (OM / sed), BPA concentration in suspended matter (BPA / s.m.) and sediment (BPA / sed) (n=11)

	OM / sed	BPA / s.m.	BPA / sed
OM / s.m.	-0.49	-0.04	-0.23
	R ² =0.24	R ² =0.00	R ² =0.05
OM / sed		-0.53	-0.24
		R ² =0.28	R ² =0.06
BPA /			-0.51
s.m.			R ² =0.26

tion in worm and cold season, similar as for the sediments, was also applied, but did not point to trend of increase or decrease in BPA concentrations. Non existing trends or seasonal distribution of BPA are probably due to BPA dependence on existing input and degradation rate in water column (YING & KOOKANA, 2003). Higher variability in BPA concentrations in suspended matter than in bay sediments are certainly consequence of the water circulation influence (Fig. 5).

Statistical analysis of linear correlation all presented data showed no significant relationship between BPA in sediment and suspended matter ($R^2 \le 0.26$) (Table 1).

The concentrations of BPA in sediment and suspended matter showed no significant relationship with OM ($R^2 \le 0.06$, $R^2 \le 0.00$), indicating that the environmental loadings of BPA to the Kaštela Bay were not associated with OM that is in accordance with results presented by PENG et al. 2007. According to same authors lack of correlation between BPA and OM seems reasonable since BPA enters to marine environment mainly through wastewaters.

CONCLUSIONS

The occurrence of BPA was investigated in the marine environment of Kaštela Bay, Adriatic Sea, Croatia, BPA was detected in all samples of suspended matter and sediments. Concentrations of BPA in sediment ranged from 5.32 µg kg⁻¹ to 15.52 µg kg⁻¹. In a suspended matter concentrations of BPA were higher and ranged from 4.66 μg kg⁻¹ to 103.06 μg kg⁻¹. Seasonal distribution pattern of BPA concentrations was not found, that probably indicates its dependence on existing input and degradation rate in water column Granulometric content of sediments indicated silt as prevailing grain fraction (64.40±8.63%). OM content in sediment and suspended matter was also determined with average content of 12.01±1.85% and 12.51±1.30%, for sediment and suspended matter, respectively. Monthly distribution of OM showed higher contents during cold season (October-March) 13.13±1.32% and lower values during warm period (April-September) 11.56±0.54%, while for the suspended matter situation was opposite.

Statistical analysis of obtained data showed no significant correlation between concentration of BPA and content of organic matter in sediments and suspended matter.

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Received: 29 Jyne 2015 Accepted: 17 August 2015

Preliminarno istraživanje bisfenola A u sedimentu i suspendiranoj tvari u Kaštelanskom zaljevu (Jadransko more, Hrvatska)

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SAŽETAK

Prisustvo te sezonska i vertikalna raspodjela BPA istraživani su u sedimentu i suspendiranoj tvari prikupljenima u Kaštelanskom zaljevu, Jadransko more, Hrvatska. U uzorcima je određena koncentracija BPA, granulometrijski sastav sedimenta te sadržaj organske tvari (OM) u sedimentu i suspendiranoj tvari. BPA je pronađen u svim uzorcima sedimenta i suspendirane tvari, a koncentracije BPA u sedimentu su bile u rasponu od 5.32 μg kg⁻¹ do 15.52 μg kg⁻¹, u suspendiranoj tvari od 4.66 μg kg⁻¹ do 103.06 μg kg⁻¹. Nije ustanovljena sezonska raspodjela BPA u uzorcima suspendirane tvari i sedimenta. Analizom udjela pojedinih veličinskih frakcija sediment je klasificiran kao pjeskoviti silt. Prosječni udio OM u sedimentu je bio 12.01±1.85%, a u suspendiranoj tvari 12.51±1.30%. Veći udjeli OM zabilježeni su u hladnom razdoblju godine (13.13±1.32%), za razliku od toplog razdoblja kada je prosječni udio OM bio 11.56±0.54%. Raspodjela OM u toplom i hladnom razdoblju u suspendiranoj tvari bila je suprotna raspodjeli ustanovljenoj za sediment. Statističkom analizom dobivenih rezultata nije ustanovljena značajna korelacija između sadržaja OM u sedimentu i suspendiranoj tvari i koncentracije BPA.

Ključne riječi: BPA, morski okoliš, sediment, suspendirana tvar, Jadransko more