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The role of phytoplankton composition, biomass and cell volume in accumulation and transfer of endocrine disrupting compounds in the Southern Baltic Sea (The Gulf of Gdansk)

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ABSTRACT

Endocrine disrupting compounds (EDCs) like bisphenol A (BPA), 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP) are introduced to the trophic webs through among others phytoplankton. This paper describes BPA, OP and NP concentrations in phytoplankton in the Gulf of Gdansk (Southern Baltic Sea) in the years 2011–2012. The assays of BPA, OP and NP in samples were performed using HPLC with fluorescence detection. The concentrations of BPA, the most commonly used of the three compounds, were over ten times higher than OP and NP concentrations. The concentrations of the studied EDCs in phytoplankton from the Gulf of Gdansk depended on anthropogenic factors and on phytoplankton properties (species composition, biomass, volume). An increase in phytoplankton biomass did not always result in an increase of BPA, OP and NP concentrations. However, the load of the studied EDCs accumulated in phytoplankton biomass increase with a rise of biomass. An increase in BPA, OP and NP concentrations was effected by biomass growth and the proportions ofciliates, dinoflagellates, diatoms and green algae. A strong positive correlation between OP and NP concentrations and negative correlation between BPA concentrations and biomass of organisms with cells measuring <1000 μm^3 in volume results from the differing properties of these compounds.

1. Introduction

Phytoplankton is the first link in the trophic chain, therefore it performs a significant function in the transfer of organic compounds between all abiotic and biotic elements of the marine environment: water, sediments and organisms. Numerous reports found in literature indicate significant accumulation in phytoplankton of such organic pollutants as polychlorinated biphenyls (PCBs) (Berglund et al., 2001; Lynn et al., 2007), polycyclic aromatic hydrocarbons (PAHs) (Wan et al., 2007; Echeveste et al., 2010a, b, c), dioxins (PCDD/F) (Wan et al., 2005) and polybrominateddiphenyl ethers (PBDEs) (Frouin et al., 2013). It was nevertheless observed that rapid growth in phytoplankton biomass usually leads to so-called "dilution" of pollutants in the phytoplankton itself (Maria et al., 2000; Nizzetto et al., 2012; Tiano et al., 2014; Todorova et al., 2015). With regards to further distribution of unwelcome compounds in the trophic chain of the pelagic zone, this can be beneficial to organisms as it reduces pollution exposure (Harding and Phillips, 1978; Taylor et al., 1991). On the other hand, however, large blooms, and then mass dying of phytoplankton may cause the pollutant load to be transferred back into the ecosystem. There are known cases of nonylphenol concentrations increasing in sediments in areas where a considerable amount of large plant organisms had died e.g. *Ulva rigida* in the Veneziana Lagoon in Italy (Marcomini et al., 1990). The pollutants thus accumulated in sediments then may be subsequently introduced to the trophic chain of benthic organisms.

The enrichment of phytoplankton in hydrophobic compounds is influenced by a number of factors related to the form and physiology of organisms: phytoplankton structure and biomass, cell size, structure of cell walls, and growth rate; but also by the properties of the compounds themselves. Physical and chemical parameters of the aquatic environment such as light, temperature, biogenic compounds or nutrient stress also have indirect influence (Nizzetto et al., 2012; Yang et al., 2012; Frouin et al., 2013; Zhao et al., 2014).

Little is known about the distribution of compounds from the group of derivatives of phenol – alkylphenols (4-nonylphenol –

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NP, 4-*tert*-octylphenol – OP) and bisphenol A (2.2-bis(4-hydroxyphenyl)propane – BPA) in phytoplankton. BPA, OP and NP have moderate hydrophobic properties (log $K_{o/w}$ 2.3–4.48) (European Commission, 2014), which indicates the possibility of them becoming adsorbed onto the body surface as well as accumulated in the cells of phytoplankton organisms. The high bioconcentration factors (BCF) (>1000) obtained by Staniszewska et al. (2014) for phytoplankton in relation to sea water from the Gulf of Gdansk, confirm the high potential of these compounds to transfer up the marine food web. BPA and alkylphenols are described as Endocrine Disrupting Compounds (EDCs). They may interfere with the hormonal activity in living organisms. The introduction of such compounds into the trophic web leads to the impairment of life processes for organisms from higher trophic levels. There are reports in literature about negative influence of bisphenol A and alkylphenols on benthic organisms (Granmo et al., 1989; Matazzo et al., 2005), fish (Du et al., 2008), mammals (Markey, 2001; Xiao et al., 2006) and humans (Welshons et al., 2006; Vanderberg et al., 2007, 2014).

The Gulf of Gdansk (Southern Baltic Sea), particularly the coastal zone, is a basin rich in nutrient salts as: sodium nitrate and phosphoric salt, favourable for the growth of photosynthesizing organisms, especially plankton algae. In recent years, air and water temperature have been caused to frequent and large blooms and to the prolongation of the vegetative season. The raised numbers of phytoplankton organisms may considerably increase the "removal" of EDCs from water and at the same time contribute to the introduction of these compounds to the trophic chain. According to Bełdowska et al. (2014), as a result of frequent and mass algal blooms brought about by climate warming, the mercury load bound in phytoplankton biomass may become up to four times larger.

The present paper characterizes the variability of bisphenol A, 4-*tert*-octylphenol and 4-nonylphenol in the phytoplankton of the Gulf of Gdansk. We hypothesized that anthropogenic factors and the properties of phytoplankton, such as the species composition, biomass and volume size, could influence on the increase of EDCs concentrations in phytoplankton in different seasons of the year.

2. Materials and methods

Samples of water and phytoplankton were taken in the coastal zone of the Gulf of Gdansk in 2011 and 2012 in spring, summer and autumn. The stations were located near to the Tricity agglomeration (Gdansk, Sopot, Gdynia) and the mouth of the largest Polish river – the Vistula. The deepest station UW (40 m) was located 7 km north-east of the mouth of the Vistula. Station GN (37 m) was located in the middle of the Gulf of Gdansk, 19 km north-west of the Vistula estuary. Three other stations were located in shallower parts: SP (17 m) 7 km north of the Kacza river mouth, GDY (12 m) 4 km from the port of Gdynia and ME (4 m) in the vicinity of the wastewater treatment plant at Dębogórze, about 1 km from the coast (Fig. 1).

Water samples were taken using methods previously described by Staniszewska et al. (2015). Phytoplankton for BPA, OP and NP analysis was collected using a WP2-type 25 μm mesh size plankton net (surface haul for about 20 min) and sieved through a 100 μm mesh size (in order to separate it from zooplankton and large suspended particles). The concentrated phytoplankton biomass was frozen until laboratory treatment.

Phytoplankton for qualitative and quantitative analysis was taken with a bathometer from a depth of 0.5 m and preserved with Lugol's solution and stored under cool and dark conditions.

2.1. Determination of BPA, OP and NP in water and phytoplankton, and method validation

All solvents obtained from Merck (water, acetonitrile and methanol) were of HPLC grade. 70% chloric acid (VII) and ammonium acetate (analytically pure) were purchased from POCh. High purity (>97%) BPA, OP and NP were obtained from SIGMA-ALDRICH®. Stock and working solutions (respectively: 1 mg cm^{-3} and 10, 25, 50, 75, 100 ng cm^{-3}) of each compound were prepared in methanol.

The determination of water samples was fully described in an earlier publication by Staniszewska et al. (2015). In order to determine BPA, OP and NP, 0.1 g of the lyophilized phytoplankton, were extracted in an ultrasonic bath. It was then purified on Oasis HLB (Waters) glass cartridges (5 ml/200 mg) according to the procedure set out by Xiao et al. (2006). The final determinations were performed using high-performance liquid chromatography with fluorescency detection (Dionex; excitation at $\lambda = 275$ nm and emission at $\lambda = 300$ nm). Chromatographic separation was conducted on a HYPERSIL GOLD reversed phase column (Thermo Scientific) with a mobile phase (acetonitrile and water) under gradient conditions.

The linear correlation coefficient (r) of the analytical standards was >0.999. The average amounts of BPA, OP and NP recovered, determined through a quintuple analysis of phytoplankton samples containing a known amount of the standard, were: 89.0% (BPA), 92.0% (OP) and 99.3% (NP). The limit of quantification (LOQ) amounted to: 2.0 (BPA), 0.8 (OP) and 1.0 ng g^{-1} d.w. (NP). The achieved accuracy (variation coefficient) was <11.0%. The obtained "background" values for BPA, OP and NP were <LOQ.

2.2. Qualitative and quantitative phytoplankton analysis

Qualitative and quantitative phytoplankton analysis was conducted with an upright Nikon E80i microscope and Nikon TMS inverted microscope according to HELCOM guidelines (2011). The size of the counting chambers (10, 20, or 50 cm^3) and the sedimentation time (24 or 48 h) depended on the abundance of phytoplanktonic organisms. The counting units (N) were cells, coenobia, or trichomes 100 μm in length. The biovolume of phytoplankton was calculated using species-specific geometric formulas and standardized size classes (Olenina et al., 2006).

2.3. Calculation methods

The statistical analysis and the graphic representation of the obtained results were carried out using Microsoft Excel 2007. Results were of a non-parametric nature, so the Kruskal–Wallis test was used to determine the significance of differences ($p < 0.05$). The dependences were determined using the Spearmann's correlation coefficient (r), adopting a confidence interval of 95%.

The bioconcentration factor (BCF) was determined according to the formulas presented by Hu et al. (2005).

3. Results and discussion

3.1. The variability of bisphenol A and alkylphenol concentration in phytoplankton

The main factor which may influence BPA, OP and NP concentrations in phytoplankton of the Gulf of Gdansk are anthropogenic sources of these compounds. BPA production in the EU is about 16 times higher than alkylphenol production (ICIS Chemical Business, 2008), which translates to higher bisphenol A concentrations in water samples and organisms inhabiting the Gulf of Gdansk. BPA