The occurrence of selected endocrine-disrupting chemicals in water and sediments from an urban lagoon in Southern Italy.

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Footnote: In memory of our wonderful colleague, Prof. Gustavo Damiano Mita, who gave his scientific input, but sadly passed away on 24th November 2019.

Running Title: *Endocrine disruptors in an urban lagoon in Southern Italy.*

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Abstract

Endocrine disrupting chemicals (EDCs) are agents able to exert perturbation toward the endocrine system via a broad array of signalling pathways. While some EDCs are natural, most are synthetic, being these generated and released into the environment as a result of antropogenic activities. Analytical surveillance plays a critical role not only in investigating the prevalence of such chemicals in environmental samples, but also in evaluating the extent at which their detected concentrations treaten the biodiversity of the species living thereby. An analytical surveillance study was carried out in a lagoon in Southern Italy. This water basin is subjected to a season-dependent anthropogenic pressure and relates to the sea through a mouth channel that allows periodic exchanges with the marine waters, making this water body a "dynamic environment". An analytical method for screening a range of fourteen EDCs in surface waters and sediments in this water body was developed and validated. This method includes a fast and cost-effective sample preparation, based on a solid-liquid (sediments) and liquid-liquid (surface waters) extraction. The quali-quantitative analysis was conducted by reversed-phase liquid chromatography tandem UV and fluorescence detection. Only four chemicals out the fourteen investigated EDCs were detected in both matrices with a frequency higher than 60%. The average concentrations of the single EDC were higher in sediments than in surface waters (range of 132 – 28.000 ng L^{-1} in water and of 730 - 155.000 ng Kg^{-1} dw in deposits). Limited to the assayed EDCs, the ecosystem has a low risk with regards to the conservation of biodiversity of the animal species living thereby, since the total estrogenic activity does not exceed 1 ng L^{-1}.

Keywords: Sediments; surface waters; toxicology, endocrine disrupting chemicals; Italian lagoon; environmental surveillance.

1. Introduction

The U.S. Environmental Protection Agency defines an endocrine-disrupting chemical (EDC) as "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process". (Diamanti-Kandarakis et al., 2009). EDCs span a wide range of compounds that are different both in terms of their chemistry but also for the biological effects they exert. Since 1993, when EDCs were categorized for the first time (Colborn, vom Saal, & Soto, 1993), the toxicology of these compounds has been systematically dissected. While these toxicants were initially thought to exert actions mainly through nuclear hormone receptors, today, the elucidation of their effects from a mechanistic perspective has substantially grown. Contemporary basic research shows that these toxicants can interact with a plethora of molecular targets, including nonnuclear steroid hormone receptors (*e.g.*, membrane ERs), nonsteroid receptors (*e.g.*, neurotransmitter receptors such as the serotonin receptor, dopamine receptor, norepinephrine receptor), orphan receptors [*e.g.*, aryl hydrocarbon receptor (AhR)—an orphan receptor] and various enzymatic pathways (Diamanti-Kandarakis et al., 2009).

EDCs are released to the environment due to industrial manufacturing processes, landfill leachate, pesticide application, and, mainly, from effluents of the industrial and municipal wastewater treatment plant. These can then contaminate the soil and the aquatic pollution and represent a risk factor for the terrestrial animal species (humans included) and for the biodiversity of marine species by triggering feminization processes (Lu, Yan, Wang, & Chen, 2011; Yan, Lu, Liu, & Jin, 2012; Yang, Yang, Liu, Chen, & Shen, 2018). The present study evaluates the EDC-related contamination levels in surface waters and sediments sampled from a lagoon located in Southern Italy. This water body was selected because it lies in a context subjected to an unprecedented season-dependent anthropogenic pressure. To fulfill is work's aim, an analytical method encompassing both the sample preparation and the simultaneous determination of 14 EDCs was developed and validated. The chosen EDCs belong to different chemical classes, aiming to offer a survey of water quality of this aquatic environment: a phthalate [(di(2-ethylhexyl) phthalate, DEHP]; two chlorobenzenes, (1,4-dichlorobenzene and 1,2,4,5-tetrachlorobenzene); three phenol derivatives (2-chlorophenol, 4-nonylphenol, and triclosan (TCS)); eight bisphenols, such as Bisphenol A (BPA) and seven of its analogs. We have included not only BPA but also its analogs since the industries are progressively replacing BPA with these in various manufacturing processes as these are considered safer than the parent compound. However, this assumption was indeed demonstrated somewhat inaccurate, as some of these analogs have instead endocrine disrupting activity superior to that of BPA as reported in the literature (Russo et al., 2018; Usman & Ahmad, 2016; Usman, Ikhlas, & Ahmad, 2019) and because the widespread pollution and the potential risks of bisphenol analogs other than BPA in the aquatic environment are still poorly understood.

BPA is a monomer widely used in the manufacture of some polymeric materials (Almeida, Raposo, Almeida-González, & Carrascosa, 2018; Mikołajewska, Stragierowicz, & Gromadzińska, 2015). Since many European and non-European countries have been restricting BPA use, particularly in food packaging (EFSA Panel on Food Contact Materials and Aids 2015), many of its analogs, classified as bisphenols (BPs), have been designed. These have replaced BPA in several industrial processes since they were deemed to exert inferior estrogenic activity. Industrial operations in which BPs have been employed include the manufacture of resins and plastics (Jin & Zhu, 2016). Critically, many of these resulted indeed in some cases even more estrogenic than BPA itself (Eladak et al., 2015; Michalowicz, Mokra, & Bak, 2015; Mokra, Wozniak, Bukowska, Sicinska, & Michalowicz, 2018; Rosenmai et al., 2014; Russo et al., 2018; Usman & Ahmad, 2016). So far, BPs have been detected in food (Cacho, Campillo, Vinas, & Hernandez-Cordoba, 2012; Fattore, Russo, Barbato, Grumetto, &

Albrizio, 2015; Grumetto et al., 2013; Liao & Kannan, 2013), house dust (Liao, Liu, Guo, et al., 2012), water and sediments of seas, rivers and lakes (Gong, Duan, Yang, Ran, & Chen, 2016; Liao, Liu, Alomirah, et al., 2012; Liu et al., 2017; Noszczynska & Piotrowska-Seget, 2018; Song et al., 2014; Sun et al., 2017), personal care products (Liao & Kannan, 2014), and thermal paper (Liao, Liu, & Kannan, 2012; Russo, Barbato, & Grumetto, 2017). Furthermore, BPs have also been detected in human biological specimens such as blood, urine, and saliva (Cobellis, Colacurci, Trabucco, Carpentiero, & Grumetto, 2009; Cunha & Fernandes, 2010; Liao, Liu, Alomirah, et al., 2012; Russo, Barbato, Mita, & Grumetto, 2019; F. Zhou et al., 2013; X. Zhou, Kramer, Calafat, & Ye, 2014).

TCS is an antimicrobial active in many personal care and consumer products, and, due to its bioaccumulation and persistence potential in the environment, has been often detected in surface, ground, and drinking water (Yuan, Hu, Li, & Yu, 2020). Phthalate esters (PEs) are chemical plasticizers used to soften polyvinyl chloride (PVC) flexibility. DEHP, the primary PE, is well studied as EDC and for its toxicological effects (Park et al., 2020). According to European legislation, REACH classified DEHP in category 1B, including toxic and hazardous substances.

We decided to screen these 14 chemicals in a lagoon (Lucrinus Lacus), considering their possible inefficient removal from the environment and the toxic effects of all these compounds on aquatic organisms and humans. The lagoon lies near the "Campi Flegrei" coast, in the Campania region, Southern Italy, and its banks are the location of various catering businesses, whose activity is significantly increased in the high season (summer). So, it is very prone to anthropogenic contamination (farming, urban waste, irrigation). Another reason motivating the choice to consider this specific water body is its relatively small size (1.5 Km length and 5 meters max depth) as this allows achievement of some reliable insights into its health state only with few sample collections and over a shorter time window.

The lagoon is a "dynamic environment" in contact with the open sea and the adjacent thermal springs by the canals. Furthermore, this site features the instability of the seabed subjected to alternating positive and negative bradyseism and small, but continuous, earthquakes due to the seismic activity of the "Campi Flegrei" area. The water body relates to the sea through a mouth channel that allows periodic exchanges with the marine waters because of tidal motions (Aucelli, Brancaccio, & Cinque, 2017). The purpose of this study is (*i*) the development of an analytical methodology suitable to screen surface water and sediments sampled from the lagoon (*ii*) the investigation of the occurrence and surface water/sediment distribution of these target EDCs, (*iii*) the rational evaluation the pollution extent of this water basin throughout the year and finally (*iv*) the evaluation of the potential estrogenic activity, using estradiol equivalents (EEQ), for a risk assessment for the aquatic species living in this water basin.

2. Materials and Methods

2.1 Reagents and chemicals

Methanol (HPLC analytical grade) and formic acid (minimum purity ≥95%) were both purchased from Sigma Aldrich (Milan, Italy). Water (18.2 $M\Omega \cdot cm^{-1}$) was purified and deionized in house via a Milli-Q plus instrument from Millipore (Bedford, New Hampshire, USA). Analytical standards such as BPF (4,4′- dihydroxydiphenylmethane, minimum purity ≥ 99.0 %), BPS (4,4′-sulfonyldiphenol, minimum purity ≥98%), BPA (2,2-bis(4-hydroxyphenyl)propane, minimum purity ≥ 99%), BADGE (2,2-Bis[4-(glycidyloxy)phenyl]propane, minimum purity ≥ 95%), 2-CP (2-chlorophenol, minimum purity ≥ 99%), 4-NP (4-Nonylphenol, minimum purity ≥ 98%), DCB (1,4 dichlorobenzene, minimum purity ≥ 99.0 %), TCB (1,2,4,5, tetrachlorobenzene, minimum purity ≥ 98.0%), DEHP (Bis(2-ethylhexyl) phthalate, minimum purity ≥ 98.0 %), TCS (5-Chloro-2-(2,4-

dichlorophenoxy)phenol, minimum purity ≥97%) and Carbamazepine (5H-Dibenz[b,f]azepine-5-carboxamide, minimum purity ≥ 99 %) were purchased from Sigma-Aldrich (UK). Other analytical standards such as BPE [(4,4'-ethylidenediphenol, minimum purity > 98%)], BPB (2,2-Bis(4-hydroxyphenyl) butane, minimum purity ≥ 99%), BPAF (hexafluoroisopropylidene) diphenol, minimum purity > 98%), BPM (4,4-(1,3-Phenylenediisopropylidene) bisphenol, minimum purity > 98%) were purchased from TCI Europe (Zwijndrecht, Belgium).

2.2 Study area and sampling locations

The lagoon is located in Campania region, Southern Italy, and separated from the Gulf of Pozzuoli by a narrow strip of land. It is limited to the east by Monte Nuovo, to the west by Baia's hills, to the north by a small land between the "Monte delle Ginestre" and the Averno Lake. It has a surface of 68,000 m^2, a perimeter of 1,250 m, and a depth max of 5 meter (Aucelli et al., 2017). A map picturing the Campania Region and below a magnification of the studied water body is shown in Figure 1. The lagoon is fed by a modest contribution of fresh spring waters from a thermal area known as "Stufe di Nerone". The locations of the sampling sites in the lagoon were chosen according to their proximity to the main anthropogenic pressures around the lagoon perimeter (five different sampling sites along the perimetric area and one in the middle of the lagoon), the geographic coordinates, as well as the water depths measured in the different sampling sites, are listed in Table 1. Two of them, identified with L1 and L4, characterized by a more significant anthropic impact as located in the proximity of catering services; other two, L2 and L3, lying next to a canal connecting the lake with the sea; one, L5 site, approaching a canal communicating with a thermal area known as "Stufe di Nerone" and the last, L6, corresponding to the center of the lagoon. Relevant physico-chemical parameters, such as water temperature, pH, and electrical conductivity in the mild spring season, are listed in Table 1 to show their dependence on the specific sampling site. Depth up to about 5 m and an average width of 120 m suggests that the tiny dimensions determine this lagoon's low thermal capacity. The slightly alkaline pH values and the high electrical conductivity values, ranging between 43 and 47 dS m^{-1}, indicate waters' marine nature. A relevant negative gradient of electrical conductivity from east to west is presumably the result of two different and opposite factors: the supply of freshwater from the discharge canal of "Stufe di Nerone", located on the west side of the lake, and the periodic tidal excursions of marine waters flowing through the canal of mouth situated at the South-East side.

Sample collection

Water and sediment samplings were collected in January, April, and July 2018 to assess the influence of the atmospheric conditions and of the intensity of agricultural and touristic activities taking place on the banks of the water body on the occurrence of the selected EDCs. Seasonal climatic parameters were as following: average temperature (11.2 °C January, 18.2 °C April, 26.8 °C July) and the average monthly precipitation (80.8 mm January, 19.4 mm April, 25.2 mm July) as reported on the website ANCE Campania (www.ancecampania.it). Campania region has been the area in Southern Italy featuring the highest touristic flows in the three years 2016-2018 (+5.17% compared to the 2014-2016-time window while the whole country's growth was +3.1%). The touristic traffic was recorded to be at its maximum during July and August.

Both grab samples of surface sediments (0–20 cm) and waters were collected from the lagoon at each sampling site, whose coordinates are detailed in Table 1 in the three times of the year (for a total of 36 samples). Water and sediment samples were simultaneously collected (500 cc each one) and stored in amber-colored wide-necked glass containers previously rinsed several times with the sample's water itself. Afterward, the box was refrigerated and delivered to the laboratory. Each sample was immediately added with 0.5 g of sodium azide to inhibit bacterial activity. The water samples were filtered to remove suspended impurities through a 0.45 µm Whatman membrane filter (from Sigma Aldrich, Milan, Italy). Filtered samples were stored up in amber glass bottles covered with aluminum foil and kept in a refrigerated room at –20°C. Sediments were collected using a Van Veen sampler sampling the first 5 cm of the lagoon bottom sediments. Each sample was extracted twice and analyzed in triplicate for the pollutants under our investigation.

2.3 Sample preparation

2.3.1 Surface waters

Different sample preparation procedures were tested for the analysis (and pre-concentration) of shallow waters, including solid phase extraction (SPE) performed on three other cartridges, *i.e.*, Strata X, Strata C18 (200 mg/3 mL both from Phenomenex, Bologna, Italy), and Oasis HLB (200 mg/3 mL from Waters, Milan, Italy). For Oasis HLB, the water was filtered, acidified with formic acid 0.1 % (v/v), loaded onto the cartridge previously conditioned with methanol, and equilibrated with water. The wash step was done with 95:5 water:methanol to remove polar compounds, possibly interfering with the analytes. The elution was accomplished with methanol, according to the instructions of the manufacturer. As to Strata X and Strata C18, identical procedures were followed except for the elution step instead of acetonitrile. The extractions were also envisaged either under *vacuum* or at atmospheric pressure. For the extraction procedure, 10.0 mL of filtered water samples were spiked with 10 µL of a 5.0 mg mL^{-1} carbamazepine solution to monitor the extraction process's efficiency and added with 50.0 mg NaCl. Then, 142 µL of chloroform were added, vortexed and centrifuged for 5 minutes at 605 x g. The chloroform droplet at the bottom of the test tube was collected, dried under a stream of nitrogen pre-concentrated by 100 folds, and dissolved in 100 µL methanol for HPLC analysis. Recovery was evaluated by spiking artificial seawater samples with all the analytes under investigation, including carbamazepine, which was used as Internal Standard (IS) to track down possible flaws, *e.g.*, injection in the LC system of air bubbles, and errors in dilution.

2.3.2 Sediments

Sediments homogenized, freeze-dried, pounded in a ceramic mortar, and then sieved with a 70-mesh sieve. The powders obtained were used for the analysis: 1.0 g of sediment was dispersed in 2.0 mL of methanol, spiked with the 10.0 µg of the IS, and vortexed for 30 minutes, to ensure contact with the surface of the sediment to attain sorption equilibrium. The suspension underwent ultrasonication for 30 minutes, after centrifugation at 605 x g (3000 rpm) for 10 min. The liquid phase was collected, filtered through 0.45 µm nylon filters (Millipore, Burlington, MA USA), and gently dried under nitrogen stream for 10 min. The dry residue was pre-concentrated by 10 folds by dissolution in 100 µL of methanol acidified with 0.1 % v/v of formic acid before LC analysis. Different ultrasonication times were tested, *i.e.*, 5, 10, 15, 30, 45, 60, 120 and 190 minutes but an ultrasonication time of 30 minutes allowed recovery (> 90%) and a more selective extraction. A portion of the collected sediment samples was analyzed before being spiked, to determine possible background concentrations of target compounds in the matrix. Only sediment samples analytically verified selected pollutants-free were used spiked with the compounds of interest for the method validation.

2.4 Analytical method and chromatographic measurements

A systematic QA/QC protocol was adopted to monitor the analysis of chemicals under investigation, including linearity, retention time, limits of detection (LOD) and quantification (LOQ), and recovery. For every batch of samples, a procedural blank and spike sample consisting of all reagents was run to check for interference and cross-contamination (Russo, Barbato, et al., 2019). The Limit of detection (LOD) indicates method’s sensitivity, and it is the lowest concentration, at which the respective compound is just measurable. In contrast, the Limit of quantitation (LOQ) is the lowest concentration with acceptable linearity, accuracy, and precision. Practically, LODs and LOQs were defined as the concentration with a signal–to–noise ratio of three/ten, respectively. It was calculated based on the standard deviation of the curve’s response (SDa) of the curve and the calibration 'curve's slope (S). The standard deviation of the response can be determined based on the standard deviation of the y-intercepts of regression lines. Procedural blanks were performed along with each batch of samples to avoid possible carryover. The latter was considered acceptable if the peak area response in the blank sample obtained after measurement of the high-concentration sample was not >20% of all of the investigated analytes' peak area responses at the lower LOQ value and 5% of the peak area response of the IS. Throughout the analyses, plastic equipment was adequately treated to avoid any possible background contamination by keeping the plastic labware in contact with a 50/50 *n*-hexane: tetrahydrofuran solution (Olivieri et al., 2012). The details regarding LODs for the target compounds in waters and sediments are reported in Table 2. For surface water, artificial seawater was used as a blank and fortified with increasing analyte concentrations under consideration. It was not done for sediments due to the intrinsic unfeasibility in recreating artificial deposits. The matrix effect was quantitatively assessed by the post extraction addition method (W. Zhou, Yang, & Wang, 2017). A sediment sample, previously analytically verified as EDC free, was used as a blank, and the extracts were fortified with increasing concentrations of the analytes under consideration. The matrix effect, calculated as the ratio of the slopes achieved in the post extracts and that instead recorded in solvent was negligible (from 0.94 to 1.08). Therefore, the LOQ and LOD values in sediments were evaluated based on accuracy data achieved from post extracts. Standard calibration curves covered the range indicated in Table 2 and had squared correlation coefficients (r^2) greater than 0.95. The separation and detection of the selected EDCs were accomplished according to an already published method (Russo, Barbato, et al., 2019). All mobile phases were filtered through nylon filters 0.45 μm in pore diameter (Millipore, Burlington, MA USA). Each sample was injected three times to test the instrument repeatability. Matrix-matched calibration curves were obtained by plotting peak areas against concentrations of the analytes. Data acquisition and integration were accomplished by Cromatoplus 2011 software. IS elution time was 16.50 ± 0.30 min. Experimental values reported in graphics are the average value of three independent measurements. The % standard deviation never exceeded 10.60% for each retention value, and the 95% confidence interval associated never exceeded 0.04.

2.5 Risk Assessment

The presence of EDCs in the aquatic environment has been proved to induce adverse endocrine effects on marine organisms. The most common observed effects are vitellogenin production, feminization, imposex, and reproductive disorders (Yan et al., 2017; Yang et al., 2018). However, considering the health impact of each pollutant individually rather than the effects of mixtures of this estrogenically active substances might be a misleading exercise. Therefore, it is way more informative to estimate the total estrogenic activity of the water samples to assess their potential risk due to the "cocktail effect," *i.e*. the capability of EDCs in mixtures to act together in an additive manner (Brian et al., 2005).

The total estrogenic activity of target EDCs was evaluated as estradiol EEQ_t, of the pollutants in water samples and calculated by using the following equation:

$$(EEQ_t) = \sum C_i \text{ x } EEF_i \qquad \text{Eq. 1}$$

C_i concentrates the individual i-th EDC (ng L^{-1}) and EEF_i the respective estradiol equivalency factor. EEF_i, in turn, is defined as $EEF_i = EC50_{E2}/EC50_i$, where $EC50_{E2}$ is the concentration that occurs half of the maximum response for the estradiol, and $EC50_i$ the concentration at which occurs half of the full response of the i-th component under the same assay method. EEFi of EDCs were taken from the literature (van Leeuwen et al., 2019).

2.6 Statistical analysis

To evaluate the statistical significance of the results a commercially available statistical package for personal computer (Microsoft Excel 2016) was used. For the calibration curve, averages of each concentration levels were generated from at least five independent measurements. Inter- and intra- day variability was also carefully evaluated over different time frames (1, 4 and 15 weeks).

3. Results and discussion

The most suitable technique for our purposes of extraction was vortex assisted liquid-liquid microextraction (VLLME) (Shalash, Makahleh, Salhimi, & Saad, 2017) described in detail in materials and method section. The extraction procedures performed by any SPE cartridge gave unreproducible recoveries that fluctuated between 40 to 102 %. Other approaches that were envisaged were dispersive liquid-liquid microextraction (DLLME) (Zuloaga, Olivares, Navarro, Vallejo, & Prieto, 2015) performed using acetone as dispersant and chloroform as an extractant in different ratios and in the presence (either 50 or 100 mg) or absence of NaCl. Albeit fast and cost-effective, this procedure led to low recoveries (32-45%) and, therefore, was discontinued. Specifically for VLLME, the attempts were made with both *n*-octanol and chloroform, but better recovery and selectivity were achieved with the latter solvent.VLLME allowed a reproducible average recovery of 75%. The matrix-matched calibration curve accomplished in artificial seawater supported a negligible (ca. 0.90) matrix effect, assessed as the ratio between the slopes of the calibration curve achieved in artificial seawater (kindly offered by the research institution Stazione Zoologica Anton Dohrn of Naples, Italy) and measured in methanol.

Different extraction solvents were tested for the sediment samples, including methanol (acidified or not with formic acid 0.1 % (v/v)), acetonitrile, and dichloromethane. Moreover, the recovery was evaluated *vs.* the increasing ultrasonication time. We indeed found out that 30 minutes was the optimal ultrasonication time window as shorter times led to lower recoveries (<60 %) and longer ultrasonication times, such as 60 and 180 minutes, led to slightly insufficient recoveries (93% and 62%, respectively) and higher noise, thus compromising the selectivity of the analysis. This information is included in Figure 2. The preliminary analyses of randomly selected sediment powders showed no presence of carbamazepine. Therefore, because of its physicochemical properties, which place it right in the middle elution window and in the lights of its absence from the environmental sample, this was selected as IS.

Six out of the fourteen pollutants screened by our method were found in the analyzed samples (Table 3). These results can be grouped into three categories. Four contaminants (BPA, BPB, BPAF, and BADGE) were detected both in the waters and in the sediments of all the sites and seasons investigated. Among those, BPB was determined with a lower and differentiated detection frequency in waters (61%) and related sediments (17%) and two pollutants (BPE and 2 CP) with discrete detection frequency in the waters but were not detected in the relative sediments. Table 3 shows the range of EDCs concentration levels in sediments and waters and their detection frequency, regardless of the sampling and site. Interestingly, the percentage composition of BPA, BPAF, and BADGE waters differed from that of sediments.

Furthermore, Table 3 shows that pollutant concentrations in sediments are at least one order of magnitude higher than in water. 2 CP belongs to the Volatile Organic Compounds (VOCs) and has a 0.308 kPa vapor pressure (Kim et al., 2019). This implies that a mass amount of this chemical can be released because of atmospheric evaporation, and this phenomenon may become more relevant at higher environmental temperatures. On the other hand, this liquid is denser than water, and its stratification in the water/air interface seems rather unlikely. Since neither BPE nor 2 CP was detected in sediments, we focused our attention only on chemicals detected in both matrices. Figures 3 shows the concentrations of the BPA, BPAF, BADGE, and BPB in waters and associated sediments regarding the sampling sites and the climatic period. In this instance, concentration values < LOQ were considered equal to zero.

Figure 3 indicates that the highest concentration values of each of the bisphenols found in our investigation occurred in the L5 and L6 sampling sites during spring and summertime. This evidence could be motivated by the aspect that L5 is closer to a canal strongly affected by the tourist-seaside activities that conveys mineral waters to the lagoon, and for L6, to its greater depth. Furthermore, the highest concentration of EDCs was measured in the mild-to-hot season. This occurrence might be due to the superior intensity of agricultural activities taking in the surrounding fruit and vegetable crops, possibly resorting to organic substances as fertilizers and pesticides. However, as already stated in sample collection, the catering services' activity during the high touristic season was increased to fulfill the demands of a higher number of visitors.

The higher concentrations in the sampling of June, both in water and in sediments, can be motivated by the increasing solubility of EDCs due to the higher environmental temperature and the lagoon's low thermal capacity (max depth $\leq$5 m). Similar results have indeed been reported by Gong *et al.* (Gong et al., 2016).

Consistently, the concentrations of EDCs in sediments are an order of magnitude higher than in waters. Indeed, as already verified by Zhang and co-workers for some organochlorine pesticides, hydrophobic pollutants undergo extensive long-term deposition and accumulation as a result of the strong affinity of these substances for colloids in porewater (Zhang, Hong, Zhou, Huang, & Yu, 2003).

Due to their hydrophobic nature (see Table 4), BPs in aqueous ecosystems tend to be absorbed onto the organic particulate and dissolve in porewater. Generally, the release from sediments to water occurs by diffusion, and a good deal of literature data (Zhang et al., 2003) suggests this process is inversely dependent on the contaminant's lipophilicity. Investigating sediments contamination is highly relevant since sediments are crucial in the transport, fate, and bioavailability of EDCs,

Figure 4 shows the mean concentration values of BPA, BPAF, BADGE, and BPB during all the monitoring sampling both in water and sediments. To the best of our knowledge, no reference to BADGE occurrence in water bodies has been reported in the literature so far. Only Ballesteros-Gomez *et al.* (Ballesteros-Gomez, Ruiz, Rubio, & Perez-Bendito, 2007), as a result of EDC environmental analyses, detected BADGE in the waters of some rivers located in the south of Spain. BADGE has been reported to generate different derivatives because of the hydrolysis of the epoxy groups (*e.g.*, BADGE × 2HCl, BADGE × HCl, BADGE × H_2O, BADGE × $2H_2O$, and BADGE×HCl×H_2O). This process has also been detailed in food processing and storage if the epoxy resins used for packaging contain BADGE (Alabi, Caballero-Casero, & Rubio, 2014). BADGE's hydrolysis rates are constant over a 5.0 – 10.0 pH range increasing significantly at higher and lower pH values. The hydrolysis can be both acid and base-catalyzed. The higher occurrence of BADGE in sediments than in water can also be motivated by BADGE's inferior tendency to be hydrolyzed when absorbed onto sediments. In Table 4, a selection of relevant physicochemical parameters for BPA, BADGE, BPAF, and BPB is listed. However, when the average concentrations in sediments and water for BPA and BADGE are considered, the ratio between sediment and water concentration suggests that the BADGE is comparatively more present in sediments than in water to BPA, although this is more water-soluble than BADGE. According to this evidence, it seems plausible that these two phases were far from equilibrium or that a detectable input might have come from an external source.

Table 5 reports the mean, median, and standard deviation (Std. Dev) of each EDC measurements, detected both in sediments and water. The average concentrations in sediments are greater than those in the waters for each detected EDCs. Indeed, sediments may act as reversible and irreversible sinks for persistent organic contaminants, and their sorption can be affected by several environmental factors. Indeed, Xu and co-workers have evidenced that pH drop, temperature decrease, and salinity reduction can increase the sorption of these chemicals in sediment (Xu, Wang, & Li, 2008). Furthermore, the sorption capability is affected by particle size distribution and the organic carbon content of sediment (Cornelissen et al., 2005).

The distribution coefficients between sediments and waters seem to be roughly inversely related to water solubility. Our research work results show a significant difference in the distribution of each EDCs in sediments and water. All these causes reflect the different percentage distribution in waters and sediments.

The composition profiles of the chemicals detected in water (wat) and sediments (sed) with a frequency higher than 60% thought the year of sampling is shown in Fig. 5. BADGE was predominant both in sediments and in water, followed by BPAF, BPA, and BPB.

Although BPA is still detected (around 22% both in sediment and water samples), other analogs significantly contributed to the lagoon’s pollution. The predominance of BADGE (77% water and 61% sediments) suggests that although BPA is still the most widely manufactured and used bisphenol in Italy, other analogues are being progressively utilized by industries and consequently in the human activities as an alternative. This is consistent with monitoring results in food and biological samples (Gonzalez et al., 2020; Gonzalez et al., 2019; Russo, Varriale, Barbato, & Grumetto, 2019). A consideration of the assessment of the potential risk for species living in this lagoon should be made taking into account the results according to the Eq. 1. Considering the single EEQ_i of three contaminants with more than 60% of detection frequency, we observe the following estrogenic activity ranking BPAF > BPA > BADGE. For risk assessment purposes, the European Commission set the concentration of E2, causing endocrine-disrupting effects at 1 ng L^{-1} (AC01769567, 1996). Therefore, since the ΣEEQ of lagoon waters (data reported in Table 6) does not exceed 1.0 ng L^{-1} (level that may cause estrogenic effects in aquatic organisms), these can be claimed to be at low risk of estrogenic activity, according to the selected EDCs of our study.

Finally, several studies have reported the concentrations of BPs in river and lake waters around the world. Indeed, Patrolecco *et al*. (Patrolecco et al., 2006) reported BPA levels as high as 40 ng L^{-1} in Tiber river crossing Rome, Italy. A very high value of BPAF was detected in both waters, *i.e.* 111 ng L^{-1}, and sediments, *i.e.*, 12 ng L^{-1} in Taihu lake, a water basin located in China. Apart from these studies, the literature reports BPA values higher than BPAF and BADGE. This evidence goes well with the more widespread use of this chemical as compared to its structural analogs (Usman & Ahmad, 2016).

No consideration can be made in the case of BADGE due to the lack of data in the literature and the substantial difference in the water bodies shown in the table, *i.e.* lake *vs* WWTP influents.

4. Conclusions

Our study assessed the occurrence in water and sediments of a selected panel of EDCs in the aquatic environment located in Campania region, near Naples, in Southern Italy. Three bisphenols, *i.e.*, BPA, BPAF, and BADGE, were detected in both sediments and water with a greater than 60% detection frequency. The occurrence of the EDCs could be related to the intensification of anthropogenic activities such as catering or vegetable farming production, nautical, fishing, and agricultural activities.

BPs concentrate more on sediments, as generally found by other authors, and the relative abundances of the single BPs are different in waters and sediments. Based on our findings, limited to the fourteen EDCs that have been determined, the potential estrogenic risk in the water samples of this water basin is estimated to be low. In fact, the total estrogenicity does not exceed 1 ng L^{-1} , which is the threshold level that may cause estrogenic effects in aquatic organisms (AC01769567, 1996). These results also suggest that BPA is still the most widely manufactured and therefore released into the environment. Nevertheless, BPAF and BADGE are also exploited mainly in Italy. These results are relevant since mixtures of EDCs can exert potential endocrine disruption effects that are superior to the mere sum of the single toxicities exerted by each pollutant taken individually as a consequence of the well-known "cocktail effect" (Naville et al., 2019). Infact, this is emphasized in the European Commission communication of November 2018 concerning EDCs (Dinu, 2019), which highlights the endocrine disruptor-related issues for the environment, and the importance of a deep knowledge of EDCs threshold-dose level and the cocktail effects. In view of these achievements, further extensive studies are encouraged to extend the range of chemicals to be investigated and review the possible risk raised by these pollutants for both the aquatic ecosystem (Kang, Asai, & Katayama, 2007) and animals (humans included).

Acknowledgments

A grant from Regione Campania-POR supported this work Campania FESR 2014/2020 "Combattere la resistenza tumorale: piattaforma integrata multidisciplinare per un approccio tecnologico innovativo alle oncoterapie-Campania Oncoterapie" (Project N. B61G18000470007). The authors gratefully acknowledge the postgraduate student Arturo Viola who helped out in both sampling and analytical determination.

Author credits:

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L.Grumetto: Conceptualization (Equal), Funding acquisition (Equal), Validation (Equal), Writing-review & editing (Equal).

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figures

Figure 1

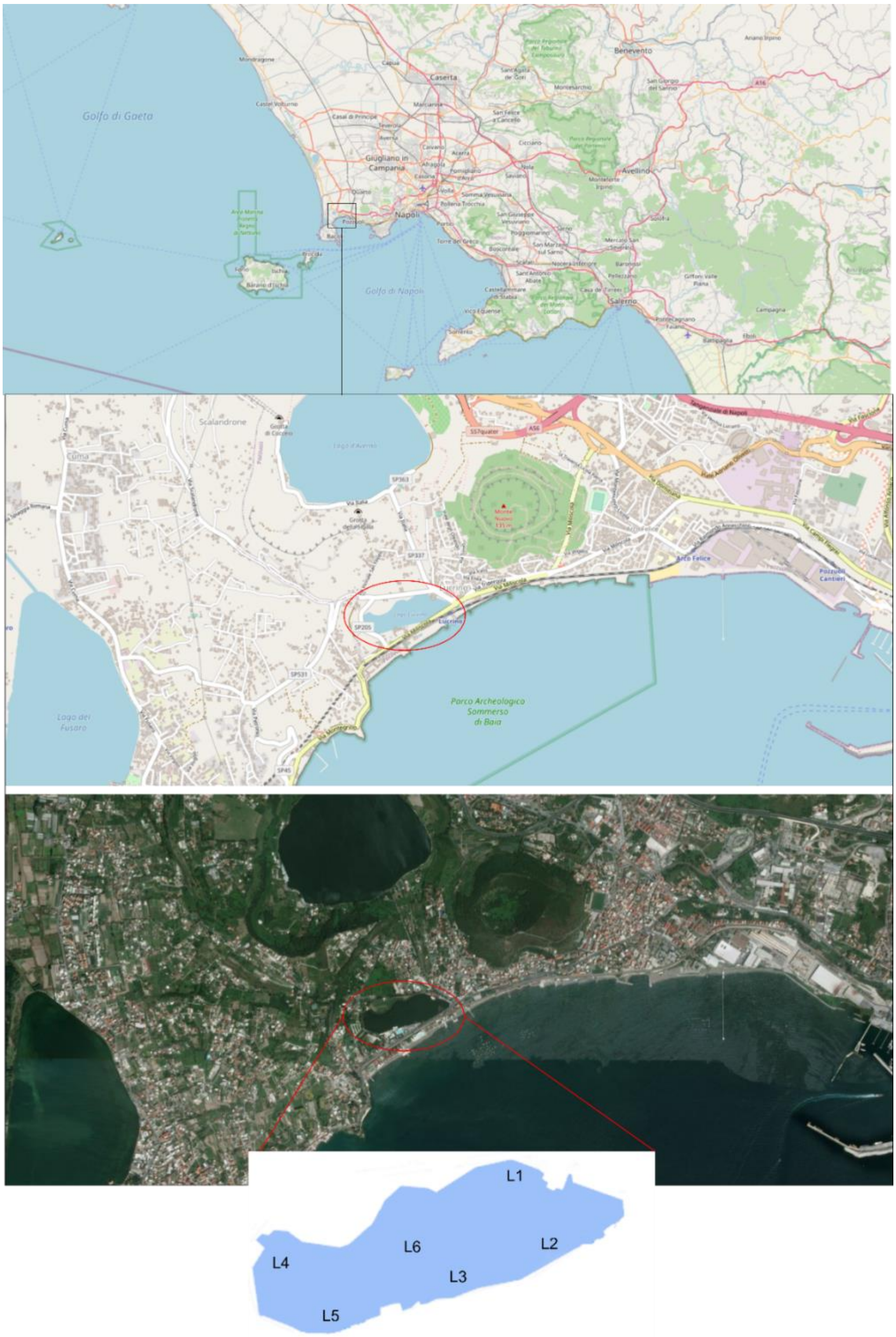


Figure 1. Map of the lagoon showing the sampling spots.

Figure 2

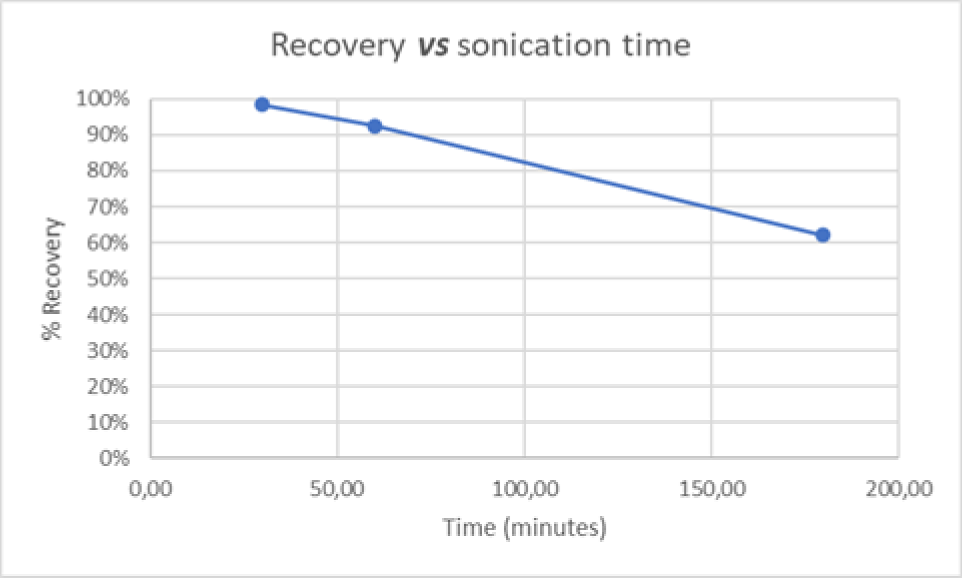
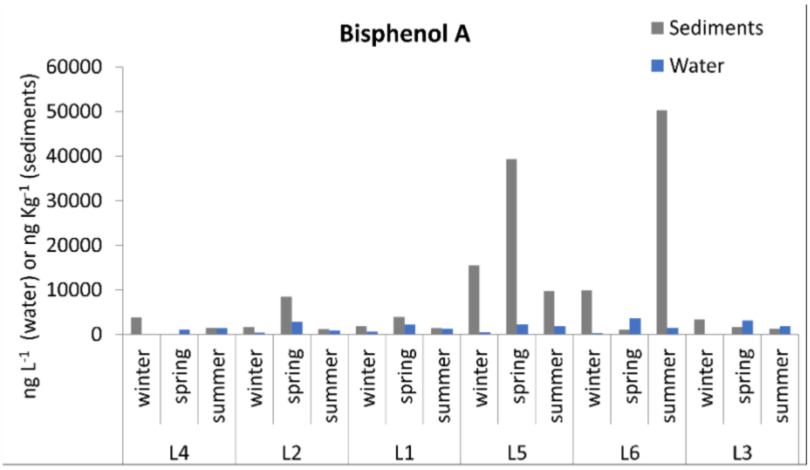
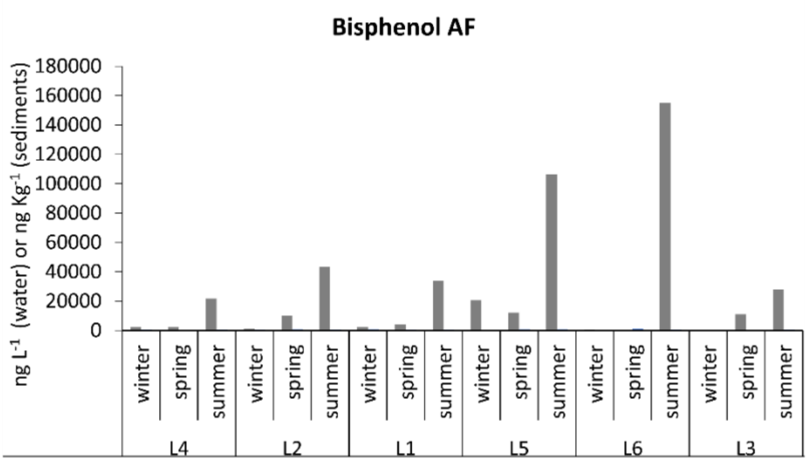
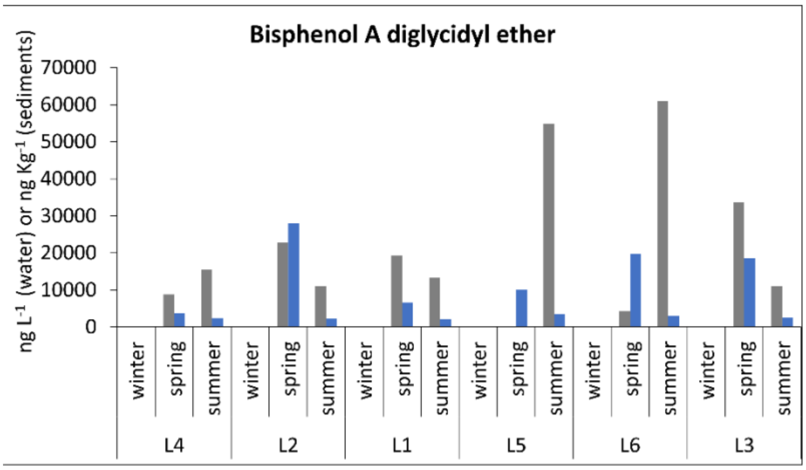


Figure 2. Average % recovery measured after 30, 60 and 180 minutes of ultrasonication time.

Figure 3


Bisphenol A


Bisphenol AF


Bisphenol A diglycidyl ether

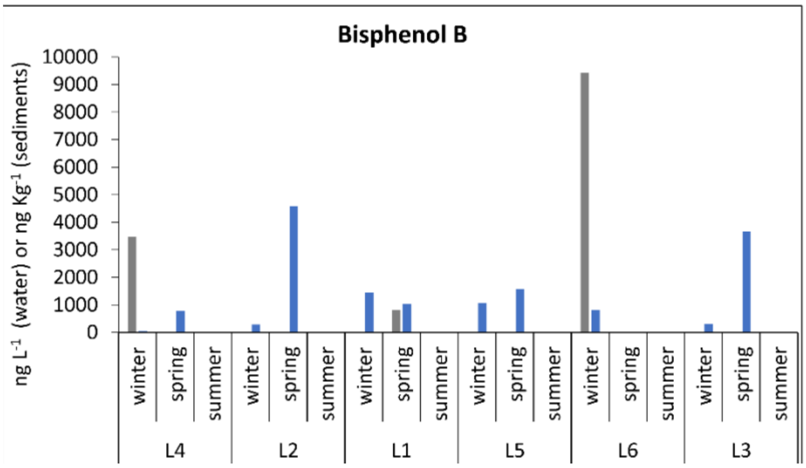

Bisphenol B

Figure 3. Histograms plotting BPA, BPAF, BADGE and BPB concentrations (ng Kg^{-1}) in sediments (grey) and (ng L^{-1}) water (blue) *vs* sampling site and sampling season.

Figure 4.

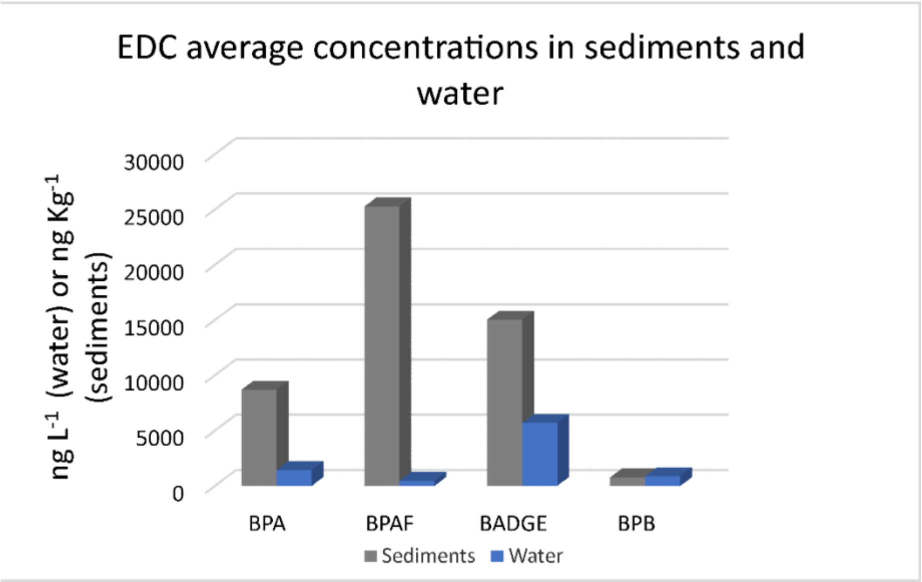


Figure 4. Mean concentration values for the EDCs BPA, BPAF, BADGE and BPB all samplings in waters and sediments, regardless the sampling time and site (range found 132 – 28000 ng L^{-1} in water and of 730 - 155000 ng Kg^{-1} dw in deposits).

Figure 5.

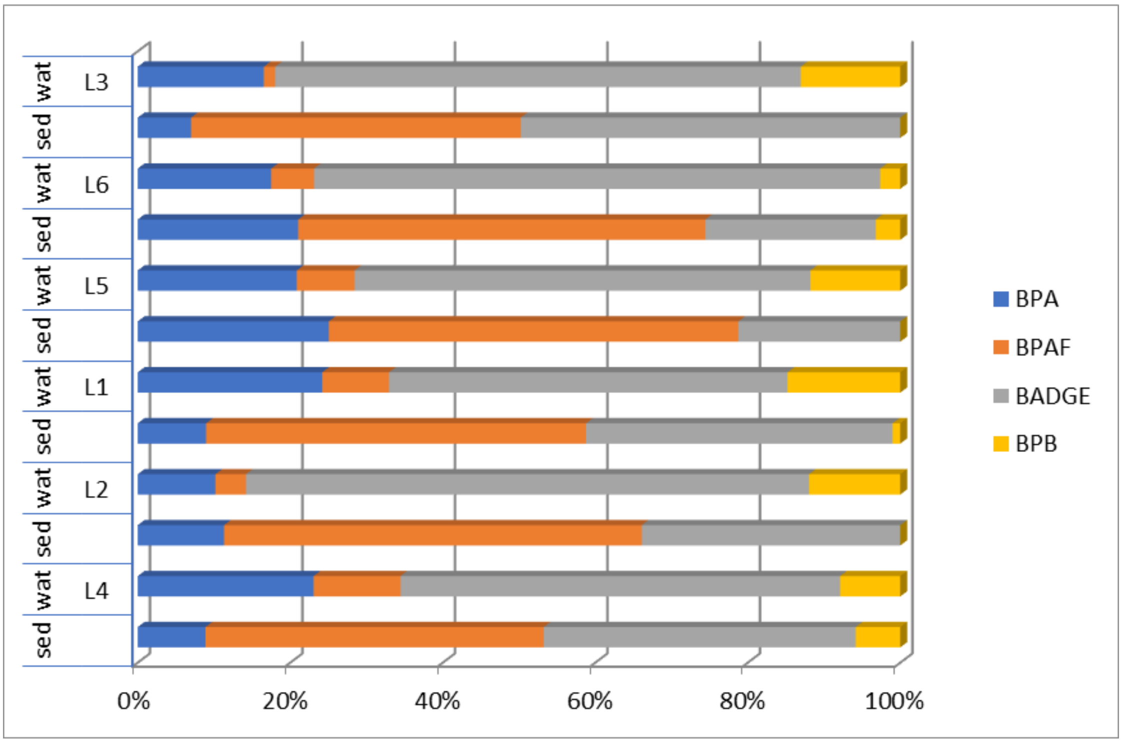


Fig. 5. Composition profiles of bisphenols detected in water (wat) and sediments (sed) with a frequency higher than 60% thoughtout the sampling year.

Tables

Table 1

Sampling site	**N-Coordinates**	**E-W Coordinates**	**Depth (m)**	**Temperature (°C)**	**pH**	**Electrical conductivity (dS m^{-1})**
L1	40.830077	14.081450	-1.30	15.5	7.97	46.3
L2	40.829651	14.082643	-0.50	15.9	7.97	46.8
L3	40.829470	14.083000	-1.30	15.5	7.97	46.3
L4	40.829240	14.077501	-0.30	15.6	8.08	43.2
L5	40.828750	14.078577	-1.32	15.2	7.94	45.1
L6	40.829505	14.081656	-2.10	15.4	7.92	46.3

Table 1. Geographic coordinates, water depth and physico-chemical parameters of waters measured in different sampling sites in spring season.

Table 2

VALIDATION PARAMETERS	FD DETECTED (BPF, BPE, BPA, BPB, BPAF, BADGE, BPM AND 4-NP)	UV DETECTED (BPS, 2-CP, DCB, TCS AND DEHP)
INSTRUMENT QUANTITATION LIMIT (RANGE)	2,300 – 14,500 ng L^{-1}	530 – 24,000 ng L^{-1}
METHOD QUANTITATION LIMIT (RANGE)	25.0 – 200.3 ng L^{-1} (surface water) 230.2- 1920.0 ng Kg^{-1} (sediments)	5.5 – 245.2 µg L^{-1} (surface water) 59.6 – 2,507.0 µg Kg^{-1} (sediments)
METHOD DETECTION LIMIT (RANGE)	7.2 – 57.8 ng L^{-1} (surface water) 70.3-568 ng Kg^{-1} (sediments)	1.9 – 75.6 µg L^{-1} (surface water) 18.7 – 736 µg Kg^{-1} (sediments)

Table 2. Main validation parameters, expressed in quantitative ranges, for the analytes under consideration divided according to whether they were detected by UV or FD.

Table 3

EDCs	**CONCENTRATION IN WATER ng L^{-1}**		**CONCENTRATION IN SEDIMENTS ng KG^{-1} DW**	
	Range	DF (%)	Range	DF (%)
BPA	298-3620	89	1140-50000	95
BPAF	132-1070	78	730-155000	89
BADGE	2140-28000	67	4290-61000	61
BPB	47-3660	61	820-9420	17
BPE	300-2770	50	n.d.	n.d.
2 CP *	100- 1910	67	n.d.	n.d.

DF= detection frequency; n.d. = not detected

* results expressed as µg L^{-1}

Table 3. Concentration levels of the EDCs detected in water and sediments

Table 4

BISPHENOL	WATER SOLUBILITY (NG ML^{-1}, 25°C)(KIM ET AL., 2019)	LOG K$_{OW}$ (RUSSO ET AL., 2018)	HALF-LIFE IN WATER (DAYS)(CALIFORNIA)	HALF-LIFE IN MARINE SEDIMENTS (DAYS)(CALIFORNIA)
BPA	120 x 10^{3}	3.67	37.5	337.5
BADGE	700 (20 °C)	3.87	60.0[a]	540.0[b]
BPAF	Negligible	2.49	180.0	1600.0
BPB	27	4.13	38.0 [a]	340.0 [b]

[a] Predicted to be persistent according to U.S. EPA criteria

[b] Predicted to be very stubborn according to U.S. EPA criteria

Table 4. Physico-chemical parameters of the EDCs retrieved most frequently retrieved in the environmental sample.

Table 5

	EDC	Mean	Median	Std. Dev.
Waters **(ng L^{-1})**	**BPA**	1430	1430	1.08
	BPAF	460	540	340
	BADGE	5680	3220	8170
	BPB	870	300	1.31
Sediments **(ng Kg^{-1}) dw**	**BPA**	8680	3570	14.0
	BPAF	25300	11500	41.3
	BADGE	15000	15500	18.9
	BPB	760	0.00	2.31

Table 5. Statistical parameters of the detected EDCs in water and sediments.

Table 6.

BISPHENOLS	EEF_I (VAN LEEUWEN ET AL., 2019)	AVERAGE CONCENTRATION IN WATER (NG L^{-1})	EEQ_T IN WATER (NG L^{-1})
BPA	$1.07 * 10^{-4}$	$1.43 * 10^{3}$	$1.53 * 10^{-1}$
BPAF	$7.23 * 10^{-4}$	$0.46 * 10^{3}$	$3.33 * 10^{-1}$
BADGE	$1.07 * 10^{-7}$	$5.68 * 10^{3}$	$6.08 * 10^{-4}$
ΣEEQ			$4.86 * 10^{-1}$

Table 6. The estrogenic activity (EEQ), for each EDC detected in water samples.

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