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Corresponding Author: Emanuele Magi

Corresponding Author's Institution: University of Genoa

First Author: Marina Di Carro

Order of Authors: Marina Di Carro, Emanuele Magi, Francesco Massa, Michela Castellano, Cristiana Mirasole, Shivani Tanwar, Enrico Olivari, Paolo Povero

Title Page with all author info

**Untargeted approach for the evaluation of anthropic impact on the sheltered
marine area of Portofino (Italy)**

Marina Di Carro^a, Emanuele Magi^a *, Francesco Massa^b, Michela Castellano^b, Cristiana Mirasole^a,
Shivani Tanwar^a, Enrico Olivari^b, Paolo Povero^b

^a Department of Chemistry and Industrial Chemistry, University of Genoa, Via Dodecaneso 31,
16146 Genoa, Italy

^b Department for the Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26,
16132 Genoa, Italy

* corresponding author

email address emanuele.magi@unige.it

Graphical abstract (optional)

Highlights (mandatory)

Abstract

1 **Untargeted approach for the evaluation of anthropic impact on the sheltered**
2 **marine area of Portofino (Italy)**

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4 Marina Di Carro, Emanuele Magi, Francesco Massa, Michela Castellano, Cristiana Mirasole,
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6
7 **Abstract**

8 Seawater passive sampling with Polar Organic Chemical Integrative Samplers (POCIS) combined
9 with Gaschromatography-Mass Spectrometry analysis were employed as a tool for screening
10 unknown contaminants in a complex Ligurian marine coastal area.

11 The untargeted approach allowed recognizing different classes of compounds, mainly hydrocarbons
12 from C₂₀ to C₃₀. Besides, two chemicals, deriving from anthropic activities, N-
13 butylbenzenesulfonamide (NBBS) and diphenyl sulfone (DPS), were identified and quantified in all
14 samples. Both analytes showed decreasing concentrations from the more confined site to the outer
15 one. The oceanographic characterization of the area performed with multiparametric probes
16 provided useful information, in agreement with chemical analyses. The presence of NBBS and DPS
17 in the site presenting lower continental inputs demonstrated the usefulness of the integrative
18 sampling approach for temporal and spatial monitoring, especially for low level and/or short-term
19 pollution events that traditional monitoring can fail to detect.

20
21
22 6 keywords

23 Passive sampling, trace contaminants, GC-MS, Seawater, Marine Protected Area, Ligurian sea

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26 **1. Introduction**

27

28 Liguria is a coastal Italian region placed in north-western Italy, consisting in a narrow strip of land
29 more than 300 km long bordered by the Ligurian Sea, the Apennines mountains, the Alps and
30 France. The coastal and the continental shelf generally descend rapidly to considerable marine
31 depths. Despite its narrow morphology, Liguria is the region with the highest coastal urbanization,
32 because of the presence of important urban and port activities and several polluted discharges
33 (Cattaneo-Vietti et al., 2010; Dapuzo et al., 2015; Magi et al., 2008; Ruggieri et al., 2011). Intense
34 urban and industrial coastal development characterized the region in the past and today it is
35 characterized by plenty economical activities such as agriculture (flowers, wine, olive oil), industry,
36 professional fishing, fish farming and definitely the tourism (in particular marine activities are
37 diving, recreational boating and sport fishing). It also hosts eight natural parks, terrestrial reserves
38 and four marine protected areas, witness of the natural heritage and biodiversity richness of the
39 region. In this context, the Portofino promontory and the Gulf of Tigullio exhibit all of the Ligurian
40 region characteristics being an important touristic location, hosting Portofino Marine Protected Area
41 (MPA) and different small marinas and harbors, but being also highly urbanized with presence of
42 agricultural and local industrial activities. Monitoring the possible anthropic impacts on a complex
43 coastal area is challenging but is mandatory for the environment and human health and a wise
44 management that can guarantee the future uses.

45 Anthropogenic activities can have a wide range of adverse impacts on coastal marine environments
46 through the discharge of chemicals, which may originate from surface runoff, untreated and
47 partially treated effluents, freshwater discharges from polluted rivers, shipping traffic and
48 contaminated atmospheric depositions (Martínez Bueno et al., 2009). The main limitation for the
49 detection of chemical residues in marine environment is the high factor of dilution, since
50 contaminants can be present at very low concentrations (ng L^{-1} or below): very sensitive techniques
51 are therefore required to assess their presence in the water samples collected. The usual approach,
52 called grab or spot sampling, often involves large volumes of water and only provides the
53 instantaneous concentrations of pollutants (Vrana et al., 2005). The main drawback of this method
54 is that contaminant concentrations vary over time, and episodic pollution events can be missed.

55 In the last few years there has been a rapid development in the use of passive sampling devices that
56 allow continuous monitoring of aqueous pollutants (Miège et al., 2015). Unlike spot sampling,
57 time-integrative passive sampling allows the determination of time-weighted average (TWA)
58 concentration of pollutants over extended sampling periods; due to the dynamic interaction with
59 high volumes of water, it also enables the in situ preconcentration of ultra-trace compounds and the
60 detection of contaminants deriving from episodic events, not always possible by spot sampling. By

61 using passive sampling, regular monitoring of the chemicals can be used to track spatial and
62 temporal trends in waters (Alvarez et al., 2014).

63 Among passive samplers, POCIS (Polar Organic Chemical Integrative Samplers) were designed to
64 sequester and concentrate polar organic chemicals with $\log K_{o/w}$ values lower than 4 (Alvarez et al.,
65 2004). The sampler contains a sorbent phase sandwiched between two microporous
66 polyethersulphone membranes: pollutants diffuse from the water through the membrane and adsorb
67 onto the sorbent phase. POCIS has been used to preconcentrate trace compounds mainly from river
68 waters, wastewater and freshwater (Alvarez et al., 2005; Bartelt-Hunt et al., 2009; Li et al., 2010;
69 Liscio et al., 2009; Mirasole et al., 2016) but also from seawater (Arditsoglou and Voutsas, 2008;
70 Martínez Bueno et al., 2016; Muñoz et al., 2010).

71 The use of passive samplers, such as the POCIS, addresses key aspects of the Marine Strategy
72 Framework Directive (MSFD) and contributes to extend the capability of ocean monitoring systems
73 and programs.

74 The traditional approach in environmental monitoring with LC-MS or GC-MS involves target-
75 compound analyses: to this aim, only few ions for each compound are monitored to maximize
76 sensitivity. The main drawback of this method is that the quantitation is limited to the target
77 analytes, with an incomplete assessment of the contamination of the real samples (Magi and Di
78 Carro, 2016). In the last decade, the use of high resolution mass spectrometry analyzers has been
79 proposed for a more comprehensive evaluation of the environmental pollution: high resolution MS,
80 although not widely employed in environmental laboratories, can provide powerful information
81 which can elucidate chemical structures. As an alternative, GC-MS in EI mode allows the
82 acquisition of library searchable spectra and therefore a (tentative) identification of the compound.

83 In this work, the latter approach was employed to investigate the POCIS eluate obtained from the
84 samplers deployed at three sites of the Portofino Promontory with different shelter degrees and
85 urbanization levels in the Gulf of Tigullio coastal area. These sites were also investigated from a
86 physico-chemical point of view to highlight the differences in the water masses, using a classical
87 survey with multiparameter probes.

88

89 **2. Materials and methods**

90 2.1. Site characterization

91 The Portofino Promontory is located about 25 km east of Genoa in the Ligurian Gulf and possesses
92 13 km of coastline along the Ligurian Sea. The peninsula is a hilly area that reaches a height of 610
93 m at the summit of Monte di Portofino. Three municipalities of Camogli, Portofino, and Santa
94 Margherita have jurisdiction over lands of the Portofino Peninsula (Salmona and Verardi, 2001).

95 The eastern part of the promontory is bathed by the Gulf of Tigullio (Figure 1) in which the main
96 coastal current flows from SE to NW and carries the continental waters towards the Portofino
97 Promontory, although a near shore eastward current sometimes occurs (Doglioli et al., 2004). Wind
98 forcing, which is known to deeply influence the surface-water movement (Astraldi and Manzella,
99 1983), may spread the continental water in the entire area or rapidly carry it offshore (Doglioli et
100 al., 2004) Various watercourses flow into the Tigullio Gulf; in particular the Entella river, one of
101 the main natural rivers of Liguria, flows into the sea close to the promontory, although with a rather
102 low discharge, less than $10 \text{ m}^3 \text{ s}^{-1}$ during summer (Tomaselli et al., 2009). The continental inputs
103 together with the high level of urbanization of the eastern coast of the promontory represent a wide
104 and notable range of potential sources of pollutants for the peculiar environment of the Portofino
105 promontory, which is legally protected by the Marine Protected Area (MPA) of Portofino. The area
106 is densely populated by both residents and tourists, resulting in comparatively high nutrient inputs
107 into coastal waters (Parravicini et al., 2013). In particular, the resident population in the Tigullio
108 Gulf area is more than 100,000 (2015, Italian Statistical Institute data), but during the summer
109 season, due to the seaside strong tourist vocation of the area, the population increases (Rapallo,
110 Chiavari, Lavagna) and in seaside Municipalities (Portofino, Santa Margherita Ligure, Zoagli,
111 Sestri Levante) reaches about 240,000 people.

112 In the Portofino MPA and the Tigullio Gulf anthropic pressure is high also in terms of recreational
113 boating, cruise ships and, to a lesser extent, traditional and recreational fishery. Because of the
114 environmental qualities and the presence of the marina of Santa Margherita together with other
115 small marinas, the Portofino MPA is the most visited among the Ligurian MPAs especially in the
116 summer periods and during the weekends (Venturini et al., 2016).

117

118 2.2. Oceanographic features

119 From July to September 2013 a survey on the seawater surface layer in the three areas was carried
120 out through multiparametric probes: temperature, salinity and dissolved oxygen were acquired by
121 an Idronaut 304 probe while fluorescence, turbidity and colored dissolved organic matter (CDOM)
122 were acquired by a C6™ Multi-Sensor Platform (Turner Designs) integrating three Cyclops-7™
123 sensors. The CDOM sensor was equipped with a LED lamp of 365 nm (excitation 325/120 nm;
124 emission 470/60 nm), the in-vivo chlorophyll-a sensor with a LED lamp of 460 nm (excitation
125 465/170 nm; emission 696/44 nm), while the turbidity sensor used a LED lamp of 850 nm
126 (excitation 850 nm; emission 850 nm). The raw data were calibrated with in-situ measurements or
127 proper standards. To calibrate the fluorometer, in-situ seawater samples were collected and
128 chlorophyll-a determined in laboratory (Holm-Hansen et al., 1965). CDOM and turbidity sensors

129 were calibrated in laboratory using quinine-sulfate (range: 0-60 ppb QS, $r=0.99$) and formazine
130 standards (range: 0-32 NTU, $r=0.99$), respectively.

131

132 2.3. Standards and reagents

133 Analytical grade diphenyl sulfone (DPS) and N-Butylbenzenesulfonamide (NBBS) were obtained
134 from Sigma-Aldrich (Milan, Italy). Both stock analyte solutions (1000 mg L^{-1}) and working
135 standards were prepared in MeOH and stored at $-20 \text{ }^\circ\text{C}$ in the dark. Standard mixtures ($0.1\text{-}5 \text{ mg L}^{-1}$)
136 of the analytes were prepared at different concentrations by appropriate dilution of the individual
137 stock solutions. A standard mixture containing all even n-alkanes from C_{10} to C_{40} was purchased
138 from Sigma-Aldrich (Milan, Italy). Dichloromethane, isopropanol, trifluoroacetic acid and
139 methanol were obtained from Merck (Darmstadt, Germany). All organic solvents were of analytical
140 or chromatographic grade. Water was purified by a Milli-Q system (Millipore, Watford,
141 Hertfordshire, UK).

142

143 2.4. POCIS preparation and field deployment

144 POCIS samplers were assembled in our laboratories in accordance with the characteristics of the
145 commercial ones (mass of the sorbent phase 200 mg and 45.8 cm^2 as sampler surface area).
146 Polyethersulfone membranes (PES, $0.1 \text{ }\mu\text{m}$ pore size) and Oasis HLB sorbent phase were purchased
147 from Pall Italia (Buccinasco, Italy) and Waters (Vimodrone, Italy), respectively. PES membranes
148 were washed before use in a $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ solution ($80:20 \text{ v/v}$) for 24 hours at room temperature
149 (RT) and 1 hour at $40 \text{ }^\circ\text{C}$. Membranes were dried in laminar hood and subsequently washed with
150 CH_3OH for 24 hours at RT and 1 hour at $40 \text{ }^\circ\text{C}$. After drying in a laminar hood, the membrane-
151 sorbent-membrane layers were compressed between two stainless-steel support rings held together
152 with three thumbscrews and stored frozen at $-20 \text{ }^\circ\text{C}$.

153 Three passive samplers were deployed in a tank filled with tap water for 14 days and served as a
154 quality control blank. The analyses of the extracts of the blanks showed no signal for DPS and
155 hydrocarbons; a small contribution for NBBS was observed and subsequently subtracted from the
156 sample area values.

157 The POCIS samplers were deployed in Ligurian seawater protected by stainless-steel grids (Figure
158 2) during summer 2013, at surface depths (2-4 m) in three different sites (Figure 1): ARION Buoy
159 (AB, 90 m seafloor depth, $44^\circ 17.737'\text{N}$; $009^\circ 11.443'\text{E}$), Paraggi Buoy (PB, 20 m seafloor depth,
160 $44^\circ 18.551'\text{N}$; $009^\circ 12.793'\text{E}$), and Santa Margherita Harbor (SMH, 5 m seafloor depth,
161 $44^\circ 19.794'\text{N}$; $009^\circ 12.840'\text{E}$). At the end of each period of sampling (72 days for AB, 45 days for
162 PB and 22 days for SMH), the POCIS were collected, rinsed with Milli-Q water, wrapped in
163 aluminum foil and transported to the laboratory under cooled conditions (approximately $4 \text{ }^\circ\text{C}$).

164 Samplers were stored at -20 °C.

165

166 2.5. POCIS extraction

167 Prior to processing, the POCIS were allowed to reach room temperature; any remaining debris was
168 rinsed away with Milli-Q water. Each POCIS was carefully dismantled and Milli-Q water was used
169 to transfer the sorbent into a 1 cm internal diameter glass syringe cartridge fitted with a Teflon frit
170 and glass wool. The solid phase was eluted with 10 mL of dichloromethane: isopropanol:
171 trifluoroacetic acid (80:20:0.1 v/v/v) in a round bottom flask. The solution was reduced to small
172 volume (around 0.5 mL), then 20 mL dichloromethane were added and it was subsequently
173 evaporated to dryness and redissolved in CH₃OH (0.5 mL). All samples were filtered before
174 analyzing in gas chromatograph coupled with mass spectrometer.

175

176 2.6. GC-MS analysis

177 Samples were analyzed by an Agilent 7890 gas chromatograph with a model 5975 mass
178 spectrometer (Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with a
179 Phenomenex ZB5 capillary column (30 m x 0.25 mm x I.D. 0.25 mm) coated with 5%
180 phenylpolysiloxane. Helium was employed as carrier gas with a constant flow of 1.2 mL min⁻¹. The
181 oven temperature was programmed as follows: 50 °C for 1 min, 10 °C/min to 300 °C, and then held
182 at 300 °C for 10 min. The injector temperature was held at 250 °C. The transfer line was set at 280
183 °C, the source at 300 °C, and the quadrupole at 150 °C. The mass spectrometer was operated in full
184 scan (SCAN) for the non-target analyses and in full scan/selected ion monitoring (SCAN/SIM)
185 mode for the quantitative analyses with an electron impact ionization of 70 eV. Full-scan spectra
186 were collected from 50 to 550 m/z. To maximize sensitivity, the fragmentation pattern of each
187 analyte was considered in choosing the most suitable fragments for the analysis in SIM. For each
188 compound, two ions were monitored to provide good specificity, using the most abundant for the
189 quantitation.

190

191

192 3. Results and discussion

193 A survey carried out in the summer period in the surface layer (<5 m) showed significant
194 differences in temperature, salinity, dissolved oxygen and also in CDOM, turbidity and chlorophyll
195 concentrations in the three sites (Table 1 and S1 in Supplementary data). Some parameters showed
196 huge minimum-maximum variations, such as seawater temperature that showed the highest values
197 in the middle of the summer and decreased in September, according to the normal seasonal cycle
198 mainly driven by the atmospheric weather. Despite the seasonal variation, the mean temperature

199 was higher in the harbor (SMH) than at the other outside stations. Salinity showed high values
200 typical of the surface waters in summer season (dry and hot), that varied along a gradient. Higher
201 salinity (>38) occurred in the southern side of the Promontory (AB) in accordance with other
202 surveys (Misic et al., 2011) while the values decreased towards the Tigullio Gulf and the harbor
203 (SMH), signal of a freshwater input. The dissolved oxygen was near the saturation only in the
204 southern side of the Promontory (AB), decreasing to values around 80% in the Gulf (BP) and the
205 harbor (SMH), suggesting a smaller water exchange in the more confined areas. CDOM, turbidity
206 and chlorophyll were higher in the harbor (SMH). The CDOM signal acquired by Cyclops-7™
207 sensors is more related to the refractory organic matter (like humic acids), which has been related to
208 continental inputs (Vignudelli et al., 2004). These kind of compounds in the southern part of the
209 Promontory are normally quite low, with the exception of the winter period (Misic et al., 2011),
210 when frequent rainfall occurs, thus, reinforcing the river discharges. The most important
211 watercourse in the area as already mentioned is Entella River, which flow rate in summer 2013
212 (July-September) was just $4.66 \pm 2.62 \text{ m}^3 \text{ sec}^{-1}$ as expected (Tomaselli et al., 2009) but four events
213 over the mean value occurred (Figure 3); in particular, in the second half of August and in the
214 second half of September flow rate was $> 10 \text{ m}^3 \text{ sec}^{-1}$ for few days.

215 The significantly higher CDOM values for the samplings in SMH indicated that the allochthonous
216 inputs (for example the small torrent that ended in the harbor and the use of running water to take
217 care of the boats) could have favored the production and accumulation of autotrophic biomass. In
218 fact during summer in oligotrophic areas, as the Ligurian Sea (Misic and Fabiano, 2006; Vidussi et
219 al., 2000), turbidity and chlorophyll concentrations in surface waters are generally low, and
220 continental inputs that can occasionally reach the eastern tip of the Promontory can add particulate
221 matter and trigger phytoplankton blooms adding inorganic nutrients to the coastal seawater (Misic
222 et al., 2011; Ruggieri et al., 2006).

223 The coastal area of the Gulf of Tigullio is more frequently reached by continental input as
224 highlighted by the occurrence of organic matter of continental origin in the shelf sediments within,
225 approximately, the 50-m isobath, i.e. at a maximum distance of 3-4 km from the coast; only during
226 the season of heavier rainfall (autumn-winter), which often causes abrupt inputs by river outfalls, a
227 widespread diffusion of fine sediment particles was observed (Misic et al., 2016).

228

229 3.1 Identification of seawater contaminants

230 Full scan GC-MS analysis of the POCIS extracts showed rather complex profiles, containing
231 several peaks; chromatograms of each site were compared among them in order to find possible
232 similarities and differences. The spectrum of each signal was considered and compared with those
233 contained in the NIST library. Chemicals belonging to different classes of compounds, mainly

234 hydrocarbons and phthalates, were identified in all samples with qualitative and quantitative
235 differences from site to site. Among all the peaks recognized by the software, only two were present
236 in all chromatograms, corresponding to N-butylbenzenesulfonamide (NBBS) and diphenyl sulfone
237 (DPS), identified with a quality match higher than 95%.

238 The former is a lipophilic compound, widely used as a plasticizer in polyacetals, polycarbonates,
239 polysulfones, and polyamides (Dzikowitzky et al., 2004), frequently found in groundwater, river
240 water and effluents from wastewater treatment sites (Franke et al., 1995; Huppert et al., 1998).
241 NBBS is considered a neurotoxic compound and may also affect liver and kidney (Marrocco et al.,
242 2015), therefore its potential leaching from polyamide plastics used as food container (Skjevraak et
243 al., 2005) and nylon-11 tubing (Gilmore et al., 2004) have raised concerns regarding potential
244 human exposure.

245 DPS is a herbicide, insoluble in water, widely employed in the past as ovicide and acaricide;
246 nowadays it is used as monomer in the polymer industry and produced as a byproduct of the
247 manufacture of phenol (Wick and Gschwend, 1998). It has been detected with high frequency in
248 two coastal Mediterranean areas, with concentration range from 15.5 to 75.6 ng L⁻¹ (Martínez
249 Bueno et al., 2009; Muñoz et al., 2010).

250 In Table 2, chemical structures and log K_{ow} of NBBS and DPS are reported.

251 To confirm the presence of these compounds in the studied samples, a solution containing both
252 analytical standards was injected in the GC-MS system. The retention times of the obtained signals
253 were compared to those of the peaks in the POCIS eluate, proving the identification of the two
254 chemicals.

255 To evaluate the concentration level of the identified pollutants, a quantitative method was
256 developed by mass spectrometry; the fragmentation pattern of each analyte was considered to
257 choose the most suitable fragments for the Selected Ion Monitoring (SIM) analysis. Two ions were
258 monitored for each compound to provide good specificity, using the most abundant for the
259 quantitation.

260 Calibration curves of DPS and NBBS were drawn by plotting the analyte peak area versus the
261 concentration. Each point of the curve (0.1, 0.2, 0.5, 1 and 5 mg L⁻¹ in CH₃OH) was the mean of
262 three replicates. Although limits of detection and quantitation (calculated as a signal to noise ratio
263 of 3 and 10, respectively) were slightly different for each analyte, the lowest point of the calibration
264 curve (0.1 mg mL⁻¹) was considered as the limit of quantitation (LOQ) for the two compounds.

265 In Table 3, LOD (limits of detection) and LOQ of NBBS and DPS are reported, together with
266 selected ions monitored for their quantitation and retention times.

267 Results obtained by POCIS analysis are usually expressed as the amount of the pollutants measured
268 in the sampler; this value can be also used to estimate the time-weighted average (TWA)

269 concentration of the contaminants over the period of POCIS exposure, derived from the fluctuations
270 of contamination levels (Alvarez et al., 2004). In fact, the amount of chemicals found in the sorbent
271 phase after deployment is correlated with their concentration in water, mediated over time, and it
272 depends on the sampling rate (R_s), i.e. the volume of water cleared in a unit of time for a given
273 molecule, according to the following equation (Greenwood et al., 2007):

$$274 \quad C_s = C_w R_s t / M_s \quad (1)$$

275 where C_s and C_w are the concentrations of the compound in the POCIS sorbent (ng g^{-1}) and in the
276 water (ng L^{-1}), respectively, t is the sampling period (days), M_s the mass of the sorbent in the
277 POCIS (g), and R_s is the specific sampling kinetic constant, known as sampling rate (L day^{-1}). R_s
278 changes from analyte to analyte and can be derived experimentally by laboratory or in-situ
279 calibrations (Di Carro et al., 2014; Morin et al., 2012).

280 In this work, to estimate the concentration of the contaminants in seawater, equation (1) was
281 employed to calculate C_w using the sampling rate of DPS reported in the literature obtained by
282 means of laboratory calibration experiments (Martínez Bueno et al., 2009). No sampling rate value
283 was found in the literature for NBBS; considering that the two analytes have similar $\log K_{o/w}$, the
284 evaluation of NBBS concentration in water was achieved using the same R_s value of DPS.

285 Results, expressed as the amount of each analyte per POCIS (ng) and as C_w (ng L^{-1}), corresponding
286 to the calculated TWA concentrations of the analytes in water, are reported in Table 4.

287 Both NBBS and DPS showed increasing TWA concentrations from the open sea (AB) to the coast,
288 being 6.9 and 6.2-fold higher in the harbor (SMH), respectively; Paraggi Buoy showed intermediate
289 concentrations for both chemicals. Moreover NBBS showed the highest concentrations in each site
290 (from 2.7 to 3.7-fold higher than DPS).

291 In general few data of the concentration of the two chemicals in seawater are available in the
292 literature, while more investigations for inland waters were carried out. However, it is interesting to
293 note that NBBS TWA concentration in SMH is comparable to the concentration (550 ng L^{-1}) found
294 in terrestrial water samples located in an industrial area (petrochemical plant and the fishery
295 factory) (Grigoriadou et al., 2008). The NBBS TWA concentration in AB is lower and present the
296 same order of magnitude reported for a marine sample in Aegean Sea (Grigoriadou et al., 2008),
297 and falls in the range of concentrations found in the Lippe river, Germany ($20\text{-}140 \text{ ng L}^{-1}$)
298 (Dsikowitzky et al., 2004), whose higher concentrations were ascribed to discharges of sewage
299 treatment plants.

300 NBBS was also found in some samples of the San Francisco Estuary with a mean concentration of
301 260 ng L^{-1} ; the maximum concentration occurred in South Bay (454 ng L^{-1}), the most impacted
302 region of the estuary with longest residence time, comparable to SMH (Oros et al., 2003).

303 As regards DPS, in the coastal area of Portofino Promontory its TWA concentrations outside the
304 harbor (PB and AB) fall in the range reported in the literature for urbanized Spanish and French
305 coastal areas (15.5–75.6 ng L⁻¹), highlighting the not negligible level of this compound (Martínez
306 Bueno et al., 2009; Martínez Bueno et al., 2016). DPS levels were higher than other detected
307 herbicides (such as simazine, atrazine, terbutryn). This result is not unexpected, since DPS is
308 widespread in the environment, enters into aquatic ecosystems through various sources and is not
309 strongly subjected to degradation (Wick and Gschwend, 1998). As regards the Santa Margherita
310 harbor (SMH), TWA concentration estimated in this work is higher than the maximum value
311 reported for the Spanish coast, confirming the higher impact of human activities on a confined area.
312 NBBS and DPS compounds can be used as anthropogenic markers to track pollution plumes
313 (Dsikowitzky et al., 2004), and in particular NBBS can be used as a trace for sewage effluents. The
314 Gulf of Tigullio area is characterized by a seasonal peak of tourism presence during the summer
315 magnifying the sewage loads (Parravicini et al., 2013) towards the treatment plants (not equipped
316 with chemical treatments at the time of this study - the outfalls are shown in Figure 1), explaining
317 the presence of NBBS in all the sites. Nevertheless, the widespread presence of NBBS can be
318 related also to other not punctual sources.

319 In general both chemicals showed that each site in the coastal area of Portofino Promontory is
320 impacted at various degrees. As expected, the highest impact occurs in the harbor, but significant
321 levels of NBBS and DPS were also found in the other two sites.

322 As described previously, the site AB is the closest to the Portofino MPA and relatively distant from
323 the possible continental inputs (rivers, outfalls, urbanized area), as highlighted by the results of the
324 multiparametric survey, showing physico-chemical features typically found in open-sea.
325 Nevertheless the estimated concentrations of the two analytes in seawater of this area were 79 and
326 29 ng L⁻¹ for NBBS and DPS, respectively. This result can be explained taking into account that
327 POCIS are integrative samplers, able to register all contamination events occurred during the
328 deployment period; oceanographic studies (Cattaneo-Vietti et al., 2015; Doglioli et al., 2004;
329 Locritani et al., 2011) demonstrated that the AB area is occasionally reached by surface currents
330 coming from the coastal area of the Gulf of Tigullio, which may cause not negligible input of
331 continental waters in the area. Moreover a rather high number of boats (ferries, recreational and
332 fishing boats) sails across the area in the summer season, in particular during weekends (Venturini
333 et al., 2016).

334 The observed attenuation gradient of analyte TWA concentrations can be attributed to a dynamic
335 dilution, as a result of the marine hydrodynamic conditions, depending on the different shelter
336 degree and depth of each site, together with the levels of the coastal and marine human activities.

337 The result obtained by POCIS monitoring is in agreement with the different water mass
338 characteristics of the three sites (i.e. the different degree in the continental inputs shown in
339 particular by salinity and CDOM) and the higher trophy in the harbor compared to the open sea
340 oligotrophy in summer season.

341 Therefore, the POCIS passive sampler approach allowed highlighting the same gradient among the
342 different stations suggested by the traditional spot investigation with a multiparametric probe. It
343 also underlined that anthropogenic compounds can reach the “pristine areas”, demonstrating the
344 usefulness of this kind of sampling approach for temporal and spatial monitoring of the anthropic
345 impact even in a complex coastal area. Moreover, POCIS has already demonstrated to be a useful
346 tool for detecting episodic or short-term pollution events (Mazzella et al., 2008), in particular when
347 the source of impact is not continuous in time and the traditional samplings (with low frequency)
348 can miss the events.

349

350 3.2 Hydrocarbons

351 As already mentioned in the previous paragraph, some of the peaks detected in the chromatograms
352 of the different samples were identified as hydrocarbons, with qualitative and quantitative
353 differences from site to site. For example, the chromatogram of ARION Buoy showed several
354 signals with high intensity at time intervals of about 0.9 min in the range between 18.1-26.0 min (as
355 shown in Figure 4). The spectrum of these peaks suggested the presence of the homologous series
356 of hydrocarbons at medium molecular weight. To confirm this evidence, a standard mixture
357 containing even hydrocarbons from C₁₀ to C₄₀ was analyzed: the chromatogram showed signals
358 with an average difference in retention time of about 1.8 min; in particular, C₂₀-C₃₀ peaks of the
359 standard solution were in the same range of AB (18.1-26.0 min), overlapping with every other peak
360 in the sample chromatogram. Therefore, in AB seawater both even and odd hydrocarbons from C₂₀
361 to C₃₀ were present. A similar behavior was observed for Santa Margherita Harbor. Chromatograms
362 like those obtained for AB and SMH show a trend comparable to the fingerprint of fuels; in
363 particular the peaks in the range C₂₀ - C₃₀ can be considered a marker of fuel oils (Bayona et al.,
364 2015), so the occurrence of these compounds in seawater is not surprising, especially in Santa
365 Margherita Harbor. On the other hand, the presence of hydrocarbons in the POCIS eluate can
366 appear unexpected. In fact, POCIS were designed to sequester rather polar organic chemicals, with
367 a logK_{o/w} lower than 4 (pharmaceuticals, polar pesticides, illicit drugs, etc.). Nonetheless, the
368 sorbent Oasis HLB, contained between the two membranes, is typically considered an universal
369 sorbent in environmental analysis due to its lack of selectivity, and for this reason has been used to
370 preconcentrate a wide variety of chemical classes from water (Andrade-Eiroa et al., 2016).

371 As already mentioned, the sampling site AB presents the lowest anthropic impact and for this
372 reason the samplers were deployed for 72 days; SMH is a much more impacted area and in this
373 case, the sampling period was shorter (22 days). POCIS sampling mechanism involves the presence
374 of a boundary layer on both sides of the membrane surface which affects the uptake of the analytes
375 by the sorbent phase. Our hypothesis is that for hydrophobic substances, the thickness of the
376 boundary layer is rather high, determining a low sampling rate value; nevertheless, POCIS can
377 accumulate hydrocarbons either when the concentration in water is relatively high, as expected in
378 SMH, or quite low, when long deployment periods are used (i.e. AB site).

379 No sampling rate for hydrocarbons is reported in the literature; Harman et al. calculated R_s values
380 for selected aquatic pollutants, including some hydrophobic compounds (Harman et al., 2008). In
381 particular, a sampling rate value of 0.024 L d^{-1} was found for pyrene ($\log K_{o/w}$ equal to 5.30):
382 considering that alkanes are even more hydrophobic than polycyclic aromatic hydrocarbons, their
383 sampling rate value is expected to be lower. For this reason, no quantitative estimation was given
384 for the chromatographic signals found in the POCIS extracts which were ascribed to alkanes from
385 C_{18} to C_{30} .

386
387

388 **Conclusions**

389 The passive sampling approach combined with untargeted analysis by means of GC-MS was
390 successfully employed in different sites of the Portofino promontory in the Ligurian coast. Two
391 compounds of anthropic origin, NBBS and DPS, were measured in all samples with TWA
392 concentrations in the range of 79-547 and 29-180 ng L^{-1} , respectively. These results are in
393 agreement with the water mass characteristics of the different stations (i.e. the diverse degree in the
394 continental inputs shown in particular by salinity and CDOM) obtained with a multiparametric
395 probe.

396 Among the different classes of identified compounds, hydrocarbons were present in all samples; in
397 particular, the pattern of the chromatographic signals in Arion Buoy and Santa Margherita Harbor
398 was ascribed to hydrocarbons from C_{20} to C_{30} , compatible with the fingerprint of fuel oils.

399 Results obtained with the present work confirm that POCIS represents a useful tool also for the
400 monitoring of low-impacted areas where long deployment time can reveal contaminants at low
401 background levels or released by episodic events.

402

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407 Entella river flow rate.

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558

Figure captions

Figure 1 - Portofino Promontory and Tigullio Gulf; location of the POCIS samplers (AB: Arion Buoy, PB: Paraggi Buoy, SMH: Santa Margherita Harbor), outlets of the sewage treatment plant, anthropic presence along the coast (solid lines) and main marine current (arrows).

Figure 2 - POCIS samplers in protective grids, before and after the deployment.

Figure 3 - Daily Entella River flow rate; period of POCIS deployment (in red PB; in green AB).

Figure 4 - Enlargement of the chromatogram obtained for the POCIS extract of Arion Buoy showing three extracted ions typical of hydrocarbon (m/z 57, 71 and 85).

Tables

Table 1. Oceanographic features in the three sites of POCIS sampler deposition

Table 2. DPS and NBBS chemical formulas and structures, CAS numbers, log K_{ow} and molecular weights

Table 3. Retention times, SIM ions (in bold the quantifier fragments), LOD and LOQ for the two analytes.

Table 4 . Amounts of analytes measured in the passive samplers expressed as ng (CsMs) and Time-weighted average concentration in seawater expressed as ng L⁻¹ (Cw) in the three sites.

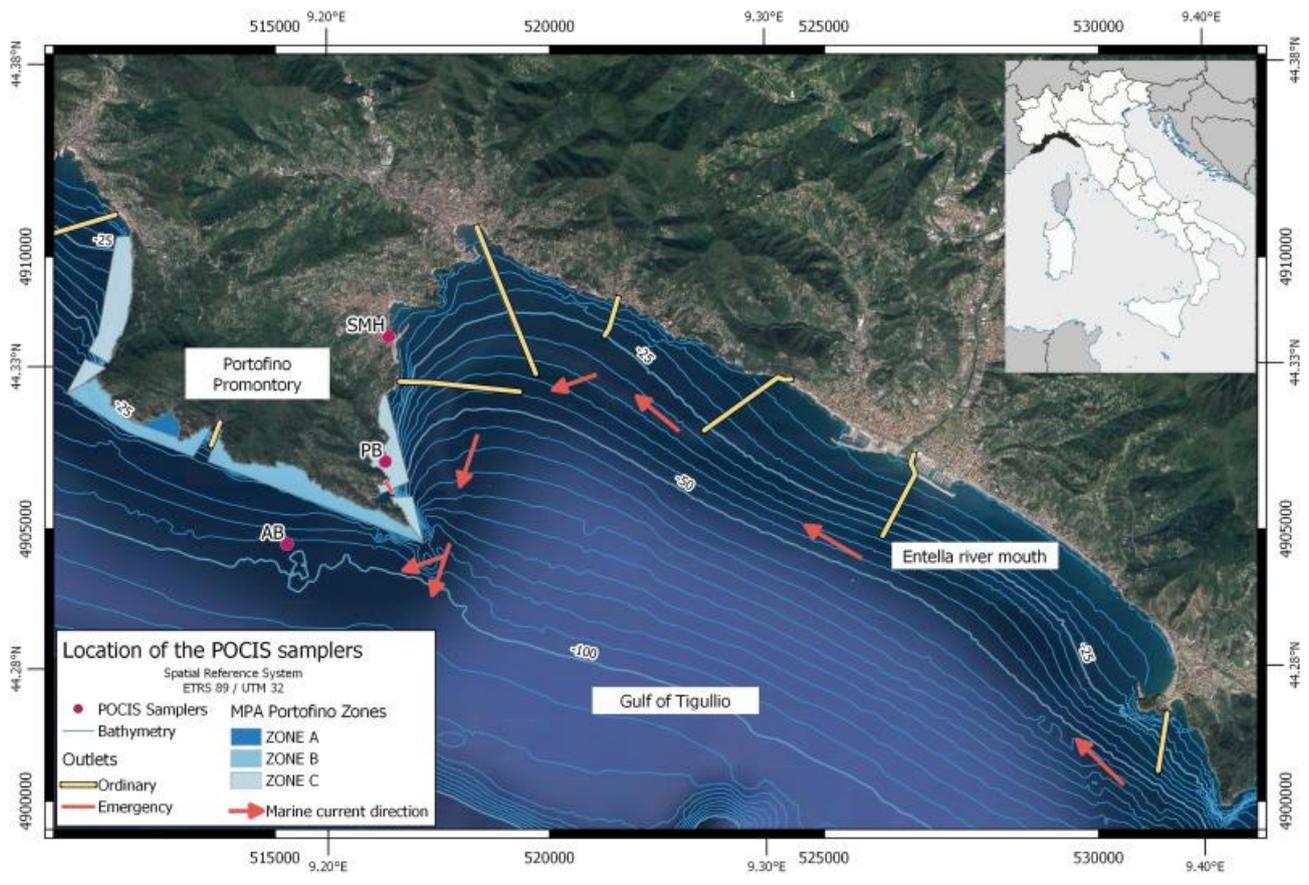


Figure 1



Figure 2

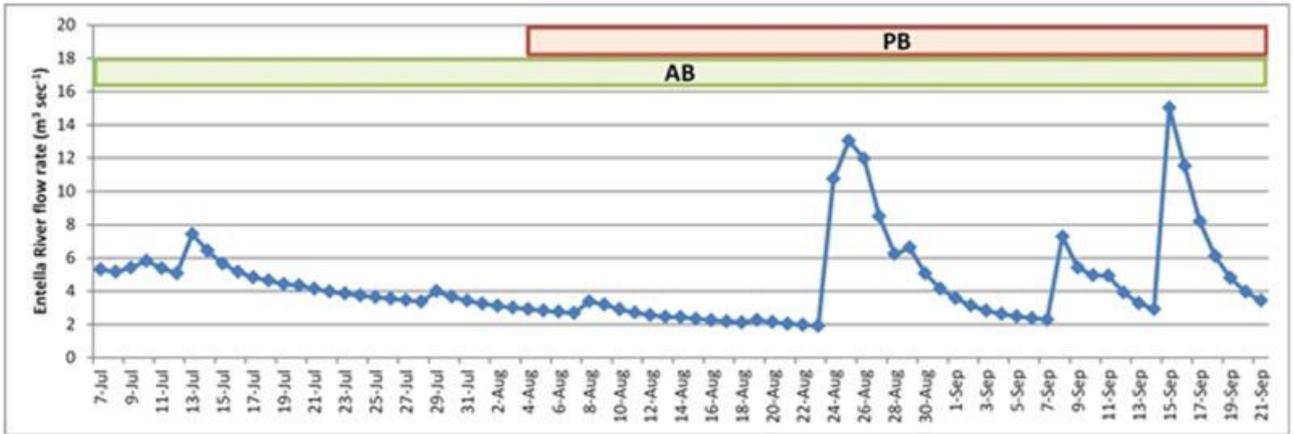


Figure 3

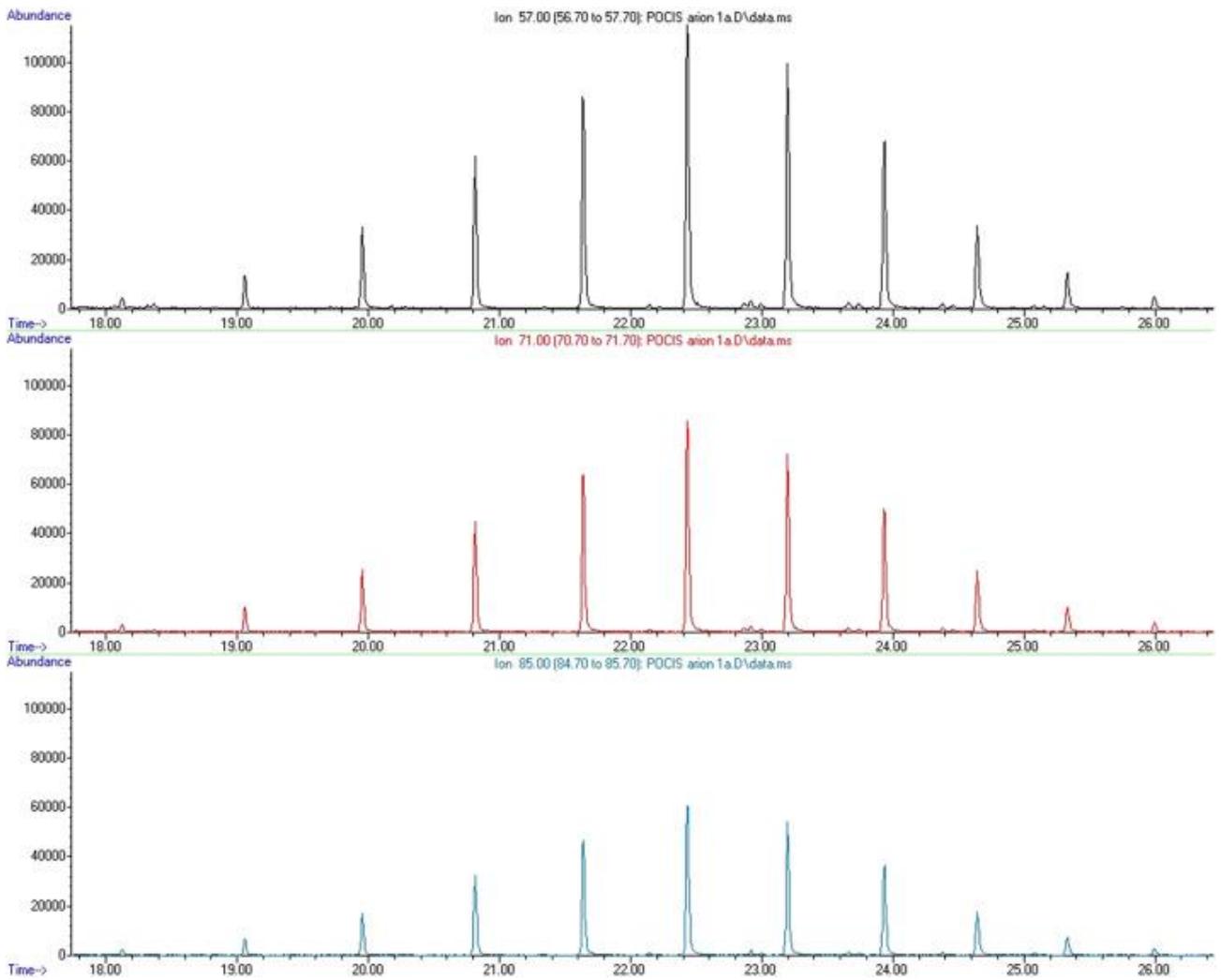
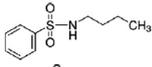
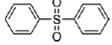


Figure 4

	S. Margherita Harbor SMH					Paraggi Buoy BP					ARION Buoy AB					
	Mean	Std. dev.	Min	Max	Median	Mean	Std. dev.	Min	Max	Median	Mean	Std. dev.	Min	Max	Median	
Temperature	25.14	1.87	22.74	28.11	24.98	24.42	1.69	22.62	27.59	24.19	24.52	1.76	22.74	27.26	23.89	
Salinity	37.92	0.05	37.74	38.08	37.91	37.98	0.07	37.82	38.13	37.94	38.04	0.04	37.92	38.14	38.02	
Oxygen	%	80.2	5.9	72.6	92.7	78.0	80.1	5.2	70.3	89.3	79.9	95.7	5.5	82.4	102.6	97.0
CDOM	$\mu\text{g QS/L}$	33.44	9.46	10.31	79.43	32.39	20.46	6.46	7.76	45.80	20.02	17.85	5.90	4.53	44.16	18.01
Tu	NTU	0.92	0.15	0.58	1.37	0.92	0.46	0.06	0.34	0.69	0.46	0.46	0.09	0.31	0.85	0.45
Chl	$\mu\text{g/L}$	1.21	0.85	0.40	3.23	0.93	0.26	0.03	0.20	0.36	0.27	0.19	0.03	0.12	0.24	0.19

Table 1

Compound	Empirical formula	Structure	CAS n.	Log $K_{o/w}$	Molecular weight (g mol^{-1})
NBBS	$\text{C}_{10}\text{H}_{15}\text{NO}_2\text{S}$		3622-84-2	2.1 ^a	213.295
DPS	$\text{C}_{12}\text{H}_{10}\text{O}_2\text{S}$		127-63-9	2.4 ^b	218.27

^a Proviron Fine Chemicals 2003.

^b Syracuse Research Corporation (2009).

Table 2

	RT (min.)	SIM ions (m/z)	LOD (mg L^{-1})	LOQ (mg L^{-1})
NBBS	16.59	141, 170	0.03	0.1
DPS	18.08	125, 218	0.03	0.1

Table 3

	NBBS		DPS	
	$C_s M_s$ (ng)	C_w (ng L^{-1}) ^a	$C_s M_s$ (ng)	C_w (ng L^{-1}) ^a
Arion Buoy (AB)	1860	79	700	29
Paraggi Buoy (PB)	3106	216	859	59
S. Margherita Harbor (SMH)	3841	547	1264	180

^a Values are calculated by means of Eq. (1) using $R_s = 0.319 \text{ L day}^{-1}$ (Martínez Bueno et al., 2009).

Table 4