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NON-TARGETED ANALYSIS BY DLLME-GC-MS FOR THE MONITORING OF POLLUTANTS IN THE MAR MENOR LAGOON

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ABSTRACT

Water from the Mar Menor lagoon (SE Spain) was analysed for the presence of organic pollutants resulting from agricultural and industrial practices, plastics and pharmaceutical products. The impact of rain on the input of 54 toxic chemical compounds and their distribution close to the shores of the Mar Menor lagoon was assessed in three sampling campaigns (a total of 42 samples). The combination of a miniaturised sample treatment based on dispersive liquid-liquid microextraction (DLLME) and gas chromatography-mass spectrometry (GC-MS) allowed the determination of the pollutants at low levels. Six deuterated compounds were used as internal standards for the quantification of different group of analytes. A total of nine targeted pollutants, dibutyl phthalate, diisobutyl phthalate, diethyl phthalate, bis(2-ethylhexyl) phthalate, anthracene, 1-methylnaphthalene, hexachlorocyclopentadiene, bis(2-ethylhexyl) adipate and oleamide were detected and quantified in the samples at concentrations that ranged from 3 to 264 µg L^{-1} for bis(2-ethylhexyl) phthalate and dibutyl phthalate, respectively.

On the other hand, the use of MS allowed the identification of nine putative compounds, (2,4-diterbutyl phenol, butyl citrate, triethyl phosphate, phthalic anhydride, ethyl oleate, erucamide, Irganox 1076, lidocaine, and oxandrolone) based on MS libraries. It was statistically shown that both targeted and untargeted

36 pollutants are affected by rainy conditions. However, there were no significant

37 differences in the compounds content concerning the sampling location.

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39 *Keywords:* organic pollutants, targeted analysis, untargeted analysis, seawater,

40 DLLME, GC-MS

1. Introduction

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43   One of the most critical environmental problems nowadays is the presence

44 of toxic compounds in aquatic environments because of their persistence, long-

45 range transportability and potentially negative effects on any form of aquatic life.

46 The input of pollutants into the aquatic systems occurs mainly through river

47 discharges, air deposition, wastewater effluents of industrial and urban origin,

48 groundwaters and surface overflows (Carratalá et al., 2017). The more important

49 anthropogenic sources of pollutants include waste from farmlands, pulp mills and

50 wood treatment facilities, accidental spillage during loading and transportation,

51 and leachates from waste sites and landfills (Baker, 2005; Yusof et al., 2009).

52 Unfortunately, conventional wastewater treatment practices face problems in

53 removing certain pollutants that may otherwise be released into rivers and the

54 sea (Happonen et al., 2016; Gómez-Gutiérrez et al., 2006; Steen et al., 2001).

55 Among the different organic pollutants, of particular concern are phenolic

56 compounds such as phenol itself, nitrophenols (NPs) and chlorophenols (CPs),

57 phthalate esters (PAEs), polychlorinated biphenyls (PCBs) and polycyclic

58 aromatic hydrocarbons (PAHs). CPs and NPs are important intermediates in the

59 production of pesticides, preservatives and dyes (Faludi et al., 2015). CPs are

60 toxic and carcinogenic compounds, which have been shown to bioaccumulate

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and be resistant to degradation, with negative effects on humans and animals (Xu et al., 2017). NPs, which are mainly used in the manufacture of paints, adhesives, explosives, pesticides and pharmaceutical products, are also toxic to humans, their effects including irritation of the eyes, skin and respiratory tract (Pastor-Belda et al., 2018). PAEs are a class of synthetic chemical compounds widely used as plasticisers in common household products, cosmetics, detergents, flame retardants, plastics, inks, adhesives and medical devices. Their entry into surface waters may come directly from the production of plastics or from leaching from PAEs contained in materials such as plastics and consumer products (Net et al., 2014).

This study focuses on an ecosystem of high biological and social value that has been severely degraded in the last decades, the Mar Menor lagoon (Murcia, SE Spain), which is one of the largest (135 km^2) Mediterranean coastal lagoons. The effects observed in the flora and fauna of the Mar Menor in recent years are signs of trophic changes and ecosystem deterioration. In 2001 it was officially acknowledged as susceptible area. Due to its ecological importance, several studies have been performed with the objective to improve the lagoon protection state (Bayo et al., 2019; Carratalá et al., 2017; Erena et al., 2019; Jiménez-Martínez et al., 2016). There have also been published several studies concerning the occurrence and evaluation of pesticides, endocrine-disrupting compounds (bisphenol A and alkylphenols), PAHs and pharmaceuticals in the Mar Menor lagoon and other areas around the Mediterranean Sea (Moreno-González et al., 2014; Salgueiro-González et al., 2019; Carratalá et al., 2017; Desbiolles et al., 2018; Gros et al., 2012).

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85 Liquid chromatography coupled to tandem mass spectrometry (LC-
86 MS/MS) (Gómez-Gutiérrez et al., 2006; Moreno-González, et al., 2015; Moreno-
87 González et al., 2014) and the combination of gas chromatography with mass
88 spectrometry (GC-MS) (Campillo et al., 2013; Moreno-González et al., 2013) are
89 the most common methodologies used for pollutant determination in waters.
90 Regarding sample preparation, different approaches have been applied, ranging
91 from conventional techniques such as liquid-liquid extraction (Haiba, 2009) and
92 solid-phase extraction (Moreno-González et al., 2014) to miniaturised techniques
93 like stir bar sorptive extraction (Campillo et al. 2013; León et al., 2018), solid-
94 phase microextraction (Huang et al., 2015; Zhang et al., 2017) and dispersive
95 liquid-liquid microextraction (DLLME) (Salgueiro-González et al., 2019; Chormey
96 et al., 2018; Wu et al., 2018). The use of techniques of sample preparation
97 applying liquid phase microextraction strategies in analytical chemistry is
98 continuously increasing due to its simplicity and economy in terms of time and
99 solvents used (Campillo et al., 2017).

100 This study evaluates the levels and distribution of a wide range of targeted
101 toxic compounds (a total of 54) from different chemical families and different
102 origins, including phenols, NPs, CPs, PAEs, PCBs, PAHs, aromatic, heterocyclic
103 and aliphatic compounds, in the Mar Menor lagoon in different rainy conditions
104 by means of GC-MS after a easy to apply preconcentration step using DLLME.
105 Considering the wide range of compounds studied, the selection of adequate
106 internal standards was considered important to achieve an accurate and precise
107 quantification of the different species. Moreover, untargeted pollutants were
108 identified in the studied samples, simultaneously with the targeted analysis, using
109 MS libraries (NIST and Willey).

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2. Materials and methods

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2.1. Materials and reagents

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The standard analyte mixture of 4-methylphenol, N-nitroso-di-n-propylamine, hexachloroethane, nitrobenzene, 3,5,5-trimethyl-2-cyclohexen-1-one, 2-nitrophenol, 2,4-dimethylphenol, bis(2-chloroethyl) ether, 2,4-dichlorophenol, 1,2,4-trichlorobenzene, naphthalene, 4-chloroaniline, hexachlorocyclopentadiene (ClCP), 4-chloro-3-methylphenol, 1-methylnaphthalene (1-MeNP), 2-methylnaphthalene (2-MeNP), cyclopentadiene, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, 1-chloronaphthalene, 3-nitroaniline, 1,4-dinitrobenzene, dimethyl phthalate, biphenylene, 1,2-dinitrobenzene, dibenzofuran, 2,4-dinitrotoluene, diethyl phthalate (DEP), fluorene, diphenylamine, azobenzene, bromophenoxy benzene, hexachlorobenzene, 2,3,4,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol, phenanthrene, anthracene, carbazole, diisobutyl phthalate (DIBP), di-n-butyl phthalate (DBP), fluoranthene, pyrene, bisphenol A, oleamide, benzyl butyl phthalate, bis(2-ethylhexyl) adipate (DHEA), benzo(a)anthracene, triphenylene, bis(2-ethylhexyl) phthalate (DHEP), di-n-octyl phthalate, benzo(a)pyrene, benzo(k)fluoranthene, benzo(g,h,i)perylene, and 1,2,7,8-dibenzophenanthrene at 1000 µg mL^{-1} in dichloromethane, as well as an internal standard (IS) mixture of 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12} at 4000 µg mL^{-1} were purchased from Restek

134   (Bellefonte, PA, USA). Working solutions at 1 µg mL^{-1} were prepared by dilution

135   of concentrated solutions in dichloromethane and kept at 4 °C in amber vials.

136   Organic solvents as methanol, acetonitrile, carbon tetrachloride,

137   dichloromethane and 1,1,2,2-tetrachloroethane (chromatographic grade) were

138   obtained from Sigma (Steinheim, Germany). Ethanol (96% v/v) was obtained

139   from Fisher Scientific (Waltham, Massachusetts, USA) and 37% v/v hydrochloric

140   acid from Panreac (Barcelona, Spain). A Milli-Q system (Millipore, Bedford, MA,

141   USA) was used for water purification. Potassium dibasic phosphate anhydrous

142   and sodium chloride purchased from Merck (Darmstadt, Germany) were used.

143   In order to avoid PAEs contamination, glassware materials were used in

144   the laboratory instead of plastic devices, being cleaned by rising with distilled

145   water and acetone. After that, the materials were dried in an oven at 60 °C. Blanks

146   were carried out between sample sets.

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148   *2.2. Apparatus and software*

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150   An Agilent 6890N gas chromatograph (Agilent, Waldbronn, Germany)

151   connected to an Agilent 5973 quadrupole mass spectrometer with an inert ion

152   source was used. Helium was used as mobile phase with a 1 mL min^{-1} constant

153   flow. Splitless injection (2 µL) was used. The samples were placed in 2 mL vials

154   with microinserts of 250 µL with polymeric feet. The analyses were performed

155   using an Agilent HP-5MS (5% diphenyl-95% dimethylpolysiloxane) capillary GC

156   column (30 m x 0.25 mm I.D., 0.25 µm film thickness). The GC initial temperature

157   was 50 °C and then it was increased to 175 °C (3 min), at 15 °C min^{-1}. Then, the

158   temperature was raised to 250 °C with a speed of 10°C min^{-1}. Finally, temperature

increased to 270 ºC at 20 ºC min^{-1}. The compounds were eluted with retention times ranging from 5.11 to 16.15 min (Table 1). The analysis time was around 20 min. The quadrupole, ion source and transfer line were set at temperatures of 150, 230 and 300 ºC, respectively. The electron-impact (EI) mode at 70 eV was used for the mass spectrometer. Chromatograms were obtained in full scan mode at m/z 35-500.

The identification of the compounds was carried out using the Wiley and NIST mass spectra libraries. The software programs used were the MSD Chemstation Data Analysis application, Version G1701EA, revision E.02.02.SP2, Minitab 19 and Simca-P 14.1 (Umetrics, Malmö, Sweden).

For the sample treatment, an LLG-uniTEXER vortex mixer (LLG-Labware, Constantí, Tarragona), an EBA 20 (Hettich, Tuttlingen, Germany) centrifuge and an IKA-KS-130-Basic orbital shaker (IKA, Staufen, Germany) were used. An AS220/C/2 balance from Radwag Wagi Elektroniczne (Radom, Poland) was used as well. Cellulose acetate filters (0.6 µm) were used for sample filtration (Filter-lab, Barcelona, Spain).

2.3. Sampling sites and sample collection

A total of 42 samples were obtained throughout three sampling campaigns. Samples were collected on 19 October 2019 (day 1, after a flood event), 22 November 2019 (day 2, rainy day) and 18 January 2020 (day 3, dry day) from various sites around the Mar Menor lagoon using 250 mL capacity low-density polyethylene bottles which were completely filled. The bottles were pre-rinsed with ultrapure water and the seawater sample before the sample collection.

184 The samples were collected at around 0.5 metres depth approximately 5 metres

185 from the shoreline. The rainfall measurements obtained for the five days

186 preceding each of the sampling times were 0.6, 5.2, and 0 mm for days 1, 2 and

187 3 (data obtained from the National Meteorology Agency, 2020). At each sampling

188 site, duplicate water samples were taken close to the same site. All the samples

189 were stored at 4 ºC before analysis, which was carried out normally within 48 h

190 following their arrival in the laboratory. Seven sampling sites were selected for

191 this study along the Mar Menor lagoon as shown in Fig. 1. Some of these

192 sampling locations have been described as potentially contaminated areas by

193 other authors (Salgueiro-González et al., 2019).

194   These sampling sites were selected to obtain information for the three

195 main areas into which the Mar Menor lagoon may be divided. The northern region

196 includes sampling points 1, 2 and 3, which are the nearest to the connexion area

197 with the Mediterranean Sea; the southern area (the most confined) which

198 includes sampling points 6 and 7; and the intermediate region (sampling points 4

199 and 5). In the intermediate region (a coastal resort town) there is a wastewater

200 treatment plant (WWTP), whose effluent discharges into the Mar Menor lagoon

201 through the Albují watercourse.

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203 *2.4. Analytical procedure*

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205   Seawater samples were filtered through 0.6 µm cellulose acetate filters.

206 For the DLLME preconcentration stage, 1 mL of ethanol (disperser solvent) and

207 125 µL of chloroform (extractant solvent) were mixed and injected quickly with a

208 microsyringe into a 10 mL sample of the seawater containing the internal

209 standard mixture at 50 µg L^{-1} previously placed in a 15 mL screw-cap glass

210 centrifuge tube with conical bottom. The cloudy solution resulting from the

211 dispersion of the fine organic droplets through the aqueous solution was

212 centrifuged for 3 min at 600 g for deposition of chloroform droplets. This solvent

213 was collected and positioned in a GC microvial insert and submitted to GC-MS.

214   The recovery study was performed using two seawater samples spiked

215 with the standards at two concentration levels (5 and 50 µg L^{-1}) and the internal

216 standard mixture at 50 µg L^{-1}.

217   The spiked samples were homogenised and left to rest for 1 h to allow the

218 analytes to disseminate regularly in the sample interacting with the matrix

219 substances, before conducting the analytical procedure.

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221 **3. Results and discussion**

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223 *3.1. Optimisation of chromatographic conditions*

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225   The separation parameters were optimized using a 1 µg mL^{-1} standard

226 mixture dissolved in chloroform, and injecting into the GC system a volume of 2

227 µL, which corresponded to the maximum allowed for this solvent. Several

228 temperature programs were studied, and the finally selected started at 50 ⁰C,

229 increasing to 175 ⁰C (3 min) at 15 ⁰C min^{-1}. A ramp of 10 ⁰C min^{-1} led the

230 temperature to 250 ⁰C, and finally to 270 ⁰C at 20 ⁰C min^{-1}, being the analysis

231 time 19.8 min. The carrier gas flow was assessed from 0.5 to the highest value

232 recommended by the manufacturer (1 mL min^{-1}). Best results were obtained with

233 a helium flow rate of 1 mL min^{-1}, allowing a good monitorization of the 54

234   pollutants in less than 20 min. The temperature of the injection port was varied in

235   the 240-300 ºC range, to achieve analyte vaporization preventing retention on the

236   glass liner. The analytical signals were greater at 280 ºC because higher

237   temperatures produce thermal degradation of some compounds.

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239   *3.2. Optimisation of DLLME conditions*

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241   The first DLLME step was to optimize the nature of the extractant solvent

242   by adding 150 µL of different extractant solvents to 10 mL of a spiked seawater

243   sample (100 µg L^{-1}) along with 1 mL of acetonitrile as disperser solvent.

244   Chloroform, carbon tetrachloride, dichloromethane and 1,1,2,2-tetrachloroethane

245   (with higher density than water) and 1-octanol (less dense than water) were

246   proved. Fig. S1 (**Supplementary Fig. S1**) shows the results obtained for eight

247   selected analytes representing the different chemical families. 1-Octanol, which

248   is the only tested extractant lighter than water, provided a low microextraction

249   efficiency. However, the chlorinated solvents permitted good extraction of the

250   analytes, and, as the extraction efficiency of chloroform was slightly higher than

251   that of carbon tetrachloride, it was selected.

252   Several solvents (acetonitrile, acetone and ethanol) which are all soluble

253   in the acceptor and donor phases were evaluated as disperser agents. The study

254   was carried out by using a 1 mL volume of the dispersant solvent and 150 µL of

255   chloroform. Ethanol was selected as the optimum dispersant agent since it gave

256   the best results in terms of sensitivity and cleanness of the enriched extracts.

257   To study the influence of the factors extractant and disperser volumes and

258   pH of the aqueous phase (each studied at three levels) on DLLME efficiency, a

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259 full factorial experimental design method was carried using a 10 mL of a spiked

260 seawater sample, at a concentration of 100 µg L^{-1} for each analyte. The volume

261 of the extractant and disperser solvents were varied in the 50-200 µL and 0.5-1.5

262 mL ranges, respectively and the effect of pH was studied between 3 and 8, for a

263 total of 10 runs. The variation in pH did not affect the response, while the volume

264 of the extractant and disperser solvents had a significant effect on the analyte

265 signals. Then, a response surface design was carried out to determine optimal

266 conditions. As shown in Fig. 2, the highest extraction efficiency expressed as the

267 total area of all the compounds was obtained with 1 mL of ethanol and 150 µL of

268 chloroform. As regards the pH, the best results were obtained when the pH was

269 left unmodified, corresponding therefore to that of the samples, which was around

270 6.

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272 *3.3. Validation of the method*

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274 Calibration curves were obtained for standard solutions prepared at six

275 concentrations, ranging from 2.5 to 400 µg L^{-1} (with the IS mixture at 50 µg L^{-1}),

276 by least-squares linear regression analysis of the analyte peak area ratio with

277 respect to IS area *versus* analyte concentration. Each IS was selected for the

278 quantification of chemical compounds showing similar retention times. In

279 addition, for use in the MS detector, the deuterated compounds were selected as

280 IS, which can be easily discriminated from the standards based on their mass

281 spectra. Therefore, the following six deuterated compounds were used. 1,4-

282 Dichlorobenzene-*d$_4$* (retention time (RT)= 5.20 min) was applied for the

283 quantification of the compounds with RTs up to 6.5 min. Naphthalene-*d$_8$* (RT=

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284   6.45 min) and acenaphtene-d_{10} (RT= 8.10 min) were applied for the quantification

285   of compounds eluting in the 6.5-8.13 and 8.2-9.68 min ranges, respectively.

286   Phenanthrene-d_{10} (RT= 9.60 min) and chysene-d_{12} (RT= 12.84 min) were added

287   for the quantification of compounds eluting from 9.7 to 12 min and from 12 to 14

288   min, respectively. Finally, perylene-d_{12} (RT=14.05 min) was used for the

289   compounds eluting above 14 min.

290   The standard additions method was applied to two different seawater

291   samples in order to evaluate any potential matrix effect. The slopes obtained by

292   the application of the standard addition method for the seawater samples and

293   those obtained bythe aqueous standard calibration graphs did not show

294   significant differences at 95% confidence level, as confirmed by an ANOVA test,

295   when the "p" values obtained were higher than 0.05 for all the compounds.

296   Therefore, quantification of the seawater samples was carried out against

297   aqueous standards.

298   The sensitivity of the method was evaluated from the limits of detection

299   (LODs) and quantification (LOQs), which were calculated using signal-to-noise

300   ratios (S/N) of 3 and 10, respectively. LODs ranged from 0.008 to 1.5 µg L^{-1} and

301   LOQs from 0.025 to 5.0 µg L^{-1} (Table 1). To evaluate the precision of the method,

302   seawater samples fortified at 50 µg L^{-1} with the analytes and the ISs were

303   submitted to ten consecutive DLLME with GC-MS analyses, which provided the

304   average relative standard deviation (RSD). The RSD values were between 5.0

305   and 12%, corresponding to fluoranthene and triphenylene, respectively

306   (**Supplementary Table S1**).

307   Since no reference materials were available, in order to evaluate the

308   accuracy of the method, experiments were carried out by fortifying two seawater

309   samples at two levels, 5 and 50 µg L^{-1}, for each compound and adding the IS

310   mixture at 50 µg L^{-1}. The recovery values for all pollutants were in the 94-105%

311   range, the average values being 97±1% (n=216) and 97±1% (n=216) for the

312   lowest and highest recovery levels, respectively.

313   Fig. 3 shows the chromatogram for a seawater sample fortified at 50 µg L^{-}

314   ^{1} for all the compounds using the optimised DLLME and GC-MS method.

315   As can be observed in the chromatogram, the first forty compounds eluted

316   between 5 and 10 min. The overlapping of certain peaks in the initial part of the

317   chromatogram (from 5 to 6 min) was solved by checking the characteristic *m/z*

318   ions of each analyte. From 10 min to the end, there was almost no overlapping

319   of the signals in the chromatogram.

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321   *3.4. Analysis of samples*

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323   *3.4.1. Targeted analysis*

324   The procedure was applied to the analysis of 42 seawater samples.

325   Identification of the fifty-four targeted compounds was based on their MS

326   spectrum libraries and retention times. The MS detector was worked in SCAN

327   mode to allow the simultaneous determination of targeted and untargeted

328   compounds, avoiding the need to carry out two different chromatographic runs.

329   The analytes were quantified using the ratio of the peak area of their

330   characteristic *m/z* ions (Table 1) and the peak area of their corresponding IS.

331   A total of nine targeted compounds were detected and quantified in the

332   lagoon samples: five PAEs (DBP, DEHA, DIBP, DEP, DEHP), two PAHs (1-

333   MeNP and anthracene), one halogenated aromatic compound (ClCP) and

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334   oleamide. The concentration of these targeted compounds ranged from 2.9 to

335   264 µg L^{-1} for DEHP and DBP, respectively, as shown in Table 2.

336   As can be observed in Fig. 4, of the nine targeted pollutants found in the

337   samples, the PAEs and oleamide were the most frequently found, with DEP and

338   DIBP present in all the samples. However, PAHs were found in low percentages

339   in positive samples (in a third of the total) from the study area.

340   Anthracene is included in the list of priority water pollutants by Directive

341   2008/105/EC of the European Parliament on environmental quality standards in

342   the field of water policy (European Commision, 2008). The maximum permitted

343   concentration for this compound in surface waters is 0.4 µg L^{-1}. The results

344   showed that this contaminant reached higher levels than those permitted in the

345   Mar Menor on the dry sampling day (day 3). The main sources of contamination

346   of this compound are industrial and urban activities, transport and chemical

347   products used in the maintenance of boats. PAHs have been detected at lower

348   levels (ng L^{-1}) by other authors in several Mar Menor water samples (Moreno-

349   González et al., 2013), while average anthracene concentrations were 3.5 ng L^{-1}

350   in waters sampled in 2010 by Campillo et al. (2013). A study carried out by Haiba

351   (2019) identified the presence of the 16 priority PAHs in the River Nile, where

352   they ranged from 14.8 to 648 ng L^{-1}.

353   The DBP levels observed in the samples on sampling day 1 (10.2-264 µg

354   L^{-1}) exceeded the environmental risk limit (10 µg L^{-1}) proposed by Van Wezel et

355   al. (2000). The higher concentration of this compound on sampling day 1 may be

356   have been due to lixiviation of this pollutant following a strong storm that had

357   occurred a few days before that sampling day. DBP, as well as the other PAEs

358   found in the samples, are mainly used as plastic additives, so that one of main

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sources of contamination of the Mar Menor would be plastic residues. Plastic contamination of the lagoon has previously been detected by Bayo et al. (2019), who quantified microplastics throughout the Mar Menor lagoon. For their part, Zhang et al. (2017) found PAEs in the Changjiang River Estuary, with DBP present in the 46.6-2412 ng L^{-1} range. Recently, four PAEs were detected in several aquatic environments in different countries, DEHP being present in the 0.02-28.4 µg L^{-1} range in some river waters of China (Heo et al. 2020).

3.4.2. Spatial distribution of targeted contaminants and effect of rain in different parts of the Mar Menor lagoon

The mean overall concentration of the targeted pollutants in the lagoon (seven sampling sites) on three different sampling days was statistically analysed using one-way ANOVA (**Supplementary Table S2**). The results showed that sampling day influenced the concentration of most of the organic pollutants ($p \leq$ 0.05) except for oleamide and DIBP. In addition, sampling day 3 was significantly different from sampling days 1 and 2 ($p \leq 0.05$). The lower concentration found on the rainy days (1 and 2) compared with those found on the dry sampling day (3), except in the case of DBP and oleamide, showed that pollutant concentrations are affected by rainfall.

However, the results indicated that sampling location does not affect the concentration of any compound in the lagoon ($p \geq 0.05$ in all cases).

Multivariate statistics using Principal Component Analysis (PCA) was also carried out on the raw data to evaluate the influence of co-variables on the observed levels and patterns of the contaminants.

383   Positions assigned in the PCA to each analysed pollutant along the two

384   main factor axes appear in Fig. 5 (t1 vs. t2), where it can be observed that

385   seawater from sampling day 3 (red) was clearly differentiated from the pollutant

386   concentrations on sampling days 1 and 2 (green and blue, respectively). The

387   location of sampling day 3 is due to the high levels of anthracene, DEHA, DEP,

388   1-MeNP and ClCP and low levels of oleamide and DBP. By contrast, the location

389   of sampling days 1 and 2 is due to the high levels of oleamide and DPB and low

390   levels of anthracene, DEHA, DEP, 1-MeNP and ClCP (**Supplementary Fig. S2**).

391   Other authors have evaluated the effect of seasonal inputs on the pollutant

392   concentrations in seawater samples, reaching several conclusions. For example,

393   Heo et al. (2020) indicated that PAEs concentrations in the seawater near

394   beaches are sensitive to rainfall events being the effect different depending on

395   each PAE analyte. Huang et al. (2015) found that the effect of rain on the PAH

396   concentrations in the Pearl River Estuary was of no significance due to the

397   different sources of this type of pollutant. It has been concluded that the input of

398   contaminants into the surface water environment depends on the

399   physicochemical characteristics of the environment (soils, pH, rainfall level) and

400   the pollutant properties. Therefore, it is difficult to generalise about the

401   conclusions (Heo et al., 2020; Moreno-González et al., 2013).

402   The spatial distribution of contaminants did not vary statistically between

403   different sites of the Mar Menor. However, the concentration of DBP, oleamide

404   and DIBP was higher in areas close to the WWTP (location 4) at the rainy day

405   (day 2). These results agree with the ANOVA statistical data shown in this section

406   (*3.4.2)* (**Supplementary Table S2**). Other authors have found that the distribution

407   of several PAEs in seawater near coastal areas was not homogeneous (Heo et

408   al., 2020).

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410   *3.4.3. Untargeted analysis. Spatial distribution and climate effect of putative*

411   *compounds along the Mar Menor watercourse*

412   A database including other potential contaminants based on the literature

413   was created (**Supplementary Table S3**). All these compounds were checked in

414   the studied seawater samples by reference to their characteristic *m/z* ions, using

415   the Wiley and NIST MS libraries for identification. A total of 9 putative compounds

416   were detected in several samples: triethyl phosphate (TEP), 2,4-ditertbutyl

417   phenol (DP), lidocaine, phthalic anhydride (PA), butyl citrate (BC), oxandrolone,

418   ethyl oleate, erucamide and Irganox 1076 (Table 3). Most of these compounds

419   are used as additives in the production of plastic devices (TEP, PA, BC, ethyl

420   oleate, erucamide and Irganox 1076) or their degradation compounds (DP).

421   These results showed plastic products to be one of the main sources of the

422   lagoon's pollution. On the other hand, lidocaine and oxandrolone are species

423   related to pharmaceutical products, which could reach the lagoon mainly in

424   wastewaters.

425   A similar statistical study was carried out to evaluate the effect of the

426   sampling location and rainy conditions on the untargeted pollutants. These

427   compounds were not quantified because of the lack of standards for them, so

428   peak area values were used as observational response for the multivariate

429   statistical evaluation.

430   The mean overall peak area for the untargeted pollutants for the different

431   points of the lagoon on the three sampling days was statistically analysed using

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432   one-way ANOVA (**Supplementary Table S4**). The results showed that the

433   sampling day influenced the concentration of most of the untargeted pollutants (p

434   ≤ 0.05) except BC, lidocaine and oxandrolone. By contrast, sampling location did

435   not affect the concentration of any compound in the lagoon ($p \geq 0.05$ in all cases).

436   Therefore, targeted and untargeted pollutants showed similar tendencies.

437   In the multivariate PCA analysis, as can be observed in Fig. 6, seawater

438   samples from sampling day 3 (red) were clearly differentiated from the those of

439   sampling days 1 and 2 (green and blue, respectively). The location of sampling

440   day 3 is due to the high levels of PA, ethyl oleate, and oxandrolone and low levels

441   of DP, erucamide, TEP and Irganox 1076 (**Supplementary Fig. S3**). By contrast,

442   most of the samples from sampling days 1 and 2 showed high levels of DP,

443   erucamide, TEP and Irganox 1076, and low levels of PA, ethyl oleate, and

444   oxandrolone.

445   As regards sampling location, no differences were observed among

446   seawater samples collected in the Mar Menor.

447

448   **4. Conclusions**

449

450   A sensitive DLLME with GC-MS method has been proposed for evaluation

451   of the effect of rain on the input of toxic chemical compounds and their distribution

452   in the coastal surface waters the Mar Menor lagoon. This allowed the

453   simultaneous quantification of 54 targeted pollutants (those expected to be

454   present in surface water) and the identification of untargeted species that may

455   also act as potential contaminants in surface water environments. The targeted

456   compounds were mainly PAEs and PAHs. Among the untargeted compounds,

19

the nine putative contaminants identified were DP, BC, TEP, PA, ethyl oleate, erucamide, Irganox 1076, lidocaine and oxandrolone. The statistical analyses carried out (ANOVA and PCA) showed that rainy conditions affect the concentration of pollutants at the different points of the lagoon sampled. However, the location of the different sampling points had no effect in this respect.

Declaration of Competing Interest

The authors declare that they have no conflict of interest. The manuscript was approved for publication by all the authors.

Acknowledgements

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Legends for the figures

Fig. 1. Map showing the sampling sites in the Mar Menor lagoon (1-7) and nearby areas. Image Source: Google maps

Fig. 2. Surface plot of the average area of the total compounds *versus* dispersant and extractant volumes.

Fig. 3. Chromatogram of a seawater sample spiked with the compounds and the internal standard mixture at 50 µg L^{-1}. The numbers on the peaks are related to the different analytes, as numbered in Table 1. The peaks named A (1,4-dichlorobenzene-d_4), B (naphthalene-d_8), C (acenaphtene-d_{10}), D (phenanthrene-d_{10}), E (chysene-d_{12}) and F (perylene-d_{12}) correspond to the internal standard compounds.

Fig. 4. Percentage of positive samples of the nine targeted compounds detected in the samples during the study period.

Fig. 5. Principal component analysis for the nine targeted pollutants defining absolute contaminant concentration at a given sampling site and in specific weather conditions. Cxy identifiers mean "x" sampling day (1-3) and "y" sampling location (1-7).

Fig. 6. Principal component analysis for the nine untargeted pollutants, defining contaminant peak area at a given sampling site and in specific weather conditions. Cxy identifiers mean "x" sampling day (1-3) and "y" sampling location (1-7).