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Occurrence, distribution and risk assessment of organic micropollutants in the Saronikos Gulf, Greece, utilizing LC-TIMS-HRMS

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This study investigates the occurrence, distribution and ecological risk of emerging contaminants (ECs) and priority pollutants (PPs) in seawater and sediments of the Saronikos Gulf and Elefsis Bay, Greece, an area continuously impacted by wastewater treatment plant (WWTP) effluents, industrial discharges and maritime traffic. Utilizing novel liquid chromatography tandem ion-mobility spectrometry and high-resolution mass spectrometry, the occurrence of more than 4000 LC-amenable organic micropollutants was investigated through wide-scope target and suspect screening. A total of 171 analytes were detected in marine samples, with pharmaceuticals identified as the most prevalent class (36% in seawater, 41% in sediments) followed by plant protection products (18% in seawater, 27% in sediments). Per- and polyfluoroalkyl substances (PFASs) were also detected in both matrices. Semi-polar ECs with higher molar mass were determined exclusively in sediments near WWTPs, possibly due to their high log_P values, reflecting their affinity for particulate matter. Additionally, the seawater circulation pattern was found to play a significant role in controlling the spatial distribution of ECs. Comparison with earlier studies in the area suggests a clear shift in pharmaceutical usage by the local population. Risk assessment, based on risk quotient calculations and environmental quality standards (EQSs) set by EU legislation, revealed that PFASs exceeded annual average environmental quality standard values in 92% of seawater samples, whereas 20 ECs in seawater and 12 in sediments exceeded predicted no-effect concentrations (PNECs), indicating potential adverse effects on marine biota.

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Environmental significance

This is the first study investigating the chemical imprint of human-related activities in the Saronikos Gulf, Greece, setting the baseline of chemical contamination of the study area, through novel high resolution mass spectrometric workflows. Detected substances were prioritized based on their persistent, bioaccumulative and toxic properties, as well as EU legislation values (where applicable). A total of 20 compounds presented high risk for studied seawater, while 12 compounds presented high risk for sediments. Among the latter, perfluorooctanesulfonic acid (PFOS) and di-(2-ethylhexyl)-phthalate (DEHP) exhibited the highest extent of predicted no-effect concentration (PNEC) exceedance in sediments. This is the first step towards unravelling the chemical imprint of major anthropogenic activities in the Eastern Mediterranean marine environment.

1. Introduction

Over the past few years, emerging contaminants (ECs) have attracted the wide interest of the scientific community, as well as national and international environmental agencies (US-EPA; EU-EEA), because of the possible risks they pose towards both human health and environmental ecological balance.^{1,2} Among

others, ECs include pharmaceutically active compounds (PhACs), personal care products, artificial sweeteners, industrial chemicals, per- and polyfluorinated alkyl substances (PFASs), plant protection products and surfactants, along with their transformation products (TPs).^{1,3,4}

Potential sources of ECs in the marine environment include a wide variety of human-related activities, such as landfill leachates, surface run-offs, atmospheric deposition, application of biosolids, metallurgy, distilleries, textile/paper/dye/pharmaceutical industries, manure of agricultural land and shipping activities. Moreover, small- and large-scale wastewater treatment plants (WWTPs) have been highlighted as major sources of ECs in the marine environment, since the complete

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removal of ECs even with tertiary treatment technologies is yet to be achieved. It is reported that in WWTPs, EC removal efficiency varies in the range of 20–50%, 30–70%, and, in some cases, >90% during the primary, secondary, and tertiary treatment steps, respectively, depending on their physicochemical properties and utilized treatment technologies.^{5,6} The main mechanisms for removal of ECs occurring during the secondary treatment at WWTPs are biological and/or chemical transformation and sorption. The most common employed processes in WWTPs are activated sludge (CAS) and membrane biological reactors (MBRs). The efficiency of a CAS system depends on the physicochemical characteristics of the substances and on the nature of the microbial community. MBRs emerged as an alternative to CAS, integrating aerobic biodegradation and membrane separation, modestly more efficient than CAS in the extent of removal of several CECs.⁵

Following the release of ECs in the marine environment, biotic and abiotic processes take place, leading to their degradation in seawater, sediments and biofilms, while their distribution depends on their varying physicochemical properties, such as water solubility, solid–liquid distribution, polarity, vapor pressure and environmental conditions like pH values, organic matter content and water temperature, leading to the formation of metabolites and TPs.⁷ These compounds can potentially pose even greater adverse effects than parent substances towards human health and marine environmental quality status.^{8,9}

Sediments constitute a significant long-term repository for moderate to high lipophilicity ECs in the marine environment considering their high $\log P$ values.¹⁰ Their accumulation in sediments may affect benthic communities, due to bioaccumulation and bio-transfer along the food chain.^{11,12} Sediment and benthic communities serve as comprehensive indicators of anthropogenic impacts in ecological and environmental status assessments, as outlined by EU directives (Marine Strategy Framework Directive – MSFD; Water Framework Directive – WFD).¹³ To that end, the environmental protection agency (EPA) of the United States has been recently collecting environmental monitoring data to develop exposure computation mechanisms, aiming to facilitate quicker strategy development and decision-making by regulatory authorities worldwide.⁴

Since 2015, the European Commission has established watch-lists of substances for Union-wide monitoring in the field of water policy regarding the occurrence of ECs in aquatic environments.^{14–16} These watch-lists are updated at least every two years, and recently, the EU Commission adopted a new watchlist of substances in surface waters (Decision EU 2025/439), suspected of posing a risk to the environment and human health.^{16,17} As of now, a total of 67 different chemicals have been included in watch-lists since 2015 (five different EU decisions, namely: 2015/495, 2018/840, 2020/1161, 2022/1307 and the latest, 2025/439). The latest update included 29 substances to be investigated in the field of water policy. Member states should monitor listed substances to provide data on their concentrations and environmental presence, thus helping regulators to determine whether these substances pose

a widespread threat. For substances posing such a risk, maximum allowable threshold values should be set, and measures should be taken to reduce or phase out their emissions. Thus, it is highly important for member states to monitor the occurrence of ECs in the marine environment. However, only a few related studies have been published to date, focusing on a limited number of compounds from specific chemical groups, like industrial chemicals and pharmaceuticals.^{18–21}

Following recent advances in high resolution mass spectrometry (HRMS), the interest of many scientific groups has shifted towards simultaneous determination of numerous ECs with various physicochemical properties, belonging to different chemical groups.^{22–24} Besides broadening the target group of ECs, HRMS provides the ability to perform suspect and non-target screening, further multiplying the number of ECs and their TPs that can be traced.^{25,26} To the best of our knowledge, published data on the occurrence of ECs in the Greek Seas are scarce, focusing either on a limited number of pharmaceuticals, along with their human metabolites and TPs in seawaters, *via* low resolution mass spectrometry (LRMS),^{27,28} and on the utilization of HRMS techniques on both seawaters and marine sediments.^{29,30}

2. Study aim

Herein, a wide-scope target screening of more than 2500 semi-polar to polar LC-amenable ECs from different chemical classes is conducted in the water column and sediments of the Saronikos Gulf and Elefsis Bay, Greece, by applying a novel analytical methodology utilizing liquid chromatography trapped ion mobility spectrometry tandem high-resolution mass spectrometry (LC-TIMS-HRMS). Additionally, a suspect screening was performed, covering a wide variety of surfactants, cleansers and antiseptic substances, some of which exhibit endocrine disrupting properties,³¹ further leveraging the capabilities of HRMS. These compounds cover a wide chemical domain with different physicochemical properties, such as molecular weight and polarity. The targeted ECs' occurrence and distribution patterns in the water column and sediments are studied in relation to anthropogenic activities, their physicochemical properties and the characteristics of the studied marine environment, facilitating the investigation of possible point sources and accumulation mechanisms. An ecological risk assessment was also conducted, aiming to prioritize detected chemicals risk-wise.

This study establishes a baseline assessment of the environmental quality of the Saronikos Gulf and Elefsis Bay regarding ECs, thus setting the baseline for future scientific research in the area. Additionally, it explores the connection between specific substances and wastewater treatment plant (WWTP) effluents. To support the discussion on EC sources, influent and effluent wastewater samples from the two major WWTPs operating in the region – Thriassio and Psyttalia – were also analyzed, simultaneously evaluating both the removal efficiencies and chemical impacts of these WWTPs on the studied marine environment.



3. Study area and sampling design

The Saronikos Gulf is located in the central Aegean Sea, northeastern Mediterranean Sea [Fig. 1]. With a coastline of ~ 270 km, a sea surface area of ~ 2866 km² and an average water depth of ~ 100 m, the gulf constitutes the coastal receptor area of the metropolitan city of Athens, capital city of Greece, with more than 5 000 000 inhabitants. The Saronikos Gulf is subjected to intense anthropogenic pressure from several point and non-point pollution sources. In detail, these include intense maritime traffic, industrial activities, oil refineries, marinas, touristic facilities, and the industrial zone of Elefsis Bay and Piraeus port (the largest port in Greece and one of the biggest in Europe), which affect the marine environment of the area *via* runoff and/or submarine groundwater discharges, as well as atmospheric deposition of ECs from adjacent urban areas^{32–34} [Fig. 1].

Most importantly, the inner Saronikos Gulf, at the northern part of its eastern basin, receives effluent wastewater from the second largest WWTP (Psyttalia island) in Europe and one of the biggest worldwide (capacity of 5 600 000 p.e. and flow of approximately 800 000 m³ per day; provision of 1 000 000 m³ per day).^{35,36} The Psyttalia WWTP carries out pre-treatment, primary and secondary treatment with advanced biological nitrogen removal, sludge treatment and cogeneration of electrical and thermal energy. Until 1994, sewage from Athens was discharged as untreated wastewater in the Saronikos Gulf, north of Psyttalia, directly affecting Elefsis Bay. During the period 1994–2004 the sewage was primarily treated, while from 2004 onwards, an advanced secondary sewage treatment plant has also been operating. The Psyttalia WWTP uses advanced secondary biological treatment based on the activated sludge process. The treated sewage effluent is dispersed into the Saronikos Gulf at a 63m depth, following the prevailing circulation, and is traced to the south, southeast and southwest of the sewage outfall,³⁷ below the pycnocline (extending between depths of ~ 40 and ~ 70 m) during the stratified period (May–November), or in the surface layer during the cold period (December–April). Lower dissolved oxygen (~ 3.00 mL L⁻¹) values were recorded near the bottom of the sampling stations located mainly southwest of Psyttalia. This decrease had been systematically observed in the past and is probably due to the oxidation of organic matter and has been associated with the treated wastewater field and its dispersion in the inner Saronikos Gulf depending on the prevailing circulation. The dissolved oxygen concentrations usually recorded in the study area during the winter-early March period are ~ 6.00 mL L⁻¹.^{13,38}

Elefsis Bay is an almost enclosed small and shallow embayment, with an area of 71.5 km² and an average water column depth of 33 m, which is connected to the Saronikos Gulf by two narrow and shallow channels [Fig. 1]. Elefsis Bay is differentiated from the Saronikos Gulf not only in its morphology, but also in the extent of the chemical pollution it receives. A significant number of industries (oil refineries, shipyards, chemical plants, food, metal and cement industries, *etc.*) are located along its northern coastline. Elefsis Bay is considered

a heavily impacted marine area, whereas the marine environment of the Saronikos Gulf is characterized as heavily (near the Psyttalia sewage outfall) to moderately polluted.¹³ The treated sewage of the Thriassio WWTP affects the eastern part of Elefsis Bay. The Thriassio WWTP has been operating since 2012 and treats wastewater of the surrounding municipalities (Aspropyrgos, Elefsis, Mandra and Magoula), as well as the surrounding industrial and craft areas (capacity of 117 000 p.e. and flow ~ 21 000 m³ per day). The Thriassio WWTP uses secondary biological treatment based on the activated sludge process.

In March 2023, the Hellenic Centre for Marine Research (HCMR) conducted a sampling campaign using the research vessel (R/V) “Aegaeo” in the wider Saronikos Gulf and Elefsis Bay. The campaign involved the collection of 13 seawater samples from various depths (surface, 50 m and near the bottom) and 4 surface sediment samples. Additionally, influent ($n = 2$) and corresponding effluent ($n = 2$) wastewater samples were obtained from both wastewater treatment plants (WWTPs) operating in the region (Thriassio and Psyttalia). The sampling followed the treatment stage timeframes at each facility, ensuring a 24-hour interval between influent and effluent collection for both WWTPs. Seawater samples were collected with 12 L Niskin bottles mounted on a rosette sampling system. In each case, 2.5 L were transferred to amber-glass bottles with Teflon-lined caps and stored in a fridge at 4 °C. Marine sediments were collected using a stainless-steel box corer with a surface area of 40 × 40 cm, wrapped in pre-combusted aluminum foil and stored in the freezer at -20 °C. Wastewater samples were received in 1 L HDPE bottles and immediately stored in the freezer at -20 °C. The sampling grid was designed primarily to assess the impact of wastewater treatment plants (WWTPs) on the marine environment. Additionally, other influencing factors, such as maritime traffic, industrial activities, and land-derived pressures, were also considered. The sampling stations were named in accordance with the National Monitoring Project for the implementation of the WFD in Greece. Sampling stations S7, S8 and S11 are positioned in the Saronikos Gulf [Fig. 1], S7 is located very close to the mouth of the pipe discharging from the Psyttalia WWTP, while stations S8 and S11 are located 4.0 nmi southwest and southeast of the WWTP. These two stations are also influenced by various anthropogenic pressures [Fig. 1]. Finally, station S1 is located at the north-eastern part of the Elefsis Bay and is affected by the Thriassio WWTP, as it is located ~ 1 nm from the sewage pipe. Stations S1-biol and S7-biol are located at the sewage pipes, receiving direct discharges from the Thriassio and Psyttalia WWTPs, respectively [Fig. 1]. Details regarding sampling stations and information for their physicochemical characteristics are presented in the SI (Section 1: Sampling stations) [Tables S1 & S2].

4. Materials and methods

4.1. Sample preparation

Upon reaching the lab, samples were immediately treated through generic sample preparation workflows, based on



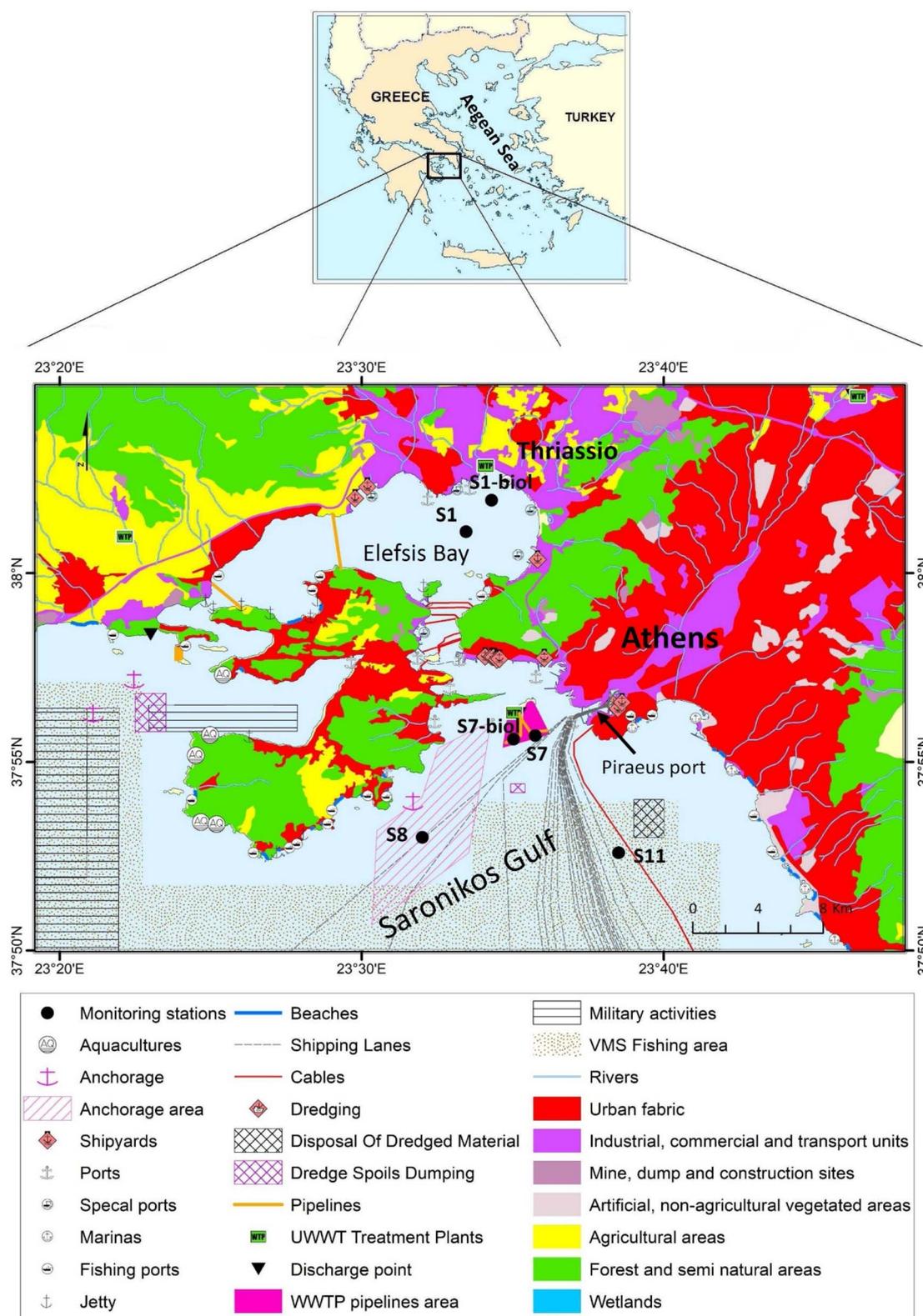


Fig. 1 Sampling stations in the Saronikos Gulf and Elefsis Bay, along with detailed anthropogenic pressures in the wider study area.

previously reported methods.^{28,29,39–42} These methods involve solid phase extraction (SPE) for seawater and wastewater samples, and an in-house developed ultrasonic assisted

extraction (UAE) method for sediments with minor modifications. The utilization of untargeted protocols aimed to extract as many LC-amenable, semi-polar to polar, thermally unstable,



organic ECs from a variety of chemical groups, along with their respective human metabolites and TPs. Studied chemical classes include coffee and tobacco related compounds, artificial sweeteners, illicit drugs, industrial chemicals, PFASs, personal care products, pharmaceuticals, plant protection products and surfactants.

In brief, pH of 1L seawater and 100 mL wastewater samples was initially adjusted to 6.5 ± 0.2 and then spiked with a mixture of deuterated internal standards (I.S.), recorded in the SI [Table S4]. Samples were then loaded onto a mixed-mode cartridge SPE system. Elution was performed with 6 mL of a basic solution, followed by 4 mL of an acidic solution. The eluents were evaporated to dryness under a gentle N_2 stream and reconstituted to a final volume of 200 μ L with a mixture of $H_2O/MeOH$ 50/50 v/v, thus attaining a 5000-fold pre-concentration factor for seawater samples and a 500-fold pre-concentration factor for wastewater samples.

Regarding marine sediment samples, 1 g of each lyophilized sample was initially transferred to a 15 mL polyethylene centrifuge tube and spiked with the same I.S. mixture as seawater samples. Afterwards, 3 mL of an extraction mixture, consisting of $H_2O/MeOH$ 50/50 v/v, 0.1% EDTA, and 0.5% formic acid, were added, and each tube was shaken by hand, then *via* vortexing, then inserted in a 50 °C ultrasonic bath for 15 min. Samples were centrifuged for 10 min at 4000 rpm, and supernatant liquids were collected in glass tubes. The whole process was repeated two more times until a final extract of 9 mL was collected for each sample. Extracts were again evaporated to dryness and reconstituted to a final volume of 200 μ L with a mixture of $H_2O/MeOH$ 50/50 v/v, thus attaining a 5-fold pre-concentration factor. In this calculation, we assumed that 1 g of sediment is equivalent to 1 mL of sample, considering the bulk density of sediments, which is close to 1 g mL^{-1} .

Further information regarding sample preparation procedures is presented in the SI (Section 2: Sample preparation).

4.2. Instrumentation

The separation of the analytes was carried out using an ultra-high performance liquid chromatograph (UHPLC) (Elute LC, Bruker, Bremen, Germany) with a $1.8 \mu\text{m}$ C18-2100 \times 2.1 column (Intensity Solo, Bruker, Bremen, Germany). A hybrid trapped ion mobility spectrometer (TIMS) coupled to a hybrid quadrupole time-of-flight mass spectrometer (QTOF-MS) was employed (timsTOF Pro 2, Bruker, Bremen, Germany). A novel ionization source, vacuum insulated probe heated electrospray ionization (VIP-HESI), was employed, aiming to enhance ionization efficiency without in-source fragmentation of thermally unstable analytes.

The HRMS system operated in both positive and negative ionization modes, producing data from data independent acquisition (DIA), in which extraction of both full scan MS and MS/MS, without pre-selection of ions, was conducted. This mode, also referred to as broad band Collision Induced Dissociation (bbCID), was utilized for quantification purposes. Autosampler injection volume was set at 1 μ L for positive and at 3 μ L for negative ionization mode, ensuring acceptable peak

intensity both for identified analytes' parent ions and their respective fragments. Samples were analyzed in TIMS-ON mode, making use of the instrumentation's added value.

4.3. QA/QC procedures

To ensure the instrumentation's proper function, a system suitability test (SST) was carried out at the beginning of the sequence. The aliquot contains a number of compounds representative of the database, which are eluted throughout the whole chromatogram, and its analysis provides data regarding elution status, retention time (RT) shift and mass error. A reference standard solution (STD) was analyzed throughout the sequence, followed by pure solvent aliquots. Analyses of said solutions facilitate the evaluation of the instrument's sensitivity and the assessment of possible carry-over effects, respectively. Compounds detected in the pure solvent mixture were not reported if their intensity surpassed 5% of the previous injection. Detailed information is available in the SI (Section 4.2. Instrumentation performance evaluation).

A field blank sample was generated by leaving a 2.5 L amber-glass bottle filled with Milli-Q water with the cap off during seawater sampling. For wastewater analysis, two 1 L HDPE bottles were filled with Milli-Q water as field blanks and placed close to the sewage pipes with the caps off during influent and effluent wastewater sampling, respectively. Three procedural blank samples were generated, one for each environmental matrix analyzed. As a seawater procedural blank, 1 L of Milli-Q water was utilized, 9 mL of pure extraction solvent mixture were used as a procedural blank for sediment analysis, while 100 mL of Milli-Q water were used as a procedural blank for wastewater analysis. Field and procedural blank samples were treated and analyzed with the same procedures and parameters as real samples. For quantification purposes, analytes with relative areas not exceeding 150% of either the blank sample's relative area were considered contaminations occurring during sampling and/or sample preparation, and thus not reported in this study.

Calibration of mass and mobility accuracy was performed before analysis. Sodium formate ion cluster masses produced in the ion source were measured and compared to theoretical values. Acceptable mass accuracy was considered only if experimental mass readings matched theoretical ones by more than 99%. After that, mass readings were corrected to match the theoretical ones. Regarding mobility calibration, CCS values of compound ions included in an IMS tuning mix were evaluated and accepted if experimental ion mobility readings matched theoretical ones by more than 99%. Again, ion mobility values, expressed as k_0^{-1} , were corrected to match theoretical ones.

Method accuracy was evaluated by calculating the recovery range for each tested matrix. To that end, matrix spiked samples were analyzed for 3 concentration levels, along with matrix-matched samples. All aliquots were also spiked with the I.S. mix to correct signal suppression or enhancement. For the seawater, sediment and wastewater matrices included herein, calculated recovery ranges were equal to 62–109%, 60–122% and 71–118%, respectively.



4.4. Data treatment parameters

All analytes determined through wide-scope target screening are included in the target screening database developed by the Laboratory of Analytical Chemistry of the NKUA.⁴³ Analytes from the surfactants' chemical group, which were determined through suspect screening, were included in a previously developed suspect screening dataset.^{44–46} Raw data were treated using the TASQ software (Bruker Daltonics). Strict identification criteria were applied to HRMS data, ensuring accuracy of analyte determination. These criteria include mass-to-charge ratio (m/z) accuracy, retention time shift, isotope profile matching, qualifier ion detection and CCS value matching. Detailed information is included in the SI (Section 5.1 Screening parameters).

4.5. Analyte quantification

For target screening quantification purposes, a 6-level spike curve was produced, and relative areas of analytes were utilized for concentration calculation, as explained in the SI (Section 5.3 Quantification procedure). Quantification of surfactant compounds included in the suspect list was realized by a semi-quantification procedure, based on available reference standards, and is further explained in the SI (Section 5.3 Quantification procedure). Consequently, signal-to-noise ratios (S/N) generated by the software were used to calculate the limits of detection (LOD) and quantification (LOQ), which correspond to S/N values equal to 3 and 10, respectively. Analyte concentrations not exceeding LOD values were not considered in quantification, while those with concentration levels between LOD and LOQ values were classified as “below quantification level (BQL)”. Statistical compromises were applied, in which BQL concentrations were replaced with LOQ/2 values, in the interest of calculating cumulative concentrations of chemical classes in each sample.

4.6. Risk assessment

To conduct a risk assessment of the study area, predicted no-effect concentrations (PNECs) in seawater and sediment samples were collected from the NORMAN database⁴⁷ [Table S5]. Risk quotient factors (RQFs) were calculated based on the following equation:

$$RQF = MEC/PNEC$$

MEC = measured concentration.

As MEC values, the average measured concentration was used to highlight the general risk factors, while maximum determined concentrations reflected the worst-case scenario. Compounds were consequently categorized based on calculated RQFs, as follows:

- (1) $RQF < 0.1$: low risk, exposure is unlikely to promote adverse effects.
- (2) $0.1 < RQF < 1$: moderate risk, signifying the need for further investigation.
- (3) $RQF > 1$: high risk, necessitating appropriate risk reduction measures.

5. Results and discussion

5.1. Chemical classification of the determined compounds

A total of 171 analytes were detected in marine samples of the Saronikos Gulf and Elefsis Bay areas, 128 of which were determined in seawater samples, while 70 were determined in sediment samples. Out of these substances, 101 were uniquely detected in seawater samples, 43 were uniquely detected in sediment samples, and 27 were distributed in both environmental compartments. Their occurrence, based on their chemical classification and sub-classification, is depicted in pie charts [Fig. 2A and B]. Regarding wastewater analysis, 189 compounds were determined in influents and 162 in effluents. Among them, 134 analytes were commonly detected both in influent and effluent wastewater, while 52 were uniquely determined in influents, and 25 were solely detected in effluents, possibly due to the transformation mechanisms of influent compounds during the cleanup process.⁴⁸ The occurrence of chemicals in wastewater samples is also presented in pie charts [Fig. 2C and D]. Detailed analysis results, regarding concentration levels of ECs, are presented in the SI [Tables S6 and S7].

In general, more polar ECs were present in aqueous matrices, in contrast to the sediment layer, which generally consists of semi-polar lipophilic ECs with higher $\log P$ values [Tables S5–S7]. A similar trend is observed regarding the molar mass of detected compounds, with low molar mass compounds being generally present in seawater and wastewater samples, while heavier ECs tend to precipitate in the sediment layer. The literature suggests that semi- to non-polar chemicals form aggregations with microplastics through sorption effects.⁴⁹ These aggregations contribute to the formation of a new sediment layer over a span of many years, further degrading sea bottom quality. A critical threat they pose is the recontamination of near-bottom seawater, as ECs are continuously freed from microplastics due to the latter's (bio)degradation and return to the aquatic or sediment compartments.

Regarding seawater samples, the largest proportion of detected compounds was pharmaceuticals (36%, $n = 46$) and plant protection products (18%, $n = 23$). It is noted that a total of 12 out of the 55 PFASs included in the wide-scope target screening database were determined (9% of total compounds). A total of 19 industrial chemicals (15%), 17 surfactants (13%), 6 coffee and tobacco related compounds (5%), 3 personal care products (2%) and 2 artificial sweeteners (2%) were also present in the study area during the sampling period [Fig. 2A and Table S6]. Comparing detected chemicals in different tested matrices, 58 compounds were simultaneously determined in wastewater effluents and seawater samples of the study area, reflecting the continuous encumbrance that local WWTPs impose on the marine environment [Fig. 3].

Regarding sediments, pharmaceuticals (41%, $n = 29$) and plant protection products (27%, $n = 19$) were the dominant chemical classes of determined analytes. A total of 9 PFASs (13%) were determined in the sampling area, along with 6 additional industrial chemicals (9%), 4 surfactants (6%), 2



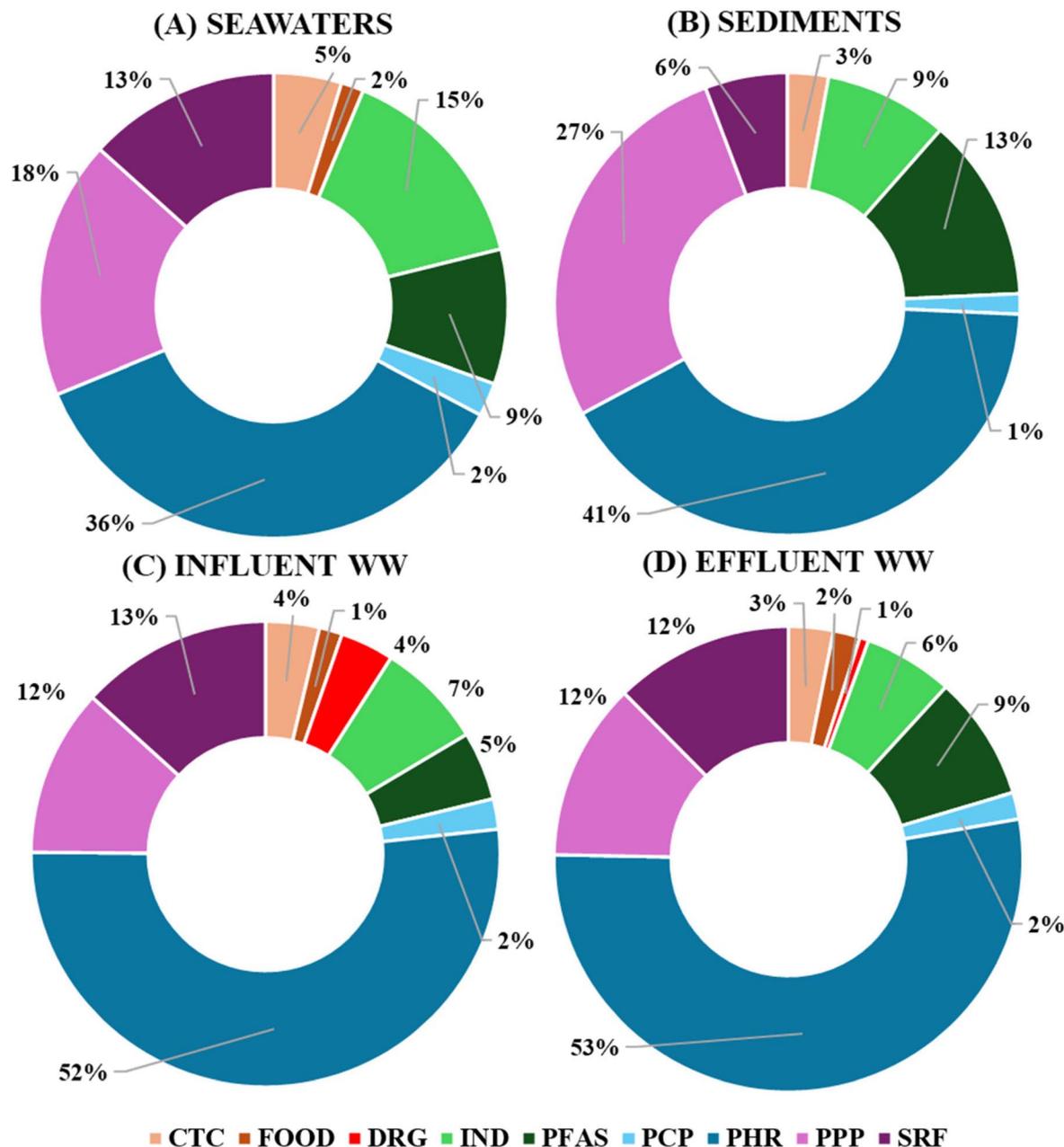


Fig. 2 Pie charts presenting the distribution of analytes per chemical class in tested matrices. Abbreviations: CTC: coffee & tobacco related compounds, FOOD: food additives, DRG: illicit drugs, IND: industrial chemicals, PFASs: per- and polyfluorinated alkyl substances, PCP: personal care products, PHR: pharmaceuticals & TPs, PPP: plant protection products & TPs, SRF: surfactants.

coffee and tobacco related compounds (3%), and 1 personal care product (1%) [Fig. 2B; Table S7]. A total of 14 compounds were simultaneously determined in wastewater effluents, seawater and sediment samples of the study area, reflecting the WWTPs' chronic chemical impact on the Saronikos Gulf and Elefsis Bay area. However, 28 substances were commonly determined in seawater and sediment samples, which could be attributed to different point sources of contamination, besides WWTP discharges [Fig. 3].

Wastewater analysis indicates similar trends, regarding determined compound classification between influents and

effluents. Specifically, proportions of detected substances in influent and effluent wastewater were equal to 52% ($n = 98$) and 53% ($n = 86$) for pharmaceuticals, 13% ($n = 25$) and 12% ($n = 20$) for surfactants, 12% ($n = 22$) and 12% ($n = 20$) for plant protection products, 7% ($n = 14$) and 6% ($n = 10$) for industrial chemicals, 5% ($n = 9$) and 9% ($n = 14$) for PFASs, 4% ($n = 7$) and 3% ($n = 5$) for coffee and tobacco related compounds, 4% ($n = 7$) and 1% ($n = 1$) for illicit drugs, 2% ($n = 4$) and 2% ($n = 3$) for personal care products, and 1% ($n = 3$) and 2% ($n = 3$) for artificial sweeteners, respectively [Fig. 2C and D and Table S8].



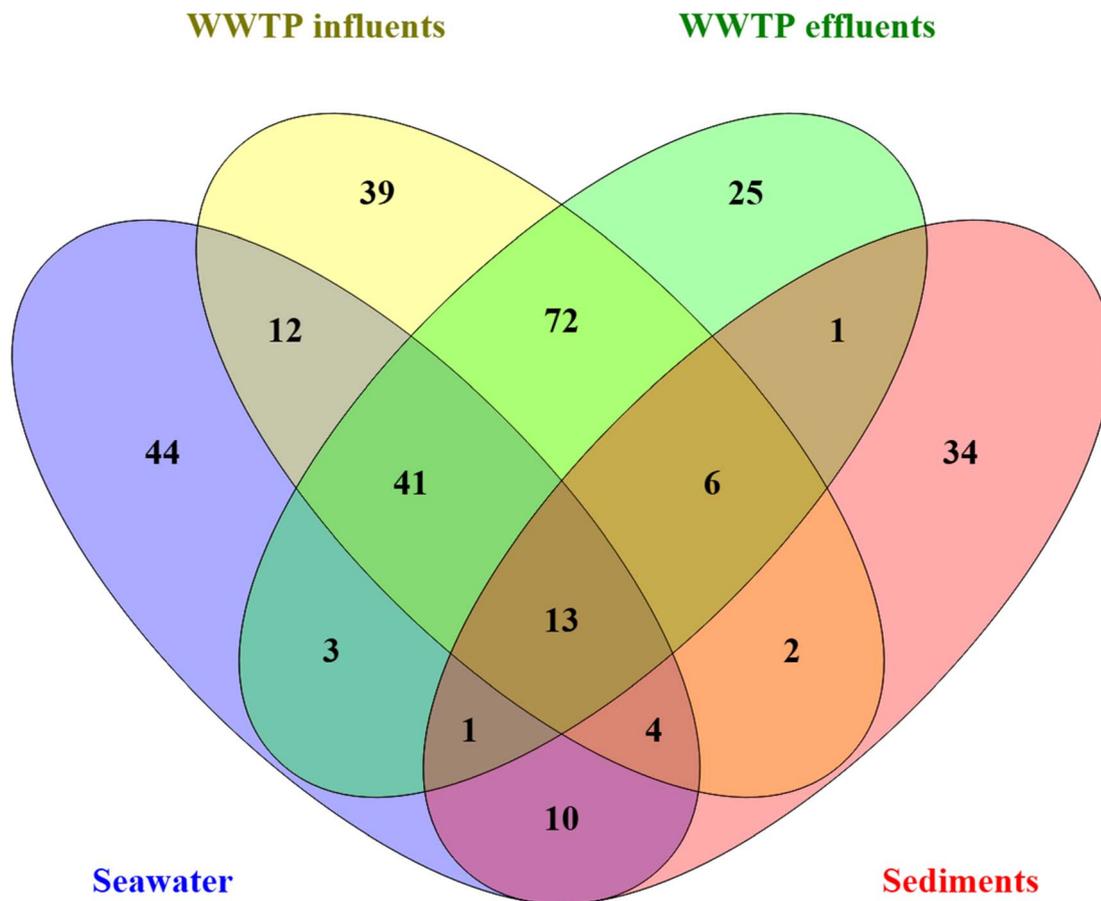


Fig. 3 Venn diagram of analytes determined in different tested matrices of the study area.

5.2. Occurrence and spatial distribution of ECs and PPs in seawater samples

Holistic results regarding seawater sample analysis can be found in the SI [Table S6]. Determined ECs and PPs per chemical class and sampling depth are visualized as a heatmap [Fig. 4]. The most chemically burdened seawater samples, in terms of cumulative concentration of compounds, were seawater collected at the sewage discharge points of the Thriassio (S1-biol) and Psyttalia (S7-biol) WWTPs. Specifically, 45 compounds amounted to a cumulative concentration of $13.8 \mu\text{g L}^{-1}$ in sampling station S1-biol, whereas 64 compounds amounted to a cumulative concentration of $12.0 \mu\text{g L}^{-1}$ in sampling station S7-biol. Subsequently, 47 analytes were detected in seawater at the surface (2 m) of station S1, with a cumulative concentration of $2.17 \mu\text{g L}^{-1}$. In seawater close to the surface (2 m) of station S7, a total of 42 compounds were detected in a cumulative concentration of 970 ng L^{-1} , while close to the bottom, 29 detected substances had a cumulative concentration of 846 ng L^{-1} . The sewage from the Psyttalia WWTP (station S7) outflows at a 63 m depth (the max depth of station S7 is $\sim 70 \text{ m}$) affecting the near-bottom layer and probably the sediment.⁵⁰ During March, prevailing hydrological conditions result in a homogenized water column, facilitating the dispersion of sewage from the Psyttalia and Thriassio WWTPs into the surface layer.⁴⁸ Although the prevailing

circulation in the inner Saronikos Gulf typically follows a southwestward direction from Phyttalia,^{35,36} surface seawater currents during the sampling days were directed southeastward (current speed: $2\text{--}12 \text{ cm s}^{-1}$). This suggests that ECs discharged from the Psyttalia WWTP would be more abundant at the surface layer of station S11, located southeast of the outfall, rather than at the surface layer of station S8, situated to the southwest. The current measurements (direction and speed) are further supported by the lower salinity and reduced% light transmission values recorded at station S11, confirming the dispersion of ECs southeast of Psyttalia [Fig. S1]. According to the results, the majority of the studied ECs were indeed dispersed toward the southeastern region of Psyttalia (Fig. 5) confirming the important role of hydrological conditions in controlling the spatial distribution of ECs in the study area. Moreover, SFCs were found at station S11, in higher concentrations than at station S7, probably indicating other sources than the WWTP sewage. Specifically, shipping and anthropogenic activities along the coastline appear to contribute to their presence in the study area. Some ECs depending on their characteristics (*e.g.* $\log P$) may be adsorbed onto particles and start sinking in deeper layers, following the prevailing circulation patterns in the near-bottom layer. ECs were distributed towards the southeast and southwest of Psyttalia at the deep water layers, as it was found that in this layer, the sewage plume



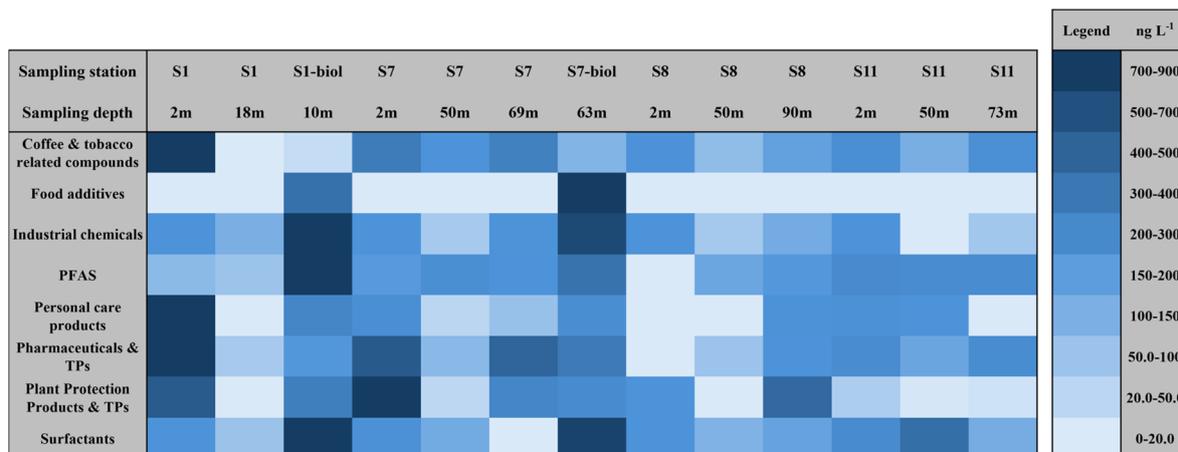


Fig. 4 Sampling station chemical encumbrance heatmap per chemical class of ECs and sampling depth for the considered seawater samples.

was dispersed towards both the southeast and southwest of Psytalia [Fig. S1]. The majority of ECs, especially those with relatively low log *P* values, appear at lower concentrations at the near-bottom layer compared to the surface. [Fig. 6].

The highest concentration levels regarding coffee and tobacco related compounds were determined in seawater close to the surface (2m) of stations S1 and S7. Stimulants caffeine

(85% FoD) and cotinine (77% FoD) were determined at concentrations ranging between 16.8 and 580 ng L⁻¹. Caffeine and its metabolites theobromine and theophylline were determined in influent wastewater from the Thriassio WWTP at 49.8, 29.5 and 8.14 μg L⁻¹, respectively. Consequently, caffeine was not detected in S1-biol seawater, while its concentration levels in S1 seawater were determined at 206 ng L⁻¹ (2 m) and

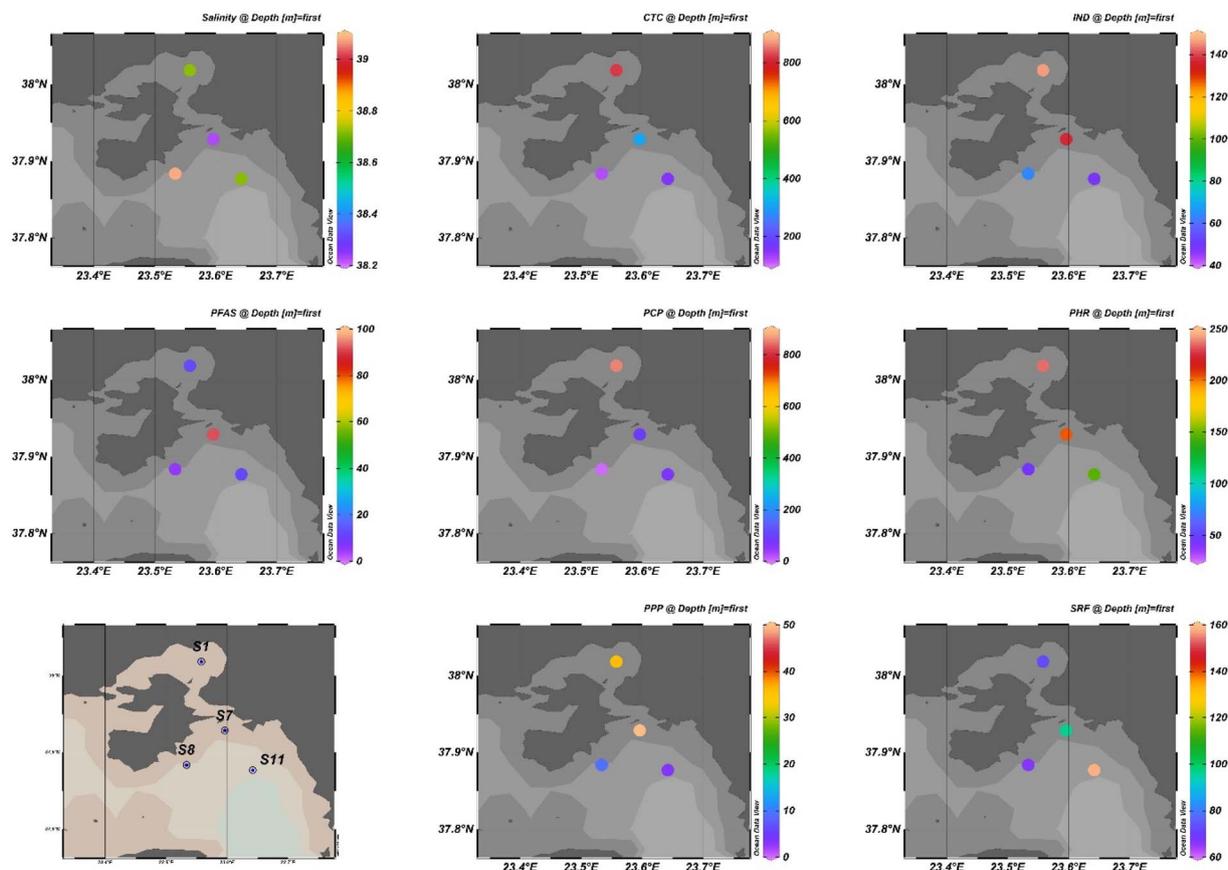


Fig. 5 Concentrations of the main ECs' categories at the surface layer of the study area, starting at a 2 m depth. Abbreviations: CTC: coffee and tobacco related compounds, IND: industrial chemicals, PFAS: per- and polyfluorinated alkyl substances, PCP: personal care products, PHR: pharmaceuticals & TPs, PPP: plant protection products & TPs, SRF: surfactants.



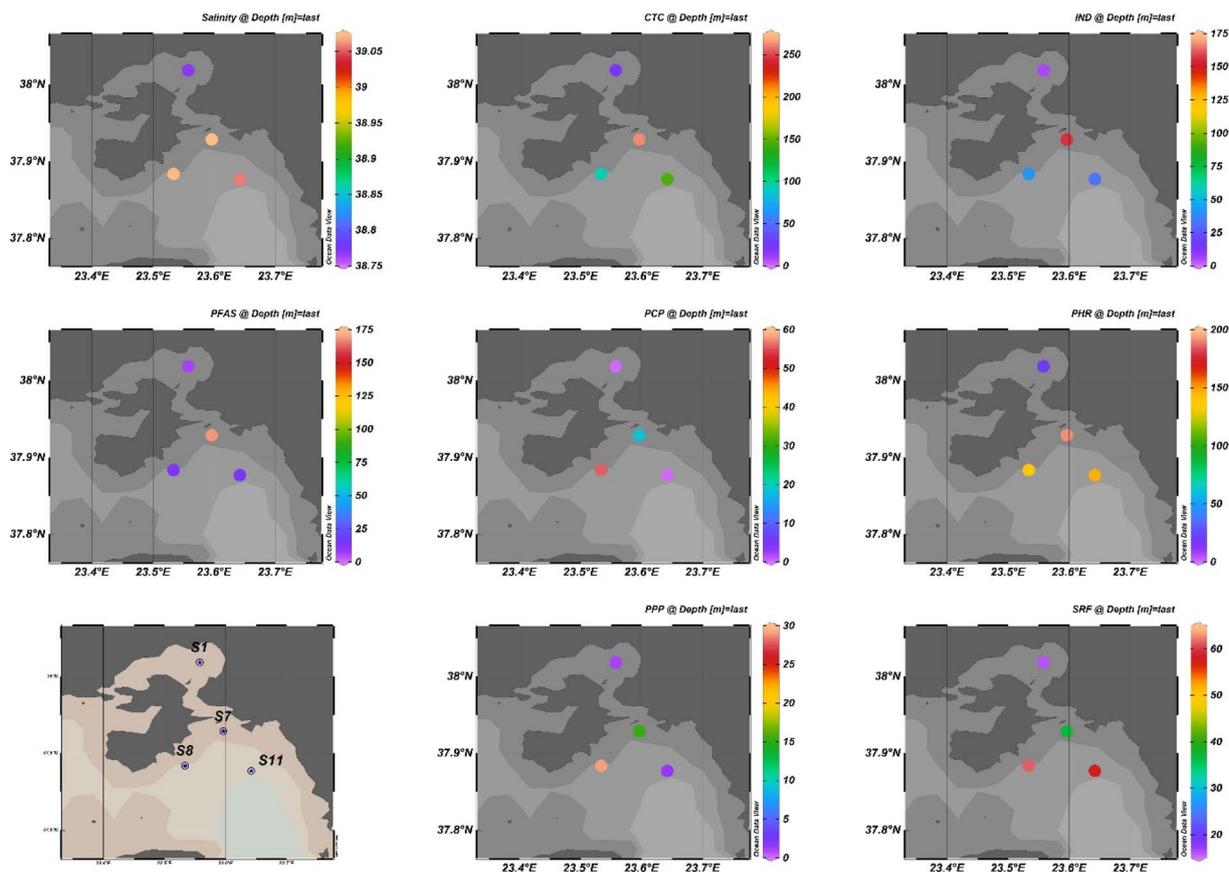


Fig. 6 Concentrations of the main EC categories at the near-bottom layer of the study area. Abbreviations: CTC: coffee & tobacco related compounds, IND: industrial chemicals, PFASs: per- and polyfluorinated alkyl substances, PCP: personal care products, PHR: pharmaceuticals & TPs, PPP: plant protection products & TPs, SRF: surfactants. Last depth = near-bottom depth of each station.

16.8 ng L⁻¹ (18 m). Theophylline was also detected in concentrations equal to 27.8 ng L⁻¹ (2 m) and 3.26 ng L⁻¹ (18 m). This could be an indication of water column contamination by external factors besides WWTP effluents, such as industrial area operations in the Elefsis Bay area. The presence of compounds linked to human habits like coffee consumption and smoking is likely indicative of the vast urbanization in the Attica region and have also been reported in the past in various aquifer datasets.^{51–54}

Corrosion inhibitors 5-methyl-benzotriazole (100% FoD) and mercaptobenzothiazole (85% FoD) were determined in concentrations ranging between 1.68 and 62.2 ng L⁻¹. These compounds are used in various industrial processes and their presence could be attributed to extensive urbanization,⁵⁵ as well as industrial activities and maritime traffic occurring in the Saronikos Gulf area. Concentration levels of 5-methyl-benzotriazole increased post-wastewater cleanup from 2.82 to 5.08 µg L⁻¹. In S1-biol seawater, it was determined in concentration levels equal to 3.40 ng L⁻¹, showing a significant reduction. However, it was determined in the surface layer (2 m) of sampling station S1 at 19.9 ng L⁻¹, while close to the bottom (18 m), a concentration of 1.68 ng L⁻¹ was measured. This increase could be due to maritime scrubber water and industrial runoffs in the wider area of Elefsis Bay, where the largest

shipyard in Greece operates, and also attests to additional point sources besides wastewater inputs. This compound has also been detected in corresponding sediments of sampling station S1, in a concentration of 0.877 µg per kg d.w. Its determination is possibly due to its lipophilicity (log *P* equal to 1.40) [Table S5] and continuous accumulation in the sediment layer of Elefsis Bay.

A total of 12 PFASs were detected in the tested seawater samples. Legacy PFAS perfluorooctanesulfonic acid (PFOS) was determined at concentration levels ranging between 1.07 and 18.4 ng L⁻¹ in the study area, in comparable levels to similar aquifers.^{56,57} Next generation PFASs were also detected throughout the sampling area, like 4,8-dioxa-3*H*-perfluorononanoic acid (ADONA), which was determined in concentration levels ranging between 2.82 and 160 ng L⁻¹. Its concentration levels close to the sea bottom (69 m) of station S7 were equal to 160 ng L⁻¹, while near the surface (2 m), it was determined at 83.5 ng L⁻¹. This could be due to its lipophilicity (log *P* value equal to 4.10), leading to sorption on suspended organic material and removal from the aqueous seawater compartment. During wastewater cleanup, longer carbon chain PFAS concentrations are generally reduced, while concentrations of smaller carbon chain PFASs are increased. For example, concentration values of PFOS and 6 : 2 fluorotelomer sulfonic



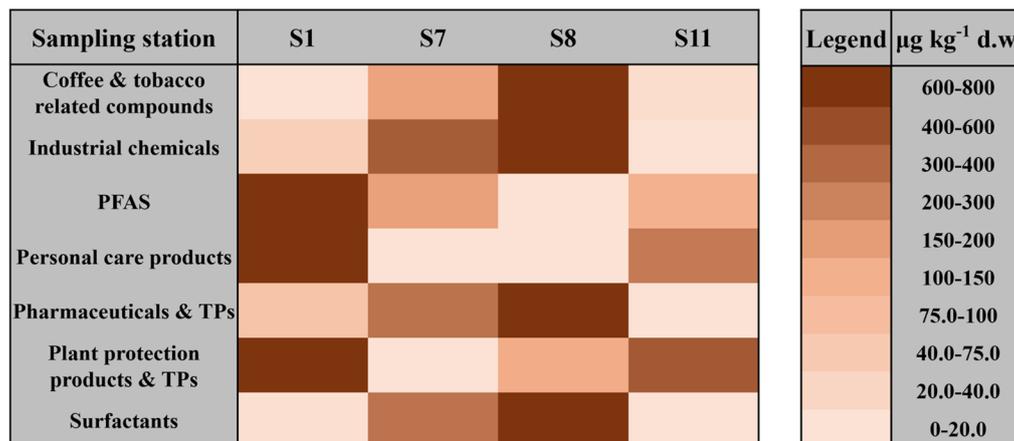


Fig. 7 Sampling station chemical encumbrance heatmap per chemical class of ECs for the considered sediment samples.

acid (6 : 2 FTS) are reduced from 375 and 108 ng L⁻¹ to 133 and 58.7 ng L⁻¹, respectively, between influent and effluent wastewater. However, perfluorobutanesulfonic acid (PFBS), perfluoropentanoic acid (PFPeA) and perfluoropentanesulfonic acid (PFPeS) concentrations increased from <LOD values to 1.63, 0.548 and 0.721 ng L⁻¹, respectively. This could be due to PFAS carbon chains breaking down during the cleanup processes. Regarding PFOS, its concentration in S1-biol seawater was calculated to be equal to 18.4 ng L⁻¹ which consequently reduced to 0.561 ng L⁻¹ in seawater close to the surface (2 m) of station S1. In particular, 6 : 2 FTS concentration continuously decreased between the S1-biol seawater and benthic seawater (18 m) of station S1, where it was determined to be 25.5 and 0.321 ng L⁻¹, respectively, whereas its concentration close to the surface (2 m) was not determined above LOD values. It seems that the only source of these PFASs in the marine environment of Elefsis Bay is effluent wastewater discharges. The difference in calculated concentrations between legacy and next generation PFASs in the seawater column could be attributed to the efficient removal rates of longer chain PFASs by WWTPs, as well as the possible breakdown of the carbon chain due to degradation occurring during the wastewater cleanup process.⁵⁸⁻⁶⁰

Antidiabetic medication metformin and antiepileptics pregabalin and carbamazepine, along with the latter's human consumption metabolite, 10-hydroxy-carbamazepine, were simultaneously determined in stations S1 and S7, at concentration levels ranging between 0.235 and 78.8 ng L⁻¹. These sampling points continuously receive effluent wastewater from the Thriassio and Psyttalia WWTPs, in which aforementioned pharmaceuticals have been previously reported,^{6,42} as also confirmed by this study. Interestingly, benzodiazepines bromazepam, citalopram and the latter's metabolite, norcitalopram, along with the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen, ketoprofen and salicylic acid, were determined in stations S1 and S7 at concentration levels ranging between 0.363 and 60.1 ng L⁻¹, and 2.18 and 76.3 ng L⁻¹, respectively. The occurrence of benzodiazepines in the marine environment could be likely attributed to their low rate of

metabolism,^{6,61} as well as their ineffective removal from the wastewater stream by WWTPs, while the presence of NSAIDs could be linked to different point sources, due to their effective removal ratio by WWTPs reported in this study [Table S8].

Regarding PPPs, a total of 23 substances were detected throughout the sampling area. Substances metolachlor, pentachlorophenol (PCP) and fluometuron were determined in the study area in detection frequencies equaling 100%, 62% and 54%, respectively. Their concentration levels ranged between 0.132 and 35.2 ng L⁻¹ in the sampling area. Fungicide azoxystrobin was also determined in seawater samples with 23% FoD and its highest measured concentration was determined in seawater close to the surface (2 m) of station S11, equal to 1.96 ng L⁻¹. Azoxystrobin was determined in the Psyttalia WWTP influent wastewater at 3.56 ng L⁻¹, while in effluents, its concentration was calculated at 2.64 ng L⁻¹. In seawater from sampling station S7 (50 m) its concentration was determined at 1.00 ng L⁻¹, while interestingly sediment samples from sampling station S8 contained azoxystrobin in concentration values equal to 0.806 µg per kg d.w., reflecting its continuous accumulation on the sediment layer at a short distance from its main point source, possibly due to sea currents and circulation patterns occurring in the Saronikos Gulf. The occurrence of these substances is linked to agricultural activities and has been previously reported in other studied marine substrates.^{62,63}

5.3. Occurrence and spatial distribution of ECs and PPs in sediment samples

The full results from sediment sample analysis are presented in the SI [Table S6]. Determined ECs and PPs per chemical class and sampling depth are visualized as a heatmap [Fig. 7]. The most chemically burdened sediments in the sampling area, in terms of cumulative concentration of ECs, were in station S8, where 43 analytes were detected in a total concentration of 1360 µg per kg d.w. and station S7, where 24 analytes were detected in a total concentration of 675 µg per kg d.w. Sediments from station S1 contained 42 compounds in a cumulative concentration of 281 µg per kg d.w., while in station S11, a total of 26



compounds were determined at 90.8 μg per kg d.w. This feature coincides with the consistently lower dissolved oxygen concentrations recorded near the bottom of station S8,^{37,38} and has been linked to the presence and dispersion of the treated wastewater plume in the inner Saronikos Gulf, southwest of Psyttalia, following the prevailing circulation pattern. To this end, the sediments serve as indicators of the long-term environmental condition, rather than capturing transient or short-term fluctuations, and reveal the link of their quality with the hydrographic characteristics of the marine environment.

The highest concentration levels of the stimulant nicotine and its metabolites anabasine and cotinine were determined at sampling station S8, with levels ranging between 12.6 μg per kg d.w. and 578 μg per kg d.w., indicating the extensive inhabitation of the surrounding Saronikos Gulf area. These substances were also found in the sediment of station S7, but at concentrations lower by an order of magnitude compared to those in S8, suggesting that the prevailing seawater circulation during recent years may have transferred Psyttalia WWTP effluents toward the seabed at station S8. This hypothesis is further supported by the presence of the four other most abundant ECs in sampling station S8 sediments, namely gabapentin (680 μg per kg d.w.), valsartan (97.5 μg per kg d.w.), apophedrin (71.9 μg per kg d.w.) and venlafaxine (67.5 μg per kg d.w.), which are closely associated with wastewater discharges, as confirmed by this study's wastewater analysis. The relatively higher concentrations of ECs linked to WWTP discharges in the analyzed sediments underscore the long-term chemical accumulation of these substances at the seabed, contributing to its ongoing quality degradation.

Corrosion inhibitors 5-methyl-benzotriazole and mercapto-benzothiazole were both determined with a frequency of detection surpassing 50% in the sampling area, at concentration levels ranging between 0.281 and 1.84 μg per kg d.w. Both compounds were determined simultaneously in sediments from stations S1, where the industrial area of Elefsis Bay is located, and S11, where maritime traffic occurs, due to the Piraeus port operations. Plasticizers di-(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP) and dimethyl phthalate (DMP) were identified with a detection frequency of 100% for DEHP and 50% for both DEP and DMP. Their cumulative concentrations ranged between 9.65 μg per kg d.w. in sampling station S11, where maritime traffic is frequent, and 84.8 μg per kg d.w. in sampling station S8, close to the Psyttalia WWTP. The passenger ship traffic in this area is very intense, considering that Piraeus port is one of the largest in the Mediterranean, and also that the biggest shipyard of Greece operates in Elefsis Bay. Reported plasticizer DEHP is regulated under EU legislation for its seawater presence,^{64,65} but limitations are yet to be imposed for its existence in sediment samples.

A total of 9 PFASs were detected in sediment samples throughout the sampling area. Legacy PFASs like PFOS and PFOA were determined at concentrations ranging between 1.19 and 7.85 μg per kg d.w. in the sampling area. These PFASs are not completely removed from the wastewater stream during cleanup, as proved by wastewater and the seawater analysis close to the WWTP pipelines, and thus the determined

quantities in the sediment layer could be attributed to their continuous discharge by local wastewater treatment facilities, as well as diffuse sources, as has been previously reported in the literature.⁵⁷ Their persistent and toxic nature has led to their gradual replacement by next generation PFASs. Perfluorobutanoic acid (PFBA) was determined to have the highest concentration among PFASs in tested sediment samples, ranging between 27.8 and 28.4 μg per kg d.w. Interestingly, perfluorohexadecanoic acid (PFHxDA), the longest carbon chain PFAS determined in this study, was solely detected in sediment samples. Its unique presence in the sediment layer could be due to its high lipophilicity ($\log P$ value equal to 10.3), leading to its sorption to suspended material and its removal from the aqueous seawater compartment [Table S5].

Antibiotics sulfadiazine and sulfisoxazole from the sulfonamide sub-class were determined at concentration levels equal to 0.378 and 0.293 μg per kg d.w., respectively, at station S8. On the other hand, antibiotics cinoxacin and norfloxacin, from the quinolone sub-class were determined in concentration levels between 0.126 and 7.65 μg per kg d.w. in all tested sediment samples. Most sulfonamide antibiotics have been replaced in terms of their prescription pattern by new quinolone antibiotics, while the ones that are still in use are being mainly administered as veterinary medications (see Section 5.4: Evidence of shift in pharmaceutical accumulation patterns). It is reported that sulfonamide sulfadiazine is currently used as an antimicrobial agent in aquacultures and not prescribed for humans anymore.^{66,67} Antihypertensive drug valsartan, which is among the most prescribed blood pressure regulators, was determined in 75% of tested sediments. In sampling stations S7 and S8, this substance was determined at concentration levels equal to 69.2 and 97.5 μg per kg d.w., respectively. These concentration levels could be attributed to the compound's high partition coefficient value ($\log P$ value of 4.40), which attests to its hydrophobic nature. Repelling effects from the aqueous seawater compartment, as well as possible precipitation mechanisms, could be the key factors in valsartan's transportation and deposition on the sediment layer.

Out of the 18 PPPs and their TPs, 10 of them were determined in sediments of station S1, close to the Thriassio WWTP, which receives urban and hospital wastewater, as well as the industrial and agricultural areas of Elefsis Bay. Their cumulative concentration was measured at 23.8 μg per kg d.w. Plant growth regulators 4-iodophenoxyacetic acid and maleic hydrazide were the most abundant PPPs in the study area, in terms of concentration levels, which were calculated between 3.51 and 6.70 μg per kg d.w. The highest detection frequencies for PPPs were observed for the plant growth regulator 4-iodophenoxyacetic acid and the fungicide azoxystrobin, the highest determined concentration of which was 0.806 μg per kg d.w. in sampling station S8. Azoxystrobin was determined in analyzed effluent wastewater samples in a concentration range of 2.64 and 3.91 μg per kg d.w. [Table S8].

Interestingly, a total of 30% ($n = 13$) of detected compounds in sampling station S8 sediments were also determined in seawater from sampling station S7. This corroborates a certain sea current direction within the Saronikos Gulf, as well as



circulation mechanisms and patterns of direct deposition towards the southwest part of the sampling area, where station S8 is located.

5.4. Evidence of shift in pharmaceutical use

A past study investigating pharmaceuticals²⁸ is the only one that has been carried out in the wider area of the Saronikos Gulf concerning LC-amenable compounds. This study focused exclusively on a dataset of 156 pharmaceutical compounds and their metabolites in seawater samples collected in 2013, which were analyzed *via* low resolution mass spectrometry. A direct comparison of the reported results provides evidence of a shift in pharmaceutical compound sources and precipitation patterns in the study area during sampling.

Specifically, antibiotic compounds like clarithromycin were only detected during the 2013 sampling campaign. This could be possibly attributed to changes in drug prescription and consumption during the last decade, which indicates a significant reduction in clarithromycin prescription rates around the world.⁶⁸ This is confirmed by its <LOD concentration values in the influent and effluent samples from the WWTPs of Thrasio and Psytalia and also in the seawater sampled close to the pipelines of the two WWTPs, reported in this study [Table S8]. In replacement of these, new types of antibiotics have emerged, specifically quinolones, such as cinoxacin and norfloxacin, which were detected only during the 2023 campaign. Antibiotic compounds sulfamethizole and sulfamethoxazole, which were only detected during the 2013 sampling campaign, belong to the chemical class of sulfonamides. Due to their adverse side effects on human health, they are now mainly utilized as veterinary drugs,⁶⁶ while more substances from the quinolone group are administered in replacement of sulfonamides. Additionally, results from Si-biol and S7-biol stations' seawater analysis indicate that sulfamethoxazole concentration levels ranged between 3.34 and 4.75 ng L⁻¹, which were significantly lower than those calculated in effluent wastewater samples from the Psytalia WWTP, equal to 725 ng L⁻¹.

Moreover, benzodiazepine drug diazepam was identified solely in 2013. Instead, different benzodiazepines were detected during the 2023 sampling campaign, namely bromazepam and citalopram, along with more mild antidepressant substances. Influent wastewater analysis from both WWTPs operating in the area reflects benzodiazepine consumption. Detected psychoactive substances from the benzodiazepine group include alprazolam, citalopram, midazolam and temazepam. This difference in determination can be attributed to possible changes in prescription patterns by modern psychiatry.^{64,69} Benzodiazepines, however, are being sparingly administered, due to concerns about their dependence and possible adverse effects.

Furthermore, the psychoactive substance venlafaxine along with its human metabolite *O*-desmethyl-venlafaxine were detected during the 2023 sampling period. Determination of both the parent compound and human metabolite provides additional confidence value to conducted research. As reported in the literature, venlafaxine is excreted from the human body as 5% unchanged and 29% metabolized to *O*-desmethyl-

venlafaxine.⁷⁰ Parent compound determination is difficult in seawater possibly due to its limited concentration levels and extensive dilution occurring in the sea. On the other hand, venlafaxine determination was accomplished in sediments sampled in 2023, where years of deposition have significantly increased its concentration, rendering it detectable *via* the employed experimental workflow and analytical technique.

The antibiotic substance amoxicillin, which belongs to the group of β -lactams, was uniquely detected during the 2013 sampling period. However, posterior research has proven that due to abiotic mechanisms taking place in the aquatic ecosystem, the β -lactam ring breaks down due to hydrolysis.^{67,68} As a result, MS and MS/MS data from amoxicillin determination do not produce mandatory fragment ions and consequently, this antibiotic was not considered as a finding in the present study.^{67,71,72}

Finally, analytical method constraints should also be considered, *e.g.*, pharmaceuticals like duloxetine and flumequine, and illicit drugs like MDMA were solely determined during the 2013 sampling period at very low concentration levels, lower than the LOD values attained in the 2023 study. These particular compounds were also not determined in concentration levels surpassing LOD values in tested wastewater samples. This could be due to better sensitivity levels achieved by conducting LC-QqQ-MS analysis employed in the 2013 samples,²⁸ compared to the LC-TIMS-QTOF-MS technique implemented herein. To that end, a complementary LRMS technique is proposed for utilization in future studies regarding illicit drug determination, as their low concentration levels in seawater samples render them difficult to detect in HRMS analysis.

5.5. Environmental implications

During the sampling period, the study area is characterized at moderate to poor eutrophication status, based on Chl-*a* concentrations (Table S2). Moreover, Particulate Organic Carbon (POC) is also high, compared to other coastal areas of Greece.³⁸ The chemical quality of European coastal waters is regulated by the Water Framework Directive (WFD). Directive 2013/39/EU⁶⁴ amended previous legislation annexes 2000/60/EC and 2008/105/EC by adding more regulated chemicals to existing law. In 2022, new proposals were submitted to the European Parliament, under which new ECs were prioritized, and specific environmental quality standards (EQSs) were set for several substances.^{64,65} Recently, the Commission has adopted a new watchlist of substances in surface waters, suspected of posing a risk to the environment and human health.¹⁷ The detection and determination of these substances is paramount to evaluate the environmental quality of the study area.

A total of 15 regulated substances were present in seawater samples, including 10 PFASs, 2 pharmaceuticals, 2 plant protection products, and 1 plasticizer. EU legislation suggests that determined PFAS concentrations are multiplied by a relative potency factor (RPF) and presented as \sum PFQA equivalents. Detected priority substances are listed in the SI, along with their



Table 1 List of determined analytes, whose concentration levels exceed recorded PNEC values in at least one tested seawater sample, along with their frequency of detection, percentage and number of seawater samples where PNEC was exceeded and RQF range

Seawater samples					
Analyte name	%FoD	PNEC exceedance	Minimum RQF	Median RQF	Maximum RQF
C12-LAS	15%	15% ($n = 2$)	0	0	48.4
Nicosulfuron	8%	8% ($n = 1$)	0	0	30.7
4-Nitrophenol	15%	15% ($n = 2$)	0	0	23.6
Fenbendazole	15%	15% ($n = 2$)	0	0	15.7
C13-LAS	15%	15% ($n = 2$)	0	0	11.4
Benzododecinium	100%	77% ($n = 10$)	0.330	1.17	11.0
<i>N</i> -Methyldodecylamine	92%	23% ($n = 3$)	0	0.548	4.35
Ibuprofen	46%	31% ($n = 4$)	0	0	4.34
<i>N,N</i> -Dimethyldodecylamine	15%	15% ($n = 2$)	0	0	3.09
Bisphenol AF	15%	15% ($n = 2$)	0	0	2.98
2-Phenylphenol	15%	8% ($n = 1$)	0	0	2.42
Benzylidimethyltetradecylammonium	100%	8% ($n = 1$)	0.0589	0.346	2.00
BAC 14	15%	15% ($n = 2$)	0	0	1.96
Octhilinone	15%	15% ($n = 2$)	0	0	1.62
1-Naphthol	15%	8% ($n = 1$)	0	0	1.25
Thiabendazole	23%	8% ($n = 1$)	0	0	1.13
Fluometuron	54%	8% ($n = 1$)	0	0.0126	1.10
Benzylidimethylhexadecylammonium	15%	8% ($n = 1$)	0	0	1.08
BAC 16	8%	8% ($n = 1$)	0	0	1.04
Dinoterb	92%	8% ($n = 1$)	0	0.0548	1.01

annual average standards (AA-EQSs), maximum allowable concentrations (MAC-EQSs) and RPFs for PFASs [Table S9].

Besides seawater close to the sea bottom (18 m) of sampling station S1 (sum of PFOA equivalent concentrations equal to 2.90 ng L^{-1}), AA-EQS value of 4.40 ng L^{-1} ^{64,65} was surpassed in every other tested seawater sample. The sum of PFOA equivalent concentration levels in the rest of the study area ranged between 4.53 and 37.3 ng L^{-1} . These findings could be attributed to the large scale of industrial activities taking place in the Saronikos Gulf and the Elefsis Bay, as well as the extent of urbanization in the area. Antiepileptic substance carbamazepine, NSAID ibuprofen and plasticizer DEHP did not exceed AA-EQS values during sampling, the maximum concentration values of which were calculated to be equal to 3.77, 4.78 and 31.6 ng L^{-1} ,

respectively. Insecticide clothianidin was also detected in the sampling area, in maximum concentration levels of 0.829 ng L^{-1} , below the proposed AA-EQS levels.⁶⁵

Following the calculation of ECs' concentrations in each tested sample, a prioritization of analytes was conducted by evaluating compounds' bioaccumulative potential, as well as calculating the extent of predicted no effect concentration (PNEC) exceedance in both environmental matrices. Ecotoxicological data are presented in the SI [Table S5].^{45,47,73} Analyte information includes lipophilic properties ($\log P$ values) and persistent bioaccumulative and toxic (PBT) data, which are reflected by the recorded lowest PNEC values. In this study, RQFs for the worst-case scenario were calculated based on the maximum MEC, as suggested in the literature. However,

Table 2 List of determined analytes, whose concentration levels exceed recorded PNEC values in at least one tested sediment sample, along with their frequency of detection, percentage and number of sediment samples where PNEC was exceeded and RQF range

Sediment samples					
Analyte name	%FoD	PNEC exceedance	Minimum RQF	Median RQF	Maximum RQF
Perfluorooctanesulfonic acid (PFOS)	100%	100% ($n = 4$)	181	231	586
Di-(2-ethylhexyl) phthalate (DEHP)	100%	100% ($n = 4$)	51.4	267	471
Octhilinone	100%	100% ($n = 4$)	1.07	1.57	1.95
2-Phenethylamine	100%	75% ($n = 3$)	0.595	1.42	3.51
Venlafaxine	75%	75% ($n = 3$)	1.53	2.27	2.27
Methyl-pirimiphos	50%	50% ($n = 2$)	8.65	8.88	9.11
Miconazole	75%	50% ($n = 2$)	0.549	1.20	4.39
Nicotine	50%	50% ($n = 2$)	1.39	8.65	15.9
Anabasine	50%	25% ($n = 1$)	0.499	3.50	6.51
Fenbendazole	50%	25% ($n = 1$)	0.211	0.901	1.59
Isoproturon	25%	25% ($n = 1$)	1.75	1.75	1.75
Phorate-sulfone	25%	25% ($n = 1$)	7.89	7.89	7.89



considering the availability of a limited number of tested samples, general risk factors were calculated based on the median and not the average concentration values. Risk evaluations for both tested marine compartments were conducted in this study [Tables 1 and 2].

Seawater analysis suggests that a total of 20 ECs exceeded PNEC values in terms of determined concentration ($RQF > 1$, high risk), in at least one tested sample [Table 1]. In detail, concentration levels of linear alkylbenzene sulfonates C12-LAS and C13-LAS exceeded seawater PNEC values in 2 tested samples each, with worst-case scenario RQFs equal to 48.4 and 11.4, respectively. The concentration levels of 7 other antiseptic and disinfectant substances exceeded seawater PNEC values in at least one tested seawater sample. In detail, benzododecinium ($n_{\text{exceedance}} = 10$), *N*-methyl-dodecylamine ($n_{\text{exceedance}} = 3$), *N,N*-dimethyl-dodecylamine ($n_{\text{exceedance}} = 2$), benzyl-dimethyl-tetradecylammonium ($n_{\text{exceedance}} = 1$), BAC-14 ($n_{\text{exceedance}} = 2$), BAC-16 ($n_{\text{exceedance}} = 1$) and benzyl-dimethyl-hexadecylammonium ($n_{\text{exceedance}} = 1$) exhibited maximum RQFs ranging between 1.04 and 11.0 throughout the study area. Fungicide othilinone exceeded PNEC values in terms of calculated concentration in 2 seawater samples and had a worst-case scenario RQF equal to 1.62. Other plant protection products nicosulfuron, fluometuron and dinoterb also exceeded PNEC values in a single tested seawater each, with maximum RQFs ranging between 1.01 and 30.7. Concentration levels of industrial chemicals 4-nitrophenol ($n_{\text{exceedance}} = 2$), bisphenol AF ($n_{\text{exceedance}} = 2$), 2-phenylphenol ($n_{\text{exceedance}} = 1$) and 1-naphthol ($n_{\text{exceedance}} = 1$) exhibited exceedance of their recorded PNEC values in tested seawater. The highest maximum RQF for 4-nitrophenol was measured at 23.6. Finally, veterinary drug fenbendazole ($n_{\text{exceedance}} = 2$), NSAID ibuprofen ($n_{\text{exceedance}} = 4$) and antifungal substance thiabendazole ($n_{\text{exceedance}} = 1$) also exceeded PNEC values in terms of measured concentrations, with their worst-case scenario RQFs ranging between 1.13 and 15.7.

In sediments, a different image is depicted through data inspection, considering the chemical classification of compounds exceeding PNEC values ($RQF > 1$, high risk) in terms of calculated concentration. A total of 12 analytes were determined in $RQFs > 1$ in at least one sediment sample [Table 2]. Extremely high RQF values were calculated for PFOS, compared to other analytes. Concentration levels of this particular PFAS exceeded sediment PNEC values by at least 181 times, with a maximum RQF equal to 586. Similar but relatively lower RQFs were calculated for plasticizer DEHP, which presented a risk quotient range between 51.4 and 471. Antifungal medication othilinone exceeded PNEC values in terms of concentration levels in all tested sediment samples, with a maximum RQF equal to 1.95, whereas pharmaceuticals 2-phenethylamine ($n_{\text{exceedance}} = 3$), fenbendazole ($n_{\text{exceedance}} = 2$) and miconazole ($n_{\text{exceedance}} = 1$) exhibited high-risk RQFs in tested sediments. The psychoactive substance venlafaxine was determined in an RQF range between 1.53 and 2.27 in all samples where it was detected. Furthermore, nicotine, along with its human metabolite anabasine, exceeded sediment PNEC values by a maximum of 15.9 and 6.51 times, respectively. Lastly, plant protection

substances methyl-pirimiphos and isoproturon were determined in concentration levels higher than their PNEC values in 100% of sediment samples, where they were detected. Their highest RQFs were equal to 9.11 and 1.75, respectively.

6. Conclusions

This study's main objective was to assess the presence, distribution and ecological risk of ECs and priority pollutants in the Saronikos Gulf and the Elefsis Bay marine environment, in relation to anthropogenic activities, utilizing a novel ion mobility HRMS analytical technique. Reported results reflect the long-term chemical burden on the study area. Due to the continuous treated wastewater discharges by local WWTPs, seawater in the area is susceptible to certain chemical stressors, associated with human-related activities, such as low metabolism percentage pharmaceuticals and plant protection products. Additionally, the prolonged deposition of ECs onto sediments has resulted in the accumulation of high-risk industrial chemicals and PFASs, as well as pharmaceuticals and coffee and tobacco related substances. Comparison of these results with previous studies conducted in the area reveals clear shifts in pharmaceutical consumption patterns by the local population.

Besides chemical identification and prioritization, this study reflects possible circulation patterns occurring in the Saronikos Gulf. Seawater samples from sampling station S7 (close to the Psytalia WWTP pipelines) were chemically encumbered mainly by pharmaceuticals, industrial chemicals and plant protection products. Sediment from sampling station S8 (southwest of the Psytalia WWTP) was determined to be the most chemically encumbered, both in number of detected ECs and in cumulative concentration of chemicals. A total of 30% ($n = 13$) of compounds detected in S8 sediments were also determined in seawater from sampling station S7, indicating circulation mechanisms and patterns of direct deposition.

Overall, the environmental quality status of the study area was not determined to be heavily burdened by anthropogenic chemicals. Risk assessment performed in studied samples showed that only a small fraction of detected substances exceed annual average values of concentrations reported in EU legislation. Regarding PNEC values, a total of 20 compounds presented high risk for studied seawater, while 12 compounds presented high risk for sediments. Among the latter, PFOS and DEHP exhibited the highest extent of PNEC exceedance in considered sediments.

This study is only the first step towards unravelling the pollution status of the Greek coastal and open sea areas. Utilization of HRMS techniques provides the ability of retrospective analysis, by performing wide-scope target screening on existing raw data, through new and enriched databases. Non-target screening protocols will also be developed and applied in the future to expand the chemical domain, which is covered by HRMS analysis of environmental samples. In the future, more seawater, sediment and wastewater samples should be analyzed to clearly recognize pollution patterns and compound degradation procedures. Marine biota samples should be analyzed as



well, in the interest of determining possible biological factors of compound degradation and transformation, and also the transportation of determined chemicals through the marine food web.

Author contributions

Lougkovoios R.: data curation, formal analysis, investigation, methodology, validation, writing – original draft, writing – review & editing. Parinos C.: conceptualization, data curation, funding acquisition, investigation, methodology, supervision, writing – original draft, writing – review & editing. Gkotsis G.: data curation, investigation, writing – review & editing. Nika M.-C.: data curation, investigation, writing – review & editing. Hatzianestis I.: conceptualization, funding acquisition, methodology, resources, supervision, writing – review & editing. Pavlidou A.: conceptualization, funding acquisition, methodology, resources, supervision, writing – original draft, writing – review & editing. Thomaidis N.: conceptualization, funding acquisition, resources, supervision, writing – review & editing.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

All data considered in this study will be available upon request.

Supplementary information (SI) is available. See DOI: <https://doi.org/10.1039/d5va00287g>.

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