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Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater

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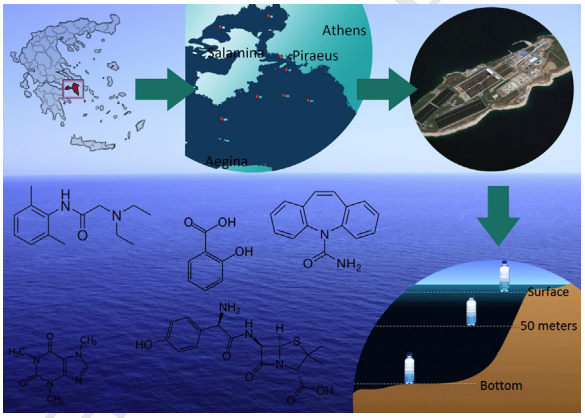
HIGHLIGHTS

- This is the largest study of emerging contaminants (158) in seawater.
- Thirty eight compounds have been detected.
- Amoxicillin, salicylic acid and caffeine showed the highest concentration levels.
- Wastewater release proved to be the major source of contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

The occurrence and spatial distribution of 158 pharmaceuticals and drugs of abuse were studied in seawater of the Eastern Mediterranean Sea (Saronikos Gulf and Elefsis Bay in central Aegean Sea). This area is affected by various anthropogenic pressures as it receives the treated wastewater of the greatest Athens area and off-shore input fluxes. This study constitutes the largest one in terms of number of analytes in this environmental compartment. It provides the first evidence on the occurrence of several pharmaceuticals in marine environment including amoxicillin, lidocaine, citalopram or tramadol, among others.

22 samples were collected at three different depths in 9 sampling stations in order to assess the presence and the spatial distribution of the target compounds. A multi-residue method based on solid phase extraction and liquid chromatography coupled to tandem mass spectrometry was developed for the determination of the 158 target substances and validated for seawater sample analysis. 38 out of the 158 target compounds were detected, 15 of them with frequencies of detection equal to or higher than 50%. The highest detected values corresponded to amoxicillin, caffeine and salicylic acid, with concentrations in the range of <5.0–127.8 ng L^{-1}; 5.2–78.2 ng L^{-1} and <0.4–53.3 ng L^{-1}, respectively. Inputs from the wastewater treatment plant (WWTP) of Athens revealed to be the main source of pollution in the Inner Saronikos Gulf, whereas, other anthropogenic pressures such as contamination from shipping activity, industrial effluents, dredging and/or inputs from land proved to be also relevant. The concentrations of some compounds varied significantly with depth suggesting that currents play an important role in the dilution of the target compounds.

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1. Introduction

A large variety of emerging organic contaminants, including pharmaceuticals, drugs of abuse and related metabolites, have been identified and reported in different environmental compartments. Although the present knowledge in the occurrence and behavior of these compounds in surface and wastewater is well documented (Li, 2014; Pal et al., 2013), such knowledge in marine waters is still very limited.

Discharges of wastewater treatment plant (WWTP) effluents constitute the main source of entry of these substances in the environment (Venkatesan and Halden, 2014). After legal or illegal administration, certain amounts of drugs and/or related metabolites are excreted and subsequently transported through the sewage system to WWTPs, where they are not completely removed (Joss et al., 2005). As a consequence, relevant amounts of these substances are released into the aquatic environment. Either through WWTPs or other routes of disposal, a fraction of these compounds ends up into the sea, which is the main receptor of land-based pollutants (Zhang et al., 2013).

Pharmaceuticals and drugs of abuse are designed to cause specific effects even at low concentrations (McEneff et al., 2014). Therefore, long term exposure to low doses of these compounds can cause adverse effects in the ecosystems (Fent et al., 2006). This becomes more evident when sewage discharges affect the area of study. An example can be found in the study conducted by Brooks et al. (2005), where fluoxetine, sertraline and their related metabolites norfluoxetine and norsertraline were detected at high levels in marine fish tissues collected downstream from an effluent discharge in Texas (USA). However, the knowledge on effects and thresholds of pharmaceutical mixtures and related substances in the marine environment is still scarce.

Most of the published literature focus on coastal marine waters collected across the shoreline, analyzing only few specific categories of pharmaceuticals, This is the case of the study performed by Lolić et al., evaluating the presence of 7 analgesics and anti-inflammatory drugs and 2 metabolites in samples collected from beaches located in the north of Portugal (Lolic et al., 2015). Another example can be found in the study carried out by Nödler et al., analyzing 43 drugs in seawater (Nodler et al., 2014). This study evaluated the presence of pharmaceuticals, corrosion inhibitors, biocides, and stimulants collected from various areas including the Mediterranean Sea, Baltic Sea and Pacific Ocean. Benotti and Brownawell studied the microbial degradation of 19 pharmaceuticals being antipyrine, carbamazepine, cotinine, sulfamethoxazole, and trimethoprim as the most refractory (Benotti and Brownawell, 2009).

Only few studies have analyzed offshore seawater. Weigel et al. analyzed 7 drugs in the North Sea and detected clofibric acid and caffeine at concentrations of 1.3 ng L^{-1} and 16 ng L^{-1}, respectively (Weigel et al., 2002). Zhang et al. evaluated seawater from the Bohai Sea and the Yellow Sea (China), analyzing 11 antibiotics. All the target compounds were detected being erythromycin, sulfamethoxazole and trimethoprim as the most ubiquitous ones with concentrations ranging between 0.1 and 16.6 ng L^{-1} (Zhang et al., 2013). Loos et al. analyzed 67 compounds, including 22 pharmaceuticals in the Northern Adriatic Sea (Loos et al., 2013). In this study samples were collected from only one sampling station (Aqua Alta Oceanographic Tower), located 16 km away from Venice and 65 out of the 67 target drugs were detected reaching concentrations up to 367 ng L^{-1} for caffeine and 36 ng L^{-1} for nitrophenol.

So far, extensive studies evaluating a large list of pharmaceuticals and drugs of abuse in offshore seawater have not been conducted. Moreover, no studies have considered the distribution of analytes at different depths of the water column with the exception of the work performed by Lara-Martín et al., who studied the changes in the concentrations of 64 pharmaceuticals at two different depths (surface and bottom water) in one single sampling point during a tidal event (Lara-Martin et al., 2014).

The aim of the present work was to study the occurrence and distribution of various pharmaceuticals and drugs of abuse in the seawater column of the inner Saronikos Gulf and Elefsis Bay, in Greece. These marine areas are affected by different anthropogenic pressures, including wastewater release, shipping activity, dredging, industrial effluents, port activities and municipal activities. For these purposes, a highly sensitive analytical method was developed and validated for the determination of 158 compounds in seawater, with a wide range of physicochemical properties, belonging to the following categories: Antidepressants, anxiolytics, antipsychotics, antibiotics, antiepileptics, analgesics, NSAIDs, diuretics, antihypertensives, anti-ulcers, anesthetics, sympathomimetics and steroids as well as the main drugs of abuse and their metabolites. To the authors' knowledge, the present study constitutes the largest one conducted in seawater in terms of number of analytes, assesses the presence of several substances for the first time in this environmental compartment under various anthropogenic pressures and it is the first one evaluating samples collected at different depths of the water column.

2. Materials and methods

2.1. Chemicals and reagents

Compound names, CAS numbers, structures, molecular formulas and other relevant physicochemical properties for all the target compounds are summarized in Table S1 (Supplementary material). All standards were of high purity grade (>90%) and were purchased mainly from Sigma-Aldrich (Athens, Greece) and LGC Promochem (Molsheim, France). Suppliers for each target analyte are also listed in Table S1. Caffeine is a stimulant. However, this substance is commonly added as an additive in many pharmaceutical products, particularly analgesics. For convenience, caffeine has been placed in the group of analgesics and not to create a special category for this compound.

All deuterated compounds were obtained from LGC Promochem (Molsheim, France): morphine-D3 (MOR-D3), codeine-D3 (COD-D3), cocaine-D3 (COC-D3), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine-D3 (EDDP-D3), 3,4-methylenedioxy-N-methylamphetamine-D5 (MDMA-D5), 3,4-methylenedioxy amphetamine-D5 (MDA-D5), and lysergic acid diethylamide-D3 (LSD-D3).

Acetonitrile (ACN) and methanol (MeOH) LC–MS grade were purchased from Merck (Darmstadt, Germany) as well as hydrochloric acid (HCl) 37%, while formic acid (FA) 99% was obtained from Sigma–Aldrich, Fluka (Buchs, Switzerland). Ammonia 25% was purchased from Panreac (Barcelona, Spain) and ammonium formate from Fluka (Buchs, Switzerland). Distilled water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Oasis-HLB cartridges (200 mg/6 mL) were purchased from (Waters, Milford, MA, USA) and RC syringe filters (4 mm diameter, 0.2 μm pore size) from Phenomenex (Torrance, CA, USA). Nylon membrane filters with a pore size of 0.45 μm were provided by Whatman International Ltd. (Maidstone, England).

About 10 mg of each individual standard was accurately weighed and placed in a 10-mL volumetric flask. Cephalosporins, penicillins and macrolides were dissolved in MilliQ-water, while all other analytes in MeOH. Stock solutions of 1.0 mg L^{-1} of each compound were obtained and stored at −20 °C. From this multi-analyte solution all working solutions were prepared daily by appropriate dilution of the mixture stock standard (1.0 mg L^{-1}) and IS solutions (1.0 mg/L) in MeOH. Calibration standards were prepared by serial dilution of the mixed working solution using Milli-Q water with 0.05% v/v formic acid resulting in individual concentrations ranging from 0.20 to 100 μg L^{-1}.

2.2. Study area and sampling

The present study was carried out in the Saronikos Gulf and the Elefsis Bay during December 2013. The Saronikos Gulf is located

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between the region of Attica and the Peloponnese Peninsula, in Greece. This area is highly influenced by the proximity of the densely populated city of Athens (3,737,550 residents according to the National Statistical Service of Greece) and its metropolitan area. The WWTP of Athens is located on Psittalia Island and is the second largest treatment plant in Europe. Apart from the treated sewage, no other potential sources of anthropogenic inputs exist in the area of the Inner Gulf (Pavlidou et al., 2014). The locations of the nine sampling stations evaluated in this study are detailed in Fig. 1. Detailed description of the study area and the sampling stations is included in the Supplementary File, Section S2.

22 seawater samples were collected during December 2013 from 9 sampling stations (7 in the inner Saronikos Gulf and 2 in the Elefsis Bay; Fig. 1) at different sampling depths (surface, 50 m and near the bottom). The sampling cruise was conducted using the R/V Aegaio. Additional information including the exact coordinates of the sampling stations, maximum depth as well as the distances from land and from the WWTP is listed in Table S2. Other physicochemical parameters of the collected water including salinity, dissolved oxygen and nutrient concentrations (phosphate, silicate, nitrate, nitrite and ammonium) are also included in Table S2.

The seawater samples were collected with Niskin type bottles attached in a rosette. Dissolved oxygen (DO), phosphate and ammonium were measured *on board*, while the samples for the determination of nitrate, nitrite, silicate were kept deep-frozen (−20 °C) until analysis. Seawater samples for the determination of the 158 pharmaceuticals and drugs of abuse were collected in 2.5 L amber glass bottles, previously rinsed with methanol and ultrapure water. Once collected and during shipment, samples were acidified to pH (3.0 ± 0.5) with 12 M HCl. Upon reception in the laboratory, samples were vacuum filtered through 0.45 μm nylon membrane filters, and an aliquot of 1 L for each sample was stored in the dark at −20 °C until analysis.

2.3. Analytical methods

2.3.1. Physicochemical parameters

Temperature, salinity, and density in the water column were measured with a CTD profiler (Sea Bird Electronics), which was equipped with pressure, temperature, and conductivity sensors. Dissolved oxygen was measured on board with the Winkler method according to Carpenter (1965a and 1965b). The determination of ammonium and phosphate was also performed on board using a 25 Lambda Perkin Elmer spectrophotometer (Koroleff, 1970; Murphy and Riley, 1962). The determination of nitrites, nitrates and phosphates was performed and certified according to ISO 17025 (Certification No. 366) biogeochemical laboratories of HCMR with a SEAL autoanalyzer III, using standard methods for silicate (Mullin and Riley, 1955), nitrate–nitrite (Strickland and Parsons, 1972) and phosphate (Murphy and Riley, 1962).

2.3.2. Pharmaceutical and drugs of abuse

The analysis of the target compounds in the collected samples were performed following a procedure based on solid phase extraction (SPE) and liquid chromatography coupled to tandem mass spectrometry. This procedure was developed by merging two previous existing methodologies for drugs of abuse and pharmaceuticals, respectively (Borova et al., 2014; Dasenaki and Thomaidis, 2015).

Briefly, seawater samples (pH 3 (previously optimized in the aforementioned methodologies) were pre-concentrated onto Oasis HLB cartridges (Waters, Millford, MA, USA), previously preconditioned with 6 mL of MeOH followed by 6 mL of deionized Milli-Q water. After loading 1.0 L of seawater under a light vacuum (400 kPa), cartridges were rinsed with 6 mL of Milli-Q water and left under a vacuum (~55 kPa) for 1 h. Elution was performed with 6 mL of MeOH (3 times × 2 mL). Extracts were evaporated to dryness under mild nitrogen stream at 40 °C and further reconstituted to a final volume of 250 μL (25% MeOH, 75%

Fig. 1. Area of study and sample locations.

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H_2O (0.05% formic acid)). The achieved enrichment factor was 4000. Final extracts were filtered through 0.2 mm RC syringe filters.

Instrumental analysis was performed with a Thermo UHPLC Accela system connected to a TSQ Quantum Access triple quadrupole mass spectrometer from Thermo Electron Corporation (San Jose, CA, USA) equipped with an electrospray ionization source (Thermo Ion Max) in both positive and negative modes. Chromatographic separation was achieved on an Atlantis T3 C18 (100 mm × 2.1 mm, 3 μm) column from Waters Corporation (Milford, MS, USA) at a constant flow rate of 100 μL min^{-1}. The mobile phase for the positive detection mode consisted of water (0.01% FA (v/v)) and MeOH for the positive detection mode and water (1 mM ammonium formate), MeOH and ACN (constant in a proportion of 5%) for the negative detection mode. Gradient elution programs are presented in Table S3A (Supplementary information) along with other relevant ESI parameters, which were obtained as a compromise using the optimum values for most compounds.

Identification and quantification were performed under selected reaction monitoring (SRM) mode, recording the transitions between the precursor ion and the two most abundant product ions for each target analyte, thus achieving 4 identification points per compound (2002/657/EC). Quantification was based on standard additions, and isotopically labeled compounds were used only for the quantification of those compounds in which isotopically analog compounds were available. SRM transitions for each compound were optimized by infusion of standard solutions at a mean concentration of 1 mg L^{-1}. The optimized ionization mode, fragmentation voltages, collision energies and chromatographic retention times for each analyte are summarized in Table S3B (Supplementary material).

2.3.3. Statistical analysis

Spearman's rank correlation tests were performed to investigate the overall correlation among the analytes and between the analytes and the rest of the studied parameters included in Table S2. These parameters included the distance from the main WWTP and the distance from land. Only compounds with a frequency of detection higher than 50% were considered for statistical analysis. Values below the limits of detection were replaced to half of the limit of detection (Farmaki et al., 2012). Statistical analysis was performed using STATISTICA, version 10 (StatSoft, Tulsa, USA).

2.4. Quality assurance and quality control

The analytical method used in the present work was evaluated under the optimized conditions in terms of linearity range, sensitivity, accuracy, and repeatability and matrix effects. Table S4 summarizes the method performance parameters. Method validation and method performance is discussed in detail in the Supplementary information, Section S4.

2.5. Risk assessment

For target compounds detected at least once toxicity data (EC_{50} or LC_{50}) for three different trophic levels (algae, daphnids and fish) have been collected either through literature search or from ECOSAR program which is used from the US EPA. In either case the lowest short period toxicity values were collected in order to take into consideration the worst case scenario. According to the Technical Guidance Document of the European Commission, the risk quotient (RQ) is calculated as the maximum measured environmental concentration (MEC) divided into the predicted no effect concentration (PNEC), which is EC_{50} or LC_{50} value divided to 1000 in case short-term toxicity data is used (Thomaidi et al., 2015).

3. Results and discussion

3.1. Levels of pharmaceuticals and drugs of abuse in seawater

Pharmaceuticals and drugs of abuse, along with many other substances enter seawater through various pathways, being particularly important WWTP release. After the entrance of these substances, two important phenomena take place: diffusion and dilution. Other processes like abiotic or biotic transformation and adsorption may occur but their study is out of the scope of the present work.

Fig. 2 summarizes the concentration levels of the detected target analytes in the different sampling points. These values as well as the frequency of detection for each compound are also listed in detail in Table S5 (only compounds detected at least in one sample have been included).

38 out of the 158 analyzed compounds were detected at least in one sample, while 120 analytes remained undetected. Out of them, there were 15 substances (Caffeine, Tramadol, Salicylic acid, Lidocaine, Amoxicillin, Carbamazepine, Amisulpride, Niflumic Acid, Norvenlafaxine, Paracetamol, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), Diclofenac, Mefenamic acid, Citalopram and Sulpiride) which showed a frequency of detection equal or above 50%, in some cases with maximum concentrations higher than 50 ng L^{-1}. The other 23 compounds were detected sporadically in the concentration range < LOD — 8.2 ng L^{-1}.

3.1.1. Analgesics

The analgesics tramadol and paracetamol, along with the stimulant caffeine, were widely detected in the evaluated sampling stations. Caffeine, although it is not an analgesic, it was included in this category for convenience, since it is widely used in combination with analgesics (and also other pharmaceuticals). Tramadol was detected at low concentration levels (<0.1–1.0 ng L^{-1}) in all the analyzed samples, including the most remote sampling station S16, showing its widespread distribution. The presence of this compound may be related to the high levels recently reported in treated water of the Psittalia WWTP (mean concentration 892 ng L^{-1}) (Dasenaki and Thomaidis, 2015). These results constitute the first ones regarding the presence of tramadol in seawater.

Caffeine was also detected in all the evaluated samples, with concentrations in the range of 5.2–78.2 ng L^{-1}. The lowest concentration level occurred in the "blank station" S16 while the maximum levels were detected at S11 and S7. The main source of contamination seems to be the Psittalia WWTP effluents, where mean concentrations of 464 ng L^{-1} have been recently detected (Dasenaki and Thomaidis, 2015). Passenger ships, with strong activity in this area, may also be an important source of contamination for this compound. Caffeine levels reported in the present study are in the same range as those reported by Weigel et al. and Magnér et al. in seawater from Tromsø (Norway) and Stockholm (Sweden), respectively, receiving inputs from WWTPs (Magner et al., 2010; Weigel et al., 2004). Higher levels (130–1400 ng L^{-1}) were detected by Comeau et al. in seawater receiving WWTP inputs in Nova Scotia (Canada) (Comeau et al., 2008).

Paracetamol was detected with a lower frequency (64%), reaching high concentrations (up to 40.5 ng L^{-1}) in some cases. S8 (surface) and S11 (bottom) were the samples with the highest levels of this compound. Paracetamol has not been previously reported in offshore seawater samples. Only Nödler et al. determined high concentrations (39–2893 ng L^{-1}) of this compound in estuarine waters from northern Aegean beaches in Greece (Nodler et al., 2014).

3.1.2. NSAIDs

Salicylic acid, diclofenac, mefenamic acid and naproxen were the only NSAIDs detected in the samples. These compounds have been previously detected at high concentrations in treated wastewater from Psittalia WWTP (Dasenaki and Thomaidis, 2015). However, other

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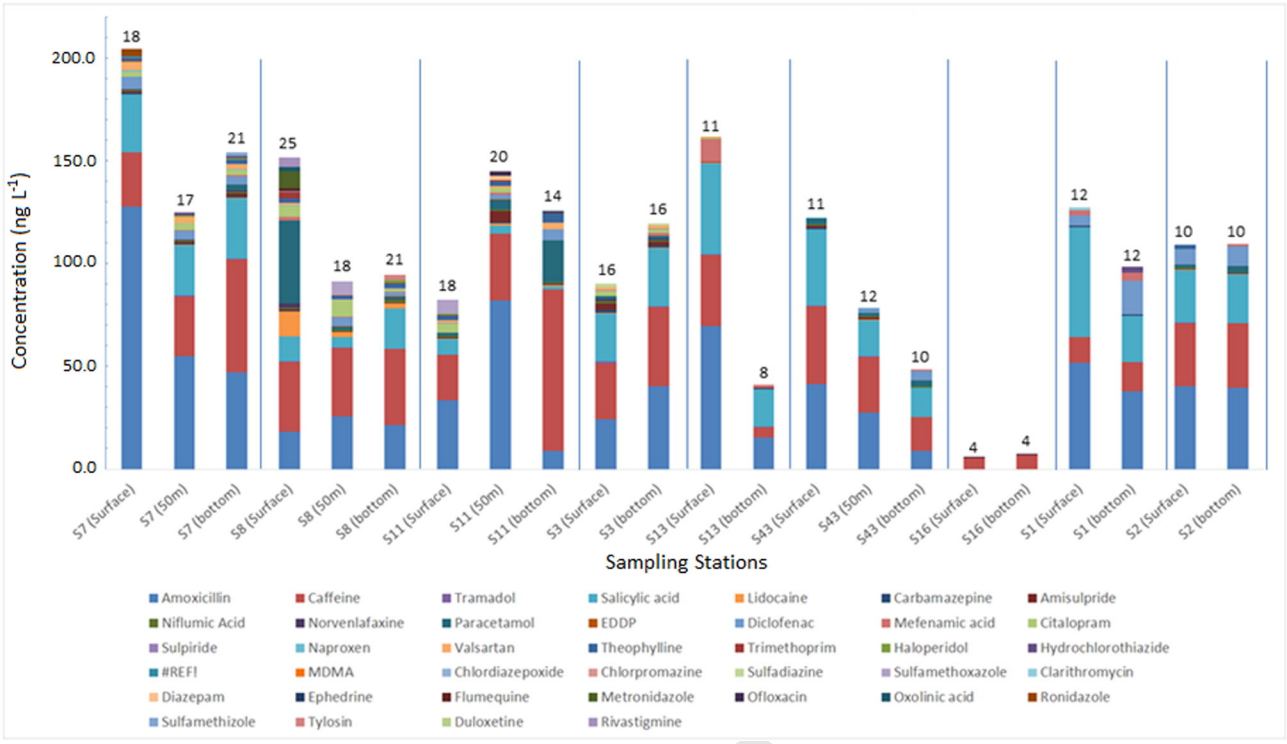

Fig. 2. Cumulative levels of the target compounds displayed as a stacked bar plot at the different sampling stations. Numbers above bars depict the number of analytes found in each station.

compounds of this family with a high rate of use, according to the high concentrations detected in the influent wastewater from Psittalia WWTP, such as ibuprofen or ketoprofen, were not detected in any sample. This behavior is related to the fact that these compounds are efficiently removed during wastewater treatment, since low concentrations were reported in the corresponding treated wastewaters. These facts show the close relationship between discharges of wastewater and emerging contaminants in seawater. Both ibuprofen and ketoprofen have been previously detected in seawater in studies carried out in Nova Scotia (Canada) and Northern Taiwan (Comeau et al., 2008; Fang et al., 2012).

Salicylic acid was detected in concentrations above LOQ in all samples except from S16, with a total frequency of detection of 90.9%. Concentration levels ranged from <0.4 to 53.3 ng L^{-1}, reaching the maximum concentration in Eleusina Gulf (S1 sampling station). These results constitute the first evidence of the presence of this compound in seawater.

Naproxen was detected at low levels, in the range of <0.01–0.8 ng L^{-1}, with a low frequency of detection (45%), reaching its maximum concentration near Psittalia WWTP (S7 sampling station). Higher levels were detected for this compound by Comeau et al. and Vidal-Dorsch et al. in seawater up to 130 and 30 ng L^{-1} respectively (Comeau et al., 2008; Vidal-Dorsch et al., 2012).

Diclofenac was detected with a frequency of detection of 59% in the range of <1.4–16.3 ng L^{-1}. Maximum concentrations were determined in Eleusina Gulf (S1 sampling station). The presence of this compound can be explained by the high concentrations determined in the effluent wastewaters from Psittalia (mean concentration 927 ng L^{-1}) (Dasenaki and Thomaidis, 2015), since this compound does not show a good elimination performance by the common processes applied during wastewater treatment (Jelic et al., 2011). The described concentration levels are comparable with those reported by Fang et al. in seawater from Taiwan (Fang et al., 2012), while other studies determined lower levels for this substance not exceeding 0.6 ng L^{-1} (Vidal-Dorsch et al., 2012).

Finally, mefenamic acid was detected in the range of <0.2–10.9 ng L^{-1}, with a frequency of detection of 59%. The reported levels are higher to those determined by McEneff et al., in the range of <0.29–0.6 ng L^{-1} (McEneff et al., 2014). In other studies carried out by Wille et al. in seawater from Belgium mefenamic acid was not detected (Wille et al., 2010).

3.1.3. Antibiotics

Amoxicillin, along with caffeine, was the compound detected at the highest levels, up to 127.8 ng L^{-1} and 78.2 ng L^{-1}, respectively, with a frequency of detection of 90.9%. Only at the sampling station S16 amoxicillin remained undetected. Maximum levels, up to 127.8 ng L^{-1}, were detected at the sampling station S7, near the WWTP. Concentrations detected at this point were the highest observed among all the studied compounds. Those draw attention that the determined levels are higher to those previously determined in treated wastewater in the study carried out by Dasenaki and Thomaidis (2015)). This fact can be explained by the seasonal consumption of some antibiotics. The sampling campaign in the aforementioned wastewater study was carried out in spring (April 2012) while seawater samples for the present study were collected in winter (December 2013). Amoxicillin is a heavily consumed antibiotic during cold months and reaches peak consumption in Greece during January (Van Boeckel et al., 2014). This data constitutes the first evidence of the presence of amoxicillin in seawater.

Clarithromycin and trimethoprim were detected sporadically at low concentration levels in the range of <1.0–1.5 and <0.4–3.4 ng L^{-1}, respectively. The rest of the studied antibiotics remained undetected. Clarithromycin, erythromycin, azithromycin and trimethoprim were detected in seawater in the study carried out by Zhang et al., reaching maximum concentration levels of 0.5, 6.7, 0.4 and 11.6 ng L^{-1}, respectively (Zhang et al., 2013). Trimethoprim was detected at higher levels (<3.3–29 ng L^{-1}) in harbor seawater by Wille et al. (2010).

Sulfonamides were generally not detected except from sulfamethoxazole and sulfadiazine. These compounds were determined at a frequency of detection of 9% in the ranges of <0.1–6.3 ng L^{-1} and <0.1–2.0 ng L^{-1}, respectively.

3.1.4. Other pharmaceuticals

Antihypertensive drugs were not detected in the present study with the exception of valsartan (frequency of detection 40.9%). This compound was detected at low levels, in the range of <0.8–3.7 ng L^{-1}, basically at the sampling station S7, close to the WWTP. Valsartan was previously detected at high levels (mean concentration of 624 ng L^{-1}) in treated wastewater from Psittalia WWTP (Dasenaki and Thomaidis, 2015), which may explain its presence.

None of the studied anti-lipidemic agents were detected in the evaluated samples. This is in agreement with previous studies which either did not detect anti-lipidemics or detected some substances (e.g. clofibric acid or gemfibrozil) at very low concentrations (Fang et al., 2012; Vidal-Dorsch et al., 2012).

From the group of anesthetics only lidocaine was detected. This compound was determined with high frequency of detection (90.9%) and concentration levels in the range of <0.01–12.8 ng L^{-1}. The highest concentration for this compound was observed at S8. Effluent wastewater analysis in Psittalia WWTP revealed mean concentrations of lidocaine of 293 ng L^{-1} (Thomaidi et al., 2015), which may justify its wide occurrence. The data here-in presented constitutes the first evidence on the presence of lidocaine in seawater.

Only one out of the screened anti-epileptic drugs, carbamazepine, was detected. Although this substance was determined at low concentration levels, with a maximum concentration of 1.4 ng L^{-1}, it seemed to be quite ubiquitous since it was detected in 17 out of the 22 evaluated samples. Carbamazepine was detected at similar concentration levels (<0.05–0.9 ng L^{-1}) in seawater from California (Vidal-Dorsch et al., 2012) but in higher levels in Ireland (<15–1710 ng L^{-1}) (McEneff et al., 2014) and Canada (4.0–26.3 ng L^{-1}) (Magner et al., 2010) and Belgium in harbor seawater (<1.65–119 ng L^{-1}) (Wille et al., 2010). Analysis of treated wastewater from the WWTP of Athens revealed carbamazepine mean concentrations of 461 ng L^{-1} (Dasenaki and Thomaidis, 2015). Thus, it seems evident that the main source of this compound in seawater occurred by WWTP release into the sea.

4 out of the 11 screened anti-psychotic drugs were detected: amisulpride, haloperidol, sulpiride and chlorpromazine. Amisulpride was detected in the range of <0.2–5.5 ng L^{-1} with a high frequency of detection (77.3%). Sulpiride presented a frequency of detection of 50% and was detected at low concentrations in the range of <0.06–0.5 ng L^{-1}. Chlorpromazine and sulpiride were only detected sporadically and at low concentration levels (<0.05–0.6 ng L^{-1} and <0.06–0.3 ng L^{-1}, respectively). All these substances, except chlorpromazine, have not been previously identified in seawater samples.

3 out of the 16 screened anti-depressants were detected. Norvenlafaxine, the main metabolite of venlafaxine, belonging to the category serotonin–norepinephrine reuptake inhibitors (SNRIs), was detected with a frequency of detection of 68.2% but in low concentration levels (<0.01–2.0 ng L^{-1}). However, the parent compound venlafaxine remained undetectable. Citalopram, with a lower frequency of detection (50%) was detected in the range of <0.06 to 8.0 ng L^{-1}. Citalopram was detected at 328 ng L^{-1} (mean concentration) in effluent wastewater from the WWTP of Athens (Dasenaki and Thomaidis, 2015) and seems to be the major source for the presence of this substance in the evaluated samples. The present study reports for the first time the occurrence of citalopram in seawater. Duloxetine was scarcely detected at low concentration levels.

Out of the 4 diuretic drugs included in the screening list, only hydrochlorothiazide was detected at concentration levels in the range of 1.3–1.4 ng L^{-1}, with low frequency detection. This compound was only detected close to the Psittalia WWTP, showing the strong relationship among the presence of this compound and the WWTP discharges. A mean concentration of 1149 ng L^{-1} was previously reported for this compound in treated wastewater (Dasenaki and Thomaidis, 2015).

3.1.5. Drugs of abuse

Out of the 17 screened drugs of abuse and related compounds only EDDP, 3,4-methylenedioxy-methamphetamine (MDMA) and ephedrine were eventually detected. EDDP, a metabolite of methadone, showed the highest frequency of detection (59.1%), similar to those corresponding to highly consumed drugs such diclofenac or mefenamic acid. However, the determined concentration levels were very low (<0.02–0.1 ng L^{-1}). EDDP was previously detected in effluent wastewater of the WWTP of Athens at a mean concentration of 40 ng L^{-1} (Thomaidi et al., 2015), being the major source of contamination of this compound in seawater. MDMA and ephedrine showed low frequencies of detection and the determined concentrations were below LOQ in all cases. MDMA was also detected in the aforementioned study in treated wastewater from Psittalia WWTP but at lower levels (8.1 ng L^{-1}), which can explain the low determined concentrations in the evaluated samples. On the contrary, ephedrine was detected at high concentrations in the effluents of Psittalia WWTP (2246 ng L^{-1}). The low concentrations determined in the seawater samples throughout the samples suggest a rapid degradation of this compound in seawater due to biotic or abiotic transformations. Overall, results showed a scarce presence of drugs of abuse in seawater of the evaluated area. As a general observation, it seems that recalcitrant and not easily biodegradable compounds were more frequently detected in the marine water, in accordance with a previous study (Benotti and Brownawell, 2009).

3.2. Spatial distribution of pharmaceuticals and drugs of abuse

The stacked bar plot (Fig. 2) summarizes the occurrence of pharmaceuticals and drugs of abuse at the different sampling stations. The graph shows that the most polluted sampling stations were S7 and S8.

The highest cumulative concentration levels occurred at the sampling station S7. This fact can be explained because this sampling station is located very close to the Psittalia WWTP and receives directly the treated wastewater discharges. The dilution effect at sampling station is much milder than in the other evaluated locations. This is in agreement with the fact that many target compounds reached their peak concentration levels in this sampling station. Among these compounds are amoxicillin, valsartan or hydrochlorothiazide, with maximum concentration values of 127.8 ng L^{-1}, 3.2 ng L^{-1} and 1.4 ng L^{-1}, respectively. Other compounds also showed high concentrations like caffeine or salicylic acid, with values up to 54.9 ng L^{-1} and 29.1 ng L^{-1}, respectively. According to the fact that WWTP is the main source of emerging pollutants in seawater, it seems reasonable that the distance of a sampling station from the WWTP outfall probably plays an important role in the concentration of the target analytes, since the dilution effect increases with the distance. The prevailing water circulation led to the dilution of the effluents in the inner Saronikos Gulf. However, after entering the sea, substances are affected by currents and those which do not suffer transformation processes can be transported to a fair long distance, being difficult to predict concentrations at a given location.

In addition, high nutrient concentrations and relatively lower DO values were detected near the bottom of the inner Saronikos Gulf, as the biochemical result of the oxidation of the organic matter which is carried by the wastewater effluents into the inner Saronikos Gulf. This was more prominent near the bottom of the stations located southwest and in a distance from the Psitallia sewage plant (~6–14 km), indicating that the organic matter which is carried by the wastewater plume followed the prevailing circulation and finally decomposed at a distance from the pipe, resulting in the DO decrease. It seems that there is a systematic variation pattern of the DO values throughout a year, with an increase during February–March, because of the homogenization of the water column and the oxygenation of the deep layers. The investigation of fecal sterols in the sediments (coprostanol values, coprostanol/cholesterol, and coprostanol/coprostanol + cholestanol ratios) confirms the sewage dispersion pathways. According to these results, although

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the whole area in a distance ~14 km from the outlet is contaminated by human wastes, the sediments in a direction southwest of Psittalia were more seriously affected than in the southeast direction (Pavlidou et al., 2014).

In accordance with this, the highest number of positive compound detections was observed at station S8. However, a relevant direct input of pharmaceuticals and other substances from the population living in the coastal areas of Aegina and Salamina Islands should also be considered. These remote populated areas, where no connection with a wastewater treatment plant is feasible, have no other option but to dispose their wastes in cesspits. Particularly in coastal areas, the compounds may escape from land and reach the seawater. Some compounds including citalopram, trimethoprim, paracetamol or theophylline showed maximum concentration levels at S8 stations. It draws attention the case of theophylline, which was not detected at S7, and supports the thesis of direct discharges from land.

The third most polluted sampling station regarding both concentration levels and number of detected compounds was S11. This station is located at 8.33 km from the WWTP (similar to S8) and also at a considerable distance from the Attica coast (5.58 km). At this sampling station some analytes detected at unusual high concentrations for such a location were amoxicillin, caffeine, paracetamol, amisulpride and diclofenac, with concentration levels up to 81.9 ng L^{-1}, 78.2 ng L^{-1}, 20.3 ng L^{-1}, 5.5 ng L^{-1} and 5.3 ng L^{-1}, respectively. These substances are widely consumed daily by a broad spectrum of the population. The main distinguishing characteristic of this sampling station is it is affected to a higher extent (along with S3) by emissions from passenger ships which travel from Athens to several Greek Islands. The passenger ship traffic in this area is very intense and it is remarkable that almost all the routes followed by ships go through this location.

The next sampling station in terms of pollution was S3. This sampling station is located very close to the land (0.5 km) and therefore very near to densely populated areas. However, apart from the loads of contaminants arriving from the WWTP discharges, it seems that the main source of contamination comes from *Keratsini*, which receives wastes from the ships. At this station, 16 compounds were detected with concentrations up to 40.3 ng L^{-1} in the case of caffeine and amoxicillin.

S43 sampling station seemed less polluted regarding the presence of pharmaceutical and drugs of abuse than the aforementioned stations. S43 showed a more similar profile in both concentration levels and number of detected analytes to the "remote" S16 station. S43 is located almost at the same distance from the WWTP than the stations S8 and S11, but with the difference that S43 is less affected from direct land inputs due to its location is in the center of Saronic Gulf.

The less polluted sampling station was S16. This fact was expected since it is located far away from the WWTP and at this point seawater is renewed with clean water of the Aegean Sea. Only caffeine and norvenlafaxine were detected at concentration levels of 5.2–6.7 ng L^{-1} and 0.05–0.06 ng L^{-1}, respectively.

S13 sampling station showed remarkable high levels for mefenamic acid (10.9 ng L^{-1}) and salicylic acid (up to 44.2 ng L^{-1}). S13 is located far from the WWTP (19.30 km) and at a significant distance from land (3.29 km). S13 may receive pharmaceutical inputs from untreated wastewater from the small populations living in the south of Salamina and in the north of Aegina, with a poor sewage management system.

Sampling stations located in Elefsis Bay, (S1 and S2), showed very similar pollution profiles. This fact seems reasonable since Elefsis Bay is a shallow, semi-closed gulf and seawater is renewed at a limited rate. Several compounds were detected including amoxicillin (37.7–51.6 ng L^{-1}), caffeine (12.6–31.5 ng L^{-1}), salicylic acid (22.4–53.3 ng L^{-1}) and diclofenac (5.2–16.3 ng L^{-1}). These stations are affected by shipping activity, industrial effluents and also by wastewater inputs from the populations located at the shores of the gulf.

No significant statistical correlation was found between the concentration levels of the analytes and the rest of the physicochemical parameters summarized in Table S2, except for the distance from Psittalia WWTP and the distance from the closest land.

Spearman's rank correlation tests revealed a strong negative correlation between the concentrations of most analytes and the distance of the sampling station from the WWTP and the distance from the land (Table S6). Absence of correlation does not imply any other phenomena (e.g. other sources), but it is simply the result of the high uncertainty of the low concentrations (values near LOQs) for the compounds with insignificant correlation with the distances.

It was found that some compounds correlated significantly, denoting a common source (i.e. WWTP). Absence of correlation does not imply different sources, but, again, it originates from the low concentrations observed (values near LOQs) and the high uncertainty of these concentrations.

3.3. Profile according to seawater depth

The distribution of the target compounds according to depth was evaluated. Patterns according to seawater depth can be found for analytes that occurred not only with high frequency of detection but also at high concentration levels. It is not possible to draw sound conclusions for compounds detected at low concentration levels, since these measurements have high uncertainty (Borecka et al., 2013). For this purpose, the major analytes in terms of frequency of detection and concentration levels, namely caffeine, amoxicillin and salicylic acid are included in Fig. 3.

In the case of the stations located in Elefsis Bay (S1 and S2), most compounds were detected at similar concentration levels in all the analyzed samples, showing a homogeneous distribution pattern, independent of the depth, since this time of the year the water column is fully homogenized. On one hand, in the sampling station located near the port of Piraeus in Keratsini (S3), most target substances including caffeine, amoxicillin and salicylic acid were detected at higher concentration levels in the bottom samples rather than at the sea surface. This trend was also observed at S8 and S11, although to a lesser extent and was related to the sewage dispersion. On the other hand, at the sampling station S13 analytes were detected at higher concentration levels in the surface layer. A similar trend was observed at the sampling station S43, located in the middle of the Saronikos Gulf, where a gradual increase in concentration levels from the bottom to the surface was observed. These facts can be explained by the location of the sampling stations. In December 2013, the vertical mixing of water column has reached almost ~60 m (Kontoyiannis, 2010). According to the data of % light transmission, it seems that some quantities of sewage were present at stations S7 (10–15 m and 65 m depth) and S8 (80 m), whereas, at all the other sampling stations the water column was fully homogenized (Kontoyiannis, 2014) and the sewages reached the surface layer due to their low density. The fact that at S8 some widely used compounds (lidocaine, norvenlafaxine and paracetamol) showed concentrations in surface water much higher than in any other analyzed sample may be related to direct inputs from land, as described in the previous section.

The station located near the WWTP (S7) showed no clear trend about the distribution of the evaluated compounds according to the depth. Amoxicillin's highest concentration was observed at S7-surface, while caffeine concentration at S7-bottom was two times the determined concentration at S7-surface. Moreover, the concentration of salicylic acid remained constant independent of the depth.

It is known that several processes may define the fate of the pharmaceuticals in the seawater column. The most important are microbially mediated degradation, sorption, chemical transformation, photodegradation and evaporation (Lahti et al., 2012). Photodegradation may play a role in the distribution of the analytes in the water column (Benotti and Brownawell, 2009). In general, the most refractory pharmaceuticals have half-lives of more than 35 days (Benotti and Brownawell, 2009). However, we assume that the vertical distribution

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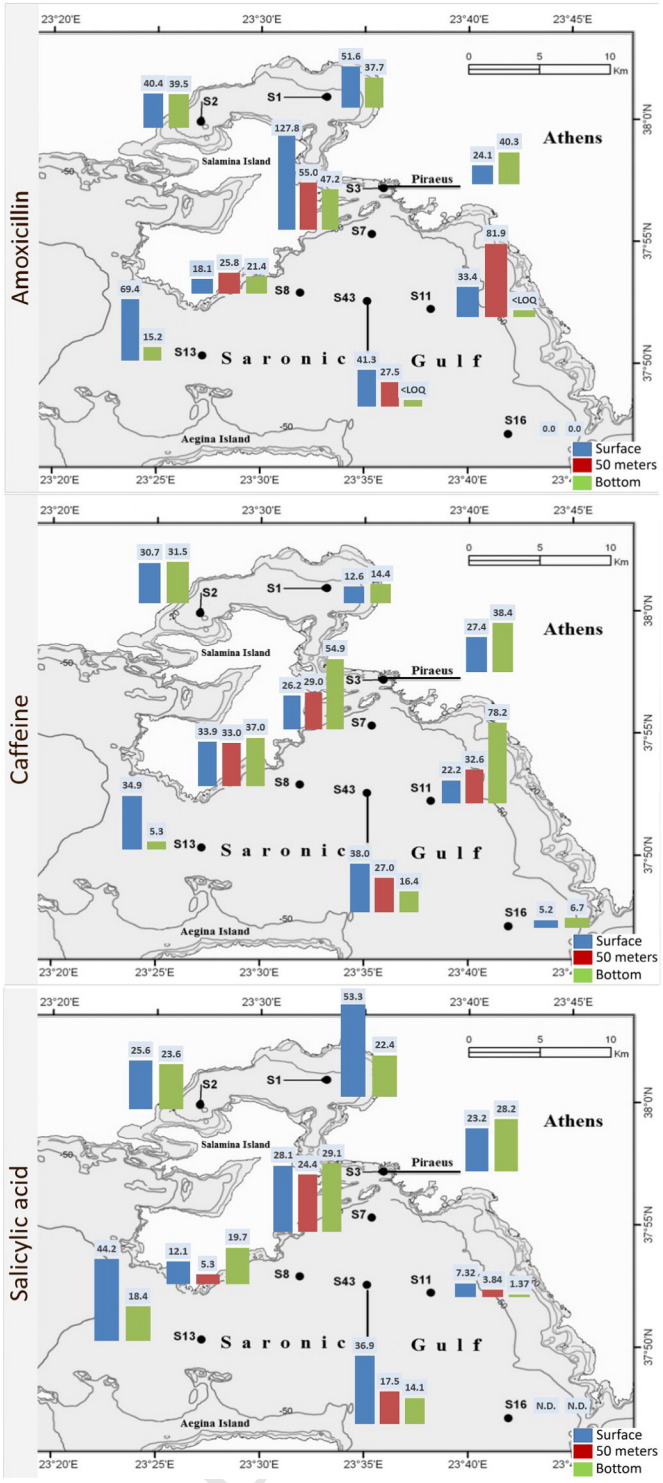

FIG. 3. Concentration levels detected at different depths for amoxicillin, caffeine and salicylic acid (concentrations in ng L^{-1}).

of the pharmaceuticals in the homogenized water column of Saronikos Gulf, where relatively high flow speeds prevail, is mainly controlled by the dispersion of the sewage plume from WWTP. Overall, though it is possible to observe some trends, due to the low number of compounds detected with a high frequency of detection and at high levels of concentration (the two factors together), it is difficult to draw sound conclusions with the available data.

3.4. Environmental risk assessment

Table S7 summarizes the toxicity data, the maximum measured concentration and the risk quotients. Risk quotients above 1 indicate potential environmental risk as already stated in various articles (e.g. (Thomaidi et al., 2015). The concentration levels of the compounds reported in this study cannot pose toxic effect either on fish or on daphnids. However, the maximum concentration level of amoxicillin and caffeine reported by this study can have a possible toxic effect on algae. The rest of the concentrations of the emerging pollutants cannot pose any risk on algae.

4. Conclusions

A large number of substances, including 158 pharmaceuticals belonging to several therapeutic groups and drugs of abuse, were investigated in offshore seawaters from the Saronicos Gulf, Greece. 38 substances were detected, being amoxicillin, salicylic acid and caffeine the ones with the highest levels, up to 128 ng L^{-1} in some cases. Treated wastewater release from the WWTP of Athens was clearly the main source of contamination for these waters. A negative correlation was found between the distance WWTP-sampling point and the concentration of the target analytes. The comparison of the levels previously detected for the compounds of interest in effluent wastewaters from this WWTP with the obtained results in the present study also highlights the effect of these discharges in seawater quality. However, pollution from shipping activity as well as direct inputs from land also proved to be important factors.

The distribution of the analytes according to the depth (3 different levels) was also assessed. Significant differences in the concentrations of several analytes at different depths were observed. Results suggest that the currents play an important role in the distribution of pollutants. However, it is hard to draw sound conclusions in this regard due to the few analytes present at high concentrations with a high frequency of detection.

The present study is the largest in terms of number of evaluated analytes and the first one analyzing samples at different depths. This study constitutes the first evidence of the presence of the substances tramadol, lidocaine, amoxicillin, amisulpride, niflumic acid, norvenlafaxine, EDDP, citalopram, sulpiride, valsartan, chlordiazepoxide, chlorpromazine, ephedrine, ronidazole, sulfamethizole, duloxetine and rivastigmine in seawater.

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Appendix A. Supplementary data

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