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Identification of emerging contaminants in greywater emitted from ships by a comprehensive LC-HRMS target and suspect screening approach *

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ABSTRACT

The increase in maritime traffic has led to substantial greywater discharges into the marine environment. Greywater, originating from sinks, showers, kitchen, and laundry facilities, contains a wide array of chemical contaminants influenced by on-board activities, ship size, and management practices. The lack of comprehensive regulations for greywater management, along with limited research on its chemical composition, highlights the need to characterize these waste streams. This study is one of the first to provide a comprehensive characterization of greywater samples from ships using advanced liquid chromatography coupled to high-resolution-mass-spectrometry (LC-HRMS) strategies, including wide-scope target and suspect screening. The target analysis detected 86 compounds, such as pharmaceuticals, stimulants, tobacco and food-related products, personal care products, UV filters, surfactants, perfluoroalkyl compounds, plasticizers, and flame retardants, many of which are rarely measured in routine monitoring programs. Furthermore, 11 additional compounds were tentatively identified through suspect screening. A novel scoring system further highlighted 25 priority compounds posing ecological risks to marine ecosystems, including pharmaceuticals such as tapentadol, dextrorphan, citalopram, or irbesartan. This study emphasizes the significant introduction of chemicals at μ g L⁻¹ levels through greywater discharges, underscoring the urgent need for improved management practices to mitigate ecological risks to the marine ecosystem.

1. Introduction

The shipping industry plays a crucial role in the global economy as it provides a cost-effective means of transporting large quantities of goods (Corbett, 2003). However, vessels also generate considerable volumes of diverse wastewater streams that can adversely affect the quality of marine environments. These ship-generated wastes include ballast water, used for maintaining stability, bilge water collected in the hull, scrubber water from exhaust cleaning systems, as well as sewage water and greywater produced by passengers and crew members. Among all these wastewater streams, greywater, comprising wastewater from dishwashing, showers, laundry, and washbasins, accounts for a substantial portion of the total volume of wastewater emitted from ships (USEPA, 2008; Vaneeckhaute and Fazli, 2020). Additionally, greywater contains a complex mixture of emerging contaminants, which may pose potential threats to marine ecosystems (Noman et al., 2019).

To mitigate the impact of ship emissions, the International Maritime Organization (IMO) has established regulations under the IMO Convention MARPOL 73/78, detailed in Annexes I-VI, to reduce pollution from international shipping. Annex IV specifically regulates the discharge of domestic waste from ships into the sea, but focuses only on sewage, leaving greywater unregulated. This regulatory gap has resulted

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in diverse management practices across the shipping industry. Companies with strong environmental commitments often invest in advanced treatment technologies or store greywater for proper disposal at port facilities, while others discharge it directly into the sea, adhering to certain distance limits. This lack of standardized regulation is sometimes addressed by specific geographic regions with their own national regulations. For instance, the Norwegian Maritime Authority (NMA, 2019) regulates greywater discharges in Norway, while in the United States, the Environmental Protection Agency (USEPA, 2013) enforces strict guidelines. However, a more thorough understanding of the chemical composition and potential hazards of greywater discharges into the sea is essential to support stronger legislation and ensure proper disposal practices.

The management of greywater discharges from ships is a critical environmental issue that has attracted growing concern due to its potential impact on marine ecosystems. Although obtaining precise data on greywater volumes discharged into the sea or onto shore is challenging, it is estimated that around 5.5 million m³ are annually released into the Baltic Sea (Ytreberg et al., 2020). Greywater often contains substantial amounts of nitrogen (N) and phosphorus (P), key nutrients that contribute to eutrophication, leading to harmful algal blooms, oxvgen depletion, and disruptions to aquatic biodiversity. Studies in the Baltic Sea have documented eutrophication effects driven by nutrient inputs from various sources, including greywater discharges (Kalnina et al., 2021; Ytreberg et al., 2020). Research specifically addressing the chemical composition of ship-generated greywater is limited. However, some studies indicate that it may be a potential source of microplastics (Jang et al., 2024; Peng et al., 2022). Existing research has primarily focused on sewage water from cruise ships, revealing the presence of various contaminants such as pharmaceuticals, personal care products, polycyclic aromatic hydrocarbons (PAHs) and pesticides (Vicente-Cera et al., 2019). Notably, some of these contaminants were found at concentrations comparable to or even higher than those in urban wastewater effluents, highlighting the need for greywater treatment to reduce contaminants to non-toxic levels (Berardi et al., 2019; Parida et al., 2021). The difference in contaminants concentration is mainly attributed to water-saving measures implemented on ships, leading to more concentrated wastewater compared to land-based equivalents (Vaneeckhaute and Fazli, 2020). The release of these higher concentrations can exert some potential toxic effects on the marine environment, including synergistic interactions that amplify their ecological impact (Syberg et al., 2017).

Concerning the analytical techniques used for a holistic characterization of greywater effluents, methods based on high-resolution-massspectrometry (HRMS) coupled with liquid chromatography (LC) are robust and highly effective tools. A key advantage of HRMS is its ability to identify a vast number of chemical contaminants even without analytical reference standards, by using suspect lists with the exact mass for hundreds or thousands of contaminants, or mass defect analysis tools (Myers et al., 2014; Samanipour et al., 2018). The identification process is further refined by incorporating additional criteria like isotopic patterns, fragment ions and plausible chromatographic retention times (Gago-Ferrero et al., 2015). However, full identification and quantification can be challenging to the unavailability of reference standards for every compound. To overcome these limitations, new computational tools have emerged, including chromatography retention time prediction models (Goryński et al., 2013; Kaliszan, 2007) and predicted ionization efficiency tools (Aalizadeh et al., 2021), which are increasingly employed in the evaluation of emerging contaminants (Castaño-Ortiz et al., 2024; Gutiérrez-Martín et al., 2023b; Kalogiouri et al., 2017; Malm et al., 2021; Tadić et al., 2022).

The main objective of this study was to characterize, for the first time, the chemical composition of greywaters discharged from ships using LC-HRMS-based methods, by integrating wide-scope target and suspect screening analytical approaches. Additionally, a prioritization strategy was developed to identify key contaminants that could pose significant risks to marine ecosystems, considering several factors such as concentration levels, ecotoxicity data, bioaccumulation potential, and estimated persistence in water.

2. Materials and method

2.1. Chemicals and reagents

All analytical standards, including the isotopically labelled standards (ILS) were purchased from Merck, LGC Standards (Barcelona, Spain) and Sigma Aldrich (Steinheim, Germany) with high purity grade (<98 %) and are summarized in Table S1 (SI-1). More details are included in section SI-1.

2.2. Sample collection and preparation

Five greywater samples were collected in May 2022 by the Baltic Sea Action Group (BSAG) from cargo ships that were docked at Helsinki port, Finland. Greywater management in these ships entails combining all waste streams from the laundry, kitchen, and accommodations into onboard tanks and subsequently discharging the greywater into port facilities upon docking. The collected greywater exhibited pH values ranging from 5.0 to 8.7, the concentration for total suspended solid (TSS) was between 120 and 760 mg L⁻¹, total nitrogen (TN) concentrations were from 53 to 730 mg L⁻¹, total phosphorous (TP) ranged from 8.8 to 56 mg L⁻¹, chemical oxygen demand (COD) from 480 to 2200 mg L⁻¹, biochemical oxygen demand (BOD5) from 430 to 880 mgL⁻¹, and fecal coliforms from $3.3 \cdot 10^5$ and $2.3 \cdot 10^7$ pmy 100 mL⁻¹, as detailed in Table S2 (SI-2).

Samples were taken in 1L amber glass bottles during the discharge phase into the port facilities and stored at 4 °C, and shipped to ICRA, where they remained stored at -20 °C until sample analysis. Samples were filtered, the pH was adjusted to 7, and 200 mL were fortified with ILS as surrogate standards prior to pre-concentrated by solid phase extraction (SPE). More details are in section **SI-2**, together with the quality control and assurance data.

2.3. Instrumental analysis

The samples were analyzed using a Vanquish ultra-high-performance liquid chromatography (UHPLC) system (Thermo) coupled to a HRMS Orbitrap Exploris 120 (Thermo Fisher Scientific), with an electrospray ionization (ESI) source, using the method developed by Gutiérrez-Martín et al. (2023a). Chromatography separation was achieved using a Cortecs C18⁺ column (2.1 × 100 mm, 2.7 µm) with a Cortecs C18 VanGuard cartridge (2.1 × 5 mm, 2.7 µm), by Waters Corporation (Milford, MA, USA). HRMS data was acquired using two different approaches: a) All Ion Fragmentation (AIF) method, which fragments all precursor ions, and b) Data Dependent Acquisition (DDA), which targets the most intense precursor ions before fragmentation to obtain cleaner MS/MS spectra. Additional instrumental conditions are provided in section SI-3.

2.4. Identification of contaminants

To identify emerging contaminants, two different approaches were used: a) wide-scope target screening, using an extensive database containing 788 compounds, and b) suspect screening, using a database of over 10,000 compounds. These databases encompass various chemical classes, such as pesticides, pharmaceuticals, industrial compounds, surfactants, etc. Further details of these identification methods are provided in the following sections.

2.4.1. Wide-scope target screening

For the target screening of greywater samples, an in-house database of 788 compounds, developed by Gutiérrez-Martín et al. (2023a), was

used. The database includes information on the molecular formulas for each compound, the exact mass of the precursor ions, their retention time (RT) under these specific chromatographic conditions and relevant fragments, providing a comprehensive identification profile for each compound. This detailed information facilitates accurate identification and quantification during the screening process.

The raw data obtained from the AIF acquisition were processed with TraceFinder 4.1 (Thermo Scientific) software. This program employs an algorithm that considers the exact masses and the RTs of the chromatographic peaks in the full scan mass spectra and compares them against a database containing compound-specific information for 582 compounds in positive ionization and 206 in negative. Each detected peak corresponding to a target compound was evaluated according to predefined parameters set in the method, including mass accuracy, RT match and isotopic fitting (Fig. 1). Extracted Ion Chromatograms (EIC) of the precursor ion were created within a mass error window of ± 5 ppm and a RT tolerance of 0.3 min. A minimum peak intensity of 10⁵ was selected as positive with a minimum of 5 points per peak. To enhance the reliability of the results, the isotope match feature was enabled with a threshold setting of 70%. However, this value was only considered as a positive confirmation and not for rejecting the occurrence of compounds, because strong matrix effects combined with low concentration levels of analytes may distort the isotopic pattern results. In addition, the appearance of at least the most intense fragment ion in the high energy function of the AIF data was required for confirming their identity.

Procedural blank filtering was then conducted to eliminate



Fig. 1. Screening and prioritization Target Workflow.

compounds with intensity areas that were not at least three times higher than those found in the blanks. The process involved a thorough manual evaluation for each compound in all samples, aiming to ensure accurate results and minimize false positive detections.

2.4.2. Suspect screening

To conduct suspect screening, samples were analyzed with Compound Discoverer 3.1 software, utilizing raw data acquired from the DDA mode. A default workflow named "Environmental w Stats Unkonwn ID w Online and Local Database Searches" was selected and modified according to the following criteria: a) mass range selection from m/z 100-1000 and RT from 0.7 to 17 min, b) chromatographic alignment with a mass tolerance of ± 5 ppm and a RT tolerance of 0.3 min, c) detection of unknown compounds with a mass tolerance of ± 5 ppm, minimum peak intensity of 10^6 and a minimum signal to noise ratio of 10, and d) grouping features detected at a mass tolerance of \pm 5 ppm and 0.3 min. Then, the identification of tentative compounds after data treatment was made by comparing the experimental and theoretical accurate masses and MS/MS ion spectra using the software-linked libraries mzCloud and Chemspider. To assess the plausibility of the suspect compounds identified, their chromatographic RTs were estimated using a Quantitative Structure-Retention Relationship (OSRR) tool, QSRR Automator (Naylor et al., 2020), and then compared to the measured RTs. A QSRR model was built using a training dataset that included compound names, the structures in form of Simplified Molecular Input Line Entry System (SMILES) and the experimental RTs from available reference standards included in the in-house database. The validated model, based on linear regression, achieved a mean absolute error of 0.14 and a coefficient of determinator (r^2) of 0.990 (Fig. S1, SI-4). A RT threshold of ± 2.8 min, based on the tool's observed error margin, was also established for the RT matching criteria. Only the compounds showing a good match (score >70%) between the experimental and theoretical spectra, confirmed through visual inspection, and RTs falling within the threshold of ± 2.8 min, were considered as tentatively identified. Detailed information regarding the QSRR model is provided in section SI-4.

The confidence in compound identification in this study followed the recently introduced Identification Points (IP) system for non-target screening methods established by Alygizakis et al. (2023). The primary identification criteria comprise accurate mass determination of the parent ion with a plausible RT (0.15 IP), a favorable isotopic fit (0.20 IP), and complete presence of all fragments within a high-quality library (0.40 IP). Penalties are imposed when only AIF data is available (-0.10)IP) and if the database for other experimental fragments, excluding the most intense ion, contains two or less fragments (-0.10 IP). To enhance clarity and consistency in compound identification, this IP system has been aligned with the widely utilized five-level scheme established by Schymanski et al. (2015). According to this scheme, level 1 (confirmed identification) requires an IP score exceeding 0.75, which can only be achieved using analytical standard. Identifications classified under level 2 (probable structure) fall within the IP score range of 0.6–0.75, while level 3 (tentative identification) corresponds to an IP score from 0.50 to 0.60. For level 4 (unequivocal molecular formula) identification, the score should be below 0.5 and higher or equal to 0.2. Finally, any identification receiving below 0.20 IP is categorized as level 5 (exact mass) identifications.

2.5. Quality assurance, quality control and quantification

Quality parameters of the analytical method were evaluated to ensure the reliability and applicability of the method to the analysis of greywater in terms of extraction recoveries, matrix effects and method detection and quantification limits. Conducting a detailed evaluation and analytical validation of the performance of all 788 compounds is impractical as it would be an extremely time-consuming task. Therefore, a subset of 88 compounds was selected, representing relevant compounds from various chemical groups analyzed, such as pharmaceuticals, personal care products, industrial chemicals among others, as listed in Table S1 (SI-1). Recovery experiments were conducted using a pool of various greywater samples, spiking with a standard mixture at a concentration level of 500 ng L⁻¹ in initial sample (200 µg L⁻¹ in extract). The precision was expressed in terms of relative standard deviation (% RSD). Six-point matrix-matched calibration curve (1–250 µg L⁻¹), and a calibration in solvent, were prepared to evaluate method linearity, matrix effect, recoveries and estimate compound concentrations. The limits of quantification (LOQs) were identified as the lowest concentration where a peak was observed on the matrix-matched calibration curve, corresponding to a signal-to-noise ratio of ≥10. The limits of detection (LODs) were estimated as 3/10 of the LOQ values. More details are provided in section SI-5.

For the instrumental performance, the Orbitrap was calibrated for mass accuracy on the day of analysis for both positive and negative ionization, following the manufacturer's guidelines. The presence of ILS was evaluated in all the injected samples to ensure the optimal performance of the LC-HRMS system. Calibration standards were measured at the beginning and the end of each sequence, and one calibration standard was measured repeatedly every 10–15 injections, as a quality control standard, to check for signal stability.

To estimate the concentrations of the compounds detected in the target screening, various approaches were used. Specifically, the compounds included in the calibration curves were quantified using both matrix-matched calibration and internal standard calibration curves. Concentrations were further refined by adjusting recovery values from spiked greywater samples when necessary. Conversely, for the compounds not included in the calibration curves, a semi-quantification approach was implemented due to the absence of analytical standards. The semi-quantification tool applies a quantitative structure-ionization relationship (QSIR)-based model developed by Aalizadeh et al. (2022) to predict the ionization efficiency (IE) of unknow compounds analyzed using LC-HRMS. This model derives experimental log IE values from individual calibration curves relative to a reference compound. To harmonize log IE values within the greywater matrix, matrix-matched calibration curves were utilized to account for variations induced by the matrix and background ions, utilizing the open platform http://tr ams.chem.uoa.gr/semiquantification. Dimethomorph and sulfadimethoxine were employed as reference compounds for positive and negative ionization modes, respectively, yielding a slope of 2.3.10⁴ and $3.2 \cdot 10^4$. The accuracy of the prediction model was assessed by comparing the concentration ratios of spiked samples using matrix-matched calibration (quantitative value) and those estimated by the tool (semiquantitative values), with more detailed information in SI-5.

2.6. Prioritization strategy

To highlight the most ecologically relevant contaminants, a prioritization strategy was applied, adapted from the ones described in Čelić et al. (2019) and Gros et al. (2017). A scoring system was implemented to the set of contaminants detected in greywater, including the following criteria: a) the maximum concentration detected in the samples defined the profile, using PNEC values as reference points; b) ecotoxicity data, based on the hazard quotient (HQs) ratios and (c) bioconcentration factors, as indicators for potential risks to marine biota and d) their estimated persistency in water (half-lives in days), as indicated in Table 1.

HQ were estimated as the ratio between the highest measured concentration in all the samples and the Predicted No-Effect Concentration (PNEC) values. In this study, priority was given to experimental PNEC data, focusing on chronic toxicity over acute toxicity data in marine water, and used provisional-PNEC (P-PNEC) when experimental data were unavailable. P-PNEC was derived through QSAR (Quantitative Structure-Activity Relationship) toxicity prediction models. All PNEC Table 1

Criteria and scoring system for prioritization of identified substances in greywater.

Criteria	Score				
	1	2	3	4	
a) Max detected conc.	${<}1~\mu g~L^{-1}$	$>\!\!1~\mu g~L^{-1}$	$>\!50~\mu g~L^{-1}$	${>}200~\mu g~L^{-1}$	
b) HQ = MC/PNEC	\leq 0.01	≥ 0.01	≥ 0.1	≥ 1	
c) BCF	<10	>10	>100	>1000	
d) $t_{1/2}$ in water (days)	<15	$\geq \! 15$	\geq 37.5	≥ 60	

values, including P-PNEC, were obtained from the NORMAN Ecotoxicology Database (NORMAN Database System, Commission, 2003). BCFs and $t_{1/2}$ values were obtained from ChemSpider database.

According to Table 1, each compound was given a score value (1–4) in each category (a-d). Compounds with the highest total score values were proposed as the most ecologically relevant and that could pose a potential threat to the marine environment.

3. Results and discussion

3.1. Method performance evaluation in greywater

The performance of the analytical method was evaluated through an examination of quality parameters within the greywater matrix. Linearity was studied in both solvent and matrix-matched calibration curves and used for the compound's validation dataset. For 88% of the compounds, r² for the matrix calibration curves exceeded 0.99, and consistently surpassed 0.98 in all cases. Linearity was observed in the range of LOQ-250 μ g L⁻¹. The LOQs for most compounds were between 5 and 50 μ g L⁻¹. Accuracy and precision were evaluated through recovery experiments involving spiked samples at a concentration level of 500 ng L^{-1} . Acceptable recovery rates were observed for 70 out of the 88 compounds analyzed, falling within the range of 55-120%. Precision was consistently below 20% in all cases, except for UV-9 (25%). The matrix effect values ranged from approximately -30% to -80%, leading to signal suppression due to the high complexity of the matrix. The specific values of LOQs, precision, accuracy, and matrix effect for each compound of the validation dataset are presented in Table S3 (SI-5).

Additionally, the potential of the semi-quantification tool is shown in Fig. 2. This figure depicts the distribution of concentration ratios between semiquantitative and quantitative values for each compound in spiked samples, represented as individual data points. The area of the violin plot indicates the density of data points within each concentration range. The results show reliable estimates, with 83% of the compounds having semi-quantitative values within an order of magnitude higher



Fig. 2. Variation between semiquantitative calculated concentration and calibration curve calculated concentration. Positive values indicate overestimation while negative values indicate underestimation.

and lower of the real value, and 67% within a range of $\pm 3x$. This level of accuracy is considered acceptable given the variability inherent in these tools, certifying the accurate approximations of the compounds' qualitative concentrations in ship-generated greywater samples. Additionally, recoveries were assumed to be 100% in the semi-quantification strategy since approximately 70% of validated compounds exhibited satisfactory recoveries (>70%).

3.2. Wide-scope screening of organic contaminants in greywater emitted from ships

A total of 86 target organic compounds were identified in at least one of the evaluated greywater samples, as detailed in Table S4 (SI-6). Among these compounds, 42 were fully validated and quantified using analytical standards. The remaining 44 target compounds, identified through their experimental RTs and fragmentation patterns, were semiquantified by predicting their concentrations using the QSIR model. Individual concentrations for all detected compounds can be found in Table S4 (SI-6), indicating the compounds quantified with standards and those with the QSIR model.

Fig. 3 illustrates the contribution of each chemical group, expressed as a percentage, to the total concentration of all detected compounds, in each ship analyzed. In the distribution pattern within greywater, stimulants (caffeine and its metabolites) emerge as the most significant compound group in terms of concentration. They are followed by tobacco and food-related chemicals, and then by surfactants and pharmaceuticals. While all ships exhibit a diverse chemical profile with some shared similarities, ship number 3 stands out as the most distinct in terms of chemical composition. In this case, tobacco-related chemicals dominate as the most abundant compound group, and pharmaceuticals make a substantial contribution to the total concentration. This variability underscores the ship-specific patterns in the prevalence of distinct chemical groups, which could be attributed to factors such as ship size, the volume of greywater produced, crew size, among other operational variables.

For the predominant chemical groups, including stimulants, tobaccorelated chemicals, and food-related chemicals, a total of 13 compounds were detected within a concentration range spanning from ng L^{-1} to mg L^{-1} . All compounds, except nicotine, were semi-quantified. Within the stimulant category, caffeine, and its metabolites (theobromine,

theophylline and paraxanthine) manifest consistent presence across all samples. Caffeine notably exhibited the highest concentration in Ship 2, 4 and 5 ranging from 1886 to 2678 μ g L⁻¹, surpassing the levels of any other detected compound. A similar pattern was observed in another study where urban greywater from residential areas in Australia was studied, although the maximum concentrations there were slightly lower (Turner et al., 2019). Moreover, significant concentrations for piperine, an alkaloid compound naturally occurring in black pepper, were also detected in the same samples, with levels ranging from 268 to $426 \ \mu g \ L^{-1}$. Comparative studies have shown piperine concentrations in wastewater samples ranging from 22 to 2020 ng L^{-1} (Wang et al., 2020), and up to 338 ng L^{-1} in river water (Nanusha et al., 2020). These findings underscore the potential ubiquity and variability of piperine levels in aquatic environments, despite not being frequently measured in routine monitoring studies. Distinctively, cotinine, recognized as another potential marker for domestic wastewater contamination, was identified in samples originating from Ship 1 and 3 at concentrations of 45 $\mu g \; L^{-1}$ and 91 $\mu g \; L^{-1},$ respectively. This compound, a metabolite of nicotine, is commonly used as an indicator of tobacco smoke exposure (Haufroid and Lison, 1998). Furthermore, alongside cotinine, nicotine itself and its derivative, 3-hydroxycotinine, were also detected at lower concentrations ranging from 4 to 22 μ g L⁻¹. The detected concentrations of nicotine and 3-hydroxycotinine were consistent with typical levels found in untreated wastewaters, ranging approximately from 1 to 10 µg L^{-1} (Buerge et al., 2008; Gago-Ferrero et al., 2020; Senta et al., 2015). However, the concentrations of cotinine were notably higher, even comparable to the highest concentrations found in Spanish wastewater, reaching up to $42 \ \mu g \ L^{-1}$ (Huerta-Fontela et al., 2008).

The category of pharmaceuticals and their metabolites emerged as the chemical group with the highest number of compounds detected, encompassing 30 identified compounds, primarily in samples from ships 1 and 3. Among them, analgesic/anti-inflammatories and antihypertensives were the most ubiquitous subclasses. Notably, tapentadol, an opioid analgesic, exhibited the highest concentration among the analgesic/anti-inflammatories category, registering its maximum concentration at 55 μ g L⁻¹. This compound was semi-quantified and consistently detected across all samples, although, in general, at lower concentrations, ranging from 1 to 2 μ g L⁻¹. Although a few studies have detected tapentadol in surface water, effluent wastewater (Campos-Mañas et al., 2019) and hospital wastewater at ng L⁻¹



Fig. 3. Contribution of each compound group to the total concentration across the sample types, with expressed as the percentage of its concentration relative to the total concentration of all detected chemicals.

(Arvaniti et al., 2023), this compound is not routinely monitored in target analytical methods. Indeed, the methods used for its identification in the cited studies rely on HRMS, highlighting their potential for detecting previously un-recognized contaminants that go beyond routine target methodologies. Conversely, within the antihypertensive subclass, telmisartan was semi-quantified and recorded the highest concentration at 15 μ g L⁻¹, exclusively detected in Ship 3. However, other antihypertensive agents such as losartan (0.1–6.3 μ g L⁻¹) and valsartan (0.3–2.2 μ g L⁻¹), as well as the diuretic compound hydrochlorothiazide (0.1–4.3 μ g L⁻¹), were detected in samples from at least three different ships. These concentrations align closely with those found in wastewater effluents (Alygizakis et al., 2019; Gago-Ferrero et al., 2020; Gros et al., 2017), except for telmisartan, whose concentration resembled that found in hospital wastewater, reaching up to 10 μ g L⁻¹ (Arvaniti et al., 2023). The presence of pharmaceuticals and their metabolites in the greywater samples was attributed to either excretion via urine in shower facilities, a potential mixture with sewage water, or disposal through the sink.

Surfactants and personal care products (PCPs) constituted another ubiquitous chemical class across all samples, with notable concentrations observed particularly in samples obtained from Ship 2, 4 and 5. Generally, PCPs were discerned within a concentration range of 0.1–1.6 μ g L⁻¹. This category includes compounds such as propylparaben and UV filters being benzophenone-4 and ensulizole the most prevalent compounds. Interestingly, while propylparaben has been recurrently detected at comparable concentrations in effluents from greywater systems and wastewater treatment plants (Archer et al., 2017; Hernández Leal et al., 2010; O'Malley et al., 2020), ensulizole has been rarely reported, particularly in LC-MS based studies. Lauryl diethanolamide, polyethylene glycols (PEGs) and polypropylene glycols (PPGs) are typical constituents in many cosmetics products. However, while they are not typically included in most targeted analytical methods, they are often incorporated into several suspect list for their detection by HRMS. These compounds were detected and semi-quantified over a concentration range from 1 to 50 μ g L⁻¹. The consistent presence of these compounds across all samples highlights their ubiquitous nature in greywater streams. Their occurrence may originate from various sources, including wash-off from skin and clothing as well as absorption into the body and subsequent excretion (Liao and Kannan, 2014).

A total of 27 compounds were identified as industrial chemicals, flame retardants, plasticizers, PFASs and other compounds utilized in diverse industrial applications. Among these groups, the highest concentrations were observed in the industrial chemicals and flame retardants categories. Industrial chemicals, predominantly found in Ships 1 and 3, mostly correspond to the corrosion inhibitors 8-Hydroxiquinolone (8-HQ), detected and semi-quantified at concentrations of 39 and 114 μ g L⁻¹. These corrosion inhibitors are commonly employed in various structural components of ships and marine structures to protect metal surfaces from deterioration, particularly within piping systems. For flame retardants, tributyl phosphate (TBP) was the predominant compound and semi-quantified, registering the highest concentration at $37~\mu g~L^{-1}$ in Ship 1. This compound is commonly encountered in materials or products like cleaning agents, degreasers, or other maintenance chemicals. However, its presence in wastewater is contingent upon specific operational practices and on onboard activities, which may explain why it was only detected in one sample. Moreover, tris(2chloroethyl) phosphate (TCIPP), tris(2-butoxyethyl) phosphate (TBOEP) and tris(2-chloroethyl) phosphate (TCEP) were consistently detected across most of the samples, albeit at lower concentrations ranging from 0.5 to $10 \,\mu g \, L^{-1}$, with a noticeable predominance observed in Ship 1. These compounds serve as plasticizers and find wide application in electronic and furniture items, thus contributing to their presence in household greywater, although typically at concentrations up to 1 µg L⁻¹ (Gros et al., 2017; Zraunig et al., 2019). Furthermore, bis (2-butoxyethyl) 2-hydroxyethyl phosphate was identified at the highest concentration in Ship 1. This compound has been recognized as a

significant metabolite derived from TCIPP and TBOEP (Van den Eede et al., 2013), elucidating its heightened presence in the same ship alongside its precursors.

Regarding plasticizers, their presence was evident in samples originating from Ships 1 and 3, with bisphenol S (BPS) and monobutyl phthalates emerging as the most representative compound at low levels. BPS has been introduced as a safer alternative to bisphenol A (BPA), which has been banned by regulatory agencies such as the European Commission and US Food and Drug Administrations, for use in plastics and as a preservative in canned foodstuffs. However, BPS has recently been detected in various environmental matrices, including surface water, sewage sludge, and urine samples, due to its high stability and widespread use as the primary BPA substitutes. Emerging evidence indicates that BPS may have similar endocrine-disrupting effects to BPA, raising concerns about its potential risks to ecosystems and human health (Qiu et al., 2019, 2018; Thoene et al., 2020).

Similarly, monobutyl phthalates, which are common metabolites from short-chain phthalates used in various products such as PPCs, detergents, paints, and adhesives, have been found at levels of ng L⁻¹ (Gutiérrez-Martín et al., 2023a; Jin and Zhu, 2016). Lastly, perfluorooctanoic acid (PFOA) was the only PFAs detected, observed in Ship 1 at a concentration of 3 ng L⁻¹. Gros et al. (2017) found PFOA between PFAs with the highest concentrations in urban greywater, at 8 ng L⁻¹.

3.3. Expanding the chemical space by suspect screening

A suspect screening approach was implemented to expand the identification of compounds beyond those included in the in-house database. With this approach, 19 additional compounds were identified. To further increase confidence in compound identification, their chromatographic RTs were estimated using the QSRR Automator, and some of them, were outside the established RT threshold of ± 2.8 min. Thus, they were discarded for positive identification, reducing the suspect hits to 14 compounds. According to the IP systems presented by Alygizakis et al. (2023), from these 14, a total of 11 suspects were identified at level 2 (IP score range of 0.6–0.75), while the other three fell under level 3 or less. Thus, only the suspects showing the highest identification level were considered and are listed in Table 2.

These newly identified compounds included stimulants, among which were rarely monitored caffeine metabolites such as 1-methylxanthine and other lesser-known derivatives like cyclo(phenylalanyl-prolyl) and 1,3,7-trimethyluric acid (Martínez-López et al., 2014; Rothwell et al., 2019). These compounds were present in all samples, except in ship 3, where caffeine concentrations were very relevant. Another interesting and rarely monitored compound found in all samples was 4-methyl-5-thiazoleethanol, which contributes to the flavour and aroma of food products. Its presence in greywater samples could be attributed to cleaning activities of containers that held food products.

Other identified compounds included PEG-like surfactants (n 6-8). Their presence is very likely as PEG n5 was found in all samples using the wide-scope target screening approach. The presence of PEGs in urban and industrial wastewater is common because they are widely used in various industries such as pharmaceutical, cosmetics and industrial cleaning due to its solubility, low toxicity and versatile properties (Eriksson et al., 2003). Moreover, other relevant compounds were sodium [dodecanoyl(methyl)amino] acetate and quadrol, which are with detergents, in Ship 1. Additionally, associated N, N-Diethylethanolamine, an industrial, surfactant and detergent related chemical, was found in Ship 1 and 3, where the majority of industrial chemicals were detected. This compound finds application in various industrial processes such as chemical manufacturing, the textile industry, and the production of personal care products. Lastly, the pharmaceutical valpromide was detected in ships 1 and 5. All these cited compounds are not typically included in routine monitoring programs, thus highlighting the potential of suspect screening methodologies for

Table 2

List of suspect compounds detected in greywater samples.

Name	Group	Molecular Formula	Reference Ion	m/z (expected)	Δ Mass (ppm)	RT (min)	RT (min) predicted	IP score ^a	Confidence level ^b
4-Methyl-5-thiazoleethanol	Food-related chemical	C ₆ H ₉ NOS	$[M+H]^{+1}$	144.0482	3.1201	1.81	2.02	0.65	2
N,N-Diethylethanolamine	Industrial chemical	C ₆ H ₁₅ NO	$[M+H]^{+1}$	118.1229	2.1539	0.69	1.46	0.65	2
Valpromide	Pharmaceutical	C ₈ H ₁₇ NO	$[M+H]^{+1}$	144.1386	2.3760	6.63	5.52	0.65	2
1-Methylxanthine	Stimulant (caffeine metabolite)	$\mathrm{C_6H_6N_4O_2}$	$[M+H]^{+1}$	167.0569	3.2484	1.90	1.05	0.63	2
Cyclo(phenylalanyl-prolyl)	Stimulant (caffeine metabolite)	$C_{14}H_{16}N_2O_2$	$[M+H]^{+1}$	245.1292	2.9873	4.73	2.86	0.75	2
1,3,7-Trimethyluric acid	Stimulant (caffeine metabolite)	$C_8H_{10}N_4O_3$	[M-H] ⁻¹	209.0679	-0.3614	2.02	0.34	0.75	2
PEG n6	Surfactant	C12H26O7	$[M+H]^{+1}$	283.1761	3.2562	2.98	4.35	0.75	2
PEG n7	Surfactant	$C_{14}H_{30}O_8$	$[M + NH_4]^{+1}$	344.2288	2.8378	3.36	4.87	0.75	2
PEG n8	Surfactant	C16H34O9	$[M+H]^{+1}$	371.2285	2.7709	3.68	5.15	0.75	2
Sodium [dodecanoyl (methyl)amino]acetate	Surfactant	$C_{15}H_{29}NO_3$	$[M+H]^{+1}$	272.2225	1.7150	9.23	9.08	0.75	2
Quadrol	Surfactant	$C_{14}H_{32}N_2O_4$	$[M+H]^{+1}$	293.2441	2.0376	0.74	-1.38	0.75	2

^a According to Alygizakis et al. (2023).

^b According to Schymanski et al. (2015).

identifying relevant and compounds that are not frequently tracked by routine target methods.

3.4. Environmental implications of greywater discharges into marine ecosystems

Greywater constitutes a complex mixture of contaminants that may pose significant risks to marine ecosystems when discharged directly into the marine environment. The release of greywater from ships contributes to nutrient pollution, chemical contamination, and microbial pollution, which can lead to eutrophication, toxicity, and the spread of pathogens. Chemical contamination, particularly from emerging contaminants, is of growing concern due to its potential to cause toxicity, bioaccumulation, endocrine disruption, and promote antibiotic resistance in marine organisms, among other negative effects. To identify the most remarkable contaminants that may pose a major risk to the marine environment, a prioritization strategy was implemented, as described in section 2.6. It is important to note that the prioritization process does not account for the potential dilution of greywater once it is discharged into the marine environment, as it is difficult to estimate due to varying environmental factors and conditions. Thus, the estimated risks are based on worst-case scenario of no dilution, which may slightly overestimate the actual ecological impact in marine environments.

According to Table 1, each compound underwent an assessment, receiving a score ranging from 1 to 4 for each category, which contributed to its total score value. Compounds with a total score equal to or higher than 10 were selected as priority contaminants to establish their potential environmental significance (Table 3). The primary criteria with the highest weight in the ranking were the HQs, as they were relatively high, due to the low PNEC values, and the high persistence values. Additionally, the presence of high concentrations, as seen with compounds like caffeine, piperine and tapentadol also significantly influenced in the ranking.

Out of the 25 compounds highlighted as priority contaminants, 12 were pharmaceuticals. Among them, the analgesic tapentadol, the antitussive dextromethorphan and its metabolite dextrorphan were identified as the top priority compounds. Moreover, antidepressants such as carbamazepine, citalopram, and mirtazapine, along with the antihypertensive drugs irbesartan, olmesartan, amlodipine and losartan, caffeine and tobacco related products (i.e. cotinine) were included in the priority list. Some of these compounds, like carbamazepine, caffeine and nicotine related compounds are well-known markers of wastewater contamination. Nevertheless, other compounds such as tapentadol, dextromethorphan, dextrorphan and mirtazapine are rarely monitored

able 3	
riority contaminants based on the scoring system used (see Table 2)	1.

Compound	Compound group	Total Score
Tapentadol**	Analgesic	13
Piperine**	Food-related chemical	13
Dextrorphan**	Antitussive (metabolite)	13
Dextromethorphan**	Antitussive	12
Carbamazepine	Antidepressant	11
Citalopram*	Antidepressant	11
Mirtazapine**	Antidepressant	11
Irbesartan*	Antihypertensive	11
Cetirizine**	Antihistaminic	11
TCEP*	Flame retardant	11
Lauryl diethanolamide*	Personal care product	11
Caffeine**	Stimulant	11
Cotinine**	Tobacco-related chemical	11
Olmesartan**	Antihypertensive	10
Amlodipine*	Antihypertensive	10
Losartan*	Antihypertensive	10
Mefenamic acid	Analgesic	10
MTBT**	Industrial chemical	10
TPHP*	Flame retardant	10
PEG n10**	Surfactant	10
PFOA*	PFAS	10
Nobiletin**	Food-related chemical	10
3-Hydroxycotinine**	Tobacco-related chemical	10
Nicotine*	Tobacco-related chemical	10
8-Hydroxyquinoline	Industrial chemical	10

*Semi-quantified through a standard solution calibration.

**Semi-quantified using QSIR model.

compounds whose occurrence may be overlooked when using routine target analytical methods. Notably, while some of these priority contaminants, including mirtazapine and dextrormethrophan, were not among the compounds found at the highest concentrations. This suggests indicating that they may exhibit significant toxicity.

Moreover, the inclusion of compounds like piperine and nobiletin in the priority list is noteworthy due to the increasing attention of phytochemicals as phytotoxins. Piperine, in particular, has garnered attention due to its known estrogenic activity (Hama et al., 2021; Zwart et al., 2018). Additionally, the flame retardants TCEP and TPHP, and the industrial chemical MTBT, and the perfluoroalkyl compound PFOA are flagged as of major concern. These compounds were likely released via laundry effluents due to a combination of clothing embedded with these compounds or dust accumulation on the clothing, being a potential source of contamination. Despite the ban of PFOA production in the US and Europe, its continued manufacture in other regions raises the possibility of global consumers exposure through imported products (Antonopoulou et al., 2024).

This comprehensive assessment highlights the potential ecotoxicological risks associated with the direct discharge of greywater from ships, as it contains chemical compounds with high toxicity, emphasizing the need for effective mitigation strategies and regulatory measures to safeguard marine ecosystems. Herein individual compounds are ranked to establish a preliminary screening for their potential environmental impact. Nevertheless, in greywater, these compounds are present as mixtures with potential synergistic or antagonistic effects. Thus, studies evaluating the potential ecotoxicological impacts of these mixtures, considering different dilution factors, are essential to fully understand the risks associated with greywater discharges from ships to marine ecosystems.

4. Conclusions

This study provides a comprehensive characterization of the chemical composition of greywater, revealing its substantial contaminant load with certain chemicals present at higher concentrations than those found in land-based wastewater. The combination of advanced widescope target and suspect screening approaches proved to be a powerful tool for a comprehensive chemical characterization of ship-generated greywater, detecting a myriad of contaminants within a single analysis. The use of the latest tools for compound semi-quantification and advanced scoring system for compound identification significantly enhanced confidence and accuracy in identifying and quantifying chemicals. Indeed, the combination of both analyses revealed the presence of 97 organic contaminants spanning several chemical classes, including pharmaceuticals, plasticizers, flame retardants, personal care products, UV filters, industrial chemicals, stimulants, food and tobaccorelated chemicals. Furthermore, these methods identified compounds that conventional target analytical methods would likely overlook. This study highlights the concentration and variety of chemical contaminants in greywater, which may be significantly influenced by ship activities, size and management practices.

The prioritization strategy identified 25 compounds as those of major ecological concern, highlighting the urgent need for targeted regulatory measures. These findings emphasize the need for regular monitoring programs and improved greywater management practices to enhance onboard treatment efficiency. Managing ship sewage presents numerous challenges due to high contaminants concentration and confined spaces, necessitating innovative solutions like source separation systems to segregate greywater from blackwater. This approach not only enhances treatment efficiency but also facilitates tailored processes to effectively remove specific contaminants. Additionally, the reuse of treated greywater for non-potable applications, such as flushing toilets or cleaning decks, could alleviate freshwater shortages and mitigate the environmental impact of greywater discharges, highlighting the importance of integrating these practices into marine operations.

CRediT authorship contribution statement

E. García-Gómez: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. R. Gil-Solsona: Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. E. Mikkolainen: Writing – review & editing, Validation. M. Hytti: Writing – review & editing, Validation. E. Ytreberg: Writing – review & editing, Validation. P. Gago-Ferrero: Writing – review & editing, Validation, Methodology. M. Petrović: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. M. Gros: Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125524.

Data availability

Data will be made available on request.

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