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Master Thesis

Monitoring of emerging contaminants in seawater, sediments and biota samples from the Black Sea by liquid chromatography coupled to high resolution mass spectrometry

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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

Προσδιορισμός αναδυόμενων ρύπων σε θαλασσινό νερό, ιζήματα και δείγματα ζωντανών οργανισμών από τη Μαύρη Θάλασσα με υγροχρωματογραφία συζευγμένη με φασματομετρία μάζας υψηλής διακριτικής ικανότητας

Βασιλάτος Δ. Κωνσταντίνος, Χημικός

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ABSTRACT

The environmental problems in the Black Sea resulting from anthropogenic activities are mainly pollution and eutrophication process, accompanied by natural variability and climatic changes. However, anthropogenic activities are with no doubt presently the powerful driver of change in its ecosystem. In the Black Sea coasts, uncontrolled industrial and domestic wastes are either deposited into or stored at a variety of land and water-based disposal sites. Environmental crisis of the Black Sea is well related to the unique characteristics of the marine environment.

The term “emerging pollutants” (EPs) or “emerging contaminants” (ECs) refers to compounds and their metabolites that are not included in regular monitoring studies, are not currently covered by existing water-quality regulations, and are thought to be potential threats to environmental ecosystems and human health and safety.

The Water Framework Directive (WFD) (2000/60/EC) sets a comprehensive management planning system to help protect and improve the ecological and chemical status of Black Sea, and in general all European water bodies. Recent advances and improvements in analytical techniques, and especially in high resolution mass spectrometry, have given the opportunity to scientific groups to detect and identify a huge number of chemical compounds, even in complex matrices. LC-HRMS allows the wide-scope screening of ECs and their (bio) TPs.

The aim of this thesis is the determination of emerging contaminants with a wide LC-ESI-Q-ToF target screening methodology of more than 2000 compounds. The sea water samples were purified with SPE method and used HLB cartridges, sediments were purified with ultrasonic extraction and biota samples with 2-days generic extraction before the analysis.Extracts were analyzed with reversed-phase liquid-chromatography coupled to quadrupole-time-of-flight mass spectrometry (RPLC-QTOF-MS) with positive and negative electrospray ionization and the data were acquired through data dependent and data independent acquisition mode (bbCID and Auto-MS scan modes).

The study area for contamination state was covered by three transects of Black Sea; the western side close to Ukraine/Romania (Danube Delta), the eastern Black Sea close to Georgia and the central side including (Open Sea) sampling points across the length of Black Sea and out of reach of any coastal city. Twenty one seawater samples (seven Open Sea, seven UA (Ukraine) and seven GE (Georgia)) were collected and analyzed. The results indicate the presence of Phthalates (including Dimethyl-phthalate and Benzyl butyl-phthalate), also eleven phenols were determined in seawater samples like 2,4-Dinitrophenol (DNP) which presented high frequency of detection among all analyzed samples. Moreover, PFCs were detected like PFOA, PFHxA and GENX but most were in low concentrations. Also 61 emerging contaminants (industrial chemicals,

pharmaceuticals & PCPs, drugs TPs & cathine, plant protection products, PPPs TPs, stimulants TPs and naturally occurring compounds) have been detected in the analyzed samples. Furthermore, four sediment samples, two from Ukraine, one from Open Sea and one from Georgia were collected for monitoring the degree of contamination in sediments of the Black Sea.
Among the seven screened phthalates, only Di (2-ethylhexyl) phthalate (DEHP) and Di-n-butyl-phthalate were detected in the samples and all screened phenols were below the detected limits in the tested samples. Fourteen ECs were detected in sediment samples and most of the detected compounds were plant protection products. Finally twenty biota samples were collected from Georgia, Ukraine and Russian Federation and analyzed in total. Among the eighteen PFCs that were screened in the biota samples, only Perfluoroctane sulfonic acid (PFOS) was detected in two fish from Georgia and two from Ukraine. Moreover, thirty six ECs were detected in the analyzed biota samples. The results indicate the presence of drugs TPs in Georgian (79.9%) and Ukrainian (72.2%) samples, whereas pharmaceutical and PCPs contribute the most (59.6%) to the total EPs pollution in the Russian Federation samples.

The aggregated target analysis results produced within EMBLAS II and EMBLAS plus projects, implemented in 2016-2017 and 2019, respectively were evaluated in this project. Apart from the determination of priority pollutants included in the WFD (2013/39/EC), unique data on the occurrence of emerging contaminants have been extracted for several seawater, biota samples and sediments, collected in coastal/shelf areas and open sea. The overall results indicate that the levels of specific contaminants or the selected sampling sites remarkably affect the overall pollution pattern of the samples. To conclude both the number and the average total concentration of emerging contaminants were lower in 2019 samples compared to the previous campaigns.

SUBJECT AREA: Environmental Analytical Chemistry

KEYWORDS: Black Sea; Emerging Contaminants; High Resolution Mass Spectrometry; Marine Environment.

ΠΕΡΙΛΗΨΗ

Τα περιβαλλοντικά προβλήματα στην Μαύρη Θάλασσα που οφείλονται σε ανθρωπογενείς δραστηριότητες είναι κυρίως η ρύπανση και ο ευτροφισμός, που συνοδεύεται από φυσική μεταβλητότητα και κλιματολογικές αλλαγές. Ωστόσο, οι ανθρωπογενείς δραστηριότητες είναι αναμφίβολα σήμερα ο ισχυρός μοχλός αλλαγής στο οικοσύστημά της. Στις ακτές της Μαύρης Θάλασσας, τα ανεξέλεγκτα βιομηχανικά και οικιακά απόβλητα είτε εναποτίθενται είτε αποθηκεύονται σε μια ποικιλία χερσαίων και υδάτινων χώρων απόρριψης. Η περιβαλλοντική κρίση της Μαύρης Θάλασσας σχετίζεται καλά με τα μοναδικά χαρακτηριστικά του θαλάσσιου περιβάλλοντος αυτής.

Ο όρος «αναδυόμενοι ρύποι» (EPs) ή «αναδυόμενοι μολυσματικοί παράγοντες» (ECs) αναφέρεται σε ενώσεις και τους μεταβολίτες τους που δεν περιλαμβάνονται σε τακτικές μελέτες παρακολούθησης, δεν καλύπτονται επί του παρόντος από τους υφιστάμενους κανονισμούς για την ποιότητα του νερού και θεωρούνται πιθανές απειλές για περιβαλλοντικά οικοσυστήματα και ανθρώπινη υγεία και ασφάλεια.

Η οδηγία πλαίσιο για τα ύδατα (ΟΠΥ) (2000/60 / ΕΚ) ορίζει ένα ολοκληρωμένο σύστημα σχεδιασμού διαχείρισης για την προστασία και τη βελτίωση της οικολογικής και χημικής κατάστασης της Μαύρης Θάλασσας, και γενικά όλων των ευρωπαϊκών υδάτινων σωμάτων. Οι πρόσφατες εξελίξεις και βελτιώσεις στις αναλυτικές τεχνικές, και ιδιαίτερα στη φασματομετρία μάζας υψηλής ανάλυσης, έδωσαν την ευκαιρία σε επιστημονικές ομάδες να εντοπίσουν και να εντοπίσουν ένα τεράστιο αριθμό χημικών ενώσεων, ακόμη και σε πολύπλοκες μήτρες. Η χρήση υγροχρωματογραφίας συζευγμένη με φασματομετρία μάζας υψηλής διακριτικής ικανότητας (LC-HRMS) επιτρέπει την ευρεία εξέταση των αναδυόμενων ρύπων καθώς και των μεταβολιτών τους. Ο στόχος αυτής της διατριβής είναι ο προσδιορισμός των αναδυόμενων ρύπων με μια μεθοδολογία ευρείας στοχευμένης διαλογής (LC-ESI-Q-ToF), για περισσότερες από 2000 ενώσεις. Τα δείγματα θαλάσσιου νερού καθαρίστηκαν με την μέθοδο εκχύλισης στερεάς φάσης(SPE) και χρησιμοποιήθηκαν φυσίγγια τύπου HLB, τα ιζήματα καθαρίστηκαν με την μέθοδο εκχύλισης με υπερήχους και τα δείγματα των ζωντανών οργανισμών με μια γενική μέθοδο εκχύλισης 2 ημερών πριν από την ανάλυση.Τα εκχυλίσματα αναλύθηκαν με υγροχρωματογραφία αντίστροφης φάσης συζευγμένη με φασματομετρία μάζας με υβριδικό τετράπολο-αναλυτή χρόνου πτήσης (RPLC-QTOF-MS) χρησιμοποιώντας δύο λειτουργίες σάρωσης (DDA, DIA). Μία βάση δεδομένων που περιείχε περισσότερους από 2.100 αναδυόμενους ρύπους χρησιμοποιήθηκε για τη στοχευμένη ανάλυση και η ανίχνευση βασίστηκε στην ακρίβεια μάζας, στο χρόνο ανάσχεσης, στο ισοτοπικό προφίλ και στα χαρακτηριστικά θραύσματα.

Η περιοχή μελέτης για την κατάσταση της ρύπανσης καλύφθηκε από τρεις διατομές της Μαύρης Θάλλασας. την δυτική πλευρά κοντά στην Ουκρανία / Ρουμανία (Δέλτα του Δούναβη), την ανατολική Μαύρη Θάλασσα κοντά στη Γεωργία και την κεντρική πλευρά, συμπεριλαμβανομένων σημείων δειγματοληψίας (Ανοιχτή Θάλασσα) σε όλο το μήκος της Μαύρης Θάλασσας και μακριά από οποιαδήποτε παράκτια πόλη. Συλλέχθηκαν και αναλύθηκαν είκοσι ένα δείγματα θαλασσινού νερού (επτά ανοιχτή θάλασσα, επτά UA (Ουκρανία) και επτά GE (Γεωργία). Τα αποτελέσματα δείχνουν την παρουσία φθαλικών (συμπεριλαμβανομένου του φθαλικού διμεθυλίου και του φθαλικού-βενζυλοβουτυλίου),επίσης έντεκα φαινόλες προσδιορίστηκαν σε δείγματα θαλασσινού νερού όπως η 2,4-δινιτροφαινόλη (DNP), η οποία παρουσίασε υψηλή συχνότητα ανίχνευσης σε όλα τα δείγματα που αναλύθηκαν. Επιπλέον, εντοπίστηκαν PFCs όπως τα PFOA, PFHxA και GENX, αλλά τα περισσότερα ήταν σε χαμηλές συγκεντρώσεις. Επίσης, 61 αναδυόμενοι ρύποι (βιομηχανικά χημικά, φαρμακευτικά προϊόντα και PCPs, μεταβολίτες φαρμάκων και η καθίνη, φυτοπροστατευτικά προϊόντα, μεταβολίτες των φυτοπροστατευτικών, μεταβολίτες των διεγερτικών και φυσικά απαντώμενες ενώσεις) έχουν ανιχνευθεί στα αναλυθέντα δείγματα. Επιπλέον, τέσσερα δείγματα ιζημάτων, δύο από την Ουκρανία, ένα από την ανοιχτή θάλασσα και ένα από τη Γεωργία συλλέχθηκαν για την παρακολούθηση του βαθμού μόλυνσης στα ιζήματα της Μαύρης Θάλασσας.

Μεταξύ των επτά φθαλικών εστέρων που ελέγχθηκαν, μόνο τα δι (2-αιθυλεξυλ) φθαλικό (DEHP) και φθαλικό-δι-ν-βουτύλιο ανιχνεύθηκαν στα δείγματα και όλες οι ελεγχόμενες φαινόλες ήταν κάτω από τα ανιχνευόμενα όρια στα δείγματα. Δεκατέσσερις αναδυόμενοι ρύποι ανιχνεύθηκαν σε δείγματα ιζημάτων. Οι περισσότερες από τις ανιχνευθείσες ενώσεις ήταν φυτοπροστατευτικά προϊόντα.

Τέλος, συλλέχθηκαν και αναλύθηκαν συνολικά είκοσι δείγματα ζωντανών οργανισμώναπό τη Γεωργία, την Ουκρανία και τη Ρωσική Ομοσπονδία. Μεταξύ των δεκαοκτώ PFCs που εξετάστηκαν στα δείγματα, μόνο το σουλφονικό οξύ (PFOS) εντοπίστηκε σε δύο ψάρια από τη Γεωργία και δύο από την Ουκρανία. Επιπλέον, τριάντα έξι αναδυόμενοι ρύποι εντοπίστηκαν στα δείγματα που αναλύθηκαν. Τα αποτελέσματα δείχνουν την παρουσία μεταβολιτών φαρμάκων σε δείγματα Γεωργίας (79,9%) και Ουκρανίας (72,2%), ενώ τα φαρμακευτικά προϊόντα και τα προϊόντα προσωπικής περιποίησης συνεισφέρουν περισσότερο (59,6%) στη συνολική ρύπανση των αναδυόμενων ρύπων στα δείγματα της Ρωσικής Ομοσπονδίας. Τα συγκεντρωτικά αποτελέσματα ανάλυσης στα έργα EMBLAS II και EMBLAS plus, που υλοποιήθηκαν το 2016-2017 και το 2019, αξιολογήθηκαν αντίστοιχα σε αυτό το έργο. Εκτός από τον προσδιορισμό των ρύπων προτεραιότητας που περιλαμβάνονται στην ΟΠΥ (2013/39 / ΕΚ), έχουν εξαχθεί μοναδικά δεδομένα για την εμφάνιση αναδυόμενων ρύπων για πολλά δείγματα θαλασσινού νερού, και ιζήματα που συλλέγονται σε παράκτιες περιοχές / ανοικτές θάλασσες. Τα συνολικά αποτελέσματα δείχνουν ότι τα επίπεδα συγκεκριμένων ρύπων ή των επιλεγμένων τοποθεσιών δειγματοληψίας επηρεάζουν σημαντικά το

συνολικό μοτίβο ρύπανσης των δειγμάτων. Κλείνοντας, τόσο ο αριθμός όσο και η μέση συνολική συγκέντρωση των αναδυόμενων ρύπων ήταν χαμηλότερα στα δείγματα του 2019 σε σύγκριση με τις προηγούμενες καμπάνιες.

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Περιβαλλοντική Αναλυτική Χημεία

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: Μαύρη Θάλασσα, Αναδυόμενοι Ρύποι, Φασματομετρία Μάζας Υψηλής Διακριτικής Ικανότητας, Θαλλάσιο Περιβάλλον

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PREFACE

This master thesis was performed at the laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens under the supervision of Professor Nikolaos S. Thomaidis.

I would like to thank my supervisor, Professor N. S. Thomaidis, for his advices, guidance, support during my master and for giving me the opportunity to work in EMBLAS PLUS 2019 project as a member of TrAMS group. I am very happy to be part of his research group and grateful for all the opportunities and trust that I received. Also, I especially thank the members of the examination committee, for their comments and remarks in regards with my master thesis.

These past two years were very important in order to develop and improve my theoretical knowledge and practical skills in the field of Chemical Analysis and Quality Control. I would like to thank all the professors for teaching and imparting their knowledge during my master. Moreover, I am deeply grateful to post-doc researcher Dr. Maria-Christina Nika for her advices, guidance and support during my master thesis and for our collaboration during the past one year. I would like to say a big thank you to each and every member of TrAMS group I met in the lab during my master.

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CHAPTER 1: INTRODUCTION

1.1 Black Sea

The Black Sea is situated in south-eastern Europe, partly bordering Asia. Its south coast is the Pontic Mountains with the Caucasus Mountains to the northeast, while the topography of the north-western (NW) coast (except for Crimea) isrelatively low and flat. Its coastline is about 4400 km long. The Black Sea is classified geo-morphologically into a shelf, the continental slope and a deep-sea depression (Fig. 1). The shelf edge slope is steep and the shelf is basically narrowexcept for the NW.Its connection to the World Ocean is through the Aegean and Mediterranean Sea, where 300 $km^3 year^{-1}$of mesohalinewater passes out through the Bosphorus Strait. As part of a two-way hydrologicalexchange, the Black Sea's cooler and less saline outflow floats over the warm, more saline Mediterranean inflow. Part of this passage include the Sea of Azov and the Strait of Kerch [1]. Although total basin precipitation is less than evaporation, the water supply from several big rivers, such as Danube, Dnieper, Rioni, etc. with a total river catchment area of about 2.4 X 106 km^2, give a positive freshwater balance,resulting in a low salinity in the upper layer of between 18 psu in the open sea to 16 psu close to the shore. The riverinput in the NW causes a drop of salinity of up to 5 psu close to river mouth regions. The amount of freshwater and nutrient input into the southern parts of the Black Sea is relatively small.

Figure 1: The Black Sea [1]

The environmental problems in the Black Searesulting from anthropogenic activities are mainly pollution and eutrophication process, accompanied by natural variability and climatic changes, which are evinced by stagy changes in its ecologicalsystem and resources. However, anthropogenic activities are with no doubt presently the powerful driver of change in the Black Sea ecosystem at all levels of organization. In the Black Sea coasts, uncontrolled industrial and domestic wastes are either deposited into or stored at a variety of landand water-based disposal sites. The need for economic development apparently either blinded ordamaged our view of what constituted a fine life. Ecosystem did perform quite well in treating somesubstances, which further contributed to our disregard for the hazards caused by wastes.Environmental crisis of the Black Sea is well related to the unique characteristics of the marine environment [2].

1.2 Priority Pollutants

The concentrations of various substances in water in dissolved, colloidal or suspended form are typically low but vary considerably. Priority Pollutants refer to a list of 126 specific pollutants that includes heavy metals and specific organic chemicals. The priority pollutants are a subset of "toxic pollutants" as defined in the Clean Water Act (USA). These 126 pollutants were assigned a high priority for development of water quality criteria and effluent limitation guidelines because they are frequently found in wastewater.

Heavy Metals (Total and Dissolved): "Heavy Metal" in the water treatment field refers to heavy, dense, metallic elements that occur only at trace levels in water, but are very toxic and tend to accumulate.
Pesticides:Pesticides comprise a large class of compounds of concern. Typical pesticides and herbicides include DDT, Aldrin, Chlordane, Endosulfan, Endrin, Heptachlor, and Diazinon. Surprisingly, concentrations of pesticides in urban runoff may be equal or greater than the pesticides in agricultural runoff.
Polycyclic Aromatic Hydrocarbons (PAHs)**:** Polycyclic Aromatic Hydrocarbons include a family of semi-volatile organic pollutants such as naphthalene, anthracene, pyrene, and benzo(a)pyrene. There are typically two main sources of PAHs: spilled or released petroleum products (from oil spills or discharge of oil production brines) and combustion products that are found in urban runoff.

***Polychlorinated biphenyls (*PCBs):** Polychlorinated biphenyls are organic chemicals that formerly had widespread use in electrical transformers and hydraulic equipment. This class of chemicals is extremely persistent in the environment and has been proven to bioconcentrate in the food chain, thereby leading to environmental and human health concerns in areas such as the Great Lakes. (source:https://corrosion-doctors.org › Natural Waters).

In Europe, the Water Framework Directive was voted by the European Parliament and the European Council in October 2000 and entered into force in December 2000. It aims to establish a legal framework for the protection of water quality in European countries (for river water, sea water, groundwater and coastal water). The directive recognizes that specific measures have to be adopted at a European level against water pollution by individual pollutants, or groups of pollutants, presenting a significant risk to the aquatic environment and water used for the production of drink water. The Directive on Priority Substances of 2008 (a daughter directive of the Water Framework Directive) also made a list of substances for which it should be investigated whether they should be included in the list of priority substances or priority hazardous substances.A list of 33 priority substances for which environmental quality standards were set in 2008, including selected existing chemicals, plant protection products, biocides, metals and other groups like Polyaromatic Hydrocarbons (PAHs) that are mainly incineration by-products and Polybrominated Biphenylethers (PBDE) that are used as flame retardants. The following 33 substances and chemical compounds are included in the list of priority substances established by the European Union. Some of these priority substances are also priority hazardous substances (last 13 of those): Alachlor, Atrazine, Benzene, Chlorfenvinphos, Chlorpyrifos, 1,2-Dichloroethane, Dichloromethane, Di(2-ethylhexyl)phthalate (DEHP), Diuron, Fluoranthene, Isoproturon, Lead and its compounds, Naphthalene, Nickel and its compounds, Octylphenols,Pentachlorophenol,Simazine, Trichlorobenzenes,Trichloromethane, Trifluralin,Anthracene, Pentabromodiphenylether, Cadmium and its compounds, $C_{10\text{-}13}$-chloroalkanes, Endosulfan, Hexachlorobenzene, Hexachlorobutadiene, Hexachlorocyclohexane, Mercury and its compounds, Nonylphenols (are part of the alkylphenol ethoxylate group of non-ionic surfactants), Pentachlorobenzene, Polyaromatic hydrocarbons and Tributyltin compounds.

Priority pollutants are ubiquitously present in aquatic systems due to their widespread application and have received increasing attention as they cause distress to aquatic life. In an aquatic sedimentary environment, these pollutants may undergo chemical transformations and can serve as a sink or secondary source of pollutants. With various biogeochemical changes like turbulences and dia-genetic processes, the persistent organic pollutants can remobilize into adjoining water columns and ultimately reach the aquatic organisms. The long-term persistence, bioaccumulation and bio-magnification potential of pesticides may pose a severe threat to the entire biosphere [3].

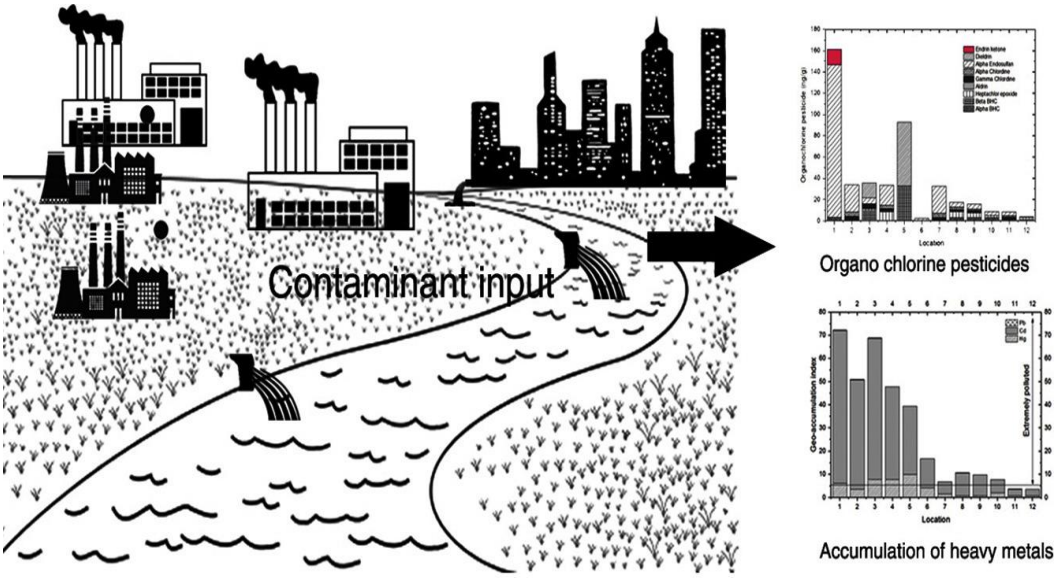


Figure 2: Distribution of priority pollutants in the sediment (source:https://www.sciencedirect.com/science/article/pii/S0025326X18303497)

Extensive commercial, industrial and domestic use ofvolatile organic chemicals (VOCs), virtually ensures thatthe general population will be exposed to these compounds to some extent. VOCs are also important environmental contaminants. Aromatic hydrocarbons, such as benzene, toluene, ethylbenzene and xylene (major aromatic VOCs,commonly called BTEX) are widely used in the chemical industry.The need for monitoring toxic VOCs is based on their adverse health effects, so reliable measurements of BTEX compounds are essential, especially since benzene is known to be a human carcinogen. Exposure to them can occur via inhalation, ingestion, eye or skin contact and, to a small extent, by absorption through the skin [4].

Over the past decades, (PAHs) have been aroused great concerns due to their widespread occurrence and toxic effect on ecosystem and human. PAHs are regarded as priority pollutants by the United States Environmental Protection Agency, the European Community, and the Chinese Government.

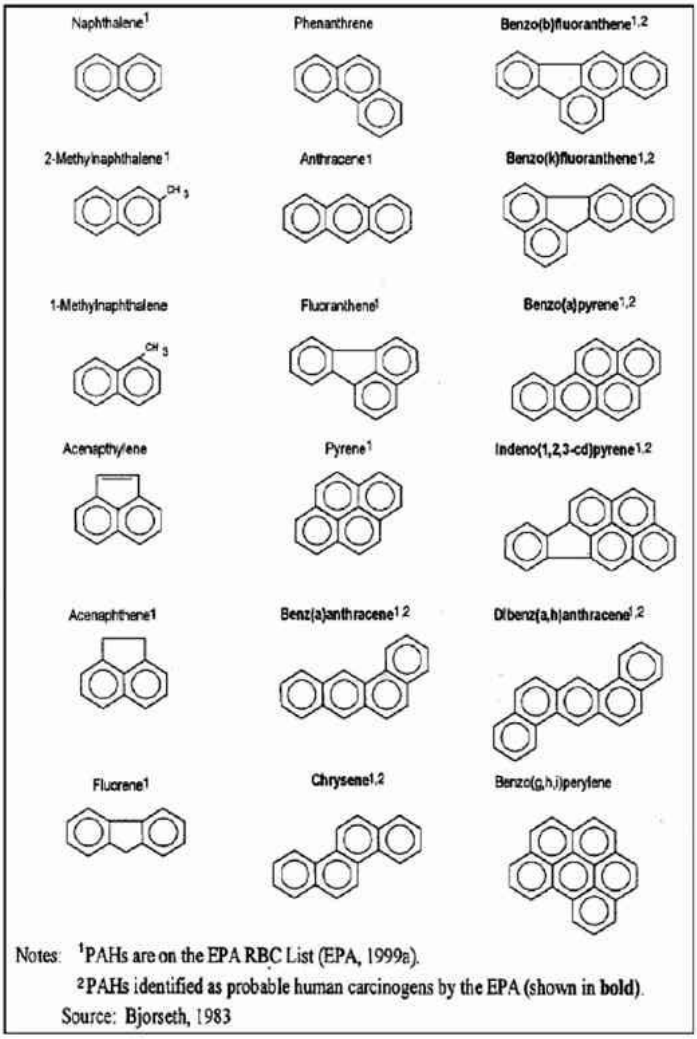


Figure 3 :The most commonly analyzed polycyclic aromatic hydrocarbons (PAHs) [5].

Studies of PAHs and their derivatives have become relevant due to their mutagenic and carcinogenic properties. Moreover, due to their hydrophobic and persistent characteristics, these substances have the ability to accumulate in sediments, bio-accumulate in aquatic organismsand, at lower concentrations, in the water. PAHs present in the sediment are a source ofPAH exposure directly to benthic organisms and indirectly, through re-suspension, to pelagic organisms. Sediment can act as an important compartment for storage, transportand subsequent release of pollutants. Due to the ease of most chemical compounds in aggregating to sediment particles, these depositscan show effects of the anthropogenic emission to the environment over time. That is, sediments can record the increase or decrease in pollution in a certain region. Thus, sediment deposition is a very useful tool to evaluate the history of the anthropic contribution, as well as the evolution of pollutants in the environment of a region.

Urban and industrial areas are affected by PAH pollution due to specific anthropogenic inputs, such as industrial wastewater, street dust discharge, deposition of fossil fuel, carbonized particles and petroleum products spills. The

individual identification and quantification of saturated and polycyclic aromatic hydrocarbons are widely used for determining sources of combustion contamination and/or degradation of oil and derivatives in the aquatic environment. The quantification of these substances indicates the environmental quality, besides diagnosing different sources. The distribution and characteristics of PAHs found in sediments provide information on their precursor sources. Although PAHs are ubiquitous pollutants, their sources in the aquatic environment can be differentiated, based on diagnostic ratios (the ratio of the less “kinetically” stable PAH isomers versus their “thermodynamically” stable isomers) or in the distributionof parent PAH composition [6].

1.3 Emerging Contaminants

The term “emerging pollutants” (EPs) or “emerging contaminants” (ECs) refers to compounds and their metabolites that are not included in regular monitoring studies, are not currently covered by existing water-quality regulations, have not been studied in depth, are overlooked in monitoring studies and are thought to be potential threats to environmental ecosystems and human health and safety (Fig.4).

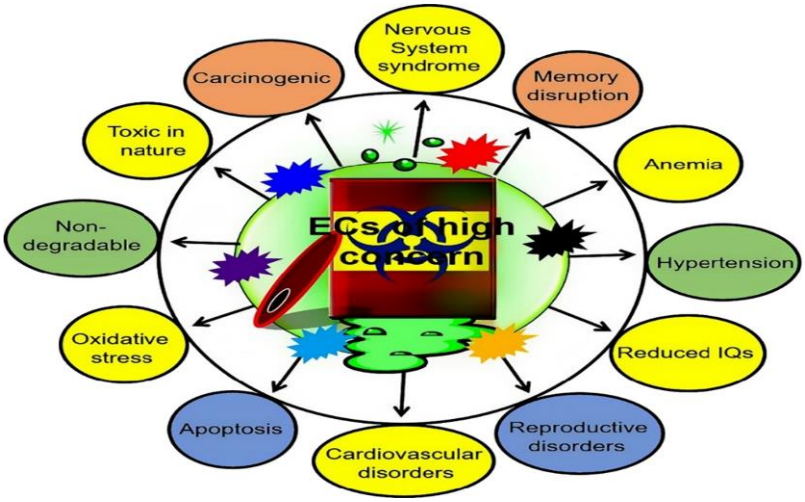


Figure. 4: Major consequences and adverse effects of ECs of high concern on human's health and the environment [7].

According to NORMAN (Network of reference laboratories, research centers and related organizations for monitoring emerging environmental substances), they are compounds that are not included in routine environmental monitoring programs and may be candidates for future legislation due to their adverse effects and/or persistency (http://www.norman-network.net/). Once released into the environment, EPs are subjected to biotic and abiotic transformation processes that are responsible for their transformation and/or elimination,

according to their persistence, transport, and ultimate destination (Fig.5). Various transformations can take place, producing compounds that, to some extent, differ in their environmental behavior and eco-toxicological profile from the parent compound. Formation of transformation products (TPs) occurs mainly through oxidation, hydroxylation, hydrolysis, conjugation, cleavage, de-alkylation, methylation and demethylation. EPs and their TPs can move vertically through the soil profile to groundwater and away from the source site with mobile groundwater. They also have the potential to reach surface water when they travel laterally as surface run-off or through sub-soil tile drains, entering streams, major rivers, reservoirs, and ultimately estuaries and oceans [8].

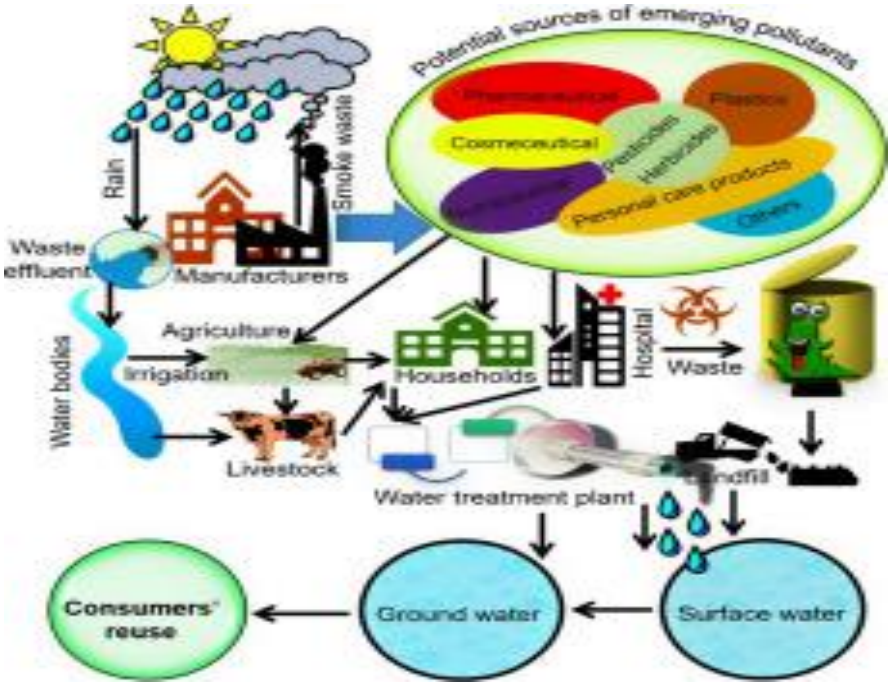


Figure 5: The transfer of pollutants in environment [7]

With the growth of urbanization and industrial development, water consumption and the associated discharge of wastewater and pollutants into the environment are increasing annually. Large quantities of wastewater carrying pollutants directly enter natural water bodies or soils may have the ability to contaminate the local environment and destroy the wildlife habitats. To address this potential problem, wastewater treatment plants (WWTPs) are built as important parts for purifying wastewater with the urbanization process all over the world. However, wastewater treatment processes cannot completely remove all pollutants, and large amounts of traditional and emerging pollutants may enter rivers or soils through effluent discharge and sludge disposal where they may threaten the health of wildlife. The occurrence and toxicological effects of heavy metals (HMs), regarded as traditional

pollutants, have been studied for many decades. Whereas, emerging pollutants (including perfluoroalkyl substances [PFASs], pharmaceutical and personal care products [PPCPs]) have more recently caused widespread concerns due to their persistence in the environment and adverse effects on both wildlife and humans In PFASs (which are synthetic organic compounds), hydrogen atoms in hydrocarbon groups are completely replaced by fluorine atoms. Hence, PFASs have the dual characteristics of hydrophobicity and lipophobicity, as well as high chemical stability, and are widely used in various coating materials and aqueous film-forming foams. Consequently, PFASs are globally distributed in water, soils, sediments, dust, and biota, particularly in the vicinities of factories and WWTPs. Another group of emerging contaminants, PPCPs, includes a broad range of chemicals which are employed to treathuman and veterinary diseases or maintain personal hygiene. However, PPCPs cannot be completely metabolized by humans or animals and therefore may enter WWTPs through sewagepipe networks. Wastewater treatment plants serve as a barrier to purify wastewater and prevent pollutants from directly entering the environment [9].

1.4 Classes of emerging contaminants

Contaminants of emerging concern or emerging contaminants (ECs) represent relatively newly discovered groups of unregulated contaminants which occur in surface and groundwater, such as pharmaceuticals and personal care compounds, and generally include compounds used in everyday life and various industrial additives. ECs are not necessarily newly created chemicals. It could be stated that term refers to three general categories. The first category includes compounds which are recently introduced into the environment (e.g., industrial additives). The second category consists of compounds that might have been present in the environment in the past for many years, but it is only during the last years that their presence was detected and their significance started to attract interest (e.g., pharmaceuticals). The third category includes compounds that are known for a longer time but their potential negative impact on humans and the environment was only recently realized (e.g., hormones). Today, it is clear that an integrated research and approach to these contaminant groups should be multidisciplinary, involving disciplines such as chemistry, biology and engineering [10].

1.4.1 Perfluorinated chemicals (PFCs)

Perfluorinated chemicals (PFCs) are man-made chemicals which have been manufactured for over 40 years and have been used indifferent commercial and industrial applications, such as:surfactants and surface protectors in paper, leather, carpets,upholstery, paints, lubricants, polishers, food packaging,and fire-fighting foams including aqueous film forming [14]. Also Perfluorinated chemicals

(PFCs) such a perfluorooctanesulfonic acid compound (PFOS), perfluorooctanoic acid (PFOA), or perfluorononanoic acid (PFNA) have been usedin numerous industrial products, known widely for their use in Teflon products, although they arealso used as additives in detergents, soaps, as surfactants, explosives, and as flame retardants .They are considered a new generation of contaminants that have arouse concern because they are globally distributed in the environment, especially in the aquatic environment , have a high bioaccumulation potential, and can have an impact onaquatic organisms. PFCs are moderately water soluble, non volatile and thermally stable, and due to a strong carbon fluorine (C–F) covalent bond, PFCs are resistant to hydrolysis, photolysis biodegradation, and metabolism. These characteristics explain the environmental persistence and bioaccumulative potential of PFCs [15].

1.4.2 Surfactants (surface-active agents)

Surfactants (surface-active agents) are a diverse group of chemicals consisting of a polar, water-solublehead group and a nonpolar hydrocarbon tail group, which is not as soluble in water. Surfactants are best known for their solubility and cleaning properties which secured them a place among detergents and other cleaning products. Massive quantities of surfactants are being used in households and industry every day, and most end up dispersed in different environmental compartments (soil, water, sediment). After use, residual surfactants are discharged into sewage systems or directly into surface waters. They also accumulate in great quantities in wastewater treatment plants. Concentrations of surfactants or their degradation products vary in surface waters, sediments, and soils amended with sludge. The elevated levels of surfactants in the environment can greatly affect the ecosystem; their toxicity to organisms from mammals to bacteria is well known [10].

1.4.3 Industrial Compounds

This group includes substances used in industrial processes and production, especially in the chemical industry. Two known EDCs, bisphenol A and the metabolite NP, the fire retardant (tri (2-chloroethyl) phosphate) and the musk galaxolide are among the most frequently detected substances, especiallyin groundwaters. Nonylphenol and bisphenol A present the highest detectionfrequency in Europe (11% and 39.6%, respectively) compared to the USA in wastewater influents andeffluents and in sewage sludge, with higher values occurring in Austria for both compounds. It is reported that wastewater treatment processes achieve high removal rates of bisphenol A (up to 99%) through

biodegradation. Polychlorinated alkanes (PCAs) are industrial chemicals detected in treated wastewater effluents at trace levels, as well as in sewage sludge. However, little is known concerning their impact on human health and the environment. MTBE (methyl tertiary-butyl ether) and BTEX compounds (benzene, toluene, ethylbenzene, m-, o-, and p-xylene) are frequently detected in groundwater. They are considered as posing potentialrisks for human health and drinking water contamination and MTBE is classified as a potential carcinogen [10].

1.4.4 Flame Retardants

These compounds are used in plastics, textiles and furnishing foam in order to reduce their flammability by interfering with polymer combustion. They can be halogenated or brominated compounds. Polybrominated diphenyl ethers (PBDEs) flame retardants are bioaccumulative and are considered as Endocrine Disrapting Chemicals(EDCs). PBDEs have been used in plastics, textiles, electronic circuitry among other materials [10].

1.4.5 Pesticides

Pesticides are among the most used chemical substances worldwide, with an annual production of over 3 million tons. Their use in agriculture has allowed increasing the quality andthe quantity of food production. However, regardless of their merits, they have been appointed as some of the most toxic substances in the environment and consequently represent a risk for ecosystems and human health. For this reason, and based on the available information, the protection of water resources and aquatic ecosystems from pesticide pollution has motivated the adoption of several regulatory measures. For instance, residues of selected pesticides that are considered priority substances in the environment must best rictly controlled in European water bodies and biota, so that their levels remain below established environmental quality standards (EQS) Research on the environmental occurrence of medium to highly polar pesticides has been very much focused in the water compartment while PBT (persistent, bioaccumulative, and toxic) pesticides have been usually targeted in biota due to their high octanol-water partition coefficients and hence capacity to partition into lipids. However, ionizable and ionic pesticides, are also likely to bioaccumulate in aquatic organisms via ion specific sorption mechanisms.The knowledge on the bioaccumulation potential of this type of pesticides is nowadays very limited but essential for proper risk assessment and pesticide regulation [18].

1.4.6 Pharmaceuticals

Human pharmaceuticals and various endocrine-disruptingchemicals (EDCs), residues of raw, and treated wastewaters have been detected in the environment. Besides ecological concerns, the ubiquity of pharmaceutically active compounds (PhACs) as environmental contaminants has raised human health-related questions in view of their potential transfer into food stuffs, such as vegetables, fish, and seafood. Concerns for human health include both direct toxicity of the chemicals and indirect effects (e.g., antibiotic-resistant bacteria). To date, no comprehensive risk assessment was derived with regards to the chronic exposure to PhAC residues through food [11]. Once administered, PhACs are metabolised to varying degrees, and their excreted metabolites and unaltered parent compounds can also undergo further modification due to biological, chemical and physical processes. The presence of pharmaceuticals in water is attributed to personal hygiene products, pharmaceutical industry raw and treated effluents, run off from agricultural fields fertilized with treated sewage sludge, hospital waste and therapeutic drugs.Data pertaining to a wide spectrum of PhACs, 118 compounds belonging to 17 different classes distinguished by their function or biological activity, were considered: 23 analgesics/anti-inflammatories, 36 antibiotics, 1 antidiabetic, 1 antifungal, 3 antihypertensives, 1 barbiturate, 12 beta-blockers, 2 diuretics, 9 lipid regulators, 10 psychiatric drugs, 6 receptor antagonists, 4 hormones, 4 beta-agonists, 3 antineoplastics, 1 topical product, 1 antiseptic and 1 contrast agent [12].

1.4.7 Illicit Drugs & Drugs of Abuse

Illicit drugs were recently listed as emerging contaminants in a review covering all the latest developments in water analysis. This class of substances has characteristics very similar to pharmaceuticals, already known as environmental contaminants, such as the source of contamination, the polar chemical structure and the similar behaviour in the environment. Like pharmaceuticals, illicit drugs are a heterogeneous group of compounds with different structures and physico-chemical properties, and are biologically active. Since about 200 million individuals worldwide are current users of cocaine, heroin, amphetamine-like stimulants, marijuana and other drugs , these substances are consumed worldwide in quantities comparable to therapeutic drugs (thousands of tons per year), and can be detected in the environment at the same levels.In analogy with pharmaceuticals, the main source of contamination forillicit drugs is human consumption, while other minor sources are uncontrolleddischarges related to the handling of these

substances. The residues of drugs of abuse persisting in consumers urine enter the sewage networks with the wastewater and are only partially removed by sewage treatment plants (STPs). As a result these substances are still detectable in treated water and contaminate the receiving surface waters. On account of their polarity or moderate lipophilic properties, illicit drugs can be expected to be distributed mostly in water, or adsorbed on suspended solids in the water phase, sludge in the STPs and sediment and soils in the environment. Despite their low volatility, these substances have been also detected in airborne particles in several of the world's cities [13].

1.4.8 Personal Care Products (PCPs)

Personal Care Products (PCPs) are used mainly to improve the quality of daily life. Over the past few years, there has been increasing awareness of the unintentional presence of PPCPs in various compartments of the aquatic environment (e.g. water, sediments and biota) at concentrations capable of causing detrimental effects to the aquatic organisms.This has become a major concern because PPCPs are extensively and increasingly used in human and veterinary medicine, resultingin their continuous release to the environment [14].

1.4.9 Artificial sweeteners

Artificial sweeteners are used worldwide as sugar substitutes in remarkable amounts in food, beverages, and also indrugs and sanitary products, such as mouth washes. They provide no or negligible energy and thus are ingredients of dietary products. Besides their useful function as wastewater markers, like other organic contaminants, some artificial sweeteners are also precursors of oxidation products which can be form edduring advanced wastewater treatment by ozone or during the ozonation processes in waterworks .Owing to their use as food additives, artificial sweeteners are extensively tested for potential adverse health effects on humans [19].

1.4.10 Hormones and Steroids

This category of ECs includes natural endogenous steroids, such as sex hormones (androgens such asandrostenedione and testosterone, estrogens such as oestrone, oestriol and progesterone), phytoestrogens, faecal indicators and plant

sterols, which are excreted from the human body. Synthetic androgens include oxandrolone, nandrolone and synthetic estrogens (xeno-estrogens) such as diethylstilbestrol, which areused as contraceptives. Natural and synthetic steroids and hormones occur in wastewater influents and effluents as free active steroids. Most of these compounds are considered as EDCs. Estrone, E2, NP and bisphenol A are the four most frequently detected compounds in groundwaters [10].

1.4.11 Transformation Products [(bio) TPs

Once released into the environment, EPs are subject to biotic and abiotic transformation processes that are responsible for their transformation and/or elimination, according to their persistence, transport, and ultimate destination. Various transformations cantake place, producing compounds that, to some extent, differ in their environmental behavior and ecotoxicological profile from the parent compound. Formation of transformation products (TPs) occurs mainly through oxidation, hydroxylation, hydrolysis, conjugation,cleavage, dealkylation, methylation and demethylation. EPs and their TPs can move vertically through the soil profile to groundwater and away from the source site with mobile groundwater.They also have the potential to reach surface water when they travel laterally as surface run-off or through sub-soil tiled rains, entering streams, major rivers, reservoirs, and ultimately estuaries and oceans .Since there is a gap in the information on the occurrence and the toxicity of TPs in the environment, we are unable to evaluate their significance in risk assessment. Standardized toxicity tests can provide quantitative information on the toxicity of the TP, compared to its parent compound, but these studies are limited .In general, TPs are less toxic and more polar than the parent compounds. However, in some cases, they may be more persistent or exhibit higher toxicity or be present at much higher concentrations [8].

1.5 Black Sea pollution-EMBLAS PLUS(2019) project

The Black Sea is one of the most vulnerable regional seas in the world given its limited exchange of water with the open oceans and the large watershed area in continental Europe. The five strongly interlinked priority trans-boundary problems of the Black Sea are: eutrophication, nutrient enrichment, changes in marine living resources, chemical pollution, biodiversity/habitat changes and marine litter.The development and improvement of a monitoring network and national marine monitoring programs is a management target of high priority in the region. Coordination in policies and legislation among the Black Sea countries is also of common interest to the EUs partners as it influences their own ability to implement

EU legislation and policies (the EU Water Framework Directive WFD and EU Marine Strategy Framework Directive - MSFD).The overall objective of EMBLAS PLUS project is to help improve protection of the Black Sea environment. Besides further enhanced monitoring by 10 out of 11 descriptors of MSFD this objective will be pursued through further technical assistance focused on marine data collection and local small-scale actions targeted at public awareness raising and education. (source: https://oceanconference.un.org/commitments/?id=15806)

CHAPTER 2: DETECTION OF EMERGING CONTAMINANTS IN BLACK SEA SAMPLES (BIOTA, SEDIMENTS AND SEAWATERS) – LITERATURE REVIEW

2.1 Introduction

The analysis of emerging contaminants, many of which were unknown until recently, is a significant environmental issue . Emerging contaminant issues have been highlighted by several recent scientific meetings on this topic and a seriesof papers that deal with these compounds in the environment.Current practice and experience dictates that investments in detection, prevention, control and elimination strategies of environmental pollution by chemical pollutants will continue to rise [10].The low concentrations (parts per billion) of ECs and PPs and the complexity of matrices especially in biota, which include a large number of other substances with different physicochemical properties make it difficult to identify them.As a result it is necessary to use advanced analytical techniques, so liquid chromatography coupled to high resolution mass spectrometry is the technique of choice for polar and semi-polar compounds because of its excellent selectivity and sensitivity.

2.2 Sample treatment

A big variety of extraction techniques is reported in environmental studies, depending on the matrix of analysis (liquid or solid), such as liquid-solid extraction (LSE), which is combined with microwave-assisted extraction (MAE) or ultrasound assisted extraction (UAE), solvent reduced techniques, [e.g. matrix solid-phase dispersion (MSPD), and liquid-phase micro extraction (LPME)], supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE)]. The common technique that is used as a clean-up step during the determination of ECs and PPs in environmental matrices is Solid Phase Extraction (SPE). The usual steps of SPE include the conditioning of the sorbent in the cartridge, the loading of the sample, where analytes interact with the sorbent and impurities pass through, the wash-up, the drying and finally the elution of the analytes. SPE is the most suitable technique for isolation of the target compounds from most of the types of matrices.The interference of matrix components during the analytical measurement causes signal suppression or enhancement due to co-eluting matrix compounds of samples during ionization in Gas and Liquid Chromatography coupled to Mass

Spectrometry (LC-MS), and mainly when using Electrospray Ionization (ESI) as source. The need for the determination of a big variety of analytes with different physicochemical properties demands the usage of a generic clean-up step before SPE.

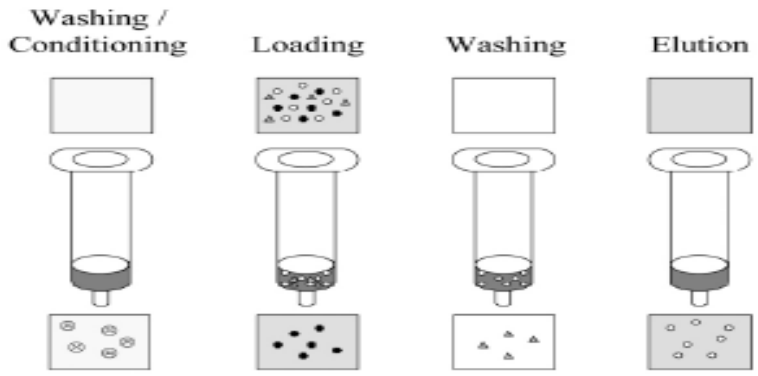


Figure 6: Steps of Solid Phase Extraction [20]

2.3 Analytical techniques – Liquid Chromatography coupled to Mass Spectrometry (LC-MS)

LC–MS is a sophisticated hyphenation of analytical techniques, which enables the determination of organic emerging contaminants in complex environmental matrices. A range of different LC-MS technologies have been put forward in recent years for the analysis of mixtures of many known and unknown compounds at low concentrations in complex matrices [21,22].

2.3.1 Reversed Phase Ultra High Performance Liquid Chromatography (RP-UHPLC)

In UHPLC, short chromatographic columns, which include small-diameter particles in the stationary phase. UHPLC fast and high resolution separation is provided, which increases LC-MS sensitivity and minimizes matrix interference arising from minimal sample preparation [23,24]. UHPLC is mainly performed in reversed-phase (RP) mode, using C18 columns. The mobile phase consists of an aqueous and an organic solvent. Methanol and Acetonitrile are commonly used as organic solvents. In some methods, the mobile phase is acidified with small percentages by volume of acetic or formic acid in order to improve

ionization of the compounds in the positive ionization mode [25]. Gradient elution programs are preferred for better and faster separations.

2.3.2 High-resolution mass spectrometry (HRMS)

Many scientific groups, which are dealing with the determination of organic contaminants in environmental samples, develop analytical methods that include liquid chromatography coupled to tandem mass spectrometry using low resolution mass analyzers, usually triple quadrupole (QqQ), because this technique is reliable for qualitative and quantitative determination of selected/known biomarkers. On the other hand, the use of liquid chromatography coupled to high-resolution mass spectrometry allows the wide-scope screening of parent compounds and (bio)TPs, which may be already known, suspect or unknown. Consequently, it can be used for the determination of the continuous growing and diverse group of ECs [26–28]. Among the possible ionization techniques in LC-MS, ESI is the most widely used, compared with atmospheric pressure chemical ionization (APCI) or the more recent atmospheric pressure photoionization (APPI) [22]. LC-HRMS has an excellent performance on qualitative applications thanks to the high mass accuracy and the selectivity in full-scan acquisition mode that ensure reliable detection and identification, while more and more studies use LC-HRMS for complete analysis, both identification and quantification [26-28]. With full-spectrum accurate-mass data, a theoretically unlimited number of analytes which are present in a sample can be identified, because the acquisitions have been made as “all ions all the time” [21]. The simultaneous determination of a broad number of compounds in one injection, with a corresponding reduction of time and costs, and even when reference standards are not available, make LC-HRMS one of the current trends in environmental analytical chemistry [29]. Moreover, investigation can be performed in a retrospective way in order to detect compounds that initially were not considered, even after years, without additional analysis of the samples. This ability is advantageous, because in some occasions, samples might already have been discarded or the analytes have been degraded [27,28].

Time-of-flight (TOF) is one of the most used HRMS analyzers and it is easily coupled to ultra high performance liquid chromatography (UHPLC). Mass resolution typically ranges from 20,000 up to 80,000 Full Width at Half Maximum (FWHM) and mass accuracy is lower than 2 ppm. Hybrid configurations, such as Quadrupole-Time-of-flight (QTOF), increase the potential of the analyzer for screening purposes and provide relevant structural information by obtaining accurate-mass product-ion spectra after MS/MS experiments [29]. Product-ion spectra can be obtained with either data

dependent acquisition or data independent acquisition, where the instrument automatically switches after a full-scan-mode acquisition to a product-ion scan mode as the second scan event in the scan cycles [21].

2.3.2.1 Data Dependent Acquisition (DDA)

In this acquisition, there is firstly a full scan which is defined as the survey scan and data are processed “on-the-fly” to determine the candidates of interest based on predefined selection criteria, such as intensity threshold or suspect inclusion list. If the selection criteria are met, MS/MS analysis is then triggered and MS/MS scans (data-dependent) are performed [22,28].
With this acquisition, ‘clean’ spectra with structural information are obtained in one injection. However, if the number of candidates of interest is big, the number of scans is decreased, so there are less data points that affect the detectability of the chromatographic peak [22].

2.3.2.2 Data Independent Acquisition (DIA)

With this acquisition, there is no need to pre-select the precursor ion. Full-scan spectra at different collision energies are obtained in one injection. This acquisition provides simultaneously accurate mass data of parent compounds and fragment ions in a single run using two scans, one at low and one at high collision energy. By applying low energy (LE) in the collision cell, no fragmentation is performed.A full-scan spectrum is obtained that provides information for the parent ion (the (de)-protonated molecule) and, in some cases, the adduct ions and the in-source fragments. By applying high energy (HE) in the collision cell, fragmentation is performed and a spectrum similar to MS/MS experiments is obtained. This approach is called all-ions MS/MS, MSE or bbCID, according to the QTOF manufacturer [22].

2.4 Data Treatment

After the sample preparation and the LC-HRMS analysis, raw data can be treated with three different approaches, target, suspect and non-target screening. A systematic workflow for all three approaches is shown in the following Figure 7.

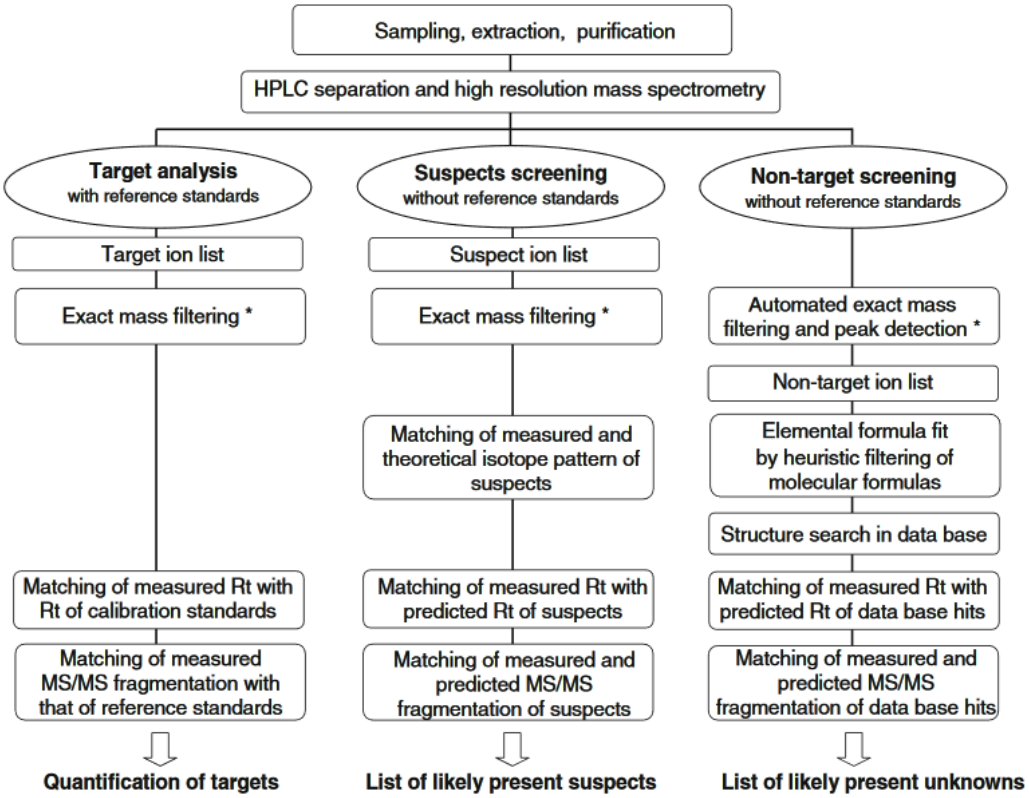


Figure 7: Systematic workflow for target, suspect and non-target screening by LC-HRMS/MS [22]

2.4.1 Target screening

In this approach, an in-house developed database is used for the screening of a large number of compounds. The information included in the database is based on the analysis of the available reference standards [29]. The reference standard is necessary for comparison of the retention time, the MS spectrum profile (precursor ion, adducts, in-source fragments), as well as the MS/MS spectrum (fragment ions and ion ratios) [30]. Quantitation can be performed in full-scan mode, but requires greater effort than in LC-LRMS methods where Single Reaction Monitoring (SRM) mode is used [29,30].

2.4.2 Suspect screening

In this approach, a list of suspect compounds that are possible to be found in specific samples is built. The screening is based only on the exact m/z of the expected ions, which, in case of the ESI source, are usually the pseudomolecular ions [M+H]+ and [M-H]-, except for some compounds which exclusively show adduct formation. Molecular formula and structure are known,

so this information can be efficiently used in the identification and confirmation process [22]. Absence from blank samples, mass accuracy, isotopic pattern, retention time prediction, ionization efficiency and information on fragment ions reported in the literature are parameters that can facilitate tentative identification of suspect candidates [22,31].

2.4.3 Non-target screening

In non-target methodologies, samples are searched for compounds without any previous information on them. These unknown compounds are actually new, unexpected or not searched ones in specific samples. Identification is a challenge in this approach, as more than one elemental formula and several plausible structures are obtained for a given unknown compound detected in a sample [27,28]. Except for the elucidation of unknowns, non-target screening is used for the identification of (bio) TPs, arising from in vivo and in vitro experiments, in-silico modeling and degradation laboratory studies [21]. In this case, the number of chemically meaningful structures, which can be assigned to an unknown peak, is limited to structures that show a close relationship with the parent compound and also, an adequate control sample or time series is available [22].

The development and the use of powerful HR-MS is the drivingforce in development of novel analytical methodologies for the identificationof TPs. Due to its sensitivity in full-scan acquisition modeand high mass accuracy, HR-MS is suitable for target and nontargetanalysis, pre- and post-acquisition processing, retrospective analysis and discovery of TPs [8].

2.4.4 Confidence in the identification procedure

2.4.4.1 Confidence in target screening

The confirmation of positive findings in target screening can be performed by attributing identification points (IPs). According to the 2002/657/EC guideline, 4 IPs are required for unequivocal confirmation, and for HRMS instruments with resolution higher than 10,000, the precursor ion earns 2 IPs and the product ions earn 2.5 IPs [32]. This means that one single HRMS/MS transition can confirm the detection of a substance, which is risky when there are several co-eluting isomers. Another fact is that resolving power may largely vary between HRMS instruments, which makes the definition of general criteria difficult [21]. More precise criteria for the use of mass accuracy and mass resolution have to

be implemented to define clearly the requirements for a reliable confirmation in LC-HRMS [22]. Bletsou et al. [30] proposed an identification points system for HRMS analysis in order to take full advantage of the capabilities of HRMS instruments.

2.4.4.2 Confidence in suspect and non-target screening

An identification strategy through five levels of confidence has been proposed for HRMS screening by Schymanski et al. [33], as described in the following Figure. Level 1 corresponds to the confirmed structure by the use of a reference standard, level 2 to a probable structure using literature or diagnostic data, level 3 to tentative candidate(s) with possible, not exact, structures, level 4 to an unequivocal molecular formula and level 5 to the exact mass. Non-target screening starts from level 5 and suspect screening from level 3 and, as identification confidence increases, they reach 'better' levels up to level 1. Target screening starts by definition from level 1. If the evidence of the sample and the evidence of the reference standard (target) or the tentative candidate (suspect) do not match, then the component associated with the target or suspect should become a 'non-target of interest' and 'downgrade' to level 5 [34].

Generally, in both suspect and non-target screening, reference standards are required for ultimate and unambiguous confirmation, but should be purchased in a final stage, when solid well-found evidence exists on the presence of the compound in the sample [22].

Moreover, complementary techniques can be used for evaluation of possible candidates, such as NMR, a powerful structure elucidation technique, although this requires sufficiently high concentrations and often an isolation of the unknown compound [22].

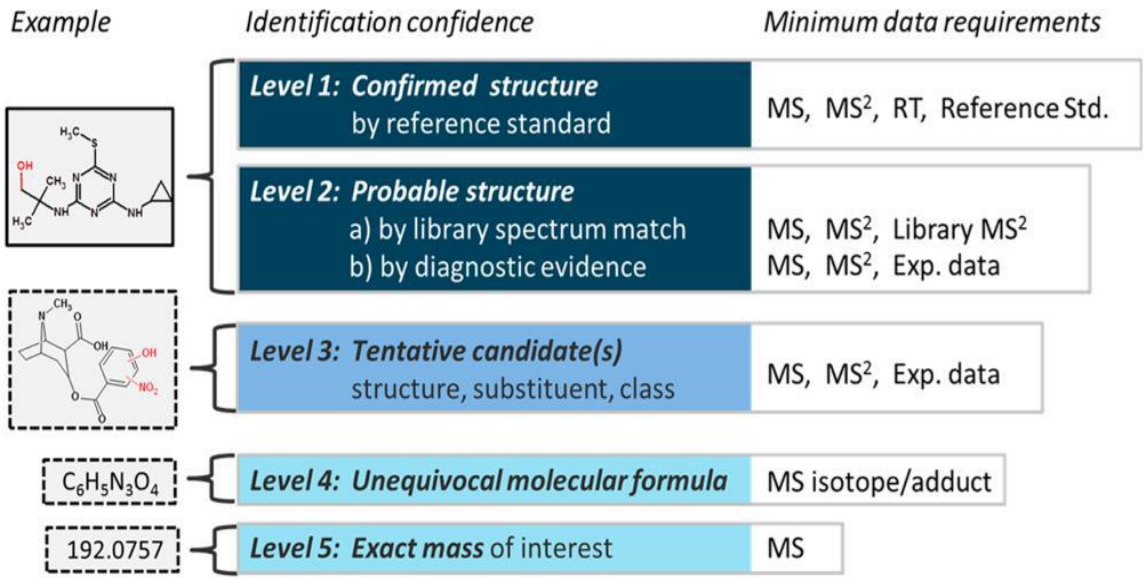


Figure 8: Identification confidence levels in HRMS [33].

2.5 Literature Review

Scientific knowledge about the course and effects of pollutants on aquatic ecosystems has improved significantly in recent years. More is now known about the aquatic environment (eg water, sediment, or living organisms) in which a pollutant may be discharged. Therefore, it is possible to detect, identify or even measure its concentration. However, for some very hydrophobic substances, which tend to accumulate in living organisms, it is difficult to detect them, even with the use of advanced analytical techniques. In recent decades, numerous studies and publications have been carried out to identify OCPs, PAHs, PCBs and many other groups of substances belonging to the emerging contaminants group like perfluoroalkyl substances [PFASs], pharmaceutical and personal care products [PPCPs] in samples of fish and other marine organisms (mussels and other shellfish, shrimp, crabs). From a variety of wetlands with residues of anthropogenic activity (urban wastewater, industrial waste, pesticides, and fertilizers from agricultural activities), with the aim of demonstrating satisfactory methods of simultaneous determination of these compounds. In Table 1 the biota matrices are presented, in Table 2 the water samples matrices are presented and finally in Table 3 the sediment matrices and the clean-up techniques for the determination of specific analytes by gas chromatography (GC) and liquid chromatography (LC) are listed, respectively.

Table 1: Biota matrices, analytes, clean-up techniques, analytical techniques and references

#	Matrices	Analytes	Clean-up Technique	Analytical Technique	Reference
1	Mussel Tissue	Polycyclic Aromatic Hydrocarbons(PAH'S)	matrix solid-phase dispersion–gel permeation chromatography	GS-MS	[35]
2	Mussel Tissue	Polycyclic Aromatic Hydrocarbons(PAH'S)	Column Chromatography	GS-MS	[36]
3	Mussel Tissue	Trace elements compounds	Microwave Assisted Extraction (MAE), Accelerated Solvent Extraction (ASE)	GS-MS	[37]
4	Mussels (Mytilus Galloprovincialis)	Polybrominated diphenyl ethers (PBDEs)	Accelerated Solvent Extraction (ASE)	GS-HRMS	[38]
5	Mussel Tissue	Polychlorinated Biphenyls (PCBs), POPs (Persistent Organic Pollutants)	Microwave Assisted Extraction (MAE), Accelerated Solvent Extraction (ASE), Soxhlet Extraction	GS-MS-MS	[39]
6	bluemussels (*Mytilus edulis*), 8 oysters (*Ostrea edulis*), 3 common cockles (*Cerastoderma edule*) and 7 surf clams (*Spisula solida*)	Polycyclic Aromatic Hydrocarbons(PAH'S)	*Accelerated Solvent Extraction (ASE) followed by two semi-automatic clean-up steps; gel permeation chromatography (GPC) on S-X3 and solid phase extraction (SPE) on pre-packed silica columns*	GS-MS	[40]
7	Mussel Tissue	polychlorinated naphthalenes (PCNs)	pressurized liquid extraction (PLE)	GS-HRMS	[41]
8	mussels *Mytilus edulis trossulus*	Non-steroidal antiinflammatory drugs and natural estrogens	*Accelerated Solvent Extraction (ASE)*	GS-MS	[42]
9	blue mussel (*Mytilus edulis*), salmon fillet (*Salmo salar*)	Polycyclic Aromatic Hydrocarbons(PAH'S)	Accelerated Solvent Extraction (ASE) followed by gel permeation chromatography (GPC)	GS-MS	[43]
10	Mussel Tissue	Tributyltin (TBT) and Triphenyltin (TPT)	Accelerated Solvent Extraction (ASE)	GS-MS	[44]
11	mussel (Mytilus galloprovincialis)	Organochlorine compounds	Column Chromatography	GS-MS	[45]
12	mussels(*Mytilus galloprovincialis*)	Butiltyn compounds	Solid-phase extraction (SPE) on Florisil pre-packed columns	GS-MS	[46]
13	fish and mussel tissue	Polybrominated diphenyl ethers (PBDEs)	Ultrasound-assisted extraction	GC-ICP-MS	[47]

14	insect larvae (Hydropsyche exocellata), zebra mussels (Dreissenapolymorpha), oysters (Crassostrea gigas), anchovies (Engraulisencrasicholus), sardines (Sardinapilchardus), and crabs (Procambarusclarkii)	Perfluorinated compounds(PFCs)	Ultrasound-assisted extraction	LC-MS-MS	[15]
15	Mollusks	Perfluorinated compounds(PFCs)	Solid-phase dispersion (MSPD)	LC-MS-MS	[14]
16	fresh fish muscle tissue	Pesticides	QuEChERS extraction	LC-MS-MS	[18]
17	Mollusks	Pharmaceuticals and various endocrine -disrupting chemicals (EDCs)	Solvent extraction	LC-ESI-MS-MS	[10]
18	Fish tissue, bivalves	Bisphenol A (BPA) and Tetrabromobisphenol A (TBBPA)	QuEChERS extraction followed by a liquid–liquid extraction (LLE)	LC-MS-MS	[48]
19	mussels (Mytilus galloprovincialis and Mytilus edulis) and croalgae (Laminaria digitata)	Diclofenac	QuEChERS extraction	LC-MS	[49]
20	Mussels and Oysters	Marine toxins		LC-HRMS/QTOF	[50]
21	Fish tissue	Hydroxylated Polybrominated diphenyl ethers (OH-PBDEs)	Liquid–liquid extraction (LLE)	LC-MS-MS	[51]
22	Marine sponge and bull shark liver	Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers	Column Chromatography	(APCI-LC-MS-MS	[52]
23	Grey mullet liver (Chelonlabrosus) and mussel (Mytilus galloprovincialis)	Perfluorinated compounds(PFCs)	Accelerated Solvent Extraction (ASE)	LC-MS-MS	[53]
24	Mussel Tissue	Polycyclic Aromatic Hydrocarbons(PAH'S)	Accelerated Solvent Extraction (ASE)	HPLC	[54]

Table 2: Water samples matrices, analytes, clean-up techniques, analytical techniques and references

#	Matrices	Analytes	Clean-up Technique	Analytical Technique	Reference
1	Water samples	Persistent Organic Pollutants	Solid-phase Micro extraction (SPME)	GC-(Q)TOF, GC–HRMS	[55]
2	Water samples	Polybrominated diphenyl ethers (PBDEs)	Solid-phase extraction (SPE)on silical pre-packed columns	GC-MS	[56]
3	Water samples	Polybrominated diphenyl ethers (PBDEs)	Liquid/liquid extraction(LLE) followed by Column Chromatography	GC-MS	[57]
4	Water samples	tetrabutyltin (TeBT), tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), triphenyltin (TPhT), diphenyltin (DPhT), monophenyltin (MPhT), tricyclohexyltin (TCyT), and dicyclohexyltin (DCyT)	Pressurised liquid extraction(PLE) .	GC–HRMS	[58]
5	Water samples	Polychlorinated biphenyls (PCBs), Polychlorinated alkanes (PCAs), Polybrominated diphenyl ethers (PBDEs), Polychlorinated dibenzo-*p*-dioxins Polychlorinated furans (PCDD/Fs)	Matrix solid-phase dispersion (MSPD)	GC–HRMS	[59]
6	Water samples	Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs)	Column Chromatography	GC–HRMS	[60]
7	Water samples	Pharmaceuticals	Column Chromatography	LC–ESI-QTOF MS	[61]
8	Water samples	Pesticides and transformation products, antibiotics and several drugs	Solid-phase extraction (SPE) on mix mode pre-packed columns	Ultra-performance liquid chromatography-QTOF (UPLC-QTF)	[62]
9	Water samples	Polar organic micro-pollutants	Solid-phase extraction (SPE) on Omnifrit columns	LC–HRMS	[63]
10	Water samples	Pharmaceuticals, surfactants, biocides, personal care products, and sweeteners enter	Solid-phase Micro extraction (SPME)	LC–HRMS	[64]
11	Water samples	Pharmaceutical drugs, disinfection agents,	Solid-phase Micro extraction (SPME)	LC-MS/MS, LC-TOF-MS	[65]

		pesticides			
12	Water samples	trace tributyltin and triphenyltin	Ultrasound-assisted extraction	LC-ESI-MS/MS	[66]
13	Water samples	Tributyltin chloride (TBT)	Molecularly imprinted polymers (MIPs)	LC-MS/MS	[67]
14	Water samples	Phthalate Ester Metabolites	Accelerated Solvent Extraction (ASE)	LC-ESI-MS/MS	[68]

Table 3: Sediment matrices, analytes, clean-up techniques, analytical techniques and references

#	Matrices	Analytes	Clean-up Technique	Analytical Technique	Reference
1	Sediment	Polychlorinated biphenyls (PCBs) Polybrominated diphenyl ethers (PBDEs)	Ultrasonic-solvent extraction	TD–GC–MS/MS	[69]
2	Sediment	Chlorinated paraffins (CPs)	Column Chromatography	SCGC/ECNI-MS	[70]
3	Sediment	Polybrominated diphenyl ethers (PBDEs)	Ultrasound-assisted leaching-dispersive solid-phase extraction followed by dispersive liquid–liquid microextraction (USAL-DSPE-DLLME)	GC–MS/MS	[71]
4	Sediment	Organochlorine pesticides (OCPs)	Graphitized carbon black solid-phase extraction(GCB-SPE)	GC–MS	[72]
5	Sediment	Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs	Solid-phase xtraction (SPE)on C-18 pre-packed columns	GC–HRMS	[73]
6	Sediment	Polychlorinated *n*-Alkanes	Accelerated Solvent Extractor (ASE)	GC–HRMS	[74]
7	Sediment	Polychlorinated biphenyls (PCBs), Organochlorine pesticides (OCPs) and chlorobenzenes	Solid-phase Micro extraction (SPME)	GC–MS/MS	[75]
8	Sediment	Pharmaceuticals, personal care products, alkyl phenols and	QuEChERS extraction	LC-ESI-MS/MS	[76]

		plasticisers			
9	Sediment	Pesticides	Solid Phase Micro Extraction (SPME)	HPLC	[77]
10	Sediment	Endocrinedisrupting chemicals	QuEChERS extraction	LC–MS/MS	[78]
11	Sediment	Ametryn	Liquid-liquid extraction(LLE)	LC–MS/MS	[79]
12	Sediment	Natural estrogens, organochlorine pesticides, parabens, polycyclic aromatic hydrocarbons (PAHs), bisphenol A , alkylphenols	Ultrasound-assisted extraction	LC–MS/MS	[80]
13	Sediment	polycyclic aromatic hydrocarbons (PAHs),	Accelerated Solvent Extraction (ASE)	GC–MS	[81]
14	Sediment	polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs (nitro-PAHs), oxygenated forms of PAHs (oxy-PAHs), hydroxy-PAHs (OH-PAHs)	Accelerated Solvent Extraction (ASE)	GC-ESI/MS, LC-ESI/MS,	[82]
15	Sediment	Polychlorinated Biphenyls (PCBs) and Organochlorine pesticides (OCPs)	Accelerated Solvent Extraction (ASE)	GC–MS/MS	[83]
16	Sediment	Synthetic musks	Accelerated Solvent Extraction (ASE)	GC–MS	[84]
17	Sediment	ultra-trace carbazoles	Accelerated Solvent Extraction (ASE)	GC–MS/MS	[85]
18	Sediment	Polybrominated diphenyl ethers (PBDEs), Polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs)	Accelerated Solvent Extraction (ASE)	GC–MS	[86]
19	Sediment	Organochlorine pesticides (OCPs)	Solid-phase extraction (SPE) on silica and Florisil pre-packed columns	GC–MS	[87]
20	Sediment	Polychlorinated naphthalenes (PCNs)	Soxhlet extraction	GC–HRMS	[88]

CHAPTER 3: SCOPE

Overwhelming evidence over many decades has shown that many organic compounds, or“emerging contaminants”, have been released in the environment due to anthropogenic activities. In the last four decades, the Black Sea has suffered important changes induced by human activities.Waste from towns and cities, farms and factories flow into the Black Sea; some come directly from the coast, but most flows relentlessly from the region's major rivers, River Danube, Dnieper and Dniester. It is not surprising that the Black Sea is one of the world's most threatened marine ecosystems.

The Water Framework Directive (WFD) (2000/60/EC) sets a comprehensive management planning system to help protect and improve the ecological and chemical status of European water bodies. This is underpinned by the use of environmental quality standards (2013/39/EU) to help assess risks to the ecological quality of water environment and to identify the scale of improvements that would be needed to bring waters under pressure back into a good condition. Recent advances and improvements in analytical techniques, and especially in high resolution mass spectrometry, have given the opportunity to scientific groups to detect and identify a huge number of chemical compounds, even in complex matrices. LC-HRMS allows the wide-scope screening of ECs and their (bio) TPs with an acquisition of accurate-mass full spectrum data. These data can be used for target, suspect and non-target screening, as well as retrospective screening, years after the treatment of samples without additional analysis of them.

This master thesis is part of EMBLAS PLUS 2019 project, funded by the United Nations Programme. (https://www.euneighbours.eu/en/east/stay-informed/projects/emblas-plus-improving-environmental-monitoring-black-sea-special).The project builds on the results of the previous EMBLAS and EMBLAS-II projects to improve protection of the Black Sea environment through further technical assistance focused on marine data collection and local small-scale actions targeted at reduction of pollution by marine litter, public awareness raising and education. The project works with key partners from research/scientific and educational institutions, and civil society organisations. The overall objective of the project is to help improve protection of the Black Sea environment. The project title is “Monitoring of priority pollutants and emerging contaminants in Black Sea”.

The aim of this master thesis is the determination of emerging contaminants with a wide LC-ESI-Q-ToF target screening methodology of more than 2000 compounds. The seawater samples were purified with SPE method and used

HLB cartridges, sediments were purified with ultrasonic extraction and biota samples with 2-days generic extraction before the analysis. LC-ESI-Q-ToFanalysis was performed on all samples with positive and negative electrospray ionization as well as with bbCID and Auto-MS scan modes.

CHAPTER 4: MATERIALS AND METHODS

4.1 Chemicals and Materials

Regarding the chemicals of the sample preparation; Methanol, Acetonitrile were HPLC grade, and were purchased from Fischer Scientific (Loughborough, UK), Formic Acid 98-100% for analysis was purchased from CARLO ERBA Reagents S.A.S. (Barcelona, Spain), while Ammonia solution 25% for analysis was purchased from CHEM-LAB NV (Zedelgem, Belgium).

All the solvents for the chromatographic analysis were hypergrade for LC-MS. Methanol and Acetonitrile were obtained from Merck (Darmstadt, Germany) and the eluent additives ammonium formate, ammonium acetate and formic acid 99% were purchased from Sigma Aldrich (Steinheim, Germany). Ultrapure water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA).

Regarding with the internal standards (IS), which were used for the analysis and the method validation, Flunixin-d3, Meloxicam-d3, Bisphenol A (BPA)-d16, Diuron-d6, Atrazine-d5, Diazepam-d5 and (±) Amphetamine-d6 were obtained from Sigma Aldrich (Steinheim, Germany), while Sulfadiazine-d4, Sulfadimidine-d4, Sulfadimethoxine-d4, Cetirizine-d3, Mefenamic Acid-d3, Diethyl-phthalate-d4, Aspartame-d3 and Sucralose-d6 were obtained from Toronto Research Chemicals (Ontario, Canada).

Isotope labeled compounds were added and spiked samples were also prepared and analyzed for ensuring the quality control of every method, for recoveries estimation and for quantitation purposes.

4.2 Sample Pretreatment

Samples were gathered in July/ August of 2019, from different locations of Black Sea region. Water samples from Ukraine coastal, closest to Danube delta, Open Sea and close to Georgia/coastal Georgia. Sediments from Ukraine, Open Sea and Georgia. Biota samples from Georgia, Ukraine and Russian federation. The sampling locations are shown in the following figure. The sampling stations for sediments are colored in brown.

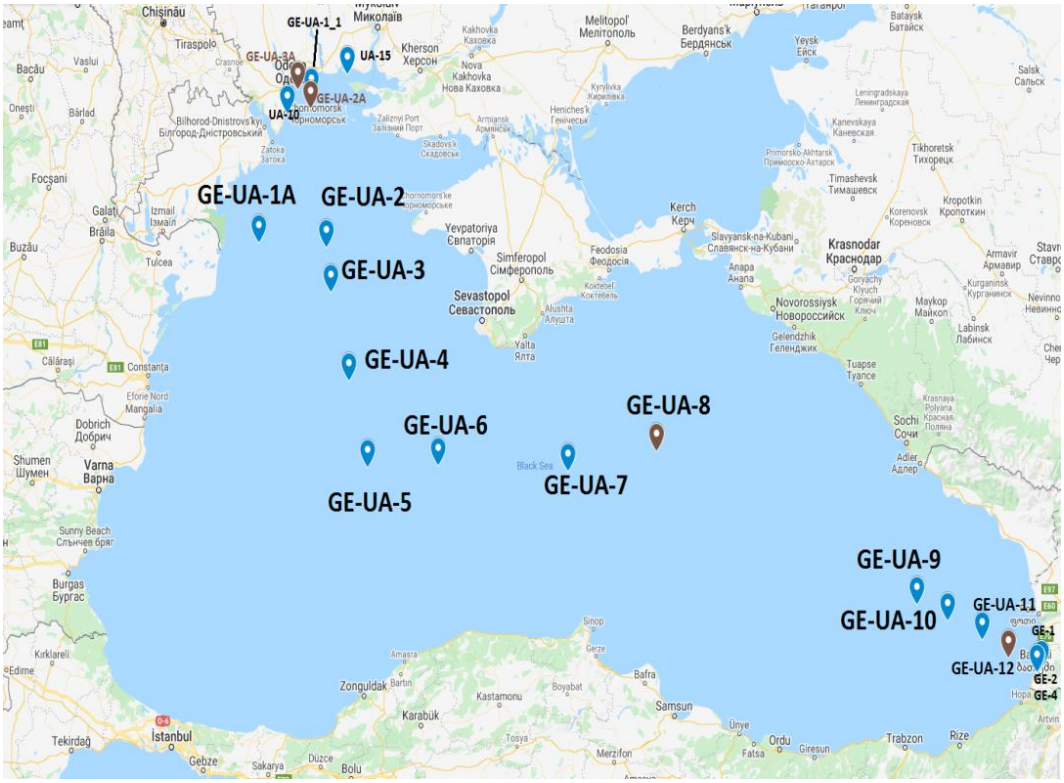


Figure 9: ***Sampling Locations*** *(source: https://tinyurl.com/y2u2xqpk, ©Alygizakis N.)*

Seawater samples were processed in Environmental Institute (Slovakia) using HORIZON SPE-DEX 4790 device (USA). The samples were cleaned up and pre-concentrated on Atlantic HLB-M Disk with 47 mm disk holder according to an automated extraction program. The extracts were evaporated using gentle stream of nitrogen and reconstituted at 50:50 methanol: water (500 uL total volume extract). The extracts were then shipped to the Laboratory of Analytical Chemistry for analysis. Wet sediments and biota samples were sent to theLaboratory of Analytical Chemistry on dry ice, according to the strict protocols. All samples were lyophilized, using the Telstar's Freeze-Dryer LyoQuest, before analysis, in order to enhance extraction efficiency, improve the precision and achieve lower detection limits. After lyophilization, the % humidity of each sample was calculated and the freeze-dried samples were homogenized using pestle and mortar. After homogenization, the samples were storage in brown glass bottles in the freezer (-80 °C) till the analysis.

Figure 10: ***Freeze-Dryer, Telstar*** *(source:https://www.telstar.com/lab-hospitalsequipment/laboratory -freeze-dryers/lyoquest/).*

All the coding and freeze-drying details for the samples are included in the following tables.

Table 4: Sea water samples coding and details

Short Coding	Sample Code	Matrices	Sampling Location	Sampling Year
SW 1	GE-UA-1	water	Ukraine coastal	2019
SW 2	GE-1	water	Georgia Coastal	2019
SW 3	GE-UA-1A	water	Danube	2019
SW 4	GE-UA-2	water	Close to Danube Delta	2019
SW 5	GE-UA-2A	water	Ukraine coastal	2019
SW 6	GE-UA-3	water	Open Sea	2019
SW 7	GE-UA-3A	water	Ukraine coastal	2019
SW 8	GE-UA-4	water	Open Sea	2019
SW 9	GE-UA-5	water	Open Sea	2019
SW 10	GE-UA-6	water	Open Sea	2019
SW 11	GE-UA-7	water	Open Sea	2019
SW 12	GE-UA-8	water	Open Sea	2019

SW 13	GE-UA-9	water	Close to Georgia	2019
SW 14	GE-UA-10	water	Close to Georgia	2019
SW 15	UA-10	water	Ukraine coastal	2019
SW 16	GE-UA-11	water	Close to Georgia	2019
SW 17	UA-11	water	Ukraine coastal	2019
SW 18	GE-UA-12	water	Close to Georgia	2019
SW 19	UA-15	water	Ukraine coastal	2019
SW 20	GE-2	water	Georgia Coastal	2019
SW 21	GE-4	water	Georgia Coastal	2019

Table 5: Sediments samples coding and details

Short Coding	Sample Code	Matrices	Sampling Location	Sampling Year
S1	JBSS-GE-UA8	sediment	Open Sea	2019
S2	JBSS-GE-UA-2A	sediment	Ukraine	2019
S3	JBSS-GE-UA-12	sediment	Georgia	2019
S4	JBSS-GE-UA-3A.M	sediment	Ukraine	2019

Table 6: Biota samples coding and details

Short Coding	Sample Code	Species	Matrices	Sampling Location	Sampling Year
BSB 1	UoA 1	Tracturustrachurus	fish	Georgia	2019
BSB 2	UoA 2	Tracturustrachurus	fish	Georgia	2019
BSB 3	UoA 3	Mullusbarbatus	fish	Georgia	2019
BSB 4	UoA 4	Mullusbarbatus	fish	Georgia	2019
BSB 5	UoA 5	Uranoscopusscaber	fish	Georgia	2019
BSB 6	UoA 6	Uranoscopusscaber	fish	Georgia	2019
BSB 7	UoA 7	Rapanavenosa	mollusks	Georgia	2019

BSB 8	UoA 8	Mytilusgalloprovincialis	mollusks	Georgia	2019
BSB 9	UoA 9	Mytilusgalloprovincialis	mollusks	Ukraine	2019
BSB 10	UoA 10	Belonebelone	fish	Ukraine	2019
BSB 11	UoA 11	Trachurusmediterraneus	fish	Ukraine	2019
BSB 12	UoA 12	Rapanavenosa	mollusks	Ukraine	2019
BSB 13	UoA 13	Merlangiusmeriangus	fish	Ukraine	2019
BSB 14	UoA 14	Neogobius melanostomous	fish	Ukraine	2019
BSB 15	UoA 15	Mytilusgalloprovincialis	mollusks	Ukraine	2019
BSB 16	UoA 16	Scorpaenaporcus	fish	RussianFederation	2019
BSB 17	UoA 17	Symphodustinca	fish	RussianFederation	2019
BSB 18	UoA 18	Scophthalmus	fish	RussianFederation	2019
BSB 19	UoA 19	Mytilusgalloprovincialis	mollusks	Russian Federation	2019
BSB 20	UoA 20	Horse mackerel	fish	RussianFederation	2019

Table 7: Sediments freeze-drying details

Lab Code	mass of wet sample (g)	mass of freeze-dried sample (g)	% humidity
S1	128.0028	25.5279	81.7
S2	158.8752	70.2798	55.6
S3	140.9890	37.1873	76.2
S4	156.3740	61.6412	57.8

Table 8: Biota freeze-drying details

Lab Code	mass of wet sample (g)	mass of freeze-dried sample (g)	% humidity
BSB-1 2019	25.7022	5.4122	78.9
BSB-2 2019	27.8301	6.3321	77.2
BSB-3 2019	28.4368	5.9420	79.1
BSB-4 2019	22.6021	4.9321	78.2
BSB-5 2019	31.5620	6.0560	80.8
BSB-6 2019	28.5610	5.3418	81.3

BSB-7 2019	29.9617	7.7216	74.2
BSB-8 2019	25.0084	4.4562	82.2
BSB-9 2019	23.5681	3.4905	85.2
BSB-10 2019	27.1658	6.9496	74.4
BSB-11 2019	32.3119	7.2669	77.5
BSB-12 2019	41.6103	12.0593	71.0
BSB-13 2019	29.6627	5.8725	80.2
BSB-14 2019	30.5462	6.5175	78.7
BSB-15 2019	29.5410	3.9090	86.8
BSB-16 2019	47.7299	12.7719	73.2
BSB-17 2019	33.5077	8.0429	76.0
BSB-18 2019	57.5704	21.7936	62.0
BSB-19 2019	48.7295	12.5279	74.3
BSB-20 2019	48.7379	12.2886	74.8

4.3 Sample preparation

Sediments and biota samples were lyophilized (temperature: -55°C, vacuum: 5×10-2 mbar) before analysis, in order to enhance extraction efficiency, improve the precision and achieve lower detection limits. In addition, the concentrations of the analytes can be expressed in both dry weight and wet weight.The samples were subjected to specific pre-treatment methods prior to the identification of the emerging contaminants with LC-HRMS target screening. These methods are refered as follows.

4.3.1 LC-HRMS target screening method in Seawater samples

Seawater samples were filtered through glass fiber filters (GFF, pore size 0.7 μm), using a pressurized filtration apparatus, immediately after sampling and 2 L of each sample were stored in the dark at 4 °C until extraction (if needed). After the adjustment of the pH of the samples to 6.5 (±0.2) with few drops of HCl 0.1 M, 25 μL of the IS mix solution were spiked in each sample. Sample clean-up and pre-concentration was realized by SPE. Layered ‘mixed bed’ cartridges consisting of Oasis HLB (200 mg) and a mixture of Strata-X-AW (weak anion exchanger), Strata-X-CW (weak cation exchanger) and Isolute ENV+ (300 mg of total mixture) were used. Conditioning of the cartridges were performed with 3 ml methanol and 3 ml water. The sample was loaded to the SPE cartridges at 10 mL/ min using a vacuum manifold. Then, the cartridges were kept refrigerated at -20 °C and delivered to the laboratory for the rest of the analysis. In the laboratory, the cartridges were rinsed with 6 mL of Milli-Q water and dried by passing air through the cartridges for 0.5 to 1 h (using vacuum on the SPE box; cartridges were visual inspected for complete dryness). The elution of the analytes from the adsorbent material was performed

by a basic solution (6 mL of ethylacetate/methanol (50/50 v/v) containing 2% ammonia hydroxide (v/v)), followed by an acidic solution (4mL of ethylacetate/methanol (50/50, v/v) containing 1.7% formic acid (v/v)). The extract was evaporated under a gentle nitrogen stream (at 45 °C) to a final volume of 50 µL and finally reconstituted to a final volume of 250mL (methanol/ Milli-Q, 50/50 v/v). Samples were sent to the laboratory after being pre-treated so the final step was that the extract filtered directly into a 2 mL vial using a syringe fitted with a 0.22 µm RC membrane filter and was ready for LC-HRMS/MS analysis.

4.3.2 LC-HRMS target screening method in Biota samples

A sample preparation method comprising of a generic extraction of the analytes from the biota sample, using a mixture of solvents and further low-temperature and hexane clean-up was followed. A 0.2 g portion of freeze-dried biota sample was weighed and placed into a 15 mL polypropylene centrifuge tube. Each sample was spiked with 20 µL of the internal standards mix solution (1 mg/ L), listed 2 mL of Milli-Q water containing 0.1% formic acid (v/v) and 0.1% EDTA (w/v), 2 mL of methanol, and 2 mL of acetonitrile added in all samples, subsequently. After the addition of each solvent, the tube was vortex-mixed for 30 s. The sample set was placed in an ultrasonic bath at 60°C for 20 min, the samples were then centrifuged at 4000 rpm for 10 min, and the supernatant was decanted into a new polypropylene centrifuge tube. The tubes were then placed in -20 °C for 12 h to precipitate the lipids and remaining proteins. After centrifuging and discarding the precipitate, a defatting step with hexane completed the sample clean-up. The extracts were evaporated to dryness under a nitrogen stream and reconstituted in 0.2 mL of methanol/Milli-Q water (50/50 v/v). Finally, the extracts were filtered through a 0.22 µm pore size RC filter (to remove matrix interferences) and were transferred to a glass vial for HPLC-HRMS/MS analysis.

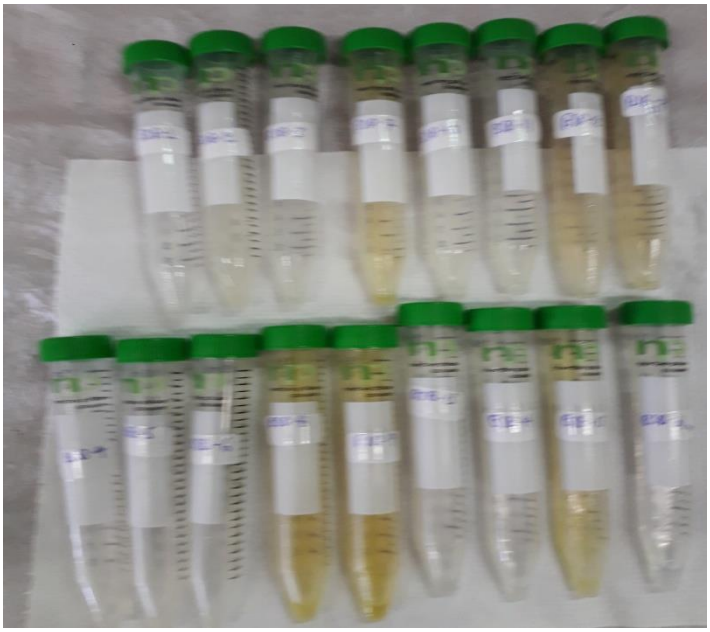


Figure 11: Biota samples before evaporation with N_2.

4.3.3 LC-HRMS target screening method in Sediment samples

0.2 g freeze-dried sediment sample was placed in a plastic centrifuge tube (15 mL), spiked with 20 µL of the internal standards mix solution and kept in contact overnight. The sample was then extracted with 2 mL Methanol–Milli-Q water (pH 2.5, formic acid 0.5 % and 0.1 % EDTA), 50:50 (v/v), by vortex (1 min), followed by ultrasonic extraction for 15 min at 50 °C. After the extraction, the extract was centrifuged for 10 min at 4000 rpm and the supernatant was collected in a glass test tube. This procedure was repeated two more times. In total 6 mL supernatant were collected. Then the total extract was evaporated to dryness under a gentle steam of N_2 at 40 °C. Reconstitution of the analytes was performed with 0.2 mL Methanol/Milli-Q water (50/50 v/v). Finally, the extract was filtered through a 0.22 µm RC syringe filter and then the samples were transferred to a glass vial for HPLC-HRMS/MS analysis.

4.4 Instrumentation

An Ultra-High Performance Liquid Chromatography (UHPLC) system (UltiMate 3000 RSLC, Thermo Fisher Scientific, Germany) coupled to a Quadrupole. Time of Flight Mass Spectrometer (QTOF-MS) (Maxis Impact, Bruker Daltonics, Bremen, Germany) was used for the analysis of the samples. The UHPLC apparatus consists of a solvent rack degasser, a binary pump with solvent selection valve (HPG-3400), an auto-sampler and a column. The QTOF-MS apparatus

consists of an Electrospray Ionization (ESI) source operating in positive and negative mode.

Figure 12: **UHPLC-QTOF-MS, Maxis Impact, Bruker Daltonics**
(source: http://www.directindustry.com/prod/bruker-daltonics/product-30029-991983.html).

In the analysis, two separate reversed-phase chromatographic runs were performed for positive and negative ESI mode. An Acclaim RSLC 120 C18 column (2.1 × 100 mm, 2.2 µm) (Dionex Bonded Silica Products, Thermo Scientific, Dreieich, Germany), preceded by an ACQUITY UPLC BEH C18 1.7 µm guard column of the same packaging material (VanGuard Pre-Column, Waters, Dublin, Ireland), and thermostated at 30°C, was used. In the positive ESI mode, the aqueous mobile phase consisted of 90% H2O, 10% CH3OH, 5 mM HCOONH4, 0.01% HCOOH and the organic mobile phase consisted of CH3OH, 5 mM HCOONH4, 0.01% HCOOH.
In the negative ESI mode, the aqueous mobile phase consisted of 90% H2O, 10% CH3OH, 5 mM CH3COONH4 and the organic mobile phase consisted of CH3OH, 5 mM CH3COONH4. The gradient elution program was the same for both ionization modes and applied changes in mobile phase and in flow rate. It started with 1.0% of organic phase (flow rate 0.200 mL/min) for 1 min, increasing to 39.0% by 3 min (flow rate 0.200 mL/min), and then to 99.9% (flow rate 0.400 mL/min) in the following 11 min. These almost pure organic conditions were kept constant for 2 min (flow rate 0.480 mL/min) and then initial conditions were restored within 0.1

min, kept for 3 min and then the flow rate decreased to 0.200 mL/min for the last minute. The gradient elution program which is used in the chromatographic analysis is also presented in the following table. The injection volume was set to 5 μL. The operating parameters of the ESI interface were the following: capillary voltage 2500 V for positive and 3000 V for negative mode, end plate offset 500 V, nebulizer pressure (N2) 2.0 bar, drying gas (N2) 8.0 L/min, drying temperature 200°C. Data were acquired through a Data Independent Acquisition (DIA) scan mode, called broad-band Collision Induced Dissociation (bbCID), which provided both MS and MS/MS spectra simultaneously using two different collision energies with a scan rate of 2 Hz and a mass range of 50-1000 Da. Low collision energy (4 eV) provided a full scan spectrum (MS) and high collision energy (25 eV) provided a spectrum where all ions were fragmented (bbCID MS/MS).

Table 9: The gradient elution program

Time (min)	Flow Rate (mL/min)	Aqueous Solvent (%)	Organic Solvent (%)
0	0,2	99.0	1.0
1	0.2	99.0	1.0
3	0.2	61.0	39.0
14	0.4	0.1	99.9
16	0.48	0.1	99.9
16.1	0.48	99.0	1.0
19.1	0.2	99.0	1.0
20	0.2	99.0	1.0

An external calibration of the QTOF mass spectrometer was performed with a sodium formate solution before analysis. Also, a calibrant injection was performed automatically at the beginning of each run and the segment of 0.1-0.25 min was used for internal calibration. The calibrant solution of sodium formate consisted of 10 mM sodium formate clusters in a mixture of water: isopropanol 1:1. The theoretical exact masses of calibration ions with formulas Na (NaCOOH)1−14 in the range of 50−1000 Da were used for calibration. The instrument provided a typical resolving power of 36,000-40,000 during calibration. Bruker's software that was used for raw data analysis was Data Analysis 5.1, TASQ Client 2.1.

4.5 Target screening for the determination of emerging contaminants

An in-house database (https://www.norman-network.com/nds/SLE/, S21 - UATHTARGETS, last visit 08/03/2020) of more than 2300 ECs and priority pollutants was used for the target screening of the samples in both positive and

negative ESI mode. The database contained compounds from different classes; personal care products, steroids & hormones, pharmaceuticals (>450), antibiotics (>50), illicit drugs and new psychoactive substances (>500), industrial chemicals (>100), pesticides (>900), sweeteners, surfactants, biocides as well as their (bio) TPs. The database contained information for the precursor ions, retention time, adducts, in-source fragments and bbCID, MS/MS fragments, as well as identifiers for the compounds (CAS number, InChI). This information was acquired from the analysis of the standard solutions of these compounds, which were available in the laboratory, with the bbCID method, or was part of the manufacturer's database, Bruker's ToxScreener 2.1, which was built with the same bbCID method.

The raw data were processed with Bruker's TASQ Client 2.1 and Data Analysis 5.1. The TASQ method in TASQ Client 2.1 created in all samples the Extracted Ion Chromatogram (EIC) of the precursor ion of the compounds included in the database with a mass error window of ±0.005 Da.

Every peak, which was detected for a target compound was evaluated according to some parameters that were set to the method and after manual inspection. The first one was the mass accuracy, which refers to the difference between the accurate mass (measured) and the exact mass (theoretical) and is expressed in mDa or ppm. The second one was the retention time shift, which refers to the difference between the measured retention time and the one that is recorded to the database. The last parameter was the isotopic fitting, which refers to the correlation between the theoretical and the experimental isotopic pattern. Its calculation is based on the standard deviation of the masses and the intensities for all isotopic peaks and is expressed by the mSigma value. Lower mSigma value indicates better isotopic fitting.The screening parameters that were set to the method in both positive and negative ESI mode were an area threshold of 1000 counts and an intensity threshold of 500 counts. Regarding the mass accuracy, peaks having this value higher than 2.0 mDa and 5 ppm were rejected. Regarding the retention time, peaks having this value higher than 0.4 min were also rejected. The mSigma threshold was set to 200. However, this value was only considered as a positive confirmation and not for rejecting peaks, because strong matrix effects combined with low concentration levels of analytes may affect the isotopic pattern results and give a bad mSigma value, although the compound may be present. In order to confirm the screening results, bbCID MS/MS fragments were examined, as well as adducts and in-source fragments in full scan MS. Apart from the EIC of the precursor ion of a compound, the TASQ method created with the same mass error window the EICs of its adducts, in-source and bbCID MS/MS fragments, so the fitting of their chromatographic profiles were inspected and evaluated. Except for TASQ Client 2.1, Data Analysis 5.1 was used for the inspection and evaluation of the bbCID mass spectra.

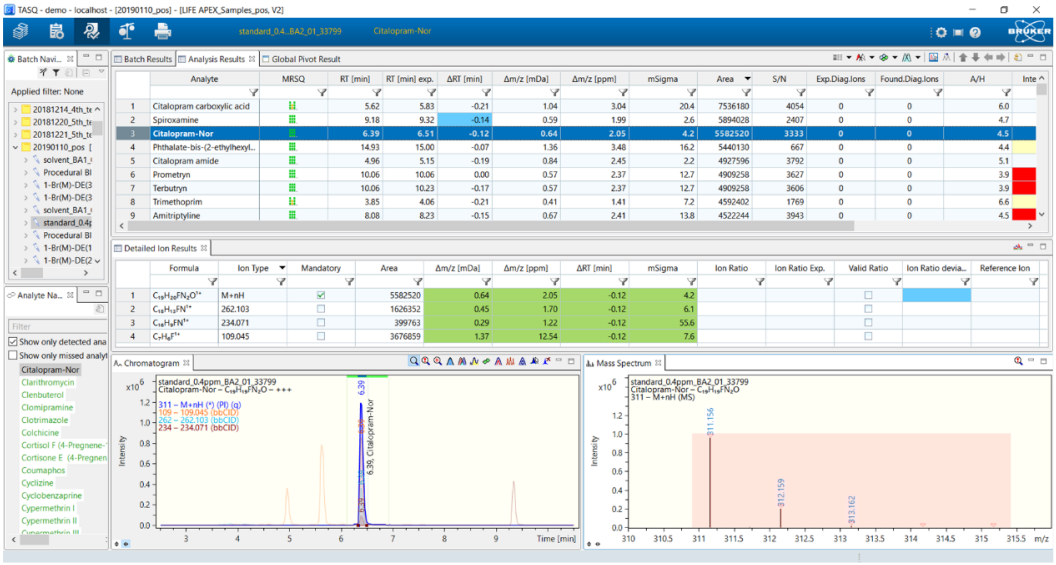


Figure 13: Data Treatment Interface - Tasq Client 2.1 (Bruker Daltonics)

For the identification and confirmation of the analytes, the Identification Points (IPs) system that has been proposed for HRMS analysis by Bletsou et al. [62] was used. Precursor ion (mass accuracy) and retention time earn together 2 IPs, while isotopic fitting earns 0.5 IP. Furthermore, each of the in-source and bbCID MS/MS fragments (mass accuracy) earns 2.5 IPs.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Introduction

The study area for contamination state was covered by three transects of Black Sea; the western sideclose to Ukraine/Romania (Danube Delta), the eastern Black Sea close to Georgia and the central sideincluding (Open Sea) sampling points across the length of Black Sea and out of reach of any coastal city.Seawater and sediment samples close to Georgia (GE) and Ukraine/Romania (UA/RU) were obtained during the National Pilot Monitoring Studies (NPMS), while the Open Sea samples during Joint Open Sea Surveys (JOSS). Twenty one seawater samples (seven Open Sea,seven UA (Ukraine)and seven GE (Georgia)) and four sediment samples (one Open Sea, two UA and one GE) in total were collected and analyzed. The sampling locations have shown in figure 9, paragraph 4.2.

The samples are not presented based on numerical alignmentin the graphs, butare lined-up from theWestern to the Eastern part of Black Sea, in order to easily visualize the influence of the nearby coastal pollution sources. The provided statistical values, refer only to samples where a substance was detected (when concentration wasbetween LOD and LOQ, it was substituted by LOQ/2 for mean/median and SD calculations). The frequency of detection, considering all tested samples, is also provided for an overview of the results.
GE-UA-15, 1, 3A, 10, 11, 2A and 1A are refered as Western BS (Black Sea) samples. The group of Open Sea samples includes GE-UA-2,3,4,5,6,7 and 8, while GE-UA-9,10,11,12 and GE-1,2,3 and 4 are refered as Eastern BS samples.

The extent of contamination in the marine organisms of Black Sea was also investigated. Fish and shellfish tissue monitoring serves as an important indicator of contaminated sediments and water, and many states routinely conduct tissue analyses as a component of their comprehensive environmental monitoring programmes.

5.2 Target screening results in seawater samples

Priority pollutants and emerging contaminants may be present at ng or even pg/L in seawater samples. Thus, the most common procedures used to carry out the determination of organic compounds in aquatic environmental matrices applied sample pre-concentration steps, such as solid-phase extraction (SPE) or liquid-liquid extraction (LLE) followed by separation and determination using

liquid chromatography (LC), coupled with mass spectrometry (LC–MS). Tandem mass spectrometry (MS/MS) offers higher performance than single-quadrupole instruments, in terms of sensitivity and selectivity.

Table 10:Classes of priority substances and emerging contaminantsdetermined in seawaters, along with the used analytical method.

Class of priority pollutants andemerging contaminants	**Matrix**	**Sample preparation method**	**Analytical Method**
Phthalates	seawater	Clean-up and pre-concentration by LVSPE, using HLB SPE Disks	LC-ESI (+/-)-QTOFMS(/MS) (bbCIDmode)**
Phenols			
Perfluorinated compound (PFCs)			
Emerging contaminants*			

** including pharmaceuticals, veterinary drugs, personal care products, pesticides, stimulants, psychotropic drugs, drugs of abuse, industrial chemicals, sweeteners, naturally occurring compounds, as well as their TPs.*
*** broadband collision-induced dissociation mode, which provides MS and MS/MS spectra at the same time, while it works at two different collision energies. At low collision energy (4 eV), MS spectra were acquired and at high collision energy (25 eV), fragmentation is taking place at the collision cell resulting in MS/MS spectra.*

Phthalates

Dimethyl-phthalate, Benzyl butyl-phthalate, Di-n-octy-phthalate and Diphenyl-phthalate were <1.25 ng/L in all tested samples, whereas Di-n-butyl-phthalate was detected in almost all tested samples. Figure 14 shows the distribution of the three detected phthalates in the seawater samples of EMBLAS plus.

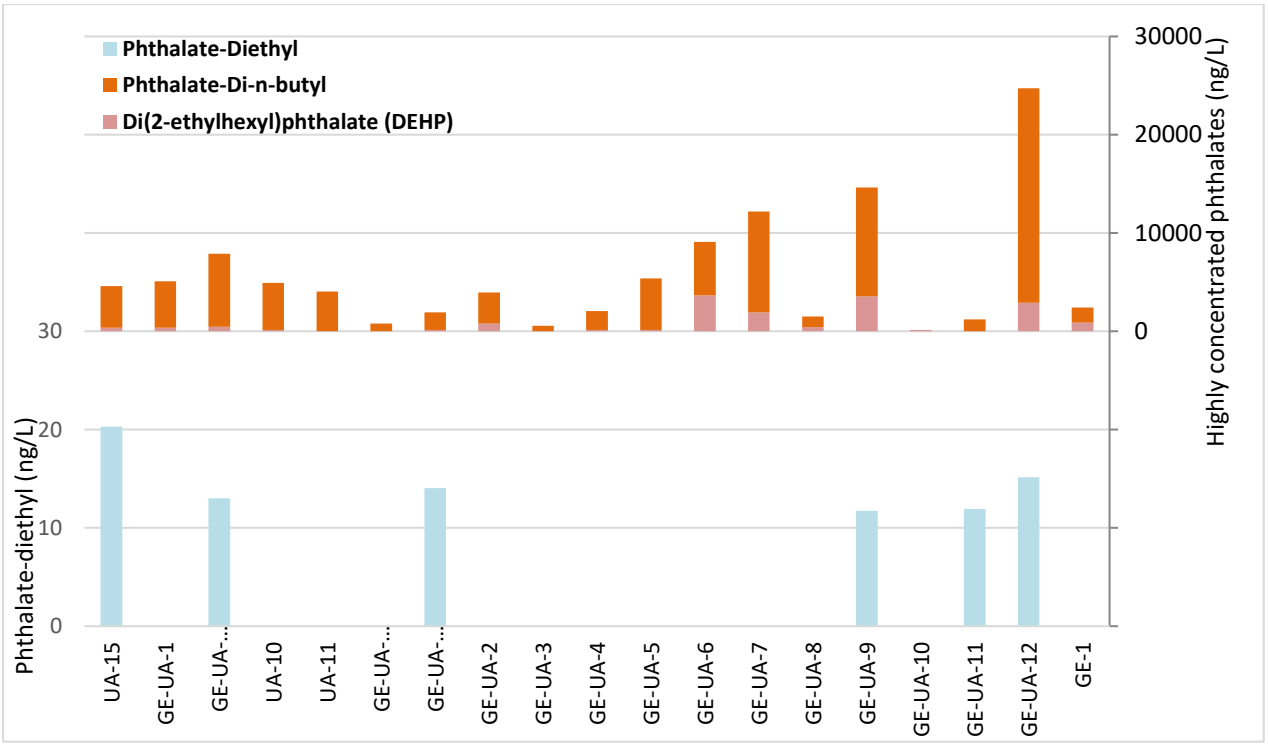


Figure 14: Phthalates(ng/L) in seawater samples from Ukrainian, Romanian, Open Sea and Georgian area, July/ August 2019.

Phenols

Eleven phenols were determined in EMBLAS plus seawatersamples. The WFD compounds (4-n-Nonylphenol (NP), Octylphenol ((4-(1,1',3, 3'-tetramethyl-butyl)-phenol)) and Pentachlorophenol) were below the respective limits of detection in all tested samples (20, 20 and 0.28 ng/L, respectively). BPA was detected only in UA-15 at 971 ng/L, while 2,4-Dinitrophenol (DNP), presented high frequency of detection among all analyzed samples (Table 11). 2,4,6-Tri-tert-butylphenol, 4-n-Nonylphenol mono-ethoxylate (NPE_1EO), 4-n-Nonylphenol di-ethoxylate (NPE_2EO), 4-n-Octylphenol-mono-ethoxylate, 4-n-Octylphenol-di-ethoxylate and Tetra-bromobisphenol A (TBBP-A) were screened but not detected with a SDL equal to 1.25 ng/L.

Table 11: Concentrations of DNP (ng/L) in seawater samples from Ukrainian, Romanian, Open Sea and Georgian area, August/September 2019.

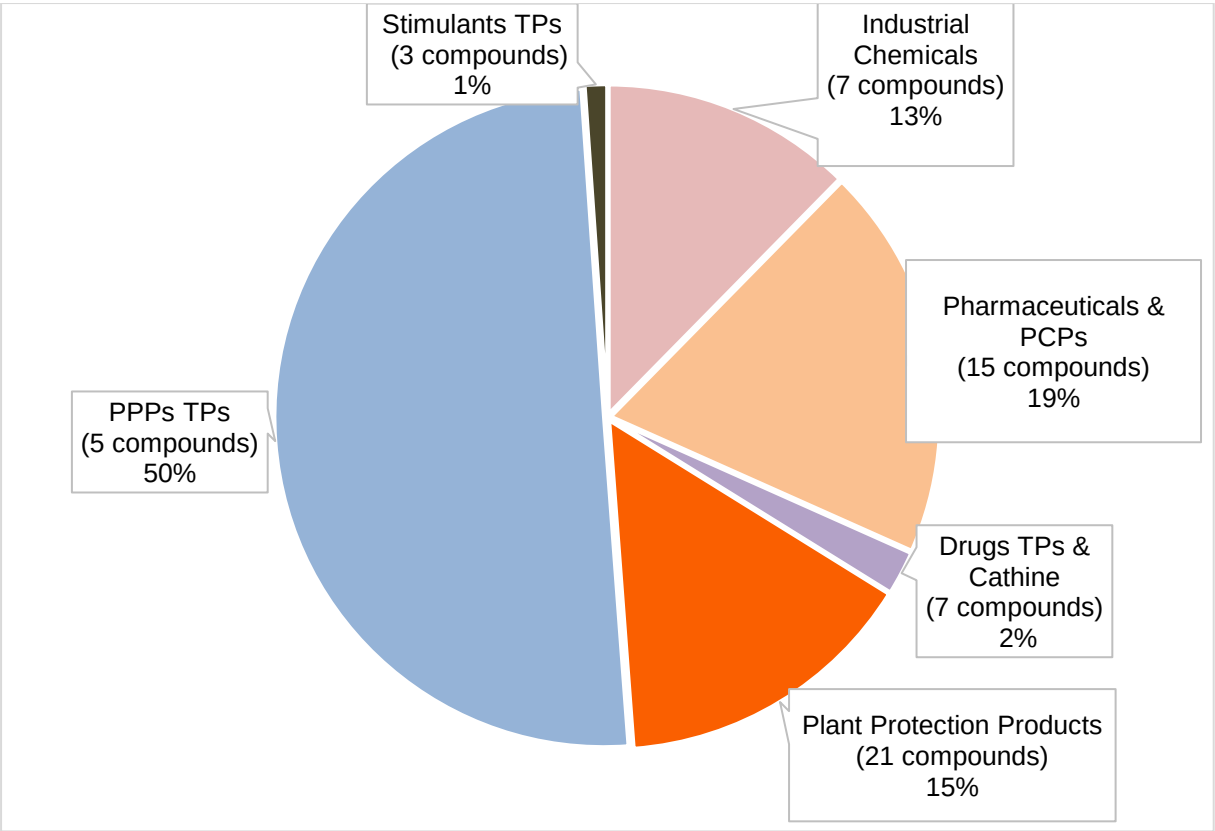
Phenols	Tested area	Mean (ng/L)	Median (ng/L)	Min (ng/L)	Max (ng/L)	StdDv (ng/L)	LOD/ LOQ (ng/L)	Frequency of detection
DNP	Western BS	9.90	9.84	6.54	13.6	2.6	1.06/3.20	100
	Open Sea	10.0	12.7	<3.20	12.9	5.6		57
	Eastern BS	6.32	2.83	<1.06	18.0	7.9		57

Perfluorinated compounds (PFCs)

Among the 30 screened PFCs, PFOA, PFHxA and GENX were detected in EMBLAS plus seawater samples. Although PFOS was below the limit of detection (0.06 ng/L) in all samples, its replacement compound GENX was detected in 4 samples (in UA-10, UA-2A and UA-2 at below LOQ levels (0.65 ng/L) and in UA-1A at 0.708 ng/L). PFHxA was detected only at below LOQ levels (3.90 ng/L) is 3 samples (UA-15, GE-UA-9 and 10). All other PFCs were below the screening detection limit of 1.25 ng/L.

Emerging contaminants

The chromatographs obtained by the LC-HR-MS/MS analysis were screened with an in-house database, comprised of 2252 ECs. In total 61 emerging contaminants, grouped into 9 main categories, (industrial chemicals, pharmaceuticals & PCPs, drugs TPs& cathine, plant protection products, PPPs TPs, stimulants TPs and naturally occuring compounds) have been detected in the analyzed samples. Cathine was the only drug of abuse detected in the samples and therefore it will be presented in the graphs along with drugs TPs. The contribution of each class of ECs in the total pollution of the tested samples, is depicted in Figure 15. Since, two naturally occurring compounds, adenine and adenosine were present in the samples in concentration levels orders of magnitude higher than the rest emerging contaminants, they were excluded from the graph. Otherwise, their contribution to the total detected concentration would be 85%. Based on the results presented in Figure 15, plant protection products and their TPs (26 compounds) contribute the most (65%) to the total pollution of EMBLAS plus seawater samples, while pharmaceuticals, PCPs, their TPs and cathine (22 compounds) follow (21%). Moreover, saccharine, the only sweetener detected in EMBLAS plus samples, was detected at <LOQ levels (10 ng/L) in UA-10, and therefore, is not presented in the graphs below.



**the naturally occurring compounds, adenine and adenosine and the sweetener saccharine were not included in the graph.*

Figure 15: The contribution of the detected classes of ECs in seawater samples from Ukrainian, Romanian, Open Sea and Georgian area, July/ August 2019.

The pollution in each tested seawater sample is illustrated in Figure 16. The highest levels of emerging contaminants were calculated in UA-10 sample, mainly attributed to the high total concentration of pharmaceuticals & PCPs. Overall, the highest median total concentrations of ECs were noticed in Western BS samples (159 ng/L), compared to Open Sea and Eastern BS ones (95 and 117 ng/L, respectively). The detection levels for Pharmaceuticals and PCPs decrease significantly in Open Sea samples, whereas plant protection products TPs total concentration, presents low variation across the tested samples, even in the ones collected from the Open Sea.

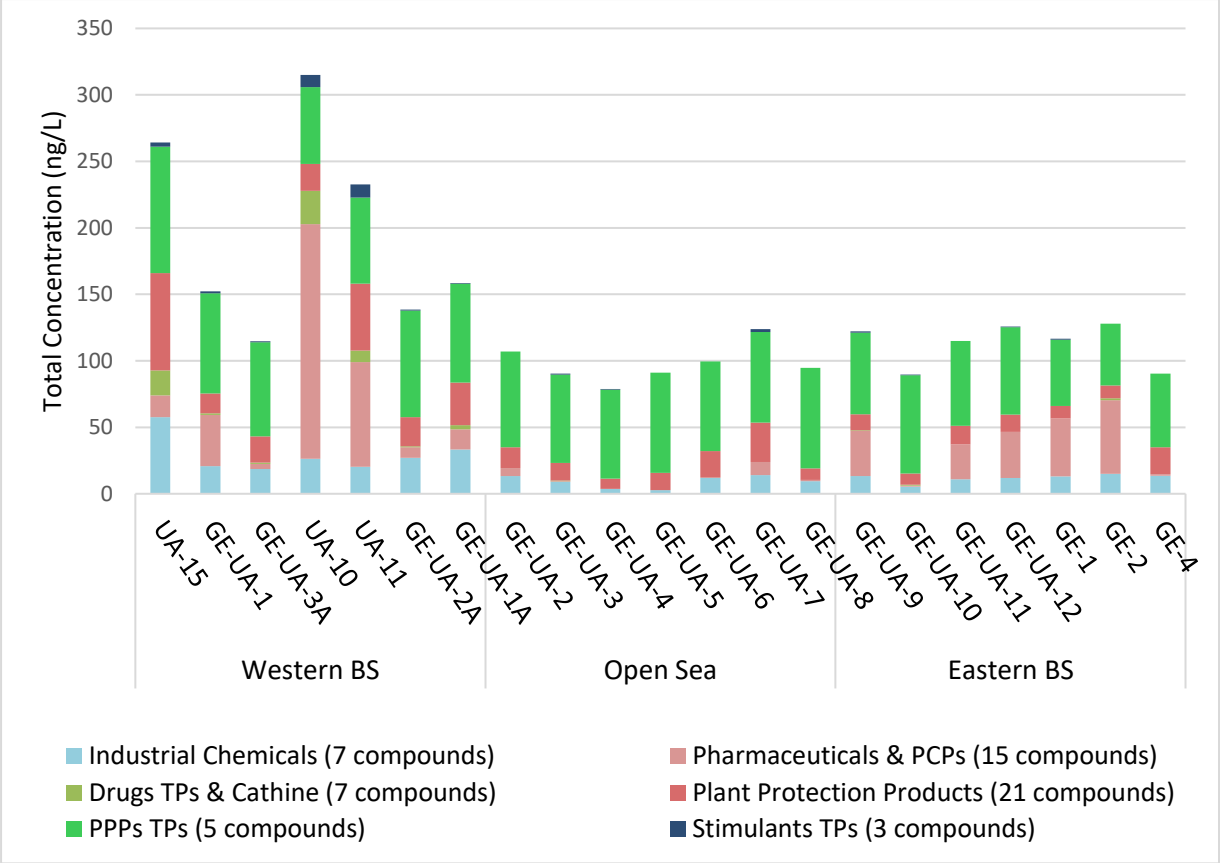


Figure 16: Emerging Contaminants (ng/L) in seawater samples from Ukrainian, Romanian, Open Sea and Georgian area, July/ August 2019.

Naturally occurring compounds

Two naturally occurring compounds (adenine andadenosine) were detected in the tested samples. The most predominant one (100% detection frequency) was adenosine, while adenine was detected in 17 out of 21 samples.The concentration levels for adenosine and adenine ranged from 4.3 to 370 and from 24.9 to 8783 ng/L, respectively.Significantly higher median concentrations of adenosine were noticed in Western BS samples.

Drugs of abuse

Cathine was the only compound detected in EMBLAS plus samples, representing the class of drugs of abuse. It was detected at <LOQ (4.00 ng/L) levels in 2 samples of Western BS, UA-15, UA-11 and in UA-1A, close to the Danube Delta.

Stimulants TPs

The metabolite of caffeine, theobromine, and nicotine's main metabolites, cotinine and Hydroxy-cotinine were present in the tested samples. Caffeine was also detected (classified as pharmaceutical), while nicotine was below the screening

detection limit. Cotinine presented the highest detection frequency (67%), with common detection trend among the different transects of BS. Hydroxy-cotinine was detected only in Western BS samples, while Theobromine was the most abundant of stimulants TPs compounds, reaching a maximum of 4.26 ng/L in UA-10.

Industrial chemicals

Sevenindustrial chemicals were detected in total, mainly from the class of benzothiazoles (2-amino-benzothiazole and 2-hydroxy-benzothiazole), benzotriazoles (benzotriazole (1-H-BTR), 4-hydroxy-benzotriazole and tolytriazole (mixture of 4- and 5-methyl-benzotriazole)) and benzene sulfonamides (toluene sulfonamide). 1-H-BTR was omnipresent in the seawater samples, while tolytriazole presented a 90.5% frequency of detection (FoA). 4-hydroxy-benzotriazole (detected only in Western BS) was the most abundant compound of the class, as it was detected at 31.7 ng/L in UA-15. Toluene sulfonamide was detected only at <LOQ levels (5.50 ng/L), while didecyldimethylammonium was present only in one Open Sea sample (GE-UA-7).

Sweeteners

Saccharine, the only representative of this class, was detected at <LOQ levels (10 ng/L) in UA-10.

Plant protection products

A number of 21 plant protection products were detected in the tested samples.Among them, the highest detection frequency was noticed for terbuthylazine, propazine, cloridazone (all 100% FoA), diphenamide (95.2%) and carbendazim (90.5%). All pesticides were detected at <10 ng/L, except for DEET, Pyrethrin I and Picolinafen, which were detected at concentration levels up to 12.1, 15.0 and 19.3 ng/L, respectively. Allethrin I, picolinafe, flutriafol, carbetamide, isoxaben and azoxystrobin, were detected only in Western BS samples. The highest levels of total pesticides were noticed in UA-15 (73.3 ng/L), a concentration level 10 times higher than the lowest pesticided-contaminated sampling site, GE-UA-4 (7.82ng/L). Moreover, WFD pesticides will be reported by JRC.

Plant protection products TPs

Five plant protection products (PPPs) TPs detected in total. Among them, there were 2 TPs of atrazine, desethyl-atrazine and 2-hydroxy-atrazine), desethyl-terbuthylazine, 2-hydroxy-propazine and metolachlor-ESA. 2-hydroxy-atrazine and 2-hydroxy-propazine were detected in all seawater samples, while desethyl-atrazine presented a 95.2% frequency of detection. Desethyl-terbuthylazine and metolachlor-ESA were detected only in Western BS samples at below LOQ levels (3.50 and 15.0 ng/L, respectively). 2-hydroxy-propazine was the most predominant

compound in terms of concentration (maximum concentration at 85.0 ng/L in UA-15) and frequency of detection, presenting no significant variation among the tested transects (70.0, 63.7 and 56.9 ng/L of median concentration in Western, Open Sea and Eastern BS samples, respectively).

Pharmaceuticals& PCPs

Pharmaceuticals was the most frequently detected class of emerging contaminants, since 21compounds in total were detected in the tested samples. The highest measured concentration of total pharmaceuticals was noticed in the Western BS samples, and especially in UA-10(176 ng/L). Carbamazepine was the pharmaceutical with the highest frequency of detection (90.5%), while chloramphenicol was the most abundant compound, reaching 58.4 ng/L at UA-10. Several compounds were present only in Western BS samples, like methylparaben, salicylic acid, chloramphenicol, lopinavir, pyrazophos, benzamidine, pregabalin, lamotrigine and valsartan. On the other hand, florfenicol was detected in only 2 samples from Georgia (GE-1 and 2).

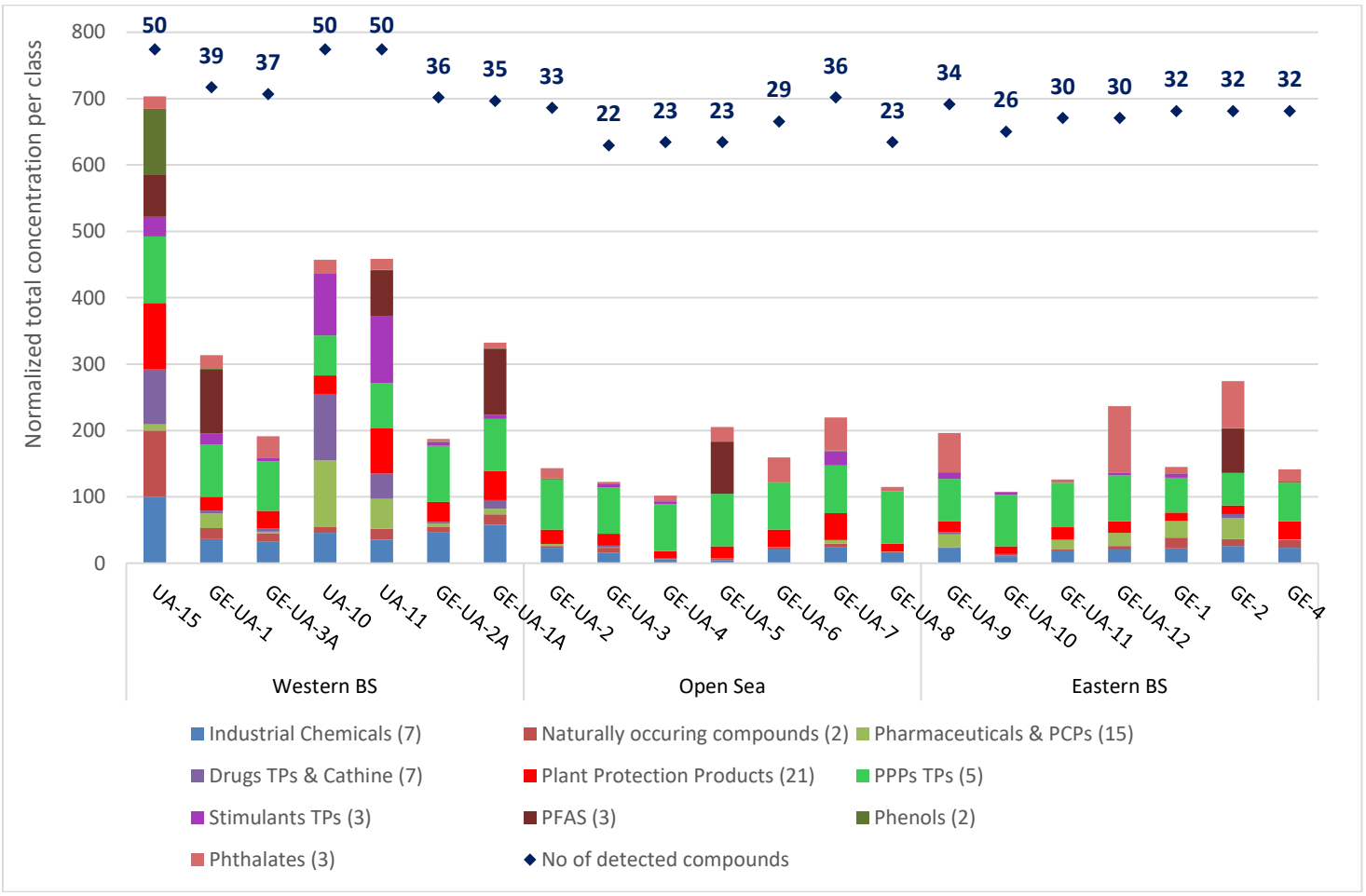
Transformation products of pharmaceuticals and related compounds

Six TPs were detectedin total. All of them were detected only in Western BS samples, except for 10.11-dihydro-10.11 dihydroxy-carbamazepine which was detected in all tested areas. 4-acetamido-antipyrine and 4-formylamino-antipyrine, two TPs of metamizole were detected with median concentrations of 4.86 and 11.7 ng/L, respectively, while 10.11-epoxide-carbamazepine and 10.11-dihydro-10.11 dihydroxy-carbamazepine were detected at maximum concentrations in UA-11 at 0.75 (LOQ/2) and 4.30 ng/L, respectively. N-oxide-lidocaine was detected only in UA-10 at <LOQ levels (4.88 ng/L), while N-oxide-tramadol was detected in 5 samples of Western BS.

Overall pollution

In order to gather the data obtained through different analysis and to combine all the pollution data from this survey, a cumulative chart was prepared (Figure 17). Not only the priority regulated pollutants, but also the detected emerging contaminants were taken into consideration. Since the concentration levels of the different classes of pollutants are distributed in a wide range, the distribution of some low concentrated classes would have been overlooked and underestimated if the total concentration of detected pollutants was the point of pollution comparison. For overcoming this, a normalization was performed. The total concentrations per class of pollutants were calculated in every sample and the maximum value was considered as 100%. The other samples were normalized according to this maximum value within the class. So, if a sample was the most

polluted one in all the reported classes of pollutants (11 in total), its normalized pollution would be 1100 (maximum sum of normalized pollution per class of pollutants). Moreover, the total number of detected pollutants per sample, is presented in the upper cluster of the graph, as a pollution marker. The maximum values per class of pollutants,used for normalization are listed in Table 12.It should be noted that metals were not measured in GE-UA-1A and GE-UA-3, thus the upper maximum normalized pollution for these samples is 1000.



** the sweetener saccharine was not included in the graph.*

Figure17: Normalized pollution cumulative chart for seawaters from Ukrainian, Romanian, Open Sea and Georgian area, July/ August 2019.

The Western BS samples were by far the most polluted ones, withUA-15 being the most polluted sample. The median number of detected compounds per transict

were: 39 for Western BS, 23 for Open Sea and 32 for Eastern BS samples. The sample close to the Danube delta (GE-UA-1A) was among the five most polluted samples and it should also taken into consideration, that metals were not measured in this sample. The pollution in Open Sea samples is mainly attributed to the presence of metals and PPPs and their TPs. Maximum median concentration of phthalates was noticed in Eastern BS samples.

Table 12: Maximum total concentrations per class of pollutants.

Class of pollutants	Maximum total concentration (ng/L)	Sample with maximum concentration
Industrial Chemicals (7)	57.7	UA-15
Naturallyoccuringcompounds (2)	6,154	UA-15
Pharmaceuticals&PCPs (15)	176	UA-10
DrugsTPs&Cathine (7)	22.6	UA-10
Plant Protection Products (21)	73.3	UA-15
PPPsTPs (5)	95.2	UA-15
StimulantsTPs (3)	9.97	UA-11
PFAS (3)	1.94	GE-UA-1A
Phenols (2)	979	UA-15
Phthalates (3)	24,730	GE-UA-12

5.3 Target screening results in sediments

Many pollutants, including a large number of hazardous compounds, are hydrophobic and their environmental behavior varies markedly between sorbed and dissolved states. Sediments are known to effectively sequester hydrophobic chemical pollutants entering water bodies such as estuaries. Apart from being a sink for pollutants, sediments are also a potential source of pollution toxicity to aquatic organisms.It is thus necessary to account for sorption reactions in analysis and prediction of the environmental transport and fate of these pollutants. Thus, four sediment samples, 2 from Ukraine (GE-UA-3A and 2A), 1 Open Sea (GE-UA-8) and one from Georgia (GE-UA-12) were collected for monitoring the degree of contamination in sediments of the Black Sea. The results of analysis per class of pollutants are presented below.The sediment samples were wet-sieved through 63 um mesh size sieve during the survey, then freeze-dried in the laboratory and the results are provided in μg/Kg (dry weight). The (%) water content in each sample was calculated and is presented in Table 13.

Table 13: (%)Water content of sediment samples.

Sediment sample names	(%)Water content
JBSS-GE-UA-8	81.7
JBSS-GE-UA-2A	55.6
JBSS-GE-UA-12	76.2
JBSS-GE-UA-3A	57.8

Phthalates

Among the seven screened phthalates, only Di (2-ethylhexyl) phthalate (DEHP) and Di-n-butyl-phthalate were detected in the samples. In particular, DEHP concentrations ranged from 233 (GE-UA-2A) to 994 µg/Kg (GE-UA-12), while Di-n-butyl-phthalate was detected with 100% frequency at 220-249µg/Kg.The rest phthalates were below the screening detection limit of 5 µg/Kg.

Phenols

All screened phenols were below the detected limits in the tested samples. The LOD for Octylphenol and 4-n-Nonylphenol was 40µg/Kg, while for Pentachlorophenol, 2,4,6-Tri-tert-butylphenol, Bisphenol A (BPA), 4-*n*-Nonylphenol mono-ethoxylate (NPE1EO), 4-n-Nonylphenol di-ethoxylate (NPE2EO),4-*n*-Octylphenol-mono-ethoxylate, 4-*n*-Octylphenol-di-ethoxylate, 2,4-Dinitrophenol (DNP) and Tetrabromobisphenol A (TBBP-A)) it was 5 µg/Kg.

Emerging Contaminants

Fourteen ECs were detected in sediment samples.Most of the detected compounds were plant protection products (7), while 3 pharmaceuticals, 2 naturally occurring compounds and 2 industrial chemicals were also detected. Moreover, transformation products of ECs were below the screening detection limit in all tested samples. As presented in Figure 18, the naturally occurring compounds contribute the most to the total detected concentration of sediments.

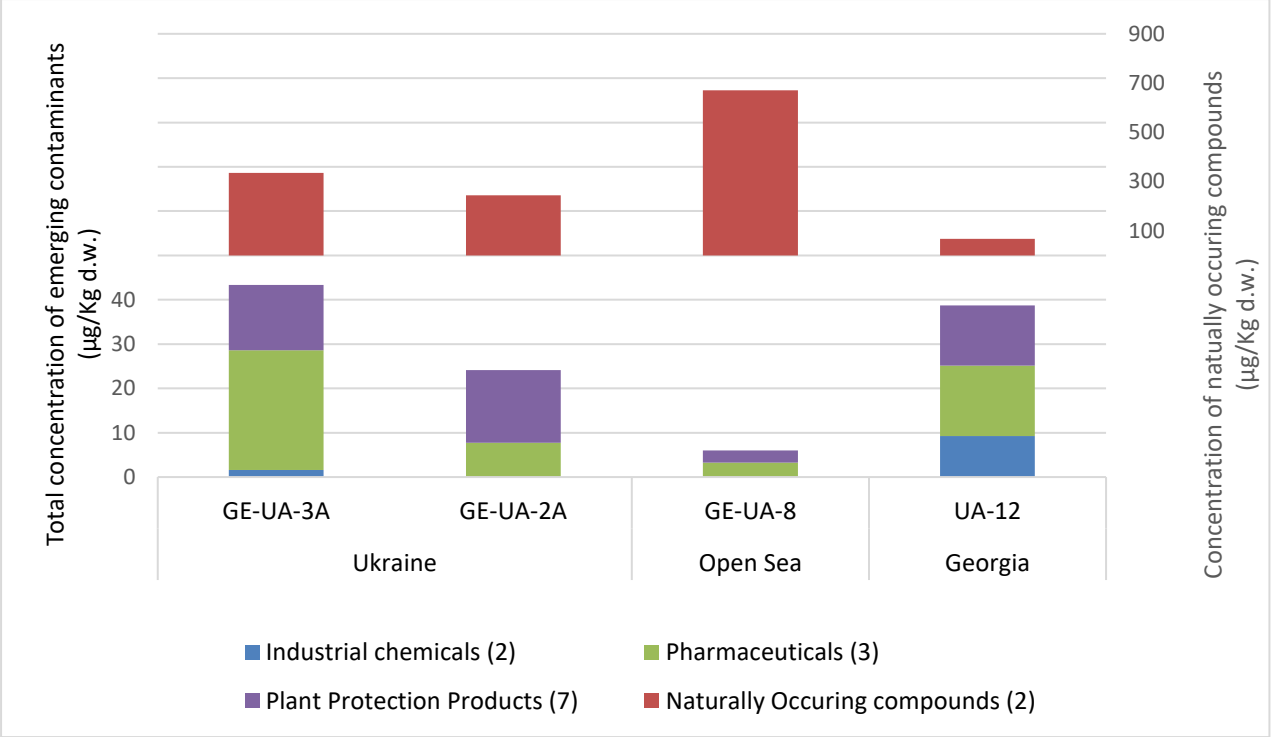


Figure 18: Emerging Contaminants (µg/Kg) in sediments from Ukrainian, Romanian, Open Sea and Georgian area, July/ August 2019.

Naturally occurring compounds

Adenine was detected in all samples, while adenosine was present in all samples except for UA-12. The maximum detected concentrations were measured in GE-UA-8 for adenine and in GE-UA-31 for adenosine (at 658 and 89.2 µg/Kg, respectively).

Industrial chemicals

Two related industrial chemicals were detected in the samples. N,N-Dimethyldodecylamine was detected only in GE-UA-3A at 1.57 µg/Kg, while N-Methyldodecylaminewas present only in UA-12 at 9.25 µg/Kg.

Plant Protection Products

Seven pesticides (carbendazim, carboxin, endothal, ethiofencarb, flonicamid, metamitron and metolcarb) in total were detected in sediments of the Black Sea. 14.8µg/Kgof pesticides was the total maximum concentration detected in GE-UA-10, whereas UA-12 and GE-UA-3 were the sample in which most pesticides were detected (5 compounds).

Pharmaceuticals

In total, 3 pharmaceuticals were present in EMBLAS plus samples. Apophedrine was detected at <LOQ levels (6.50 μg/Kg) in all tested samples, levetiracetam was present in all coastal sediments at concentrations 4.5-22.8 μg/Kg, while desomorphine was detected only in GE-UA-3 at <LOQ levels (2.00 μg/Kg).

Overall Pollution

The cumulative graph of sediment pollution burden, including priority substances and ECs, is presented in Figure 19. The total concentrations per class of pollutants were calculated in every sample and the maximum value was considered as 100. The other samples were normalized according to these maximum values. So, if a sample was the most polluted one in all ofthe reported classes of pollutants (7 in total), its normalized pollution would be 700 (maximum sum of total normalized pollution). Moreover, the total number of detected pollutants per sample, is presented in the upper cluster of the graph, as a pollution marker. The maximum values per each class of pollutants, according to which the normalization was performed, are listed in Table 14. GE-UA-3A sediment, sampled from Ukraine was the most polluted, where the maximum number of pollutants (21 in total) and the maximum normalized pollution (496) were noticed. Based on the results, coastal and shelf sediment samples are more polluted compared to open sea samples.

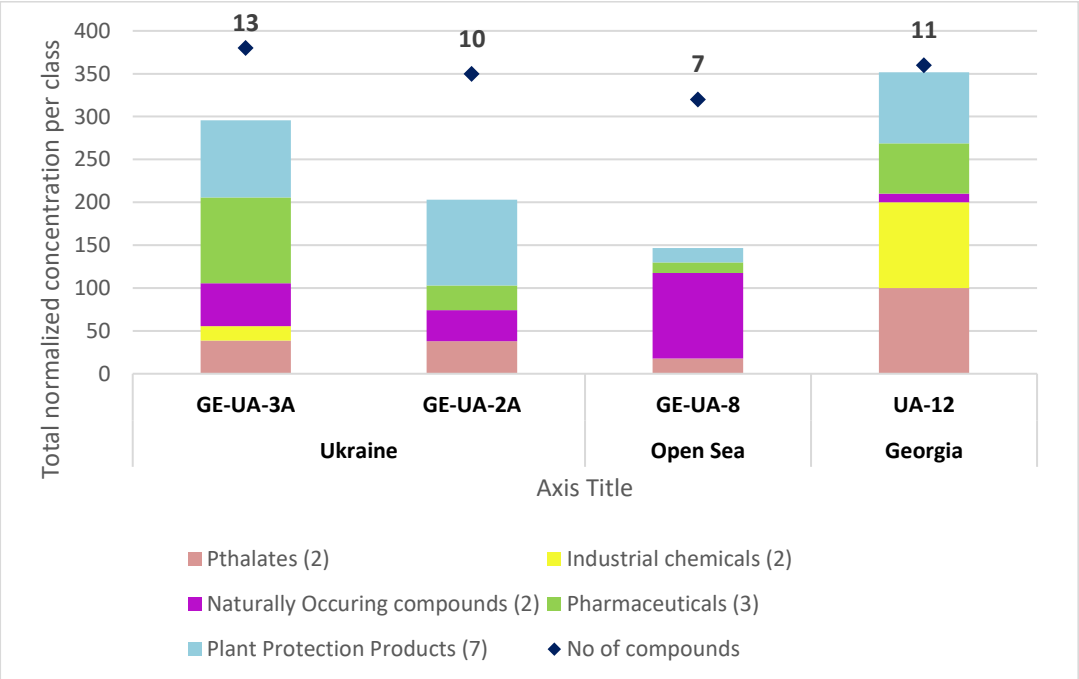


Figure 19: Normalized pollution cumulative chart for sediments from Ukrainian, Romanian,Open Sea and Georgian area, July/ August 2019.

Table 14: Maximum total concentrations per class of pollutants.

Class of pollutants	Maximum total concentration (µg/Kg)	Sample with maximum concentration
Phthalates	1,243	UA-12
Industrial chemicals	9.25	UA-12
Naturallyoccuringcompounds&Steroids	671	GE-UA-8
Pharmaceuticals	27.0	GE-UA-3A
Plant Protection Products	16.4	GE-UA-2A

5.4 Target screening results in biota samples

The aim of this survey was to document priority substances and emerging contaminants' concentrations in biota samples (fish and mollusks). Twenty biota samples were collected and analyzed in total. The samples were freeze-dried before analysis and results are provided in µg/Kg of wet weight.The %of water content of every sample and the matrix of analysis, arelisted in Table 15.

Table 15: Water content (%), speciesand matrix of analysis for the tested biota samples.

Sample	Species	Country	Code	Water content (%)
Fish	*Trachurustrachurus*	Georgia	UoA1	78.9
Fish	*Trachurustrachurus*	Georgia	UoA2	77.2
Fish	*Mullusbarbatus*	Georgia	UoA3	79.1
Fish	*Mullusbarbatus*	Georgia	UoA4	78.2
Fish	*Uranoscopusscaber*	Georgia	UoA5	80.8
Fish	*Uranoscopusscaber*	Georgia	UoA6	81.3
Mollusks	*Rapanavenosa*	Georgia	UoA7	74.2
Mollusks	*Mytilusgalloprovincialis*	Georgia	UoA8	82.2
Mollusks	*Mytilusgalloprovincialis*	Ukraine	UoA9	85.2
Fish	*Belonebelone*	Ukraine	UoA10	74.4
Fish	*Trachurusmediterrfneus*	Ukraine	UoA11	77.5
Mollusks	*Rapanavenosa*	Ukraine	UoA12	71.0
Fish	*Merlangiusmeriangus*	Ukraine	UoA13	80.2
Fish	*Neogobiusmelanostomus*	Ukraine	UoA14	78.7
Mollusks	*Mytilusgalloprovincialis*	Ukraine	UoA15	86.8
Fish	*Scorpaenaporcus*	RussianFederation	UoA16	73.2
Fish	*Symphodustinca*	RussianFederation	UoA17	76.0

Fish	*Scophthalmus*	RussianFederation	UoA18	62.0
Mollusks	*Mytilusgalloprovincialis*	RussianFederation	UoA19	74.3
Fish	*Horsemackerel*	RussianFederation	UoA20	74.8

For enabling results' presentation, the tested samples were grouped by country and matrix:

-Ukraine, fish (4 samples): UoA10, 11, 13 and 14

-Ukraine, mollusks (3 samples):UoA9, 12 and 15

-Russian Fed., fish (4 samples):UoA16, 17, 18 and 20

-Russian Fed., mollusk (1 sample):UoA19

-Georgia, fish (6 samples):UoA1, 2, 3, 4, 5 and 6

-Georgia, mollusks (2 samples):UoA7 and 8

A short description of the used sample preparation and analytical methods, are presented in Table 16.

Table 16: Classes of priority substances and emerging contaminants determined in biota samples, along with the analytical method used.

Class of priority substances& emerging contaminants	Sample preparation method	Analytical method
Perfluorinated compounds (PFCs)	Ultrasonic assisted extraction with EDTA (0.1% w/v, 0.1% v/v formic acid)/MeOH/ACN, followed by protein precipitation and defatting processes	LC-ESI (+/-)-QTOFMS(/MS)
Emerging contaminants*		

*including pharmaceuticals, veterinary drugs, personal care products, pesticides, stimulants, psychotropic drugs, drugs of abuse, industrial chemicals, sweeteners, naturally occurring compounds, as well as their TPs.

Perfluorinated compounds (PFCs)

Among the 18 PFCs that were screened in the biota samples, only Perfluoroctane sulfonic acid (PFOS) was detected in two fish from Georgia and two from Ukraine, in concentration ranging from 4.87 (UoA1) to 8.99 μg/Kg w.w. (UoA13). The LODsand LOQs of PFCs for biota, are presented in Table 17.

Table 17: LODs and EQS of PFCs in biota samples.

PFCs	LOD/ LOQ (µg/Kg)
Perfluoropentanoicacid (PFPeA)	3.0/8.9
Perfluorohexanesulfonate (PFHxS)	
Perfluorodecanesulfonate (PFDS)	6.0/18
Perfluorobutanesulfonate (PFBS)	
Perfluorooctanoicacid (PFOA)	
Perfluorodecanoicacid (PFDA)	
Perfluorohexanoixacid (PFHxA)	
Perfluorononanoicacid (PFNA)	
Perfluoroctanesulfonicacid (PFOS)	0.802/2.43
Perfluoroheptanoicacid (PFHpA)	9.7/32
Perfluorododecanoicacid (PFDoA)	
Perfluorotridecanoicacid (PFTrDA)	
N-ethylperfluorooctane sulfonamide (N-EtFOSA)	
Perfluorotetradecanoicacid (PFTeDA)	19.4/64.1
Perfluoroundecanoicacid (PFUdA)	11.9/35.6
Perfluorooctanesulfonamide (PFOSA)	
Perfluoroheptanesulfonate (PFHpS)	
N-methylperfluorooctane sulfonamide (N-MeFOSA)	49/160

Emerging Contaminants

In total, thirty six ECs were detected in the analyzed biota samples.As presented in Figure 19, the sample with the highest total ECs concentration was UoA13, a fish sample from Ukraine, at 1.23 mg/Kg.The highest number of detected ECs was noticed in UoA8 sample, mollusks from Georgia, in which 15 compounds were detected. 4-Formyl-antipyrine was the contaminant that was detected in the highest concentration levels and with the highest detection frequency (80%). The median concentration of ECs in biota was significantly higher in the samples from the Russian Federation (463 µg/Kg), compared to Georgia and Ukraine (163 and 113 µg/Kg, respectively). Moreover, the median concentration for all tested fish (14, samples, 192 µg/Kg) was more than 2 times higher than that of mollusks (6 samples, 87.4 µg/Kg). EMBLAS plusbiota samples were dominated by the presence of drugs TPs (56.7 % of EPs pollution). The same trend was also observedfor the pollution in Georgian and Ukrainian samples, in which 79.9 and 72.2%, respectively is attributed to the presence of drugs TPs, whereas pharmaceutical and PCPs contribute the most (59.6%) to the total EPs pollution in the Russian Federation samples.The highest number of detected pollutants, 15 in total, was noticed in UoA8 and 9.

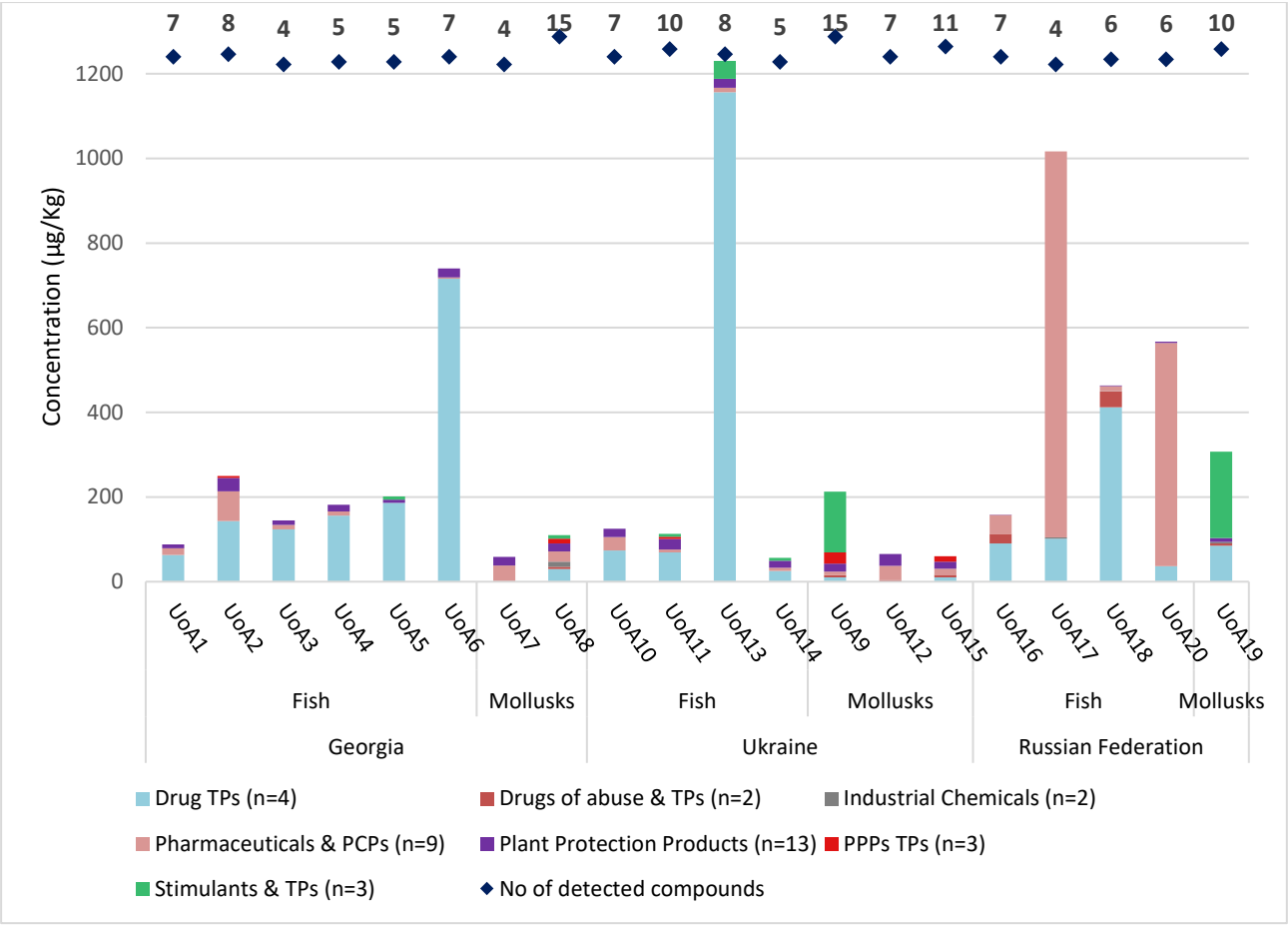


Figure 20: Emerging Contaminants (µg/Kg) in biota samples from Ukraine, Georgia and the Russian Federation, July/ August 2019.

Pharmaceuticals & PCPs

Overall, 9 pharmaceuticals & PCPs (trimethoprim, albuterol, caffeine, dropropizine, lidocaine, phenazone, methylparaben, salicylic acid and gentamycin) were detected in all tested samples. Salicylic acid has been detected in the highest concentration levels (898µg/Kg), while methylparaben was the most frequently detected compound (50% frequency of detection). The highest total pharmaceutical concentration was measured in UoA17, a fish sample from the Russian Federation. In generall, the levels for pharmaceuticals were significantly higher in the biota from the Russian Federation.

Drugs TPs

Among the detected drugs TPs, 4-formylamino-antipyrine was the most frequently detected compound, 80% of detection frequency, and the most abundant one, reaching up to 1156µg/Kg in UoA13. Moreover, an additional TP of metamizole, 4-formyl-antipyrine, was also detected with high frequency (25%). Atenonol acid was detected only in UoA1 at 4.33µg/Kg, while amisulpride-N-oxide was present in 3 mollusks from Georgia and Ukraine.

Drugs of abuse& TPs

Fenproxen was detected in 6 samples at concentration ranges from<11.1 (LOQ) to 32.2 μg/Kg (UoA18). Moreover, ritalinic acid, the major metabolite of methylphenidate, was detected only in UoA12 (mollusks from Ukrain) at <LOQ levels (8.86 μg/Kg).

Stimulants &TPs

Nicotine and its metabolites, anabasine and anatabine were detected in EMBLAS plus biota samples. The most frequently detected compound of this class was nicotine (detected in 5 samples), whereas anabasine was the most abundant one, reaching up to 144μg/Kg in UoA9. UoA19, mollusks from the Russian Federation, was by far the most polluted sample concerning stimulants & TPs (204 μg/Kg).

Plant protection products

Pesticides was the most frequently detected class of EPs, since 13 compounds (propamocarb, propoxur, dicrotophos, bioallethrin, deet, myclobutanil, metolachlor, fenobucarb, metamitron, glufosinate, dinoterb, dioxacarb and diethofencarb) were detected in at least one biota sample. No pesticides were detected in Uo17 and 18, while in general the concentration levels for pesticides in the Russian Federation biota, was significantly lower compared to those detected in Georgia and Ukraine. Dinoterb was the most frequently detected compound, as it was detected in 15 samples (omnipresent in Georgian and Ukrainian samples). The highest total concentration of plant protection products was measured in UoA2, at 31.7 μg/Kg, whereas up to 9 compounds were detected in UoA9, mollusks form Ukraine.

Plant protection products TPs

Three TPs of plant protection products were detected in EMBLAS plus biota samples. Propachlor-OXA, Sulfone-ethiofencarb and Desisopropyl-atrazine were detected in 1, and 5 samples, respectively. All compounds were detected at their maximum concentrations in UoA9, mollusks from Ukraine (propachlor-OXA: 5.72, ethiofencarb-sulfone: 4.49 and desisopropyl-atrazine: 15.7 μg/Kg).

Industrial chemicals

Only two industrial chemicals were detected in the analyzed biota samples. Phosphate-triethyl was detected only in UoA17 at <LOQ levels (4.65μg/Kg). Didecyldimethylammonium was detected in 3 samples from Georgia and the

Russian Federation at concentrations ranging from <0.78 (LOQ) to 12.2 µg/Kg (UoA8).

Overall pollution

The cumulative graph of biota pollution, including priority substances and ECs, is presented in Figure 21. The total concentrations per class of pollutants were calculated in every sample and the maximum value was considered as 100. The rest samples were normalized according to this maximum value. So, if a sample was the most polluted one in all the reported classes of pollutants (13 in total), its normalized pollution would be 1300 (maximum sum of normalized pollution per class of pollutants). It should be highlighted that two classes of compounds, dioxins and dioxin like compounds and PBDEs were not determined in UoA15 and 17, so the maximum sum of normalized pollution for these three samples is equal to 1100.The total number of detected pollutants per sample is presented in the upper cluster of the graph as a pollution marker. The maximum values per class of pollutants are listed in Table 18.

UoA12, mollusks from the Ukraine was the most polluted sample in terms of total normalized concentration (483), whereas the highest number of pollutants (64) was detected in UoA9, mollusks form Ukraine.

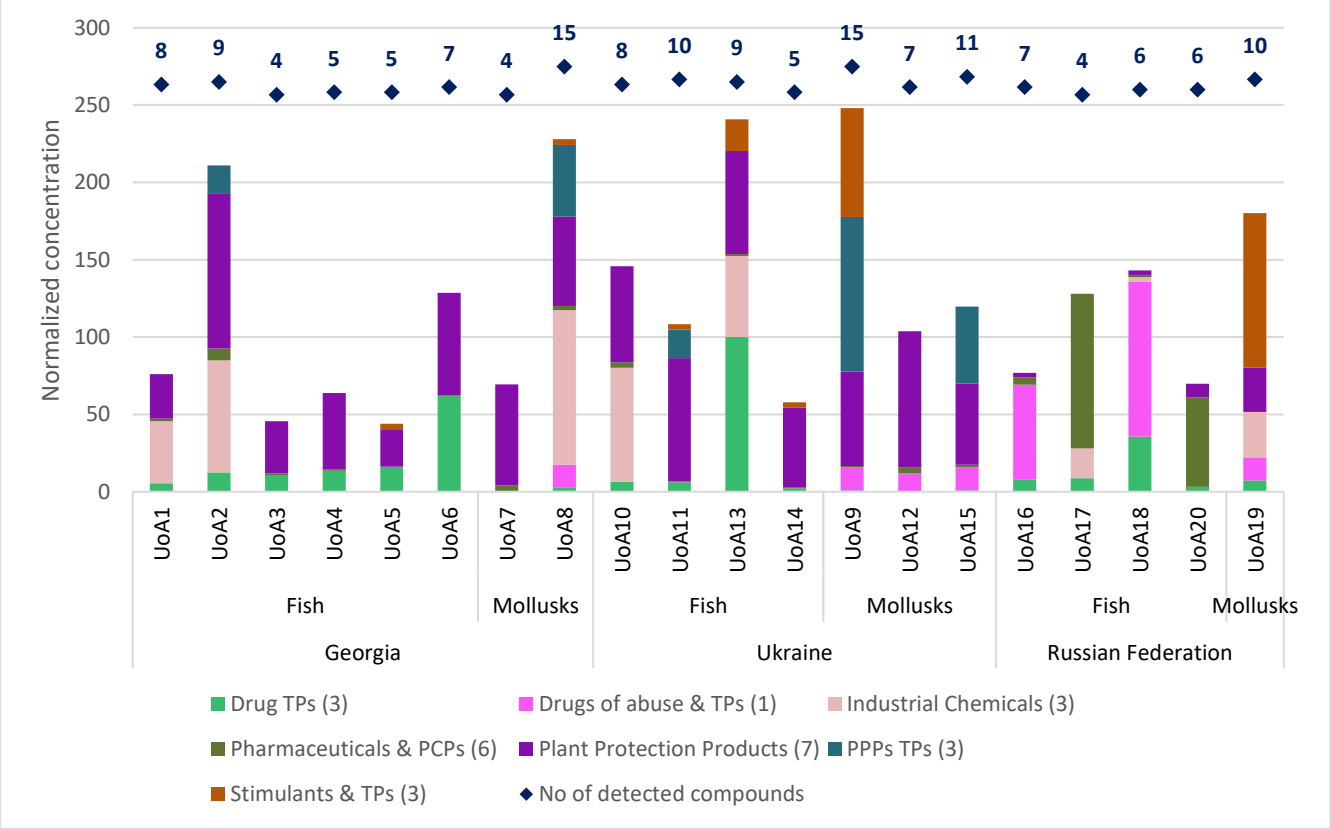


Figure 21: Normalized pollution cumulative chart for biota samples from Ukraine, Georgia and the Russian Federation, July/ August 2019.

Table 18: Maximum total concentrations per class of pollutants.

Class of pollutants	Maximum total concentration (μg/Kg)	Sample with maximum concentration
Drugs of abuse & TPs (1)	37.2	UoA18
Industrial Chemicals (3)	12.2	UoA8
Pharmaceuticals&PCPs (6)	912	UoA17
Plant Protection Products (7)	31.7	UoA2
PPPsTPs (3)	25.9	UoA9
Stimulants&TPs (3)	204	UoA19

CHAPTER 6: COMPARISON BETWEEN EMBLAS II and EMBLAS plus PROJECTS (2016-2017 and 2019)

6.1 Introduction

EMBLAS II and EMBLAS plus projects, funded by the EU and UNDP, were implemented in 2016-2017 and 2019, respectively, aiming to perform integrated environmental monitoring studies in order to assess the water quality of the Black Sea. The Laboratory of Analytical Chemistry of NKUA has undertaken the analysis of several classes of priority pollutants, as well as the determination of thousands of emerging contaminants and their transformation products, in seawater, sediments and biota, through the application of wide-scope target, suspect and non-target screening methodologies.

The overall objectives of employing chemicals analysis in the Black Sea were to implement extended monitoring environmental surveys for gathering occurrence data of priority pollutants and emerging contaminants in different environmental matrices, to apply several novel analytical methodologies and to exploit the capabilities of high resolution mass spectrometric (HRMS) techniques through wide-scope target suspect and non-target screening. In total 74 seawater samples and 28 sediments were analyzed during the three campaigns. Table 19 presents the samples of the three surveys and their distribution in 3 main transects of the BS, for comparison purposes. Western BS covers samples withdrawn from Ukrainian and Romanian areas (including Danube river estuaries), Open Sea samples that were collected out of reach of any coastal city, and Eastern BS which includes samples close to Georgian coast.

Table 19:Allocation of seawater and sediment samples of EMBLAS campaigns

Transects	EMBLAS II		EMBLAS plus	EMBLAS II		EMBLAS plus
	2016	2017	2019	2016	2017	2019
	Seawater samples			Sediments		
Western BS	9	6	8	8	3	2
Open Sea	10	7	6	5	2	1
Eastern BS	14	7	7	6	0	1
All samples	***33***	***20***	***21***	***19***	***5***	***4***

Moreover, 44 biota samples, including fish, mollusks and dolphins were analyzed during the three campaigns. The respective matrix of analysis and country of origin

are listed in Table 20. For selected fish, both muscle tissue and whole fish were analyzed.

Table 20: Biota samples of EMBLAS campaigns

Country	EMBLAS II		EMBLAS plus	EMBLAS II		EMBLAS plus	EMBLAS II
	2016	2017	2019	2016	2017	2019	2017
	Fish-muscle tissue (+whole fish)			Mollusks-soft body			Dolphins-muscle
Ukraine	4 (3)	4 (2)	4	1	2	3	2
Georgia	1 (1)	6 (1)	6	2	1	2	-
Russian Fed.	-	1	4	-	-	1	-

6.2 Results for seawater samples

Phenols

Eleven phenols were determined in all EMBLAS samples. Among them, 4-n-nonylphenol (NP), octylphenol, bisphenol A (BPA) and 2,4-dinitrophenol (DNP) were present in the samples, whereas pentachlorophenol, which is included in WFD, was not detected (LOD: 0.280 ng/L). NP was detected with high frequency of appearance (FoA) of 94% only in 2016 seawater samples with median concentration at 60.4 ng/L. Even its maximum detected concentration (145 ng/L, in Eastern BS) was more than 2-fold times lower than the respective EQS. Moreover, octylphenol was present at BQL levels (<30 ng/L) in both EMBLAS II campaigns with high FoA of 73 and 60%, in 2016 and 2017, respectively. Although, the endocrine disrupting compound BPA presented low FoA (2016: 9, 2017: 35 and 2019: 5%), it was detected in quite high median concentrations in all campaigns (2016: 541, 2017: 207 and 2019: 970 ng/L). The most frequently detected phenol was DNP. The statistical data of its detection are provided in Table 21 The detection trend of octylphenol, NP and BPA, significantly affected the pollution pattern of total phenols, with median concentrations at 83.1 ng/L in 2016, 25.6 in 2017 and 6.53 in 2019.

Table 21: Occurrence data of 2,4-Dinitrophenol within EMBLAS projects.

Statistical values	EMBLAS II		EMBLAS plus
	2016	2017	2019
%FoA	33	95	71
Median Concentration (ng/L)	5.30	5.25	9.84
Concentration range (ng/L)	<3.00 (LOQ)-7.30	<3.80 (LOQ)-34.9	<3.20 (LOQ)-18.0

Perfluorinated compounds (PFCs)

Among the 18 PFCs that were screened in EMBLAS samples in 2016 and 2019, perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA) were present in the samples, whereas perfluoroctane sulfonic acid (PFOS), a WFD compound, was not detected (LOD: 0.06 ng/L). PFOA presented high FoA, 45 in 2016 and 81% in 2019, while the average concentrations were 1.33 and 0.87 ng/L,

respectively. Moreover, although PFOA was <LOD in all Western BS samples in the first sampling campaign, in 2019 it was detected in 71% of Ukrainian/Romanian samples. New PFCs and replacement compounds were added in the target database of NKUA in 2019, enabling the screening of 30 PFAS compounds in total in the EMBLAS plus samples. GenX, a PFOS and PFOA replacement compound that was introduced more than a decade ago, was detected in 4 samples (mainly of Western BS) at concentrations ranging from <0.650 (LOQ) to 0.708 ng/L. This finding may be linked to the decreasing trend of PFOA detection over the years. In order to gain a holistic view of PFCs occurrence in all EMBLAS samples, retrospective screening of the additional PFCs in the acquired HRMS data of 2016 and 2017 is highly recommended.

Emerging Contaminants

The in-house database of NKUA was used for screening thousands of emerging contaminants (ECs) in EMBLAS samples. The list of ECs used within EMBLAS II included 2041 compounds, while additional 211 compounds were added in the list that was used for screening EMBLAS plus samples. Table 22 summarizes the statistics of ECs detection in the three campaigns.

Table 22:Statistics on emerging contaminants detectionin EMBLAS seawater samples.

Statistical values	EMBLAS II		EMBLAS plus
	2016 (n=33)	2017 (n=20)	2019 (n=21)
	No of detected emerging contaminants		
Total	108	117	61
Median (all tested samples)	19	25	20
Average (all tested samples)	23	30	22
Median (Western & Eastern BS samples)	21	41	23
Average (Western & Eastern BS samples)	27	36	25
	Frequency of appearance		
No. of ECs with %FoA<10	61	35	23
No. of ECs with %FoA>40	19	23	19
Most frequently detected class of ECs (%FoA)*	Pharm.- DoA & their TPs (44%)	Pharm.- Psycho. their TPs (56%)	PPPs & their TPs (43%)
	Detected Concentrations (ng/L)**		
Median (all tested samples)	139	79	116
Average (all tested samples)	288	233	129
Median (Western & Eastern BS samples)	161	350	126
Average (Western & Eastern BS samples)	323	331	154

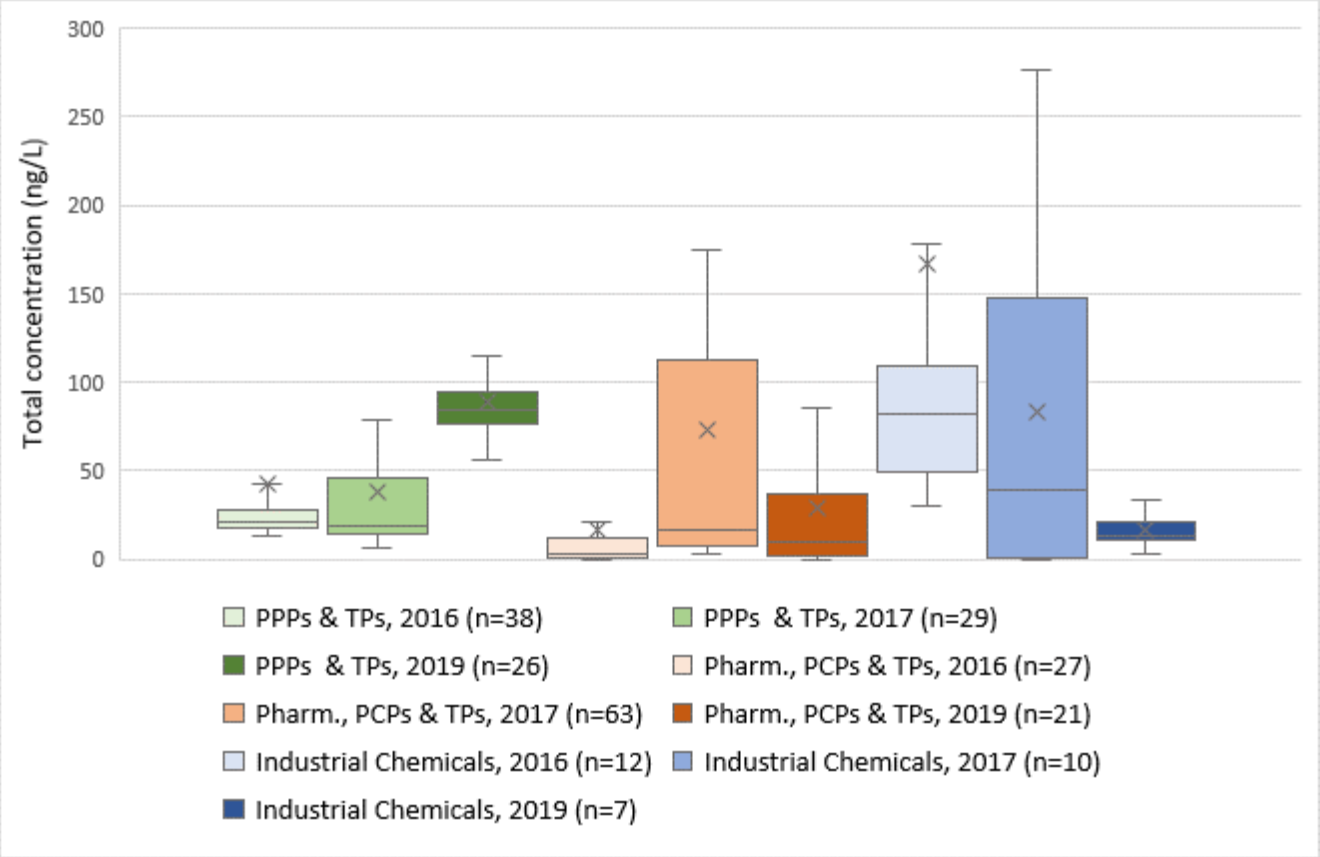
**Pharm: pharmaceuticals, DoA: Drugs of abuse, Psycho.: psychotropic drugs, TPs: Transformation Products, PPPs: Plant Protection Products; **Naturally occurring compounds were not included in the calculations*

Although the list of screened emerging contaminants increased in 2019, significantly less compounds (n=61) were detected in EMBLAS plus samples compared to those of EMBLAS II (n=108 and 117). This is mainly attributed to the significant contribution of samples collected close to the Danube river estuaries. Samples UA-7 and UA-5 in 2016 and 1A and 1-C in 2017, were collected close to the Danube estuaries and were by far the most polluted samples in terms of total

number and total concentration of emerging contaminants. In EMBLAS plus, GE-UA-1 sample was the one closest to the Danube estuaries, but its coordinates are remarkably far from the coast, and therefore less pollution was noticed. Median and average values of the detected ECs are provided for all tested samples and only for Western/Eastern BS samples in Table 22, in order to evaluate how coastal/shelf samples, affect the overall pollution patterns. Although the statistical values for all tested samples do not vary significantly among the tested campaigns, this pattern is not followed, when Open Sea samples are excluded. The average number of detected ECs in Western and Eastern BS samples was observed in 2017 (n=36). Concerning the FoA of detected ECs, in 2016 considerably more compounds presented low distribution in the tested samples, as 61 ECs were detected in less than 10% of the analyzed samples, compared to 35 and 23, in 2017 and 2019, respectively. On the contrary, the number of the most ubiquitous compounds (FoA >40%) per campaign varies from 19 to 23. Pharmaceuticals/ psychotropic drugs/ drugs of abuse and their TPs were the most predominant class of ECs in the first 2 campaigns, while plant protection products dominated the matrix of EMBLAS plus samples. Moreover, although the average total concentration of ECs in 2016 and 2017 samples does not present significant variation, EMBLAS plus samples present 44-46% lower levels. In order to further investigate this pattern, box-plots for the most important classes of pollutants, pharmaceutical & PCPs, plant protection products and industrial chemicals, as well as their TPs, were created, as presented in Figure 22**.** The concentrations of pharmaceuticals and industrial chemicals in 2017 presented high deviation, which is mainly attributed to their high variation in Western/Easter samples for pharmaceuticals and all transects for industrial chemicals (data not shown). PPPs presented an increasing detection trend over the years, since the average concentration was doubled, from 43 in 2016 to 88 ng/L in 2019. This is mainly attributed to the most abundant PPP detected in EMBLAS plus samples, propazine-2-hydroxy, with 100% FoA and average concentration at 61.8 ng/L. The average concentration of pharmaceuticals varied from 16 (2016) to 73 ng/L (2017). The significantly higher number of detected pharmaceuticals in 2017 (n=63) compared to the rest campaigns (n=27 and 21), is mainly associated with the high contamination of 1A and 1C samples, which are highly affected by the Danube river estuaries. The higher levels of industrial chemicals in 2016 and 2017 are mainly attributed to the detection of benzothiazole -2-OH (average C in 2016: 311 ng/L) and melamine and toluenesulfonamide (average C in 2017: 74 and 52 ng/L, respectively).

The summary of ECs detected in samples from different sampling campaigns is presented in Table 23.The classification, %frequency of appearance and average detected concentrations are listed. Compounds that present an alarming trend of occurrence over the years are marked (the darker the color, the highest the FoA/Concentration). The compounds included in this list, will be further

investigated through the assessment of their ecotoxicological risk, in order to conclude if they will be included in “Black Sea Specific Contaminants” list.



**Outliers are not presented in the graph; Pharm: pharmaceuticals, TPs: Transformation Products, PPPs: Plant Protection Products*

Figure 22:Total concentrations of selected classes of ECs in seawater samples over the years.

Table 23: Occurrence trends for emerging contaminants detected in more than 1 sampling campaigns in seawater samples.

Classification	Emerging Contaminant*	%FoA			Average C (ng/L)		
		2016 (n=33)	2017 (n=20)	2019 (n=21)	2016	2017	2019
Industrial Chemicals	Benzenesulfonamide	100	40	ND	20.1	25.1	ND
	Benzothiazole- 2-Amino	9	25	33	42.6	<0.978 (LOQ)	1.61
	Benzothiazole -2-OH	33	ND	5	311	ND	<15.0 (LOQ)
	Benzotriazole (BTR)	15	50	100	1.8	6.78	5.05
	Benzotriazole-Hydroxy	6	ND	10	7.50	ND	18.3
	Didecyldimethylammonium (DADMAC (C10:C10))	33	25	5	1.6	<15.3 (LOQ)	2.34
	Melamine	3	40	ND	16.4	73.5	ND
	Phosphate-triethyl	27	20	ND	0.443	5.79	ND
	Toluenesulfonamide	100	55	52	33.1	51.9	<5.50 (LOQ)
	Tolytriazole	6	45	90	42.7	11.2	8.32
Pharmaceuticals & PCPs	Amantadine	6	35	ND	<1.30 (LOQ)	0.921	ND
	Caffeine	9	40	38	13.2	62.5	43.4
	Carbamazepine	9	85	90	13.2	8.38	1.92
	Florfenicol	3	ND	10	<0.800 (LOQ)	ND	5.98
	Gabapentin	3	15	ND	<19.3 (LOQ)	<7.82 (LOQ)	ND
	Lamotrigine	12	15	29	0.990	10.0	1.14
	Lidocaine	6	5	ND	1.21	5.88	ND
	Metformin	6	10	ND	72	<7.40 (LOQ)	ND
	Metoprolol	6	10	ND	3.14	5.23	ND
	Pindolol	55	55	ND	1.33	11.2	ND
	Salicylamide	ND	70	19	ND	13.0	5.34
	Salicylic acid	ND	15	14	ND	3.63	3.44
	Telmisartan	12	5	52	14.6	4.99	1.31
	Tramadol	18	25	ND	0.592	2.63	ND
	Valsartan	24	5	5	7.26	21.3	18.7
	Venlafaxine	6	10	ND	1.57	<1.49 (LOQ)	ND
Drugs TPs	Antipyrine- 4-Acetamido	6	30	10	<19.4 (LOQ)	17.4	4.86
	Antipyrine- 4-Formylamino	6	45	14	<19.4 (LOQ)	10.5	9.13
	Carbamazepine -10-Hydroxy	3	5	ND	<3.71 (LOQ)	7.36	ND
	Carbamazepine-10.11-epoxide	6	5	5	<3.71 (LOQ)	<4.05 (LOQ)	<1.50 (LOQ)
	Tramadol-N-oxide	ND	10	24	ND	<0.714 (LOQ)	<0.700 (LOQ)
	Tramadol-O-Desmethylnor	3	15	ND	<0.484(LOQ)	4.20	ND
Psychotropic dugs	Cathine	12	20	14	3.72	10.4	<4.00 (LOQ)
	Sulpiride	6	45	ND	0.782	1.34	ND
	Tiapride	9	25	ND	2.39	1.36	ND
Plant Protection	Azoxystrobin	3	10	24	<1.65 (LOQ)	<0.869 (LOQ)	0.346
	Bentazone	24	15	ND	2.62	10.3	ND

Products	Carbendazim	100	50	90	<1.27 (LOQ)	1.45	1.39
	Carboxin	64	100	*ND*	1.92	4.18	*ND*
	Chloridazone	100	35	100	1.23	2.35	4.61
	DEET (Diethyltoluamide)	61	45	71	3.70	3.12	3.08
	Dinoterb	6	35	33	<1.37 (LOQ)	0.684	0.418
	Imidacloprid	6	*ND*	14	0.637	*ND*	3.50
	Isoxaben	*ND*	5	5	ND	<1.33 (LOQ)	4.74
	Metolachlor	27	40	38	28.9	1.45	1.61
	Picaridin (Icaridin)	*ND*	10	5	*ND*	<2.13 (LOQ)	0.934
	Prometryn	100	90	38	2.4	<0.547 (LOQ)	2.51
	Propazine	100	100	100	2.75	3.25	1.42
	Tebuconazole	15	*ND*	33	7.28	*ND*	<2.00 (LOQ)
	Terbuthylazine	6	30	100	29.1	<2.47 (LOQ)	2.08
PPPs TPs	Atrazine-2-hydroxy	100	35	100	<12.6 (LOQ)	7.58	3.45
	Atrazine-desethyl	ND	65	95	*ND*	<5.90 (LOQ)	0.606
	Chloridazone-methyl-desphenyl	12	5	*ND*	3.41	0.817	*ND*
	Dimethachlor-ESA	3	10	*ND*	<63.1 (LOQ)	3.14	*ND*
	Metolachlor-ESA	12	30	24	47.0	43.3	<15.0 (LOQ)
	Metribuzin-Desamino (DA)	18	10	*ND*	4.19	<2.09 (LOQ)	*ND*
	Terbuthylazine-desethyl	6	35	19	<16.9 (LOQ)	7.61	<3.50 (LOQ)
	Thiacloprid-amide	3	5	*ND*	<2.67(LOQ)	<3.43 (LOQ)	*ND*
Stimulants	Nicotine	36	40	*ND*	8.30	14.0	*ND*
Stimulants TPs	Cotinine	64	45	67	<1.92 (LOQ)	5.00	1.23
	Cotinine-Hydroxy	9	10	10	<1.92 (LOQ)	0.841	1.62
	Theobromine	15	*ND*	19	<46.0 (LOQ)	*ND*	2.69
Sweeterners	Saccharine	94	40	5	8.07	9.30	<10.0 (LOQ)
	Sucralose	15	20	*ND*	11.8	2.47	*ND*

**Naturally occurring compounds are not included; ND: not detected*

6.3 Results for sediments

Phenols

Octylphenol, 4-n-nonylphenol di-ethoxylate (NPE_2EO) and 2,4-dinitrophenol (DNP) were detected in EMBLAS II sediments, whereas all phenols were <LOD in 2019 samples. Although octylphenol presented high %FoA (74 and 60, in 2016 and 2017, respectively), it was detected only at BQL levels. All samples of 2016 were positive for the presence of NPE_2EO (average C: <52.8 (LOQ)), while in 2017 it was detected in 60% of the analyzed samples, at BQL levels. DNP was present at 29.4 μg/Kg only at UA-15 (old fraction) in 2016. Since most of the detected phenols were detected in trace levels (<LOQs), no specific trend over the years could be concluded.

Emerging Contaminants

Overall, 23, 29 and 13 ECs were detected in the sediments of 2016, 2017 and 2019, respectively, while the average number per sample did not vary significantly, when all samples (including Open Sea) were considered, as presented in Table 24. The statistic values for EMBLAS 2019 samples were significantly higher due to the contribution of JOSS-1 from Ukraine, which was remarkably contaminated (high number of ECs and high total C). In 2019, 19 compounds were present in more than 50% of the analyzed samples, whereas, 12 compounds were detected only once. The most frequently detected classes of ECs in the coastal/shelf samples are in line with the observations for seawaters. Pharmaceuticals & their TPs dominate EMBLAS II samples, while PPPs are the main contributors in 2019 sediments. The highest median total concentration was noticed in 2017 and the most recent coastal/shelf analyzed samples seemed to be less contaminated compared to the samples of 2016 and 2017. The naturally occurring compounds adenine, adenosine, 2-phenethylamine and tyramine were not considered for the extraction of statistics.

Table 24:Statistics on emerging contaminants detectionin EMBLAS sediments

Statistical values	EMBLAS II		EMBLAS plus
	2016 (n=19)	2017 (n=5)	2019 (n=4)
	No of detected emerging contaminants		
Total	23	29	13
Average (all tested samples)	6	13	7
Average (Western & Eastern BS samples)	7	19	8
	Frequency of appearance (Western & Eastern BS samples)		
No. of ECs detected in 1 sample	7	12	5
No. of ECs with %FoA>50	3	19	7
Most frequently detected class of ECs (%FoA)	Pharm.- Psycho. & their TPs (43%)	Pharm. & their TPs (72%)	PPPs (58%)
	Detected Concentrations (µg/Kg)		
Median (all tested samples)	29	165	42
Median (Western & Eastern BS samples)	106	195	56

**Pharm: pharmaceuticals, Psycho.: psychotropic drugs, TPs: Transformation Products, PPPs: Plant Protection Products; FoA: frequency of appearance **Naturally occurring compounds were not included in the calculations*

Nine compounds were commonly detected in samples from different campaigns and their occurrence data are provided in Table 25. The pharmaceutical apophedrin was the only compound that was detected in sediments from all tested years, with an increasing trend in %FoA, but decreasing trend in concentration levels. Moreover, levetiracetam was more frequently detected in 2019 and in significantly higher levels.

Table 25: Occurrence trends for emerging contaminants detected in more than 1 sampling campaigns in sediments.

Classification	Emerging Contaminant	%FoA			Average C (µg/Kg)		
		2016 (n=19)	2017 (n=5)	2019 (n=4)	2016	2017	2019
Plant Protection Products	Ametryn	11	20	ND	<1.80 (LOQ)	<1.30 (LOQ)	*ND*
	Carbendazim	16	*ND*	25	<5.60 (LOQ)	*ND*	<5.20 (LOQ)
Industrial Chemicals	Amino benzimidazole (2-)	57	40	*ND*	<0.488 (LOQ)	<1.35 (LOQ)	*ND*
	Benzotriazole (BTR)	21	40	*ND*	<0.738 (LOQ)	<0.761 (LOQ)	*ND*
Pharmaceuticals & TPs	Apophedrin	42	60	100	89.3	39.5	<6.50 (LOQ)
	Levetiracetam	5	*ND*	75	<5.66 (LOQ)	*ND*	13.3
	Oxprenolol	21	40	*ND*	<0.643 (LOQ)	<0.957 (LOQ)	*ND*
	Salicylamide	5	40	*ND*	3.57	<2.05 (LOQ)	*ND*
	Salicylamide-N-Isopropyl	5	40	*ND*	<3.41 (LOQ)	<2.05 (LOQ)	*ND*

**FoA: Frequency of appearance; TPs: transformation products; ND: not detected; Naturally occurring compounds were not included*

6.4 Results for biota samples

Perfluorinated compounds (PFCs)

The WFD compound Perfluoroctane sulfonic acid (PFOS), was the most frequently detected compound of this class with FoA of 67, 6 and 20% in 2016, 2017 and 2019, respectively. Although it was always detected in levels below its EQS (9.1 μg/Kg) in fish, an excessive concentration of 204 μg/Kg was measured in a dolphin from Ukraine. Moreover, perfluorodecanesulfonate (PFDS) and perfluorooctanoic acid (PFOA) were also present in 2016 samples at average concentration at BQL levels (0.80 and 64.2 μg/Kg, respectively) and perfluoroheptanoic acid (PFHpA) was detected in 2017 in 3 samples at <32 μg/Kg. Although 30 additional PFCs were added in NKUA database in 2019, they were all <LOD in EMBLAS plus biota.

Emerging Contaminants

All EMBLAS biota samples were screened using the in-house database of NKUA. The list of ECs used within EMBLAS II included 2041 compounds, while additional 211 compounds were added in the list that was used for screening EMBLAS plus samples. Naturally-occurring compounds in biota matrices, like vitamins and steroids, are not reported as emerging contaminants. Table 25 summarizes the statistics of ECs detection in the three campaigns.

The overall number of detected ECs in biota samples ranged from 36 in the most recent campaign to 80 in EMBLAS-II 2017 samples. The average number of compounds detected in fish samples (muscle tissue) presented no remarkable variation within the three campaigns, ranging from 6 in 2019 to 9 in 2016. Mollusks of 2019 were less contaminated, considering the average number of detected ECs (n=10), compared to EMBLAS-II biota samples (15 and 17, in 2016 and 17, respectively). Although the overall number of detected ECs in 2017 samples were outstanding, most of compounds presented low FoA, since 45 contaminants were detected in less than 10% of the analyzed samples. The number of the most ubiquitous compounds (FoA> 40%) ranged from 5 (in 2017 and 2019) to 13 (in 2016). The most frequently detected class of pollutants in EMBLA-II campaigns were pharmaceuticals, (%FoA of 42 and 61), whereas plant protection products and their TPs was the most dominant class (44%) in EMBLAS-plus biota samples. This trend is in agreement with seawater and sediments findings. The highest average cumulative concentration of ECs was noticed in fish collected in 2017 and in mollusks of 2016. In general, the average concentration of all analyzed samples decreased in 2019, compared to the previous campaigns.

Table 26: Statistics on emerging contaminants detectionin EMBLAS biota samples

Statistical values	EMBLAS II		EMBLAS plus
	2016 (n=12)	2017 (n=19)	2019 (n=20)
	No of detected emerging contaminants		
Total	45	80	36
Average (all tested samples)	12	10	8
Average (fish-muscle tissue)	9	7	6
Average (mollusks)	15	17	10
Average (dead dolphins)	-	18	-
	Frequency of appearance		
No. of ECs with %FoA<10	16	45	11
No. of ECs with %FoA>40	13	5	5
Most frequently detected class of ECs (%FoA)*	Pharm. (42)	Pharm. (61)	PPPs & TPs (44)
	Detected Concentrations (μg/Kg)**		
Average (all tested samples)	497	393	307
Average (fish-muscle tissue)	465	484	291
Average (mollusks)	491	141	136
Average (dead dolphins)	-	734	-

**Pharm: pharmaceuticals, TPs: Transformation Products, PPPs: Plant Protection Products*

In total 24 contaminants, listed in Table 27, were commonly detected in biota samples of more than one sampling campaign. Among them DEET, nicotine and fenproporex (NARL), presented increasing FoA in 2019 samples. Although, salicylic acid was detected in significantly less samples in the most recent campaign, the detected average concentration was 2 orders of magnitude higher compared to EMBLAS II samples. Moreover, the detected levels for the stimulants nicotine and anabasine in 2019 were higher compared to those of previous years.

Table 27: Occurrence trends for emerging contaminants detected in more than 1 sampling campaigns in biota samples

Classification	Emerging Contaminants	%FoA			Average C (μg/Kg)		
		2016 (n=19)	2017 (n=5)	2019 (n=4)	2016	2017	2019
Plant Protection Products & TPs	Atrazine-desisopropyl	8	21	25	<50.8 (LOQ)	97.4	9.58
	Chlordimeform	58	21	*ND*	<18.1 (LOQ)	4.54	*ND*
	DEET (Diethyltoluamide)	17	16	50	<0.455 (LOQ)	11.8	1.74
	Glufosinate	50	58	*ND*	17.1	12.8	*ND*
	Imazamox	42	11	*ND*	<79.6 (LOQ)	<22.7 (LOQ)	*ND*
	Metalaxyl	17	5	*ND*	1.42	<0.121 (LOQ)	*ND*
	Methfuroxam	17	21	*ND*	19.8	5.42	*ND*
	Myclobutanil	42	42	45	<35.1 (LOQ)	0.35	<5.53 (LOQ)
	Propamocarb	*ND*	5	5	*ND*	4.31	12.8

Pharmaceuticals & TPs	Antipyrine- 4-Formylamino	33	37	25	21.4	9.79	10.8
	Cytarabin	42	74	*ND*	26	18.1	*ND*
	Ephedrine	25	5	*ND*	0.529	2.42	*ND*
	Gemfibrozil	67	21	*ND*	<232 (LOQ)	<23.6 (LOQ)	*ND*
	Guaifenesin	25	5	*ND*	12.6	<8.30 (LOQ)	*ND*
	Lidocaine	*ND*	5	5	*ND*	59.5	<0.665 (LOQ)
	Oxfendazole	50	11	*ND*	<0.674 (LOQ)	2.34	*ND*
	Salicylic acid	100	63	15	2.69	9.03	485
	Sulfamethoxazole	8	5	*ND*	34.5	<3.94 (LOQ)	*ND*
	Trimethoprim	8	21	15	<8.73 (LOQ)	25.7	10.6
Stimulants & TPs	Anabasine	17	5	10	<2.98 (LOQ)	<6.32 (LOQ)	110
	Nicotine	17	*ND*	25	11.3	*ND*	36.8
Drugs of abuse	DMT (Dimethyltryptamine)	17	5	*ND*	<7.74 (LOQ)	<1.16 (LOQ)	*ND*
	Fenproporex (NARL)	17	5	30	34.1	<1.16 (LOQ)	13.7
Industrial Chemicals	Tributylamine	17	5	*ND*	4.65	<2.40 (LOQ)	*ND*

CHAPTER 7: CONCLUSIONS

Many countries around the world increasingly are concerned with the protection of their coastal regions and estuaries against pollution and other harmful effects of human activities. A case in point is the Black Sea regionwhich is suffering from continuing anthropogenic stress. Governments have initiated a regional approach to the management and protection of the marine environment that is supported by research at national and international levels.

The Black Sea Convention (BSC) provides a regional cooperation framework to protect against pollution. It entered into force in 1994. Black Sea became the focus of various EU policies, both thematic (e.g. Fishery, Integrated Coastal Zone Management (ICZM), Marine Strategy Framework Directive (MSFD), Water Framework Directive (WFD), Habitat and Birds Directives.

The ECs encompass a diverse group of compounds, however, only a small proportion of the chemical compounds have been sufficiently monitored in the water bodies.The development of high resolving power mass analyzers (HRMS) has contributed a lot towards the wide-scope screening of emerging contaminants. HRMS full scan acquisition offers the possibility of retrieving all the information concerning these compounds. The application of a generic sample treatment for the extraction of analytes, along with the data dependent and data independent acquisition mode by LC-HRMS allowed the wide-scope target screening for the detection of polar and semi-polar emerging contaminants in sediments, seawater and biota samples from Black Sea region.

Wide-scope target screening using a database with more than 2,100 emerging contaminants, priority pollutants, as well as their (bio)transformation products, was applied based on some performance criteria; mass accuracy, retention time, isotopic pattern and MS/MS information, were attributed in order to facilitate confidence.

Twenty one seawater samples (seven Open Sea, seven UA (Ukraine) and seven GE (Georgia)) in total were collected and analyzed.The results indicate that Phthalate-Di-n-butyl was detected in almost all tested samples and 2,4-Dinitrophenol (DNP) presented high frequency of detection among them. Although PFOS was below the limit of detection (0.06 ng/L) in all samples, its replacement compound GENX was detected in fourof them at below LOQ levels (0.65 ng/L) and in one at 0.708 ng/L.Sixty one emerging contaminants have been detected in the analyzed samplesand Cathine was the only drug of abuse among them. Plant protection products and their TPs (26 compounds) contribute the most (65%) to the total pollution of seawater samples, while pharmaceuticals, PCPs, their TPs (22 compounds) follow (21%). Furthermore four sediment samples (one Open Sea, two UA and one GE) were collected and analyzed.Regarding the results, among the seven screened phthalates only Di (2-ethylhexyl)phthalate (DEHP) and di-n-butyl-phthalate were detected in the samples and all screened phenols were below

the detected limits in the tested samples. Moreover, fourteen ECs were detected in sediment samples, it seems that the naturally occurring compounds adenine and adenosine) contribute the most to the total detected concentration of sediments. Finally twenty biota samples were collected and analyzed. The results indicate that thirty six ECs were detected and 4-Formyl-antipyrine was the contaminant with the highest concentration levels and with the highest detection frequency (80%).

The aggregated target analysis results produced within EMBLAS II and EMBLAS plus projects, implemented in 2016-2017 and 2019, respectively were evaluated in this project.Both the number and the average total concentration of emerging contaminants in seawater samples and sediments were lower in 2019 samples compared to the previous campaigns. The levels of specific contaminants or the selected sampling sites remarkably affect the over pollution pattern of the samples. In general pharmaceuticals and their TPs presented a decrease in their total average concentration over the years, whereas plant protection products were most abundant in the most recent campaign in both seawater and sediments. Furthermore, in biota samples the number of detected emerging contaminants in mollusks was constantly higher compared to fish in all campaigns, while the average cumulative concentration of all biota samples withing the same campaign ranged from 307 in 2019 to 497 μg/Kg in 2016.

As EMBLAS plus is an ongoing project there are existed also, future perspectives. Firstly, to assess the potential ecotoxicological risk of the detected analytes based on the Environmental Quality Standards (EQS) of the Water Framework Directive (2013/39/EC), experimental Predicted No Effect Concentrations (PNECs) and the use of advanced toxicity prediction chemometrics tools. Moreover, to prioritize the detected compounds based on their occurrence trends and ecotoxicity. Finally,to upload the results in the on-line database system of NORMAN and store the acquired chromatograms to the Digital Sample Freezing Platform (DSFP) for future retrospective screening purposes andto propose a list of Black Sea Specific Contaminants that should be included in future regular monitoring campaigns.

ABBREVIATIONS – ACRONYMS

Atmospheric Pressure Chemical Ionization	**APCI**
Atmospheric Pressure Photoionization	**APPI**
Benzene, Toluene and Xylenes	**BTX**
broad-band Collision Induced Dissociation	**bbCID**
Data Dependent Acquisition	**DDA**
Data Independent Acquisition	**DIA**
Digital Sample Freezing Platform	**DSFP**
Electrospray Ionization	**ESI**
Emerging Contaminants	**ECs**
Emerging Pollutants	**EPs**
Endocrine-Disrupting Chemicals	**EDCs**
Environmental Quality Standards	**EQS**
Extracted Ion Chromatogram	**EIC**
Full Width at Half Maximum	**FWHM**
Heavy Metals	**HMs**
High Energy	**HE**
Identification Points	**IPs**
Joint Open Sea Surveys	**JOSS**
Liquid Chromatography coupled to Mass Spectrometry	**LC-MS**
Liquid-Liquid Extraction	**LLE**
Liquid-Phase Micro Extraction	**LPME**
Liquid-Solid Extraction	**LSE**
Low Energy	**LE**
Marine Strategy Framework Directive	**MSFD**
Matrix Solid-Phase Dispersion	**MSPD**
Methyl Tertiary-Butyl Ether	**MTBE**
Microwave-Assisted Extraction	**MAE**
National Pilot Monitoring Studies	**NPMS**
Perfluorinated Chemicals	**PFCs**
Perfluoroalkyl Substances	**PFASs**
Perfluorononanoic Acid	**PFNA**
Perfluorooctanesulfonic Acid Compound	**PFOS**
Perfluorooctanoic Acid	**PFOA**
Persistent, Bioaccumulative, and Toxic	**PBT**
Personal Care Products	**PCPs**
Pharmaceutical and Personal Care Products	**PPCPs**
Pharmaceutically Active Compounds	**PhACs**
Plant Protection Products	**PPPs**
Polybrominated Biphenylethers	**PBDE**
Polychlorinated Alkanes	**PCAs**
Polycyclic Aromatic Hydrocarbons	***PAHs***
Predicted No Effect Concentrations	**PNECs**

Pressurized Liquid Extraction	**PLE**
Priority Pollutants	**PPs**
Quadrupole-Time-of-Flight	**QTOF**
Reversed Phase Ultra High Performance Liquid Chromatography	**RP-UHPLC**
Reversed-Phase	**RP**
Screening Detection Limit	**SDL**
Sewage Treatment Plants	**STPs**
Single Reaction Monitoring	**SRM**
Solid Phase Extraction	**SPE**
Supercritical Fluid Extraction	**SFE**
Time-of-flight	**TOF**
Transformation Products	**TPs**
Ultra High Performance Liquid Chromatography	**UHPLC**
Ultrasound Assisted Extraction	**UAE**
Volatile Organic Chemicals	**VOCs**
Wastewater Treatment Plants	**WWTPs**
Water Framework Directive	**WFD**

REFERENCES

[1] N. Todorova, S.V. Alyomov, B.C. Chiotoroiu, B.Fach, T.S. Osadchaya, M.Rangelov, B.Salihoglu, V.lVasilev, Black Sea, *The Black Sea—The Past, Present, and Future Status*, Elsevier, 2019, pp. 209-223

[2] L. Bat, A. Öztekin, F. Şahin, E. Arıc, U. Özsandık, An overview of the Black Sea pollution in Turkey, *Mediterranean Fisheries and Aquaculture Research*, vol. 2, 2018, pp. 67-86

[3] S.N. Sruthia, S. Chandran , M. Mohana, E.V. Ramasam, Distribution of priority pollutants in the sediment of Vembanad Estuary(Peninsular India), *Marine Pollution Bulletin*, vol. 133, 2018, pp. 294–303

[4] A. Campos-Candel, M. Llobat-Estellés, A.R. Mauri-Aucejo,Desorption of BTEX from activated charcoal using accelerated solvent extraction: evaluation of occupational exposures, *Analytical and Bioanalytical Chemistry*, vol. 387, 2007, pp. 1517–1523

[5] H.I. Abdel-Shafy, M.S.M. Mansour, A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation*, Egyptian Journal of Petroleum*, vol. 25, 2016, pp. 107-123

[6] L.O. Santos, A.G. Santos, J.B. de Andrade, Methodology to examine polycyclic aromatic hydrocarbons (PAHs) nitratedPAHs and oxygenated PAHs in sediments of the Paraguaçu River (Bahia,Brazil), *Marine Pollution Bulletin*, vol. 136, 2018, pp. 248–256

[7] T.Rasheed, M. Bilal, F.Nabeel, M.Adeel, H.M.N. Iqbal, Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection, quantification, and treatment, *Environment International*, vol. 122, 2019, pp. 52–66

[8] A.A. Bletsou , J. Jeon , J. Hollender , E. Archontaki, N.S. Thomaidis, Targeted and non-targeted liquid chromatography-massspectrometric workflows for identification of transformation productsof emerging pollutants in the aquatic environment, *Trends in Analytical Chemistry*, vol. 66, 2015, pp. 32-44

[9] Y. Zhou, J. Meng, M. Zhang, S. Chen, B. He, H. Zhao, Q. Li, S. Zhang, T. Wang, Which type of pollutants need to be controlled with priority in wastewater treatment plants: Traditional or emerging pollutants?, *Environment International*, vol. 131, 2019, 104982

[10] A. Stefanakis, J.A. Becker, A review of emerging contaminants in water: Classification, sources and potential risks, *Impact of Water Pollution on Human Health and Environmental Sustainability*, IGI Global, 2017

[11] S. Bayen, E.S. Estrada, G. Juhel, B.C. Kelly, Direct injection of tissue extracts in liquid chromatography/tandem mass spectrometry for the determination of pharmaceuticals and other contaminants of emerging concern in mollusks, *Analytical and Bioanalytical Chemistry*, vol. 15, 2015, pp. 8760-9

[12] P. Verlicchi , M.A. Aukidy, E. Zambello, Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass loadand environmental risk after a secondary treatment—A review,*Science of the Total Environment*, vol. 429, 2012, pp. 123–155

[13] S. Castiglioni, E. Zuccato, Illicit Drugs as Emerging Contaminants, ACS. Symposium Series, 2010, pp. 119–136

[14] A.J. Ebele, M.A. Abdallah, S. Harrad, Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment, *EmergingContaminants*, vol. 3, 2017, pp. 1–16

[15] E. Villaverde-de-Sáa, J.B. Quintana, R.Rodil, R. Ferrero-Refojos, E.Rubí, R.Cela, Determination of perfluorinated compounds in mollusks by matrix solid-phase dispersion and liquid chromatography–tandem mass spectrometry, *Analytical and Bioanalytical Chemistry*, vol. 402, 2012, pp. 509–518

[16] M. Fernández-Sanjuan, J. Meyer, J. Damásio, M. Faria, C. Barata, S. Lacorte, Screening of perfluorinated chemicals (PFCs) in various aquatic organisms, *Analytical and Bioanalytical Chemistry*, vol. 398, 2010, pp. 1447–1456

[17] T. Ivanković, J. Hrenović, Surfactants in the environment, *Archives of Industrial Hygiene and Toxicology*, vol. 61, 2010, pp. 95–110

[18] M.V. Barbieri, C. Postigo, N. Guillem-Argiles , L.S. Monllor-Alcaraz, J.I. Simionato , E. Stella, D. Barceló, M. López de Alda, Analysis of 52 pesticides in fresh fish muscle by QuEChERS extraction followed by LC-MS/MS determination, *Science of the Total Environment*, vol.653, 2019, pp. 958–967

[19] F.T. Lange, M. Scheurer, H. Brauch, Artificial sweeteners — a recently recognized class of emerging environmental contaminants: a review, *Analytical and Bioanalytical Chemistry*, vol. 403, 2012, pp. 2503–2518

[20] V. Camel, Solid phase extraction of trace elements, *Spectrochimica ActaPart B*, vol. 58, 2003, pp. 1177–1233

[21] J. Aceña, S. Stampachiacchiere, S. Pérez, D. Barceló, Advances in liquid chromatography–high-resolution mass spectrometry for quantitative and qualitative environmental analysis, *Analytical and Bioanalytical Chemistry*, vol. 407, 2015, pp. 6289–6299

[22] M. Krauss, H. Singer, J. Hollender, LC–high resolution MS in 111 environmental analysis: from target screening to the identification of unknowns, *Analytical and Bioanalytical Chemistry*, vol. 397, 2010, pp. 943–951

[23] M. Ibáñez, J.V. Sancho, D. McMillan, R. Rao, F. Hernández, Rapid non-target screening of organic pollutants in water by ultra performance liquid chromatography coupled to time-of-light mass spectrometry, *Trends in Analytical Chemistry*, vol. 27, 2008, pp. 481–489

[24] S.D. Richardson, Water Analysis: Emerging contaminants and current issues, *Analytical Chemistry*, vol. 79, 2007, pp. 4295–4324

[25] C. Postigo, M.J.L. de Alda, D. Barceló, Analysis of drugs of abuse and their human metabolites in water by LC-MS2: A non-intrusive tool for drug abuse estimation at the community level, *Trends in Analytical Chemistry*, vol. 27, 2008, pp. 1053–1069

[26] R. Cariou, E. Omer, A. Leon, G. Dervilly-Pinel, B. Le Bizec, Screening halogenated environmental contaminants in biota based on isotopic pattern and mass defect provided by high resolution mass spectrometry profiling*, Analytica Chimica Acta*, vol. 936, 2016, pp. 130–138

[27] B. Du, J.M. Lofton, K.T. Peter, A.D. Gipe, C.A. James, J.K. McIntyre, N.L. Scholz, J.E. Baker, E.P. Kolodziej, Development of suspect and non-target screening methods for detection of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass spectrometry, *Environmental Science: Processes & Impacts*, vol. 19, 2017, pp. 1185–1196

[28] A.L. Heffernan, M.M. Gómez-Ramos, C. Gaus, S. Vijayasarathy, I. Bell, C. Hof, J.F. Mueller, M.J. Gómez-Ramos, Science of the Total Environment Non-targeted, high resolution mass spectrometry strategy for simultaneous monitoring of xenobiotics and endogenous compounds in green sea turtles on the Great Barrier Reef, *Science of the Total Environment*, vol. 599–600, 2017, pp. 1251–1262

[29] J.A. Baz-Lomba, M.J. Reid, K. V. Thomas, Target and suspect screening of psychoactive substances in sewage-based samples by UHPLC-QTOF, *Analytica Chimica Acta*, vol. 914, 2016, pp. 81–90

[30] A.A. Bletsou, D.E. Damalas, P.G. Ferrero, E.L. Schymanski, H.P. Singer, J. Hollender, N.S. Thomaidis, Wide-scope quantitative target screening of 2327 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS, in: Proc. 14th Int. Conf. Environ. Sci. Technol. Rhodes, Greece, 3-5 Sept. 2015, 2015

[31] P. Gago-Ferrero, E.L. Schymanski, A.A. Bletsou, R. Aalizadeh, J. Hollender, N.S. Thomaidis, Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS, *Environmental Science and Technology*, vol. 49, 2015, pp. 12333–12341

[32] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities, 2002, pp. 1–29

[33] E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H.P. Singer, J. Hollender, Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence*, Environmental Science and Technology*, vol. 48, 2014, pp. 2097–2098

[34] E.L. Schymanski, H.P. Singer, J. Slobodnik, I.M. Ipolyi, P. Oswald, M. Krauss, T. Schulze, P. Haglund, T. Letzel, S. Grosse, N.S. Thomaidis, A. Bletsou, C. Zwiener, M. Ibáñez, T. Portolés, R. de Boer, M.J. Reid, M. Onghena, U. Kunkel, W. Schulz, A. Guillon, N. Noyon, G. Leroy, P. Bados, S. Bogialli, D. Stipaničev, P. Rostkowski, J. Hollender, Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis, *Analytical and Bioanalytical Chemistry*, vol. 407, 2015, pp. 6237–6255

[35]V. Fernandez-Gonzalez, E. Concha-Gran, S.Muniategui-Lorenzo, L. Mahıa,E. Fernandez-Fernandez, D. Prada-Rodrıguez, A matrix solid-phase dispersion–gel permeation chromatography–programmed temperature vaporisation–GC-MS procedure for the analysis of polycyclic aromatic hydrocarbons in mussel samples, *Journal of Separation Science*, vol 33, 2010, pp. 3741–37

[36] V. Fernɑndez-Gonzɑlez, S. Muniategui-Lorenzo, P. Lσpez-Mahva, D. Prada-Rodrvguez, Development of a programmed temperature vaporization-gas chromatography–tandem mass spectrometry method for polycyclic aromatic hydrocarbons analysis in biota samples at ultra-trace levels, *Journal of Chromatography A*, vol. 1207, 2008, pp. 136–145

[37] G.Bajger, P.Konieczka, J.Namieśnik, Speciation of trace element compounds in samples of biota from marine ecosystems, *Chemical Speciation and Bioavailability*, vol. 12, 2015, pp. 80-233

[38] G. Bianco, G. Novario, G. Anzilotta, A. Palma, A. Mangoned , T.R.I. Cataldid, Polybrominated diphenyl ethers (PBDEs) in Mediterranean mussels (*Mytilusgalloprovincialis*) from selected Apulia coastal sites evaluated by GC–HRMS, *Journal of Mass Spectometry*, vol. 45, 2010, pp. 1046–1055

[39] N. Carro, I. Garcia, M. Ignacio & A. Mouteira, Optimization of Soxtec, Extraction for the Determination of Polychlorinated Biphenyls (PCBs) in Mussel and Comparison with Soxhlet Extraction, Accelerated Solvent Extraction, and Microwave Assisted Extraction, *Analytical Letters*, vol. 45, 2012, pp. 2161-2175

[40] L.Duedahl-Olesen, F.Ghorbani, Optimizationof large volume injection for improved detection of polycyclic aromatic hydrocarbons (PAH) in mussels, *Polycyclic Aromatic Compounds*, vol. 28, 2008, pp. 4-5, 282-301

[41] R. Lega, D. Megson, C. Hartley, P. Crozier, K. Mac Pherson, T. Kolic, P.A. Helm, A. Myers, S.P. Bhavsar, E.J. Reiner, Congener specific determination of

polychlorinated naphthalenes in sediment and biota by gas chromatography high resolution mass spectrometry, *Journal of Chromatography A*, vol. 1479, 2017, pp. 169–176

[42] D.l Wolecki, M. Caban, K. Pazdro, E. Mulkiewicz, P. Stepnowski, J. Kumirska, Simultaneous determination of non-steroidal anti-inflammatory drugs and natural estrogens in the mussels Mytilus edulis trossulus, *Talanta*, vol. 55, 2019, pp. 81-712

[43] L.Liguori , K.Heggstad, H.T. Hove, K.Julshamn, An automated extraction approach for isolation of 24 polyaromatic hydrocarbons (PAHs) from various marine matrixes, *Analytica Chimica Acta*, vol. 573-574,2006, pp. 181–188

[44] P.W. Looser, M.l. Berg, K. Fent, J. Muhlemann, R.P. Schwarzenbach, Phenyl- and Butyltin Analysis in Small Biological Samples by Cold Methanolic Digestion and GC/MS, *Analytical Chemistry*, vol. 72, 2000, pp. 5136-5141

[45] P. Licata, D. Trombetta, M. Cristani, D. Martino, F. Naccari, Organochlorine compounds and heavy metals in the soft tissue of the mussel Mytilus galloprovincialis collected from Lake Faro (Sicily, Italy), *Environment Internationa*l, vol. 30, 2005, pp. 805-810

[46] E. Magi, C.Liscio, M. Di Carro, Multivariate optimization approach for the analysis of butyltin compounds in mussel tissues by gas chromatography–mass spectrometry, *Journal of Chromatography A*, vol. 1210, 2008, pp.99-107

[47] P.Novak, T.Zuliani, R.Milacic, J.Scancar, Development of an analytical method for the determination of polybrominated diphenyl ethers in mussels and fish by gas chromatography—Inductively coupled plasma mass spectrometry, *Journal of Chromatography A*, vol. 1524, 2017, pp. 179-187

[48] S.C. Cunha, C. Oliveira, J.O. Fernandes, Development of QuEChERS-based extraction and liquid chromatography-tandem mass spectrometry method for simultaneous quantification of bisphenol and tetrabromobisphenol in seafood: fish, bivalves and seaweeds, *Analytical and Bioanalytical Chemistry*, vol. 21, 2016, pp 9980-3

[49] S.C. Cunha, A. Pena, J.O. Fernandes, Mussels as bio-indicators of diclofenac contamination in coastalenvironments, *Environmental Pollution*, vol.225, 2017, pp. 354-360

[50] I. Dom , R.Biré, V.Hort , G.Lavison-Bompard , M. Nicolas, T. Guérin, Extended Targeted and Non-Targeted Strategies forthe Analysis of Marine Toxins in Mussels and Oystersby (LC-HRMS), *Toxins*, vol.10, 2018, pp.375-397

[51] D. Hu, X. Hu, W. Chen, H. Wang, H. Wang, K. Zhu, Bin Wu, C. Lin, Determination of Hydroxylated Polybrominated Diphenyl Ethers in Chinese Aquatic Products byLC–MS/MS, *Journal of Chromatographic Science*, vol. 51, 2017, pp. 1-8

[52] Y. Kato, S. Okada, K.Atobe,T. Endo, F. Matsubara, T. Oguma, K.Haraguchi,Simultaneous Determination by APCI-LC/MS/MS of Hydroxylated and Methoxylated Poly-brominated Diphenyl Ethers Found in Marine Biota, *Analytical Chemistry*, vol. 81, pp. 5942–5948

[53] I. Zabaleta, E. Bizkarguenaga, A. Prieto, M. Ortiz-Zarragoiti, L.A. Fernández, O. Zuloag, Simultaneous determination of perfluorinated compounds and their potential precursors in mussel tissue and fish muscle tissue and liver samples by liquid chromatography–electrospray-tandem mass spectrometry, *Journal of Chromatography*, vol. 1387, 2015, pp. 13-23

[54] V. Yusa, O. Pardo, P. Marti, A. Pastor, Application of accelerated solvent extraction followed by gel performance chromatography and high-performance liquid chromatography for the determination of polycyclic aromatic hydrocarbons in mussel tissue, *Food Additives and Contaminants*, vol. 22, 2005, pp. 482–489

[55] P. Plaza-Bolanos, N.M. Valera-Tarifa, A.G. Frenich, Current Applications of GC-(Q)TOF and GC–HRMS for the Determination of Persistent Organic Pollutants in Water and Sediments Samples, *Advances in High Resolution and Accurate Mass GC–MS*, vol. 6, 2018, pp. 431-452

[56] O. Komolafe, B. Bowler, J. Dolfing, W. Mrozik, R.J. Davenport, Quantification of poly-brominated diphenyl ether(PBDE) congeners in wastewater by gas chromatography with electron capture detector (GC-ECD), *Analytical Methods,* vol. 11, 2019, pp. 3474–3482

[57] X. Hu, D. Hu, W. Chen, B. Wu, C. Lin, Simultaneous Determination of Methoxylated Polybrominated Diphenyl Ethers and Polybrominated Diphenyl Ethers in Water, Soil and Sediment from China by GC–MS, *Journal of Chromatographic Science*, vol. 53, 2015, pp.1239-1249

[58] M.G. Ikonomou, M.P. Fernandez, T. He, D. Cullon, Gas chromatography–high-resolution mass spectrometry basedmethod for the simultaneous determination of nine organotincompounds in water, sediment and tissue, *Journal of Chromatography A*, vol. 975, 2002, pp. 319–333

[59] S.P.J. van Leeuwen, J. de Boer, Advances in the gas chromatographic determination of persistentorganic pollutants in the aquatic environment, *Journal of Chromatography A*, vol. 1186, 2008, pp. 161–182

[60] M. Vecchiato, S. Zambon, E. Argiriadis, C. Barbante, A. Gambaro, R. Piazza, Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers(PBDEs) in Antarctic ice-free areas: Influence of local sources on lakesand soils, *Micro chemical Journal*, vol. 120, 2015, pp. 26–33

[61] G.Nürenberg, M. Schulz, U. Kunkel, T.A. Ternes, Development and validation of a generic non-target method based on liquid chromatography – high resolution mass spectrometry analysis for the evaluation of different wastewater treatment options, *Journal of Chromatography A*, vol. 1426, 2015, pp. 77-90

[62] M. Ibanez, J.V. Sancho, D. McMillan, R. Rao, F. Hernandez, Rapid non-target screening of organic pollutants in water by ultra performance liquid chromatography coupled to time-of flight mass spectrometry, *Trends in Analytical Chemistry*, vol. 2, 2008

[63] M. Ruff, M.S. Mueller, M. Loos, H. P. Singer,Quantitative target and systematic non-target analysis of polar organicmicro-pollutants along the river Rhine using high-resolution mass spectrometry Identification of unknown sources and compounds, *Water Research*, vol. 87, 2015, pp. 145-154

[64] C. Hug, N. Ulrich, T. Schulze, W. Brack , M. Krauss, Identification of novel micro-pollutants in wastewater by a combination of suspect and non target screening, *Environmental Pollution*, vol.184, 2014, pp. 25-32

[65]M.E.T. Padrón, C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, Micro extraction Techniques Coupled to Liquid Chromatography with Mass Spectrometry for the Determination of Organic Micro pollutants in Environmental Water Samples, *Molecules*, vol.19, 2014, pp. 10320-10349

[66] Q. Sun, Z. Chen, D. Yuan, M.Megharaj, R. Naidu, On-line solid-phase extraction coupled with liquidchromatography/electro spray ionization mass spectrometry for the determination of trace tributyltin and triphenyltin in water samples, *Rapid Communications in Mass Spectrometry*, vol. 23, 2009, pp. 3795–3802

[67] S. Zhu, F. Hu, T. Yang, N. Gan, D. Pan, Y. Cao, D. Wu, Synthesis and characterization of a molecularly imprinted polymer for the determination of trace tributyltin in seawater and seafood by liquidchromatography–tandem mass spectroscopy, *Journal of Chromatography B*, vol.921-922, 2013, 21-26

[68] O. Blair, M.G .Ikonomou , B. C. Kelly, B. Surridge , F.A.P.C. .Gobas, Ultra-Trace Determination of Phthalate Ester Metabolites in Seawater, Sediments, and Biota from an Urbanized Marine Inlet by LC/ESI-MS/MS, *Environmental Science & Technology*, vol. 43, 2009, pp. 6262–6268

[69] P.Tolgyessy, B. Vrana, K. Silharova, An Improved Method for Determination of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Sediment by Ultrasonic Solvent Extraction Followed by Stir Bar Sorptive Extraction Coupled to TD–GC–MS, *Chromatographia*, vol. 76, 2013, pp. 177–185

[70] P. Stejnarova, M. Coelhan , R. Kostrhounova, H. Parlar, I. Holoubek, Analysis of short chain chlorinated paraffins insediment samples from the Czech Republic by short-column GC/ECNI-MS, *Chemosphere,* vol. 58, 2005, pp. 253–262

[71] A.R. Fontana, N.B. Lana, L.D. Martinez, J.C. Altamirano, Ultrasound-assisted leaching-dispersive solid-phase extraction followed byliquid–liquid micro extraction for the determination of polybrominated diphenylethers in sediment samples by gas chromatography–tandem mass spectrometry, Talanta, vol. 82, 2010, pp.359–366

[72] M.S. Kim, T. Kang, H. Pyo, J. Yoon,K. Choi, J. Hong, Determination of organochlorine pesticides in sedimentusing graphitized carbon black solid-phase extraction andgas chromatography/mass spectrometry,*Journal of Chromatography A*, vol. 1208, 2008, pp. 25–33

[73] M. Nichkova, E.K. Park, M.E. Koivunen , S.G. Kamita, S.J. Gee, J. Chuang, J. M. Van Emon, B.D. Hammock, Immunochemical determination of dioxins in sediment and serum samples, *Talanta*, vol. 63, 2004, pp. 1213–1223

[74] G.T. Tomy ,G.A. Stern, W. Lockhart, D. Muir, Occurrence of C10-C13Polychlorinated *n*-Alkans in Canadian Midlatitude and Arctic Lake Sediments, *Environmental Science & Technology*, vol. 33, 1999, pp. 2858-2863

[75] H. Zhang, S. Bayen, B.C. Kelly, Multi-residue analysis of legacy POPs and emerging organiccontaminants in Singapore's coastal waters using gaschromatography–triple quadrupole tandem mass spectrometry, *Science of the Total Environment,*vol. 523, 2015, pp. 219–232

[76] A. Berlioz-Barbier, A. Vauchez, L. Wiest, R. Baudot, E. Vulliet , C. Cren-Olivé, Multi-residue analysis of emerging pollutants in sediment using QuEChERS-based extraction followedby LC-MS/MS analysis, *Analytical and Bioanalytical Chemistry,* vol. 406, pp. 1259–1266

[77] J. S. Aulakh , A. K. Malik , V. Kaur, P. Schmitt-Kopplin, A Review on Solid Phase Micro Extraction—High Performance Liquid Chromatography (SPME-HPLC) Analysis of Pesticides, *Critical Reviews in Analytical Chemistry*, vol. 35, 2005, pp. 71-85

[78] D.M. dos Santosa, L.Buruaem, R.M. Gonçalves, M. Williams, D.M.S. Abessa, R.Kookanad, M.R.R. de Marchi, Multi-residue determination and predicted risk assessment of contaminantsof emerging concern in marine sediments from the vicinities of submarine sewage outfalls, *Marine Pollution Bulletin*, vol. 129, 2018, pp. 299–307

[79] A.E. Jacomini, P.B. de Camargo, W.E.P. Avelar, P.S. Bonato, Determination of Ametryn in River Water, River Sediment and Bivalve Mussels by Liquid Chromatography-Tandem Mass Spectrometry, *Journal of the Brazilian Chemical Society*, vol. 20, 2009, pp. 107-116

[80] S. Kinani, S. Bouchonnet , N. Creusot , S. Bourcier, P. Balaguer , J.M. Porcher, S. Aıt-Aıssa, Bioanalytical characterization of multiple endocrine- and dioxin-like activities in sediments from reference and impacted small rivers, *Environmental Pollution*, vol. 158, 2010, pp. 74–83

[81] B. Pérez-Fernández, L. Viñas, J. Bargiela, Occurrence and toxicological assessment of polycyclic aromatic hydrocarbons (PAHs) in marinesediments under mussel farming influence, *US National Library of Medicine National Institutes of Health*, vol 4, 2018, pp. 15862–15872

[82] M. Han, J. Kong, J. Yuan, H. He, J. Hu, S. Yang, S. Li, L. Zhang, Cheng Sun, Method development for simultaneous analyses of polycyclic aromatic hydrocarbons and their nitro-, oxy-, hydroxyl derivatives in sediments, *Talanta*, vol. 205, 2019, pp. 120128

[83] M. Y.Azis, L. Asia, A. Piram, B. Buchari, P. Doumenq, H. Setiyanto, Matrix effects on organic pollutants analysis in marine sediment, *Journal of Physics*, vol. 1013, 2018, pp. 012193

[84] J.S. Che , R.P. Yu , Q.J. Song, L.P. Wang, S.F. Wu, Determination of synthetic musks in the sediment of the Taihu lake by using accelerated solvent extraction (ASE) and GC/MS, *International Journal of Environmental Analytical Chemistry*, vol.91, 2011, pp.387-399

[85] W.L. Chen, Z. Xi, H. Wolschke, J. Gandrass, D. Kotke, M. Winkelmann, R. Ebinghaus, Quantitative determination of ultra-trace carbazoles in sediments in the coastal environment , *Chemosphere*, vol. 150, 2016, pp. 586-595

[86]X. Zhao, T. Cui, R.Guo, Y. Liu, X. Wang, Y. An, X. Qiao, B. Zheng, A clean-up method for determination of multi-classes of persistent organic pollutants in sediment and biota samples with an aliquot sample, *Analytica Chimica Acta*, vol. 1047, 2018, pp. 71-80

[87] M.S. Kim, T. Kang, H. Pyo, J. Yoon,K. Choi, J. Hong, Determination of organochlorine pesticides in sediment using graphitized carbon black solid-phase extraction andgas chromatography/mass spectrometry, *Journal of Chromatography A,* vol. 1208, 2008, pp. 25–33

[88] R .Leg, D. Megson, C. Hartley, P. Crozier, K. Mac Pherson, T. Kolic, P.A. Helma, A. Myers, S.P. Bhavsar, E.J. Reiner, Congener specific determination of polychlorinated naphthalenes in sediment and biota by gas chromatography high resolution mass spectrometry, *Journal of Chromatography A*, vol.1479, 2017, pp.169–176