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## Impact of the rivers on the Black Sea ecosystem

Common borders. Common solutions.

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## Acronyms

AAS-ET	atomic absorption spectrometry
AMBI	AZTI Marine Biotic Index
ANEMONE	Assessing the vulnerability of the Black Sea marine ecosystem to human pressures
ANOVA	Analysis of variances
BEAST	Black Sea Eutrophication Assessment Tool
BG	Bulgaria
BOD	Biological Oxygen Demand
BS	Black Sea
BSC	Black Sea Commission (Commission on the Protection of the Black Sea Against Pollution)
BSIMAP	Black Sea Integrated Monitoring and Assessment Programme
CHASE	Contaminants Status Assessment Tool
CIS	Cooled Injection System
COD	Chemical Oxygen Demand
CR	contamination ratio
CRAN	statistical package for R software
CTD	Instrument for measuring conductivity, temperature, and depth
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DIN	Dissolved Inorganic Nitrogen

DIP	Dissolved Inorganic Phosphorus
DIVA	Data-Interpolating Variational Analysis
DMA	Direct Mercury Analyzer
DO	Dissolved oxygen
DRB	Danube River Basin
EAC	Environmental Assessment Criteria
EBM	Ecosystem-Based Management
EEA	European Environmental Agency
EEI	Ecological Evaluation Index
EF	enrichment factors
EG	ecological groups
EI	electron ionization
EQR	Ecological Quality Ratio
EQS	Environmental Quality Standard
ERL	Effect Range Low
ERM	Effect Range Medium
ES	Environmental standard
EU	European Union
EUR	European
EUT_Ratio	Eutrophication ratio
GAM	Generalized Additive Model
GAMM	Generalized Additive Mixed Models
GC-ECD	Gas chromatography with an electron-capture detector
GC-MSMS	Gas Chromatography - Tandem Mass Spectrometry
GES	Good Environmental Status
GF-AAS	graphite furnace - atomic absorption spectrometry method
HAB	Harmful Algal Blooms
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HEAT	HELCOM Eutrophication Assessment Tool
HELCOM	The Baltic Marine Environment Protection Commission
HF	hydrogen fluoride
IAEA	International Atomic Energy Agency
IBI	phytoplankton-integrated index (Phyto-IBI)
ICES	The International Council for the Exploration of the Sea
ICP-MS	inductively Coupled Plasma Mass Spectroscopy
ICPDR	International Commission for the Protection of the Danube River
IO-BAS	Institute of Oceanology - Bulgarian Academy of Science
IOC	International Oceanographic Commission
IUCN	International Union for Conservation of Nature
JRC	Joint Research Center
KED	Kinetic Energy Discrimination
LAT	Latitude
LBS	Land Based Sources
LONG	Longitude
LUSI	Land Uses Simplified Index
M-AMBI	AZTI Marine Biotic Index
MAC	Maximum allowable concentration
MISIS	MSFD Guiding Improvements in the Black Sea Integrated Monitoring System
MODIS	Moderate Resolution Imaging Spectroradiometer
MPC	Maximum permissible concentration
MPS	MultiPurpose Sampler
MSFD	Marine Strategy Framework Directive
NASA	National Aeronautics and Space Administration
NATO	North Atlantic Treaty Organization
NEAT	Nested Environmental Status Assessment Tool
NIMRD	National Institute for Marine Research and Development "Grigore Antipa", Romania
NIVA	Norwegian Institute for Water Research
NMDS	Non-metric multidimensional scaling
NOAA	National Oceanic and Atmospheric Administration
NPMS	National Preventive Mechanisms
NPOC	Non Purgeable Organic Carbon
NWBS	Northwest Black Sea
OCP	Organochlorine pesticides
ODV	Ocean Data View - software
OECD	Organization for Economic Co-operation and Development

OOAO	One-Out-All-Out-principle
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic (the 'OSPAR Convention')
PAH	Polyaromatic hydrocarbons
PCB	Polychlorinated biphenyls
PLE	accelerated pressure extraction unit
PRIMER	Plymouth Routines in Multivariate Ecological Research - software
PSU	Practical Salinity Units
PTFE	Politetrafluoroetilena
PTV	programmable temperature vaporizing inlet
RBD	River basin district
RIA	river-sea impact area
RO	Romania
ROSCI	Romanian Site of Community Interest
RV	Research Vessel
SAU	Spatial Assessment Unit
SDD	Secchi disk depth
SE	Standard error
SIMPER	Similarity Percentage analysis
SNU-FF	Sinop University Fisheries Faculty
TBT	Tributyltin
TDS	Thermal Desorption System
TDU	Thermal Desorption Unit
TM	Trace metals
TN	Total Nitrogen
TNMN	TransNational Monitoring Network
TOC	Total organic carbon
TP	Total Phosphorus
TPH	Total Petroleum Hydrocarbons
TR	Turkey
TRIX	Trophic Index
TSS	Total suspended solids
TUBI	Turkish Benthic index
TÜBİTAK	Scientific and Technological Research Council of Turkey (Turkish: Türkiye Bilimsel ve Teknolojik Araştırma Kurumu, TÜBİTAK)
TÜBİTAK-MRC	Scientific and Technological Research Council of Turkey - Marmara Research Center
TUDAV	Turkish Marine Research Foundation
UA	Ukraine
UM	Unit of Measure
UNDP	United Nations Development Programme
UNEP MAP	United Nations Environment Programme / Mediterranean Action Plan
UNESCO	United Nations Educational, Scientific and Cultural Organization
US-EPA	United States Environmental Protection Agency
USA	United States of America
UV-VIS	Ultraviolet-visible spectroscopy
UWWT	Urban Waste-Water Treatment
UWWTD	Urban Waste-Water Treatment Directive
WB	Water Bodies
WC	Water Column
WFD	Water Framework Directive
WORMS	World Register of Marine Species

## Executive summary

Across the Black Sea region, environmental monitoring is fragmented, and the ANEMONE project set out to enable an environmental and methodological framework for future coordination and implement an integrated approach to environmental monitoring within the Black Sea basin. Hence, in ANEMONE, we performed (one of the) first assessments of the pressures and impacts simultaneously for seven rivers from northern (Dnieper, southern Bug), northwestern (Dniester and Danube), western (Kamchia), and southern (Sakarya and Yesilirmak) Black Sea. We aggregated data from four countries (from north to south - Ukraine, Romania, Bulgaria and Turkey), six cruises and a sampling network of 62 stations. Thus, the monitoring and assessment related provisions of the Bucharest (Black Sea) Convention and its Protocols, taking into account existing regional (BSIMAP) and national monitoring and assessment programs, the best practices of other Regional Sea Conventions, and last, but not least, Marine Strategy Framework Directive (MSFD) principles, aiming to contribute further to harmonization of methodologies and filling in of knowledge gaps identified in the region.

The outcomes represent the databases for chemistry - water, sediment and biota, pelagic habitats components - phytoplankton, zooplankton, and benthic habitats - zoobenthos. Apart from the analytical results themselves, new monitoring data regarding the neighbouring area, we applied tools for integrated assessments. Only some were applied before (E-TRIX and BEAST), but others were entirely new for the Black Sea. We learned to apply the new tools (e.g. CHASE, NEAT) through workshops organized in the project's lifetime and held by their authors and developers, all from the European area. Consequently, the information ensures the establishment and improvement of the regional pool of data that allows the production of common indicator assessment reports in an integrated manner, which ensures comparability across the Black Sea region due to agreed indicators of good environmental status.

Therefore, the deliverable consists of the comparative assessment of rivers impact on the Black Sea ecosystem quality through pilot case studies carried out in marine areas in front of seven rivers' discharging into the Black Sea and following the ecosystem approach. The scope is to assess the Black Sea ecosystem vulnerability to the human pressures resulting from rivers discharge as an important tool for decisions.

Following the River-Sea cruises (2019-2020) in ANEMONE project was identified, using integrated tools, a high risk of eutrophication (BEAST) and chemical contamination (CHASE) in the rivers neighbouring areas. The risk was decreasing from N-NW (southern Bug, Dnieper, Dniester, Danube) to W (Kamchia) and slightly increased again in S (Sakarya and Yesilirmak) and was not particularly correlated with the average rivers' flows but more with the basin's area and activities.

In our study, the nutrients and contaminants enrichment led to "moderate"- "poor"- "bad" status in most of the areas, highlighting the risk to not achieve the Good Environmental status at least for descriptors 5 and 8. The integrated tool's results could be used as governance performance indicators - evaluating the success of policies developed to effectively manage the coastal and marine environment. For example, the Danube's Mouths are classified as potential problem areas which represents an encouraging case for the Black Sea waters quality improvement. Implementing the Danube basin's program of measures (ICPDR) (e.g. TransNational Monitoring Network (TNMN), phosphate detergents ban) might lead to the improvement of the Black Sea waters quality in other rivers catchments.

On the other hand, the atmospheric deposition on the entire basin and the seawater circulation are considered of major importance. Thus, because they were neglected in the present study, we consider that the next development of such assessments should consider both strains.

# Introduction

The Black Sea is the endpoint of four “top 10” rivers in Europe (the Danube, Dnieper, Don, Dniester), totalling more than 8 000 km length journeys across its drainage basin (2 000 000 km<sup>2</sup>). Consequently, about 160 million people, almost half of non-coastal countries, are using and impacting the Black Sea ecosystem indirectly and without realising it. We do not have a price for this “product” yet. Shiny attempts to monetise it, but nobody succeeded to value all “benefits”.

Comparable with human health prevention, it is crucial to plan (assess), do (minimise), check (monitor) and act (review) for the Black Sea environmental condition. The process should be continuous and dynamic with a unique objective - the healthiest sea as numerous marine organisms' living place. Otherwise, the cost is catastrophic. The Black Sea almost “died” once, and the action of the Danube’s riparian countries saved it, but not at all.

Like everywhere in the Black Sea basin the human activities and needs such as agriculture, transportation, energy production or urban development exert pressures on the water environment that are in need to be assessed for managing each river basin and for taking decisions on adequate measures for addressing and reducing these pressures (ICPDR, 2015).

Interdependencies and interactions that occur at the land-sea interphases steadily increase under the current complex global scenarios. The geomorphological and ecological interdependent patterns (e.g., sediment transfer, nutrient flows from catchment areas) also relate to social, economic, and technological linkages, like the ones present at developing infrastructures or harvesting agriculture, produce heavily reliant on well-functioning maritime logistics. Under this perspective, marine systems' governance needs to be interwoven, and communication, participation, and joint planning need to occur. This necessity remains, however, a significant challenge for this day. Sustainability issues and vulnerabilities are differently perceived since world views are fundamentally different<sup>1</sup>.

River discharges are critical factors affecting the marine ecosystem functioning. Land-originated inflows carrying fresh, nutrient or pollutants rich water are the factor responsible for creating new physical and biochemical conditions, which can create a favourable medium for many marine organisms to run their biological cycles within. Like in the other basins, in the Black Sea, land-

The infographic is a vertical stack of five colored boxes. The top box is yellow with a blue border and contains the number '7' above the word 'RIVERS' in blue, and a circular illustration of a river flowing through a canyon. The second box is yellow with a blue border and contains the words 'DANUBE', 'DNIEPER', and 'DNIESTER' in blue, followed by a blue wavy line representing water, and the text '3D TOP 10' in blue. The third box is yellow with a blue border and contains a map of Europe with several countries highlighted in different colors, and the text 'LEGISLATION EU NATIONAL' in blue. The fourth box is yellow with a blue border and contains the text 'REGIONAL BLACK SEA COMMISSION' in blue, and a blue square containing several white dolphins. The bottom box is blue with a yellow border and contains the text 'TAKE "MEASURES FOR PRESSURES"' in yellow.

<sup>1</sup> [https://www.oceangov.eu/working\\_groups/land-sea-interactions/](https://www.oceangov.eu/working_groups/land-sea-interactions/)

originated water inflows are associated with the nutrients' enrichment, eutrophication and pollution being one of the factors, which trigger these processes<sup>2</sup>.

Hydrological and hydro-chemical characteristics of coastal water bodies of the Black Sea differ significantly. The coastal marine environment has really and quickly changed in space and time, comparing with shelf waters. The near-shore phytoplankton community depends not only on river flow but also on local coastal runoff, including sewage and agricultural drains, other forms of human activities, local wind-driven surface currents causing upwelling and downwelling, and other natural and anthropogenic factors.

The pressures' management at this level is complex. Hence, besides the interaction of tangible entities, the interaction, cross-fertilisation, and distinct separation of different modes of organising, decision-making, of governance play out along the coast, challenged by population density, shifting materialities and weak, partly outdated, or little-enforced governance mechanisms (Assche et al., 2020). Indeed, the distinction between governance and government has been made and remade naturally in coastal environments, as coasts often represent political entities' borders. Policies can focus on the sea or turn their back to it, but can never ignore the, in times of rapid environmental change, decreasingly reliable boundary between land and sea. Coasts are, therefore, place of institutional rupture and consecutive co-evolution (Assche et al., 2020).

The Black Sea ecosystem has no internal borders. Thus, riparian countries inhabitants, and direct beneficiaries of the resources, are responsible for the ecosystem's health more than anybody. Understanding that what is going in is transported everywhere will end in a healthier Black Sea shared by us.

We, as scientists, want to reveal the impact of river discharges as an essential pressure when managing activities and continental waters, as all such discharges, influence the total marine productivity of the Black Sea.

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<sup>2</sup> <https://www.icpdr.org/main/danube-basin/black-sea>

# 1 Material and methods

According to the deliverable's aim, we divided abiotic and biotic data to describe the pelagic and benthic habitats in front of each river influence area.

Therefore, for pelagic habitats were first discussed the biological (phytoplankton, zooplankton) characterisation and the water column physico-chemical characteristics (temperature, salinity, pH, dissolved oxygen), nutrients (phosphate, silicate, nitrate, nitrite, ammonium) and organic matter (biological oxygen demand, total phosphorus, total nitrogen, total organic carbon, total suspended solids) and contaminants (heavy metals, total petroleum hydrocarbons (TPH), polyaromatic hydrocarbons (PAHs), pesticides and polycyclic biphenyls (PCBs)).

We continued, for benthic habitats, to describe the biological features (macrozoobenthos and meiobenthos) and the sediments' chemistry (heavy metals, total petroleum hydrocarbons (TPH), polyaromatic hydrocarbons (PAHs), pesticides and polycyclic biphenyls (PCBs)).

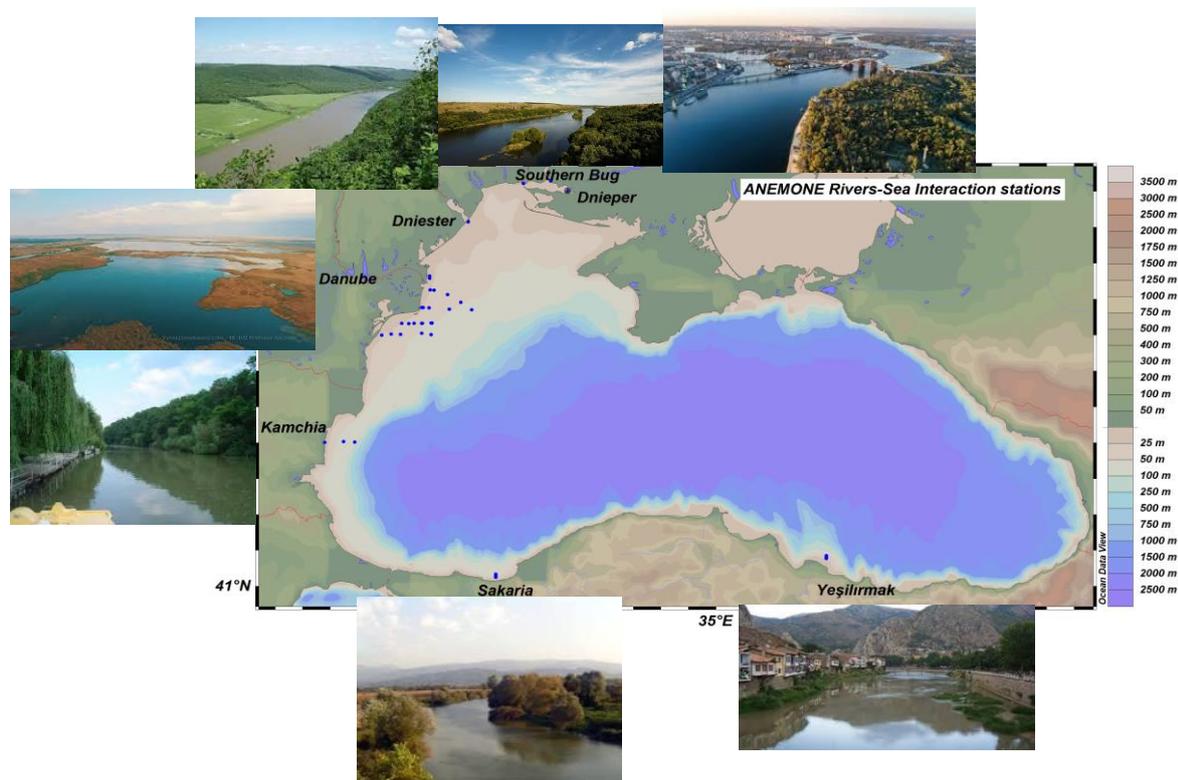


Figure 1.1- Map of stations

After presenting the results, with the purpose of the harmonised investigation were applied integrated tools for diverse assessments - Biodiversity - NEAT (Nested Environmental status Assessment Tool), Eutrophication - E-TRIX (Trophic Index), BEAST (Black Sea Eutrophication Assessment Tool), pollution - CHASE (Contaminants Status Assessment Tool), NEAT (Nested Environmental status Assessment Tool) and cumulative pressures (EcolImpact Mapper). Some were used for the first time in the Black Sea area (CHASE, NEAT and EcolImpact Mapper).

Finally, we statistically analysed all common parameters (abiotic and biotic) for testing the hypotheses that each river discharge has a distinct influence on the Black Sea ecosystem. Data were analysed with Microsoft Excel, Ocean Data View, Statistica, Primer 7.

For Kamchia river influence, Generalized Additive Mixed Models (GAMM) were employed to study the relationships between the in situ environmental variables and phytoplankton and mesozooplankton response metrics. Analysis of Variance (ANOVA) was used to test the models and smooth terms of statistical significance. Statistical analyses and graphic representations were undertaken in R 3.6.2 (R Core Team, 2019) CRAN package (Wood, 2017) and CRAN package (Peterson and Carl, 2014).

We did the maps with Ocean Data View (ODV) software version 4.7.3; a computer program for the interactive exploration that displays data in two basic ways: either by showing the original data at the data locations as coloured dots of user-defined size or by projecting the original data onto equidistant or variable resolution rectangular grids and then displaying the gridded fields. The gridded fields of method 2 are data products, and that small scale or extreme features in the data may be modified or lost because of the gridding procedure (DIVA gridding). All ODV representations done within the scope of this assessment have used method 2 (data products). For calculation of AMBI and m-AMBI\*(n) was used freeware software available on [www.azti.es](http://www.azti.es), for structural indexes (S, iChao1, H', IMg), PAST 3.14 and MS Excel. Details of sampling and analytical methods are in Annex B.

## 2 Rivers' catchment and features description

### 2.1 Dnieper River

From the spring to the mouth, the Dnieper flows through the three states' territory: Russia, Belarus, and Ukraine and serve as a natural border between countries. The Dnieper irrigates 13 densely populated areas, one in Russia (Smolensk Region), three in Belarus (Vitebsk, Mogilev, Gomel Regions), and nine in Ukraine (Chernigov, Kiev, Cherkasy, Kirovograd, Poltava, Dnepropetrovsk, Zaporizhia, Kherson and Nikolaev Regions). More than fifty cities and towns lie on the riverbanks, including the capital of Ukraine - Kiev.

Along the Ukrainian section of the Dnieper are located 25 cities with more than 7.5 million inhabitants. The main are Kiev (4130000), Kremenchug (232000), Kaminsky (273700), Dnipro (1040000), Zaporozhye (786000) and, Nikopol (128369) (Figure 2.1).

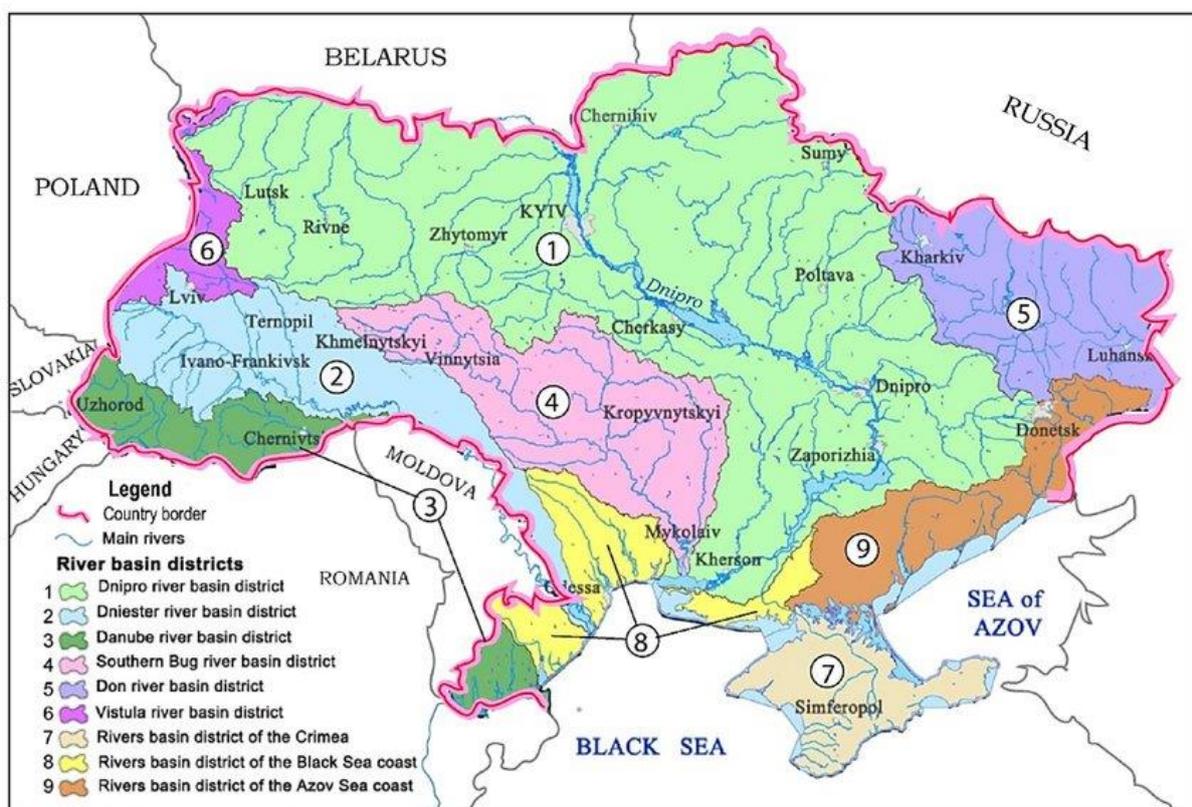


Figure 2.1 - The main rivers of the northwestern Black Sea region

The Dnieper is a flat river with a slow and calm course. It has a winding channel, form sleeves, rifts, islands, channels, and shallows. It is divided into three parts: the upper reaches of the river - from the source to Kiev (1320 km), the middle - from Kiev to Zaporozhye (555 km) and the lower - from Zaporozhye to the Black Sea (325 km).

The direction of the current changes several times: from the headwaters to Orsha, the Dnieper flows southwest, then to Kiev - directly south, from Kiev to the Dnieper southeast. In Zaporozhye, there is a second, shorter (90 km length) southward stretch of the river. Further to its estuary, it flows in a southwestern direction. Thus, the Dnieper forms on the territory of Ukraine a semblance of a large bow facing east, which doubles the route along the Dnieper from Central Ukraine to the Black Sea: the distance from Kiev to the mouth of the Dnieper in a straight line is 450 km while along the river is 950 km. The width of the river valley is up to 18 km. The floodplain is up to 12 km wide, and the Delta area has 350 km<sup>2</sup>.

The intensive use of the Dnieper, especially since the 20<sup>th</sup> century, has led to several serious environmental problems:

- Pollution of the Dnieper waters with industrial and domestic wastewater and intensively applied agrochemicals in the catchment (fertilisers, pesticides). In recent years, the pollution of the banks of the river and tributaries, including by vacationers, has also increased.
- Noncompliance with the rules of development and use of the Dnieper coastal protection zone.
- Swamp formation on the Dnieper: due to the building of hydropower plants' cascade, the middle and lower river's reaches have turned into a chain of lakes with almost stagnant water. Also, unique river landscapes and ecosystems, particularly the Zelenyi Lug (Green Meadow), were lost because the reservoirs flooded the Dnieper floodplain.

### Pressures<sup>3</sup>

In all three Dnieper's riparian countries, many domestic waste dumps and industrial waste storage facilities are in the Dnieper basin. Following 2001 estimates, industrial waste accumulation is 8.5 billion tons in waste storage facilities (up to 50 % accumulated in Ukraine, up to 10% in Belarus, and about 40% in the Russian Federation). There is an estimated annual increase in accumulated industrial waste of 8% to 10%. The storage facilities contain up to 40% of especially hazardous industrial waste, including salts of heavy and non-ferrous metals (lead, cadmium, nickel, chromium) and oil products (up to 2.5%). After the Chernobyl catastrophe, a large amount of radioactive caesium was deposited in the reservoir sediment.

### Transboundary impact

Discharges of insufficiently treated municipal and industrial wastewaters and pollution from waste disposal sites and agriculture harm the Dnieper River's water quality and its major transboundary tributaries.

### Trends

Hydropower stations, nuclear power stations and manufacturing industries have caused ecological damage at a sub-regional scale. Large-scale development of timberland, draining of waterlogged lands for agriculture, and intensive growth of cities where sewage treatment is insufficient to worsen the environmental and human health problems both in the Dnieper river basin and Black Sea region.

## 2.2 Southern Bug River

The southern Bug has its entire catchment area in Ukraine. It originates on the Volyn-Podolsky plateau in Kholodets village of Khmelnytsky Region, from there flows east through Vinnitsa, near which it changes direction to the southeast and flows into the Bug estuary, then with the Dnieper (Dnieper estuary) flows into the Black Sea via Dnieper-Bug estuary. It runs through the southwest of Ukraine, within Khmelnytsky Region (122 km), Vinnitsa Region (324 km), Kirovograd Region (70 km) and Odessa Region (40 km; along the border with Kirovograd Region), as well as Nikolaev Region (250 km). The total length of the river is 813.6 km. The total area of the southern Bug basin is 64 300 km<sup>2</sup> (Figure 2.1).

The southern Bug's main tributaries are Bolshaya Vys, Rotten Tikich, Wolf, Mountain Tikich, Zgar, Ingul, Kodyma, Mertvovod, Row, Savranka, Sinyukha, Sob, Black Tashlyk, Chicheklya, Yatran. The largest of them, the Sinyukha River (its basin area has 16,804 km<sup>2</sup> - 26% of the total), is formed at the Tikich and Bolshaya Vys Rivers' confluence. The longest tributary (342 km) is the Ingul River.

Several reservoirs used mainly for power production have been created on the southern Bug River: Shchedrivske, Ladyzhenskoye, Sabarovskoye, Glubochanskoye, Gayvoronskoye, Pervomayskoye and Aleksandrovskoye.

Replenishment with water happens due to snow and rainfalls (prevails), as well as due to groundwater runoff. High water is from late February to mid-April - early-May, low water - from June to February and floods are rare. Significant variability characterises the river flow. Average water consumption (132 km from the mouth) near the village Alexandrovka is 92.1 m<sup>3</sup>/s (the highest - 5320 m<sup>3</sup>/s, the lowest - 2.6 m<sup>3</sup>/s). The share of spring runoff (in the annual runoff) is 61 %, in summer - 9 %, in autumn - 12 %, and in winter - 18 %. The river flow is slightly regulated. The southern Bug does not have large hydropower stations (such as the Dnieper and Dniester). It freezes over almost regularly

<sup>3</sup> <https://unece.org/fileadmin/DAM/env/water/blanks/assessment/black.pdf>

in November (December) - February, opens by mid-March; the ice regime is not constant; often in winter, the ice is melting and freezing again. The lower reaches do not freeze in warm winters. The mineralisation of water near the village Aleksandrovka (in the south Ukrainian Nuclear Power Station area) is spring flood - 600 mg/dm<sup>3</sup>; summer-autumn low water - 674 mg/dm<sup>3</sup>; winter low water - 701 mg/dm<sup>3</sup>.

#### Pressures<sup>4</sup>

Wastewater discharges and diffuse pollution from agriculture are the main factors contributing to the entry of pollutants into the southern Bug basin. According to the data of the regional departments of ecology, a significant excess of the content of pollutants in the river was recorded. The level of water pollution in the lower reaches of the southern Bug River corresponds to class 4 out of 6 - polluted.

#### Impact

Discharges of inadequately treated wastewater and pollution from along coastal developments and agricultural waste are damaging the water quality of the southern Bug River and its main tributaries.

#### Trends

Nuclear power plants continue to cause environmental damage on a subregional scale. The lack of water and the slowing down of the flow of the southern Bug River is associated with the creation of 197 reservoirs and 7 thousand ponds, where 40 % of the river basin's runoff is concentrated, and inadequate wastewater treatment exacerbates environmental and human health problems in the river basin.

## 2.3 Dniester River

The Dniester is a river in Eastern Europe along which the state border between the Republic of Moldova and Ukraine goes. It flows from the northwest to the southeast within Ukraine and Moldova's territory into the Black Sea. The length of the Dniester is 1362 km, and the basin area has 72100 km<sup>2</sup>(Figure 2.1).

Sixty-two cities and 95 urban-type settlements are in the Ukrainian part of the basin, two municipalities and 41 cities within the Moldavian part. More than 7 million people inhabit Ukrainian and Moldavian areas adjacent to the river. The river spring is in the Ukrainian Carpathians, on the slopes of Mount Chentyeveka at an altitude of 870 m, flows into the Dniester estuary connected to the Black Sea in the Odessa Region. The average downstream flow rate is 310 m<sup>3</sup>/s. The volume of annual runoff is 10.2 km<sup>3</sup>. The slope of the river is 0.666 m/km.

In its upper reaches, the Dniester flows in a deep narrow valley and has the character of a fast mountain river. The current velocity in this region is 2-2.5 m/s. Here, many tributaries flow into the Dniester, originating from the Carpathians' slopes, mainly on the right. The largest tributary in this section is the Stryi. Below the Galich city (Ivano-Frankivsk Region), the flow becomes calmer, but the valley remains narrow and deep.

In the middle course, tributaries flow only to the left: the Zolotaya Lipa, the Strypa, the Seret, the Zbruch, the Smotrych, the Muksha (from the territory of Ternopil, Khmelnytsky and Vinnitsa Regions). The Dniester reservoir is located on the territory of Ukraine (Khmelnytsky, Chernivtsi, and Vinnitsa Regions). It was formed during the Dniester hydropower station's construction (677.7 km from the mouth of the Dniester, Novodnistrovsk, Chernivtsi Region).

The length of the Dniester within Moldova is 660 km. The area of the basin within Moldova is 19,070 km<sup>2</sup>, which is 57 % of its territory. Below the city of Mogilev-Podolsky (Vinnitsa region, Ukraine), the valley expands, but up to Vykhatintsy of Rybnitsky District, the valley expands, Dniester still flows in a narrow and deep canyon-like valley with high steep and rocky coasts cut by ravines.

The current environmental state of the Dniester basin can be characterized as tense, with a whole range of problems regarding the quantitative and qualitative characteristics of water bodies, the reduction of biological resources and diversity, and manifestations of water's destructive effect. The river basin has a diversified and complex economy characterized by a high density of environmentally hazardous activities - mining (e.g., potassium salts, sulphur, gas, oil, building materials), chemical industry, oil refining, engineering, food, and energy production. One of the first places in terms of

<sup>4</sup> <https://unece.org/fileadmin/DAM/env/water/blanks/assessment/black.pdf>

environmental impact on the Dniester is hydropower production. In the middle reaches of the Dniester, two-channel reservoirs were constructed - the Dubossary Reservoir and the Dniester Reservoir, which influenced the decrease in river flow, which led to a change in structural communities and the predominance of cyanobacteria and euglena algae, as well as sedimentation. Almost 67 % of the Dniester basin area within Ukraine is under agricultural activities. The share of arable land in farmland reaches 66 % in Ukraine. On the territory of Moldova, agricultural land occupies 76 % of the Dniester basin and only 9 % by forests. Because of the existing land use structure, diffuse pollution sources are among the most significant factors of anthropogenic load on the basin. According to the conditions of water regime, physical and geographical features, the Dniester basin is divided into three parts: the Carpathian (upper) part with a highly developed hydrographic network and about 70% of the river flow is formed in this part; Podolsk (middle) part of the basin with a well-developed hydrographic network. Large riverbed Dniester and Dubossary Reservoirs were built on this site, which caused significant changes to the river's hydrological and thermal regime. That resulted in significant adverse effects on biological resources of the basin below the dam of the Novodnistrovskaya hydropower station; the lower part of the basin has a poorly developed hydrographic network and a well-developed flooded massif, which is significantly influenced by economic activity: part of this massif is drained, and part is separated under pond farms.

The priority environmental problems of the Dniester basin are:

- The destructive effect of water: water erosion, bank destruction, catastrophic floods in the upper part of the basin.  
Unsatisfactory water quality, including drinking water supply sources.
- Unsatisfactory sanitary-ecological and hydrological conditions of small rivers in the basin.
- Depletion and scarcity of water resources in the basin.
- Eutrophication
- Reduction of biological diversity in aquatic ecosystems of the basin.
- Reduction of hydrobiological resources.

### Pressures<sup>5</sup>

The Dniester flows through a densely populated area with a highly developed industry (mining, wood-processing, and food). Aquaculture, discharges of municipal wastewaters and diffuse pollution from agriculture are other main pressure factors introducing pollutants as nitrogen compounds, heavy metals, oil products, phenols, and copper. During the warm season, a deficit of dissolved oxygen and increased BOD<sub>5</sub> levels occur additionally. Microbiological pollution is also of concern. Petrol mining and chemical industries (e.g., oil refining) cause water pollution by phenols and oil products. Their key sources are in the basin's upper part, where petroleum mining occurs, and oil refineries are located. Due to the high migration ability of phenols and oil products, elevated concentrations are also found in the Middle Dniester.

### Transboundary impact

Moldova assesses that the upper and middle Dniester basin is moderately polluted, whereas the Lower Dniester and the Dniester tributaries are assessed as substantially polluted. Recently, the technical status of wastewater treatment plants in Moldova substantially decreased. Although wastewater treatment plants in cities continue to decrease efficiency, most of the other treatment plants are out of order. For some cities (e.g., Soroki), new treatment plants are to be constructed. Also, there is a great challenge to plan, create and correctly manage water protection zones in Moldova, including abolishing non-licensed dumpsites in rural areas.

### Trends

Although there was an improvement in water quality over the last decade, mainly due to the decrease in economic activities, the water quality problems remain to be significant. A further decrease in water quality related to nitrogen and phosphorus compounds and the microbiological and chemical status is to be expected. In both countries, the construction of wastewater treatment plants and the enforcement of water protection zones are of utmost importance.

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<sup>5</sup> <https://unece.org/fileadmin/DAM/env/water/blanks/assessment/black.pdf>

## 2.4 Danube River

The Danube River Basin (DRB) covers more than 800000 km<sup>2</sup> - 10 % of continental Europe - and extends into 19 countries' territories, being the most international river basin in the world. The Danube River Basin can - based on its gradients - be divided into three sub-regions: the upper basin, the middle basin, and the lower basin (including the Danube Delta). The Upper Basin extends from the source of the Danube in Germany to Bratislava in Slovakia.

The Middle Basin is the largest of the three sub-regions, extending from Bratislava to the Iron Gate Gorge's dams on the border between Serbia and Romania.

The lowlands, plateaus and mountains of Romania and Bulgaria form the Lower Basin of the River Danube. Finally, the river divides into three main branches, forming the Danube Delta, which covers an area of about 6750 km<sup>2</sup>.

Over 80 million people live in this basin, with many depending on the Danube for drinking water, energy production, agriculture, and transport. Its ecological diversity, from plant and animal species to critical habitats, is also highly valued. However, the increasing human impacts, pressure, and severe pollution from agriculture, industry, and municipalities affect the water supply for communities, irrigation, hydropower generation and industry, transportation, tourism, and fishing opportunities. The Danube River and many of its tributaries form the spawning grounds for many fish. They also receive various degrees of treated wastewater from many different sources, ultimately ending up in the Black Sea, affecting the nutrient levels in a significant portion of its waters<sup>6</sup> (Figure 2.2).



Figure 2.2 - Danube River basin (from ICPDR)

The estimated basin-wide nutrient emissions for 2009–2012 are 605000 t/y TN and 38500 t/y TP. Diffuse pathways contributed with 84 % (TN) and 67 % (TP). For N, groundwater (base flow and interflow) is the most important diffuse pathway, with 54 %. In the case of P, soil erosion (32 %) and urban runoff (18 %) generate the highest emissions. Regarding the sources, agriculture (N: 42 %, P: 28 %) and urban water management (N: 25 %, P: 51 %) are responsible for most nutrient emissions. The untreated wastewater discharges significantly influence the total point source emissions - 28 % (TN) and 39 % (TP). The long-term average (2003–2012) observed river loads estimated from measured river discharge and nutrient concentration data at the river mouth (TNMN station Reni) are 490000

<sup>6</sup><https://www.icpdr.org/flowpaper/app/services/view.php?doc=drbmp-update2015.pdf&format=pdf&page={page}&subfolder=default/files/nodes/documents/>

t/y (TN) and 25 000 t/y (TP), a significant reduction in the transported nutrient fluxes to the Black Sea being detected. However, the input is still considerably higher than those of the early 1960s, representing river loads under low pressures (TN: ca. 300000 t/y, TP: ca. 20000 t/y).

At the basin scale, the urban wastewater sector generates about 255000 t/y Biological Oxygen Demand (BOD), and 550000 t/y Chemical Oxygen Demand (COD) discharges into the surface water bodies of the Danube Basin (reference year: 2011/2012). From an overall COD emission of approximately 610 000 t/y, the urban wastewater sector releases 90 %. More than 60 % of the BOD surface water emissions via urban wastewater stem from agglomerations with existing sewer systems but without treatment.

The main sectors with nutrient pollution from the industrial sector are releasing in total, 7200 t/y nitrogen and 220 t/y phosphorus were released in the reference year. For nitrogen, the chemical industry has the highest importance emitting almost 45 % of the total discharges. In the case of phosphorus, food production and energy sectors have the highest share with 39 % and 24 %. The reported industrial emissions are relatively small compared to those of the urban wastewater, only 8 % (TN) and 2 % (TP).

Out of a 28836 km network in the Danube River Basin, good ecological status or ecological potential is achieved for 7107 km (25 %); good chemical status for 20 380 km (70.7 %) without data on mercury in biota, which is a decisive element for assessing the chemical status because in all surface water bodies exceeded its Environmental Quality Standard (EQS) and caused bad chemical status.

According to the data of the Hydrometeorological Service of Ukraine, the flow of the main rivers into the Black Sea in 2018 are as follows:

- Danube near Reni (54 miles) - 206.4 km<sup>3</sup>.
- Dnieper in the lower section of the Kakhovskaya hydroelectric power station - 39.2 km<sup>3</sup>.
- Dniester near Mogilev Podolskiy - 7.11 km<sup>3</sup>.
- Southern Bug near the village of Aleksandrovka - 1.06 km<sup>3</sup>.

Based on the results of the monitoring performed by UkrSCES in 2019 in the lower reaches of these rivers, the annual load has been calculated (Table 2.1, Table 2.2 and Table 2.3).

**Table 2.1 - Intake of nutrients and suspended solids from the main Rivers into the Black Sea**

Parameters	P min.	P org.	P total	N min.	N org.	N total	Si	TSS
Danube load t/y	12900	2400	15300	263760	76470	340230	479200	33952800
Dnieper load t/y	3850	180	4030	56320	780	57100	100500	203050
Dniester load t/y	430	227	657	9700	8450	18150	20600	150000
Southern Bug load t/y	71	19	90	2683	37	2720	2780	11340

**Table 2.2 - Intake of trace metals from the main Rivers into the Black Sea**

Parameters	Cu	Cd	Pb	Ni	As	Hg	Zn	Fe
Danube load t/y	527	33	396	58	355	18	206	49380
Dnieper load t/y	271	21.5	56	0	62	0	90	430
Dniester load t/y	44	1.2	65	0	0	1.1	0	43
Southern Bug load t/y	2.80	0.1	2.1	1.0	0	0	2.8	52

**Table 2.3 - Intake of POPs from the main Rivers into the Black Sea**

Parameters	HCH total	DDT total	PCBs total	PAHs total	Of these, the largest contribution		
					Naphthalene	Phenanthrene	Fluoranthene
Danube load t/y	0.27	1.03	1.80	5.97	2.47	2.15	0.38
Dnieper load t/y	0.05	0.47	1.27	0.23	0.06	0.05	0.06
Dniester load t/y	0.01	0.08	0.33	0.01	0	0	0
Southern Bug load t/y	0	0.01	0.02	0.01	0	0	0

## Pressures<sup>7</sup>

More than 81 million people living in the Danube River basin significantly affect the basin's natural environment, causing pressure on water quality, water quantity, and biodiversity.

<sup>7</sup> <https://unece.org/fileadmin/DAM/env/water/blanks/assessment/black.pdf>

The most significant pressures fall into the following categories: organic pollution, nutrient pollution, pollution by hazardous substances, and hydro-morphological alterations. Insufficient treatment of wastewater from major municipalities is a significant cause of organic pollution. In parts of the Middle and Lower Danube, wastewater treatment plants are missing, or the treatment is insufficient. CODCr, ammonium-nitrogen and orthophosphate phosphorus reach the highest values in the Lower Danube. The chemical, food, and pulp and paper industries are prominent industrial polluters, and wastewaters from these plants raise the levels of nutrients, heavy metals, and organic micro-pollutants in the river network.

Cadmium and lead can be considered as the most severe inorganic microcontaminants in the Danube River basin. Especially critical is cadmium, for which the target value under the transnational monitoring network (TNMN) is substantially exceeded in many locations downstream of river kilometre 1071 (values are in many cases 2-10 times higher than the target value). The pollution of the Lower Danube by cadmium and lead can be regarded as a significant problem.

Agriculture has long been a significant source of income for many people, and it has also been a source of pollution by fertilisers and pesticides. Many tributaries, such as the rivers Prut, Arges, Russenski Lom, Iskar, Jantra, Sio, and Dyje, are considered as rather polluted by nitrogen compounds. Most of these are in the lower part of the Danube.

Like many large rivers, the impact of the high transboundary river nutrient loads in the Danube river basin is the most critical in the receiving coastal waters of the Black Sea; however, pressures from the coastal river basins directly affecting the coastal waters of the Danube RBD also need to be considered.

A substance of particular concern in the lower Danube is p,p'-DDT. Here, the very low target values of the TNMN are often exceeded in the order of two magnitudes. It means that, despite a high analytical uncertainty, the level of p,p'- DDT is significant and gives a strong indication of the potential risk of failure to reach good status. For lindane, the results of the TNMN classification are not so alarming.

#### **Transboundary impact**

In the Danube basin, there are areas in “high and good status”, but there are also stretches of river which falls under “heavily modified water bodies” and has been assessed as “polluted”. As analysed in the above section, cadmium, lead, mercury, DDT, lindane and atrazine are among the most serious pollutants.

The Upper Danube, where chains of hydropower plants exist, is mainly impacted by hydro-morphological alterations, and many water bodies have also been provisionally identified as “heavily modified water bodies”. The Middle Danube is classified as “possibly at risk” due to hazardous substances. The Lower Danube is “at-risk” due to nutrient pollution and hazardous substances, and in large parts, due to hydro-morphological alterations. It is “possibly at-risk” due to organic pollution.

#### **Trends**

The Danube basin's water quality has improved significantly during the last decade, with improvements in the general environmental conditions in the Danube basin.

Improvements in water quality can be seen at several TNMN locations. A decrease in biodegradable organic pollution is visible in the Austrian-Slovakian section of the Danube and a lower section downstream at Chiciu/Silistra.

As for nutrients, ammonium-nitrogen decreases are evident in locations of the upper part of the Danube. Nitrate-nitrogen decreases in several locations of the German-Austrian part of the Danube River and some Lower Danube locations. Nitrate-nitrogen decreases have also been seen in the tributaries Morava, Dyje, Vah and Drava, and in the Sava River at the Una River's confluence Jasenovac.

A decrease of orthophosphate phosphorus has been observed at Slovak monitoring locations, at Danube Szob, and at most downstream locations on the Danube River starting from the Reni Chilia/Kilia arm. An improvement can also be seen in the tributaries to the upper part of the river and further in the rivers Drava, Siret, and the Sava/Una rivers' monitoring site at Jasenovac.

Despite the last decade's achievements, water and water-related ecosystems in the Danube River basin continue to be at risk from pollution and other harmful factors.

More intensive farming, especially in the new EU member States' fertile areas in the basin, may increase agricultural pollution. This calls for developing a long-term strategy to address pollution problems, predominantly diffuse pollution from agriculture. As is the case in other basins, the frequency of severe flood events due to climatic changes could increase, which may cause substantial economic, social, and environmental damage in combination with unsustainable human practices.

## 2.5 Kamchia River

The Kamchia River, entirely located in Bulgaria, is one of the largest (245 km length) and highly anthropogenic loaded river systems in the Bulgarian Black Sea basin watershed (Shtereva et al., 2006). The river catchment (5358 km<sup>2</sup>) covers 40% of the Bulgarian Black Sea catchment area. It contributes to about half the freshwater discharge by national rivers, ranging between  $179.3 \cdot 10^6$  m<sup>3</sup>/y and  $1475.3 \cdot 10^6$  m<sup>3</sup>/y (about 19.25 m<sup>3</sup>/s), collecting urban effluents of 230 settlements, populated by 310000 inhabitants, including untreated sewage discharges (Shtereva et al. 2010, Dineva, 2011). It drains mid-altitude (mean altitude: 300-800 m asl) catchment, having downstream lowland river sections. The average slope of the system is 2.9 %, and the average altitude - 327 m. The river has two main branches, the Golyama-Kamchia in the north considered the main stem and the Luda-Kamchia in the south (Figure 2.3). The river system is highly regulated with 26 reservoirs larger than 106 m<sup>3</sup>; only three of them - Kamchia, Ticha and Tsonevo, have a total volume of 870 mln m<sup>3</sup>, which is about 92 % of the total volume of water. Two reservoirs are located along Luda Kamchia (Kamchia and Tzoveno), and a third one (Ticha) is formed through the confluence of three Golyama river tributaries. The estimated average annual discharge of Kamchia increases from 1.14 m<sup>3</sup>/s at Ticha village to 26.3 m<sup>3</sup>/s at the mouth (Soufi, Uzunov, 2008). The Kamchia reservoir provides most of the drinking water for Burgas and Varna's cities (storage capacity: 229 Mm<sup>3</sup>). After the reservoir construction, the total flow decreased from 0.87 km<sup>3</sup>/year to 0.61 km<sup>3</sup>/y.

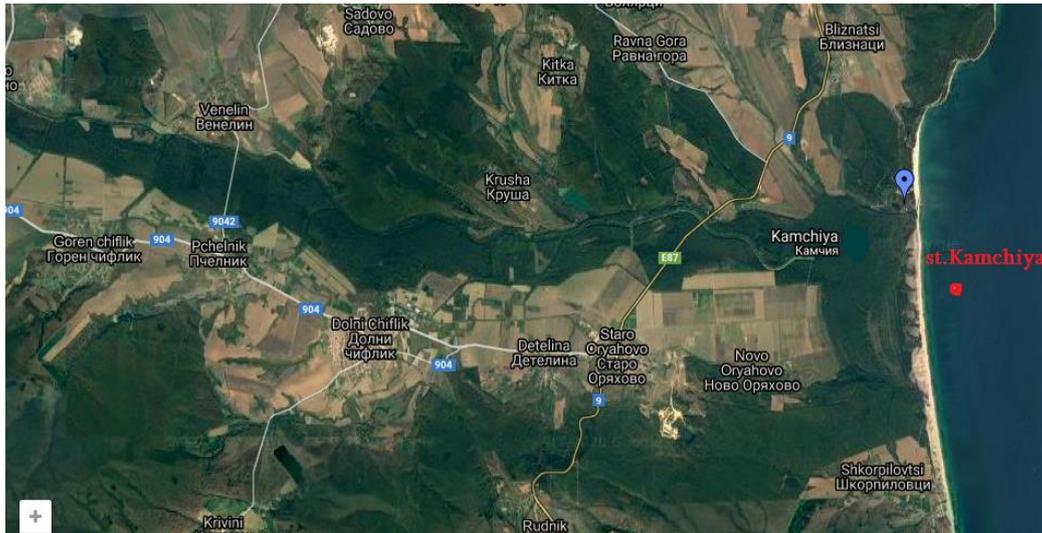
The anthropogenic inputs (industry, agriculture, and urbanisation) are of significant importance for the high content of organic matter and nutrients in the riverine waterflow (Shtereva et al., 2006, Shtereva et al., 2007, Shtereva et al. 2010), exerting pressure on the coastal ecosystem nutrient regimes (Shtereva, Krastev, 2009, Dineva, 2011).

Kamchia annually receives ~1.85 Mm<sup>3</sup> industrial and 15.3 Mm<sup>3</sup> municipal wastewater (Mihailov et al., 2005). The region is under the extensive impact of the tourism industry. Out of the total organic pollution discharged into the Bulgarian Black Sea by the national rivers (measured by BOD<sub>5</sub>) in the range 2000 - 7158 t/y, the share of the Kamchia river was between 608 t/y and 4146 t/y (1998-2005), from the total nitrate-nitrogen between 885 t/y and 5098 t/y, the Kamchia River's contribution was from 520 t/y to 3278 t/y and from the total orthophosphate-phosphorus discharge in the ranges from 65 t/y to 1141 t/y, the Kamchia River's rate was between 36 t/y and 222 t/y (Dineva, 2011).

The size of the impacted area, related to the advection of the river plume, depends strongly on the volume of discharged waters, as well as on the winds and current system in this part of the western Black Sea, the southeasterly and easterly winds spreading the eutrophic waters towards the coast (Shtereva et al., 2010a, Truhchev et al., 2010).

One of the significant factors in the dynamic of river runoff and sediment discharge is climate change, the intensity of heavy rains and storms and warmer and dry periods. During the last decade, the flood frequency in Bulgaria has increased, with almost 70 % due to river overflows associated with heavy rainfalls, snow melting and mismanagement of riverbeds and dikes (Vasileva et al., 2019). During 2004-2018 intense floods have been registered in 2005-2006, 2007, 2009, 2010 and 2012, the strongest one in the Varna region and Kamchia area observed in 2014 (Romanova et al., 2012, Rusinov et al., 2014). The river has an intensely dynamic seasonal regime originally of maximum flow in February/March and minimum in October (Tockner et al., 2010).

The high fluvial discharge resulting from increased precipitation rates during rainy periods may be one of the major, if not the most critical factor, for controlling the structure and seasonal dynamics of estuarine/marine coastal waters phytoplankton assemblages due to its effect on turbidity, salinity, nutrient concentrations, and water residence time (Noriega et al., 2013; Saeck et al., 2013). Riverine nutrients act in concert with local hydrographic conditions to create distinct ecological niches for phytoplankton communities across river-sea continuums (Gomes et al., 2018) and further impact the coastal processes.



**Figure 2.3 - Map of the Kamchia River (red dot -sampling station at the river mouth in the coastal Black Sea area)**

The Kamchia reserve is under the protection of UNESCO. The total area of the protected habitats in reserve, together with Kamchia Sands Protected Area, adds up to 1200 ha. The area around the mouth and lower course of the river are remarkable for their variety of habitats. Besides, the Kamchia River mouth is a crucial site for birds situated on the migratory route Via Pontica (Shtereva et al., 2006).

#### Pressures

The main drivers in the Kamchia River region (catchment and coastal zone) are industry, agriculture, and urbanization, which cause significant pressure on the aquatic systems. The river collects effluents of 230 settlements in the catchment area, populated by 310000 inhabitants, including untreated sewage discharges (Soufi, Uzunov, 2008). It is an attractive touristic area but lacking adequate treatment facilities. Kamchia annually receives ~1.85 Mm<sup>3</sup> industrial and 15.3 Mm<sup>3</sup> municipal wastewater (Mihailov et al., 2005). Therefore, the riverine water is affected by a high organic matter content and nutrients (most frequently for nitrite) exceeding the Bulgarian Water quality standards. The river system is highly regulated, with 26 reservoirs larger than 10<sup>6</sup> m<sup>3</sup>, only the three of them (with a total volume of 870 mln m<sup>3</sup>), constituting about 92 % of the total volume of accumulated water.

#### Trends

The flow regulation has caused a degradation of riparian vegetation and local habitat loss. In contrast, the higher discharge resulting from the increased frequency of rainy periods in the area, associated with the recent climatic changes, emerging as one of the critical factors controlling the structure and seasonal dynamics of estuarine/marine coastal processes.

## 2.6 Sakarya and Yesilirmak Rivers

The total area of Turkey's Black Sea basin is 246525 km<sup>2</sup>. Overall, there are 18 rivers in the basin area and five large (Sakarya, Filyos, Kızılırmak, Yeşilirmak, and Çoruh) rivers. Five main river basins are discharging to the Black Sea, including Sakarya (63273 km<sup>2</sup>) and Yesilirmak (39693 km<sup>2</sup>) (Figure 2.4). Despite the considerable inter-annual variability in loads and flows, the reported annual average flows are 6.4 km<sup>3</sup>/y for Sakarya and 6.1 km<sup>3</sup>/y for Yesilirmak (DSİ, 2016). The total freshwater inflow along the Turkish coastline into the Black Sea is 923 m<sup>3</sup>/s (TDA, 2008); thus, the total flow of these two rivers (~ 500 m<sup>3</sup>/s) constitutes approximately half of the total.



Figure 2.4 - Sakarya and Yesilirmak drainage areas

In the Black Sea, there are low salinity areas distributed along the south coast in response to the river discharges, including Sakarya and Yesilirmak. These freshwater contributions delineate the peripheral zone with salinity around 17.5-18.0 PSU separated by the interior water mass of 18.2-18.5 PSU by a well-defined, narrow frontal zone (MarinTurk Project report, 2014).

The results of the monitoring project, undertaken in the period 2014-2016, indicate that Sakarya River on the western Black Sea basin and Yeşilirmak rivers on the eastern Black Sea basin exert a significant pollution effect on the Black Sea ecosystem (MoEU-DGEIAPİ and TUBİTAK-MRC, 2017 report).

Nutrient and organic matter enrichment is high in Turkish Black Sea coastal areas. Insufficient wastewater treatment, marine outfall discharges and river inputs are the principal sources of input. Besides, solid wastes (storage areas by the coast) are causing problems in the coastal areas. High sedimentation rates at several fishing ports mean that dredging is a frequent activity, releasing sediment-trapped nutrients back into the water column. Some localised activities, such as agriculture (with associated erosion), sand/gravel extraction, industry and aquaculture, also contribute to eutrophication along the Black Sea coast.

Widespread agriculture and animal farming, untreated domestic and industrial wastewaters, solid waste dumping sites, erosion around the dam lakes and rivers, sand and gravel mining on riverbeds are the main activities creating pressure in the Sakarya River Basin. Total pollution (point and diffuse sources) loads from Sakarya RB were calculated as 47000 t/y TN and 5500 t/y TP (MoFW and TUBİTAK MRC, 2013).

Organic pollution in the Black Sea coastal areas is high due to the wastewater treatment facilities and river input. The landscapes of the Black Sea region are not suitable to construct wastewater treatment facilities. Some cities use the sewerage system directly disposing of deep marine outfalls, but most small settlement areas use septic tanks or package biological treatment. However, present sewerage systems also show such a variety as combined and/or separate systems. Ordu, Giresun city centres have separate sewerage systems, whereas Sinop, Trabzon, and Zonguldak have combined systems; only Samsun has both combined and separate systems draining the city (Bakan et al., 1996). Besides, solid wastes deposited in coastal areas cause pollution problems.

Eutrophication based pressure-impact analysis in the Black Sea was undertaken using the Land Uses Simplified Index (LUSI) (Flo et al., 2011) and the LUSIVAL methodology (Romeo et al., 2013) under the DEKOS Project (2014) (TUBITAK MRC and MoEU-GDEM 2014, Ediger et al., 2015). Chlorophyll *a* concentration was used as an indicator of impact, representing one of the descriptor 5 (Eutrophication) criteria. The water bodies under the Yeşilirmak river and Samsun province's impact were identified as the highest-pressure areas. In contrast, the water body under Sakarya's impact was defined as a "moderate" pressure area. These coastal waters (water bodies) are also designated as "sensitive" areas (following the UWWTD implementation) in 2015. Sensitivity was assessed based on physico-chemical, hydro-morphological and biological characteristics, together with exposure to land-based sources of pollution (TUBITAK SINHA Project 2015, TUBITAK-MRC and MoFWA 2015). These coastal areas are defined as mesotrophic/eutrophic according to their average DIN, TP, bottom water oxygen saturation and chlorophyll *a* concentration. They have a relatively limited exchange/mixing with waters further offshore, with phyto-benthos and zoobenthos results suggesting moderate impact levels. Additionally, the water bodies are threatened by nutrient inputs from the Sakarya and Yesilirmak Rivers. Phyto- and zoo-benthos communities are showing clear signs of degradation. Thus, these water bodies under the impact of Sakarya and Yeşilirmak were designated as "sensitive areas" (SINHA Project -TUBITAK-MRC and MoFWA, 2015). Marine benthic macrophytes are used as indicators for the assessment of ecological status. The ecological status of stations along the Black Sea coast was assessed (MoEU-DGEI-API and TUBITAK-MRC, 2014, 2015) using the Ecological Evaluation Index (EEI, Orfanidis et al., 2011;). According to the Black Sea coasts' ecological status levels, the lowest values were usually observed at the stations nearby Sakarya discharge due to abundant opportunistic algal species (e.g., *Ulva* sp. *Cladophora* sp. and *Ceramium* sp.) in the shallow sub-littoral zone. The station of Yeşilirmak was classified as moderate status (SINHA Project-TUBITAK-MRC and MoFWA 2015).

In the Black Sea region of Turkey, contaminants arise from numerous anthropogenic sources such as land-based industrial and agricultural activities, pollution by ship, atmospheric deposition and mineral exploration and riverine inputs. They include synthetic compounds, such as pesticides, and non-synthetic compounds, such as metals, dispersed by industrial processes, and polycyclic aromatic hydrocarbons, dispersed by combustion and oil spills. Generally, industrial facilities are low in number in the Black Sea region and concentrated in Zonguldak and Samsun. Copper (in Murgul and Samsun) and iron/steel production (Samsun) are essential in the eastern Black Sea region. Zonguldak and Samsun harbours are important transportation centres for these industries and the fertiliser industry in Samsun. The main industrial sectors located in the Sakarya and Yesilirmak River Basins are plastics, rubber and synthetic resins, mineral products other than metals, food processing, metal products, chemicals and chemical products (LBS NAP, 2017).

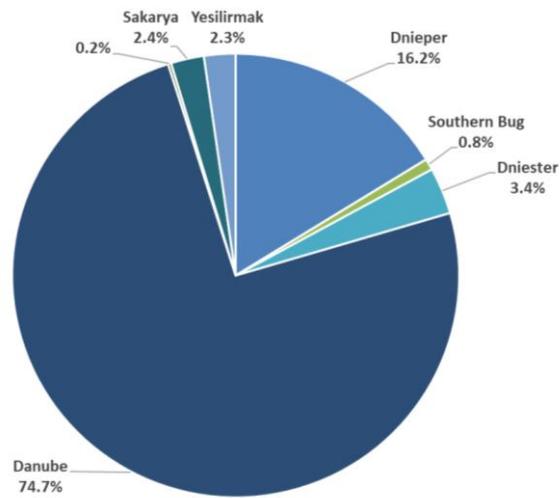
## Pressures

Besides direct discharges, spills of contaminants leaching from land, together with atmospheric deposition, large quantities of contaminants, notably those deriving from agricultural activities, are carried to the Black Sea via Sakarya and Yesilirmak rivers. Some Pesticides sourced from agricultural activities were found above Environmental Quality Standards (EQS) (BIKOP Project 2012-2014). Amasya and Sakarya provinces were chosen as pilot areas for the Black Sea Region due to the significant agricultural activity in these provinces.

Hence, we set out to analyse the impact of seven rivers in the N-NW-W-S areas of the Black Sea, of which three are in the top 10 longest rivers in Europe (Danube, Dnieper, and the Dniester). The rivers cross amount to over 1500000 km<sup>2</sup> so that when discharged into the Black Sea, they can bring significant quantities of suspensions, nutrients, organic matter, and pollutants. The average flow of the Danube exceeds about three times all the others. Taken together, the Danube and Dnieper bring approx. 91 % of the freshwater supply 1320000 km<sup>2</sup>, 88 % from the total (Figure 2.5). We also calculated the flow per catchment unit area as a pressure indicator (Table 2.4).

*Table 2.4 - Black Sea rivers main characteristics*

River basins	Area of basin	Annual discharge	Flow per catchment unit area
	10 <sup>3</sup> km <sup>2</sup>	km <sup>3</sup> /y	(km <sup>3</sup> /y)/ (10 <sup>3</sup> km <sup>2</sup> )
Danube	817.0	200.00	0.245
Dnieper	503.0	43.50	0.086
Dniester	72.1	9.10	0.126
Southern Bug	63.7	2.20	0.035
Sakarya	63.3	6.40	0.101
Yesilirmak	36.1	6.10	0.169
Kamchia	5.0	0.61	0.122



*Figure 2.5 - Share of average flows - Black Sea rivers*

# 3 Structure, functions, and processes of marine ecosystems with relevance for rivers-sea interaction assessment - cruises ANEMONE results

## 3.1 Pelagic Habitats

Pelagic ecosystems face numerous exogenous stressors, which threaten the sustainability of their current functions. Numerous human activities cause eutrophication, habitat degradation, biological and geological removals and different activities and pressures, climate change, ocean acidification. The potential impacts of these stressors on the pelagic habitats, much less their interaction and cumulative effects, are largely unknown. However, a study indicates that there remain no places in the ocean, no matter how deep, distant or dynamic, that are not affected by human activities (Halpern et al. 2008). In response to these intensifying threats, there have been increasing calls for better management, conservation, and marine biodiversity protection (Dunn et al., 2011).

Pelagic habitats have faster dynamics and lower predictability levels than terrestrial and marine benthic habitats (Ray, 1991; Gray, 1997; Hyrenbach et al., 2000). Living in liquid is different from living in gas, and the vital rates of all marine organisms are controlled to a great degree by the properties of and processes occurring within the sea (Purcell, 1977; Andersen et al., 2015; Manderson, 2016). Bertrand et al. (2014) describe the pelagic ecosystem as where the “substrate” consists of constantly moving water masses. Pelagic habitats are also defined by the frontal structures and subsides created and delivered by divergent and convergent flows (Tew Kai et al., 2009, Della Penna et al., 2017, Dickey-Collas et al., 2017).

The water’s transport is the primary mechanism for direct impacts of onshore human activities on the nearshore marine environment at the rivers’ mouths. The adverse effects of excess nutrients and sediments carried by rivers to coastal waters have been well-described worldwide. Sediments and nutrients typically co-occur in the freshwater runoff, even though those two pressures’ dynamics and impacts are not identical. Nutrients and other dissolved matter are transported further than sediments, so the freshwater plume containing dissolved nutrients may encompass a smaller sediment plume (Fredston et al., 2016). Nutrient additions to coastal and marine ecosystems can lead to increased phytoplankton abundance, including harmful algal blooms. In extreme cases, the bacterial decomposition of these phytoplankton blooms depletes dissolved oxygen levels to such an extent that most marine life cannot survive, creating anoxic “dead zones” in coastal waters (Fredston et al., 2016). The influence of rivers on the shelf waters depends on many factors, but the main is the volume of river flow, which varies from season to season and year to year.

Measurements of chlorophyll *a*, used as an estimate of phytoplankton biomass, are included in most eutrophication monitoring programs. Chlorophyll *a* represents the biological eutrophication indicator with the best geographical coverage at the European level (EEA, 2019).

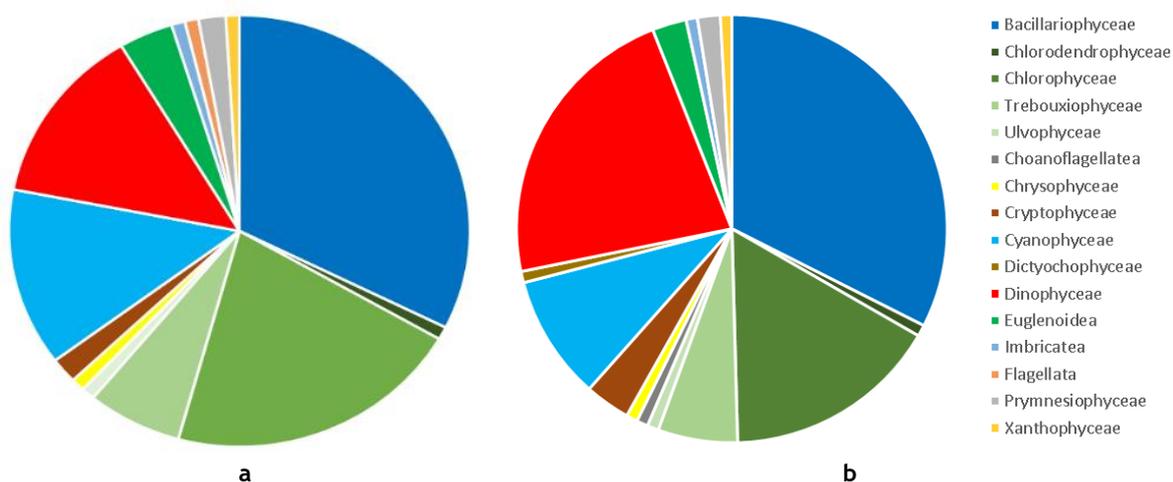
### 3.1.1 Phytoplankton

Phytoplankton is the leading producer of primary production and the basis of the water trophic chains. Phytoplankton communities, consisting of fast-growing, short-cyclical organisms, are the first to respond to environmental conditions changes by coherent rearrangement of their structural and functional organization. Both Water Framework Directive (Directive 2000/60/EC) and Marine Strategy Framework Directive (Directive 2008/56/EC) consider the phytoplankton a necessary component of assessing water bodies’ ecological status. Various marine phytoplankton indicators can provide valuable information on ecological processes essential for coastal countries’ quality of life and economy. Structural indicators of phytoplankton, immediately revealing the changes in nutrient concentration in the water column, have the advantage in analysing the eutrophication.

Chlorophyll *a* is one of the most frequently determined biochemical parameters, an indicator of biomass and primary productivity. Because of its importance in the marine ecosystem and measured more easily than phytoplankton biomass, chlorophyll *a* was included on the indicator list for “Eutrophication” in the EU “Water Framework Directive” as one of the impact parameters to be monitored.

### Dnieper River, southern Bug River, Dniester River and Danube (UA) River

In the coastal waters, we found 146 taxa belonging to 15 classes. Bacillariophyceae (47), Chlorodendrophyceae (1), Chlorophyceae (27), Choanoflagellata (1), Chrysophyceae (1), Cryptophyceae (4), Cyanophyceae (15), Dictyochophyceae (1), Dinophyceae (29), Euglenoidea (5), Imbricatea (1), Prymnesiophyceae (2), Trebouxiophyceae (8), Ulvophyceae (1), Xanthophyceae (1), and uncertain taxon Flagellata (1). In **summer** were observed 105 taxa belonging to 14 classes, diatom-green complex of species with the great contribution of dinoflagellates and cyanobacteria. We registered 105 taxa belonging to 14 classes: Bacillariophyceae (34), Chlorodendrophyceae (1), Chlorophyceae (22), Trebouxiophyceae (7), Ulvophyceae (1), Chrysophyceae (1), Cryptophyceae (2), Cyanophyceae (14), Dinophyceae (14), Euglenoidea (4), Imbricatea (1), Flagellata (1), Prymnesiophyceae (2), Xanthophyceae (1). In **autumn**, we noted 117 taxa belonging to 15 classes. The dominant classes were Bacillariophyceae and Dinophyceae, but green algae and cyanobacteria remained high. We noted 117 taxa belonging to 15 classes: Bacillariophyceae (38), Chlorodendrophyceae (1), Chlorophyceae (19), Trebouxiophyceae (7), Ulvophyceae (1), Choanoflagellata (1), Chrysophyceae (1), Cryptophyceae (4), Cyanophyceae (11), Dictyochophyceae (1), Dinophyceae (26), Euglenoidea (3), Imbricatea (1), Prymnesiophyceae (2), Xanthophyceae (1) (Figure 3.1).



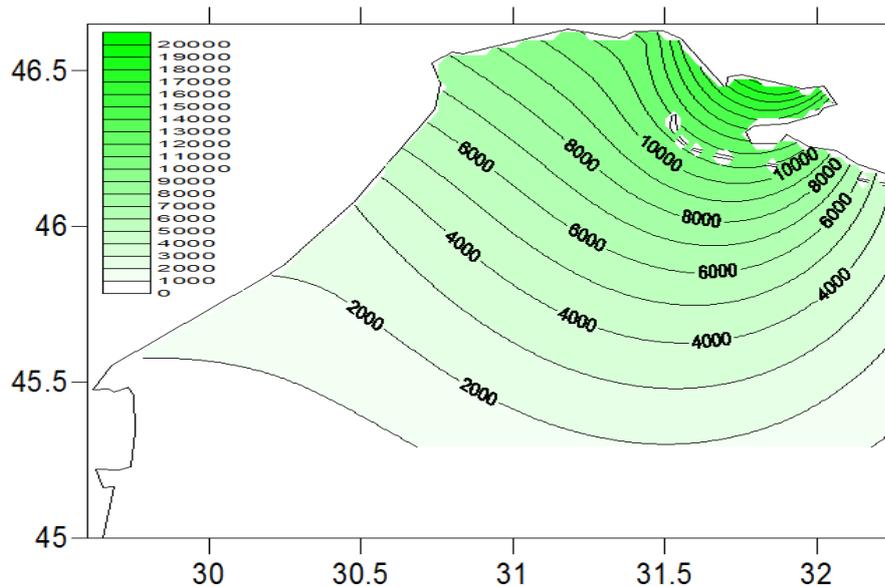
**Figure 3.1 - Taxonomic structure of phytoplankton community in the coastal waters of N-NW Black Sea in summer (a) and autumn (b), 2019**

The Shannon index of biodiversity for coastal waters varies from 1.59 to 1.97 (summer) and 0.53-2.69 (autumn), with averages of 1.77 and 1.76, respectively. The highest values of biodiversity index were in Zatoka waters (St. 3), at the exit of Dniester estuary.

In summer, the coastal waters near the estuaries' exits were characterized by high values of quantitative characteristics. The average abundance was  $11.03 \cdot 10^6$  cells/L, and the average biomass,  $2.34 \text{ g/m}^3$ . The highest values were observed in the surface waters near Ochakov (St. 6) at the exit of Dnipro-Buh estuary ( $59 \cdot 10^6$  cells/L and  $12.3 \text{ g/m}^3$ , respectively) due to simultaneous blooms of 4 cyanobacteria *Microcystis aeruginosa*, *Nodularia spumigena*, *Jaaginema kisselevii*, *Dolichospermum flosaquae*. The lowest values were observed in the Danube region's coastal waters (TW5), at the near-bottom layer close to the Kiliya arm ( $45 \cdot 10^3$  cells/L and  $44 \text{ mg/m}^3$ , respectively).

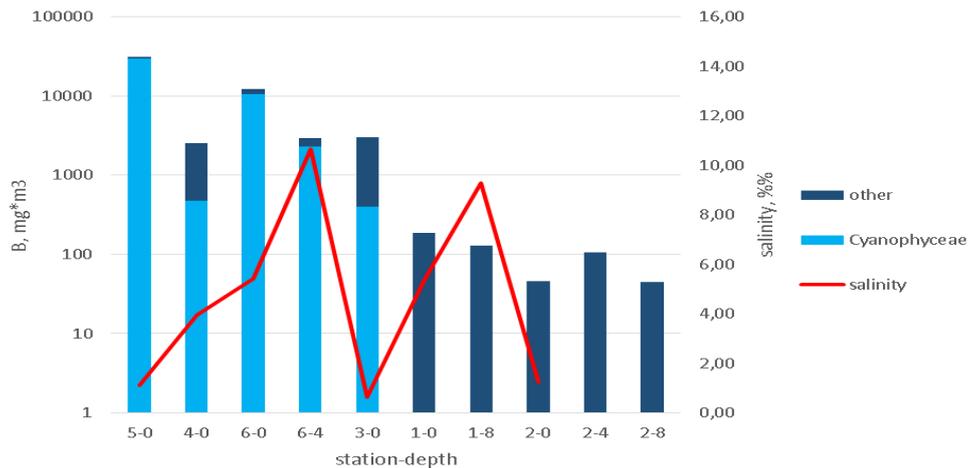
In autumn, the quantities and densities of phytoplankton communities were much lower than in summer. The average abundance was  $3.36 \cdot 10^6$  cells/L, and the average biomass was  $1.18 \text{ g/m}^3$ . As in summer, the highest values were observed in the surface waters near Ochakov, at the exit of Dnipro-Buh estuary ( $21 \cdot 10^6$  cells/L and  $5.57 \text{ g/m}^3$ , respectively), due to the bloom of freshwater species *Monactinus simplex* (by biomass) and filamentous cyanobacteria *J. kisselevii*. At the near-bottom layer close to the Kiliya arm ( $76 \cdot 10^3$  cells/L and  $55 \text{ mg/m}^3$ , respectively) were the lowest values in the Danube region's coastal waters.

In summer, we observed the tremendous simultaneous bloom of four cyanobacteria *M. aeruginosa*, *N. spumigena*, *J. kisselevii* and *D. flosaquae*. Comparative sampling in Dnieper and Bug rivers showed that this bloom started in the Bug River, where the quantities were the highest, with the share of the Cyanobacteria species more than 94 %. Due to the Rim Current, a narrow counter-clockwise flowing, basin-wide current which runs along the Black Sea coast and in particular is responsible for water transition in northwestern Black Sea shelf from its northeastern part to its southwestern part (Staneva et al., 2001), this bloom spread along the coastal line. The spatial distribution of the phytoplankton biomass occurs because of the expansion of blooming freshwater from Bug mouth along the northwestern shore of the Black Sea up to the exit of Dniester estuary (Figure 3.2).



**Figure 3.2 - Spatial distribution of phytoplankton biomass ( $\text{mg}/\text{m}^3$ ) in the surface layer of the coastal waters of N-NW Black Sea, June 2019**

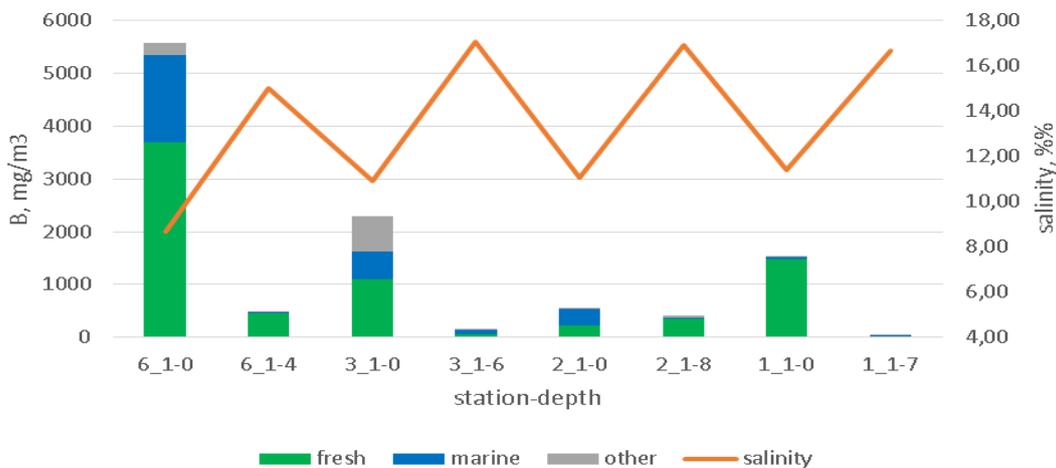
Freshwater spread over marine waters, so the surface layer's biomass was much higher than in the near-bottom layer. In the Dnieper-Bug and Dniester coastal areas, the biomass values were related to the share of cyanobacteria (Figure 3.3). In the coastal Black Sea waters, the highest biomass was in the surface waters near Ochakov ( $12.3 \text{ g}/\text{m}^3$ ), with the share of Cyanobacteria more than 85 %. The Danube region's coastal waters were not affected by the cyanobacteria bloom, and the biomass did not exceed  $200 \text{ mg}/\text{m}^3$ , with less than 1 % cyanobacteria. The lowest values were observed in the Danube region's coastal waters, at the near-bottom layer near the Kiliya arm's exit ( $44 \text{ mg}/\text{m}^3$ ). All stations except for St. 2, had the highest biomass in the surface layer and coincided with the minimum salinity, and a negative correlation was observed ( $r=-0.49$ ). We also detected a high positive correlation of biomass with pH ( $r=0.79$ ) and water temperature ( $r=0.56$ ). As for nutrients, we found a slight positive correlation with DIP ( $r=0.37$ ) and a negative with DIN ( $r=-0.38$ ).



**Figure 3.3 - Spatial and vertical distribution of salinity (PSU), phytoplankton biomass (mg/m<sup>3</sup>) and share of Cyanobacteria (% of total biomass) in the coastal waters, N-NW Black Sea, June 2019**

We also observed a strong negative correlation between phytoplankton biomass and salinity ( $r=-0.79$ ), at the coastal stations, in autumn. High values of quantitative characteristics coincide with a high share of freshwater species (Figure 3.4). We also observed a very high correlation with pH ( $r= 0.94$ ). Biomass was very slight positive correlated with DIP ( $r= 0.36$ ), slight negative correlated with DIN ( $r=-0.20$ ) and the correlation with temperature was absent ( $r=-0.11$ ).

As in summer, the highest values were observed in the surface waters near Ochakov, at the exit of Dnieper-Bug estuary ( $21 \cdot 10^6$  cells/L and  $5.57$  g/m<sup>3</sup>, respectively), due to the bloom of freshwater colonial species *M. simplex* and filamentous cyanobacteria *J. kisselevii*.

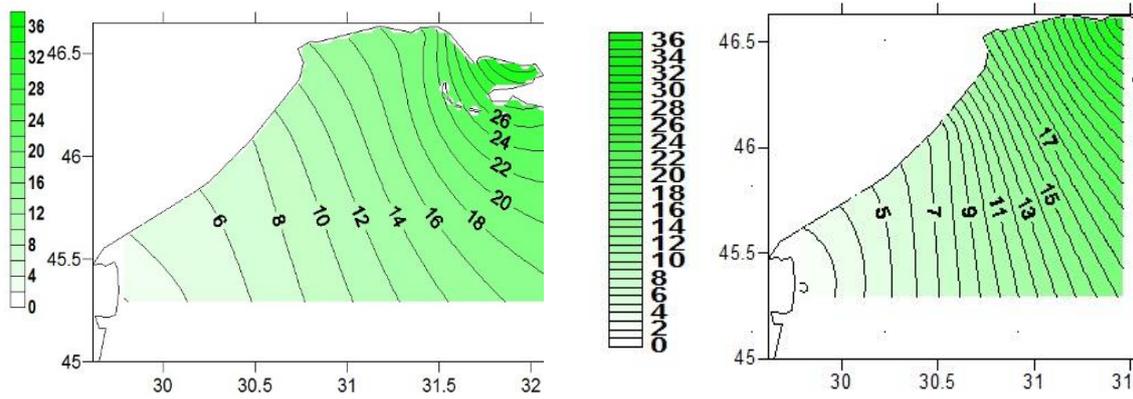


**Figure 3.4 - Spatial and vertical distribution of salinity (PSU), phytoplankton biomass (mg/m<sup>3</sup>), and the species' contribution with different salinity preferences to the total biomass of phytoplankton in the coastal waters of N-NW Black Sea, September 2019**

The quantitative characteristics of phytoplankton near the Danube mouth were much lower. The lowest values were also observed in the Danube region's coastal waters, at the near-bottom layer near the exit of Kiliya arm ( $76 \cdot 10^3$  cells/L and  $55$  mg/m<sup>3</sup>, respectively).

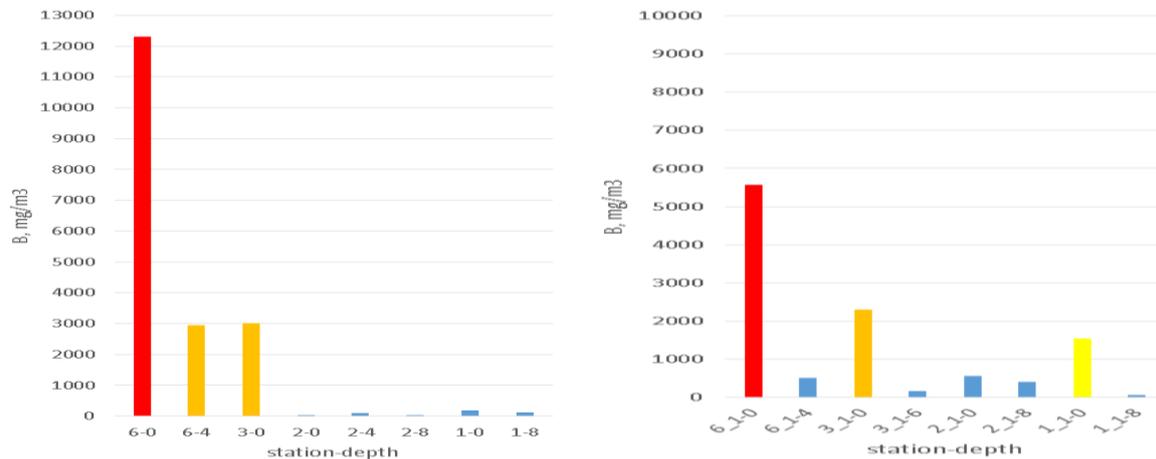
So, we assume that in the summer and autumn, the Danube river's negative effect on the coastal marine environment (according to phytoplankton indicators) was significantly lower than Dnieper, Bug and Dniester rivers.

The values of chlorophyll *a* in the coastal waters in summer vary from  $1.49$  µg/L to  $49.0$  µg/L, in autumn from  $1.85$  µg/L to  $29.8$  µg/L. The highest values for phytoplankton biomass were observed in the Dnieper-Bug estuary and decreased along the Danube mouth's coastal line. The spatial distribution of chlorophyll *a* concentration in surface waters is shown in Figure 3.5.



**Figure 3.5 - Spatial distribution of chlorophyll a concentration ( $\mu\text{g/L}$ ) in the N-NW Black Sea (left - spring, right - autumn)**

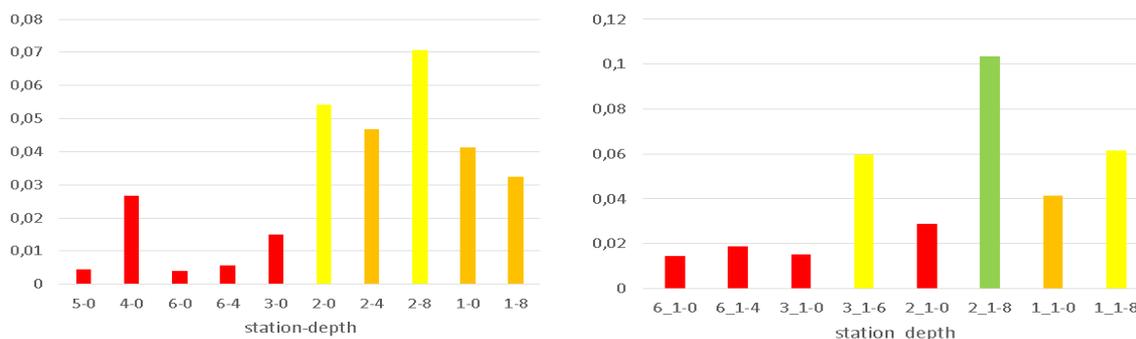
One of the principal indicators for the assessment of marine water quality is the phytoplankton biomass. We have developed this indicator's thresholds for all the main regions of the NW Black Sea for every season. Thus, the coastal waters in summer are assessed as “bad” and “poor” for the stations in Dnieper-Bug and Dniester regions, and “high” in the Danube region (Figure 3.6).



**Figure 3.6 - Water quality assessment of coastal waters by phytoplankton biomass (left - summer, right - autumn)**

In autumn, in the surface waters of the Dnieper-Bug and Dniester region, the quality of water was “bad” and “poor”, whilst in the surface waters of the Danube region near the exit of Bistroe arm, it was “moderate”. The near-bottom layer of all regions and surface layer near the exit of the Bistroe arm had a “high” status.

The water quality assessment results by the Menhinick index are shown in Figure 3.7, but we underline that the thresholds are defined for the present regions. We used the scale used in the EMBLAS project for water quality assessment (Moncheva, 2016). According to this scale, in summer, GES results were absent for the coastal samples, and in autumn, only 18 % of samples were GES (green).



**Figure 3.7 - Water quality assessment of the coastal waters by Menhenick index (left - summer, right - autumn)**

### Conclusions

In summer, in the coastal waters, the species diversity of microalgae was formed by the diatom-green complex of species with a great contribution of dinoflagellates and cyanobacteria. There was observed the bloom of cyanobacteria, which started from the Dnieper-Bug River system and spread along the most of NW coastal line. The share of cyanobacteria in total phytoplankton biomass was more than 94 % in the estuary and up to 85 % in the coastal waters. Only in the Danube area, the phytoplankton biomass was lower than GES limits.

In autumn, the dominant classes in microalgal biodiversity were Bacillariophyceae and Dinophyceae, but the part of green algae and cyanobacteria also remained high. Quantitative characteristics were much lower than in summer. The general tendency was the decrease of phytoplankton biomass from the exit of Dnieper-Bug estuary along the coast in the southeast direction, with the “spots” of increase near the rivers’ mouths, where the dominant are freshwater cyanobacteria and green algae. The spatial distribution of chlorophyll *a* concentration mainly coincides with the distribution of phytoplankton biomass. For the coastal waters, the maximum of chlorophyll *a* was noted in the surface layer. The highest values were observed in the Dnieper-Bug estuary and decreased along the coastal line towards the Danube mouth.

Coastal marine ecosystems of the NW Black Sea shelf were strongly affected by the Dniester and especially Dnipro and Bug River systems. The blooms start in the Dnieper and Bug rivers and due to the Rim Current spread along the significant part of NW Black Sea shore. The Danube river's negative effect on the Danube area is significantly lower than the other Ukrainian rivers.

In the coastal water bodies, the good ecological status was assessed in the Danube region but not in the Dnieper-Bug and Dniester region in summer. In autumn, most stations' surface waters are not in good ecological status, unlike the near-bottom waters of all regions, which are in good status. According to phytoplankton indicators, the ecological status increased along the coast from the exit of the Dnieper-Bug estuary to the Danube region, and the surface waters were of a worse ecological class than near-bottom waters.

### Danube River (RO)

The superficial layer of freshwater near the Danube's mouths can spread over large areas of the sea, also spreading the suspensions and plankton it contains. The surface and their mixing speed depend on the wind regime and the water mixing. Another important aspect is the impact that the violent contact between two unique living environments has. Freshwater species die in suddenly contact with marine water, as do the few marine elements found in the freshwater. Marine waters are much more transparent, so for marine phytoplankton, a harmful factor is not only the lower salinity but a large amount of suspensions. Suspensions can also act directly in the destruction of phytoplankton by attaching to them, preferably those with long shapes or setae (*Chaetoceros*, *Skeletonema*, *Nitzschia*), sometimes covering them almost completely. Thus, their buoyancy is reduced, causing them to fall to the bottom, where they accumulate (Băcescu, 1965).

With the osmotic shock to which they are subjected by the sudden change of salinity, especially in the surface horizon, phosphorus is released first, then nitrates. It results that in this area, both the Danube and the sea continuously transport large quantities of phytoplankton, which is destroyed by the violent contact between the two environments, release significant quantities of biogenic elements. Under these conditions, a series of species with brackish tendencies or with greater tolerance to the saline regime can develop intensely. Due to winds and currents, the waters near the

Danube's mouths are then entrained in various directions, especially to the south, along the Romanian coast. Thus, near the coast, it creates favourable conditions for phytoplankton development and, therefore, for trophic zooplankton's massive correlative development, especially copepods (Băcescu, 1965).

The phytoplankton abundance and biomass are highly variable around the Danube mouths, is primarily regulated by the nutrient supply and the organic matter and the light availability, water stratification, and mixing.

We identified 131 species, varieties and forms of phytoplankton communities belonging to 12 taxonomic classes. Dinoflagellates (35 %), diatoms (23 %), chlorophytes (18 %) and cyanophytes (8 %) represented 84 % of the total number of species. Among dinoflagellate species (46), those from genera *Gymnodinium* (5), *Protoperidinium* (5), *Prorocentrum* (4) and *Gyrodinium* (4) were the most diverse. Among diatom species (30), genera *Chaetoceros* (7), *Thalassiosira* (3), *Cyclotella* (2) and *Skeletonema* (2) showed the highest richness. A relatively high number of other groups species were identified for Chlorophyceae (24), Cyanophyceae (11), Trebouxiophyceae (6) and Cryptophyceae (5), while the classes Ebriophyceae, Euglenoidea, Prymnesiophyceae, Chrysophyceae, Dictyochophyceae and Ulvophyceae were represented by few species only (Figure 3.8).

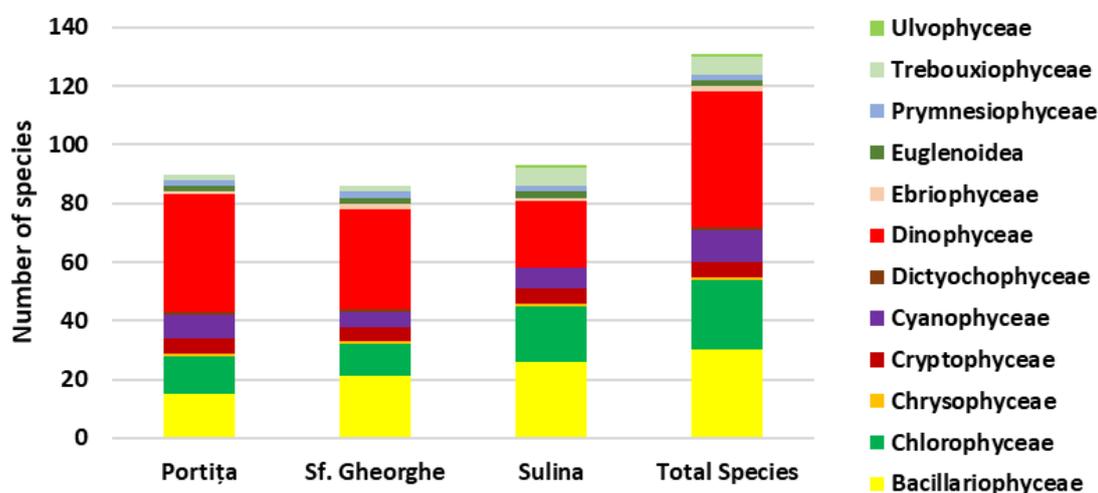


Figure 3.8 - Taxonomic composition of phytoplankton - Danube's mouths, May 2019

Comparing the biodiversity on the three transects under the influence of the Danube, we observed that, even though it was not a significant difference in the total number of species, the proportion of freshwater species was higher on Sulina (42 %) than on other transects (26-27 %) (Figure 3.9).

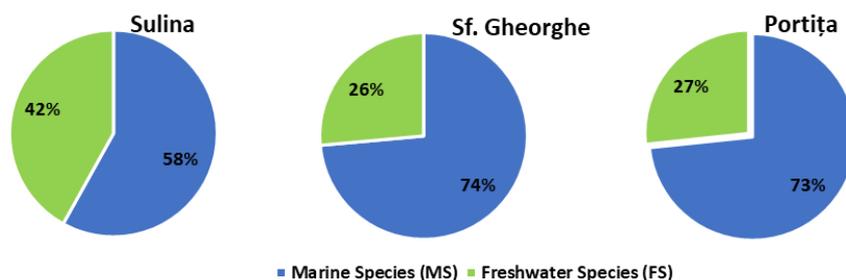


Figure 3.9 - The proportion of marine and freshwater - Danube's mouths, May 2019

The phytoplankton was composed of 84 marine and 47 freshwater species (Annex Cfannex). Numerous species of dinoflagellates (46) and diatoms (24) dominated the marine phytoplankton. Most of the dinoflagellates' species were found at Portița (40), their number dropping to half (23) at Sulina, one of the Danube's mouths. Alternatively, most of the marine diatoms' species were found at Sulina (20), and their number dropped at Portița (13). Chlorophytes with the highest diversity at Sulina (19) represented half of the total species number of freshwater phytoplankton. Their number dropped at

Sf. Gheorghe (11) and Portița (13). The freshwater phytoplankton community was represented as well by species from Cyanophyceae (8), Trebouxiophyceae (6) and Bacillariophyceae (7) classes (Figure 3.10).

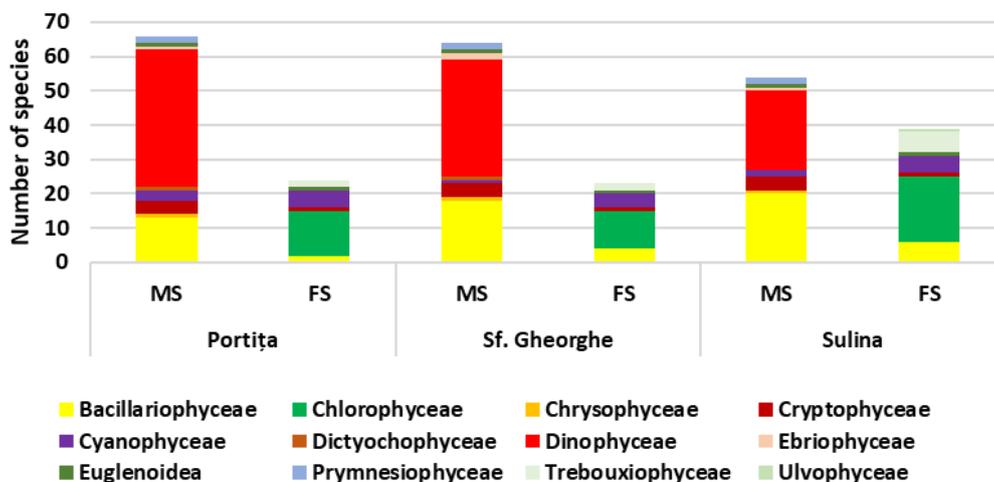


Figure 3.10 - Distribution of marine and freshwater phytoplankton species - Danube's mouths, May 2019

The total abundance of phytoplankton varied between  $4.48 \cdot 10^3$  cells/L and  $3.80 \cdot 10^6$  cells/L and the total biomass, between  $1 \text{ mg/m}^3$  and  $2367 \text{ mg/m}^3$  (Figure 3.11). The development of the phytoplankton showed a common distribution with maximum values ( $2.72 \cdot 10^6$ - $3.80 \cdot 10^6$  cells/L and  $1729$ - $2367 \text{ mg/m}^3$ ) in the surface layers (0-10 m) and minimum ( $4.48 \cdot 10^3$  - $17.92 \cdot 10^3$  cells/L and  $1$ - $44 \text{ mg/m}^3$ ) in the near-bottom layers (50-60 m).

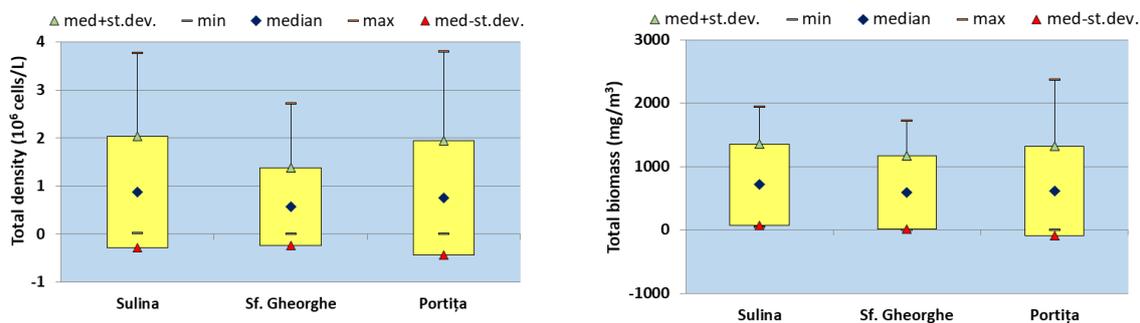
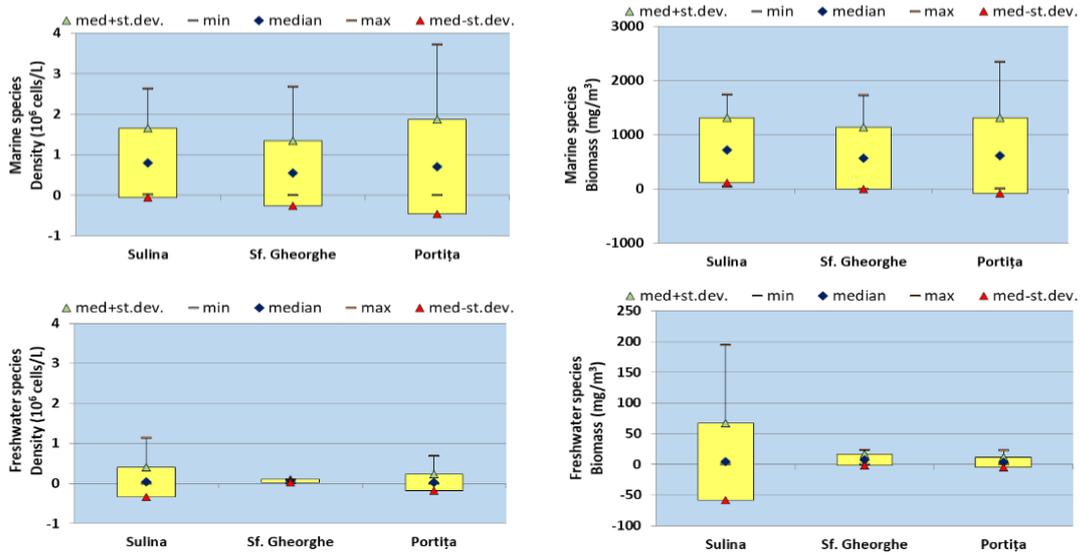


Figure 3.11 - Spatial variation of total phytoplankton abundance and biomass - Danube's mouths, May 2019

On Sulina and Sf. Gheorghe transects, under direct Danube's influence, the maximum density of marine phytoplankton was up to  $2.62 \cdot 10^6$  cells/L, respectively,  $2.67 \cdot 10^6$  cells/L, the maximum biomass was  $1700 \text{ mg/m}^3$  on both transects. On Portița transect, at a distance from the Danube direct discharge, the maximum values were with  $1 \cdot 10^6$  cells/L and  $600 \text{ mg/m}^3$ .

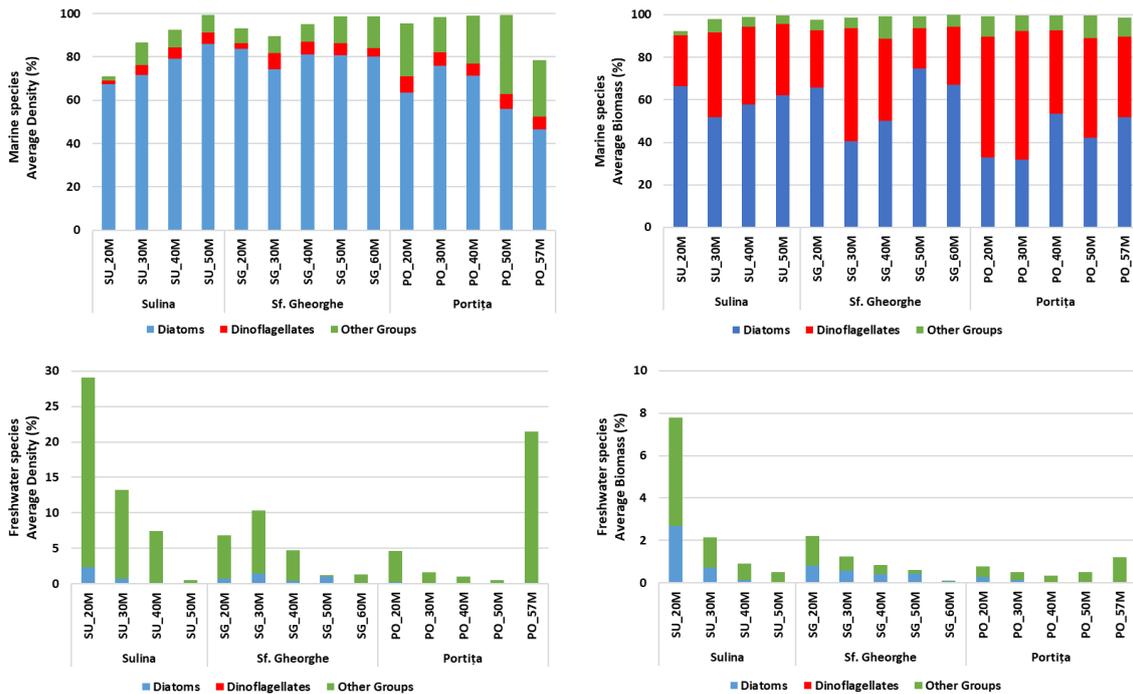


**Figure 3.12 - Spatial variation of marine and freshwater species total abundance and biomass - Danube's mouths, May 2019**

The freshwater phytoplankton quantities were up to  $1.15 \cdot 10^6$  cells/L and  $195 \text{ mg/m}^3$ , and both recorded on the Sulina profile. On Sf. Gheorghe and Portița transects, the maximum values recorded were even lower,  $107.80 \cdot 10^3$  cells/L, respectively,  $688 \cdot 10^3$  cells/L and  $24 \text{ mg/m}^3$  on both transects (Figure 3.12).

The average abundance of phytoplankton varied between  $463.10 \cdot 10^3$  cells/L and  $1.97 \cdot 10^6$  cells/L, and the average biomass - between  $385 \text{ mg/m}^3$  and  $1256 \text{ mg/m}^3$ . The marine species average abundance and biomass were up to  $1.40 \cdot 10^6$  cells/L, respectively,  $1158 \text{ mg/m}^3$ , representing between 71 % and 99 % of the total average density and 92-100 % of the total average biomass (Figure 3.13). Marine diatoms were the most important group that contributed to both the average density and biomass with 46-86 %, respectively, 32-75 % of the total. The dinoflagellates, mostly marine species, had a lower contribution in density (2-8 %) but reached 61 % of the total average biomass on Portița 30m station. The Other groups' species represented between 2 % and 37 % in average density and between 2 % and 10 % in average biomass. The freshwater species average abundance and biomass were up to  $573.76 \cdot 10^3$  cells/L, respectively,  $98 \text{ mg/m}^3$ , representing between 1 % and 29 % of the total average density and up to 8 % of the total average biomass. Species from other groups, such as chlorophytes, cyanophytes, trebouxiphytes and ulvophytes, mainly represented freshwater phytoplankton, together contributing up to 27 % of the total average density (Sulina 20 m) and 5 % of the total average biomass. The freshwater diatoms represented about 2-3 % of the total average density and biomass.

The chlorophyll *a* concentration varied between  $0.07 \text{ } \mu\text{g/L}$  and  $23.81 \text{ } \mu\text{g/L}$ . The highest concentration was recorded in the surface layer, at Sulina 20m, which corresponded to the development of the diatom *Skeletonema subsalsum* ( $2.30 \cdot 10^6$  cells/L and  $690.55 \text{ mg/m}^3$ ) and a group of freshwater species from Chlorophyceae and Cyanophyceae classes ( $1.15 \cdot 10^6$  cells/L and  $195 \text{ mg/m}^3$ ). The values gradually decreased to  $0.13$ - $6.17 \text{ } \mu\text{g/L}$  with depth and distance from the shore.



**Figure 3.13 - Phytoplankton taxonomic structure based on marine and freshwater average abundance and biomass at Danube's mouths, May 2019**

On Sf. Gheorghe profile, concentrations of 7-8  $\mu\text{g/L}$  were in the surface layer and decreased to 1-3  $\mu\text{g/L}$  at 20-30 m depth and below 1  $\mu\text{g/L}$  at 40-50 m depth.

The chlorophyll *a* concentration on the Portița profile was between 0.07  $\mu\text{g/L}$  and 2.61  $\mu\text{g/L}$ , the maximum value is recorded on the 30 m station, in the surface layer. In the deeper layers, the chlorophyll *a* concentration was below 1  $\mu\text{g/L}$  (Figure 3.14).

Bloom densities were recorded in 93 % (13/14) stations with values between  $1.07 \cdot 10^6$  cells/L and  $3.80 \cdot 10^6$  cells/L. *Chaetoceros curvisetus*, *Skeletonema subsalsum*, and *Pseudo-nitzschia delicatissima* mainly produced blooms by recording each over 1 million cells/L in six stations. Several species from other classes developed together with diatoms in the other seven stations and exceeded 1 million cells/L (Table 3.1).

*Pseudo-nitzschia delicatissima* is a marine diatom geographically widely distributed, producing domoic acid in cultures. Domoic acid may be a worldwide threat on temperate coasts, at least. However, action can be taken to alleviate the potential danger by monitoring the phytoplankton and utilizing temporary closings of selected fisheries in target areas when necessary for the duration of a bloom (Carmelo, 1997). *P. delicatissima* is listed as harmful in `IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae` (Moestrup et al., 2009). *P. delicatissima* was observed in four of these stations in densities between  $1.08 \cdot 10^6$  cells/L and  $1.81 \cdot 10^6$  cells/L, representing 47-63 % of the total. Also, *P. delicatissima* contributed with 2-60 % of the total density in other stations that recorded bloom densities. *P. delicatissima* blooms exclusively emerged at a distance from the Danube direct discharge and influence (Figure 3.15).

*Skeletonema subsalsum* is a brackish water diatom with usually extremely short linking structures, which lengths vary with salinity. *S. subsalsum* was observed in two of these stations in densities between  $1.35 \cdot 10^6$  cells/L and  $2.30 \cdot 10^6$  cells/L, representing between 49 % and 61 % of the total. *S. subsalsum* contributed 0.75-50 % of the total density in 9 from 13 stations with bloom densities. *S. subsalsum* is a fresh to brackish water diatom, occurring in salinity up to 15 PSU in rivers, lakes, inland seas, coastal waters, and marshes, and often associated with eutrophic conditions (Kipp et al., 2020). *S. subsalsum* proliferated in front of Sulina arm where salinity was the lowest and waters silicate-rich.

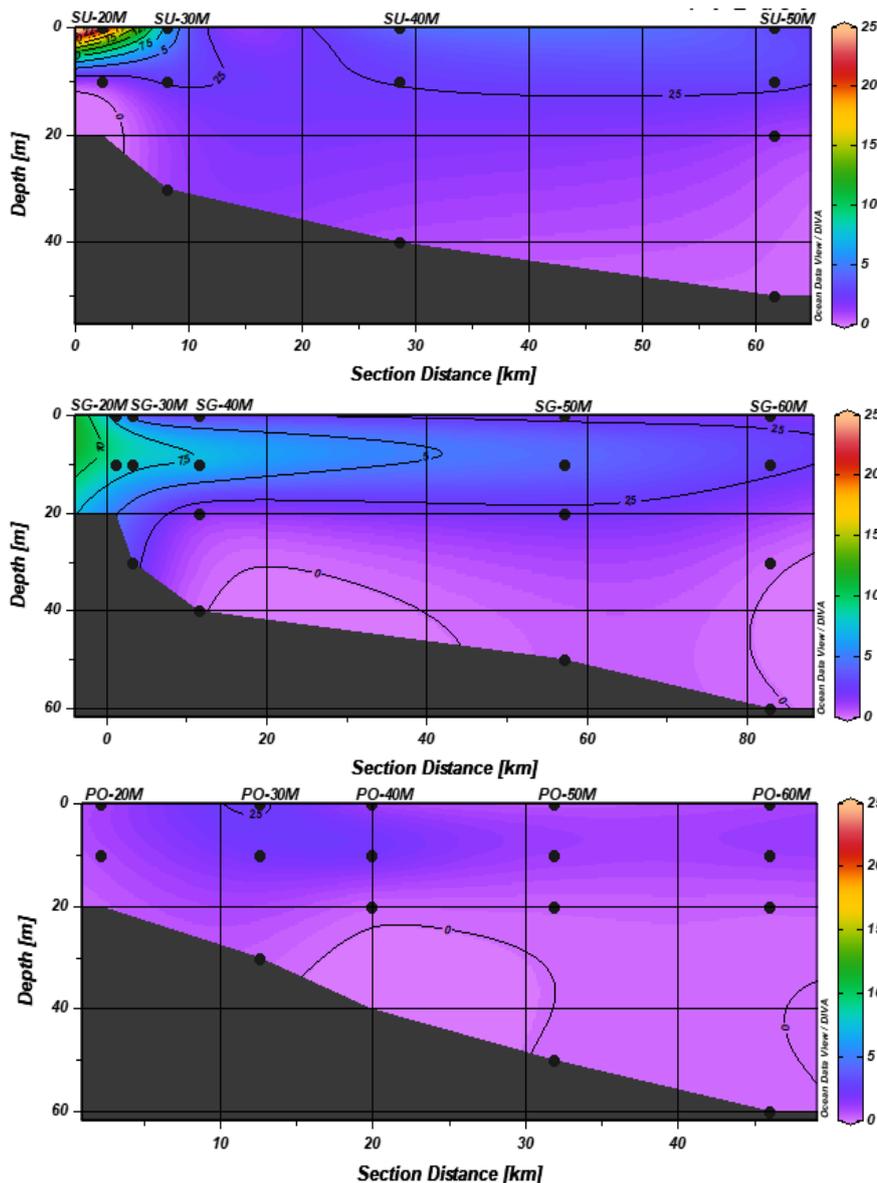


Figure 3.14 - Water column distribution of chlorophyll a concentration - Danube's mouths, May 2019

Another diatom only observed in one station with a density above 1 million cells/L was *Chaetoceros curvisetus* ( $1.13 \cdot 10^6$  cells/L), representing 42 % of the total. *C. curvisetus* is known to have a high nitrate and phosphate removal capacity (Karthikeyan et al., 2013). *C. curvisetus* contributed with up to 44 % of all stations that recorded bloom densities. The centric diatom *Chaetoceros sp.* is harmful algae though it does not secrete any harmful toxins. The diatom's setae are easily broken, and if large quantities lodge in the gills of a fish, they may kill the fish. The secondary spines anchor the setae to the sensitive gill tissue causing irritation, and the fish react by producing mucus. Eventually, it dies from suffocation. Fish mortality due to this species is reported from aquaculture farms and pen cultures (Begum et al., 2015).

These three diatoms were accompanied by other species that together reached bloom densities, such as *Emiliania huxleyi* (representing up to 36 %, being present in 12 stations), *Pseudanabaena limnetica* (representing between 1 % and 41 %, in 7 stations) and *Komma caudata* (up to 9 %, in 13 stations).

Table 3.1 - Stations with bloom densities at Danube's mouths, May 2019

Nr. Station Crt.	Sample Depth (m)	<i>Pseudo-nitzschia delicatissima</i>	<i>Chaetoceros curvisetus</i>	<i>Skeletonema subsalsum</i>	<i>Emiliania huxleyi</i>	<i>Pseudanabaena limnetica</i>	<i>Komma caudata</i>	Other species	Total Abundance (10 <sup>3</sup> cells/L)
1 SU-20M	0	●	●	●	●	●	●	●	3778
2 SU-30M	0	●	●	●	●	●	●	●	2772
	10	●	●	●	●	●	●	●	1071
3 SU-50M	0	●	●	●	●	●	●	●	1420
	10	●	●	●	●	●	●	●	1207
4 SG-20M	10	●	●	●	●	●	●	●	1514
5 SG-30M	0	●	●	●	●	●	●	●	1232
6 SG-40M	0	●	●	●	●	●	●	●	1929
	10	●	●	●	●	●	●	●	1705
7 SG-50M	10	●	●	●	●	●	●	●	1825
8 SG-60M	10	●	●	●	●	●	●	●	2718
9 PO-20M	0	●	●	●	●	●	●	●	1321
10 PO-30M	0	●	●	●	●	●	●	●	3801
11 PO-40M	0	●	●	●	●	●	●	●	1577
12 PO-50M	0	●	●	●	●	●	●	●	2340
	10	●	●	●	●	●	●	●	1327
13 PO-57M	0	●	●	●	●	●	●	●	3164
	10	●	●	●	●	●	●	●	1594



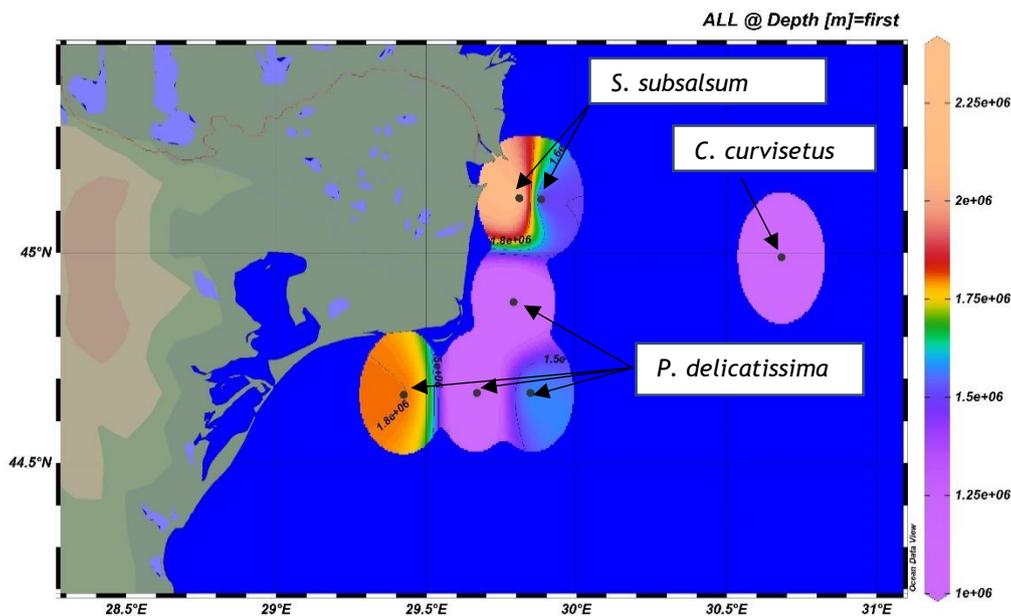


Figure 3.15 - Distribution of the main bloom species along Danube's mouths

## Conclusions

Qualitatively, we identified 64 % marine and 36 % freshwater phytoplankton species. The freshwater species were found more on Sulina profile, under the Danube's direct influence, than on the other transects. Marine species represented between 71 % and 99 % of the total average density and 92 % - 100 % of the total average biomass. Marine diatoms were the most important group, contributing to both the average density and biomass with 46 % - 86 %, respectively, 32 % - 75 % of the total. Other classes such as chlorophytes, cyanophytes, trebouxiophytes and ulvophytes mainly represented the freshwater phytoplankton, which together contributed up to 27 % of the total average density and only 5 % of the total average biomass.

The chlorophyll *a* concentration varied between 0.07 µg/L and 23.81 µg/L. The values gradually decreased with depth and distance from the shore.

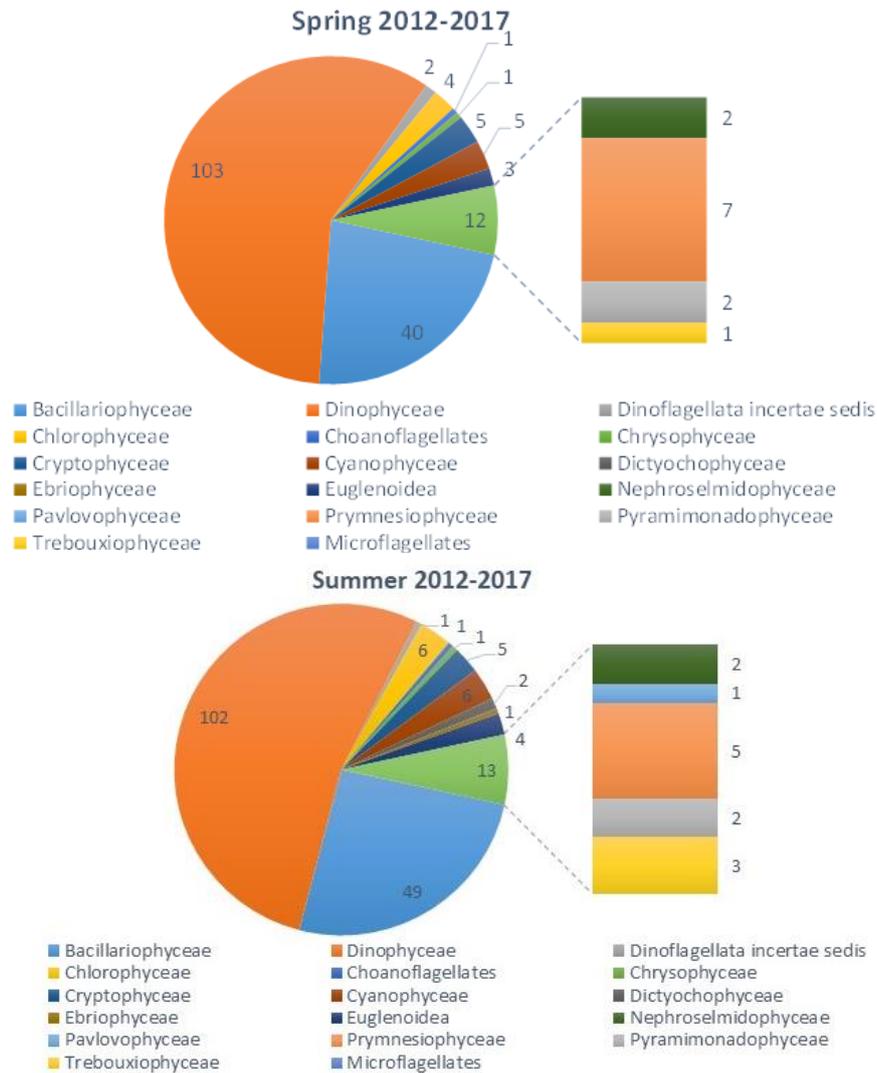
Bloom densities were recorded in 13 from 14 sampling stations with values between  $1.07 \cdot 10^6$  cells/L and  $3.80 \cdot 10^6$  cells/L. *Chaetoceros curvisetus*, *Skeletonema subsalsum* and *Nitzschia delicatissima*, each recording over 1 million cells/L, mainly produced blooms in six of these stations. Several species from Other groups developed together with the diatoms and exceeded 1 million cells/L in the other seven stations.

The river discharge provided an optimal environment for both brackish and marine species. Most phytoplankton species have a broad spectrum of tolerance to differences in salinity and a high capacity to use the Danube's nutrients by creating blooms along the area.

## Kamchia River

The effect of Kamchia River on the ecological status of coastal Black Sea ecosystem is investigated based on a spring-summer seasonal dataset of short time series (2012-2019) to represent the level of discharge impacts low in the food chain comparing two scenarios of dry (2013, 2019) and wet (2014, 2016) years.

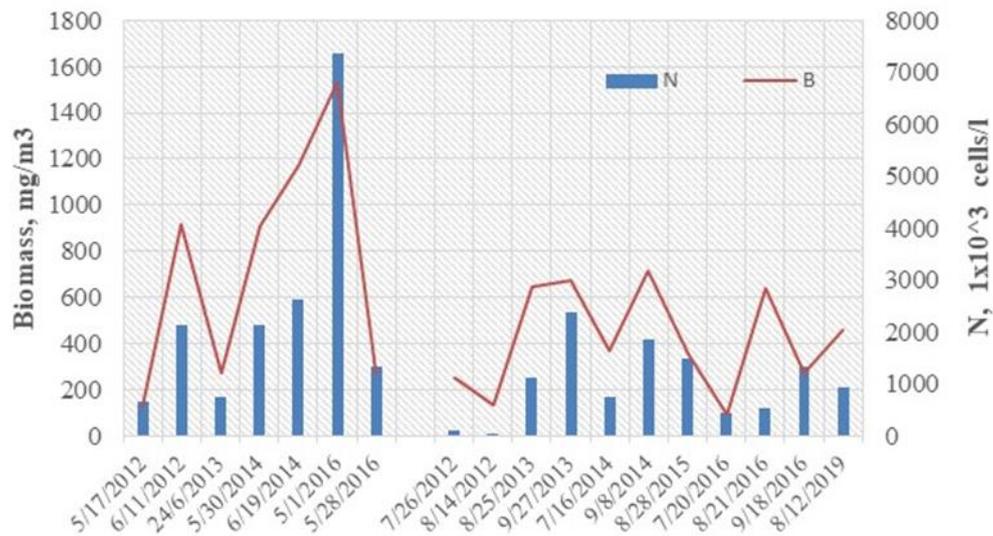
During spring-summer (2012-2019), the phytoplankton community of Kamchia water body was featured by high species richness and taxonomic diversity - the number of species fluctuating between 176 and 191 and varieties from 17 classes, including a great number of unidentified microflagellates. In summer 2019 (August), the number of species was also very high - 130 species from 17 classes (Figure 3.16).



**Figure 3.16 - Taxonomic structure of phytoplankton communities in the Kamchia river Water body in spring and summer, 2012-2017**

The highest species diversity was observed among dinoflagellates (102-103 in the spring-summer 6 years period and 83 species in August 2019), followed by diatoms represented by about two times low species richness, especially in 2019 (19 species). The remaining groups were represented by a far low number of species (between 1-7) but frequently dominating the structure of the phytoplankton communities such as *Emiliana huxleyi* (Prymnesiophyceae), *Pyramimonas* sp. (Pyramimonadophyceae), Cryptophyceae (*Hemiselmis* sp., *Plagioselmis* sp.), *Nephroselmis astigmatica* (Nephroselmidophyceae).

The total numerical metrics varied in wide ranges depending on the environmental conditions (season, dry-wet scenarios and hydrodynamics), for the abundance between  $662.9 \cdot 10^3$  cells/L and  $7359 \cdot 10^3$  cells/L in spring and between  $52.1 \cdot 10^3$  cells/L and  $2364 \cdot 10^3$  cells/L in summer and the biomass between  $127.52 \text{ mg/m}^3$  and  $1534.09 \text{ mg/m}^3$  in spring and  $92.60 \text{ mg/m}^3$  and  $717.04 \text{ mg/m}^3$  in summer, respectively (Figure 3.17). Similarly, the chlorophyll *a* concentration greatly fluctuated, between  $0.9 \text{ mg/m}^3$  and  $6.6 \text{ mg/m}^3$  in spring and  $0.2 \text{ mg/m}^3$  and  $3.4 \text{ mg/m}^3$  in summer.



**Figure 3.17 - Variation of phytoplankton - Total Abundance (cells/L) and Biomass (mg/m<sup>3</sup>) in Kamchia water body, (spring-summer 2012-2019)**

The average spring abundance was 2.4 times higher than that in summer ( $2.4 \cdot 10^6 \pm 2.3 \cdot 10^6$  cells/L versus  $0.99 \cdot 10^6 \pm 0.7 \cdot 10^6$  cells/L), the biomass about 1.7 times ( $741.85 \pm 530$  mg/m<sup>3</sup> versus  $431.33 \pm 230$  mg/m<sup>3</sup>) and chlorophyll *a* about two times ( $2.6 \pm 2.1$  mg/m<sup>3</sup> versus  $1.3 \pm 0.9$  mg/m<sup>3</sup>). The difference between the wet-dry scenario was much higher, about ten times in abundance and more than five times in the biomass (Figure 3.18 b).

Like the total abundance and total biomass, a specific feature in the dynamic of the taxonomic profile of phytoplankton assemblages was the high variability, exhibiting alternating community structure, which makes it challenging to extract distinct trends, typical for estuarine/marine coastal mixing zones (Raateoja, Kauppila, 2019). A relatively sensible presence of the freshwater/brackish species *Pyramimonas* sp. (class Pyramimonadophyceae) and microflagellates both in spring (up to 17.1-34.5 %) and clearer in summer (up to 34.1-40.5 %, respectively) is noted over the entire period, in terms of total abundance, with a minor contribution in the total biomass due to their small size.

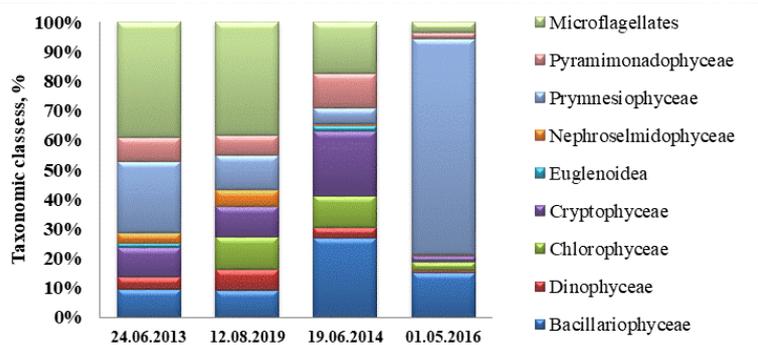
The rainy period (2014) was marked by an increased proportion (~ 34%) of freshwater/brackish species from the classes Chlorophyceae (*Monoraphidium contortum*), Cryptophyceae (*Hemiselmis* sp., *Plagioselmis* sp.) and Euglenoidea (*Euglena acusformis*), concomitant with an elevated share of diatoms (61.8 % of the total abundance). The maximum diatom abundance ( $1.3 \cdot 10^6$  cells/L) and spring biomass ( $466.941$  mg/m<sup>3</sup>) observed in May 2014 were contributed specifically by the indicator species of eutrophication *Pseudo-nitzschia delicatissima*, *Cyclotella choctawhatcheana*, *Thalassiosira nordenskiöldii*. For comparison, these proportions for the selected dry scenarios were below 18 % for the three classes and between 8.0-8.4 % ( $61 \cdot 10^3$ - $63 \cdot 10^3$  cells/L) for diatoms at the much lower total abundance and biomass. The dinoflagellates biomass peak ( $515.04$ - $529.63$  mg/m<sup>3</sup>) was also measured in 2014 (the rainy year) in June and September after high diatom development.

As riverine waters change nutrient loads, so does the stoichiometry of the coastal waters, resulting in changes of N:P supply and consequent variations in algal communities (Glibert, Burkholder, 2011), increased algal growth and development of high biomass, as well as shifts in species diversity could be observed. Usually, the nitrate in this source water exists at sub-Redfield ratios and hence is quickly drawn down by enhanced growth of coastal diatoms that benefit from the DIP and Si-rich water (Stukel et al., 2014). For example, a highly dense and mixed bloom of diatoms comprising primarily *Skeletonema* sp. *Pseudo-nitzschia* spp., *Thalassiosira* sp., and *Chaetoceros* spp. was reported to occupy the low salinity core of the plume fuelled by riverine derived silicate and cross-shore and upward flux of DIN into the surface layers (Gomes et al., 2018) and similar to our results nanoplankton such as Cryptophytes, Prasinophytes and Haptophytes were also observed in large numbers in low salinity plume waters. As many dinoflagellates are mixotrophic, it is possible that this mixing of trophic levels allows them to exist and adapt to all situations.

Even if the frequency of total densities exceeding  $1 \cdot 10^6$  cells/L was high (>55 % of the sampling cases) no monospecific or a 2-3 species cohort outbursts were observed except the bloom of *Emiliania huxleyi* in September 2013 (abundance  $1.3 \cdot 10^6$  cells/L) and especially in May-June 2016, in this case

originating from the northwest as a regional bloom and controlled by the basin-wide current patterns (Figure 3.18 a). It was a particular case in which the bloom density was locally sustained to reach a cell abundance of  $5.2 \cdot 10^6$  cells/L (Figure 3.18 b), more than three times higher than that in the northern Bulgarian coastal WBs.

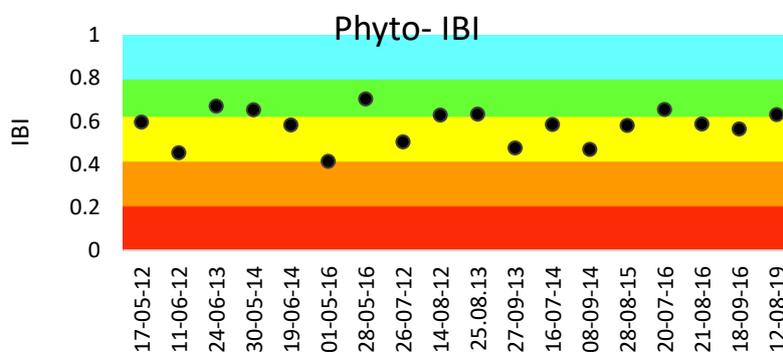
Accordingly, based on the phytoplankton-integrated index (Phyto-IBI), the ecological status of Kamchia WB associated with the latter event was in category poor (Figure 3.18 c). During spring-summer 2012-2019, the Phyto-IBI predominantly fluctuated within the categories moderate-good; however, no consistent trend towards achieving good ecological status was observed, most likely modulated by the impact of the Kamchia river as shown by the Generalised Additive Mixed Model (GAMM) approximations (Figure 3.19, Figure 3.20).



(a)

Metric/Date	24.06.2013	19.06.2014	01.05.2016	12.08.2019
N, cells/l	731752	2622784	7359550	790376
B, mg/m <sup>3</sup>	270.403	1169.515	1534.086	455.500

(b)



(c)

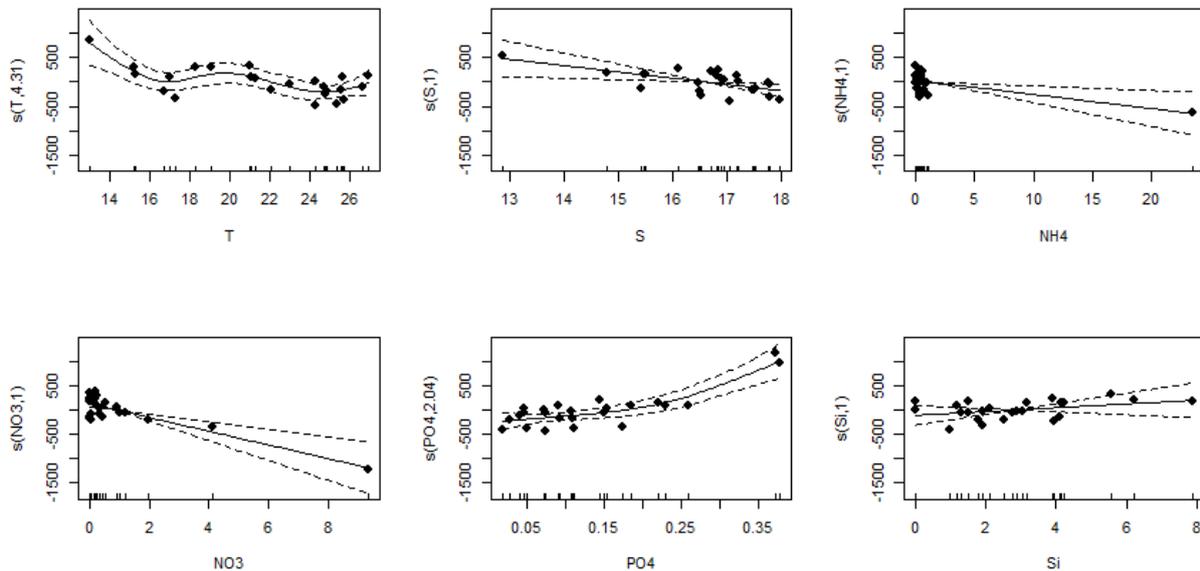
**Figure 3.18 - Taxonomic profile of phytoplankton communities abundance (in %) under the three different scenarios (a) dry scenario (24.06.2013 and 12.08.2019), wet scenario (19.06.2014) wet scenario + regional bloom (01.05.2016); (b) Total abundance, N (cells/l) and Biomass, B (mg/m<sup>3</sup>) and (c) Variation of the ecological status of WB Kamchia based on the integrated phytoplankton index Phyto-IBI (spring-summer 2012-2019); colour-codes correspond to the WFD classification system**

GAMM was applied to model phytoplankton biomass and the integrated index Phyto-IBI as an alternative to GAM (due to limitations in data availability observations,  $n=24$ ) as a flexible instrument to detect nonlinearities in data and its robustness and flexibility in terms of distributional assumptions. The basic GAMM model was used without implementation of correlation structure - as data shows no autocorrelations in and between the data series (max. lag=0.4); therefore,

autocorrelation is not accounted in the model; nevertheless, standardised residuals are checked for autocorrelation to ensure that the models result are not misinterpreted.

Phytoplankton biomass was modelled by the implementation of the following GAMM:  $\text{PhytoplanktonB} = \alpha + f_1(T) + f_2(S) + f_3(\text{NH}_4^+) + f_4(\text{NO}_3^-) + f_5(\text{PO}_4) + f_6(\text{Si})$ .

The model outcome (Figure 3.19 and Table 3.2) clearly shows statistically significant linear effects of temperature, salinity, and ammonia and nonlinear effects of nitrate and phosphates concentrations. The overall effect on the response (phytoplankton biomass) is rather complex, influenced by multiple factors.



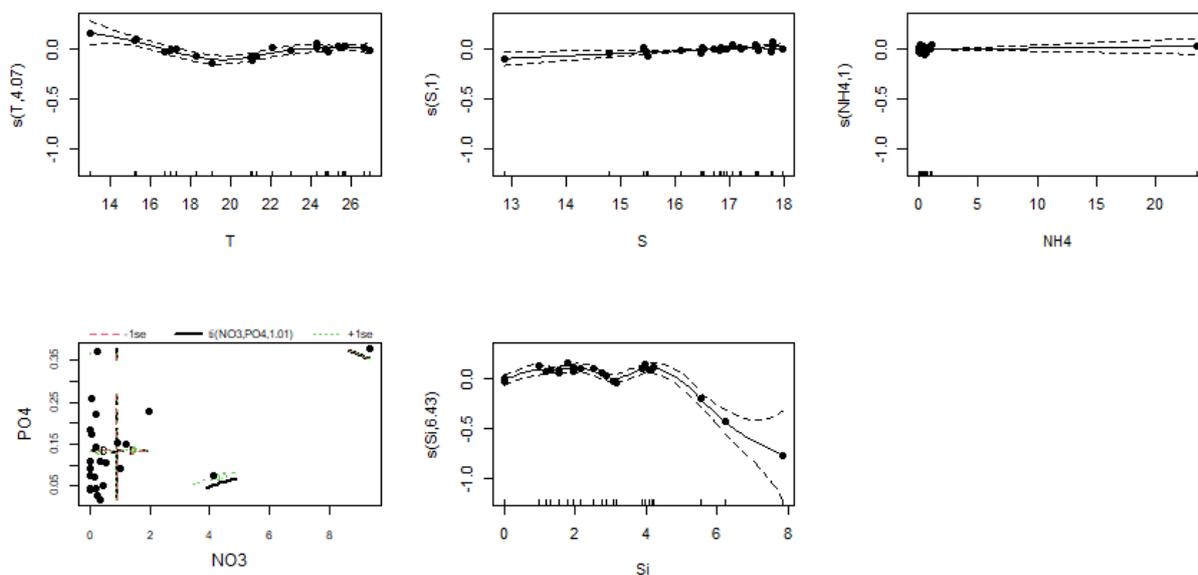
**Figure 3.19 - GAMM terms fit showing the effect of various environmental variables on phytoplankton biomass. Locations of observations are shown as vertical lines on the x-axes; the dots represent the observations partial residuals. Solid lines are the estimates of the smooths; the dashed lines indicate 95% confidence intervals; in situ data - T(temperature, °C), S (salinity, PSU), NH<sub>4</sub> ( ammonium, μM), NO<sub>3</sub> (nitrate, μM), PO<sub>4</sub> (phosphate, μM) and SiO<sub>4</sub> (silicate, μM)**

**Table 3.2 - GAMM ANOVA results on the effect of various environmental variables on phytoplankton biomass**

Family: Gaussian, Link function: identity, Formula: $\text{PhytoB} \sim s(T) + s(S) + s(\text{NH}_4) + s(\text{NO}_3) + s(\text{PO}_4) + s(\text{Si})$					
Parametric coefficients:					
	Estimate	Std. Error	t value	Pr(>  t )	
(Intercept)	585.38	40.95	14.3	3.51e-09	***
Approximate significance of smooth terms:					
	edf	Ref.df	F	p-value	
s(T)	4.306	4.306	4.997	0.012738	*
s(S)	1	1	6.764	0.021979	*
s(NH4)	1	1	8.597	0.011671	*
s(NO3)	1	1	19.985	0.000634	***
s(PO4)	2.044	2.044	17.362	0.000205	***
s(Si)	1	1	1.226	0.288354	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
R-sq.(adj) = 0.744, Scale est. = 38561 n = 24					

IBI GAMM terms have been selected following the underlying ecology concept, i.e. the expectations that the eutrophication drives low Phyto-IBI values associated with "poor" ecological status; hence a two-way interactions effects (included in the model as tensor product) were studied aiming at assessment of their potential effect on the response of the integrated index. The model results

(Figure 3.20 and Table 3.3) highlighted the statistically significant combined linear effect of nitrate and phosphate concentrations and salinity and nonlinear effect of temperature and ammonia.



**Figure 3.20 - GAMM terms fit showing the effect of various environmental variables on IBI. Locations of observations are shown as vertical lines on the x-axes; the dots represent the observations partial residuals. Solid lines are the estimates of the smooths; the dashed lines indicate 95% confidence intervals; in situ data - T (temperature, °C), S (salinity, PSU), NH<sub>4</sub> (ammonium, μM), NO<sub>3</sub> (nitrate, μM), PO<sub>4</sub> (phosphate, μM) and SiO<sub>4</sub> (silicate, μM)**

**Table 3.3 - GAMM ANOVA results on the effect of various environmental variables on IBI**

Family: Gaussian, Link function: identity, Formula: IBI ~ s(T) + s(S) + s(NH4) + ti(NO3, PO4) + s(Si)					
Parametric coefficients:					
Estimate Std. Error t value Pr(> t )					
(Intercept) 0.56191 0.01178 47.7 1.29e-12 ***					
Approximate significance of smooth terms:					
	edf	Ref.df	F	p-value	
s(T)	4.075	4.075	7.144	0.008299	**
s(S)	1	1	7.237	0.024788	*
s(NH4)	1	1	0.598	0.459343	
ti(NO3,PO4)	1.012	1.012	11.803	0.007343	**
s(Si)	6.43	6.43	18.026	0.000156	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
R-sq.(adj) = 0.892, Scale est. = 0.0010186 n = 24					

Other studies (reviewed in Masotti et al., 2018) have also reported a positive relationship between increased river discharge, nutrient input, and high phytoplankton biomass in adjacent coastal areas to river mouths. For example, in coastal waters off Japan, chlorophyll *a* concentration from ocean colour satellite estimates, was two times higher during periods of high river discharge than periods of low discharge. Similarly, high chlorophyll *a* level was observed in winter and early spring, coinciding with an abrupt precipitation increase in northwestern Florida's Apalachicola River. Other runoff-related changes have also noted that variation in salinity, silica, and total nitrogen were the main driver of phytoplankton community structure and productivity (Barroso et al., 2016), with further potential implications to the ecological status of the coastal marine environment (Masotti et al., 2018).

Although the Kamchia River's water discharge is low compared to the big rivers discharging into the NW Black Sea, its influence on coastal water quality should not be ignored. Small rivers (i.e., rivers with small drainage basins and small annual discharges) affect adjacent coastal waters to a limited extent under average climatic conditions, while under certain climatic conditions, their cumulative

discharge can increase in response to precipitation events and heavy rains (Mertes, Warrick, 2001; Saldias et al., 2016, Osadchiev, Korshenko, 2017). Our data shows that the rainfall regime along the Bulgarian coast during the last decade manifests high year to year variability but is maintained above the average (1960-1990) in over 70%. A remarkable for Bulgaria rainfall, particularly in the research area, significantly exceeding the mean norm (approx. 10 folds) was reported in spring-summer 2014 (Drenovski, Kastreva, 2017), with maximum extremes measured in June (211mm - 458 %). Subsequently, an increase in the river discharge rate was measured in the Kamchia watershed ( $Q_{max}=200 \text{ m}^3/\text{s}$ ), resulting in salinity lower than 13PSU in the coastal area's mixing zone. The precipitation in 2016 (spring, about four times higher than the norm) was considerably lower than in 2014, but similar environmental changes were observed.

## Conclusions

Our results suggest that the high river runoff (wet season) enhance the biological activity near the river mouth (increased total phytoplankton abundance, biomass and chlorophyll *a*), sustaining higher local bloom densities in case of regional blooms (the case of *E.huxleyi* bloom), causing alteration of phytoplankton taxonomic structure.

What is noteworthy is the marked interannual variability in the wet-dry conditions and consequent phytoplankton community structure, modulated by the extent of the Kamchia River and its inputs.

Apart from the peak flow events, the River Kamchia impact is traceable throughout the inner coastal area only, located mainly in the one-mile coastal zone, as documented by previous studies (Truhchev et al. 2010, Shtereva et al., 2010a).

## Sakarya River and Yesilirmak River

In July 2019, a total of 62 species were identified from six taxonomic classes. The bulk of the species pool was composed of Dinoflagellates - 42 species, 17 genera (68 % of the total number) among which the genus *Protooperidinium* (13 species), *Prorocentrum* and *Dinophysis* (5 species) were the most diverse. Among diatoms (17 species, 11 genera), genus *Chaetoceros* (4 species) and genus *Coscinodiscus* showed the highest species richness. A few species belonging to the classes Prymnesiophyceae and Dictyochophyceae have been identified (Figure 3.21).

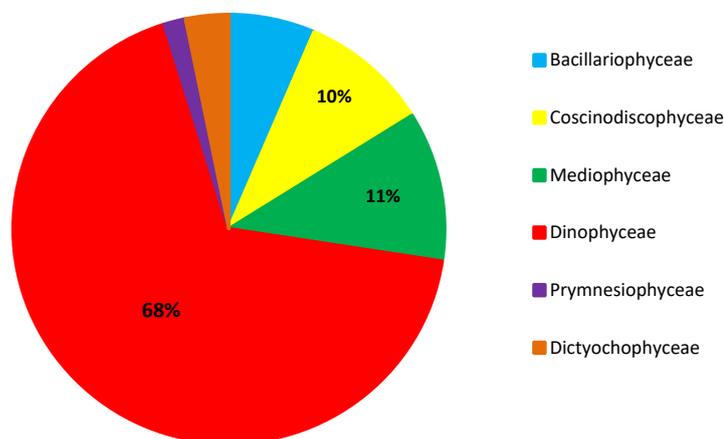


Figure 3.21-Proportional distribution of phytoplankton classes, July 2019

Species belonging to 11 classes were recorded in January 2020 (Figure 3.22). A total of 63 species were determined in which Bacillariophyceae was represented by 5 genera 5 species; Coscinodiscophyceae by 4 genera and 6 species; Mediophyceae by 6 genera and 14 species; Dinophyceae by 12 genera and 29 species; Dictyochophyceae by 2 genera and 3 species and the other classes by 1 genera and 1 species. From the total number of species, 46 % were represented by dinoflagellates, 39 % by diatoms and the remaining 13 % by other classes. The bulk of the species pool was composed of Dinoflagellates among which the genus *Protooperidinium* (8 species), *Tripos* (5 species) *Dinophysis* (4 species) and *Prorocentrum* (3 species) were the most diverse. Among diatoms,

*Chaetoceros* (8 species) showed the highest species richness. A few species belong to the classes Prymnesiophyceae, Cyanophyceae, Cryptophyceae, Dictyochophyceae, Noctilucophyceae, Trebouxiophyceae and Thecofilosea have been identified.

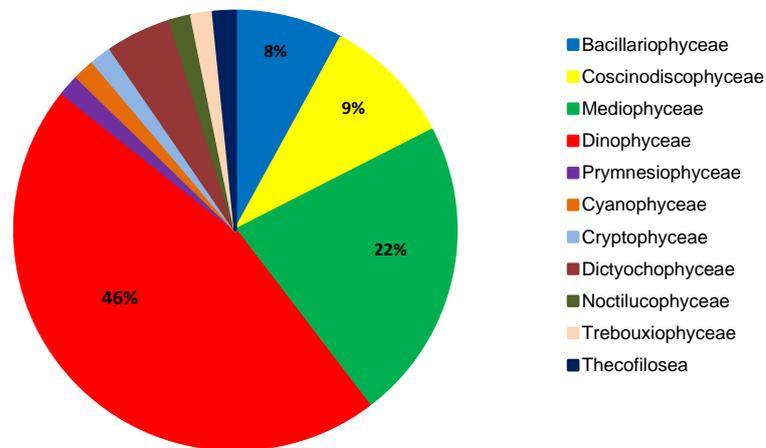


Figure 3.22 - Proportional distribution of phytoplankton classes, January 2020

In July 2019, the average total abundance of phytoplankton for the entire area varied between  $8.8 \cdot 10^3$  cells/L and  $7.1 \cdot 10^4$  cells/L and the average biomass between 59.35 mg/m<sup>3</sup> and 345.92 mg/m<sup>3</sup>. Dinoflagellates dominated 89 % of the total phytoplankton biomass sampled from all depths of the stations. 55 % of the total phytoplankton abundance obtained from all sampling stations' depths was dominated by dinoflagellates (Figure 3.23). The highest abundance value was detected at the station named SAK07. We observed that the members of *Emiliana huxleyi* were dominant in this sampling station. Also, this species was shown to be dominant in sampling stations SAK08 and YSL10. However, it was found that the diatom abundance values in this period are low (Figure 3.24).

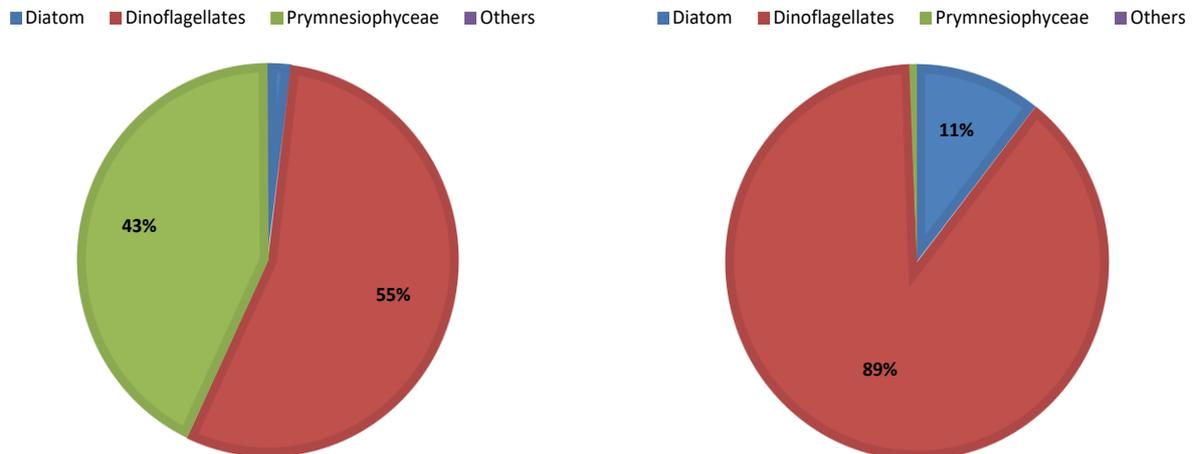


Figure 3.23 - Distribution (%) of average total abundance (left) and average total biomass (%) proportion of phytoplankton groups, July 2019

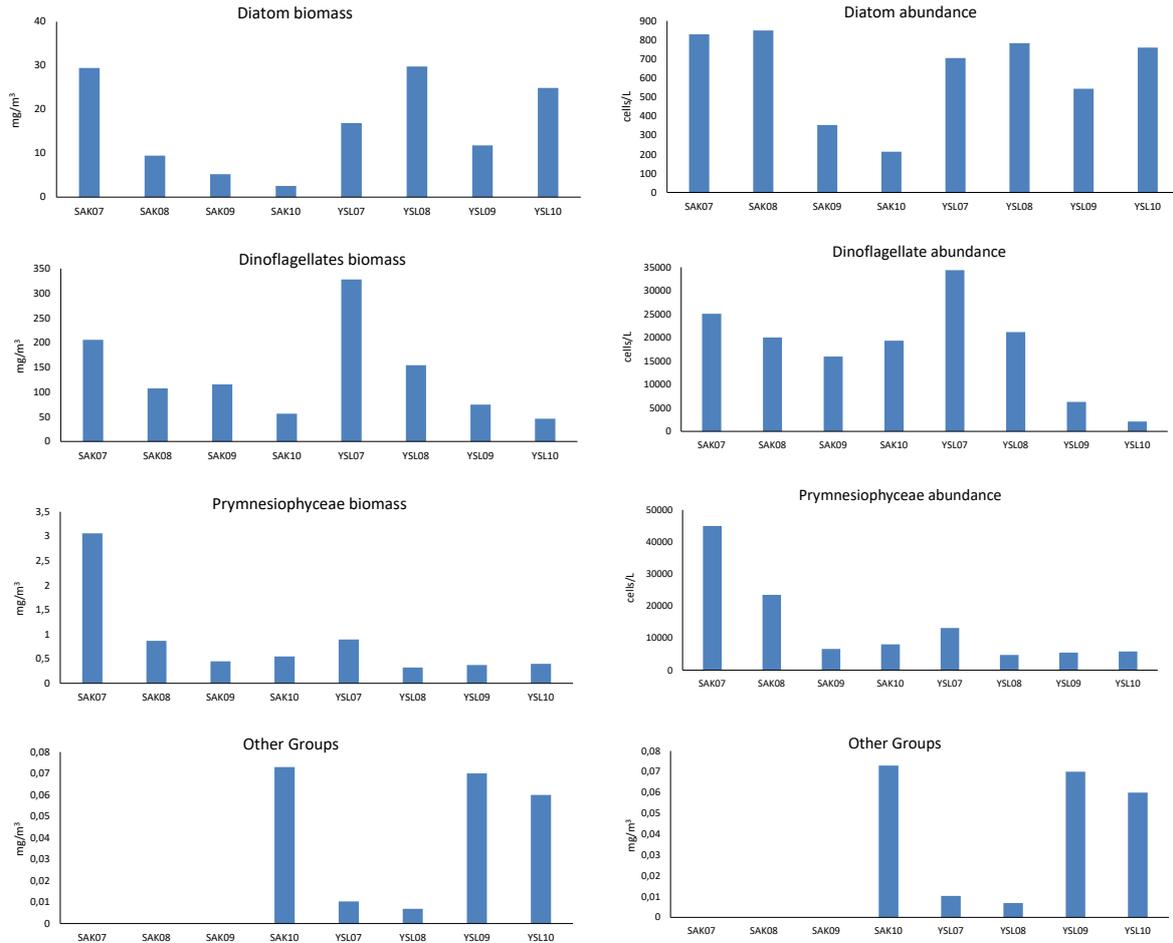


Figure 3.24 - Total biomass and abundance values of phytoplankton groups, July 2019

In January 2020, the average total abundance of phytoplankton for the entire area varied between  $7.4 \cdot 10^3$  cells/L and  $4.3 \cdot 10^4$  cells/L and the average biomass between 44.35 mg/m<sup>3</sup> and 509.2 mg/m<sup>3</sup>. The highest value of phytoplankton abundance was at the station YSL07. Also, *Oscillatoria* sp. were dominant in stations SAK07 and SAK08. However, dinoflagellate species dominated in other stations. They dominated 43 % of the total phytoplankton abundance of all depths of the sampling stations. The biomass was similar to the stations' abundance. It was computed that dinoflagellates dominated the biomass values except for stations SAK07 and SAK08. Dinoflagellates dominated 83 % of the total phytoplankton biomass sampled from all stations' depths (Figure 3.25, Figure 3.26).

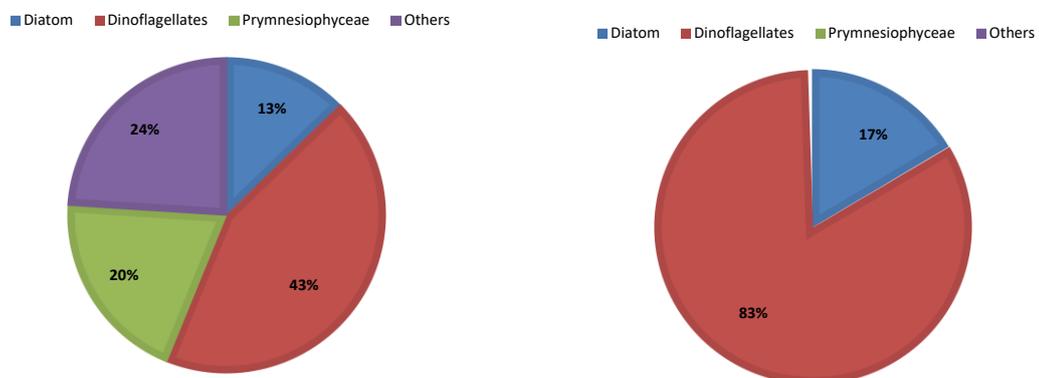


Figure 3.25 - Distribution (%) of average total abundance (left) and average total biomass (%) proportion of phytoplankton groups, January 2020

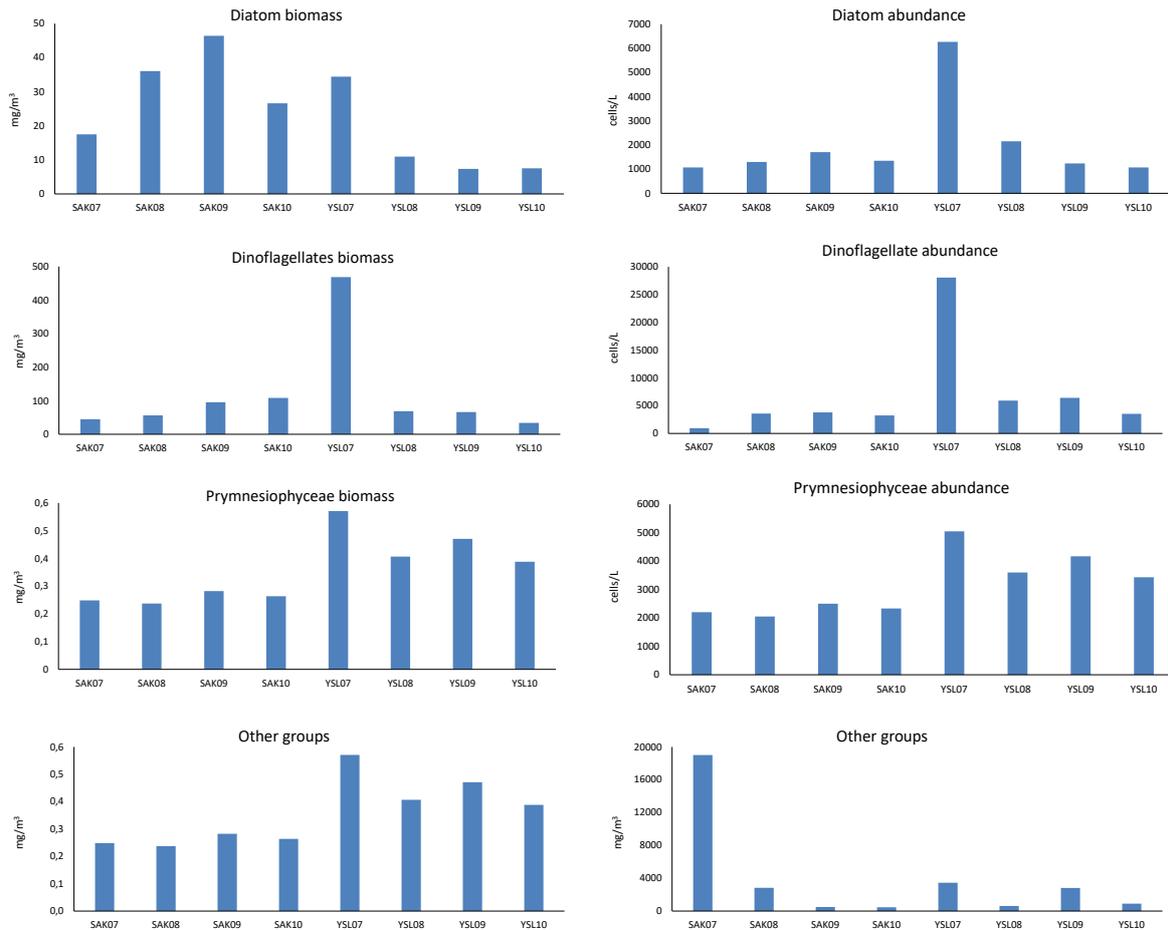


Figure 3.26 - Total biomass and abundance values of phytoplankton groups, January 2020

## Conclusions

Nutrients (N, P) transported to the Black Sea through rivers have increased steadily through the years. Because large dams that have been built to control the flow of rivers reduce the silicate load carried to the sea by the rivers, the Si/N ratio in the surface layer of the Black Sea has decreased drastically and the abundance of diatoms that consume silicate has reduced significantly and dramatic changes have occurred in the distribution of dominant species (Dekos, 2014). The dominance of dinoflagellates detected in the study area during the sampling periods has confirmed this situation.

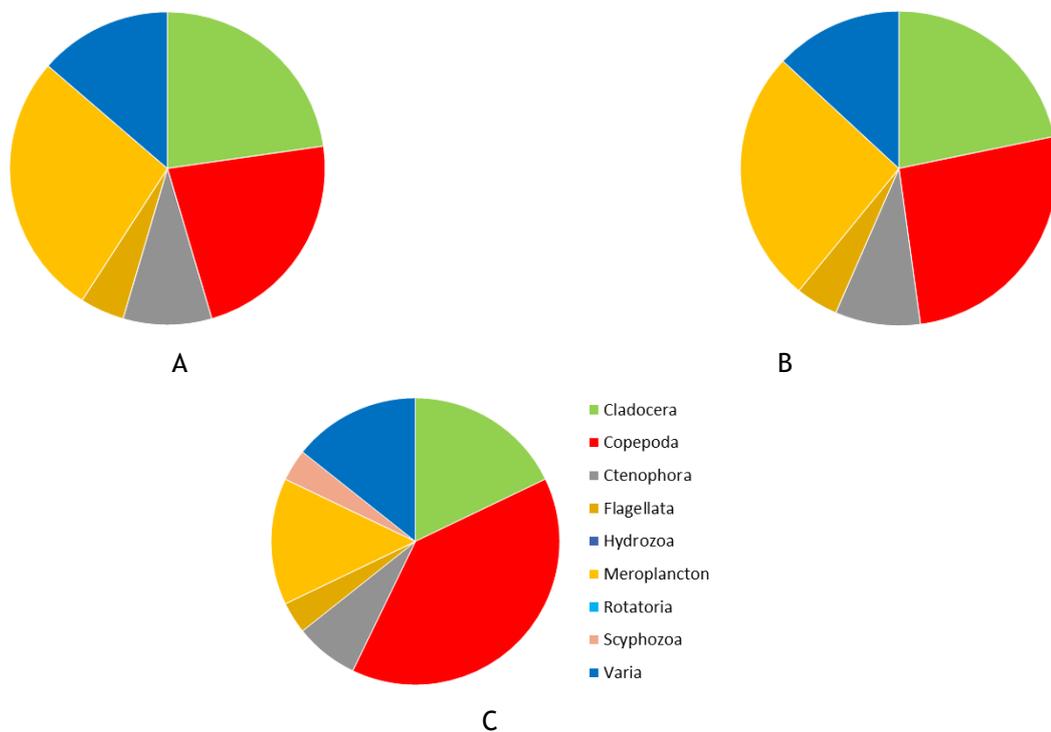
### 3.1.2 Zooplankton

Zooplankton is an essential part of marine ecosystems. It is an essential link in the food chain. Additionally, due to the sensitivity of zooplankton organisms to environmental changes, the zooplankton community's state and structure may indicate the ecosystem's state. The Marine Strategy Framework Directive (Directive 2008/56/EU) of the European Union defines zooplankton as an essential component of assessing water bodies' ecological state. Various marine zooplankton indicators can provide valuable information on ecological processes important for coastal countries' quality of life and economies. The state of zooplankton and its structural characteristics are of particular interest because, in contrast to short-cycle phytoplankton, which reflects mainly momentary changes, and long-cycle macrozoobenthos, which has a large inertia, zooplankton is the only one reflecting the state of the environment in the medium term.

## Dnieper River, southern Bug River, Dniester River and Danube (UA) River

For this report, we used the ANEMONE project materials and national monitoring performed by UkrSCES. During the shelf studies, we observed four areas of NWBS: the part of Zernov Phyllophora field that belonged to Mixed Waters region (ShW\_UA\_5) influenced by Dnipro-Bug and Dniester waters, surroundings of Zmejinyj island, which belonged to Danube region (ShW\_UA\_1), Odessa dumping region, which belonged to Dnipro-Bug region (ShW\_UA\_3) and the central NWBS (Central Waters region, ShW\_UA\_7). For the study of coastal waters, we observed marine waters near the Danube mouth (Kiliya and Bystryj arm), near Zatoka (Dniester estuary area), and near Ochakov (Dnipro-Buh estuary area) with riverine waters of Dnipro and Buh near their mouths.

In the coastal zone of the NWBS, we identified 49 taxa. The high taxonomic diversity was due to the presence of brackish and freshwater species. The species composition was based on Copepoda (15) and Cladocera (12); Rotifera (6) was also diverse in freshened areas. Nine taxa represented the meroplankton organisms. Non-forage zooplankton consisted of jellyfish (2) and flagellates (1). The rest of the taxa (2) did not significantly contribute to total species diversity (Figure 3.27).



**Figure 3.27 - Taxonomic structure of the zooplankton community in the coastal waters of the Dnieper-Bug region (A), Dniester region (B) and Danube region (C), 2019**

In the coastal area, the Shannon-Weaver index varied from 1.40 bit/ind in the Dnieper-Bug estuary to 2.18 bit/ind in the Dniester estuary zone and averaged  $1.79 \pm 0.26$  bit/ind. Shannon-Weaver index's higher values are a usual situation because of the greater biodiversity due to freshwater and brackish species' contribution. In the Dnieper-Bug region's coastal zone, it averaged  $1.72 \pm 0.18$  bit/ind, in the Dniester region,  $1.75 \pm 0.61$  bit/ind and in the Danube  $1.89 \pm 0.35$  bit/ind (Figure 3.28).

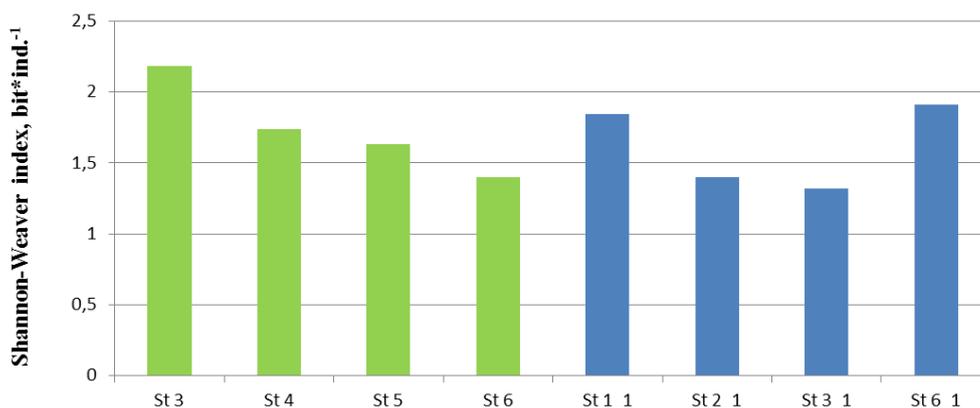


Figure 3.28 - Shannon-Weaver index by zooplankton abundance in the coastal waters (■ - September, ■ - July)

In the coastal zone, the abundance varied from 7788 ind/m<sup>3</sup> near the town of Ochakov to 149 000 ind/m<sup>3</sup> in the Dnieper estuary mouth (Dnieper-Bug region). On average, in the study region, it was 43161±41333 ind/m<sup>3</sup>. On average for the regions, it was 58714 ± 42240 ind/m<sup>3</sup> in the Dnieper-Bug region, 18295 ± 9383 ind/m<sup>3</sup> in the Dniester region and 18699 ± 16269 ind/m<sup>3</sup> in the Danube region. The biomass varied from 43.80 mg/m<sup>3</sup> near the city of Ochakov to 9611.246 mg/m<sup>3</sup> in the Dnieper estuary. On average, in the research area, it was 1156.62 ± 2694.60 mg/m<sup>3</sup>. The average biomass in the regions was 1807.36 ± 3478.35 mg/m<sup>3</sup> in the Dnieper-Bug region, 122.46 ± 41.65 mg/m<sup>3</sup> in the Dniester region and 178.14 ± 227.67 mg/m<sup>3</sup> in the Danube region.

Depending on the level of salinity, freshwater, brackish, or marine species dominated the samples. Thus, in the Dnieper-Bug estuary, near the town of Ochakov, the copepod *Acartia tonsa*, the larvae of Cirripedia and rotifers of the genus *Brachionus* dominated. In the Dnieper-Bug estuary, near the Dnieper's mouth, *Eudiaptomus gracilis* and the genus *Acanthocyclops* were dominant among the copepods; among the cladocerans, the genus *Bosmina*; rotifers of the genera *Asplanchna* and *Brachionus* were also numerous. However, the marine complex dominated.

Riverine transport is the primary mechanism for the direct impact of onshore human activities on the coastal marine environment. The negative impacts of excess nutrients and sediments carried by rivers into coastal waters have been well studied worldwide and are gaining the immediate attention of conservationists and resource managers. Sediments and nutrients are commonly found in freshwater runoff, although the two loads' dynamics and impacts are not the same. Nutrients and other dissolved matters are transported farther than sediments, so a freshwater plume containing dissolved nutrients can wrap around a smaller plume of sediment (Fredston et al., 2016). The addition of nutrients to coastal and marine ecosystems leads to eutrophication that led to a sharp increase in the number of phytoplankton up to the water bloom, which can provoke the formation of oxygen-free zones and death of hydrobionts from hypoxia. Also, during eutrophication, the proportion of short-cycle species, which include jelly species, increases. They eat up the food supply and compete with valuable commercial fish. Eutrophication also changes marine algal communities, providing a competitive advantage for some species of macroalgae. Loading of nutrients and sediment can also lead to increased turbidity and decreased availability of light, which in turn affects species such as benthic macroalgae. Sediment can also physically strangle sensitive habitats and impair fish larval development (Fredston et al., 2016).

In July and September 2019, the river runoff was relatively low, and the share of freshwater species in the diversity of zooplankton did not exceed 7%. The only exceptions are the samples taken in the Dnieper-Bug estuary, where the contribution of freshwater and brackish water species was 50%-70% of the total diversity, and the mouth of the Dniester estuary, where in summer it was 26% (Figure 3.29).

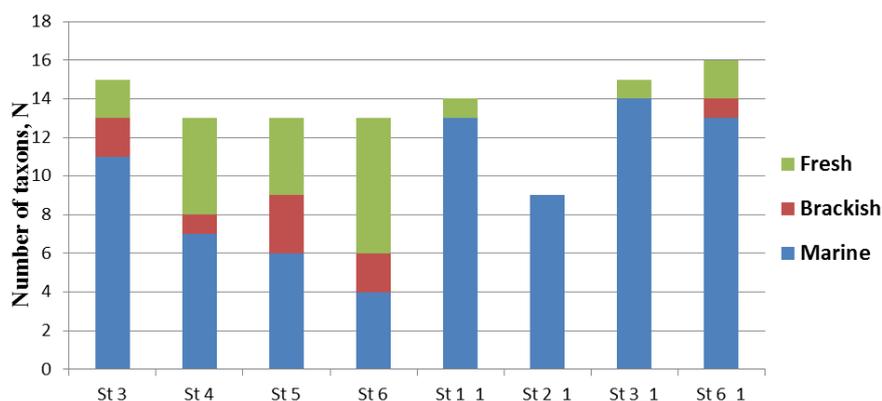


Figure 3.29 - The distribution of salinity preferences of the zooplankton species at the coastal waters

Freshwater zooplankton is represented mainly by rotifers of the genera *Brachionus* (4), *Filinia* (1), *Asplanchna* (1), copepods *Eudiaptomus gracilis* and *Acanthocyclops vernalis*, cladocerans of the genera *Bosmina*, *Daphnia*, *Diaphanasoma*, *Moina*. Brackish-water zooplankton are mainly represented by the cyclopoids *Halycyclops negletus* and *Calanipeda aquae-dulcis* and various cladocerans (*Cercopagis pengoi*, *Cornigerius maeoticus*, *Leptodora kindtii*). Interestingly, certain species of brackish-water organisms were registered only at certain stations. Thus, the cyclopoid *H. negletus* was recorded only around the Dniester estuary, and brackish-water cladocerans were recorded only in the Dnieper-Bug estuary.

The contribution of brackish and freshwater organisms to the abundance and biomass of zooplankton in the coastal zone was higher, but still, they do not constitute the majority at most stations. The only exceptions are the Dnieper-Bug estuary, where the contribution to the abundance reached 78 % and to biomass up to 91 % (Figure 3.30), and the Dniester estuary's mouth (2 % and 20 %, respectively).

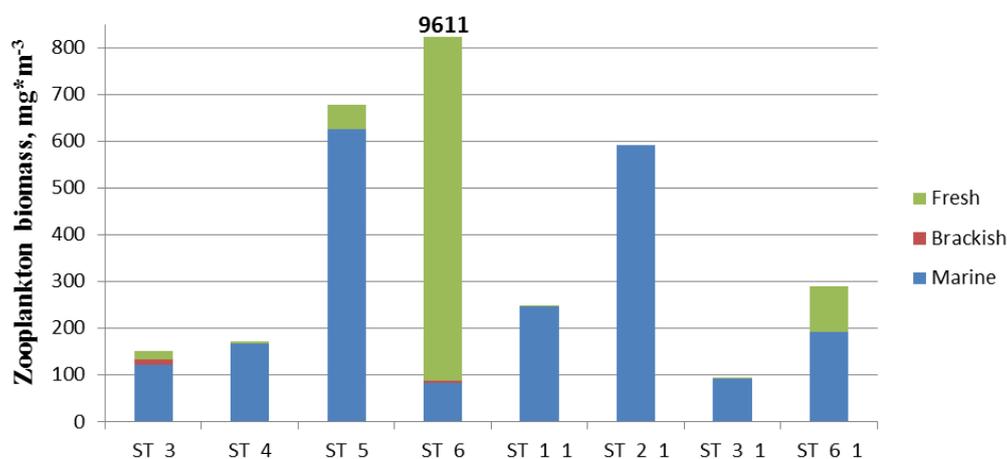


Figure 3.30 - The contribution of the species with different salinity preferences to zooplankton's total biomass in the coastal waters

There is no significant Pearson correlation between biomass and salinity ( $r=-0.19$ ), biomass and pH ( $r=-0.38$ ), biomass and nitrogen-containing compounds ( $r=-0.28$ ), phosphorus-containing compounds ( $r=-0.46$ ) on coastal stations.

We used several quantitative characteristics to assess the environment's quality by zooplankton indicators - the total biomass of zooplankton, the Shannon-Weaver diversity index (by abundance), the share of copepods (by biomass), and the proportion of *Noctiluca scintillans* in the biomass. We have developed threshold values for these indicators for all major regions of the N and NW Black Sea (Table 3.4).

A five-point scale is used to assess the ecological status of coastal waters according to the WFD. Most coastal stations in 2019 had high biomass. The high biomass may indicate an increase in the level of anthropogenic eutrophication. However, at all stations, a low level of development of *N. scintillans* was observed. It should be noted that *N. scintillans* is a marine species and is rare and scarce in

significantly freshened regions. It indicates the need to develop new assessment methods, which would be more suitable for use in estuarine zones and freshened areas of the sea. There was high biodiversity by the Shannon-Weaver index at all stations, and at almost all stations, there was a high proportion of copepods, which indicated a good ecological state. Generally, in summer, the Dniester region's ecological status was assessed as "Good", and the Dniester-Bug region as "Moderate". In autumn, the Dniester-Bug region's state was assessed as "Moderate", and for Dniester and Danube areas, as "Good".

**Table 3.4 - Water quality assessment of the coastal waters by the zooplankton metrics, 2019**

River influence	Metrics				General assess
	Total biomass mg/m <sup>3</sup>	<i>N.scintillans</i> , %	Shannon index, bit/ind. <sup>1</sup>	Copepoda %	
July					
Dnieper-Bug (ShW_UA_7)	3487.04 ±5309.7	0	38.24 ±43.98	1.59±0.17	Moderate
Dniester (ShW_UA_2)	151.909 ±151.909	0	49.08 ±49.08	2.18±2.18	Good
September					
Dnieper-Bug (ShW_UA_3)	547.599 ±682.054	0.01±0.02	37.77 ±16.73	1.82±0.11	Moderate
Dniester (ShW_UA_2)	93.009 ±93.009	0±0	74.45 ±74.45	1.32±1.32	Good
Danube_Bistroe (ShW_UA_1)	327.677 ±234.537	0.91±1.58	49.83 ±37.5	1.8±0.38	Good

During the study, we identified 49 zooplankton taxa of marine, brackish-water and freshwater complexes. Copepoda was the basis of species diversity in all observation regions; Cladocera and Rotatoria were also diverse.

High abundance and biomass were in coastal waters. Depending on the level of salinity, existed both freshwater, brackish-water and marine species. Thus, in the Dnieper-Bug estuary, near the town of Ochakov, the copepod *Acartia tonsa*, larvae of Cirripedia, and rotifers of the genus *Brachionus* dominated. In the Dnieper-Bug estuary, near the Dnieper's mouth, among the copepods, *Eudiaptomus gracilis* and genus *Acanthocyclops* were dominant, among cladocerans, the genus *Bosmina*, among rotifers, the genera *Asplanchna* and *Brachionus* were also numerous. However, the marine complex mainly dominated.

The influence of rivers on marine ecosystems is mainly expressed in the supply of freshwater with large quantities of nutrients and freshwater species to the sea. However, we did not find a clear correlation between salinity and zooplankton biomass. Freshwater species also do not have a significant contribution to marine zooplankton communities. However, we observed that the closer to the river estuary zone, the increased abundance of the biomass. Changes in quantitative characteristics may be explained by the fact that smaller organisms have a shorter life cycle, which is a sign of increased eutrophication. However, for a more accurate assessment, further research is needed.

It is noteworthy that this is not strictly related to the overall productivity of the stations. When calculated the ratio of biomass to abundance, we saw that biomass was decreasing faster, which indicates an increase in the proportion of small species in the community.

Certain species of brackish-water organisms were registered only at certain stations. Thus, the cyclopoid *H. negletus* was recorded only in the Dniester estuary and brackish-water cladocerans (*Cercopagis pengoi*, *Cornigerius maeoticus*, *Leptodora kindtii*) were recorded only in the Dnieper-Bug estuary.

Most coastal stations in 2019 had high biomass. A very low level of development of *N. scintillans* was also observed at all stations. However, it should be noted that *N. scintillans* is a marine species and is rare and scarce in significantly freshened regions. This indicates the need to develop new assessment methods that would be more suitable for use in estuarine zones and freshened areas of the sea. At all stations was a high diversity according to the Shannon-Weaver index.

## Conclusion

At almost all stations, was a high proportion of copepods, which indicates a good ecological state. In general, in summer, the Dniester and Dnieper-Bug regions' ecological status may be assessed as "Good". In the fall, the Dnieper-Bug region's state may be assessed as "Moderate", Dniester and Danube - as "Good".

## Danube River (RO)

### Microzooplankton communities

In the Romanian Black Sea waters were identified a total number of 13 species of tintinnids, apart from the four families. The highest species richness was on the Sf. Gheorghe profile (12), while on the Portița profile, was recorded the lowest (9) (Annex C). Regarding the vertical distribution of species richness was observed that in the surface layer was registered a higher diversity (between 9-11 species) than in the 10 m layer (between 5-7 species) on all three investigated profiles (Figure 3.31).

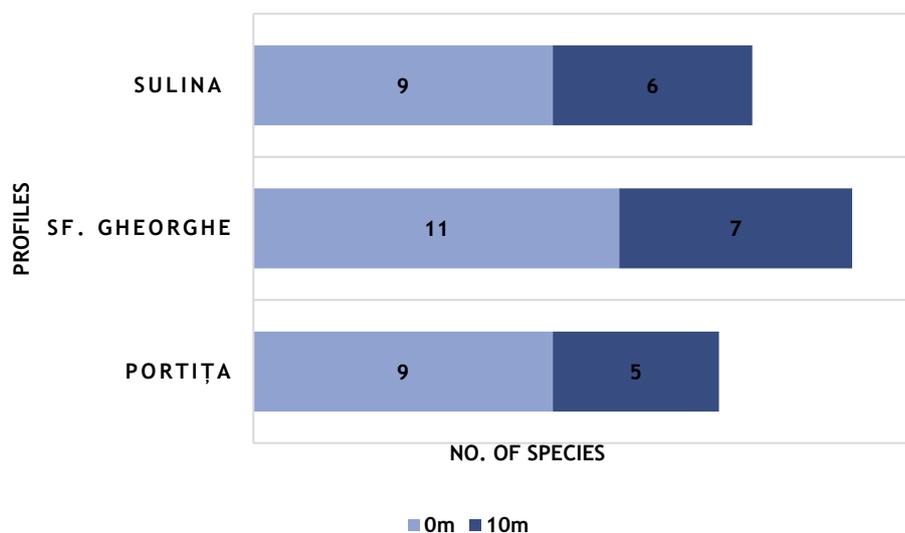
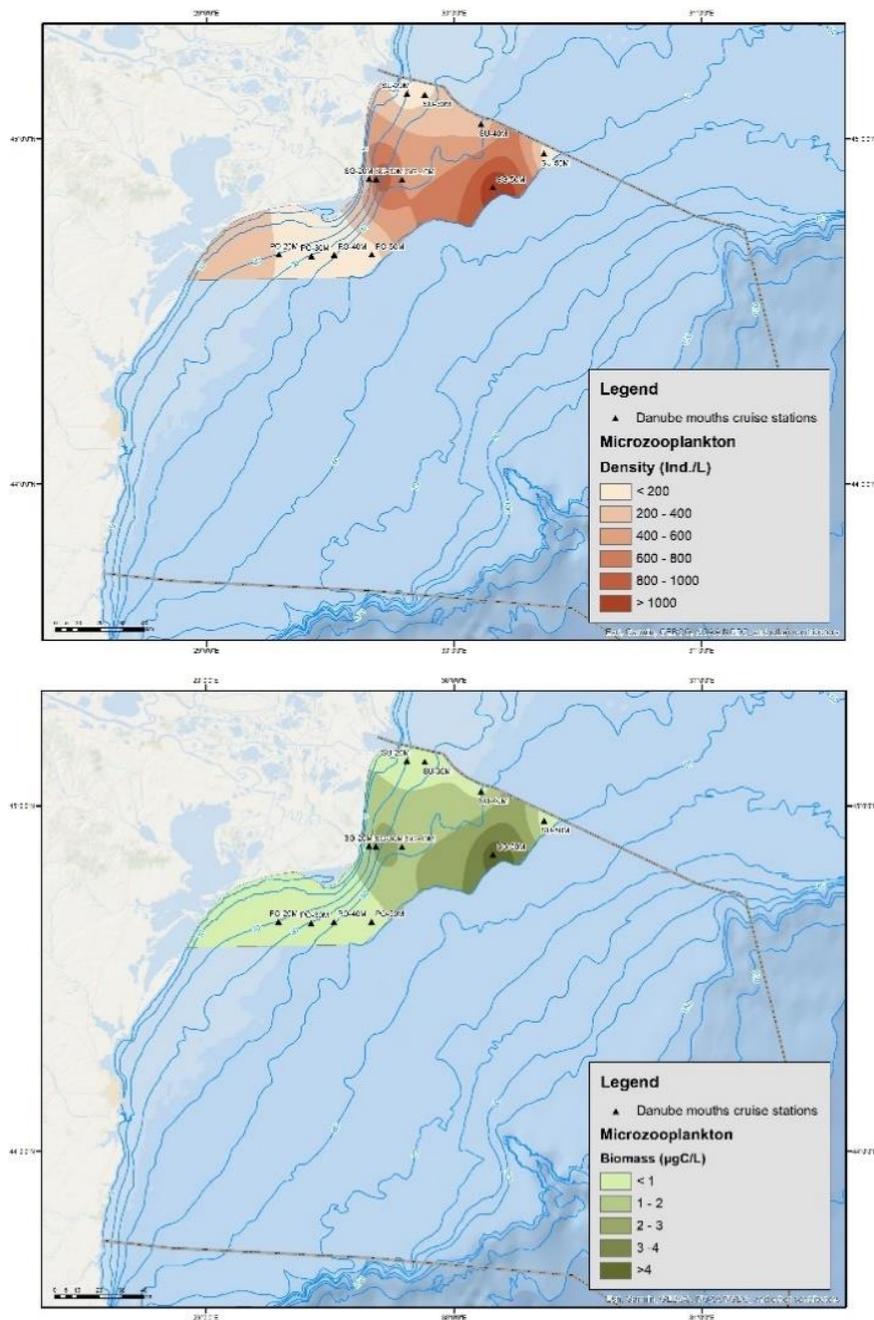


Figure 3.31 - The diversity of tintinnids in the Danube's mouths area - Romanian Black Sea, May 2019

Regarding the species distribution, we can note that from the total of 13 identified species, only 4 (*Tintinnopsis baltica*, *T. beroidea*, *T. karajacensis* and *Stenosemella ventricosa*) are present in all layers of the three investigated profiles, all being characteristic species for the recorded temperatures. The species *Codonella cratera* was present only in 10 m layer, while the species *Tintinnopsis compressa*, *T. lobiancoi*, *T. urnula*, *Tintinnidium mucicola* and *Metacylis mediterranea* were present exclusively in the surface layer. *Tintinnidium mucicola* is the species present only on the Sulina profile. *Tintinnopsis campanula* and *Metacylis mediterranea* were found only on the profiles of Sf. Gheorghe and Portita missing from the Sulina profile. *Codonella cratera* was found only on the profiles of Sulina and Sf. Gheorghe missing from the Portita profile.

The highest abundance and biomass of tintinnids from the Sulina profile were recorded in SU-40m station (238 ind/L and 0.65  $\mu\text{gC/L}$ ), probably due to the higher quantity of nutrients (phosphates and nitrates), while in station Sulina 20 m, they recorded values of about four times lower (60 ind/L and 0.15  $\mu\text{gC/L}$ ) (Figure 3.32). For quantitative analysis, the dominant species on this profile were *Tintinnopsis beroidea* in the surface layer and *T. karajacensis* in the 10 m layer (Figure 3.33). *T. beroidea* recorded density and biomass values of 218 ind/L, and 0.42  $\mu\text{gC/L}$  and *T. karajacensis* recorded 250 ind/L and 1.1  $\mu\text{gC/L}$ .

On the Sf. Gheorghe profile, the highest abundance and biomass of tintinnids was recorded in the 50 m station (1224 ind/L and 4.78  $\mu\text{gC/L}$ ) while in station 20 m they recorded the minimum (387 ind/L and 1.17  $\mu\text{gC/L}$ ) (Figure 3.32). From the point of view of the quantitative dominance of the species, they were *Tintinnopsis karajacensis* (0 m) and *T. beroidea* (10 m), the situation reversed from Sulina (Figure 3.33). *T. karajacensis* recorded the density and biomass of 2102 ind/L and 9.29  $\mu\text{gC/L}$  while *T. beroidea* recorded 1056 ind/L and 3.38  $\mu\text{gC/L}$ .



**Figure 3.32 - Community structure of tintinnids community in the Danube mouths area- density (left side) and biomass (right side), May 2019**

The highest mean values of density and biomass were in the Portita 20 m and the lowest in the Portita 30 m (Figure 3.32). The species that dominated the community of tintinnids from the Portița profile were *Tintinnopsis beroidea* in the surface layer and *T. karajacensis* in 10 m layer; the situation was like the one on the Sulina profile (Figure 3.33). *T. beroidea* recorded the density and biomass values of 112 ind/L and 0.22 µgC/L while *T. karajacensis* recorded 226 ind/L and 0,49 µgC/L, in the layers mentioned above.

The outliers occurred at Sulina 20m and Portita 30 m, respectively Sf. Gheorghe 30 m and 50 m (Figure 3.33) from all the analysed northern areas.

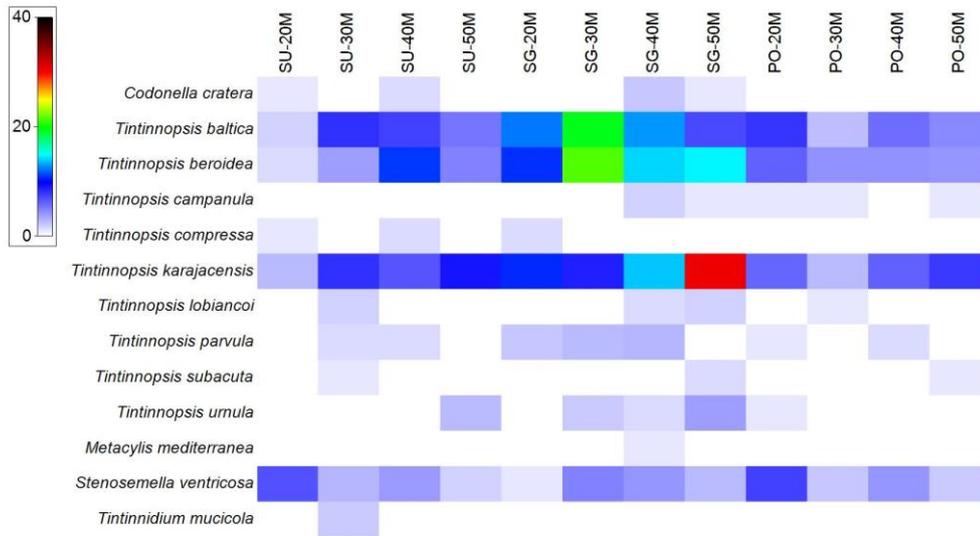


Figure 3.33 - Density (ind./L) of tintinnids community (transformed values) in the Danube mouths area (integrated layers), May 2019

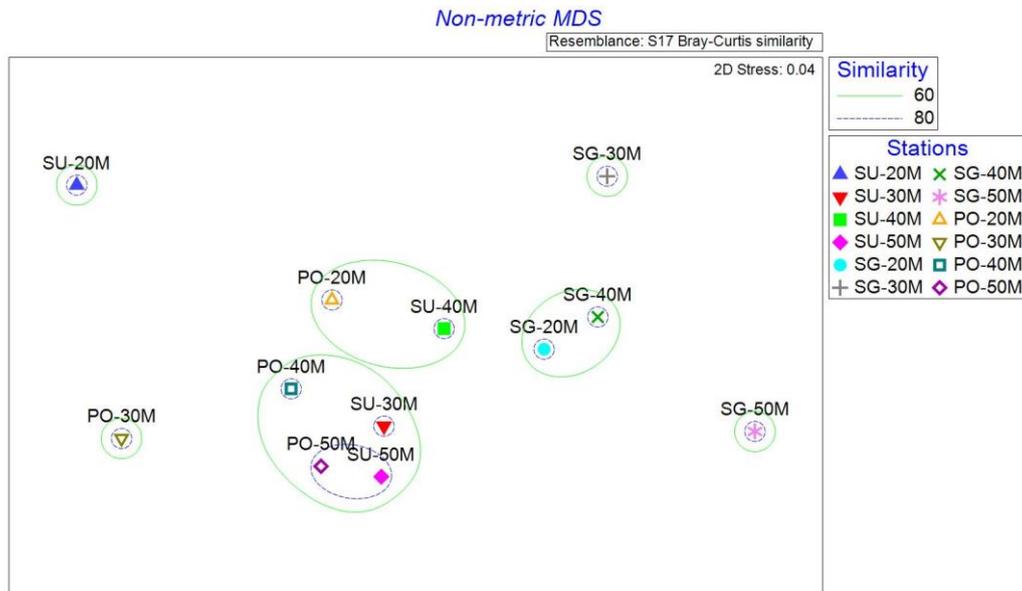


Figure 3.34 - nMDS analysed of microzooplankton from the Danube mouths area, May 2019

### Conclusions

From a total of 13 species identified in the northern part of the Romanian Black Sea, a diversity of 12 species were observed in the surface layer, respectively 8 species in the 10 m layer, a situation which corresponds to the ecology of these organisms. *Tintinnopsis compressa*, *T. lobiancoi*, *T. urnula*, *Tintinnidium mucicola*, *Metacylis mediterranea* are the species found exclusively in the surface layer, while *Codonella cratera* is the species found exclusively in the 10 m layer.

*Tintinnopsis baltica*, *T. beroidea*, *T. karajacensis* and *Stenosemella ventricosa* are the species found in all the profiles and layers analysed. They are also dominant in terms of quantity, being species that prefer colder waters.

The tintinnids community registered maximum values both from a qualitative and quantitative point of view on Sf. Gheorghe's profile, because the Danube discharges have the most significant influence (highest values of nutrients and the total suspended solids content, also the lowest average salinity). The minimum number of species and abundance of tintinnids community was registered on the Portita profile.

Following the microzooplankton component analysis, we can conclude that the Danube discharges influence it.

### Mesozooplankton communities

We identified a total of 21 mesozooplankton species, Copepoda being the best represented (8), followed by Cladocera (5) and meroplankton (4) (Annex C).

Among the marine zooplankton, copepods are the most familiar and dominant constituent since they comprise around 55-95 % of the total zooplankton abundance in the marine pelagic system (Angara, 2013).

Copepods *Acartia clausi* and *Pseudocalanus elongatus* were most abundant, representing the bulk of the community. *Acartia clausi* reached the highest density values in station SG-20M and SU-50M and *Pseudocalanus elongatus* in SU-50M, SU-40M, SG-40M. *Oithona similis*, another copepod with high-density values, was best represented in PO-20M, SG-60M and SU-50M.

*Pleopis polyphemoides*, belonging to Cladocera, appeared mainly in all stations, the other four species being identified only in some sampling stations (Figure 3.35). *Bosmina longirostris* and *Chydorus sphaericus* appeared only in stations located under the direct influence of the Danube (SU-20M, SU-30M). The small-bodied *C. sphaericus* often appears as a common plankter in eutrophic waters where extensive Cyanobacteria blooms are prevalent (Gannon, 1972). The cladoceran species *Bosmina longirostris* is widely distributed throughout the world in temperate and tropical climates, having the capability to colonise freshwater ecosystems at the phase of environmental change, including warming, eutrophication, salinization, and acidification (Adamczuk et al., 2019).

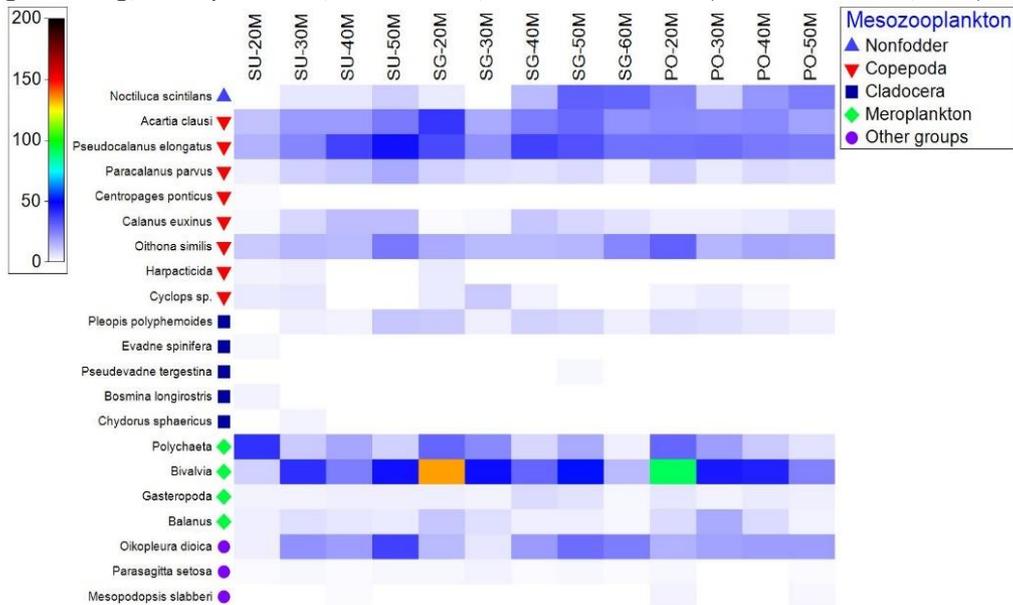


Figure 3.35 - Qualitative structure of mesozooplankton species - NW Romanian Black Sea, May 2019

Polychaeta and Bivalvia were best represented from the meroplanktonic component, with high densities in sampling stations near the coast (SG-20M, PO-20M).

Other groups recorded lower density values, *Oikopleura dioica* being the only species better represented from the quantitative point of view.

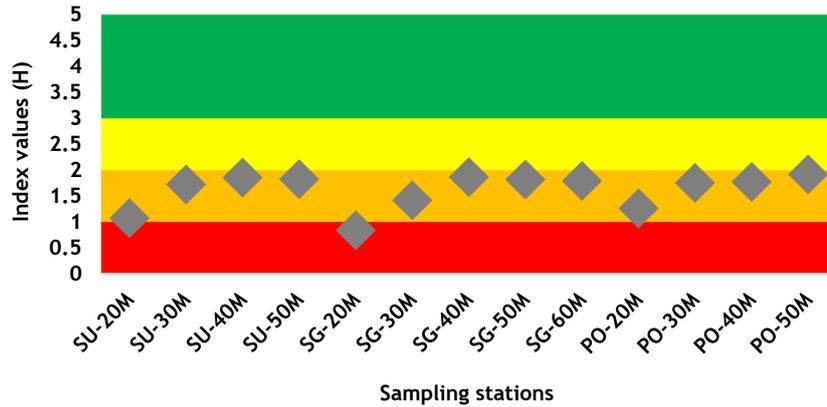


Figure 3.36 - Shannon diversity index - NW Romanian Black Sea, May 2019

*Noctiluca scintillans* recorded higher values in SG-50M, SG-60M, PO-50M, PO-20M and PO-50M, in the other stations reaching lower density values (Figure 3.35).

The Shannon diversity index ( $H'$ ) is commonly used to characterize species diversity, richness, and evenness of the community's species. In biological communities, the Shannon-Wiener diversity index varies from 0 to 5. Values less than one characterize heavily polluted conditions, and values in the range of 1-2 are characteristics of moderately polluted conditions, while values above 3 show stable environmental conditions (Shah et al., 2013). Taking into consideration, the Shannon index calculated for samples ranged from 0.8 to 1.9. In station SG-20M the biological oxygen demand and total suspended solids content reached the highest values, influencing the species diversity, therefore the low value of Shannon diversity index for mesozooplankton, indicating a heavily polluted condition (Figure 3.36).

Regarding the mesozooplankton's community structure, the fodder component was dominant; the highest densities were in Sf. Gheorghe 20m and the lowest in Sulina 20m. The non-fodder component represented by *Noctiluca scintillans* recorded high-density values only in stations SG-50M, SG-60M and PO-40M, in the other sampling stations reaching lower values (Figure 3.37).

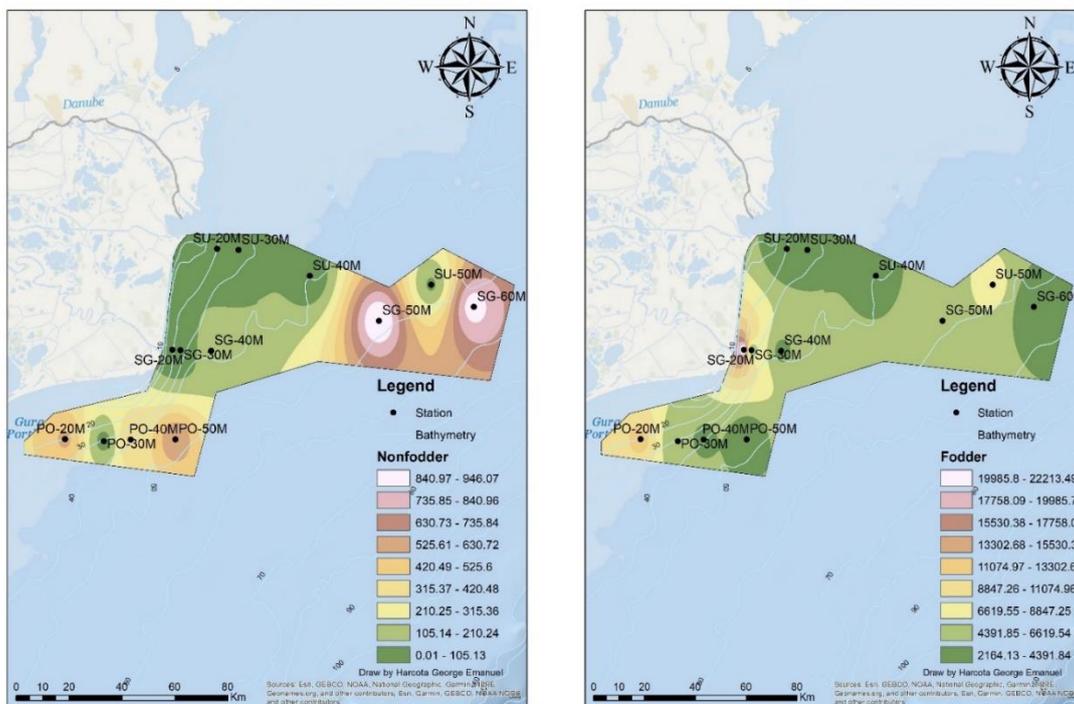
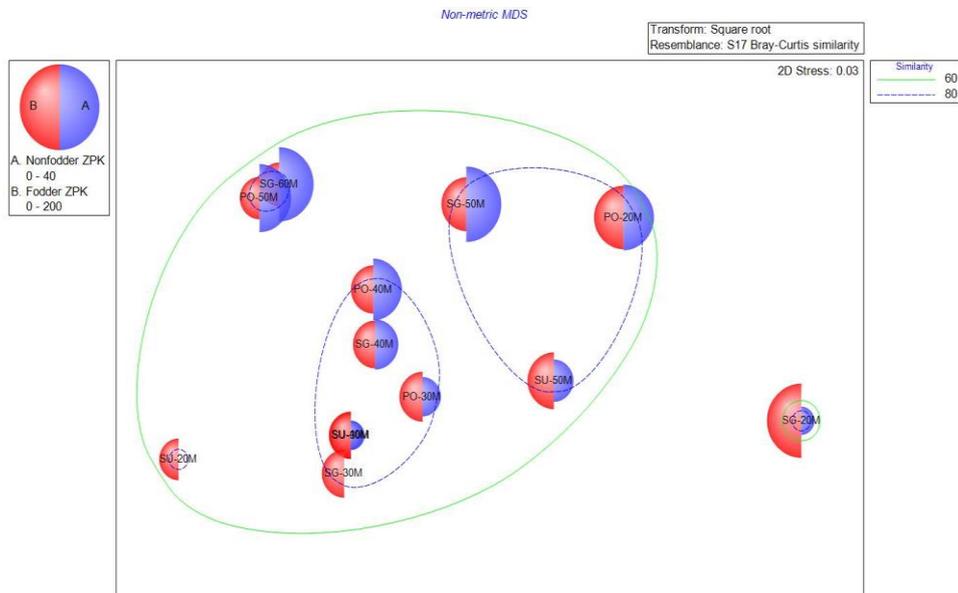


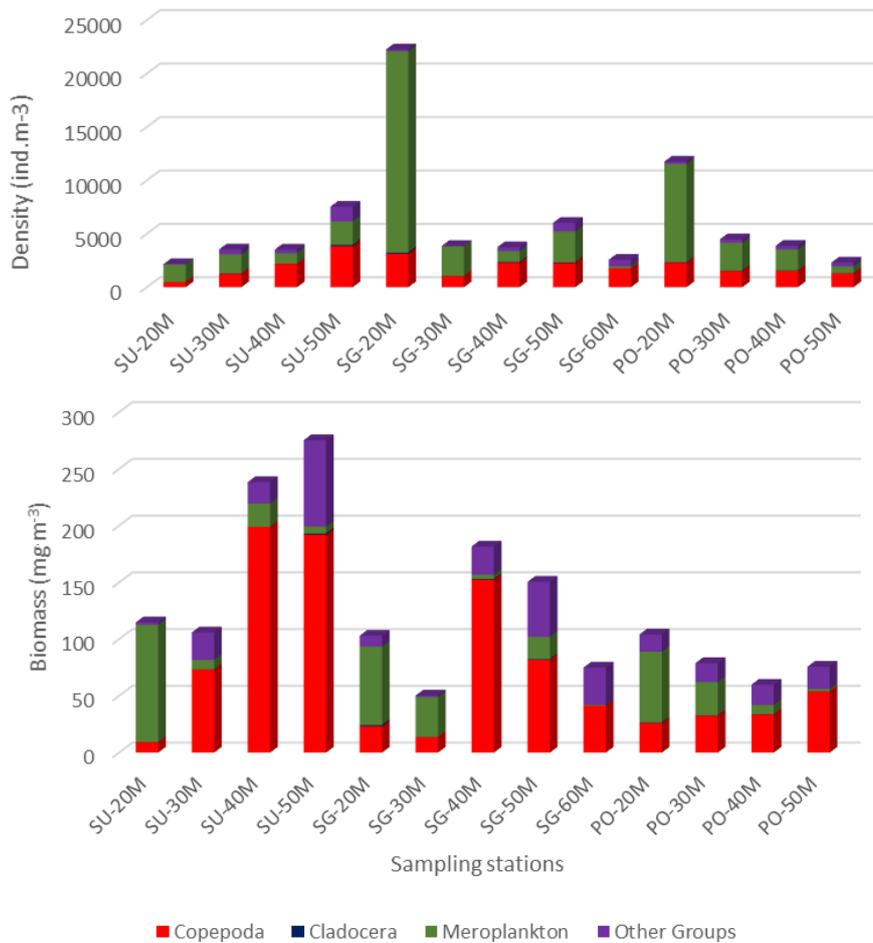
Figure 3.37 - Distribution of non-fodder (left) and fodder (right) mesozooplankton densities - NW Romanian Black Sea, May 2019

The non-metric MDS performed for the fodder, and non-fodder densities showed a similarity of 80% for stations PO-40M-SG-40M-PO-30M-SG-30M-SU-30M-SU-40M, forming a single cluster. For stations SU-30M and SU-40M, the similarity was 95% due to the close values recorded for both fodder and non-fodder components. Similarities of 80% were between stations SG-50M-PO-20M-SU-50M and between SG-50M-PO-50M. A particular case was station SG-20M which did not record any similarity with the other sampling stations due to the biological oxygen demand, and total suspended solids content reached the highest values (Figure 3.38).



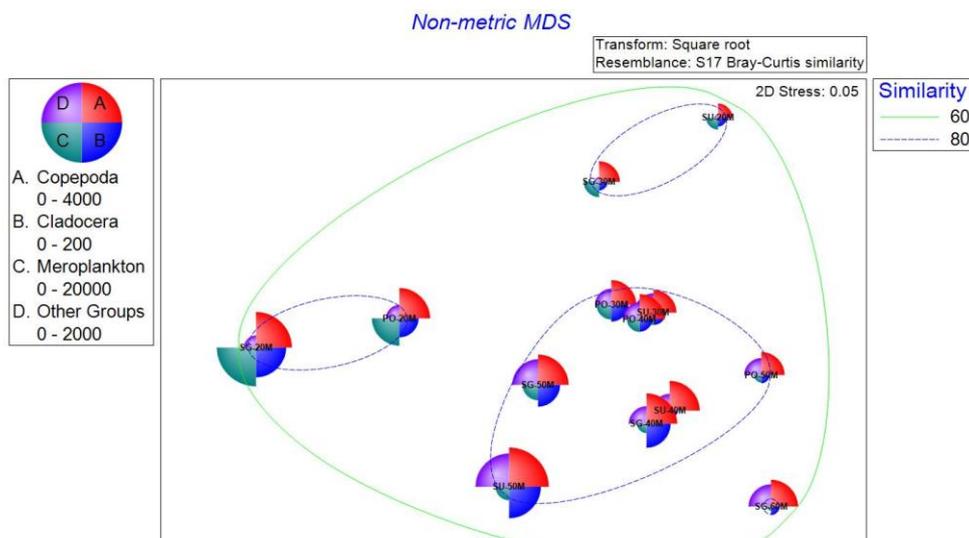
**Figure 3.38 - Non-metric multidimensional scaling (NMDS) ordination based on fodder and non-fodder density values- NW Romanian Black Sea, May 2019**

As far as the fodder component is concerned, Copepoda represented the community's bulk with the maximum density and biomass in SU-50m (3844 ind/m<sup>3</sup>, 192 mg/m<sup>3</sup>), being followed by SG-20m (Figure 3.39). Meroplankton recorded high densities and biomasses with the maximum in SG-20M (18843 ind/m<sup>3</sup>, 70 mg/m<sup>3</sup>).



**Figure 3.39 - Quantitative structure of fodder mesozooplankton - NW Romanian Black Sea, May 2019**

Analysing the distribution between the stations based on mesozooplankton's fodder densities, a high similarity was observed between samples that are under the direct influence of the Danube (SG-30M-SU20-M, SG-20M-PO-20M), which formed two clusters. The other stations that are far from the Danube influence formed a single cluster, including stations with depths between 30 m and 50 m (Figure 3.40).



**Figure 3.40-Non-metric multidimensional scaling (NMDS) ordination based on fodder densities - NW Romanian Black Sea, May 2019**

## Conclusions

The Danube's influence is observed by the presence of the species *Bosmina longirostris* and *Chydorus sphaericus*, which recorded higher density values in stations SU-20M and SU-30M.

Shannon diversity index recorded variations, indicating heavily pollution in the areas near the Danube mouths.

The sampling stations located in the vicinity of the Danube mouths (20m and 30m bathymetric strips) were characterised from the quantitative point of view by the mesozooplankton's trophic component, the Danube providing the nutritional support for this component. Therefore, stations in front of the Danube mouths recorded the highest similarities.

*Noctiluca scintillans*, the non-fodder mesozooplankton component, recorded lower density and biomass values and did not register blooms that could have led to exceeding the target for D5-Eutrophication (HAB).

## Macrozooplankton communities

During the cruise, two macrozooplankton species were identified: scyphozoa *Aurelia aurita* and the ctenophore *Pleurobrachia pileus*. The number of jellyfish species was low due to cold water temperature and month of sampling (May) which represents the beginning of their development.

In the northern part of the Romanian continental shelf, in Sulina profile represented by three stations, the species *A. aurita* reached maximum density, 0.52 ind/m<sup>3</sup> in front of the Sf. Gheorghe Danube's mouth (SG-30M) and maximum biomass 15988.13 mg/m<sup>3</sup> in the same station. The minimum density of 0.42 ind/m<sup>3</sup> (SG-20M) and the minimum biomass (2690.71 mg/m<sup>3</sup>) were recorded at SG-40M (Figure 3.41).

At Portita (four stations), the species *A. aurita* reached the maximum density and biomass concentrated on the offshore area, PO-60M, 0.69 ind/m<sup>3</sup> and 53239.21 mg/m<sup>3</sup>. The minimum density, 0.26 ind/m<sup>3</sup>, was found in PO-30M and the minimum biomass, 2400.64 mg/m<sup>3</sup> in the PO-20M (Figure 3.41).

The species *A. aurita* prefers waters with low temperature, having high densities where salinity is lower (<18 PSU) and less dense surface waters similar to the distribution of *Pleurobrachia pileus* (Mutlu E., 2009). The species distribution is heterogenous, the values oscillating over the entire analysed area. The density is very high in the PO-40M station, being influenced by the high depth and the lower water temperature in the water column.

Additionally, many scientists suggest that disturbed environments are more susceptible to invasion. In the case of *A. aurita*, overfishing of native pelagic fish has made establishment and success rates much higher (Richardson et al., 2009). Other researchers point out that eutrophication influences the establishment and success of the jellyfish. Excess nutrient runoff encourages phytoplankton blooms. This shift in plankton size from large to small means that larger species higher up in the food web are inefficiently consuming a less nutritious diet causing declines in predator populations. Anthropogenic habitat may also be responsible for success in *A. aurita*'s invasion (Richardson et al., 2009).

At Sf.Gheorghe profile, the species *P. pileus* reached maximum density, 16.48 ind/m<sup>3</sup> in front of the Danube's mouth (SG-30M) and the maximum biomass, 454.74 mg/m<sup>3</sup> in the SG-20M. The minimum abundance and biomass, 5.86 ind/m<sup>3</sup>, 194.48 mg/m<sup>3</sup>, respectively, were measured in SG-40M (Figure 3.42).

At Portita profile, the species *P. pileus* reached the maximum density concentrated in the open sea area, 28.58 ind/m<sup>3</sup> (PO-60M), and the maximum biomass 688.61 mg/m<sup>3</sup> in the same station. The minimum density, 0.26 ind/m<sup>3</sup> was found in PO-30M and the minimum biomass, 2400.64 mg/m<sup>3</sup> in PO-20M (Figure 3.42). The occurrence of HABs and 'fish kill' could be related to the abundance of Ctenophore *P. pileus* (Bu-Olayan and Bivin, 2006). Gelatinous zooplankton outbreaks could exacerbate the fish stock decline and may lead to trophic dead ends by channelling the flow of energy to "waste" (in the sense of lost fish production) (Sclutter et al., 2010).

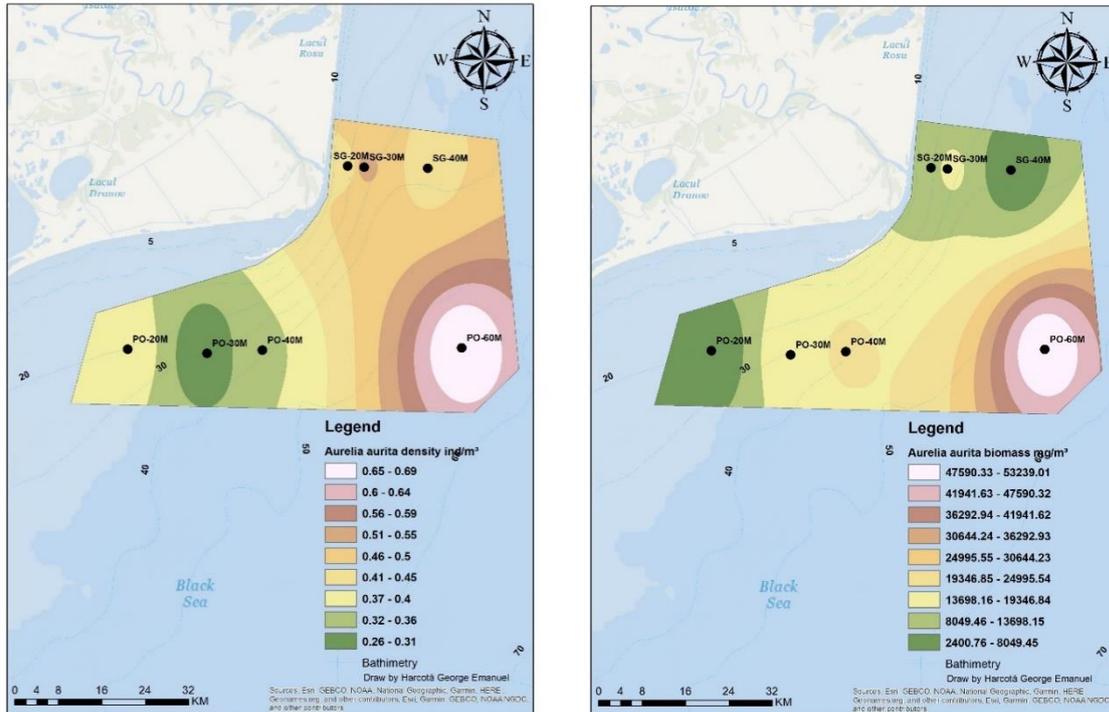


Figure 3.41 - *A. aurita* density (left) and biomass (right), May 2019

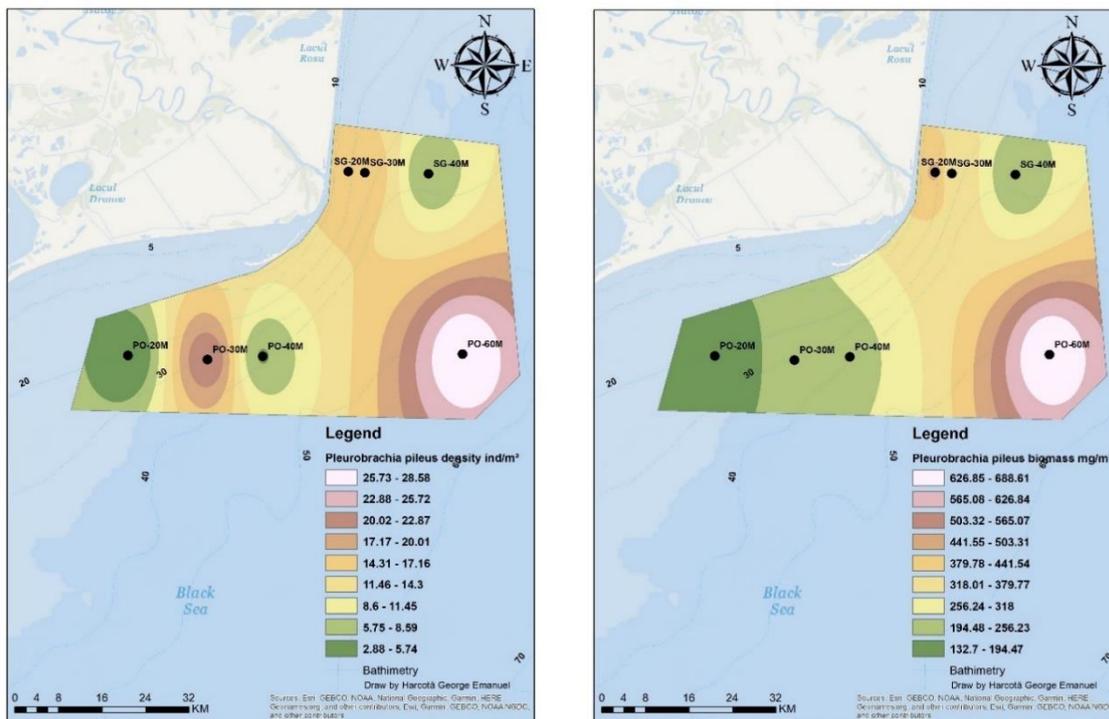


Figure 3.42 - *P. pileus* density (left) and biomass (right), May 2019

In the area under the direct influence of the Danube, the species *P. pileus* registered a non-uniform distribution, with high densities (station SG-30m) on SG profile, in the area in which the Danube River discharges into the Black Sea being characterized by a strong water current and low salinity values. In the offshore station (SG-40M), the density value decreases (Figure 3.42). In the Portita profile, the

distribution is non-uniform, while in the offshore area where the bottom depth is higher, and the Danube's influence is reduced, a higher density concentration was observed.

## Conclusions

Species variability was reduced due to sampling at the beginning of the jellyfish species season. In the transitional and marine water bodies, the density of species *P. pileus* was dominant compared to *A. aurita*.

According to our data, the Danube's freshwater input does not influence the distribution of gelatinous organisms.

The exact effects of eutrophication continue to be challenging to distinguish, although the Black Sea constitutes an example of large pelagic coelenterate populations in eutrophic conditions (Purcell et al., 2000).

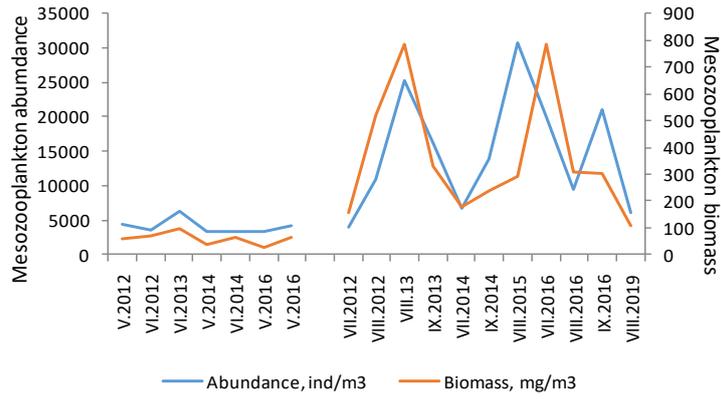
## Kamchia River

Mesozooplankton showed a remarkable variation in density (3271-30690 ind./m<sup>3</sup>) and biomass (24.47-787.13 mg/m<sup>3</sup>). The variability of abundance and biomass in spring among the years was relatively small, about 2-4 folds, while the summer is characterized with huge numerical metric deviation (min.- 3864 ind./m<sup>3</sup>, max- 30690 ind./m<sup>3</sup> and within 156.86-787.13 mg/m<sup>3</sup>, respectively) (Figure 3.43). The lowest abundance/biomass and species number observed during the study (June 2014) coincided with a large rain event in the watershed. The zooplankton community was composed of 22 species/taxa (15 classes), varying from 14 to 22 - poor species richness and taxonomic diversity. Freshwater/brackish species were not registered. Copepoda was the richest species group (11), followed by Cladocera (5) and Meroplankton (8 taxa), Ctenophora (3) and Sciphozoa (1). The different species/taxa frequency did not show any major changes between environments (scenarios). *Acartia clausi* prevailed in both wet and dry seasons with 32 % and 17 %. The species that contributed to the period differences were *Pleopis polyphemoides* (15 % in wet and 10 % in dry season), *Penilia avirostris* (dry scenario - 14 %), Cirripecia larvae (13 % - wet season). The development of *N. scintillans* in spring and *M. leidy* in summer reflected indirectly on mesozooplankton biomass since both negatively correlated with the biomass of planktonic fauna.

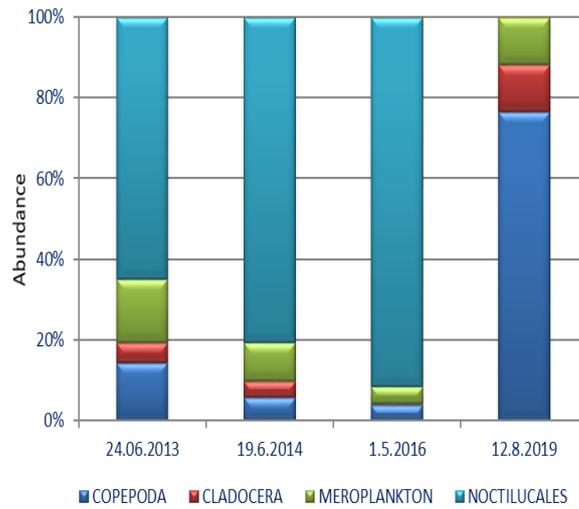
During the wet period, the abundance and biomass of *N. scintillans* overwhelmed the other-zooplankton abundance/biomasses with more than 80 %. Dry years demonstrated opposite community structure with Copepods prevalence and equivalent cladoceras and benthic larvae presence ((c)

Figure 3.43). Statistical analysis confirmed the negative correlation of mesozooplankton abundance with Noctiluca density in spring, well pronounced in the wet season. Like the phytoplankton community pattern in May 2016, Noctiluca was positively affected by regional and local river discharge. The same phenomenon of the appearance of *N. scintillans* during the flood season was found by Cardoso (2012) concluding that the mass development of Noctiluca was a result of freshwater input.

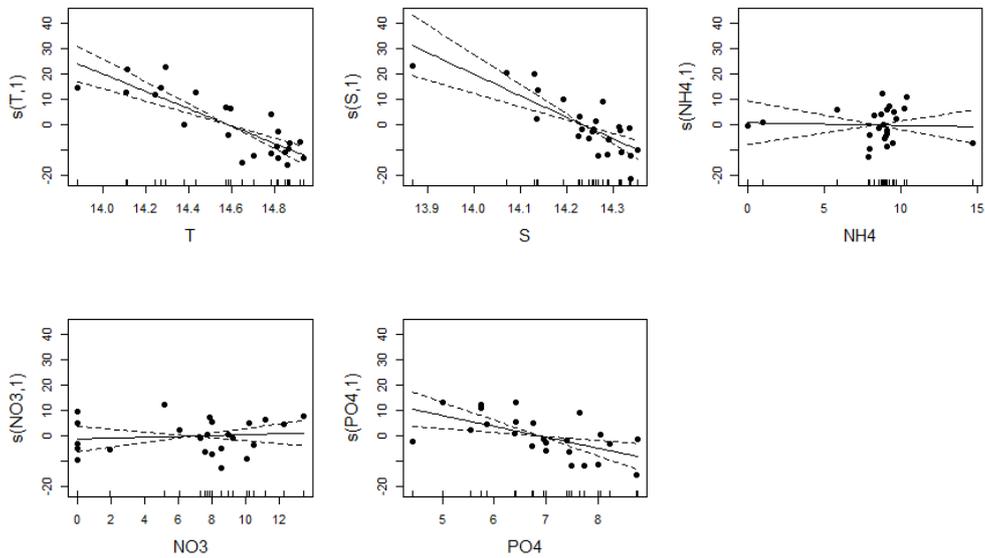
*N. scintillans* is recognized to play an essential role in the population dynamics of the zooplankton community by feeding on their eggs and competing for food resources (Nakamura, 1998). Therefore, its response to environmental factors was also modelled, providing statistically significant patterns in its abundance variations, associated with single or a multiplex of environmental variables. GAMM of *N. scintillans* abundance provided statistically significant model approximation ( $R^2_{adj}=0.652$ ), showing statistically significant negative linear effects of temperature ( $p=4.00e-7$ ,  $df=1$ ), salinity ( $p=3.36e-7$ ,  $df=1$ ) and PO<sub>4</sub> concentrations ( $p=0.006$ ,  $df=1$ ) Figure 3.43 and Table 3.5).



(a)



(b)



(c)

Figure 3.43 - Mesozooplankton quantity metrics (a), zooplankton community structure (b) and GAMM fits between *N. scintillans* abundance and environmental variable (in situ data) (c); T (temperature, °C), S (salinity, PSU), NH<sub>4</sub> ( ammonia, μM), NO<sub>3</sub> (nitrate, μM), PO<sub>4</sub> (phosphate, μM) and SiO<sub>4</sub> (silicate, μM)

Table 3.5 - GAMM ANOVA results

Family: Gaussian, Link function: identity, Formula: NScintillans ~ s(T) + s(S) + s(NH4) + s(NO3) + s(PO4)					
Parametric coefficients: Estimate Std. Error t value Pr(> t )					
(Intercept)	12.291	1.357	9.054	4.03e-08	***
Approximate significance of smooth terms:					
	edf	Ref.df	F	p-value	
s(T)	1	1	47.261	1.09E-06	***
s(S)	1	1	25.735	7.81E-05	***
s(NH4)	1	1	0.024	0.87763	
s(NO3)	1	1	0.115	0.73818	
s(PO4)	1	1	9.324	0.00684	**
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
R-sq.(adj) = 0.65, Scale est. = 42.381 n = 24					

According to the mesozooplankton biomass indicator, the Kamchia water body's ecological status was “poor” to “moderate”. During spring-summer 2012-2019, mesozooplankton biomass indicator fluctuated within the categories poor-moderate, predominantly in the “poor” state during spring (wet) season with *N. scintillans* prevalence and in moderate - in summer (Figure 3.44).

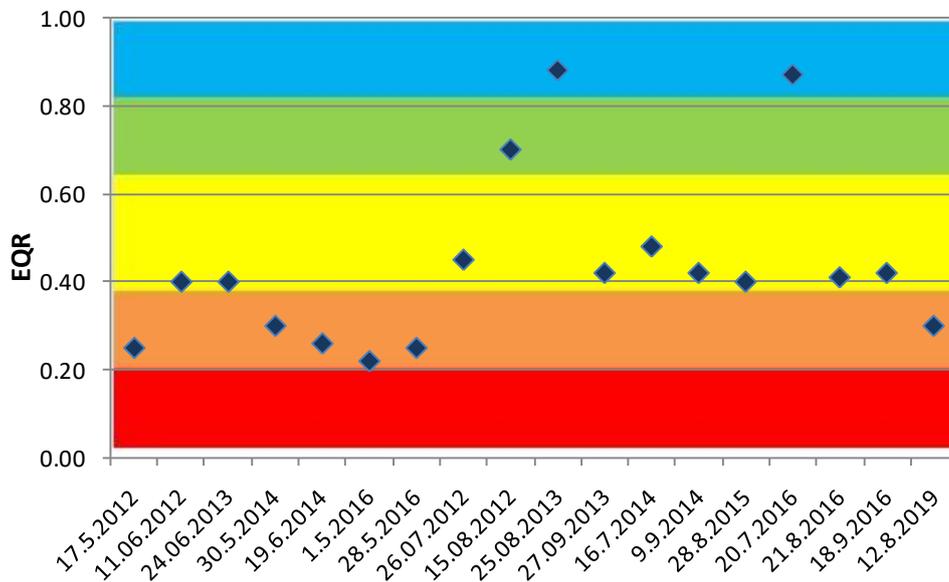


Figure 3.44 - Variation of the ecological status of WB Kamchia based on the mesozooplankton biomass indicator (spring-summer 2012-2019); colour codes correspond to the WFD classification system

## Conclusions

The results emphasize that albeit the interannual and seasonal variability, the zooplankton has not shown a clear community pattern in the discrete scenarios (dry-wet), most likely because of time-lagging behind the phytoplankton response.

The River Kamchia impact (wet scenario), similar to the response of phytoplankton, is apparent on the numerical development of *Noctiluca scintillans*, spatially throughout the inner coastal area only (the one-mile coastal zone), as documented by previous studies (Truhchev et al. 2010, Shtereva et al., 2010a).

## Sakarya River and Yesilirmak River

During the study, Sakarya and Yeşilirmak Rivers had in total, 30 mesozooplankton taxa. Ten species of the subclass Copepoda (*Acartia (Acartiura) clausi* (Giesbrecht, 1889), *Acartia (Acanthacartia) tonsa* Dana, 1849, *Acartia* sp. Dana, 1846 *Calanus euxinus* (Hulsemann, 1991), *Centropages ponticus*

(Karavaev, 1895), *Oithona davisae* (Ferrari F.D. & Orsi, 1984), *Oithona similis* (Claus, 1866), *Paracalanus parvus* (Claus, 1863) *Pseudocalanus elongatus* (Boeck, 1865) and *Pontella mediterranea* (Claus, 1863), four species of the superorder Cladocera (*Evadne spinifera* (P. E. Müller 1867), *Penilia avirostris* (Dana, 1849), *Pleopis polyphemoides* (Leuckart, 1859) and *Pseudevadne tergestina* (Claus, 1877)), one species of the phylum Chaetognatha (*Parasagitta setosa* (Müller, 1847)), one species of the class Appendicularia (*Oikopleura (Vexillaria) dioica* Fol, 1872)), and eleven groups belonging to meroplankton were in the sampling area.

Average abundance and biomass values in both Sakarya and Yeşilırmak were higher in July than in January. Mesozooplankton average abundance and biomass values in July in Sakarya (6130 ind/m<sup>3</sup> and 132 mg/m<sup>3</sup>) and Yeşilırmak (5368 ind/m<sup>3</sup> and 137 mg/m<sup>3</sup>) were similar. Mesozooplankton average abundance and biomass values in January were higher in Sakarya (2245 ind/m<sup>3</sup> and 47 mg/m<sup>3</sup>) than Yeşilırmak (1287 ind/m<sup>3</sup> and 18 mg/m<sup>3</sup>).

The mesozooplankton abundance and biomass values varied between 3363 ind/m<sup>3</sup> (station YSL 10) - 8581 ind/m<sup>3</sup> (station SAK 08) and 78 mg/m<sup>3</sup> (station YSL 10) - 209 mg/m<sup>3</sup> (station YSL 07) in July 2019, 729 ind./m<sup>3</sup> (station YSL 09) - 2642 ind/m<sup>3</sup> (station SAK 07) and 8 mg/m<sup>3</sup> (station YSL 09) - 59 mg/m<sup>3</sup> (station SAK 10) in January 2020, respectively (Figure 3.45).

The abundance and biomass are more than 2.5 times higher in summer compared to winter in Sakarya River. The abundance 4 times and biomass are more than 7.5 times higher in summer compared to winter in Yeşilırmak River. The mean abundance 3 times and the mean biomass is four times higher in summer compared to winter.

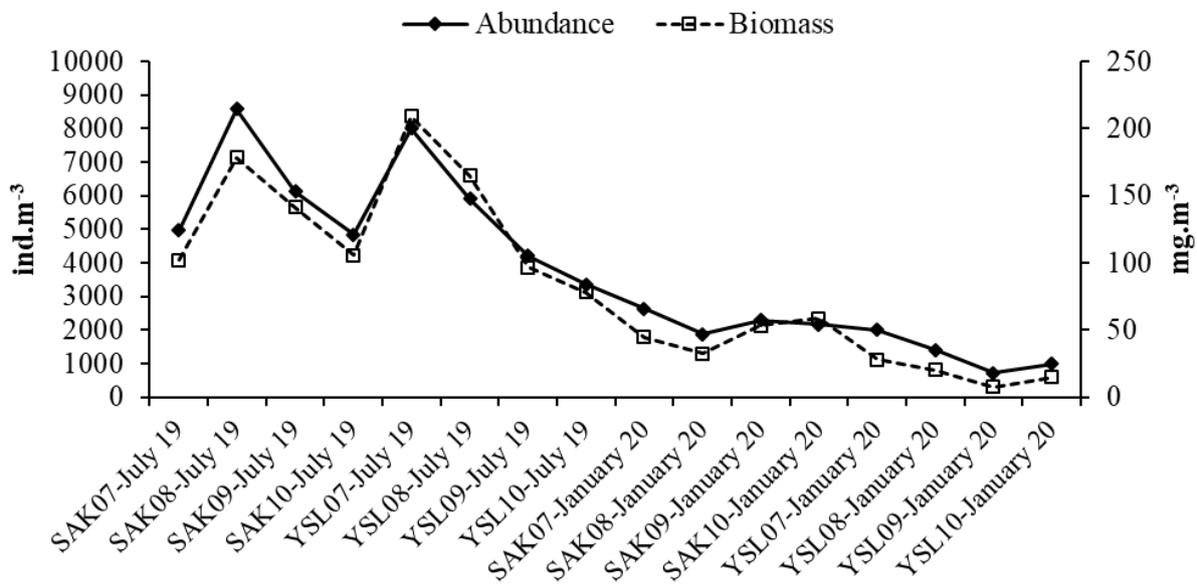


Figure 3.45 - The abundance (ind./m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) values of mesozooplankton at sampling stations in the Sakarya and Yeşilırmak Rivers (absent Noctiluca)

In terms of relative mesozooplankton abundance and biomass, Copepoda had high percentages in all stations during the study (54 % SAK09 July 2019 - 94 % SAK10 January 2020, abundance and 54 % SAK09 July 2019 - 88 % SAK07 January 2020, biomass) (Figure 3.46).

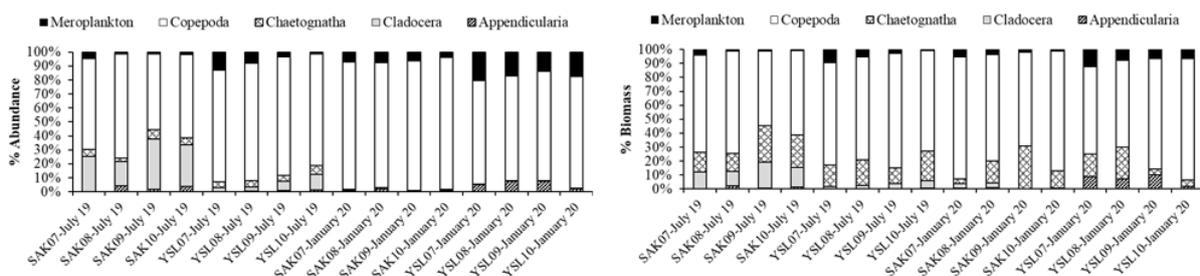


Figure 3.46 - The relative abundance and biomass of the mesozooplankton groups in Sakarya River and Yeşilırmak River (absent Noctiluca)

The lowest number of taxa was recorded in January 2020 at YSL07 and YSL09 (11 taxa or groups), and the highest was recorded in July 2019 at the SAK10 (21 taxa or groups). The maximum Shannon diversity index was found in July 2019 SAK10 (3.09). The minimum Shannon diversity index was determined in January 2020 SAK10 (1.89). This decrease in diversity was due to the numerical dominance of *P. parvus* and *A. clausi* (Figure 3.47).

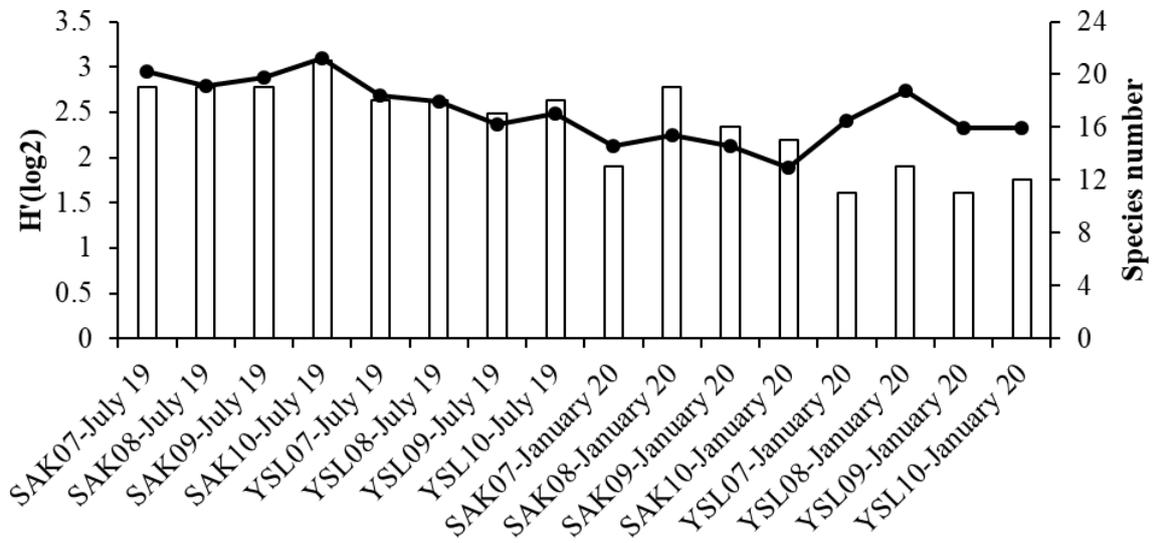


Figure 3.47 - The Shannon diversity index ( $H'$ ) for mesozooplankton for each month and sampling stations (absent *Noctiluca*)

#### *Noctiluca scintillans*

The abundance and biomass values of *N. scintillans* were between 5.6 ind/m<sup>3</sup> (station SAK09) and 457 ind/m<sup>3</sup> (station YSL10) in July 2019 and from 0.5 mg/m<sup>3</sup> (station SAK09) to 40 mg/m<sup>3</sup> (station YSL10) in January 2020. This species was not present in samples from July 2019 in Station YSL08 and YSL09. Abundance and biomass values of *N. scintillans* were higher in January than in July.

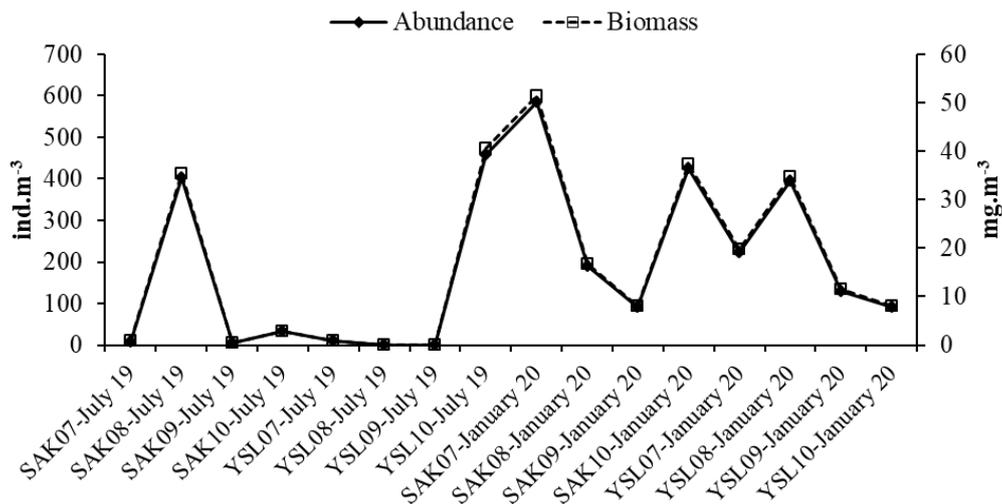


Figure 3.48 - The abundance (ind./m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) of *Noctiluca scintillans* at sampling stations in the Sakarya and Yeşilirmak Rivers

#### Conclusions

The species identified in the present study were marine zooplankton organisms common to the Black Sea (İşinibilir et al., 2017). Unidentified Cladocera species detected in Sakarya River (station SAK07) have characterized the river effect in January 2020.

*Pontella mediterranea* is highly sensitive to pollution at the microfilm layer of surface waters (Kideys et al., 2000). This species was only observed in the Yeşilırmak River in July 2019 (station YSL 07). Cladocera is one of the important nutritional zooplankton groups of coastal marine ecosystems and its presence is influenced by environmental factors (Üstün et al., 2019). Coastal ecosystems are under the influence of terrestrial inputs (anthropogenic activities) and meteorological phenomena (e.g. rain) (Resmi et al. 2011). These factors affect the amount of inorganic nutrients and phytoplankton in the aquatic environment and many other physiochemical properties, which may have an impact on the distribution and abundance of Cladocera. In the Black Sea, the presence of Cladocera is characterised as extremely seasonal - they reach maximum densities in the summer and early autumn (Lebedeva et al. 2015; Üstün et al., 2018). In this study, *Pseudevadne tergestina* and *Penilia avirostris* of marine cladocera dominated both rivers in July 2019. Cladocera species were higher in Sakarya River in terms of abundances and biomasses, and it was determined as the dominant species of *P. tergestina*.

In the present study, *Acartia clausi*, warm water species *Acartia tonsa* and *Centropages ponticus* were determined at high values in July 2019. *Paracalanus parvus* was the most dominant Copepoda species in January 2020. *C. ponticus* showed maximum abundance and biomass in Yeşilırmak River, whereas *A. clausi*, *A. tonsa* and *P. parvus* displayed high values of abundance and biomass in Sakarya River. These species are the dominant species in the coastal waters of the southern Black Sea (Yıldız and Feyzioğlu 2016; Üstün et al., 2018)

Cirripedia larvae were found in high value in July 2019 in both rivers. Decapoda larvae, Gastropoda larvae, Polychaeta larvae were determined at higher values in Yeşilırmak River than Sakarya River in July 2019. Bivalvia larvae became the dominant group in January 2020 in both rivers.

Alexandrov et al. (2014) stated organisms that show improved environmental conditions: *Pontella mediterranea*, *Centropages ponticus*, *Pseudevadne tergestina* and Decapoda larvae. These groups are the predominant species in our study.

### 3.1.3 Physical and chemical characteristics - water column

General hydro-physical and hydro-chemical characteristics of waters are the main indicators of their quality and the marine ecosystem's state, which is influenced by anthropogenic and natural factors associated with climate change. The Black Sea ecosystem's main anthropogenic factors include toxic and biotic pollution and eutrophication (Zaitsev, 1998; Yunev et al., 2007; Tokarev et al., 2007). Eutrophication results from the increase in nutrients loads that leads to phytoplankton's rapid growth, the so-called algal bloom. Algal blooms, "green tides", "red tides" lead to fish and other marine organisms' death and threaten the population's health. Many eutrophication studies worldwide have shown a significant role in this phenomenon of nitrogen (N) and phosphorus (P). Large quantities of nutrients (N, P, Si and some organic compounds) can cause undesirable consequences such as structural and functional changes in marine ecosystems and their stability. The reduction of the anthropogenic load of nutrients and eutrophication of waters is the subject of the MSFD (Marine Strategy Framework Directive 2008/56/EU) as one of the main pressures.

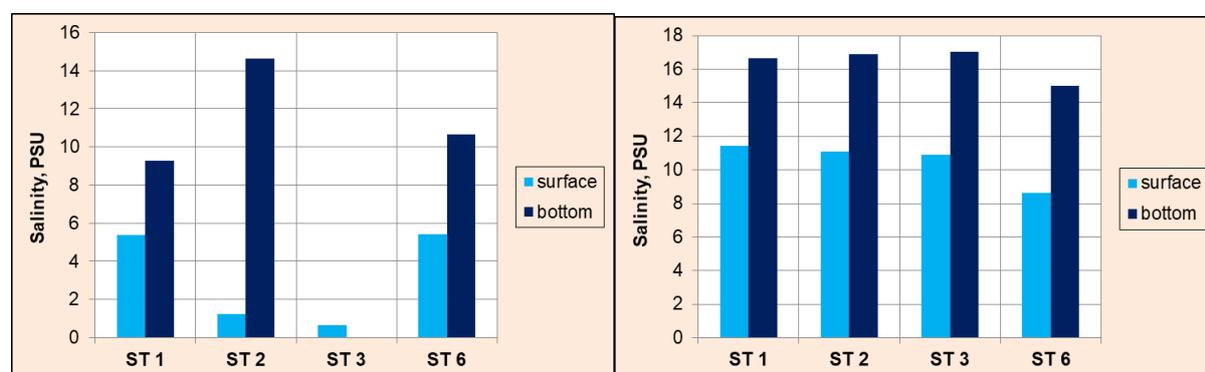
#### Dnieper River, southern Bug River, Dniester River and Danube River (UA)

During the coastal cruises in the zones of the mouths of the northwestern shelf's main rivers - Danube, Dniester, Dnieper, southern Bug, we observed in June 2019 a significant variability of water temperature in the surface layer. In September, the water temperature exceeded the typical monthly values by 3-4 °C, both in the northern shelf and Danube areas.

In June, the surface layer's salinity from the Danube area was in the range of 1.24-5.39 PSU (Figure 3.49, St. 1 and 2). According to WFD, coastal waters are classified as oligohaline for 0.5-5 PSU and as transitional waters (mesohaline marine type) for salinity 5-18 PSU. The salinity increased with depth, and in the bottom (8.6-8.9 m) in the halocline layer, it was 9.27-14.64 PSU. In autumn, with the minimum flow of the Danube, which usually occurs in September (Simonov AI, Altman AI, 1991), salinity increased, and in the surface layer, was within 11.08-11.1 PSU, while in the bottom layer (8.5-9.0 m), increased to 16.65-16.91 PSU (Figure 3.49, St. 1 and 2).

In the Dniester estuary area, in the Tsaregradsky (June), the surface salinity was 0.63 PSU (Figure 3.49, St. 3), which indicates an increased runoff of Dniester. Dnieper estuary's salinity (0m, June) was 1.12 PSU, and at the entrance to the Bug estuary, 3.96 PSU. Thus, the waters of the estuary were oligohaline. At the estuary's exit (Ochakov area), the surface waters had a salinity of 5.41 PSU, which increased with depth and amounted to 10.65 PSU in the bottom layer at 5 m depth (Figure 3.49, St. 6). Thus, the estuary waters are of the transitional type in the oligohaline open lagoon's upper part and in the lower part of the mesohaline open lagoon (Iarochevitch, 2017).

In September, as on the Danube seaside, with a decrease in the Dniester and Dnieper's runoff, the salinity of coastal waters at the Dniester outlet and Dnieper-Bug estuaries in the surface and bottom layers increased. Thus, on the Dniester estuary's seashore, 2 miles northeast of the Tsaregradsky mouth on the sea surface, salinity was at the level of 10.92 PSU, and in the bottom layer (4.5 m) it increased to 17.06 PSU (Figure 3.49, St. 3).



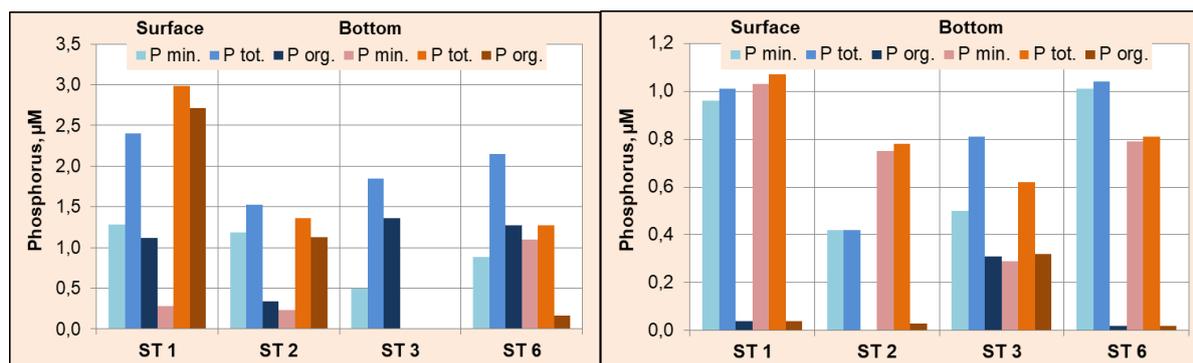
**Figure 3.49 - Salinity of sea areas near the main rivers of the northwest shelf of the Black Sea in June (left) and in September (right), 2019**

In the Ochakov area, at the exit from the Dnieper-Bug estuary, salinity (0 m, September) increased to 8.66 PSU and at a depth of about 5 m in the bottom layer reached 15.00 PSU (Figure 3.49, St. 6).

In the near-mouth zones, the variability of marine waters' physicochemical characteristics is predominantly determined by the river flow and the wind regime.

pH on the northwestern shelf in the estuarine areas under study varied in June in the range of 8.26-9.15 and exceeded the maximum allowable value of 8.5 established by Resolution no. 431 of the Cabinet of Ministers of Ukraine (2002). The maximum allowable level was in the Danube area (8.69), at the Bug estuary's entrance (9.10) in the surface layer and on the seashore of the Dnieper-Bug Estuary in the Ochakov area, both in the surface and in the bottom layer (5m) with pH of 9.15 and 8.53, respectively. In the autumn (September), with a general decrease in river runoff, the water pH slightly improved, exceeding (8.86) being noted only at the outlet from the Dnieper-Bug estuary (0 m). In the rest of the estuarine areas, seawater's pH varied in the range 8.28-8.43.

The dissolved inorganic phosphorus concentration (DIP) in June 2019 varied in a wide range from 0.23  $\mu\text{M}$  to 1.28  $\mu\text{M}$  (average, 0.8  $\mu\text{M}$ ).



**Figure 3.50 - DIP in front of the main rivers of the north - northwest shelf of the Black Sea in June (left) and in September (right), 2019**

In June, in front of the Danube, increased phosphate concentrations (DIP), 1.28  $\mu\text{M}$ , and 1.19  $\mu\text{M}$  were observed at the sea surface layer and decreased with depth. In the bottom layer (8.9 m and 8.6 m), the DIP concentration was 0.28  $\mu\text{M}$  and 0.23  $\mu\text{M}$ , respectively (St. 1 and 2). In the coastal area of the Dniester estuary (Tsaregradskiy mouth), the concentration of phosphate (0 m) was 0.49 (St. 3). Increased phosphate content was at the Dnieper-Bug estuary outlet in the surface layer, 0.88  $\mu\text{M}$ , and at the bottom, 1.10  $\mu\text{M}$  (Figure 3.50).

In September, with a general decrease in river runoff, a decrease in phosphates' concentration is also noted, on average by more than 1.5 times. In front of the Danube, a decrease in the phosphate concentration (0 m) to 0.96  $\mu\text{M}$  and 0.42  $\mu\text{M}$  was observed, in the bottom layer (8.5 m and 8.0 m), an increase in concentrations was noted to 1.03  $\mu\text{M}$  and 0.75  $\mu\text{M}$ , respectively (St. 1 and 2). The minimum 0.29  $\mu\text{M}$  was observed in the Dniester estuary in the bottom layer at a depth of 4.5 m, and in the surface layer, it increased to 0.50  $\mu\text{M}$  (St. 3). In the Dnieper-Bug estuary (Ochakov area), increased phosphate content persisted in September (1.01  $\mu\text{M}$  in the surface layer) and 0.79  $\mu\text{M}$  in the bottom layer (St. 6), (Figure 3-50b).

The concentrations of total phosphorus (TP) followed the general trend of DIP. A relatively increased TP concentration was observed in June with an average value of 1.94  $\mu\text{M}$  during increased river flow and decreased to 0.82  $\mu\text{M}$  in September, with a general decrease in river flow. In June, a relatively increased TP concentration is in the surface layer on the Dnieper-Bug estuary seashore, 2.15  $\mu\text{M}$ , and in front of the Danube, 2.40-2.99  $\mu\text{M}$  in the surface and bottom layers, respectively. In September, the concentration of total phosphorus decreases, but areas with a relatively increased TP concentration do not change. In September, at the Dnieper-Bug estuary's exit, the increased TP concentration was 0.81  $\mu\text{M}$  (bottom) and 1.04  $\mu\text{M}$  (surface); on the Danube area opposite at the Bistroe's arm was 1.01  $\mu\text{M}$  (surface) and 1.07  $\mu\text{M}$  (bottom). In September, the average concentration of total phosphorus decreased relative to its average concentration in June more than two times.

Generally, the total phosphorus content was dominated by dissolved mineral phosphorus, which accounted for 56 % of the total. In June, with a relatively increased flow of rivers, the TP share was 40.2 % - mineral phosphorus and 59.8 % - organic phosphorus. In September, with relatively low rivers flow, the organic form of phosphorus's contribution decreased to 12.2 %.

Concentrations of nitrites in June varied in the range of 0.54-2.10  $\mu\text{M}$ , and the average value was 1.01  $\mu\text{M}$ . The highest content of nitrites (0.86-2.10  $\mu\text{M}$ ), with a maximum concentration of 2.10  $\mu\text{M}$ , exceeding the environmental standard (ES) for the quality of the marine environment (ES = 0.714  $\mu\text{M}$

NO<sub>2</sub>) was observed in waters on the Danube area (St. 2). In other areas of observation, opposite the Dniester River and at the exit from the Dnieper-Bug estuary, the concentration of nitrites was at the level of 0.54-0.69  $\mu\text{M}$ , not exceeding ES (Figure 3.51).

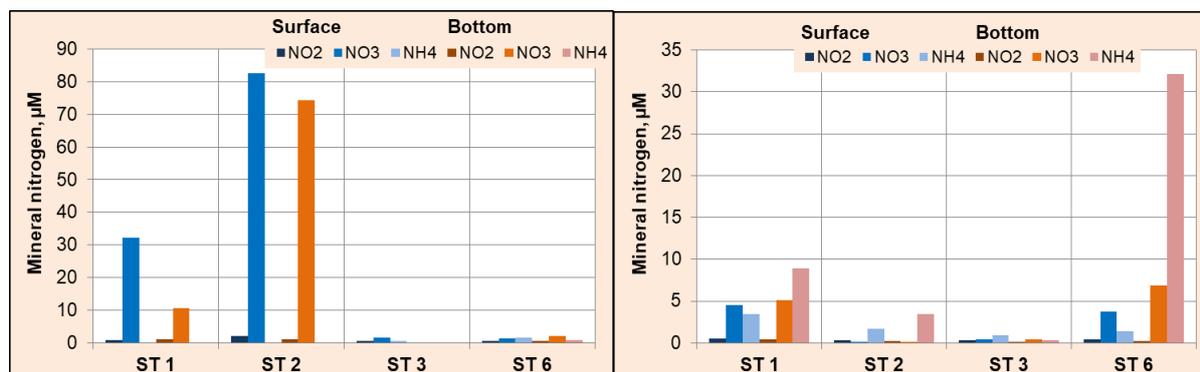


Figure 3.51 - DIN in seawaters near the main rivers of the northwest shelf of the Black Sea in June (left) and in September (right), 2019

During the autumn, the nitrites concentration varied in the range of 0.07-0.56  $\mu\text{M}$  (average, 0.31  $\mu\text{M}$ ). Generally, in September, due to a decrease in river runoff, the nitrites concentration decreased three times than in June. A relatively increased concentration of nitrites (0.45-0.56  $\mu\text{M}$ ) in September was noted on the Danube seaside opposite at the Bistroe arm (St.1). In other areas, the concentration was in the range of 0.07-0.39  $\mu\text{M}$ .

In June, the concentration of nitrates varied over a very wide range of 1.41-82.5  $\mu\text{M}$  (average, 29.3  $\mu\text{M}$ ). The maximum values, 10.64-82.53  $\mu\text{M}$ , exceeding the environmental standard ES = 7.14  $\mu\text{M}$ , were observed at the Danube area. In the rest of the regions, near rivers Dniester and Dnieper-Bug, the nitrate content in June varied between 1.41  $\mu\text{M}$  and 2.17  $\mu\text{M}$ .

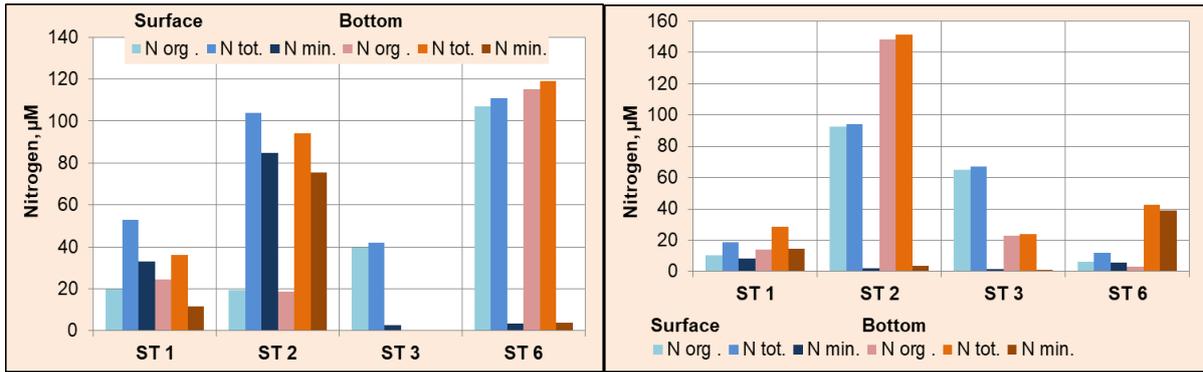
In September, the nitrates levels were an order of magnitude less than June, 0.02-6.85  $\mu\text{M}$  (average, 2.62  $\mu\text{M}$ ). Relatively increased values, 4.50-5.09  $\mu\text{M}$ , (St. 1) and 3.74-6.85  $\mu\text{M}$  (St. 6), were observed at the Bistroe arm and the exit from the Dnieper-Bug estuary, respectively. In other areas, the concentration of nitrates did not exceed 0.38  $\mu\text{M}$  (Figure 3.51).

Ammonium in June was in the range 0.04-1.66  $\mu\text{M}$  (average, 0.52  $\mu\text{M}$ ). The maximum was recorded in Ochakov's coastal waters, 1.66  $\mu\text{M}$  (surface). In September, the ammonium concentration was in the range 0.30-32.13  $\mu\text{M}$ . In contrast to nitrites and nitrates, the concentrations of which decreased in autumn, the ammonium's average concentration increased to 6.53  $\mu\text{M}$ . The maximum, 32.13  $\mu\text{M}$  was noted at the outlet of the Dnieper-Bug estuary (Ochakov area), bottom layer (4.6 m) (St. 6). Increased ammonium values, 3.48-8.93  $\mu\text{M}$  and 1.70-3.40  $\mu\text{M}$ , were in the coastal waters of the Danube Bistroe arm and Kiliya, respectively (St. 1 and 2).

The sum of mineral forms of nitrogen (DIN) in June was on average 30.8  $\mu\text{M}$  and varied in the range of 2.78 - 84.63  $\mu\text{M}$ . Relatively increased values, 11.73 - 33.13  $\mu\text{M}$  and 75.43 - 84.63  $\mu\text{M}$  were in the coastal waters of the Danube Bistroe arm and Kiliya, respectively. In other areas, the DIN concentration in June varied in the range of 2.78-3.72  $\mu\text{M}$ .

DIN decreased in September to an average of 9.47  $\mu\text{M}$ . The DIN concentration varied in the range of 0.75-39.2  $\mu\text{M}$ . Increased concentrations, 8.54-14.47  $\mu\text{M}$  and 5.53-39.20  $\mu\text{M}$  were observed in the Danube Bistroe arm's coastal waters and the Ochakov coastal area at the outlet of the Dnieper-Bug estuary, respectively. The maximum concentration of DIN in the Ochakov coastal area was noted in the bottom layer (39.20  $\mu\text{M}$ ). The relative contribution to the total mineral forms of nitrogen in June was 3.2 % nitrites, 95.4 % nitrates and 1.4 % ammonium. In September, the relative contribution changed and amounted to 3.3 % nitrites, 27.7 % nitrates and 69 % ammonium.

Concentrations of total nitrogen (TN) varied within the range of 36.3-118.9  $\mu\text{M}$  in June, with a maximum in the Ochakov coastal area's bottom layer (St. 6). The average concentration in June was 79.9  $\mu\text{M}$  and exceeded the environmental standard EN = 71.39  $\mu\text{M}$ . In September, during low river runoff, TN concentrations decreased and were in the range of 11.91-151.57  $\mu\text{M}$  with an average of 54.76  $\mu\text{M}$ . The maximum TN concentrations in September with an exceeding ES were observed only at the Danube arm Kiliya, 94.4-151.6  $\mu\text{M}$  with a maximum in the bottom layer at 8.5 m depth (St. 2). Organic and mineral nitrogen contribution to the total (TN) was in June 62 % - organic nitrogen and 38 % - DIN, and in September 83 % - organic nitrogen and 17 % - DIN (Figure 3.52).



**Figure 3.52 - Concentrations of nitrogen forms in seawaters near the main rivers of the northwest shelf of the Black Sea in June (left) and in September (right), 2019**

Silicate concentrations in June were in the range 1.13-66.6  $\mu\text{M}$  (average, 36.7  $\mu\text{M}$ ). Increased values were in the Dnieper-Bug estuary, 43.08-66.6  $\mu\text{M}$  and in the surface layer in front of the Danube area, 47.0-48.1  $\mu\text{M}$ .

Like most hydro-chemical characteristics, silicate concentrations decreased in September, in the range of 7.5-50.3  $\mu\text{M}$ , (average, 18.9  $\mu\text{M}$ ). Relatively increased silicate levels were in the Dniester coastal area, 11.8-22.5  $\mu\text{M}$ , and at the outlet from the Dnieper-Bug estuary, 20.2 - 50.3  $\mu\text{M}$ .

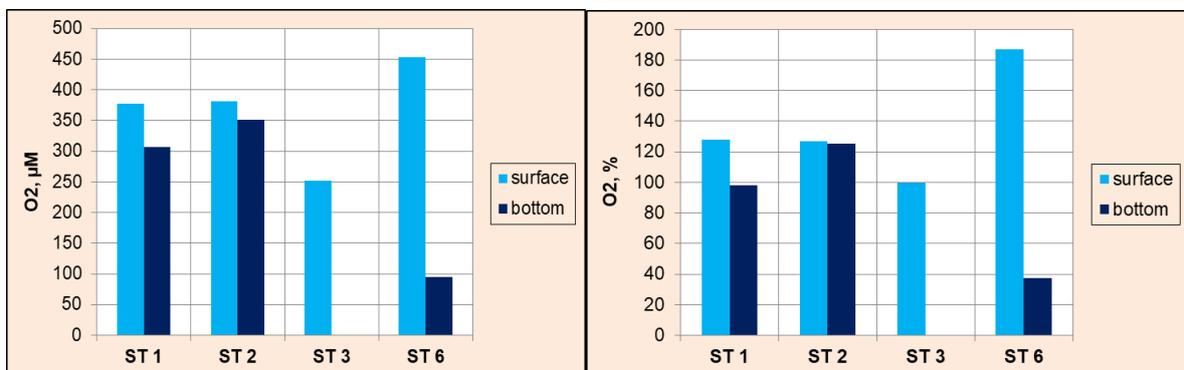
The average ratio of nutrients to the observation period was:

- in June DIN/DIP = 39.5; Si/DIN = 1.2
- in September DIN/DIP = 13.2; Si/DIN = 2.0

The optimal ratio for the phytoplankton's development, the so-called Redfield ratio, is DIN/DIP = 16:1 and Si/DIN = 1:1 (Redfield, 1958). Thus, the ratios indicate an excess of mineral nitrogen in June and its deficiency in September, which is unfavourable for diatoms growth.

The dissolved oxygen content in the water and its dynamics is one of the defining criteria of the marine ecosystem status and indicator of organic matter's primary production intensity and biochemical oxidation.

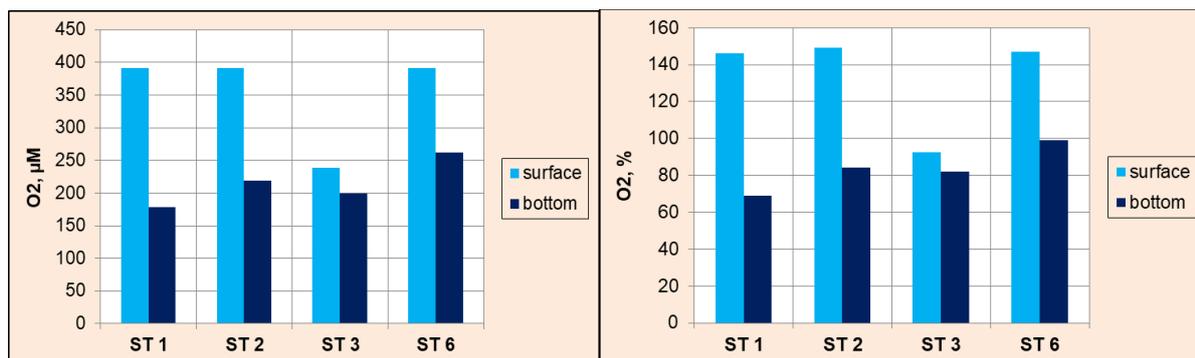
The concentration of dissolved oxygen in the north-northwestern shelf of the Black Sea in June varied over a very wide range from 95  $\mu\text{M}$  (37.4 %) to 453.8  $\mu\text{M}$  (187.0 %) with an average oxygen content of 316.6  $\mu\text{M}$  (114.6 %). The most environmentally unpleasant water conditions were noted in the Dnieper-Bug estuary, where the maximum oxygen concentration, 453.8  $\mu\text{M}$  (Figure 3.53). was observed in the surface layer with a relative saturation of water with oxygen - 187 % (St. 6), which indicates the development of phytoplankton blooms and eutrophication processes. Because of these processes in the coastal waters in the Ochakov area, hypoxia was noted in the bottom layer at a depth of 4.6 m (95  $\mu\text{M}$ ), (Figure 3.53) and saturation 37.4 % (St. 6). Increased oxygen content in the surface layer of 376.9-381.9  $\mu\text{M}$  (125-128 % saturation) was noted on the Danube seaside (St. 1 and St. 2), (Figure 3.53).



**Figure 3.53 - Concentrations of oxygen dissolved (left) and saturation percentages (right) in front of the main rivers of the north-northwestern shelf of the Black Sea, June 2019**

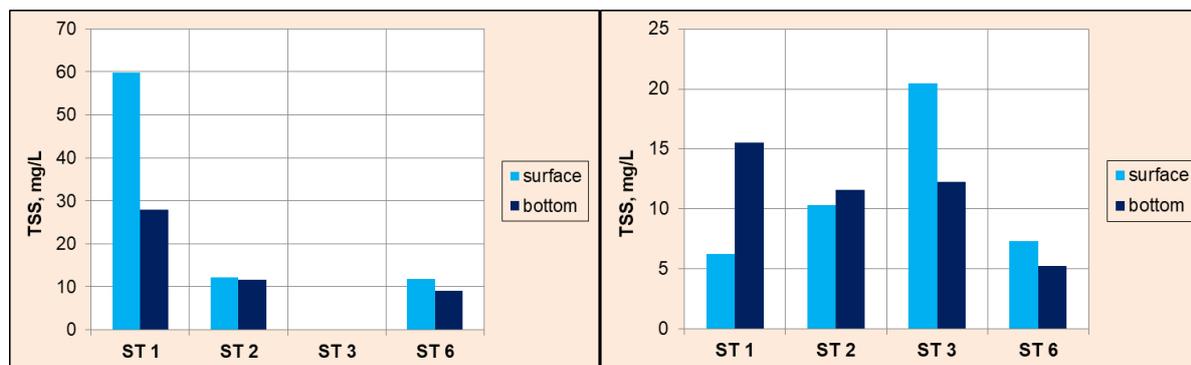
In September, the oxygen concentration varied within 178.8 - 390.6  $\mu\text{M}$  (69-149 %). The average was 283.8  $\mu\text{M}$  (saturation 108.6 %). Increased dissolved oxygen levels, 390.6  $\mu\text{M}$  (146-149 %) were in the

surface layer at two stations on the Danube seaside (St. 1 and St. 2) and in the coastal waters of the Ochakov area in the zone of the outlet from the Dnieper-Bug estuary (Figure 3.54).



**Figure 3.54 - Dissolved oxygen (left) and saturation (right) in front of the main rivers of the north-northwestern shelf of the Black Sea, September 2019**

The content of total suspended solids (TSS) in seawater was maximum in June in the surface and bottom layers in the Danube region at station 1 (59.7 mg/L and 28.0 mg/L, respectively) in front of Bistroe arm (St. 1), (Figure 3.55).



**Figure 3.55 - Total suspended solids (TSS) content in front of the main rivers of the north-northwestern shelf of the Black Sea, June (left) and September (right), 2019**

In September, the content in total suspended solids decreased in the range of 5.22-20.5 mg/L. The maximum (20.5 mg/L) was noted in the surface layer in the area of “Zatoka” at the outlet of the Dniester River waters (Figure 3.55).

## Conclusion

Among the regions under study, the most unfavourable conditions of the marine environment are noted for many indicators of assessing the state of eutrophication of waters at the outlet from the Dnieper-Bug estuary and in the Danube region.

## Danube River (RO)

The seawater temperature recorded typical values for the beginning of the warm season, outlining an increasing gradient from north to south. Maximum, 16.87 °C, was measured at the surface, Portita 50 m (Figure 3.56). The thermocline began to shape in the surface layer (0-10 m), although temperatures above 10 °C were in the superficial layer (0-5 m). Minimum 6.93 °C, was found at the bottom (Sulina 40 m) (Figure 3.57).

Overall, salinity did not show a particular gradient at the surface, only some extremes and outliers corresponding to the Danube’s direct discharge points, Sulina (9.41 PSU) and Sf. Gheorghe (6.21 PSU) (Figure 3.58). The variability profile of the water column was similar to temperature but reverse. Thus, the most substantial rise was observed in the 0-10 m layer with an enhancement in the

superficial layer 0-5 m. A homogenous bottom layer was observed with maximum salinity between 18.28 PSU and 18.36 PSU (Figure 3.59).

The dissolved oxygen content was highest at the surface (308.6 - 363.5  $\mu\text{M}$ ) due to the atmospheric exchange and biological production confirmed by a maximum from the water column (10 m) at Periboina 60 m. It also outlines the decreased gradient with depth as a typical feature of the Black Sea waters (Figure 3.60). Thus, bottom oxygen saturation reached its minimum, 64.8 %, at Sf. Gheorghe 60m (Figure 3.61).

The lowest pH was measured at the surface, Sulina 20 m, and represents an outlier. The surface layer has the highest variability, 7.91-8.71 (standard deviation 0.19) (Figure 3.62).

The biological oxygen demand ( $\text{BOD}_5$ ) recorded an outlier (4.87  $\text{mgO}_2/\text{L}$ ) in front of Sf. Gheorghe mouth highlighting a potential increase in organic discharge (Figure 3.63).

Phosphate and silicate concentrations were significantly correlated with salinity ( $r=-0.71$  and  $r=-0.95$ ) and followed the same pattern at the surface. Thus, in front of Sf. Gheorghe discharge point (surface) were both highest concentrations due to the Danube discharge (Figure 3.64 and Figure 3.65).

The inorganic nitrogen species had different behaviour. Therefore, nitrate revealed a riverine input explained by the significant correlation with salinity ( $r=-0.62$ ). The maximum was at the surface, Sf. Gheorghe 30 m. Nitrite and ammonium concentrations were not significantly correlated with salinity. It is to note that nitrite levels were extreme at the surface in many stations, located beyond the direct influence of the Danube's discharge (Figure 3.66). Ammonium reached its highest concentrations in the water column. One intermediate peak was observed at 10 m, while the maximum at the bottom (50 m).

The total suspended solids content (TSS) reached the maximum in front of Sf. Gheorghe arm under the direct influence of the Danube discharge (Figure 3.67).

Total nitrogen (TN) concentrations were higher in the front of Sulina and Sf. Gheorghe mouths being significantly correlated with salinity ( $r=-0.92$ ), phosphate ( $r=0.81$ ), silicate ( $r=-0.94$ ) and nitrate ( $r=-0.76$ ), emphasizing the riverine input of nutrients and organic matter (Figure 3.68). Unlike total nitrogen, the organic carbon content was rather a consequence of biological productivity, confirmed by the significant correlation with dissolved oxygen concentrations ( $r=0.59$ ) (Figure 3.69). Total phosphorus (TP) concentrations were also indicated the riverine inputs due to high correlations of the surface layer content with salinity ( $r=-0.85$ ), phosphate ( $r=0.84$ ), silicate ( $r=0.88$ ), total nitrogen ( $r=0.85$ ). However, the maximum level was found for the 10 m depth at Portita 20 m (Figure 3.70).

## Conclusions

The influence of the Danube in the area under study is well known and documented (Gomoiu, 1992, Humborg et al., 1997, Mihailov et al., 2013). Nowadays, the Danube's discharge pressure is mainly observed for the nutrients input. Thus, phosphate, silicate, nitrate, total nitrogen, and total phosphorus were significantly correlated with salinity. Moreover, in 95 % and 42 % cases, GES was not achieved for DIN and DIP, respectively.

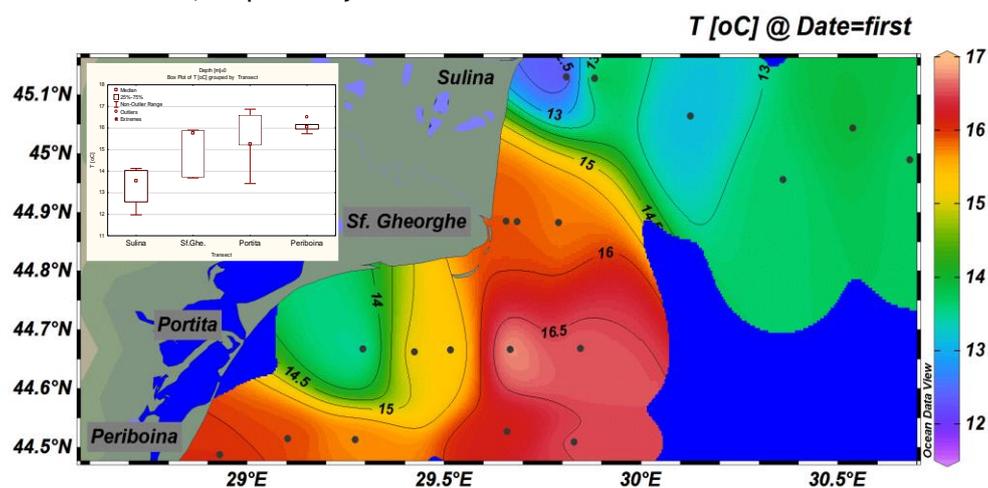


Figure 3.56- Surface seawater temperature, May 2019

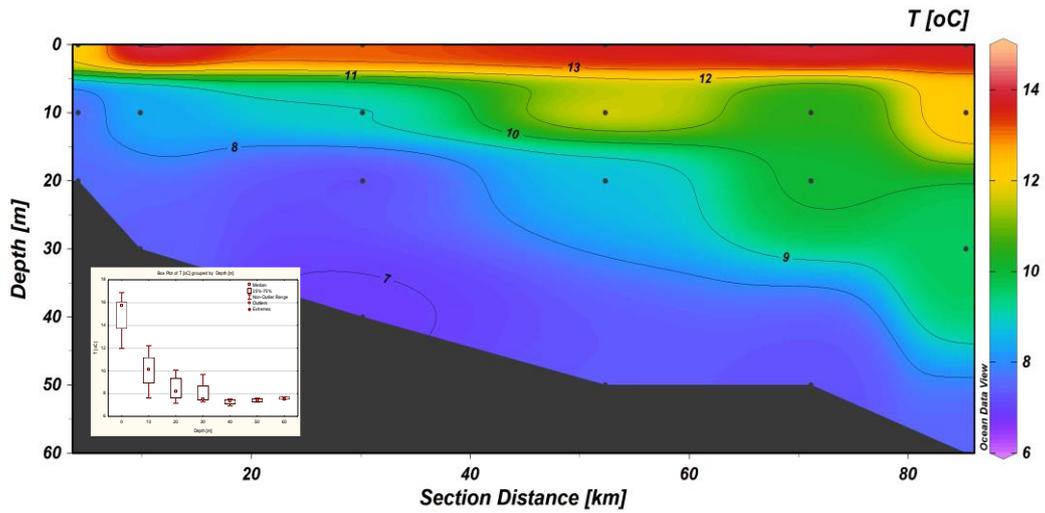


Figure 3.57 - Water column temperature, Sulina transect, May 2019

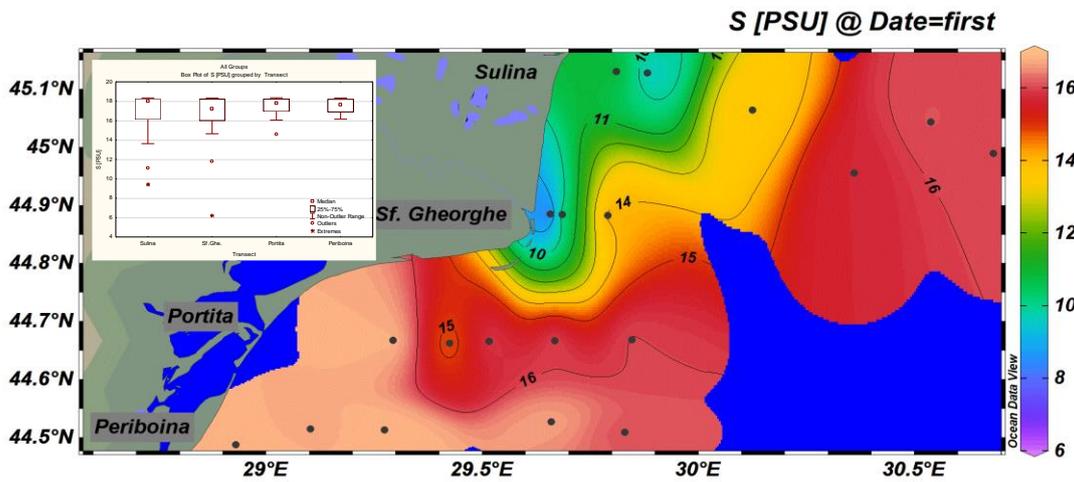


Figure 3.58 - Surface salinity, May 2019

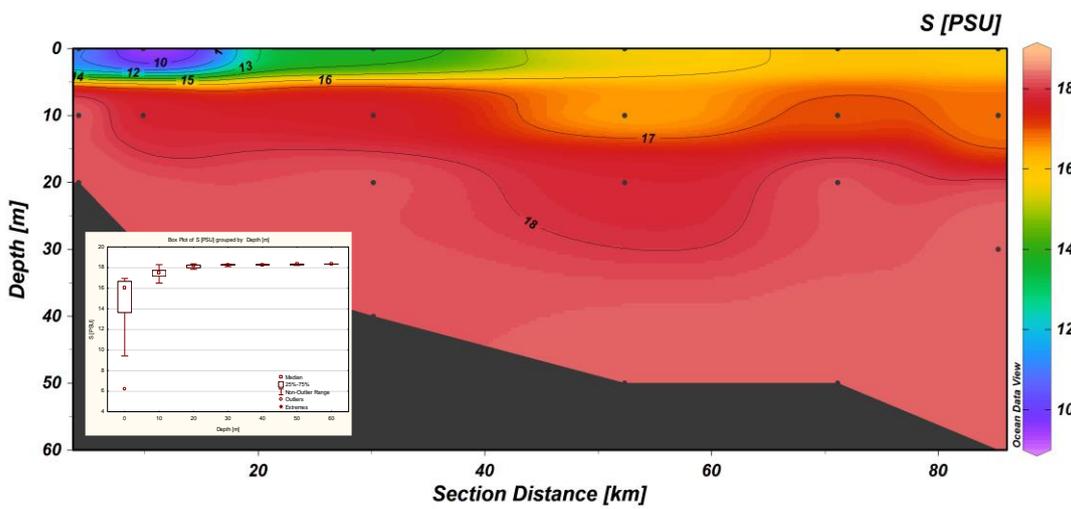


Figure 3.59 - Water column salinity, Sulina transect, May 2019

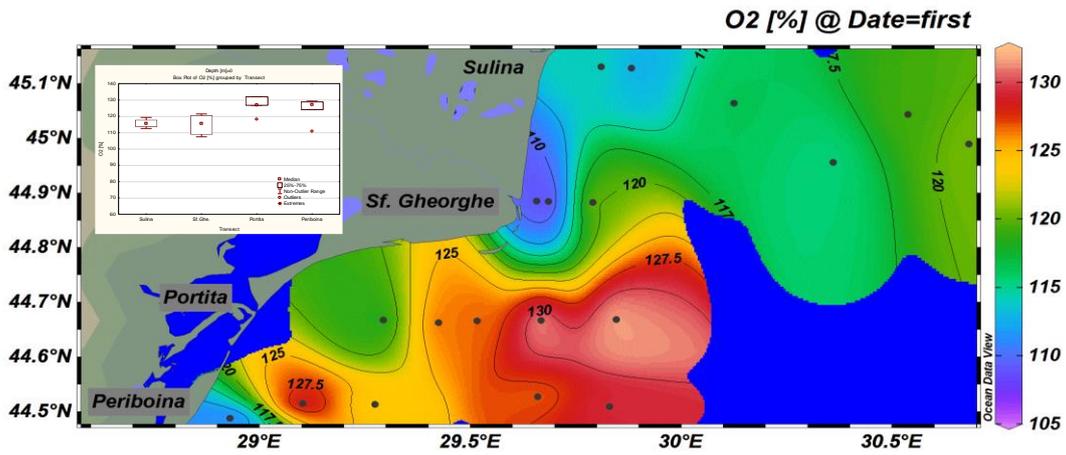


Figure 3.60 - Surface Dissolved oxygen saturation, May 2019

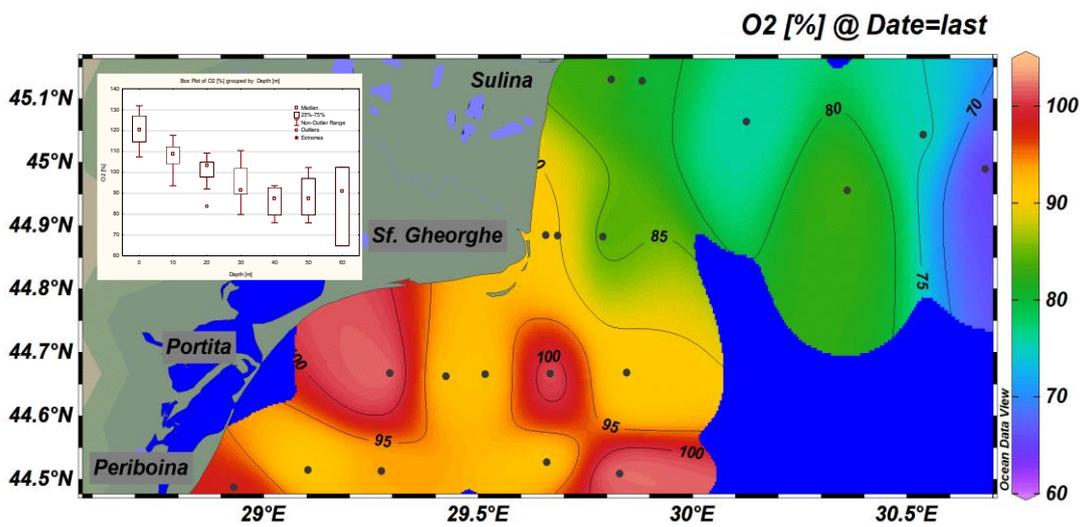


Figure 3.61 - Bottom Dissolved and water column oxygen saturation, May 2019

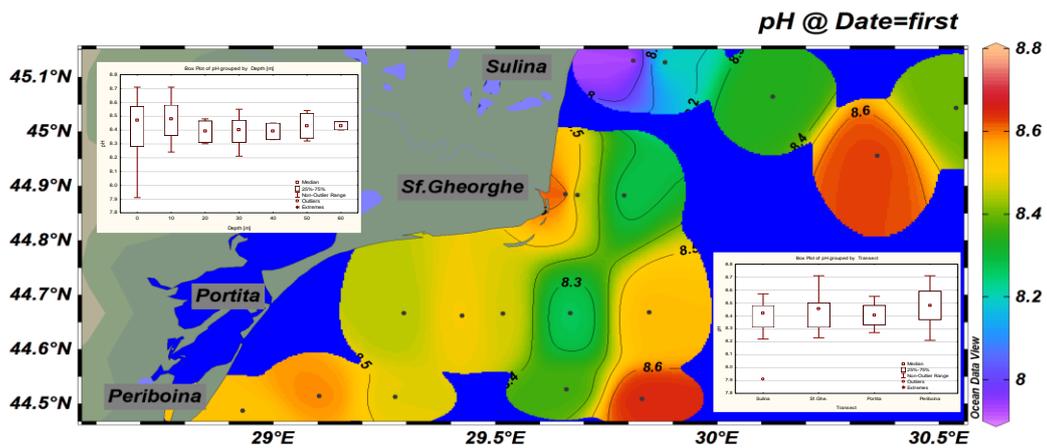


Figure 3.62 - pH variability (0 m, water column and by transect), May 2019

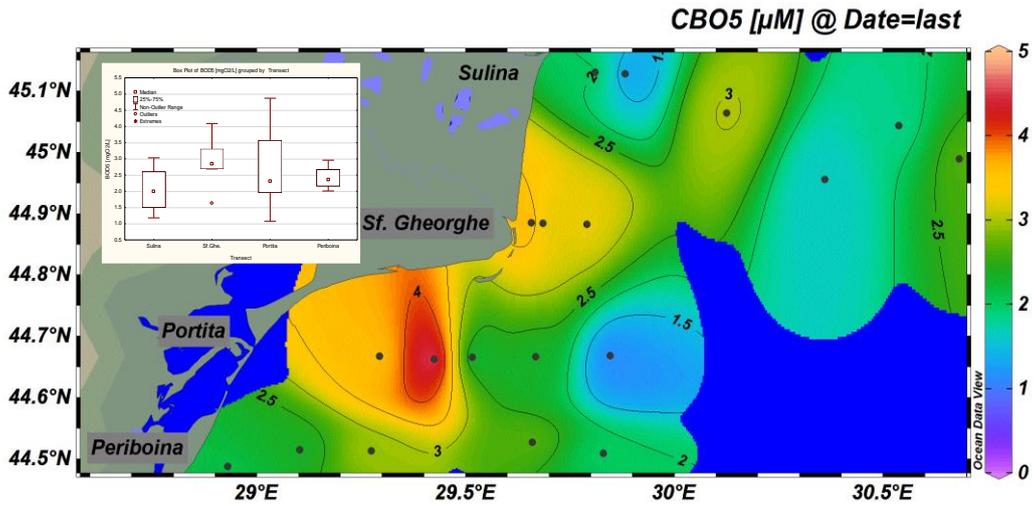


Figure 3.63 - Biological Oxygen Demand variability (0 m), May 2019

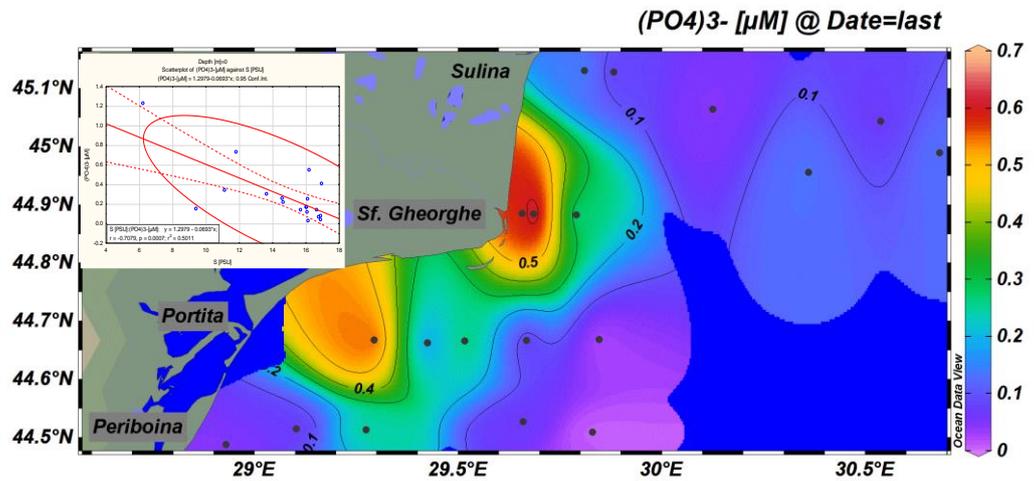


Figure 3.64 - Phosphate concentrations spatial distribution (0 m) and correlation with salinity (0 m), May 2019

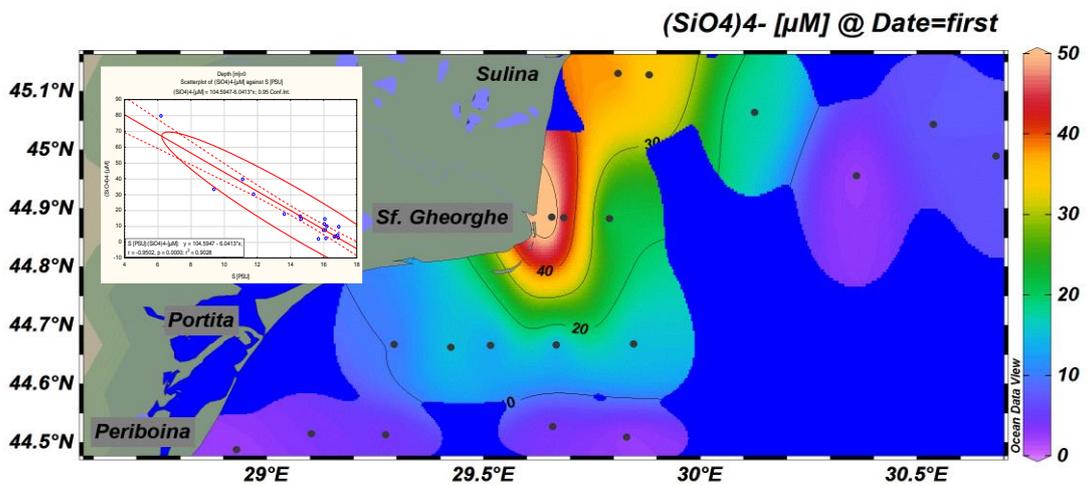


Figure 3.65 - Silicate concentrations spatial distribution (0 m) and correlation with salinity (0 m), May 2019

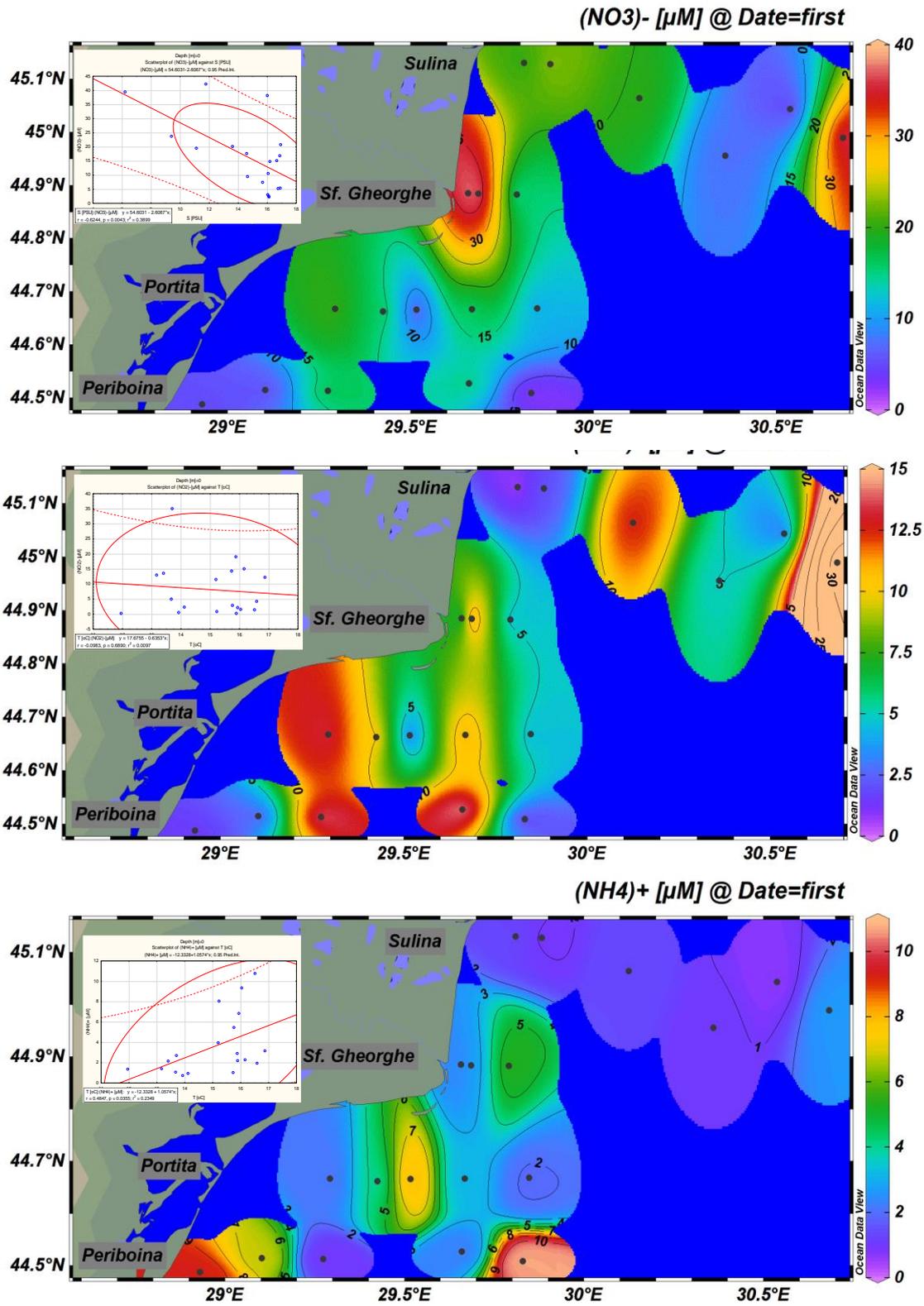


Figure 3.66 - Inorganic nitrogen forms (nitrate, nitrite, and ammonium) concentrations spatial distribution and correlations with salinity (0 m), May 2019

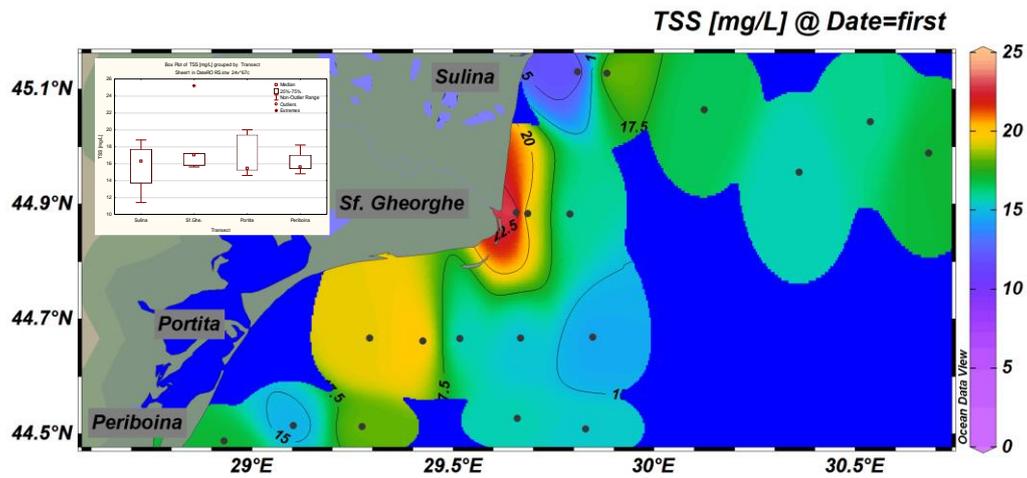


Figure 3.67 - Total Suspended Solids (TSS) spatial distribution and by transect (0 m), May 2019

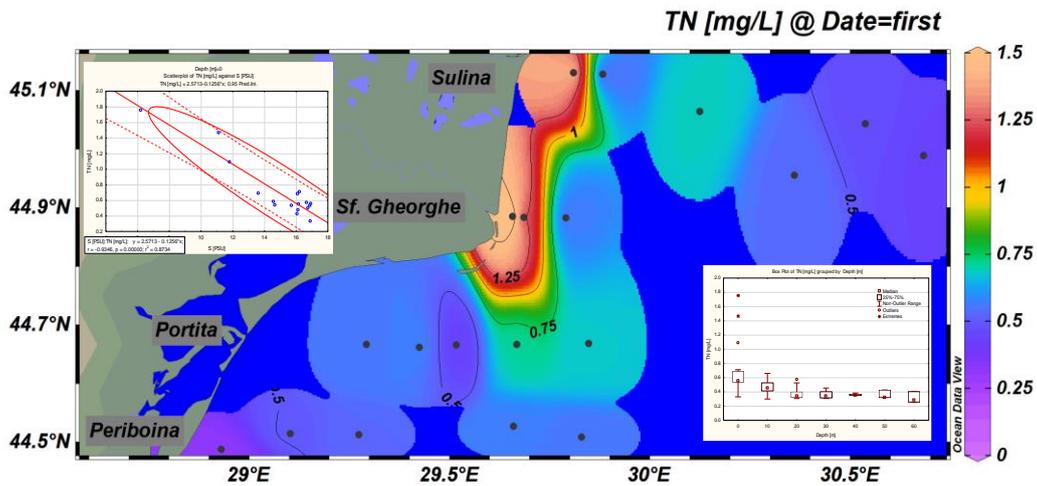


Figure 3.68 - Total Nitrogen (TN) variability (0 m and water column) and correlation with salinity, May 2019

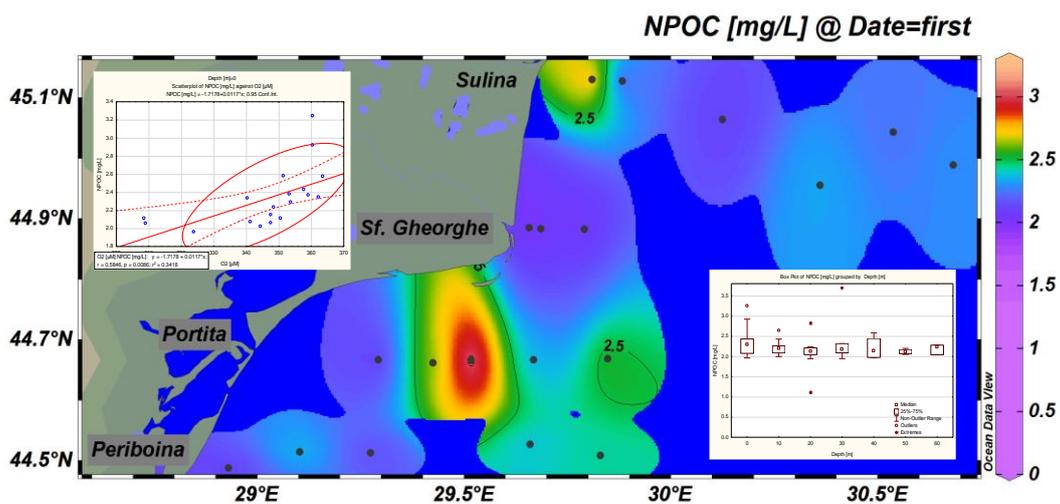


Figure 3.69 - Total Organic Carbon as Non Purgeable Organic Carbon (NPOC) variability (0 m and water column), and correlation with dissolved oxygen concentrations at surface, May 2019

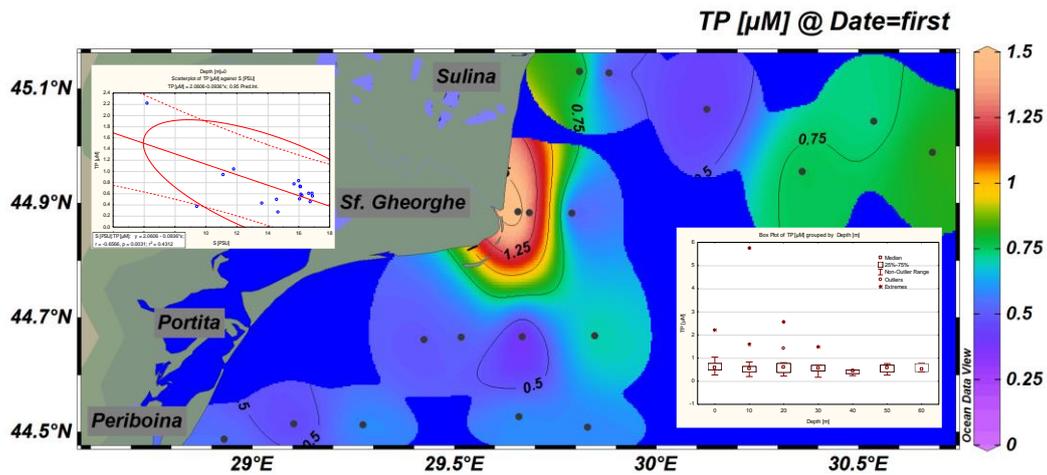


Figure 3.70 - Total Phosphorus (TP) variability (0 m and water column), and correlation with salinity, May 2019

### Kamchia River

According to the Koppen climate classification, the study area lies in a warm oceanic climate/humid subtropical zone (Cfa) with hot and humid summers<sup>8</sup> [6]. The precipitation seasonality mainly controls the water discharge distribution. The wet season shows a water discharge increase in May and June. The rainfall regime during the last decade manifests high year to year variability but is maintained above the average (1960-1990) in more than 70 % (Figure 3.72 a, b). A remarkable for Bulgarian rainfall, particularly in the research area, significantly exceeding the mean norm (approx. 10 folds) was reported in spring-summer 2014 (Drenovski and Kastreva, 2017), with maximum extremes measured in June (211mm - 458 %) (Figure 3.72b). Subsequently, an increase in the river discharge rate was measured in the Kamchia watershed ( $Q_{max}=200 \text{ m}^3/\text{s}$ ), resulting in salinity lower than 13 PSU in the coastal area's mixing zone. The precipitation in 2016 (spring, about four times higher than the norm) was considerably lower than in 2014, but similar environmental changes were observed. Pearson correlation analysis was used to highlight a statistically significant association of river discharge with seawater nutrient content.

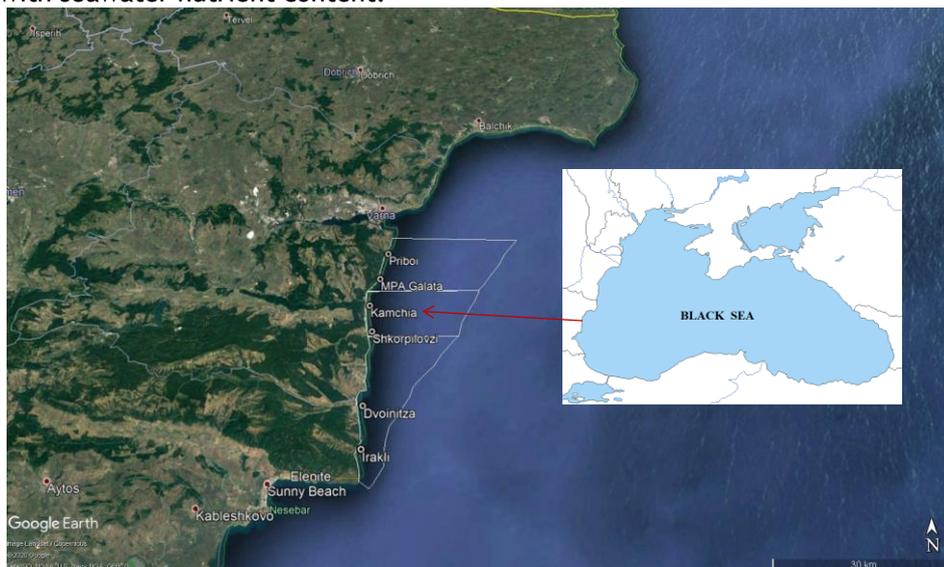
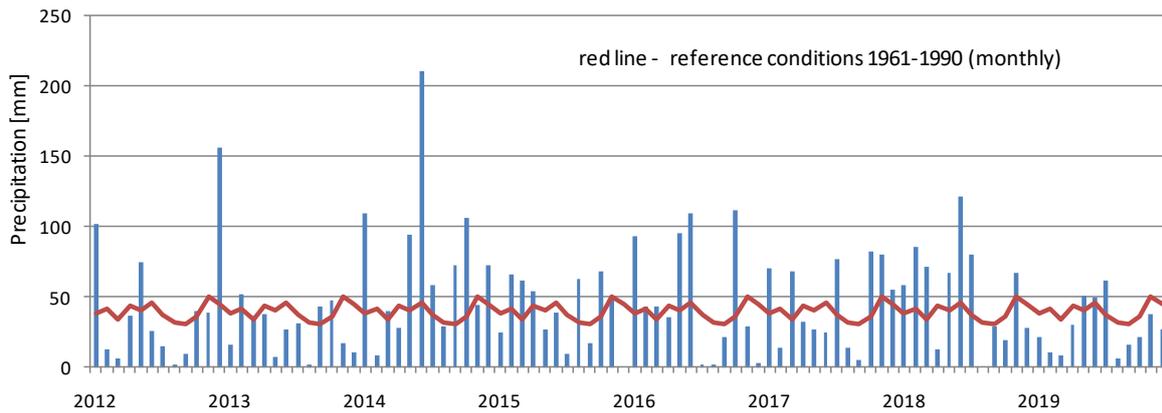


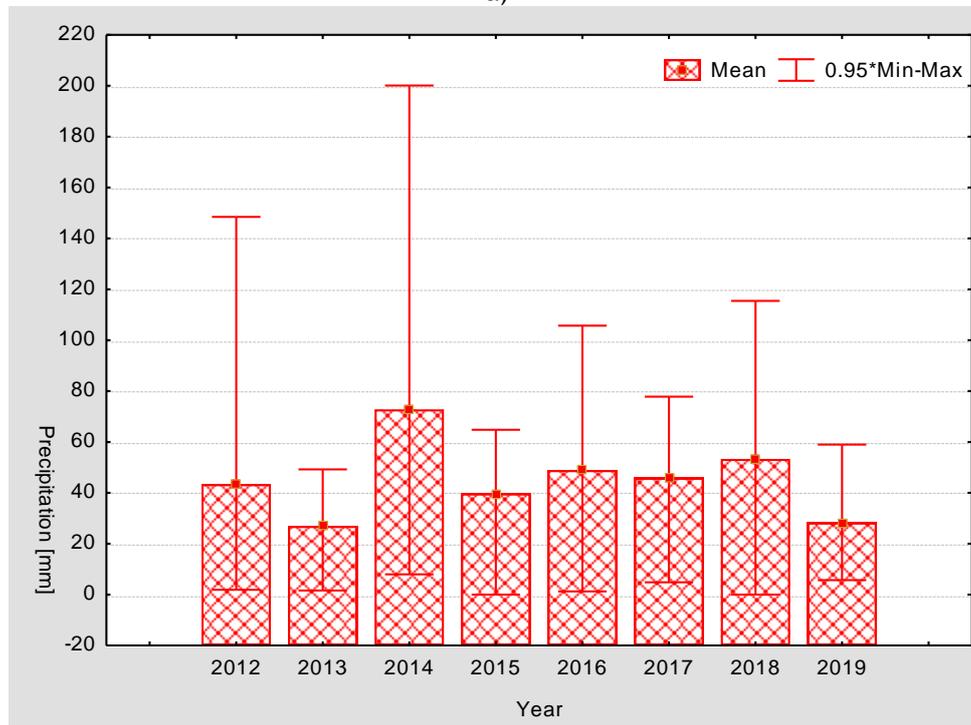
Figure 3.71 - Location of sampling station Kamchia (red arrow) in front of the river mouth

<sup>8</sup> <https://willkommen-in-germany.tumblr.com/post/140352779606/germany-by-k%C3%B6ppenclimate-classification-map=pdf&page={page}&subfolder=default/files/nodes/documents/>

Initially, rivers' capability to export nutrients is controlled by water discharge, which is a function of climate, topographic relief, water retention properties of the soils, and the geologic structure of the basin. Episodic but extreme rainfall events can create pulsed riverine discharge events, increasing both nutrients and sediments. Hydrographic input of rivers is related to seasonal variations in precipitation with mild, wet winters and cool, dry summers (Peterson et al. 1984). Riverine control of the plume spread is demonstrated by satellite imagery (Figure 3.73). Due to the intensive rainfall, the river discharge carried substantial nutrients loads (phosphates and silicates), directly impacting the coastal zone (Figure 3.74). Their concentrations varied between 0.02  $\mu\text{M}$  to 0.38  $\mu\text{M}$  and 1.30  $\mu\text{M}$  to 7.8  $\mu\text{M}$ , respectively, with a maximum in spring associated with the higher river run-off. Ammonium concentrations varied from 0.2  $\mu\text{M}$  to 23.8  $\mu\text{M}$ , with higher values occurring during summer when heterotrophic activity is at its maximum. Phosphate ranged from 0.03  $\mu\text{M}$  to 0.38  $\mu\text{M}$  with higher values tending to occur during spring (wet 2012), although high values also occurred in July 2016.



a)

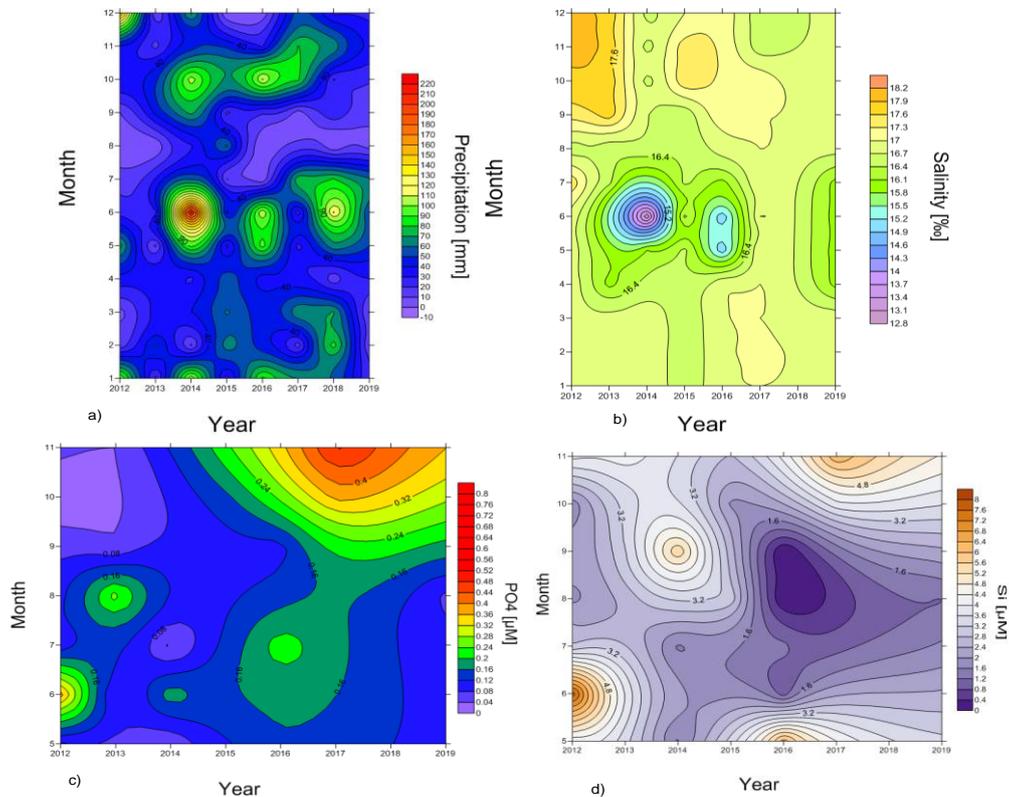


b)

**Figure 3.72 - Precipitation regime during 2012 - 2019 (a) monthly rainfall, red line - reference conditions 1961-1990; b) annual mean with minimum and maximum concentrations)**

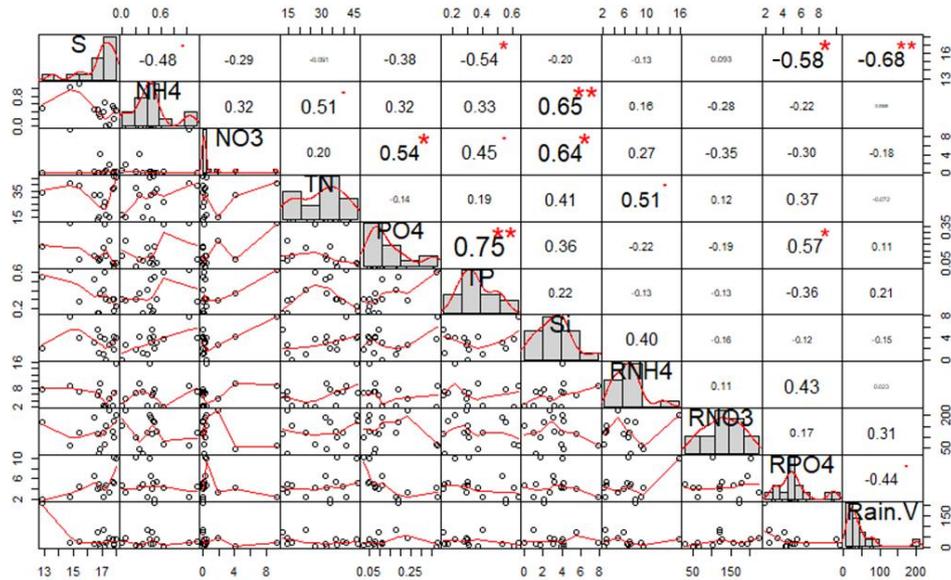


**Figure 3.73 - River plume size during the selected dry (2013, 2019) and wet scenario (2014, 2016); 2016 demonstrated double effect of phytoplankton bloom at basin scale and river outflow (Terra/MODIS True color images, 250m resolution, <https://wvs.earthdata.nasa.gov/>)**



**Figure 3.74 - Environmental variables co-variation (a) precipitation and salinity, b) PO<sub>4</sub> and Si at Kamchia monitoring station (Varna region), 2012-2019**

Correlation analysis of in-situ coastal water nutrients (PO<sub>4</sub>, TP, NH<sub>4</sub>, NO<sub>3</sub>, TN, Si) and salinity against river discharge loads (RPO<sub>4</sub>, RNH<sub>4</sub>, RNO<sub>3</sub>) and monthly average precipitation (Varna region) highlighted moderate to strong negative correlation ( $r=-0.68$ ,  $p=0.0003$ ) of rainfall with sea surface salinity, a moderate negative correlation of river discharge phosphates ( $r=-0.58$ ) with salinity and moderate positive correlation of river discharge ammonia concentrations with in situ total nitrates (Figure 3.75), indicating the association of environmental conditions to riverine pressures.



**Figure 3.75 - Correlation matrix plot of in situ environmental and river discharge variables: in situ nutrients,  $\mu\text{M}$  ( $\text{PO}_4$ ,  $\text{TP}$ ,  $\text{NH}_4$ ,  $\text{NO}_3$ ,  $\text{TN}$ ,  $\text{Si}$ ) and  $S$ - salinity; riverine discharge nutrients,  $\mu\text{M}$  ( $R_{\text{PO}_4}$ ,  $R_{\text{NH}_4}$ ,  $R_{\text{NO}_3}$ ) and precipitation in Varna, mm ( $\text{Rain.V}$ )**

## Conclusions

The Kamchia river discharge directly influenced the coastal waters near the mouth, as most of the main parameters, such as suspended solids, nitrite and ammonia nitrogen and phosphates, measured at the majority of monitoring points along the stream of the river were reported to exceed the ecological standards before entering into the Black Sea (Truhchev et al., 2010).

The results emphasize that the nutrient condition in the river - coastal gradient was decisively dependent on the extent of the river discharge (dry-wet scenario), the riverine inflow's interaction with the waters of marine origin, and the topography of mixing and current patterns.

Apart from the peak flow events, the River Kamchia impact is traceable throughout the inner coastal area only, located mainly in the one-mile coastal zone, as documented by previous studies (Truhchev et al. 2010, Shtereva et al., 2010a).

## Sakarya and Yesilirmak Rivers

In front of the Sakarya River, in the summer cruise were multiple layers (Figure 3.76). There was a warm ( $\sim 26^\circ\text{C}$ ) and less saline ( $\sim 16$  PSU) water mass at the uppermost top due to atmospheric heating and river input. Between 5 m and 25 m depth were sharp halocline and thermocline layers. With these layers' combination, the potential density of water increased from  $9 \sigma\text{-t}$  to  $14 \sigma\text{-t}$  drastically. The water temperature and salinity at 25m depth were  $\sim 10^\circ\text{C}$  and  $\sim 18.5$  PSU, respectively. Below this depth, the temperature gradient was slight along with the depth, and the minimum was  $8.6^\circ\text{C}$ . Alternatively, there was a second halocline layer observed. This layer started at 80 m depth until the sea bottom. The salinity and density increased to  $\sim 21.2$  PSU and  $16.4 \sigma\text{-t}$ , respectively. This multiple-layered structure also affected the dissolved oxygen content of the water column. At the surface, the dissolved oxygen was  $250.1 \mu\text{M}$ , whereas the highest content of dissolved oxygen ( $\sim 312 \mu\text{M}$ ) was between 20 m and 85 m, where the temperature of the water was low. The dissolved oxygen below 85 m depth dramatically decreased, and  $\sim 150$  m depth it was depleted. The peak values of the fluorescence ( $\sim 2.5 \text{ mg/m}^3$ ) at all stations were measured at 20 m depth which is fully supported with high water column chlorophyll *a* concentrations measured at about 20 m during the summer season. Dissolved oxygen values also indicate comparative high values of primary productivity at that depths.

In winter, cold water at the surface increased the sea's density by at least  $+3 \sigma\text{-t}$  and at most  $+4.5 \sigma\text{-t}$  (Figure 3.77). Alternatively, the water column's stratification was primarily dominated by the water's salinity since the temperature was between  $8.9 \text{ }^\circ\text{C}$  (bottom) and  $11.8 \text{ }^\circ\text{C}$  (surface). The uppermost layer's salinity ranged between 16 PSU and 18 PSU because of the current direction of the Sakarya River in the Black Sea during the measurements. The outflow was moving to the northwest after leaving the river mouth; therefore, some stations should be considered less affected by the river. For example, the surface salinity of the SAK15 was 18.1 PSU, whereas for SAK05 was 16 PSU. Below 5 m depth, the river input diminished, and the salinity of all stations remained  $\sim 18.5$  PSU up to 95 m depth. From this depth, it was identified the halocline layer until the bottom. The maximum salinity at the bottom was 21.2 PSU. Dissolved oxygen was  $281.4 \mu\text{M}$  from the surface to the halocline. However, within the halocline layer, the dissolved oxygen content was consumed and depleted at the 140 m depth. The fluorescence values were less than  $1 \text{ mg/m}^3$  at all stations and the peak values generally located at the top. This means that the winter primary production levels are at maximum levels at the surface which was also supported with the distribution of chlorophyll-a concentrations measured at discrete depths of the water column.

At the Yeşilırmak River's mouth were three layers in summer (Figure 3.78). The first one was the mixed layer (0-25 m). A sharp pycnocline was just below the mixed layer up to  $\sim 30$  m depth. The last layer was a slight pycnocline where the salinity increased until the bottom.

In the mixed layer, the temperature, salinity and density were  $25.5 \text{ }^\circ\text{C}$ , 18 PSU, and  $10.4 \sigma\text{-t}$ . At the surface, only one station was under the strong influence of the river input in this dry season. YSL11 station had 1 PSU lower salinity up to 2 m depth than the others. In contrast with the surface, physical parameters were  $8.7 \text{ }^\circ\text{C}$ , 20.6 PSU and  $15.9 \sigma\text{-t}$ , at the bottom. Dissolved oxygen was  $240.7 \mu\text{M}$  at the mixed layer, increased up to  $356.4 \mu\text{M}$  at the thermocline, and hereafter decreased with depth. The fluorescence showed two different peaks (at most  $2.5 \text{ mg/m}^3$ ) in the water column. Some stations which were generally close to the river mouth had higher values at the surface while the others had their peaks at 45 m depth. When the deeper stations' water column chlorophyll *a* concentration are examined, maximum values were found at about 40-45 m depths having concentrations in the range of  $0.8\text{-}1.0 \mu\text{g/L}$  supporting the in-situ measurements. This is a feature of the summer season for the eastern basin marine waters. At shallower depths, however, because of river impact on the coastal environment and less light transparency through the water column, maximum values were found at the surface with higher concentrations.

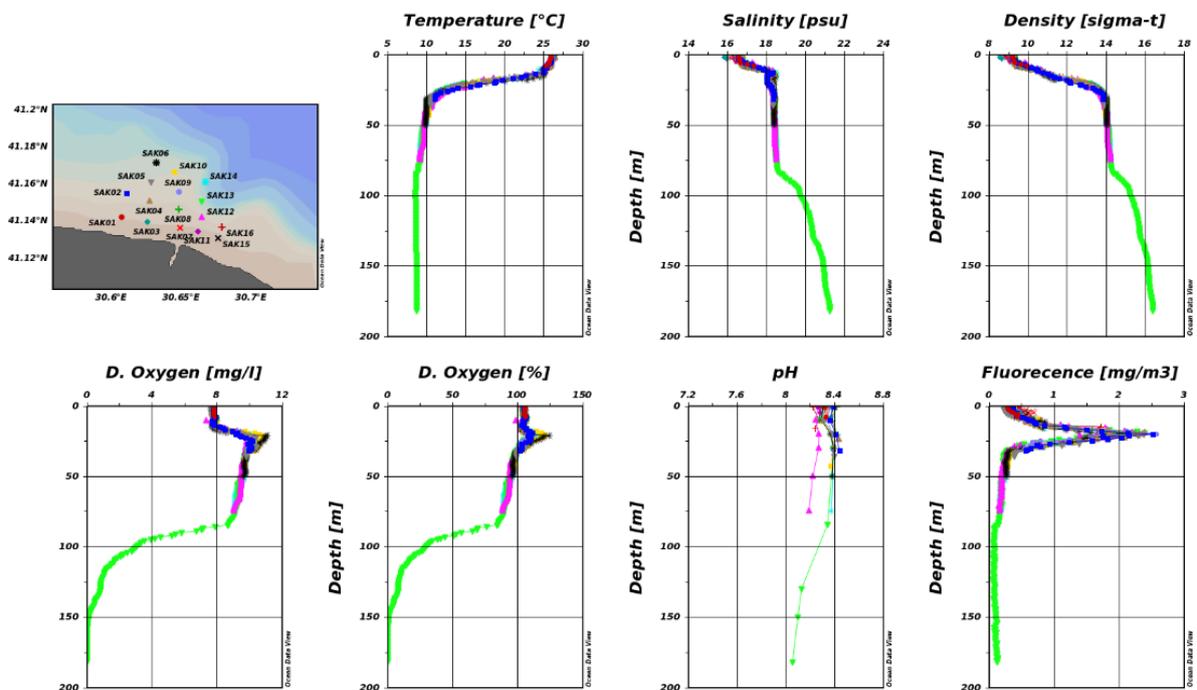


Figure 3.76 - Temperature, salinity, density, dissolved oxygen, pH and fluorescence summer - Sakarya River, July 2019

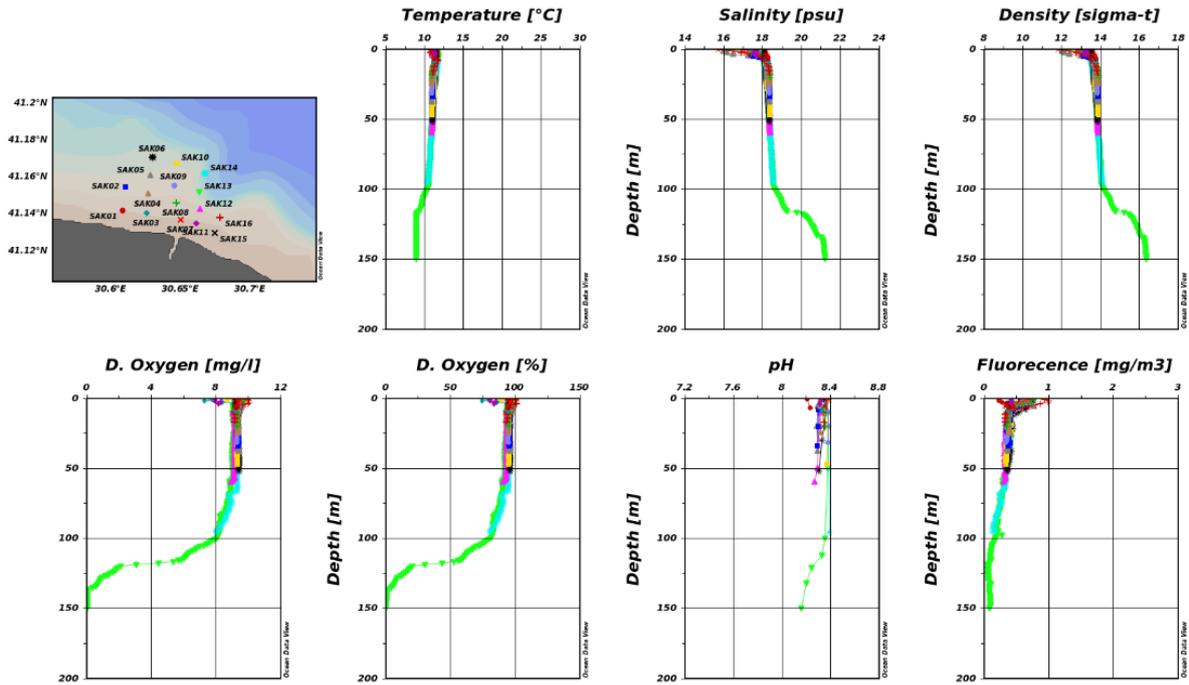


Figure 3.77 - Temperature, salinity, density, dissolved oxygen, pH and fluorescence winter - Sakarya River, January 2020

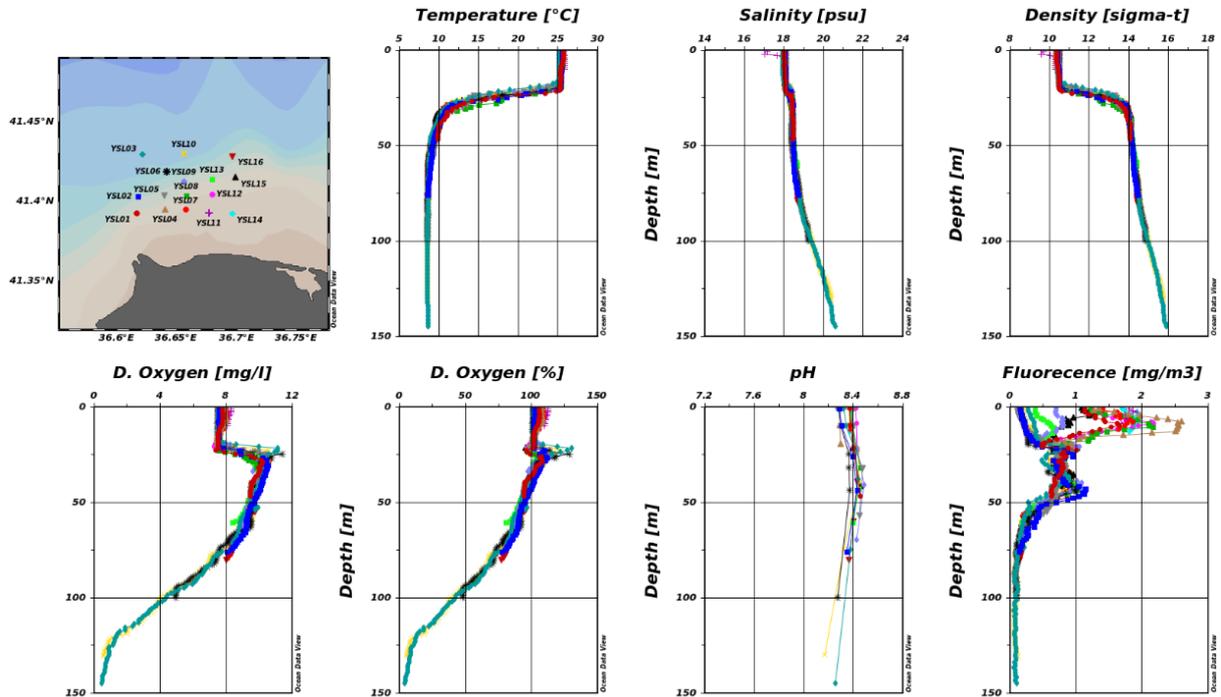
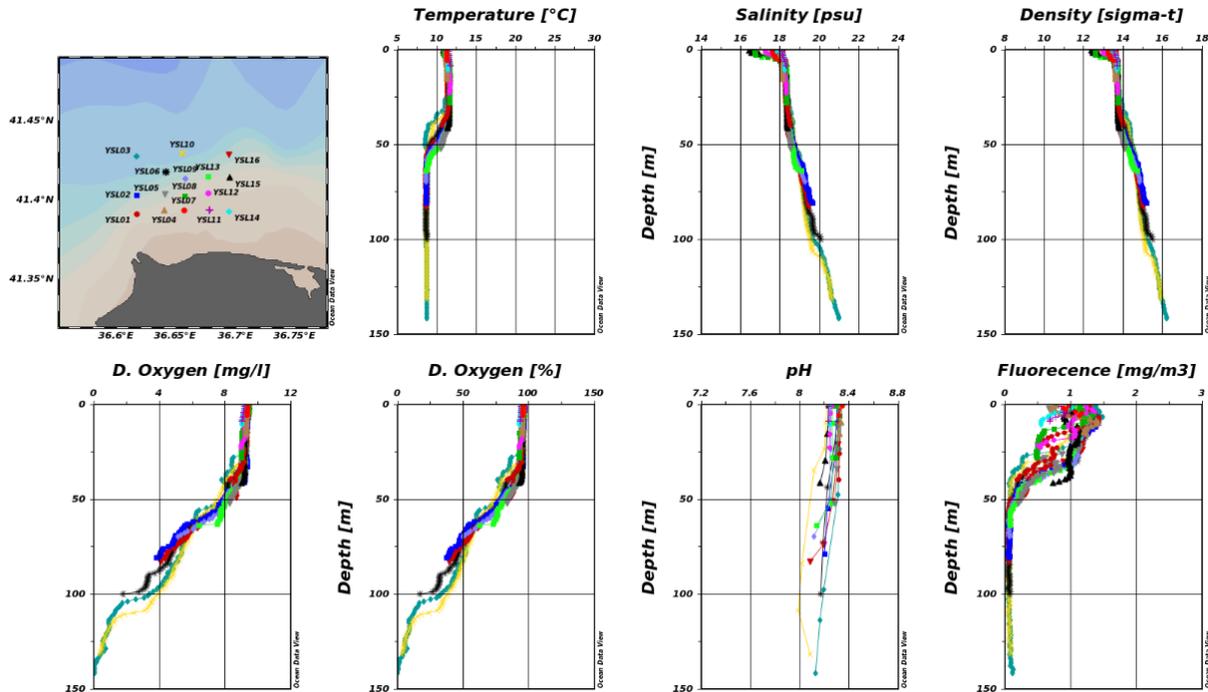


Figure 3.78 - Temperature, salinity, density, dissolved oxygen, pH and fluorescence summer, Yeşilirmak River, July 2019

In winter (Figure 3.79), the surface water density was greater than in summer due to cold water. However, the salinity was the main parameter leading the density to increase since the temperature was between 8.5 °C and 11.7 °C. According to salinity, the mixed layer had 40 m thickness at the surface; below this layer was located the halocline layer, where salinity increased to 21 PSU. Moreover, more stations, which were generally the eastern stations, were under a strong influence

of the river input in this rainy season. These stations had up to 1.5 PSU lower salinity values than the western ones had. The dissolved oxygen was constant in the mixed layer, and it was  $\sim 287.2 \mu\text{M}$ . However, it was decreased in the pycnocline layer and consumed at the bottom. The peak values of fluorescence in the water column were consistently observed in the mixed layer; however, all of them were less than  $1.5 \text{ mg/m}^3$ . Therefore, the primary production in winter could be less than in summer, for the considered studies.



**Figure 3.79** Temperature, salinity, density, dissolved oxygen, pH and fluorescence winter, Yeşilırmak River, January 2020

TNOx concentrations of the surface mixed layer waters varied from  $0.16 \mu\text{M}$  to  $11.40 \mu\text{M}$  (mean,  $2.12 \mu\text{M}$ ) in winter (Figure 3.80) and from  $0.05 \mu\text{M}$  to  $9.56 \mu\text{M}$  (mean,  $1.44 \mu\text{M}$ ) in summer (Figure 3.81) for the coastal waters of the Sakarya River. For Yeşilırmak River influence area, in the surface waters, TNOx concentrations were between  $0.30 \mu\text{M}$  and  $5.08 \mu\text{M}$  (mean,  $1.82 \mu\text{M}$ ) in winter and changed between  $0.05 \mu\text{M}$  and  $1.58 \mu\text{M}$  (mean,  $0.20 \mu\text{M}$ ) in summer. The winter and summer values of TNOx in front of the Sakarya River were close to each other. However, for the Yeşilırmak River, winter values were higher compared to summer. Since Sakarya River basin has a higher level of nitrogen inputs compared to Yeşilırmak River, we assume that the high urban and industrial pressures on Sakarya River resulted in pollution of its coastal and open sea areas, which is higher compared to the Yeşilırmak River. Higher terrigenous inputs from the Sakarya River might also be affecting the higher nitrogen concentrations.

Ammonium concentrations in front of Sakarya and Yeşilırmak Rivers showed a similar profile to TNOx concentrations. The highest values were in front of Sakarya River stations.

Orthophosphate ( $\text{PO}_4$ ) concentrations in the surface waters of Sakarya River influence area varied between  $0.07 \mu\text{M}$  and  $0.64 \mu\text{M}$  ( $0.10\text{-}0.69 \mu\text{M TP}$ ) in winter and between  $0.02 \mu\text{M}$  and  $0.44 \mu\text{M}$  ( $0.33\text{-}1.20 \mu\text{M TP}$ ) in summer. In Yeşilırmak River influence area,  $\text{PO}_4$  concentrations were in the range  $0.07\text{-}0.19 \mu\text{M}$  ( $0.07\text{-}0.46 \mu\text{M TP}$ ) in winter and  $0.02\text{-}2.97 \mu\text{M}$  ( $0.09\text{-}3.07 \mu\text{M TP}$ ) in summer. The highest phosphorus concentrations were especially in summer at the Yeşilırmak River influence area.  $\text{PO}_4$  and TP concentrations are much higher at Yeşilırmak River, as expected, and these high levels of  $\text{PO}_4$  and TP concentrations were associated with the agricultural activities in the river basin.

The silicate ( $\text{SiO}_2$ ) content of the coastal waters rises because of the terrigenous input. Si concentrations in the surface waters were in the range of  $1.68\text{-}14.63 \mu\text{M}$  (winter) and  $1.86\text{-}14.80 \mu\text{M}$  (summer) in the Sakarya River influence's coastal waters. In the Yeşilırmak River influence area, Si concentrations were between  $0.40 \mu\text{M}$  and  $13.13 \mu\text{M}$  (winter) and  $0.40\text{-}5.39 \mu\text{M}$  (summer). The average silicate concentrations were approximately the same in winter for both rivers; however, at Sakarya River area were about three times higher than at Yeşilırmak River in summer.

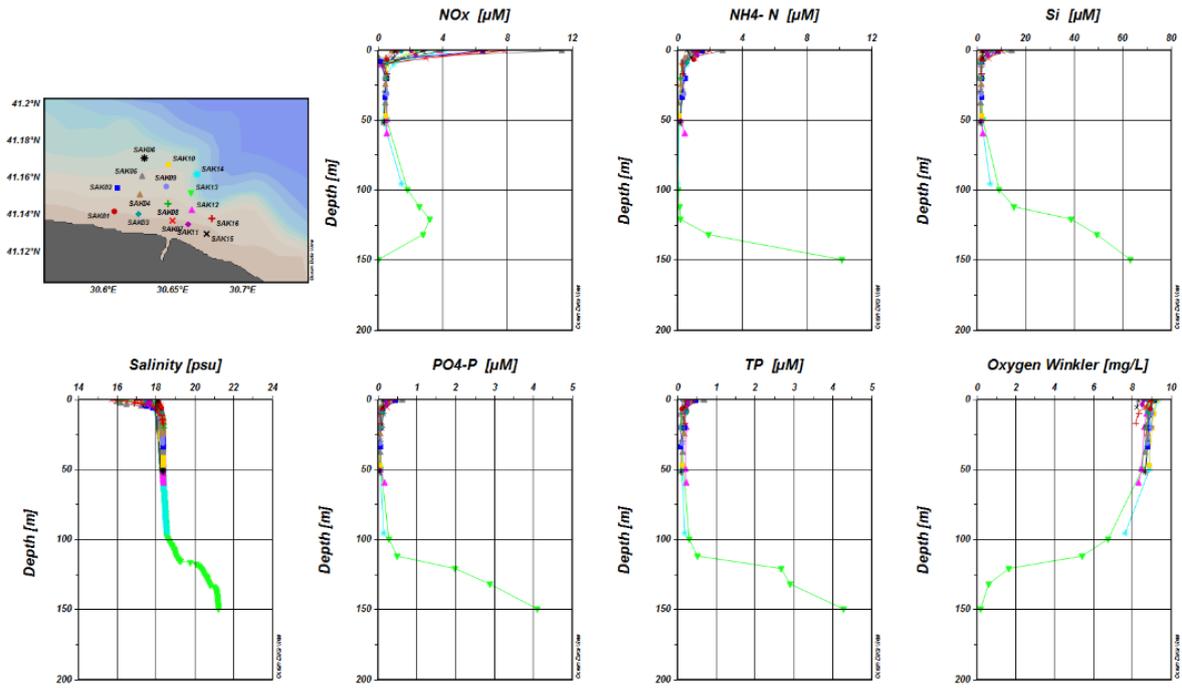


Figure 3.80 - Water column variability of total oxidised nitrogen (TNOx), phosphate ( $\text{PO}_4^{3-}$ ), silicate (Si), and dissolved oxygen (DO), Sakarya River, January 2020

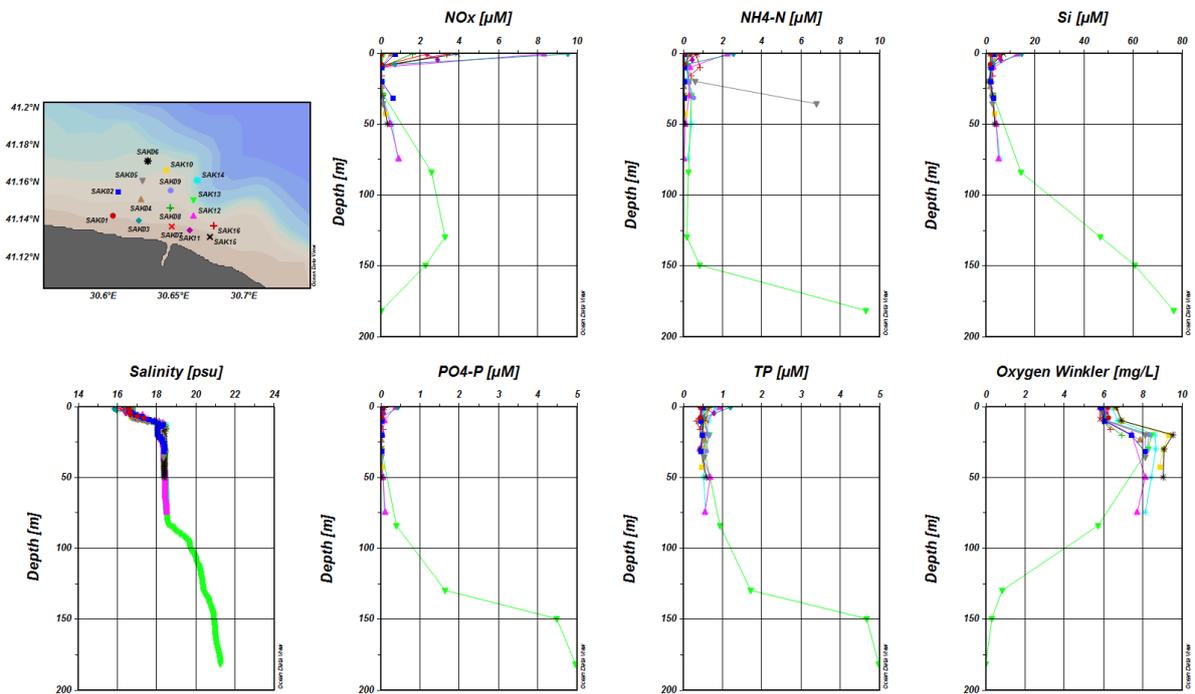


Figure 3.81 - Water column variability of total oxidised nitrogen (TNOx), phosphate ( $\text{PO}_4^{3-}$ ), silicate (Si), and dissolved oxygen (DO), Sakarya River, July 2019

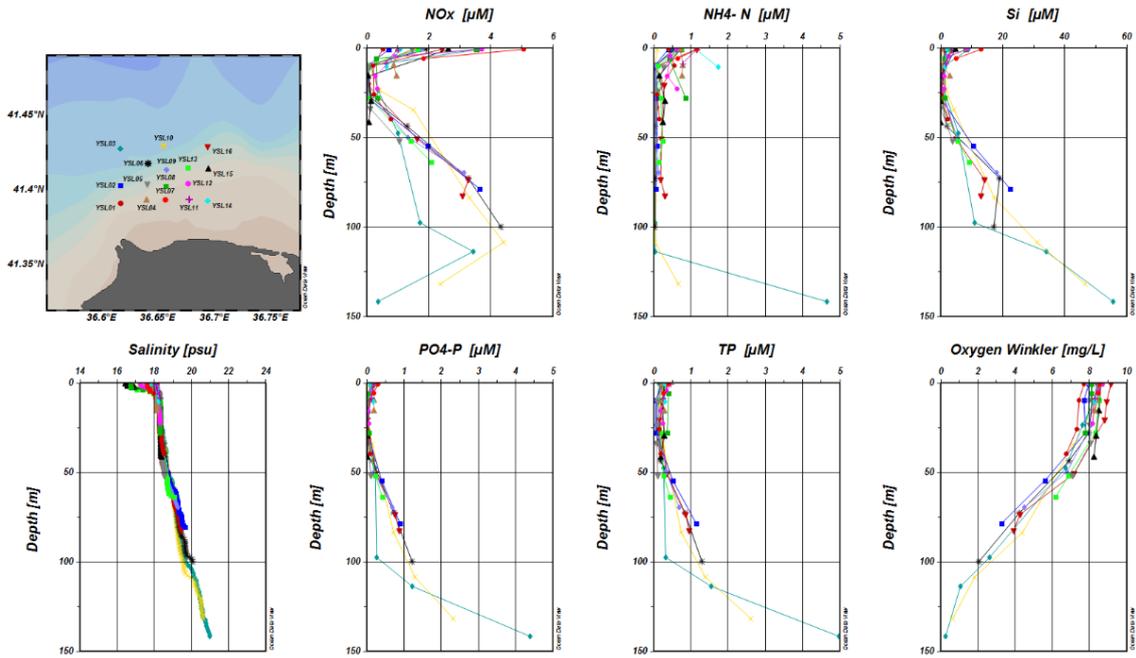


Figure 3.82 - The winter measurements of nitrogen ( $\text{NO}_2^+ \text{NO}_3^- \text{N}$ ), phosphorus ( $\text{PO}_4^{3-}$ ), silicate (Si), and dissolved oxygen (DO), Yeşilırmak River, January 2020

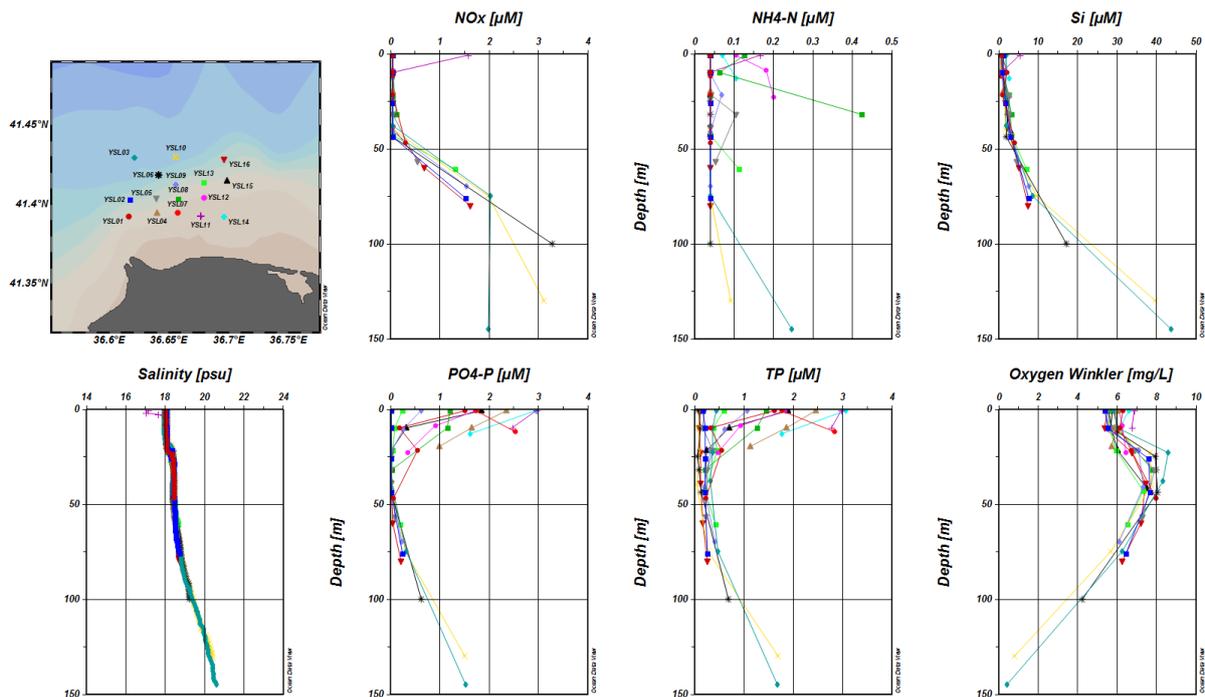


Figure 3.83 - The summer measurements of nitrogen ( $\text{NO}_2^+ \text{NO}_3^- \text{N}$ ), phosphorus ( $\text{PO}_4^{3-}$ ), silicate (Si) and dissolved oxygen (DO), Yeşilırmak River, July 2019

The surface mixed layer chlorophyll *a* concentrations ranged between 0.16  $\mu\text{g/L}$  and 1.57  $\mu\text{g/L}$  (winter) and 0.05  $\mu\text{g/L}$  and 0.93  $\mu\text{g/L}$  (summer) at Sakarya River influence area. Yeşilırmak River influence area had chlorophyll *a* values ranging between 0.32  $\mu\text{g/L}$  and 2.34  $\mu\text{g/L}$  (winter), and 0.17-4.05  $\mu\text{g/L}$  (summer). Yeşilırmak River chlorophyll *a* concentrations were higher than at Sakarya River in both periods, especially during summer; this seems to be related to the higher inputs of phosphorus at Yeşilırmak area during this period.

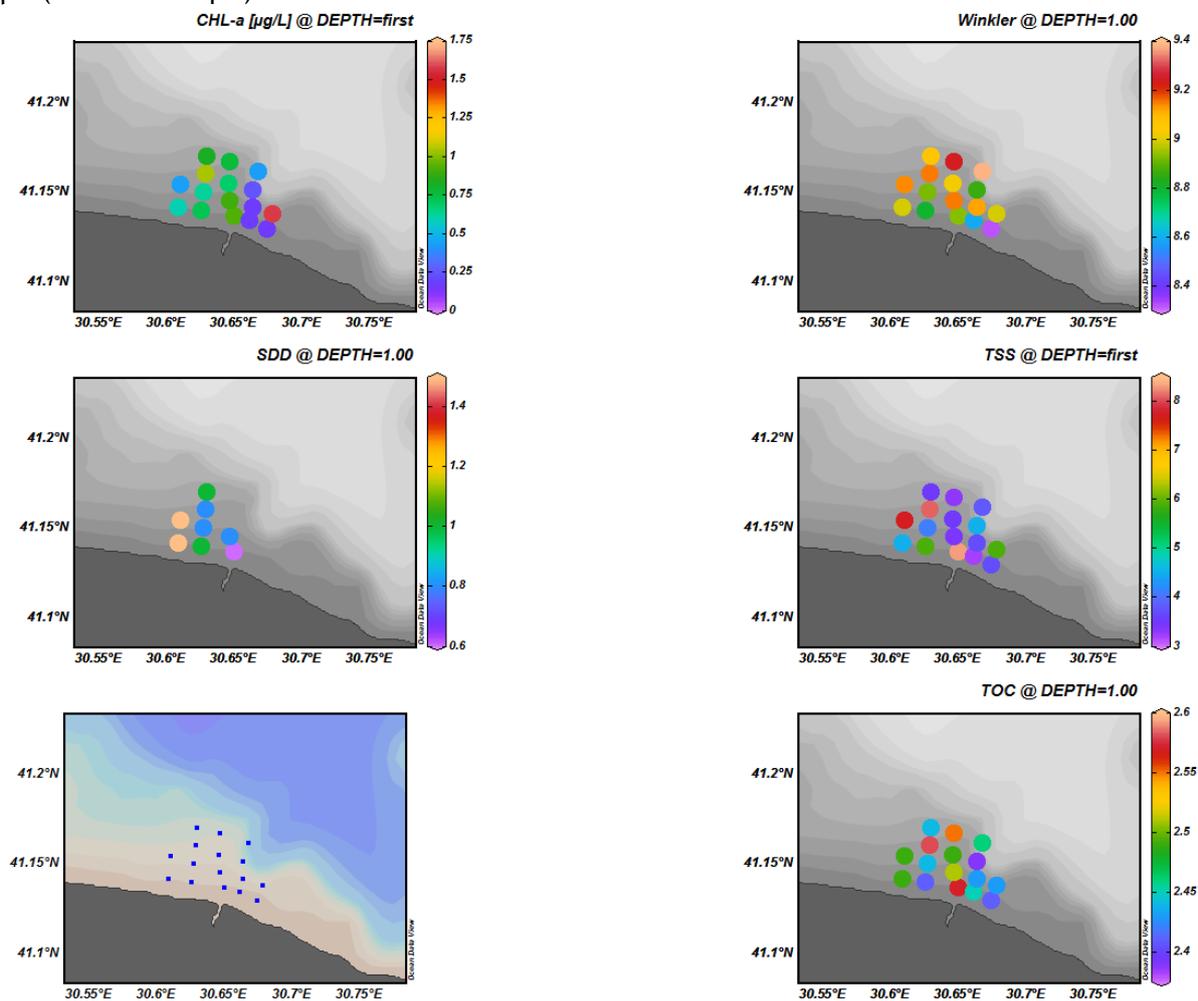
Seawater transparencies (Secchi Disc Depth) in the summer were very close to each other and ranged between 2 m and 8.5 m (Figure 3.84 and Figure 3.85). The measurements could not be performed in

winter at some stations in the Sakarya river influence area because of the rough sea. The winter values ranged between 0.5-6 m being lower at the Sakarya river influence area.

High values of total suspended solids were measured in front of the Sakarya river in winter, 3.2-8.3 mg/L and compared to 0.7-4.0 mg/L found in front of the Yeşilirmak river. In summer, the total suspended solids content was in the range of 0.1-2.7 mg/L, with relatively higher values in the shallow stations of the Yeşilirmak area.

The total organic carbon (TOC) contents varied from 2.22 mg/L to 5.48 mg/L, with an average of 3.06 mg/L; values in summer were higher than in winter, especially at the Yeşilirmak area.

The surface dissolved oxygen (DO) concentrations were measured in the range of 241.9-293.1  $\mu\text{M}$  in winter at both sites, and summer values were expectedly measured lower in the range of 168.4-208.1  $\mu\text{M}$ . Oxygen deficiency in the bottom layer waters has been considered as an indirect effect of eutrophication (MSFD, 2017) available at stations with bottom depth up to 50m. Therefore, bottom layer values are also evaluated (Figure 3.88). Sakarya area is represented with stations having depths of 6-230 m and near bottom DO values were measured < 0.25-301.7  $\mu\text{M}$  (mean: 215.9  $\mu\text{M}$ ) in summer and 6.3-280.3  $\mu\text{M}$  (mean: 250.9  $\mu\text{M}$ ) in winter. Near bottom DO values at the Yeşilirmak area stations having depths of 8-152 m were measured as 14.1-249.1  $\mu\text{M}$  (mean: 190  $\mu\text{M}$ ) in summer and 8.75-265.6  $\mu\text{M}$  (mean: 180.6  $\mu\text{M}$ ) in winter.



**Figure 3.84 - Surface chlorophyll a, dissolved oxygen, transparency (SDD), total suspended solids (TSS) and total organic carbon (TOC), Sakarya River, January 2020**

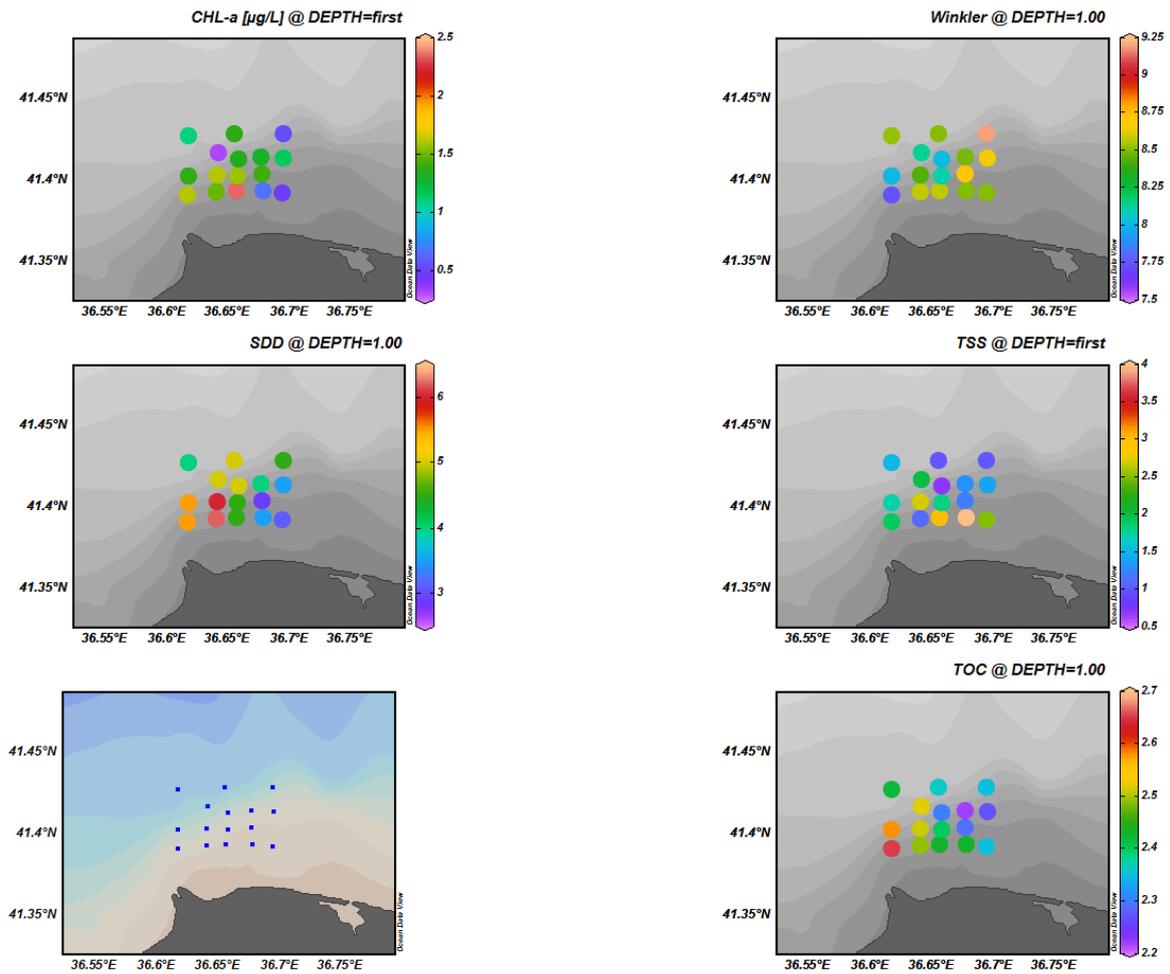


Figure 3.85 - Surface chlorophyll a, dissolved oxygen, transparency (SDD), total suspended solids (TSS) and total organic carbon (TOC), Yeşilırmak River, January 2020

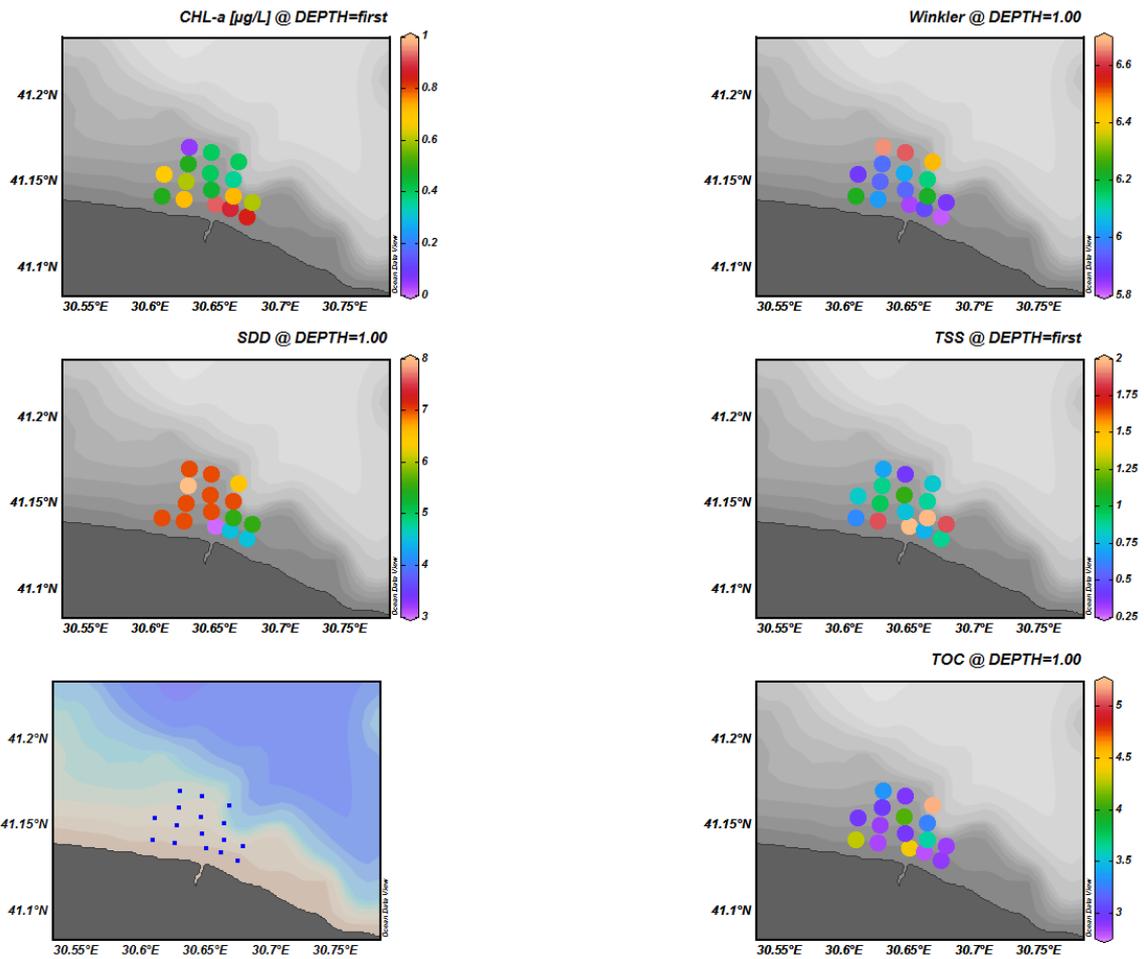
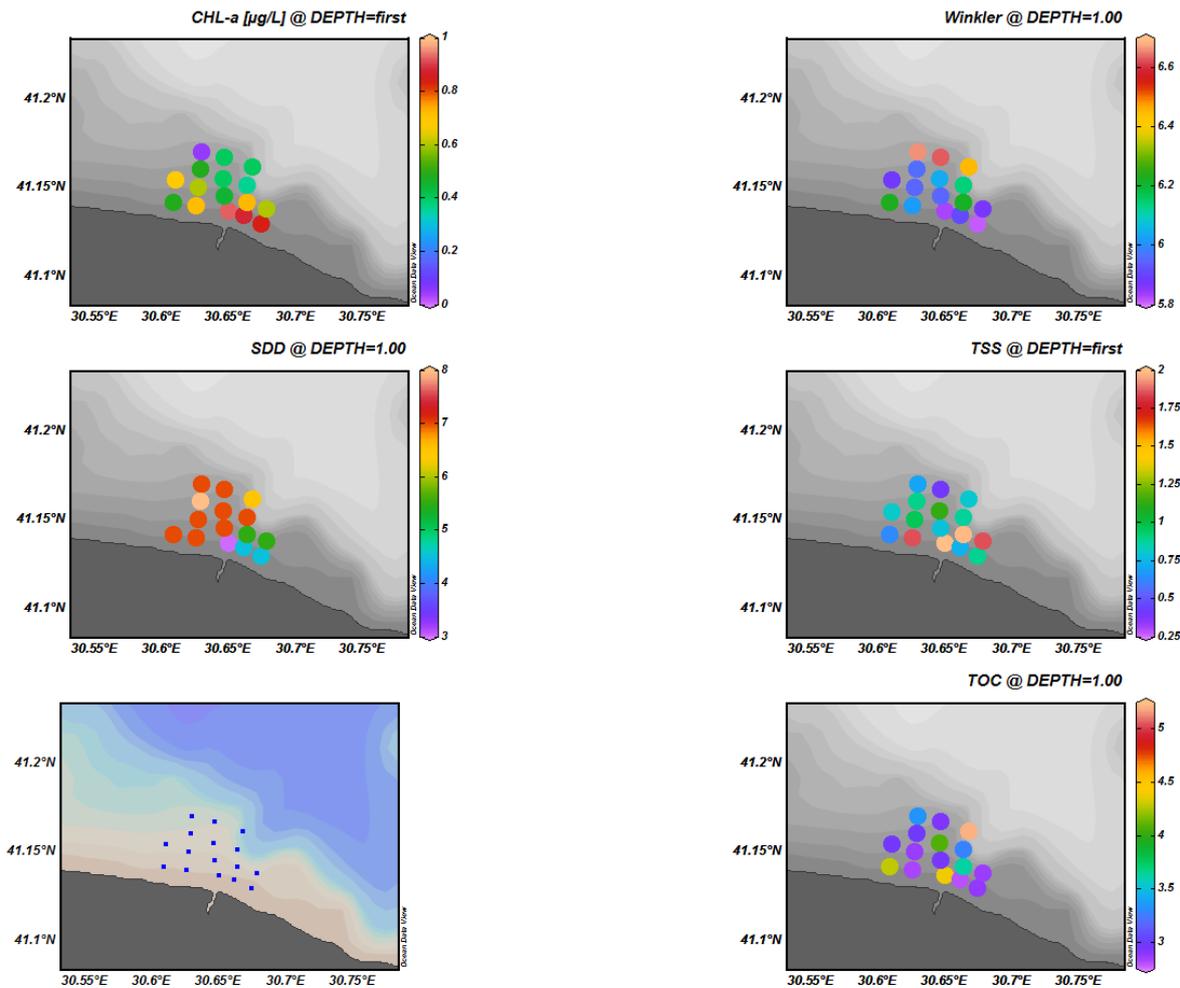


Figure 3.86 - Surface chlorophyll a, dissolved oxygen (Winkler), transparency (SDD), total suspended solids (TSS) and total organic carbon (TOC), Sakarya River, July 2019



**Figure 3.87 - Surface chlorophyll a, dissolved oxygen, transparency (SDD), total suspended solids (TSS) and total organic carbon (TOC), Yeşilırmak River, July 2019**

When surface layer data of all pelagic variables are correlated using Spearman's correlation method, significant relationships between few variables were obtained, changing seasonally and regionally. For example, chlorophyll a is not correlated with any other variable during winter at both sites; however, it has shown significant positive correlations with inorganic nutrients at the Sakarya River site in summer. It has a positive correlation with temperature, phosphorus, total suspended solids (TSS) and total organic carbon at Yeşilırmak site during summer, but inverse relation with salinity, transparency (SDD), and silicate whereas there is no relation with nitrogen. Almost all nutrients are inversely correlated with salinity at both sites during both seasons, emphasizing the effect of river inputs on the higher concentrations of nutrients. Unsurprisingly, SDD is positively correlated with salinity at both sites and seasons (Table 3.6).

Deeper layers -pycnocline and the underneath waters- exhibited an increasing trend of nutrients and decreased dissolved oxygen concentrations. Additionally, TNOx values usually start decreasing at around 100 m at both sites and periods, sometimes completely or almost depleted because of denitrification taking place at hypoxic and anoxic conditions. In this situation, phosphate, ammonium, and silicate show rapid increases till the bottom.

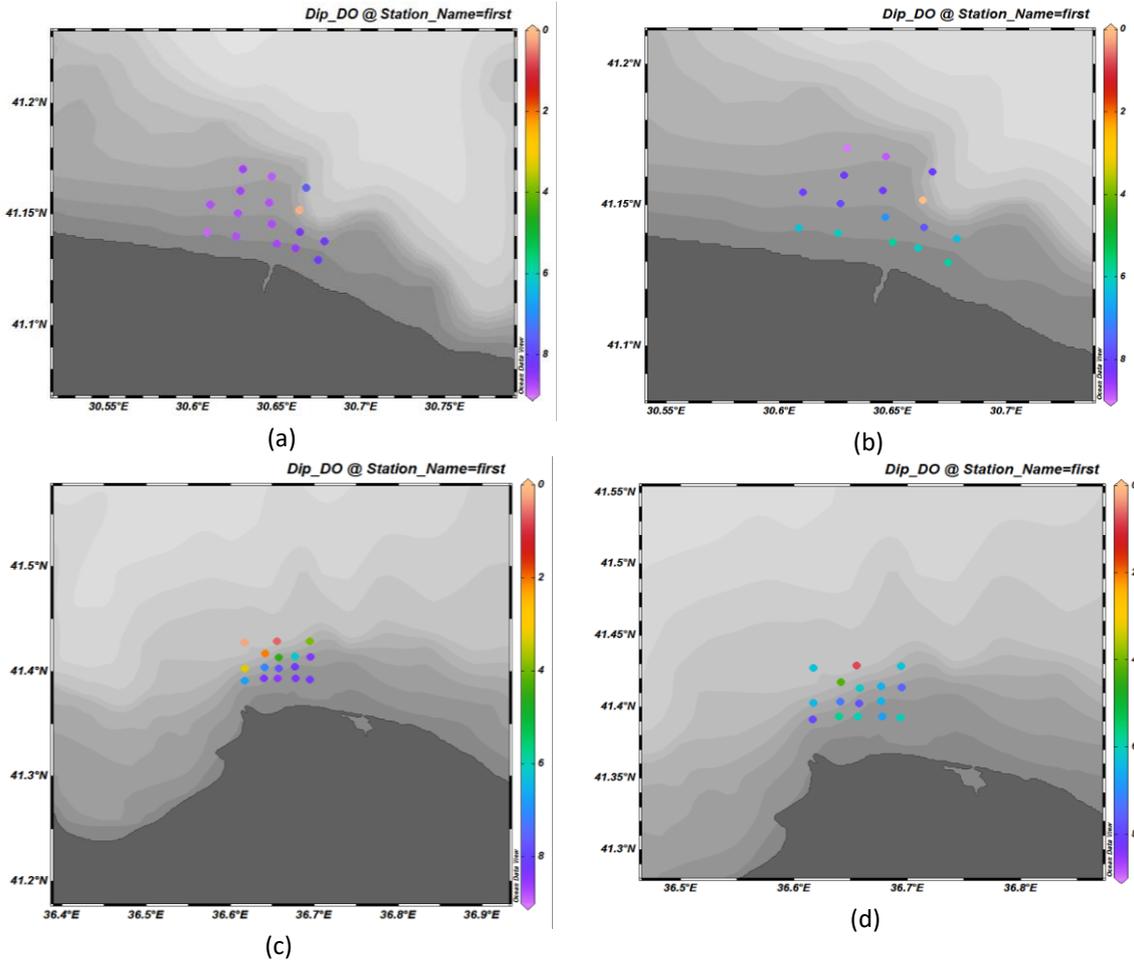


Figure 3.88 - Near bottom dissolved oxygen concentrations in front of the Sakarya River (a: winter, b: summer) and Yeşilırmak River (c: winter, d: summer)

Table 3.6 - Spearman's correlation of nutrients, chlorophyll and Secchi depth with salinity at Sakarya and Yeşilırmak river sites during summer and winter sampling periods

Parameter		Secchi	(PO <sub>4</sub> ) <sup>3-</sup>	TP	(SiO <sub>4</sub> ) <sup>4</sup>	(TNOx)	(NH <sub>4</sub> ) <sup>+</sup>	Chl_a	DIN
Sakarya River (Winter)									
S [PSU]	R <sup>2</sup>	0.75	-0.81	-0,70*	-0.79	-0.78	-0.74	-0.28	-0.77
	N	8	31	31	31	31	31	28	31
Sakarya River (Summer)									
S [PSU]	R <sup>2</sup>	0.72	-0.53	-0.46	-0.58	-0.82	-0.35	-0.60	-0.74
	N	15	32	32	32	32	32	20	32
Yeşilırmak R. (Winter)									
S [PSU]	R <sup>2</sup>	0.51	-0.58	-0.60	-0.81	-0.87	-0.50	-0.06	-0.84
	N	16	28	28	28	28	28	28	28
Yeşilırmak R. (Summer)									
S [PSU]	R <sup>2</sup>	0.68	-0.57	-0.62	-0.09	-0.41	-0.33	-0.49	-0.41
	N	16	29	29	29	29	29	22	29

\*Correlation is significant at the 0.05 level (2-tailed), \*\*Correlation is significant at the 0.01 level (2-tailed)

In this study, surface waters (<10 m) were under the Sakarya and Yeşilırmak Rivers' influence, and nutrient levels varied between the stations. It has been more homogeneous in the depth contour between 10-50 m, and nutrient salt changes remain in a narrow range. At the stations which are deeper than 40-50 m, the Black Sea water nutrient changes depending on the density throughout the water column.

### 3.1.4 Contaminants

Contaminants are defined in the EU legislation as, “substances (e. g., chemical elements and compounds) or group of substances that are toxic, persistent and liable to bio-accumulate and other substances or group of substances, which give rise to an equivalent level of concern” (Water Framework Directive, 2006). This definition is like the hazardous substances defined in OSPAR, HELCOM and Barcelona Conventions. Safe chemical contaminant concentrations are essential in achieving healthy, biologically diverse, and productive seas in the MSFD context (Law et al. 2010). MSFD considers synthetic and non-synthetic contaminants. The non-synthetic contaminants are naturally occurring chemicals such as trace metals found in the earth’s crust or polyaromatic hydrocarbons (PAH), resulting from fossil fuels’ combustion and organic materials. Synthetic contaminants are man-made products such as polychlorinated biphenyls (PCBs), pesticides, brominated flame retardants, dioxins and organotin (e.g. tributyltin - TBT) and introduced into the marine environment through human activities.

The sources of contamination are identical - if contaminants (whether naturally occurring or synthetic) enter the marine environment, they will be taken up by biota (via ingestion and adsorption) and passed up the food chain) to primary predators, including humans. In the Black Sea region of Turkey, contaminants arise from numerous anthropogenic sources such as land-based industrial and agricultural activities, pollution by ship, atmospheric deposition and mineral exploration and riverine inputs. They include synthetic compounds, such as pesticides from agricultural activities, and non-synthetic compounds, such as metals, dispersed by industrial processes, and polycyclic aromatic hydrocarbons, dispersed by combustion and oil spills.

Contaminants adsorbed to particulate matter are deposited in the water column and stored in the sediment (Sigg et al., 1987; Hakanson, 1977; Thomas, Meybeck, 1996). Depending on natural or physical conditions, it acts as a source of contamination for aquatic organisms that feed on particulates through resuspension or release into the water phase through desorption. The contamination above a certain level lead to negative consequences such as loss of biodiversity (Föstner, Whitman, 1981, Kruopiene, 2007; Brils, 2008; Ozkan, Buyukisik, 2012).

Metal pollution has been accepted as one of the anthropogenic pressures for the marine environment. Besides its persistence, metals in aquatic systems have toxic effects on organisms, as determined in many studies (Chatterjee et al., 2007; Cukrov et al., 2011). In addition to natural events such as precipitation and erosion, the transport of metals to the marine environment is due to human-induced activities. The rivers under the pollution pressure transfer their heavy metal load on the coastal marine environment.

Persistent organic pollutants (POPs) constitute a diverse group of organic substances, which are toxic, persistent, bioaccumulative and prone to long-range transport. They have different intrinsic physical-chemical properties, which dictate their environmental behaviour (Lohmann et al., 2007).

Most POPs do not occur in nature but are synthetic chemicals released because of anthropogenic activities. All environmental media can become contaminated by POPs once they are released into the environment. For instance, spraying persistent pesticides onto crops can contaminate vegetation and soils, direct discharges from POPs manufacturing facilities may contaminate rivers and release POPs from the stacks of incinerators, and industrial facilities contaminated air. Consequently, POPs can contaminate local areas close to where they are released (Allsopp et al., 2001).

PAHs represent a complex mixture of compounds (a family of more than 100 organic molecules comprising at least two aromatic cycles) originating from the incomplete combustion of organic matter classified as persistent organic pollutants. Combustion of fossil fuels, waste incineration, and oil spills are the potential sources of PAHs in the environment (Sakan et al., 2017).

The recognition of hazardous substances coming from rivers and their distribution and storage in the intermediate layers are of great interest in preserving the ecological integrity of the Black Sea (Miladinova et al., 2020).

#### **Dnieper River, southern Bug River, Dniester River and Danube (UA)**

The status of seawater was assessed for trace metals (TM), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs). In 2019, 2 expeditions were conducted in the sea areas adjacent to the deltas of the main rivers of Ukraine (Danube, Dniester, Dnieper and southern Bug).

We used the pollution factor (Kz) to assess seawater’s ecological state in areas affected by contaminated river runoff. Kz reflects the concentration of all pollutants of the same type in a certain

period in a given area. This factor represents the sum of the ratios of the concentration of each pollutant to its maximum permissible concentration, following EU Directive 2013/39/EU (MAC-EQS), or the maximum permissible concentration according to Ukrainian legislation (MPC), to the number of measurements performed in a given period. The accuracy of displaying the region's state using Kz depends on the monitoring stations nature and the number of observations over the past period. The overall assessment of the ecological condition of water in the study area is determined by the worst assessment of the pollutants' group.

Calculation formulas:

$$CR = \frac{C_{mon}}{C_{Threshold}}$$

$$Kz = \frac{1}{n} \sum_{i=0}^n CR_i$$

Where: CR is the contamination ratio, C<sub>mon</sub> is the monitored concentration, C<sub>threshold</sub> is the threshold value.

According to Kz value, the ecological status of seawater is:

Kz < 0.5	Very good
Kz = 0.5-1.0	Good
Kz = 1.0-2.5	Satisfactory
Kz = 2.5-5.0	Bad
Kz > 5.0.	Very bad

We present the seawater's ecological condition in areas affected by river runoff by group (Table 3.7) and individual (Table 3.8) pollutants.

**Table 3.7- Kz calculated for groups pollutants in seawater**

Station	Depth [m]	Kz PCB`s individual	Kz TM	Kz OCP`s	Kz PCB`s (Ar1254 and Ar1260)	Kz PAH`s
June 2019						
ST 1	0	19.1	0.13	0.27	0	0
ST 1	8.9	31.4	0.11	0.39	0	0
ST 2	0	13.4	0.05	0.12	0	0
ST 2	8.6	9.14	0.05	0.18	0	0
ST 3	0	25.1	0.14	0.19	0	0.03
ST 4	0	28.4	0.12	0.29	0.01	0.03
ST 5	0	25.3	0.06	0.13	0	0.04
ST 6	0	15.5	0.01	0.14	0.01	0
ST 6	4.6	31.6	0.15	0.17	0	0.08
September 2019						
ST 1	0	11.9	0.10	14222.35	0.05	0.97
ST 1	8.5	9.95	0.12	0.14	0.02	0.47
ST 2	0	6.48	0.16	18555.65	0.03	0.44
ST 2	8	10.5	0.11	0.12	0	0.14
ST 3	0	27.4	0.18	0.25	0.05	0.38
ST 3	4.5	16.9	0.37	55611.24	0.01	0.32
ST 6	0	24.5	0.20	0.22	0.04	0.54
ST 6	4.6	13.1	0.23	0.17	0.02	0.4

The ecological status of the Black Sea waters is “very bad” due to the increased content of individual PCBs. The maximum pollution by these compounds was noted in June in the bottom at station 6 in the Ochakov area (exit from the Dnieper-Bug estuary) and station 1 in the Danube Delta area (exit from the Kiliya arm) (Table 3.7).

In September, extreme pollution with organochlorine pesticides, particularly heptachlor, was recorded in the Black Sea's surface layer of sea waters at station 3 (Dniester region) and at stations 1, 2 (Danube region).

In September, increased content of benzo(g,h,i)perylene was also recorded at all stations with a maximum in the Danube and Dnieper-Bug regions (Table 3.8). Concentrations of toxic metals in all study areas were negligible.

Table 3.8 - Kz individual pollutants in seawater - heavy metals

Station	Depth [m]	Kz Cd	Kz Pb	Kz Ni	Kz Hg
June 2019					
ST 1	0	0.22	0.24	0.06	0
ST 1	8.9	0.10	0.29	0.03	0
ST 2	0	0	0	0.03	0.17
ST 2	8.6	0	0	0	0.20
ST 3	0	0.28	0.23	0.04	0
ST 4	0	0.37	0.10	0	0
ST 5	0	0.06	0.14	0.03	0
ST 6	0	0.05	0	0	0
ST 6	4.6	0.16	0.46	0	0
September 2019					
ST 1	0	0.28	0.12	0	0
ST 1	8.5	0.26	0.22	0	0
ST 2	0	0.12	0.14	0	0.37
ST 2	8.0	0.05	0.11	0	0.27
ST 3	0	0.34	0.12	0	0.27
ST 3	4.5	0.59	0.13	0	0.77
ST 6	0	0.10	0.12	0	0.59
ST 6	4.6	0	0.09	0	0.84

Table 3.9 - Kz individual pollutants in seawater - PAHs

Station	Depth [m]	Kz Naphthalene	Kz Anthracene	Kz Fluoranthene	Kz Benzo[b]fluoranthene	Kz Benzo[k]fluoranthene	Kz Benzo[a]pyrene	Kz Benzo (g,h,i) perylene
June 2019								
ST 1	0	0	0	0	0	0.01	0.01	0
ST 1	8.9	0	0	0	0	0	0.01	0
ST 2	0	0	0	0	0	0	0.02	0
ST 2	8.6	0	0	0	0	0	0	0
ST 3	0	0	0	0.01	0	0	0.01	0.18
ST 4	0	0	0	0.01	0	0.01	0.01	0.18
ST 5	0	0	0	0.02	0	0.01	0.01	0.21
ST 6	0	0	0	0.01	0	0	0.01	0
ST 6	4.6	0	0.14	0.02	0	0	0.03	0.35
September 2019								
ST 1	0	0	0	0.03	0.46	0.32	0.07	5.88
ST 1	8.5	0	0	0.01	0.25	0.17	0.03	2.87
ST 2	0	0	0	0.01	0.21	0.14	0.02	2.71
ST 2	8	0	0	0	0.08	0.04	0.01	0.85
ST 3	0	0	0	0.02	0	0.25	0	2.41
ST 3	4.5	0	0	0.01	0.18	0.13	0.01	1.91
ST 6	0	0	0	0.04	0.38	0.33	0	3.06
ST 6	4.6	0	0	0.02	0.3	0.29	0	2.22

Table 3.10 - Kz individual pollutants in seawater - Pesticides

Station	Depth[m]	Kz HCB	Kz HCH total	Kz Heptachlor	Kz Ciclodiene total	Kz DDT	Kz DDT total	Kz AR-1254	Kz AR-1260
June 2019									
ST 1	0	0	0.08	0	0	1.12	0.45	0.21	0.01
ST 1	8.9	0	0.51	0	0	1.29	0.52	0.32	0.01
ST 2	0	0	0.01	0	0	0.51	0.21	0.11	0
ST 2	8.6	0	0.06	0	0	0.73	0.29	0.12	0.01
ST 3	0	0	0.04	0	0	0.8	0.32	0.22	0.01
ST 4	0	0	0.06	0	0	1.21	0.48	0.38	0.01
ST 5	0	0	0.05	0	0	0.53	0.21	0.28	0.01
ST 6	0	0	0.06	0	0	0.57	0.23	0.14	0
ST 6	4.6	0	0.08	0	0	0.67	0.27	0.26	0
September 2019									
ST 1	0	0	0.01	85333	0	0.55	0.22	0.11	0
ST 1	8.5	0	0.11	0	0	0.50	0.20	0.11	0
ST 2	0	0	0.02	111333	0	0.37	0.15	0.06	0
ST 2	8	0	0.07	0	0	0.45	0.18	0.10	0
ST 3	0	0	0.03	0	0	1.07	0.43	0.24	0
ST 3	4.5	0	0.13	333666	0	0.46	0.18	0.11	0
ST 6	0	0	0.08	0	0	0.89	0.36	0.22	0.01
ST 6	4.6	0	0.12	0	0	0.63	0.25	0.13	0

Table 3.11 - Kz individual pollutants in seawater - PCBs

Station	Depth [m]	Kz PCB101	Kz PCB118	Kz PCB153	Kz PCB138	Kz PCB180
June 2019						
ST 1	0	39.0	56.15	0	55.5	2.75
ST 1	8.9	67.5	89.23	0.11	81.0	6.25
ST 2	0	39.0	27.69	0.05	19.5	0.40
ST 2	8.6	17.5	28.08	0	18.5	0
ST 3	0	51.5	73.46	0.39	71.5	1.10
ST 4	0	0	140.38	0.99	142	1.25
ST 5	0	27.0	98.46	0.83	89.5	1.00
ST 6	0	34.0	43.08	0.09	56.0	0
ST 6	4.6	73.0	84.23	0.67	73.0	1.10
September 2019						
ST 1	0	22.5	36.54	0.12	29.5	0.50
ST 1	8.5	18.0	31.54	0.09	46.5	0
ST 2	0	13.5	18.85	0	28.5	0
ST 2	8.0	22.0	30.0	0.15	40.5	0
ST 3	0	51.5	85.0	0.45	53.0	0
ST 3	4.5	54.5	30.0	0.10	30.5	0
ST 6	0	49.5	71.92	0.61	61.5	0.75
ST 6	4.6	24.0	41.54	0	36.0	0.25

## Danube (RO)

### Heavy Metals

The marine environment's heavy metal contamination may be correlated with urban or industrial sources such as factories, thermoelectric plants, port activities, and sewage treatment stations. The influence of rivers on coastal areas is significant, constituting a major source of metals, especially in particulate forms, extreme hydrological events (floods) contributing to this input's intensification (Sakson et al., 2018). Atmospheric fluxes, demonstrating both natural and anthropogenic influences, are also considered to have an important contribution to European seas, both in coastal and basin

areas, depending on the variability of the meteorological and local climatic conditions. Biogeochemical processes and natural levels of metals in the marine environment depend on numerous factors, such as sedimentary rock type, oxygen content, currents, salinity, pH. The spread of metals in water, sediment, and atmosphere results from their presence in the earth crust. In their natural concentrations, metals play an essential role in many biochemical processes in living organisms, but any concentration that exceeds the background can become toxic because of anthropogenic activities (OSPAR, 1992).

Over time, the pressures suffered by the Black Sea make it an environmentally vulnerable unit, especially because this sea is semi-closed and too small to self-balance ecologically. Thus, the saturation point of contaminants discharged into the sea will be achieved faster than in the case of oceans. For example, high levels of heavy metals measured in the Mediterranean indicate non-stationary geochemical cycles resulting from an increase in external inputs (Saliot A., 2005). Also, the almost total absence of tides does not allow the dilution of contaminants and prevents natural purging phenomena encountered in larger water bodies (e.g., in the oceans). The Black Sea, like the Mediterranean Sea, shows a deficiency in the movement of deep-sea water masses and surface currents that "turn into circles" in these almost closed basins. The consequence of these specific characteristics is that the response of small semi-closed seas to environmental disruptions resulting from anthropogenic pressures is faster than in the oceans.

Metals fall into the category of non-degradable pollutants and, by this persistent character, can sometimes quite strongly alter the natural biogeochemical balance in contaminated environments. Processes that remove metals from seawater primarily include active biological absorption and passive deposition (e.g., the combined process of superficial adsorption on a wide variety of high-affinity surfaces associated with the particulate material, followed by particle deposition). Much of this particulate material (along with associated metals) is recycled either in the water column or in the superficial sediments. Weakly bound metals may be released from the surface of the depositing particles, replenishing the stock of dissolved metals. Marine sediments can also act as a source of metals by releasing them back into the water column. Primary flow processes between sediments and water column are re-suspension and deposition, bioturbation, advection, upwelling/downwelling, diagenetic processes and diffusion. Due to these remobilization processes, the effects of metal pollution on the local environment can be substantial and long-lasting, even in the case of restoration efforts (Richir and Gobert, 2016).

Metals concentrations in surface seawater from all transects were characterized by high variability, within the following ranges: 2.09-44.93 µg/L Cu; 0.16-1.33 µg/L Cd; 9.37-27.65 µg/L Pb; 10.39-27.65 µg/L Ni; 0.17-19.69 µg/L Cr. Data obtained during this cruise for the area impacted by the Danube are slightly higher in comparison with typical ranges reported for Black Sea marine waters (except for cadmium), for instance, the limit of predominant values (75<sup>th</sup> percentile of 2012-2017 monitoring data) being as follows: 6.31 µg/L Cu; 1.14 µg/L Cd; 7.43 µg/L Pb; 3.78 µg/L Ni; 3.21 µg/L Cr (Oros A., 2019).

Numerous studies on biogeochemical processes and the distribution of heavy metals in the NW Black Sea have demonstrated the importance of the metals input from the Danube and other rivers, together with the influence of the redox cycles of the Mn and Fe complexes. For example, Cu and Ni were found in higher concentrations in the Black Sea continental shelf than in the deep-sea basin's oxic layer, reflecting the significant impact of rivers and anthropogenic inputs on this semi-enclosed sea. High concentrations of dissolved lead observed in surface waters in the open sea area were attributed to atmospheric intakes combined with less efficient metal capture in these waters poorer in particle matter (Tankere S.P.C., 2001).

Copper, lead, and chromium concentrations in surface waters presented a pronounced decreasing gradient from north to south, their values being higher along Sulina and Sf. Gheorghe transects, in comparison with Portita and Periboina. Meanwhile, cadmium had a different behaviour, with a decreasing gradient from coastal to open sea (beyond 40 m depth) that was noticed for all four transects. Nickel presented higher concentrations at Sulina and Sf. Gheorghe transects, but in stations beyond 50 m depth, and along Portita transect (Figure 3.89).

Once entered the marine system, trace metals are removed from the surface water body by internal fluxes like sedimentation on biogenic or terrigenous particles, by diffusive exchange of dissolved species across interfaces or by advective vertical transport. As has been demonstrated by Pohl et al. (2006) in the brackish semi-enclosed Baltic Sea, the accumulation in sediments is the only noteworthy sink of heavy metals due to prolonged water residence time. Consequently, heavy metals that are particle reactive, like Pb, have very low residence time, vertical sedimentation (sinking associated with particles) and lateral transport, as atmospheric input is in the same order of magnitude. In contrast, the metals (Cd, Cu, Zn) with "nutrient-like" behaviour have a residence time of several

decades primarily due to their coupling to biological processes, in their case the lateral transport being more important than vertical sedimentation. (Pohl et al., 2006). This demonstrates that the system reacts very fast for particle reactive elements like Pb, while for Cu and Cd sedimentation processes are not the preferential sink and can be neglected (Pohl et al., 2006).

The data from the area influenced by the Danube indicated a moderate level of trace metal pollution, since concentrations of copper, lead and nickel in surface waters surpassed recommended environment quality standards (EQS) in 34 %, 41 % and, respectively, 29 % of analysed samples, whereas cadmium had low levels, below EQS (Directive 2013/39/EU: 1.5 µg/L Cd, 14 µg/L Pb, 34 µg/L Ni; national legislation: 30 µg/L Cu) (Figure 3.89).

Compared to available monitoring data (2015 - 2018) from the same area, the results in 2019 were generally maintained between similar wide variability ranges especially observed in 2015 and 2018, with no significant increasing or decreasing trends. Only cadmium presented in 2019 a lower median than the previous period (2015 - 2018), while for nickel, the median was higher in 2019 (Figure 3.90).

## **Conclusions**

Metals concentrations in surface seawater indicated a moderate level of trace metal pollution, as between 30-40 % of samples surpassed recommended EQS values for Cu, Pb and Ni, whereas Cd values were below EQS.

Generally, a decreasing gradient from the northern to the southern area was noticed for most analysed metals, reflecting Danube influence upon receiving zone.

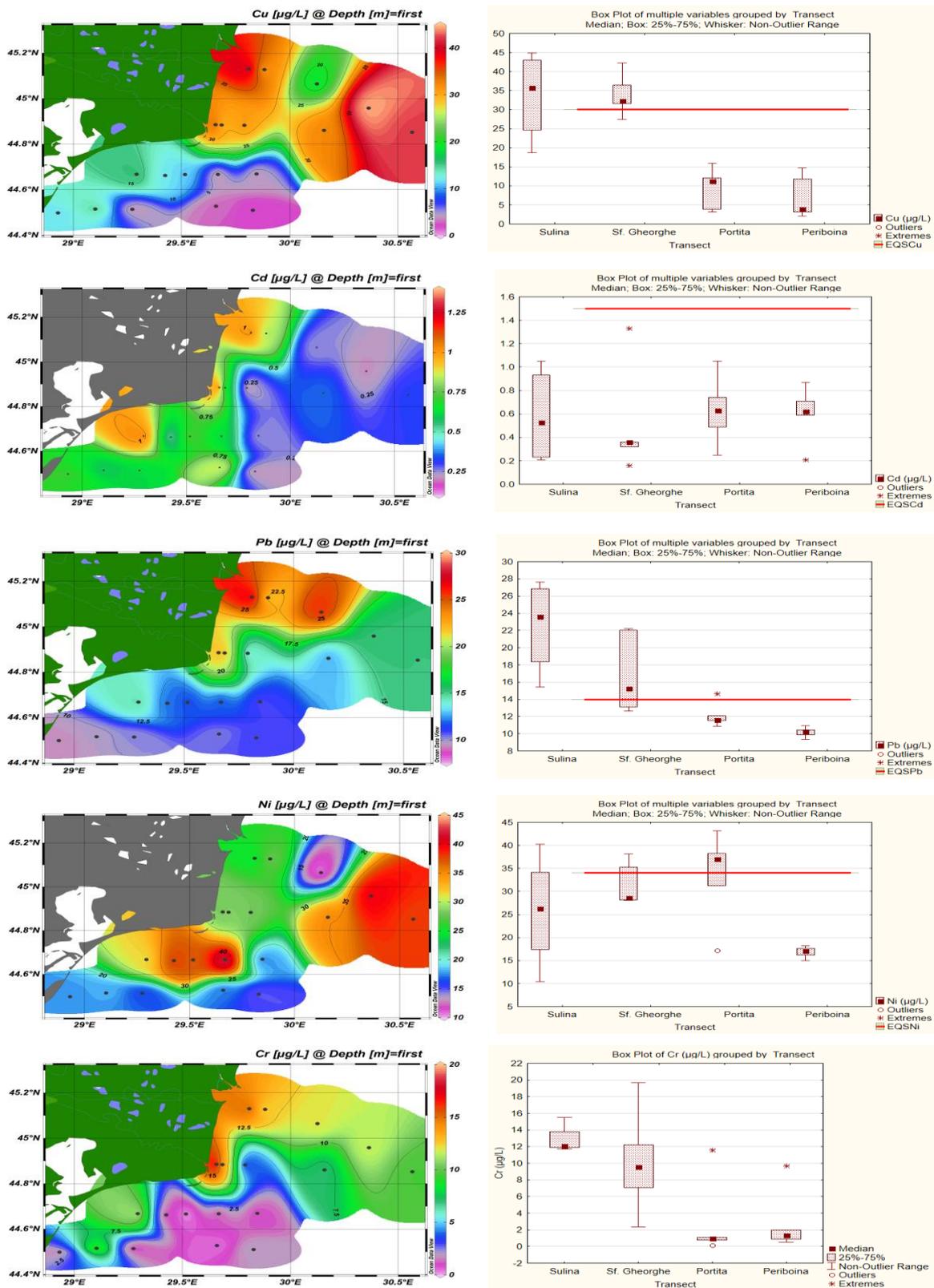
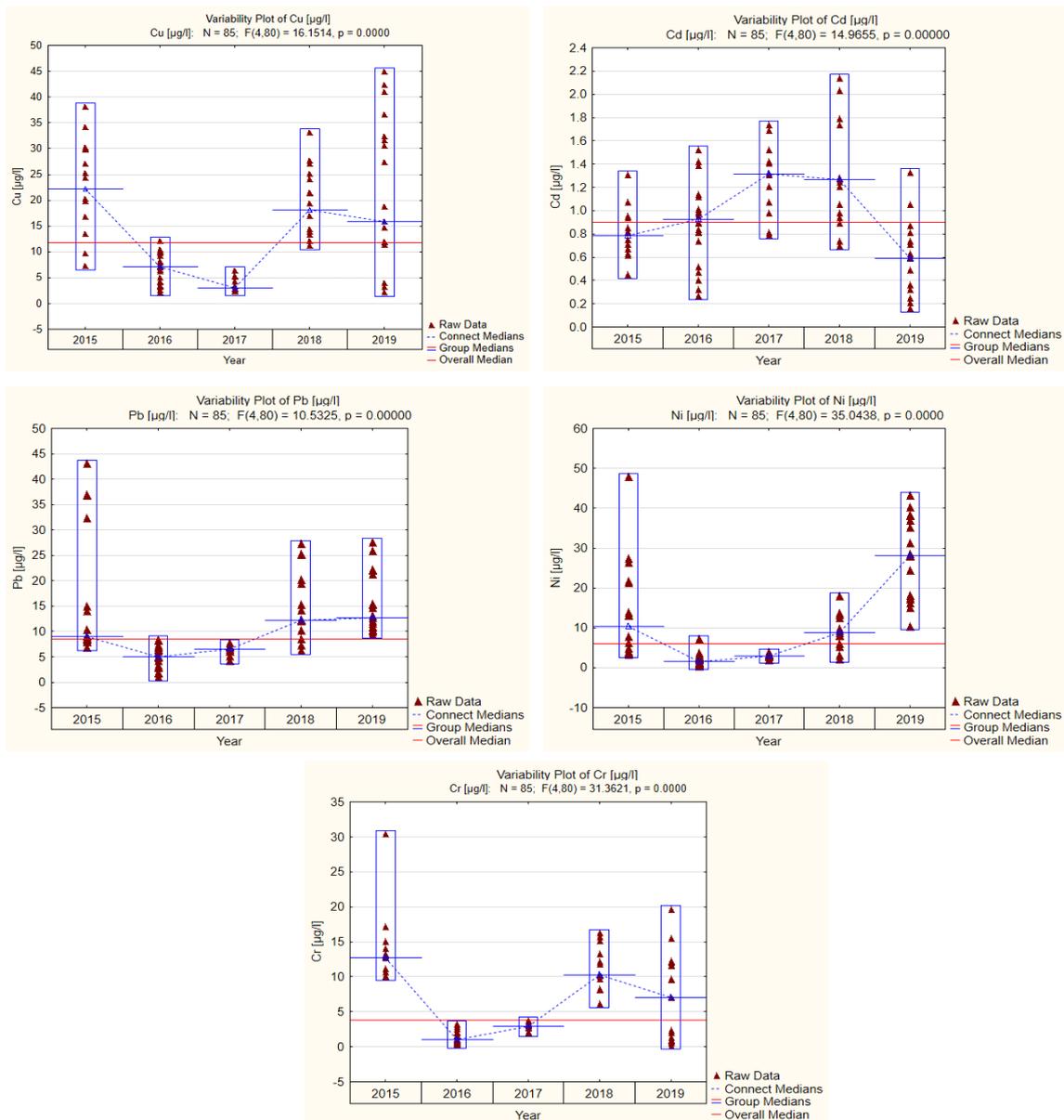


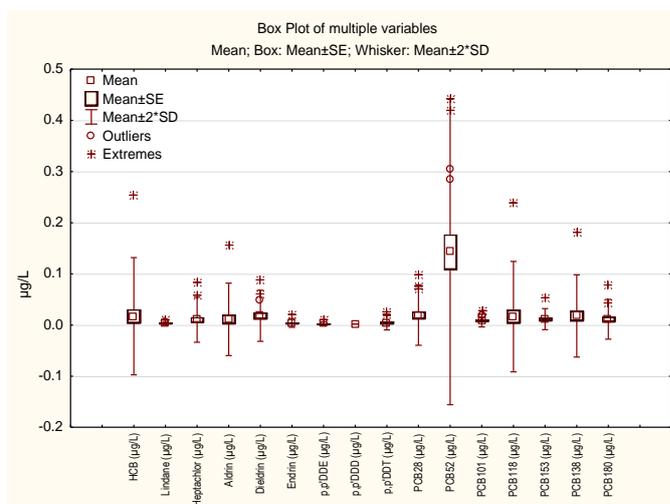
Figure 3.89 - Spatial distribution of heavy metals concentrations in surface waters in the marine area under the influence of Danube, May 2019



**Figure 3.90 - Trends of heavy metals concentrations in surface waters from the marine area under the influence of Danube, 2015 - 2019**

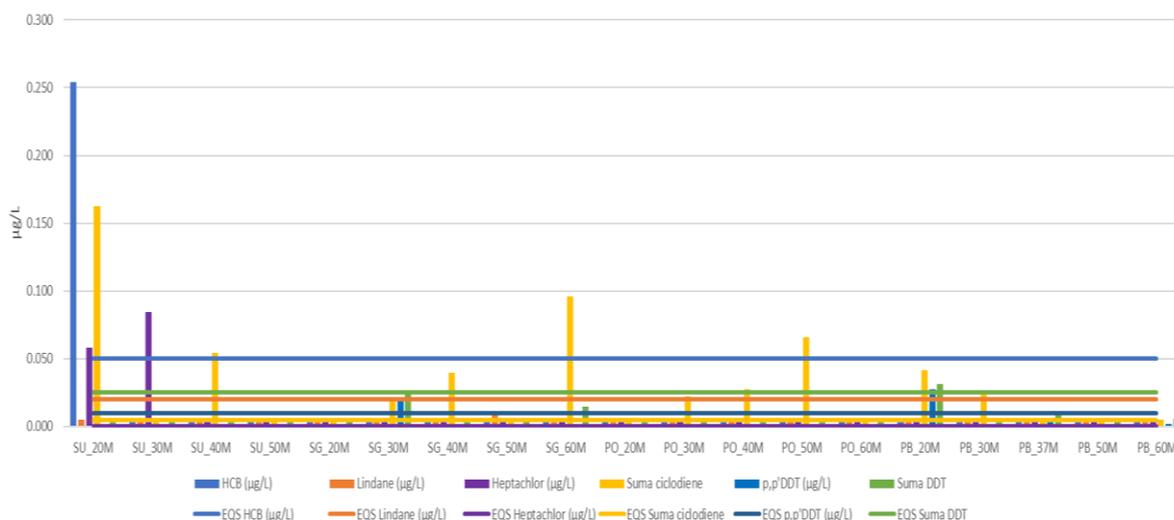
### Organic pollutants

Organic pollutants concentration in surface seawater varied in large limits. Except for dieldrin, PCB 28 and PCB 52, the chlorinated compounds had the 75<sup>th</sup> percentile lower than the detection limit. Besides this, most of the pollutants recorded extreme values. HCB, heptachlor, aldrin, dieldrin, PCB 28, PCB 52, PCB 118, and PCB 138 noted high concentrations (Figure 3.91). However, these values fall within the study area's normal range of variability (Nicolaev et al., 2019; Nicolaev et al., 2017).



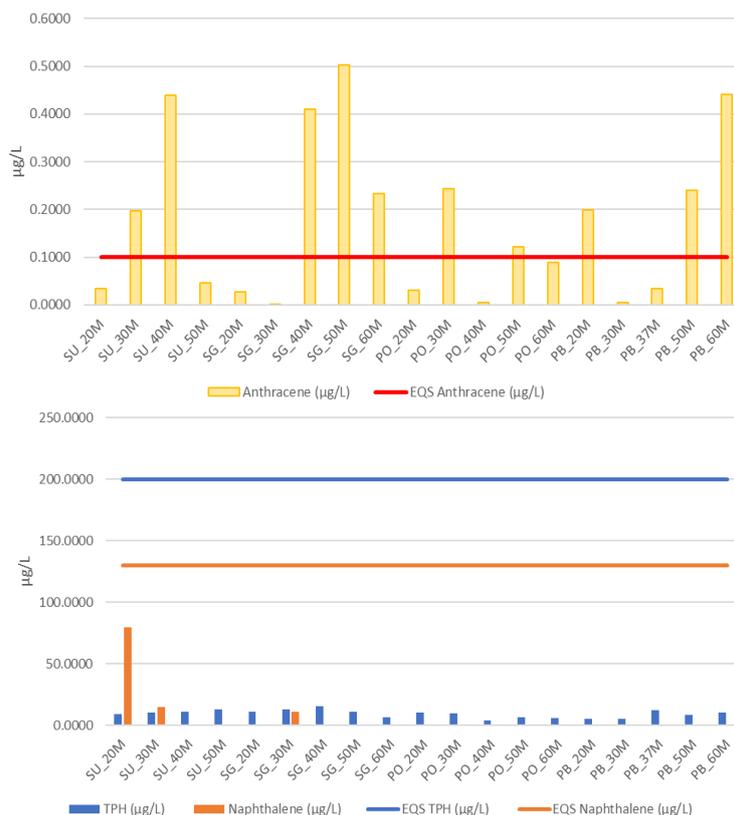
**Figure 3.91 - Variability of organochlorinated compounds in surface waters in the marine area under the Danube's influence, May 2019**

The results indicate a moderate level of organic pollution in the area influenced by the Danube. The concentration of cyclodiene pesticides (aldrin, dieldrin, endrin) exceeded the threshold values proposed for water to define good ecological status (according to Directive 2013\_39\_EU) in 53 % of the analysed samples. The other regulated compounds exceeded the threshold values as follows: HCB - 5 %, heptachlor, sum of DDTs (DDT and metabolites) and p,p' DDT - 10 % (Figure 3.92). Except for cyclodiene pesticides, organochlorinated compounds were in good status according to the methodology developed to assess the status of the Black Sea ecosystem in respect to MSFD (Boicenco et al., 2018).



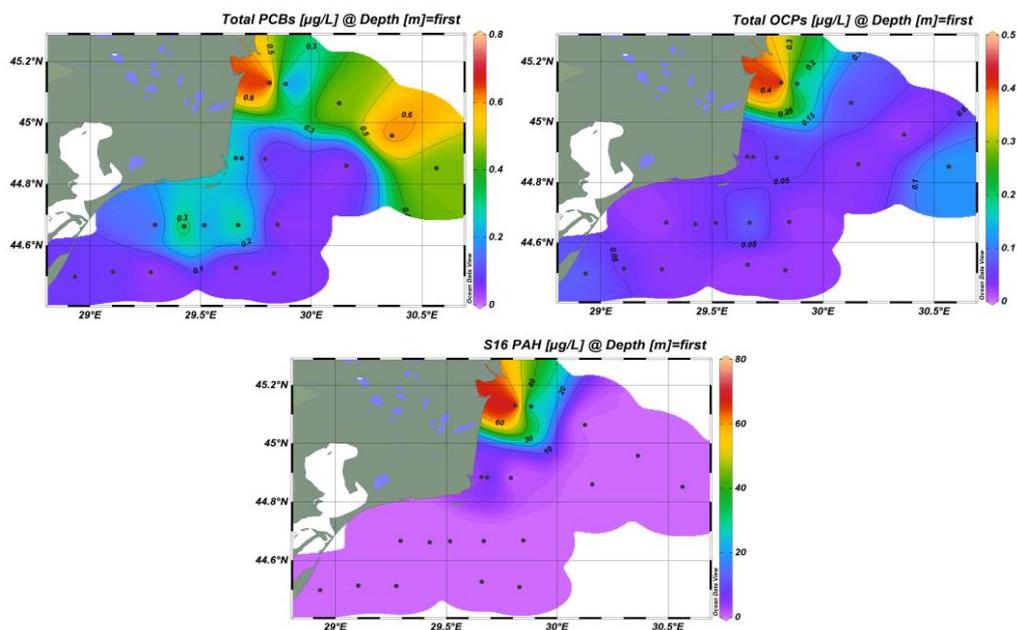
**Figure 3.92 - Concentrations of organochlorinated pesticides in surface waters in the marine area under the influence of Danube compared to the proposed values to define good environmental status, May 2019**

TPHs values ranged between 4.25 µg/L and 15.62 µg/L and were much lower than the maximum admissible value (200 µg/L) stipulated by national legislation (Order no. 161/2006). The PAHs analysis highlighted the presence of four of the sixteen investigated compounds: naphthalene, acenaphthylene, phenanthrene and anthracene. Anthracene was the only regulated compound that exceeded the threshold values proposed for water to define good ecological status, according to Directive 2013\_39\_EU, in 53 % of the samples (Figure 3.93).



**Figure 3.93 - Concentrations of hydrocarbons in surface waters in the marine area under the influence of Danube compared to the proposed values to define good environmental status, May 2019**

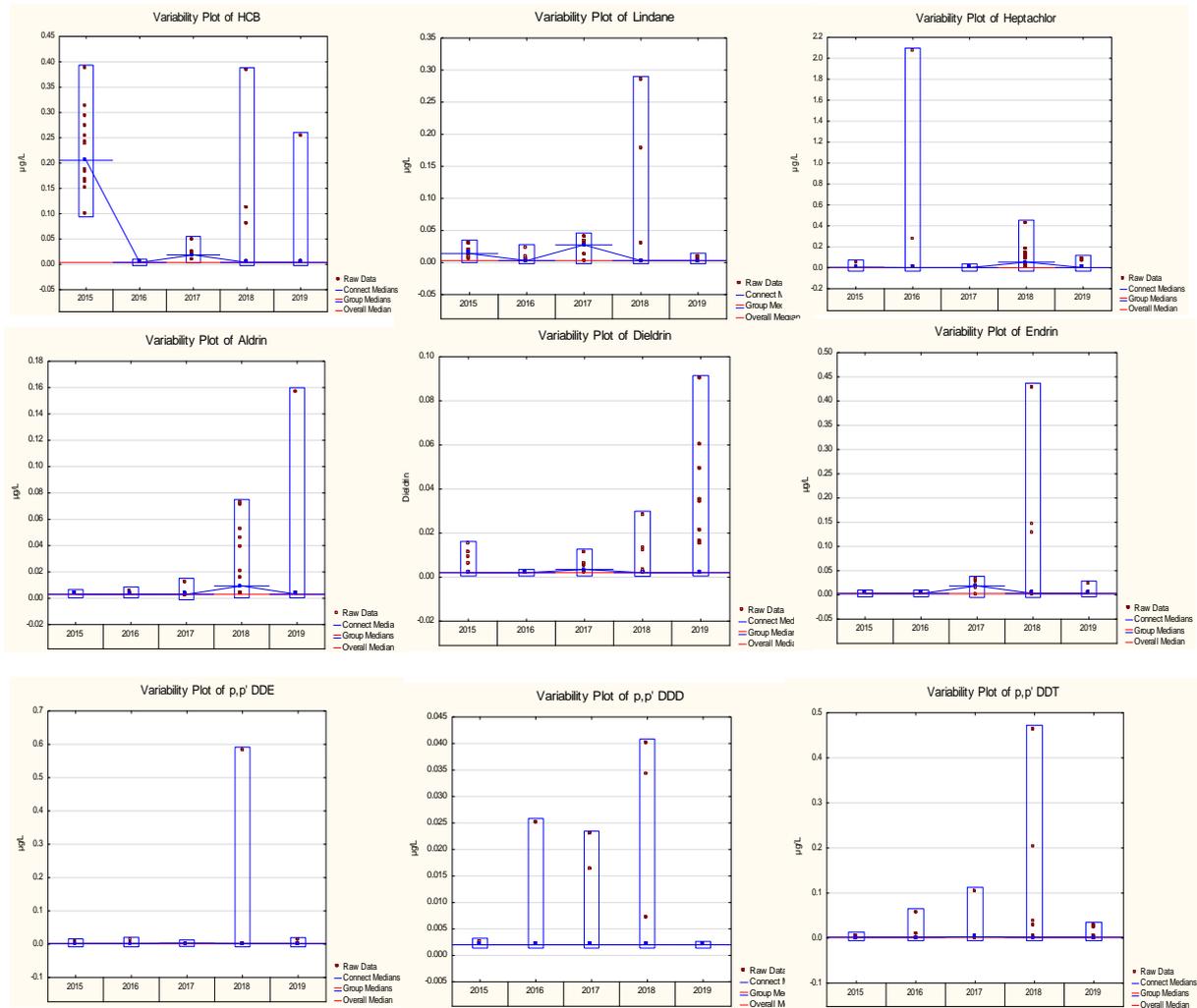
However, the Danube influence was observed mainly in front of river mouths (Sulina), both for the chlorinated compounds and polyaromatic hydrocarbons (Figure 3.94).



**Figure 3.94 - Spatial distribution of organic pollutants concentrations in surface waters in the marine area under the Danube's influence, May 2019**

The results of organochlorinated compounds in 2019 had similar variability ranges or even lower for HCB, lindane, heptachlor, endrin, p,p' DDE, p,p' DDD, p,p' DDT, PCB 52, PCB 101, PCB 180 in comparison with monitoring data (2015 - 2018) from the same area. The median value had the same level as in the previous period, but higher values were recorded in 2018 for most compounds. Only aldrin and dieldrin had in 2019 some extreme values even higher than in 2018 (Figure 3.95, Figure 3.96).

The fourth polyaromatic hydrocarbons and the sum of the sixteen investigated compounds and total petroleum hydrocarbons were generally similar with wide variability ranges observed in 2015-2018. Only acenaphthylene presented in 2019 a median value lower than the previous period (Figure 3.97).



**Figure 3.95 - Trends of organochlorine pesticides concentrations in surface waters from marine area under the influence of Danube, 2015 - 2019**

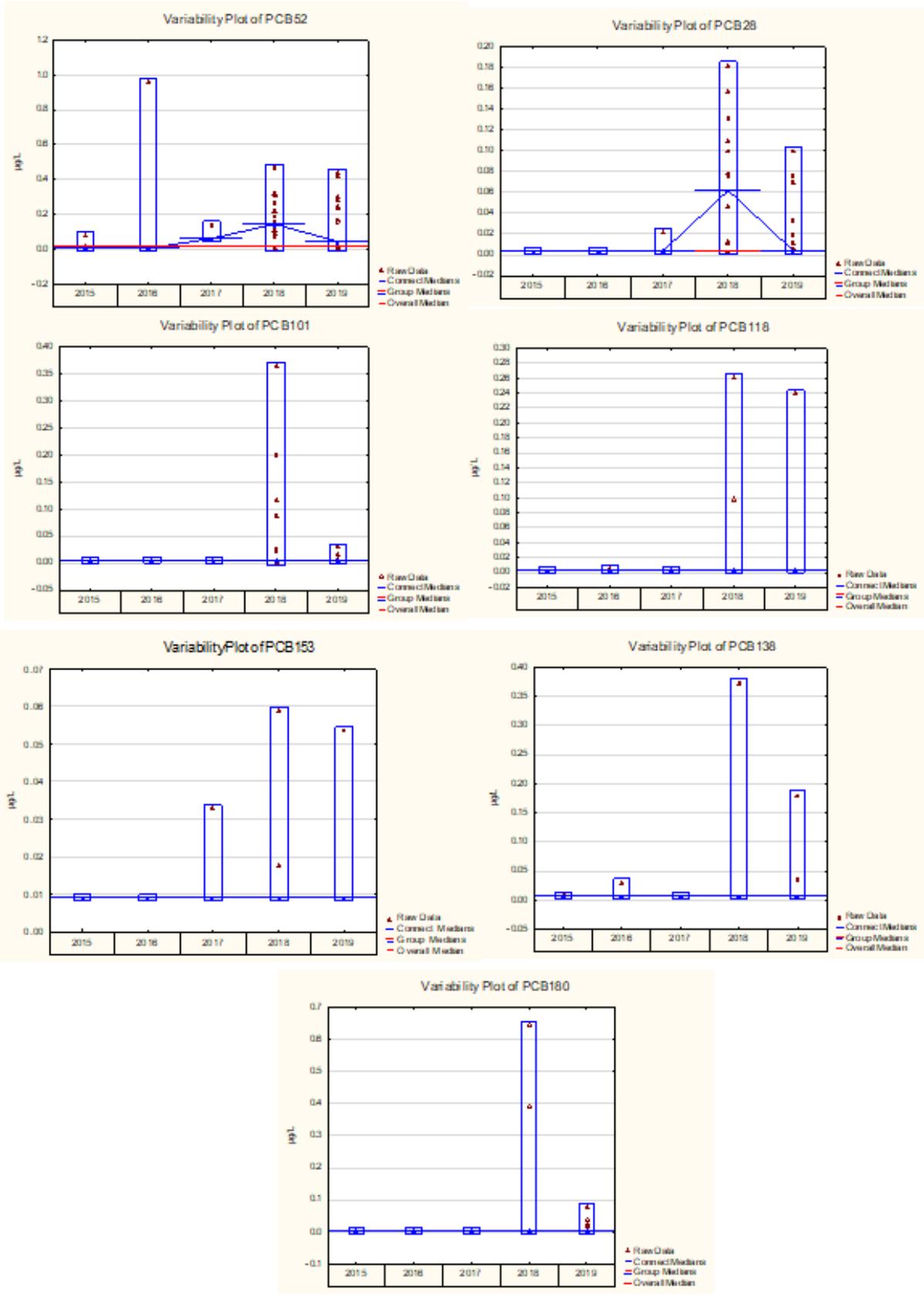
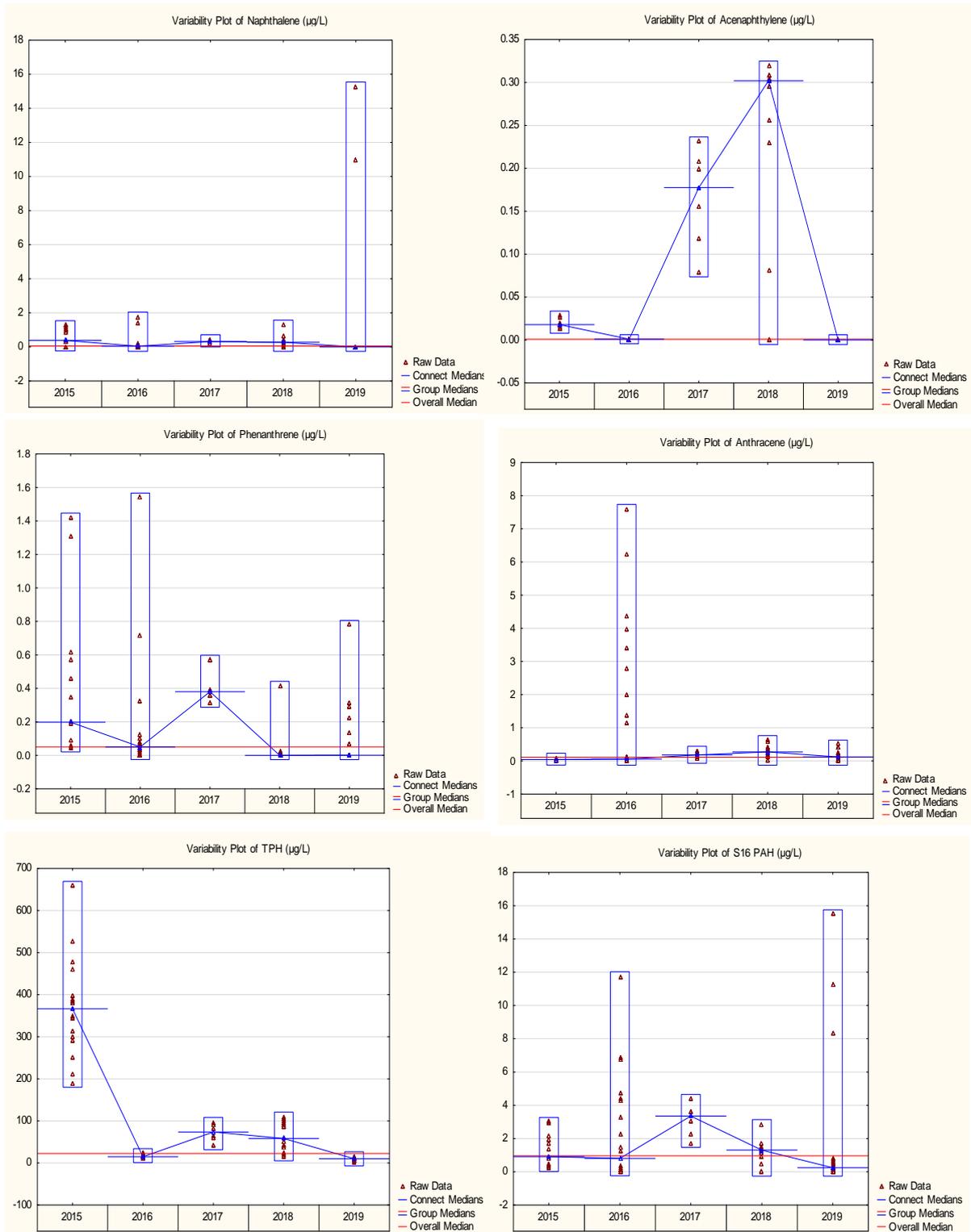


Figure 3.96 - Trends of polychlorinated biphenyls concentrations in surface waters from marine area under the influence of Danube, 2015 - 2019



**Figure 3.97 - Trends of hydrocarbons in surface waters from the marine area under the influence of Danube, 2015 - 2019**

Concentrations of organic pollutants in surface seawater indicated a moderate level of organic pollution, as only the sum of cyclodiene pesticides and anthracene surpassed recommended EQS values in over 50 % of samples. The other compounds level corresponded to good ecological condition. The higher levels of total chlorinated compounds and polyaromatic hydrocarbons were observed in front of the river mouth, reflecting Danube influence upon receiving zone.

## Sakarya River and Yesilirmak River

Although little is known about the sedimentology of the shallow shelves along the southern Black Sea (Duman et al. 2006), it is generally accepted that most of the sediments accumulated on continental shelves are generated inland and transported by fluvial systems (Lericolais et al. 2010), while a contribution from the local organic matter production and deposition of the planktonic debris is also an important factor (Tezcan et al. 2017).

In the southwestern Black Sea shelf, sediments are mainly transported by the two major rivers: the Sakarya River and Filyos River (Duman et al. 2006). The grain size analysis shows that the surface sediments in the mouth of major rivers (Sakarya, Kızılırmak, and Yeşilirmak) characterized by relatively high mud portions mainly due to the high sediment loads from these rivers (Yücesoy and Ergin, 1992; Duman et al. 2006).

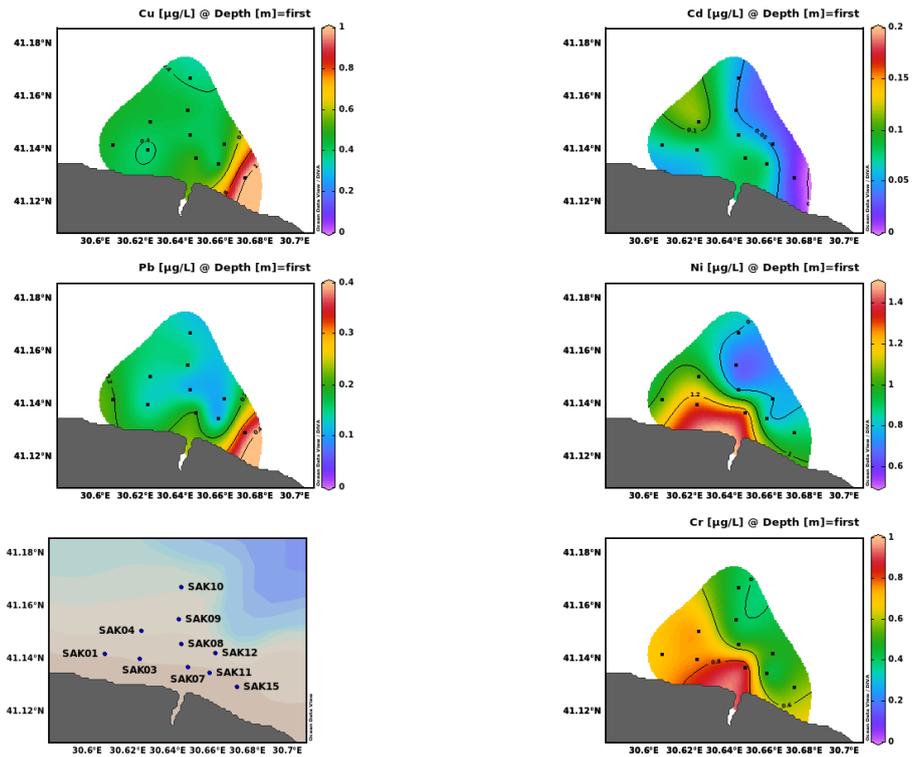
Recently, the results from the monitoring project, undertaken within 2014-2016, indicates that Sakarya River on the western Black Sea basin and Yeşilirmak rivers on the eastern Black Sea basin exert a significant pollution effect on the Black Sea ecosystem (MoEU-DGEIAPI and TUBITAK-MRC, 2017).

### *Heavy metals*

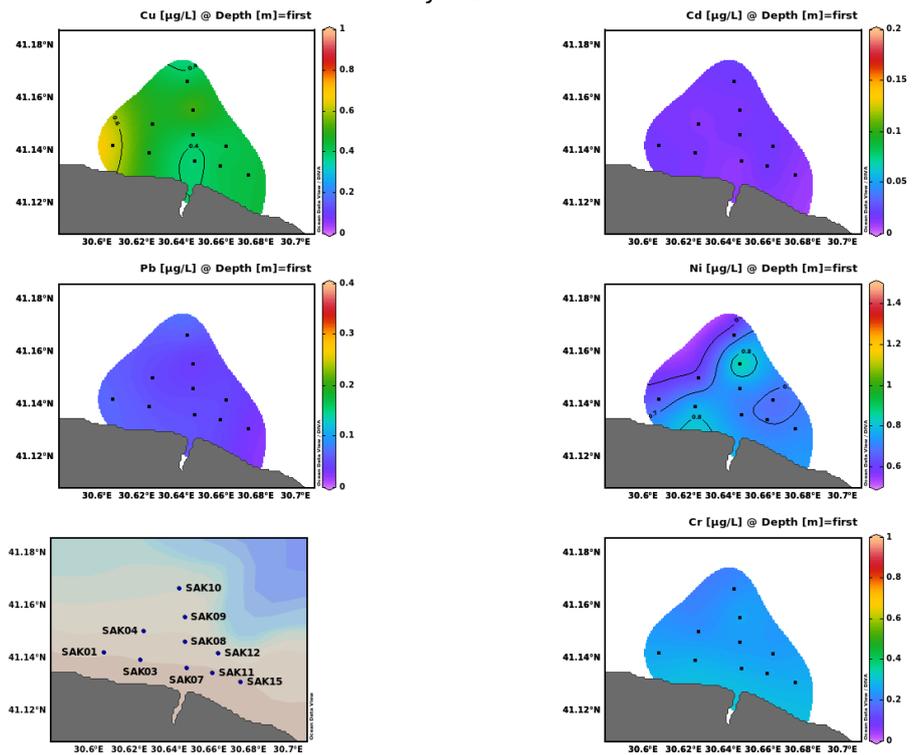
Important sources of heavy metals located near the Yeşilirmak River are a fertilizer plant and copper smelter, an iron and steel complex industry, and a thermal power plant in the eastern coastal site of the Sakarya River. Furthermore, the secondary sources of high metal levels are the erosion products of mineralized zones in these rivers' drainage basin.

Previous studies carried out by the Balkis et al. 2007, showed that Cd and Pb concentrations in the water matrix were lower than the detection limit (0.01 µg/L) in Yeşilirmak, Kızılırmak, and Sakarya sediment samples, whereas Co, Cr, Ni, Zn, Fe and Mn contents were higher than the shale average. The highest amounts of Co, Cr, Zn, Fe, and Mn were found outside (a) than inside (b) of the Yeşilirmak and Sakarya Rivers. The result showed that Yeşilirmak and Sakarya mouths and shelf areas were more contaminated than the other river mouth sediment samples (Balkis et al., 2007). A comparison of the studies conducted on Turkey's Black Sea coast is given in a very recent review (Bat et al., 2021).

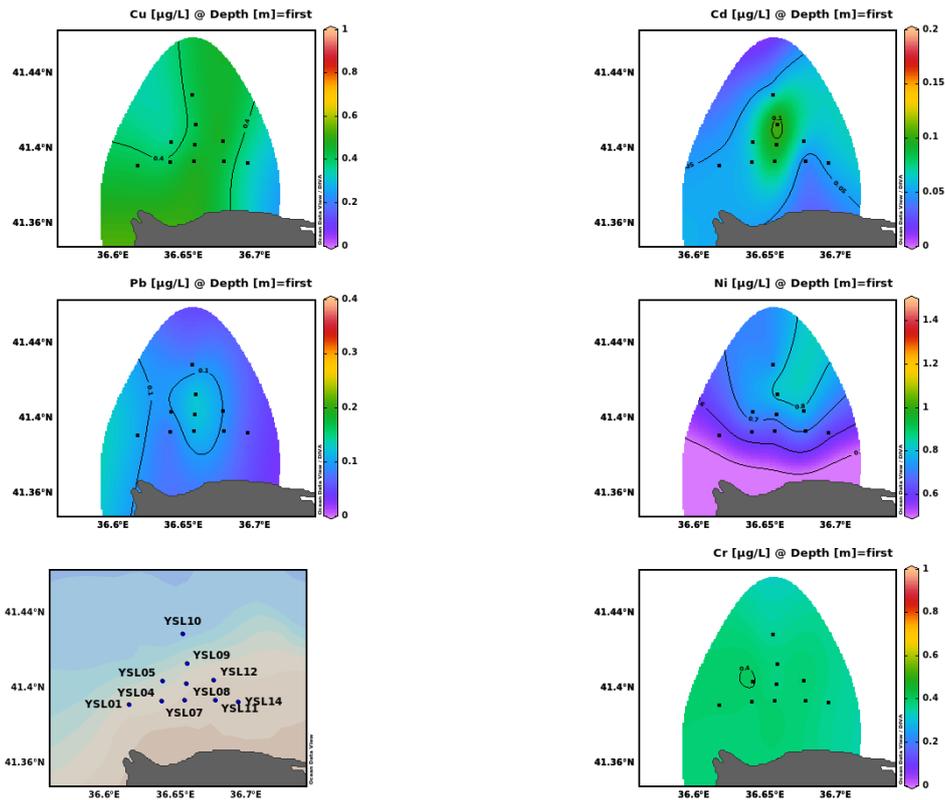
The present work was conducted in the marine areas in front of the Sakarya river and Yeşilirmak river mouths (river-sea Impact areas) to understand their contribution to organic and inorganic pollution. The statistical analysis of the data (mean, min-max values and 75th percentiles) of both rivers are given in Annex D. Cd had concentrations between 0.01 µg/L and 0.20 µg/L. In stations SAK 4 (winter) and YSL14 (summer), Cd had the maximum concentrations. Pb concentrations were detected between 0.03 µg/L and 0.35 µg/L. The maximum concentrations were at SAK15 and YSL 14 (winter). Ni concentrations were between 0.56 µg/L and 1.32 µg/L. Maximum concentrations were at station SAK7 (summer).



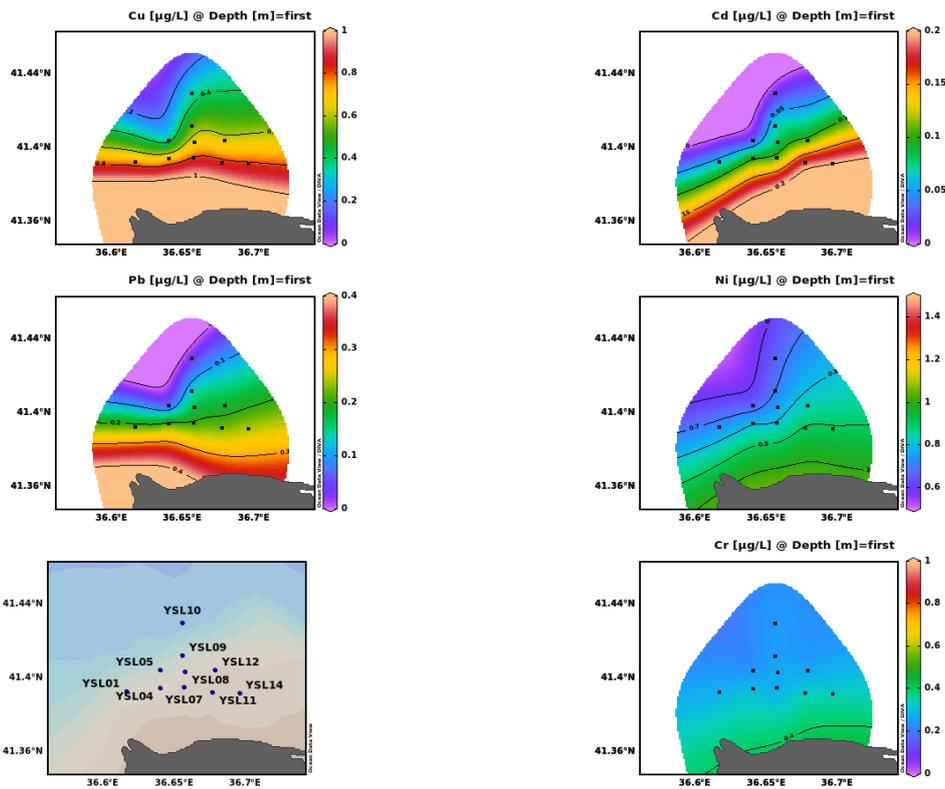
a-Sakarya RIA winter



b-Sakarya RIA summer



c- Yesilirmak RIA winter



d- Yesilirmak summer

Figure 3.98 - Spatial distribution of the metals in seawater (a-Sakarya winter 2020; b- Sakarya summer 2019; c-Yesilirmak winter 2020; d-Yesilirmak summer 2019)

The spatial distribution map shows that higher concentrations of the metals in the water matrix were dominated at the stations near the mainland or river mouths (Figure 3.98).

Concentrations of metals in surface water samples (oxic layer) collected from all stations were detected below the EQS levels established by Directive 2013/39/EU and the National Surface Water Management Regulation of Turkey (2016) (Figure 3.99).

Most of the elements were found in higher concentrations in winter except for the Sakarya river impact area. However, the seasonal difference was not significant in the Yesilirmak River impact area except for the summer's higher Zn concentrations.

Similarly, there was no major difference in all samples' metal contents except for the higher values of As in the Yesilirmak river impact area (Figure 3.99).

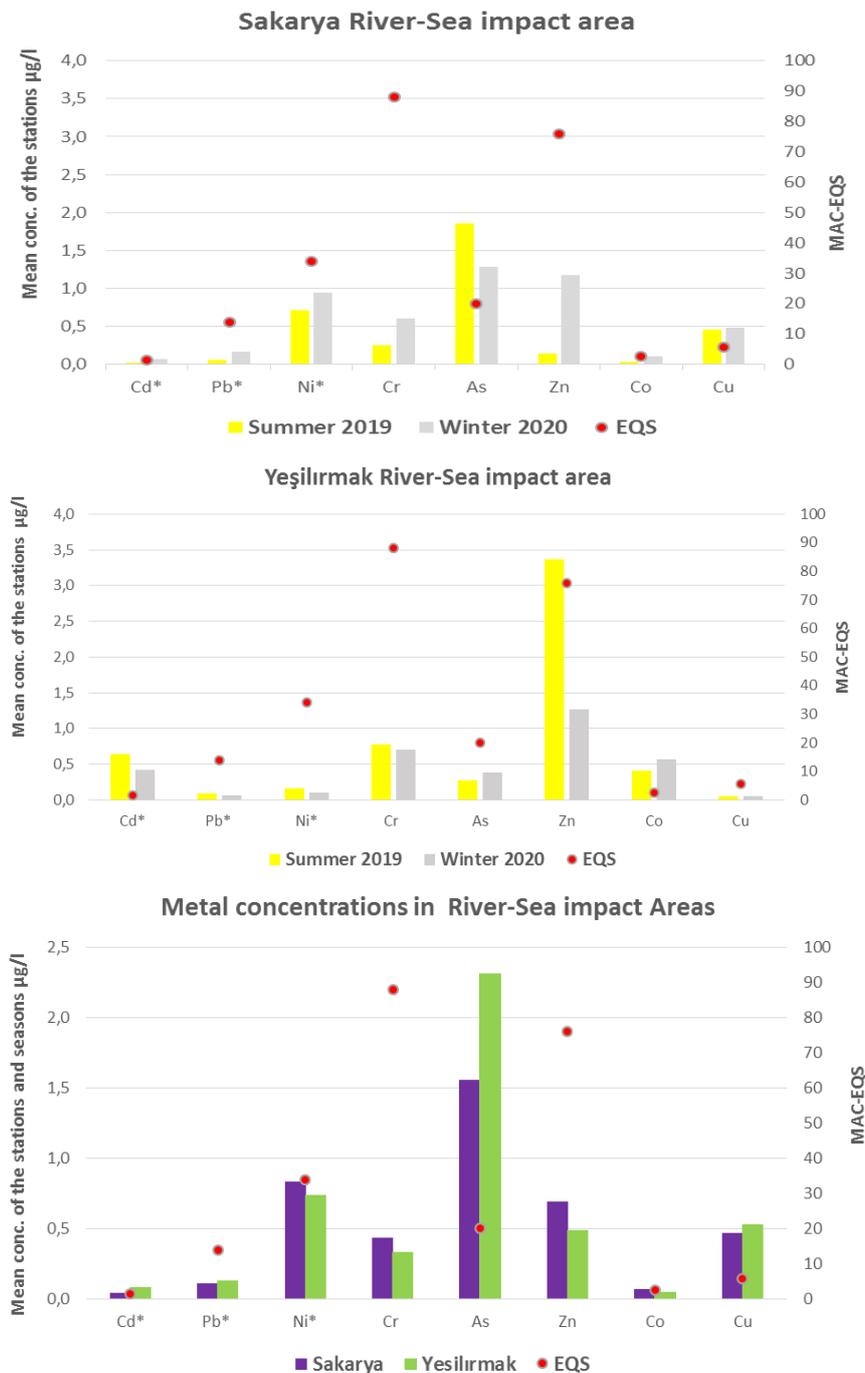


Figure 3.99 - Heavy Metals concentrations - water - summer and winter

Metal's concentrations in surface seawater from July 2019 and January 2020 indicated a low level of trace metal pollution, as 100 % of samples were below recommended EQS values for all measured metals, such as Cu, Pb, Ni, Cd. Generally, a decreasing gradient from the river mouth to the open area was noticed for most analysed metals, reflecting river influence upon receiving zone. Seasonal differences were observed in Sakarya samples in terms of higher metal content in winter.

### Organic pollutants

Previous studies carried out at the southern Black Sea coastal area have provided important information about metal pollution. However, few studies were concentrated on POPs pollution (SoE Report, Moncheva and Boicenco, 2014).

The contribution of POPs from river discharges is especially of great interest for the regional MSFD assessments. In this study, Yesilirmak and Sakarya riverine impact areas are investigated in terms of POPs under ANEMONE Project.

Total Petroleum Hydrocarbons (TPH) in seawater values ranged between 0.019 µg/L and 0.960 µg/L and 0.055 µg/L and 1.014 µg/L in Sakarya and Yesilirmak river impact areas, respectively. These values are lower than the Max-EQS value (100 µg/L) stated in the National Surface Water Management Regulation (2016) (Figure 3.100).

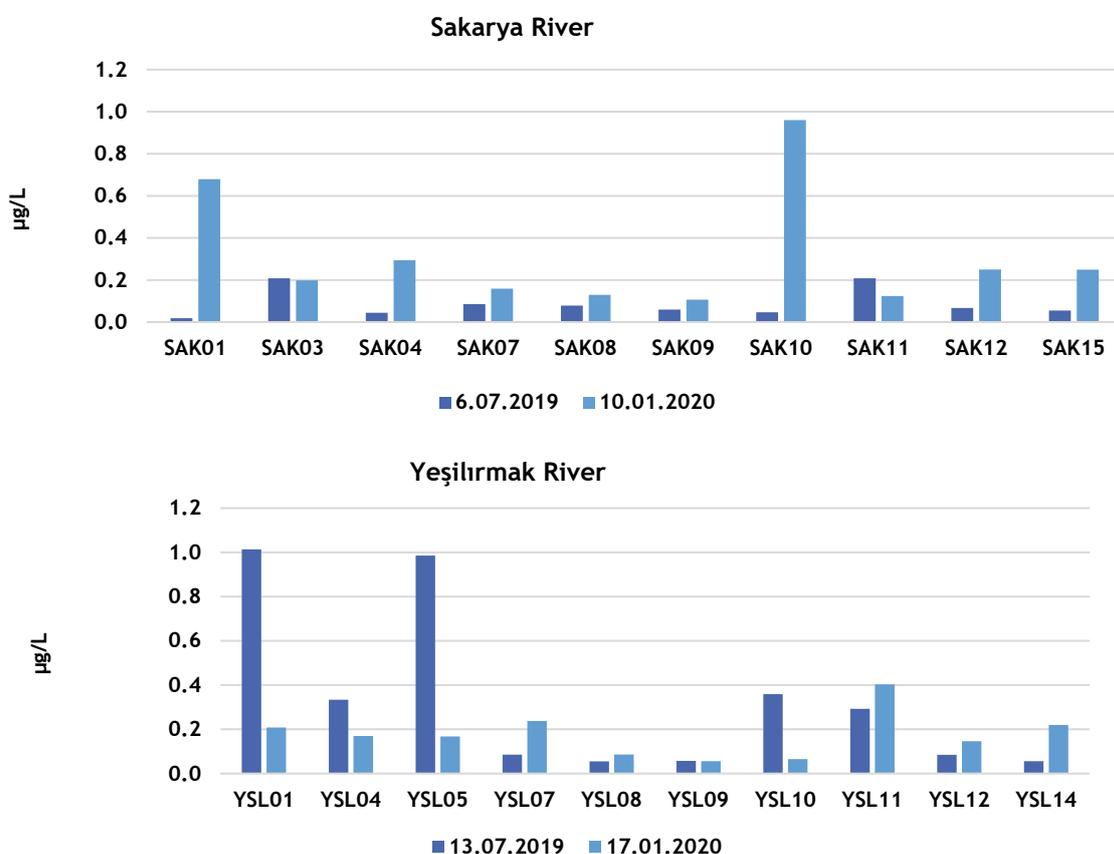
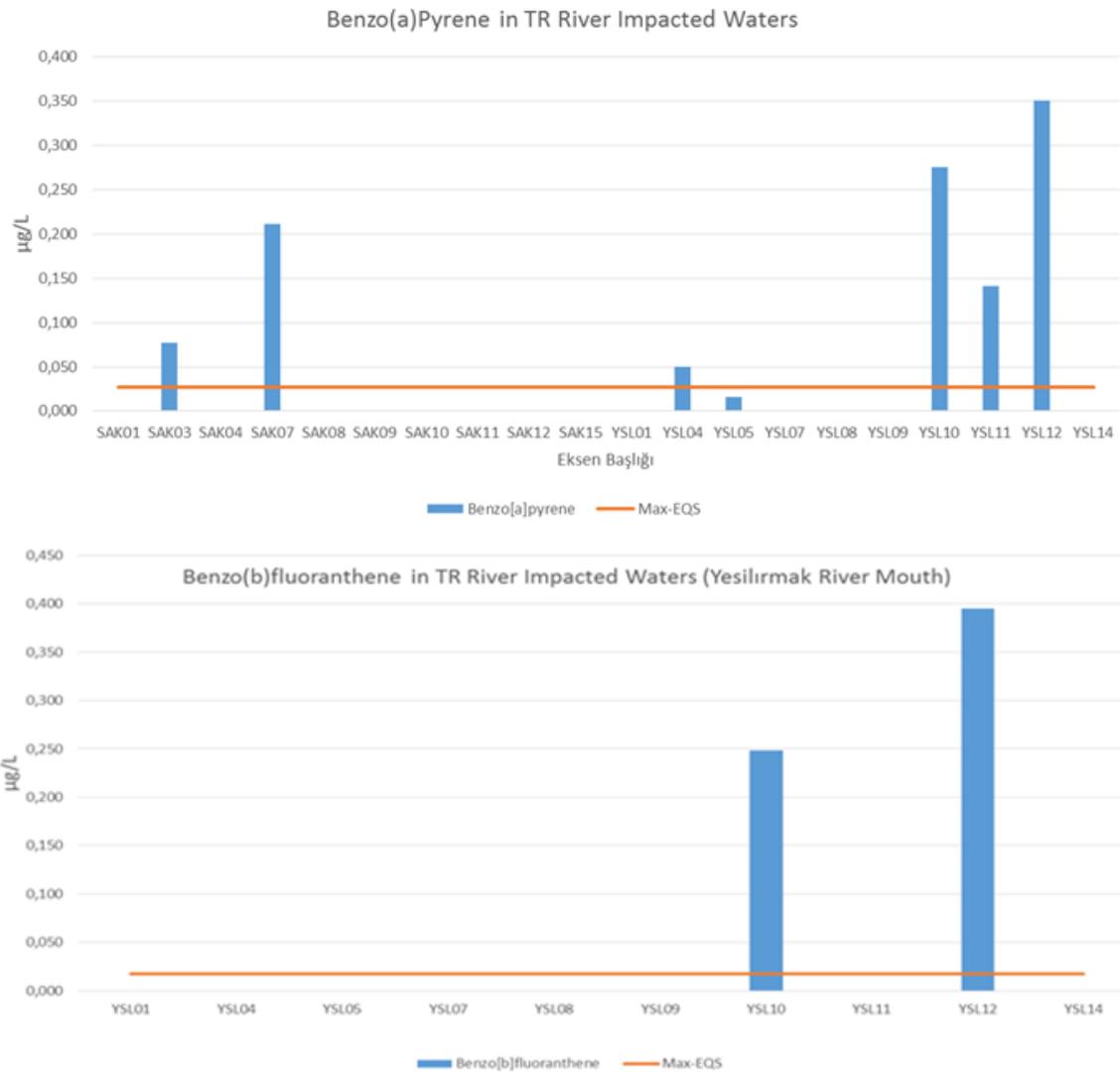


Figure 3.100 - Concentrations of Total Petroleum Hydrocarbons (TPH) in surface waters, in the marine area under the influence of Sakarya River and Yesilirmak River

Concentrations of most of the priority organic substances were below the Max-EQS (Directive 2013/39/EU) except Benzo(a)Pyrene (BaP), one of the 16 polycyclic aromatic hydrocarbons. The BaP levels were found higher than the Max-EQS (0.027 µg/L) in the winter season at two stations of Sakarya (SAK07 and SAK03: 0.211 µg/L and 0.078 µg/L) and four stations of Yesilirmak river mouths (YSL4, YSL10, YSL11 and YSL 12: 0.050 µg/L, 0.276 µg/L, 0.141 µg/L and 0.351 µg/L respectively). Benzo(b)fluoranthene concentrations were also higher in the two stations of Yesilirmak (0.248 µg/L and 0.395 µg/L at YSK 10 and YSK 12) than the threshold value (Max-EQS 0.017 µg/L) (Directive 2013/39/EU) (Figure 3.101). PCBs and Pesticides concentrations in seawater were below the detection limit.



**Figure 3.101 - Concentrations of benzo(a)pyrene and benzo(b)fluoranthene in surface waters, in the marine area under the influence of Sakarya and Yesilirmak in relation to the proposed value to define good environmental status, January 2020**

TPH values in the surface seawater are lower than the Max-EQS value (100 µg/L) stated in the National Surface Water Management Regulation (2016). Concentrations of most of the priority organic substances were below the Max-EQS (Directive 2013/39/EU) except Benzo(a)Pyrene (BaP), one of the 16 polyaromatic hydrocarbons. PCBs and Pesticides in seawater measured below the detection limit.

### Conclusions

Metal's concentrations in surface seawaters collected from Sakarya and Yesilirmak Rivers' impact areas in July 2019 and January 2020 indicated a low level of trace metal pollution, as 100 % of samples were below recommended EQS values for all measured metals - Cu, Pb, Ni, Cd. Generally, a decreasing gradient from the river mouth to the open area was noticed for most analysed metals, reflecting river influence upon receiving zone. Seasonal differences were observed in Sakarya samples in terms of higher metal content in winter. TPH values were lower than the Max-EQS value (100 µg/L) for both rivers' impact areas. The concentrations of most of the priority organic substances were measured below the Max-EQS (Directive 2013/39/EU) except Benzo(a)Pyrene (BaP), one of the 16 polyaromatic hydrocarbons. Similarly, PCBs and Pesticides in seawater were measured below the detection limit.

## 4 Benthic habitats

### 4.1 Zoobenthos communities

Benthic habitats play an essential role in some of the key ecosystem processes (e.g., primary production, food webs, recycling), but they are subject to many human pressures, putting at risk their functionality (Claudet & Fraschetti, 2010).

The European Marine Strategy Framework Directive (MSFD, 2008) requires the European Member States to achieve a Good Environmental Status (GEnS) by 2020 (Borja, 2006, Borja et al., 2011b and Borja et al., 2013).

The AZTI Marine Biotic Index (AMBI; Borja et al., 2000) and M-AMBI (Muxica et al., 2007) are widely used in assessing the quality of the benthic environment all over the world, and they were also reported as suitable approaches to assess the benthic ecological quality in the Black Sea. M-AMBI\*(n) (Sigovini et al., 2013) simplified the original method M-AMBI. It was proposed as one of the indicators for assessing the good environmental status of marine habitats in the Romanian and Bulgarian marine waters (Todorova et al., 2013, 2018; Abaza et al., 2018).

The assessment of benthic habitats' condition is one of the evaluation criteria both in the WFD (as the biological quality element) and in the MSFD descriptors (Benthic Habitat - D1, D4, D6). To describe the structure and functional conditions of the macrozoobenthos community under D4 used the following classification of organisms after (Macdonald et al. 2010). This classification includes the type of 1) Food source collecting type (EPibenthic, Surface, SS-subsurface) and 2) Feed Mode (Deposit feeder (ingests sediment; De), Detritus feeder (ingests particular matter only, without sediment; Dt), Suspension/Filter feeder (strains particles from the water, Su), Predator (eats live animals only; Pr), Scavenger (carrion only; Sc), Suctorial parasite (Sp), Chemosynthetic (with symbiotic bacteria, Ch), Lignivorous (eats wood, Li), Grazer (feeds by scraping, either on algae or sessile animals, Gr), and Browsing (feeds by tearing or gathering particular items, Br)), 3) Food size (Macdonald et al. 2010).

#### 4.1.1 Dnieper River, southern Bug River and Dniester River

The total number of species found in the area under study was 47 (Figure 4.1). The share (%) of main taxa was the same as for general NW Black Sea - Bivalvia, Polychaeta, and Crustacea were the most abundant. The most frequent species were *Alitta succinea* (Leuckart, 1847), *Anadara kagoshimensis* (Tokunaga, 1906), *Nephtys hombergii* Savigny in Lamarck, 1818, *Lentidium mediterranean* (O. G. Costa, 1830), *Cerastoderma glaucum* (Bruguière, 1789), *Chamelea gallina* (Linnaeus, 1758), *Chironomus salinarius* (Kieffer, 1915), *Monodacna colorata* (Eichwald, 1829), *Mytilus galloprovincialis* (Lamarck, 1819), *Amphibalanus improvises* (Darwin, 1854), and formed up to 90 % of total community biomass (Figure 4.2).

The reception of the polychaete *Aonides paucibranchiata*, *Capitella capitata europaea* Wu, 1964, *Eteone* sp., *Prionospio cirrifera* accounted for 51 % of the population. One of the main factors of rivers influence on the Black Sea is the salinity decrease in contact zones, which makes possible the penetration of brackish and freshwater species, such as *Chelicorophium nobile* (G.O. Sars, 1895) and *Chironomus plumosus* (Linnaeus, 1758). Euryhaline species, which prefer estuarine conditions, Such as *Parvicardium* spp., *Theodoxus euxinus* (Clessin, 1886), *Corophium volutator* (Pallas, 1766) and others, are also distributed in these zones. It should be noted that biomass and abundance could vary significantly between stations.

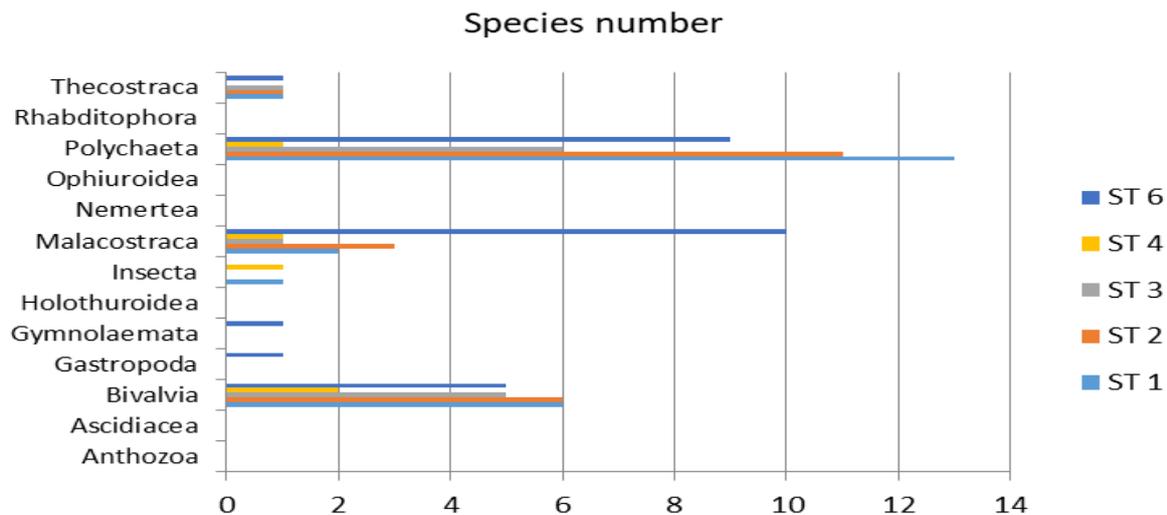


Figure 4.1 - Taxa composition within "River-Sea border" area

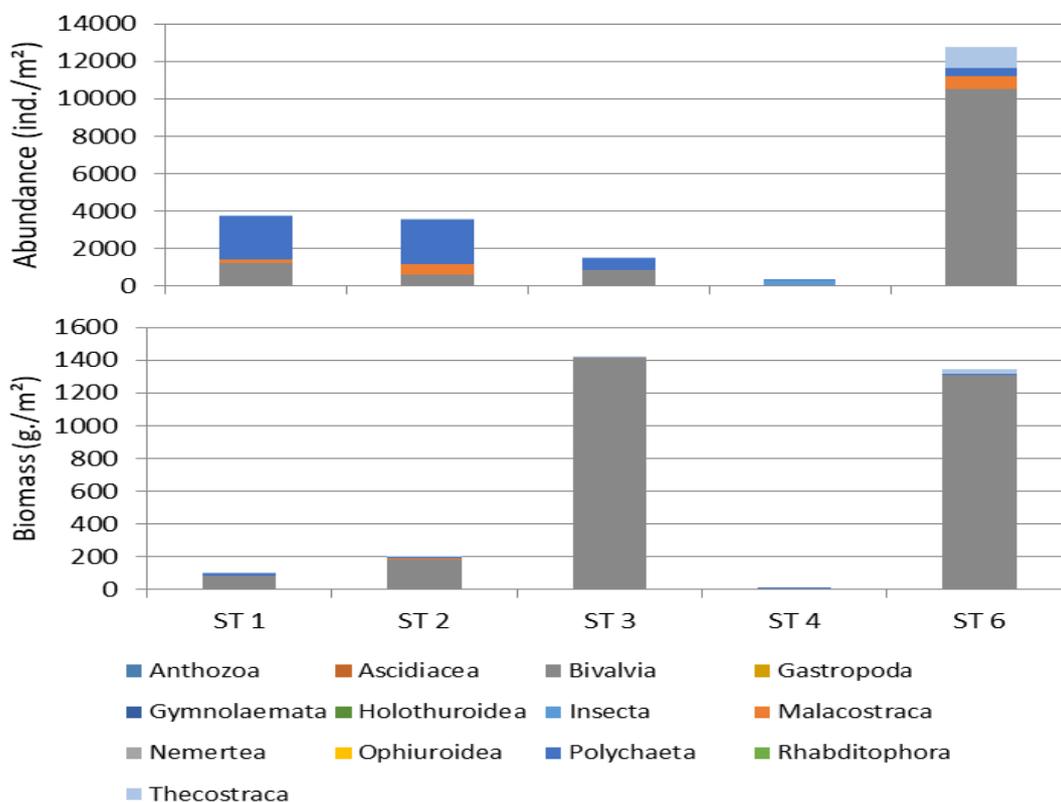
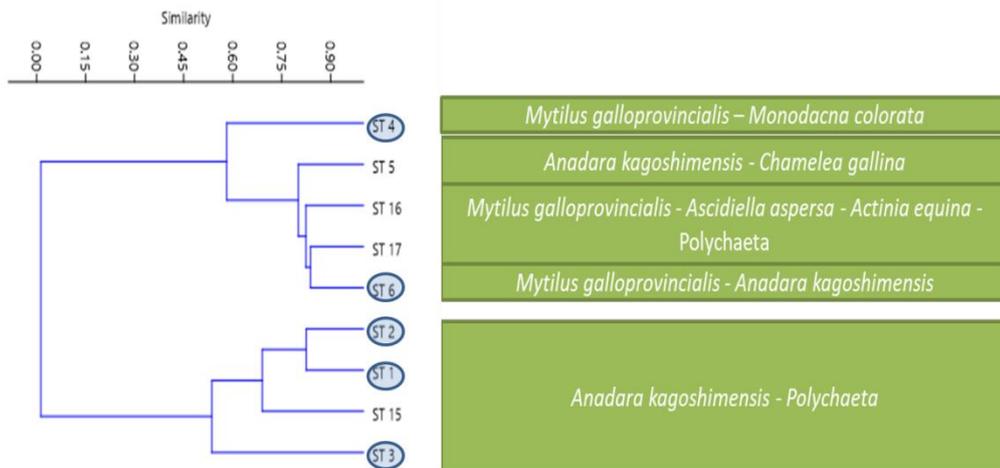


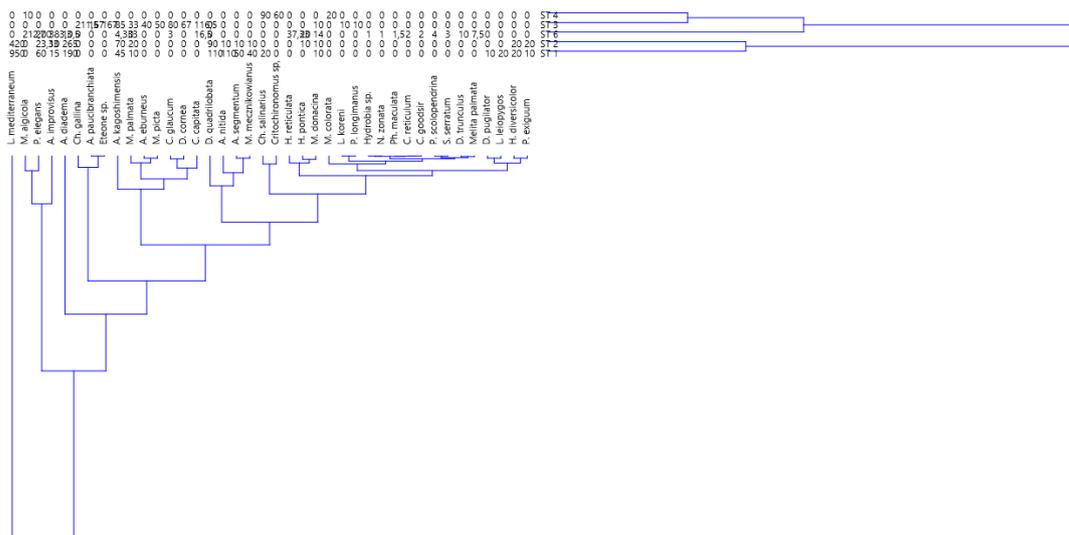
Figure 4.2 - Abundance and biomass of different taxa within "River-Sea border" area

Minor groups represented by Porifera, Cnidaria (*Sertularella polyzonias* (Linnaeus, 1758)), and Platyhelminthes are in the contact zone, unlike fully marine habitats with Bryozoa, Chordata, Cnidaria (*Actinia equina*), Echinodermata and Nemertea. Alternatively, a lot of fully marine organisms are absent in contact zones. Widespread marine euryhaline species form the fauna of macrozoobenthos in these zones. The two main groups of communities could be distinguished on the seabed basing on taxa composition and biomass conditions: 1) *Mytilus galloprovincialis* mainly on circalittoral mixed sediments and 2) *Anadara kagoshimensis* mainly on Circalittoral mud and offshore circalittoral mixed sediments

Bray Curtis similarity of macrozoobenthos based on % biomass and abundance (ind./m<sup>2</sup>) data comparing “river-sea border” stations (in circles) and total marine stations within the Ukrainian region of the Black Sea (Figure 4.3, Figure 3.4).



**Figure 4.3** Bray Curtis similarity of macrozoobenthos based on % biomass data comparing “river-sea border” stations (in circles) and total marine stations within the Ukrainian region of the Black Sea



**Figure 4.4** - Bray Curtis similarity of macrozoobenthos based on abundance (ind./m<sup>2</sup>) data comparing “river-sea border” within the Ukrainian region of the Black Sea

One of the features of contact zones is a high amount of organic matter, washed by river flow, which may increase the biomass of detritofags, especially Annelids and species specialised on subsurface predation meiobenthos (Figure 4.5).

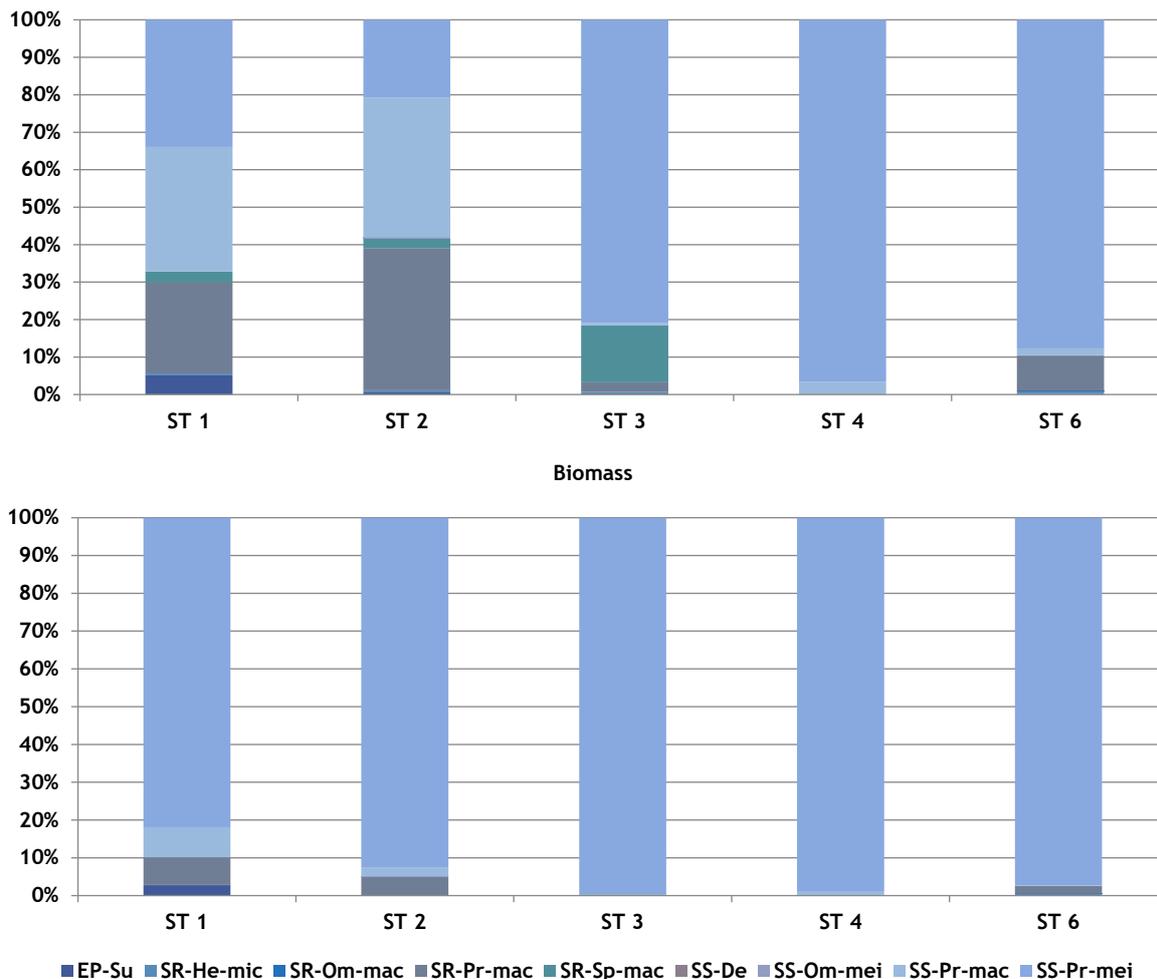


Figure 4.5 - Shares (%) in abundance and biomass of different functional feeding groups within “river-sea border” of the Ukrainian region of the Black Sea

Specific conditions in contact zones lead to changes in biodiversity. The number of species richness decreases in comparison with fully marine habitats. As we can see by iChao1 values, the lower bound of potential species richness in contact zones is generally lower than in shelf zones. The same pattern is observed on diversity indices (Table 4.1).

Table 4.1- Alpha diversity of studied samples

Station	River	S	iChao1	H'	I <sub>Mg</sub>
1	Danube	23	23	2.325	2.674
2	Danube	20	20	2.116	2.322
3	Dniester	13	13	2.124	1.635
6_1	Dnipro-Bug	17	19.25	0.6068	1.767
6_2	Dnipro-Bug	13	13	0.9741	1.437

The Environmental Status was assessed with indexes required by MSFD - AZTI Marine Biotic Index - AMBI (Dauvin and Ruellet, 2007; Albayrak et al., 2006; Muxika, Borja, and Bonne, 2005; Borja et al., 2004; Van Hoey et al., 2010; Соn, 2008; G. R. Phillips, A. Anwar, L. Brooks, L. J. Martina, A. C. Miles, 2014). While the Black Sea waters are the mesohaline type, the maximum values for indexes for the Black Sea were taken from the Water Framework Directive intercalibration technical report (Part 3) (Carletti and Heiskanen, 2009). M-AMBI\*(n) was calculated after (Sigovini, Keppel, and Tagliapietra, 2013).

The formula for AMBI calculation is:

$$\text{AMBI} = ((0 * \%GI) + (1.5 * \%GII) + (3 * \%GIII) + (4.5 * \%GIV) + (6 * \%GV))/100$$

GI - EG I the disturbance-sensitive species,

GII - disturbance-indifferent species,

GIII - disturbance-tolerant species,

GIV - second-order opportunistic species,

GV - first-order opportunistic species.

A comparison of AMBI values revealed that most of the stations were slightly disturbed (Table 4.2). The only undisturbed place was the Snake Island region in the central part of the NWBS shelf.

*Table 4.2 - AMBI and M-AMBI*

Type	Station	AMBI	Disturbance	M-AMBI(n)	Class
Danube	1	2.687	Slightly disturbed	0.908318	High
Danube	2	2.732	Slightly disturbed	0.832653	Good
Dniester	3	2.606	Slightly disturbed	0.741825	Good
Dnipro-Bug	6_1	2.692	Slightly disturbed	0.584388	Moderate
Dnipro-Bug	6_2	2.706	Slightly disturbed	0.576109	Moderate

Two main community types were found over the investigation of the NWBS, 1) *Mytilus galloprovincialis* mainly on circalittoral mixed sediments, and 2) *Anadara kagoshimensis* mainly on Circalittoral mud and offshore circalittoral mixed sediments. *Anadara kagoshimensis* with polychaetes was the most common for community type with river-sea border zone, with a muddy bottom and lower salinity.

The number of species was comparable in river-sea border (47) and shelf zone (50); the poorest species composition was shown under anthropogenic impact within hot spot area (10).

The trophic structure was typical for selected communities type. The hot-spot area trophic structure was closer to the river-sea border zone's trophic structure devote to soft muddy sediments there.

The ecological quality of river-sea contact zones, in general, was lower than in the central part of the shelf zone. Estimation of multimetric index M-AMBI(n) revealed the high class in all central parts of the NWBS shelf.

The conditions in the Dnieper-Bug estuary were "moderate" both in Summer and Autumn season.

## 4.1.2 Danube River

The study of Danube's River influence on the marine sector was based on a set of 48 macrozoobenthos samples collected at depths between 20 m and 60 m.

Macrozoobenthos data were analysed using abundance, species richness (S - as the number of taxa per sample), Shannon's diversity index (H'), AMBI index and its five ecological groups (EG) as single metrics. For this purpose, AZTI's AMBI software (<http://ambi.azti.es>) was used. As a multimetric or multivariate method, M-AMBI\*(n) was applied.

Reference conditions for the three M-AMBI \*(n) parameters (AMBI, diversity and richness) were calculated using the 95<sup>th</sup> percentile of richness (S) and diversity (H) values and the 5<sup>th</sup> percentile of AMBI values, using the available data.

Also, to determine the influence of freshwater input on benthic communities, statistical analysis using PRIMER package software v.7 was applied to the fourth root transformed abundance data (Clarke et al., 2014).

The marine habitats in front of the Danube's Mouths are under permanent stressful conditions due to freshwater input. The life of benthic organisms is influenced not only by different aspects of the muddy substrate but also by water dynamics. The Danube has an important influence on sedimentation in the predeltaic marine sector. The populations of benthic organisms that live here are well adapted, and some of them are recognized as indicator species that prefer such areas. In normal meteorological conditions, sedimentation does not affect benthic populations. However, the percentage of sedimentation increases during floods, which leads to the destruction of organisms, especially filter feeders. This was confirmed by the occurrence of many dead young molluscs in thanatocoenosis (Bacescu et al., 1971).

According to literature, at depths between 20m and 60m, muds inhabited by the bivalve *Mytilus galloprovincialis* were thoroughly described as having a primarily alluvial origin, different colours, depending on the distance from the Danube, varying from grey-yellowish to dark grey and including large quantities of dead shells, favouring the settlement of *Mytilus* youngsters. Deeper, starting from 60m, the mud changes its colour and consistency to lighter grey as they are inhabited by other bivalve species (*Modiolula phaseolina*). This type of mud also contains dead shells of different sizes, representing a mixture of sediments (Bacescu et al., 1971, Abaza et al., 2018).

The area under study comprised shallow circalittoral (20-40m depth) and deep circalittoral (40-60m depth). These two subunits of circalittoral are divided based on a temperature gradient characteristic of the Black Sea. This zone is also included in two marine protected areas: between 20m and 40m ROSCI 0066 (Danube Delta, marine part) and between 40m and 60m ROSCI 0413 (the South Lobe of the Zernov's Phyllophora field).

In term of diversity, in our samples, 92 macrozoobenthic species were found belonging to 10 groups. In terms of species number, the dominant group was Crustacea (31 %), followed by Polychaeta (29 %) and Mollusca (21 %). The Other groups accounted for less than 7 % of the total species (Figure 4.6).

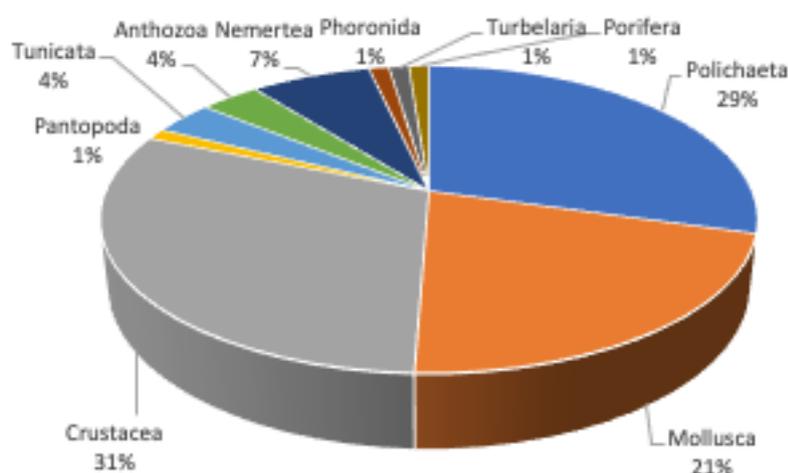


Figure 4.6 - Percentages of zoobenthic groups based on species number- in front of the Danube River, May 2019

SIMPER analysis highlighted that at 20 m depth, six macrozoobenthic species had a high contribution to the group's similarity. The first one was *Heteromastus filiformis* with 17.29 %, followed by *Capitella capitata* (14.77 %), *Alitta succinea* (13.87 %), *Melinna palmata* (10.03 %), *Abra prismatica* (9.07 %) and *Polidora cornuta* (6.10 %). At 30 m depth, five species had 71 % contribution. Among these, *M. palmata* (27.35 %) and *Nephtys hombergii* (18.21 %) had the highest contribution. At 37 m depth, three species contributed with 75 %: *M. palmata* (41.84 %), *Diadumene lineata* (17.74 %) and *N. hombergii* (16.07 %). At 40 m depth, thirteen species contributed with 71 %. Among them, the most typical species were *H. filiformis* (12.16 %) and *M. palmata* (11.87 %). At 50 m and 60 m, *Terebelides stroemii* and *Phtisica marina* dominated the communities.

We observed different dominant species according to depth range:

- At 20 m depth, the dominant species were the opportunistic polychaetes *H. filiformis* and *Capitella capitata*. *Capitella capitata*, the second dominant species from 20 m, is considered in many studies, the most common indicator of high organic matter content in sediments (Dean, 2008).
- Between 30 m and 40 m, *M. palmata* dominated the benthic communities. Deposit feeder, *M. palmata*, is an opportunistic species preferring soft-sediment rich in detritus and considered an indicator of areas with relatively high sedimentation rate.
- Between 50 m and 60 m, *T. stroemii* and *P. marina* dominated. Living individuals of *Modiolula phaseolina* appeared only at 60 m, except Sulina 50 m.

We identified three broad habitat types: circalittoral coarse and mixed sediments (Figure 4.7), circalittoral terrigenous muds (Figure 4.8) and deep circalittoral coarse mixed sediments (Figure 4.9).



Figure 4.7 - Circalittoral coarse and mixed sediments with diverse faunal assemblages (*Spisula*, *Capitella*, *Heteromastus* etc.) - in front of the Danube River, May 2019



Figure 4.8 - Circalittoral terrigenous muds with *Melinna palmata* and *Nephtys hombergii* - in front of the Danube River, May 2019



Figure 4.9 - Deep circalittoral coarse mixed sediments with *Modiolula phaseolina* and polychaetes - in front of the Danube River, May 2019

Using SIMPER analysis, we observed the dissimilarities according to depth range. The dissimilarity between 20 m and 30 m was 63.40 %. High dissimilarity (76.19 %) was also registered between 30 m and 50 m.

According to the Non-metric Multi-Dimensional Scaling (nMDS), dimension two stress, 0.09, abundance has a clear trajectory from 20 m to 60 m on each transect (Figure 4.10).

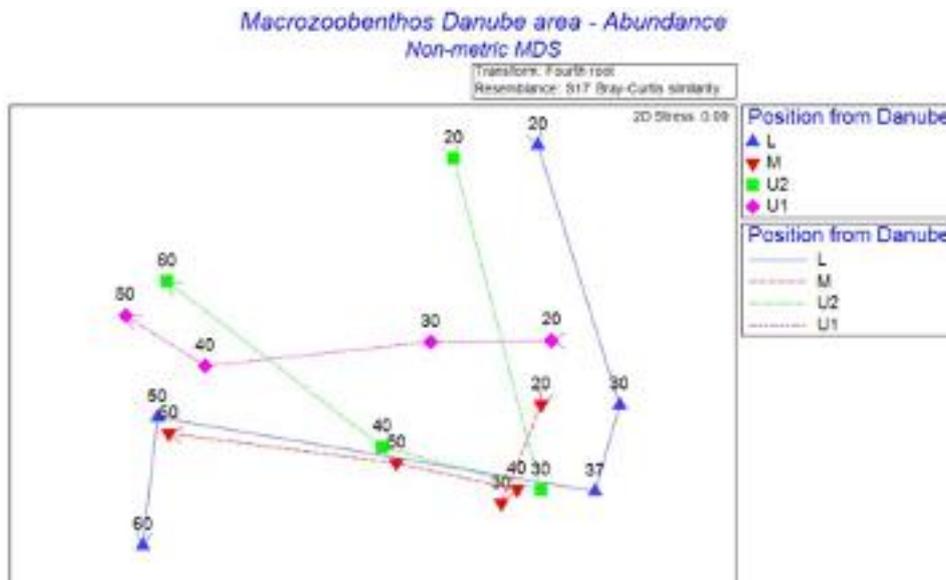


Figure 4.10 - Macrofaunal abundance trajectory - in front of the Danube River, May 2019

An essential factor determining the distribution pattern of macrozoobenthic fauna was depth, respectively habitat type, and not the stations' position from the Danube's Mouths (Figure 4.11). The best explanation of this distribution is that Danube's River input influences the entire sector (Sulina-Periboina), and no gradient can be observed. The highest abundances were recorded by *Melinnia palmata* at depths from 30 m to 40 m (sometimes even 50 m).

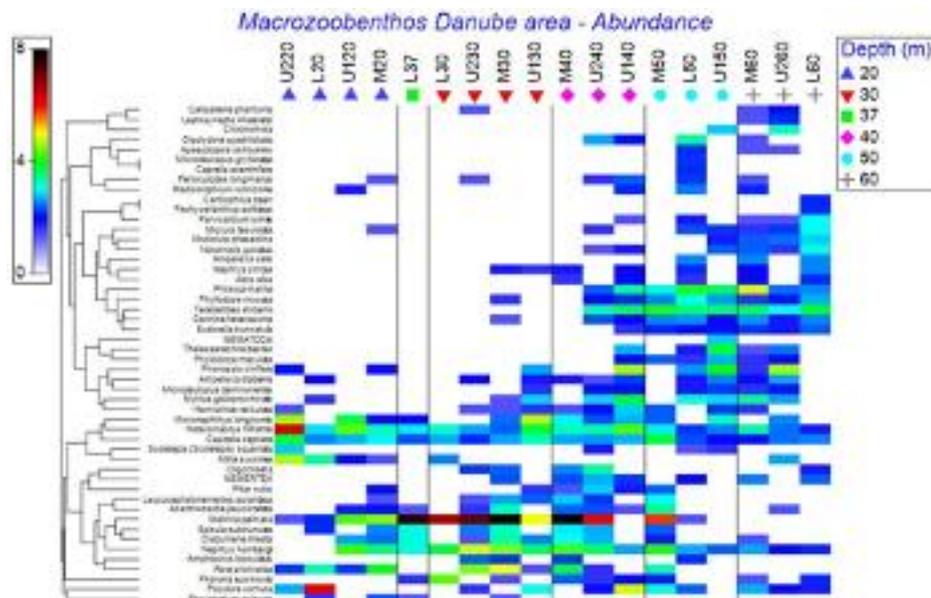
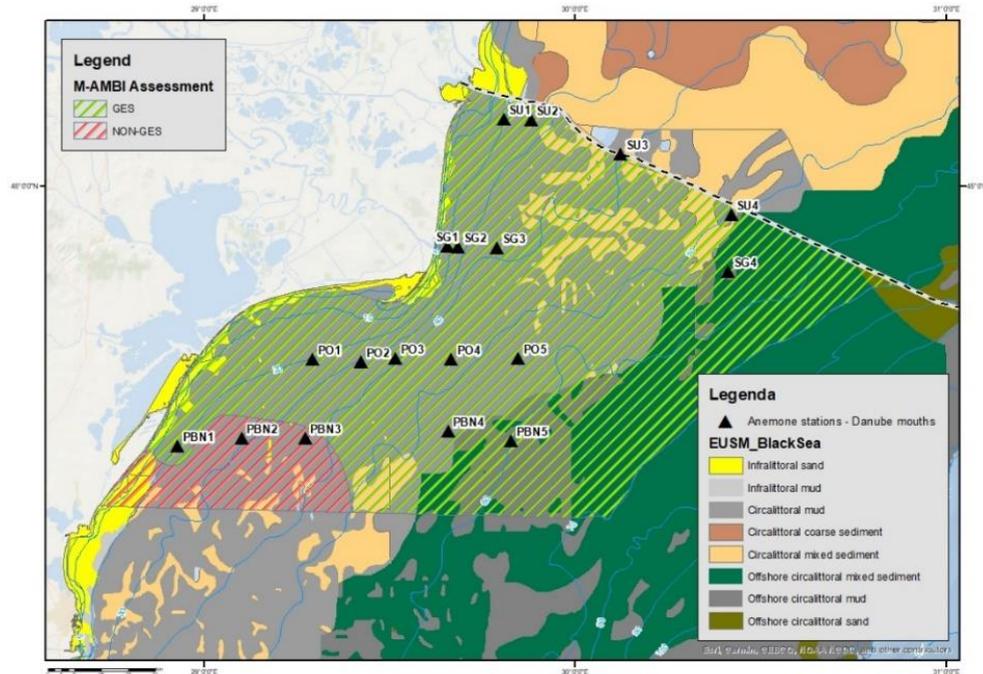


Figure 4.11 - Shade plot of macrozoobenthos abundance depending on: position (U - in front of the Danube Mouths; M-medium distance from the Danube Mouths, L-lower position the Danube Mouths) and depth

The macrobenthic communities are considered an excellent tool to evaluate the ecological status of aquatic ecosystems. The ecological status assessment of the macrozoobenthic communities, using M-

AMBI\*(n), showed that 89 % (16 stations) of the stations were GES and 11 % (2 stations) were Non-GES (Figure 4.12). Non-GES stations are situated in the southern part of the area at 30 m and 37 m (PBN2 and PBN3). The EQR values for these two stations were 0.62 (PBN2), respectively, 0.61 (PBN 3). The boundary between GES and Non-GES was 0.68. A low diversity was observed in Non-GES stations compared to other stations from the same depth.



**Figure 4.12 - Map of the studied area, sampling stations and ecological status of communities evaluated using M-AMBI\*(n) - in front of the Danube River, May 2019**

We identified a total of 92 macrozoobenthic species in the entire area. In general, opportunistic species adapted to live in areas with a high sedimentation rate dominated the benthic communities. M-AMBI\*(n) multimetric index applied to benthic species densities showed good ecological status for 89 % of the stations.

Compared to other stations from the same depth, low diversity was observed in the Non-GES stations (PBN2 and PBN3).

## Conclusion

The Danube delta species composition is close to those of the Dnieper, south Bug and Dniester. The main factor determining the distribution pattern of macrozoobenthic fauna was depth, respectively habitat type.

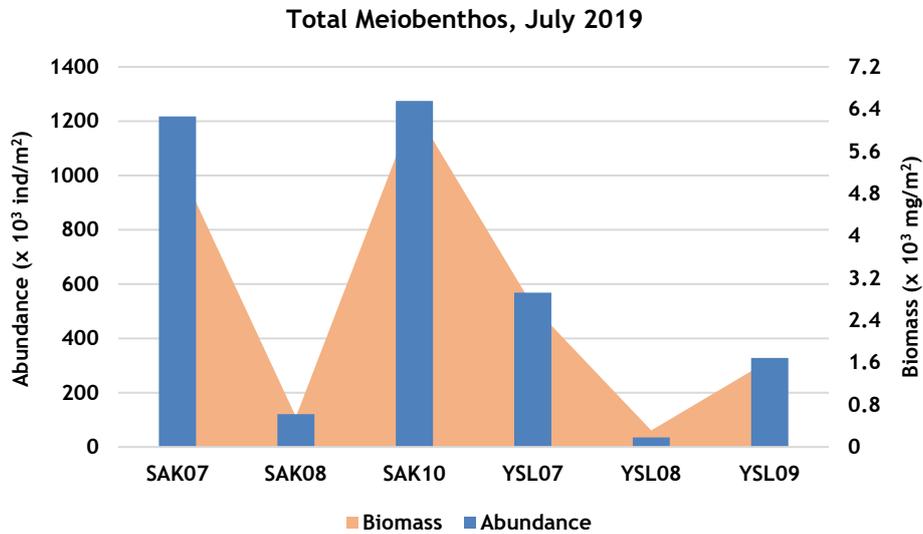
### 4.1.3 Sakarya River and Yesilirmak River

#### Meiobenthos communities

As a result of the analyses of meiobenthic material collected during the samplings at the survey area, samples revealed 13 362 individuals (July 2019) and 6 563 individuals (January 2020). We recorded and counted ten higher taxonomic groups (Nematoda, Harpacticoida, Polychaeta, Oligochaeta, Turbellaria, Ostracoda, Kinorhyncha, Amphipoda, Bivalvia, Acarina) (Annex C).

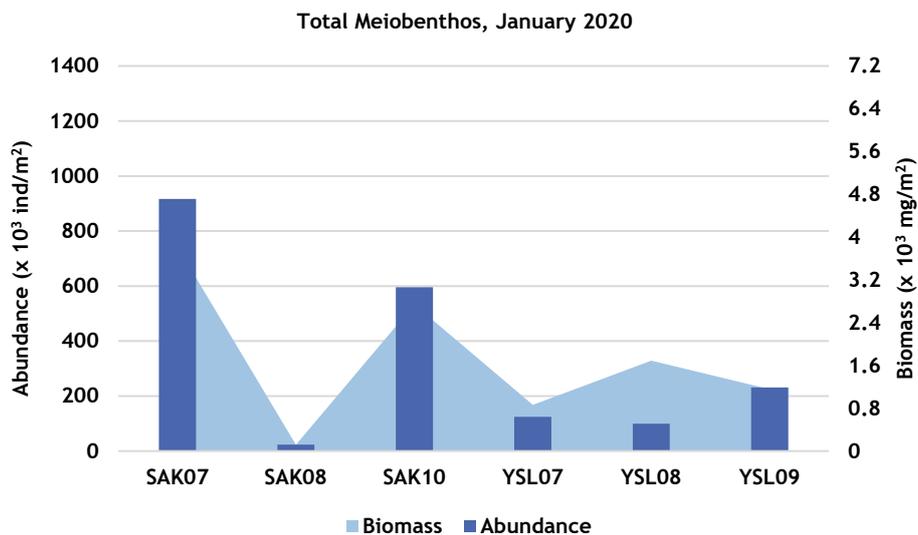
The distribution of taxa among stations showed that the diversity of the station SAK10 was high ranking first with ten taxonomic groups, followed by YSL09 with eight groups. Stations SAK07, SAK08, SAAT04 and YSL08 had the lowest number of groups. As of rarely found taxa, several specimens of Kinorhyncha were encountered only at stations SAK10 and YSL09.

The total mean meiobenthos of the stations ranged between  $35 \cdot 10^3$ - $1\,274 \cdot 10^3$  ind./m<sup>2</sup> in July 2019. The highest value was recorded at SAK10, followed by SAK07. The lowest value was recorded at YSL08. The stations' total mean biomass ranged between 313.1 mg/m<sup>2</sup> and 6 405.2 mg/m<sup>2</sup> and fluctuated in parallel to the abundance values (Figure 4.13).



**Figure 4.13 - Spatial variation of abundance and biomass of total meiobenthos - in front of the Sakarya and Yesilirmak Rivers, July 2019**

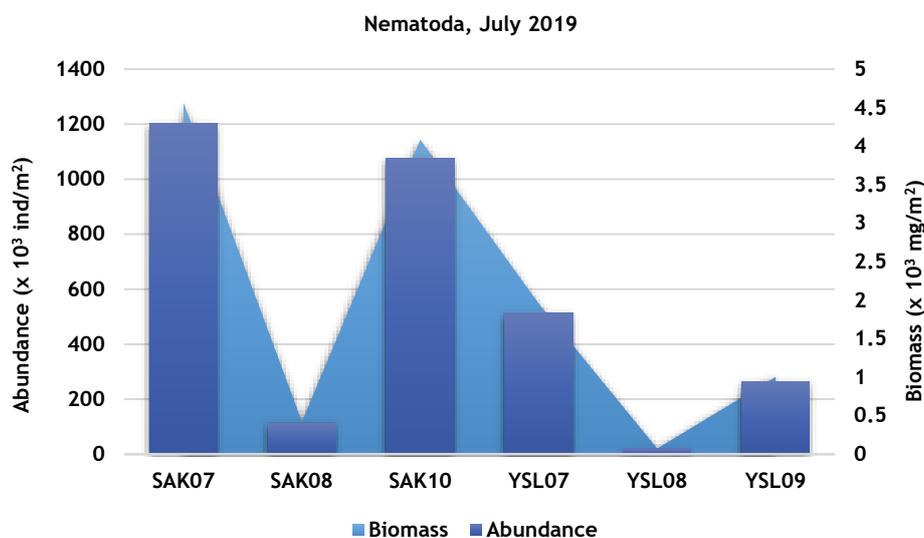
The total mean meiobenthos prominently decreased at almost all stations and ranged between  $23 \cdot 10^3$  ind./m<sup>2</sup> and  $916 \cdot 10^3$  ind./m<sup>2</sup>, in January 2020. The highest value was recorded at SAK07 ( $595 \cdot 10^3$  ind./m<sup>2</sup>), followed by SAK10. The lowest value was recorded at SAK08. The stations' total mean biomass ranged between 118.2 ind./m<sup>2</sup> and 3 819.3 mg/m<sup>2</sup> and fluctuated in parallel to the abundance values except for YSL08 due to polychaetes ostracods and juvenile bivalves, contribution (Figure 4.14).



**Figure 4.14 - Spatial variation of abundance and biomass of total meiobenthos - in front of the Sakarya and Yesilirmak Rivers, January 2020**

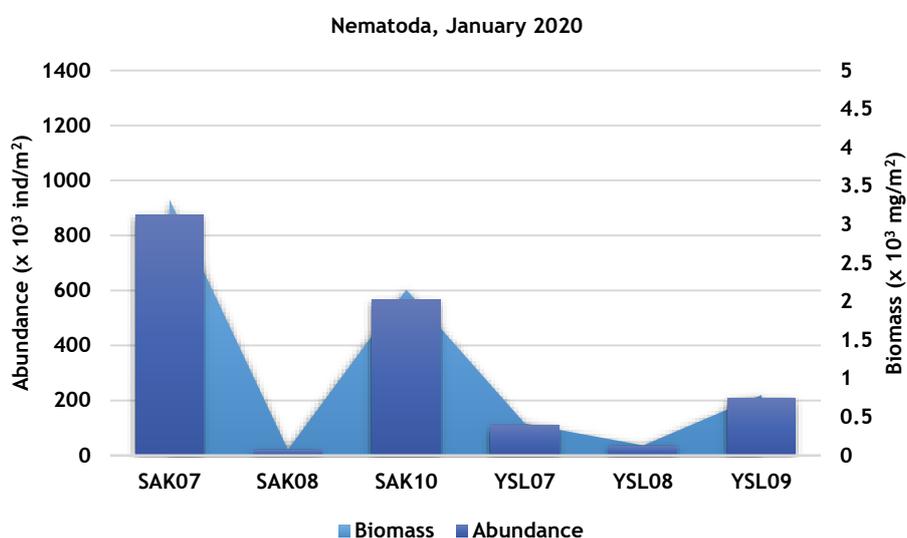
As the most significant component of meiofauna, free-living marine nematodes were found at all stations. Nematode abundance was considerably high at SAK07 ( $1\,201 \cdot 10^3$  ind./m<sup>2</sup>), followed by SAK10

( $1\ 074 \cdot 10^3$  ind./m<sup>2</sup>) at the river impact stations. The lowest values were recorded at YSL08 ( $19 \cdot 10^3$  ind./m<sup>2</sup>). Biomass fluctuated in parallel to the abundance values and ranged between 74.6 mg/m<sup>2</sup> (YSL08) and 4 567.4 mg/m<sup>2</sup> (SAK07) (Figure 4.15).



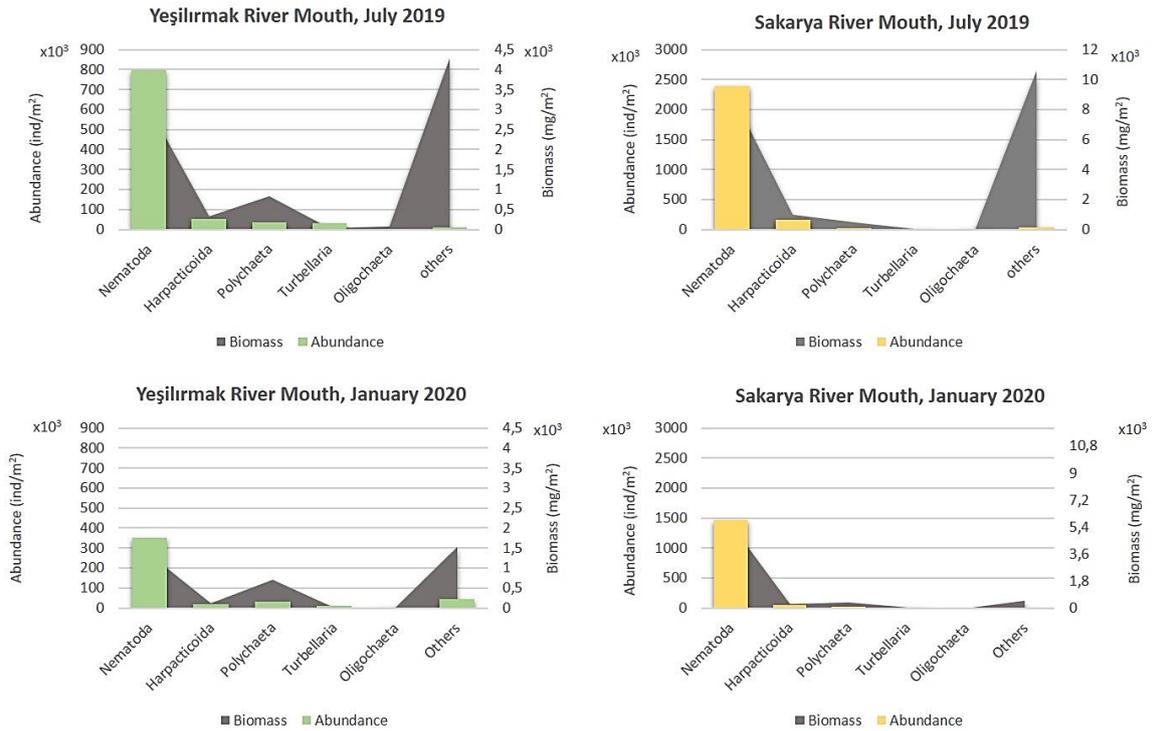
**Figure 4.15 - Spatial variation of abundance and biomass of Nematoda - in front of the Sakarya and Yesilirmak Rivers, July 2019**

Nematode abundances considerably decreased in winter and ranged between  $20 \cdot 10^3$  ind./m<sup>2</sup> and  $875 \cdot 10^3$  ind./m<sup>2</sup> in January 2020. The lowest values were recorded at SAK08, followed by YSL08 ( $34 \cdot 10^3$  ind./m<sup>2</sup>). Biomass values fluctuated in parallel to the abundance and ranged between 76.6 mg/m<sup>2</sup> (SAK08) and 3 325.2 mg/m<sup>2</sup> (SAK07) (Figure 4.16).



**Figure 4.16 - Spatial variation of abundance and biomass of Nematoda - in front of the Sakarya and Yesilirmak Rivers, January 2020**

The structure of major meiofaunal taxa showed differences between Yeşilirmak and Sakarya river mouths. Nematode were more abundant at Sakarya river mouth stations, whereas abundance and biomass of Polychaeta were higher at Yeşilirmak river mouth stations. Seasonal changes were also prominent, and almost all values decreased in winter (Figure 4.17).



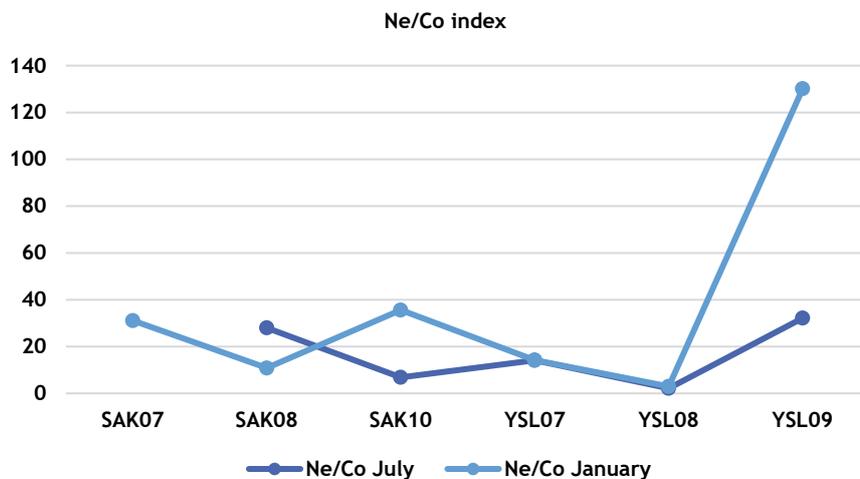
**Figure 4.17 - Total mean abundance and biomass of major meiofaunal taxa - in front of the Sakarya and Yeşilirmak Rivers, July 2019, and January 2020**

Abundance percentages showed that nematodes had the highest share at all stations except YSL08, where they had a low share of 35 % in January 2020 (Figure 4.18). The second dominant group was others (27 %), mostly including Ostracoda, followed by Polychaeta (19 %) and then Harpacticoida (11 %). It was clear that meiobenthos of SAK07 was almost composed of nematodes during both seasons (98 % and 95 %, for summer and winter, respectively). Percentages' biomass showed a higher contribution of Polychaeta as the individuals are larger than the other meiobentic groups. Likewise, the percentages of the group "Others" at the stations were high since this group included juvenile bivalves and ostracode.



**Figure 4.18 - Percentages of meiobenthic groups based on abundance and biomass - in front of the Sakarya and Yesilirmak Rivers, July 2019, and January 2020**

Raffaelli and Manson (1981) suggested using Ne/Co index in pollution studies as a fast, easy, and reliable tool to monitor the effect of organic matter enrichment. This index was supported by the different pollution tolerance of these two main meiobenthic taxa; hence a higher Ne/Co value might indicate a polluted site. The abundance of nematodes can decline after pollution events but have shown higher tolerance to pollution than copepods (e.g., Gee et al., 1985). Moreover, considering the difficulty and time-consuming process of taxonomic identification of nematodes and copepods, the use of this index became popular. Raffaelli and Manson (1981) proposed 100 as a pollution threshold; however, Warwick (1981) recommended other values for sand (Ne/Co > 10) and mud (Ne/Co > 40) seabed. The ratio ranged between 2.17 (YSL08- July 2019) and 130.16 (YSL09- January 2020) at the river mouth stations. The highest Ne/Co value, above 100, was at YSL09 in January 2020 (Figure 4.19).



**Figure 4.19 - Ne/Co Index values - in front of the Sakarya and Yesilirmak Rivers, July 2019 and January 2020**

## Conclusions

Our meiobenthic research at the river impact area revealed ten higher taxonomic groups (Nematoda, Harpacticoida, Polychaeta, Oligochaeta, Turbellaria, Ostracoda, Kinorhyncha, Amphipoda, Bivalvia, Acari) at Sakarya and Yeşilirmak rivers mouths. As of rarely found taxa, several specimens of Kinorhyncha were encountered only at stations SAK10 and YSL09. Kinorhynchans are marine micrometazoans, usually representing 1-8 % of the total meiofauna (Neuhaus 2012) and rarely encountered ecological meiobenthos studies since some specific methods are generally needed for their recruitment from the sediment (Sanchez et al. 2012).

The distribution of taxa among stations showed that the diversity of the station SAK10 was high ranking first with ten taxonomic groups. The main reason might be the biotope different from the other stations, with lots of shell fragments, providing more interstitial space for different meiobenthic taxa to inhabit. The highest value also recorded at SAK10 where the meiobenthic diversity is high. From a seasonal point of view, the total mean meiobenthos of the stations prominently decreased at almost all winter stations. It is probably the result of the reproduction periods during spring, causing high abundance values in summer.

As the most essential component of meiofauna, free-living marine nematodes were found at all sampling stations in the research area except YSL08, where harpacticoids and polychaetes significantly contributed to the total meiobenthos of the station. Since nematodes are at the lower step of the benthic chain than polychaetes, we might assume that nematodes became a food source for polychaetes at this station.

When we consider the ecological quality of Yeşilirmak, a higher share of harpacticoids at YSL08 indicated a relatively better-defined habitat compared to the other stations, also supported by the lowest Ne/Co index both in summer and winter. YSL08 had the lowest Ne/Co scores, 2.17 and 2.97, thus representing a better benthic quality than the other stations in both seasons. On the contrary, the abundance of nematodes was 130 times more than that of harpacticoid copepods at the station YSL09 in winter, showing a tendency for a polluted environment (Ersoy-Karacuha et al., 2011, Semprucci et al., 2016). YSL09 (Yeşilirmak) showed the highest index value in January, representing organic pollution. For Sakarya mouth stations evaluation, SAK07 and SAK08 with sandy biotopes represented polluted sites in January and July, respectively, since the index was above 10 (Warwick, 1981). Alternatively, SAK10 revealed good conditions, also supported by high taxonomic diversity. In addition to all these, more experiments are necessary to fix the Ne/Co index threshold for the Black Sea because of the complex answer of meiofauna to sediment changes. We might say that Ne/Co index may help masses the health status of the benthic environment. On the other hand, it may not be used as a single method for biomonitoring. Still, it can be a supporting index parallel to other meiofauna indices such as Maturity Index, which is based on nematode genera and copepode values. Nevertheless, the maturity Index requires much more time, usually not applicable to biomonitoring studies or short-term projects (Raffaelli & Manson, 1981; Sezgin et al., 2013; Ürkmez et al., 2017).

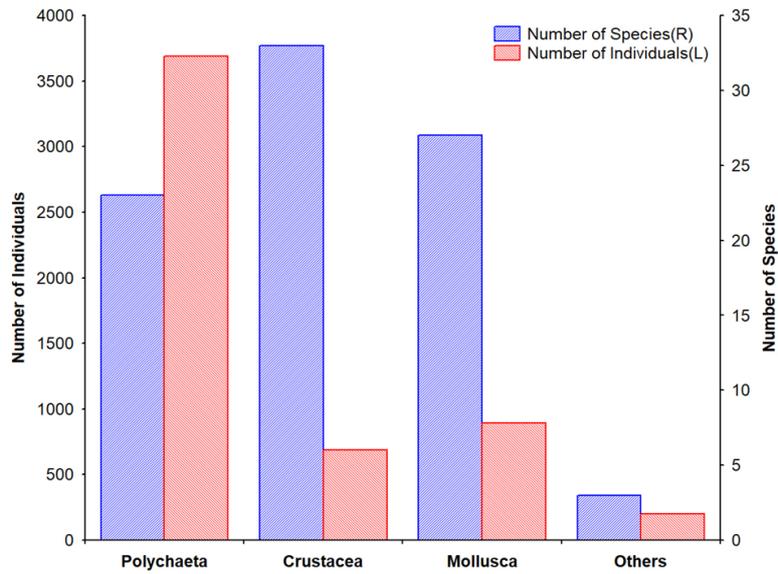
## Macrozoobenthos communities

As a result of the analyses of benthic material collected during the samplings in July 2019 at the survey area, a total of 86 species belonging to 6 taxonomic groups (Nemertea, Oligochaeta, Polychaeta, Crustacea, Mollusca and Echinodermata) were identified (Annex C).

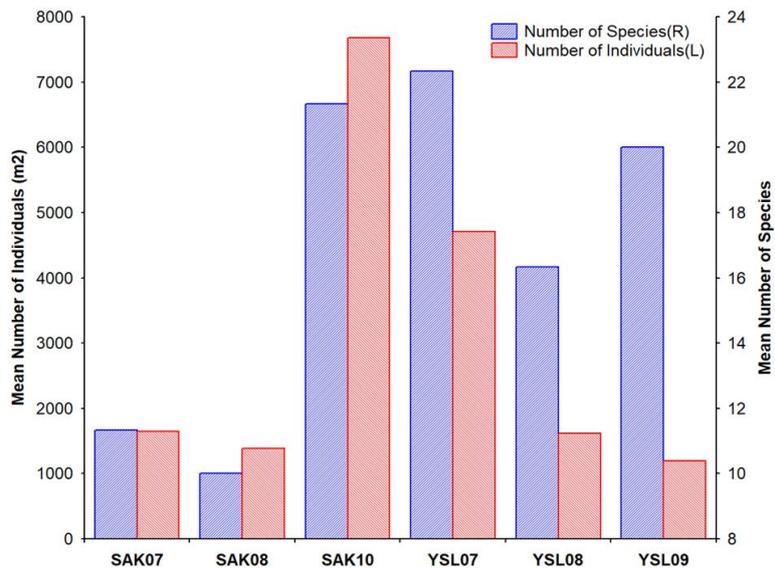
The species' distribution among taxonomic groups was examined; Crustacea ranked first with 33 species and followed by Mollusca (27 species), Polychaeta (23 species). Other groups were represented by a fewer number of species (Figure 4.20).

We found the highest mean of species number at station YSL07 (22 species) and the lowest at station SAK08 (10 species). Regarding the individuals, station SAK10 had the highest mean (7 680 ind./m<sup>2</sup>), while station YSL09 the lowest densities (1 196.7 ind./m<sup>2</sup>) (Figure 4.21).

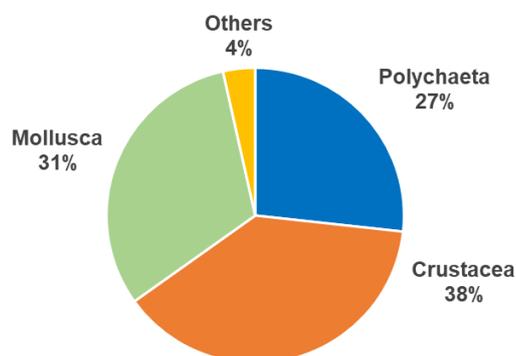
The dominant taxon was Crustacea (38 %), followed by Mollusca (31 %) and Polychaeta (27 %). Other groups represented 4 % dominance (Figure 4.22).



**Figure 4.20 - Distribution of the number of species and mean of individuals among taxonomic groups - in front of the Sakarya and Yesilirmak Rivers, July 2019**



**Figure 4.21 - Distribution of the mean number of species and individuals among stations - in front of the Sakarya and Yesilirmak Rivers, July 2019**



**Figure 4.22 - Dominance (%) of zoobenthic taxa based on the number of species - in front of the Sakarya and Yesilirmak Rivers, July 2019**

The most abundant group was Polychaeta (67 %), followed by densities of Mollusca (16 %), Crustacea (13 %) and others (4 %), respectively (Figure 4.23).

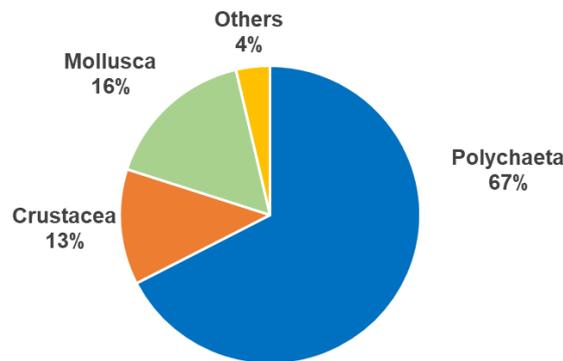


Figure 4.23 - Dominance (%) of zoobenthic taxa number of individuals - in front of the Sakarya and Yesilirmak Rivers, July 2019

According to the Soyer's Frequency Index, the most constant taxon with an 83 % frequency index value was polychaete *Heteromastus filiformis* in summer. The other frequent species were polychaetes *Micronephyts longicornis* (67 %), *Prionospio maciolekae* (56 %), mollusks *Calyptraea chinensis* (56 %), and *Abra alba* (50 %).

The highest mean diversity index value among the stations was at station YSL09 ( $H' = 3.56$ ), and the lowest at SAK10 ( $H' = 1.53$ ). Evenness index values ranged between ( $J'$ ) 0.31 (sta SAK10) and 0.91 (sta YSL09) (Figure 4.24). In the station SAK10 with the lowest species diversity, the evenness index value was also found to be the lowest. When the species diversity index value is low and there is single-species dominance, the evenness index value is also low; the evenness index value is high at stations with higher species diversity.

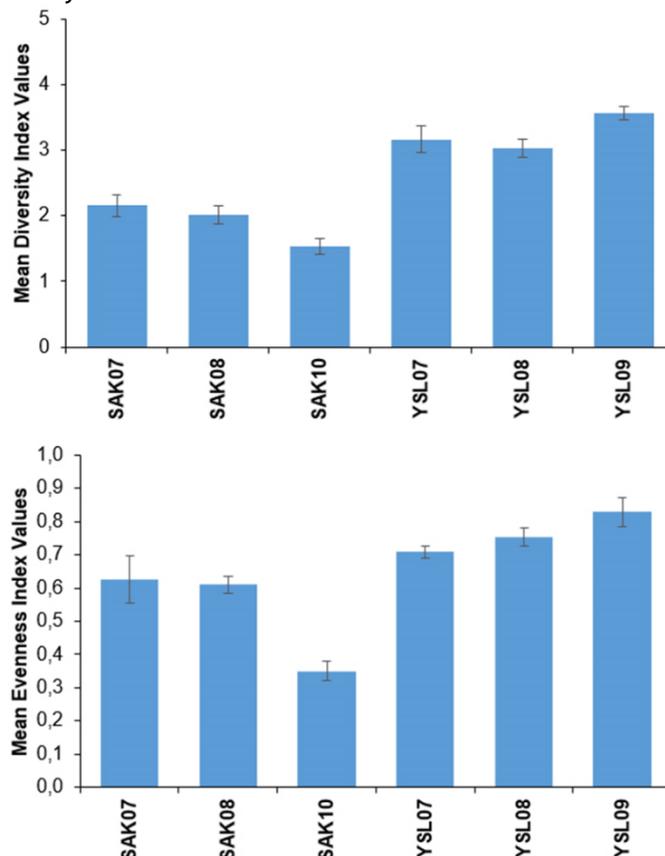


Figure 4.24 - Diversity and evenness index (mean  $\pm$  SE) - in front of the Sakarya and Yesilirmak Rivers, July 2019

Turkish Benthic index TUBI values among the stations ranged between 1.64 (SAK08) and 3.67 (YSL07). The station SAK08 had the lowest TUBI scores in the area, thus classifying the water body's benthic quality status as "poor"; four stations possessed TUBI scores that indicated "moderate" ecological status. Only station YSL07 had a high mean TUBI value ( $3.5 \pm 0.1$ ), indicating the "high" ecological status (Figure 4.25).

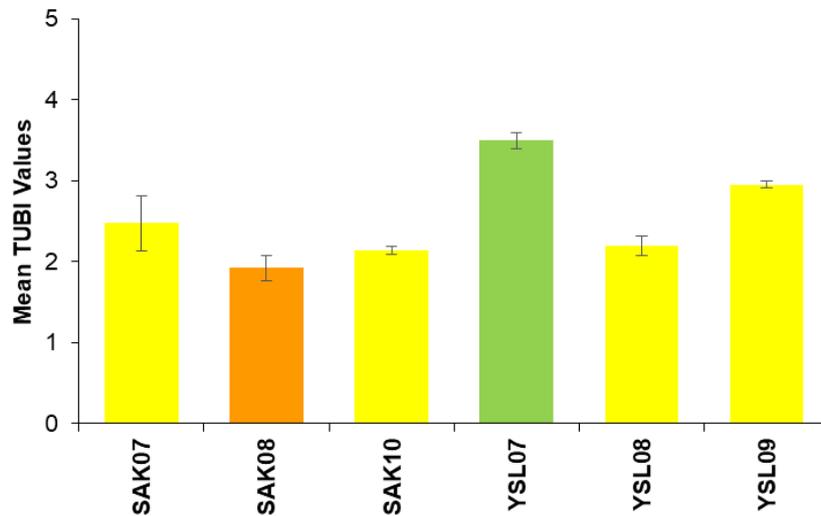


Figure 4.25 - TUBI (mean  $\pm$  SE), - in front of the Sakarya and Yesilirmak Rivers, July 2019

In winter, we identified a total of 85 species belonging to 9 taxonomic groups (Cnidaria, Nemertea, Oligochaeta, Polychaeta, Phoronida, Crustacea, Mollusca, Echinodermata and Tunicata (Annex C). The community structure of soft-bottom zoobenthos of the area is described seasonally using some ecological analyses. Mollusca ranked first with 31 species, followed by Crustacea (27) and Polychaeta (20). "Other" groups were represented by a smaller number of species (Figure 4.26).

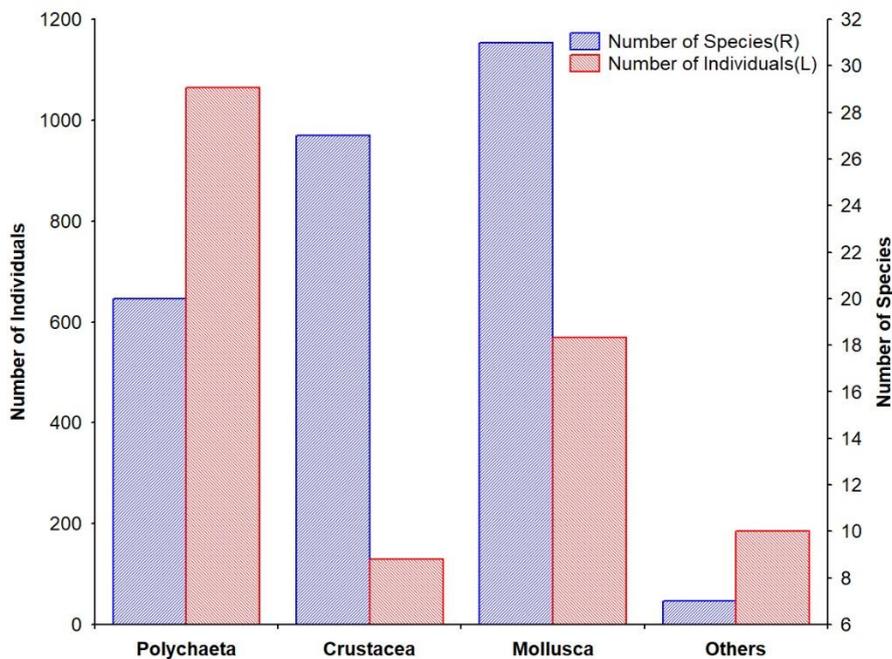
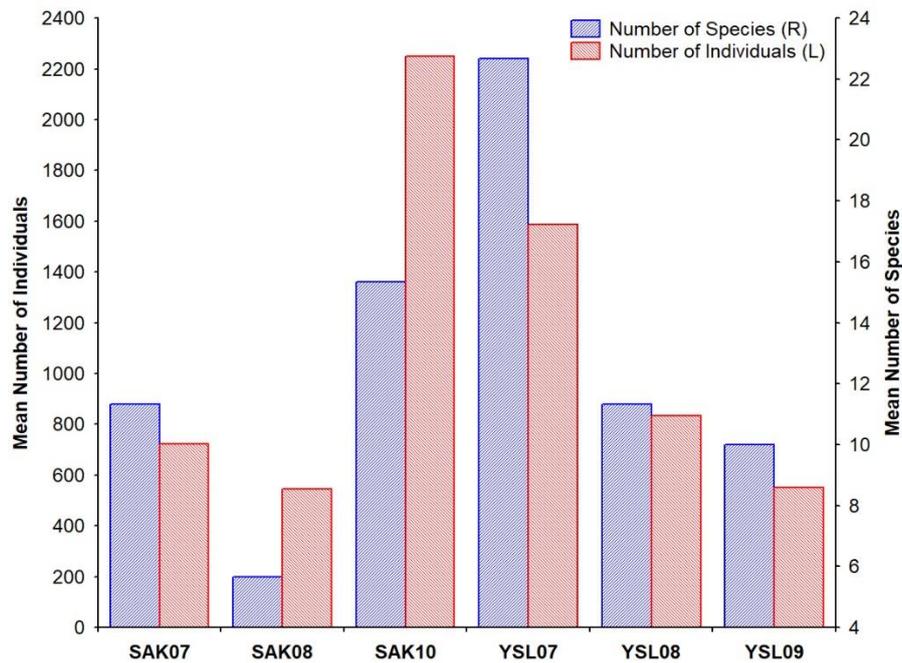


Figure 4.26 - Distribution of the number of species and mean of individuals among taxonomic groups- in front of the Sakarya and Yesilirmak Rivers, January 2020

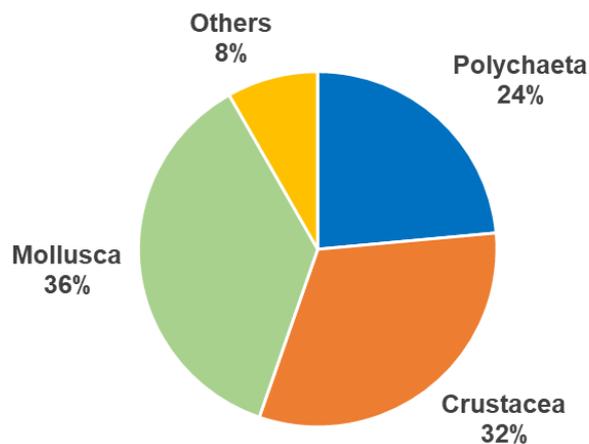
The highest mean of species number was determined at station YSL07 (23 species) and the lowest at station SAK08 (6 species). The highest mean of individuals was encountered at station SAK10 (2 250

ind./m<sup>2</sup>), and station SAK08 (546.7 ind./m<sup>2</sup>) were observed to have the lowest number of individuals (Figure 4.27).



**Figure 4.27 - Distribution of the mean number of species and individuals among stations- in front of the Sakarya and Yesilirmak Rivers, January 2020**

As the proportion of species, Mollusca was found to be the dominant taxon (36 %), followed by Crustacea (32 %) and Polychaeta (24 %). Other groups were represented by 8 % dominance (Figure 4.28).



**Figure 4.28 - Dominance (%) of zoobenthic taxa based on the number of species - in front of the Sakarya and Yesilirmak River, January 2020**

Based on the number of individuals, Polychaeta was found as the dominant group (55 %) followed by Mollusca (29 %), Crustacea (7 %) and others (9 %), respectively (Figure 4.29).

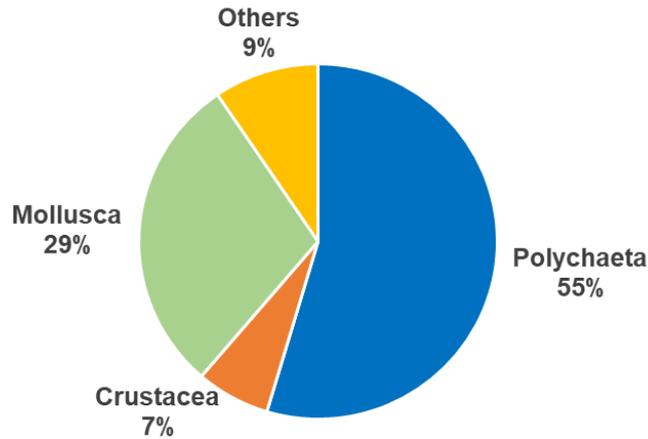


Figure 4.29 - Dominance (%) of zoobenthic taxa number of individuals- in front of the Sakarya and Yesilirmak Rivers, January 2020

In winter, polychaete *Nemertea* (sp.) was detected to be the most constant taxon with a frequency index of 78 %. The other frequent species were determined as polychaetes *Heteromastus filiformis* (61 %) and *Micronephyts longicornis* (56 %).

The highest mean diversity index value among the stations was found at station YSL09 ( $H' = 2.65$ ), and the lowest at station SAK08 ( $H' = 1.80$ ). Evenness index values ranged between ( $J'$ ) 0.60 (sta SAK10) and 0.80 (sta YSL09 and SAK07) (Figure 4.30). In stations with low species diversity, species showed heterogeneous distribution.

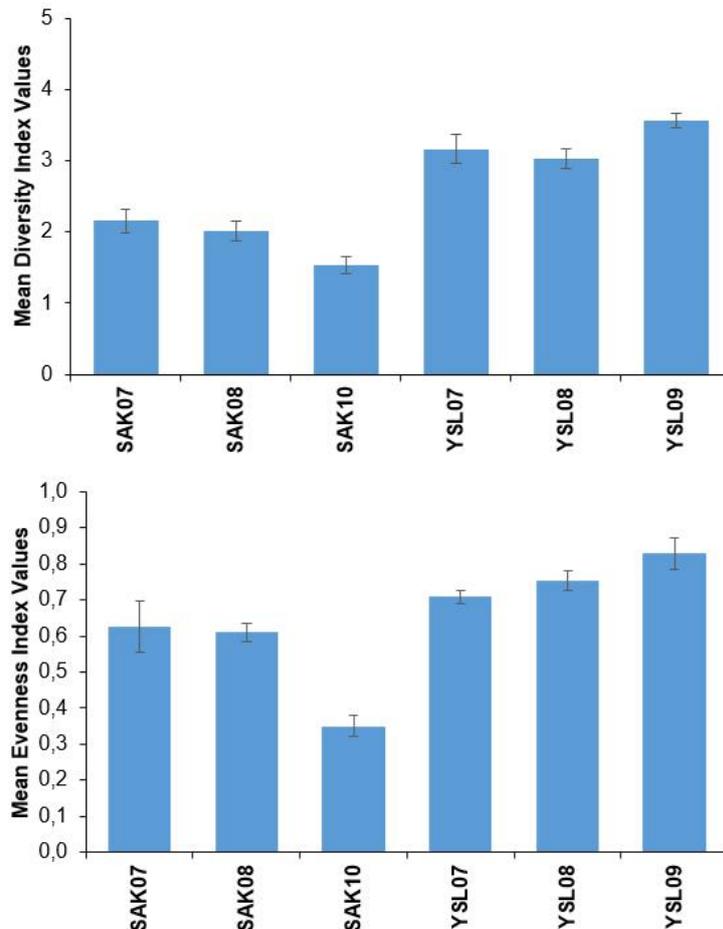


Figure 4.30 - Diversity and evenness index (mean  $\pm$  SE) - in front of the Sakarya and Yesilirmak Rivers, January 2020

Turkish Benthic index TUBI values among the stations ranged between 1.80 (SAK08) and 3.61 (YSL07). The station SAK08 had the lowest TUBI scores in the area, thus classifying the water body's benthic quality status as “poor”; four stations possessed TUBI scores that indicated “moderate” ecological status. Only station YSL07 had a “high” mean TUBI value ( $3.6\pm 0.1$ ), indicating high ecological status (Figure 4.31).

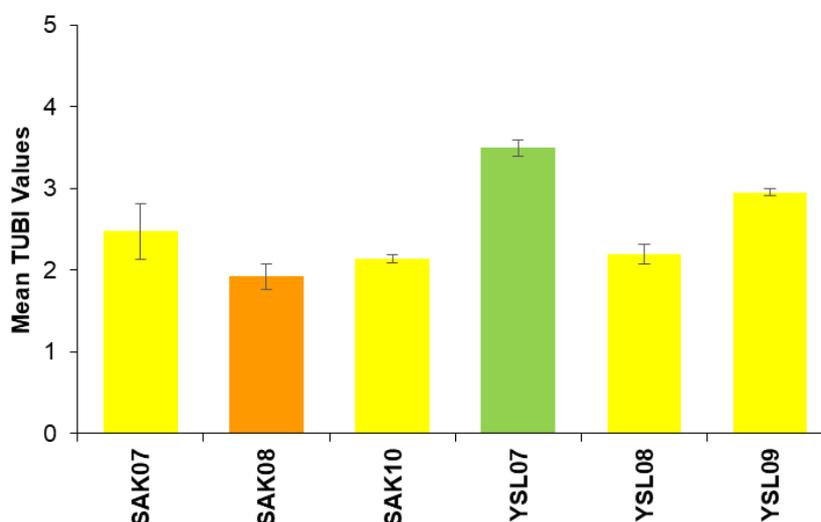


Figure 4.31 - TUBI (Mean  $\pm$  SE), January 2020

## Conclusions

A total of 115 macrozoobenthic species belonging to 9 taxonomic groups (Cnidaria, Nemertea, Oligochaeta, Polychaeta, Phoronida, Crustacea, Mollusca, Echinodermata and Tunicata) were determined in the research area. Among species, three alien species, polychaete *Polydora cornuta*, molluscs *Anadara kagoshimensis*, and *Rapana venosa* were found. Considering the number of individuals of alien species, the ecosystem is not considered endangered in terms of alien species. Characteristic species for the area were polychaetes *Heteromastus filiformis*, *Prionospio maciolekae*, *Aricidea claudiae* and mollusk *Abra alba*. *Heteromastus filiformis* is known as first-order opportunistic species. Others are also known as tolerant species to organic enrichment. In the stations, Mollusca represented a high number of species and Polychaeta many individuals in the sampling periods. The distribution of the species and individuals by stations show that the highest mean number of species in the summer and winter period was found at station YSL07 (22, 23 species, respectively) and the lowest mean number of species at station SAK08 (10, 6 species, respectively). Regarding individuals, station SAK10 had the highest average (7 680 ind./m<sup>2</sup>, 2 250 ind./m<sup>2</sup>, respectively) in both periods, while station YSL09 had the lowest densities in summer (1196.7 ind./m<sup>2</sup>) and station SAK08 in winter (546.7 ind./m<sup>2</sup>). In the assessment of the ecological quality of stations, Turkish Benthic Index TUBI developed by Çinar et al. (2015) was used. The stations' ecological quality conditions were determined to be the same in the summer and winter seasons. SAK08 was found in "poor" condition, while YSL09 was "good" ecological condition. The ecological quality status of the stations was moderate in most of the stations. However, threshold values of TUBI need to be calibrated for the Black Sea.

## 4.2 Chemical characteristics - sediments

Measurements of heavy metals only in marine water are insufficient for assessing the ecosystem's state due to high variability, fluctuating inputs, and low residence time. With a combined action of adsorption, hydrolysis, and co-precipitation, only a minor part of the free metal ions remains dissolved in water, while a large amount of them is stored in sediments. However, when environmental conditions change, sediments can be converted from heavy metal deposits into sources for the water column. Therefore, the content of heavy metals in sediments is measured to provide vital information for the assessment of environmental risks in the long term (Zhuang W, Gao X, 2014). Sediment is a matrix of materials comprised of detritus, inorganic, and organic particles and relatively heterogeneous in terms of its physical, chemical, and biological characteristics (Hakanson, 1992). On entering marine ecosystems, many POPs quickly become bound to particulate matter in the water and, in time, sink to the seabed to be deposited in sediment. In this way, sediments act as a sink for POPs deposited in the marine environment (Allsopp et al., 2001). However, due to various diagenetic processes, the sediment-bound metals pollutants may remobilize and be released back to overlying water sand, imposing adverse effects on terrestrial and aquatic organisms (Bhattacharya et al., 2003).

### 4.2.1 Dnieper River, southern Bug River and Dniester River

Trace metals (TM), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) were the base for the assessment of the bottom sediments' status. The evaluation was done by the UkrSCES methodology using Maximum Allowable Concentrations (MAC) from the Ecological Standards of Marine Environment Quality.

The ecological condition of bottom sediments is estimated using Kz average:

for TM:		for organic compounds:
Kz < 0.5	Very good	Kz < 0.2
Kz = 0.5 - 1.0	Good	Kz = 0.2 - 1.0
Kz = 1.0 - 1.25	Satisfactory	Kz = 1.0 - 5.0
Kz = 1.25 - 2.5	Bad	Kz = 5.0 - 25.0
Kz > 2.5	Very bad	Kz > 25.0

The assessment of bottom sediment' ecological condition in areas affected by river runoff by groups of pollutants (Table 4.3) and individual pollutants (Table 4.4) resulted from Kz.

The bottom sediments' ecological status ranges from "Very good" to "bad" quality (Table 4.3). In June, station 6 (Ochakov area, exit from the Dnieper-Bug estuary) had an increased content of organochlorine pesticides. It is also available at station 3 (outlet of the Dniester waters) and at station 6 (Ochakov region in September). As a result, in these areas, the integral quality class is assessed as "bad".

Several metals had high levels in bottom sediments (Table 4.4). So, because of nickel at station 1 and at stations 1 and 2 (Danube region) and mercury - at stations 2 (Danube region) and 6 (Ochakov region), the ecosystem status was "bad".

In September, high concentrations of nickel, chromium and copper were also recorded in the Danube region.

From individual PAHs, naphthalene caused the "bad" status at Station 6 in September.

Organochlorine pesticides caused the most significant pollution. In June, due to increased lindane concentrations at station 6, DDT at station 2, and dieldrin at station 3, bottom sediments' quality is assessed as "very poor". In September, these pesticides' concentrations were even higher: lindane and dieldrin - at station 3, the sum of DDT and its metabolites - at station 6 (Table 4.6).

**Table 4.3 - Kz groups pollutants in bottom sediments - in front of the Dnieper-Dug, Dniester, and Danube Rivers, 2019**

Station	Kz TM	Kz OCP`s	Kz PCB`s (Ar1254 and Ar1260)	Kz PAH`s
June 2019				
ST 1	0.74	1.24	0.29	0.18
ST 2	0.75	4.60	1.51	0.07
ST 3	0.28	4.73	3.15	0.19
ST 6	0.32	9.74	4.18	0.33
September 2019				
ST 1	0.97	1.20	0.21	0.55
ST 2	0.86	0.82	0.38	1.03
ST 3	0.37	21.3	1.94	0.68
ST 6	0.24	5.28	1.68	1.59

**Table 4.4- Kz - individual pollutants in sediment - Heavy metals - in front of the Dnieper-Dug, Dniester, and Danube Rivers, 2019**

Station	Kz Cu	Kz Cd	Kz Pb	Kz Ni	Kz Cr	Kz Zn	Kz Co	Kz As	Kz Hg
June 2019									
ST 1	0.89	0.52	0.31	1.77	0.83	0.76	0.57	0.34	0.65
ST 2	0.63	1.05	0.26	0.93	0.52	0.59	0.51	0.35	1.92
ST 3	0.35	0.33	0.12	0.13	0.37	0.33	0.44	0.20	0.31
ST 6	0.33	0.21	0.09	0.02	0.13	0.19	0.18	0.18	1.53
September 2019									
ST 1	1.24	0.41	0.36	2.25	1.92	0.93	0.71	0.34	0.55
ST 2	1.38	0.38	0.28	2.28	1.23	0.89	0.54	0.30	0.47
ST 3	0.41	0.60	0.13	0.46	0.37	0.31	0.30	0.20	0.56
ST 6	0.28	0.26	0.10	0.33	0.23	0.47	0.23	0.18	0.11

**Table 4.5 - Kz individual pollutants in sediment - PAHs in front of the Dnieper-Dug, Dniester, and Danube Rivers, 2019**

Station	Kz Naphthalene	Kz Phenanthrene	Kz Anthracene	Kz Fluoranthene	Kz Benzo[a]anthracene	Kz Crysenene	Kz Benzo[k]fluoranthene	Kz Benzo[a]pyrene	Kz Benzo[ghi]perylene	Kz Indeno[1,2,3-cd]perylene
June 2019										
ST 1	0.12	0.02	0.02	0.35	0.23	0.24	0.15	0.16	0.26	0.29
ST 2	0.11	0.05	0	0.13	0.16	0.08	0.03	0.03	0.09	0
ST 3	0.19	0.24	1.04	0.01	0.16	0.03	0.01	0.17	0.01	0
ST 6	0.17	0.44	0.05	0.72	0.27	0.30	0.52	0.24	0.29	0.30
September 2019										
ST 1	0.72	1.11	0.11	1.12	0.39	0.45	0.23	0.35	0.48	0.52
ST 2	0.23	1.04	0.30	3.06	1.05	1.07	0.63	0.80	1.03	1.14
ST 3	1.33	3.02	0.22	0.77	0.27	0.26	0.17	0.19	0.24	0.29
ST 6	12.5	0.43	0	0.94	0.29	0.39	0.43	0	0.35	0.64

**Table 4.6 - Kz individual pollutants in sediment - Pesticides in front of the Dnieper-Dug, Dniester, and Danube Rivers, 2019**

Station	Kz HCB	Kz α-HCH	Kz β-HCH	Kz Lindane	Kz HCH total	Kz Heptachlor	Kz Aldrin	Kz Dieldrin	Kz DDT total	Kz AR-1254	Kz AR-1260
June 2019											
ST 1	0	0	0	4.60	0.05	0	0	0	6.51	0.49	0.09
ST 2	0	0	0	8.00	0.08	0	0	0	33.3	2.53	0.50
ST 3	3.82	0	0	8.20	0.08	0	0	27.6	2.88	6.25	0.06
ST 6	0	0	0.41	58.0	0.66	6.04	0	10.8	11.7	8.15	0.21
September 2019											
ST 1	0	0	5.59	0	1.12	3.33	0.18	0	0.60	0.38	0.05
ST 2	0	0	0	4.40	0.04	0	0.02	0	2.88	0.73	0.03
ST 3	0	0	0.14	72.4	0.75	0	0	102	16.8	3.70	0.18
ST 6	0	0	0	10.0	0.10	0	0	0	37.4	3.35	0.01

## Conclusions

The highest levels of pollution of bottom sediments with TMs and PAHs were observed in the Danube region both in July and September 2019. For these groups of substances, the Danube River has the greatest anthropogenic impact on the northwestern part of the Black Sea.

OCP and PCB contamination levels were high in all marine regions adjacent to river deltas in both June and September 2019. Sources of their input: agricultural fields (with rain runoff), settlements and enterprises located on the banks of rivers.

### 4.2.2 Danube River

#### Heavy Metals

Concentrations of heavy metals (Cu, Cd, Pb, Ni, Cr) measured in surface sediments were characterised by some degree of variability, reflecting the impact of various anthropogenic inputs, the mineralogical diversity and granulometric characteristics of sediments.

The following variation ranges were observed: 17.39-50.13 µg/g Cu; 0.20-1.35 µg/g Cd; 3.48-13.75 µg/g Pb; 37.57-83.36 µg/g Ni; 13.08-48.95 µg/g Cr (Annex D). Data for the area impacted by the Danube are comparable with typical ranges reported for Black Sea marine sediments (even lower, in case of chromium), for instance, the limit of predominant values (75<sup>th</sup> percentile of 2012-2017 monitoring data) being as follows: 51.08 µg/g Cu; 1.15 µg/g Cd; 15.09 µg/g Pb; 78.09 µg/g Ni; 69.74 µg/g Cr (Oros A., 2019).

Sediments are an important repository for various pollutants and play a significant role as sensitive indicators for monitoring contaminants in aquatic systems (Ozkan, Buyukisik, 2012). Sediments are an important carrier and a sink of heavy metals in the hydrological cycle, reflect the system's current quality, and provide information on pollution sources' impact (Kruopiene, 2007). The distribution of heavy metals in sediments is influenced by natural and anthropogenic sources' contribution and depends on sediments' mineralogic and granulometric characteristics. Sediments with a finer texture and a higher organic content tend to accumulate higher concentrations of heavy metals than coarse sediments. This depends on specific hydrodynamic conditions that influence the fine particle (silt and clay) distribution (Naifar et al., 2018). In marine areas characterized by low depositional energy, the accumulation of fine particles and pollutant is facilitated. In contrast, coastal areas are characterized by high depositional energy (wave, currents), sediments are dominated by coarse-grained particles (sand).

Most heavy metals concentrations in surface sediments presented an increasing tendency at higher depths (>40 m). Higher concentrations were also noticed at Periboina 20 m station (Cd, Pb, Ni) and along Portita transect (Pb, Ni). No evident accumulation of metals in front of Danube mouths was noticed, except for Ni and Cr in front of Sf. Gheorghe discharge (Figure 4.32).

Data from the area influenced by the Danube indicated a low level of trace metal pollution since concentrations of copper and cadmium in surface sediments surpassed recommended values (EQS) in 18 % and 1.6 %, respectively, of analysed samples, whereas Pb and Cr had levels below EQS (ERLs: 1.2 µg/g Cd, 47 µg/g Pb, 81 µg/g Cr; national legislation: 40 µg/g Cu, 35 µg/g Ni) (Figure 4.32).

Most Ni concentrations were higher than the recommended threshold. Still, it should be mentioned, however, that especially in the case of this element, the concentrations characterizing the natural background can typically be higher in marine sediments from the Black Sea area (Secieru, 2002).

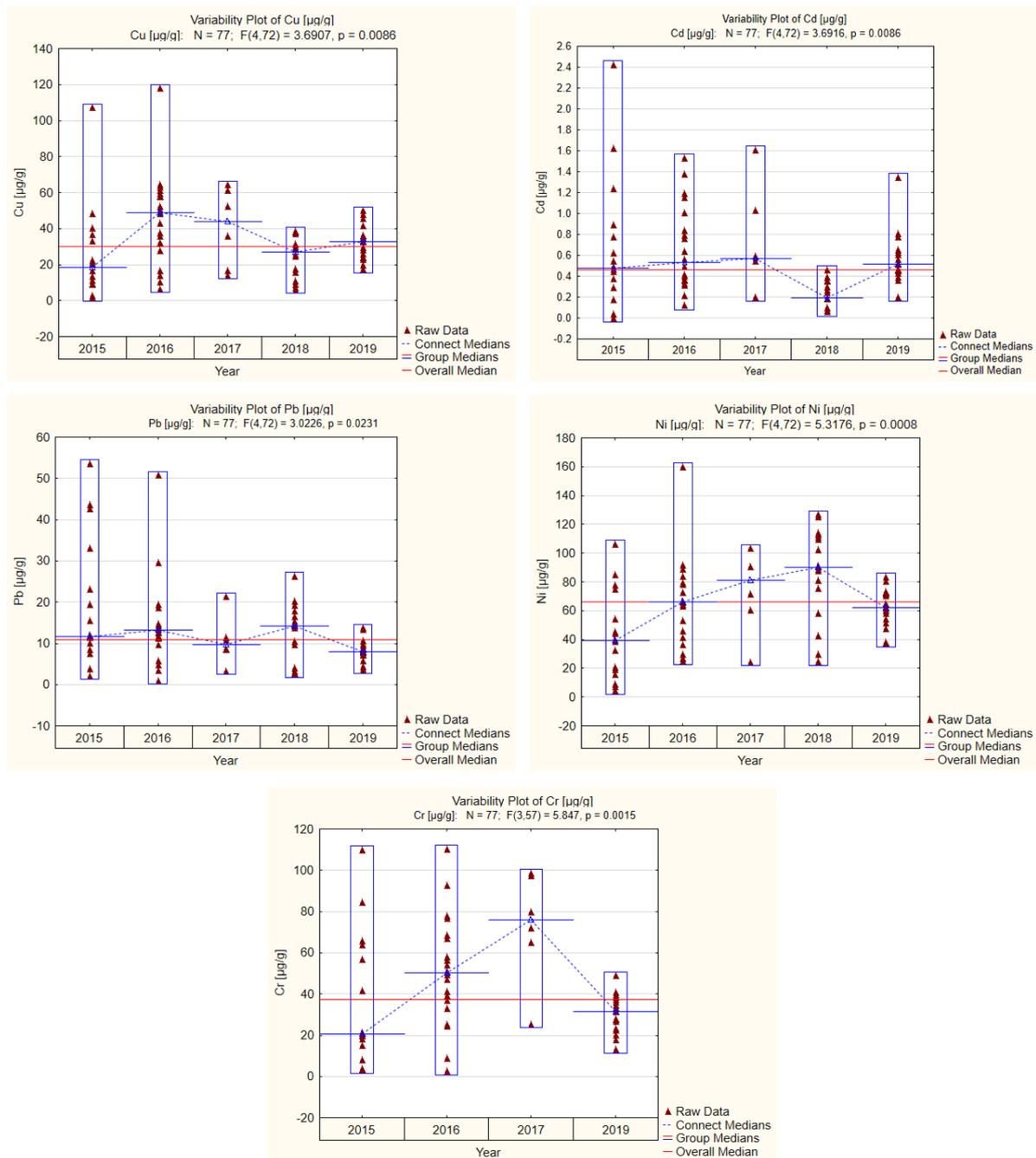
In comparison with available monitoring data (2015 - 2018) from the same area, in 2019, the results were generally maintained between similar variation ranges, with slight decreasing trends for Pb, Ni and Cr (Figure 4.33).

## Conclusions

Metal's concentrations in surface sediments indicated a low level of trace metal pollution, as between 1.6 - 18 % of samples surpassed recommended EQS values for Cu and Cd. In contrast, Pb and Cr values were below EQS.

In 2019 the results were generally maintained between similar variation ranges compared with the previous period (2015-2018), with slightly decreasing Pb, Ni and Cr trends.





**Figure 4.33 - Trends of heavy metals concentrations in surface sediments from the marine area - in front of the Danube River, 2015 - 2019**

### Organic pollutants

Organic pollutants concentration in sediment varied from detection limits to 380 ng/g dry weight (dw) (Annex D).

The median concentrations were under the detection limit, except for heptachlor, dieldrin, p,p' DDT, PCB 28, naphthalene and phenanthrene. Highest concentrations were recorded for lindane (140.93 ng/g dw), p,p' DDD (258.44 ng/g dw), PCB 28 (188.60 ng/g dw), PCB 52 (156.84 ng/g dw), PCB 118 (156.32 ng/g dw), PCB 138 (379.79 ng/g dw). The values fall within the study area's normal range of variability (Nicolaev et al., 2019; Nicolaev et al., 2017).

The total petroleum hydrocarbon concentrations ranged between 33.29  $\mu\text{g/g}$  and 150.42  $\mu\text{g/g}$ , while the Polyaromatic hydrocarbons analysis highlighted the presence of six of the sixteen investigated compounds: naphthalene, acenaphthylene, acenaphthene, phenanthrene, chrysene and dibenzo(a,h)anthracene. POPs are generally hydrophobic, have low degradation rates with high

chemical stability, and effectively adsorb onto sediments (Ribeiro et al. 2016; Montuori et al. 2016). Due to their high stability and resistance to degradation in the aquatic environment, these contaminants can accumulate in sediments over time. Studies have shown that physicochemical properties may influence POPs and PAHs' distribution in different bays (Yang et al. 2011; Alegria et al. 2016).

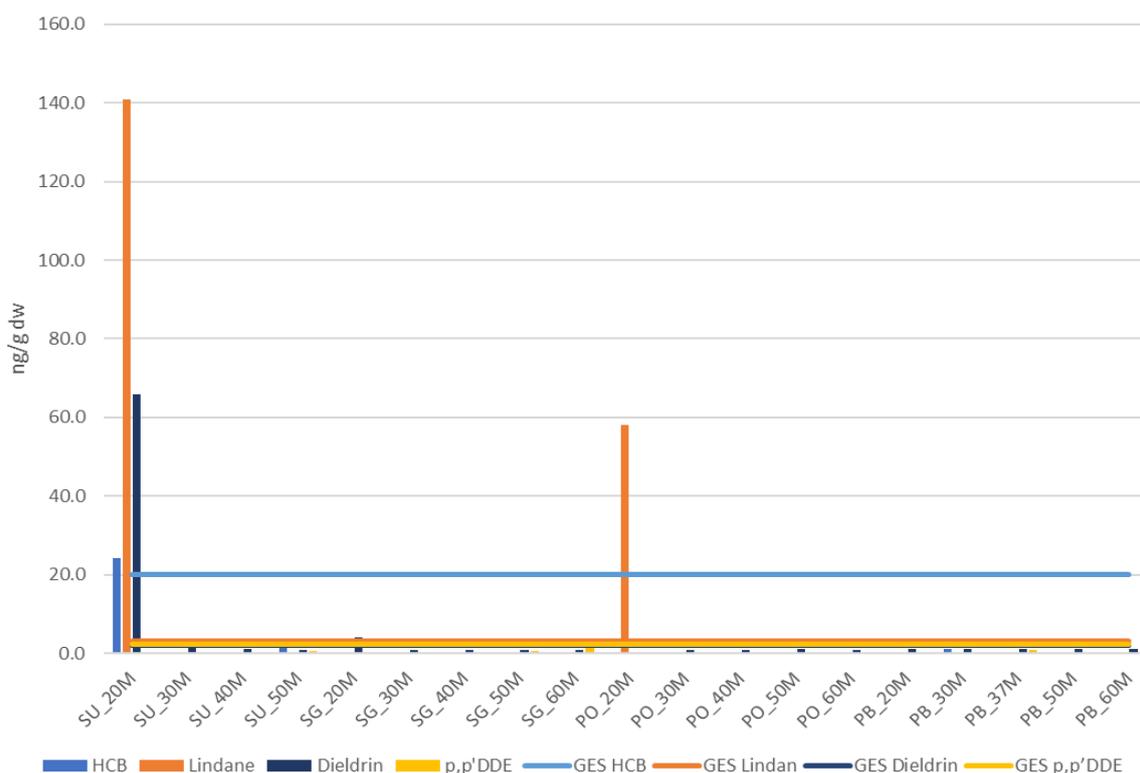
The results from the area influenced by the Danube indicate a moderate level of organic pollution in sediment. Except PCB 28, which exceeded the threshold values proposed for sediment to define good ecological status in 58 % of the samples, the other chlorinated compounds exceeded the threshold values in different proportion between 0 % and 16 % (Figure 4.34, Figure 4.35 ).

Total petroleum hydrocarbons exceeded the maximum admissible value (100 µg/g) stipulated by national legislation<sup>9</sup> in 10 % of the samples. From the PAHs group, phenanthrene was the only regulated compound that exceeded the threshold proposed for sediment to define good ecological status in 10 % of the samples (Figure 4.36).

Except PCB 28, organic pollutants were in good status according to the methodology developed to assess the status of the Black Sea ecosystem in respect to MSFD (Boicenco L. et al., 2018).

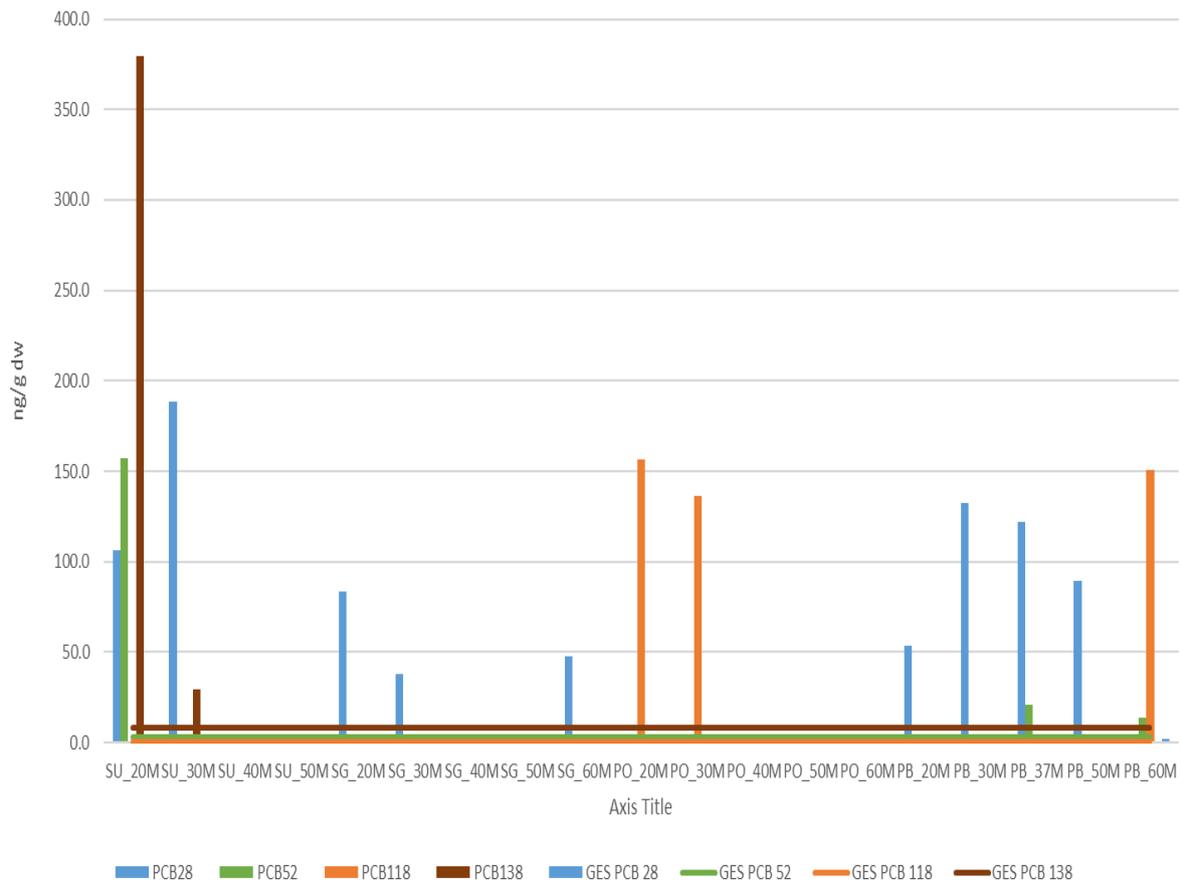
In most aquatic systems, the silt/clay fraction (< 63 µm) is the main transporter of adsorbed chemical substances, such as polycyclic aromatic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls. Many potentially toxic organic compounds, especially chlorinated ones found in many pesticides, are strongly associated with sediment, particularly with organic carbon transported as part of river sediment loads (Botello et al., 2018).

The Danube influence was observed in front of river mouths for chlorinated compounds. No evident accumulation of polyaromatic hydrocarbons was noticed (Figure 4.37).

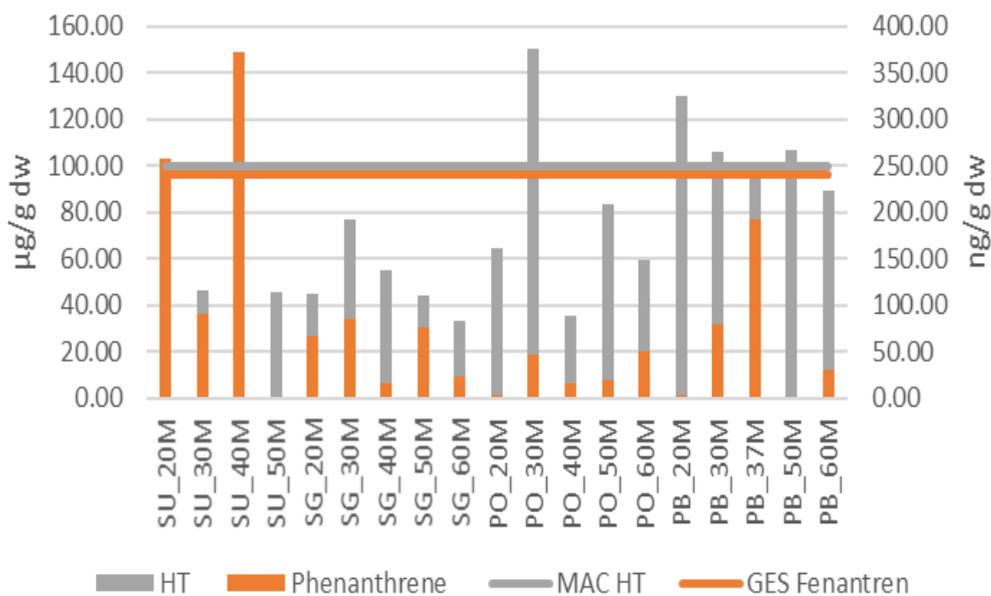


**Figure 4.34 - Concentrations of organochlorinated pesticides in sediment in the marine area under the influence of Danube in relation to the proposed values to define good environmental status - in front of the Danube River, May 2019**

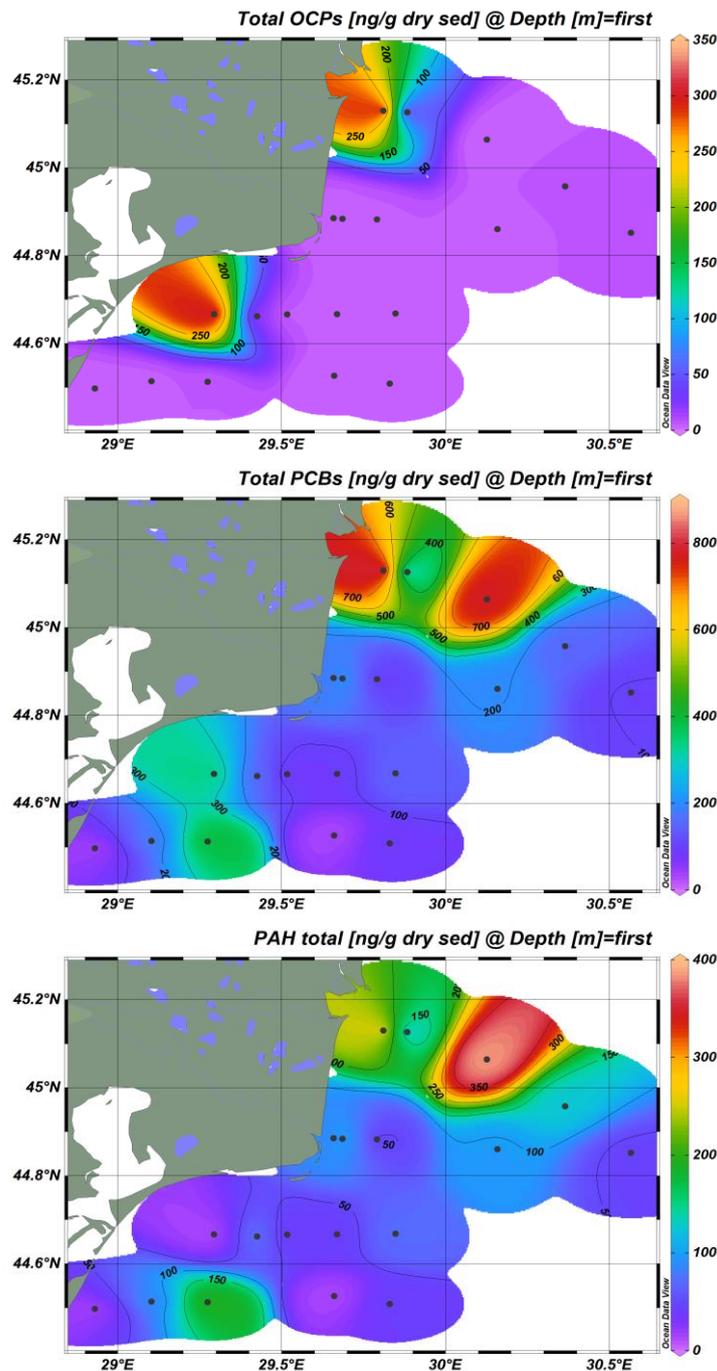
<sup>9</sup>Order no. 756/1997



**Figure 4.35 - Concentrations of polychlorinated biphenyls in sediment in the marine area under the influence of Danube in relation to the proposed values to define good environmental status- in front of the Danube River - in front of the Danube River, May 2019**



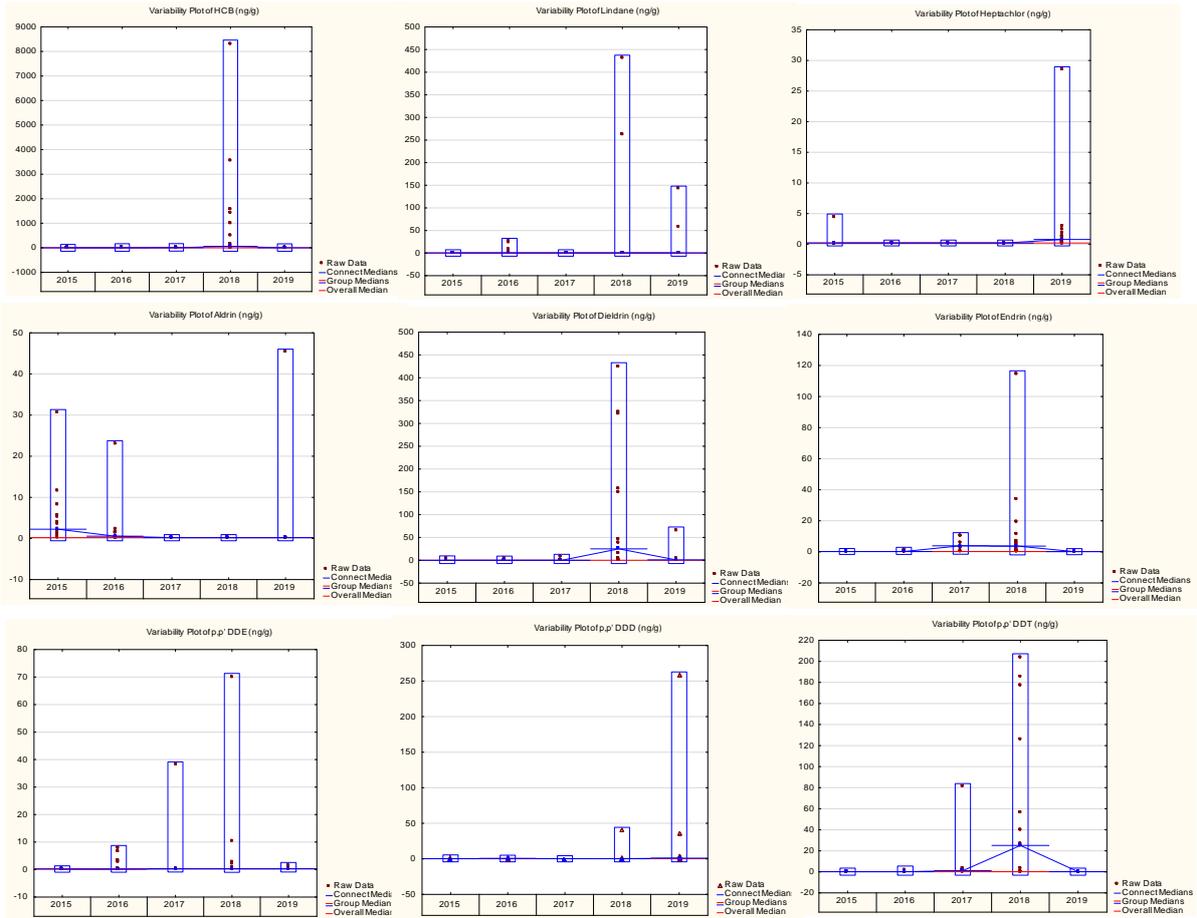
**Figure 4.36 - Concentrations of phenanthrene and total petroleum hydrocarbons in sediment in the marine area under the influence of Danube in relation to the proposed values to define good environmental status - in front of the Danube River, May 2019**



**Figure 4.37 - Spatial distribution of organic pollutants concentrations in sediment in the marine area under the influence of Danube - in front of the Danube River, May 2019**

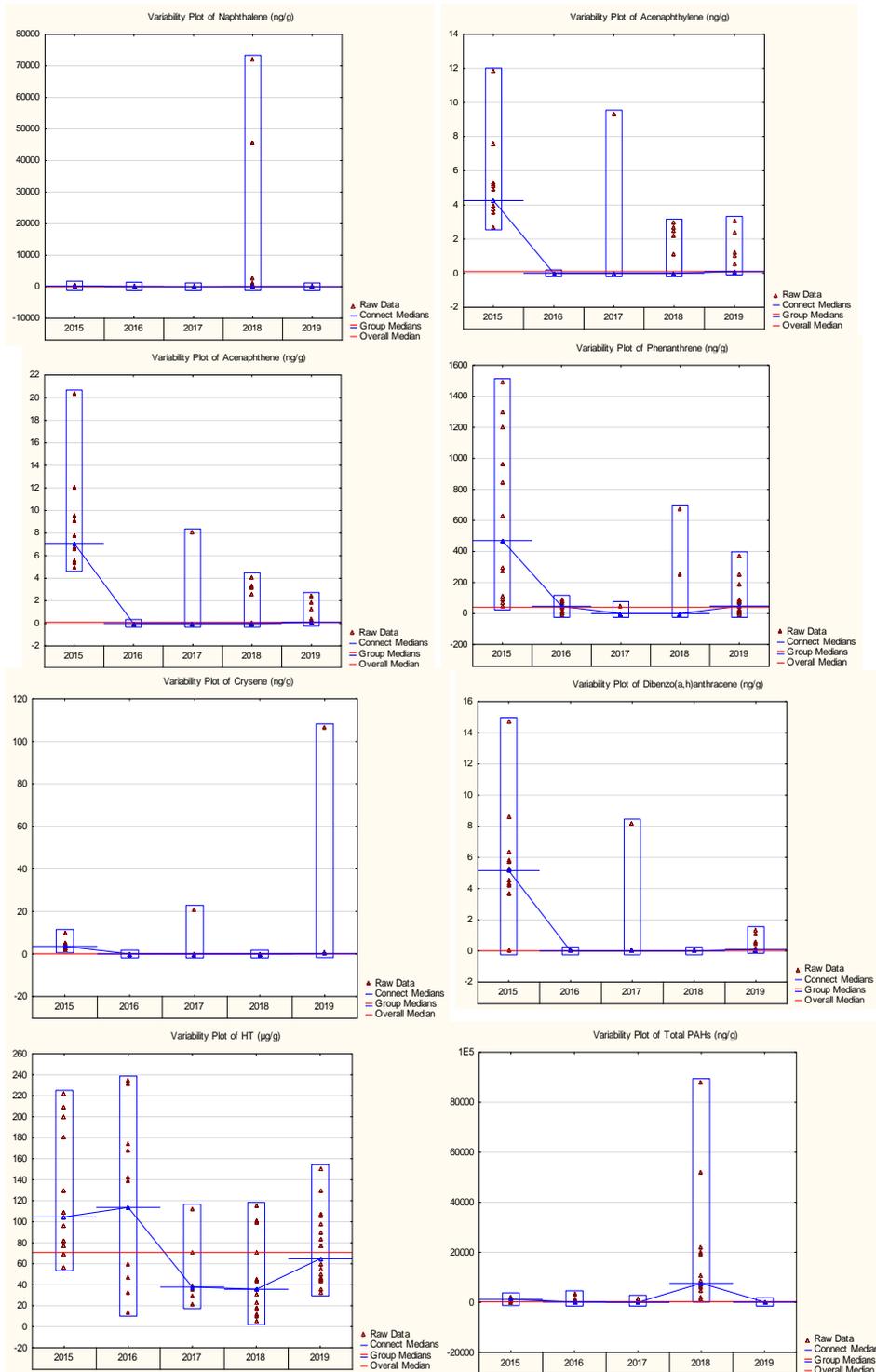
In comparison with monitoring data (2015-2018) from the same area, in 2019, the results of organochlorinated compounds were maintained between similar variability ranges or even lower for HCB, lindane, dieldrin, endrin, p,p' DDE, p,p' DDT, PCB 52, PCB 101, PCB 153, PCB 180. While the median was at the same level as in the previous period, higher values were recorded in 2019 for most organochlorinated pesticides. Heptachlor, aldrin, p,p' DDD, PCB 28, PCB 118 and PCB 138 had in 2019 some extreme values, higher than 2015 -2018 (Figure 4.38, Figure 4.39).

The results of the six polycyclic aromatic hydrocarbons identified in 2019, as for the sum of the sixteen investigated compounds and total petroleum hydrocarbons, were maintained between similar wide variability ranges observed in 2015-2018 or even lower. Only chrysene presented in 2019 higher values than the previous period (Figure 4.40).



**Figure 4.38 - Trends of organochlorine pesticides concentrations in sediment from the marine area - in front of the Danube River, 2015 - 2019**





**Figure 4.40 - Trends of hydrocarbons in sediment from the marine area - in front of the Danube River, 2015 - 2019**

### Conclusions

The concentration of organic pollutants in sediment indicated a moderate level of organic pollution, as, except for PCB 28, organic pollutants level corresponded to good ecological condition. The Danube influence was observed in front of river mouths for chlorinated compounds. No evident accumulation of polyaromatic hydrocarbons was noticed.

### 4.2.3 Sakarya River and Yesilirmak River

#### Heavy Metals

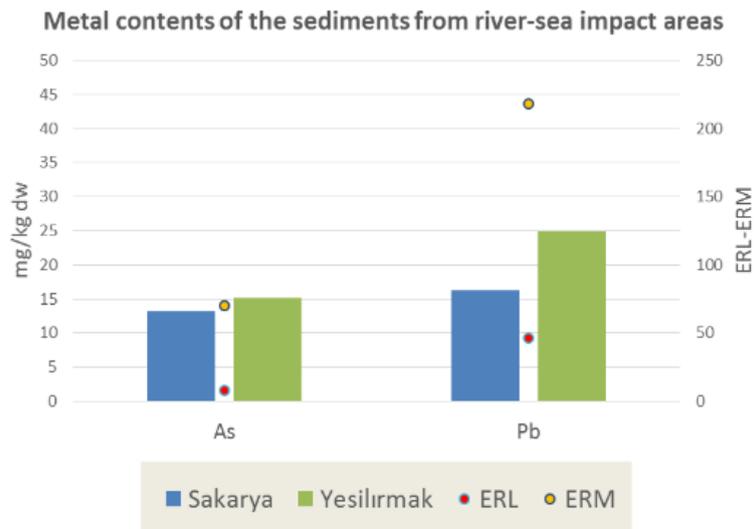
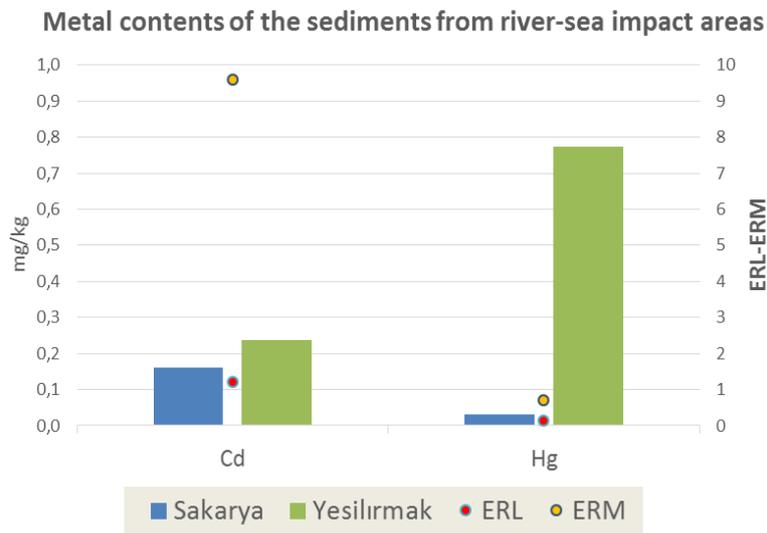
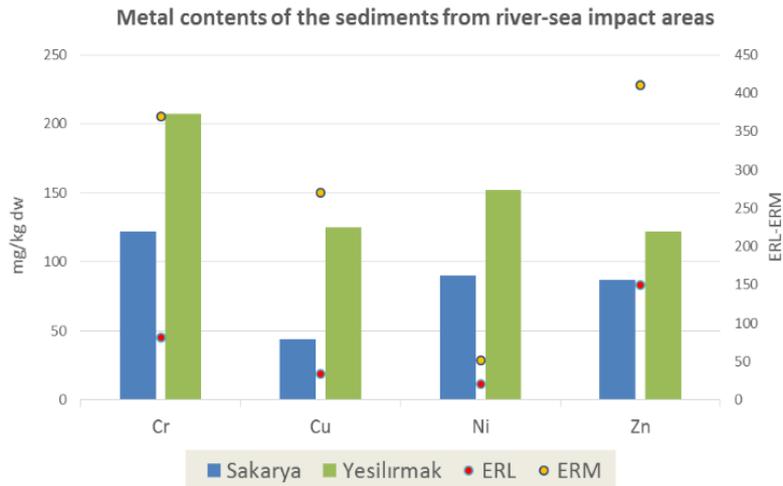
The heavy metal concentrations (As, Cu, Cr, Cd, Pb, Ni, Hg) measured in surface sediments collected from river-sea impact areas in June 2019 reflect the impact of various anthropogenic inputs and the diversity of the mineralogical and granulometric properties of the sediment.

The following variation ranges (in dry weight) were observed: 7.84-18.51 µg/g As; 22.77-196.68 µg/g Cu; 0.08-0.58 µg/g Cd; 36.35-358.92 µg/g Cr; 9.59-52.26 µg/g Pb; 32.61-216.20 µg/g Ni; 38.62-184.42 µg/g Zn (Annex D).

Metal contents (mean values) of the sediments collected from Yeşilirmak and Sakarya river impact areas were compared with the ecosystem impact threshold values (ERL: Effect Range Low, ERM: Effect Range Medium) developed by weight of evidence approach (MacDonald et al., 1996; Long et al., 1995). It is observed that mean Ni and As values of both RIA sediment samples are higher than and about the ERM values, respectively. The mean Hg and Cr values of Yesilirmak RIA samples are more elevated than and similar to the ERM value, respectively (Figure 4.41).

Higher heavy metals contents of surface sediments are noticed close to the river discharge of Sakarya (Cu, Cd, Ni and Cr) and deeper locations (>40m) of Yesilirmak (Ni, Pb, Cd) (Figure 4.42).

Data obtained during this cruise for the area impacted by the Yesilirmak and Sakarya river impact areas are generally comparable with typical ranges reported for Black Sea marine sediments, for instance, the limit of predominant values (75<sup>th</sup> percentile of 2017-2018 monitoring data) being as follows: 14.67 µg/g As; 56.40 µg/g Cu; 0.17 µg/g Cd; 22.91 µg/g Pb; 41.307 µg/g Ni; 74.57µg/g Cr. However, the dominance of high Ni, Cu, Cr, and Hg contents in the Yeşilirmak river-impact area samples are higher than the 75<sup>th</sup> percentile values of 2018 monitoring values. Similarly, it has been observed that the metal contents of the sediment samples collected from the Yesilirmak river-sea impact area (RIA) are higher than the Sakarya RIA samples (Figure 4.43).



**Figure 4.41 - Comparison of the heavy metals in sediments - in front of the Sakarya and Yesilirmak Rivers, June 2019**

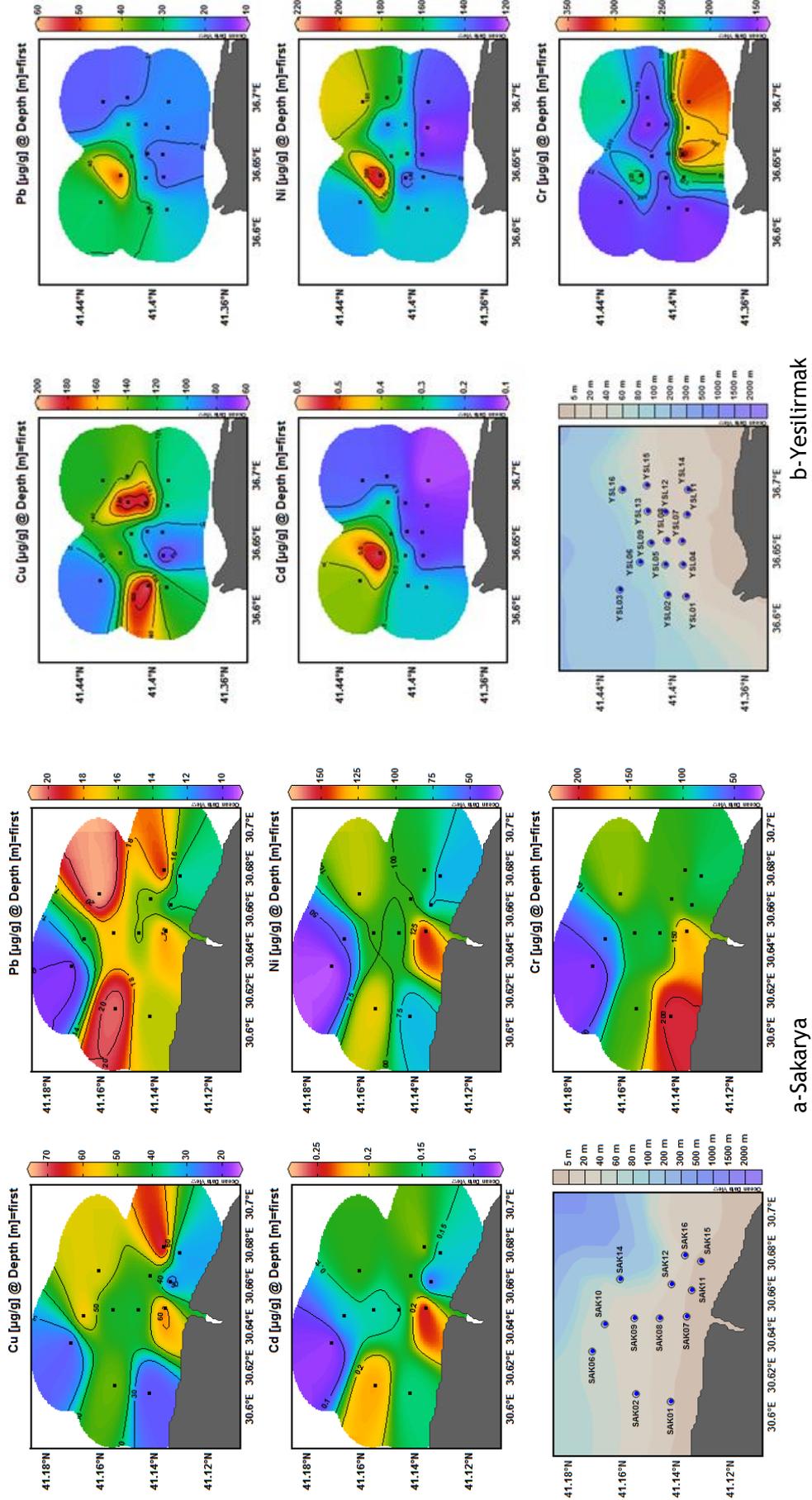
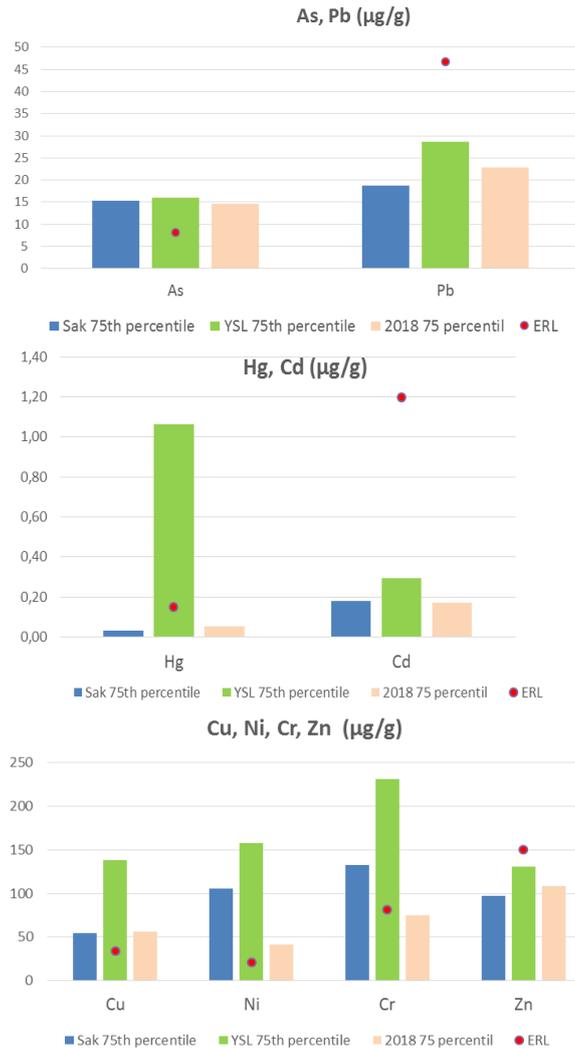


Figure 4.42 -Spatial distribution of heavy metals concentrations in surface sediments in the marine area - in front of the Sakarya and Yesilirmak Rivers, June 2019



**Figure 4.43 - Comparison of 75 percentiles of sediment metal contents (present and 2017 monitoring) - in front of the Sakarya and Yesilirmak Rivers**

Assessment of sediment metal contamination by comparing only with the threshold values can be misleading, especially in the absence of background values. For this reason, in addition to the ERL comparison given above, an evaluation was conducted using the enrichment factors (EF). Proportioning element's amounts calculate the enrichment factor in the sample and the earth's crust (Krauskopf, 1985). In this calculation, values are normalized with Al contents of samples and shale. Enrichment values are classified according to six categories (<1, 1-3, 3-5, 5-10, 10-25, 25-50 and >50) (Sakan et al., 2014). In this classification, an enrichment factor of 1 indicates that the element in question is of lithogenic origin. EF values above 25 and 50 indicate severe and very severe enrichments, respectively. EF classifications are shown in Figure 4.44 for Sakarya and Yesilirmak River Impact area sediments.

The EF values of Sakarya river impact area (RIA) sediments are lower than the Yesilirmak RIA sediments. In Sakarya RIA sediment samples, only SAK10 from 12 stations show "severe enrichment" in Arsenic (8.3 % of the stations). Among the Yeşilirmak RIA sediment samples, 6.7 % of the stations have "very severe enrichment" in Cu, Ni and Cd. "Severe enrichments" are observed in 20 % of the stations for Hg, and Cr; 13 % of the stations in As and 6.7 % of the stations in Cu, Ni and Pb.

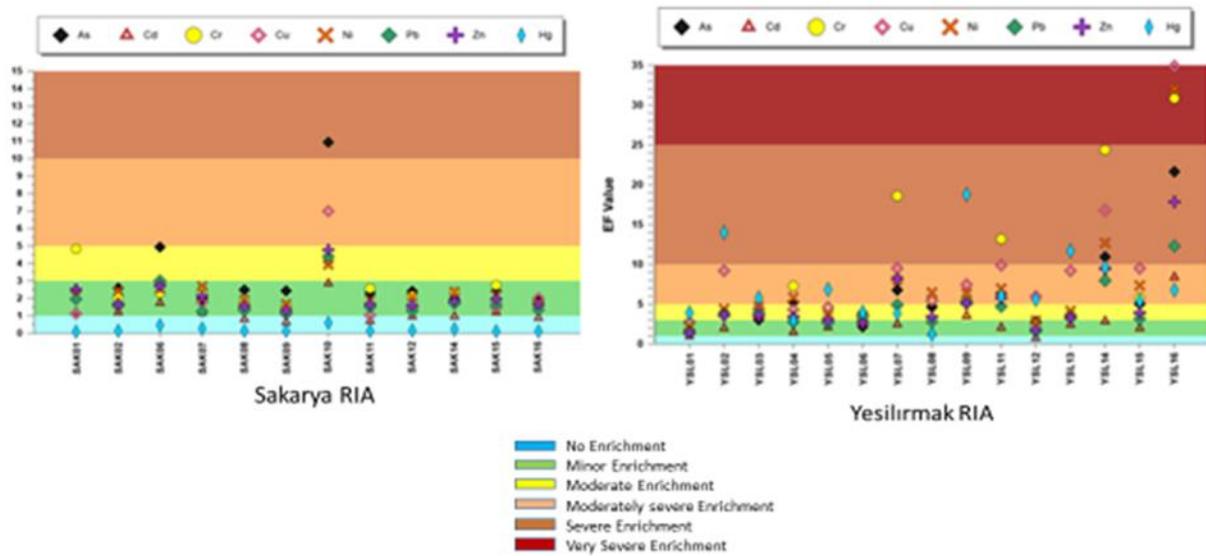


Figure 4.44 - Metal Enrichment Factors - in front of the Sakarya and Yesilirmak Rivers

The dominance of the EF values (75 percentiles) for Sakarya RIA indicate “no enrichment” for all metals. However, the dominance of the EF values means “moderately severe enrichment” of Cu, Ni and Hg, “moderate enrichment” of As, Cr, Pb and Zn and “minor enrichment” of Cd, for Yesilirmak RIA.

### Conclusions

Ni and As mean concentrations of both rivers impacted areas sediment samples are higher than those of the ERM values. The Hg and Cr mean contents of Yesilirmak RIA samples are more elevated than and similar to the ERM value, respectively.

In general, it has been observed that the metal contents of the sediment samples collected from the Yesilirmak river-sea impact area (RIA) are higher than the Sakarya RIA samples. The Ni, Cu, Cr, and Hg contents in the Yesilirmak river-impact area samples are higher than the 75<sup>th</sup> percentile of 2018 monitoring values.

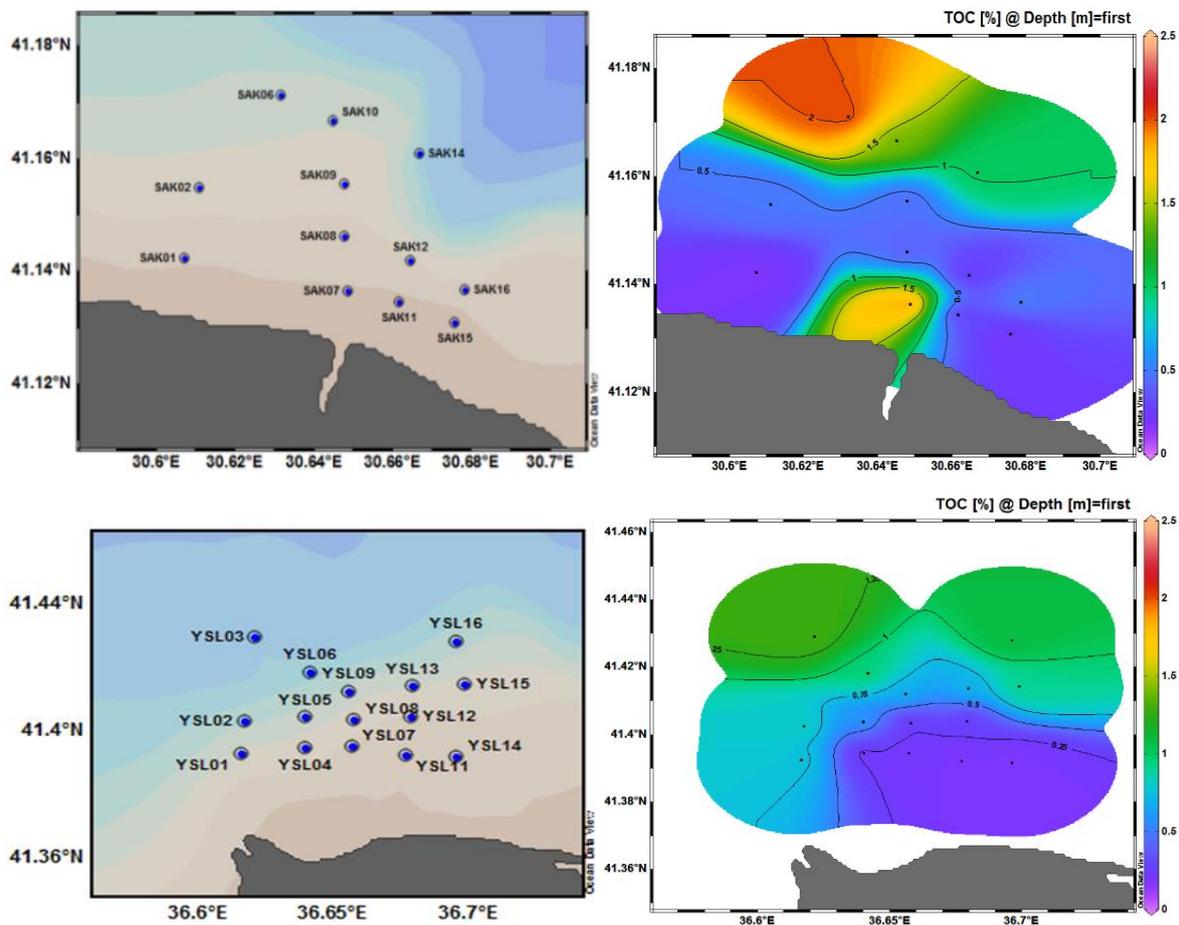
Similarly, the EF values’ dominance (75<sup>th</sup> percentile) for Sakarya RIA indicates “no enrichment” for all metals. However, the dominance of the EF values means “moderately severe enrichment” of Cu, Ni and Hg, “moderate enrichment” of As, Cr, Pb and Zn and “minor enrichment” of Cd, for Yesilirmak RIA.

### Organic pollutants

Organic carbon is one of the main parameters showing organic matter pollution in sediments. Organic matter entering the marine environment or naturally occurring (production) decomposes as long as it remains in the water column. In this process, the water column oxygen is used because of biochemical reactions in the environment. Depending on its residence time in the water column, either before it reaches the sediment, it completely decomposes or accumulates in it. The content of sedimentary organic carbon is related to the sediment grain size. A higher organic content correlates with increasing clay-silt ranges due to an increased surface area (Tyson, 1995). Wind-driven currents and waves also influence the spatial distribution and transport of sediments and organic matter (Magniet et al., 2002). An area with low hydrodynamic energy will favour the accumulation of fine sediments due to enhanced settlement of silt-clay particles. By contrast, areas exposed to higher hydrodynamic energy levels will be characterised by coarser sediments (Ergin and Bodur, 1999). The depth of the stations where sediment samples are taken is a factor affecting the organic carbon contents (due to residence time) in oxic conditions, should also be considered when interpreting the carbon values.

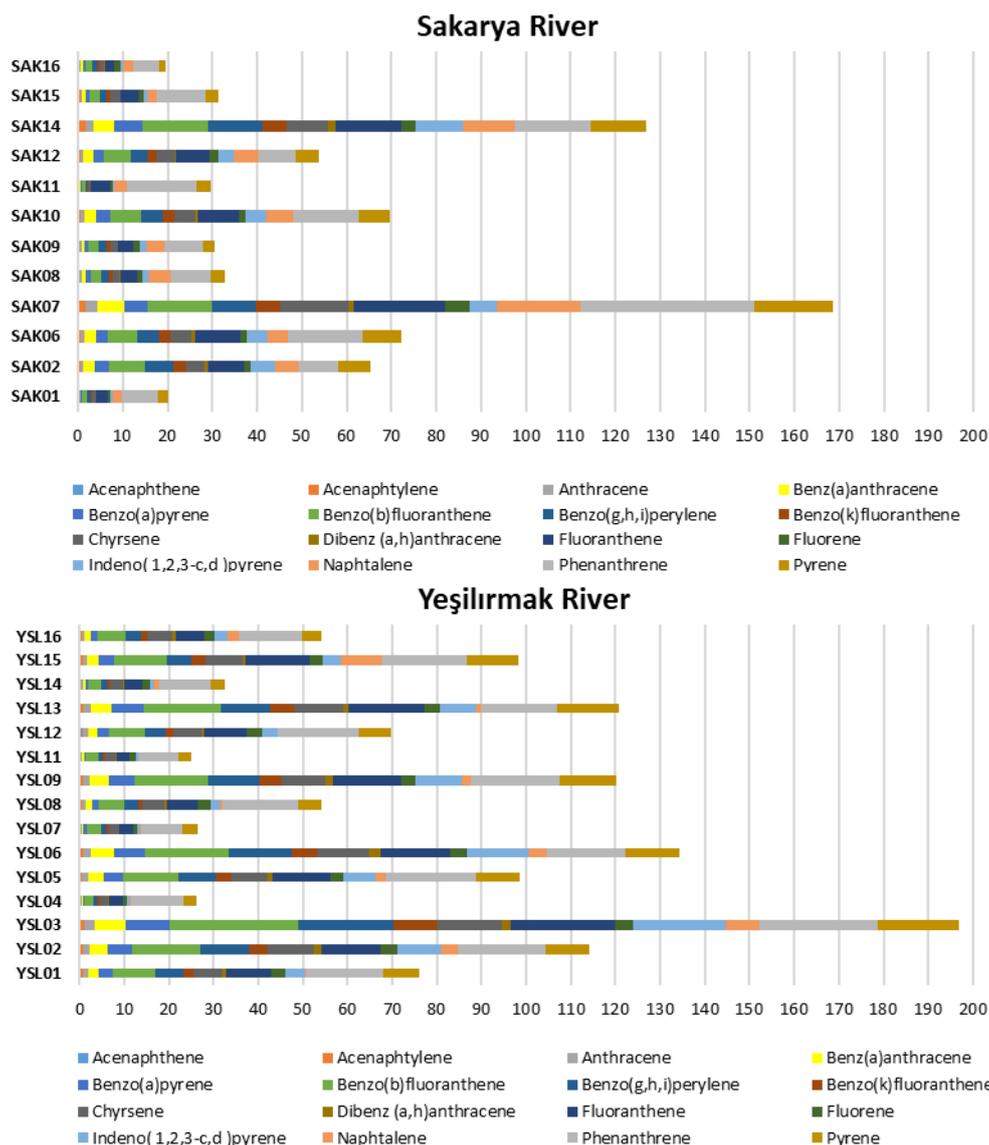
The total organic carbon (TOC) content (Figure 4.45) ranged from 0.2 % to 2% (mean 0.76 %) and 0.2 % to 1.3 % in Sakarya and Yesilirmak river-sea impact areas, respectively. Maximum value occurred

in SAK 6 and SAK 7 stations. Since the SAK6 station has a larger grain size, its organic content is expected to be lesser than SAK7, which contains higher muddy particles. As was mentioned, stations with a coarse material predominance (such as SAK 6 and SAK10) were considered in the contamination assessment.



**Figure 4.45 - Spatial distribution of the Total Organic Carbon - in front of the Sakarya and Yeşilirmak Rivers, July 2019**

In front of the Sakarya river, the Total Petroleum Hydrocarbons (TPH) concentration in sediment varied from 0.27  $\mu\text{g/g dw}$  to 8.96  $\mu\text{g/g dw}$ . TPH concentration was measured between 0.41  $\mu\text{g/g dw}$  and 8.11  $\mu\text{g/g dw}$  in the Yeşilirmak River influence area. Total Polyaromatic Hydrocarbons (PAH) concentrations varied between 19.4-168.5 ng/g in sediments affected by the Sakarya river and between 24.7-196.8 ng/g in those impacted by Yeşilirmak river. Total PAH levels remained far lower than the NOAA residue quality guideline value for the Low Effect Range (ERL) of 4000 ng/g (Long et al., 1990; Long et al., 1995). PAH components concentrations in the Sakarya and Yeşilirmak-influenced sediments are shown in Figure 4.46 and Annex D.

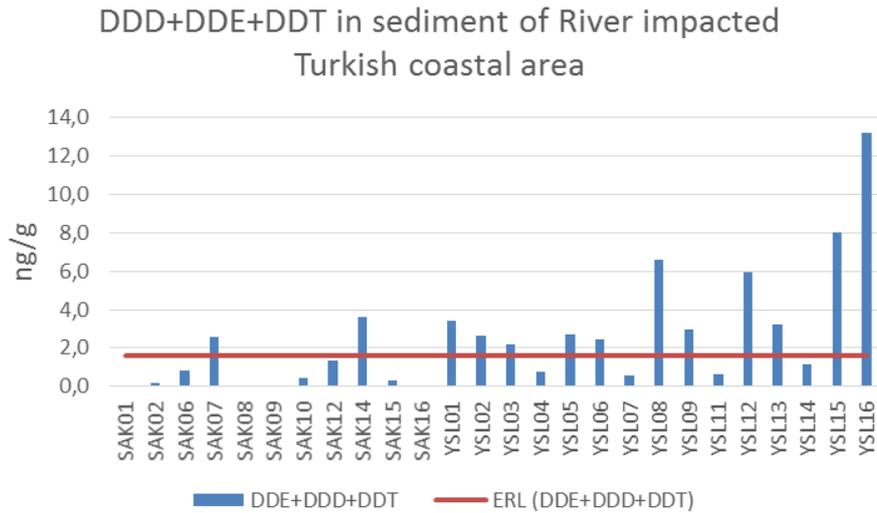


**Figure 4.46 - Distribution of PAH components in sediments - in front of the Sakarya and Yesilirmak Rivers, July 2019**

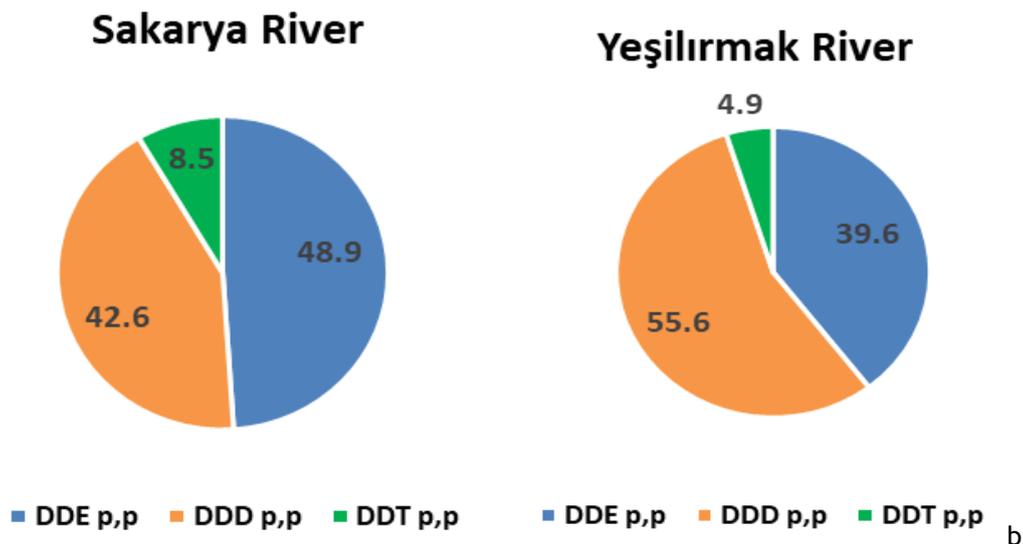
$\alpha$ -HCH, lindane, heptachlor, aldrin, dieldrin, endrin, p,p' DDT components, the median concentrations were at the detection limit (Annex D).

The total PCB values in all sediment samples are below the ERL value (22.7 ng/g dw). In sediment, the sum of the DDT's including metabolites (DDE+DDD+DDT), exceeded the threshold values (1.58 ng/g) by approximately 18 % of the Sakarya and 73 % of Yesilirmak samples (Figure 4.47 and Figure 4.48). Concentrations of other organochlorinated pesticides and polychlorinated biphenyls were below the threshold values in all stations of both study sites.

The average values of DDT and its metabolites detected in Sakarya River sediment samples (p'p- DDT 8.5 % > p'p- DDE 48.9 % > p'p- DDD 42.6 %) and Yeşilirmak River sediment samples (p'p- DDT 4.9 % > p'p- DDE 39.6 % > p'p- DDD 55.6 %) indicated that DDT metabolites, p'p- DDE and p, 'p- DDD were dominant. The distribution of DDT and its metabolites (%) in sediment are shown in Figure 4.47.



**Figure 4.47 - Concentrations of DDD+DDE+DDT in surface sediments of the marine area under the influence of Sakarya and Yesilirmak Rivers, in relation to the proposed value to define good environmental status, July 2019**



**Figure 4.48 - Distribution of DDT and its metabolites (%) in sediment- in front of the Sakarya and Yesilirmak Rivers**

Pesticide derivatives ( $\alpha$ -HCH,  $\beta$ -HCH, heptachlor, aldrin, dieldrin and endrin) were measured at trace quantity or below the detection limit.

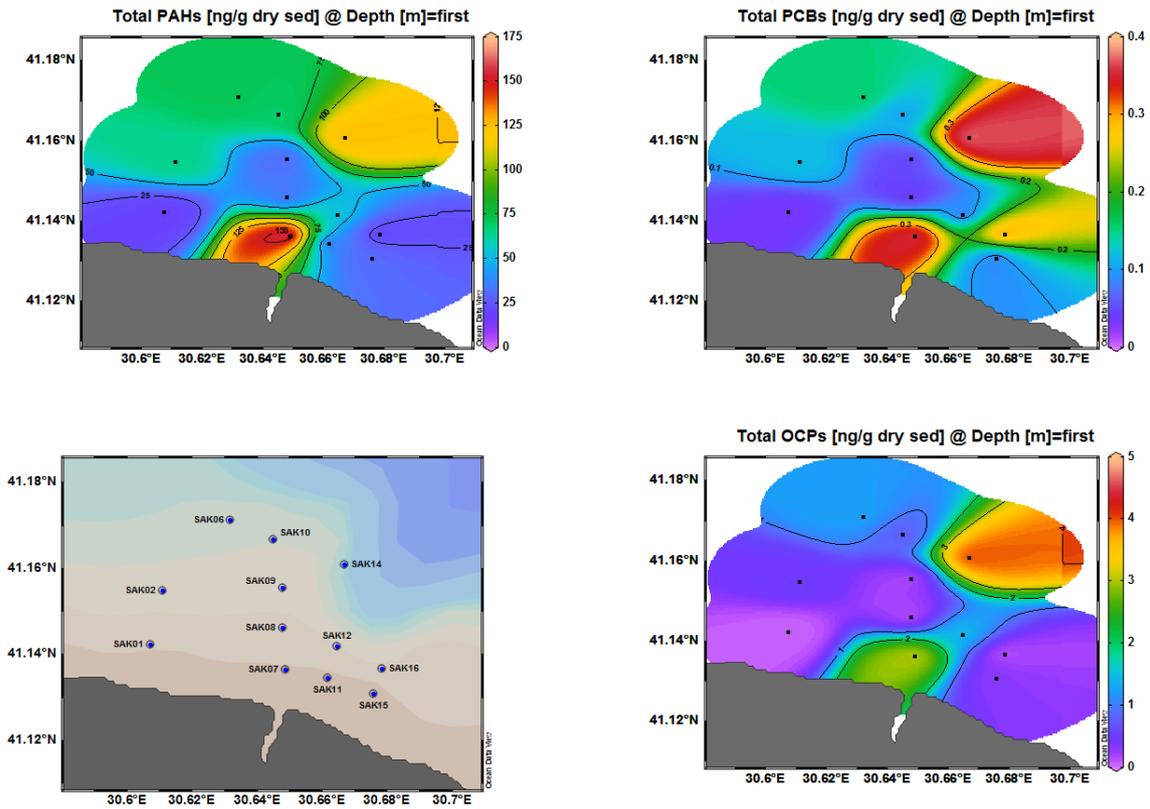


Figure 4.49 - Spatial distribution of organic pollutants concentrations in sediment in the marine area under Sakarya river's influence, July 2019

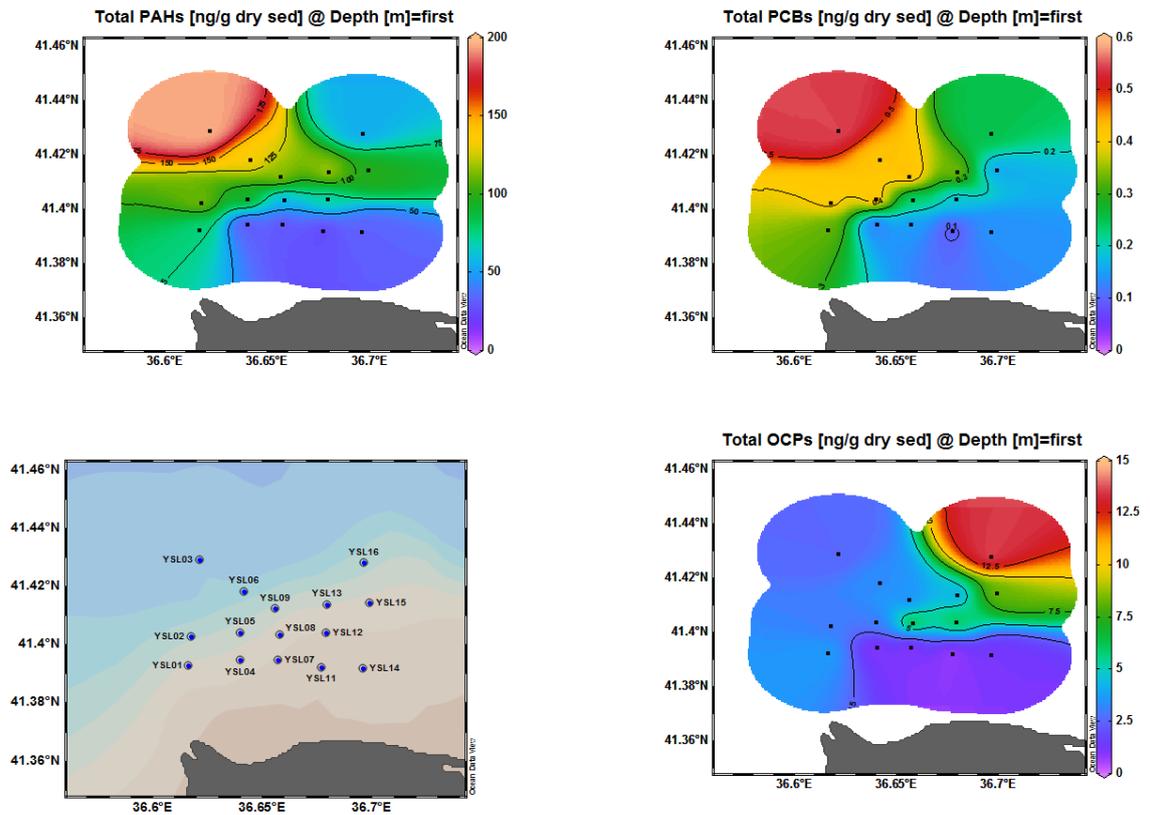


Figure 4.50 - Spatial distribution of organic pollutants concentrations in sediment in the marine area under Yeşilirmak river's influence, July 2019

In July 2019, most of the DDT and its metabolites concentrations in surface sediments presented an increasing tendency at higher depths (>80 m) in the open of the Sakarya River and east of the Yeşilirmak river (Figure 4.49 and Figure 4.50).

## **Conclusions**

Concentrations of other organochlorinated pesticides, polychlorinated biphenyls (PCBs) and Polyaromatic hydrocarbons (PAHs) in sediment were below the threshold values in all stations. The sum of the DDT's including metabolites (DDE+DDD+DDT), exceeded the threshold values (1.58 ng/g) in approximately 18 % of the Sakarya and 73 % of Yesilirmak samples. DDE is dominant in sediments affected by the Sakarya River, and DDD is prevalent in the Yeşilirmak River. It means that DDTs presence is due to historical degradation. DDT can biodegrade to DDE under aerobic conditions and to DDD under anaerobic conditions.

## 5 Activities and pressures linked to rivers discharge

Marine ecosystems are under high demand for human use, giving concerns about how pressures from human activities may affect their structure, function, and status (Korpinen et al., 2020). One of the most significant pressures is from rivers runoff, which causes the marine ecosystem's deterioration because of the input of nutrients, organic matter, and contaminants representing the anthropogenic pressures (Table 5.1).

**Table 5.1 - Anthropogenic pressures on the marine environment (amending MSFD directive 845/2017-citation) - with relevance for rivers-sea interaction assessment - cruises ANEMONE**

Theme	Pressure	
Biological	Input or spread of non-indigenous species	✓
	Input of microbial pathogens	✗
	Input of genetically modified species and translocation of native species	✗
	Loss of, or change to, natural biological communities due to cultivation of animal or plant species	✗
	Disturbance of species (e.g. where they breed, rest and feed) due to human presence	✗
Physical	Physical disturbance to seabed (temporary or reversible)	✗
	Physical loss (due to permanent change of seabed substrate or morphology and to extraction of seabed substrate)	✗
	Changes to hydrological conditions	✗
Substances, litter, and energy	Input of nutrients – diffuse sources, point sources, atmospheric deposition	✓
	Input of organic matter – diffuse sources and point sources	✓
	Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides) – diffuse sources, point sources, atmospheric deposition, acute events	✓
	Input of litter (solid waste matter, including micro-sized litter)	✗
	Input of anthropogenic sound (impulsive, continuous)	✗
	Input of other forms of energy (including electromagnetic fields, light and heat)	✗
	Input of water – point sources (e.g. brine)	✗

**Table 5.2 - Main activities in Black Sea's River Basins**

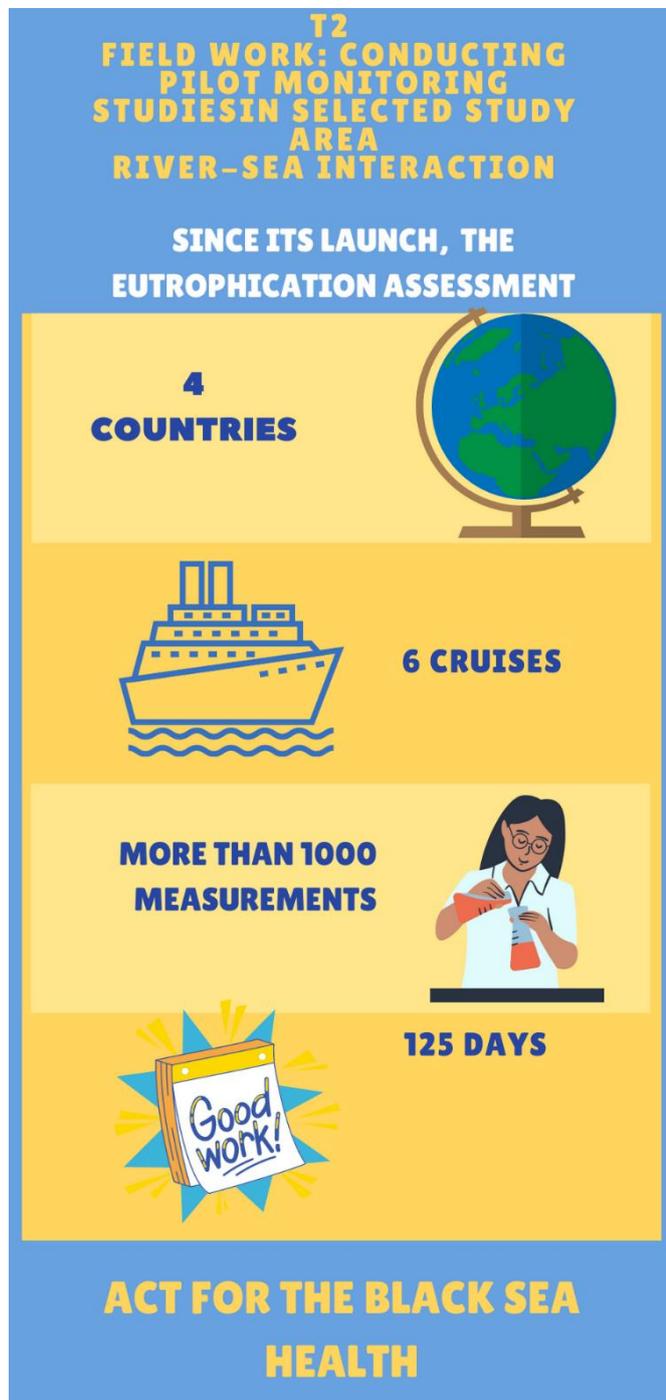
Black Sea River basins	Main activities	Pressures (analyzed) Substances, litter and energy theme
Dnieper	Agriculture / Industry / Urbanization / Ports	Input of nutrients and organic matter Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides)
Southern Bug	Agriculture / Industry / Urbanization	Input of nutrients and organic matter Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides)
Dniester	Agriculture / Industry / Urbanization / Ports	Input of nutrients and organic matter Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides)
Danube	Agriculture / Industry / Urbanization / Ports	Input of nutrients and organic matter Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides)
Sakarya	Plastics, Rubber and Synthetic Resins / Manufacture of mineral products other than metals / Food processing / Manufacture of factory-made metal products / Manufacture of chemicals and chemical products / Metal industry	Input of nutrients and organic matter Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides)
Yesilirmak	Manufacture of mineral products other than metals / Metal industry / Fertilizer industry / Food processing / Plastics, Rubber and Synthetic Resins	Input of nutrients and organic matter Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides)

## 5.1 Introduction of nutrients and organic matter - Eutrophication

Black Sea eutrophication has been an indispensable topic since at least the 80s. Major components of the ecosystem had begun to collapse as early as 1973 when records showed significant areas of summer hypoxia on the northwestern shelf due to eutrophication (Mee, Friedrich, Gomoiu, 2012). Unfortunately, due to the absence of measures, eutrophication effects continued from year to year, and considerable changes in the pelagic ecosystem at a basin-wide scale became noticeable in the second half of the 1980s and the beginning of the 1990s (Yunev, Moncheva and Carstensen, 2005). Thus, during the 1980s and early 1990s, the Black Sea ecosystem was in a catastrophic condition (Kideys, 2002). Laying at the Danube's discharge mouths, the NW coast was particularly affected by eutrophication due to the river's increased nutrients input from point and diffuse sources of pollution (agriculture, untreated waters, industry, atmospheric deposition). The cascade effects followed soon; a general increase in phytoplanktonic production; disturbances in oxygen condition and appearance of hypoxia and anoxia phenomena; mass mortalities of benthic organisms; the reduction of species diversity and simplification of community structure; the exuberant development of opportunistic species and significant qualitative and quantitative fluctuations within the population (Gomoiu, 1992). After 1992-1993, the nutrient limitation abruptly shifted from nitrogen to phosphorus, which then severely reduced plankton production, and the system maintained low biomass of bacterioplankton, zooplankton, and total marine living resources but moderate *Noctiluca scintillans* and gelatinous biomass (Oguz, Velikova, 2010). During the decade following the regime shift of the 1990s, fish stocks gradually improved as a result of good recruitment and a possibly favourable climate, shrinking fishing effort and diminishing *Mnemiopsis leidyi* biomass.

The outcome was a partial recovery to pre-shift conditions (Daskalov et al., 2017) considered as an alternative pristine state dominated by jellies and opportunistic species than the fish-dominated healthy pristine state (Oguz, Velikova, 2010) (Lazar et al., 2019).

The coastal waters' nutrients enrichment has been addressed in the European legislation since 1991 (Urban Waste-Water Treatment-UWWT and Nitrates Directives) (Palialexis et al., 2014). In 2000, the European Commission put into practice the Water Framework Directive (WFD) to reach a "Good Ecological Status" (GECs) in all water bodies of the member states. In terms of eutrophication, for transitional and coastal waters, Member States indicated the phytoplankton, macroalgae, and



angiosperm biological quality elements were most likely to be used for the assessment of ecological status concerning nutrient pressure and that macroinvertebrates and fish (in transitional waters only) were most likely to be used in relation to oxygen depletion (WFD-CIS, 2009). Since WFD was mainly focused on catchments, with only small assessment areas along the coast (up to 1 NM distance from shore), it was extended towards the marine environment by the Marine Strategy Framework Directive (MSFD) in 2008. MSFD requires EU Member States to achieve and maintain “Good Environmental Status” (GES) of their marine waters, i.e., the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas that are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a sustainable level, thus safeguarding the potential for uses and activities by current and future generations (PCEU, 2008). Hence, the WFD and the MSFD constitute legislative frameworks to combat eutrophication in European seas (Greenwood et al., 2019), including the Black Sea (Boicenco et al., 2018). Both directives have a common conceptual approach but different criteria to implement it. In addition to EU legislation, there are several international conventions on river basin management e.g., for the protection of the Danube River (ICPDR) as well as conventions for the protection of the marine environment, e.g. for the Black Sea (Bucharest Convention) (Ibisch et al., 2016). In line with European legislation, the Regional Sea Convention, Black Sea Commission identified the eutrophication reduction as one of the ecological quality objectives, EcoQO 3 (BSC, 2009) coordinated also with the Black Sea Integrated Monitoring and Assessment Program (BSIMAP) for 2017-2022 (2016) (Lazar et al., 2019). All these international frameworks are supplemented by national legislation.

### 5.1.1 E-TRIX

A universal method for assessing the level of eutrophication of marine waters and generally accepted manuals for practical assessment does not exist to date. For each study on this problem, a subjective author’s approach prevails, which usually determines the choice of indicators and their number when calculating various environmental indices. Usually, the proposed assessment methods are limited with the number of measured hydro-chemical and biological parameters and indicators of the marine environment. The most frequently recommended for scientific research and use in monitoring programs for the state of the natural marine environment is the calculated E-TRIX index, which has been widely used in recent years.

E-TRIX is an integral indicator related to the characteristics of the primary production of phytoplankton and nutritional factors. E-TRIX formula is composed of the following indicators of the ecosystem: concentration of chlorophyll *a*, which replaces the index of phytoplankton autotrophic biomass; the deviation of oxygen saturation from 100% - an indicator of the primary production intensity of the system, which covers the phase of active photosynthesis and the phase of respiration predominance; the concentration of total phosphorus and inorganic nitrogen-indicators of the presence of the nutrients (Vollenweider, 1998).

The following formula calculates E-TRIX:

$$TRIX = [\log(Ch \cdot D\%O \cdot N \cdot P) + 1.5]/1.2$$

Where,

Ch - chlorophyll *a* concentration, µg/L.

D%O - deviation in absolute values of dissolved oxygen from 100% saturation.

N - concentration of the sum of inorganic nitrogen dissolved forms, µg/L.

P - concentration of total phosphorus, µg/L.

According to the water trophic status, E-TRIX changes in the range 0-10 (Table 5.3).

Table 5.3 - Characteristics of seawater quality according to TRIX

MSFD	Water quality	E-TRIX	Trophic level	Characteristics of water
GES*	High	0 - ≤4	Low	High transparency of water, lack of colour anomalies of water, lack of satiety and lack of saturation of dissolved oxygen
	Good	>4 - ≤5	Moderate	Occasional cases of reducing the water transparency, lack of water colour anomalies, hypoxic bottom waters.
Not GES	Moderate	>5 - ≤6	High	Low water transparency, water colour anomalies, hypoxia of bottom waters, and occasional cases of anoxia.
	Bad	>6 - ≤10	Very high	High water turbidity, large areas of colour anomalies of water, regular hypoxia over a large area and frequent anoxia of bottom waters, death of benthic organisms

\* - Good Environmental Status

Methodological aspects in determining the E-TRIX index by the averaged individual measurements' data and calculating the initial data and subsequent averaging of index values were already discussed (Ukrainsky, 2010). In the calculation, the formula uses common and most frequently measured hydro-chemical and hydrobiological characteristics of marine waters; the number of parameters does not change, making it possible to compare E-TRIX values for different sea areas and oceans.

For assessing the trophic status and water quality with the E-TRIX in the zones of influence of river waters, we used all data from river-sea cruises.

In winter, in the area of influence of Sakarya and Yeşilırmak Rivers, water quality was "Good" (Figure 5.1). Only at station YSL07, the status was "Moderate" water quality due to the increased concentration of chlorophyll *a* (2.34 µg/L). The water quality improved to "High" water quality and "Low" trophic level at the more seaward station. In general, in this region, water quality may be assessed as "Good" and trophic level as "Moderate", except for YSL07 ("Moderate" Water quality).

In the area of Sakarya River's influence, E-TRIX was slightly higher than in the Yeşilırmak River zone and indicated "Good" Water quality and "Moderate" Trophic level. At the mouth station SAK07, E-TRIX marked "Moderate" Water quality and "High" Trophic level. The same situation was in the more seaward stations SAK05 and SAK16, where the E-TRIX value was 5.54 and 5.24 ("Moderate" Water quality and "High" Trophic level). At all three stations, an increased concentration of nutrients was also noted, with a low chlorophyll *a* concentration.

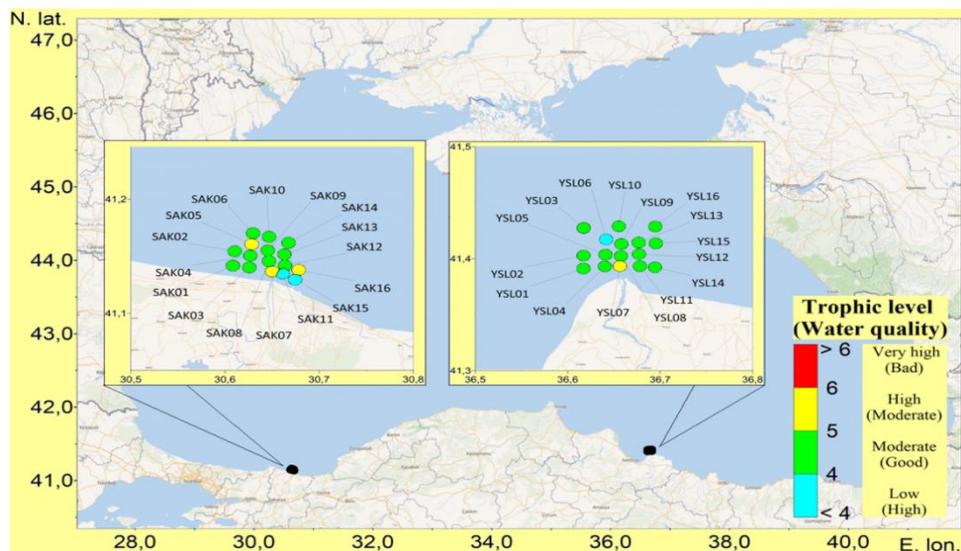


Figure 5.1- Black Sea (0 m) eutrophication assessment, E-TRIX, Winter (2020)

In spring, the water quality in the areas where rivers flow into the Black Sea is significantly deteriorating, associated with river runoff, precipitation and meltwaters, which wash away the substances from the soil surface, thereby enriching sea waters with nutrients. In spring, most stations

in the area of river influence, according to E-TRIX, demonstrated “Bad” water quality and “Very high” trophic level (Figure 5.2).

In the Dnieper and Bug Rivers mouths and Dnieper-Bug estuary (at stations 4, 5, 6), the average E-TRIX value was 7.1. For all the stations, E-TRIX value was greater than 6.0, which indicated “Bad” Water quality and “Very high” Trophic level. A high concentration of total phosphorus (66.6-102.83 µg/L) and chlorophyll *a* (20.5-49.0 µg/L) was noted at these stations.

In the Dniester’s estuary, E-TRIX was 3.58, which pointed out “High” water quality and “Low” Trophic level.

In the area of influence of the Danube River, the average E-TRIX indicated “Bad” water quality and “Very high” trophic level. In the areas of direct influence of river waters (stations 1, 2 and Sulina 20 m, Sf. Gheorghe 20 m, Sf. Gheorghe 30 m, Portita 20 m), the average E-TRIX was 6.53; thus, water quality was assessed as “Bad” and trophic level as “Very high”. The maximum value of E-TRIX was noted at station Portita 20 m, where the maximum concentration of total phosphorus was also recorded (327.70 µg/L), with low chlorophyll *a* concentration (0.94 µg/L). At more seaward stations, away from the coastline, the average E-TRIX was 5.75 that indicated “Moderate” water quality and “High” trophic level. As an exception, high values of E-TRIX were registered at the three most seaward stations. At the station Sf. Gheorghe 60 m that is farthest from the coastline, E-TRIX was 6.25, this station also had the highest concentration of mineral nitrogen (1061.29 µg/L), and the chlorophyll *a* concentration 1.79 µg/L, so the water quality was assessed as “Bad” and trophic level as “Very high”. At the stations Periboina 50 m and Periboina 60 m, E-TRIX was 6.13 and 6.06, respectively, which indicated “Bad” Water quality and “Very high” trophic level. At these stations, high chlorophyll *a* concentration of 3.19 µg/L and 5.44 µg/L were also noted. This distribution is associated with the hydrological conditions and climate of this area.

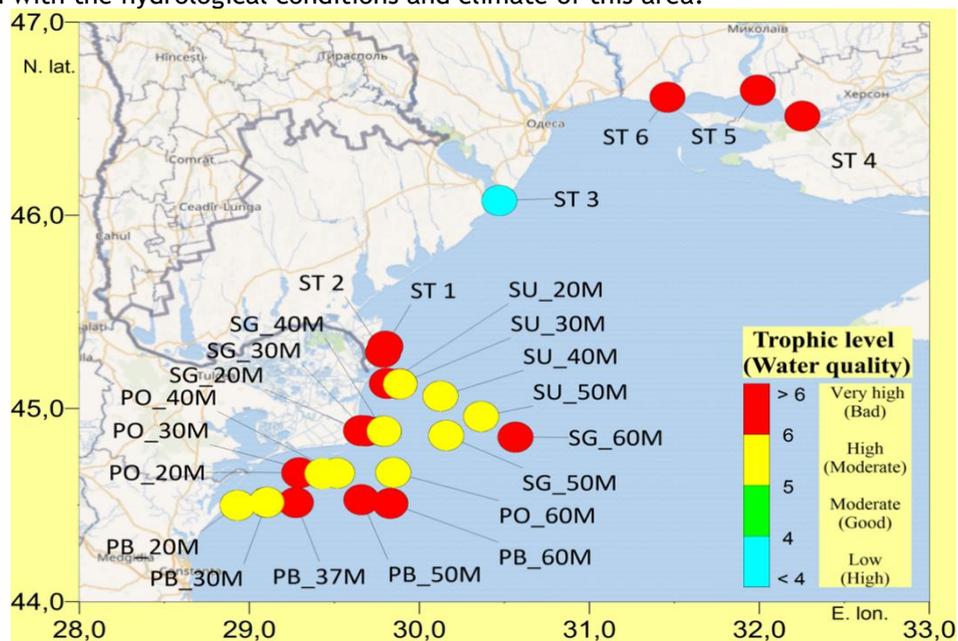


Figure 5.2 - Black Sea (0 m) entrophication assessment, E-TRIX, Spring (2019)

According to the E-TRIX assessment in the summer, the water quality is similar to the spring (Figure 5.3). In the area where the Yesilirmak River flows into the Black Sea, the average E-TRIX was 2.13 in the whole region, which indicated “High” water quality and “Low” trophic level. At station YSL11, E-TRIX was 5.5, which marked “Moderate” water quality and “High” trophic level. At this station, we registered the high concentration of total phosphorus (92.46 µg/L) and inorganic nitrogen (24.41 µg/L), with an average concentration of chlorophyll of 1.7 µg/L.

In the area of influence of the Sakarya River, for the entire region, the average E-TRIX was 3.3, which indicated “High” water quality and “Low” trophic level. At the stations closest to the coastline, E-TRIX was in the range of 4.24-4.68, which pointed out the “Good” water quality and “Moderate” trophic level. At 2 stations, SAK03 and SAK12, E-TRIX was 5.31 and 5.26, which demonstrated “Moderate” water quality and “High” trophic level. At the same stations, there was registered increased total phosphorus concentrations (37.25 µg/L and 29.03 µg/L) and inorganic nitrogen (170.0 µg/L and 148.76 µg/L), but the chlorophyll *a* concentration was low, 0.72 µg/L.

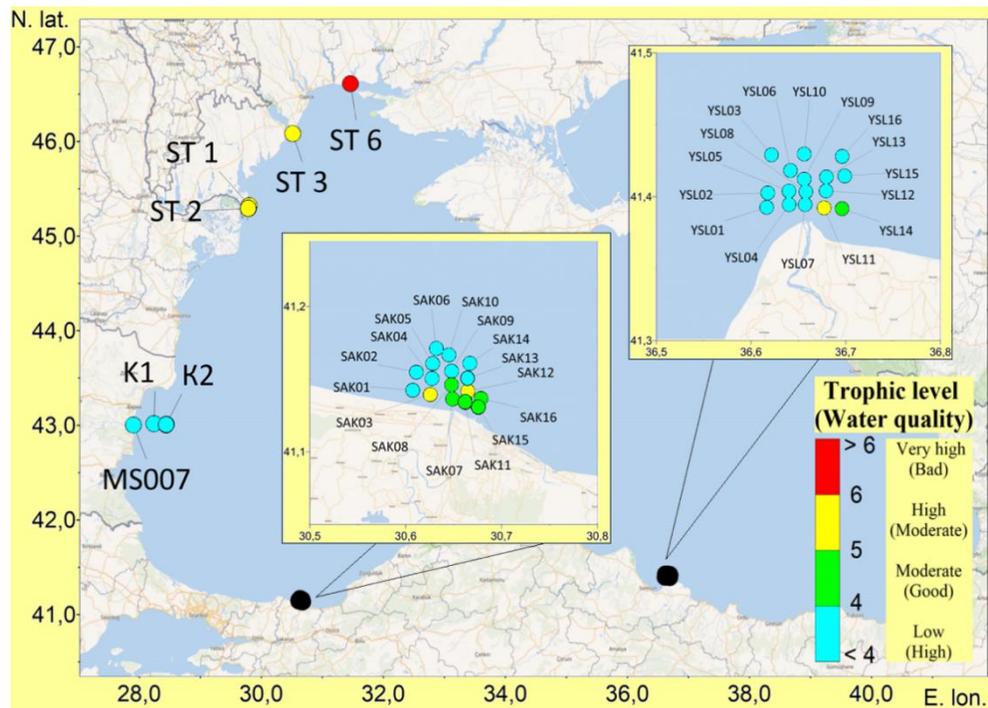


Figure 5.3 - Black Sea (0 m) eutrophication assessment, E-TRIX, Summer (2019)

E-TRIX remains high in the marine waters at Dnieper-Bug estuary's mouth area, 6.76, which indicated "Bad" water quality and "Very high" trophic level. There were noted high chlorophyll *a* (29.8 µg/L) and total phosphorus (32.21 µg/L) levels. Near the Dniester and Danube Rivers mouths, E-TRIX was in the range of 5.0-5.94, which pointed to "Moderate" water quality and "High" trophic level. The Dniester River area was also characterized by a high chlorophyll *a* concentration, 7.44 µg/L. In the areas influenced by the Kamchia River, E-TRIX was in the range of 3.36-3.66 that demonstrating the "High" water quality and "Low" trophic level. In general, according to E-TRIX, the water quality in the summer in the zone of influence of the rivers became better than in the spring, except for the Dnieper - Bug rivers region, where the water quality was assessed as "Bad" and trophic level as "Very high".

## Conclusions

In winter, in the zone of influence of Sakarya and Yeşilırmak Rivers, the average E-TRIX indicated Good Water quality and Trophic level - Moderate. In the area of influence of the Yeşilırmak River, the water quality was "Good" and the trophic level "Moderate". The exception was st. YSL07, with "Moderate" water quality and "High" trophic level.

In the zone of Sakarya River influence, E-TRIX indicated "Good" Water quality and "Moderate" Trophic level. At the mouth station SAK07 and two more seaward stations SAK05 and SAK16, E-TRIX indicated "Moderate" Water quality and "High" Trophic level. At all three stations, an increased concentration of nutrients was noted, with a low concentration of chlorophyll *a*.

In the areas of the river's influence, the highest E-TRIX was in the spring. The maximum was in the areas where the Dnieper and Bug Rivers emerge. Also, high E-TRIX were in the Danube River influence area, which indicated "Bad" water quality and "Very high" trophic level. At more seaward stations of the Danube area, away from the coastline, E-TRIX decreases, pointing out "Moderate" water quality and "High" trophic level. At the seaward stations, where was the maximum amount of inorganic nitrogen and high chlorophyll *a* concentration, E-TRIX exceeded 6.0. This distribution is associated with the hydrological conditions and climate of this area. E-TRIX indicated "High" water quality and "Low" trophic level in the Dniester river area.

In general, the water quality in the summer in the zone of influence of the rivers became better than in the spring, except for the area under the Dnieper - Bug influence, where the water quality was "Bad" and trophic level "Very high".

In the areas where the Dniester and Danube Rivers flow in the Black Sea, water quality was "Moderate" and the trophic level "High".

In the Kamchia River influence area, E-TRIX marked "High" Water quality and "Low" trophic level. In the area of Sakarya River influence, the average E-TRIX indicated "High" water quality and "Low" trophic level. At the stations closest to the coastline, E-TRIX showed "Good" water quality and "Moderate" Trophic level, and only at two stations, SAK03 and SAK12, E-TRIX marked "Moderate" water quality and "High" trophic level.

In the Yeşilirmak mouth area, E-TRIX indicated "High" water quality and "Low" trophic level, except for the YSL11 station with "Moderate" Water quality and "High" Trophic level.

In general, according to the results of the E-TRIX assessment, the Black Sea shelf waters quality matched GES for both surface and near-bottom waters. The exception is two stations in the Ukrainian part of the shelf, where the water quality was lower than GES. In the central area of NPMS at st. UA-16, the surface water quality was not GES; the E-TRIX value is 5.17, which pointed out "Moderate" Water quality and High Trophic level.

## 5.1.2 BEAST

BEAST (Black Sea Eutrophication ASsessment Tool) is an integrative tool for the eutrophication assessment proposed by the Black Sea Commission through the Baltic2Black project, and similar to HEAT (HELCOM, 2009) which runs on MS Excel. The eutrophication assessment of the Black Sea in respect to the descriptor's 5 (MSFD) and BEAST (Black Sea Eutrophication ASsessment Tool) requirements uses a core set of indicators (Lazar et al., 2016)) (Table 1).

BEAST categories have three criteria:

C1 - causes of eutrophication,

C2 - direct effects and

C3 - indirect effects, indicating the main cause-effect relationships in the eutrophication development.

Each criterion could have a set of indicators (based on availability and expert's choice). Within the criteria, BEAST takes a weighted mean (according to the significance of the parameter or the data quality), evenly distributed. The result of each indicator status is the EUT\_Ratio. Simultaneously, between the categories, the One-Out-All-Out-principle (OOAO) is applied (the worst assessment of a quality element determines the overall assessment result). The result is qualitative, the "Final eutrophication status": high, good, moderate, poor, and bad. With the scope of data visualization, we assigned a value to each qualitative result - 1-High (blue), 2-Good (green), 3-Moderate (yellow), 4-Poor (orange), 5-Bad (red).

For this assessment, we used as core indicators (due to their availability, reference conditions availability and relevance) as follows:

C1 - causative factors - surface nutrients concentrations - comprises ten nutrient indicators, though they are not used together in any assessment units.

C2 - direct effects - phytoplankton blooms - surface chlorophyll *a* (as an estimate of the Total biomass) or the total phytoplankton biomass (UA), and Secchi depth.

C3 - indirect effects - bottom dissolved oxygen (%) (effective only for coastal and shelf waters up to 50m bottom depth due to the natural features of the Black Sea).

According to BEAST, in front of rivers discharging mouths, 36% of total stations are not at risk of eutrophication, while 64 % highlighted the moderate to poor ecosystem status. From the latter, the poor took the most significant share (Figure 5.4). The nutrients enrichment was the cause of the Dnieper and Danube rivers, whereas the direct effects of nutrients enrichment emerged in front of Dnieper, Bug, Dniester, Sakarya and Yesilirmak. Kamchia did not show any influence during this study. With one exception (St.6 exit from Dnipro-Bug estuary), no indirect effects were influencing the total results (Figure 5.5).

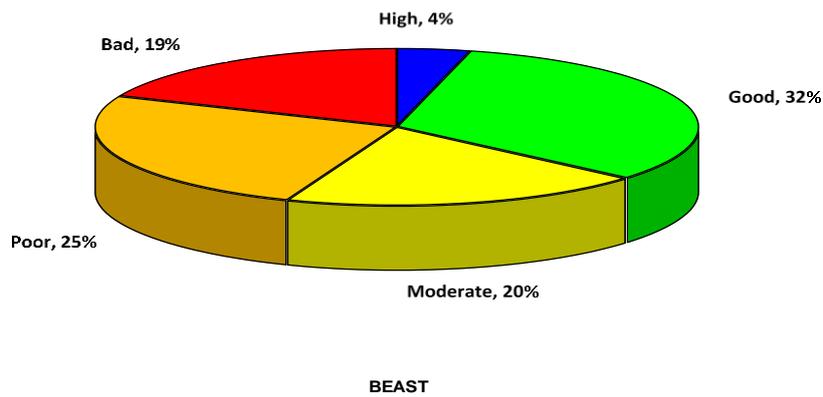


Figure 5.4 - Black Sea ecosystem status - eutrophication - stations percentage from the total, ANEMONE River-Sea cruises, 2019-2020

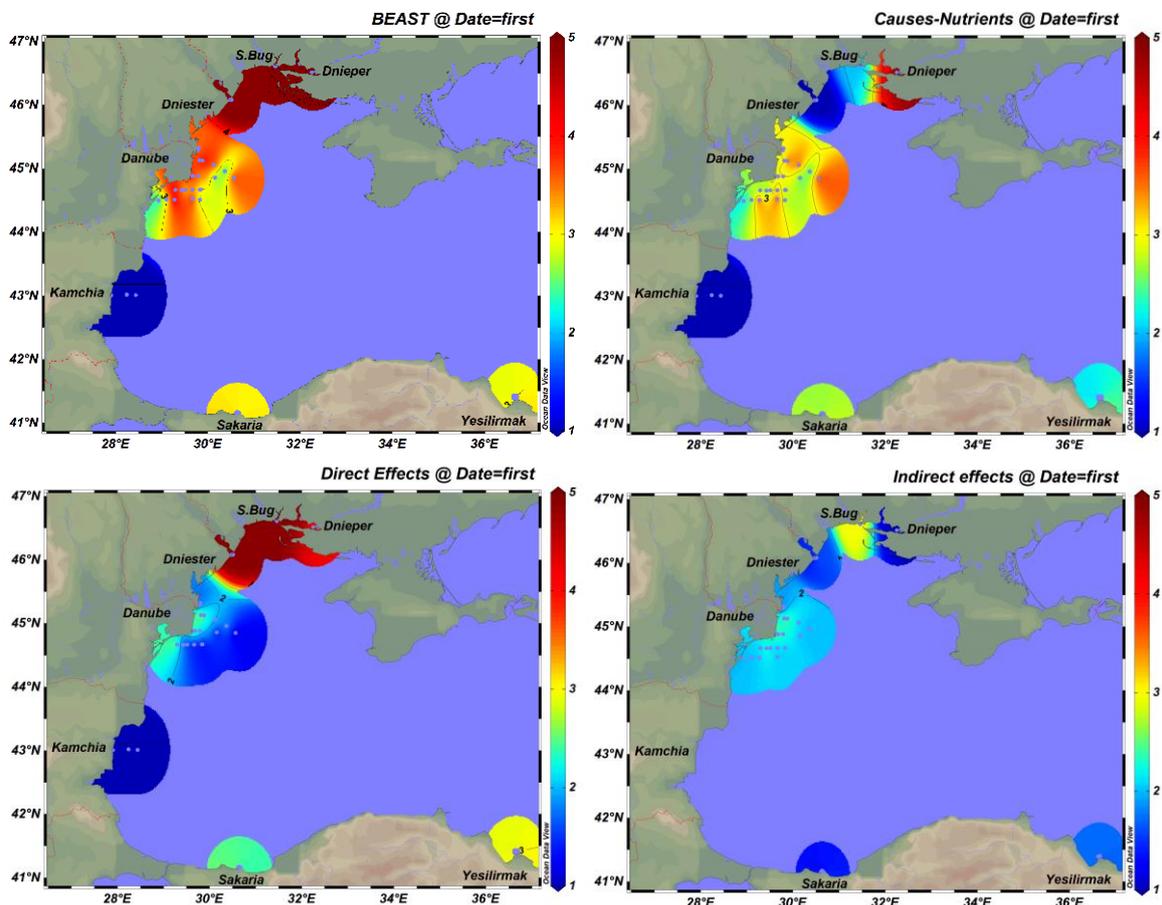


Figure 5.5 - Black Sea eutrophication status according to BEAST and each criterion, ANEMONE River Sea interaction cruises, 2019-2020

One of the tool's important applicability is related to the problem areas identified and links with activities and their generated pressures. The primary pressure for eutrophication is introducing nutrients from point and diffuse sources in the marine environment. Thus, following the OSPAR approach, BEAST results were categorized as follows:

- areas showing an increased degree of nutrient enrichment accompanied by direct and/or indirect/other possible effects are "problem areas".

b. areas which may show direct effects and/or indirect or other possible effects, when there is no evident increased nutrient enrichment, for example, because of the transboundary transport of (toxic) algae and/or organic matter arising from adjacent/remote areas. These areas are "problem areas".

c. areas with an increased degree of nutrient enrichment where: (i) either there is firm, scientifically based evidence of the absence of (direct, indirect, or other possible) eutrophication effects - these are classified initially as 'non-problem areas', although the increased degree of nutrient enrichment in these areas may contribute to eutrophication problems elsewhere; (ii) or there is not enough data to perform an assessment or where the data available is not fit for the purpose - these are classified initially as "potential problem areas";

d. areas without nutrient enrichment and related (in)direct/other possible effects are "non-problem areas" Andersson, 2015) (Figure 5.6).



Figure 5.6 - BEAST (average) related to rivers' influence (green-no problem area, orange-potential problem area, red-problem area)

Thus, for Kamchia influence, BEAST identified „no problem areas “without nutrients enrichment and any effects. The „potential problem areas “are defined in front of the Danube (UA and RO), Sakarya and Yesilirmak rivers. This assessment classified the Dnieper, Dnieper-Bug estuary, Bug, and the Dniester as problem areas similar to TRIX results.

Eutrophication is still a significant problem in the northwestern Black Sea because of the increased nutrients riverine input. It is also true that their drainage basins are vast (both as surface and population) compared to the western and southern areas. Therefore, in this context, the effort of nutrients enrichment reduction is crucial for the ecosystem 's health.

Danube's case showed that the cooperation for the protection and sustainable use of the River resulted in an improved Black Sea's status. Thus, for over two decades of countries' cooperation, were taken measures to reduce the Black Sea pollution loads from sources in the Danube River Basin. To improve the water quality, an ambitious programme of measures for the whole Danube River Basin District has been agreed under the EU WFD. To assess trends in water quality, the ICPDR manages the TransNational Monitoring Network (TNMN). The network carefully monitors physical, chemical, and biological conditions in the Danube and its tributaries and provides in TNMN Yearbooks an annual overview of pollution levels and long-term trends for water quality in the basin<sup>10</sup>.

BEAST showed worst results than E-TRIX because of their definition. In contrast to TRIX, BEAST considers the reference values for each parameter. TRIX still needs a parametrization for the formula's constants which in original were developed for the Adriatic Sea. However, all results are dependent on the number of samples, sampling frequency, monitoring design - gradient, seasonality, and rivers flows and loads.

According to our data, acquired during the ANEMONE project, the Black Sea eutrophication is a problem in the neighbouring area of six of seven's studied rivers where due to an increased degree

<sup>10</sup> <https://www.icpdr.org/main/issues/water-quality>

of nutrient enrichment were observed their direct and indirect effects. Thus, more than half stations were under “moderate” and “poor” status, representing a non-GES condition.

Future improvement efforts should be focused on activities related to nutrients input from point and diffuse sources, mainly wastewater, agriculture runoff and atmospheric deposition.

## 5.2 Input of other substances (e.g., synthetic substances, non-synthetic substances, radionuclides) - CHASE

A good ecological and environmental status has as a prerequisite condition a good chemical condition. It is one of the most topical challenges facing policymakers, water managers, and scientists (Laane et al., 2012).

The recognition of hazardous substances coming from rivers and their distribution and storage in the intermediate layers are of great interest to preserve the ecological integrity of the Black Sea. Coordinating Black Sea protection measures requires a good understanding of river flow's fate into the sea (Miladinova et al., 2020).

Indicators are tools for evaluating marine environments' status in relation to management targets or thresholds. Application of the widely used “one-out, all-out” principle could easily result in an entirely negative overall evaluation for all objectives. A drawback of this approach is that a few strongly negative indicator values could shadow the potentially generally positive state of a given ecological objective. It would make any progress towards improving the environmental status invisible, as long as at least one indicator shows poor performance (Ojaveer, Eero, 2011).

The assessment of river impacts on the Black Sea coastal environmental status was done using an integrated hazardous substances assessment tool (CHASE) as a common approach for the Black Sea region and to avoid misleading conclusions. Pollutants concentrations were evaluated against threshold values that define good environmental status in each region, using the HELCOM integrated hazardous substances assessment tool (CHASE) developed by NIVA Denmark (Andersen et al., 2016). This tool integrates data on hazardous substances in different matrices and bio-effect indicators, if available and is based on a substance- or bio-effect-specific calculation of a ‘contamination ratio’ being the ratio between an observed concentration and a threshold value. Values <1.0 indicate areas potentially ‘unaffected’, while values >1.0 indicate areas potentially ‘affected’. These ratios are combined within matrices, i.e., for water, sediment, and biota and biological effects. The integrated assessment provides a final status for an assessment unit, placing it in one of five classes: bad, poor, moderate, good, and high. Thus, this classification system is essentially binomial (unaffected vs. affected) and is distinguished by a threshold value. The other classes are based on defined deviations from the unaffected/affected boundary. While the threshold between the good and moderate status equals 1.0 (reflecting the use of contamination ratios), the high-good threshold is 0.5, the moderate-poor threshold is 5.0 and the poor-bad threshold is 10.0. The overall assessment uses a “one out - all out” principle regarding each matrix (Andersen et al., 2016). To better view the environmental status in each region, the graphic representation was done using the program Ocean Data View, so for each status class, it has assigned a value, from 1 - High to 5 - Bad.

The results were compared with the assessment done using the method in place in each region to figure out the benefit of using CHASE tool.

In Ukraine, the national methodology to assess the ecological state is by calculating a pollution factor,  $K_z$ , which reflects the concentration of all pollutants of the same type in a certain period in a given area. This factor represents the sum of the ratios of the concentration of each pollutant to its maximum permissible concentration, in accordance with EU Directive 2013/39 (MAC-EQS) for water, even the implementation of MSFD is not obligatory or the maximum permissible concentration according to Ukrainian legislation for sediment, to the number of measurements performed in a given period. Like CHASE, there are five quality classes (very good, good, satisfactory, bad, and very bad). The overall assessment of the water or sediments' ecological condition is determined by the group of pollutants' worst quality.

In Romania, the status of the Black Sea ecosystem in respect to MSFD is assessed by evaluating the 75<sup>th</sup> percentile of the data in the assessment unit in a given period against threshold values that define good environmental status (MAC-EQS) following European legislation (EU Directive 2013/39) in water or ERL and EAC values (Effect Range Low and Environmental Assessment Criteria) developed by US EPA and OSPAR for assessing the ecological significance of sediment concentrations (OSPAR, 2008; UNEP MAP, 2011; US EPA, 1998; Long et al., 1998). As a result, the “Good” or “Bad” status for each substance is obtained for each matrix; the overall result is given by the worst-case using the

"one-out, all-out principle" (Boicenco et al., 2018). In Turkey, the implementation of MSFD is not obligatory yet. Using. Some pilot studies are carried out to assess contamination in the water matrix according to the WFD (EU Directive 2000/60), using Max-EQS (EU Directive 2013/39). To decide each station's chemical status, the "one-out all-out principle" is applied for both matrices (except heptachlor which has an EQS below the detection limit) and overall assessment.

The results obtained in the Romanian area influenced by the Danube River revealed some are exceeding the threshold values that define good environmental status.

Except for cyclodiene pesticides, heptachlor and anthracene, organic pollutants were in good status in water in the Danube's influence area, according to the methodology developed in Romania to assess the status of the Black Sea ecosystem with respect to MSFD (Boicenco et al., 2018). As for heavy metals, Cu, Pb and Ni surpassed recommended EQS values, whereas Cd values were below EQS. In sediment, only PCB 28, copper and cadmium were in bad status. The other contaminants had levels below thresholds that define good ecological status.

Based on the "one out - all out" principle the sediment status was evaluated as "Bad" in all station in sediment and water (Table 5.4, Table 5.5) and in consequence, the overall status was evaluated as "Bad".

Based on the "one-out - all-out" principle, the sediment status was "Bad" in all stations (Table 5-4, Table 5-5), and the overall assessment was "Bad" in all stations.

The evaluation done using the integrated hazardous substances assessment tool (CHASE) in each station pointed out states of the chemical status from "Moderate" to "Bad" in sediment, "Bad" in water (Table 5.4 and Table 5.5) and the overall assessment was "Bad" in all stations.

The results are the same for water, and some differences are noted for sediment. These differences are the result of the different approach: two quality classes of local methodology and five for the integrated tool. As an overall result, the two assessment concluded the same quality for the area.

In the Ukraine area, influenced by Danube, Dniester, Dnieper, and southern Bug Rivers, the overall assessment was similar. In water, the results obtained for heavy metals correspond to a "Very good" and "Good" ecological state on the heavy metals pollution. High levels of individual PCBs, organochlorine pesticides and benzo(g,h,i)perylene were recorded. Consequently, the seawater' ecological status corresponded to the quality class - "Very bad" (Table 5.6).

In sediment, the increased content of organochlorine pesticides was especially noted in stations 3 and 6. Also, naphthalene was in bad status at station 6. Heavy metals status was generally good and very good, except for mercury at stations 2 and 6, nickel at station 1 and 2, copper at station 2 and chromium at station 1. As a result, in stations 2, 3 and 6, the overall quality class was assessed as bad and satisfactory in station 1 (Table 5.7).

**Table 5.4 - Romania sediment status according to CHASE and national methodology assessment**

Station	Matrix	CHASE status	National methodology evaluation status
SU_20M	Sediment	5-Bad	Bad
SU_30M	Sediment	5-Bad	Bad
SU_40M	Sediment	3-Moderate	Bad
SU_50M	Sediment	3-Moderate	Bad
SG_20M	Sediment	5-Bad	Bad
SG_30M	Sediment	4-Poor	Bad
SG_40M	Sediment	3-Moderate	Bad
SG_50M	Sediment	3-Moderate	Bad
SG_60M	Sediment	4-Poor	Bad
PO_20M	Sediment	5-Bad	Bad
PO_30M	Sediment	5-Bad	Bad
PO_40M	Sediment	3-Moderate	Bad
PO_50M	Sediment	3-Moderate	Bad
PO_60M	Sediment	3-Moderate	Bad
PB_20M	Sediment	4-Poor	Bad
PB_30M	Sediment	5-Bad	Bad
PB_37M	Sediment	5-Bad	Bad
PB_50M	Sediment	5-Bad	Bad
PB_60M	Sediment	5-Bad	Bad

**Table 5.5 - Romania water status according to CHASE and national methodology assessment**

Station	Matrix	CHASE score/status	National methodology evaluation status
SU_20M	Water	5-Bad	Bad
SU_30M	Water	5-Bad	Bad
SU_40M	Water	5-Bad	Bad
SU_50M	Water	5-Bad	Bad
SG_20M	Water	5-Bad	Bad
SG_30M	Water	5-Bad	Bad
SG_40M	Water	5-Bad	Bad
SG_50M	Water	5-Bad	Bad
SG_60M	Water	5-Bad	Bad
PO_20M	Water	5-Bad	Bad
PO_30M	Water	5-Bad	Bad
PO_40M	Water	5-Bad	Bad
PO_50M	Water	5-Bad	Bad
PO_60M	Water	5-Bad	Bad
PB_20M	Water	5-Bad	Bad
PB_30M	Water	5-Bad	Bad
PB_37M	Water	5-Bad	Bad
PB_50M	Water	5-Bad	Bad
PB_60M	Water	5-Bad	Bad

The evaluation done using the integrated hazardous substances assessment tool (CHASE) in each station pointed out states of the chemical status “Moderate”, “Poor” and “Bad” in sediment and “Bad” in water and the overall assessment was “Bad” in all stations.

**Table 5.6 - Ukraine water status according to CHASE and national methodology assessment**

Station	Matrix	CHASE status	National methodology evaluation status
ST 1	Water	5-Bad	Very bad
ST 2	Water	5-Bad	Very bad
ST 3	Water	5-Bad	Very bad
ST 4	Water	5-Bad	Very bad
ST 5	Water	5-Bad	Very bad
ST 6	Water	5-Bad	Very bad

**Table 5.7 - Ukraine sediment status according to CHASE and national methodology assessment**

Station	Matrix	CHASE status	National methodology evaluation status
ST 1	Sediment	3-Moderate	Satisfactory
ST 2	Sediment	3-Moderate	Bad
ST 3	Sediment	5-Bad	Bad
ST 6	Sediment	4-Poor	Bad

The results were the same in water and similar in sediment. The two approaches are the same using five quality classes, even if their definition is slightly different. As an overall result, the two assessments concluded the same quality for the area.

Measurement results of the organic compounds such as petroleum hydrocarbons, PAHs, PCBs and OCPs and heavy metals in water and sediment matrices indicate relatively less contamination of the Turkish coastal areas under the influence of rivers.

In water, concentrations of most of the priority organic substances were found below the Max-EQS (Directive 2013/39/EU) except benzo(a) pyrene (BaP), and benzo(b)fluoranthene, two of the 16 polyaromatic hydrocarbons. Besides iron that surpassed the maximum admissible levels in more than 50% of the samples, the heavy metals were in good status.

In sediment, the sum of the DDT's including metabolites (DDE+DDD+DDT) exceeded the threshold values. The concentrations of the other organochlorinated pesticides and polychlorinated biphenyls were below the threshold values in all stations of both study sites. Some of the heavy metals like copper, nickel, chromium, arsenic and in some cases mercury, also, surpassed the threshold values.

The assessment made for water using national assessment shows that 65% of stations have bad, and the others have good water quality. For sediments, the national assessment concluded that one station is in “Good” status and the others in “Bad” status. There are many differences between the studied areas regarding indicator substances or threshold values used in the assessment. Still, the Black Sea quality seems to be better in the southern part, where the status was moderate compared with the other areas in bad status. Therefore, we identified the chemical pressure coming from organic pollutants input from northwestern rivers. The results can contribute to evaluating the efficiency of the measures in the Black Sea region. A commonly agreed set of indicators and threshold will give a better understanding of the pressures of the Black Sea (Table 5.9).

According to the CHASE results, the assessment made for the water matrix shows that all stations are in “Moderate” status. The CHASE results warn about the general status in the assessment area: if a chemical is in bad status, you must take measures to protect the ecosystem against its effects. Still, the five quality classes allow for prioritization between different areas. Some of them are more affected than others (even if we are talking about the levels of pollutants or the number of contaminants exceeding the thresholds). Any classification below “Good” status requires adequate measures to reduce pollution (Table 5.8), and for sediments, 22 % of the stations are in “Poor” and the others in “Moderate” status. There are many differences between the studied areas regarding indicator substances or threshold values used in the assessment. Still, the Black Sea quality seems to be better in the southern part, where the status was moderate compared with the other areas in bad status. Therefore, we identified the chemical pressure coming from organic pollutants input from northwestern rivers. The results can contribute to evaluating the efficiency of the measures in the Black Sea region. A commonly agreed set of indicators and threshold will give a better understanding of the pressures of the Black Sea (Table 5.9).

Based on these assessments, we can say that the use of the CHASE tool makes a better separation in the chemical status. National classification based on the “one-out, all-out” principle can only create two categories that may not be useful for the coastal managers.

As the overall assessment, CHASE uses the ‘one-out, all-out principle’, so the global status was evaluated to “Bad” for the northwestern and western part of the Black Sea. In contrast, the southern area was evaluated to “Moderate” in most of the stations (Figure 5.7), even if in sediment, the evaluation concluded a better quality than in the water in the northwestern and western area (Figure 5.8 and Figure 5.9).

**Table 5.8 - Turkey water status according to CHASE and national methodology assessment**

Station	Matrix	CHASE status	National methodology evaluation status
SAK01	Water	3-Moderate	Bad
SAK03	Water	3-Moderate	Bad
SAK04	Water	3-Moderate	Bad
SAK07	Water	3-Moderate	Bad
SAK08	Water	3-Moderate	Bad
SAK09	Water	3-Moderate	Bad
SAK10	Water	3-Moderate	Good
SAK11	Water	3-Moderate	Bad
SAK12	Water	3-Moderate	Bad
SAK15	Water	3-Moderate	Bad
YSL01	Water	3-Moderate	Good
YSL04	Water	3-Moderate	Bad
YSL05	Water	3-Moderate	Good
YSL07	Water	3-Moderate	Good
YSL08	Water	3-Moderate	Good
YSL09	Water	3-Moderate	Good
YSL10	Water	3-Moderate	Bad
YSL11	Water	3-Moderate	Bad
YSL12	Water	3-Moderate	Bad
YSL14	Water	3-Moderate	Good
YSL16	Water	3-Moderate	Good

The CHASE results warn about the general status in the assessment area: if a chemical is in bad status, you must take measures to protect the ecosystem against its effects. Still, the five quality classes allow for prioritization between different areas. Some of them are more affected than others

(even if we are talking about the levels of pollutants or the number of contaminants exceeding the thresholds). Any classification below “Good” status requires adequate measures to reduce pollution. There are many differences between the studied areas regarding indicator substances or threshold values used in the assessment. Still, the Black Sea quality seems to be better in the southern part, where the status was moderate compared with the other areas in bad status. Therefore, we identified the chemical pressure coming from organic pollutants input from northwestern rivers. The results can contribute to evaluating the efficiency of the measures in the Black Sea region. A commonly agreed set of indicators and threshold will give a better understanding of the pressures of the Black Sea.

**Table 5.9 - Turkey sediment status according to CHASE and national methodology assessment**

Station	Matrix	CHASE status	National methodology evaluation status
SAK01	Sediment	3-Moderate	Bad
SAK02	Sediment	3-Moderate	Bad
SAK06	Sediment	3-Moderate	Bad
SAK07	Sediment	3-Moderate	Bad
SAK08	Sediment	3-Moderate	Bad
SAK09	Sediment	3-Moderate	Bad
SAK10	Sediment	3-Moderate	Bad
SAK11	Sediment	3-Moderate	Bad
SAK12	Sediment	3-Moderate	Bad
SAK14	Sediment	3-Moderate	Bad
SAK15	Sediment	3-Moderate	Bad
SAK16	Sediment	3-Moderate	Bad
YSL01	Sediment	3-Moderate	Bad
YSL02	Sediment	4-Poor	Bad
YSL03	Sediment	3-Moderate	Bad
YSL04	Sediment	3-Moderate	Bad
YSL05	Sediment	3-Moderate	Bad
YSL06	Sediment	4-Poor	Bad
YSL07	Sediment	3-Moderate	Bad
YSL08	Sediment	3-Moderate	Bad
YSL09	Sediment	4-Poor	Bad
YSL11	Sediment	3-Moderate	Bad
YSL12	Sediment	4-Poor	Bad
YSL13	Sediment	4-Poor	Bad
YSL14	Sediment	3-Moderate	Good
YSL15	Sediment	3-Moderate	Bad
YSL16	Sediment	4-Poor	Bad

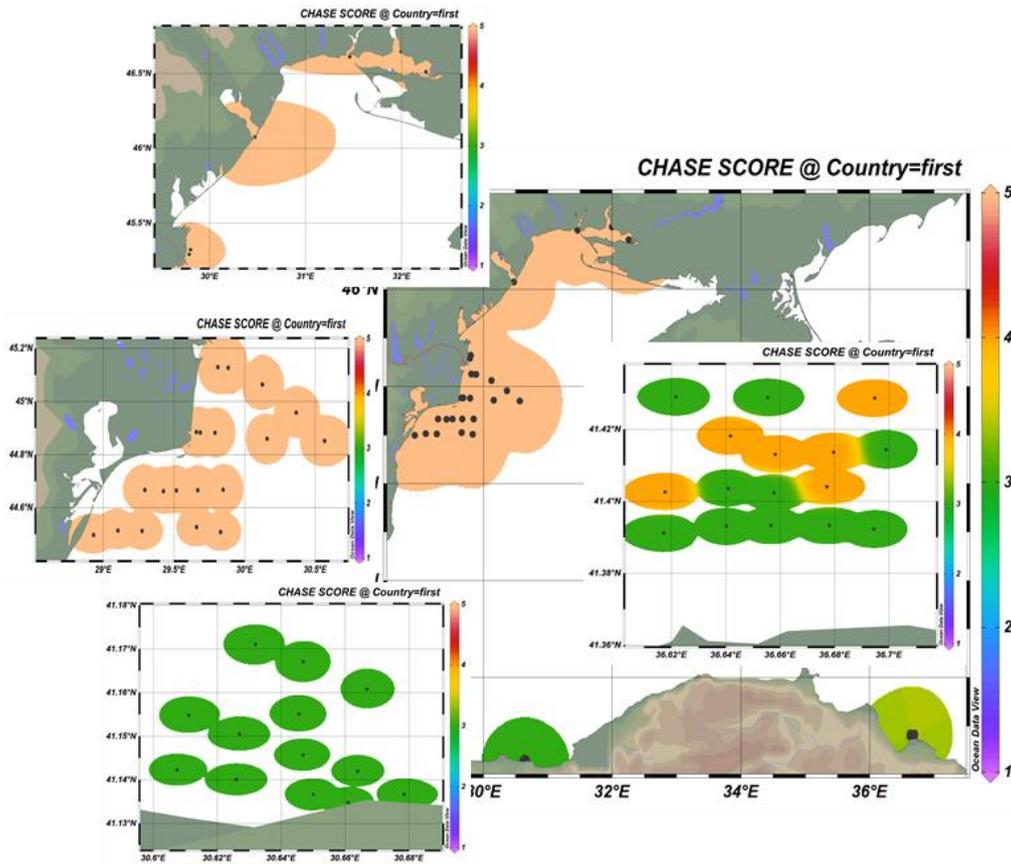


Figure 5.7 - Overall, CHASE results in the rivers influenced area of the northwestern, western, and southern part of the Black Sea

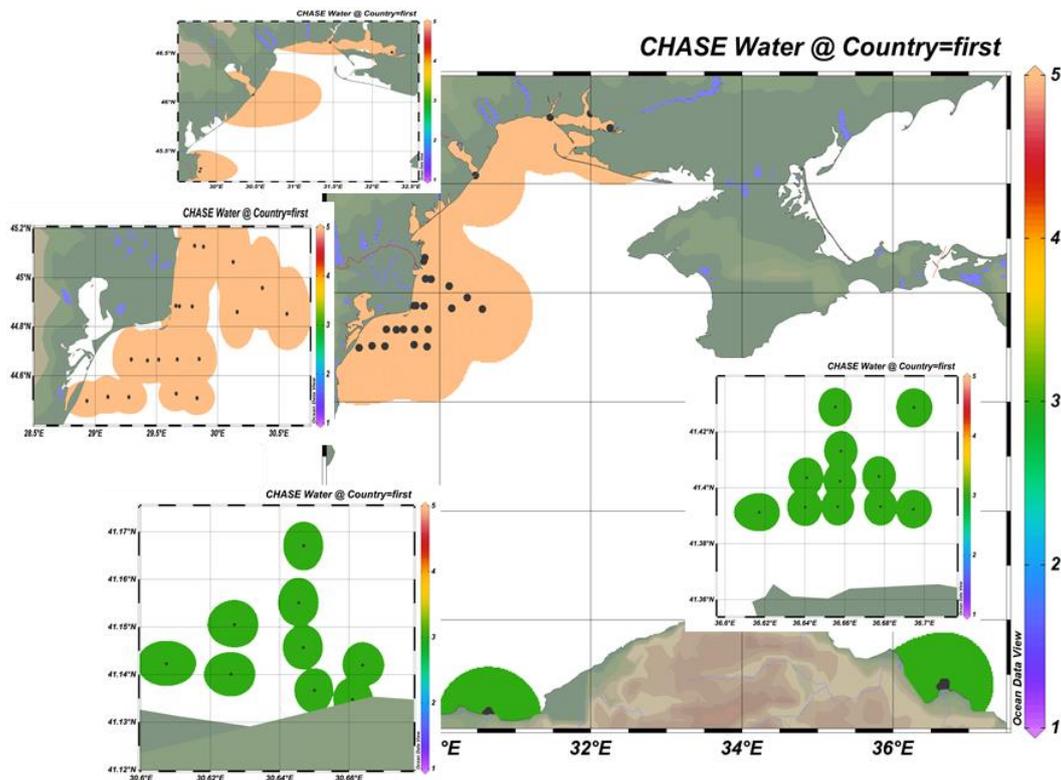


Figure 5.8 - Water CHASE results in the rivers influenced area of the northwestern, western, and southern part of the Black Sea

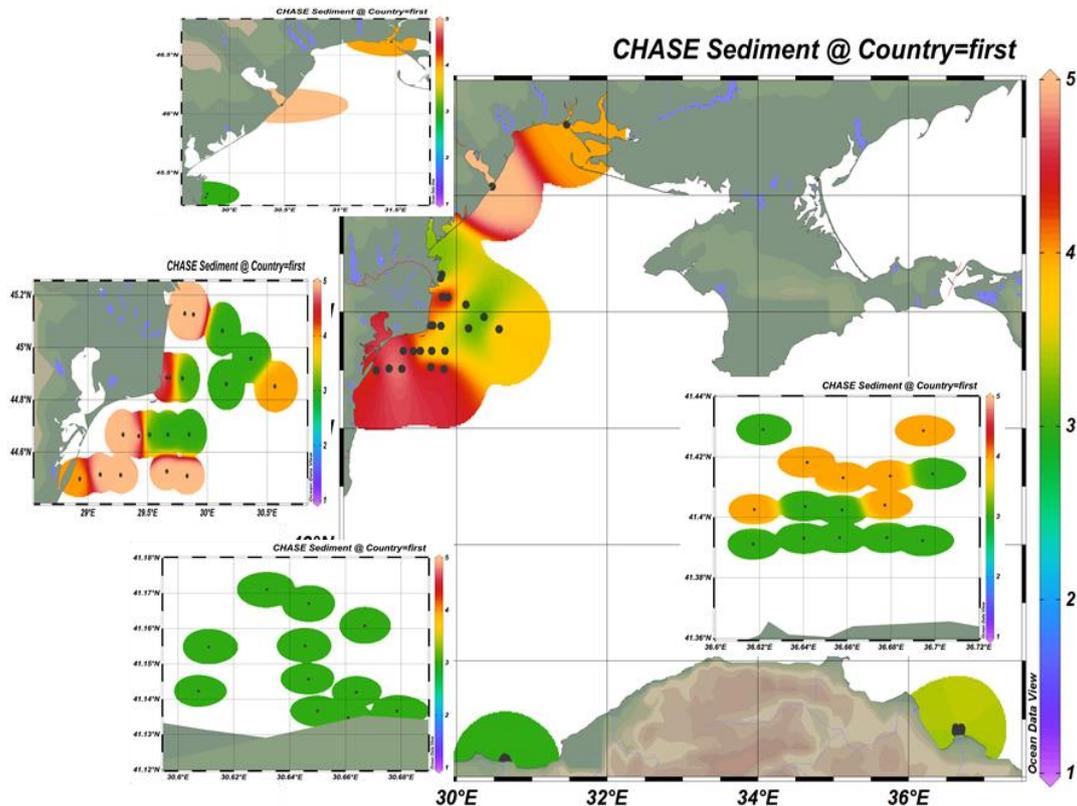


Figure 5.9 - Sediment CHASE results in the rivers influenced area of the northwestern, western, and southern part of the Black Sea

## Conclusions

The integrated assessment tools CHASE makes a clearer image of the pollution level, being more beneficial for the coastal managers.

Even though there are many differences between areas regarding indicator substances or threshold values used in assessment, the Black Sea quality is better in the southern part where the status was generally moderate, comparative with the other areas which were in bad status.

A commonly agreed set of indicators and threshold will give a better understanding of the pressures of the Black Sea.

## 5.3 NEAT

Human-induced pressures on the marine environment may also affect human well-being and economic services, such as food production and nutrient cycling (Costanza et al., 1997; Pavlidou et al., 2019). Ecosystem-Based Management (EBM) framework for human activities in the marine environment, such as Marine Strategy Framework Directive (MSFD) and as adopted by the Regional Seas Conventions, provide a coherent approach for use and management of marine and coastal resources (Pavlidou et al., 2019). This policy aims to implement an integrated approach to manage pressure activities and to achieve Good Environmental Status (GES) in the marine environment.

Lately, there are few methods efficiently used to assess environmental status in an integrative way. Assessment tools such as Ocean Health Index (OHI; Halpern et al., 2012), HELCOM Eutrophication Assessment Tool (HEAT; HELCOM, 2014), Black Sea Eutrophication Assessment Tool (BEAST; Baltic2Black Project, 2010), and an innovative method, recently developed, is the Nested Environmental Status Assessment Tool (NEAT) (Borja et al., 2016).

The NEAT software is a flexible and user-friendly desktop application implementing the biodiversity assessment tool developed as an output of the DEVOTES project <http://www.devotes-project.eu> (NEAT User Manual, Vers. 1.4). The used method is hierarchical consisting of a nested structure of spatial assessment units and habitats. It runs several steps to make an ecosystem-based assessment. These are:

The order of these hierarchies is such that the assessment begins with the nested SAUs. (e.g., a regional sea or an individual bay)

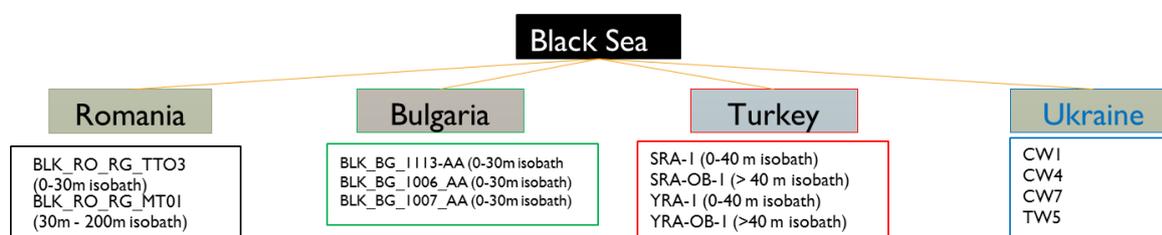
Assign habitats and ecosystem components that are associated with indicators.

Select indicators. Each indicator requires a “bad”, “high” and a “target” value. Also, reference values should be entered in five quality classes (high, good, moderate, poor and bad).

Weighting procedure can be applied to ensure no individual branch (SAUs, habitats, etc.) dominates the quality of the others.

NEAT value is the weighted average of all indicators belonging to a specific group with an uncertainty propagation.

In this study, we have applied NEAT to river interaction areas for assessing the environmental status of fourteen Black Sea spatial units (Figure 5.10) and test the assessment tool’s performance. Coastal areas were identified as spatial units of 0-30 m depth interval by Romania, Bulgaria and Ukraine whereas it was identified as 0 - 40 m by Turkey. Areas deeper than the coastal boundary isobaths was considered as marine areas (30-200 m for Romania, 40-230 m for Turkey).



**Figure 5.10 - Spatial Assessment Units identified by Romania, Bulgaria, Turkey and Ukraine**

Spatial units of Romania were the largest having 90% of all, others shared the rest 10 %. Turkish SAUs were the smallest having both coastal and marine environment based on the depth isobaths (Table 5.10, Figure 5.11).

**Table 5.10 - Areal distribution of SAUs of RO, BG, TR, UA assigned in ANEMONE**

Spatial Assessment Unit (SAU)	Area (km <sup>2</sup> )
BLK_RO_RG_TTO3 (0-30m isobath)	1359
BLK_RO_RG_CT (0-30m isobath)	1041
BLK_RO_RG_MT01 (30m isobath - 200m isobath)	20165
BLK_BG_1113-AA (0-30m isobath)	269
BLK_BG_1006_AA (0-30m isobath)	195
BLK_BG_1007_AA (0-30m isobath)	235
SRA-1 (0-40 m isobath)	16.72
SRA-OB-1 (> 40 m isobath)	15.22
YRA-1 (0-40 m isobath)	16.73
YRA-OB-1 (>40 m isobath)	41.1
CW1	29,6
CW4	47.1
CW7	1025.4
TW5	468.5

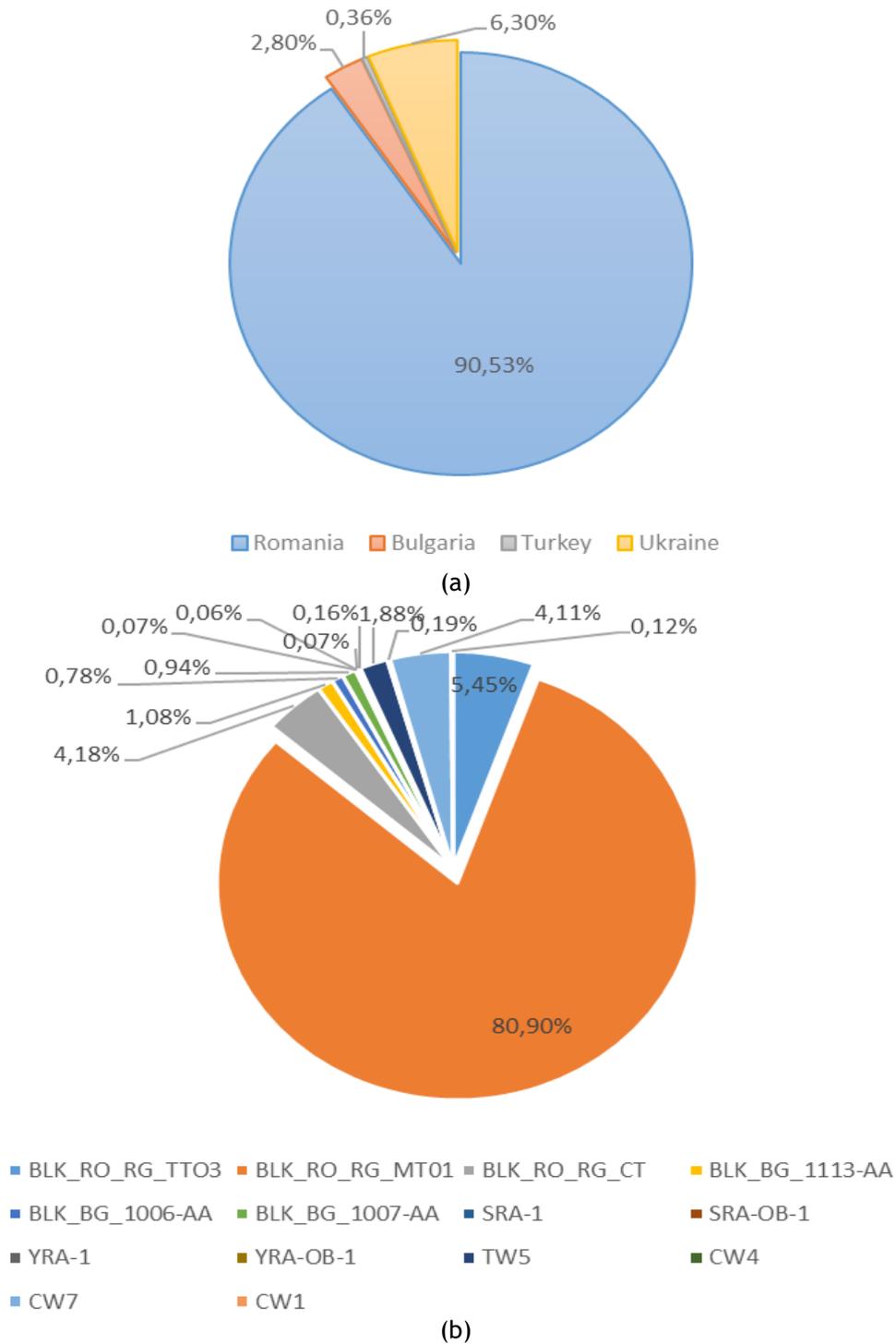


Figure 5.11 - River interaction SAU distributions (%) (a) by country, (b) by area

In the NEAT software, after the identification of SAUs and the assignment of their related habitats and ecosystem elements, indicators have to be selected. In this respect, three major habitats; rocky and sedimentary for benthic habitats and pelagic habitats were included in the ANEMONE-NEAT test. Rocky habitats were split into two groups whereas sedimentary had six sub-groups. Ecosystem components were defined in five major groups; only contaminants as one of the major components were grouped in three as contaminants in biota, sediments and water (Figure 5.12).

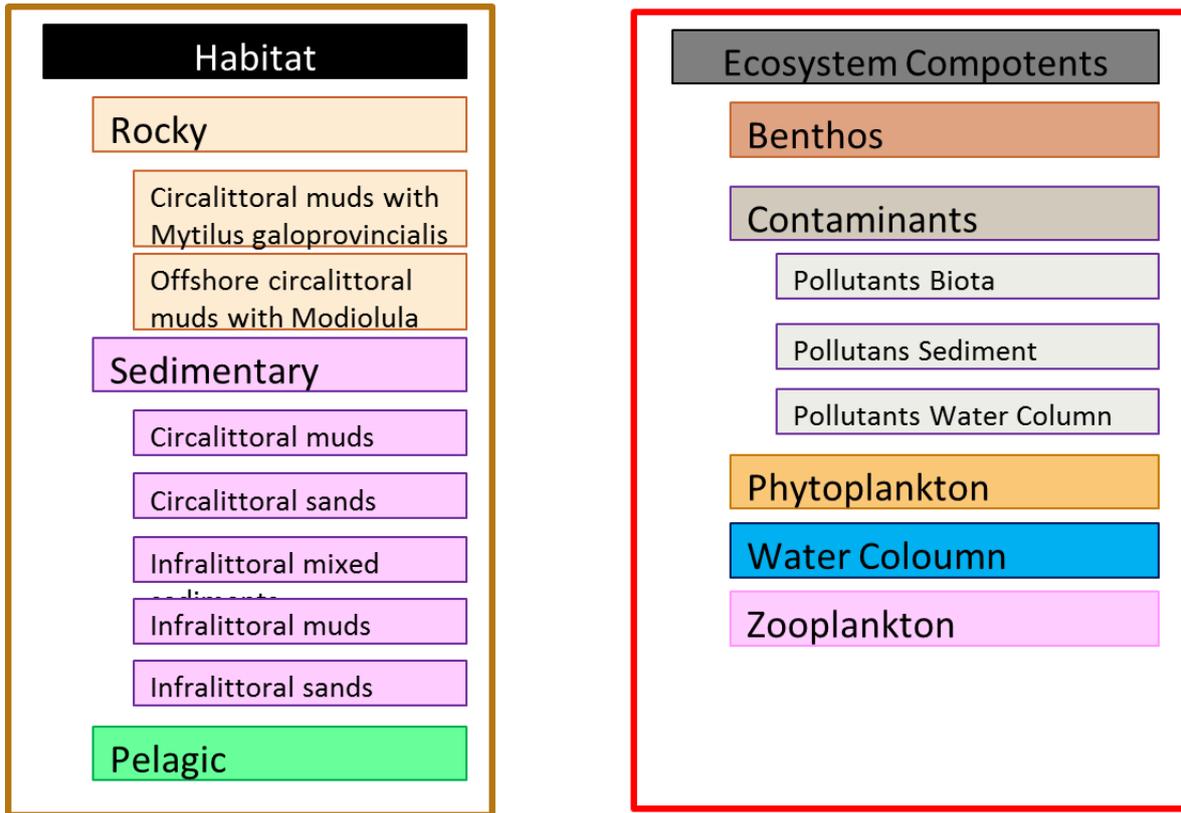


Figure 5.12 - Major and sub-grouping of habitats and ecosystem components used in ANEMONE-NEAT test

Figure 5.13 shows a possible set of indicators (as measured variables or calculated values and indices) referred by each country, including sampling periods and ecosystem components. While putting the identified indicators into the NEAT software it is also necessary to put boundary values of “high”, “moderate” and “bad” status for each.

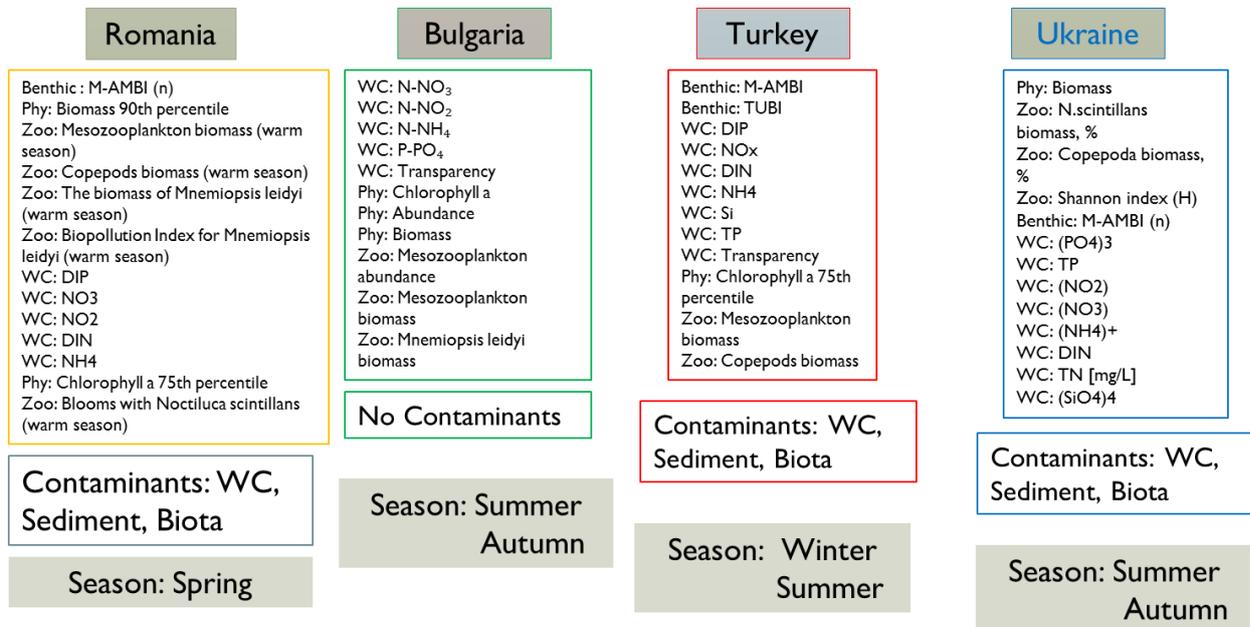


Figure 5.13 - Measured variables/assigned indicators, sampling periods and ecosystem components referred by each country (Phy: Phytoplankton, Zoo: Zooplankton, WC: Water Column)

NEAT final assessment is done in 5-classes as presented in Table 5.11 (Torsten et al., 2019). In the final assessment, it is possible to choose a weighted/not-weighted approach for different scales of SAUs or even independent of the SAU size, to include different MSFD GES descriptors and the required habitat types (Torsten et al., 2019).

**Table 5.11 - NEAT Classification scale.**

Boundary values	Color code
0.8-1.0	High
0.6-0.8	Good
0.4-0.6	Moderate
0.2-0.4	Poor
0.0-0.2	Bad

In this study, two scenarios were tested for the assessment of river-sea interaction sites: (i) all parameters' data for all seasons and (ii) all parameters' data but only for common seasons. If it was required to work only with common parameters, it would ultimately be an eutrophication assessment which was not aimed in the scope of this study.

NEAT analysis was performed both with weighted and not-weighted approaches for SAUs. Mostly the results were obtained as good/high in both scenario (Table 5.12 - Table 5.15) where there were no distinct differences in the NEAT values. This might be mainly related to the good/high results obtained for all components of the contaminants.

When the analysis is only focused on eutrophication indicators, then the status of SAUs of Turkey and Ukraine declines to moderate. However, the overall NEAT score was still good since no matter weighted or not weighted by SAUs. This is because Romania is much bigger than the others.

The results were found highly confident except for two areas in BG waters (BLK\_BG\_1113-AA and BLK\_BG\_1006-AA) which was 58% confident exhibiting moderate/good status. The reason is the high deviation among the measured values and the area was represented with a limited number of indicators.

**Table 5.12 - NEAT results of Scenario 1 (all indicators & all sampling periods) with weighting according to SAUs**

SAU	NEAT value	Confidence	WC	Phyto	Zoo	Benthos	Pollutant WC	Pollutants Sed.	Pollutants Biota
DT2.1_River_BS	0.737	100	0.539	0.635	0.748	0.685	0.739	0.859	0.847
Romania	0.733	100	0.435	0.626	0.739	0.686	0.734	0.870	0.848
BLK_RO_RG_TTO3	0.701	100	0.351	0.686	0.521	0.722	0.730	0.796	
BLK_RO_RG_MT01	0.747	100	0.445	0.821	0.752	0.682	0.734	0.876	0.848
BLK_RO_RG_CT	0.504	100		0.305		0.702			
Bulgaria	0.751	100	0.783	0.719	0.727				
BLK_BG_1113-AA	0.613	58,3	0.670	0.573	0.559				
BLK_BG_1006-AA	0.806	52,2	0.838	0.719	0.859				
BLK_BG_1007-AA	0.862	99,7	0.862	0.885	0.840				
Turkey	0.708	100	0.617	0.797	0.370	0.480	0.958	0.798	0.960
SRA-1	0.675	100	0.517	0.717	0.666	0.340	0.969	0.857	0.954
SRA-OB-1	0.689	100	0.473	0.677	0.620	0.430	0.984	0.864	
YRA-1	0.704	100	0.539	0.710	0.681	0.535	0.948	0.801	0.970
YRA-OB-1	0.730	100	0.737	0.901	0.048	0.532	0.946	0.756	
Ukraine	0.779	99,9	0.604	0.195	0.857		0.841	0.800	0.775
TW5	0.759	99,9	0.720	0.195	0.879		0.753	0.767	
CW4	0.811	61,2	0.721		0.897		0.784	0.843	
CW7	0.787	97,6	0.492		0.837		0.856	0.813	
CW1	0.780	79,3					0.825	0.775	

**Table 5.13 - NEAT results of Scenario 1 (all indicators & all sampling periods) with the not weighted approach**

SAU	NEAT value	Confidence	WC	Phyto	Zoo	Benthos	Pollutant WC	Pollutants Sed.	Pollutants Biota
DT2.1_River_BS	0.724	100	0.697	0.593	0.744	0.597	0.838	0.815	0.797
Romania	0.651	100	0.385	0.367	0.644	0.703	0.732	0.832	0.848
BLK_RO_RG_TTO3	0.701	100	0.351	0.686	0.521	0.722	0.730	0.796	
BLK_RO_RG_MT01	0.747	100	0.445	0.821	0.752	0.682	0.734	0.876	0.848
BLK_RO_RG_CT	0.504	100		0.305		0.702			
Bulgaria	0.761	99,4	0.792	0.725	0.742				
BLK_BG_1113-AA	0.613	56,2	0.670	0.573	0.559				
BLK_BG_1006-AA	0.806	52	0.838	0.719	0.859				
BLK_BG_1007-AA	0.862	100	0.862	0.885	0.840				
Turkey	0.699	100	0.569	0.755	0.496	0.460	0.963	0.817	0.960
SRA-1	0.675	100	0.517	0.717	0.666	0.340	0.969	0.857	0.954
SRA-OB-1	0.689	100	0.473	0.677	0.620	0.430	0.984	0.864	
YRA-1	0.704	100	0.539	0.710	0.681	0.535	0.948	0.801	0.970
YRA-OB-1	0.730	100	0.737	0.901	0.048	0.532	0.946	0.756	
Ukraine	0.784	100	0.661	0.195	0.871		0.810	0.808	0.775
TW5	0.759	99,9	0.720	0.195	0.879		0.753	0.767	
CW4	0.811	60	0.721		0.897		0.784	0.843	
CW7	0.787	97	0.492		0.837		0.856	0.813	
CW1	0.780	81,5						0.825	0.775

**Table 5.14 - NEAT results of Scenario 2 (all indicators & common seasons) with weighting according to SAUs**

SAU	NEAT value	Confidence	WC	Phyto	Zoo	Benthos	Pollutant WC	Pollutants Sed.	Pollutants Biota
DT2.1_River_BS	0.736	100	0.549	0.633	0.753	0.685	0.736	0.856	0.848
Romania	0.733	100	0.435	0.626	0.739	0.686	0.734	0.870	0.848
BLK_RO_RG_TTO3	0.701	100	0.351	0.686	0.521	0.722	0.730	0.796	
BLK_RO_RG_MT01	0.747	100	0.445	0.821	0.752	0.682	0.734	0.876	0.848
BLK_RO_RG_CT	0.504	100		0.305		0.702			
Bulgaria	0.751	99,9	0.783	0.719	0.727				
BLK_BG_1113-AA	0.613	56	0.670	0.573	0.559				
BLK_BG_1006-AA	0.806	51,9	0.838	0.719	0.859				
BLK_BG_1007-AA	0.862	99,8	0.862	0.885	0.840				
Turkey	0.713	100	0.501	0.721	0.370	0.480	0.957	0.798	0.960
SRA-1	0.699	100	0.462	0.630	0.666	0.340	0.968	0.857	0.954
SRA-OB-1	0.716	100	0.423	0.622	0.620	0.430	0.984	0.864	
YRA-1	0.721	99,9	0.426	0.565	0.681	0.535	0.947	0.801	0.970
YRA-OB-1	0.715	100	0.570	0.852	0.048	0.532	0.945	0.756	
Ukraine	0.776	99,5	0.700	0.195	0.859		0.766	0.789	
TW5	0.749	99,5	0.701	0.195	0.876		0.634	0.804	
CW4	0.819	79	0.459		0.824		0.860	0.858	
CW7	0.787	88,8	0.711		0.854		0.785	0.779	

In this study, two scenarios were tested. Final assessment results of NEAT for two scenarios are given in the Table 5.16. The NEAT results showed that if one area is much larger than the others, the larger areas' parameters are weighted more to the results. In addition, when some of the selected indicators are in good and very good status, it raises NEAT results to a higher quality class than they should be. Thus, these effects were hidden the true NEAT results.

**Table 5.15 - NEAT results of Scenario 2 (all indicators & common seasons) with the not weighted approach**

SAU	NEAT value	Confidence	WC	Phyto	Zoo	Benthos	Pollutant WC	Pollutants Sed.	Pollutants Biota
<b>DT2.1_River_BS</b>	0.727	100	0.691	0.580	0.750	0.597	0.845	0.817	0.904
Romania	0.651	100	0.385	0.367	0.644	0.703	0.732	0.832	0.848
BLK_RO_RG_TTO3	0.701	100	0.351	0.686	0.521	0.722	0.730	0.796	
BLK_RO_RG_MT01	0.747	100	0.445	0.821	0.752	0.682	0.734	0.876	0.848
BLK_RO_RG_CT	0.504	100		0.305		0.702			
Bulgaria	0.761	99,1	0.792	0.725	0.742				
BLK_BG_1113-AA	0.613	58,1	0.670	0.573	0.559				
BLK_BG_1006-AA	0.806	52,9	0.838	0.719	0.859				
BLK_BG_1007-AA	0.862	100	0.862	0.885	0.840				
Turkey	0.713	100	0.473	0.669	0.496	0.460	0.962	0.817	0.960
SRA-1	0.699	100	0.462	0.630	0.666	0.340	0.968	0.857	0.954
SRA-OB-1	0.716	100	0.423	0.622	0.620	0.430	0.984	0.864	
YRA-1	0.721	100	0.426	0.565	0.681	0.535	0.947	0.801	0.970
YRA-OB-1	0.715	100	0.570	0.852	0.048	0.532	0.945	0.756	
Ukraine	0.785	99,5	0.648	0.195	0.858		0.801	0.813	
TW5	0.749	99,4	0.701	0.195	0.876		0.634	0.804	
CW4	0.819	77,4	0.459		0.824		0.860	0.858	
CW7	0.787	87,8	0.711		0.854		0.785	0.779	

**Table 5.16 - Assessment of both scenarios**

SAU	WSAU		DNWSAU	
	S1	S2	S1	S2
<b>BS : ALL SAU</b>	0.737	0.736	0.724	0.727
Romania	0.733	0.733	0.651	0.651
BLK_RO_RG_TTO3	0.701	0.701	0.701	0.701
BLK_RO_RG_MT01	0.747	0.747	0.747	0.747
BLK_RO_RG_CT	0.504	0.504	0.504	0.504
Bulgaria	0.751	0.751	0.761	0.761
BLK_BG_1113-AA	0.613	0.613	0.613	0.613
BLK_BG_1006-AA	0.806	0.806	0.806	0.806
BLK_BG_1007-AA	0.862	0.862	0.862	0.862
Turkey	0.708	0.713	0.699	0.713
SRA-1	0.675	0.699	0.675	0.699
SRA-OB-1	0.689	0.716	0.689	0.716
YRA-1	0.704	0.721	0.704	0.721
YRA-OB-1	0.730	0.715	0.730	0.715
Ukraine	0.779	0.776	0.784	0.785
TW5	0.759	0.749	0.759	0.749
CW4	0.811	0.819	0.811	0.819
CW7	0.787	0.787	0.787	0.787
CW1	0.780		0.780	

The existing practice showed us that NEAT is a strong ecosystem assessment tool. However, the assessment units, habitats and indicators including the sampling seasons need to be designed from the beginning for more reliable NEAT results. For example, the SAUs could be comparable in the area (km<sup>2</sup>) and the same indicators even with larger number could be used for the same periods. It could also be suggested that, especially for the large assessment units, a pressure analysis can be made and sub- assessment units might be identified.

## 6 Conclusions and Recommendations

In ANEMONE, we performed (one of the) first assessments of the pressures and impacts simultaneously for seven rivers from N (Dnieper, southern Bug), NW (Dniester and Danube), W (Kamchia), and S (Sakarya and Yesilirmak) Black Sea.

We aggregated data from four countries (from N to S - Ukraine, Romania, Bulgaria and Turkey), six cruises and a sampling network of 62 stations. The outcomes represent the databases for chemistry - water, sediment and biota, pelagic habitats components - phytoplankton, zooplankton, and benthic habitats - zoobenthos. Apart from the analytical results themselves, new monitoring data regarding the neighbouring area, we applied tools for integrated assessments. Only some were applied before (E-TRIX and BEAST), but others were entirely new for the Black Sea. We learned to apply most of the tools (CHASE, NEAT, Ecolmpact mapper) through workshops organized in the project's lifetime and held by their authors and developers, all from the European area.

Following the River-Sea cruises (2019-2020) in ANEMONE project was identified, using integrated tools, a high risk of eutrophication (BEAST) and chemical contamination (CHASE) in the rivers neighbouring areas. The risk was decreasing from N-NW (southern Bug, Dnieper, Dniester, Danube) to W (Kamchia) and slightly increased again in S (Sakarya and Yesilirmak) and was not particularly correlated with the average rivers' flows but more with the basin's area and activities.

The dominant land use in the basin is agricultural with 65 % of coverage according to MODIS Land Cover (NASA, 2001) and the greatest sources of diffuse pollution are agricultural, and households not connected to sewer systems (European Environmental Agency (EEA), 2010) (Rouholahnejad et al., 2014). Thus, the introduction of nutrients from the upstream watershed is a significant issue in the studied area, mainly in the N-NW and S Black Sea.

The problems are caused by a complex chain of events and vary from site to site, but the fundamental driving force is the accumulation of nitrogen and phosphorus in freshwater on its way to the sea. For instance, runoff from agricultural land, animal feeding operations, and urban areas plus discharge from wastewater treatment plants and atmospheric deposition of compounds released during fossil-fuel combustion all add nutrients to freshwater before it reaches the sea (National Research Council, 2000).

The nutrient inputs to an estuary are essential knowledge for nutrient over-enrichment management, and nutrient budgets should be prepared, including the atmospheric deposition. Perhaps the greatest uncertainty with estuary nitrogen budgets concerns the contribution of atmospheric deposition (National Research Council, 2000), which was completely unnoticed in our study. Available evidence (although constrained by limited monitoring) indicates that direct deposition onto the water surface alone (not including the contribution of nitrogen which falls on the landscape and is then exported to estuaries) contributes between 1 % and 40 % of the total nitrogen input to an estuary—depending in large part on the relative area of the estuary and its watershed (Nixon et al., 1996; Valigura et al., 2001). In estuaries where the ratio of the area of the estuary to the area of its watershed is greater than 0.2, direct atmospheric depositions usually make up 20 % or more of the total nitrogen loading (Valigura et al., 2001). Where the ratio of the estuarine area to the area of its watershed is less than 0.1, atmospheric deposition directly onto the water surface generally makes up less than 10 % of the total nitrogen input (Valigura et al. 2001). For estuaries that have relatively large watersheds, the deposition of nitrogen from the atmosphere onto the landscape with subsequent runoff into the estuary is probably greater than the deposition of nitrogen directly onto the water surface. Unfortunately, the magnitude of this flux is poorly characterized for most estuaries, including the Black Sea. In addition to receiving nutrient inputs from land and atmospheric deposition, estuaries can receive nutrients across their boundary with the ocean. This term is often ignored but can be substantial (Nixon et al., 1996) due to the physical circulation pattern of an estuary which is a major determinant in the importance of nutrient import to the estuary from offshore sources.

The atmospheric transport of chemicals to the ocean has been investigated for over a century. With time, was found that the atmosphere is a critical source of nutrients, toxins and acids. It was also found that there no region of the oceans escapes the influence of human action and that this influence will increase in the future as both the human population and the per capita use of resources continue to grow (Duce et. al, 2009).

The deposition onto the landscape can be estimated for most watersheds, although the error associated with these estimates can be considerable due to inadequate monitoring and the difficulty with measuring dry deposition. The larger problem, however, is with determining what portion of the nitrogen deposition is retained in the landscape and what portion is exported to rivers and the coast.

The two major approaches for making this determination are to use statistical models or to use process-based models on nitrogen retention in the watershed. In their application to estuaries, both approaches are quite recent and are relatively untested. There is an urgent need for further development and evaluation of these techniques; however, it appears that the statistical approaches have led to more reliable estimates (National Research Council, 2000).

In our study, the nutrients and contaminants enrichment led to “moderate”- “poor”-“bad” status in most of the areas. The integrated tool's results might be used as governance performance indicators - evaluating the success of policies developed to effectively manage the coastal and marine environment. For example, the Danube's Mouths are classified as potential problem areas which represents an encouraging case for the Black Sea waters quality improvement. Implementing the Danube basin's program of measures (ICPDR) (e.g., TransNational Monitoring Network (TNMN), phosphate detergents ban) might lead to the improvement of the Black Sea waters quality in other rivers catchments.

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# 7 ANNEXES

## ANNEX A Network stations and cruises

### Ukraine

During the study, we observed three areas of the Black Sea shelf - Zernov Phyllophora field, which lies in the region of mixed waters but is influenced by the waters of the Dnieper-Bug River system, the surroundings of Zmeinyj island, which belongs to the Danube region, and Odessa damping area, which is close to Odessa Bay and belongs to Dnieper-Bug region. For the study of coastal waters, we observed the coastal areas of the Danube region (the exits of Kiliya arm and Bustryj arm), the waters of Zatoka (the exit of the Dniester estuary), and the waters near Ochakov (the exit of Dnipro-Buh estuary) (Figure 7.1). For the report, we used the materials of ANEMONE project, and national monitoring of UkrSCES.

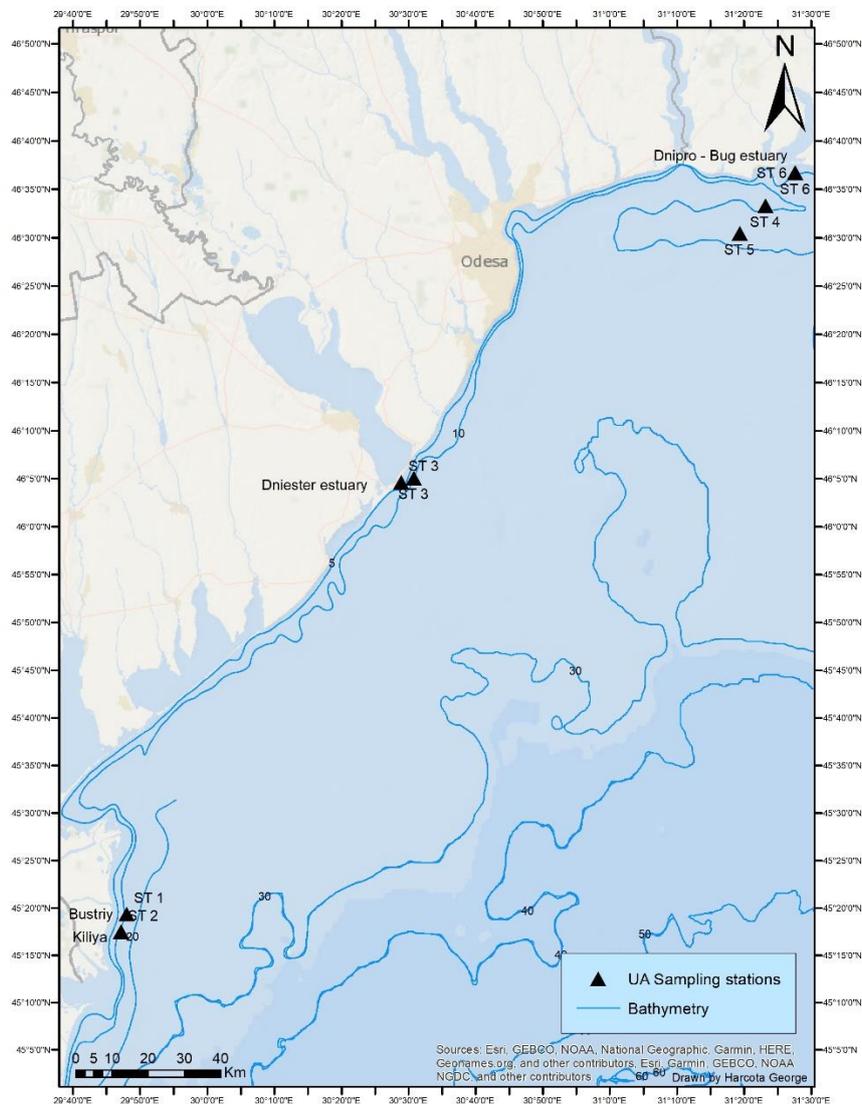


Figure 7.1 - Ukraine sampling stations

## Romania

A cruise was carried out in front of the Danube's river on board of R/V "Steaua de Mare" on 10-15 May 2019. 19 stations were sampled for water, sediment and biota (Figure 7.2).

Temperature and salinity were measured with the CTD CastAway.

The water samples were taken with a bucket from the surface. The dissolved oxygen and BOD<sub>5</sub> were sampled in special bottles, Winkler. The dissolved oxygen was fixed with specific reagents.

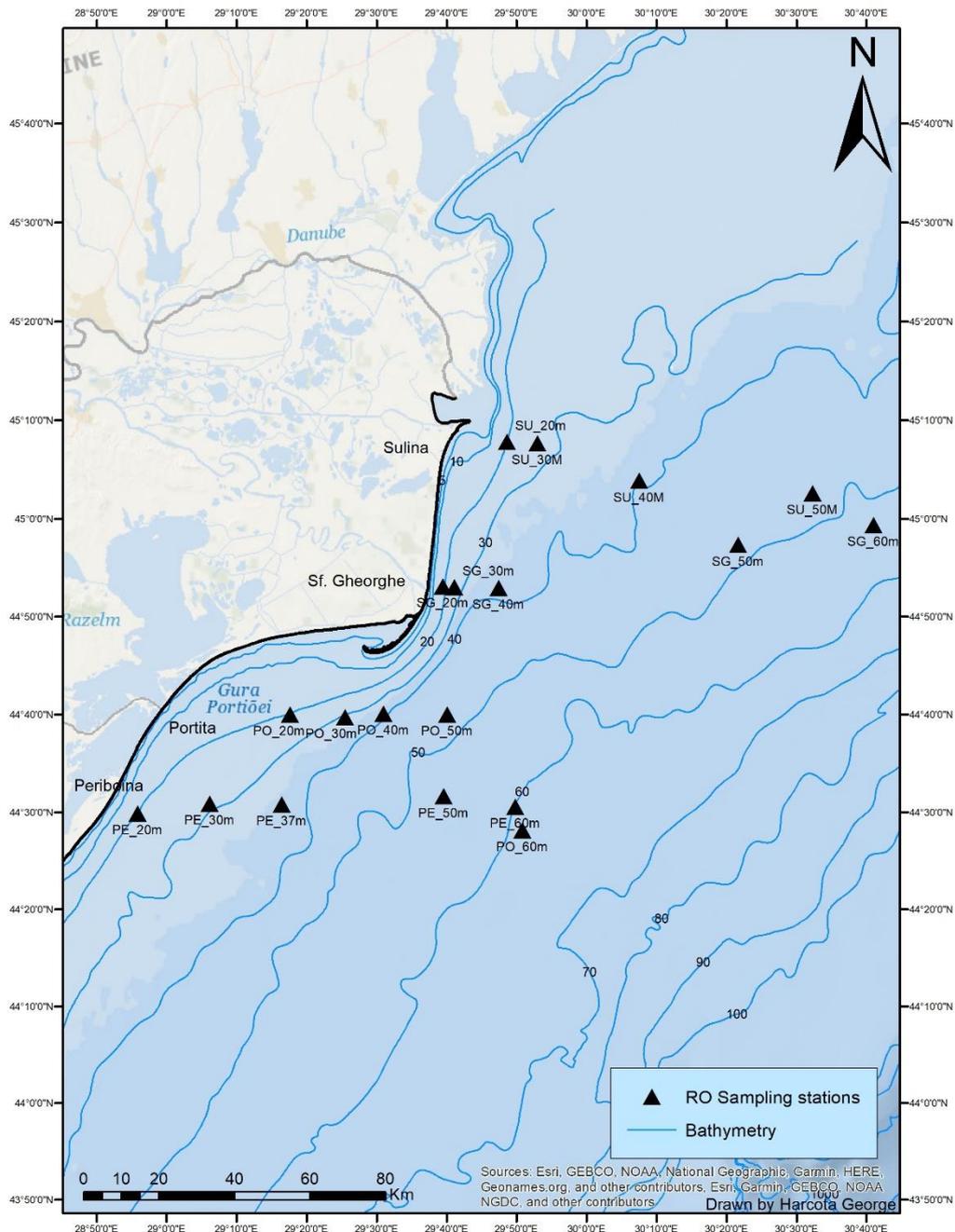


Figure 7.2 - Romania sampling stations

## Turkey

The national expedition activities were conducted in July 2019 and January 2020 onboard the R/V “TÜBİTAK Marmara”.

The seawater samples were collected from 20 stations between 6 - 140 m depths (the YSL01, YSL04, YSL05, YSL07, YSL08, YSL09, YSL10, YSL11, YSL12, YSL14 stations from the Yeşilırmak river mouth and SAK01, SAK03, SAK04, SAK07, SAK08, SAK09, SAK10, SAK11, SAK12 and SAK15 stations from the Sakarya river mouth).

The sediment samples were collected using Van Veen grab, from 27 stations between 6 - 152 m depths (the YSL01, YSL02, YSL03, YSL04, YSL05, YSL06, YSL07, YSL08, YSL09, YSL11, YSL12, YSL13, YSL14, YSL15 and YSL16 stations from the Yeşilırmak river mouth and SAK01, SAK02, SAK06, SAK07, SAK08, SAK09, SAK10, SAK11, SAK12, SAK14, SAK15 and SAK16 stations from the Sakarya river mouth). The upper 2-3 cm of sediment was subsampled with a polyethylene spoon. The subsamples were kept both in clean polyethylene bags for grain size analysis and in glass jars for TOC and metal analysis. Glass bottles containing sediments were freeze-dried and then well homogenized, coarse fragments (> 0.5 mm) were removed by sieving.

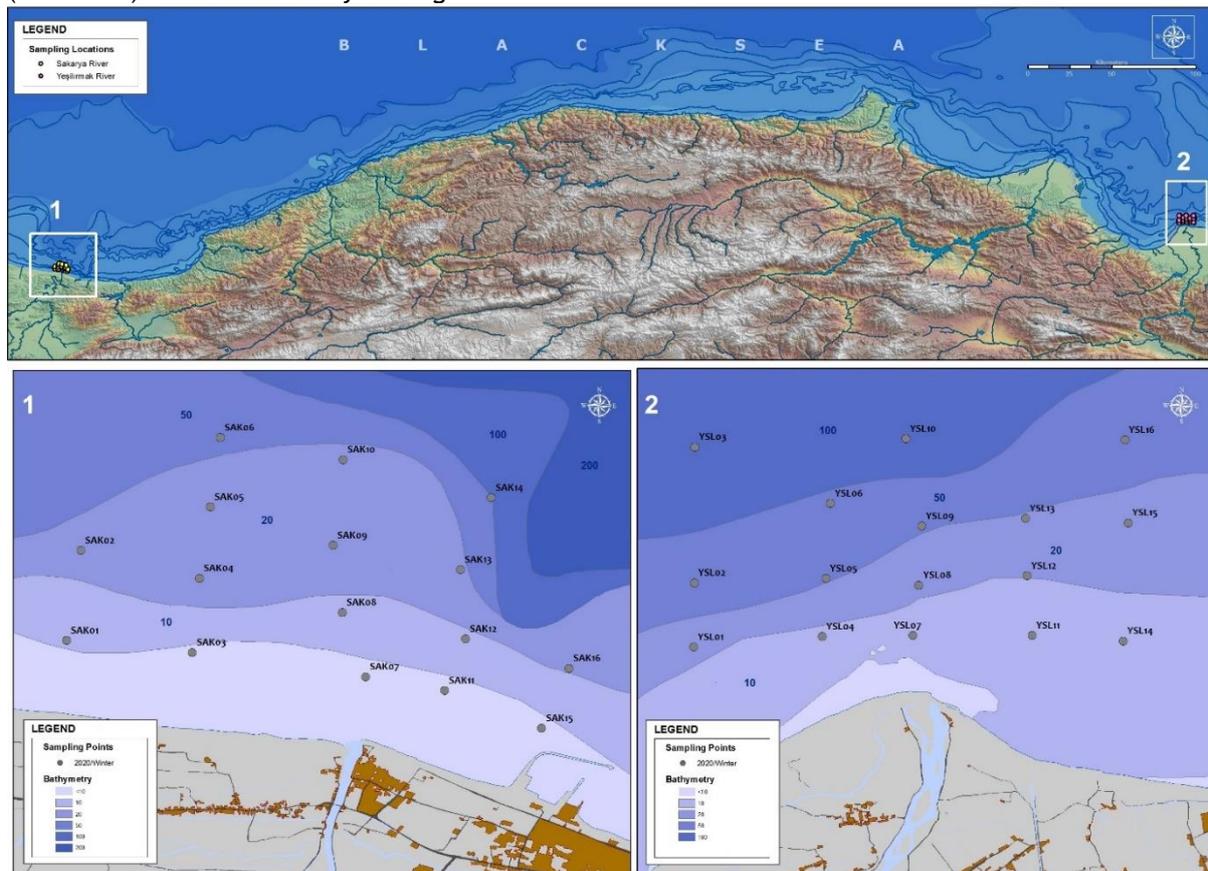


Figure 7.3 - Map of sampling stations for Sakarya river and Yeşilırmak river, July 2019 and January 2020

# Biology

## Phytoplankton

### Ukraine

Phytoplankton samples were collected by vertical series consisting of several sampling depths. The depths were chosen with the idea to collect material from main hydrophysical layers. On the shelf, the samples were collected from upper mixed layer, upper thermocline layer, lower thermocline layer, near bottom layer and chlorophyll maximum (if this maximum did not coincide with other sampling layers). In the coastal waters with the depth less than 3 m, we collected only surface water. For collecting were used 5L Niskin bottles, attached to CTD rosette system. Each sample consisted of 1-2 L of water, fixed with 40% buffered formaldehyde up to the final concentration of 2% in a sample and carried to the laboratory. Then phytoplankton cells were allowed to settle for 2 weeks and after that, the samples were slowly decanted to 30-40 ml. Shelf and open sea samples were concentrated on board by the funnel of inverted filtration to the volume of 50-100 ml and then also fixed with 40% buffered formaldehyde up to the final concentration of 2% in a sample. Before counting, the concentrated samples were concentrated one more time, down to 10-20 cm<sup>3</sup> by slow decantation. Identification of species and counting of cells were carried out under a light microscope LOMO (Russia) with magnifications of 600x in the drop with the volume of 0.05 ml. The wet biomass was calculated by the method of geometric similarity, equating shapes of cells to corresponding geometrical shapes and assuming that the cell density is equal to 1. For species identification there were used the appropriate key-books: Schiller (1937), Kiselew (1950), Proshkina-Lavrenko (1955), Tsarenko (1990), Carmelo (1997), Steidinger and Tangen (1997), Cronberg and Annadotter (2006), Krakhmalny (2011), and the taxonomic nomenclature is according to the on-line database of World Register of Marine Species (WORMS).

### Romania

A total of 45 samples have been collected along 3 transects (Sulina, Sf. Gheorghe and Portița) from 14 stations distributed over the pelagic habitats of Romanian waters. Samples from the surface were collected with a bucket. Samples from different depths (10-60 m) were collected with 5l Teflon Niskin bottles. The sampling depths were selected according to the CTD profile and the in-situ readings: surface, temperature/salinity gradient (thermocline) and 1 m above the station depth. Taxonomic composition and cell counts were done under inverted microscope connected to a video-interactive image analysis system at 400x magnification by the Utermöhl (1958) method and counting chambers (Utermöhl chambers). The individual cell biovolume ( $V$ ,  $\mu\text{m}^3$ ) was derived by measurements through the approximation of the cell shape of each species to the most similar regular solid, calculated by the respective formulas used routinely in the lab. Cell bio-volume was converted to weight ( $W$ , ng) following Hatchinson (1967). Species identification was mainly after Schiller (1937), Kiselew (1950), Proshkina-Lavrenko (1955), Carmelo (1997), Fukuyo (2000) and the taxonomic nomenclature according to the on-line database of World Register of Marine Species (WoRMS).

## Zooplankton

### Ukraine

Zooplankton was collected with Juday plankton net (0.1 m<sup>2</sup> opening, 150  $\mu\text{m}$  mesh size). In the shallow area samples were taken from the bottom to surface and in more deeper places, samples were collected from the upper mixed layer, thermocline layer and under the thermocline. Zooplankton samples were preserved using 4% formaldehyde buffered to pH 8.0-8.2 with disodiumtetraborate (borax) ( $\text{Na}_2\text{B}_4\text{O}_3 \cdot 10 \text{H}_2\text{O}$ ) formalin solution (1 part 40% formaldehyde solution and 9 parts water - sample) and stored in plastic containers. In the laboratory, the samples were concentrated to 100-200 ml and processed samples according to standard methodology (Alexandrov, 2016). A Bogorov's chamber was used for quantitative assessment (abundance and biomass calculation, using species individual weight) and qualitative (taxonomic structure) processing of samples. For species identification there were used the appropriate key-books: Mordukhai - Boltovskoy (1968), Mordukhai - Boltovskoy (1969), Mordukhai - Boltovskoy (1972), Murina V.V. (2005),

Alekseeva V.R., Tsalokhina S.Ya. (2010), and the taxonomic nomenclature is according to the on-line database of World Register of Marine Species (WORMS). The biomass was calculated according to standard weights and according to allometric equation of length (Alimov, 1989).

## Romania

To analyse the microzooplankton component, particularly the loricate ciliate community, the samples were taken from the 0 m and 10 m layers. Samples were collected in 500 ml labelled plastic containers, from Niskin bottles and preserved with formalin 4%. In the laboratory, the samples were concentrated to a final volume of 10 ml by repeated sedimentation. The final volume was analysed by the inverted microscope (Olympus XI 51) with magnification factors of 200× and 400×. The taxonomic identification of tintinnids was made according to the shape and dimensions of the lorica, indicated by literature. For qualitative and quantitative analysis, both empty tintinnids and those with protoplasm were considered because mechanical and chemical disturbances associated with collection and fixation procedures have been demonstrated to cause cell detachment (Thompson & Alder, 2005). The density of organisms was expressed as individual species/liter (ind./L). The lorica volume was calculated according to the total length and aboral diameter of the lorica, and to the geometric form assumed for each species, respectively. Biomass was expressed as carbon biomass ( $\mu\text{gC/L}$ ) using the specific biovolume conversion formula for formalin conserved biological material (Verity & Langdon, 1984).

Mesozooplankton samples (N=33) were collected from the northern area of the Romanian Black Sea coast (Sulina, Sf. Gheorghe and Portita profiles) from different layers, in May 2019, onboard of RV Steaua de Mare 1. The samples were collected using a Juday net (0.1 m<sup>2</sup> mouth opening area, 150  $\mu\text{m}$  mesh size) by vertical hauls. The samples were stored in 500 ml plastic jars and preserved with 4% buffered formaldehyde solution and were further analysed under the binocular magnifying glass. According to the methodology, the sample was homogenised, and quantitative and qualitative processing was performed in the Bogorov chamber. In the subsample(s) all plankters were counted until each of the three dominant taxonomic groups reached 100 individuals. For estimation of large animals' numbers, the whole sample was observed. All species were identified taxonomically to the species level except of the meroplankton larvae. The number of individuals and mean individual weights were used for estimating the density as ind./m<sup>3</sup>, respectively the biomasses as mg/m<sup>3</sup> wet weight (Alexandrov et al., 2011).

### Jelly fish

During 12-14.05.2019, an expedition took place on the Black Sea (Danube Rivers) where 7 macrozooplankton samples (N=7) were collected to determine the status of the macrozooplankton population. The macrozooplankton was taken onboard of the research vessel "Steaua de Mare", which allowed the proper and safe handling of the net, but at the same time provided the stability conditions necessary for the analysis of the samples immediately after sampling. Macrozooplankton sampling is performed with a Hansen-type net with a diameter of 70cm and a sieve eye of 300 $\mu\text{m}$ . The biological material is obtained by towing the net vertically in the water mass (from 2m above the seabed to the surface), at a low speed (0.5-1 m/s), to prevent damage to gelatinous organisms or clogging of the net. After collection, the net is washed with a seawater hose to remove organisms or mucus from them.

The organisms in the collecting glass are carefully moved to a bucket and immediately identified, counted, and measured. The big specimens are washed with seawater, above the container in which the sample was extracted from the net. All organisms in the sample are measured (depending on the species: width, aboral length, respectively total length). The measurements shall be carried out using a ruler, by positioning them directly on the laboratory table or graph paper (in the case of large organisms of the species *Aurelia aurita*). In the case of small specimens, a checkered Petri dish, filled with water, in which the bodies are suspended, is used to allow their measurement without the occurrence of body deformation.

The density and wet biomass of gelatinous organisms were expressed as ind./m<sup>3</sup> and mg/m<sup>3</sup>, respectively. The calculation of these parameters was performed according to the recommendations of the Macroplankton (or Gelatinous Plankton) Monitoring Guide<sup>11</sup>.

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<sup>11</sup> Black Sea Monitoring Guidelines Macroplankton (Gelatinous plankton) - <http://emblasproject.org/wp-content/uploads/2017/01/Macroplankton-findraft-March2015-PA3.pdf>.

## Turkey

The sampling of zooplankton was carried out in July 2019 and January 2020 at eight stations in front of Sakarya River and Yeşilirmak River. Zooplankton samples were collected vertically tows using UNESCO WP2 net (mesh size: 200 µm, mouth diameter: 57 cm) from bottom to surface (Table 1). After collection, the zooplankton samples were immediately fixed in a 4% formalin-seawater solution for quantitative and qualitative taxonomic analyses. In the laboratory, two subsamples were taken from a container of known volume using a Stempel pipette (1 ml). Samples were analysed under a stereomicroscope with a zooplankton counting apparatus. Finally, the whole sample was examined for rare organisms and large organisms which were counted and recorded (Postel et al. 2000). The biomass transformations were based on individual wet weights according to Petipa (1957) and Niermann et al. (1995). The abundance and biomass results were given in ind./m<sup>3</sup> and mg/m<sup>3</sup>, respectively. The mean abundance and biomass of the species/groups of mesozooplankton are presented as mean ± standard error of the mean (SE). Taxa of Cladocera, Copepoda, Appendicularia and Chaetognatha were identified at the species level. All other taxa were identified to the phylum, class or order levels. The main references used to identify the major zooplanktonic groups were Bradford-Grieve et al. (1999) and Conway et al. (2003). Systematic classification and the nomenclature of zooplankton species was done according to WoRMS (2020).

To interpret the mesozooplankton quantitative data, the Shannon-Weaver diversity index (H) and the number of species were applied to the species abundance data using PRIMER 5 software.

## Zoobenthos

### Ukraine

The macrozoobenthos sampling followed the protocol described in Todorova & Konsulova, 2005. Thus, all samples have been collected with an “Ocean” Van Veen grab and square frames, washed through a 0.5 mm mesh size sieve, fixed with formaldehyde 4% buffered with seawater and finally stored in plastic jars. In the laboratory, the organisms were identified to the lowest possible taxonomic level. Thus, all macrozoobenthos samples were collected with 0.1 m<sup>2</sup> van Veen grab or benthic frame 0.01 m<sup>2</sup>. The macrozoobenthos sampling followed the protocol described in Todorova & Konsulova, 2005. Thus, all samples have been collected with an “Ocean” Van Veen grab and square frames, washed through a 0.5 mm mesh size sieve, fixed with formaldehyde 4% buffered with seawater and finally stored in plastic jars. In the laboratory, the organisms were identified to the lowest possible taxonomic level.

Assessment of macrozoobenthic communities on the northwestern part of the Black Sea (Ukrainian part) was done based on 8 samples (total 14 subsamples) taken on 8 stations in the 2019 year (Table 7.1)

*Table 7.1 - Ukraine zoobenthos sampling stations*

Type	Station	Name	Date	Sampling device	Replicates	Maximum Depth, m
WP2.3 The central part of NWBS shelf	15	Zmeiny island region	03.09.19	Van Veen grab	0.1	26
				Frame 10*10	0.03	26
	16	Zernov`s Phyllophora Field	31.08.19	Van Veen grab	0.1	24
				Frame 10*10	0.03	24
	17	Zernov`s Phyllophora Field	31.08.19	Van Veen grab	0.1	41
				Frame 10*10	0.03	41
WP2.1 River-Sea border	1	Exit from aim “Bustriy”, Danube aria	11.09.19	Van Veen grab	0.1	9.4
				Frame 10*10	0.03	9
	2	Exit from aim “Kiliya”, Danube aria	12.06.19	Van Veen grab	0.1	9.1
						8.5
	3	Zatoka, exit from Dniester estuary	13.09.19	Van Veen grab	0.1	5
				Frame 10*10	0.03	5
	6	Ochakov, exit from Dnipro-Bug estuary	18.06.19	Van Veen grab	0.1	5.1
				16.09.19	Frame 10*10	0.03

## Romania

The study of Danube's River influence on the marine sector was based on a set of 48 macrozoobenthos samples collected at depths between 20 and 60m. According to the methodology agreed in the Black Sea region (Todorova and Konsulova, 2005), samples on soft bottoms were collected using a Van Veen grab on 0.1m<sup>2</sup>. A pre-washing of the samples through 0.5mm mesh size sieves for sediments excess removal was performed onboard. A macro-visual description of each sample was done before preserving. The preservation was done with formaldehyde 4% buffered with seawater. The samples were stored in labelled plastic containers until their subsequent examination in the laboratory. In the laboratory, the samples were washed again through 1mm and 0.5mm mesh sieve and all the organisms were identified to the lowest taxonomic level.

After the organisms were identified and determined under the stereomicroscope, quantitative parameters were calculated.

Macrozoobenthos data were analysed using abundance, species richness (S - as number of taxa per sample), Shannon's diversity index (H'), AMBI index and its five ecological groups (EG) as single metrics. For this purpose, the AZTI's AMBI software (<http://ambi.azti.es>) was used. As multivariate or multivariate method, M-AMBI\*(n) was applied. Reference conditions for the three M-AMBI \*(n) parameters (AMBI, diversity and richness) were calculated using the 0.95 percentile of richness (S) and diversity (H) values and the 0.05 percentile of AMBI values, using the available data.

Also, to determine the influence of freshwater input on benthic communities, statistical analysis using PRIMER package software v.7 was applied on fourth root transformed abundance data (Clarke et al., 2014).

## Turkey

Soft-bottom samples were collected by a Van Veen Grab (sampling an area of 0.1 m<sup>2</sup>) in July 2019 and January 2020. Subsampling with a tube corer was applied to the retrieved material on board to take meiobenthic samples. Three replicates of sediment were taken from each grab sample. The samples were preserved as soon as possible by using 75% ethanol. The samples brought to the laboratory were washed through sieves of 500 mm, 250 mm and 63-micron mesh sizes. Rose Bengal was used to stain the organic material in the suspended material. Samples were examined under a stereo binocular dissecting microscope using modified Bogorov counting chambers and meiobenthic organisms were counted and sorted into higher systematic groups. All the specimens were stored in 2 ml vials containing 75% ethanol (Giere, 2009).

Meiobenthos analyzes were performed for 6 stations at two different river mouths as Sakarya (SAK07, SAK08, SAK10) and Yeşilirmak (YSL07, YSL08, YSL09). The biotope types determined according to the material obtained from the stations and the information of stations are given in Table 7.2.

*Table 7.2 - Turkey zoobenthos station details*

Station No	Latitude	Longitude	Depth (m)	Biotope
SAK07	41.1364	30.6485	8	Fine sand+Clay
SAK08	41.1460	30.6477	22	Fine sand+Clay
SAK10	41.1671	30.6443	50	Mollusk shells+Fine sand
YSL07	41.3947	36.6565	7.5	Fine sand + Alive bivalves
YSL08	41.4030	36.6588	31	Fine sand+Clay+Phytodetritus
YSL09	41.4123	36.6535	72	Fine sand+Clay+Phytodetritus

Macrozoobenthos sampling was performed at 10 stations in July 2019 and January 2020 in Sakarya River Mouth, Samsun Port, Samsun Wastewater Treatment Plant and Yeşilirmak River Mouth to determine macrozoobenthic species diversity and their abundance and ecological quality of the area. Soft-bottom samples were collected by a Van Veen Grab (sampling an area of 0.1 m<sup>2</sup>) with three replicates. Benthic samples were sieved with a 0.5 mm mesh and the retained fauna were put in jars containing 4% seawater-formalin solution. The samples brought to the laboratory were washed through 1 mm and 0.5 mm sieve mesh sizes. The material obtained was examined under stereo binocular dissecting microscope and zoobenthic organisms were sorted into higher systematic groups. These samples were delivered to the concerned specialists for taxonomic identifications.

Community parameters such as number of species, number of individuals, Shannon-Weaver's diversity index (log<sub>2</sub> base) (H') and Pielou's evenness index (J') were calculated. The Soyer's Frequency Index

(F) was applied to the abundance data to determine the characteristic species of the area. TUBI (Turkish Benthic Index) was calculated to assess the ecological quality status of the stations (Çinar et al. 2015).

## Chemistry - Water

### Ukraine

#### Analytical methods for trace metals

Surface water samples collected for metals analysis were filtered through the membrane with pore size 0,45 µm. Metals dissolved have been determined in seawater samples, acidified up to pH=2 with Ultrapure HNO<sub>3</sub>.

Instrumental analysis and quantification: metals were analysed by electrothermal furnace atomic absorption spectrometry (AAS-ET Analytik Jena AG ZEENIT 650P). Concentration of metals calibration was performed with working standards for each element, starting from stock solutions of 1000 µg/L (Sigma-Aldrich). At least 3 instrumental readings have been performed for each sample, with average value reported. The work domains are as follows: water Cd 0-1 µg/L; other metals 0-40 µg/L;

#### Analytical methods for organic pollutants

Water samples were taken from the surface layer (0,01 m below the surface) from 10-liter Niskin bottles of the Rosette system. For determination of organic pollutants, 5 liters of seawater were poured into a polypropylene tank, which were sent to the laboratory for analysis. Internal standards PCB29 and Phenanthrene-d10 were added to the water sample prior to extraction. Extraction was carried out with hexane using a high-speed stirrer; the organic phase was separated from water in a separatory funnel. The extraction was followed by concentration in a turbo evaporator under nitrogen flow.

Concentration of PAHs was determined by gas chromatography-mass spectrometry on gas chromatograph 7890A (Agilent, USA) with mass-detector 5975C equipped with PTV injection and capillary column DB-5MS (30 m 0.25mm 0.25 µm). The carrier gas was helium at a flow rate of 1,2 ml/min. Injector starting temperature was 50 °C, ventilation of the solvent during 1 minute, volume of the sample was 15 µl, final temperature of injector was 300 °C, rate of temperature elevation was 600 °C; onset temperature of incinerator was 60 °C, hold up time 7 minutes, temperature rise to 200 °C at the rate of 10 °C/min, hold on during 1 minute, temperature rise to 310 °C at the rate of 7 °C/min, hold on during 5 minutes. Mass detector in the mode SIM (search for target weight), temperature MS Source 230 °C, MS Quad 150 °C. Analytical standards of naphthalene, anthracene, fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, benzo(b)fluoranthene, phenanthrene, benzo(a)anthracene, chrysene, fluorene, acenaphthene, pyrene (Supelco, USA), indeno(1,2,3cd)pyrene and dibenzo(a,h)anthracene (ULTRA Scientific, USA) were used for calibration. ChemStation (Agilent, USA) and AMDIS software were used for data analysis.

Concentration of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) was determined on gas chromatograph 7890B (Agilent, USA) with electron capture detector (15 millicuries of nickel 63 G2397A ECD) equipped with split less injector and capillary column HP-5 (30 m 0.32mm 0.25 µm). The carrier gas was helium at a flow rate of 2 ml/min, ECD gas was nitrogen at a flow rate of 30 ml/min, injector temperature 250 °C, volume of the sample was 1 µl; onset temperature of incinerator was 70 °C hold on during 1 minute, temperature rise to 150 °C at the rate of 10 °C/min, hold on during 0 min, temperature rise to 240 °C at the rate of 4 °C/min, hold on during 10 min. Analytical standards of α-HCH, β-HCH, γ-HCH (Sigma-Aldrich, USA), PCBs total AR-1254, PCBs total Ar-1260 (Supelco, USA), PCB-8, PCB-18, PCB-28, PCB-31, PCB-52, PCB-49, PCB-44, PCB-66, PCB-110, PCB-149, PCB-118, PCB-153, PCB-138, PCB-183, PCB-174, PCB-177, PCB-180, PCB-170, PCB-199, PCB-194 (Dr. Ehrenstorfer, Germany), PCB-101 (ULTRA Scientific, USA) were used for the calibration. ChemStation (Agilent, USA) software was used for the data analysis.

### Romania

Water samples were collected from the surface layer (1 m below the surface) from the 5 L Niskin bottles of the Rosette System.

Seawater nutrients were quantified by spectrophotometric analytical methods validated into the laboratory and having reference manual "Methods of Seawater Analysis" (Grasshoff, 1999) with the following detection limits and extended relative uncertainties, coverage factor, 95.45 %:

Parameter	Units	Limit of detection ( $\mu\text{mol}/\text{dm}^3$ )	Extended relative uncertainty, U(c), k=2, coverage factor 95.45%
(NO <sub>3</sub> ) <sup>-</sup>	$\mu\text{M}$	0.12	c x 0.08 $\mu\text{mol}/\text{dm}^3$
(NO <sub>2</sub> ) <sup>-</sup>	$\mu\text{M}$	0.03	c x 0.06 $\mu\text{mol}/\text{dm}^3$
(NH <sub>4</sub> ) <sup>+</sup>	$\mu\text{M}$	0.12	c x 0.10 $\mu\text{mol}/\text{dm}^3$
(PO <sub>4</sub> ) <sup>3-</sup>	$\mu\text{M}$	0.01	c x 0.12 $\mu\text{mol}/\text{dm}^3$

It was used a Shimadzu UV-VIS spectrophotometer, measuring range: 0-1000 nm. Salinity was measured with CTD and dissolved oxygen using the Winkler method.

Total metals (dissolved and acid-soluble suspended forms) have been determined in unfiltered seawater samples, acidified up to pH=2 with Ultrapure HNO<sub>3</sub>.

The analytical determination of the copper, cadmium, lead, nickel, and chromium was carried out by graphite furnace - atomic absorption spectrometry method (GF-AAS).

Seawater (about 1L) was transferred into glass bottles, which were stored at refrigerator temperature until their subsequent analysis in the laboratory. Extraction from the water was done with hexane/dichloromethane (3/1) mixture in separating funnel, purification on florisil column for organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), respectively silica/alumina column for polyaromatic hydrocarbons (PAHs).

Samples were analysed by gas chromatography. GC-ECD method was used for OCPs and PCBs and GC-MS method for PAHs.

The total petroleum hydrocarbons (TPHs) were analysed by the fluorescence method.

## Turkey

Heavy metals defined as Priority Substances (Cd, Pb and Ni) and Specific Pollutants (As, Cr, Cu, Co, Zn) in WFD (Directive 2000/60/EC) were analyzed for their concentrations in the water samples which are collected at 10 stations in both seasons. The water samples for metal analysis were collected from the surface layer (1 m below the surface) from the 5 l Niskin bottles of the Rosette System. The collected samples were stored in plastic bottles (250 ml).

Total metals (dissolved and acid-soluble suspended forms) have been determined in unfiltered seawater samples, acidified up to pH=2 with Ultrapure HNO<sub>3</sub>. The analytical determination of the trace elements (such as copper, cadmium, lead, nickel, and chromium) was carried out by inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (Perkin Elmer- Neixon300x model).

The accuracy of trace metal determination was indicated by a good agreement between our values and those reported for the certified reference materials (IAEA-158 Marine Sediment and QTM271SW). Recoveries of the metals were changed between 97-104 and 84-115 % for sediment and seawater samples respectively.

Seawater samples taken for PAH, PCB and pesticide were extracted on board with twister using methanol and internal standard (stir bar sorptive extraction method). Before extraction, each water sample (100 mL) was filtered through a glass fiber filter (1.2  $\mu\text{m}$ ). The sample was then placed in an Erlenmeyer flask which had been rinsed prior to use with Milli-Q water and methanol and then dried in an oven at 110 °C. Next, 10 mL of methanol was added to prevent the adhesion of compounds to the Erlenmeyer flask glass wall. Before use, all Twisters were conditioned overnight at 280 °C in a thermal desorption system (TDS) using a Gerstel Tube Conditioner with 200 mL/min nitrogen flow. A 100  $\mu\text{L}$  aliquot of internal standard mixture was added to all prepared flasks. For sample extraction, a Twister with dimensions of 1.0 mm (thickness) x 20 mm (length) was used. The sample was stirred at 850 rpm for 2 h with the Gerstel stirrer at room temperature. After the extraction was completed and stored in the refrigerator. Twister bars were taken from the glassware, rinsed with Milli-Q water, dried with lint-free paper and inserted into thermal desorption unit (TDU) liners for GC injection. Calibration injection sets were prepared in the same manner as the sample extraction procedure. An Agilent 7890B gas - chromatograph coupled with a 7000D triple quadrupole detector was used. The system was equipped with a CIS-4 Cooled Injection System with a programmable temperature vaporizing inlet (PTV), on a Thermal Desorption Unit (TDU), and autosampler (MultiPurpose Sampler [MPS]) to introduce Twister bars into the system. The triple quadrupole was operated in electron

ionization (EI) mode, 300 °C temperature ion source, 150 °C for both quadrupoles, with acquisition mode set to dMRM (dynamic multiple reaction monitoring). An HP-5ms UI 30 m× 0.25 mm× 0.25 µm) column was used as the analytical column, and a 0.7m× 0.25mm column was used as the backflush column. TDU / GC-MSMS method was used for OCPs and PCBs and PAHs.

The seawater sample for TPH was extracted with hexane on board. The extract was stored in the refrigerator. The total petroleum hydrocarbons (TPHs) were analysed by fluorescence method.

## Chemistry - Sediments

### Ukraine

Sediments samples for heavy metals and organic pollutants were collected with a Van Veen bodengreifer. Sediments were freeze-dried and then well homogenized, and large fragments (> 0.1 mm) were removed by sieving.

Then for metals analysis 0,22 g the sediment samples were treated with a mixture of ultrapure acids HNO<sub>3</sub>, HCl, after which HF was added.

Instrumental analysis and quantification: metals were analysed by electrothermal furnace atomic absorption spectrometry (AAS-ET Analytik Jena AG ZEENIT 650P). Concentration of metals calibration was performed with working standards for each element, starting from stock solutions of 1000 µg/L (Sigma-Aldrich). At least 3 instrumental readings have been performed for each sample, with average value reported. The work domains are as follows: Cd 0-2 µg/L; other metals 0-80 µg/L.

For organic pollutants, extraction 3,0 g sample bottom sediment was carried out on an accelerated pressure extraction unit (PLE) with a hexane/dichloromethane/methanol mixture (60% /20% /20%). Internal standards PCB29 and Phenanthrene-d10 were added to the bottom sediment sample prior to extraction. Extraction was followed by purification on a silica gel column and concentration in a turbo evaporator under nitrogen flow. Persistent organic pollutants were analysed by gas chromatography. GC-ECD (Agilent 7890B) was used for OCPs and PCBs, and GC-MS (Agilent 7890A with MS 5975C) was used for PAHs.

Quality control of the analysis results is carried out by the method of control analysis of reference materials.

### Romania

Sediments samples for heavy metals and organic pollutants were collected with a Van Veen bodengreifer. Sediments were freeze-dried and then well homogenized, and the coarse fragments (> 0.5 mm) were removed by sieving.

Sediments for heavy metals analysis were treated with concentrated acid (HNO<sub>3</sub> 65%) followed by the process of digestion in the microwave oven. At the end of mineralization, the samples were resumed in the 100 ml flask, with deionized water.

The analytical determination of the copper, cadmium, lead, nickel, and chromium was carried out by graphite furnace - atomic absorption spectrometry method (GF-AAS).

Sediments were subjected to microwave extraction with a mixture of hexane/acetone (1:/1). The extraction was followed by purification on florisil column for organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), respectively silica/alumina column for polyaromatic hydrocarbons (PAHs) and concentration using the Kuderna-Denish concentrator and nitrogen flow.

Samples were analysed by gas chromatography. GC-ECD method was used for OCPs and PCBs and GC-MS method for PAHs.

The total petroleum hydrocarbons (TPHs) were analysed by fluorescence method.

### Turkey

The modified EPA-3052 method was used for the preparation of the metal analysis. About 0.1 g of the homogenized sediment samples were put into a closed Teflon vessel with 4 mL of HNO<sub>3</sub> (Merck), 2 mL of HCl (Merck) and 1 mL of HF acids (Merck) for the complete digestion of the metal samples. A microwave acid digestion system (Milestone Ultrawave) was used for the digestion at 120 °C for 35 min. Teflon vessels were left to cool, and 0.3 g boric acid was added to permit the complexation of fluoride to protect the quartz plasma torch from excess hydrofluoric acid. Then the same microwave digestion procedure was reapplied. After cooling, the vessel contents were filtered and then diluted to 50 mL with deionized water. The diluted samples were preserved in polyethylene bottles for

analysis. Sample solutions and blanks were analysed for the metals (Al, Fe, As, Cu, Cr, Cd, Ni, Pb, Zn) using the ICPMS instrument (Perkin Elmer Nexion 3000x) utilizing a Kinetic Energy Discrimination (KED) mode. The mercury (Hg) content of the samples was determined using the Milestone DMA-80 Direct Mercury Analyzer.

The total organic carbon (TOC) content of the samples was determined according to the High Temperature Combustion Method (Thermo Finnigan Flash EA 1112 Series - CHNS analyzer) after removal of inorganic carbon. Grain size analysis of sediment samples was carried out using standard sieves (granulometric method). Results were assessed according to the procedure outlined by Folk (1974).

The accuracy of trace metal determination was indicated by a good agreement between our values and those reported for the certified reference materials (IAEA-158 Marine Sediment and QTM2715W). Recoveries of the metals were changed between 97-104 and 84-115 % for sediment and seawater samples respectively.

For sediment samples, the extraction was conducted using a microwave oven. Approximately 5 g portion of freeze-dried sediment samples was put in the teflon tube (PTFE) of the reactor with 30 ml hexane: acetone (1:1 v/v). Various internal standards were added to the sediment for quantifying the overall recovery of the analytical procedures: Chrysene-d12, Acenaphthene-d10, Napthalene-d8, Perylene-d12 and Phenathrene-d10 for the aromatic hydrocarbon fraction; PCB29 and PCB198 for the organochlorine compounds. The extraction was carried out at 120 °C for 35 min. Sulphur was removed used activated elemental copper. The extracts were concentrated using a rotary evaporator. Extraction was followed by purification on florisil column for organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), respectively silica column for polyaromatic hydrocarbons (PAHs) and concentration using the rotary evaporator and nitrogen flow (Table 7.3).

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbon (PAHs) were analyzed by gas chromatography (GC-MS MS). The total petroleum hydrocarbon (TPHs) were analyzed by fluorescence method.

**Table 7.3 - Organic pollutants analysis methods in sediment**

Parameter	Method	Device	Reference	Unit
OCPs and PCBs	Microwave Extraction (Acetone-hexane) Removal of sulfur by copper Clean up technique: Florisil column (three fractions)	GC/MS MS	UNEP/IOC/IAEA EPA 8081 B EPA 8121 EPA 8270 C EPA 3545 A	ng/g
PAH	Microwave Extraction (Acetone-hexane) Removal of sulfur by copper Clean up technique: Florisil column (two fractions)	GC/MS MS	UNEP/IOC/IAEA No:20; 1992 EPA 3630C Silica Gel Cleanup	ng/g
TPH	Ultrasonic Extraction (with THF)	Luminisans Spectrofluorometer	According to Chrysene Ex: 310nm ve Em: 360 nm	µg/L
TOC	High-Temperature Combustion Method (TOC)	CHNS Analyzer	In-House Method	%

## ANNEX B Lists of species

Table 7.4 - List of phytoplankton taxa identified during river sea cruises

Species / Group	UA	RO	BG	TR
<b>Bacillariophyceae</b>				
<i>Achnanthes</i> sp. Bory, 1822	+			
<i>Achnanthes parvula</i> Kützing, 1844	+			
<i>Amphora</i> sp. C.G. Ehrenberg ex F.T. Kützing, 1844	+	+		
<i>Amphora ovalis</i> (Kützing) Kützing, 1844			+	
<i>Amphora proteus</i> Gregory, 1857	+			
<i>Asterionella formosa</i> Hassall, 1850		+		
<i>Asterionella frauenfeldii</i> Grunow, 1863			+	
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen, 1979	+			
<i>Aulacoseira italica</i> (Ehrenberg) Simonsen 1979	+			
Bacillariophyceae Haeckel, 1878	+			
<i>Cerataulina bergonii</i> Ostefeld, 1903	+	+	+	
<i>Ceratoneis closterium</i> Ehrenberg, 1839	+			
<i>Ceratoneis fasciola</i> Ehrenberg, 1839				+
<i>Chaetoceros affinis</i> Lauder, 1864		+		+
<i>Chaetoceros compressus</i> Lauder, 1864			+	+
<i>Chaetoceros curvisetus</i> P.T. Cleve, 1889	+	+		+
<i>Chaetoceros danicus</i> Cleve, 1889				+
<i>Chaetoceros decipiens</i> Cleve, 1873				+
<i>Chaetoceros lorenzianus</i> Grunow, 1863			+	
<i>Chaetoceros peruvianus</i> Brightwell, 1856				+
<i>Chaetoceros rigidus</i> Ostefeld, 1902		+		
<i>Chaetoceros septentrionalis</i> Cleve, 1896		+		
<i>Chaetoceros similis f. solitarius</i> Proschkina-Lavrenko, 1955		+		
<i>Chaetoceros similis</i> Cleve, 1896				+
<i>Chaetoceros simplex</i> Ostefeld, 1902				+
<i>Chaetoceros socialis</i> H.S.Lauder, 1864		+		
<i>Chaetoceros wighamii</i> C.G. Ehrenberg, 1844		+		
<i>Cocconeis</i> sp. C.G. Ehrenberg, 1837		+	+	
<i>Cocconeis pediculus</i> Ehrenberg, 1838	+			
<i>Cocconeis scutellum</i> Meunier, 1910	+	+		
<i>Coscinodiscus</i> sp. C.G. Ehrenberg, 1839			+	
<i>Coscinodiscus angustelineatus</i> Schmidt in Schmidt et al., 1878				+
<i>Coscinodiscus centralis</i> Ehrenberg, 1844				+
<i>Coscinodiscus granii</i> Gough, 1905				+
<i>Coscinodiscus perforatus</i> Ehrenberg, 1844				+
<i>Coscinodiscus radiatus</i> Ehrenberg, 1841	+			
<i>Cyclotella choctawhatcheeana</i> Prasad, 1990	+	+	+	
<i>Cyclotella meneghiniana</i> Kützing, 1844	+	+		
<i>Cylindrotheca closterium</i> (Ehrenberg) Reimann & J.C.Lewin, 1964			+	+
<i>Cymbella</i> sp. C.A. Agardh, 1830	+			
<i>Diatoma tenue</i> C. Agardh, 1812		+		
<i>Ditylum brightwellii</i> (T.West) Grunow, 1885				+
<i>Gyrosigma</i> sp. A.H. Hassall, 1845	+			
<i>Halamphora coffeaeformis</i> (C.Agardh) Levkov, 2009		+		
<i>Halamphora hyalina</i> (Kützing) Rimet & R. Jahn in Rimet et al., 2018	+			
<i>Hannaea arcus</i> (Ehrenberg) R.M.Patrick, 1961	+			
<i>Lauderia pumila</i> Castracane, 1886				+
<i>Leptocylindrus minimus</i> Gran, 1915	+	+		
<i>Licmophora ehrenbergii</i> (Kützing) Grunow, 1867	+			
<i>Melosira moniliformis</i> (O.F. Müller) C. Agardh, 1824	+			
<i>Navicula</i> sp. J.B.M. Bory de Saint-Vincent, 1822	+	+	+	
<i>Navicula cryptocephala sensu</i> W. Smith, 1853		+		
<i>Navicula palpebralis</i> de Brébisson, 1853	+			
<i>Navicula pennata</i> A. Schmidt, 1876	+			
<i>Navicula peregrina</i> (Ehrenberg) Kützing, 1844	+			
<i>Navicula reinhardtii</i> (Grunow) Grunow in Van Heurck, 1880	+			

Species / Group	UA	RO	BG	TR
<i>Navicula salinarum</i> Grunow in Cleve, Grunow, 1880	+			
<i>Nitzschia</i> sp. A.H. Hassall, 1845	+		+	
<i>Nitzschia acicularis</i> Frenguelli, 1923	+	+		
<i>Nitzschia delicatissima</i> Cleve, 1897	+	+	+	+
<i>Nitzschia longissima</i> (Brébisson) Ralfs, 1861	+		+	
<i>Nitzschia tenuirostris</i> Mereschkowsky	+	+		
<i>Paralia sulcata</i> (Ehrenberg) Cleve, 1873			+	
<i>Pinnularia</i> sp. C.G. Ehrenberg, 1843	+			
<i>Pleurosigma elongatum</i> W.Smith, 1852	+			+
<i>Proboscia alata</i> (Brightwell) Sundström, 1986	+	+	+	
<i>Pseudo-nitzschia seriata</i> (P.T. Cleve, 1883) H., M. Peragallo, 1900	+		+	
<i>Pseudosolenia calcar-avis</i> (Schultze) B.G.Sundström, 1986	+		+	+
<i>Rhizosolenia fragilissima</i> f. <i>fragilissima</i> Bergon, 1903				+
<i>Rhizosolenia setigera</i> Brightwell, 1858				+
<i>Rhizosolenia styliformis</i> T.Brightwell, 1858				+
<i>Skeletonema costatum</i> (Greville) Cleve, 1873		+		+
<i>Skeletonema subsalsum</i> (Cleve-Euler) Bethge, 1928		+		
<i>Sphenella parvula</i> Kützing, 1844	+			
<i>Stephanodiscus hantzschii</i> Grunow, 1880	+			
<i>Synedra</i> sp. C.G. Ehrenberg, 1830	+			
<i>Synedra acus</i> var. <i>ostenfeldii</i> Krieger, 1927		+		
<i>Synedra nitzschii</i> f. <i>nitzschiioides</i> Grunow, 1862	+	+	+	+
<i>Synedra ulna</i> var. <i>subcontracta</i> Østrup in Héribaldi et al., 1920	+			
<i>Tabularia fasciculata</i> (C.Agardh) D.M.Williams & Round, 1986			+	
<i>Thalassiosira</i> sp. P.T. Cleve, 1873 emend. Hasle, 1973	+		+	
<i>Thalassiosira aestivalis</i> Gran, 1931		+		
<i>Thalassiosira baltica</i> (Grunow in P.T. Cleve, Grunow) Ostenfeld, 1901	+			
<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve, 1904			+	+
<i>Thalassiosira minima</i> Gaarder, 1951			+	
<i>Thalassiosira parva</i> Proshkina-Lavrenko, 1955	+	+		
<i>Thalassiosira rotula</i> Meunier, 1910			+	
<i>Thalassiosira subsalina</i> Proshkina-Lavrenko, 1955		+		
<i>Triceratium favus</i> Ehrenberg, 1839			+	
<i>Trieres mobiliensis</i> (J.W.Bailey) Ashworth & E.C.Theriot in Ashworth, Nakov & E.C.Theriot, 2013				+
<b>Dinophyceae</b>				
<i>Akashiwo sanguinea</i> (K.Hirasaka) Gert Hansen & Moestrup, 2000		+	+	+
<i>Alexandrium</i> sp. Halim, 1960		+	+	
<i>Alexandrium catenella</i> (Whedon & Kofoid) Balech, 1985				+
<i>Alexandrium minutum</i> Halim, 1960			+	
<i>Amphidinium</i> sp. Claparède & Lachmann, 1859		+	+	
<i>Amphidinium acutissimum</i> Schiller, 1933			+	
<i>Amphidinium crassum</i> Lohmann, 1908		+	+	
<i>Amphidinium extensum</i> Wulff, 1919		+		
<i>Amphidinium flagellans</i> Schiller			+	
<i>Amphidinium longum</i> Lohmann, 1908			+	
<i>Archaeperidinium minutum</i> (Kofoid) Jørgensen, 1912			+	
<i>Aureodinium pigmentosum</i> Dodge, 1967			+	
<i>Azadinium</i> sp. Elbrächter & Tillmann, 2009			+	
<i>Azadinium spinosum</i> Elbrächter & Tillmann, 2009			+	
<i>Biecheleria cincta</i> (Siano, Montresor & Zingone) Siano, 2012			+	
<i>Chimonodinium lomnickii</i> (Woloszynska) S.C. Craveiro, A.J.Calado, N.Daugbjerg, Gert Hansen & Ø.Moestrup, 2011	+			
<i>Cochlodinium archimedes</i> (Pouchet) Lemmermann, 1899		+		
<i>Cochlodinium pupa</i> Lebour, 1925			+	
Dinoflagellates cysts		+	+	
Dinophyceae Fritsch, 1927	+			
<i>Dinophysis acuminata</i> Claparède & Lachmann, 1859		+	+	+
<i>Dinophysis acuta</i> Ehrenberg, 1839			+	
<i>Dinophysis caudata</i> Saville-Kent, 1881	+			+
<i>Dinophysis fortii</i> Pavillard, 1924				+
<i>Dinophysis odiosa</i> (Pavillard) Tai & Skogsberg, 1934			+	

Species / Group	UA	RO	BG	TR
<i>Dinophysis sacculus</i> F.Stein, 1883		+	+	+
<i>Diplopsalis lenticula</i> Bergh, 1881	+	+	+	+
<i>Durinskia agilis</i> (Kofoid & Swezy) Saburova, Chomérat & Hoppenrath, 2012	+			
<i>Ensiculifera</i> sp. Balech ex K.Matsuoka, S.Kobayashi & G.Gains, 1990			+	
<i>Glenodiniopsis uliginosa</i> (A.J.Schilling) Woloszynska, 1928			+	
<i>Glenodinium</i> sp. Ehrenberg, 1836			+	
<i>Glenodinium paululum</i> Lindemann, 1928		+	+	
<i>Glenodinium pilula</i> (Ostenfeld) Schiller, 1935		+	+	
<i>Goniodoma sphaericum</i> Murray & Whitting, 1899			+	
<i>Gonyaulax ceratocoroides</i> Kofoid, 1910		+	+	+
<i>Gonyaulax minima</i> Matzenauer, 1933			+	
<i>Gonyaulax monacantha</i> Pavillard, 1916				+
<i>Gonyaulax polygramma</i> F.Stein, 1883			+	
<i>Gonyaulax scrippsae</i> Kofoid, 1911	+			
<i>Gonyaulax verior</i> Sournia, 1973			+	
<i>Gymnodinium</i> sp. F. Stein, 1878	+	+	+	
<i>Gymnodinium agiliforme</i> Schiller, 1928		+		
<i>Gymnodinium albulum</i> Er.Lindemann, 1928			+	
<i>Gymnodinium aureolum</i> (E.M.Hulburt) Gert Hansen, 2000			+	
<i>Gymnodinium catenatum</i> H.W.Graham, 1943			+	
<i>Gymnodinium fuscum</i> (Ehrenberg) F.Stein, 1878			+	
<i>Gymnodinium fusus</i> Schütt, 1896		+		
<i>Gymnodinium hamulus</i> Kofoid & Swezy, 1921			+	
<i>Gymnodinium lachmannii</i> Kent, 1881			+	
<i>Gymnodinium lantzschii</i> Utermöhl, 1925			+	
<i>Gymnodinium latum</i> Skuja, 1948			+	
<i>Gymnodinium najadeum</i> J.Schiller, 1928	+	+	+	
<i>Gymnodinium nanum</i> Schiller, 1928			+	
<i>Gymnodinium opressum</i> Conrad, 1926			+	
<i>Gymnodinium pulchrum</i> J.Schiller, 1928			+	
<i>Gymnodinium punctatum</i> Pouchet, 1887			+	
<i>Gymnodinium rubrum</i> Kofoid & Swezy, 1921			+	
<i>Gymnodinium simplex</i> (Lohmann, 1911) Kofoid, Swezy, 1921	+			
<i>Gymnodinium uberrimum</i> (G.J.Allman) Kofoid & Swezy, 1921			+	
<i>Gymnodinium verruculosum</i> P.H.Campbell, 1973			+	
<i>Gymnodinium wulfii</i> J.Schiller, 1933	+	+		
<i>Gyrodinium</i> sp. Kofoid & Swezy, 1921			+	
<i>Gyrodinium flagellare</i> Schiller, 1928			+	
<i>Gyrodinium fusiforme</i> Kofoid & Swezy, 1921	+	+	+	+
<i>Gyrodinium helveticum</i> (Penard) Y.Takano & T.Horiguchi, 2004		+	+	
<i>Gyrodinium lachryma</i> (Meunier) Kofoid & Swezy, 1921		+	+	+
<i>Gyrodinium pingue</i> (Schütt) Kofoid & Swezy, 1921		+	+	
<i>Gyrodinium spirale</i> (Bergh) Kofoid & Swezy, 1921			+	
<i>Herdmania litoralis</i> J.D.Dodge, 1981			+	
<i>Heterocapsa</i> sp. Stein, 1883			+	
<i>Heterocapsa circularisquama</i> Horiguchi, 1995			+	
<i>Heterocapsa minima</i> A.J.Pomroy, 1989			+	
<i>Heterocapsa niei</i> (Loeblich III) Morrill & Loeblich III, 1981			+	
<i>Heterocapsa rotundata</i> (Lohmann) Gert Hansen, 1995		+	+	+
<i>Karenia</i> sp. G.Hansen & Moestrup, 2000			+	
<i>Karlodinium veneficum</i> (D.Ballantine) J.Larsen, 2000			+	
<i>Katodinium glaucum</i> (Lebour) Loeblich III, 1965			+	
<i>Kryptoperidinium triquetrum</i> (Ehrenberg) U.Tillmann, M. Gottschling, M.Elbrächter, W.-H.Kusber & M.Hoppenrath, 2019	+	+	+	+
<i>Lepidodinium chlorophorum</i> (M.Elbrächter & E.Schnepf) Gert Hansen, Botes & Salas, 2007			+	
<i>Lessardia elongata</i> Saldarriaga & F.J.R.Taylor, 2003		+	+	
<i>Lingulodinium polyedra</i> (F.Stein) J.D.Dodge, 1989		+	+	+
<i>Margalefidinium citron</i> (Kofoid & Swezy) F.Gómez, Richlen & D.M.Anderson, 2017		+		
<i>Mesoporos perforatus</i> (Gran) Lillick, 1937		+		
<i>Nematodinium armatum</i> (Dogiel) Kofoid & Swezy, 1921			+	
<i>Oblea rotunda</i> (Lebour) Balech ex Sournia, 1973			+	

Species / Group	UA	RO	BG	TR
<i>Oxyrrhis marina</i> Dujardin, 1841			+	
<i>Oxytoxum caudatum</i> Schiller, 1937			+	
<i>Pentapharsodinium dalei</i> Indelicato & Loeblich III, 1986			+	
<i>Peridiniella danica</i> (Paulsen) Y.B. Okolodkov & J.D. Dodge, 1995			+	
<i>Peridiniella globosa</i> (P.A. Dangeard) Okolodkov, 2006			+	
<i>Peridinium</i> sp. Ehrenberg, 1830			+	
<i>Peridinium cinctum</i> (O.F. Müller) Ehrenberg, 1832	+			
<i>Peridinium</i> vegetative stages (<40 µm)		+		
<i>Peridinium</i> vegetative stages (>40 µm)		+		
<i>Phalacroma rotundatum</i> (Claparède & Lachmann) Kofoid & J.R. Michener, 1911		+	+	+
<i>Polykrikos kofoidii</i> Chatton, 1914		+	+	+
<i>Polykrikos schwartzii</i> Bütschli, 1873				+
<i>Preperidinium meunieri</i> (Pavillard) Elbrächter, 1993		+		
<i>Pronoctiluca pelagica</i> Fabre-Domergue, 1889				+
<i>Pronoctiluca spinifera</i> (Lohmann) Schiller, 1932			+	
<i>Prorocentrum compressum</i> (Bailey) T.H. Abé ex J.D. Dodge, 1975		+	+	+
<i>Prorocentrum cordatum</i> (Ostenfeld) J.D. Dodge, 1975	+	+	+	+
<i>Prorocentrum micans</i> Ehrenberg, 1834	+	+	+	+
<i>Prorocentrum scutellum</i> Schröder, 1900		+		+
<i>Protoceratium reticulatum</i> (Claparède & Lachmann) Bütschli, 1885			+	
<i>Protodinium simplex</i> Lohmann, 1908		+	+	
<i>Protoperidinium bipes</i> (Paulsen, 1904) Balech, 1974	+	+		+
<i>Protoperidinium brevipes</i> (Paulsen, 1908) Balech, 1974		+	+	+
<i>Protoperidinium claudicans</i> (Paulsen, 1907) Balech, 1974				+
<i>Protoperidinium conicum</i> (Gran) Balech, 1974				+
<i>Protoperidinium crassipes</i> (Kofoid, 1907) Balech, 1974				+
<i>Protoperidinium curtipes</i> (Jørgensen, 1912) Balech, 1974				+
<i>Protoperidinium depressum</i> (Bailey, 1854) Balech, 1974		+	+	+
<i>Protoperidinium divergens</i> (Ehrenberg) Balech, 1974			+	+
<i>Protoperidinium grande</i> (Kofoid, 1907) Balech, 1974				+
<i>Protoperidinium granii</i> (Ostenfeld) Balech, 1974		+		+
<i>Protoperidinium leonis</i> (Pavillard, 1916) Balech, 1974				+
<i>Protoperidinium oblongum</i> (Aurivillius) Parke & Dodge, 1976				+
<i>Protoperidinium pellucidum</i> Bergh, 1881			+	+
<i>Protoperidinium pentagonum</i> (Gran, 1902) Balech, 1974			+	
<i>Protoperidinium pyriforme</i> (Paulsen, 1905) Balech, 1974			+	
<i>Protoperidinium steinii</i> (Jørgensen, 1899) Balech, 1974			+	+
<i>Pyrocystis lunula</i> (Schütt) Schütt, 1896				+
<i>Pyrophacus horologium</i> F. Stein, 1883				+
<i>Scrippsiella acuminata</i> (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S. Soehner, Kirsch, Kusber & Gottschling, 2015	+	+	+	+
<i>Scrippsiella spinifera</i> G. Honsell & M. Cabrini, 1991			+	
<i>Speroidium fungiforme</i> (Anisimova) Moestrup & Calado, 2018			+	
<i>Torodinium robustum</i> Kofoid & Swezy, 1921		+	+	+
<i>Torodinium teredo</i> (Pouchet) Kofoid & Swezy, 1921			+	
<i>Triadinium polyedricum</i> (Pouchet) Dodge, 1981			+	
<i>Tripes extensus</i> (Gourret) F. Gómez, 2013				+
<i>Tripes furca</i> (Ehrenberg) F. Gómez, 2013	+		+	+
<i>Tripes fusus</i> (Ehrenberg) F. Gómez, 2013			+	+
<i>Tripes muelleri</i> Bory de Saint-Vincent, 1827		+	+	+
<i>Protoperidinium pyriforme</i> subsp. <i>breve</i> (Paulsen) Balech, 1988		+	+	
<b>Chlorophyceae</b>				
<i>Ankistrodesmus arcuatus</i> Korshikov, 1953		+		
<i>Ankistrodesmus falcatus</i> (Corda) Ralfs, 1848	+			
<i>Carteria</i> sp. Diesing, 1866		+		
<i>Chlamydomonas</i> sp. Ehrenberg, 1833		+	+	
Chlorophyceae Wille, 1884	+	+	+	
<i>Coelastrum microporum</i> Nägeli, 1855		+		
<i>Desmodesmus</i> sp. (R. Chodat) S.S. An, T. Friedl & E. Hegewald, 1999	+			
<i>Desmodesmus bicaudatus</i> (Dedusenko) P.M. Tsarenko, 2000	+			
<i>Desmodesmus communis</i> (E. Hegewald) E. Hegewald, 2000	+	+		
<i>Desmodesmus intermedius</i> (Chodat) E. Hegewald, 2000	+			

Species / Group	UA	RO	BG	TR
<i>Desmodesmus opoliensis</i> (P.G.Richter) E.Hegewald, 2000	+	+		
<i>Desmodesmus perforatus</i> (Lemmermann) E.Hegewald, 2000	+			
<i>Desmodesmus spinosus</i> (Chodat) E.Hegewald, 2000		+		
<i>Golenkinia radiata</i> Chodat, 1894		+		
<i>Hyaloraphidium contortum</i> Pascher & Korshikov, 1931	+			
<i>Kirchneriella lunaris</i> (Kirchner) Möbius, 1894	+	+		
<i>Monactinus simplex</i> (Meyen) Corda, 1839	+			
<i>Monoraphidium contortum</i> (Thuret) Komárková-Legnerová, 1969	+	+		
<i>Monoraphidium griffithii</i> (Berkeley) Komárková-Legnerová, 1969	+	+		
<i>Monoraphidium irregulare</i> (G.M.Smith) Komárková-Legnerová, 1969		+		
<i>Monoraphidium komarkovae</i> Nygaard, 1979	+			
<i>Monoraphidium lunare</i> G.Nygaard, J.Komárek, J.Kristiansen & O.M.Skulberg, 1986			+	
<i>Monoraphidium minutum</i> (Nägeli) Komárková-Legnerová, 1969		+		
<i>Pediastrum</i> sp. Meyen, 1829	+			
<i>Pediastrum duplex</i> Meyen, 1829	+			
<i>Pseudopediastrum boryanum</i> (Turpin) E.Hegewald, 2005	+			
<i>Raphidocelis danubiana</i> (Hindák) Marvan, Komárek & Comas, 1984	+			
<i>Scenedesmus</i> sp. Meyen, 1829	+			
<i>Scenedesmus ecornis</i> (Ehrenberg) Chodat, 1926		+		
<i>Scenedesmus obtusus</i> Meyen, 1829	+			
<i>Scenedesmus quadricauda</i> var. <i>ellipticus</i> West & G.S.West, 1895	+			
<i>Schroederia</i> sp. Lemmermann, 1898		+		
<i>Schroederia setigera</i> (Schröder) Lemmermann, 1898		+		
<i>Schroederia spiralis</i> (Printz) Korshikov, 1953		+		
<i>Selenastrum gracile</i> Reinsch, 1867	+			
<i>Stauridium tetras</i> (Ehrenberg) E.Hegewald, 2005		+		
<i>Tetradesmus</i> sp. G.M.Smith, 1913	+			
<i>Tetradesmus lagerheimii</i> M.J.Wynne & Guiry, 2016		+		
<i>Tetradesmus obliquus</i> (Turpin) M.J.Wynne, 2016		+		
<i>Tetraedron</i> sp. Kützing, 1845	+			
<i>Tetraedron caudatum</i> (Corda) Hansgirg, 1888		+		
<i>Tetraedron minimum</i> (A.Braun) Hansgirg, 1888	+	+		
<i>Tetrastrum staurogeniiforme</i> (Schröder) Lemmermann, 1900		+		
<i>Treubaria</i> sp. C.Bernard, 1908	+			
<b>Chlorodendrophyceae</b>				
<i>Tetraselmis inconspicua</i> Butcher, 1959	+			
<b>Cryptophyceae</b>				
<i>Chroomonas</i> sp. Hansgirg, 1885		+	+	
<i>Cryptomonas</i> sp. Ehrenberg, 1831		+		
Cryptophyceae Fritsch, 1927	+		+	
<i>Hemiselmis</i> sp. Parke, 1949			+	
<i>Hillea fusiformis</i> (J.Schiller) J.Schiller, 1925	+	+	+	+
<i>Komma caudata</i> (L.Geitler) D.R.A.Hill, 1991		+		
<i>Leucocryptos marina</i> (Braarud) Butcher, 1967			+	
<i>Plagioselmis</i> sp. Butcher ex G.Novarino, I.A.N.Lucas & S.Morrall, 1994	+		+	
<i>Plagioselmis prolonga</i> Butcher ex G.Novarino, I.A.N.Lucas, S.Morrall, 1994	+			
<i>Rhodomonas marina</i> (P.A.Dangeard) Lemmermann, 1899			+	
Microflagellates	+	+	+	
<i>Teleaulax</i> sp. Hill, 1991			+	
<b>Cyanophyceae</b>				
<i>Anabaena</i> sp. Bory de Saint-Vincent ex Bornet & Flahault, 1886		+	+	
<i>Anabaenopsis</i> sp. V.V.Miller, 1923	+			
<i>Aphanizomenon flosaquae</i> Ralfs ex Bornet & Flahault, 1886	+			
<i>Aphanizomenon gracile</i> (Lemmermann) Lemmermann, 1907			+	
<i>Chroococcus minimus</i> (von Keissler) Lemmermann, 1904	+			
<i>Chroococcus minor</i> (Kützing) Nägeli, 1849	+			
<i>Chroococcus minutus</i> (Kützing) Nägeli, 1849		+		
Cyanophyceae Schaffner, 1909	+	+		
<i>Dolichospermum flosaquae</i> (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, 2009	+			
<i>Dolichospermum spiroides</i> Klebahn, 1895	+			
<i>Glaucospira laxissima</i> (G.S.West) Simic, Komárek & Dordevic, 2014	+			

Species / Group	UA	RO	BG	TR
<i>Gloeocapsa</i> sp. Kützing, 1843			+	
<i>Gomphosphaeria</i> sp. Kützing, 1836	+			
<i>Jaaginema</i> sp. Anagnostidis & Komárek, 1988	+			
<i>Jaaginema kisselevii</i> (Anissimova) Anagnostidis & Komárek, 1988	+			
<i>Limnolyngbya circumcreta</i> (G.S.West) X.Li & R.Li., 2016		+		
<i>Merismopedia glauca</i> (Ehrenberg) Kützing, 1845		+		
<i>Merismopedia minima</i> G.Beck, 1897	+	+		
<i>Merismopedia tenuissima</i> Lemmermann, 1898		+		
<i>Microcystis aeruginosa</i> (Kützing) Kützing, 1846	+			
<i>Nodularia spumigena</i> Mertens in Jürgens, 1822	+			
<i>Oscillatoria</i> sp. Vaucher ex Gomont, 1892	+	+		+
<i>Phormidium hormoides</i> Setchell & N.L.Gardner, 1918		+		
<i>Pseudanabaena limnetica</i> (Lemmermann) Komárek, 1974		+		
<i>Romeria</i> sp. Koczwara, 1932			+	
<i>Spirulina</i> sp. Turpin ex Gomont, 1892		+		
<b>Euglenoidea</b>				
<i>Euglena</i> sp. Ehrenberg, 1830	+			
<i>Euglena viridis</i> (O.F. Müller) Ehrenberg, 1832	+			
<i>Eutreptia</i> sp. Perty, 1852	+		+	
<i>Eutreptia globulifera</i> Goor, 1925			+	
<i>Eutreptia lanowii</i> Steuer, 1904	+	+	+	
<i>Lepocinclis acus</i> (O.F.Müller) B.Marin & Melkonian, 2003		+		
<b>Prymnesiophyceae</b>				
<i>Acanthoica quattropsina</i> Lohmann, 1903		+		
<i>Braarudosphaera bigelowii</i> (Gran & Braarud) Deflandre, 1947			+	
<i>Calyptrorphaera oblonga</i> Lohmann, 1902			+	
<i>Chrysochromulina</i> sp. Lackey, 1939			+	
<i>Coccolithus</i> sp. E.H.L.Schwarz, 1894			+	
<i>Emiliana huxleyi</i> (Lohmann) W.W.Hay & H.P.Mohler, 1967	+	+	+	+
<i>Holococcolithophora sphaeroidea</i> (Schiller) J.W.Jordan, L.Cros & J.R.Young, 2005			+	
<i>Syracosphaera</i> sp. Lohmann, 1902	+			
<b>Chrysophyceae</b>				
<i>Dinobryon balticum</i> (Schütt) Lemmermann, 1901		+		
<i>Ochromonas</i> sp. Vysotskii [Wissotsky], 1887			+	
<i>Ollicola vangoorii</i> Vørs, 1992	+			
<b>Dictyochophyceae</b>				
<i>Apedinella radians</i> (Lohmann) P.H.Campbell, 1973		+	+	
<i>Dictyocha fibula</i> Ehrenberg, 1839				+
<i>Octactis octonaria</i> (Ehrenberg) Hovasse, 1946			+	+
<i>Octactis speculum</i> (Ehrenberg) F.H.Chang, J.M.Grieve & J.E.Sutherland, 2017			+	+
<b>Ebriophyceae</b>				
<i>Ebria tripartita</i> (J.Schumann) Lemmermann, 1899		+		+
<i>Hermesinum adriaticum</i> O.Zacharias, 1906		+	+	
<b>Trebouxiophyceae</b>				
<i>Actinastrum hantzschii</i> Lagerheim, 1882	+	+	+	
<i>Crucigenia fenestrata</i> (Schmidle) Schmidle, 1900	+	+		
<i>Crucigenia tetrapedia</i> (Kirchner) Kuntze, 1898	+	+		
<i>Golenkiniopsis solitaria</i> (Korshikov) Korshikov, 1953	+			
<i>Lagerheimia genevensis</i> (Chodat) Chodat, 1895	+	+		
<i>Micractinium</i> sp. Fresenius, 1858	+			
<i>Micractinium pusillum</i> Fresenius, 1858	+	+		
<i>Oocystis</i> sp. Nägeli ex A.Braun, 1855	+	+		
<i>Oocystis lacustris</i> Chodat, 1897			+	
<b>Nephroselmidophyceae</b>				
<i>Nephroselmis astigmatica</i> Inouye & Pienaar, 1984			+	
<i>Nephroselmis pyriformis</i> (N.Carter) Ettl, 1982			+	
<b>Pyramimonadophyceae</b>				
<i>Polyblepharides amyliifera</i> (Conrad) H.Ettl, 1982			+	
<i>Pyramimonas</i> sp. Schmarda, 1849			+	
<b>Synurophyceae</b>				
<i>Synura</i> sp. Ehrenberg, 1834			+	
<i>Trochiscia</i> sp. Kützing, 1834			+	+

Species / Group	UA	RO	BG	TR
<b>Ulvophyceae</b>				
<i>Binuclearia lauterbornii</i> (Schmidle) Proschkina-Lavrenko, 1966	+	+		
<b>Xanthophyceae</b>				
<i>Meringosphaera mediterranea</i> Lohmann, 1902	+			
<b>Imbricatea</b>				
<i>Paulinella ovalis</i> (A. Wulff) P.W. Johnson, P.E. Hargraves & J.M. Sieburth, 1988	+			
<b>Choanoflagellata</b>				
<i>Acanthoecca</i> sp. W.N. Ellis, 1930	+			

Table 7.5 - List of zooplankton taxa identified during river sea cruises

Species / Group	UA	RO	BG	TR
<b>Oligotrichea</b>				
<i>Codonella cratera</i> Leidy, 1887		+		
<i>Tintinnopsis baltica</i> Brandt, 1896		+		
<i>Tintinnopsis beroidea</i> Stein, 1867		+		
<i>Tintinnopsis campanula</i> Ehrenberg, 1840		+		
<i>Tintinnopsis compressa</i> Daday, 1887		+		
<i>Tintinnopsis karajacensis</i> Brandt, 1896		+		
<i>Tintinnopsis lobiancoi</i> Daday, 1887		+		
<i>Tintinnopsis parvula</i> Jörgensen, 1912		+		
<i>Tintinnopsis subacuta</i> Jörgensen, 1900		+		
<i>Tintinnopsis urnula</i> Meunier, 1910		+		
<i>Tintinnidium mucicola</i> (Claparède & Lachmann, 1858) Daday, 1887		+		
<i>Metacylis mediterranea</i> (Mereschkowsky, 1880) Jörgensen, 1924		+		
<i>Stenosemella ventricosa</i> (Claparède & Lachmann, 1858) Jörgensen, 1924		+		
<b>Appendicularia</b>				
<i>Oikopleura (Vexillaria) dioica</i> Fol, 1872	+	+	+	+
<b>Bivalvia</b>				
Bivalvia (Linnaeus, 1758)	+	+	+	+
<b>Branchiopoda</b>				
<i>Bosmina (Bosmina) longirostris</i> O.F. Müller, 1785	+	+		
<i>Cercopagis pengoi</i> Ostroumov, 1891	+			
<i>Chydorus sphaericus</i> O.F. Müller, 1776		+		
Cladocera Latreille, 1829				+
<i>Cornigerius maeoticus</i> Pengo, 1879	+			
<i>Daphnia cucullata</i> G.O. Sars, 1862	+			
<i>Diaphanosoma brachium</i> Liévin, 1848	+			
<i>Evadne spinifera</i> P.E. Müller, 1867	+	+	+	+
<i>Leptodora kindtii</i> Focke, 1844	+			
<i>Penilia avirostris</i> Dana, 1849	+		+	+
<i>Pleopsis polyphemoides</i> Leuckart, 1859	+	+	+	+
<i>Podonevadne trigona</i> G.O. Sars, 1897	+	+	+	+
<i>Pseudevadne tergestina</i> Claus, 1877		+	+	+
<b>Dinophyceae</b>				
<i>Noctiluca scintillans</i> (Macartney) Kofoid & Swezy, 1921		+	+	+
<b>Gastropoda</b>				
Gastropoda Cuvier, 1795	+	+	+	+
<b>Hexanauplia</b>				
<i>Acanthocyclops vernalis vernalis</i> Fischer, 1853	+			
<i>Acartia (Acanthacartia) tonsa</i> Dana, 1849	+		+	+
<i>Acartia (Acartiura) clausi</i> Giesbrecht, 1889	+	+	+	+
<i>Acartia</i> sp. Dana, 1846			+	+
<i>Balanus</i> Costa, 1778	+	+	+	+
<i>Calanipeda aquaedulcis</i> Krichagin, 1873	+			
<i>Calanus euxinus</i> Hulsemann, 1991		+	+	+
<i>Centropages ponticus</i> Karavaev, 1895	+	+	+	+
<i>Centropages spinosus</i> Krichagin, 1873	+			
Copepoda Milne Edwards, 1840	+			+
<i>Cyclops</i> Müller O.F., 1785	+	+		
<i>Eudiaptomus gracilis</i> Sars G.O., 1863	+			

Species / Group	UA	RO	BG	TR
<i>Eurytemora velox</i> Lilljeborg, 1853	+			
<i>Halicyclops neglectus neglectus</i> Kiefer, 1935	+			
Harpacticoida Sars G.O., 1903	+	+	+	
<i>Oithona davisae</i> Ferrari F.D. & Orsi, 1984	+		+	+
<i>Oithona similis</i> Claus, 1866	+	+	+	+
<i>Paracalanus parvus parvus</i> Claus, 1863		+	+	+
<i>Pontella mediterranea</i> Claus, 1863			+	+
<i>Pseudocalanus elongatus</i> Brady, 1865		+	+	+
<b>Malacostraca</b>				
Decapoda Latreille, 1802		+	+	+
Gammaridae Leach, 1814			+	
<i>Mesopodopsis slabberi</i> Van Beneden, 1861	+	+		
<b>Polychaeta</b>				
<i>Neanthes</i> Kinberg, 1865	+			
Polychaeta Grube, 1850	+	+	+	+
<i>Polygordius neapolitanus</i> Fraipont, 1887			+	
Spionidae Grube, 1850	+			
<b>Sagittoidea</b>				
<i>Parasagitta setosa</i> J. Müller, 1847	+	+	+	+
<b>Phoronida</b>				
Phoronis Wright, 1856	+		+	
Phoronida Hatschek, 1888				+
<b>Bryozoa</b>				
Bryozoa			+	+
<b>Hydrozoa</b>				
<i>Sarsia tubulosa</i> M. Sars, 1835			+	
<b>Leptocardii</b>				
<i>Branchiostoma lanceolatum</i> Pallas, 1774			+	
<i>Branchiostoma</i> Costa, 1834				+
<b>Nuda</b>				
<i>Beroe ovata</i> Bruguière, 1789			+	
<b>Scyphozoa</b>				
<i>Aurelia aurita</i> Linnaeus, 1758			+	
<b>Tentaculata</b>				
<i>Pleurobrachia pileus</i> O. F. Müller, 1776			+	
<i>Mnemiopsis leidyi</i> A. Agassiz, 1865			+	
<b>Pisces</b>				
Pisces (ova, larvae)	+		+	
<b>Ctenophora</b>				
Ctenophora Eschscholtz, 1829			+	
<b>Ostracoda</b>				
Ostracoda Latreille, 1802	+			
<b>Asciacea</b>				
Ascidia Linnaeus, 1767			+	
Asciacea Blainville, 1824				+
<b>Nematoda</b>				
Nematoda			+	
<b>Eurotatoria</b>				
<i>Asplanchna priodonta</i> Gosse, 1850	+			
<i>Filinia longiseta</i> Ehrenberg, 1834	+			
<i>Brachionus quadridentatus</i> Hermann, 1783	+			
<i>Brachionus c. caliciflorus</i> Pallas, 1766	+			
<i>Brachionus c. amphiceros</i> Pallas, 1767	+			
<b>Globothalamea</b>				
<i>Ammonia beccarii</i> Linnaeus, 1758	+			
<b>Rotifera</b>				
Rotifera Cuvier, 1817	+			

Table 7.6 - List of zoobenthos taxa identified during river sea cruises

Species / Group	UA	RO	TR
<b>Calcarea</b>			
<i>Sycon ciliatum</i> Fabricius, 1780		+	
<b>Anthozoa</b>			
<i>Anthozoa</i> sp. Ehrenberg, 1834			+
<i>Diadumene lineata</i> Verrill, 1869		+	
<i>Pachycerianthus solitarius</i> Rapp, 1829		+	
<i>Sagartiogeton undatus</i> Muller, 1778		+	
<b>Nemertea</b>			
<i>Nemertea</i> sp. Schultze, 1851		+	+
<i>Carinina heterosoma</i> Muller, 1965		+	
<i>Micrura fasciolata</i> Ehrenberg, 1828		+	
<i>Tetrastema bacescui</i> Bacescu & Carausu		+	
<i>Amphiporus bioculatus</i> Verrill, 1892		+	
<i>Tetrastema</i> sp.		+	
<b>Gastropoda</b>			
<i>Rapana venosa</i> Valenciennes, 1846			+
<i>Brachystomia eulimoides</i> Hanley, 1844			+
<i>Calyptrea chinensis</i> Linnaeus, 1758		+	+
<i>Cerithidium submammillatum</i> de Rayneval & Ponzi, 1854			+
<i>Chrysallida</i> sp.			+
<i>Ecrobia ventrosa</i> Montagu, 1803		+	+
<i>Hydrobia</i> sp.	+		
<i>Pusillina lineolata</i> Michaud, 1830			+
<i>Retusa</i> sp.			+
<i>Retusa truncatula</i> Bruguière, 1792		+	+
<i>Retusa umbilicata</i> Montagu, 1803			+
<i>Tragula fenestrata</i> Jeffreys, 1848			+
<i>Tritia neritea</i> Linnaeus, 1758			+
<i>Tritia reticulata</i> Linnaeus, 1758		+	
<i>Trophonopsis</i> sp.			+
<b>Bivalvia</b>			
<i>Abra alba</i> W. Wood, 1802		+	+
<i>Abra</i> cf. <i>alba</i> W. Wood, 1803			+
<i>Abra</i> cf. <i>nitida</i> O.F. Müller, 1776			+
<i>Abra nitida</i> O.F. Müller, 1776	+		+
<i>Abra prismatica</i> Montagu, 1808		+	
<i>Abra segmentum</i> Récluz, 1843	+		
<i>Abra</i> sp. Lamarck, 1818			+
<i>Acanthocardia</i> cf. <i>paucicostata</i> G.B. Sowerby II, 1934			+
<i>Acanthocardia paucicostata</i> G.B. Sowerby II, 1934		+	+
<i>Alitta succinea</i> Leuckart, 1847	+		
<i>Anadara kagoshimensis</i> Tokunaga, 1906	+	+	+
<i>Cerastoderma</i> cf. <i>glaucum</i> Bruguière, 1789			+
<i>Cerastoderma edule</i> Linnaeus, 1758		+	
<i>Cerastoderma glaucum</i> Bruguière, 1789	+		
<i>Chamelea gallina</i> Linnaeus, 1758	+		+
<i>Donacilla cornea</i> Poli, 1791	+		
<i>Donax trunculus</i> Linnaeus, 1758	+		
<i>Donax venustus</i> Poli, 1795			+
<i>Ecrobia ventrosa</i> Montagu, 1803			+
<i>Fabulina fabula</i> Gmelin, 1791			+
<i>Hemilepton nitidum</i> W. Turton, 1822			+
<i>Lentidium mediterraneum</i> O.G. Costa, 1830	+		+
<i>Lucinella divaricata</i> Linnaeus, 1758			+
<i>Lucinidae</i> sp. J. Fleming, 1828			+
<i>Macomangulus tenuis</i> da Costa, 1778			+
<i>Modiolula phaseolina</i> Philippi, 1844		+	
<i>Modiolus adriaticus</i> Lamarck, 1819			+
<i>Moerella donacina</i> Linnaeus, 1758	+		
<i>Monodacna colorata</i> Eichwald, 1829	+		

Species / Group	UA	RO	TR
<i>Mya arenaria</i> Linnaeus, 1758		+	
<i>Mytilaster lineatus</i> Gmelin, 1791			+
<i>Mytilus galloprovincialis</i> Lamarck, 1819	+	+	+
<i>Papillicardium cf. papillosum</i> Poli, 1791			+
<i>Papillicardium papillosum</i> Poli, 1791			+
<i>Parvicardium exiguum</i> Gmelin, 1791	+	+	
<i>Parvicardium simile</i> Milaschewitsch, 1909		+	
<i>Parvicardium</i> sp.			+
<i>Pitar rudis</i> Poli, 1795		+	+
<i>Polititapes aureus</i> Gmelin, 1791		+	+
<i>Rissoa splendida</i> Eichwald, 1830		+	
<i>Spisula cf. subtruncata</i> da Costa, 1778			+
<i>Spisula subtruncata</i> da Costa, 1778		+	+
<i>Tellina tenuis</i> da Costa, 1778			+
<i>Thracia</i> sp.			+
<i>Tritia neritea</i> Linnaeus, 1758			+
<b>Clitellata</b>			
<i>Oligochaeta</i> sp. Grube, 1850		+	+
<b>Polychaeta</b>			
<i>Alitta succinea</i> Leuckart, 1847	+	+	
<i>Aonides paucibranchiata</i> Southern, 1914	+		
<i>Aricidea (Acmira) catherinae</i> Laubier, 1967			+
<i>Aricidea (Strelzovia) claudiae</i> Laubier, 1967			+
<i>Capitella capitata</i> Fabricius, 1780	+	+	
<i>Capitella capitata europaea</i> Wu, 1964	+		
<i>Diogenes pugilator</i> P. Roux, 1829	+		
<i>Dipolydora quadrilobata</i> Jacobi, 1883	+	+	
<i>Eteone</i> sp.	+		
<i>Euchone cf. pseudolimnicola</i> Giangrande & Licciano, 2006			+
<i>Eulalia viridis</i> Linnaeus, 1767		+	
<i>Eunereis longissima</i> Johnston, 1840			+
<i>Exogone naidina</i> Örsted, 1845		+	+
<i>Glycera</i> sp. Haswell, 1879			+
<i>Harmothoe impar</i> Johnston, 1839		+	
<i>Harmothoe reticulata</i> Claparède, 1870	+	+	
<i>Harmothoe</i> sp. Kinberg, 1856			+
<i>Hediste diversicolor</i> O.F. Müller, 1776	+	+	+
<i>Heteromastus filiformis</i> Claparède, 1864		+	+
<i>Lagis koreni</i> Malmgren, 1866	+	+	
<i>Leiochone leiopygos</i> Grube, 1860	+		
<i>Lindrilus flavocapitatus</i> Uljanin, 1877		+	
<i>Magelona mirabilis</i> Johnston, 1865			+
<i>Melinna palmata</i> Grube, 1870	+	+	+
<i>Micronephthys longicornis</i> Perejaslvtseva, 1891		+	+
<i>Microspio mecznikowianus</i> Claparède, 1869	+		
<i>Mysta picta</i> Quatrefages, 1865	+		+
<i>Nephtys cirrosa</i> Ehlers, 1868		+	
<i>Nephtys hombergii</i> Savigny in Lamarck, 1818	+	+	+
<i>Nephtys</i> sp. Cuvier, 1817			+
<i>Nereiphylla rubiginosa</i> de Saint -Joseph, 1888		+	+
<i>Nereis zonata</i> Malmgren, 1867	+		
<i>Notomastus profundus</i> Eisig, 1887		+	
<i>Perinereis cultrifera</i> Grube, 1840			+
<i>Pholoe inornata</i> Johnston, 1839			+
<i>Phyllodoce maculata</i> Linnaeus, 1767	+	+	
<i>Phyllodoce mucosa</i> Örsted, 1843		+	+
<i>Platynereis dumerilii</i> Audouin & Milne Edwards, 1833	+		
<i>Polydora cornuta</i> Bosc, 1802		+	+
<i>Polydora limicola</i> Annenkova, 1934	+		
<i>Polynoe scolopendrina</i> Savigny, 1822	+		
<i>Prionospio cirrifera</i> Wirén, 1883	+	+	
<i>Prionospio maciolekae</i> Dagli & Çinar, 2011			+

Species / Group	UA	RO	TR
<i>Pygospio elegans</i> Claparède, 1863	+		
<i>Scolecopsis squamata</i> O.F. Muller, 1806		+	
<i>Sigambra tentaculata</i> Treadwell, 1941			+
<i>Sphaerosyllis bulbosa</i> Southern, 1914		+	
<i>Sphaerosyllis taylori</i> Perkins, 1981			+
<i>Spio decoratus</i> Bobretzky, 1870			+
<i>Spio filicornis</i> O.F. Müller, 1776	+		
<i>Spirobranchus triqueter</i> Linnaeus, 1758		+	+
<i>Terebellides stroemii</i> Sars, 1835		+	
<b>Arachnida</b>			
<i>Thalassarachna basteri</i> Johnston, 1836		+	
<b>Picnogonidae</b>			
<i>Callipallene phantoma</i> Dohrn, 1881		+	
<b>Gymnolaemata</b>			
<i>Conopeum reticulum</i> Linnaeus, 1767	+		
<b>Hexanauplia</b>			
<i>Amphibalanus improvisus</i> Darwin, 1854		+	
<i>Balanus</i> sp. Costa, 1778			+
<b>Malacostraca</b>			
<i>Ampelisca diadema</i> Costa, 1853	+	+	+
<i>Ampelisca pseudosarsi</i> Bellan-Santini & Kaim-Malka, 1977			+
<i>Ampelisca pseudospinimana</i> Bellan-Santini & Kaim-Malka, 1977			+
<i>Ampelisca sarsi</i> Chevreux, 1888		+	
<i>Ampelisca</i> sp.			+
<i>Amphipoda</i> sp. Latreille, 1816			+
<i>Apocorophium acutum</i> Chevreux, 1908			+
<i>Apocorophium</i> sp.			+
<i>Apseudopsis latreillii</i> Milne Edwards, 1828			+
<i>Apseudopsis ostroumovi</i> Bacescu & Carause, 1947		+	
<i>Bathyporeia guilliamsoniana</i> Spence Bate, 1857			+
<i>Brachynotus sexdentatus</i> Risso, 1827			+
<i>Brachyura</i> sp.			+
<i>Caprella acanthifera</i> Leach, 1814		+	+
<i>Carcinus aestuarii</i> Nardo, 1847		+	
<i>Cardiophilus baeri</i> GO Sars, 1896		+	
<i>Chondrochelia savignyi</i> Kroyer, 1842			+
<i>Crangon crangon</i> Linnaeus, 1758		+	
<i>Cumacea</i> sp. Kroyer, 1846			+
<i>Cumacea</i> sp.			+
<i>Cumella pigmaea</i> Bacescu, 1950		+	
<i>Cumopsis goodsir</i> Van Beneden, 1861	+		
<i>Deflexilodes gibbosus</i> Chevreux, 1888		+	
<i>Dexamine spinosa</i> Montagu, 1813		+	
<i>Diogenes pugilator</i> Roux, 1829			+
<i>Eudorella truncatula</i> Bate, 1856		+	+
<i>Hyale pontica</i> Rathke, 1847	+		
<i>Iphinoe elisae</i> Bacescu, 1950		+	
<i>Iphinoe maeotica</i> Sowinskyi, 1893		+	
<i>Iphinoe</i> sp.			+
<i>Iphinoe tenella</i> Sars, 1878		+	+
<i>Iphinoe trispinosa</i> Goodsir, 1843			+
<i>Leptocheirus</i> sp.			+
<i>Liocarcinus holsatus</i> Fabricius, 1780		+	
<i>Liocarcinus navigator</i> Herbst, 1794			+
<i>Medicorophium rotundirostre</i> Stephensen, 1915			+
<i>Medicorophium runcicorne</i> Della Valle, 1893		+	+
<i>Medicorophium</i> sp.			+
<i>Megaluropus massiliensis</i> Ledoyer, 1976			+
<i>Megamphopus brevidactylus</i> Myers, 1976			+
<i>Megamphopus cornutus</i> Norman, 1869			+
<i>Melita palmata</i> Montagu, 1804	+		
<i>Microdeutopus algicola</i> Della Valle, 1893	+		+

Species / Group	UA	RO	TR
<i>Microdeutopus anomalus</i> Rathke, 1843			+
<i>Microdeutopus damnoniensis</i> Bate, 1856		+	
<i>Microdeutopus gryllotalpa</i> Costa, 1853		+	
<i>Microdeutopus</i> sp.			+
<i>Microdeutopus versiculatus</i> Spence Bate, 1857			+
<i>Nototropis guttatus</i> Costa, 1853		+	
<i>Nototropis massiliensis</i> Bellan-Santini, 1975			+
<i>Orchomene humilis</i> Costa, 1853		+	
<i>Periculodes longimanus</i> Spence Bate & Westwood, 1868	+	+	+
<i>Periculodes</i> sp. G.O. Sars, 1892			+
<i>Phtisica marina</i> Slabber, 1769		+	+
<i>Pseudocuma (Pseudocuma) longicorne</i> Bate, 1858			+
<i>Sphaeroma serratum</i> Fabricius, 1787	+		
<i>Stenosoma capito</i> Rathke, 1837		+	
<i>Synchelidium haplocheles</i> Grube, 1864			+
<i>Synchelidium maculatum</i> Stebbing, 1906		+	
<i>Synchelidium</i> sp.			+
<i>Upogebia pusilla</i> Petagna, 1792		+	
<b>Ophiuroidea</b>			
<i>Amphiura stepanovi</i> Djakonov, 1954		+	
<i>Ophiuroidea</i> sp. Gray, 1840			+
<i>Ophiuroidea</i> sp. Müller & Troschel, 1840			+
<b>Holothuroidea</b>			
<i>Holothuridae</i> sp. Burmeister, 1837			+
<i>Leptosynapta inhaerens</i> O.F.Müller, 1776		+	
<b>Phoronida</b>			
<i>Phoronis euxinicola</i> Selys-Longchamps, 1907		+	
<i>Phoronis</i> sp. Wright, 1856			+
<b>Ascidiacea</b>			
<i>Ascidiella aspersa</i> Müller, 1776		+	
<i>Ciona intestinalis</i> Linnaeus, 1767		+	
<i>Eugyra adriatica</i> Drasche, 1884		+	
<b>Pilidiophora</b>			
<i>Leucocephalonemertes aurantiaca</i> Grube, 1855		+	
<i>Micrura fasciolata</i> Ehrenberg, 1828		+	
<b>Rhabditophora</b>			
<i>Leptoplana</i> Ehrenberg, 1831		+	
<b>Thecostraca</b>			
<i>Amphibalanus eburneus</i> Gould, 1841	+		
<i>Amphibalanus improvisus</i> Darwin, 1854	+		
<b>Tunicata</b>			
<i>Tunicata</i> sp. Lamarck, 1816			+
<b>Insecta</b>			
<i>Chironomus salinarius</i> Kieffer, 1915	+		
<i>Critochironomus</i> sp.	+	+	

## ANNEX C Descriptive Statistics - Chemistry

### Water

#### Ukraine

*Table 7.7 - Nutrient content in June and September 2019 on the north and northwestern shelf of the Black Sea in the coastal waters of river mouths - descriptive statistics.*

Indicator	DIP	TP	N(NO <sub>2</sub> )	N(NO <sub>3</sub> )	N(NH <sub>4</sub> )	DIN	TN	Si(SiO <sub>4</sub> )
	µM	µM	µM	µM	µM	µM	µM	µM
June 2019								
Number of observations	7	7	7	7	7	7	7	7
Average	0.78	1.94	1.01	29.3	0.52	30.8	79.9	36.7
Maximum	1.28	2.99	2.10	82.5	1.66	84.63	119	66.6
Minimum	0.23	1.27	0.54	1.41	0.04	2.78	36.3	1.13
Std. Dev.	1.12	0.62	0.53	35.3	0.61	35.6	35.0	21.7
September 2019								
Number of observations	8	8	8	8	8	8	8	8
Average	0.72	0.82	0.31	2.62	6.53	9.47	54.8	18.86
Maximum	1.03	1.07	0.56	6.85	32.13	39.20	152	50.32
Minimum	0.29	0.42	0.07	0.02	0.30	0.75	11.9	7.50
Std. Dev.	0.28	0.22	0.16	2.73	10.69	12.83	47.8	13.68

*Table 7.8 - Summary statistic of Trace metals content in June and September 2019 on the north and northwestern shelf of the Black Sea in the coastal waters of river mouths - descriptive statistics.*

Indicator	Cu	Cd	Pb	Ni	Cr	As	Hg	Zn	Fe	Co
	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[mg/L]	[µg/L]
June 2019										
Number of observations	9	9	9	9	9	9	9	7	9	9
Average	3.80	0.21	2.27	0.73	0.38	0.55	0	2.90	444	0.30
Maximum	7.56	0.55	6.38	1.98	2.16	3.34	0.01	5.10	2220	1.50
Minimum	1.58	0	0	0	0	0	0	1.10	10.0	0
Std. Dev.	2.10	0.19	2.19	0.75	0.79	1.17	0.01	1.80	793	2.10
September 2019										
Number of observations	8	8	8	8	8	8	8	8	8	8
Average	2.17	0.33	1.81	0	0.11	0.13	0.03	20.0	25.0	0
Maximum	10.7	0.88	3.03	0	0.86	1.02	0.06	91.0	74.0	0
Minimum	0	0	1.22	0	0	0	0	0	0	0
Std. Dev.	3.78	0.29	0.53	0	0.30	0.36	0.02	29.0	23.8	3.78

**Table 7.9 - Summary statistic of other pollutants content in June and September 2019 on the north and northwestern shelf of the Black Sea in the coastal waters of river mouths - descriptive statistics.**

Indicator	June 2019					September 2019				
	No. of obs.	Average	Max.	Min.	Std. Dev.	No. of obs.	Average	Max.	Min.	Std. Dev.
TPH (µg/L)	9	0.11	0.17	0.07	0.03					
Corg [mg/L]	9	5.4	8.6	2.5	2	8	2.3	4.07	0.7	1.3
Naphthalene (µg/L)	9	1.33	2.52	0	0.75	8	3.46	6.39	0.76	2.16
Acenaphthylene (µg/L)	9	0	0	0	0	8	0	0	0	0
Acenaphthene (µg/L)	9	0	0	0	0	8	0.03	0.22	0	0.08
Fluorene (µg/L)	9	0	0.2	0	0.1	8	0.1	0.4	0	0.1
Phenanthrene (µg/L)	9	2.12	10.7	0	3.39	8	9.24	16.9	7.01	3.16
Anthracene (µg/L)	9	1.6	14	0	4.7	8	0.1	0.5	0	0.2
Fluoranthene (µg/L)	9	1.12	2.71	0.39	0.84	8	2.12	4.49	0.57	1.34
Pyrene (µg/L)	9	0.4	0.8	0.2	0.2	8	0.8	1.6	0.2	0.5
Benzo[a]anthracene (µg/L)	9	0.15	0.05	0	0.17	8	1.75	2.86	0.39	0.99
Crysene (µg/L)	9	0.1	0.2	0	0.1	8	1.5	3.1	0	1.2
Benzo[b]fluoranthene (µg/L)	9	0	0	0	0	8	3.95	7.88	0	2.59
Benzo[k]fluoranthene (µg/L)	9	0.1	0.2	0	0.1	8	3.6	5.06	0.8	1.8
Benzo[a]pyrene (µg/L)	9	0.29	0.81	0	0.23	8	0.46	1.89	0	0.64
Benzo (g,h,i)perylene (µg/L)	9	0.1	0.3	0	0.1	8	2.3	4.8	0.7	1.2
Dibenzo(a,h)anthracene (µg/L)	9	0.04	0.39	0	0.13	8	4.39	11.2	0	3.35
Indeno(1,2,3-c,d)pyrene (µg/L)	9	0	0.3	0	0.1	8	8.5	22	0	8.2
HCB (µg/L)	9	0	0	0	0	8	0	0	0	0
α-HCH (ng/l)	9	0	0	0	0	8	0	0	0	0
β-HCH (ng/l)	9	1.8	10	0.1	3.2	8	1.4	2.5	0.3	0.9
Lindane (µg/L)	9	0.29	0.45	0.11	0.13	4	0.12	0.14	0.09	0.07
HCH total	9	2.1	10.2	0.29	3.08	8	1.42	2.6	0.29	0.91
Heptachlor (µg/L)	9	0	0	0	0	8	2	10	0	3.5
Aldrin (µg/L)	9	0	0	0	0	8	0	0	0	0
Dieldrin (µg/L)	9	0	0	0	0	8	0	0	0	0
Σ ciclodiene	9	0	0	0	0	8	0	0	0	0
p,p'DDE (µg/L)	9	0	0	0	0	8	0	0	0	0
p,p'DDD (µg/L)	9	0	0	0	0	8	0	0	0	0
p,p'DDT (µg/L)	9	8.26	12.9	5.13	3.03	8	6.14	10.7	3.68	2.42
DDT total	9	8.3	13	5.1	3	8	6.1	11	3.7	2.4
Atrazine (ng/l)	9	0.72	2.87	0	0.9	8	1.97	5.4	0	1.72
Dursban (ng/l)	9	0.7	1.5	0.1	0.4	8	5.2	9.8	0.6	3.5
AR-1254 (ng/l)	9	22.5	38.3	10.8	9.34	8	13.6	23.7	6.16	6.15
AR-1260 (ng/l)	9	0.7	1.4	0.2	0.3	8	0.3	0.5	0.1	0.1
PCB 8 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 18 (ng/l)	9	1	6.2	0	2	8	0.2	0.4	0	0.2
PCB 31 (ng/l)	9	0	0	0	0	8	0.03	0.19	0	0.07
PCB28 (µg/L)	9	0.3	0.8	0	0.3	8	0.1	0.3	0	0.1
PCB52 (µg/L)	9	0.55	0.97	0.2	0.29	8	0.32	0.51	0.19	0.11
PCB 49 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 44 (ng/l)	9	0.17	0.84	0	0.28	8	0.04	0.12	0	0.05
PCB 66 (ng/l)	9	1.6	2.6	0.8	0.7	8	0.9	1.5	0.6	0.4
PCB 77 (ng/l)	9	5.11	9.05	2.69	2.25	8	2.97	5.61	1.3	1.51
PCB101 (µg/L)	9	0.77	1.46	0	0.46	8	0.64	1.09	0.27	0.34
PCB 110 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 149 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB118 (µg/L)	9	1.85	3.65	0.72	0.96	8	1.12	2.21	0.49	0.6
PCB153 (µg/L)	9	0.35	0.99	0	0.39	8	0.19	0.61	0	0.22
PCB 105 (ng/l)	9	1.62	3.12	0.62	0.81	8	0.6	1.52	0	0.5
PCB 187 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 126 (ng/l)	9	0.23	0.94	0	0.31	8	0.05	0.1	0	0.04
PCB 128 (ng/l)	9	0.26	0.56	0.14	0.13	8	0.14	0.35	0	0.12
PCB 196 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 206 (ng/l)	9	0	0	0	0	8	0.02	0.15	0	0.05
PCB138 (µg/L)	9	1.35	2.84	0.37	0.75	8	0.82	1.23	0.57	0.24
PCB 183 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 174 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 177 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB180 (µg/L)	9	0.31	1.25	0	0.39	8	0.04	0.15	0	0.06
PCB 170 (ng/l)	9	0.1	0.26	0	0.1	8	0.03	0.15	0	0.05
PCB 199 (ng/l)	9	0	0	0	0	8	0	0	0	0
PCB 194 (ng/l)	9	0.99	2.69	0.28	0.85	8	0.42	0.55	0.19	0.12
PCB 209 (ng/l)	9	0.03	0.29	0	0.1	8	0.02	0.15	0	0.05

## Romania

**Table 7.10 - Descriptive statistics for physico-chemical parameters and nutrients - ANEMONE River-Sea interactions cruise, Romania, May 2019.**

	No. of obs.	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Percentile 10 <sup>th</sup>	Percentile 90 <sup>th</sup>	Std. Dev.
T [°C]	67	10.56	9.67	6.93	16.87	7.61	13.42	7.41	15.90	3.19
S [PSU]	67	16.98	17.73	6.21	18.36	16.76	18.23	14.65	18.33	2.19
O <sub>2</sub> [μM]	67	328.6	339.0	215.3	368.5	308.6	350.6	277.8	359.1	30.7
O <sub>2</sub> [%]	67	105.5	107.3	64.8	131.9	95.0	114.6	83.6	126.7	14.4
pH	67	8.43	8.45	7.91	8.71	8.33	8.50	8.28	8.59	0.13
BOD <sub>5</sub> [mgO <sub>2</sub> /L]	19	2.56	2.35	1.08	4.87	1.99	3.17	1.17	4.87	0.95
(PO <sub>4</sub> ) <sup>3-</sup> [μM]	67	0.21	0.14	0.01	1.23	0.06	0.26	0.02	0.56	0.24
TP [μM]	66	0.72	0.56	0.17	5.75	0.43	0.73	0.27	1.04	1.42
(SiO <sub>4</sub> ) <sup>4-</sup> [μM]	67	16.4	13.9	2.0	79.7	7.3	23.3	3.7	33.3	12.9
(NO <sub>2</sub> ) <sup>-</sup> [μM]	67	2.71	0.33	0.03	35.00	0.17	1.48	0.07	12.21	5.89
(NO <sub>3</sub> ) <sup>-</sup> [μM]	67	8.36	4.69	0.01	42.17	2.95	10.49	2.01	19.44	8.95
(NH <sub>4</sub> ) <sup>+</sup> [μM]	67	4.21	2.28	0.71	19.42	1.21	6.27	0.96	10.73	4.42
DIN [μM]	67	15.26	11.44	3.69	75.77	6.67	18.56	4.45	32.34	13.27
NPOC [mg/L]	66	2.23	2.20	1.12	3.69	2.07	2.32	2.00	2.58	0.33
TN [mg/L]	63	0.49	0.41	0.26	1.76	0.35	0.54	0.31	0.66	0.25
TSS [mg/L]	19	16.8	16.0	11.4	25.2	15.4	18.5	14.8	20.0	2.8

**Table 7.11 - Heavy metals concentrations in water samples from the area under the influence of Danube, May 2019.**

	No. of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Coef. Var.
Cu (μg/L)	19	20.38	15.94	2.09	44.93	3.86	32.31	72.62
Cd (μg/L)	19	0.58	0.59	0.16	1.33	0.25	0.81	57.93
Pb (μg/L)	19	15.12	12.66	9.37	27.65	10.86	21.28	38.12
Ni (μg/L)	19	26.97	28.14	10.39	43.12	17.24	37.01	36.83
Cr (μg/L)	19	6.92	7.09	0.17	19.69	0.98	12.08	89.08

**Table 7.12 - Organochlorine pesticides concentrations in water samples from the area under the influence of Danube, May 2019.**

	No. of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
HCB (μg/L)	19	0.017	0.004	0.004	0.254	0.004	0.004	0.057
Lindane (μg/L)	19	0.003	0.003	0.003	0.010	0.003	0.003	0.002
Heptachlor (μg/L)	19	0.010	0.003	0.003	0.085	0.003	0.003	0.022
Aldrin (μg/L)	19	0.011	0.003	0.003	0.157	0.003	0.003	0.035
Dieldrin (μg/L)	19	0.018	0.002	0.002	0.090	0.002	0.034	0.025
Endrin (μg/L)	19	0.004	0.003	0.003	0.022	0.003	0.003	0.004
p,p'DDE (μg/L)	19	0.002	0.002	0.002	0.010	0.002	0.002	0.002
p,p'DDD (μg/L)	19	0.002	0.002	0.002	0.002	0.002	0.002	0.000
p,p'DDT (μg/L)	19	0.004	0.002	0.002	0.027	0.002	0.002	0.007

**Table 7.13 - Polychlorinated biphenyls (PCBs) concentrations in water samples from the area under the influence of Danube, May 2019.**

	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev
PCB28 (µg/L)	19	0.019	0.004	0.004	0.100	0.004	0.021	0.029
PCB52 (µg/L)	19	0.143	0.046	0.006	0.442	0.010	0.253	0.149
PCB101 (µg/L)	19	0.009	0.006	0.006	0.030	0.006	0.007	0.006
PCB118 (µg/L)	19	0.016	0.004	0.004	0.239	0.004	0.004	0.054
PCB153 (µg/L)	19	0.011	0.009	0.009	0.054	0.009	0.009	0.010
PCB138 (µg/L)	19	0.018	0.007	0.007	0.181	0.007	0.007	0.040
PCB180 (µg/L)	19	0.011	0.003	0.003	0.079	0.003	0.004	0.019

**Table 7.14 - Total petroleum hydrocarbons (TPH) and polyaromatic hydrocarbons (PAHs) concentrations in water samples from the area under the influence of Danube, May 2019.**

	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev
TPH (µg/L)	19	9.55	10.21	4.25	15.63	6.46	11.46	3.10
Naphthalene (µg/L)	19	5.5662	0.0001	0.0001	79.5017	0.0001	0.0001	18.3876
Acenaphthylene (µg/L)	19	0.0001	0.0001	0.0001	0.0009	0.0001	0.0001	0.0002
Phenanthrene (µg/L)	19	0.0997	0.0005	0.0001	0.7793	0.0001	0.1365	0.1942
Anthracene (µg/L)	19	0.1735	0.1219	0.0001	0.5033	0.0302	0.2434	0.1693
S16 PAH (µg/L)	19	5.8407	0.2449	0.0058	79.8488	0.0476	0.6713	18.4033

## Turkey

**Table 7.15 - Metal contaminants in water matrix (two seasons).**

10 stations, summer 2019 and winter 2020 (N=20)			Sakarya Reiver-Sea impact area					Yeşilirmak River-Sea impact area				
Contaminants in water matrix (µg/l)	EQS		mean	Std.	min	Max.	75 perc.	mean	Std.	Min.	Max.	75 perc.
EU-priority subst. * (2013/39)	Cd*	1.5	0.04	0.03	0.01	0.12	0.07	0.08	0.05	0.02	0.20	0.67
	Pb*	14	0.11	0.08	0.03	0.35	0.14	0.13	0.05	0.03	0.24	0.10
	Ni*	34	0.83	0.20	0.62	1.32	0.87	0.74	0.10	0.56	0.91	0.19
Tr-Specific Poll. (2016/08)	Cr	88	0.44	0.21	0.22	0.91	0.52	0.33	0.06	0.23	0.41	0.80
	As	20	1.55	0.32	1.03	2.04	1.86	2.32	1.30	1.07	4.74	0.39
	Zn	76	0.69	0.83	0.02	3.32	0.73	0.49	0.21	0.03	0.84	3.34
	Co	2.6	0.07	0.04	0.03	0.14	0.10	0.05	0.01	0.03	0.08	0.60
	Cu	5.7	0.47	0.13	0.38	0.99	0.48	0.53	0.17	0.31	0.83	0.06

# Sediment

## Ukraine

*Table 7.16 - Summary statistic of Trace metals content in June and September 2019 on the north and northwestern shelf of the Black Sea in the bottom sediments of river mouths - descriptive statistics.*

Indicator	Cu (µg/g)	Cd (µg/g)	Pb (µg/g)	Ni (µg/g)	Cr (µg/g)	Zn (µg/g)	Mn (µg/g)	Co (µg/g)	As (µg/g)	Al (µg/g)	Hg (µg/g)	Fe (µg/g)
June 2019												
Number of observations	4	4	4	4	4	4	4	4	4	4	4	4
Average	19.3	0.42	16.7	24.9	46.0	66.0	650	8.05	7.74	45050	0.33	18925
Maximum	31.1	0.84	26.5	61.8	82.6	107	850	11.0	10.1	65100	0.58	28500
Minimum	11.5	0.17	7.71	0.76	13.2	27.0	558	3.60	5.17	19000	0.09	9150
Std. Dev.	9.28	0.30	9.01	28.4	29.1	36.0	134	3.40	2.62	20400	0.22	8495
September 2019												
Number of observations	4	4	4	4	4	4	4	4	4	4	4	4
Average	29.0	0.33	18.4	46.6	93.8	91.0	699	8.80	7.37	53650	0.13	30625
Maximum	48.4	0.48	30.2	79.8	192	130	1027	14.0	9.92	78400	0.17	47800
Minimum	9.78	0.21	8.87	11.7	23.1	43.0	328	4.50	5.09	15600	0.03	14400
Std. Dev.	19.7	0.11	10.2	37.8	79.0	43.0	307	4.40	2.28	27470	0.06	18580

**Table 7.17 - Summary statistic of other organic pollutants content in June and September 2019 on the north and northwestern shelf of the Black Sea in the bottom sediments of river mouths - descriptive statistics.**

Indicator	June 2019					September 2019				
	No. of obs.	Ave r.	Max.	Min.	Std. Dev.	No. of obs.	Aver.	Max.	Min .	Std. Dev.
TPH (µg/g dry sed)	4	31.5	56	0	26.2	1	148			
Phenols (mg/kg dry sed)	3	0.9	1	0.7	0.1	4	1	1.4	0.8	0.3
Naphthalene (ng/g dry sed)	4	2.18	2.82	1.58	0.6	4	55.8	187	3.44	88.1
Acenaphthylene (ng/g dry sed)	4	2.93	6.21	0.22	2.99	4	14	17.8	10.8	3.64
Acenaphthene (ng/g dry sed)	4	0.52	1.51	0.08	0.68	4	4.19	9.95	0.05	4.29
Fluorene (ng/g dry sed)	4	1.7	5.85	0.12	2.77	4	31.6	106	1.89	49.8
Phenanthrene (ng/g dry sed)	4	8.47	20	0.8	8.9	4	63.1	136	19.3	50.5
Anthracene (ng/g dry sed)	4	13.8	52	0.17	25.5	4	7.75	14.8	0.05	6.43
Fluoranthene (ng/g dry sed)	4	4.55	10.9	0.22	4.7	4	22.1	45.9	11.6	16
Pyrene (ng/g dry sed)	4	3.97	8.87	0.1	3.83	4	19.4	40.7	9.12	14.9
Benzo[a]anthracene (ng/g dry sed)	4	4.09	5.43	3.13	1.11	4	10	21	5.46	7.41
Crysene (ng/g dry sed)	4	3.28	6.08	0.59	2.62	4	10.8	21.3	5.16	7.2
Benzo[b]fluoranthene (ng/g dry sed)	4	3.4	7.65	0	3.92	4	9.37	21.4	0	9
Benzo[k]fluoranthene (ng/g dry sed)	4	4.39	13	0.15	5.96	4	9.1	15.7	4.22	5.17
Benzo[a]pyrene (ng/g dry sed)	4	3.76	5.94	0.78	2.16	4	8.36	19.9	0	8.49
Benzo (g,h,i)perylene (ng/g dry sed)	4	3.19	5.73	0.12	2.69	4	10.5	20.6	4.76	7.01
Dibenzo(a,h)anthracene (ng/g dry sed)	4	4.35	9.14	0	3.74	4	6.88	16.2	2.47	6.45
Indeno(1,2,3-c,d)pyrene (ng/g dry sed)	4	3.73	7.51	0	4.26	4	16.2	28.5	7.3	8.98
HCB (ng/g dry sed)	4	2.39	9.56	0	4.78	4	0	0	0	0
α-HCH (µg/kg)	4	0	0	0	0	4	0	0	0	0
β-HCH (µg/kg)	4	0.1	0.41	0	0.21	4	1.43	5.59	0	2.77
Lindane (ng/g dry sed)	4	0.99	2.9	0.23	1.28	4	1.09	3.62	0	1.7
HCH total	4	1.09	3.31	0.23	1.48	4	2.52	5.59	0.22	2.6
Heptachlor (ng/g dry sed)	4	3.78	15.1	0	7.55	4	2.08	8.33	0	4.17
Aldrin (ng/g dry sed)	4	0	0	0	0	4	0.13	0.46	0	0.22
Dieldrin (ng/g dry sed)	4	4.81	13.8	0	6.52	4	12.7	50.8	0	25.4
p,p'DDE (ng/g dry sed)	4	0	0	0	0	4	8.18	32.7	0	16.4
p,p'DDD (ng/g dry sed)	4	2.09	8.36	0	4.18	4	1.68	5.02	0	2.37
p,p'DDT (ng/g dry sed)	4	31.9	83.2	7.19	35.7	4	26.2	93.6	1.51	45
DDT total	4	34	83.2	7.19	34	4	36.1	93.6	1.51	42.3
Atrazine (ng/g dry sed)	4	2.77	9.96	0	4.81	4	0.94	2.17	0	0.9
Dursban (ng/g dry sed)	4	33.5	115	1.09	54.6	4	11.2	21.4	2.03	8.24
AR-1254 (ng/g)	4	87.1	163	9.74	69.6	4	40.8	73.9	7.62	34.5
AR-1260 (ng/g)	4	4.27	9.93	1.15	3.99	4	1.3	3.52	0.1	1.52
PCB 8 (ng/g dry sed)	4	0.11	0.44	0	0.22	4	0	0	0	0
PCB 18 (ng/g dry sed)	4	1.42	2.3	0	1	4	0.79	3.16	0	1.58
PCB 31 (ng/g dry sed)	4	7.11	25.3	0	12.2	4	0.53	2.13	0	1.07
PCB28 (ng/g dry sed)	4	2.69	7.48	0	3.47	4	0.11	0.42	0	0.21
PCB52 (ng/g dry sed)	4	3.69	7.73	0	3.26	4	2.32	5.79	0.48	2.37
PCB 49 (ng/g dry sed)	4	0	0	0	0	4	0.71	1.63	0	0.84
PCB 44 (ng/g dry sed)	4	1.69	4.28	0	2.09	4	0.8	2.08	0	1
PCB 66 (ng/g dry sed)	4	10.4	15	0.67	6.56	4	7.74	20.9	1.99	8.96
PCB 77 (ng/g dry sed)	4	27.1	47.3	1.3	19.5	4	46.4	169	1.18	82
PCB101 (ng/g dry sed)	4	7.55	12.5	0.62	5.42	4	2.7	9.79	0	4.75
PCB 110 (ng/g dry sed)	4	0	0	0	0	4	0	0	0	0
PCB 149 (ng/g dry sed)	4	0.61	1.31	0	0.71	4	0.71	2.85	0	1.43
PCB118 (ng/g dry sed)	4	11.6	18.2	1.29	7.22	4	6.92	26.9	0	13.3
PCB153 (ng/g dry sed)	4	4.13	10.8	0.54	4.62	4	1.19	4.45	0	2.18
PCB 105 (ng/g dry sed)	4	6.54	10.9	0.25	4.51	4	20.7	82	0	40.8
PCB 187 (ng/g dry sed)	4	0.4	0.92	0	0.47	4	0.04	0.07	0	0.04
PCB 126 (ng/g dry sed)	4	0.56	0.9	0.15	0.32	4	0.28	0.62	0	0.26
PCB 128 (ng/g dry sed)	4	1.21	2.13	0	1.02	4	6.51	24.8	0	12.2
PCB 196 (ng/g dry sed)	4	0.2	0.61	0	0.29	4	0	0	0	0
PCB 206 (ng/g dry sed)	4	0	0	0	0	4	0	0	0	0
PCB138 (ng/g dry sed)	4	6.91	14.7	0.44	5.89	4	8.4	32.8	0	16.3
PCB 183 (ng/g dry sed)	4	0	0	0	0	4	0	0	0	0
PCB 174 (ng/g dry sed)	4	0	0	0	0	4	0	0	0	0
PCB 177 (ng/g dry sed)	4	0	0	0	0	4	0	0	0	0
PCB180 (ng/g dry sed)	4	1.62	4.38	0	1.92	4	0.7	1.96	0	0.86
PCB 170 (ng/g dry sed)	4	0.78	1.71	0.08	0.69	4	0.65	2.53	0	1.25
PCB 199 ng/g dry sed)	4	0	0	0	0	4	0	0	0	0
PCB 194 (ng/g dry sed)	4	0.73	1.57	0	0.69	4	0.08	0.14	0	0.06
PCB 209 (ng/g dry sed)	4	0	0	0	0	4	0	0	0	0

## Romania

**Table 7.18 - Summary statistics of heavy metals concentrations in sediments samples from the area under the influence of Danube May 2019**

	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Coef. Var.
Cu (µg/g)	19	32.072	32.680	17.390	50.130	24.180	36.450	29.531
Cd (µg/g)	19	0.575	0.518	0.203	1.345	0.431	0.658	42.515
Pb (µg/g)	19	8.086	8.020	3.480	13.750	7.110	9.430	33.720
Ni (µg/g)	19	63.003	62.300	37.570	83.360	54.590	72.840	20.522
Cr (µg/g)	19	31.024	31.550	13.080	48.950	23.210	37.370	28.937

**Table 7.19 - Summary statistics of organochlorine pesticides concentrations in sediment samples from the area under the influence of Danube, May 2019**

	No.of obs.	Mean	Median	Min.	Max.	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
HCB (ng/g dw)	19	1.76	0.30	0.30	24.13	0.30	3.33	5.46
Lindane (ng/g dw)	19	10.74	0.30	0.30	140.93	0.30	57.95	34.18
Heptachlor (ng/g dw)	19	2.36	0.78	0.20	28.50	1.37	2.87	6.38
Aldrin (ng/g dw)	19	2.58	0.20	0.20	45.29	0.20	0.37	10.34
Dieldrin (ng/g dw)	19	4.49	1.03	0.00	65.91	1.10	4.11	14.89
Endrin (ng/g dw)	19	0.20	0.20	0.20	0.20	0.20	0.20	0.00
p,p'DDE (ng/g dw)	19	0.42	0.30	0.28	1.42	0.39	0.78	0.28
p,p'DDD (ng/g dw)	19	16.38	0.94	0.20	258.44	1.36	35.91	59.17
p,p'DDT (ng/g dw)	19	0.20	0.20	0.20	0.20	0.20	0.20	0.00

**Table 7.20 - Summary statistics of polychlorinated biphenyls concentrations in sediment samples from the area under the influence of Danube, May 2019**

	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
PCB28 (ng/g dw)	19	45.93	3.72	0.40	188.60	89.64	132.68	58.17
PCB52 (ng/g dw)	19	10.32	0.30	0.30	156.84	0.30	21.08	35.90
PCB101 (ng/g dw)	19	0.60	0.60	0.60	0.60	0.60	0.60	0.00
PCB118 (ng/g dw)	19	23.68	0.40	0.40	156.32	0.40	150.85	55.35
PCB153 (ng/g dw)	19	0.60	0.60	0.60	0.60	0.60	0.60	0.00
PCB138 (ng/g dw)	19	22.15	0.70	0.70	379.79	0.70	29.16	86.85
PCB180 (ng/g dw)	19	0.30	0.30	0.30	0.30	0.30	0.30	0.00

**Table 7.21 - Summary statistics of total petroleum hydrocarbons and polyaromatic hydrocarbons concentrations in sediment samples from the area under the influence of Danube, May 2019**

	No. of obs.	Mean	Median	Min.	Max.	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
TPH (µg/g dry sed)	19	73.527	64.762	33.292	150.422	97.969	130.099	33.079
Naphthalene (ng/g dry sed)	19	16.301	17.280	1.860	24.660	19.820	23.730	5.920
Acenaphthylene (ng/g dry sed)	19	0.516	0.100	0.100	3.128	0.566	2.435	0.873
Acenaphthene (ng/g dry sed)	19	0.404	0.100	0.100	2.394	0.397	1.851	0.667
Phenanthrene (ng/g dry sed)	19	75.442	47.684	0.100	373.435	84.994	257.546	98.379
Crysene (ng/g dry sed)	19	5.700	0.100	0.100	106.493	0.100	0.100	24.408
Dibenzo(a,h)anthracene (ng/g dry sed)	19	0.288	0.100	0.100	1.312	0.425	1.139	0.370
S16 PAH (ng/g dry sed)	19	98.340	76.060	12.145	388.117	109.834	276.176	95.711

Turkey

**Table 7.22 - Summary statistics of heavy metals concentrations in sediments samples from the area under the influence of Sakarya and Yeşilirmak, June 2019**

	As	Cd	Cr	Cu	Ni	Pb	Zn	Hg
<b>Sakarya River (µg/g dry sediment) (N=12)</b>								
mean	13.27	0.16	121.82	43.69	89.96	16.21	87.12	0.03
Std.dev.	3.41	0.05	44.10	16.92	34.39	3.39	26.12	0.01
Min.	7.84	0.08	36.35	22.77	32.61	9.59	38.62	0.01
Max.	18.50	0.26	202.69	74.09	158.56	20.84	142.26	0.06
25 <sup>th</sup> Percentile	10.20	0.14	109.80	26.94	66.66	13.96	69.04	0.02
75 <sup>th</sup> percentile	15.25	0.18	132.22	54.11	105.36	18.82	97.06	0.03
<b>Yeşilirmak River (µg/g dry sediment) (N=15)</b>								
mean	15.09	0.24	207.05	124.99	152.11	24.92	121.60	0.77
Std.dev.	1.59	0.13	67.85	40.95	24.18	9.03	22.49	0.56
Min.	11.83	0.11	150.31	66.88	122.38	17.43	96.06	0.12
Max.	18.28	0.58	358.92	196.68	216.19	52.26	184.42	1.79
25 <sup>th</sup> Percentile	14.17	0.14	158.49	97.79	137.56	18.60	108.10	0.33
75 <sup>th</sup> percentile	16.10	0.29	231.08	138.53	157.92	28.71	130.62	1.06

**Table 7.23 - Summary statistics of organochlorine pesticides concentrations in sediment samples from the area under the influence of Sakarya River and Yeşilirmak River, July 2019**

	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
<b>Sakarya River</b>								
α-HCH (ng/g dw)	11	0.051	0.050	0.050	0.056	0.050	0.050	0.002
β-HCH (ng/g dw)	11	0.154	0.134	0.050	0.417	0.077	0.198	0.104
Lindane (ng/g dw)	11	0.109	0.050	0.050	0.267	0.050	0.172	0.086
p,p'DDE (ng/g dw)	11	0.448	0.180	0.029	1.795	0.035	0.406	0.673
p,p'DDD (ng/g dw)	11	0.390	0.094	0.050	1.809	0.050	0.445	0.555
p,p'DDT (ng/g dw)	11	0.078	0.050	0.050	0.353	0.050	0.050	0.091
<b>Yeşilirmak River</b>								
α-HCH (ng/g dw)	15	0.082	0.082	0.050	0.166	0.050	0.096	0.033
β-HCH (ng/g dw)	15	0.243	0.274	0.045	0.454	0.077	0.351	0.133
Lindane (ng/g dw)	15	0.101	0.050	0.050	0.500	0.050	0.050	0.138
p,p'DDE (ng/g dw)	15	1.499	1.483	0.229	3.912	0.475	2.119	0.953
p,p'DDD (ng/g dw)	15	2.107	1.204	0.285	7.714	0.581	3.638	2.199
p,p'DDT (ng/g dw)	15	0.184	0.050	0.050	1.590	0.050	0.075	0.399

**Table 7.24 - Summary statistics of polychlorinated biphenyls concentrations in sediment samples from the area under the influence of Sakarya River and Yeşilirmak River, July 2019**

	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
<b>Sakarya River</b>								
PCB28 (ng/g dw)	11	0.020	0.017	0.008	0.034	0.013	0.029	0.010
PCB52 (ng/g dw)	11	0.022	0.020	0.007	0.065	0.010	0.027	0.017
PCB101 (ng/g dw)	11	0.025	0.018	0.008	0.081	0.012	0.032	0.021
PCB118 (ng/g dw)	11	0.026	0.015	0.010	0.068	0.012	0.043	0.019
PCB153 (ng/g dw)	11	0.034	0.023	0.010	0.097	0.010	0.046	0.028
PCB138 (ng/g dw)	11	0.028	0.010	0.010	0.101	0.010	0.030	0.036
PCB180 (ng/g dw)	11	0.016	0.010	0.010	0.042	0.010	0.012	0.013
<b>Yeşilirmak River</b>								
PCB28 (ng/g dw)	15	0.041	0.039	0.015	0.067	0.025	0.052	0.016
PCB52 (ng/g dw)	15	0.028	0.029	0.014	0.046	0.022	0.032	0.008
PCB101 (ng/g dw)	15	0.040	0.040	0.017	0.060	0.028	0.051	0.013
PCB118 (ng/g dw)	15	0.044	0.036	0.016	0.085	0.027	0.059	0.021
PCB153 (ng/g dw)	15	0.061	0.044	0.015	0.128	0.030	0.096	0.037
PCB138 (ng/g dw)	15	0.057	0.047	0.010	0.116	0.010	0.103	0.043
PCB180 (ng/g dw)	15	0.021	0.013	0.004	0.045	0.010	0.033	0.014

**Table 7.25 - Summary statistics of total petroleum hydrocarbons and polyaromatic hydrocarbons concentrations in sediment samples from the area under the influence of Sakarya River and Yeşilirmak River, July 2019**

Descriptive Statistics	No.of obs.	Mean	Median	Minimum	Maximum	Percentile 25 <sup>th</sup>	Percentile 75 <sup>th</sup>	Std. Dev.
<b>Sakarya River</b>								
Naphthalene (ng/g dw)	12	5.82	4.89	1.79	18.87	2.30	5.94	4.88
Acenaphthylene (ng/g dw)	12	0.50	0.34	0.19	1.48	0.24	0.50	0.43
Acenaphthene (ng/g dw)	12	0.17	0.12	0.10	0.41	0.10	0.23	0.09
Fluorene (ng/g dw)	12	1.76	1.40	0.63	5.26	1.14	1.89	1.28
Phenanthrene (ng/g dw)	12	13.50	10.04	5.71	38.68	8.34	16.26	8.79
Anthracene (ng/g dw)	12	0.73	0.49	0.09	2.58	0.33	0.86	0.72
Fluoranthene (ng/g dw)	12	7.47	5.80	2.20	20.45	3.41	9.88	5.55
Pyrene (ng/g dw)	12	6.13	4.32	1.52	17.56	2.55	8.30	4.79
Benzo[a]anthracene (ng/g dw)	12	2.00	1.57	0.20	5.91	0.55	2.60	1.81
Crysene+Triphenylene (ng/g dw)	12	4.08	2.96	0.57	15.31	1.14	4.60	4.27
Benzo[b]fluoranthene (ng/g dw)	12	5.61	4.17	0.78	14.62	1.71	7.92	4.88
Benzo[k]fluoranthene (ng/g dw)	12	2.04	1.49	0.30	5.55	0.55	2.85	1.82
Benzo[a]pyrene (ng/g dw)	12	2.18	1.76	0.20	6.29	0.51	3.01	1.98
Benzo (g.h.i) perylene (ng/g dw)	12	4.13	2.80	0.43	12.14	1.26	5.99	3.75
Dibenzo (a.h)anthracene (ng/g dw)	12	0.58	0.39	0.05	1.80	0.15	0.87	0.54
Indeno (1.2.3-c.d)pyrene (ng/g dw)	12	3.32	2.32	0.30	10.66	0.90	5.25	3.08
PAH total (ng/g dw)	12	59.96	43.32	19.41	168.52	29.81	71.57	45.88
TPH (µg/g dw)	12	2.90	1.94	0.27	8.96	0.74	3.76	3.03
<b>Yeşilirmak River</b>								
Naphthalene (ng/g dw)	15	2.32	1.01	0.13	9.37	0.13	3.59	2.81
Acenaphthylene (ng/g dw)	15	0.38	0.38	0.08	0.79	0.20	0.49	0.19
Acenaphthene (ng/g dw)	15	0.25	0.26	0.12	0.39	0.15	0.33	0.09
Fluorene (ng/g dw)	15	2.72	3.04	0.92	4.14	1.80	3.43	1.05
Phenanthrene (ng/g dw)	15	16.57	17.36	8.82	26.43	11.93	19.66	4.71
Anthracene (ng/g dw)	15	1.07	1.15	0.23	2.39	0.39	1.49	0.62
Fluoranthene (ng/g dw)	15	10.55	10.03	3.00	23.19	4.06	15.50	6.07
Pyrene (ng/g dw)	15	8.27	7.89	2.81	18.32	3.40	11.92	4.72
Benzo[a]anthracene (ng/g dw)	15	2.64	2.30	0.33	6.81	0.54	4.16	2.02
Crysene+Triphenylene (ng/g dw)	15	7.07	6.42	1.81	14.44	2.79	10.54	3.91
Benzo[b]fluoranthene (ng/g dw)	15	10.76	9.67	1.98	28.77	3.20	16.38	7.54
Benzo[k]fluoranthene (ng/g dw)	15	2.97	2.27	0.37	9.82	0.60	4.96	2.65
Benzo[a]pyrene (ng/g dw)	15	3.60	3.06	0.37	9.76	0.70	5.88	2.91
Benzo (g.h.i) perylene (ng/g dw)	15	7.01	5.61	0.95	21.35	1.38	11.04	5.88
Dibenzo (a.h) anthracene (ng/g dw)	15	0.98	0.75	0.01	2.58	0.26	1.72	0.77
Indeno(1.2.3-c.d)pyrene (ng/g dw)	15	5.95	4.00	0.55	20.71	0.94	9.87	5.78
PAH total (ng/g dw)	15	83.06	76.01	24.77	196.88	32.50	120.05	49.36
TPH (µg/g dw)	15	3.65	3.39	0.41	8.11	1.02	5.66	2.52



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