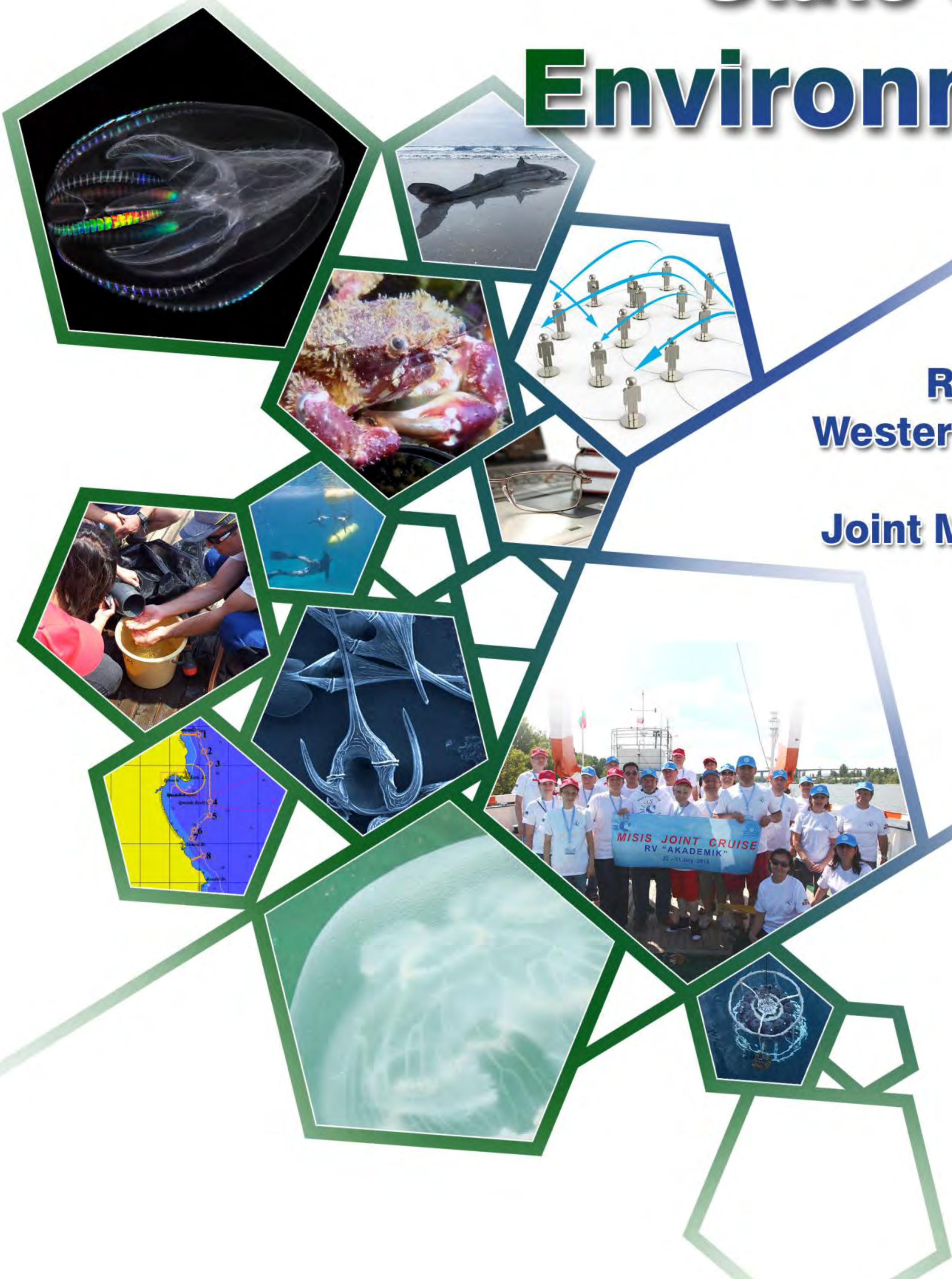
State of the Environment



Report of the Western Black Sea based on Joint MISIS cruise

State of Environment Report of the Western Black Sea based on Joint MISIS cruise

This report is based on the activities of the MISIS project (MSFD Guiding Improvements in the Black Sea Integrated Monitoring System) with the financial support from the EC DG Env. Programme "Preparatory action – Environmental monitoring of the Black Sea Basin and a common European framework programme for development of the Black Sea region/Black Sea and Mediterranean 2011".

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For bibliographic purposes this document should be cited as:
MISIS Joint Cruise Scientific Report, 2014. “State of Environment Report of the Western Black Sea based on Joint MISIS cruise” (SoE-WBS), Moncheva S. and L. Boicenco [Eds], Ed. ExPonto, 401 pp.

Design and layout: Tudor Fulga
Number of pages: 401
ISBN: 978-606-598-367-0

Contents

Abbreviations

A/H	Autotrophs/Heterotrophs
ALLO	Alloxanthin
AMBI	AZTI Marine Biotic Index
B(a)P_{eqv}	Total equivalent of toxicity by benzo(a) pyrene
BaP	Benzo(a)pyrene
BEAST	Black Sea Eutrophication ASsessment Tool
BG –	Bulgaria
BG IAR	Initial Assessment Report of Bulgaria
BOD	Biological Oxygen Demand
BS SAP	Black Sea Strategic Action Plan
BSBD	Black Sea Basin Directorate
BSC	Black Sea Commission
BG	Bulgaria
CB%	Biomass of copepods
CBD AG	Advisory Group on Conservation of Biological Diversity
CCA	Canonical correspondence analysis
Chl a	Chlorophyll a
CIL	Cold Intermediate Layer
CO	Coastal
COD	Chemical Oxygen Demand
CPAHs	Carcinogenic poly aromatic hydrocarbons
CTD	Conductivity Temperature Depth
D1	Descriptor 1 Biodiversity
D2	Descriptor 2 Non –Native species
D6	Descriptor 6 Sea floor integrity
DA	Domoic acid
DCM	Deep Chlorophyll Maximum
DDF%	Dominance after density of foraminiferans %
DDN%	Dominance after Density of Nematoda %
DE%	Proportion of r strategy dinoflagellates
DIADINO	Diadinoxanthin
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphorus
DSP	Diarrhetic shellfish poisoning
DTX1	Dinophysistoxin 1
DTXs	Dinophysis toxins
EAC	Environmental Assessment Criteria
EACs	Environmental Assessment Concentrations
EC	European Commission
EcoQ	Ecosystem Quality Objective
EcoQS	Ecological Quality State
ERL	Effect Range – Low
ESD	Equivalent Spherical Diameter
EU	European Union
EU MSFD	European Union Marine Strategy Framework Directive
EUNIS	European Nature Information System

FAO	Food and Agriculture Organization
FUC	fucoxanthin
G&R	Gaps and Recommendations
GC	ECD – Gas chromatography – electron capture detector
GCMS	Gas chromatography – mass spectrometer
GEnS	Good Environmental Status
GEOECOMAR/ GEM	National Institute Research Development for Marine Geology and Geoecology
GES	Good Environmental Status
GF	AAS – Graphite furnace – atomic absorption spectrometry
GTXs	Gonyautoxins;
H’	Shannon -Wiener community diversity index,
HAB –	Harmful algal blooms
HEX	19’Hexonoyloxyfucoxanthin
HM	Heavy metals
HMW	High Molecular Weight
HPLC	Hugh Pressure Liquid Chromatography
HS	Hot Spot
IA	Initial Assessment
IAR	Initial Assessment Report
ICC	International Coastal Clean up
IMO	International Maritime Organization
IO- BAS	Institute of Oceanology – Bulgarian Academy of Sciences
IOC UNES	Intergovernmental Oceanographic Commission
K -W	Statistical test Kruskal- Wallis
L	Left
LBS	Land Based Sources
LMW	Low Molecular Weight
M	AMBI – multivariate AMBI
MEC%	Microflagellate, Euglenophyceae, Cyanophyceae
ML –	Marine litter
µM	micromoles per cubic centimetres
MSFD	Marine Strategy Framework Directive
MVSP	Multi Variate Statistical Package
M- W	Statistical test Mann- Whitney
N	Number of samples
N.sci%	Noctiluca scintillans biomass
N/P	Nitrogen to Phosphorus ratio
NGO	Non governmental organization
NIMH	Institute of Hydrology and Meteorology
NIMRD	National Institute for Marine Research and Development
NIS	Non indigenous species
NSI	National Statistical Institute
NW	North West
O	Open sea
OA	Okadaic acid
OCP	Organo chlorine pesticides
ODV	Ocean Data View
OOAO	One out - All out principle: the worst assessment of a quality element determines the overall assessment result

OSPAR	East Atlantic Convention for the Protection of the Marine Environment
PAH	Poly- aromatic hydrocarbons
PCB	Poly - chlorinated biphenyls
PER	Peridinin
PFTs	Functional Phytoplankton types
POPs	Persistent organic pollutants
PSP	Paralytic shellfish poisoning
PTP	Potentially toxic phytoplankton species
PTXs	Pectenotoxins;
R	Right
RAMSAR	Convention on Wetlands of International Importance
RO	Romania
ROV	Remote Operational Vehicle
SAP	Strategic Action Plan
SH	Shelf
Si/N	Silicon -Nitrogen ratio
Si/P	Silicon -Phosphorus ratio
SigT	Measure of the density of seawater at a given temperature
SINOP	University SINOP
S	Species richness
Std.Dev.	Standard Deviation
TE	Toxicity equivalent
TNOx	Sum of Nitrite and Nitrate
TOC	Total organic carbon
TPH	Total petroleum hydrocarbons
TR	Turkey
UNEP	United Nations Environment Programme
US EPA	United States Environment Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization
WoRMS	World Register of Marine Species
WQ	Water Quality
WW	Waste Waters
WWTP	Waste Water Treatment Plant
YTX	Yessotoxin
ZEA	Zeaxanthin
β Car	β carotin

Executive summary

The "State of Environment Report of the Western Black Sea based on Joint MISIS cruise" (SoE-WBS) has been prepared under the MISIS Project 'MSFD Guiding Improvements in the Black Sea Integrated Monitoring System (**www.misisproject.eu,** EC DG Env. Project MISIS: No.07.020400/2012/616044/SUB/D2.) The Project is financed by EC as an activity under the EC DG Env. Programme 'Preparatory action – Environmental monitoring of the Black Sea Basin and a common European framework programme for development of the Black Sea region/Black Sea and Mediterranean 2011'. MISIS is an integral part of the overall ongoing process of harmonization of Black Sea region policy, in compliance to relevant European policy in the field of marine environment protection.

According to the Marine Strategy Framework Directive (MSFD) all member states have to achieve Good Environmental Status (GEnS) on a regional Seas basis by 2020 (EC, 2008[1]). Targets for GEnS for each of the 11 Descriptors[2] of environmental status should have been set by each member state by July 2012, and programmes of measures to achieve these targets are to be put in place by 2015. The descriptors of GEnS are further refined in the commission decision on Descriptors (EC, 2010[3]).

The directive mandates that existing regional seas agreements be used to implement these environmental objectives. Many of the MSFD descriptors are interrelated and overlapping (e.g. fish resources , food web structure, eutrophication, biodiversity, non-indigenous species and pollution) with the 4 strategic ecological quality objectives (ECOQs) set by the Black Sea Strategic Action Plan adopted in 2009 (BSSAP'2009[4]). Although the ECOQs are broader and less specific there is a clear potential synergy between the MSFD and BSSAP.

In the frame of MSFD implementation roadmap, MISIS Project aimed to assist the Member States of the Black Sea (Bulgaria, Romania, including

[1] EC 2008a Directive number 56 of 2008, Official Journal of 17 June 2008

[2] 11 descriptors for GES of MFSD: 1-Biological diversity, 2-Non-indigeneous sps, 3-Commercial fish and shellfish, 4-Food webs, 5-Eutrophication, 6-Sea-floor integrity, 7-Hydrographical conditions, 8-Contaminants and effects, 9-Contaminants in sea food, 10-Marine litter, 11-Energy and underwater noise.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:164:0019:0040:EN:PDF

[3]Commission Decision of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters. EC 2010 Decision number 477 of 2010, Official Journal of 2nd September 2010

[4] Strategic Action Plan for the Environmental Protection and Rehabilitation of the Black Sea Adopted in Sofia, Bulgaria, 17 April 2009

Turkey) to improve their national monitoring systems and to contribute to the development of a target oriented integrated monitoring system for the Black Sea.

One of the core activity of MISIS Project towards supporting the on-going process for revision of monitoring programmes stipulated in the MSFD due in 2014 for the EU-member states at national and regional Black Sea level, according to the Project DoW - PA2: "Initial testing of the revised monitoring programmes (field and laboratory work), management of data & assessments" includes:

- Organization of Joint Black Sea Survey for collecting additional data and producing homogenous data sets for the Black Sea based on a single sampling procedure and laboratory analysis of specified determinants and biological quality elements
- Organizing inter-comparison exercises to evaluate the performance of laboratories involved
- Screening for new priority pollutants
- Carrying out ecological assessment of the Black Sea, taking into consideration the requirements in the WFD and the descriptors of the MSFD

The "State of Environment Report of the Western Black Sea based on Joint MISIS cruise" was produced by the collective contribution of scientist from MISIS partner institutes, under the coordination of IO-BAS as MISIS PA2. Deliverable.

The time/duration of the cruise, polygons and parameters, methodology of data acquisition and processing and indicators for GEnS assessment were selected in compliance and relevance to MSFD and BSSAP'2009, aligned to the findings and recommendations formulated in the Diagnostic Report II "Guiding improvements in the Black Sea integrated monitoring system (including capacity building and utilization of equipment), data management, and assessments" [5] prepared in the first phase of the Project.

The Joint cruise, conducted between 22 -30 July, 2013 on board RV Akademik, comprised 3 sampling polygons and 18 stations, selected so as to cover coastal, shelf and open sea pelagic habitats and similar benthic habitats of each partner country, defined according to the Initial Assessment Reports (IAR) of Bulgaria and Romania, and comparable approach followed arbitrary for the transect in front of Turkey – Fig.1.

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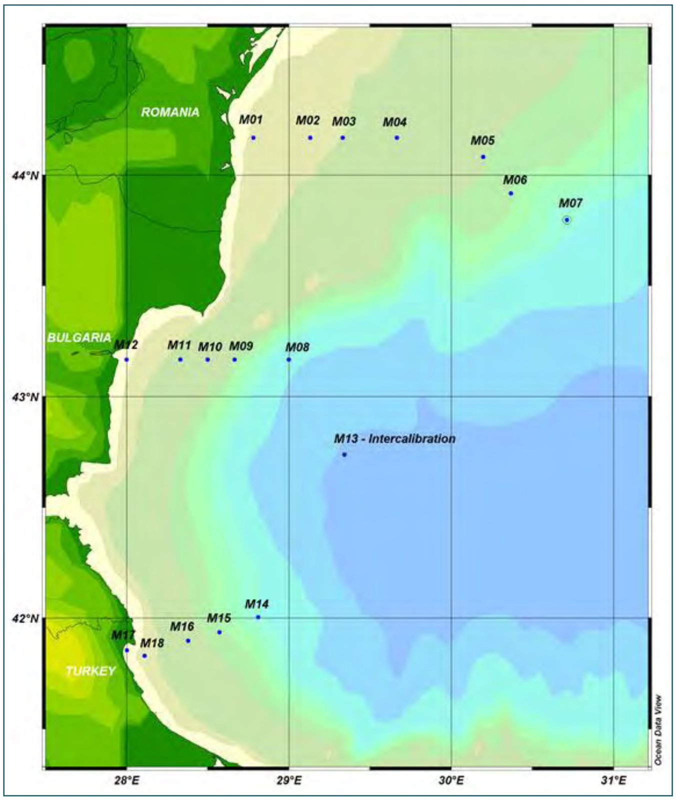
[5] Velikova V., Boicenco L., Beken-Polat C., Moncheva S., Levent B., Sezgin M., Begun T., Oros A. 2013. Diagnostic Report II guiding improvements in the Black Sea monitoring system. EC DG Env. MISIS Project Deliverables. http://misisproject.eu/



Habitat	RO	BG	TR
Costal	M1	M12	M18
Shelf	M2, M3, M4, M5	M9, M10, M11	M15, M16, M17
Open Sea	M6, M7	M8, M13	M14

Figure 1. Map of sampling stations of MISIS Joint cruise and distribution by habitats.

Summary of stations coordinates and depths is presented on Table 1.

Table 1. Stations coordinates and depths.

Stations	Date	Longitude [degrees_E]	Latitude [degrees_N]	St.Depth [m]	Sampling Depth[m]
MO1	23.07.2013	28.7833	44.1667	33	0, 14, 32
MO2	23.07.2013	29.1333	44.1667	47	0,5,16,46
MO3	23.07.2013	29.3333	44.1667	54	0,8,25,52
MO4	24.07.2013	29.6667	44.1667	65	0,15,40,63
MO5	24.07.2013	30.1983	44.0800	100	0,16,41,76,96
MO6	24.07.2013	30.3683	43.9167	500	0,15,42,87
MO7	25.07.2013	30.7150	43.7967	1000	0,16,46,87,113
MO8	27.07.2013	29.0000	43.1667	1167	0,12,39,65,85,107
MO9	27.07.2013	28.6667	43.1667	92.7	0,21,43,65,90
MO10	26.07.2013	28.5000	43.1667	76.1	0,11,25,50,74
MO11	26.07.2013	28.3333	43.1667	39.9	0,15,25,38
MO12	26.07.2013	28.0000	43.1667	23.2	0,12,22
MO13	28.07.2013	29.3433	42.7372	2015.5	0,14,43,49
MO14	29.07.2013	28.8129	42.0042	1118	0,19,65,100
MO15	29.07.2013	28.5725	41.9362	101	0,20,34,74,96
MO16	29.07.2013	28.3795	41.8972	75.6	0,24,65,74
MO17	30.07.2013	28.0043	41.8539	53	0,21,50
MO18	30.07.2013	28.1129	41.8299	25	0,16,25

Additional criteria for the selection of sampling stations was the intensity of human pressure based on information from the IARs.

In order to achieve max harmonization of field and laboratory methods an inventory of in-house routines was performed prior to the cruise, while in order to evaluate the performance of partners labs with the aim to assure that the results of the analysis could be statistically correctly combined in one data set, an intercalibration exercise was completed at two stations (M18 and M13) during the cruise for the chemical and biological parameters (*see Annexes for details*).

An impressive number of water and sediment physical, chemical (including pollutants) and biological samples (1246), related to 125 parameters were measured during the cruise (Fig.2) of relevance for indicator based assessment of the WBS environmental status.

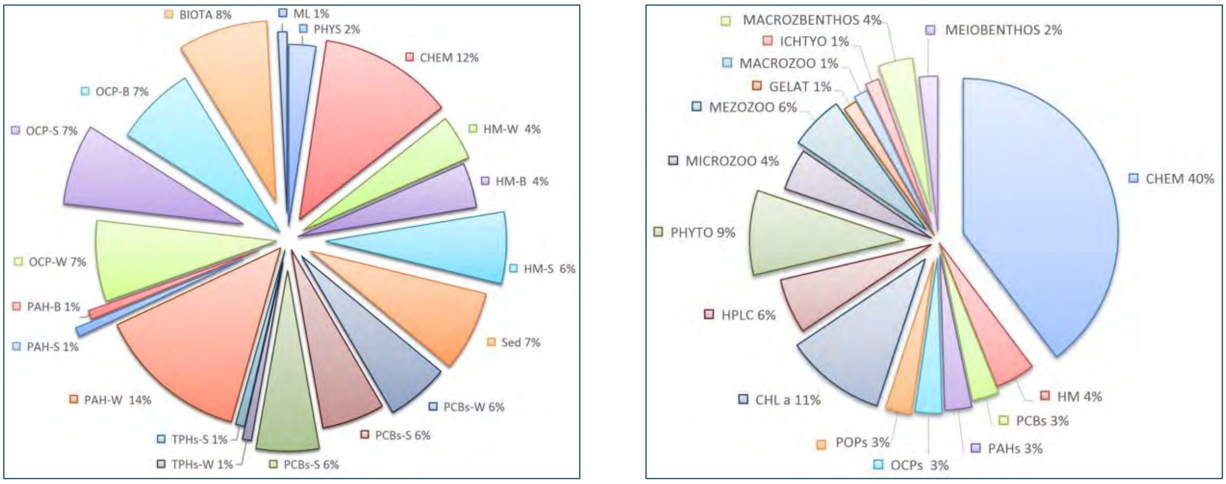


Figure 2. Number of parameters (left) and number of samples (right)

PHYSICS	CHEM	HM-W	HM-B	HM-S	Sediments	PCBs-W	PCBs-S	PCBs-B	TPHs-W	TPHs-S	PAH-W	PAH-S	PAH-B	OCP-W	OCP-S	OCP-B	BIOTA
Secchi [m]	pH	Cu	Cu	Ni (µg/g)	CaCO3 (%)	PCB28	PCB28	PCB28			PAH total	PAH total	PAH total	p,pDDT	p,pDDT	p,pDDT	chlorophyll a
T [oC]	O_2 [µM]	Cd	Cd	MnO (%)	TOC (%)	PCB52	PCB52	PCB52			Naphthalene (µg/L)			p.pDDD	p.pDDD	p.pDDD	HPLC
S [PSU]	$(PO_4)^{3-}$[µM]	Pb	Pb	Cr (µg/g)	Fe2O3 (%)	PCB101	PCB101	PCB101			Acenaphthylene (µg/L			p,pDDE	p,pDDE	p,pDDE	phytoplankton
	TP [µM]	Ni	Ni	V (µg/g)	TiO2 (%)	PCB118	PCB118	PCB118			Acenaphthene (µg/L)			Aldrin	Aldrin	Aldrin	microzooplankton
	$(SiO_4)^{4-}$[µM]	Cr	Cr	Co (µg/g)	Zr (µg/g)	PCB153	PCB153	PCB153			Fluorene			Dieldrin	Dieldrin	Dieldrin	mezozooplankton
	$(NO_2)^{-}$[µM]			Pb (µg/g)	Ba (µg/g)	PCB138	PCB138	PCB138			Phenanthrene			Endrin	Endrin	Endrin	gelatinous
	(TNO_x) [µM]			Cu (µg/g)	Sr (µg/g)	PCB180	PCB180	PCB180			Anthracene (µg/L)			HCB	HCB	HCB	macrozooplankton
	$(NO_3)^{-}$[µM]			Cd (µg/g)	Rb (µg/g)						Phenanthrene (µg/L)			Lindane	Lindane	Lindane	ichtioplankton
	$(NH_4)^{+}$ [µM]				Zn (µg/g)						Benzo[a]anthracene (µg/L)			Heptaclor	Heptaclor	Heptaclor	macrozoobenthos
	TSS [mg/L]										Fluoranthene						meiobenthos
	NPOC [mg/l]										Benzo(b)fluoranthen, µg.dm-3						
	TN [µM]										Benzo(k)fluoranthen, µg.dm-3						
	DIN [µM]										Benzo(a)pyren µg.dm-3						
	H2S										Ideno(1,2,3-c,d)pyren, µg.dm-3						
	TOC										Benzo(g,h)perylen, µg.dm-3						
											Crysene						
											Pyrene						

Most of the indicators applied in the SoE-WBS originate from the IARs of Bulgaria and Romania, some of them were discussed and agreed during the Joint AG CBD – MISIS Project meeting organized by the Black Sea Commission in Istanbul in 2013 and in addition a number of new potential indicators were tested (functional phytoplankton groups, size structure and potentially toxic species, Shannon 95 biodiversity index; microzooplankton and meiobenthos related indicators etc). For the first time bottom marine litter was quantified in the Black Sea (at 3 coastal and 3 shelf stations) following the MSFD GES TSG-ML Guidelines[6]; for the first time the BEAST tool[7] was applied for integrated assessment of eutrophication status at WBS basin scale, this is the first synchronized assessment of pollutants at WBS scale.

SoE-WBS is organized in eight Chapters. The General Hydrographic conditions of the WBS and the specific Hydrographic conditions during the cruise are discussed in Chapter I. Out of the 11 Descriptors of MSFD, indicator based assessments of environmental status are provided for 7 descriptors with dedicated Chapters: Chapter II. Biodiversity (D1) and Habitat Integrity (D6) covering the biological components phytoplankton, zooplankton and zoobenthos; Chapter III. Non-indigenous species (D2); Chapter IV. Eutrophication (D5); Chapter V. Contaminants (D8); Chapter VI. Contaminants in biota (D9) and Chapter VII. Marine Litter. Special Chapter is dedicated to the formulation of Gaps and Recommendation (Chapter VIII) stemming from the analysis by descriptors.

Although not exhaustive (data from a single cruise only), as the first report based on harmonized indicators the SoE-WBS is expected to contribute to the improvement of national monitoring programs in Bulgaria, Romania and Turkey in compliance to MSFD implementation, as well as assist the Black Sea Commission in the effort to develop integrated monitoring system for the Black Sea at basin-wide scale.

Acknowledgements. The MISIS team would like to thank the Project coordinators of EMBLAS (Violeta Velikova - scientific advisor), PERSEUS (Vangelis Papathanassiou), DEVOTES (Angel Borja), IRIS-SES (Popi Pagou) for sharing experience and results and their valuable contribution to the formulation of the Gaps and Recommendations, the Black Sea Commission for the support of the Project, the technicians for analyzing the numerous samples, the Captain and Crew of RV "Akademik "for the technical assistance during the cruise.

[6] MSFD GES Technical Subgroup on Marine Litter (TSG-ML), 2013. Monitoring Guidance for Marine Litter in European Seas. DRAFT REPORT, July 2013. https://circabc.europa.eu/sd/a/b627cfb6-cece-45bc-abc1-e4b3297adb91/DRAFT%20MSFD%20Monitoring%20Guidance%20TSG-ML%2011072013.pdf (accessed January 22, 2014).

[7] BEAST - Black Sea Eutrophication assessment Tool developed in the frame of Baltic2Black project based on the HELCOM Eutrophication Assessment Tool (HEAT 2.0)

Prof. Snejana Moncheva
Dr. Laura Boicenco

I. Hydrographic description

Completed by:
Maria-Emanuela Mihailov, Anatoli Apostolov

List of figures

INTRODUCTION

General hydrographic description of the Black Sea

The Black Sea, located between 28° - 42°E longitudes and 41° - 46°N latitudes, is about 2000m deep basin with zonal and meridional dimensions of ~1000 km and ~400 km, respectively (Fig. I.1.1). Semi-enclosed mid-latitude marginal sea, it has only a narrow opening to the shallow Bosporus Strait (less than 75m deep) restricting exchange with the Mediterranean Sea. Characterized by a flat abyssal plain, with a maximum depth of about 2200 m in the central area, the continental shelf varying offshore extension between 5 km (off the eastern Turkish and Caucasian coasts) to near 200 km in the northwestern area.

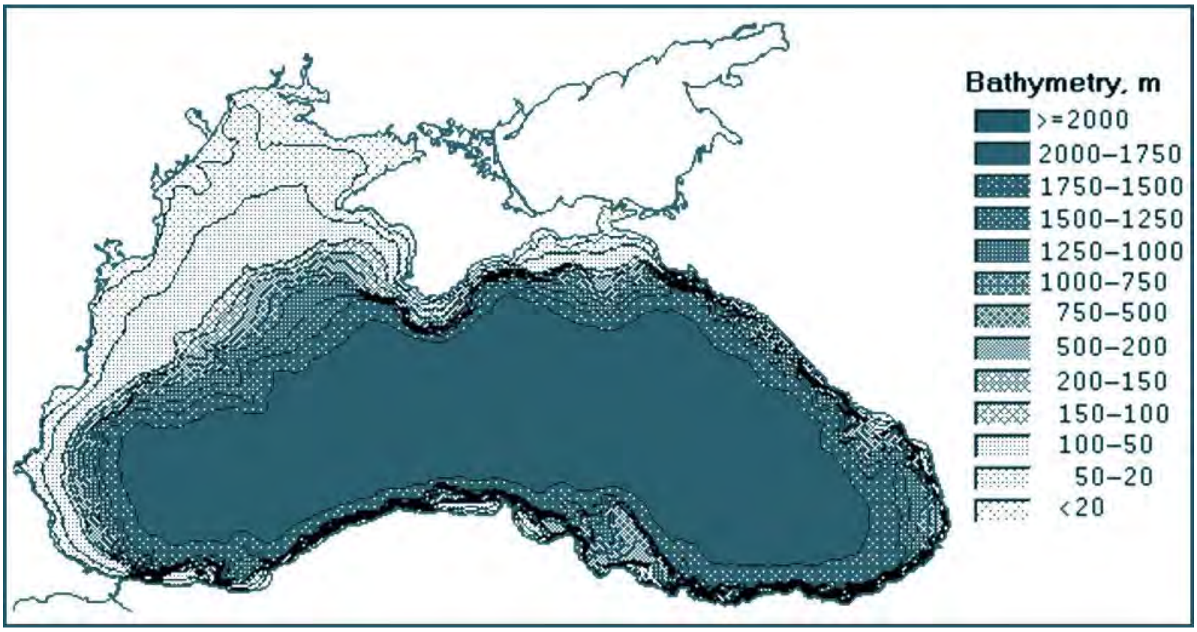


Figure I.1.1. Black Sea bathymetry
http://www.grid.unep.ch/bsein/images/bs_bathy.gif

Unlike the Mediterranean, the Black Sea is an estuarine type basin due to the large river discharge, especially on its northwestern shelf area. According to the observations performed during 1860-1989, the annual Danube River flow varied from 4×10^3 to 9×10^3 m^3/s (Bondar, 1989) and, according to Tomalzin (1985) and Ragueneau et al. (2002), the Danube's water influx is approximately 70 % of total freshwater load to the Black Sea. In addition, this flow undergoes substantial inter-annual fluctuations. The Danube's inflow is an important variable of the hydrologic and biologic evolution on the western Black Sea shelf.

Due to the rivers discharge, the northwestern Black Sea superior quasi-homogenous layer (SQL) is characterized by low salinity (generally between

16.0 – 18.0 PSU) compared with the Mediterranean Sea (~38.0 PSU). The time needed for low salinity waters to be transported from the Danube River mouths to the Bosphorus Strait lies in the range of 1-2 months. The low salinity surface waters of riverine origin overlay salty deep waters of Mediterranean origin, along the Black Sea basin (Poulain et al., 2005).

According to Ozsoy and Unluata (1997), the outflow of the Black Sea water through the Bosphorus Strait equals to 20×10^3 m^3/s, in contrast with the inflow of the Marmara Sea water estimated to be 10×10^3 m^3/s. The exchange flows at any instant of time greatly differ from these estimates, as a result of the time-dependent meteorological and hydrological forcing originating from the adjacent basins.

Black Sea circulation

The commonly assumed scheme of the Black Sea general circulation (Knipovich, 1933; Neumann, 1942; Bogatko et al., 1979; Ovchinnikov and Titov, 1990; Altman et al., 1990; Oguz et al., 1993; Titov, 1999; Korotaev et al., 2001, 2003) includes a cyclonic basin-scale boundary current flowing along the continental slope (the Main Black Sea Current), western and eastern cyclonic gyres in the open sea, and near-shore anticyclonic eddies (NAEs) between the Rim Current and the shore (Fig. I.1.2).

The Rim Current is driven mainly by the mean cyclonic wind pattern that prevails over the sea and by strong buoyancy input (Stanev, 1990; Oguz et al., 1995) and is locked to the steep continental slope with a 40–80 km wide slope current (Korotaev et al., 2001). Changes in bottom slope and coastline orientation along the Turkish and Caucasian coasts generate Rim Current meanders and instability features on a wide range of space and time scales (Oguz et al., 1993).

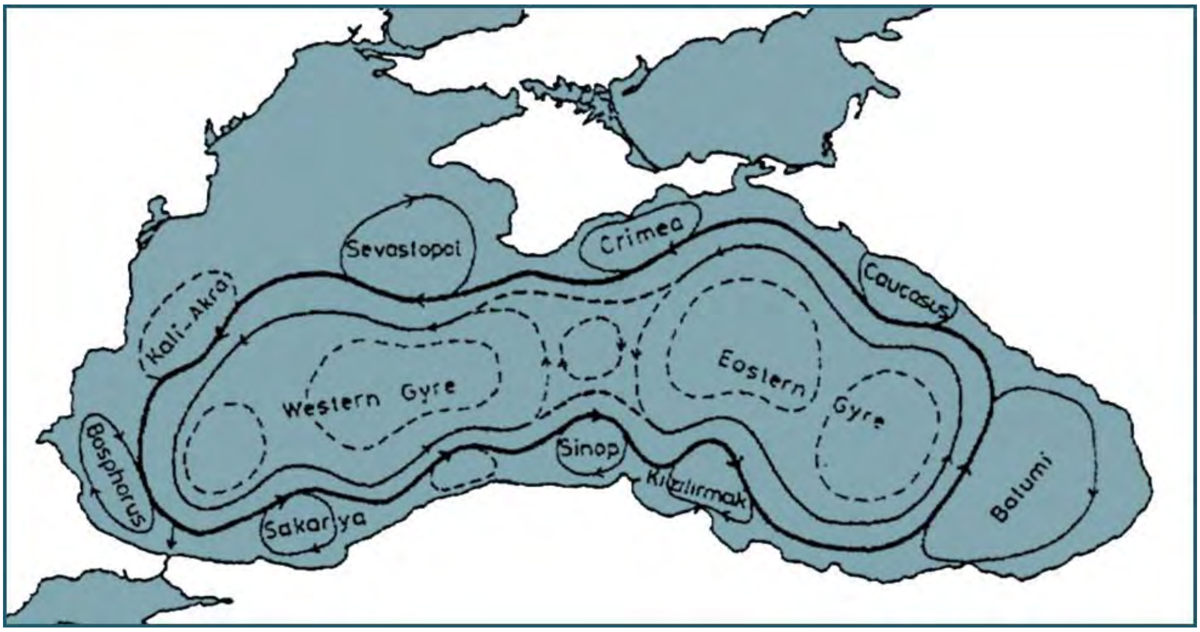


Figure I.1.2. Schematic view of the main features of the upper layer general circulation based on a synthesis of past and recent studies. Solid (dashed) lines indicate quasi-permanent (recurrent) features of the general circulation (Oguz et al., 1993).

It is known that, owing to the conservation of the potential vorticity, large-scale boundary currents flow mainly along the depth contours (Koblinsky, 1990). Since eddies are one of the main mechanisms of the interaction of the near-shore zone with the deep sea (Zatsepin et al., 2003), the character and intensity of the water exchange over the slope is affected by the topography of the continental slope. The wind effect is the most important factor that determines both the structure and the intensity of the horizontal water circulation. Owing to the mechanism of Ekman excitation (Zatsepin et al., 2002), favorable conditions are realized for the onset of the total horizontal water circulation in the basin, which have the same sign as the friction stress vorticity. The annual climatologic mean vorticity of the wind field in the Black Sea is positive (Stanev, 1990) and the total water circulation is cyclonic (Blatov et al., 1984).

In the northwestern region, where the continental slope is gentle and rather wide, anticyclonic eddies move southwestward (cyclonically) strictly following the depth contours and do not deviate toward the abyssal zone (Ginzburg et al., 2002).

The Rim Current main core is trapped to the continental slope in water depths ranging between 400 and 1800 m and at distances from the coast varying between 10 km (off central Turkey and the Caucasus area) and 40 km (off Bulgaria and eastern Turkey) (Fig. I.1.2). Its width varies from 20 to 80 km, which agrees with the values reported by Korotaev et al. (2001). The Rim Current is faster (maximum value of 40–50 cm/s of mean long-shore current), thinner and closer to the coast (maximal speed less than 30 km from the coast) off western Turkey and off the Caucasian coast (Fig. I.1.2).

The general and mesoscale circulation have a large impact on the distribution of nutrients and oxygen in the Black Sea. Some physical phenomena, such as summer upwelling have great impact on local variability of chlorophyll-a, as main indicator of primary productivity. Despite the more common assumptions, regarding the deep water as the main reserve of nutrients supplied to the photic zone, some results emphasize the relative contribution of riverine sources to new production (Murray et al., 1991), the around – basin transport by the cyclonic Boundary Current and the cross – shelf transport by frontal and jet instabilities determine the pattern of primary production in most parts of the Black Sea.

Temperature and salinity features in the Black Sea basin.

Hydrologic and hydrodynamic features of the Western Black Sea are the results of interaction processes of local meteorological events and the medium scale processes of continental slope, as a result of coast line configuration and due to specific features of coastal bathymetry. Seasonal and inter-annual temperature and salinity variations occur in the SQL and pycnocline layer. The thin mixed layer with less saline waters (about 18.0 PSU) responds strongly to seasonal temperature variations at the surface (Ivanov et al., 1997).

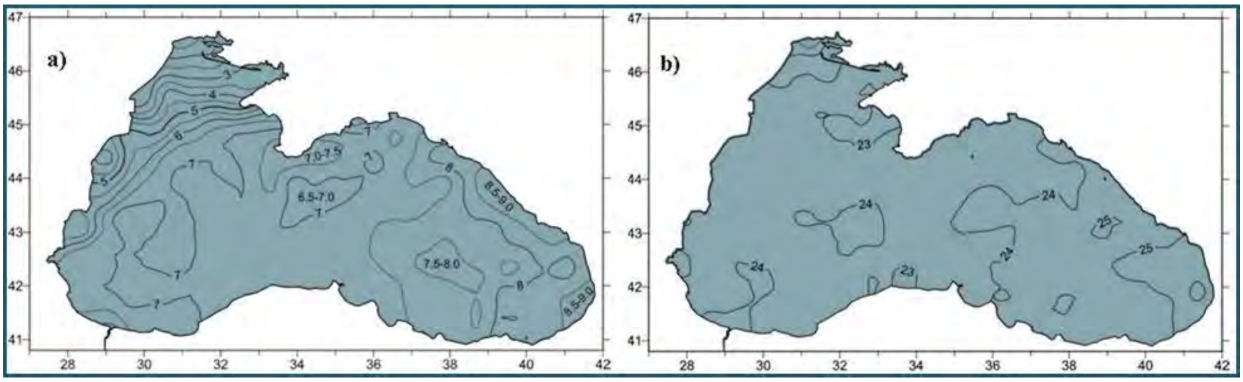


Figure I.1.3. Climatic fields of the seawater temperature (ºC) of SQL in the Black sea in February (a) and August (b) (Tuzhilkin, 2008).

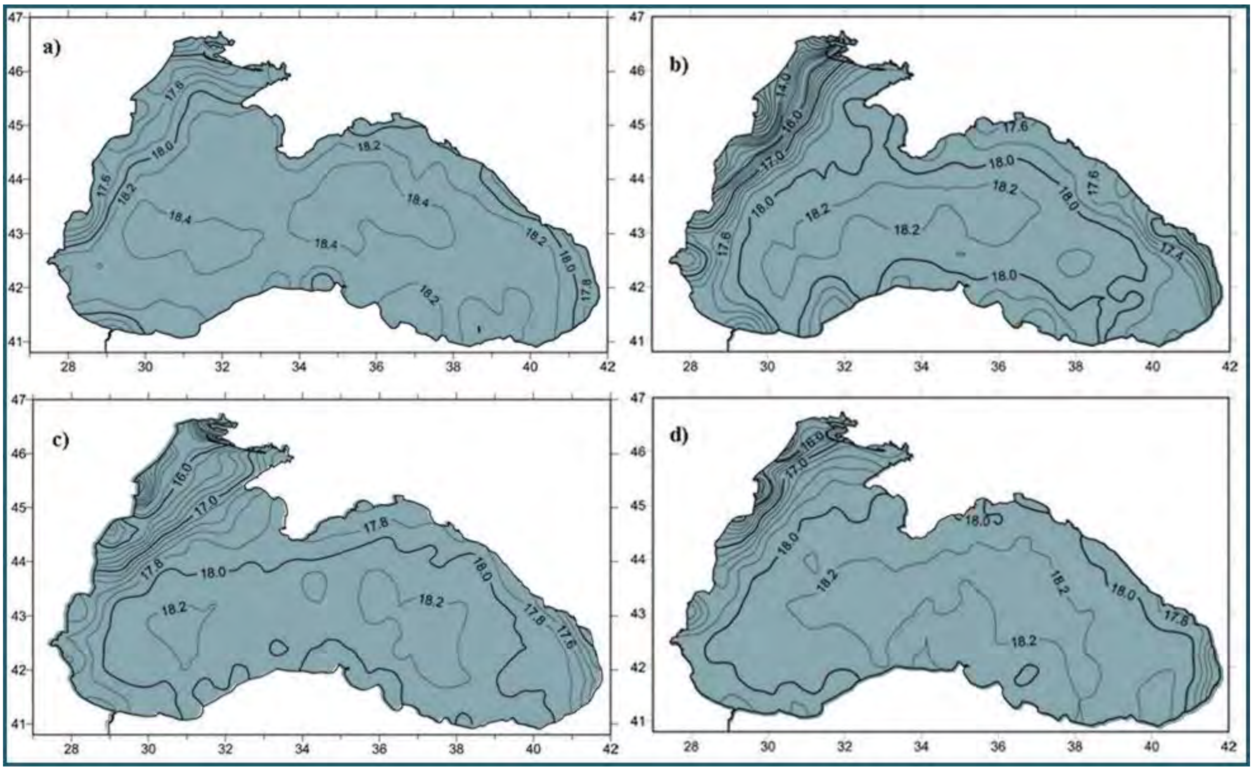


Figure I.1.4. Climatic fields of seawater salinity (PSU) of SQL in the Black Sea in February (a), May (b), August (c) and November (d) (Tuzhilkin, 2008).

The water temperature of SQL of the Black Sea over the year raises from northwest to southeast. The largest horizontal heterogeneity occurs in the winter (Fig. I.1.3) that is during a season of the minimal values, the lower - in the summer (Fig. I.1.3) when warming up of SQL is maximal.

Areas of maximal freshening of waters of SQL are located in northwest coastal zones of the Black Sea (Fig. I.1.3). From those areas, the wedges of the lowered salinity spread along the western and northeast coasts of the Black Sea, respectively (Fig. I.1.4).

Meteorological and hydrological description of the main physical parameters during the MISIS JOINT CRUISE.

DATA and METHODS

The main characteristics and the identification of the Western Black Sea waters are performed using core physical parameters analysis (currents, temperature and salinity) as well as the correlation with the atmospheric circulation (wind). The graphical distributions of the air and sea physical parameters are obtained using Golden Software (Surfer and Grapher).

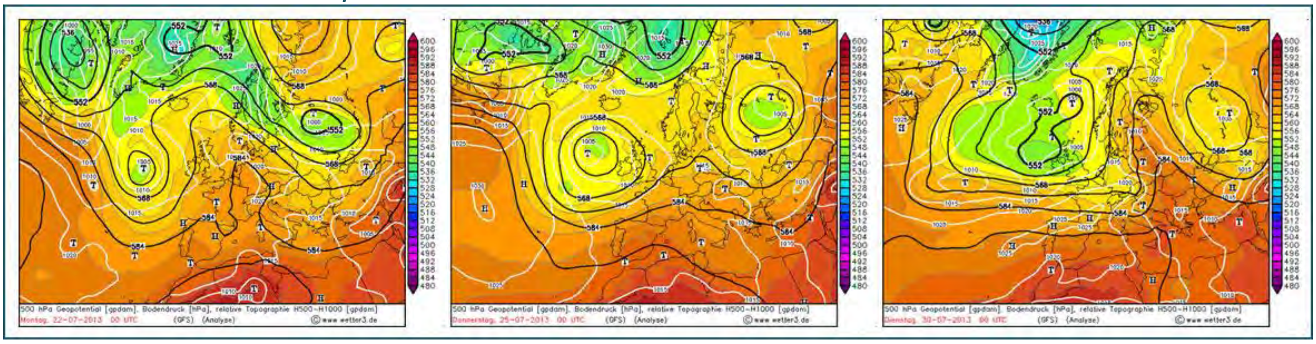
The computing, graphics and the statistical analysis were performed using *R version 3.0.3*, a free software environment under Windows platform. The statistics on Columns/Rows operation were performed on column-wise/row-wise descriptive statistics on the selected MISIS cruise data. Using cumulative frequency histograms were reported: the sample size (N), missing number, mean and geometry mean, standard deviation and geometry, standard error, upper and lower confidence intervals, variance, sum, skewness, kurtosis, coefficient of variation, mean absolute deviation, mode, sum of weights, as well as the quartiles such as minimum, maximum, median, quartiles, inter-quartile range and range. The *Frequency Count* operation allow users to specify the binning parameters 'from Minimum', 'to Maximum', the step by increment or interval number and reports bin centers, bin end points, frequency counts, cumulative counts, relative frequency, and cumulative frequency of the selected data. (http://www.r-project.org).

MyOcean daily mean fields of the sea surface currents. The basin-scale model is used for continuous analysis and forecast of the Black Sea circulation and stratification. The model output includes dynamical sea level, three-dimensional fields of current velocity, temperature and salinity. The basin-scale model assimilates satellite altimetry data provided by SL TAC, sea surface temperature provided by OSI TAC, and TS profiles provided by INSITU TAC. Model couples with bio-optical model to specify better parameterization of absorption of the short-wave radiation. The data of atmosphere forcing come from SKIRON MFSTEP Atmospheric Modeling and Weather Forecasting Group, University of Athens, Greece (*www.myocean.eu*).

RESULTS and DISCUSSIONS

Heating characteristics of the superior mixing layer and the process of forming the seasonal thermocline depends not only on the intensity of the thermal exchanges at sea-air interface, but also on the peculiarity of wind regime. In the western Black Sea shelf, generally, the sea breeze may be observed along the coast from May until September.

a)



b)

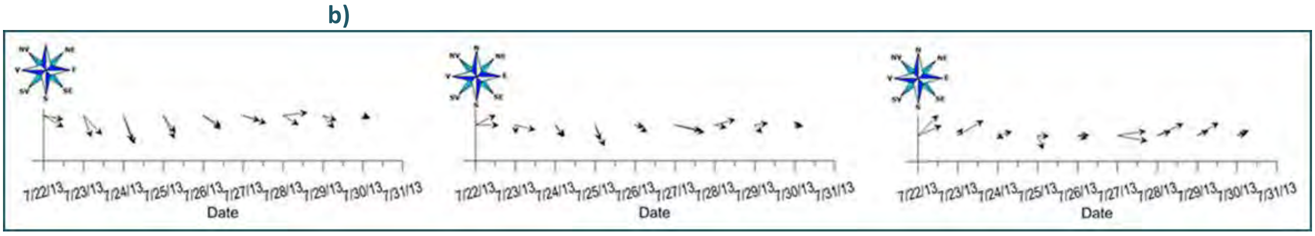


Figure I.1.5. a) Pressure system over Europe in a) 22.07, b) 25.07 and c) 30.07) (www.wetter3.de); d) Wind speed and direction (U10 – 10m above the sea) in the western Black Sea shelf, during the MISIS cruise (available meteorological data for investigated area, from: disc.sci.gsfc.nasa.gov/Giovanni)

The western Black Sea was under the influence of the Azores High ridge (also known as the Azores anticyclone) on 22th of July, 2013. The northwestern predominant air circulation advected at the ground level a mass of cold air of polar origin. In contrast, on the eastern Black Sea basin closes a core of 1010 hPa. The northwestern circulation, generally, determine in the western Black Sea, waves with limited fetch (Fig. I.1.5).

After three days the baric configuration was different in western Black Sea. On 25th of July, 2013 was observed a low pressure corridor that extends from the northwestern Russia to the northern Africa, that includes the entire Black Sea basin. The wind direction changed under the predominant north-eastern sector air circulation. The northeastern circulation on the western Black Sea determines waves higher than if the movement would be achieved from the NW at comparable wind speeds. Thermally, the cold air mass were stationary in the area of interest (Fig. I.1.5).

On July 30, at the ground level highlights a core depression centered over southern Poland and in the Baltic Sea, and a much smaller nucleus near the Aegean Sea had been observed. The air circulation changed and the predominant direction was from southern or south-western sector, which allowed the warm air mass penetration of tropical origin over the southeastern Europe, including the western Black Sea (Fig. I.1.5).

Direction and wind strength over the basin are determined by the type of movement produced by synoptic processes. During the survey, the predominant winds in the western and central Black Sea were from the northwest to west directions (Fig. I.1.5). Due to the pressure system in the Eastern Europe during 22-27.07.2013, as well as the topography and the line-coast configuration, the prevailing winds were from the northwestern and western dirrections on the Romanian (during 22 – 27.07.2013) and Bulgarian (between 23 and 27 of August 2013) shelfs, and southwestern to northwestern for the Turkish marine area (Fig. I.1.5).

In the sea surface mixed layer, sensitive to the specific air temperatures, the temperature distribution was rather homogeneous on the entire shelf, with high values (Fig. I.1.6, 7, 8, 11) down to 10m depth (SST varied within 22.9 - 26.2^{0}C range).

In July, the shelf upper cold-water boundary shifts downwards because of heating of the surface waters and the vertical mixing was suppressed by intense stratification. The sea temperature and salinity measured during the survey were consistent with warm season features (Fig. I.1.3, 4).
Seasonal thermocline, located between 10 and 25m depth on the entire section (Fig. I.1.6, 7) was well defined. The maximum of the temperature gradient was located at 15m down to 21m depth: between 4.0^0 and 5.6^{0}C/m in the open waters and 3.3^{0}C/m in the shallow waters (Fig. I.1.6, 7).

On the entire western shelf, the salinity spatial distribution depended on the fresh water inflow, meteorological events (precipitation) and circulation regime (Fig. I.1.2, 10). The limit between surface and the cold intermediate layer (CIL - defined by the 18.0 PSU salinity) was found at 30 m depth (Fig. I.1.5, 6), and the CIL upper limit deepened seaward, from the 30m down to 40m (Fig. I.1.6, 7).

Salinity ranged at the surface between 13.83 - 15.2 PSU and at 10m depth between 14.4 – 15.47 PSU on the Romanian and Bulgarian shallow waters (Fig. I.1.6, 7, 8, 10). The recorded values follow the climatological values (Fig. I.1.4) and there was no deviation detected for the summer season on the entire western Black Sea basin.

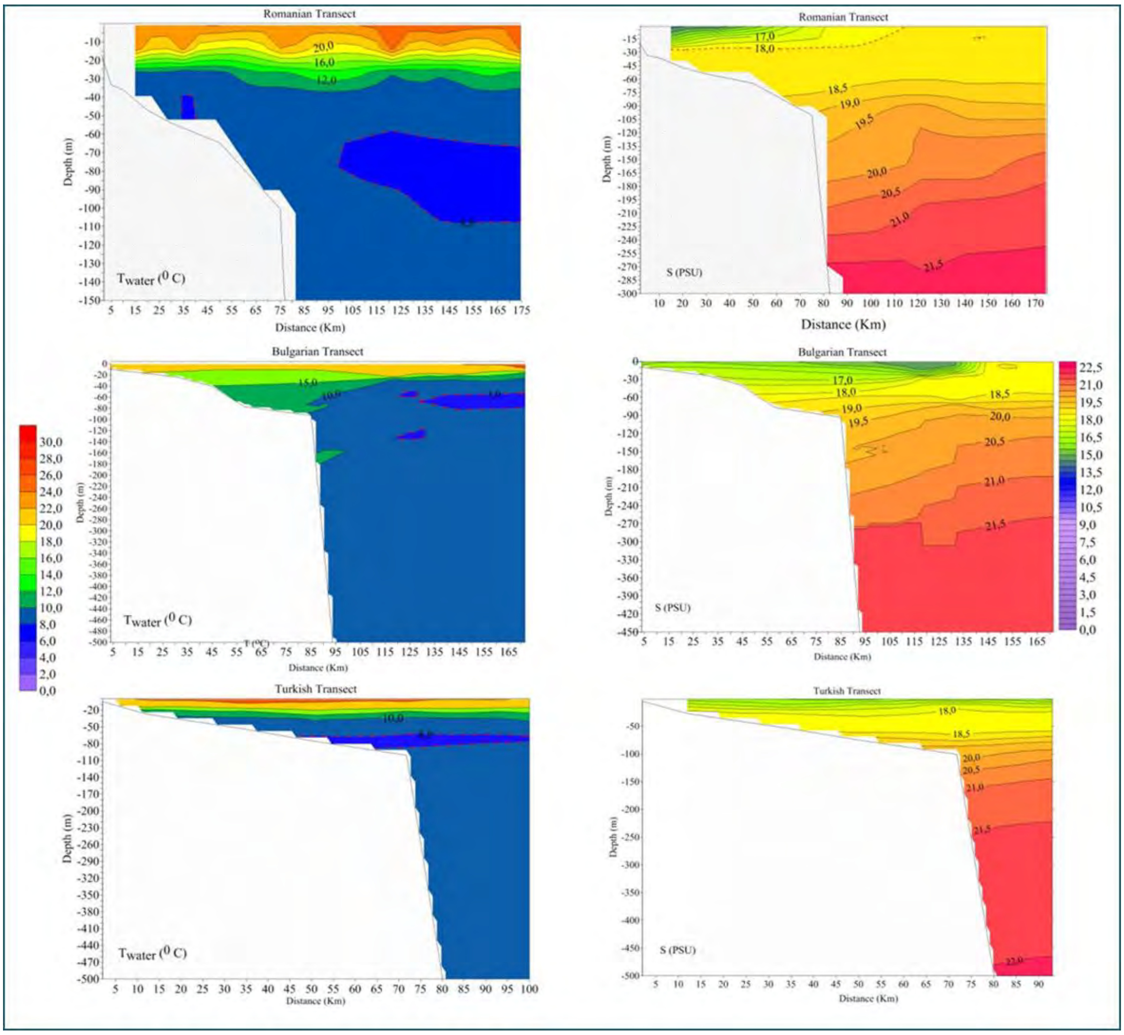


Figure I.1.6. Distribution of the sea temperature (0C) and the salinity (PSU) at the Romanian, Bulgarian and Turkish transect, during the survey.

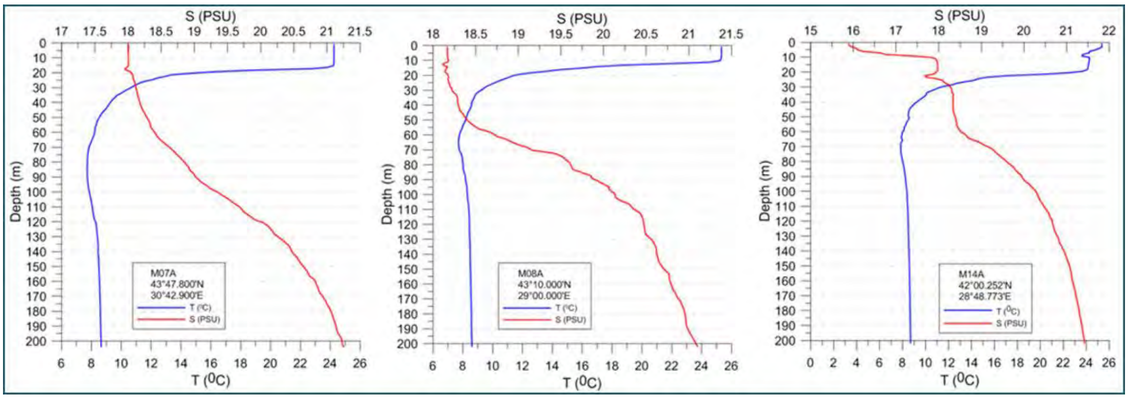


Figure I.1.7. Vertical profiles of the sea temperature (0C) and the salinity (PSU) with depth, on the Romanian (M07A), Bulgarian (M08A) and Turkish (M14A) offshore waters, during the MISIS JOINT CRUISE (22 – 31.07.2013).

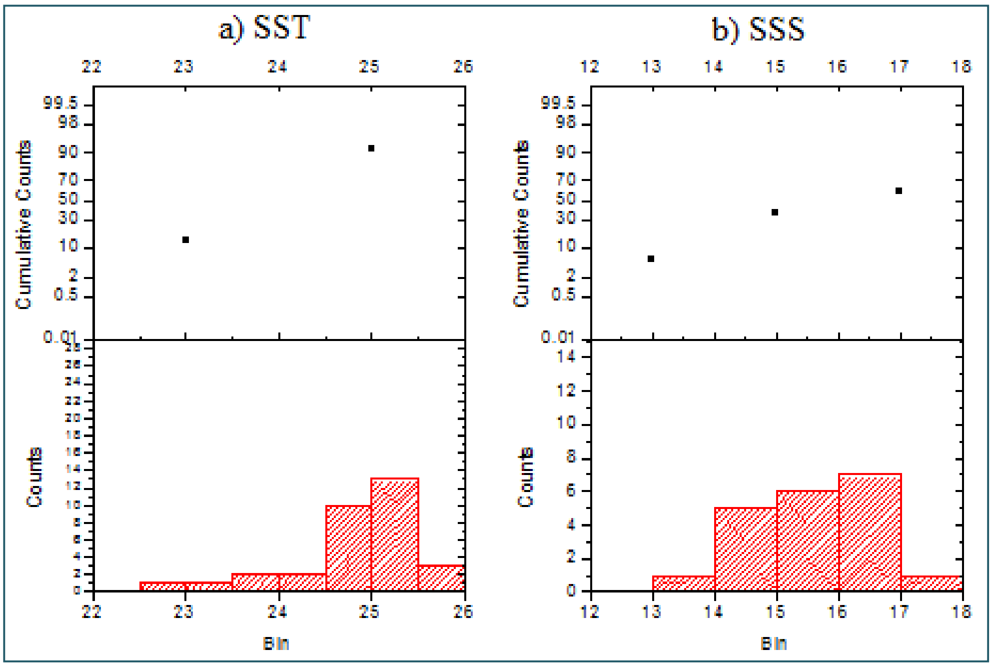


Figure I.1.8. Statistical analysis (histogram and probabilities) for the study area:
a) sea surface temperature
b) sea surface salinity

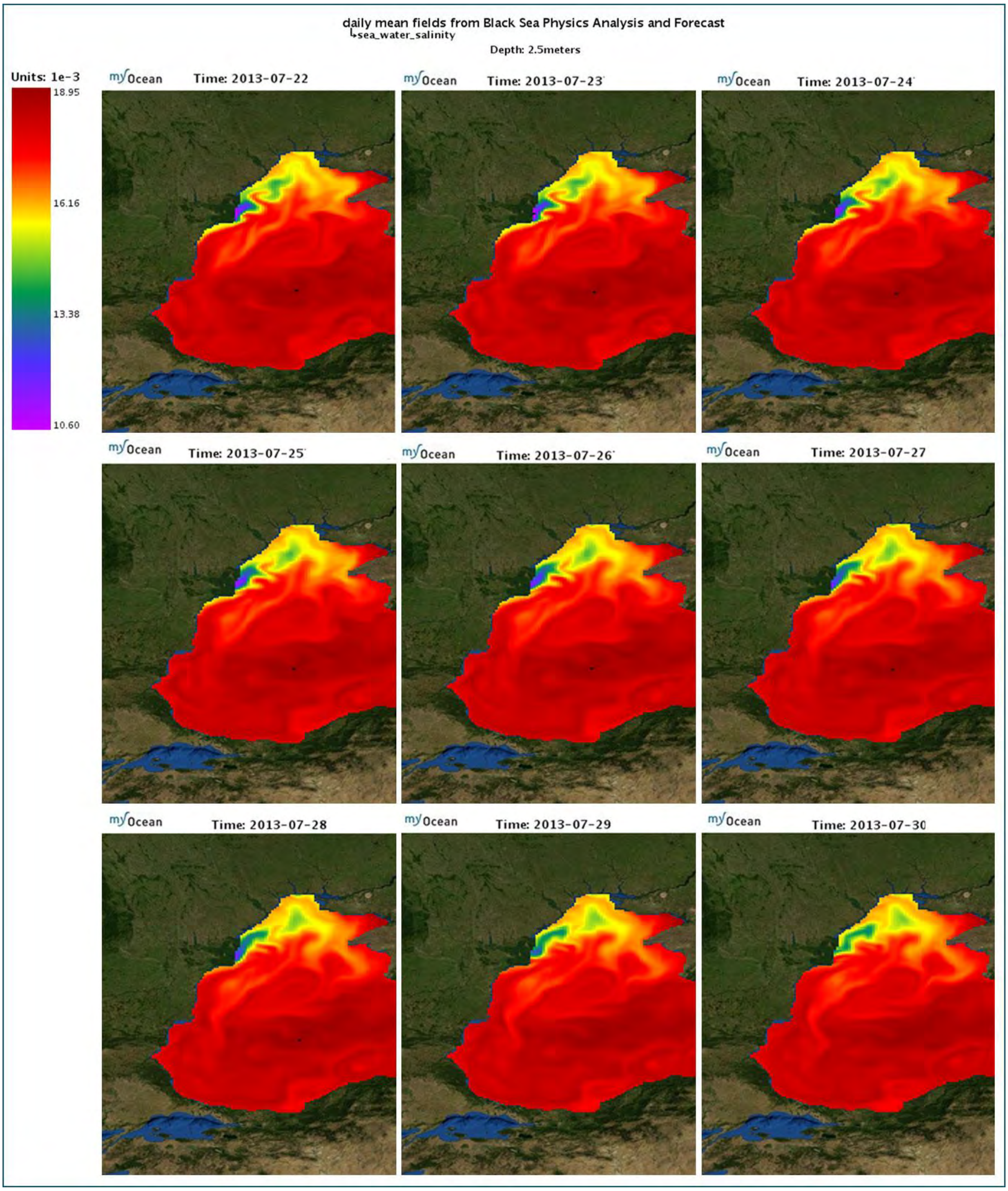


Figure I.1.9. Daily mean fields of the sea surface salinity (2.5m) during 22-31.07-2013 (MyOcean Black Sea Physics Analysis and Forecast
http://www.myocean.eu/web/69-myocean-interactive-catalogue.php).

The rivers have a significant influence on the shallow waters. Less saline waters from the northern and western Black Sea coast are pushed towards the northern shelf by the wind driven surface currents oriented south to north (Fig. I.1.6, 10). Afterwards, are trained and mixed by the offshore velocities, toward south, down to 20m in the central western Black Sea (Bulgarian offshore waters).

Predominant sea surface currents along the western shelf were oriented south to north with maximum velocities about 0.35 m/s. Nearshore currents followed the line coast and bathymetry configuration and drove the southern saline waters (from the Turkish shelf) towards north. The offshore circulation was strongly influenced by the Danube river velocities (that have a fan distribution at the river mouth into the sea). The quasi-permanent anticyclonic eddies (Danube and Sevastopol) as well as the main RIM current widespread east - southward low salinity waters (Fig. I.1.10).

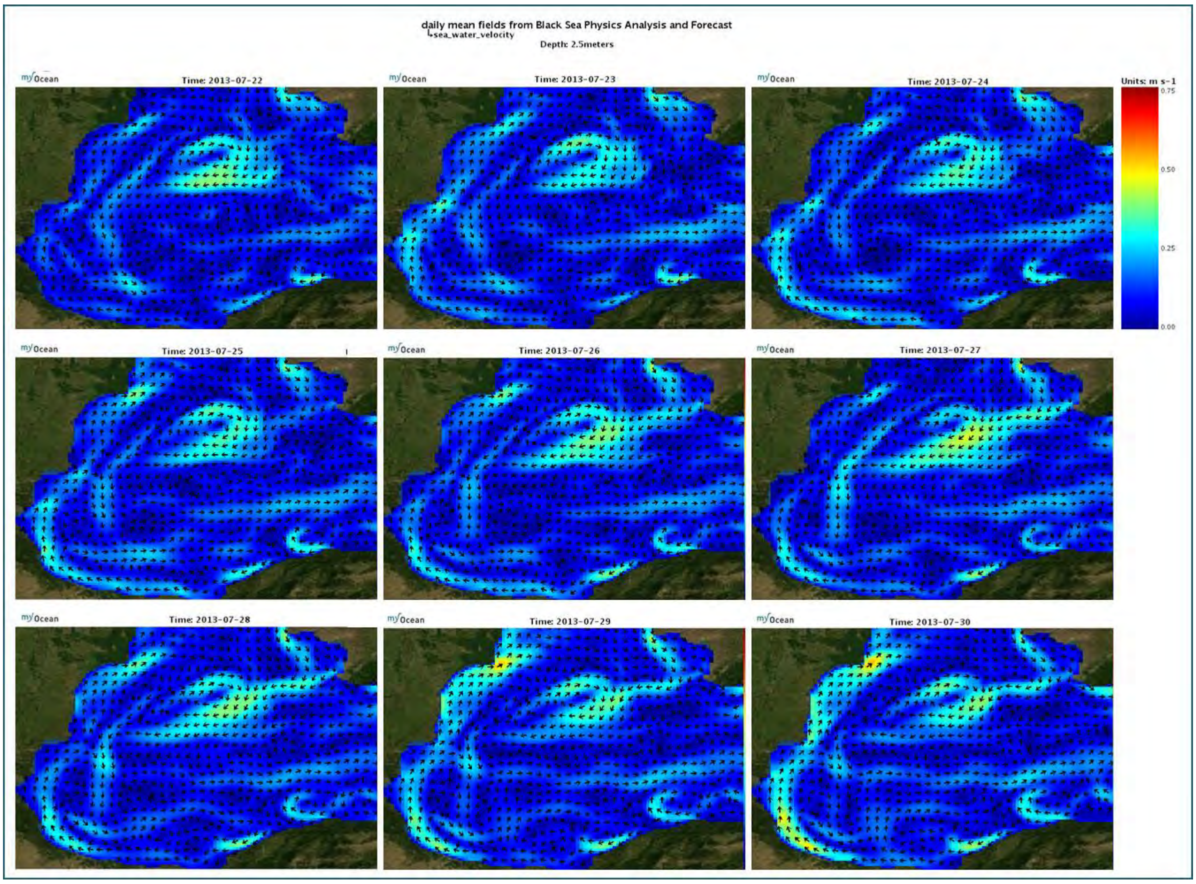


Figure I.1.10. Daily mean fields of the sea surface currents (2.5m) during 22-31.08-2013 (MyOcean Black Sea Physics Analysis and Forecast
http://www.myocean.eu/web/69-myocean-interactive-catalogue.php).

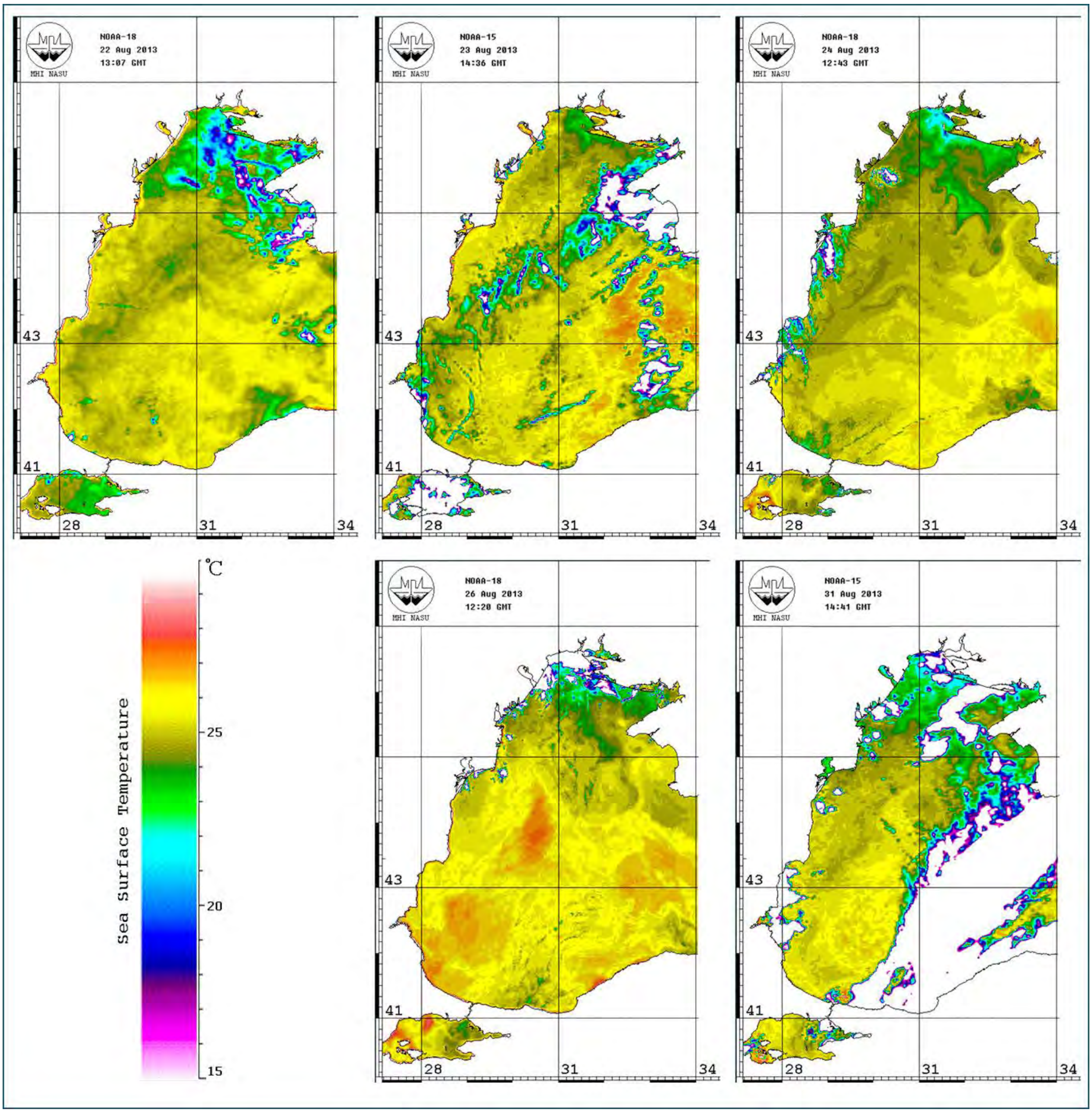


Figure I.1.11. Available NOAA satellite sea surface temperature data during the survey (http://dvs.net.ua/mp/data/main.shtml).

CONCLUSIONS

Based on hydrographic data gathered during the summer of 2013, the water thermohaline structure and the influence of freshwater discharge into the western Black Sea were analyzed.
The prevailing winds on the Black Sea during the MISIS Joint Cruise had northwestern and western dirrections on the Romanian as well as on Bulgarian shelfs and predominant southwestern to northwestern for the Turkish area.
The wind driven sea surface currents were oriented south to north and the offshore circulation induced the low values of salinity on the Romanian and Bulgarian shelf. The northern and western fresh waters influence were observed down to 20m in the central western Black Sea and near the surface in the southern region.
The well-defined seasonal thermocline was found at the regular seasonal depth (10 - 25m) on the entire sections. The maximum of the sea temperature gradient in the water column was located at 15m down to 21m depth.
The sea temperature and salinity measured during the cruise were consistent with climatological and hydrological data and no sensible variations have been detected.

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***** disc.sci.gsfc.nasa.gov/Giovanni

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II. DESCRIPTOR 1: Biodiversity Assessment

II. 1. Phytoplankton

Completed by:
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INTRODUCTION

Marine plankton are the base of ocean food web as organic matter producers and integral part of biogeochemical cycles, providing an essential ecological function for all aquatic life. The Water Framework Directive (Directive 2000/60/EC) and the Marine Strategy Framework Directive (Directive 2008/56/EC) of the European Union, as well as the Black Sea Commission Strategic Action Plan (SAP, 2009) refer to phytoplankton as an important biological component to be addressed in the assessment of the ecological status of the sea. Plankton indicators can provide valuable information about the condition and distribution of the pelagic habitats for D1 – Biodiversity.

The phytoplankton Indicators/Criteria relevant to Descriptor1 according to MSFD are related to Criteria 1.4, 1.6 and 1.7 - Table II.1.1.

Table II.1.1 - Phytoplankton related Indicators/Criteria relevant to MSFD Descriptor1 Biodiversity (C(2010) 5956)/(2010/477/EU).

Criterion		Indicators
1.4. Habitat distribution	1.4.1. Distribution range	
	1.4.2. Distribution pattern	
1.6. Habitat condition	1.6.1. Condition of the typical species and communities	Dominant species, Biodiversity indices
	1.6.2. Relative abundance and or biomass	Phytoplankton abundance and biomass
1.7. Ecosystem structure	1.7.1. Composition and relative proportion of ecosystem components	Phytoplankton Functional groups

Although phytoplankton diversity is extremely important for the stability and functioning of the marine ecosystem and biogeochemical cycles (Ptacnik et al. 2008) the indicator role bear a number of constrains. On the one hand our knowledge of marine phytoplankton biodiversity is limited due to both methodological constrains of species identification techniques (Venter et al., 2004) the effort and expense of gaining appropriate data sets by traditional microscopic methods (Irigoien et al. 2004, Cermeño et al., 2013) and mismatches between sampling and the scales of phytoplankton natural variability, for which species identity concepts within Biodiversity Ecosystem Functioning are rather vague. On the other hand mechanisms regulating patterns of phytoplankton biodiversity still remain debated and largely unexplored (Garmendia et al., 2012, Cermeno et al., 2013). Albeit the great effort to explain the factors that determine the distribution, community assembly, blooms, and succession of species the macroecological and morphospecies approaches are not properly scaled to the ecophysiology and niche requirements of the phytoplankton phylogenetic groups and species present (Smayda, 2011). Cloern & Dufford (2005) conceive phytoplankton diversity as hyperdimensional domain, whereby communities are assembled by selective forces operating on variation in algal size, motility, behavior, life cycles, biochemical specializations, nutritional mode, chemical and physiological tolerances, and dispersal processes.

Litchman et al. (2010) proposed trait-based approach as an effective way to link species diversity and community structure in phytoplankton by providing mechanistic explanations of why certain species are found under given environmental conditions and although how traits evolve in response to different selective pressures is still poorly resolved (Hairston et al. 2005, Litchman & Klausmeier, 2008) the concept of Phytoplankton Functional Types (PFTs) seems promising. Functionality includes variable properties such as similar growth and mortality rates, response to temperature and light conditions, nutritional mode - autotrophs, mixo-heterotrophs (Le Qu´er´e et al., 2005), production of toxic substances (Luckas et al., 2005), cell size (pico, nano and micro-phytoplankton) that all play an important role in the overall ecosystem processes and resilience (Moulin, 2008).

Community body size largely determines the types and strengths of flows of energy and materials in ecosystems (Legendre & Le Fe`vre, 1991). Pelagic food webs in which phytoplankton are of low average size tend to be sustained by regenerated production, where a large fraction of energy loops through microbial components. Conversely, food webs in which phytoplankton are of high average size, have a greater potential to transfer primary production to higher trophic levels, including fish (Cullen et al., 2002). Phytoplankton community size structure has the potential to reflect changes in pelagic ecosystems (Sabetta et al., 2005).

Harmful algal blooms (HAB) have a pronounced impact not only on water quality, but on species diversity, community structure by their traits (best expressed in the toxic species) or by their abundance (hypoxia conditions and associated benthic species mass mortality), impairment of reproduction (chemical biomediation) and many ecosystem functions (GEOHAB, 2001, Paerl & Huisman, 2009). Hazardous conditions occur when into the phytoplankton community with a very low cell concentration a highly toxic species is present (Sellner et al., 2003; Glibert et al., 2005; Anderson & Donald, 2007). Phytoplankton toxic species have the capacity to produce potent toxins and can have a negative effect, causing severe economic losses to aquaculture, fisheries and tourism operations and having major environmental and human health impacts (Hallegraeff et al., 1995). The range of toxins produced by HABs is quite extensive, including brevetoxins, saxitoxins, okadaic acid, domoic acid, azaspiracid and numerous others

(Landsberg, 2002; Patricia et al., 2005). Furthermore, blooms of toxic, microscopic algae lead to illness and death in humans, fish, seabirds, dolphins and other sea life. (Work et al., 1993; Scholin et al., 2000; Landsberg, 2002; Glibert, et al., 2005; Anderson & Donald, 2007; Anderson, 2008; Ferrante, 2012).

Black Sea phytoplankton checklist contains 38 species of harmful effect (BSPC Editorial Board, 2014). Recently the number of studies documenting occurrence of phytoplankton toxic species in the Black Sea have increased (Vershinin & Kamnev, 2000; Vershinin et al., 2006; Morton et al., 2007; Besiktepe et al., 2008; Morton et al., 2009, Alexandrov et al, 2010). In 2000 for the first time DSP toxicity of *Dinophysis caudata* and *Dinophysis rotundata* in the Anapa region of the Black Sea was confirmed (Vershinin & Kamnev, 2000). Investigation in Caucasian Black Sea Coast of the Russian Federation has shown presence of yessotoxin (YTX), 45-hydroxy-yessotoxin (45-OH-YTX) and homoyessotoxin (homoYTX) from shellfish hepatopancreas harvested. Concurrent with the mussel intoxication, the dinoflagellates *Lingulodinium polyedrum* and *Gonyaulax spinifera* were found in high concentrations (Morton et al., 2007). The first record of a DA producing diatom (*Pseudo-nitzschia calliantha*) isolated from the Black Sea was registered in 2008 (Besiktepe et al., 2008). This provide evidence for the exceptional importance of phytoplankton toxic species monitoring and the need to include these metrics as an indicator for ecological status in the MSFD.

An inherent feature of phytoplankton composition is the high spatio-temporal natural variability; there are no species (the occurrence or abundance of which) that can be used as universal indicators and there is no unique fixed assemblage of species each with its own abundance that is representative enough of a given ecological state of the environment. Their abundance and distribution is a function of both top-down and bottom up controls, physical, chemical, and biological parameters, such as hydrodynamics and circulation, light, nutrient availability and stoicheometry, temperature and salinity, and biotic interactions to which the responses are non-linear.

All these underline the complexity in definition and application of robust phytoplankton related indicators (Garmendia et al., 2012).

The MISIS cruise data offer the opportunity to explore the natural environmental gradients in the North-Western Black Sea sampling area and in addition to the phytoplankton metrics included in the WFD classification system for the Black Sea (Moncheva, Boicenco 2010) and those proposed in the Romanian (RO IAR, 2013) and Bulgarian (BG IAR, 2013) Initial Assessment Reports to apply a number of potential indicators for scaling ecological state: PFTs (autotrophs/heterotrophs, potentially toxic species, size structure) as well as the HPLC pigment analysis for assessment of phytoplankton taxonomic structure.

MATERIAL and METHODS

A total of 110 samples have been collected along 3 transects from 18 stations distributed over the coastal, shelf and opens sea pelagic habitats of each country by Romanian (41 samples, including inter-comparison stations) Bulgarian (39 samples, including inter-comparison stations) and Turkish (30 samples, inter-comparison stations included) teams – Fig. 1, Table 1.

Samples were collected by 5l Teflon Niskin bottles attached to CTD - SBE 911 - Rosette System equipped with in situ fluorometer (Chelsea Minitraca). The sampling depths were selected according to the CTD profile and the in situ fluorometer readings: surface, temperature/salinity gradient (thermocline), fluorescence max (deep sea chlorophyll) and 1 m above the station depth. Details of sampling, fixation and laboratory analysis are given in *Annex I.* For the purpose of combining data into a common data set a statistical evaluation of comparability of the results produced by each team was performed via specifically designed intercalibration exercise (see Intrcalibration Report –phytoplankton).

Phytoplankton abundance, biomass and dominant species

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 (Sedgwick Rafter, Utermol). 400 cells were counted from each sample, while rare and large species were checked in the whole sample (Moncheva & Parr, 2005, updated 2010).

The individual cell biovolume (V, μm^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 respective lab. The average of at least 10 measurements per species was agreed to be used for the biovolume calculation. Cell bio-volume was converted to weight (W, ng) following Hatchinson (1967).

Species identification was mainly after Schiller (1937), Kisselew (1950), Proshkina-Lavrenko (1955), Carmelo (1997), Fukuyo (2000) and the taxonomic nomenclature according to the on-line data-base of World Register of Marine Species (WoRMS) and the Black Sea check-list http://phyto.bss.ibss.org.ua.

Dominance of the species show the proportion of species contribution to the total abundance of the community, and we considered the dominance >60% in the abundance by a single species or two species.

Phytoplankton Functional types (PFTs)

Autotrophs/Heterotrophs

The phytoplankton functional authotrophs/heterotrophs list was composed from the taxonomic list of the MISIS Joint Cruise based on the IO-BAS database (trophic preferences specified for 140 species); NIMRD database (trophic preferences specified for 867 species), the Checklist of Baltic Sea Phytoplankton Species (Guy Hällfors, 2004) and in reference to the international databases available online (http://nordicmicroalgae.org/, http://www.eos.ubc.ca/, http://eol.org/).

Size structure

The routine phytoplankton methods employed in this study do not allow for correct identification and quantification of picophytoplankton that is why the analysis was based on nano- and microphytoplankton composition of phytoplankton assembly. Three size classes were derived from estimated Equivalent Spherical Diameter (ESD) for each species (Bosak et al., 2012): nanophytoplankton 1 (nano-1) with ESD < 5µm; nanophytoplankton 2 (nano-2) with ESD between 5 µm and 20 µm and microphytoplankton (micro) with ESD > 20 µm, corresponding to individual biovolumes ranges between 10-600 μm^3, 700 - 28000 μm^3 , and 32000- 140 000 μm^3 respectively. The proportion of these 3 groups in the total phytoplankton abundance and biomass was estimated for phytoplankton samples integrated for the upper surface layer down the thermocline for each sampling station to construct size structure matrix. Given the Secchi disk depths (between 4-8 in the coastal and most of the shelf stations), the integration down to thermocline only (12-21m) was assumed to represent better the response of phytoplankton to nutrient gradients of the pelagic domain preventing the interference of other environmental parameters that could limit phytoplankton growth such as light, temperature, zooplankton grazing, regenerated nutrients, etc.

The size structure matrix was correlated to environmental matrix to test the assumption that spatial body size–abundance distribution patterns of marine phytoplankton were affected by environmental nutrients and the variations in size–abundance distribution were not determined by selection processes at the species level.

The abiotic matrix (habitat template) includes 8 environmental variables (phosphates, silicates, total oxidized nitrogen, ammonium, dissolved inorganic nitrogen and nutrient ratios - N/P, Si/P, Si/N) and the biotic matrix - an array of 11 phytoplankton variables representing phytoplankton community size and taxonomic traits - Table II.1.2

Table II.1.2.List of phytoplankton and environmental parameters.

Biological matrix		Environmental matrix	
Dependent Variables	**Abbreviation**	**Independent Variable**	**Abbreviation**
Nanophytoplankton (ESD < 5µm) biomass [mg/m^3]	Nano1B	Phosphates -[µM/l]	$(PO_4)^{3-}$
Nanophytoplankton (ESD < 5 µm > 20 µm) biomass [mg/m^3]	Nano2B	Silicates -[µM/l]	$(SiO_4)^{4-}$
Nanophytoplankton ESD < 20 µm biomass [mg/m^3]	Nano12 B	Total oxidized nitrogen -[µM/l]	(TNO_x)
Microplankton ESD > 20 µm biomass [mg/m^3]	MicroB	Ammonium [µM/l]	$(NH_4)^+$
Total Phytoplankton Biomass [mg/m^3]	PhytoB	Dissolved inorganic nitrogen -[µM/l]	DIN
Bacillariophyceae biomas[mg/m^3]	BacB	N/P	N/P
Dinophyceae biomass [mg/m^3]	DinB	Si/P	Si/P
*Other classes biomass [mg/m^3]	OthersB	Si/N	Si/N

*"Other" classes is a composite group integrating all phylla present in the phytoplankton composition excluding Bacillariophyceae and Dinophyceae

Statistical relationships between the selected phytoplankton community metrics and the environment variables (nutrients) were explored. A number of multiple regressions models were constructed to examine the correlations between phytoplankton metrics in order to determine the possible controlling environmental variables. Data were first assessed for normality (Kolmogorov–Smirnov test) and Box-Cox transformed (Box & Cox, 1964). In addition to R square, Adjusted R-square was used to summarize the best fit as it takes into account the number of variables in the model. ANOVA test and t-test were used to determine the statistically significant environmental predictors ($P< 0.05$). Statistical evaluations and other data manipulations were conducted in IMB SPSS Statistics.

Canonical correspondence analysis (CCA) was performed on the same data matrices (log transformed) by Multi - Variate Statistical Package program (MVSP, version 3.1). The CCA generated output was used to trace the phytoplankton community traits spatial response to environmental factors and assess the pattern of site distribution (site scores) within the coastal-open sea habitats.

Potentially toxic phytoplankton species (PTP)

The potentially toxic species were extracted from the phytoplankton taxonomic list of the MISIS Joint Cruise based on the IOC-UNESCO Taxonomic reference list of harmful micro algae (Moestrup et al., 2009), composed of 116 species and the Black Sea phytoplankton checklist harmful effect species list (BSPC Editorial Board, 2014), numbering 38 species.

Phytoplankton Indicators

The MSFD indicators were selected so as to be consistent with the classification system developed for the WFD in RO and BG for the coastal habitats (Moncheva, Boicenco, 2010, Commission Decision (Com.Des./20 September 2013). For BG and RO shelf and open sea pelagic habitats the GES thresholds were set following the same concept of 37% deviation from the baseline conditions where appropriate (BG IAR, 2013; RO IAR, 2013). For TR BG classification system was applied as their national system is still not available.

The metrics and the WFD classification systems are presented on Table II.1.3 and the MSFD related indicators for GEnS - on Table II.1.4.
In addition to Menhinik biodiversity index the Shannon"95 index (Uusitalo et al., 2013) was also tested. Shannon"95 index is a derivative of the classical Shannon's diversity index (Shannon, 1948) and was computed using the Shannon index equation on biomass data of the taxa that together constitute 95% of the total recorded biomass. In this initial test of its applicability the threshold for GEnS was assumed at values > 2 (MARMONI Project Report, 2012).

Table II.1.3. Metrics and classification systems for the coastal pelagic habitat (WFD) colour coding correspond to WFD categories (after Moncheva & Boicenco, 2010, Com Dec).

N[cells/L]					
CWT	High	Good	Moderate	Poor	Bad
1	400-500	501-800	801-1500	1501-3000	>3000
2	400-500	501-800	801-1500	1501-3000	>3000
EQR	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

B[mg/m^3]					
CWT	High	Good	Moderate	Poor	Bad
1	400-700	701- 950	951- 2500	2501– 5000	>5000
2	400-700	701- 950	951- 2000	2001– 4000	>4000
EQR	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

Chl. a [mg/m^3]					
CWT	High	Good	Moderate	Poor	High
1	<0.9	0.9-1.5	1.5-3.1	3.1-7.0	>7.0
2	<0.7	0.7-1.2	1.2-2.5	2.5-5.5	>5.5
EQR	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

MEC %					
CWT	High	Good	Moderate	Poor	High
	2(5)-20	20-35	35-55	56-75	>75
EQR	1-0.80	0.8 - 0.65	0.65-0.45	0.45-0.25	>0.25
EQR n	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

DE%					
CWT	High	Good	Moderate	Poor	High
	2(5)-20	20-35	35-55	56-75	>75
EQR	1-0.80	0.8 - 0.65	0.65-0.45	0.45-0.25	>0.25
EQR n	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

Menhinick (1964)					
CWT	High	Good	Moderate	Poor	High
1	0.19-0.15	0.15-0.09	0.09-0.05	0.05-0.03	0.03-0.01
EQR	1.0-0.75	0.75-0.55	0.55-0.35	0.35-0.25	0.25-0.0
EQR n	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

Sheldon (1969)					
CWT	High	Good	Moderate	Poor	High
	0.96- 0.78	0.77-0.49	0.48- 0.32	0.31-0.21	0.20-009
EQR	1.0-0.75	0.75-0.55	0.55-0.35	0.35-0.25	0.25-0.0
EQR n	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

IBI					
CWT	High	Good	Moderate	Poor	High
EQR	1-0.80	0.80-0.63	0.63-0.43	0.43-0.23	0.23-0.0

Table II.1.4. Metrics and GEnS values for the shelf and open sea pelagic habitats (summer).

METRIC	SHELF	OPEN SEA
BG-Biomass [mg/m^3]	460 - 600	100 - 150
RO-Biomass [mg/m^3]	500-800	150-250
Index Menhinick	> 0.09	> 0.09
Index Sheldon	>0.49	>0.49
DE%	<35%	<35%

RESULTS and DISCUSSIONS

1. Species composition and biodiversity

In July 2013 a total of 264 species, varieties and forms were identified in the study area, from 12 taxonomic classes. The bulk of the species pool was composed by Dinoflagellates - 158 species (60% of the total number of species) among which the genus Gymnodinium (33 species), Protoperidinium (18 species) and Glenodinium (14 species) were the most diverse. Among diatoms (43 species) genus Thalassiosira (9) and Chaetoceros (7 species) along with genus Pseudo-nitzschia, Nitzschia and Thalassionema showed highest species richness. Relatively high number of species were identified for Chlorophyceae (18 species), Cyanophyceae (10 species), Cryptophyceae (9 species) and Prymnesiophyceae (9 species), while the classess Prasinophyceae (5), Dictyochophyceae (3) and Euglenophyceae (3) were represented by few species only. The highest species diversity was found at the shelf stations (almost 90% of the total species pool) and open waters (about 75% of the total number of species). Lowest diversity (half of all species) was observed in the coastal area - Fig. II.1.1.

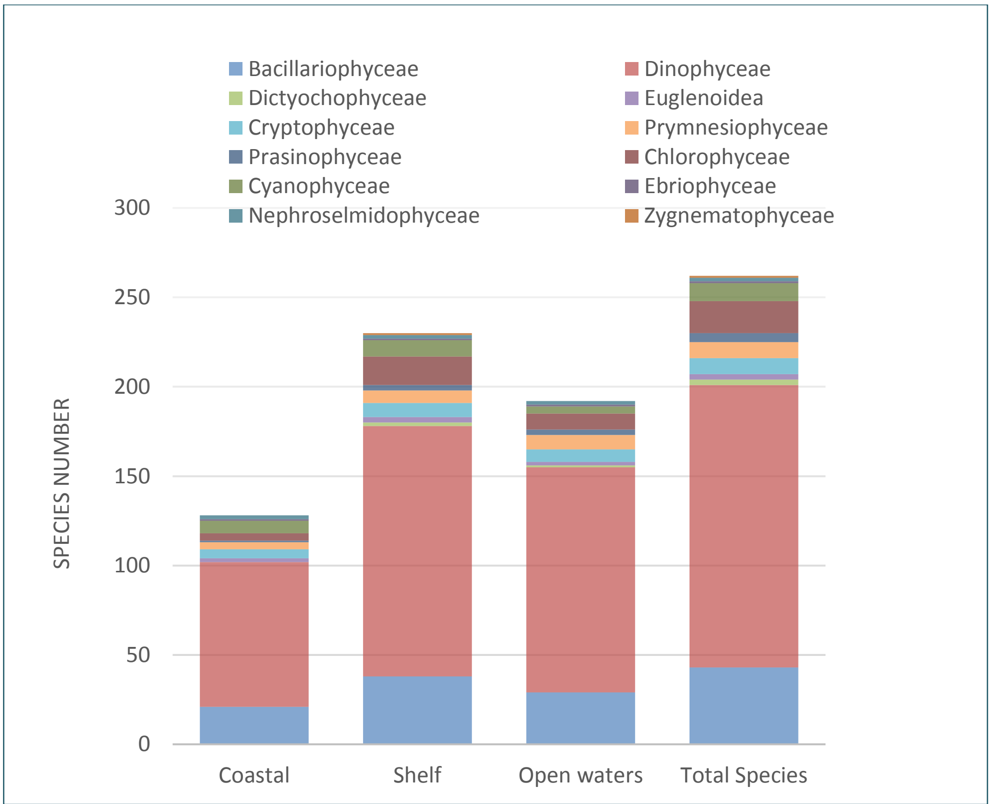


Figure II.1.1. Phytoplankton taxonomic composition in July 2013 (RO-BG-TR transects).

2. Phytoplankton community structure (abundance, biomass by taxonomic groups)

The average abundance of phytoplankton for the entire area varied between $10 \cdot 10^3$ and $1.16 \cdot 10^6$ cells/L and the average biomass - between 9 and 601 mg/m^3.

By pelagic habitats the average cell density and biomass in the coastal habitats exceeded ~ 2 times the shelf average (633 – $240 \cdot 10^3$ cells/L and 315-160 mg/m^3) and ~ 10 times the open waters average (56·10^3 cells/L and 31 mg/m^3).
By transects, phytoplankton **average abundance** in the Bulgarian coastal station (M012, $1.16 \cdot 10^6$ cells/L) was ~2 times higher than the Romanian

coastal station (M03, 633·10^3 cells/L), while in Igneada coastal habitat it was very low (M017, 36·10^3 cells/L) – Fig. II.1.2.

The pattern of **average biomass** distribution over all 3 transects was similar to the abundance distribution - decreasing from coastal to open waters off Constanta (M01 - 426 mg/m^3, M03 – 267 mg/m^3) and Galata (M012 mg/m^3 - 315 mg/m^3, M010 – 137 mg/m^3) and from the open sea to the shore in Igneada (M014 – 477 mg/m^3, M016 – 601 mg/m^3, M017 – 312 mg/m^3.

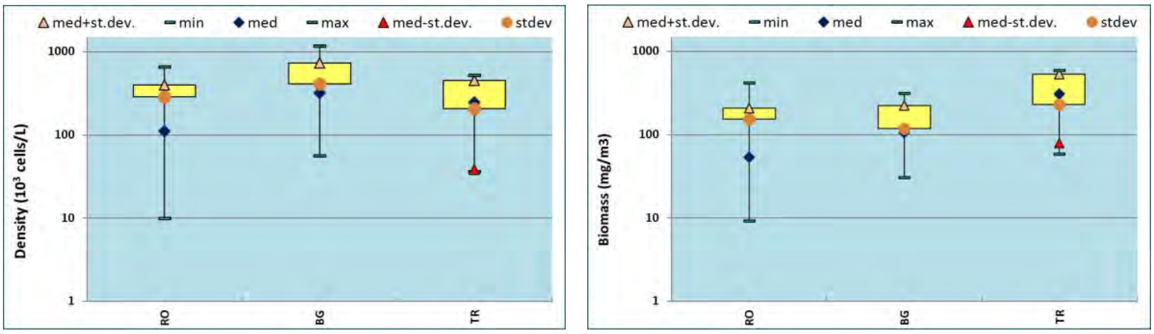


Figure II.1.2. Spatial variation of average abundance and biomass in July 2013: BG (Bulgarian), RO (Romanian) and TR (Turkish) transects.

The trend of **total abundance and biomass** in pelagic habitats decreased from coastal areas to shelf and open waters in Constanta and Galata stations most likely related to the observed spatial nutrients gradient due to the anthropogenic land-based inputs and the harbours activities. In Igneada, the reversed pattern could be associated to type of shoreline and the currents preconditioning high phytoplankton accumulation at the shelf and open waters – Fig. II.1.3.

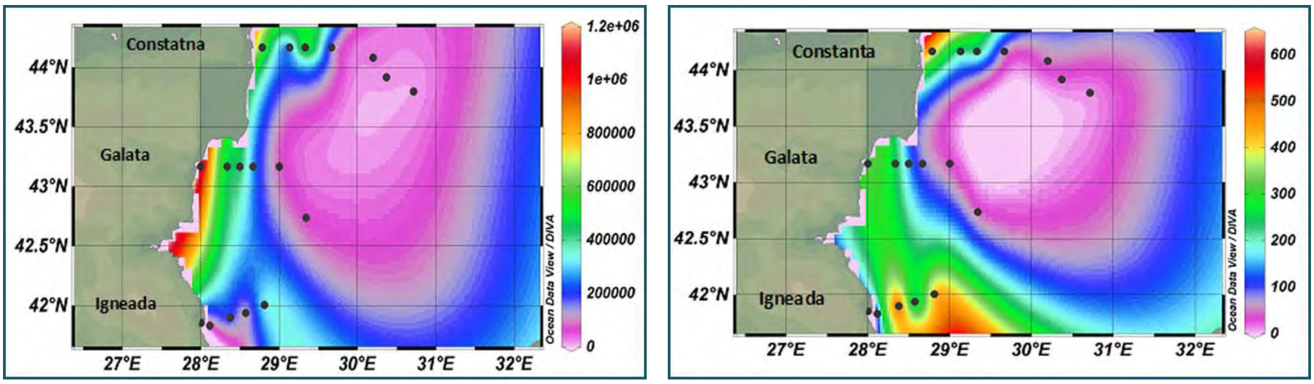


Figure II.1.3. Distribution of total phytoplankton average abundance (cells/L, left) and biomass (mg/m^3, right) during July 2013.

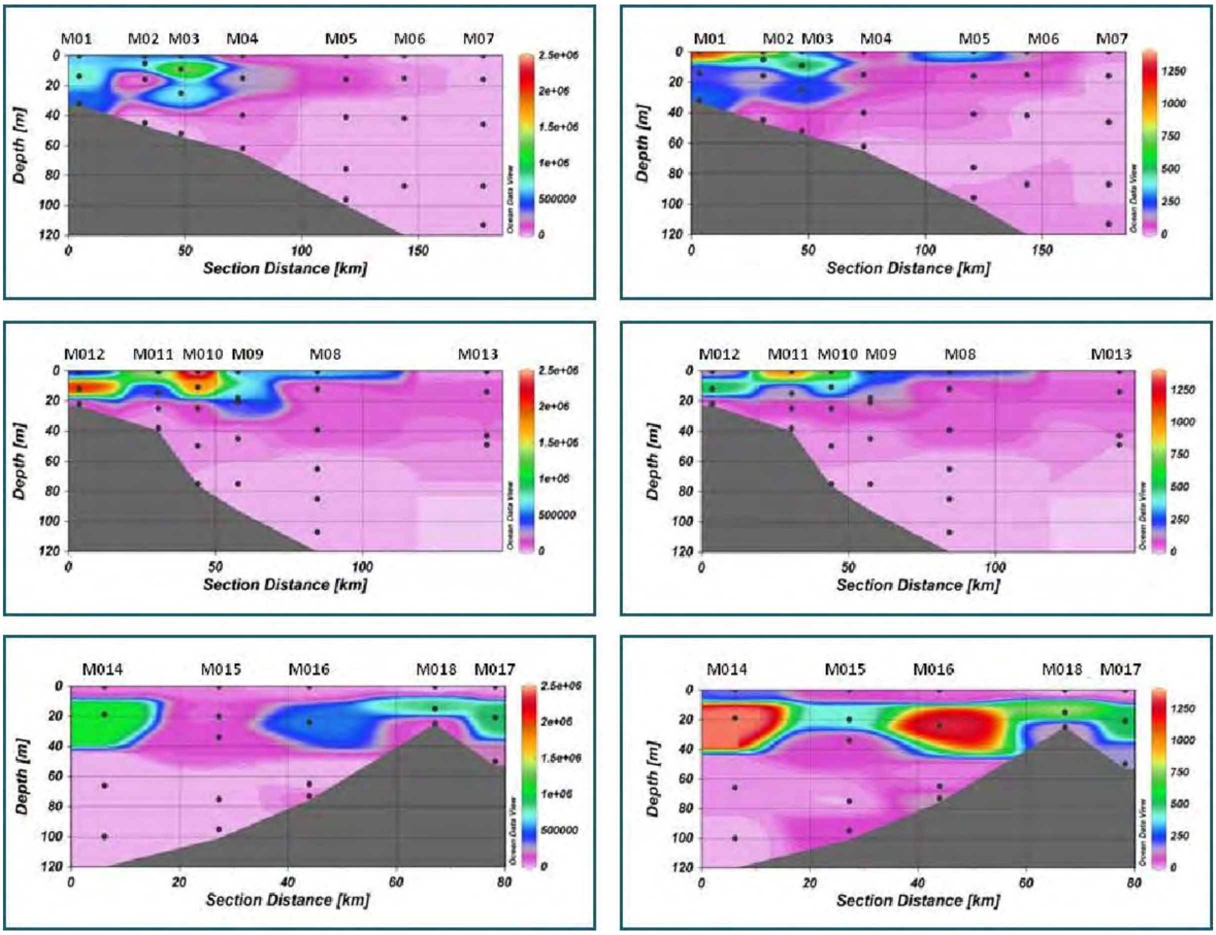


Figure II.1.4. Vertical distribution of phytoplankton abundance (cells/L, left) and biomass (mg/m^3, right) during July 2013.

The highest values of total abundance and biomass were distributed in the surface homogenouos layer (SHL) down to the thermocline (0-25 m) where conditions were favorable for phytoplankton growth, while a pronounced decrease with depth and with distance to the shore was observed - Fig.II.1.4. At some deep stations a deep chlorophyll a max was observed (*Emiliania huxleyi* of aprox. 42·10^3 cells/L at station M04 - DCM at 40m).

Phytoplankton communities taxonomic structure along coastal, shelf and open water habitats was featured by the dominance of species from “other” classes (as Cryptophyceae, Prymnesiophyceae, Prasinophyceae) in the abundance (contributing to 93%) while in the **biomass** dinoflagellates represented the bulk of the assembly (~40%), “other” ranked second (~33%) and diatoms accounting for ~27%.

Along **Constanta** transect (Fig. II.1.5.), “other” classes contributed to more than 80% in the total average **abundance**, with maximum in the coastal

(M01, 706·10^3 cells/L) and shelf area (M03, 1.3·10^6 cells/L) surface layer (0-15m), followed by diatoms -12% (M01, 470·10^3 cells/L) and dinoflagellates (M02, 74·10^3 cells/L). However, in the **biomass** diatoms (30%) and dinoflagellates (48%) co-shared dominance with maximum (M02, 770 mg/m^3, M01, 638 mg/m^3, respectively) of more than 2 times higher than the maximum accounted by “other” species biomass (M03, 208 mg/m^3).

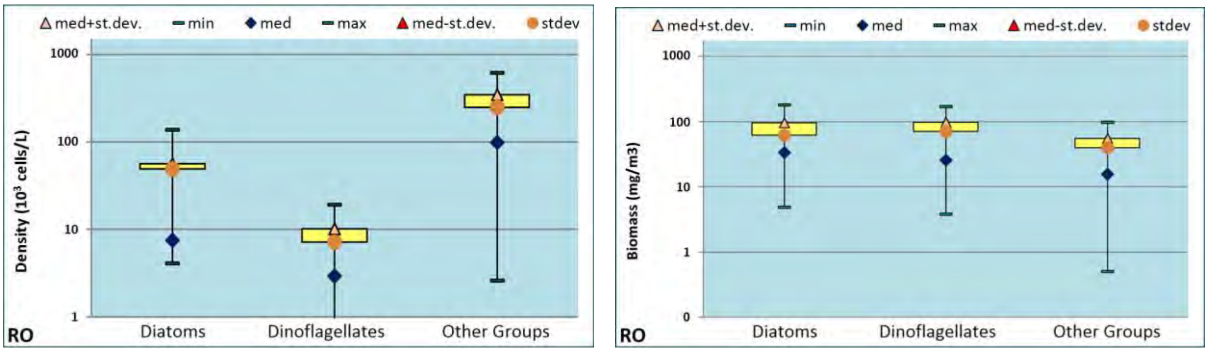


Figure II.1.5. Phytoplankton taxonomic structure on the Romanian transect based on average abundance and biomass in July 2013.

Along **Galata** transect (Fig. II.1.6) similar pattern was observed with species from “other” classes contributing to ~96% in the abundance with maximum at the shelf habitat (M010, 2.4·10^6 cells/L) exceeding by about two fold the maxima found along the other 2 transects. The difference between maxima observed in the shelf area accounted by “other “ species and dinoflagellates was about an order of magnitude (90·10^3 cells/L) and ~ 2 orders of magnitude as compared to diatoms (25·10^3 cells/L). Dinoflagellates dominated the **biomass** (~55%) a maximum measured at the shelf station, M011 - 713 mg/m^3.

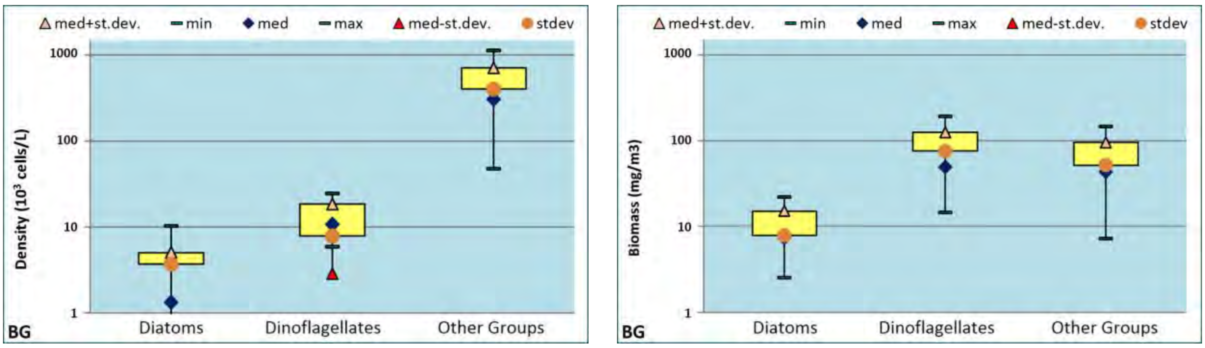


Figure II.1.6. Phytoplankton taxonomic structure along the Galata transect (BG) based on average abundance and biomass in July 2013.

In **Igneada** (Fig. II.1.7), Primnesiophyceae (again “other”) composed the bulk of the **density** (~98%), while diatoms and dinoflagellates represented a much lower proportion. Here dinoflagellates contribution (26%) to total average **biomass** was lower than that of diatoms and "other" (36, 38%, respectively). The maximum biomass was due to diatoms in the shelf area (M016, 837 mg/m^3).

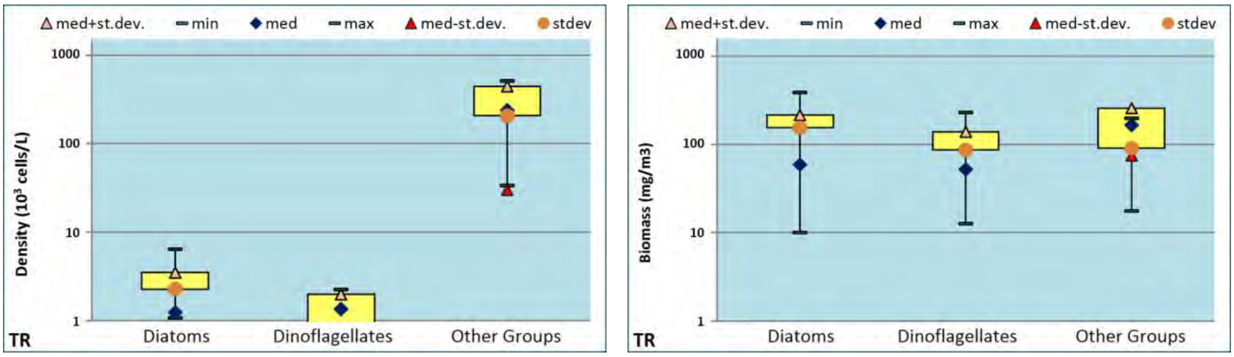


Figure II.1.7. Phytoplankton taxonomic structure on the Igneada transect based on average abundance and biomass in July 2013.

3. Dominant species

On the **Romanian** transect, 4 species were dominant in abundance (*Emiliania huxleyi, Skeletonema costatum, Gymnodinium* sp. and *Scrippsiella trochoidea*) and 7 species in biomass (*Emiliania huxleyi, Skeletonema costatum, Pseudosolenia calcar-avis*, *Prorocentrum micans, Protoperidinium granii, Ceratium furca* and dinoflagellates vegetative stages), most of them found in the shelf area (Table II.1.5.).

At **Galata** stations, there were 5 dominant species in abundance (*Emiliania huxleyi, Hemiselmis* sp., *Coccolithus* sp., *Nephroselmis pyriformis* and microflagellates*)*, 4 of them found in the open water habitat. Dinoflagellates were represented in the biomass with 7 out of 11 dominant species (*Prorocentrum micans, Ceratium furca, Gonyaulax spinifera, G. polygramma, Gymnodinium nanun, Archaeperidinium minutum* and *Peridinium* sp.).

At **Igneada** stations, only one species was dominant in abundance (*Emiliania huxleyi,* above 90% in all the habitats), while in biomass, 5 dinoflagellates co-shared the dominant phytoplankton asssesmblage (*Prorocentrum micans, Pseudosolenia calcar-avis, Ceratium furca, Lingulodinium polyedrum, Protoperidinium steinii, Gyrodinium lachryma*), the diatom *Pseudosolenia calcar-avis*, contrubuted 51% in the shelf area.

In July 2013 the only species observed in a density above $1 \cdot 10^6$ cells/L was the prymnesiophyte *Emiliania huxleyi.* In Bulgaria, *Emiliania* reached the maximum abundance in the coastal area ($1.8 \cdot 10^6$ cells/L). In the shelf area of Galata and Constanta, the concentration slightly decreased ($1.3 \cdot 10^6$ cells/L), but it was higher than the maximum density measured in Igneada open waters ($1 \cdot 10^6$ cells/L).

Table II.1.5. Dominant and common species in the phytoplankton community in July 2013.

RO	**Abundance (cells/L)**	**Biomass (mg/m^3)**
Coastal	*Emiliania huxleyi*	*Pseudosolenia calcar-avis*
	Skeletonema costatum	*Prorocentrum micans*
		Emiliania huxleyi
		Skeletonema costatum
Shelf	*Gymnodinium* sp.	*Pseudosolenia calcar-avis*
		Emiliania huxleyi
		Prorocentrum micans
		Protoperidinium granii
		Ceratium furca
Open waters	*Scrippsiella trochoidea*	*Pseudosolenia calcar-avis*
	Ulotrix zonata	*Protoperidinium granii*
		Peridinee vegetative stages
		Ceratium furca
		Skeletonema costatum
BG	**Abundance (cells/L)**	**Biomass (mg/m^3)**
Coastal	*Emiliania huxleyi* *Coccolithus* sp.1 *microflagellates*	*Pyramimonas amylifera* *Gymnodinium nanum* *Ceratoneis closterium* *Archaeperidinium minutum* *Peridinium* sp.
Shelf	*Emiliania huxleyi* *Hemiselmis* sp. *Coccolithus* sp. *Pyramimonas* sp.	*Emiliania huxleyi* *Prorocentrum micans* *Pseudosolenia calcar-avis* *Ceratium furca* *Gonyaulax spinifera*
Open waters	*Emiliania huxleyi* *Coccolithus* sp. *Microflagellates* *Nephroselmis pyriformis*	*Gonyaulax polygramma* *Pyramimonas amylifera* *Ceratoneis closterium* *Peridinium* sp. *Glenodinium* sp.
TR	**Abundance (cells/L)**	**Biomass (mg/m^3)**
Coastal	*Emiliania huxleyi*	*Emiliania huxleyi* *Prorocentrum micans*
Shelf	*Emiliania huxleyi*	*Pseudosolenia calcar-avis* *Emiliania huxleyi*
Open waters	*Emiliania huxleyi*	*Emiliania huxleyi* *Pseudosolenia calcar-avis*

Table II.1.6.Dominant species in Romanian Black Sea waters during 2000-2012 summer.

Habitat	Coastal	Shelf
Species	*Cyclotella choctawhatcheeana*	*Pseudo-nitzschia delicatissima*
	Emiliania huxleyi	*Emiliania huxleyi*
	Microcystis pulverea	*Cerataulina pelagica*
	Prorocentrum minimum	*Leptocylindrus danicus*
	Pseudo-nitzschia delicatissima	*Cyclotella choctawhatcheeana*
	Nitzschia tenuirostris	*Thalassionema nitzschioides*
	Cerataulina pelagica	*Skeletonema costatum*

The dominant species in the summer phytoplankton community during the recent period (Table II.1.6) in both coastal and shelf Romanian waters were mostly diatoms (*Cyclotella choctawhatcheeana, Pseudonitzschia delicatissima, Nitzschia tenuirostris, Cerataulina pelagica, Leptocylindrus danicus, Thalassionema nitzschioides, Skeletonema costatum*), but also the cyanobacteria *Microcystis pulverea* and the dinoflagellate *Prorocentrum cordatum*. The prymesiophyte *Emiliania huxleyi* (the dominant species in this study) was constantly present (maximum density in summer 2002 - $644 \cdot 10^3$ cells/L).

In Romanian waters**,** none of this species reached a bloom density (over 1 million cells/L) neither the dominant species from the MISIS Cruise, that were found usually at levels lower than $100 \cdot 10^3$ cells/L during the recent surveys.

In Bulgarian waters in summer in the 1 nm coastal area a high proportion of DE% in abundance along with species form “other” classess (MEC%) including the coccolithophorid *Emiliania huxleyi* featured the phytoplankton community and as a result almost all coastal water bodies (WFD) for the period 2006-2011 were classified in category “moderate” (Moncheva, 2013). Among diatoms (~ 80% in 2008 in the coastal area) and ~60% in the open sea the dominant species were *Cerataulina pelagica* (max 1761.74 mg/m^3) *Thalassisosira subsalina, Pseudonitzshia delicatissima* and *Pseudosolenia calcar - avis* . The common large – size diatom *Pseudosolenia calcar-avis* represented a high fraction of the cumulative biomass all down the water column especially in late summer, associated to its unpalatability for consumers and accumulation of biomass due to lack of grazing (Stelmaкh et al, 2009). The contribution of dinoflagellates in the biomass varied between 40-50% (common species *Prorocentrum cordatum*, *Scrippsiella trochoidea, Protoperidinium divergens, Ceratium fusus, Prorocentrum micans* and a number of small size Gymnodinium/Gyrodinium species) co-shared by diatoms. Diatoms *Chaetoceros diadema, Hemiaulas hauckii,* the dinoflagellate *Glenodinium danicum,* the chlorophyte *Nephroselmis astigmatica* and the prasinophyte *Pyramimonas sp.* although native for the area were found in higher levels than normally reported (September 2011) over the entire area. Co-dominance of several species persisted as a specific feature of the phytoplankton assemblages over all pelagic habitats that structured the dominant phytoplankton seasonal (summer) communities.

Similar to Romanian waters almost none of the typical dominant species was observed to proliferate to bloom densities in summer (2006-2013) with few exceptions only *Cerataulina pelagica* (max 1761.74 mg/m^3) in late September, 2008; *Prorocentrum cordatum* (2384.55 mg/m^3, coastal Galata and 3379.47 mg/m^3 at Galata shelf) in late June 2012 and *Pseudonitzschia delicatissima* (3.49·10^6 cells/L) in late June 2012 (Galata coastal), *Emiliania huxleyi* (9.34·10^6 cells/L at Galata coastal, late June 2012) and in September 2013 (1.3·10^6 cells/L -coastal), *Lennoxia faveolata* (2.2·10^6 cells/L, coastal August 2013 found for the first time in bloom density in Bulgarian waters).

4. Functional phytoplankton groups as Potential indicators (A/H ratio)

In July 2013, the phytoplankton community was represented by 264 species of which 55% were autotrophs. On all 3 transects the ratio of autotroph to heterotroph biomass was higher in the surface layer (0-20m, between 1.8 and 34.6) and decreased with depth (0.62 at 46m) (Table II.1.7.).

Table II.1.7. The ratio of autotroph to heterotroph biomass (yellow, A/H>1; red, A/H<1).

Station / Depth	M01	M02	M03	M04	M05	M06	M07	M08	M09	M10	M11	M12	M13	M14	M15	M16	M17	M18
0	2.38	0.80	0.87	3.80	34.65	2.01	7.01	4.13	7.51	1.80	0.84	2.22	6.78	32.58	13.98	6.04	11.82	9.07
5		1.53																
9			0.99															
11										1.99								
12								1.79				1.69						
14	3.28												2.88					
15				3.19		1.91					1.63							
16		1.61			2.07		6.86											
18									3.10									
19														20.15				
20															14.76			
21									1.57								13.31	
22												1.32						
24																18.14		
25			3.96							1.66	0.46							1.47
32	1.08																	
34															10.47			
38											0.51							
39								2.50										
40				0.59														
41					0.67													
42						0.80												
45		0.77							1.15									
46							0.62											
49													2.35					
50										1.25							2.66	
52			1.25															
65																4.63		
66														1.74				
75															1.96			

On the Romanian transect (M05, 0m), the autotrophic species biomass (diatoms – *Pseudosolenia calcar-avis, Thalassionema nitzschioides, Skeletonema costatum*; dinoflagellates – *Gymnodinium sp., Ceratium fusus, Ceratium furca*; prymnesiophytes – *Emiliania huxleyi* etc.) was of more than 30 times higher than the heterotrophic species (dinoflagellates – *Gymnodinium najadeum, Gyrodinium fusiforme, Scrippsiella trochoidea etc*.), similar to the one from Igneada station (M14) surface layer.
On the Bulgarian station, M09 - 0m, the autotrophic species biomass (diatoms - *Pseudosolenia calcar-avis*, cryptophytes - *Hemiselmis sp*., dinoflagellates - *Ceratium furca*, prymnesiophytes - *Emiliania huxleyi* etc.) exceeded ~ 7 times the heterotrophic species biomass (dinoflagellates - *Protoperidinium divergens*, *Prorocentrum compressum*, *Goniodoma sp.* etc.) The autotrophs to heterotrophs abundance ratio by stations was more balanced than the ratio based on biomass - Table II.1.8.

On the Romanian transect in coastal and open waters above thermocline the autotroph microalgae were dominant compared to the shelf area where the heterotrophs took over. Beyond thermocline, a reversed mode was observed with the exception of the shelf waters where autotrophs had a higher contribution.
On the Bulgarian transect, only in open waters both above and beyond thermocline the ratio was in favor of autotrophs.
In the shelf and open waters of the Turkish transect autotrophs were represented by a high proportion above thermocline – Fig. II.1.8 and II.1.9.

Table II.1.8. The ratio of autotrophic to heterotrophic species abundance (yellow, A/H>1; red, A/H<1).

Station / Depth	M01	M02	M03	M04	M05	M06	M07	M08	M09	M10	M11	M12	M13	M14	M15	M16	M17	M18
0	1.38	0.91	0.54	1	1.6	0.67	1.5	1.5	1.2	1.097	1.853	1.182	1.333	1	0.5	0.86	0.6	1
5		1.21																
9			0.93															
11										0.96								
12								0.966				0.703						
14	0.58												1.212					
15				1.11		1.07					0.784							
16		0.77			0.79		2.8											
18									1.167									
19														0.64				
20															0.47			
21									1.067								0.8	
22												1.083						
24																0.71		
25			1							0.879	0.931							1.08
32	2																	
34															0.4			
38											0.935							
39								1.154										
40				1														
41					0.88													
42						1												
45		1.90							0.967									
46							0.92											
49													1.484					
50										1.1							0.82	
52			1.25															
65								1.273								1.17		
66														1				
75															1.25			

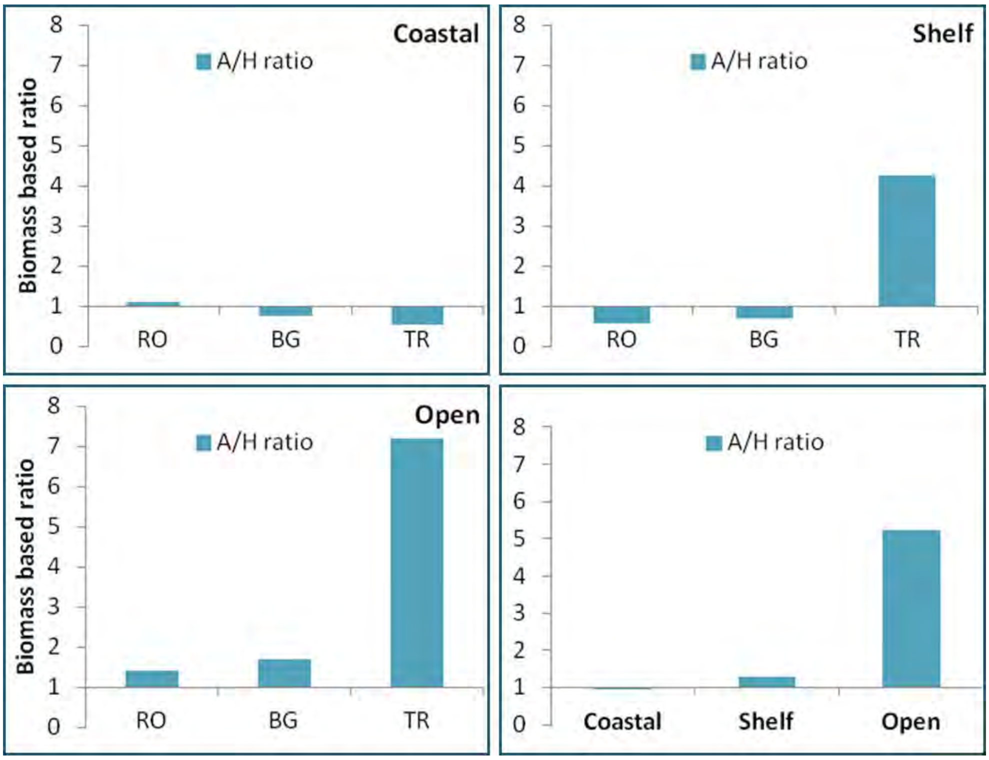


Figure II.1.8. Authotrophs/heterotrophs (A/H) ratio based on average biomass above thermocline.

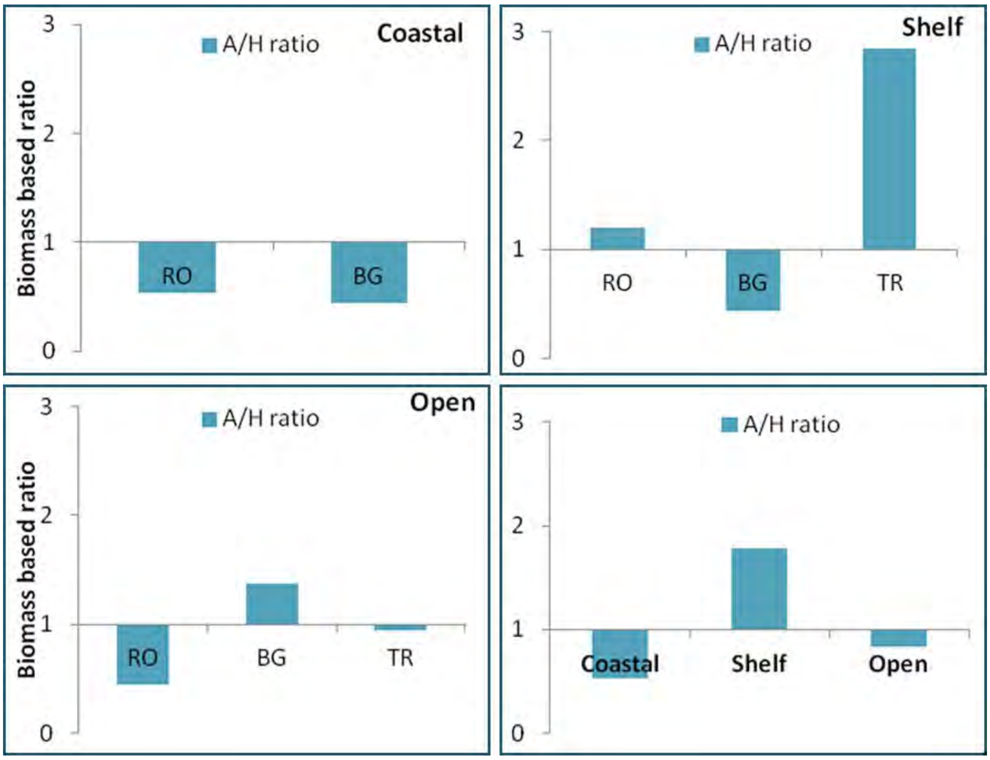


Figure II.1.9. Authothrophs/heterotrophs (A/H) ratio based on average biomass bellow thermocline.

5. Potentially toxic species (PTS)

Within the MISIS Joint Cruise data set 12 potentially toxic phytoplankton species were identified, contributing 5% of the total number of species (8 species in RO transect, 12 - in BG transect and 9 - in TR transect) - Table II.1.9. Dinoflagellates were represented by 10 species: *Prorocentrum cordatum*, *Gonyaulax spinifera*, *Lingulodinium polyedrum*, *Phalacroma rotundatum*, *Protoceratium reticulatum* and 5 species from genus *Dinophysis* (*Dinophysis acuminata*, *Dinophysis acuta*, Dinophysis *caudata*, *Dinophysis fortii*, *Dinophysis sacullus*). Among Class Bacillariophyceae 2 species from genus *Pseudo-nitzschia* (*Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia seriata*) were identified.

Table II.1.9. Potentially toxic phytoplankton species along Romanian, Bulgarian and Turkish transects.

Species	RO	BG	TR	Harmfull effect in Black Sea*	Blooming species Black Sea**
Class Bacillariophyceae					
Pseudo-nitzschia delicatissima	x	x	x		x
Pseudo-nitzschia seriata		x			x
Class Dinophyceae					
Dinophysis acuminata		x	x	x	
Dinophysis acuta	x	x			
Dinophysis caudata	x	x	x	x	
Dinophysis fortii		x	x	x	
Dinophysis sacullus	x	x	x		
Goniaulax spinifera	x	x	x	x	
Lyngulodinium polyedrum		x	x	x	x
Phalacroma rotundatum	x	x	x	x	
Prorocentrum cordatum	x	x	x		
Protoceratium reticulatum	x	x			

* *Harmful effect was reported in the Black Sea* (BSPC Editorial Board, 2014; Vershinin, Orlova, 2008)
*******Black Sea blooming species - Pseudo-nitzschia delicatissima, Lingulodinium polyedrum, Pseudo-nitzschia seriata, Prorocentrum cordatum* (Moncheva S. et. al., 2001; Velikova et al., 1999; Nesterova et al., 2008).

The average abundance of phytoplankton PTS varied between 117 and 19,206 cells/L and the average biomass between 0.376 and 107.305 mg/m^3. PTS total abundance was relatively high in RO shelf (station M02-T – 19,206 cells/L, max in the entire area) and BG shelf (stations M10-T – 18 672 cells/L and M11-T – 15,671 cells/L), while in the TR area it was low (shelf station M15-T – 1,600 cells/L) – Fig. II.1.10 and II.1.11. Dinoflagellates *Prorocentrum cordatum* (stations M02-T (RO) – 14,224 cells/L and M10-T (BG) – 13,540 cells/L) and the diatom *Pseudo-nitzschia delicatissima* (stations M02-T (RO) – 4,664 cells/L and M10-T (BG) – 3,125 cells/L) accounted to the relatively high total abundance of PTS over RO and BG Black Sea habitats. Albeit the low total abundance of PTS along the TR transect a maximum was measured at station M15-T, due to the proliferation of *Prorocentrum cordatum* (stations M15-T (TR) – 1,458 cells/L).

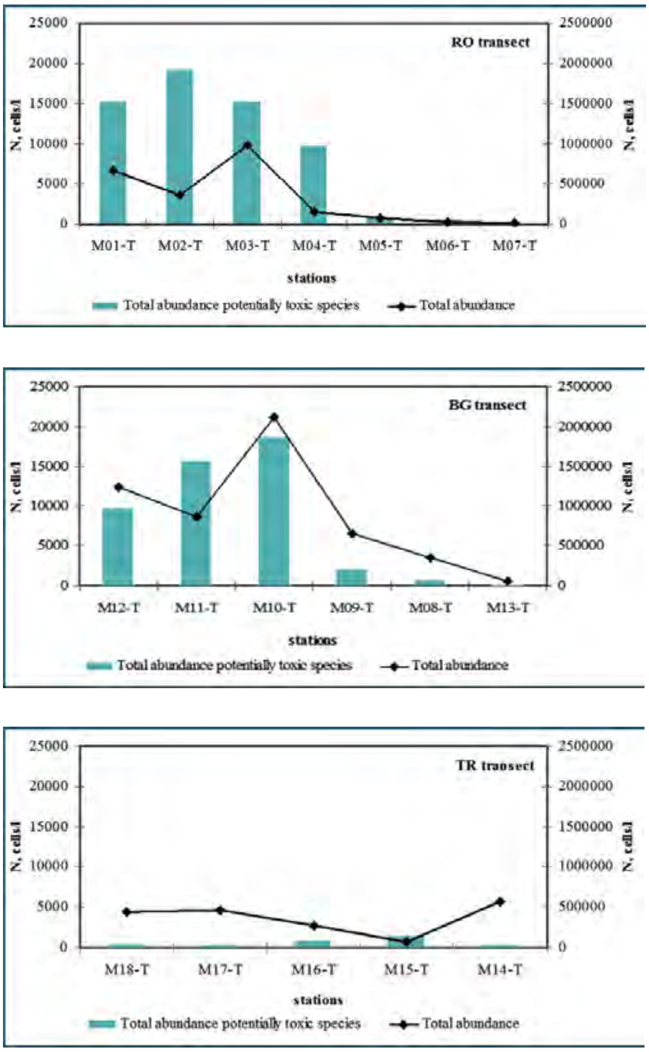


Figure II.1.10. Total abundance of the potentially toxic phytoplankton species along the Romanian, Bulgarian and Turkish transects (attention to scale difference).

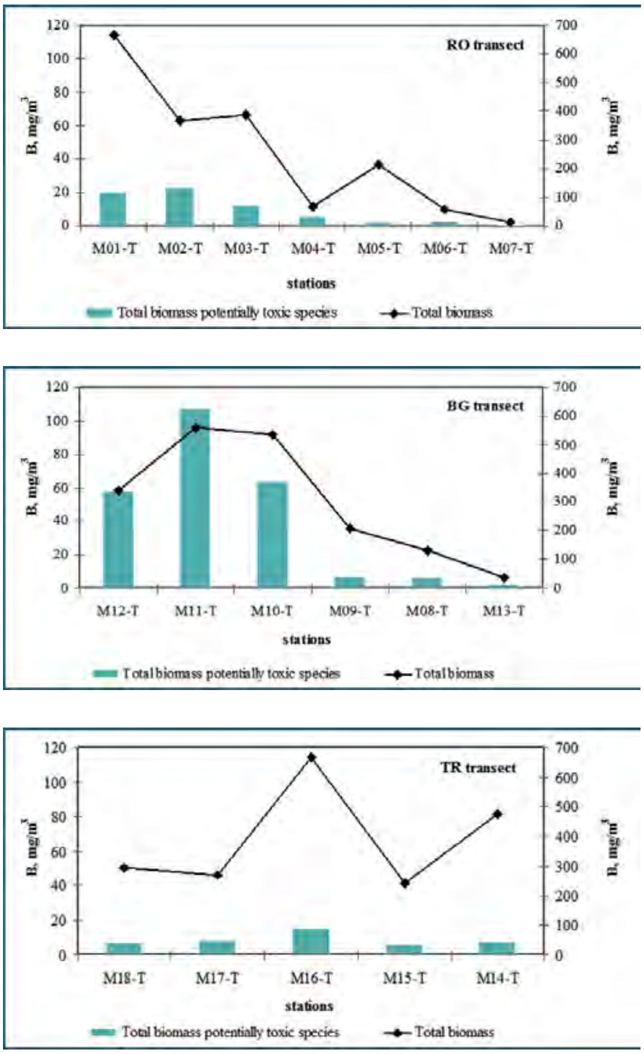


Figure II.1.11. Total biomass of potentially toxic phytoplankton species along the Romanian, Bulgarian and Turkish transects (attention scale difference).

A specific pattern distinguished the PTSs abundance and biomass spatial distribution along the three transects – a decrease from the coastal to the open sea along the RO transect, relatively high values measured in coastal and shelf stations along the BG pelagic habitats, whereas no trend and lowest densities observed in the TR waters. A pronounced decline of total PTS abundance occurred from north to south most evident in coastal waters – Fig. II.1.12 and II.1.13.

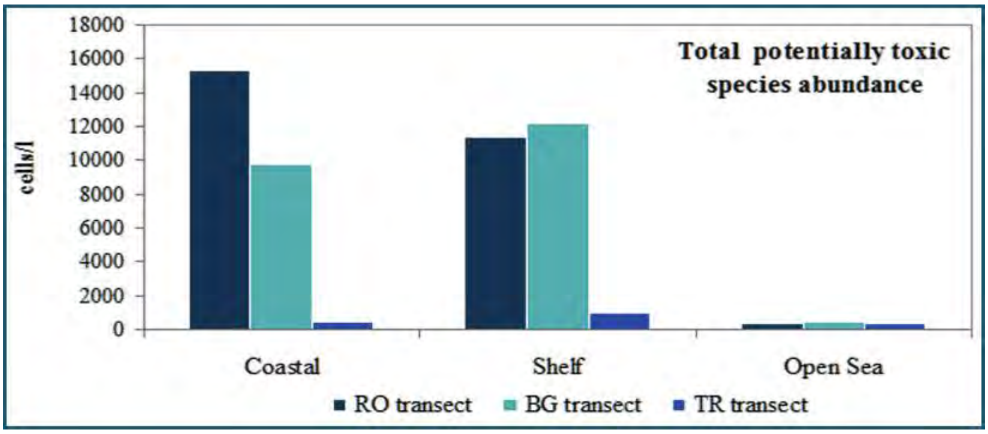


Figure II.1.12. Distribution of PTSs average abundance by pelagic habitats.

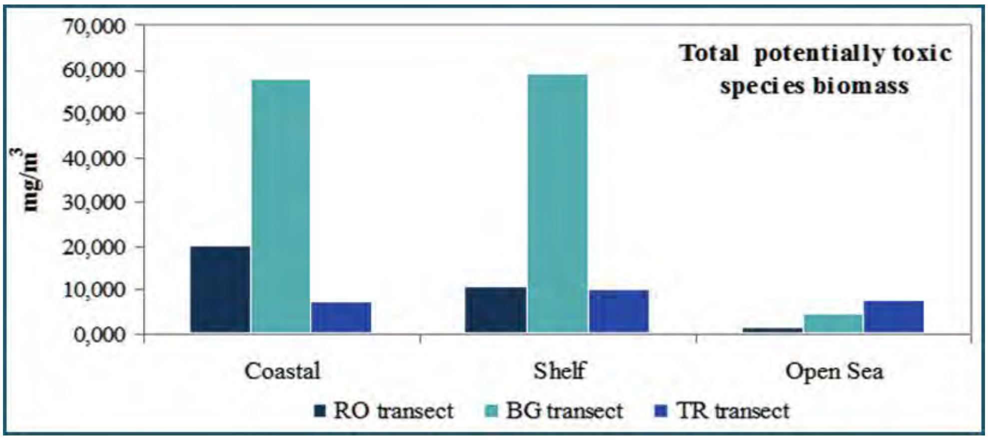


Figure II.1.13. Distribution of PTSs average biomass by pelagic habitats.

Over the RO pelagic habitats the max PTS biomass measured at the shelf station M02-T (22,996 mg/m^3) was dominated by *Prorocentrum cordatum* (14,764 mg/m^3), while *Lingulodinium polyedrum* (10,908 mg/m^3) accounted to the max biomass in Turkish waters, observed at the shelf station M15-T (15,750 mg/m^3) - Fig. II.1.14, II.1.15 and II.1.16.

PTSs cumulative biomass max in the BG waters (Fig. II.1.13) at the shelf station M11-T – 107,305 mg/m^3 (19% of the total biomass – 558,237 mg/m^3), was composed by the dinoflagellates *Gonyaulax spinifera* (shelf stations M11-T – 58,328 mg/m^3, M10-T – 28,620 mg/m^3), *Lingulodinium polyedrum* (coastal stations M12-T – 30,216 mg/m^3) and *Prorocentrum cordatum* (shelf stations M10-T – 16,529 mg/m^3, M11-T – 14,511 mg/m^3).

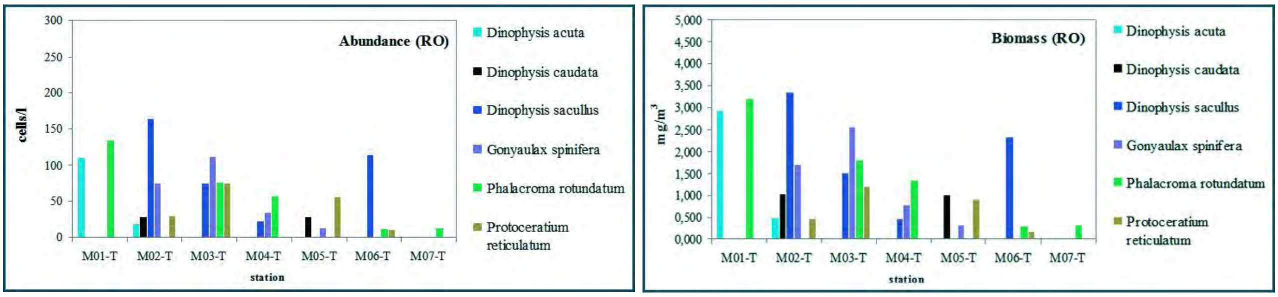


Figure II.1.14. Abundance and biomass of PTSs along the Romanian transect.

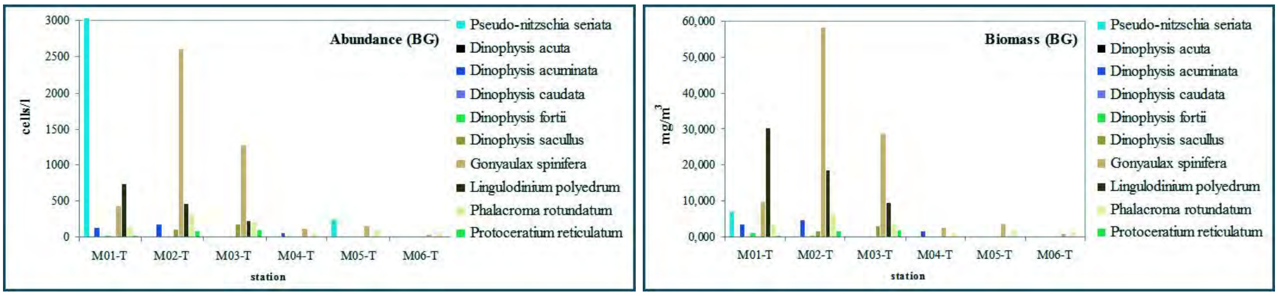


Figure II.1.15. Abundance and biomass of PTSs along the Bulgarian transect.

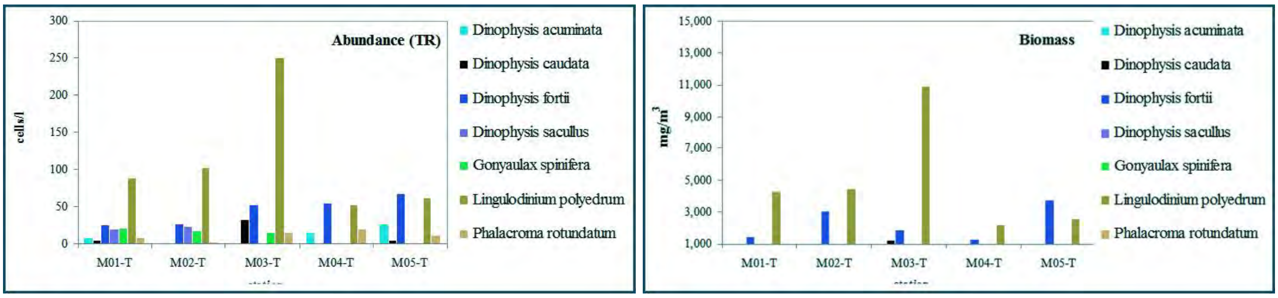


Figure II.1.16. Abundance and biomass of PTSs along the Turkish transect.

High abundance of *Prorocentrum cordatum* was a common feature of the entire pelagic domain, whereas *Pseudo-nitzschia delicatissima* maxima were observed mostly in the Romanian coast and shelf – Fig. II.1.17 and II.1.18.

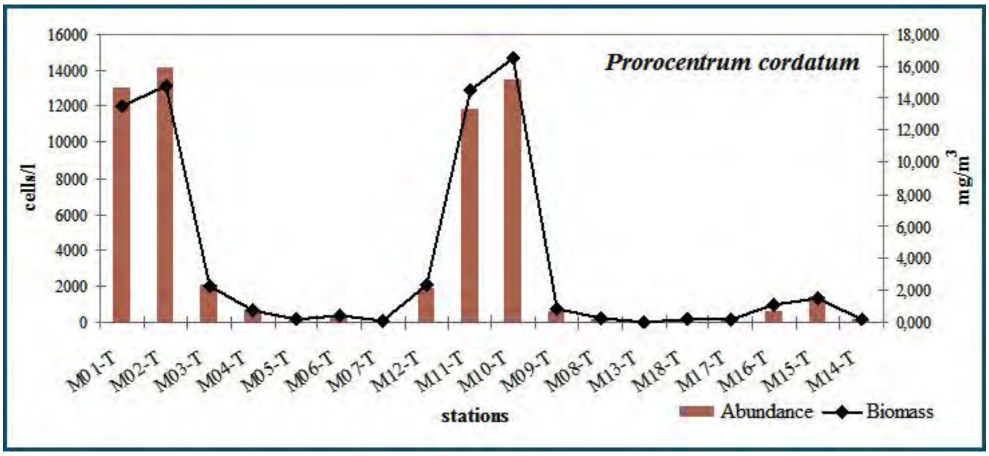


Figure II.1.17. ***Prorocentrum cordatum*** **abundance and biomass distribution along the Romanian, Bulgarian and Turkish transects.**

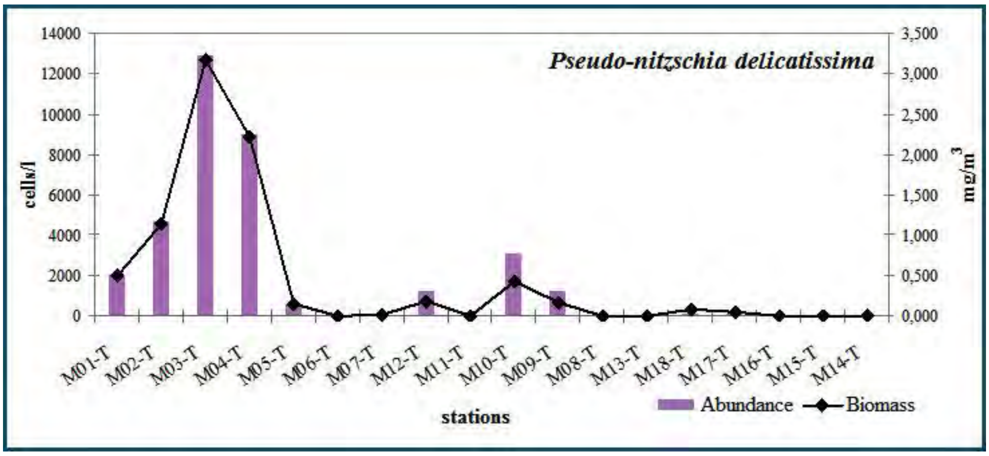


Figure II.1.18. ***Pseudo-nitzschia delicatissima*** **abundance and biomass distribution along the Romanian, Bulgarian and Turkish transects.**

The dinoflagellate *Prorocentrum cordatum* was a common and recurrent blooming agent during the most eutrophicated period of the Black Sea for the NW part (Moncheva et al, 1995; Moncheva et al., 2001; Nesterova et al., 2008), associated to widespread mass mortality events especially in summer with max biomass of ~ 500 g/m^3 observed in 1986. Toxicity test of *Pseudo-nitzschia delicatissima* by receptor binding assay and LC-MS were negative (Vershinin & Orlova, 2008), so does the toxicity mouse bioassay of *P. cordatum* (Moncheva, 1991).

However the comparison of the cell densities observed in this study with those associated to toxicity events reported for other Black Sea areas reveal higher concentrations measured for some species – Table II.1.10 and II.1.11.

Table II.1.10. PTSs toxicity reported for the Black Sea.

PTS toxic evidence	Location	Year	Abundance [cells/L]	Toxins	Method	Reference
Alexandrium tamarense	mussel hepatopancreas	2002, 2003	300-4 500	PSP toxins GTX2, GTX3 and GTX5	HPLC	Vershinin *et al.*, 2006
Pseudo-nitzschia calliantha	Domoic acid (DA) in batch culture		80000-240000	Domoic acid (DA)	HPLC	Besiktepe *et al.*, 2008
Dinophysis caudata		2002	250	OA DTX1, PTX2, PTX2sa	LCMS/MS	Morton *et al.*, 2009
Dinophysis caudata	mussel hepatopancreas Anapa region	1996-1999	200-600	DSP toxicity DTX2	HPLC	Vershinin & Kamnev, 2000
Gonyaulax spinifera	shellfish hepatopancreas (the Caucasian Black Sea Coast)	2002	375	YTX, 45-OH-YTX, homoYTX		Morton *et al.*, 2007
Lingulodinium polyedrum	shellfish hepatopancreas (Caucasian Black Sea Coast)	2002	300	YTX, 45-OH-YTX, homoYTX		Morton *et al.*, 2007
Prorocentrum lima	DSP-toxicity in hepatopancreas	2001	300			Morton *et al.*, 2009

Abbreviations: DA - domoic acid; DTX1 - Dinophysistoxin-1; DTXs - dinophysis toxins;GTXs - gonyautoxins; GTXs - gonyautoxins; OA - okadaic acid; PTXs – pectenotoxins; YTX – yessotoxins;PSP - Paralytic shellfish poisoning; DSP - Diarrhetic shellfish poisoning.

Table II.1.11. PTSs abundance measured in this study and during toxic events reported for the Black Sea.

PTSs of toxic evidence	Abundance [cells/L]	This study			
		Abundance [cells/L]	BG	BG	BG
		Habitat	coastal	shelf	shelf
		Stations	M12-T	M11-T	M10-T
Gonyaulax spinifera	375		**436**	**2608**	**1280**
Lingulodinium polyedrum	300		**750**	**465**	236
Phalacroma rotundata	200-600		154	**324**	**214**

A historical review and recent inventory (1999-2013) of PTSs in Bulgarian waters (Dzhembekova & Moncheva, 2014) confirm the presence and proliferation to blooming concentration of the three most common species (*P. delicatissima, P. seriata, P. cordatum*). Comparison between maximum densities reported for some species and toxicity evidence observations for other Black Sea regions indicate that along the Bulgarian part of the sea PTSs reach ten times higher abundance (*Alexandrium tamarense* - 6.5 times higher; *Dinophysis caudata* - 23 times higher; *Gonyaulax spinifera* - 30 times higher; *Lingulodinium polyedrum* - 28 times higher; *Phalacroma rotundatum* - 29 times higher), suggesting a potential threat of toxic events do exist.

There is evidence that nutrients can play a major role in the regulation of toxicity in some HAB species, and this can have significant implications to toxin monitoring programs and public health decisions (Anderson et al., 2002). In some cases, toxicity can increase or decrease dramatically depending on the limiting nutrient. Saxitoxin production by *Alexandrium tamarense* can be 5–10-fold higher in P-limited versus N-limited cells (Boyer et al., 1987; Anderson et al., 1990). Likewise, domoic acid production by *Pseudo-nitzschia multiseries* was inversely correlated with the ambient Si concentration in batch culture (Pan et al., 1996a). Anderson *et al*. (2002) found out that cells started accumulating toxin only when the division rate declined as a result of partial or total depletion of silica. When cultures were N-limited, no toxin was produced. Toxin production was greatly enhanced under P-deficient conditions in continuous cultures (Pan et al., 1996b). Literature results also suggest that Fe limitation can enhance toxicity in *Pseudo-nitzschia* spp. (Rue and Wells, unpublished data). For some HAB species a similar picture emerges: toxin production varies significantly with different degrees and types of nutrient limitation. The dinoflagellate *Dinophysis acuminata* produced elevated levels of the DSP toxin, okadaic acid, under both N and P limitation, but the enhancement was 6-fold larger with N-limitation (Johansson et al., 1996). In a similar although inverse mode, *Chrysochromulina polylepis* was 6-fold more toxic under P enrichment, than N-limited conditions (Johansson & Graneli, 1999a). Another prymnesiophyte, *Prymnesium parvum*, increased toxicity under N-limited or P-limited conditions (Johansson & Graneli, 1999b).

The chemical form of the nutrient supplied to the HAB species can also affect toxicity, although this is an area that has received relatively little attention. Due to the nutrient enrichment, HAB cells might be more abundant, but because of the altered nutrient ratios, their cellular toxicity could be higher or lower than with non-eutrophic conditions. Depending on the species, the net effect could thus be an increase, decrease, or no change in overall toxicity from a public health, fisheries, or ecosystem impact perspective. This is an area of obvious importance, but further research is needed before useful insights about nutrient form and HABs can be provided to coastal resource managers (Anderson et al., 2002) and for the implementation of the Marine strategy framework directive.

6. Size structure

The different size-classes showed quite different cross- phylletic composition – Table II.1.12. The nano-1 group was the most phylletically diverse, including almost all species from the composite group "others". Dinophyceae represent the bulk of nano-2 group along with diatoms and few "others" species, while the microphytoplankton group was composed from diatoms and dinoflagellates only, of much lower diversity as compared to the nano-2 class.

Table II.1.12. Taxonomic list of species by size classess.

Nano -1	**Nano-2**	**Micro**
Species	**Species**	**Species**
	Bacillariophyceae	
Chaetoceros septentrionalis	*Aulacoseira italica*	*Coscinodiscus granii*
Cyclotella caspia	*Cerataulina pelagica*	*Pseudosolenia calcar-avis*
Nitzschia tenuirostris	*Chaetoceros affinis*	*Thalassiosira eccentrica*
Pseudo-nitzschia delicatissima	*Chaetoceros curvisetus*	
Skeletonema costatum	*Chaetoceros similis*	
Thalassiosira parva	*Proboscia alata*	
	Thalassionema nitzschioides	
	Thallassiosira eccentrica	
	Triceratium sp.	
	Dinophyceae	
Amphidinium acutissimum	*Akashiwo sanguinea*	*Akashiwo sanguinea*
Glenodinium paululum	*Alexandrium* sp. *(24/21)*	*Dinophysis acuta*
Glenodinium sp.*(12/10)*	*Amphidinium crassum*	*Dinophysis caudata*
Glenodinium sp.*(9,8/7,1)*	*Amphidinium extensum*	*Dinophysis fortii*
Glenodinium sp.*(14/11,2)*	*Amphidinium longum*	*Diplopsalis lenticula*
Gymnodinium agiliforme	*Amphidinium* sp.	*Goniodoma* sp.
Gymnodinium hamulus	*Archaeperidinium minutum*	*Gonyaulax polygramma*
Gymnodinium lantzschii	*Cochlodinium archimedes*	*Gonyaulax scrippsae*
Gymnodinium nanum	*Cochlodinium citron*	*Gymnodinium rubrum*
Gymnodinium ostenfeldii	cyst 16	*Gyrodinium fusus*
Gymnodinium profundum	cyst 24	*Lingulodinium polyedrum*
Gymnodinium pulchrum	cyst 36	*Ceratium furca*
Gymnodinium punctatum	*Dinophysis acuminata*	*Ceratium fusus*
Gymnodinium semidivisum	*Dinophysis acuta*	*Phalacroma acutum*
Gymnodinium simplex	*Dinophysis sacculus*	*Protoperidinium claudicans*
Gymnodinium sp. (9,7/9,3)	*Diplopsalis lenticula*	*Protoperidinium depressum*
Gymnodinium wulffii	*Ensiculifera carinata*	*Protoperidinium divergens*
Heterocapsa rotundata	*Glenodiniopsis steinii*	*Protoperidinium granii*
Katodinium fungiforme	*Glenodinium bernardinense*	*Protoperidinium pallidum*
Lessardia elongata	*Glenodinium pilula*	*Protoperidinium solidicorne*
Protoperidinium bulla	*Glenodinium* sp.*(16,9/14,6)*	*Protoperidinium steinii*
	Glenodinium sp.5 *(17/12)*	*Pyrophacus horologicum*
	Gonyaulax cochlea	*Triadinium sphaericum*

Nano -1	Nano-2	Micro
Species	**Species**	**Species**
	Gonyaulax digitale	
	Gonyaulax grindleyi	
	Gonyaulax minima	
	Gonyaulax monacantha	
	Gonyaulax polygramma	
	Gonyaulax spinifera	
	Gymnodinium helveticum	
	Gymnodinium incertum	
	Gymnodinium lachmannii	
	Gymnodinium lacustre	
	Gymnodinium najadeum	
	Gymnodinium sp. *(18,9/17,5)*	
	Gymnodinium sp.	
	Gymnodinium sp.*(19/17)*	
	Gyrodinium flagellare	
	Gyrodinium fusiforme	
	Gyrodinium lachryma	
	Gyrodinium ovum	
	Heterocapsa triquetra	
	Lingulodinium polyedrum	
	Oblea rotunda	
	Peridinee cyst	
	Peridinee vegetative stage	
	Peridiniella catenata	
	Peridinium granii f. *mite*	
	Peridinium quinquecorne	
	Phalacroma pulchellum	
	Phalacroma rotundatum	
	Preperidinium meunieri	
	Pronoctiluca pelagica	
	Prorocentrum compressum	
	Prorocentrum micans	
	Prorocentrum minimum	
	Prorocentrum scutellum	
	Protoceratium reticulatum	
	Protoperidinium breve	
	Protoperidinium brevipes	
	Protoperidinium conicum	
	Protoperidinium globulus	
	Protoperidinium pellucidum	
	Protoperidinium subinerme	
	Scrippsiella trochoidea	
	Scrippsiella trochoidea (18/13)	
	Torodinium robustum	
	Chlorophyceae	

Nano -1	Nano-2	Micro
Species	**Species**	**Species**
Actinastrum hantzschii	*Chlamydomonas* sp.	
Ankistrodesmus arcuatus	*Crucigenia tetrapedia*	
Chlorophyceae sp.		
Coelastrum sp.		
Monoraphidium arcuatum		
Monoraphidium griffitii		
Monoraphidium sp.		
round cell (4,1)		
Scenedesmus quadricauda		
Scenedesmus spinosus		
undentified round cell (4,1)		
Cryptophyceae		
Chroomonas caudata	*Chroomonas caudata*	
Chroomonas sp.	*Cryptomonas* sp.	
Cryptophyceae sp.	*Rhodomonas marina*	
Hemiselmis sp.		
Hillea fusiformis		
Plagioselmis sp.		
Cyanophyceae		
Anabaena sp.		
Monoraphidium irregulare		
Phormidium hormoides		
Pseudanabaena limnetica		
Spirulina sp.		
Romeria sp.		
Synechococcus sp.		
Euglenoidea		
Astasia sp.	*Eutreptia lanowii*	
Lepocinclis acus		
Nephroselmidophyceae		
Nephroselmis astigmatica		
Nephroselmis pyriformis		
Prasinophyceae		
Pseudoscourfieldia marina		
Pyramimonas amylifera		
Pyramimonas sp.		
Ulotrix zonata		
Prymnesiophyceae		
Coccolithus sp.	*Calyptrosphaera oblonga*	
Emiliania huxleyi	*Coccolithus* sp.	
Padlova sp.		
Dictyochophyceae		
	Dictyocha fibula	
Ebriophyceae		
	Ebria tripartita	
Microflagellates		
microflagellates		

The size-classes proportions in phytoplankton community abundance were very homogenously distributed, the nano-1 class overwhelming at all stations with contribution between 83-99% - Fig. II.1.19. The ultimate dominant species was the prymnesiophyte *Emiliania huxleyi,* at most of the stations contributing to 80-90% of the total abundance, while at selected stations (M03, M10, M12, M14) found in bloom densities (> $1{\cdot}10^6$ cells/L).

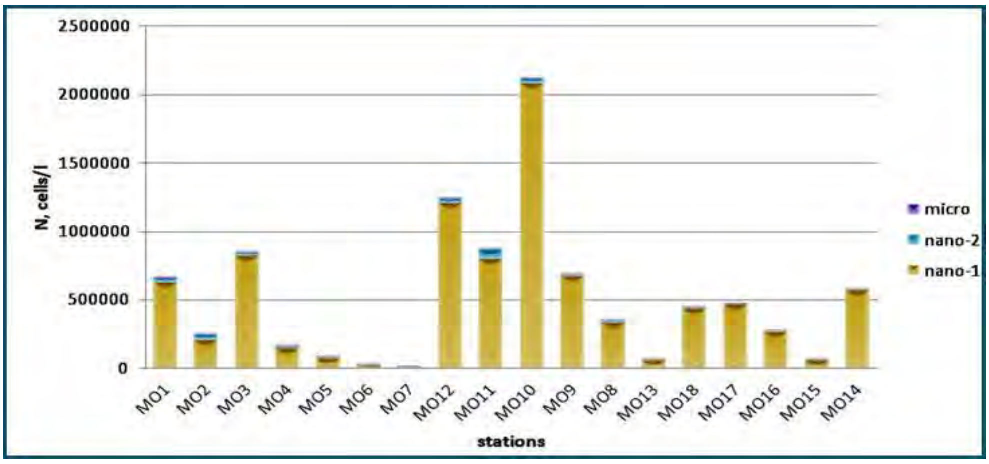


Figure II.1.19. Size-classes proportion in the total phytoplankton abundance by stations.

The presence of cyanophytes (*Pseudanabaena limnetica, Spirulina* sp.*, Monoraphidium arcuatum, Anabaena* sp.*)* in the RO shallow stations (M01, M02), Pseudo-*nitzschia delicatissima* and *Skeletonema costatum* in the shelf –open habitats (M05, M06) *added* to the diversity within the group and contributed between 3-20%.

The share of Microflagellates, Nephroselmidophyceae (*Nephroselmis pyriformis*), Cryptophyceae (*Hemiselmis* sp.*)* Prasinophyceae *(Pyramimonas* sp.*)* varied between 20-45 % in the BG shelf stations (M09, M10), while along the TR transect this size class was represented solely *by E. huxleyi* . No trend along the coastal-open pelagic habitats could be extracted.

In contrast a remarkable heterogeneity in the size-classes proportion in the phytoplankton biomass was evident, the nano-1 class contributing to more than 60% in some coastal (M18) shelf (M10, M17) and open sea (M14) stations. The nano-1 class biomass represented mostly the “others” biomass (R^2 = 0.98, correlation not shown). The micro size class high share was associated mainly with coastal (M01) and shelf habitat stations (M02, M05, M16) – Fig.II.1.20.

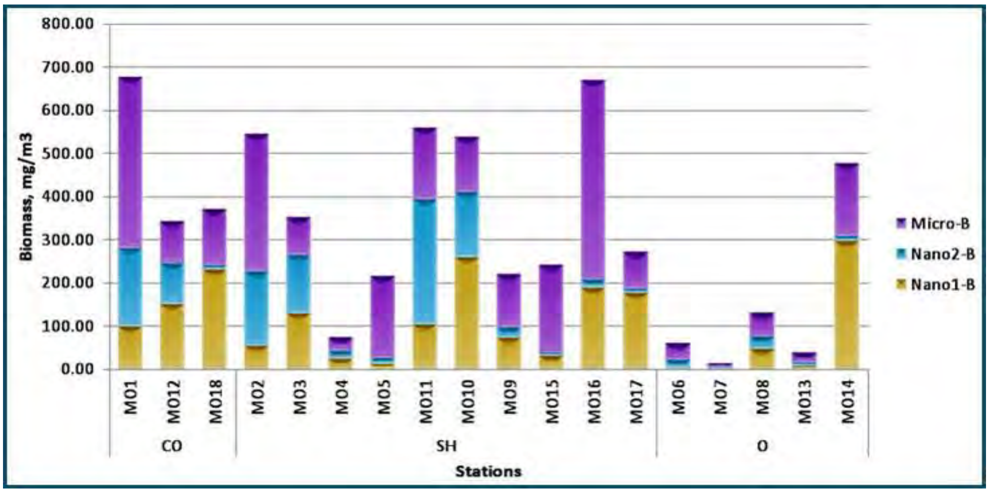


Figure II.1.20. Size-classes proportion in the total phytoplankton biomass by stations and habitats (CO-coastal, SH-shelf; O-open sea habitats).

While on average the three size classes biomass showed similar decreasing trend along the coastal-open sea habitats coinciding with the total biomass decrease (Fig. II.1.21), the trends of their proportion in the total biomass were different – more apparent in the nano-1 size class (an increase from 34 to 50% from the coast to the open sea) and a decrease of the other two size classes, although the dispersion of the data was very high (high stdev), reflecting the general pattern of nutrients spatial distribution.

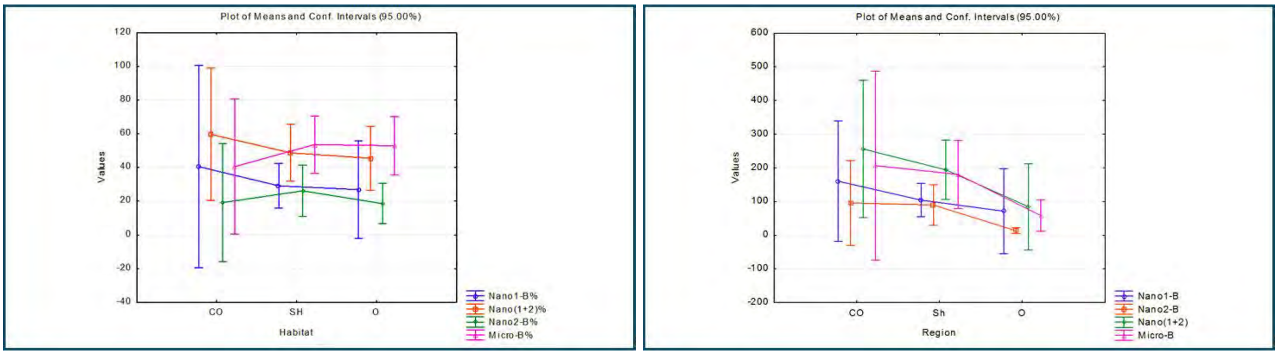


Figure II.1.21. Average size-classes proportion in the total phytoplankton biomass by habitats (CO-coastal, SH-shelf; O-open sea habitats).

Based on multiple regression analysis, 67% of the variation in the biomass of nano-1 size class was explained by nutrients (similar R^2 for "others" biomass – 0.65) - Table II.1.13. The regression model for this dependent variable has the highest R square and Adjusted R square.

Table II.1.13. Models summary statistical results.

	R	R Square	Adjusted R Square	Std. Error of the Estimate
Nano1B	,821	,673	,597	,634
Nano2B	,419	,176	-,017	1,008
Nano1-2B	,599	,358	,209	,889
MicroB	,619	,384	,240	,871
PhytoB	,672	,451	,323	,822
BacB	,633	,400	,260	,860
DinB	,375	,140	-,060	1,029
OthersB	,810	,656	,576	,651

The correlation (R^2) between nutrients and total Phyto biomass and Bacillariophyceae biomass was lower, very close to that of nano-1-2 and micro-biomass – Table II.1.13. According to ANOVA test (F-test) and significance (p-value associated with it) the multiregression models show good fit for the data - Table II.1.14.

Table II.1.14. ANOVA test goodness-of-fit for the data (in gray-statistial significant results, P<0.05).

Model		Sum of Squares	df	Mean Square	F	Sig.
	Nano1B	24,912	7	3,559	8,833	,000[b]
	Nano2B	6,500	7	,929	,913	,510[b]
	Nano12	13,255	7	1,894	2,393	,045[b]
	MicroB	14,195	7	2,028	2,668	,029[b]
	PhytoB	16,690	7	2,384	3,522	,007[b]
	BacB	14,804	7	2,115	2,859	,021[b]
	DinB	5,190	7	,741	,699	,672[b]
	OthersB	24,272	7	3,467	8,173	,000[b]

Based on the significance levels of regression coefficients determined by the *t - tests*, the predictors (nutrients) with highest impact and significance, ranked by their weight in the model, were silicates (negative coefficient), S:N ratio, total inorganic nitrogen (oxidized nitrogen plus ammonium) and Si:P ratio - Table II.1.15. The statistically significant predictors for nano-1 biomass and for others biomass (negative correlation) were phosphates, silicates and Si:N and Si:P ratios. Nutrients were responsible for 45% of the Total Biomass variation and accounted for 40% of the variation in Bacilariophyceae biomass with statistically significant predictors Silicates (negative coefficient), Si:N and dissolved inorganic nitrogen.

Table II.1.15. Statistically significant environmental variables (p <0.05).

Parameter	Correlation	$(PO_4)^{3-}$[µM]	$(SiO_4)^{4-}$[µM]	NOx [µM]	Si/P	Si/N
Nano1B [mg/m³]	Sig.	0.015	0.000		0.003	0.000
	t-test	2.576	-4.734		3.253	4.711
Nano(12) B [mg/m³]	Sig.		0.011			0.023
	t-test		-2.729			2.395
MicroB [mg/m³]	Sig.		0.015	0.009		0.023
	t-test		-2.575	2.803		2.404
Phyto Btot [mg/m³]	Sig.		0.003	0.009		0.006
	t-test		-3.223	2.809		2.938
BacB [mg/m³]	Sig.		0.033	0.004		0.02
	t-test		-2.23	3.159		2.459
OthersB [mg/m³]	Sig.	0.021	0.000		0.004	0.000
	t-test	2.439	-4.476		3.096	4.462

The CCA ordination axes (i.e. the synthetic gradients extracted by CCA) explained 55 % of the cumulative variance in phytoplankton data, 33% accounted for environmental axis 1, while environmental axes 2 explained 22% - Table II.1.14. On the basis of interset correlations, dissolved inorganic nitrogen, phosphates and silicates had the strongest correlation to axis 1, while nutrient ratios (Si:P, Si:N –negative correlation) to axis 2, providing a good representation of the major environmental factors controlling phytoplankton size structure – Table . II.1.16, Table II.1.17.

Table II.1.16. CCA statistical output.

	Axis 1	Axis 2
Eigenvalues	0.008	0.005
Percentage	32.824	21.760
Cum. Percentage	32.824	54.584
Cum.Constr.Percentage	0.409	0.588
Spec.-env. Correlations	0.859	0.839

Table II.1.17. Interset correlations between environmental variables and site scores.

	Envi. Axis 1	Envi. Axis 2
(PO4)3-	0.494	-0.095
(SiO4)4-	0.473	-0.204
(TNOx)	0.633	0.118
(NH4)+	0.029	0.299
DIN	0.325	0.249
N/P	-0.240	0.570
Si/P	-0.304	-0.426
Si/N	0.225	-0.448

The phytoplankton community metrics and environmental variables showed correlation values of 0.86 and 0.84 on canonical axes 1 and 2, respectively, suggesting a strong relationship – Table II.1.17.

The biplot generated by CCA and the derived CCA scores (Fig. II.1.22) showed a separation of 4 regions by environmental gradients and spatial distribution of phytoplankton parameters.

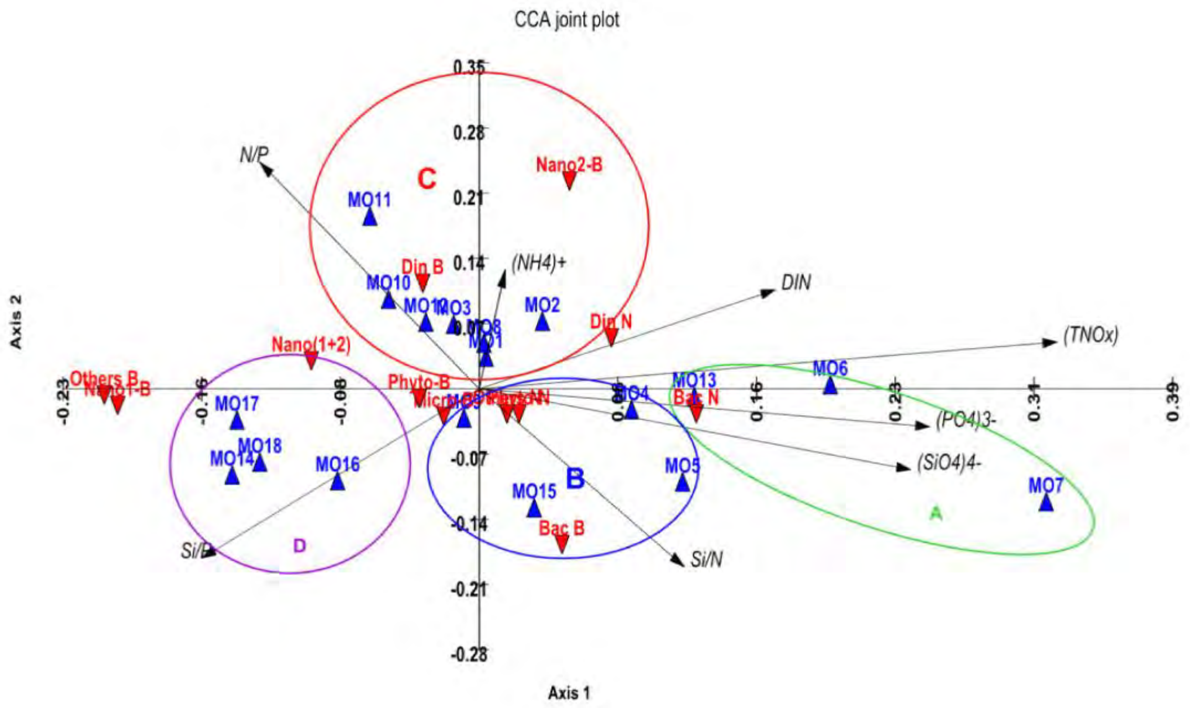


Figure II.1.22. CCA ordination joint plot

CCA ordination joint plot showing environmental gradients (arrows) - nutrients in µM/l: PO4 – phosphates, SiO4 – silicates, (TNO)x - total oxidized nitrogen, NH4 – ammonium, DIN – dissolved inorganic nitrogen and nutrient ratios - N/P, Si/P, Si/N. Phytoplankton variables – red triangles: size classes biomass [mg/m^3], (nano1-B , nano 2-B , nano 1-2, micro B); Bac B-Bacillariophyceae Biomass [mg/m^3], Bac N - Bacillariophyceae Abundance [cells/L], Din B - Dinophyceae Biomass [mg/m^3], Dn-N - Dinophyceae Abundance [cells/L], Others B – other phylla biomass [mg/m^3], Others N - Other phylla Abundance [cells/L]. Sample stations – blue triangles. Environmental arrows represent a gradient, where the mean value is located at the origin, and the arrow points in the direction of its increase, while the length and orientation indicate their relative importance to each axis. Each sample point lies at the centroid of the points for specific phytoplankton variable that occur in those samples.

Group A was composed of open sea stations, located along the ambient nutrient gradients, and centered around Bacillariophyceae (Abundance).
Group B represented mainly the deep shelf stations positioned along Si:N

ratio where Bacillariophyceae (Biomass) was the discriminating phytoplankton variable.

In Group C (shallow shelf and coastal habitat in front of Romania and Bulgaria) the stations were located along the gradients of N:P ratio and ammonium. The environment habitat selection gave preference to nano-2 size class along ammonium gradient as a main factor, concomitant to Dinophyceae biomass along N:P gradient.

Group D comprise the stations located in the southern- most transect (TR) along Si:P gradient, centered collectively around the nano size classes (nano-1, nano1-2 and Others) as associated with environment of high Si:P ratio.

Although applied to a limited single cruise dataset the results support the hypotheses that most of the observed nano- and micro-phytoplankton size structure heterogeneity could be explained by environmental constraints and occurred independently of niche apportionment of phytoplankton taxa along the environmental gradients, thus emphasizing the relevance of phytoplankton size structure as an intrinsic property of pelagic communities, functionally depending on environment trophic factors and intra-guild coexistence relationships, e.g. the size structure show a potential as a functional biomarker and complementary to phytoplankton taxomonic composition, abundance and biomass - for environment state assessment. However further efforts are necessary to fully explore the capacity of the size structure concept, analyzing patterns of phytoplankton body shapes response, surface to biovolume ratios (Stanka et al., 2013) to suggest relevant indicators (Vadrucci et al., 2013; Moncheva et al., 2007) for the marine environment.

Confidence of the analysis

The analysis was biased by the different depth and breadth of taxonomic identification of the samples as well as by some methodological constrains. Only one lab was reporting microflagellates that constitute a large portion in the nano-1 size class, some of the labs do not analyze “other” classes species with sufficient details (see *Intercalibration Report-phytoplankton*).

7. Phytoplankton indicator based Ecological state assessment

As the coastal habitat sensu MSFD is overlapping with the WFD monitoring stations the ecological status is assessed based on the Integrated Phytoplankton Index (IBI).

A common feature for the three transects was that most of the single quantitative metrics (abundance, biomass and chlorophyll a) classify the coastal habitats in high/good ecological status, with the exception of abundance (BG) and chlorophyll a (RO) corresponding to category moderate. Among the taxonomic based indicators the proportion of the cumulative abundance of Microflagellates, Euglenophytes and Cyanobacteria from the total community abundance (MEC %) corresponds to category "high", while the share of r-strategy mixotrophs in the total Dinophyceae abundance (DE %) was high (> 70%) in RO and BG coastal environment (category poor) and only in TR water corresponded to category good. According to IBI all stations complied with good ecological state (IBI> 0.64), but it should be underlined that the values were very close (BG) or even at (RO) the boundary good/moderate – Table II.1.18. For TR the IBI is conditional based on the BG classification.

For the shelf and open sea pelagic habitats the indicators and their thresholds suggested in the RO and BG IARs have been used, limited to total phytoplankton biomass, DE% and the recently proposed Shannon'95 biodiversity index tested first time in the Black Sea in this particular study.

Based on phytoplankton biomass, in conformity to the IBI defined categories, the ecological status in the coastal waters corresponded to category "good" in the three transects. The biomass variation in all RO habitats was within category "good", a trend almost portrayed in the BG pelagic domain, but it is worth noting that at the two shelf stations (M11-M10) the values were very close to the good/moderate threshold. At TR transect the open sea station and the mid-shelf station biomass outrange the category "good" – Fig.II.1.23.

Table II.1.18. Color coded categories of composite phytoplankton metrics of the Integrated Phytoplankton Index (IBI) (coastal habitat *sensu* WFD).

Station	N [cells/L]	B [mg/m$^{3]}$	Chl1 [mg/m$^{3]}$	Menh	Sheld	MEC [%]	DE [%]	IBI
M01	670365	666.237	2.6	0.05	0.05	1.04	69.11	0.64
M12	1242574	342.592	1.2	0.07	0.04	4.02	77.04	0.66
M18	444974	293	0.8	0.07	0.02	0	34.07	0.77

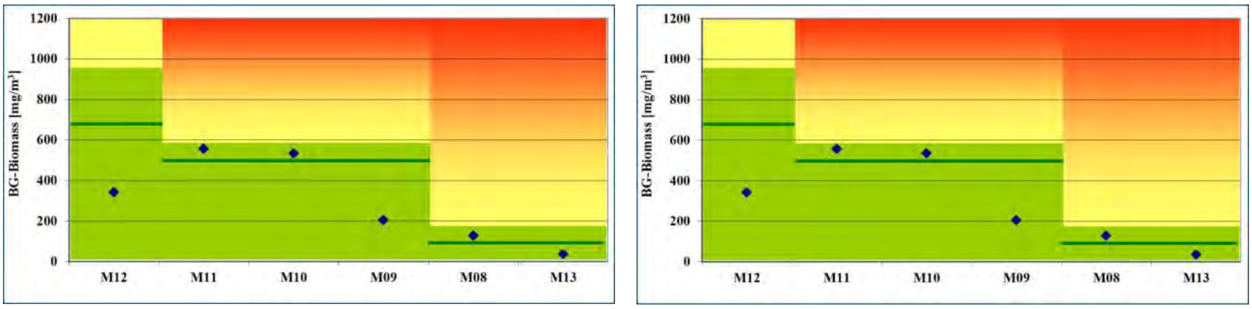


Figure II.1.23. Ecological state assessment based on phytoplankton biomass (thresholds for GEnS suggested in the RO&BG IA Report, for TR the BG values were applied) (GEnS in green).

The taxonomically based indicator (DE %) and biodiversity indices (Menhinik and Shannon'95) showed rather contrasting results – Fig.II.1.24 and II.1.25.

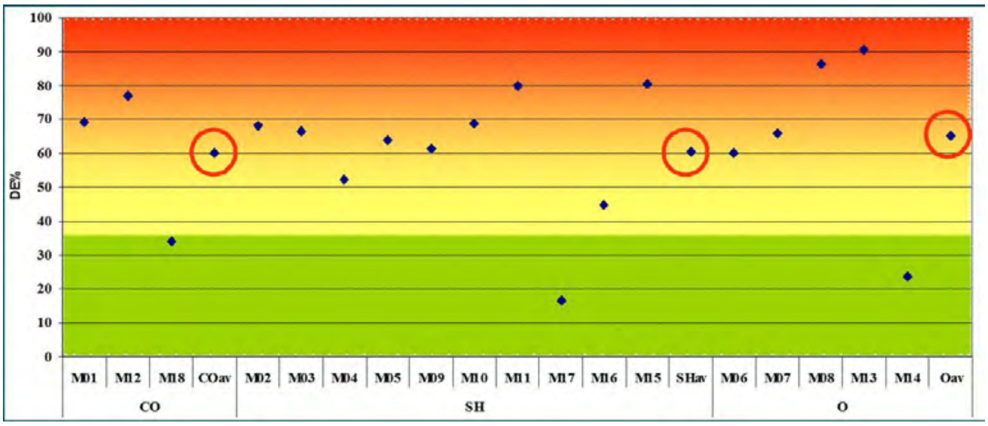
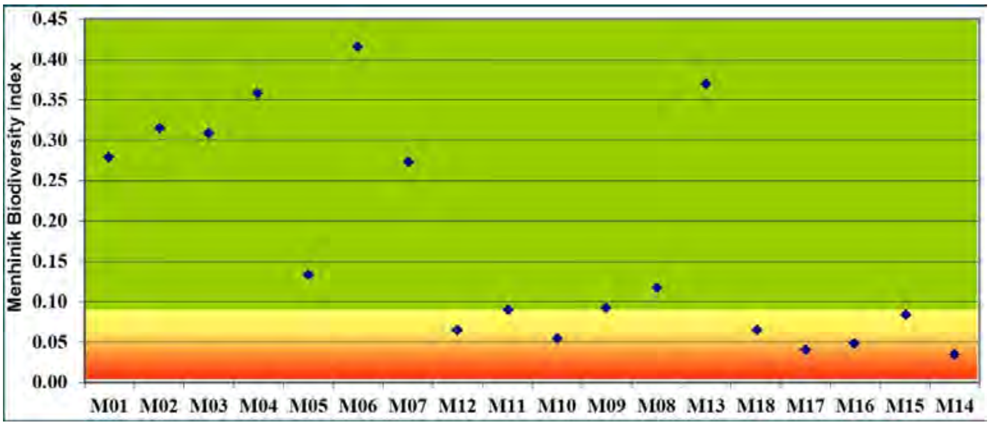


Figure II.1.24. Ecological status assessment based on DE% by sampling stations and habitats: CO-coastal; SH-shelf; open sea (red circles- average DE% for the habitat) (GEnS in green).

The average DE% by habitats (red circles) for the entire pelagic survey area as well as at the majority of the stations was above the boundary for "good" GEnS – Fig. II.1.25.

The exceptional 3 stations in the TR transect that conform to class "good" being distributed in different pelagic habitats do not allow to extract patterns and should be considered accidental.

While for the Menhinik index (based on the abundance) the WFD good/moderate class boundary was used, for Shannon'95 we assume the classical threshold >2 as a GEnS boundary, suggesting a limit of at least 20 species to represent the 95% of the biomass. With the exception of TR where the two indices present similar results (a departure from category "good") the patterns in BG and RO were distinctive (Fig.II.1.25) associated most likely to species disproportional distribution in the total community abundance and biomass discussed above. The Menhinik index and the taxonomic based biodiversity indicators contrary to the quantitative metrics suggest a disturbance in the phytoplankton community taxonomic structure, a signature quite in line with the well documented recent trends (Nesterova et al., 2008; Mikaelyan et al., 2013, Moncheva et al., 2012).



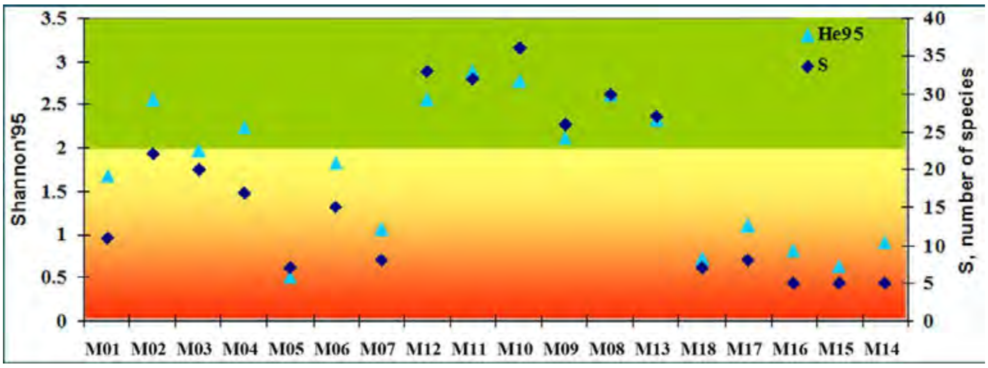


Figure II.1.25. Environmental state categories based on phytoplankton biodiversity indices: Menhinik (upper) and Shannon' 95 (lower) by stations (GEnS in green).

Albeit the IBI upward trend at the BG coastal station to category "good" during 2006-2013, the variation is around the very boundary of "good/moderate" class, e.g. the trend is rather unstable - Fig. II.1.26.

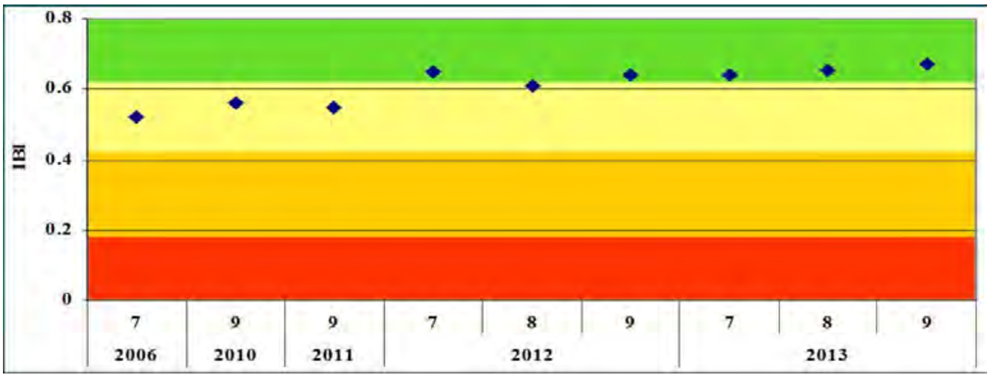


Figure II.1.26. IBI variation at the BG coastal station in summer (2006-2013).

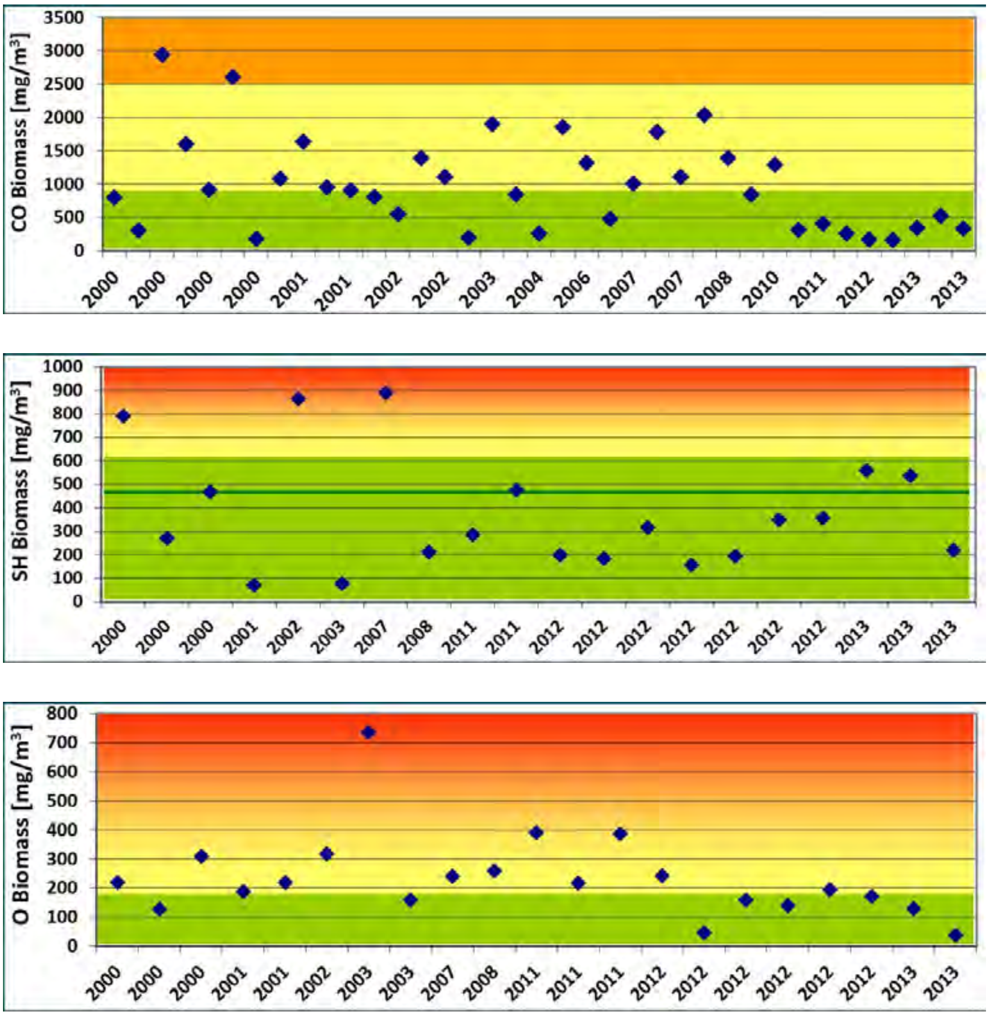
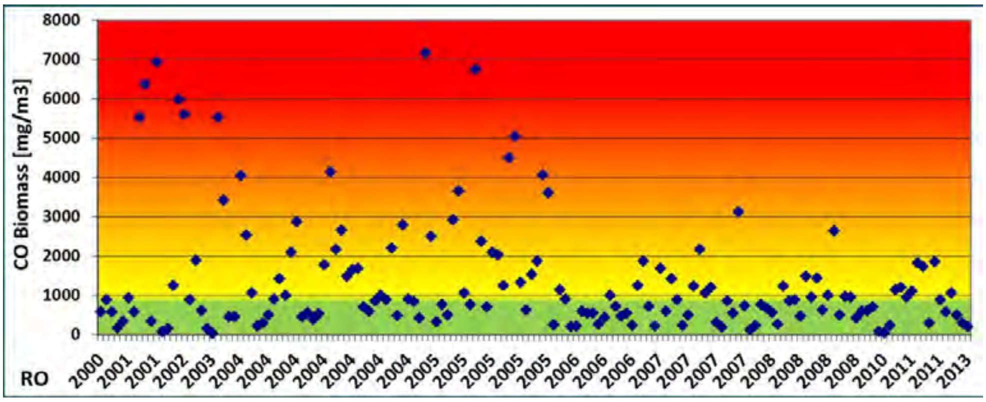


Figure II.1.27. Biomass variation in BG pelagic habitats: C- coastal, SH-shelf, O-open sea (GEnS in green).



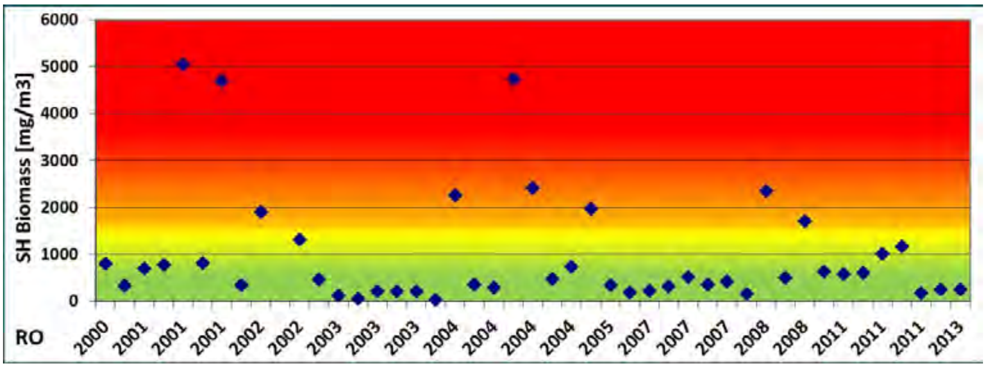


Figure II.1.28. Biomass variation in RO pelagic habitats: C- coastal, SH-shelf (GEnS in green).

Phytoplankton summer biomass (2000-2013) showed a decline in all pelagic habitats (Fig. II.1.27, Fig. II.1.28), a common trend for BG and RO waters. To a certain extend this trend could be associated to the reduction of nutrients inputs into the Black Sea basin and nutrients ratios alteration as outlined in the Euthrophication Chapter (this report). However the demonstrated high year to year and within - season variability, the concomitant shifts in the species diversity and community taxonomic and FTS structure under the conditions of pronounced climatic changes during the last 15 years cast doubt on the stability of this trend and the impact on the ecosystem functioning. Long-term temperature change in ocean waters associated with climate trends has been shown to affect phytoplankton abundance (Richardson & Schoeman, 2004), phenology (Edwards & Richardson, 2004) and shifts in taxonomic composition (Leterme et al., 2005). Beaugrand et al. (2010) document that global warming has been accompanied by an increase in the taxonomic biodiversity of phytoplankton and zooplankton in the North Atlantic Ocean on the expence of average size reduction, suggesting that these structural modification could bring about an alteration to the carbon sink in the North Atlantic with an implication for top predators (subarctic fish such as cod). A multi-decadal analysis of Baltic Sea phytoplankton point to a biodiversity increase resulted in a corresponding median increase of algal biomass per unit Total Phosphorous and Total Nitrogen by a factor of 1.4 and 1.2, respectively, implying that management decisions and associated recovery scenarios should take such shifting baselines into account when assessing the effects of pressure–response relationships (Oli et al., 2014).

8. HPLC marker pigments

Microscopic examination has been the classical method in phytoplankton studies. It involves the identification and estimation of cell abundance and biomass (Utermöhl, 1958; Booth, 1993). Alternatively, analysis of water samples using HPLC allows phytoplankton characterization by chemotaxonomic study of photosynthetic pigments. Total phytoplankton standing stock is estimated as chlorophyll-a concentration and algal classes are identified from the presence of marker pigments. Furthermore, the biomass of each taxon is calculated as a proportion of total chl-a using chl-a:marker pigment ratios (Wright & Jeffrey, 1987; Millie et al., 1993). The characteristic signatures of pigments for the identification of phytoplankton groups have been summarized in various papers (Jeffrey & Hallegraeff, 1987; Gieskes, 1991; Millie et al., 1993) but taxonomic identification from pigment signatures is not yet a straightforward task. The sole use of pigment signatures without concurrent microscopic verification could even sometimes be misleading (Gieskes, 1991; Millie et al., 1993). On the other hand, microscopic studies are often plagued by problems such as poor fixation or small size, making identification difficult. Thus a combination of both approaches has been recommended (Hallegraeff, 1981; Jeffrey & Hallegraeff, 1987; Tester et al., 1995). However, recent field studies have tended to rely mostly on pigment chemo-taxonomy using the HPLC analysis mainly because of shorter analysis time (Everit et al., 1990; Barlow et al., 1993, Letelier et al., 1993; Tester et al., 1995; Roy et al., 1996; Peeken, 1997). Furthermore, the biomass of each taxon is calculated as a proportion of total chl-a using chl-a / carotenoid pigment ratios (Everit et al., 1991; Millie et al., 1993, Jeffrey et al., 1999). Comparison of microscopic and chemotaxonomic techniques of phytoplankton identification has demonstrated that HPLC pigment analysis is a valuable tool for phytoplankton studies (Tester et al., 1995; Bidigare et al., 1996; Rodriquez et al., 2002).
Main objectives of this study was to determine the possible relationship between phytoplankton assemblages, and their marker pigments in the western part of Black Sea.
Pigments from water samples were separated and detected by the use of the sensitive Reverse Phase–HPLC method. Chlorophylls a, b, c2 and div-a, the

photosynthetic carotenoids such as peridinin (PER), fucoxanthin (FUC) and 19'Hexonoyloxyfucoxanthin (HEX), photoprotecting carotenoids such as diadinoxanthin (DIADINO), alloxanthin (ALLO), zeaxanthin (ZEA), β-carotene (β-CAR) were detected in the samples.

Concentrations of surface chl-a were generally low compared to sub surface layer - Fig. II.1.29. Chlorophyll-a concentrations were in the range of 0.1-1.94 and 0.34-2.35 μg/L in surface and sub-surface layer of stations respectively. Sub-surface layer were located between 5 and 43m depth depending on station and called also chlorophyll maximum layer.

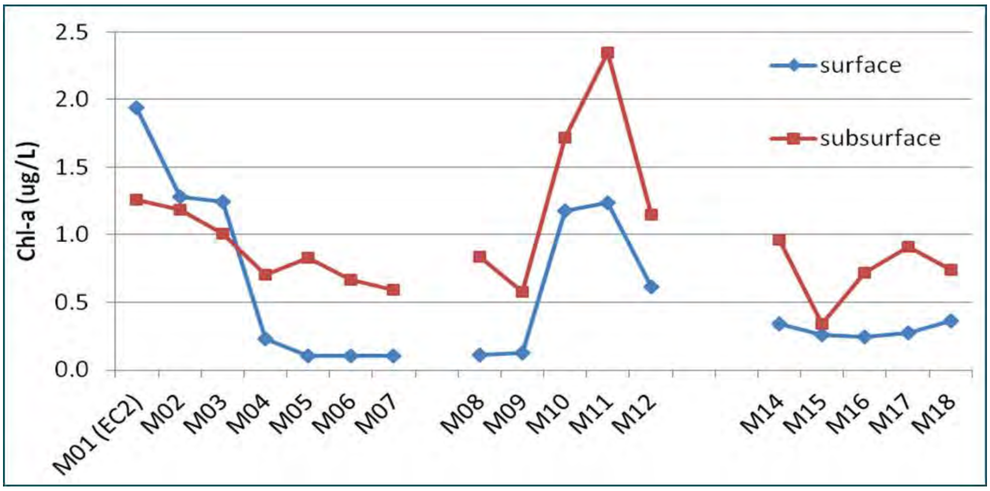
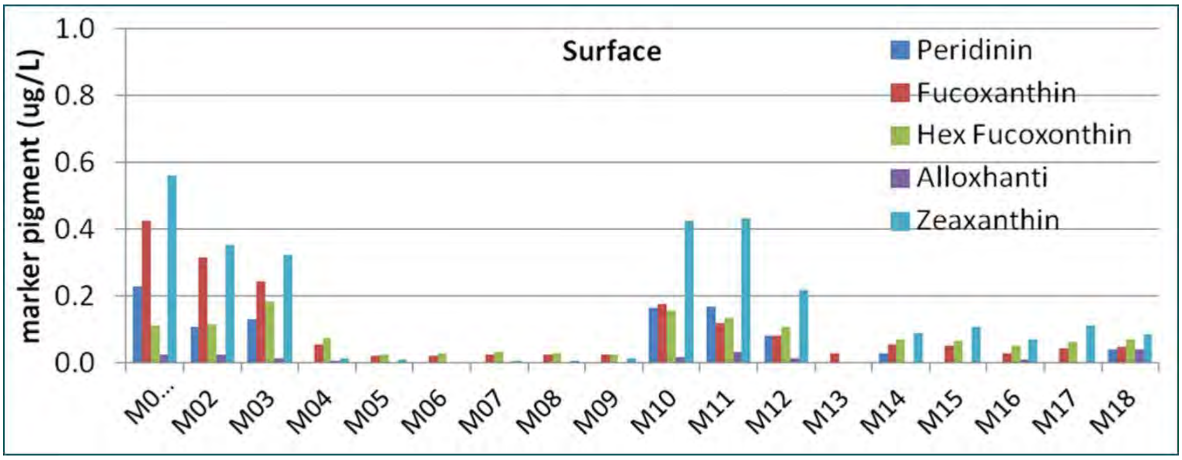


Figure II.1.29. Concentrations of chlorophyll-a along the RO, BG and TR transects.

Concentrations of detected phytoplankton pigments peridinin, fucoxanthin, 19'Hexonoyloxyfucoxanthin, alloxanthin, zeaxanthin were given in Fig.II.1.30. Most marker pigments were present at all stations but their concentrations varied greatly. Zeaxanthin was dominant throughout the surface layer, however, fucoxanthin was dominant in chlorophyll maximum layer. Peridinin, fucoxanthin, 19'-hex., alloxanthin and zeaxanthin are an indicator of dinoflagellates, diatoms, prymnesiophytes, cryptophytes and cyanophytes. All these phytoplankton groups and their marker pigments were observed in this study.



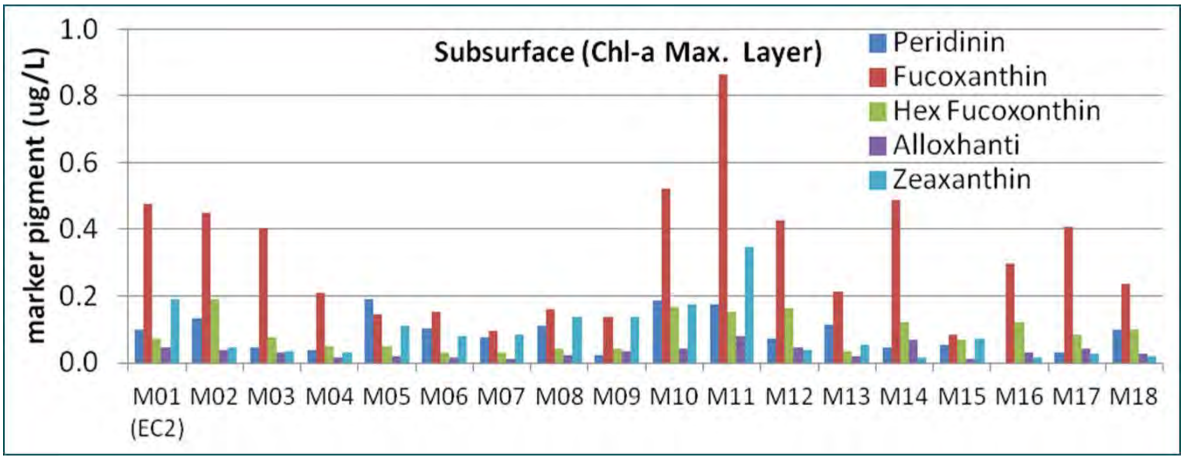


Figure II.1.30. Marker Pigment Concentrations along the RO, BG and TR transects.

There were a reasonably strong correlations ($p<0.05$-$p<0.0001$), using simple linear regression between the biomass, C biomass and abundance of dinoflagellates, prymnesiophytes and cyanophytes and their marker pigments concentrations of samples (Fig. II.1.31.). However, there was no any significant correlation between the biomass and abundance of diatoms and cryptophytes and their marker pigments. This is probably due to low fucoxanthin concentrations as well as quantification difficulties of broken large diatom cells (e.g. *Pseudosolenia* spp.). Llewellyn et al. (2005) suggested that stationary growth phase of diatoms and low light conditions can result in discrepancies between microscopy and chemotaxonomy. Another possible reason of differences between microscopic and chemotaxonomic techniques is difficulties in differentiation of living and dead diatoms under microscope. In addition, the relationship can be light and nutrient-dependent (Humphrey, 1983; Buma et al., 1991).

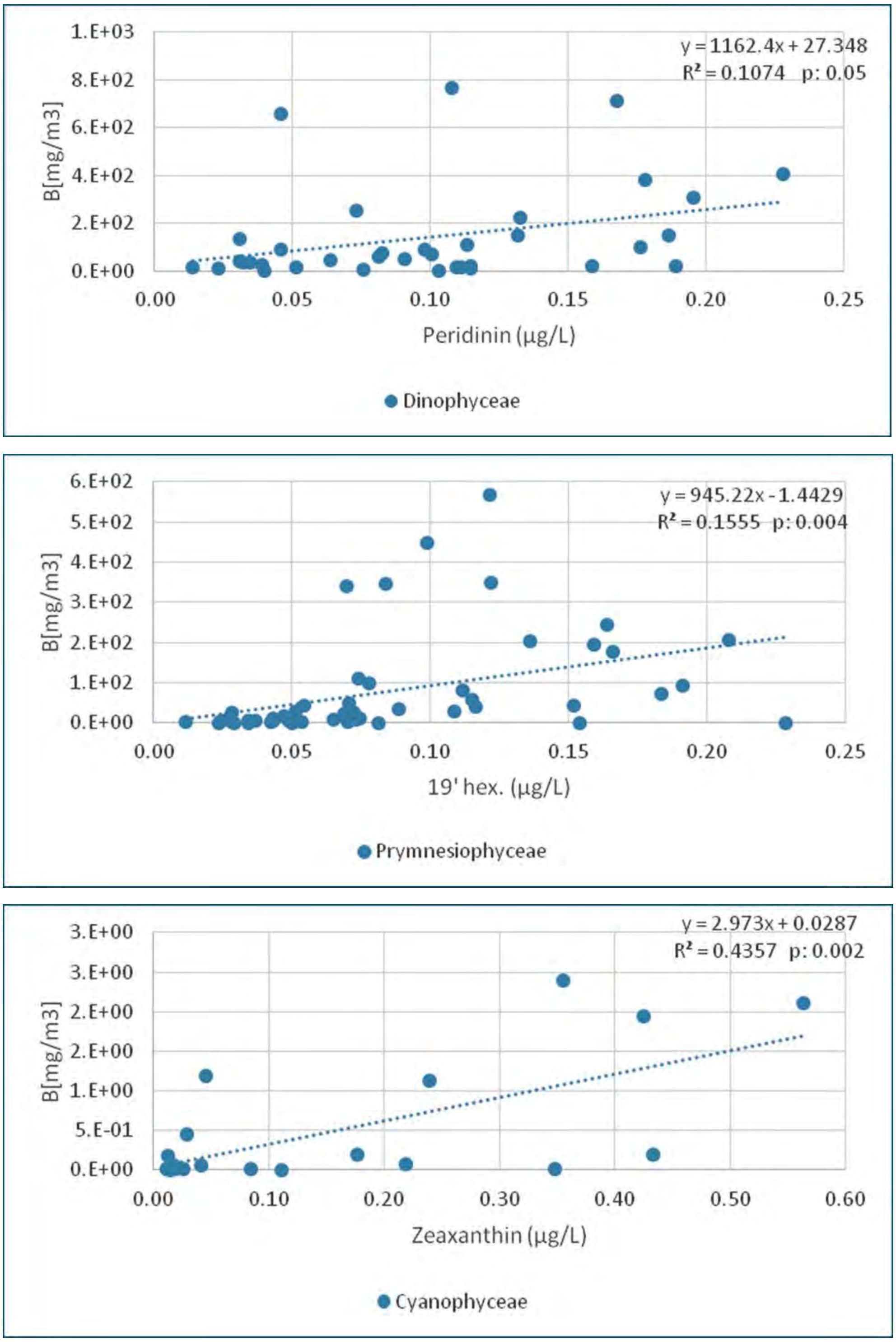


Figure II.1.31. Linear regression analyses between phytoplankton biomass and their marker pigment.

To determine the quantitative contribution of the different algal groups to the total chlorophyll-a measured, multiple linear regression analysis can be used to determine conversion factors (Gieskes et al., 1988; Barlow et al., 1993; Tester et al., 1995).

In order to estimate the contribution of the four selected algal classes to the total phytoplankton biomass, multiple regression analysis was used to determine the ratio of chlorophyll to accessory pigment for each group. Conversion of pigment data into relative quantities of various algal groups was done by estimating the contribution by various pigment markers characteristic of different algal groups to the total chl-a. Thus, since dinoflagellates have a certain chl-a: peridinin ratio, the peridinin concentration found is multiplied by this ratio and expressed relative to total chl-a to give their relative contributions to algal biomass and similar procedure was repeated for diatoms, prymnesiophytes and cyanophytes. Thus, the total

chl a= constant+a(marker1)+b(marker 2)+…
where a,b…represent various chl a: marker x ratios.

This approach was used successfully in earlier studies (Gieskes & Kraay 1983; Gieskes et al., 1988; Everitt et al., 1990; Letelier et al., 1993; Roy et al., 1996; Tester et al., 1995).

Multiple regression analysis produced the following equation:

Chl-a = -0.01 + (2.23*peridinin) + (1.7*fucoxanthin) + (0.22*19’-hex.) + (1.05*zeax.)

The r^2 for this regression was 0.98 and all the regression coefficients were significant ($p<0.001$).

In order to assess the validity of estimated conversion factors, estimated group-specific chlorophyll-a values were compared with the directly measured chlorophyll-a (Fig. II.1.32 and II.1.33). Results generally show a good fit between them.

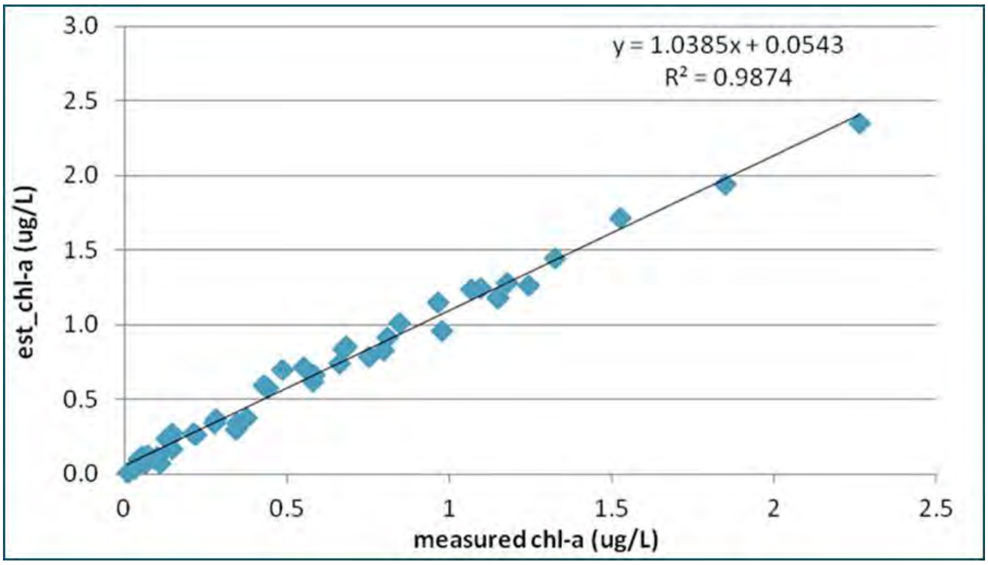


Figure II.1.32. Comparison of estimated and measured chl-a values.

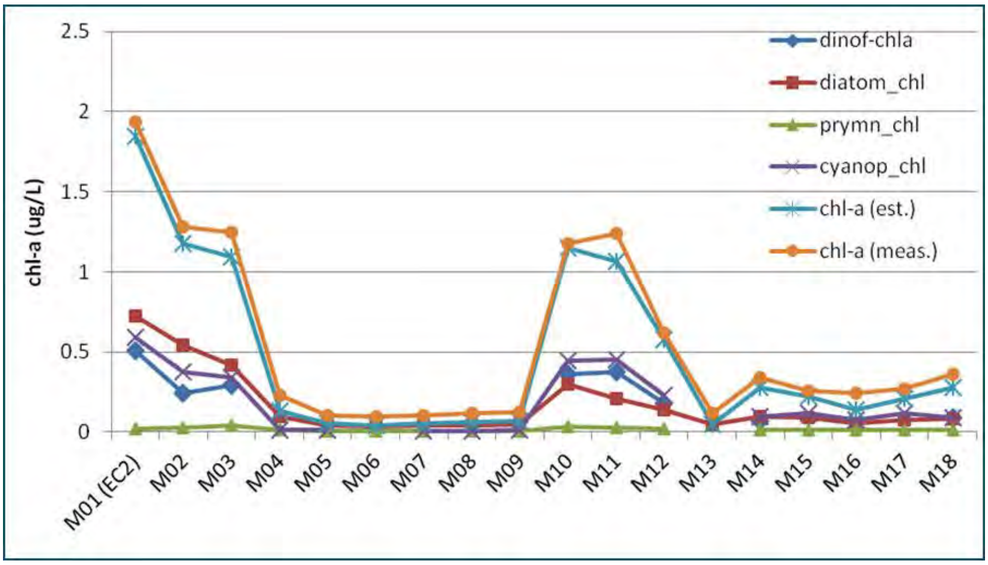


Figure II.1.33. Surface distribution of chlorophyll-a (measured), the estimated group specific chl-a and the sum of the group specific chl a (estimated) in the study area.

Chemotaxonomic method with microscopy to analyze phytoplankton composition in the Western Black Sea results found here are encouraging for further studies. However, still more research (frequent sampling) is needed to assess the correct application of the chemotaxonomical approach to natural phytoplankton assemblages in the study area.

CONCLUSIONS

Although the assessment is based on just a single snapshot survey and the results could not be considered preclusive, there is ground to make the following conclusions:

- According to the WFD classification system the coastal habitats of Romania, Bulgaria and Turkey are classified in good ecological status
- Phytoplankton biomass averaged over the pelagic habitats corresponded to the GEnS category (exception of 2 stations in Turkish waters) in conformity to the declining trends observed during the period 2006-2012.
- The taxonomically based indicator (DE%) and biodiversity indices (Menhinik and Shannon'95) by habitats for the entire pelagic survey area as well as at the majority of the stations depart from the GEnS category, suggesting a disturbance in the phytoplankton community taxonomic structure, supported also by the composition of dominant species assembly. The coocolithophorid *Emiliani huxleyi* , typically proliferating in May-June was found in blooming densities at selected stations, less common for the Black Sea species (Hemiselmis, Pyramimonas etc) entered the dominant species list.
- The highest diversity and species richness was found in the shelf habitats (almost 90%) and open waters (about 75%) of the total species record
- The trend of increased portion of taxa "other" than the habitual for the Black Sea basin species pool (microflagellates, Cryptophyceae, Prymnesiophyceae, Prasinophyceae) documented after 2000 was observed also in the present study: Cryptophyceae, Prymnesiophyceae, Prasinophyceae contributed to 93% of the abundance, and to ~33% of the biomass while dinoflagellates - to 40%, and diatoms to 27%.
- Cell densities of some potentially toxic species observed in this study were higher than those associated to toxicity events reported for different Black Sea areas, alarming for a potential threat of toxic events
- Out of the 264 species identified in this study 55% were autotrophic. Overall, the autotrophs were dominant in the water column above thermocline with the exception of the shelf waters.
- Phytoplankton size biomass partitioning show good fit as a functional biomarker selective to nutrients and nutrient ratios and a potential for environment state assessment
- The chemotaxonomic results of phytoplankton composition verified with the microscopic analysis are encouraging although further research (more frequent sampling) is needed to be refined for routine application to natural phytoplankton assemblages

GAPS

- The analysis of available data underline the need for adequate monitoring frequency and extent to capture the natural time-spatial variability of phytoplankton communities as a prerequisite to better understand the associated key drivers and pressures
- Albeit pico fraction is reported as a major component in the phytoplankton community especially in summer and its crucial role in the ecosystem processes, the investigations in the Black Sea are very limited (Uysal, 2001; Uysal, 2006, Feyzioglu et al., 2004; Akoglu et al, 2012).
- There is a lack of advanced genetic and genomic investigations for taxonomic revisions including phytoplankton, microbes and viruses.
- Despite the Black Sea regional initiatives such as Black Sea GOOS (ARGO floats and other drifters in the Black Sea), the bio-ecological operational oceanography is still poorly developed and the results inefficiently explored
- There is not enough knowledge of the capacity of Black Sea harmful algae to produce toxins, a lack of official statistics on possible phycotoxin poisonings due to lack of adequate monitoring

RECOMMENDATIONS

- The results of the intercalibration exercise call for more frequent ring tests and intercalibration campaigns in order to improve the comparability of generated data as an important prerequisite for adequate ecological state assessment at basin-wide scale
- In the context of global climate change it is now recognised that a partition of the marine phytoplankton species pool into a suite of functional types, would increase our understanding of the role of phytoplankton in the global carbon cycle and biogeochemistry (Naira et al., 2008) and with the emerging of new methods it is already possible to map some of the phytoplankton functional types (Kostadinov et al, 2010) also in the Black Sea (Churilova et al., 2013, Churilova et al., 2014).
- Satellite-borne remote sensing is crucial in providing the necessary spatio-temporal scales of observation required to proper measure and understand phytoplankton time-spatial patterns of distribution and variability and overcome scale mismatches, options that should be more efficiently exploited and employed in the Black Sea regional monitoring. However the importance in situ data should not be ignored as they prove essential for validation and thus the advance of the new emerging technologies.

- Infrastructure improvements and effective introduction of less applied approaches such as remote sensing, Continuous Plankton Recorders, Ship of opportunity / FerryBox system, should be considered as overarching and critical issues for implementation of the MSFD.
- The results give ground to suggest that as phytoplankton functional types (PFTs) are relevant proxies of ecosystem functioning, incorporation of PFTs into the monitoring programs and biogeochemical models may improve our predictive capabilities and the capacity for a better ecosystem management.
- Integration of pressure data (fluxes of nutrients from various sources including non-point sources) and relevant meteorological information is crucial for validation of the classification systems and targets set for the indicators and the adequacy of the assessments.

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II. 2. Zooplankton

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INTRODUCTION

Zooplankton play a pivotal role in aquatic ecosystems and global biogeochemical cycles, integral to aquatic productivity. They function as prey for economically important fish, grazers of primary production (zooplankton package planktonic primary production into forms available for whales, seabirds, fishes and humans), and drivers of carbon and nutrient cycles (Zooplankton manual, 2000). Characteristic of plankton communities is their natural variability (from daily to inter-annual variations), complicating the assessment of the condition. Moreover, the impact on zooplankton is diverse and largely depends on climatic signals with concurrent changes in the phytoplankton, physico-chemical conditions of the environment as a result of eutrophication and the introduction of alien species. Their population and community dynamics, including their growth, mortality, distribution, and diversity structure the ecosystem. At the same time, a changing environment influences their dynamics. Climate change is profoundly impacting marine ecosystems through changes in zooplankton (Keister and Bonnet, 2012). Despite their fundamental role zooplankton assemblages have not been widely used as indicator of ecosystem condition (Stemberger and Lazorchak, 1994) and are not included as a relevant quality element for the assessment of ecological status within Water Framework Directive.

We consider a possibility of using zooplankton as indicator for habitat ecological level reflecting composition of zooplankton community and quantitative metrics (abundance/biomass).

MATERIAL and METHODS

Study area and sample collection

The sampling campaign was performed at three transects, located in front of Constanta-East, c.Galata (travers Varna) and Igneada (Turkey waters). Samples were collected by vertical plankton Juday net, 0.1 m^2 mouth opening area, 150 µm mesh size, from 2 meters above the bottom or from the lower boundary of the oxic layer to the surface at discrete sampling layers. The length and angle of the wire were taken under consideration for calculation of the wire length to get the target horizon. A total number 62 zooplankton samples (except those for intercalibration) were collected during Joint cruise from 18 stations. The stations M01 (RO), M12 (BG) and M18 (TR) were determined as coastal, M02, M03, M04, M05 (RO), M11, M10, M09 (BG), M15, M16, M17 (TR) – as shelf and M06, M07 (RO), M08, M13 (BG), M14 (TR) as open sea (Table 1).

Samples for macrozooplankton were collected by Hensen egg net (diameter 70 cm and mesh size 300 µm). Gelatinous species (*Aurelia aurita, Pleirobrachia pileus, Mnemiopsis leidyi, Beroe ovata*) were measured, counted and recorded on board for size structure, abundance and biomass determination.

Sample preparation, preservation and storage

Before mesozooplankton sample preservation on board, the gelatinous species (*Aurelia aurita, Mnemiopsis leidyi, Beroe ovata* and *Pleorobrachia pileus*) were removed. The samples were preserved in 4% formaldehyde buffered to pH 8-8.2 with disodiumtetraborate (borax) ($Na_2B_4O_3$ •10 H_2O) formalin solution (1 part 40% formaldehyde solution and 9 parts water-sample) and stored in plastic containers (Korshenko&Aleksandrov, 2011).

Laboratory analysis

In the laboratory, the samples were concentrated to 100-150 cm^3 before being divided into sub-samples. A Bogorov's chamber was used for quantitative assessment (abundance and biomass calculation, using species individual weight) and qualitative (taxonomic structure) processing of sub-samples (Petipa 1957; Alexandrov&Korshenko, 2011). The sub-samples were examined by using Stereoscopic Zoom Microscope. At least 100 organisms from each of three dominated species were counted in each sub-sample (Alexandrov&Korshenko, 2011). The precision of calculated abundance for organisms of the first three groups, counted up to 100 specimens, amounts to 20%. The estimation of abundance for other groups ("tail") is less precise (Cassie 1971, HELCOM 1988). The data for taxa numbering less than 10 specimens were considered as qualitative. Species were identified according to Morduhay-Boltovskoy (1968, 1969 and 1972).

Our study was focused on groups and species of different size spectrum: meso (0.2 mm-2 cm) and macro (2-20 cm). The structure of zooplankton community has been analysed in terms of taxonomic composition and key groups, total and average abundance and biomass. Statistical analyses were performed by applying PRIMER 5 of PRIMER-E Ltd, Plymouth (2001).

Potential zooplankton indicators

Candidate indicators were developed and proposed in the Initial Assessment report of Bulgaria, 2012. GEnS thresholds based on long-term zooplankton data (1967-2006) available for c.Galata transect and reference period (1967-1973) were applied in Romania and Turkey waters.

Indicators are relevant to Descriptor 1 (Biodiversity) Habitat level Criteria 1.6. Habitat condition, Indicator: 1.6.2. Relative abundance and or biomass.

Biomass of copepods (CB %) - contribution of copepods biomass to total mesozooplankton biomass. Copepods are a key group contributes significantly to the diet of planktivorus fish (sprat and anchovy, partly horse mackerel), reflects composition of zooplankton community and food availability for zooplanktivorous fish. State indicator relevant to two descriptors – D1 (1.6. Habitat condition, Indicator: 1.6.2. Relative abundance and/ or biomass) and D4. To establish baselines and acceptable variability for specific indicators, trends and reference periods were evaluated. For CB% mean values and 95% confidence intervals (CI) the upper and lower 95% limits were used (HELCOM, 2012). In the context of GES for CB (CB%), upper limit is not relevant, or GEnS boundary for CB (CB%) is the lower 95% CI limit. CB (CB %) should not decline below its GES boundary (Fig. II.2.1). The GEnS boundary was estimated to be **42%** based on the reference period 1968-1973, when anchovy and sprat stocks were relatively stable and had high weight-age and body condition. The threshold is relevant for coastal, shelf and open sea.

Copepods are mostly herbivores or omnivores, therefore, this indicator would be indirectly impacted by eutrophication (via changes in primary productivity and phytoplankton composition), whereas direct impacts are expected from climatic changes, predation, introduction of synthetic compounds (at point sources) and invasive species – *Mnemiopsis leidyi* (via predation). Both positive and negative responses can result from changes in thermal regime and salinity (HELCOM, 2012).

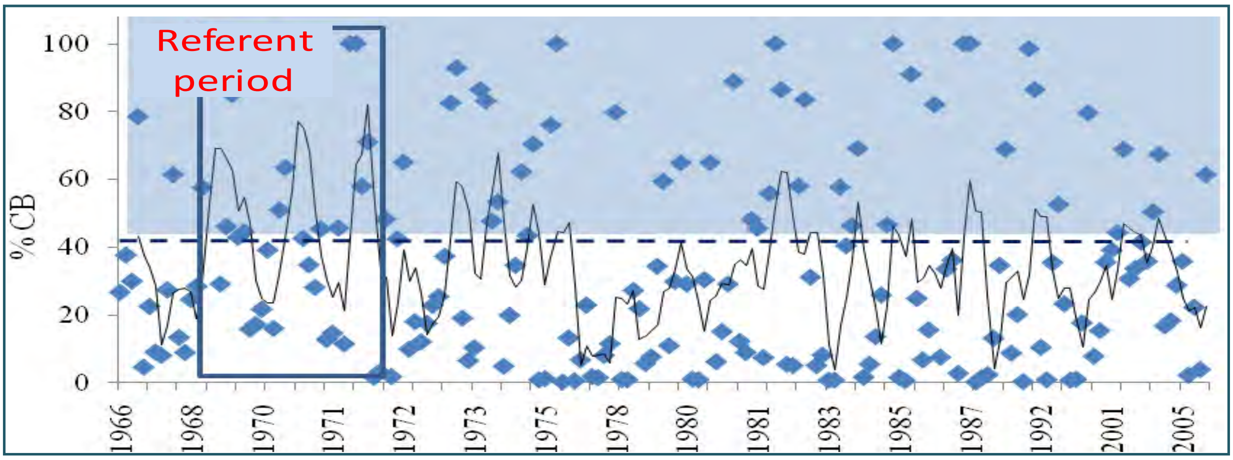


Figure II.2.1. Long-term changes in copepod contribution to the total mesozooplankton biomass (CB %) in c.Galata transect (1966-2006).
According to the trend observed, CB% >42% indicate good conditions (blue area).

Mesozooplankton biomass - Biomass is calculated using abundance of species/taxa present in mesozooplankton community and their individual weights. The indicator reflects composition of zooplankton community. Mezozooplankton indirectly exposed to eutrophication process (in case the amount of food composition and size) and catches of commercially exploited fish (through changes in the pelagic food chain), while the direct impact is shaped by climate change (temperature and salt mode), predation on fish and gelatinous plankton. Development of *M.leidyi* and *N.scintillans* reflected directly respectively indirectly on mesozooplankton biomass, since both negatively correlated with the biomass of planktonic fauna. Biomass of mesozooplankton includes an information for major key groups, forming the structure of the planktonic fauna, particular groups Copepoda, Cladocera, Meroplankton, and species *O. dioica* and *S. setosa.* Zooplankton metrics exhibit strong variability in time and space under the influence of natural and anthropogenic factors, which reflects to the total mesozooplankton biomass. In this case the indicator not only gives a threshold above or below GEnS. It is fact that high mesozooplankton biomass suggest higher trophic environment (HELCOM, 2012) and increasing concentrations of the planktonic fauna is an indirect indicator of the food ability in the water column respectively eutrophic conditions. On the other hand mesozooplankton biomass reduction indicates enhanced predator pressure in the food chain (jellyfish, ctenophores and small pelagic fish). Therefore, neither high nor low values of biomass would affect the GEnS.

Approach for defining GEnS: Baseline condition, trends and the reference period (1967-1972) were assessed using the 75 percentile for obtaining upper and lower (25 percentile) range of values to determine good environmental status. Proposed thresholds in summer are as follows: **coastal (550-280 mg.m^{-3}), shelf (300-130 mg.m^{-3})** and **open sea (150-50 mg.m^{-3}**).

***Noctiluca scintillans* biomass (N.sci %)** - contribution of *N.scintillans* biomass to total mesozooplankton biomass. The wide feeding spectrum (phytoplankton, zooplankton and detritus) of the species, development in high bloom concentrations, usually after the mass development of phytoplankton, determines its ecological importance for the pelagic ecosystem (Kiørboe, Titelman 1998; Dela-Cruz et al., 2003). *N.scintillans* density is usually higher in coastal areas where maximum phyto- and zooplankton were registered. For detection of classification limits the period of intensive eutrophictaion (1980-1993) was selected as “low” ecological state.

Approach for defining GEnS. To establish a baseline condition and acceptable variability of the indicator, trends and the reference period (1968-

1973) were assessed using the mean and 95% confidence interval (CI) (Fig. II.2.2). Because the variables were not normally distributed, the standard deviation was calculated by applying the transformation (arcsin) and then back-transformation of the data to determine the upper and lower limit of the 95% confidence interval (HELCOM, 2012). In the context of achieving good ecological status for% N.sci upper limit of 95% confidence interval is taken as the limit of good and poor condition, respectively, ie % N.sci should not be over the boundary for the good environmental status. Established threshold for good environmental status is **% N.sci <30%.** The threshold is relevant for three areas – coastal, shelf and open sea.

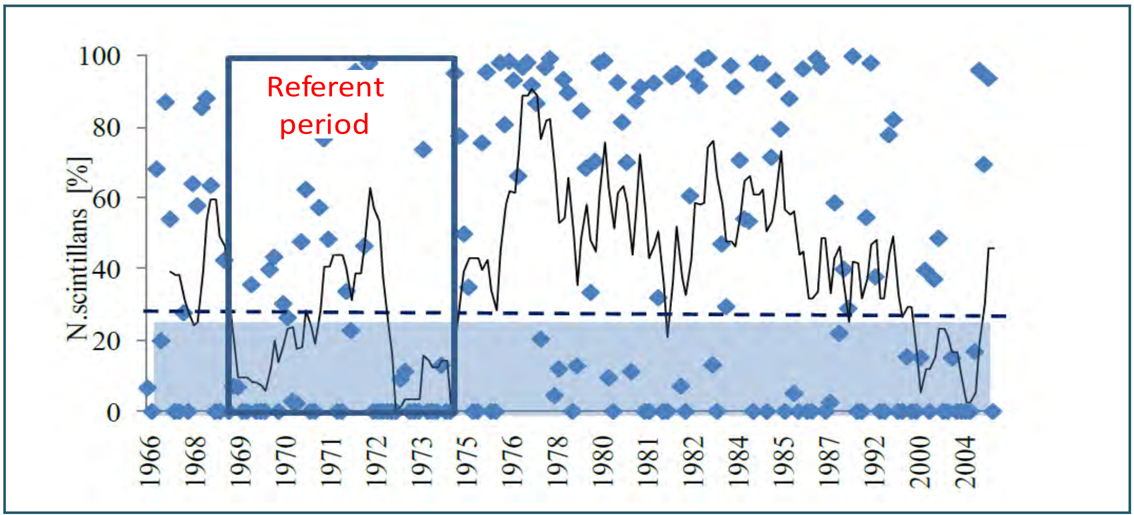


Figure II.2.2. Long-term changes in N.scintillans contribution to the total mesozooplankton biomass (%N.sci) in c.Galata transect (1966-2006). According to the trend observed, %N.sci<30% indicate good conditions (blue area).

Shannon-Weaver index – reflects the number of species in a dataset, taking into account how evenly the basic entities (such as individuals) are distributed among species. The values of a diversity index depend both the number of species and evenness increase. For a given number of species, the value of a diversity index is maximized when all species are equally abundant. Using long term species list for the western Black Sea, specific diversity index was calculated as a ratio of maximum numbers of species observed for the entire period 1966-2012 and species number actually registered in the area. The indicator reflects changes in taxonomic diversity in the area, potentially could be used for indication of bioinvasions both planktonic and benthic communities, as mezozooplanktona includes larvae of benthic organisms (meroplankton) (HELCOM, 2012). Furthermore, it can be indirectly affected by eutrophication, climate change, predation, which reduce species diversity or dominate of certain species and disproportion in the community occurred. The boundary for good status was accepted 3 bit·ind^{-1} for coastal and shelf habitats, while 2.5 bit·ind^{-1} was for open sea. The index is strictly area-specific and differentiation was required to avoid the risk of not achieving good status due to higher defined threshold. The number of species is generally lower in offshore due to the specific conditions.

RESULTS and DISCUSSIONS

Mesozooplankton abundance and biomass distribution

Mesozooplankton metrics manifested huge variability in the study area, while the numerical abundance ranged from 876 to 20001 ind·m^{-3} (about 23 times), the biomass varied 11 folds (min=27.21; max=294.05mg·m^{-3}, within average of 109.63 mg·m^{-3}±75.66). Statistical analysis ANOVA ($p < 0.05$) was applied to test the differences between the three regions coastal, shelf and open sea (Fig. II.2.3). Differentiation of the regions, presented in Initial assessment report of Bulgaria, 2012, was according to satellite data for Chlorophyll a and sea surface anomaly. Made differentiation was analogous to defined areas in previous studies (Kopelevich et al., 2002; Finenko et al., 2010) based on anthropogenic impacts, surface currents, production and bathymetry. Thus, coastal region is under the strong influence of the land-based pressures which affects considerably the community structure and distribution of planktonic communities and their large variability, while the effect to the sea is weakened. By transects, the results revealed average abundance 5223±5234 ind·m^{-3} and biomass 129.63 mg·m^{-3}±88.40 in Romania waters, mean density 7423±7738 ind·m^{-3} and biomass 228.53 mg·m^{-3}±87.52 in front of Bulgaria and one order of magnitude lower density 4628±2759 ind·m^{-3} and biomass 78.61±33.50 (approx. 2 fold) in Turkey waters (Fig. II.2.4). The mesozooplankton abundance and biomass decreased as a function of distance from the coast with an exception of shelf stations M11 (BG) and M17 (RO) (Fig. II.2.5). Corresponding figure was pronounced from North to South. As a consequence of local eutrophication and urban influence, mesozooplankton standing stock at coastal and inner shelf stations in the entire area were characterized with higher values than offshore. Also, reducing impact of the Danube river with the distance and weaker anthropogenic activities in Turkey area led to mesozooplankton biomass two time lower than Romania and Bulgaria transects.

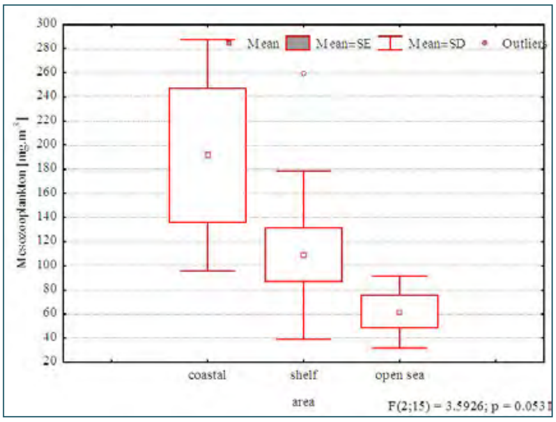


Figure II.2.3. Box-Whiskers plots, reflecting variations within the group and statistically significant differences between regions (coastal, shelf and open sea) of mesozooplankton biomass (p<0.01).

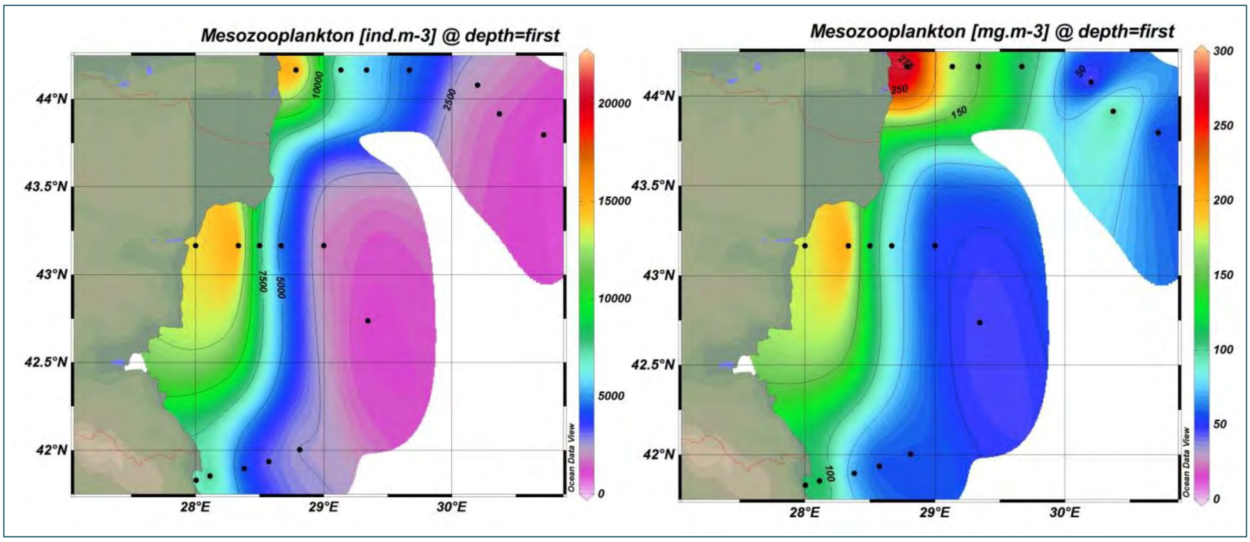


Figure II.2.4. Mesozooplankton abundance (left panel) and biomass (right panel) distribution.

Species diversity and taxonomic structure

Totally of 26 zooplankton species and taxa were identified in front of the Romanian, Bulgarian and Turkey coasts distributed among to phyla Myzozoa, Cnidaria, Ctenophora, Annelida, Arthropoda, Mollusca, Chaetognatha, Chordata. The class Crustacea was the most diverse (13 species and 4 taxa). The non-fodder part of the community was presented by *Noctiluca scintillans* and gelatinous *Aurelia aurita, Pleurobrachia pileus, Mnemiopsis leidyi* and *Beroe ovata*. Diversity richness was due to the presence of copepoda species *Acartia clausi, A. tonsa*, *Paracalanus parvus, Centropages ponticus, Calanus euxinus, Pseudocalanus elongatus, Oithona similis, O. davisae*, and cladoceras *Pleopis polyphemoides, Penilia avirostris* and *Pseudevadne tergestina, E. spinifera*. The obtained results revealed insignificant differences of investigated areas in terms of zooplankton diversity and community structure with small exceptions in the frequency of occurrence factor.

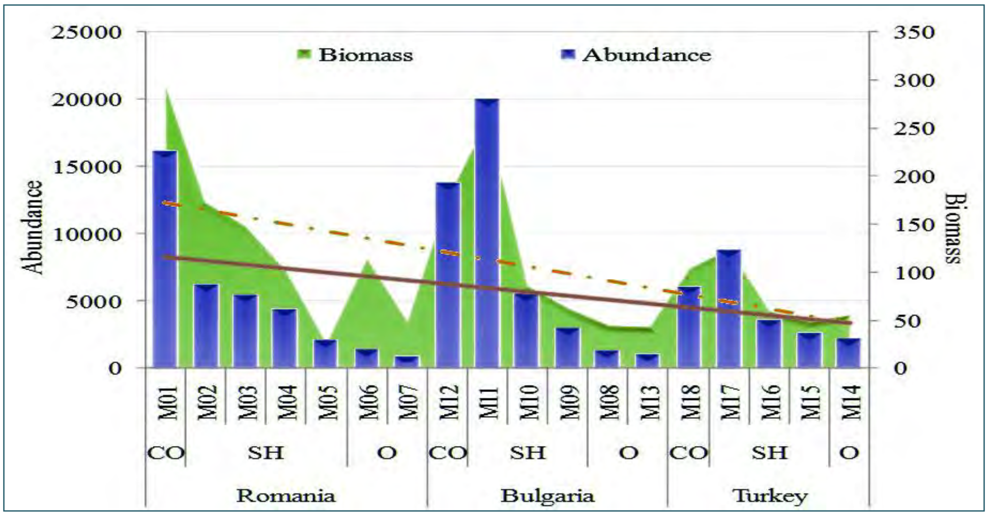


Figure II.2.5. Abundance [ind·m-3] and biomass [mg·m-3] variability and their distribution gradient (blue line – trendline in abundance; red line – trendline in biomass and CO – coastal, SH - shelf, O - open sea).

Copepods accounted for 29-90% of the mesozooplankton population according to biomass. Chaetognatha ranked second in importance with 1-28% (in average 15%), almost equal were Cladocerans with 1-33% (average 12%) and Appendicularia with 1-45% (average 10%). It is evident from the Fig. II.2.7a that the absolute biomass of *Noctiluca scintillans* is consistently higher at stations along Romanian shelf M03 (1031.470 mg·m^{-3}), M02 (336.182 mg·m^{-3}), M04 (264.315 mg·m^{-3}) than stations situated to the South and open sea. Despite of decreasing trend from North to South the highest biomass values were registered in each transect at the shelf habitats M10 (448.05 mg·m^{-3}) in Bulgaria waters and M17 (271.239 mg·m^{-3}) in Turkey, probably due to the currents regime and fresh water rivers input (average salinity 15 ‰).

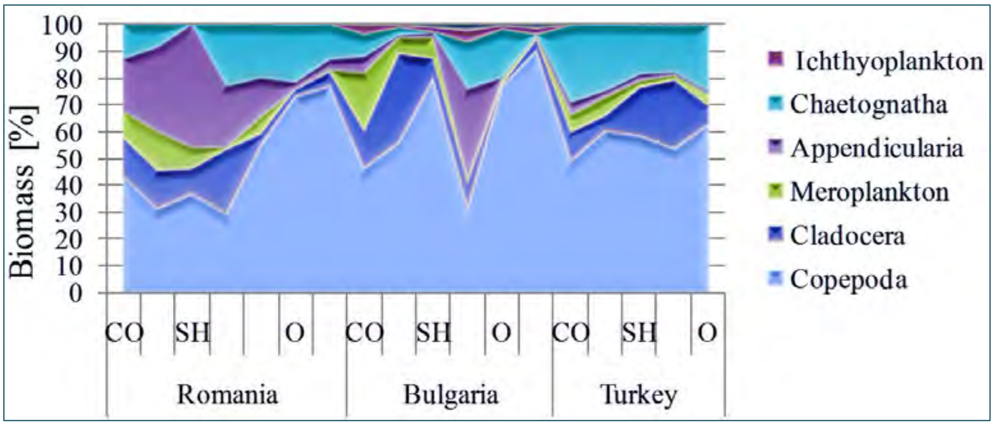


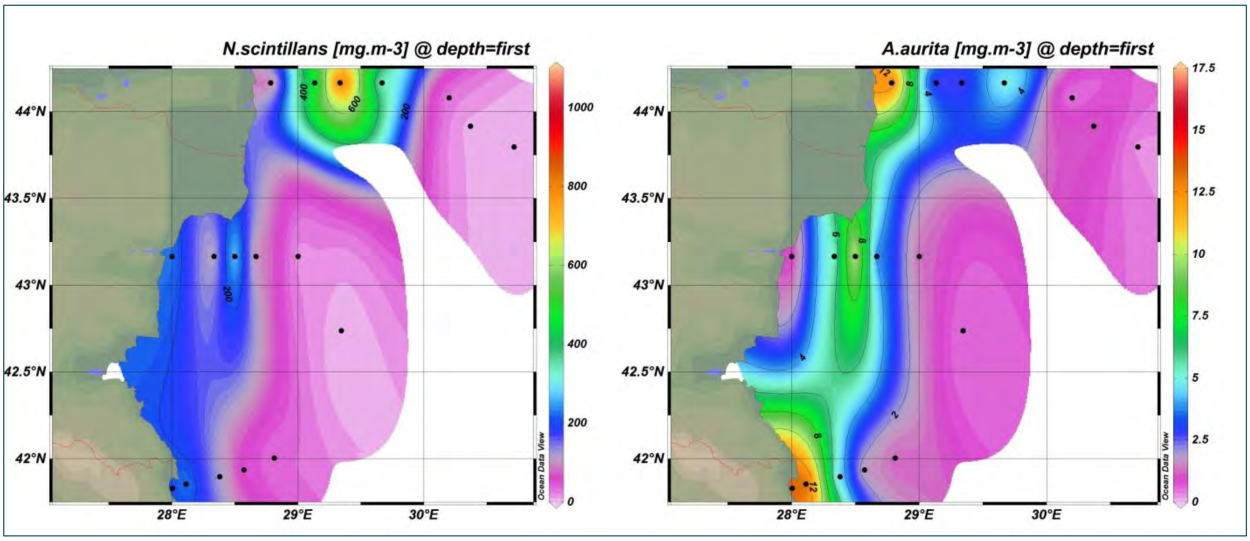
Figure II.2.6. Zooplankton taxonomic structure by biomass presented as percent at the habitats CO – coastal, SH - shelf, O -open sea.

Mnemiopsis and Aurelia are registered in higher concentrations in comparison with other gelatinous species. Aurelia presented patchiness in its distribution with hot spot in coastal area in Romania and Turkey (Fig. II.2.7b). The highest *A.aurita* biomass in c.Galata transect was registered at the shelf station (M10), characterized with maximum of *N.scintillans* as well. Comparing transects according the *M.leidyi* and *A.aurita* distribution Mnemiopsis was dominated coastal area in Romania and Bulgaria transects with decreasing trends from north to south (Fig. II.2.8). According to the literature the amount of *M.leidyi* increases when the temperature is above >22 °C and the salinity is about 15 ‰ (Shiganova 1998; Purcell et al., 2001; Mihneva, 2011). Favorable environmental factors recorded during the cruise especially in the north led to growth of Mnemiopsis population. As competitors, the quantity of *A. aurita* drops drastically with the increase of *M. leidyi* biomass above 300 $g{\cdot}m^{-2}$ (Mihneva, 2011). Using converter factor to find biomass in $g{\cdot}m^{-2}$ we observed that the entire area was presented with biomass more than threshold mention above, only with two exceptions at stations M06-open sea (Romania) and M12-coastal (Bulgaria). Only at offshore stations the impact of gelatinous was insignificant.

Habitat ecological state according to candidate indicators

Mesozooplankton biomass

Mesozooplankton biomass showed stable ecological status regarding the proposed limits in the open sea only. Expectantly, coastal and shelf area presented ambiguous results. Stations (Romania – M04, M05; Bulgaria – M12, M10, M09 and Turkey – M18, M19) characterized with domination of *N.scintillans*, *M.leidyi* and *A.aurita* which pressed mesozooplankton biomass did not reach GEnS boundaries (Table II.2.1). However, uncertainty in assessment of ecological state in Romania and Turkey waters regarding to mesozooplankton biomass is sufficient. As a consequence, applied indicator thresholds were defined and based on historical data of c.Galata transect only. In addition, different productivity of both areas, high in front of Romania but the opposite in Turkey, also various land-base pressures northward in comparison with South which reflects the mesozooplankton community structure and standing stock were not considered. How was mentioned above we tried to compare habitats and areas supported by the uniform indicators, indicator limits and approach.



a) b)

Figure II.2.7. Noctiluca scintillans (a) and Aurelia aurita (b) biomass distribution.

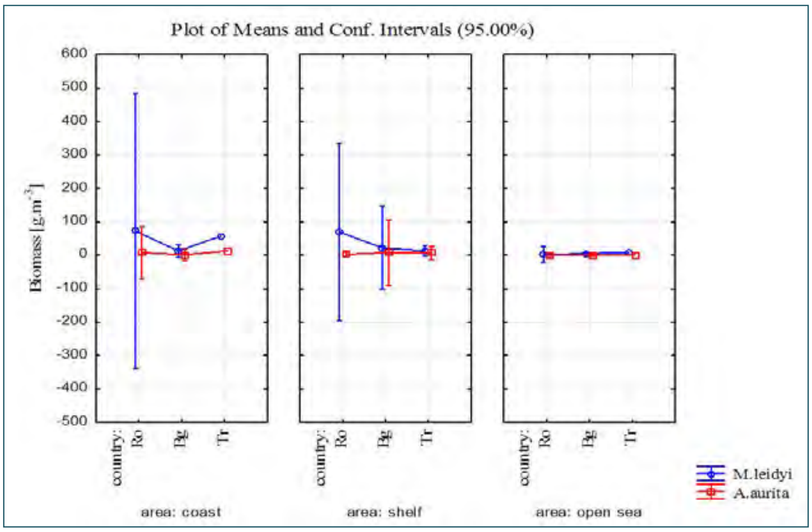


Figure II.2.8. Plot Average of M.leidyi and A.aurita biomasses and conf.interval by habitats and area.

Biomass of copepods (%CB)

Copepods are key group and potential indicator not only for food resource of planctivorous fish but they are presented all year round and well distributed within the three habitats. The share of copepods in the coastal habitats varied between 18-38% (GEnS >42 %CB), while along the shelf between 5-55% (Table II.2.1). Copepdos species in coastal and shelf habitats contributed together with cladoceras and benthic larvae to the mesozooplankton community. Copepods prevalence was well pronounced in the open sea where the indicator exceeded target value of >42%CB (with range 55-89%CB). Six Calanoida and two Cyclopoida species presented copepods. The ultimate dominant species at coast and shelf was *A.clausi* (70%) and *C.euxinus* (68%) at deep waters.

***Noctiluca scintillans* biomass (%N.sci)**

Although after 2000 heterotrophic dinoflagellate maintained lower concentrations *Noctiluca* expanded in bloom concentrations under favorable conditions (calm weather, phytoplankton blooms, low salinity) (Adnan Al-Azri et al., 2007). In July 2013 the high share was associated mainly with shelf habitat in Constanta and Igneada transects - over 66% N.sci (Table II.2.1). In 64% of cases indicator values surpassed the threshold of 30%. *N.scintillans* was scarce presented in the open sea of Romania and Bulgaria (1-2%N.sci) and Turkey (29%N.sci).

Shannon-Weaver diversity index

The rather arbitrary nature of delineating an ecological community, and the difficulty of positively identifying all of the species present, species diversity itself has two separate components: 1) the number of species present (species richness), and 2) their relative abundances/biomass (termed dominance or evenness) (Magurran, 2004). Diversity index comes from the information theory in ecology, a theoretical measure of relative abundance/biomass of each species if their share in the community would be equalized. However, under natural conditions and multiple factors, could not be expected equally contribution of species within the community**.**

The species number is relatively high (totally 26) with increase to the south but lower Shennon index (Fig. II.2.9) reflected of certain species dominance (*N.scintillans, A.clausi*, *Pleopis polyphemoides* , etc) within the plankton community. As a rule, coastal and shelf stations in the whole study area were characterized with index values below the threshold defined (3 bit·ind^{-1}) with small exceptions (M05, M11, M15) while the indices at open sea stations (M06, M07, M08, M14) were in the reference limit of good environmental state.

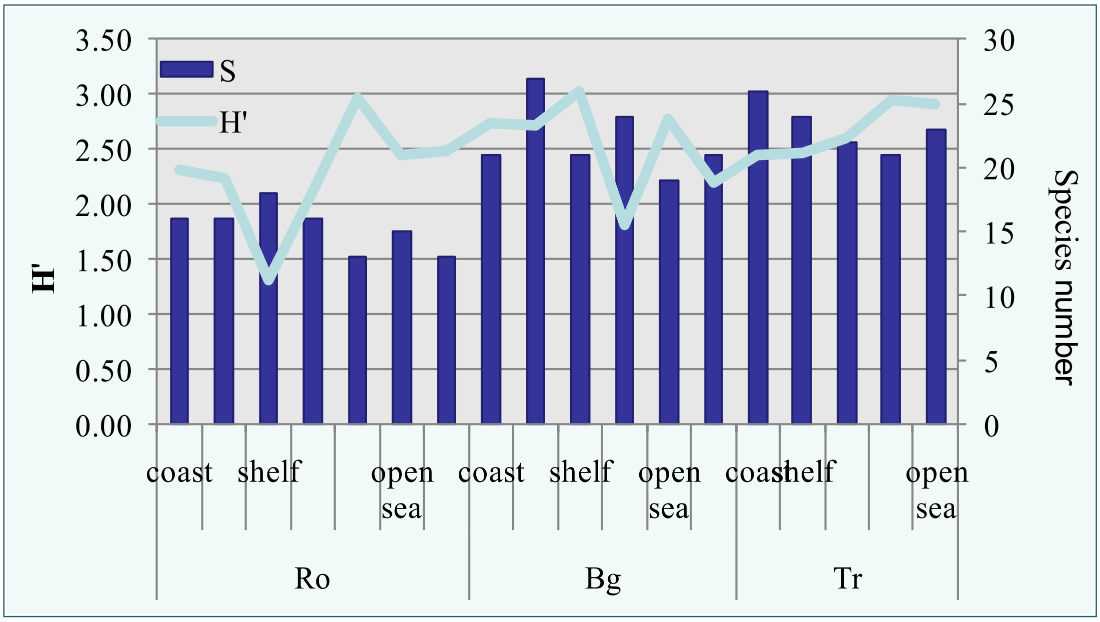


Figure II.2.9. Species number and Shannon diversity index along the transects

Table II.2.1.Ecological state regarding the indicator mesozooplankton biomass [mg·m-3] and thresholds for GEnS by habitats.

	Habitats	Biomass	State	%CB	State	%N.sci	State	H'	State
Romania	coastal	294.050		38		11		2.3	
	shelf	172.067		11		66		2.2	
		147.275		5		88		1.3	
		101.560		8		72		2.1	
		27.511		36		34		3.0	
	open sea	114.683		71		2		2.5	
		50.270		76		1		2.5	
Bulgaria	coastal	176.485		20		58		2.7	
	shelf	259.735		55		1		2.7	
		85.593		32		84		1.8	
		62.697		13		1		3.0	
	open sea	45.000		76		2		2.8	
		43.501		89		2		2.2	
Turkey	coast	104.07		18		63		2.5	
	shelf	124.38		40		26		2.5	
		59.36		19		69		2.6	
		49.12		19		68		3.0	
	open sea	56.11		45		29		2.9	

CONCLUSIONS

Coastal and shelf habitats are characterized by a tight physical-biological coupling, which means that variations in environmental variables together with land-based sources are propagated to the biological structure, thereby leading to their non-steady character (Gilabert, 2001). Zooplankton community in about of 60% of occasions is in “lower” quality in respect of candidate indicators. Opens sea habitats revealed a rather homogenous picture of zooplankton community composition and standing stock corresponding to GEnS characteristic.

GAPS

- development of new reliable indicators along with the validation of proposed reference levels and thresholds of candidate indicators of Bulgaria
- indicators are usually area-specific and unambiguous thresholds are needed to be developed (for Romania and Turkey)
- development of integrated index
- delimited the shelf habitat in inner and outer shelf
- approach in determination of the final evaluation of the ecological state is still under debate

RECOMMENDATIONS

- to provide regular monitoring of zooplankton community with relevant frequency (seasonal), including all components of plankton fauna - micro-, meso- and macrozooplankton
- harmonize assessment methodologies, analytical techniques, reporting formats at regional level
- sustain monitoring of transects with long-terms observations
- further development of guidelines, inter-comparison exercises in taxonomy and methodology

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II. 3. Benthic habitats

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INTRODUCTION

Aquatic ecosystems suffer from long-term, human induced damages, which are often irreversible (e.g. species introductions, species extinctions and contamination of sediments) (Frid et al., 1999; Bolam & Whomersley, 2005). In the last decade in Europe, consciousness regarding these threats induced the launch of two umbrella regulations; i.e. the Marine Strategy Framework Directive (MSFD; 2008/56EC) for the protection of the marine waters and the European Water Framework Directive (WFD; 2000/60/EC) for the protection of the rivers, lakes, transitional and coastal water bodies.

The MSFD establishes a framework in which the Member States have to take appropriate measures to achieve or maintain a Good Environmental Status (GEnS) in the marine environment by 2020. Their main target is the conservation and sustainable use of the marine ecosystem by reducing the pressure on natural marine resources where those are too high. MSFD requires ecosystem-based environment management. One of the important ecosystem components within the ecosystem-based approach to management are macro-invertebrate species (benthos). Due to their sessile, sedentary and relatively long life, the benthic organisms are well considered as suitable and sensitive indicators of natural and anthropogenic variations (Pearson & Rosenberg, 1978). Macrobenthos proved to be a good indicator of temporal and chronic disturbances as well (Dauer, 1993).

Meiobenthos was regarded by many researchers as a tool for ecological assessment and environmental monitoring (Alves et al., 2013), based on the facts that they reflect changes occurring in the trophic state of benthic ecosystems, but also supported by their direct benthic development, their sessile life habit, high species diversity, short generation time, and ubiquitous distribution.
Especially, nematode communities were often considered for ecological quality assessment and environmental monitoring. Heip et al. (1985) recommended the use of nematode (and meiofauna in general) as a possible tool for detecting pollution. Moreno et al. (2011) proposed nematodes as indicators for assessing marine ecological quality within the Water Framework Directive and they used c–p scaling, Maturity Index and availability of selected indicator nematode species. However the proposed thresholds are for the Mediterranean Sea, but tested at small scale in the Black Sea and provided promising results (Ürkmez et al., 2014).

Tools that were commonly used in the past with meiofauna as pollution indicators are the nematode: copepod ratio, diversity indices or graphical methods (K dominance curve) for representing the biodiversity, biomass spectra (Vanaverbeke et al., 2003), the Maturity index (Bongers et al., 1991; Bongers, 1999) or multivariate community analysis from a lower to a higher taxa level (Heip et al., 1985).The harpacticoids are considered suitable because of their spatially and temporally low variability regarding density, biomass, and diversity. The comparative analysis of harpacticoids taxocoenosis from different locations (Castel & Lasserre, 1977) showed a relative similarity of species distribution and composition, allowing the determination of the response of species to different environmental gradients/conditions. In meiobenthic communities, harpacticoids are regularly ranked second after nematodes in terms of abundance.

Nematodes, though overwhelmingly abundant, are more variable and less easily determined, but their overall density is probably a quite stable parameter (Heip, 1980). However, nematode populations could sometimes not be sensitive to pollution, but rather tolerant and different from taxa to taxa.

The other meiobenthic taxa often very abundant and frequent are foraminiferans. However, the foraminiferan's abundance distribution based on very large spatially distanced samples is quite difficult. Studies showed that foraminiferans have a patchy distribution forming sometimes small aggregations of high abundances, but similarly distributed when looking at bigger scales in the same habitat. This is due often to differential food preferences or physical-chemical parameters. For example, Montagna et al. (1983) showed that occurrences of diatoms and other meiofauna were partly determined by physical factors (salinity, temperature and redox depth). It is also reported that microphytobenthic biofilms disruption due to different climatic variation (e.g., wind stress in the littoral zone) may lead to communities' shifts. Microphytobenthos (the main foraminiferal food source) is reported to occur in patches of 2-100 cm^2 in muddy sediments (Seuront & Spilmont, 2002; Jesus et al., 2005) and in patches of 30-190 cm^2 in sandy sediments (Sandulli & Pinckney, 1999). Bacteria can also occur in patches on a centimeter scale in near-coastal sediments (Seymour et al., 2004). Additionally, Blanchard (1990) found a correlation between the patchy distribution of microphytobenthos and meiofauna and hypothesized that spatial and temporal variations in the abundance of meiofauna is caused by food availability. Harpacticoid copepods are also shown to be distributed spatially according to distribution of diatoms and bacteria (Decho & Castenholtz, 1986). A weaker relationship was revealed between macrofauna composition shifts and foraminiferan composition.

Foraminiferans were found to be very sensible to pollution as their shells deform in the presence of chemical pollution, including oil, and they have a very short life span — about 30 days. The rapid turnover means that living forams indicate the current state of pollution, while cores through sediment can reveal past history of pollution (Sanders, 2010). So, even simply visual examination could provide us useful information about the environmental state. Several indices (diversity indices Shannon-Wiener index (H', log2) and Hurlbert index (ES (100)) (Hurlbert, 1971) were tested to assess EcoQS (e.g., in the Northern Sea). Correlation between foraminiferal diversity and [O_2], as well as correlation between community data and [O_2], suggest that benthic foraminifera represent an efficient bio-monitoring tool to evaluate EcoQS. A clear pattern from "bad" to "high" EcoQS was established using the strong link between the benthic foraminiferal diversity and the bottom-water oxygen gradient (Bouchet et al., 2012).

In this context the widely-advocated ecosystem-based management demands the development of adequate tools to evaluate the status of ecosystems at large scales (Frid, 2003). Improved knowledge of the habitats and associated biological communities overlapped with human uses is essential (Kenny et al., 2003; Pickrill & Todd, 2003; Holmes et al., 2008). This information is of high priority in the European context, as the implementation of several EU strategies (on Biodiversity, MSFD, etc.) requires a comprehensive classification of marine habitats and their correspondent biodiversity (Barberá et al., 2012).

The assessment of the condition of benthic habitats is one of the evaluation criteria both in the WFD (as biological quality element) and in the MSFD descriptors (D1 - biodiversity & D6 - sea floor integrity).
An assessment procedure for determining the condition of soft-sediment benthic habitats requires the following aspects:

- habitat assignation of the samples (habitat approach),
- reference or target conditions for the benthic parameters, and
- the selection of indicator tools to assess the relative quality status (indicator approach).

One of the main objectives of the MISIS (**M**SFD GUIDING **I**MPROVEMENTS IN THE BLACK **S**EA **I**NTEGRATED MONITORING **S**YSTEM) project is the carrying out the ecological assessment of the Black Sea, taking into consideration the requirements of the WFD and the descriptors of the MSFD and hence providing general overview on the status of habitats. In order to respond to the objectives raised by the project, an expedition in the western part of the Black Sea was organised, with which occasion macrozoobenthos samples were collected.

MATERIAL and METHODS

Macrozoobenthos

Ecological assessments of the macrozoobenthic associations on the western Black Sea continental shelf were done on the basis of 47 samples taken at 13 stations, which cover the following sectors:

- Romanian transect, Constanta-East;
- Bulgarian transect - Galata transect (travers Varna);
- Turkish transect (Igneada).

The disribution of samples on depth intervals were as follows:

- 20 -30 m: 2 stations;
- 31 – 50 m: 3 stations;
- 51 m - 100 m: 6 stations;
- >100 m: 2 station (M05 and M15).

The common protocol of sampling foreseen that all team to use the same instrument of collecting. Thus, all macrozoobenthos samples were collected by helping of a Van Veen grab with surface of $0.135 m^2$. At each station, the teams collected 3 replicates.

Totally, a number of 30 macrozoobenthos samples were collected by GeoEcoMar from the Romanian and Bulgarian transects (at coastal and shelf stations), and also from inter- comparison stations. The NIMRD team collected 6 samples (3 samples from each inter-comparison station), while the Sinop team collected 12 samples from the Turkish transect (coastal and shelf stations), and the inter-comparison station M 10.

The benthos samples collected by GeoEcoMar were tagged and photos were taken when the sample was onboard. There were taken only the first top 20 cm of the sample (depends of the substrate type; at some stations the sample was collected integrally). The inter-comparison samples were integrally collected by all teams, while the SINOP team collected integral samples every time.

GeoEcoMar and NIMRD performed a pre-washing of the samples through 0.5 mm mesh size sieves for sediments excess removal. The preserving was done with formaldehyde 4% buffered with seawater and the samples were stored in labeled plastic bags and containers until their subsequent examination in laboratory. A macro-visual description of each sample was done before preserving and the main communities and species were identified on spot. After sieving, all organisms were identified to the lowest possible taxonomic level (species level).

The samples collected by the Sinop team were pre-sieved with a 0.5 mm mesh and the retained fauna was put in jars containing 10% 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 macrozoobenthic organisms were sorted into higher systematic groups. These samples were delivered to specialists for taxonomic identifications.

The structure of the macrozoobenthic community was analysed in terms of species composition, density, dominance, frequency, diversity and biomass. The diversity was calculated by the Shannon-Wiener diversity index (*H'*) on a log 2 base (Shannon & Weaver, 1963). The AZTI Marine Biotic Index, AMBI (Borja et al., 2000), and the multivariate AMBI, M-AMBI (Muxika et al., 2007) were calculated using the freeware program available on www.azti.es.
Multivariate analysis (Bray-Curtis similarity) was performed with fourth square-root transformed data using PRIMER package programme version 5.2.4 (Clarke and Warwick, 2001). Furthermore, SIMPER analysis was performed to identify the percentage contribution of each species to the overall similarity (dissimilarity) within each of the groups identified from the cluster analysis.

Meiobenthos

Overall, 13 samples of meiobenthos were taken during MISIS JOINT CRUISE (Table II.3.1), of which 4 samples on each Bulgarian (Galata) and Turkish (Igneada) transects at depths between 23 and 101 m, and 5 samples on the Romanian transect (Constanta), between 33 and 101 m depth were obtained.

The habitats corresponding to the meiobenthos sampling points were considered the same as in the macrozoobenthos case, due to the fact that the depths sampled coincided with the macrobenthos collecting sites. The meiobenthic community is rarely found in the absence of macrobenthic communities, except those formed in very harsh conditions (low oxygen, very high or low temperatures, very polluted or disturbed environments) inappropriate for macrobenthos life.

Two meiobenthic replicates were sampled at each station, using a metallic push-corer of 12.54 cm^2. The samples have been immediately preserved with formaldehyde 4% buffered with seawater (GeoEcoMar) and alcohol 95% and Rose Bengal (Sinop), respectively.

RESULTS and DISCUSSIONS

As a result of sample processing in laboratory, 135 macrozoobentic taxa were identified in the studied zones of the western part of the Black Sea continental shelf, most of them being taxonomically determined at species level and some of them being considered only at supraspecific level (ANNEX 1). The results of the macrozoobenthic analyses are presented in Table II.3.1. The composition of meiobenthic community was assessed based on abundance of individuals. These pertained to 13 higher meiobenthic groups. The average density and biomass are given for each sample analyzed (Table II.3.1).

Table II.3.1. General sinoptic situation for all stations performed for benthos in Akademic Cruise, 2013.

Team	Transect	ID Stations	Depth, m	Macrozoobenthos			Meiobenthos	
				S	Davg ind·m^{-2}	Bavg g·m^{-2}	Davg ind·m^{-2}	Bavg g·m^{-2}
GeoEcoMar	Romanian transect, Constanta-East	M01_A	33.3	19	2,930.4	13,74	174,722	747.83
		M01_C		19	2,168.2	84,93		
		M02_A	47	12	466.2	5.09	609,736	1,294.29
		M02_B		5	421.8	2.72		
		M02_C		5	214.6	1.48		
		M03_A	54	18	1,539.2	79.72	392,428	470.88
		M04_A	64.7	18	976.8	9.31	109,848	55.66
		M04_B		17	503.2	6.66		
		M04_C		16	518.0	5.67		
		M05_A	101	12	259.0	10.72	15,522	46.38
		M05_B		9	214.6	10.23		
		M05_C		11	303.4	7.68		
	Bulgarian transect - Galata transect	M12_A	23.2	14	4,092.2	19.42	783,662	5,911.81
		M12_B		30	3,433.6	251.38		
		M12_C		30	4,440.0	159.71		
		M11_B	39.9	21	2,997.0	52.92	1,978,856	28,081.76
		M11_C		17	2,715.8	109.78		
		M09_A	92.7	14	1,820.4	26.72	124,186	3,900.45
		M09_B		3	103.6	0.15		
		M09_C		5	96.2	0.33		
SINOP	Turkish transect - Igneada	M17_A	53.3	13	2,116.4	450.44	235,218	1,475.20
		M17_B		12	2,686.2	194.88		
		M17_C		4	614.2	71.60		
		M16_A	75.6	17	4,395.6	658.52	515,410	5,757.69
		M16_B		11	3,515.0	514.59		
		M16_C		18	6,275.2	753.18		
		M15_A	101	15	6,896.8	610.70	131,340	486.58
		M15_B		15	1,591.0	110.59		
		M15_C		18	5,661.0	457.62		
GeoEcoMar	Galata – Intercalibration station	M10_A	76.1	14	858.4	2.04	1,071,814	12,814.74
		M10_B		15	481.0	83.25		
		M10_C		8	621.6	4.22		
SINOP		M10_A		8	2,664.0	273.36		
		M10_B		9	4,380.8	601.66		
		M10_C		13	9,124.2	1,494.84		
NIMRD		M10_A		10	241.0	8.1		
		M10_B		7	156.0	5.0		
		M10_C		7	203.0	1.9		
GeoEcoMar	Igneada – Intercalibration station	M18_A	27.2	26	3,359.6	14.0	99,102	1,070.36
		M18_B		38	3,840.6	67.1		
		M18_C		33	2,271.8	46.7		
SINOP		M18_A		23	83,087.2	3,293.8		
		M18_B		35	97,317.4	3,643.8		
		M18_C		36	15,5614.6	7,698.4		
NIMRD		M18_A		13	1,341.0	166.10		
		M18_B		14	733.0	52.71		
		M18_C		14	1,237.0	108.33		

Description of Benthic habitats

Within the MISIS project we aimed to assess the ecological state of benthic habitats, which were classified after the European Nature Information System (EUNIS; Davies et al., 2004). EUNIS is considered as one of the most suitable classification system for the marine biodiversity from Europe, being already a formal part of the official regulation system used in the process of selection of conservation areas and in environmental assessments. More of that it has the most integrated and relevant methodology from ecological point of view of all existent European systems, and has extended in many countries and geographical regions over the time. EUNIS, unlike the classification system of habitats NATURA 2000, it is a permissive/flexible one, all types of habitats being placed into the system or could be added in.

Currently, in the Black Sea there are two classification systems of habitats in different development stages: NATURA 2000 and EUNIS. NATURA 2000 is the most used, but given the general European tendency to classify all habitats after EUNIS, it gives us more reasons to analyze and align to EC requirements. Therefore, within this study we tried to complete with existing literature, archives, and databases of partner Research Institute the data coming out from the samples collected on the three transects (Constanta East, Galata and Igneada) in order to assess the status of major habitats.

Five benthic habitats have been found in the study area based on samples analysis:

- Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata,*
- Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys,* etc,
- *Mytilus galloprovincialis* beds on mud and sandy mud,
- Circalittoral shelly silt with *Modiolula phaseolina* (include *Terebellides*, *Amphiura*, *Pachyceriantus* and large tunicates (*Ascidiella*, *Ciona*)).
- Lower circalittoral mud with *Terebellides stroemi*

As a result of specific features of the analyzed areas, the assessment of macrozoobenthic ecological status has been performed differentiated on each of the three transects, while the meiobenthic ecological status was assessed on each habitat for all three transects.

Macrozoobenthic Ecological Status

1. Constanta East

The Bray Curtis similarity analysis on the presence/absence data at 12 samples differentiated three types of communities typical of the Upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud and Circalittoral shelly muds with *Modiolula* (Fig. II.3.1).

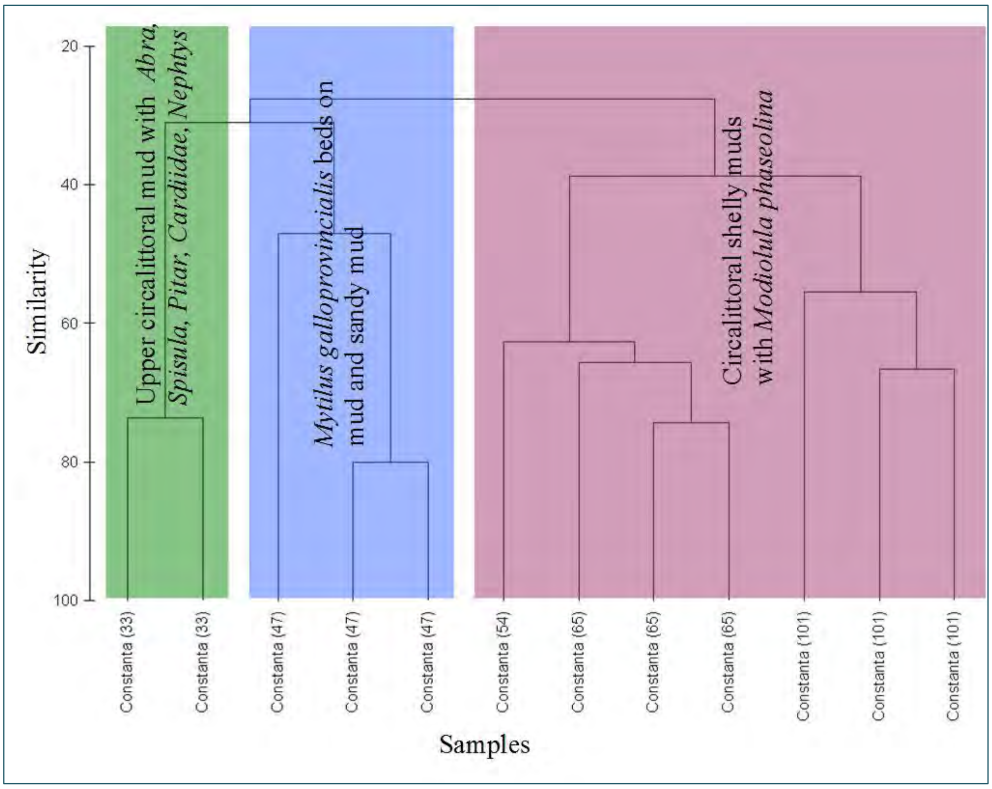


Figure II.3.1. Bray Curtis similarity of Constanta East data. The similarity groups are classified according to the EUNIS habitats classification under MSFD.

A. Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys*

Descriptor 1

1.4. Habitat distribution

This habitat lies between 23 – 25 m depth and from Danube mouths to Kaliakra Cape. The sediments are represented by mixed mud with fine sands in the northern part of the Romanian littoral and shelly muds in the southern one.

1.6. Habitat condition

1.6.1 Species state and communities

A total of 24 taxa belonging to 10 systematic groups were found in the assemblages (Annelida – 8, Mollusca - 7, Crustacea - 5, others - 4). The mean abundance of the macrozoobenthic populations was 2,550 ind·m^{-2} and 49.3 g·m^{-2} as biomass. *Oligochaeta* had the highest individuals (Davg - 1,360 ind·m^{-2}) (Table II.3.2 and II.3.3).

The characteristic species of this habitat are mollusks *Abra prismatica, Spisula subtruncata, Pitar rudis* and *Cardiidae*. The local circular currents determine a concentration of detritus sediments in the area. Thus, a rich organic muddy substrate of weak consistency and low percent of coarse material is formed here. On this substrate could be found *Abra prismatica* along with oligochaets and polychaets could be found, among the latter the most frequent being *Nephthys hombergii* and in addition *Alita succinea, Melinna palmata, Heteromastus filiformis.*

Table II.3.2. Summary of the results from the univariate statistical analysis of the macrozoobenthos from Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys* at Romanian transect, Constanta-East.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M01 (33m)	24	3.7	1357.9	2549.3	235
Biomass g·m^{-2}					
M01 (33m)	24	0.0009	34.73	49.35	7.18

Table II.3.3. Ecological characterization of macrozoobenthos from Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys* at Romanian transect, Constanta-East (D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Spisula subtruncata (da Costa, 1778)	14.8	34.743
Pitar rudis (Poli, 1795)	7.4	8.288
Nephtys hombergii Savigny in Lamarck, 1818	107.3	1.961
Abra prismatica (Montagu, 1808)	29.6	0.381
Oligochaeta	1357.9	0.222
Heteromastus filiformis (Claparède, 1864)	595.7	0.512
Mytilus galloprovincialis Lamarck, 1819	214.6	0.215
Prionospio cirrifera Wirén, 1883	55.5	0.039
Medicorophium runcicorne (Della Valle, 1893)	51.8	0.021
Phtisica marina Slabber, 1769	33.3	0.057
Iphinoe elisae Băcescu, 1950	14.8	0.007
Nemertini indet.	14.8	0.030
Phyllodoce mucosa Örsted, 1843	7.4	0.026
Obelia longissima (Pallas, 1766)	7.4	0.114
Actinothoe clavata (Ilmoni, 1830)	3.7	0.185
Alitta succinea (Leuckart, 1847)	3.7	0.002
Harmothoe impar (Johnston, 1839)	3.7	0,002
Melinna palmata Grube, 1870	3.7	0.011
Abra alba (W. Wood, 1802)	3.7	0.004
Acanthocardia paucicostata (G. B. Sowerby II, 1834)	3.7	0.037
Mya arenaria Linnaeus, 1758	3.7	2.405
Phoronis euxinicola Selys-Longchamps, 1907	3.7	0.003
Microdeutopus gryllotalpa Costa, 1853	3.7	0.001
Upogebia pusilla (Petagna, 1792)	3.7	0.074

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality (Species richness, Shannon, AMBI and M-AMBI)

Assessing benthic quality status of marine and transitional water habitats requires to set up both:

- indices to assess the relative quality of the considered habitat, and
- reference conditions for which such indices can be computed and used to infer the absolute ecological status of the considered habitat.

As reference value for good ecological status will be considered the period 1950 – 1970, characterized by lack of major impacts, so called the „ecological equilibrium period" (Băcescu et al., 1971). In that period there have been performed detailed studies on benthic biocoenosis in the northwestern Black Sea.

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.4). Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.4 indicate moderate status for the station at 33 m depth - upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys* at Romanian transect, they do not meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos (Table II.3.5). Clearly, the different indices did not produce the same ecological classifications for the zone.

The actually method of intercalibration used by the European Union is not suitable for comparing the results obtained with all the water bodies studied. Because some inconsistencies in the classification have been detected, the metrics require further validation for better understanding of the community response to different natural and/or anthropogenic pressures.

Table II.3.4. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys* at Romanian transect, S-species richness (Reference conditions), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD	S	H`	AMBI	M-AMBI	MSFD
High	S ≥ 35	H`>4	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	GES
Good	35> S ≥ 27	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	**0.85 > M-AMBI ≥ 0.55**	
Moderate	**27 > S ≥ 20**	**2 < H`≤ 3**	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Non - GES
Poor	20 > S ≥ 10	1 < H`≤ 2	**4.3 < AMBI ≤ 5.5**	0.39 > M-AMBI ≥ 0.20	
Bad	S < 10	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Table II.3.5. Ecological state of macrozoobenthos in the Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys* at Romanian transect according to average diversity and biotic indices.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M01 (33m)	24	2.20	4.38	0.61	Moderate (Not good)

B. *Mytilus galloprovincialis* beds on mud and sandy mud

Descriptor 1

1.4. Habitat distribution

The typical deep mussel bed ranges between 30 - 50 m depth. However, it can occupy an area between 15 - 70 m depth. The muddy and muddy sand substrata are typical for this habitat. The total surface of mussels` beds in front of the Romanian coast is about 7000 km^2, being the second largest community after those of *Modiolula* (Fig. II.3.2) (Băcescu et al., 1971).

Figure 2. ***Mytilus galloprovincialis***

1.6. Habitat condition

1.6.1 Species state and communities

During eutrophication period (`70s-`90s) this community suffered major changes in terms of number of species and abundances of dominant species decreasing.

In the investigated area 25 taxa belonging to 9 systematic groups were found in the assemblages (Annelida - 10, Mollusca - 5, Crustacea - 3, others - 7) (Table II.3.6 and II.3.7). The typical species found in this habitat are detritivorous polychaets *Nephtys hombergii, Terebellides stroemii, Aricidea claudiae, Heteromastus filiformis,* molustele *Mytilus galloprovincialis, Abra alba, A. prismatica,* crustaceans *Caprella acanthifera, Perioculodes longimanus.* The mean abundance of the macrozoobenthic populations was 660.45 ind·m^{-2} and 22.3 g·m^{-2} as biomass. The minimum density recorded was 1.85 ind·m^{-2}, while the biomass was 0.0005 g·m^{-2} (Table II.3.6 and II.3.7).

However, the number of samples collected is not considered enough high to reflect the real situation of the habitat, keeping in mind that 25 taxa identified with this occasion accounted for no more than 20% of total number of species found by GeoEcoMar in the last period. According to Teaca et al. (2013) the maximum biodiversity of 128 species occurred in the central part of the mussels bed. The analysis of species distribution on geographic sectors indicated a clear demarcation between the northern sector (under the Danube influence) and the southern one. In the southern part, the polichaet *Aricidea claudiae* (max. 2.700 ind·m^{-2}) is the characteristic species for the transition interval between littoral coenosis and the typical *Mytilus* one (Teacă et al., 2013).

Analysis of the *Mytilus* community on bathymetric intervals revealed the importance of this coenotic unity as a biodiversity reservoir. The spatial distribution of the *Mytilus* presents 2 transition zones (20-30 m and 56-70 m), one situated at the limit with the littoral communities and the other with the deep ones, with the true mussels' community (30-50 m) in between (Teacă et al., 2013). In the central part of the *Mytilus* beds, between 30-50 m depth the mussels and *Mya arenaria* reached 54% of total biomass, followed by *Nephtys hombergii* (Teacă et al., 2013).

Table II.3.6. Summary of the results from the univariate statistical analysis of the macrozoobenthos from *Mytilus galloprovincialis* bed on mud and sandy mud at Romanian transect, Constanta-East.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M02 (47m)	13	2.47	130.7	367.5	29.6
M03 (54m)	18	7.4	606.8	1539.2	103.2
TOTAL	**25**	**1.85**	**186.8**	**660.5**	**47.6**
Biomass g·m^{-2}					
M02 (47m)	13	0.0009	2.32	3.09	0.63
M03 (54m)	18	0.002	67.5	79.7	15.8
TOTAL	**25**	**0.0005**	**16.9**	**22.26**	**3.4**

Table II.3.7. Ecological characterization of macrozoobenthos from *Mytilus galloprovincialis* beds on mud and sandy mud at Romanian transect, Constanta-East
(D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Mytilus galloprovincialis Lamarck, 1819	12.95	16.872
Nephtys hombergii Savigny in Lamarck, 1818	111	2.054
Terebellides stroemii Sars, 1835	24.05	0.888
Heteromastus filiformis (Claparède, 1864)	186.85	0.161
Oligochaeta	138.75	0.028
Nemertini indet.	38.85	0.078
Micrura fasciolata Ehrenberg, 1828	37	0.130
Amphiura stepanovi Chernyavskii, 1861	29.6	0.511
Polycirrus jubatus Bobretzky, 1869	22.2	0.685
Abra prismatica (Montagu, 1808)	7.4	0.065
Leptosynapta inhaerens (O.F. Müller, 1776)	7,4	0.250
Perioculodes longimanus (Bate & Westwood, 1868)	5.55	0.001
Prionospio cirrifera Wirén, 1883	5.55	0.004
Phyllodoce mucosa Örsted, 1843	5.55	0.037
Caprella acanthifera Leach, 1814	3.7	0.007
Oriopsis armandi (Claparède, 1864)	3.7	0.002
Retusa truncatula (Bruguière, 1792)	3.7	0.013
Phoronis euxinicola Selys-Longchamps, 1907	3.7	0.003
Amphiporus bioculatus McIntosh, 1874	1.85	0.004
Dipolydora quadrilobata (Jacobi, 1883)	1.85	0.002
Amphinema dinema (Péron & Lesueur, 1810)	1.85	0.001
Aricidea claudiae Laubier, 1967	1,85	0.001
Megamphopus cornutus Norman, 1869	1.85	0.001
Abra alba (W. Wood, 1802)	1.85	0.278
Modiolula phaseolina (Philippi, 1844)	1.85	0.185

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

As in the previous described habitat, using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.8 indicate moderate status for the stations 47 m and 54 m depth - the *Mytilus galloprovincialis* beds on mud and sandy mud at Romanian transect, they do not meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos (Table II.3.9).

Table II.3.8. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the *Mytilus galloprovincialis* beds on mud and sandy mud at Romanian transect, S-species richness (Reference conditions), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD	S	H`	AMBI	M-AMBI	MSFD
High	S ≥ 55	H`>4	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	GES
Good	55> S ≥ 45	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	0.85 > M-AMBI ≥ 0.55	
Moderate	45 > S ≥ 25	2 < H`≤ 3	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Non - GES
Poor	25 > S ≥ 15	1 < H`≤ 2	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 15	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Table II.3.9. Ecological state of macrozoobenthos in the *Mytilus galloprovincialis* beds on mud and sandy mud at Romanian transect according to average diversity and biotic indices.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M02 (47m)	13	2.36	3.56	0.56	Moderate (Not good)
M03 (54m)	18	3.07	3.42	0.71	Moderate (Not good)

C. Circalittoral shelly silt with *Modiolula phaseolina* (include *Terebellides*, *Amphiura*, *Pachyceriantus* and large tunicates (*Ascidiella*, *Ciona*))

Descriptor 1

1.4. Habitat distribution

Along the Romanian continental shelf, *Modiolula phaseolina* was the most characteristic species on the bottoms from 55-60 m to 120 m. Called by Borcea (1931) *"phaseolinoid facies of the deep littoral zone"*, the *Modiolula phaseolina* biocoenosis (Fig.II.3.3) alone covers a stretch of about 10,000 km^2 along the Romanian shelf (Băcescu et al., 1971). It covers 40% of the shelf extended up to the limit of the oxic-anoxic layer (Băcescu, 1963; Băcescu et al., 1971; Gomoiu & Ţigănuş, 1977). Its maximum development took place between the Sulina – Sf.Gheorghe (pre-Danubian area) and Mangalia (southern) sectors (Băcescu et al., 1971).

The substratum, generally flat, pretty regular surface, is muddy, mixed with a great quantity of shells.

The substratum, generally flat, pretty regular surface, is muddy, mixed with a great quantity of shells.

Figure II.33. Image of the sediment surface with Circalittoral shelly silt with *Modiolula phaseolina* on the Romanian continental shelf of the Black Sea (depth – 61.3 m)
(Photo: Tim Stevens, taken during R/V Poseidon Cruise 363, 2008)
The substratum is covered by *Modiolula* shells togheter with sponges *Mycale syrinx* (1), *Haliclona* sp. (2), *Sycon ciliatum* (3), hydroid *Corymorpha nutans* (4) and tunicates *Ascidiella aspersa* (5) and C*iona intestinalis* (6).

1.6. Habitat condition

1.6.1 Species state and communities

Research conducted in 1970 indicated high density and biomass and a good trophic base for benthifagous fish (Băcescu et al., 1971; Gomoiu & Ţigănuş, 1977). Measurements performed between 1970 and 1980 did not show any appreciable changes in the *Modiolula phaseolina* muddy bottom, and it was the only stable biocoenoses as compared with shallow waters biocoenoses (Gomoiu & Ţigănuş, 1977). The *Modiolula* biocoenosis was however degraded after 1980 that was identified by the reduction of macrobenthic organisms, particularly those less tolerant to pollution, from 85 in 1960-1970 to 33 in 1991-1995 and 23 in 2000-2001 (Băcescu et al., 1971; Dumitrache, 1996-1997; Abaza & Dumitrache, 2008). The mean abundance and biomass of the deep macrobenthic communities decreased from 7.800 ind·m^{-2} and 233 g·m^{-2} in 1981-1982 approximately five times in 2000-2001 (Abaza & Dumitrache, 2008).

In the investigated area have been identified 31 taxa belonging to 13 systematic groups were found in the assemblages (Annelida - 15, Mollusca - 3, Crustacea - 2, others - 11) (Table II.3.11). Also in this habitat, there have been found only 32% of all species identified in the last period (2006-2010) by GeoEcoMar (Begun et al., 2010). The difference is due to small number of samples collected in the habitat. The mean abundance of the macrozoobenthic populations was 462.50 ind·m^{-2} and 8.4 g·m^{-2} as biomass. The minimum density was of 1.23 ind·m^{-2}, while the biomass was 0.0007 g·m^{-2} (Table II.3.10). According to Begun et al. (2010) the average density of macrozoobenthos from circalittoral shelly silt with *Modiolula phaseolina* was about 5,249.11 ind·m^{-2} while their average biomass was 257.76 g·m^{-2}. Our analyses on long-term changes in the composition and structure of macrobenthos showed that the system seemed to be slightly recovering from the environmental changes occurred during the 1980 - 2000 period. The most important changes observed during that period were the increase of diversity and abundance of the Polychaeta and Crustacea groups within *Modiolula* habitat.

Table II.3.10. Summary of the results from the univariate statistical analysis of the macrozoobenthos from circalittoral shelly silt with *Modiolula phaseolina* on the Romanian transect, Constanta-East.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M04 (65m)	25	2.47	172.67	666.0	39.9
M05 (101m)	18	2.47	59.2	259.0	19.4
TOTAL	**31**	**1.23**	**109.7**	**462.5**	**22.5**
Biomass g·m^{-2}					
M04 (65m)	25	0.001	2.29	7.21	0.54
M05 (101m)	18	0.0002	4.49	9.54	1.27
TOTAL	**31**	**0.0007**	**2.82**	**8.37**	**0.64**

Table II.3.11. Ecological characterization of macrozoobenthos from circalittoral shelly silt with *Modiolula phaseolina* on the Romanian transect, Constanta-East
(D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Modiolula phaseolina (Philippi, 1844)	29.60	0.876
Terebellides stroemii Sars, 1835	109.77	2.822
Nephtys hombergii Savigny in Lamarck, 1818	64.13	0.407
Pachycerianthus solitarius (Rapp, 1829)	20.97	2.245
Heteromastus filiformis (Claparède, 1864)	41.93	0.036
Abra alba (W. Wood, 1802)	3.70	0.555
Amphiura stepanovi Chernyavskii, 1861	19.73	0.530
Leptosynapta inhaerens (O.F. Müller, 1776)	12.33	0.426
Syllides longocirratus (Örsted, 1845)	29.60	0.027
Oligochaeta	20.97	0.004
Dipolydora quadrilobata (Jacobi, 1883)	17.27	0.047
Exogone (Exogone) naidina Örsted, 1845	12.33	0.001
Eudorella truncatula (Bate, 1856)	11.10	0.003
Prionospio cirrifera Wirén, 1883	9.87	0.007
Aonides paucibranchiata Southern, 1914	9.87	0.069
Phyllodoce mucosa Örsted, 1843	9.87	0.058
Capitella capitata (Fabricius, 1780)	8.63	0.001
Micrura fasciolata Ehrenberg, 1828	6.17	0.019
Suberites carnosus (Johnston, 1842)	3.70	0.075
Nemertini indet.	3.70	0.007
Polycirrus jubatus Bobretzky, 1869	2.47	0.012

Species	Davg	Bavg
Apseudopsis ostroumovi Bacescu & Carausu, 1947	2.47	0.005
Clytia hemisphaerica (Linnaeus, 1767)	2.47	0.002
Parvicardium simile (Milaschewisch, 1909)	1.23	0.086
Molgula appendiculata Heller, 1877	1.23	0.049
Melinna palmata Grube, 1870	1.23	0.004
Amphiporus bioculatus McIntosh, 1874	1.23	0.002
Sycon ciliatum (Fabricius, 1780)	1.23	0.001
Phoronis euxinicola Selys-Longchamps, 1907	1.23	0.001
Harmothoe impar (Johnston, 1839)	1.23	0.001
Oriopsis armandi (Claparède, 1864)	1.23	0.001

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.12 indicate good status for the stations 65 m and 101 m depth - the circalittoral shelly silt with *Modiolula phaseolina* at Romanian transect, meeting the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos (Table II.3.13).

Table II.3.12. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the circalittoral shelly silt with *Modiolula phaseolina* on the Romanian transect, S-species richness (Reference conditions), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD	S	H`	AMBI	M-AMBI	MSFD
High	S ≥ 35	H`>4	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	GES
Good	35> S ≥ 25	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	0.85 > M-AMBI ≥ 0.55	
Moderate	25 > S ≥ 16	2 < H`≤ 3	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Non - GES
Poor	16> S ≥ 10	1 < H`≤ 2	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 10	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Table II.3.13. Ecological state of macrozoobenthos in the circalittoral shelly silt with *Modiolula phaseolina* on the Romanian transect according to average diversity and biotic indices.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M04 (65m)	25	3.60	2.47	0.92	Good
M05 (101m)	18	3.16	1.21	0.86	Good

2. Bulgarian transect - Galata transect (travers Varna)

The Bray Curtis similarity analysis on the presence/absence data distributed the 9 replicate samples collected at station Galata (depth 76 m) in three within-team groups of similarity, the three teams involved in the study indicated as GEM, SINOP and NIMRD on Fig. II.3.4, instead of forming a common similarity group as predictable. The low between-team similarity suggests significant taxonomic expertise variation between the three teams, rather than true taxonomic composition variability.

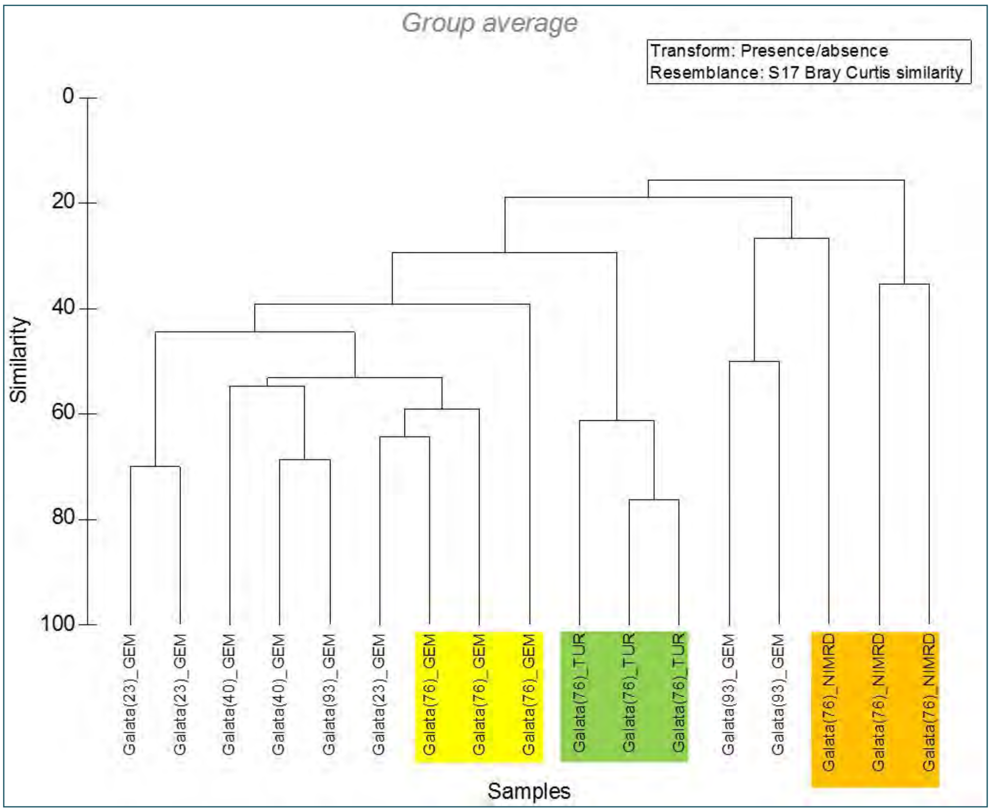
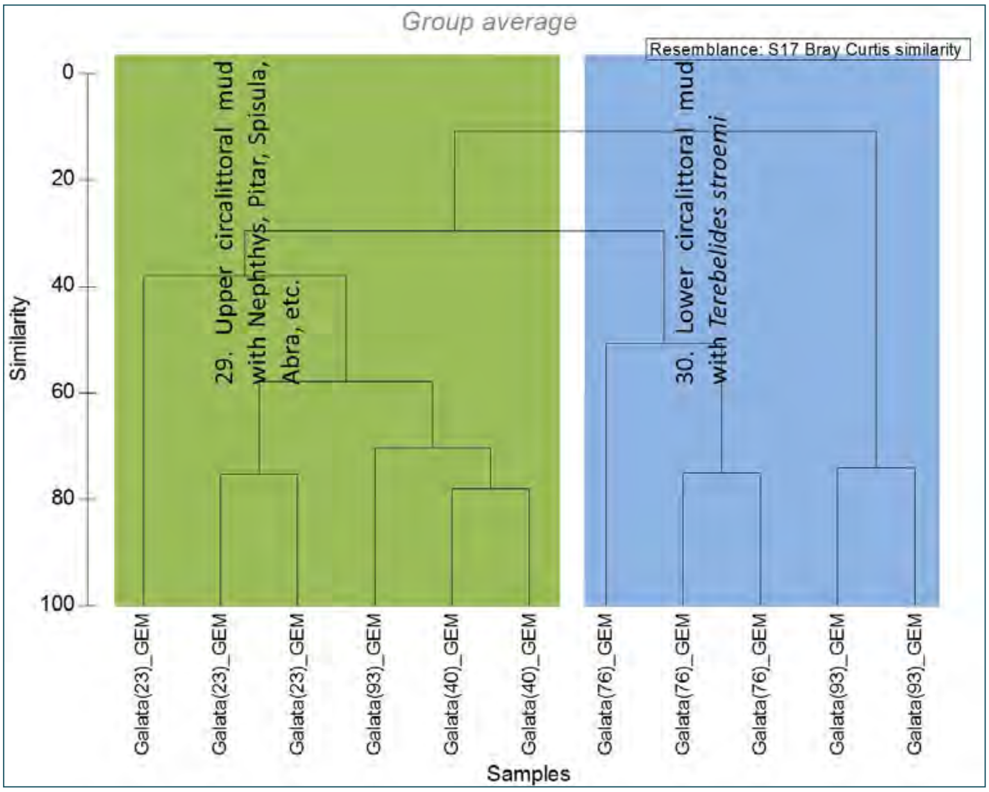


Figure II.3.4. Bray Curtis similarity of zoobenthos along Galata transect based on presence/absence data. Depth rounded to the nearest m is indicated in brackets, teams are indicated as follows: GEM – GeoEcoMar, TUR – SINOP, NIMRD – NIMRD Grigore Antipa.

Galata data produced only by GeoEcoMar were further analyzed for community differentiation, due to being internally consistent in terms of taxonomic identification. Similarity analysis differentiated two types of



communities typical of the upper and lower circalitoral mud respectively (Fig. II.3.5). One of the replicate samples at depth 93 m falls within the upper circalittoral group. This sample being inconsistent with the general community pattern is considered an outlier.

Figure II.3.5. Bray Curtis similarity of GeoEcoMar data. The similarity groups are classified according to the national habitats classification under MSFD Initial Assessment.

The classification of the above differentiated communities is in line with the national benthic habitats classification made under MSFD Initial assessment (Table II.3.14).

Table II.3.14. Bulgarian benthic habitats identified within MISIS survey classified according to the national habitats classification under MSFD Initial Assessment (Todorova et al., 2013a).

MSFD predominant habitats	National biotopes in the Bulgarian Black Sea
10. Shelf sublittoral mud	29. Upper circalittoral mud with Nephthys, Pitar, Spisula, Abra, etc. 30. Lower circalittoral mud with *Terebelides stroemi*

A. Upper circalittoral mud with Nephthys, Pitar, Spisula, Abra, etc.

Descriptor 1

1.4. Habitat distribution

This habitat lies between 20 – 40 (50) m depth from Kaliakra Cape to Maslen Cape, the sediments are represented by mud and sandy mud (Todorova et al., 2013a).

1.6. Habitat condition

1.6.1 Species state and communities

The typical species of this habitat were the detrivor polychaets *Nephtys hombergii, Melinna palmata, Aricidea claudiae, Heteromastus filiformis, Polydora cornuta, , Oriopsis armandi, Allita succinea, Prionospio cirrifera*, molusk *Pitar rudis, Spisula subtruncata, Abra alba, A. prismatica, Parvicardium exiguum,* crustaceans *Ampelisca sarsi, Phtisica marina, Iphinoe elisae* (Table II.3.16). In the study period were found 51 macrobenthic species, the highest diversity being encountered at 37 m (station M12), where they formed abundant populations (Tabel II.3.15). The average density of macrozoobenthos was about 3,550.52 ind·m^{-2} while their average biomass was 118.6 g·m^{-2}. The polychaet *Aricidea claudiae* and oligochaets represented 67% of total macrozoobenthic abundance.

Table II.3.15. Summary of the results from the univariate statistical analysis of the macrozoobenthos from Upper circalittoral mud with Nephthys, Pitar, Spisula, Abra, etc on the Bulgarian transect, Galata.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M12 (23m)	37	2.47	1,724.2	4,013.27	303.16
M11 (40m)	25	3.7	1,406.0	2,856.40	288.14
TOTAL	**51**	**1.48**	**210.2**	**3,550.52**	**232.5**
Biomass g·m^{-2}					
M12 (23m)	37	0.0002	92.79	143.50	14.79
M11 (40m)	25	0.001	51.84	81.35	10.54
TOTAL	**51**	**0.0001**	**55.71**	**118.6**	**8.35**

Table II.3.16. Ecological characterization of macrozoobenthos from Upper circalittoral mud with Nephthys, Pitar, Spisula, Abra, etc on the Bulgarian transect, Galata
(DAVG – Density - ind·m-2, BAVG - Biomass g·m-2).

Species	Davg	Bavg
Nephtys hombergii Savigny in Lamarck, 1818	156.88	2.383
Pitar rudis (Poli, 1795)	19.24	20.735
Abra prismatica (Montagu, 1808)	116.92	0.532

Species	Davg	Bavg
Mytilus galloprovincialis Lamarck, 1819	108.04	55.709
Spisula subtruncata (da Costa, 1778)	1.48	0.148
Aricidea claudiae Laubier, 1967	1258.00	0.418
Oligochaeta	1110.00	0.175
Heteromastus filiformis (Claparède, 1864)	210.16	0.181
Iphinoe elisae Băcescu, 1950	136.16	0.051
Melinna palmata Grube, 1870	118.40	1.980
Amphibalanus improvisus (Darwin, 1854)	50.32	5.313
Polydora cornuta Bosc, 1802	38.48	0.038
Prionospio cirrifera Wirén, 1883	31.08	0.022
Phoronis euxinicola Selys-Longchamps, 1907	29.60	0.029
Perioculodes longimanus (Bate & Westwood, 1868)	26.64	0.005
Nemertini indet.	20.72	0.858
Ampelisca sarsi Chevreux, 1888	10.36	0.062
Chamelea gallina (Linnaeus, 1758)	8,88	7.740
Lagis koreni Malmgren, 1866	7.40	1.495
Harmothoe impar (Johnston, 1839)	7.40	0.004
Acanthocardia paucicostata (G. B. Sowerby II, 1834)	5.92	1.274
Alitta succinea (Leuckart, 1847)	5.92	0.150
Nereiphylla rubiginosa (Saint-Joseph, 1888)	5.92	0.021
Capitella capitata (Fabricius, 1780)	5.92	0.001
Upogebia pusilla (Petagna, 1792)	4.44	0.163
Abra alba (W. Wood, 1802)	4.44	0.014
Mytilaster lineatus (Gmelin, 1791)	4.44	0.010
Carinina heterosoma Müller, 1965	4.44	0.009
Phtisica marina Slabber, 1769	4.44	0.008
Synchelidium maculatum Stebbing, 1906	4.44	0.001
Calyptraea chinensis (Linnaeus, 1758)	2.96	0.207
Parvicardium simile (Milaschewisch, 1909)	2.96	0.089
Anadara kagoshimensis (Tokunaga, 1906)	1.48	8.288
Liocarcinus navigator (Herbst, 1794)	1.48	7.415
Nassarius reticulatus (Linnaeus, 1758)	1.48	2.324
Cyclope neritea (Linnaeus, 1758)	1.48	0.414
Actinothoe clavata (Ilmoni, 1830)	1.48	0.148
Terebellides stroemii Sars, 1835	1.48	0.089
Amphiura stepanovi Chernyavskii, 1861	1.48	0.059
Retusa truncatula (Bruguière, 1792)	1.48	0.050
Gouldia minima (Montagu, 1803)	1.48	0.015
Pholoe inornata Johnston, 1839	1.48	0.004
Haliclona (Reniera) cinerea (Grant, 1826)	1.48	0.001
Pusillina lineolata (Michaud, 1830)	1.48	0.001
Clytia hemisphaerica (Linnaeus, 1767)	1.48	0.001
Gonothyraea loveni (Allman, 1859)	1.48	0.001
Obelia longissima (Pallas, 1766)	1.48	0.001
Podocoryna carnea M. Sars, 1846	1.48	0.001
Harmothoe imbricata (Linnaeus, 1767)	1.48	0.001
Medicorophium runcicorne (Della Valle, 1893)	1.48	0.001
Parvicardium exiguum (Gmelin, 1791)	1.48	0.000

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.17).

Table II.3.17. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the upper circalittoral mud with Nephthys, Pitar, Spisula, Abra, etc. at Bulgarian Black Sea (Todorova et al., 2013b). S-species richness, H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005).

WFD Ecological state	S	H'	AMBI	M-AMBI	MSFD Ecological state
High	S ≥ 39	≥ 3.3	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	Good
Good	39 > S ≥ 31	3.3 > H' ≥ 2.5	1.2 < AMBI ≤ 3.3	0.85 > M-AMBI ≥ 0.55	
Moderate	31 > S ≥ 22	2.5 > H' ≥ 1.8	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Not good
Poor	22 > S ≥ 13	1.8 > H' ≥ 0.7	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 13	H' < 0.7	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.18 indicate good ecological status for the station at 23 m depth, they do meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos.

Table II.3.18. Ecological state of macrozoobenthos at transect Galata, the Bulgarian Black Sea according to average diversity and biotic indices by team: GEM – GeoEcoMar, TUR – Sinop, NIMRD – NIMRD Grigore Antipa.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
Upper circalittoral mud with Nephthys, Pitar, Spisula, Abra, etc.					
M12 (23m)_GEM	37	2.75	3.51	0.68	Good
M11 (40m)_GEM	25	2.40	4.38	0.46	Moderate (Not good)

B. Lower circalittoral mud with *Terebelides stroemi*

Descriptor 1

1.4. Habitat distribution

The associations with *Modiolula phaseolina* distributed on large part of the Romanian platform, there are replaced by those of *Terebelides stroemi* beginning with Kaliakra Cape. Lower circalittoral mud with *Terebelides stroemi* lies on the entire Bulgarian shelf at depths between 40 – 100 m (Todorova et al., 2013a).

1.6. Habitat condition

1.6.1 Species state and communities

The typical species of this habitat were the polychaets *Terebellides stroemii, Nephtys hombergii, N. paradoxa, Aricidea claudiae,* molustele *Abra alba, Retusa truncatula* etc. The total number of species found in this habitat was 43, albeit many of them were accidental ones (ANNEX 1, Table II.3.20). The average density of macrozoobenthos was about 432.16 ind·m^{-2} while their average biomass was 17.9 g·m^{-2}. The results of univariate statistical analysis showed that the macrozoobenthic populations within this habitat recorded numerical abundances (653.6 ind·m^{-2}) and biomass (29.8 g·m^{-2}) greater in the central part of the area of distribution of the association than at the superior limit of the habitat (Table II.3.19), thus, respecting the distribution pattern of macrozoobenthos on depth gradient.

Table II.3.19. Summary of the results from the univariate statistical analysis of the macrozoobenthos from lower circalittoral mud with *Terebelides stroemi* on the Bulgarian transect, Galata.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M10 (76m)	23	2.46	367.5	653.6	75.8
M09 (93m)	16	3.70	66.6	99.9	24.8
TOTAL	**43**	**1.48**	**223.5**	**432.2**	**45.8**
Biomass g·m^{-2}					
M10 (76m)	23	0.0002	23.0	29.8	4.7
M09 (93m)	16	0.001	0.15	0.24	0.06
TOTAL	**43**	**0.0001**	**13.8**	**17.9**	**2.7**

Table II.3.20. Ecological characterization of macrozoobenthos from Lower circalittoral mud with *Terebelides stroemi* on the Bulgarian transect, Galata
(D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Terebellides stroemii Sars, 1835	13.32	0.09472
Nephtys hombergii Savigny in Lamarck, 1818	93.24	0.59200
Abra alba (W. Wood, 1802)	4.44	0.50468
Oligochaeta	223.48	0.04469
Heteromastus filiformis (Claparède, 1864)	19.24	0.01655
Aricidea claudiae Laubier, 1967	11.84	0.00438
Obelia longissima (Pallas, 1766)	10.36	0.00829
Iphinoe elisae Băcescu, 1950	8.88	0.00435
Nephtys (cf) paradoxa Malm, 1874	7.40	0.01480
Mytilus galloprovincialis Lamarck, 1819	5.92	13.8084
Phoronis euxinicola Selys-Longchamps, 1907	5.92	0.00474
Abra prismatica (Montagu, 1808)	4.44	0.60680
Nemertini indet.	2.96	0.00592
Amphinema dinema (Péron & Lesueur, 1810)	2.96	0.00237
Upogebia pusilla (Petagna, 1792)	1.48	2.1756
Amphiura stepanovi Chernyavskii, 1861	1.48	0.0444
Acanthocardia paucicostata (G. B. Sowerby II, 1834)	1.48	0.0296
Actinothoe clavata (Ilmoni, 1830)	1,48	0.01184
Polycirrus jubatus Bobretzky, 1869	1.48	0.00696
Phyllodoce mucosa Örsted, 1843	1.48	0.00518
Retusa truncatula (Bruguière, 1792)	1.48	0.00503
Phtisica marina Slabber, 1769	1.48	0.00252
Suberites carnosus (Johnston, 1842)	1.48	0.00148
Oriopsis armandi (Claparède, 1864)	1,48	0.00065
Perioculodes longimanus (Bate & Westwood, 1868)	1.48	0.00029
Sphaerosyllis bulbosa Southern, 1914	1.48	0.00015

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.21).

Table II.3.21. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the Bulgarian Black Sea (Todorova et al., 2013b). S-species richness, H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005).

WFD Ecological state	S	H'	AMBI	M-AMBI	MSFD Ecological state
Lower circalittoral mud with *Terebelides stroemi*					
High	S ≥ 28	≥ 3.3	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	Good
Good	28 □ **S ≥ 22**	3.3 □ **H' ≥ 2.6**	1.2 < **AMBI ≤ 3.3**	0.85 > **M-AMBI ≥ 0.55**	
Moderate	22 O S ≥16	2.6 □ H' ≥ 1.9	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Not good
Poor	16 □ S ≥ 9	1.9 □ H' ≥ 1.1	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 9	H' < 1.1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.22 indicate moderate status for the stations at 76 and 93 m depth, they do not meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos.

Table II.3.22. Ecological state of zoobenthos at transect Galata, the Bulgarian Black Sea according to average diversity and biotic indices by team: GEM – GeoEcoMar, TUR – Turkish institute - SINOP, NIMRD – NIMRD Grigore Antipa.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
Lower circalittoral mud with *Terebelides stroemii*					
M10(76m)_GEM	23	2.43	4.23	0.49	Moderate (Not good)
M10(76m)_TUR	15	2.21	2.26	0.51	Moderate (Not good)
M10(76m)_NIMRD	18	3.10	1.93	0.74	Good
M09(93m)_GEM	16	2.39	2.74	0.52	Moderate (Not good)

Due to the demonstrated taxonomic identification inconsistency in the dataset, the ecological state assessment involves high uncertainty and the conclusions shall be trusted with caution. Thus for example NIMRD data indicate good status for the station at 76 m, while GeoEcoMar and SINOP data show moderate status.

3. Turkish transect (Igneada)

The Bray Curtis similarity analysis on the presence/absence data at 15 samples differentiated four types of communities typical of the:

- Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata* ,
- Upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud and
- Lower circalittoral mud with *Terebelides stroemii*
- Circalittoral shelly muds with *Modiolula* (Fig.II.3.6).

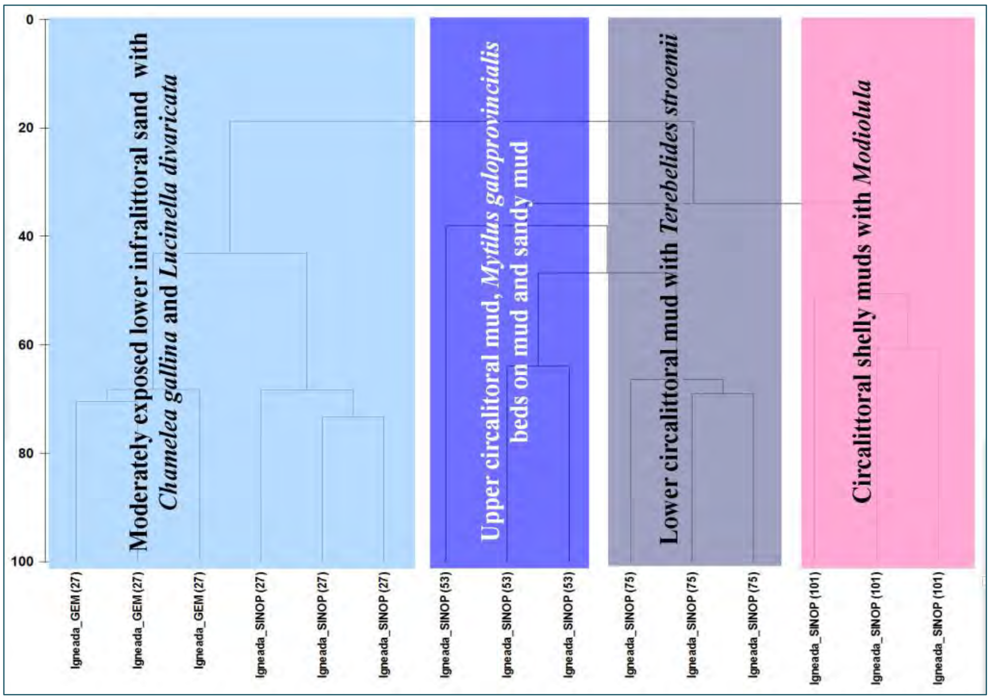


Figure II.3.6. Bray Curtis similarity of Igneada data. The similarity groups are classified according to the EUNIS habitats classification under MSFD.

A. Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata*

Descriptor 1

1.4. Habitat distribution

According to Băcescu et al. (1970) this habitat lies between Igneada (at border with Bulgaria) and Istanbul. In the present study, on the Igneada transect at 27 m depth the habitat Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata* was found. This habitat situated in coarse sandy sediments, is characterised by a relative high species richness, diversity and densities. The sediments type consisted of gray mixture of terrigenous and biogenous medium coarse sand with some silt fraction; very few small shels: *Spisula, Chamelea, Mytilus.*

1.6. Habitat condition

1.6.1 Species state and communities

After analysis of samples performed by GeoEcoMar and Sinop 71 macrobenthic taxa have been identified, pertaining to 11 major taxonomic groups (Table II.3.23 and II.3.24). The samples processed by NIMRD have been excluded from the samples analysis because of relatively high number of taxa identified at the genus level only. About 41% out of total species are mollusks, 34% - polichaets, 18% - crustaceans and 7% the other groups of organisms. The typical species in this habitat were the mollusks *Chamelea gallina, Lucinella divaricata, Pitar rudis, Bittium reticulatum,* polichetele *Aricidea claudiae, Spio decoratus,* crustaceele *Upogebia pusilla, Pseudocuma longicorne s. a.*

The average density recorded by the macrozoobenthic populations was of 57,581.87 ind·m^{-2}, out of which the mollusks represented 95% of the total. The dominant species after density were the mollusks *Bittium reticulatum* - 43% and *Chaecum trachea* – 29%. The average biomass of macrozoobenthos recorded 2,460.64 g·m^{-2}, of which the molluscs represented 99%.
From taxonomically point of view the fauna identified by the two specialists teams was quite similar but the abundances, as for example the average density of macrozoobenthos in M18_GEM was of 3,157.33 ind·m^{-2}, while in M18_Sinop of 112,000.640 ind·m^{-2} (Table II.3.23). These differences appeared from several reasons among which the natural variability of the substrate, the patchy distribution of the populations and the human factor are among the most important ones.

Table II.3.23. Summary of the results from the univariate statistical analysis of the macrozoobenthos from moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata* on the Turkish transect, Igneada.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M18 (27m)_GEM	49	2.47	1,166.7	3,157.33	175.6
M18 (27m)_Sinop	46	2.47	49,942.6	112,006.40	8,754.3
M18 Total	**71**	**1.23**	**24,971.3**	**57,581.87**	**3,554.5**
Biomass g·m^{-2}					
M18 (27m)_GEM	49	0.00002	13.71	42.61	2.54
M18 (27m)_Sinop	46	0.00020	2,116.25	4,878.67	405.25
M18 Total	**71**	**0.00001**	**1,064.9**	**2,460.64**	**165.1**

Table II.3.24. Ecological characterization of macrozoobenthos from Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata* at Turkish transect, Igneada (D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Chamelea gallina (Linnaeus, 1758)	1160.57	1064.98
Lucinella divaricata (Linnaeus, 1758)	2557.93	71.80
Pitar rudis (Poli, 1795)	152.93	121.98
Bittium reticulatum (da Costa,1778)	24971.30	908.93
Calyptraea chinensis (Linnaeus, 1758)	1425.73	70.63
Spisula subtruncata (da Costa, 1778)	842.37	49.54
Caecum trachea (Montagu,1803)	16875.70	11.81
Tricolia pullus (Linnaeus, 1758)	2377.87	37.41
Pusillina lineolata (Michaud, 1830)	2208.90	0.66
Aricidea claudiae Laubier, 1967	583.37	0.33
Ampelisca sarsi Chevreux, 1888	542.67	3.17
Aricidea catherinae Laubier, 1967	452.63	0.20
Ecrobia ventrosa (Montagu,1803)	446.47	8.50
Retusa truncatula (Bruguière, 1792)	328.07	1.22
Pseudocuma longicorne (Bate, 1858)	304.63	0.07
Spio decoratus Bobretzky, 1870	250.37	0.12
Cyclope neritea (Linnaeus, 1758)	247.90	39.32
Chrysallida interstincta (J.Adams,1797)	223.23	0.36
Heteromastus filiformis (Claparède, 1864)	157.87	0.09
Nephtys (cf) paradoxa Malm, 1874	133.20	0.20
Tellina tenuis da Costa, 1778	128.27	19.45
Rissoa splendida (Eichwald,1830)	113.47	0.83
Nassarius reticulatus (Linnaeus, 1758)	101.13	6.29
Apseudopsis ostroumovi Bacescu & Carausu, 1947	101.13	0.22
Turbonilla pusilla (Philippi,1844)	99.90	0.13
Epitonium commune (Lamarck, 1822)	86.33	0.45
Capitella capitata (Fabricius, 1780)	72.77	0.01
Cerastoderma glaucum (Bruguière, 1789)	69.07	13.75
Mangelia coarctata (Forbes,1840)	67.83	0.08
Bela nebula (Montagu,1803)	67.83	0.03
Prionospio cirrifera Wirén, 1883	53.03	0.04
Mytilaster lineatus (Gmelin, 1791)	37.00	0.17
Perioculodes longimanus (Bate & Westwood, 1868)	34.53	0.005
Gouldia minima (Montagu, 1803)	29.60	0.29
Melinna palmata Grube, 1870	25.90	0.07
Mytilus galloprovincialis Lamarck, 1819	24.67	0.38
Upogebia pusilla (Petagna, 1792)	23.43	24.55
Leiochone leiopygos (Grube, 1860)	23.43	0.17
Microdeutopus versiculatus (Bate, 1856)	18.50	0.10
Caulleriella bioculata (Keferstein, 1862)	18.50	0.02
Nemertini indet.	16.03	0.07
Iphinoe tenella Sars, 1878	16.03	0.008
Abra alba (W. Wood, 1802)	14.80	0.35
Diogenes pugilator (Roux, 1829)	12.33	0.34
Corophium sp.	11.10	0.007
Plathelminthes indet.	8.63	0.008

Species	Davg	Bavg
Amphibalanus improvisus (Darwin, 1854)	8.63	0.03
Iphinoe maeotica Sowinskyi, 1893	8,63	0.005
Nephtys hombergii Savigny in Lamarck, 1818	4.93	0.60
Exogone (Exogone) naidina Örsted, 1845	4.93	0.0005
Iphinoe elisae Băcescu, 1950	3.70	0.002
Microphthalmus similis Bobretzky, 1870	3.70	0.0004
Carinina heterosoma Müller, 1965	2.47	0.005
Glycera sp.	2.47	0.13
Papillicardium papillosum (Poli, 1791)	2.47	0.07
Magelona mirabilis (Johnston, 1865)	2.47	0.02
Thracia phaseolina (Lamarck, 1818)	1.23	0.53
Anadara transversa (Say, 1822)	1.23	0.05
Acanthocardia paucicostata (G. B. Sowerby II, 1834)	1.23	0.01
Lagis sp.	1.23	0.006
Phylo sp.	1.23	0.006
Chaetozone caputesocis (Saint-Joseph, 1894)	1.23	0.005
Phyllodoce mucosa Örsted, 1843	1.23	0.004
Eunice vittata (Delle Chiaje, 1829)	1.23	0.001
Polydora cornuta Bosc, 1802	1.23	0.001
Lagis koreni Malmgren, 1866	1.23	0.001
Phoronis euxinicola Selys-Longchamps, 1907	1.23	0.001
Aricia sp.	1.23	0.0005
Aonides paucibranchiata Southern, 1914	1.23	0.0002
Megaluropus massiliensis Ledoyer, 1976	1.23	0.0002
Leptinogaster histrio (Pelseneer, 1929)	1.23	0.0000

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.25).

Table II.3.25. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata* on the Turkish transect, S-species richness (Expert judgment), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD Ecological state	S	H'	AMBI	M-AMBI	MSFD Ecological state
High	S ≥ 50	H`>4	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	Good
Good	50> S ≥ 35	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	0.85 > M-AMBI ≥ 0.55	
Moderate	35 > S ≥ 20	2 < H`≤ 3	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Not good
Poor	20> S ≥ 10	1 < H`≤ 2	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 10	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.26 indicate good status for the station M18 (27 m depth), they do meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos.

Table II.3.26. Ecological state of macrozoobenthos in the moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata* at transect Igneada, the Turkish Black Sea according to average diversity and biotic indices by team: GEM – GeoEcoMar, TUR – Sinop.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M18 (27m)_GEM	49	3.63	1.60	0.91	Good
M18 (27m)_Sinop	46	2.49	0.07	0.87	Good

B. Upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud

Descriptor 1

1.4. Habitat distribution

According to data obtained in this study, on the Igneada transect at 53 m depth, it has been found the upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud habitat. This habitat situated in mud sediments, is characterised by a relative low species richness and diversity and densities.

1.6. Habitat condition

1.6.1 Species state and communities

After analysis of samples in this habitat 17 macrobenthic taxa have been identified, pertaining to 9 major taxonomic groups (Table II.3.27 and II.3.28). The typical species inhabiting this habitat are the molusks *Mytilus galloprovincialis, Pitar rudis, Spisula subtruncata,* polichaets *Aricidea pseudoarticulata, Nephtys (cf) paradoxa,* crustaceans *Iphinoe elisae.*

The average density recorded by the macrozoobenthic populations was of 1,805.6 ind·m^{-2}, of which the polichaets and oligochaets represents 43% of total. The dominant species after density was *Aricidea pseudoarticulata* - 32% and *Cerastoderma glaucum* – 28%. The average biomass was of macrozoobenthos was of 238.9 g·m^{-2}, out of which the molusks represented 96% (Table II.3.27 and II.3.28).

Table II.3.27. Summary of the results from the univariate statistical analysis of the macrozoobenthos from upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud on the Turkish transect, Igneada.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M17 (53m)	17	4.9	584.6	1,805.6	173.3
Biomass g·m^{-2}					
M17 (53m)	17	0.003	109.6	238.9	32.5

Table II.3.28. Ecological characterization of macrozoobenthos from upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud at Turkish transect, Igneada (D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Mytilus galloprovincialis Lamarck, 1819	37.0	88.297
Pitar rudis (Poli, 1795)	135.7	16.201
Aricidea pseudoarticulata Laubier, 1967	584.6	0.176
Nephtys (cf) paradoxa Malm, 1874	71.5	1.417
Oligochaeta	46.9	0.003
Spisula subtruncata (da Costa, 1778)	37.0	3.187
Amphiura stepanovi Chernyavskii, 1861	37.0	1.258
Neanthes caudata (Delle Chiaje, 1828)	34.5	5.862
Iphinoe elisae Băcescu, 1950	34.5	0.044
Heteromastus filiformis (Claparède, 1864)	19.7	0.019
Actinothoe clavata (Ilmoni, 1830)	17.3	1.048
Porifera indet.	17.3	0.017
Anthozoa indet.	17.3	0.017
Terebellides stroemii Sars, 1835	9.9	0.020
Nemertini indet.	4.9	0.189
Cerastoderma glaucum (Bruguière, 1789)	510.6	109.631
Cyclope neritea (Linnaeus, 1758)	189.9	11.586

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.29).

Table II.3.29. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud on the Turkish transect, S-species richness (Expert judgment), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD Ecological state	S	H'	AMBI	M-AMBI	MSFD Ecological state
High	S ≥ 35	H`>4	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	Good
Good	35> S ≥ 25	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	0.85 > M-AMBI ≥ 0.55	
Moderate	25 > S ≥ 15	2 < H`≤ 3	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Not good
Poor	15> S ≥ 10	1 < H`≤ 2	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 10	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.30 indicate good status for the station M17 (53 m depth), they do meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos.

Table II.3.30. Ecological state of macrozoobenthos in the upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud at transect Igneada, the Turkish Black Sea according to average diversity and biotic indices.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M17 (53m)	17	2.88	1.57	0.64	Good

C. Lower circalittoral mud with *Terebelides stroemii*

Descriptor 1

1.4. Habitat distribution

On Igneada transect at 76 m depth, according to data obtained the habitat characteristic is the lower circalittoral mud with *Terebelides stroemi.* This habitat situated in mud sediments, is characterised by a relatively low species richness and diversity and densities.

1.6. Habitat condition

1.6.1 Species state and communities

The samples analysis revealed 24 macrozoobenthic taxa, belonging to 10 taxonomic major groups (Table II.3.31 and II.3.32). The typical species in this habitat the polychaets *Terebellides stroemii, Nephtys* (cf) *paradoxa,* molusks *Retusa truncatula, Trophonopsis breviata*, crustaceans *Eudorella truncatula, Iphinoe elisae, Perioculodes longimanus*, echinoderms *Amphiura stepanovi* (Table II.3.32).

The macrozoobenthic populations recorded an average density of 4,728.6 ind·m^{-2}, while the average biomass was of 642.1 g·m^{-2}, among which the molusks represented 68% of total density and 95% of total biomass. In general, the numerical and weight abundances are low in this habitat, but exceptionally in this case the agglomerations of littoral species (mollusks *Cerastoderma glaucum, Tellina tenuis*), increased their values. Excluding these species, the average densities were of 2,200 ind·m^{-2}, and the biomass of 68.7 g·m^{-2}.

Table II.3.31. Summary of the results from the univariate statistical analysis of the macrozoobenthos from lower circalittoral mud with *Terebelides stroemii* on the Turkish transect, Igneada.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind.m^{-2}					
M16(76m)	24	2.47	1,825.3	4,728.6	398.6
Biomass g.m^{-2}					
M16 (76m)	24	0.0002	391.5	642.1	86.2

Table II.3.32. Ecological characterization of macrozoobenthos from lower circalittoral mud with *Terebelides stroemii*at Turkish transect, Igneada
(D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Terebellides stroemii Sars, 1835	81.40	3.496
Amphiura stepanovi Chernyavskii, 1861	656.13	22.723
Oligochaeta	320.67	0,019
Retusa truncatula (Bruguière, 1792)	271.33	0.133
Nephtys (cf) paradoxa Malm, 1874	167.73	1.938
Trophonopsis breviata (Jeffreys, 1882)	135.67	1.571
Eudorella truncatula (Bate, 1856)	71.53	0.042
Holothuroidea indet.	37.00	4.553
Stereoderma kirchsbergii (Heller, 1868) Panning, 1949	37.00	1.761
Iphinoe elisae Băcescu, 1950	37.00	0.022
Perioculodes longimanus (Bate & Westwood, 1868)	34.53	0.004
Aricidea pseudoarticulata Laubier, 1967	19.73	0.001
Synchelidium maculatum Stebbing, 1906	17.27	0.002
Phoronis euxinicola Selys-Longchamps, 1907	12.33	0.0004
Heteromastus filiformis (Claparède, 1864)	4.93	0.002
Erinaceusyllis erinacea (Claparède, 1863)	4.93	0.0004
Nemertini indet.	2.47	0.009
Harmothoe impar (Johnston, 1839)	2.47	0.0004
Phyllodoce mucosa Örsted, 1843	2.47	0.0004
Aricia sp.	2.47	0.0002
Syllides fulvus (Marion & Bobretzky, 1875)	2.47	0.0002
Cerastoderma glaucum (Bruguière, 1789)	1825.33	391.5
Tellina tenuis da Costa, 1778	710.40	181.8
Pitar rudis (Poli, 1795)	271.33	32.4

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.33).

Table II.3.33. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the lower circalittoral mud with *Terebelides stroemii* on the Turkish transect, S-species richness (Expert judgment), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD Ecological state	S	H'	AMBI	M-AMBI	MSFD Ecological state
High	S ≥ 30	H`>4	0.0 < AMBI ≤ 1.2	M-AMBI ≥ 0.85	Good
Good	30> S ≥ 24	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	0.85 > M-AMBI ≥ 0.55	
Moderate	24 > S ≥ 15	2 < H`≤ 3	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Not good
Poor	15> S ≥ 10	1 < H`≤ 2	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 10	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.34 indicate good status for the station M16 (76 m depth), they do meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos.

Table II.3.34. Ecological state of macrozoobenthos in the lower circalittoral mud with *Terebelides stroemiiat* transect Igneada, the Turkish Black Sea according to average diversity and biotic indices.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M16 (76m)	24	2.93	2.11	0.64	Good

D. Circalittoral shelly muds with *Modiolula*

Descriptor 1

1.4. Habitat distribution

According to results obtained in this study, at 53 m depth on Igneada transect, the characteristic habitat is upper circalitoral mud, *Mytilus galoprovincialis* beds on mud and sandy mud. This habitat situated in mud sediments, is characterised by a relatively low species richness and diversity and densities.

1.6. Habitat condition

1.6.1 Species state and communities

There were 27 species found in this habitat, belonging to 9 major taxonomic groups (Table II.3.35 and II.3.36). The typical species are the mollusks *Modiolula phaseolina, Trophonopsis breviata, Spisula subtruncata,* polichaets *Terebellides stroemii, Aonides paucibranchiata, Nephtys (cf) paradoxa,* crustaceans *Ampelisca sarsi, Apseudopsis ostroumovi,* echinoderms *Amphiura stepanovi* and holoturian *Stereoderma kirchsbergii.*

The average density recorded of the macrozoobenthic populations recorded was 4,716.3 ind·m^{-2}, while the average biomass was 392.9 g·m^{-2}, among which 87% of total density and 98% of total biomass were covered by the mollusks. (Table II.3.35 and II.3.36). The presence of a highly diversity and abundant Mollusca indicates a good state of the fauna in this habitat.

Table II.3.35. Summary of the results from the univariate statistical analysis of the macrozoobenthos from circalittoral shelly muds with *Modiolula* on the Turkish transect, Igneada.

	N. sp.	Min	Max	Sum	Stand. dev
Density ind·m^{-2}					
M15 (101m)	27	2.47	2,464.2	4,716.3	476.1
Biomass g·m^{-2}					
M15 (101m)	27	0.0002	232.4	392.9	48.7

Table II.3.36. Ecological characterization of macrozoobenthos from circalittoral shelly muds with *Modiolula* at Turkish transect, Igneada
(D_{AVG} – Density - ind·m^{-2}, B_{AVG} - Biomass g·m^{-2}).

Species	Davg	Bavg
Modiolula phaseolina (Philippi, 1844)	2464.20	232.3723
Trophonopsis breviata (Jeffreys, 1882)	236.80	20.4536
Spisula subtruncata (da Costa, 1778)	281.20	14.3412
Pusillina lineolata (Michaud, 1830)	402.07	0.1184
Ecrobia ventrosa (Montagu,1803)	182.53	3.4681
Ampelisca sarsi Chevreux, 1888	111.00	0.6561
Stereoderma kirchsbergii (Heller, 1868) Panning, 1949	71.53	3.1968
Erinaceusyllis erinacea (Claparède, 1863)	61.67	0.0037
Amphiura stepanovi Chernyavskii, 1861	54.27	1.8944
Ampelisca pseudospinimana Bellan-Santini & Kaim-Malka, 1977	54.27	0.2343
Microdeutopus versiculatus (Bate, 1856)	54.27	0.2146
Nephtys (cf) paradoxa Malm, 1874	34.53	0.3483
Oligochaeta	34.53	0.0020
Anthozoa indet.	24.67	1.7716
Phyllodoce mucosa Örsted, 1843	19.73	0.0419
Apseudopsis ostroumovi Bacescu & Carausu, 1947	17.27	0.0296

Species	Davg	Bavg
Orchomene similis (Chevreux, 1912)	17.27	0.0099
Perioculodes longimanus (Bate & Westwood, 1868)	17.27	0.0025
Aonides paucibranchiata Southern, 1914	12.33	0.0054
Terebellides stroemii Sars, 1835	9.87	0.2509
Heteromastus filiformis (Claparède, 1864)	9.87	0.0121
Pholoe inornata Johnston, 1839	4.93	0.0005
Syllides fulvus (Marion & Bobretzky, 1875)	4.93	0.0005
Nemertini indet.	2.47	0.0005
Capitomastus minima (Langerhans, 1880)	2.47	0.0002
Mysta picta (Quatrefages, 1865)	2.47	0.0002
Cerastoderma glaucum (Bruguière, 1789)	527.87	113.5407

Descriptor 6

6.2 Condition of benthic community

6.2.2 Multi-metric indexes assessing benthic community condition and functionality

Two ecological state classification systems are developed under MSFD definition of GES for each of the above differentiated habitats based on historical baseline (Table II.3.37).

Table II.3.37. Ecological state classification system based on diversity and biotic indices of macrozoobenthos in the circalittoral shelly muds with *Modiolula* on the Turkish transect, S-species richness (Expert judgment), H' – Shannon-Wiener community diversity index, AMBI – AZTI Marine Biotic Index (Borja et al., 2000), M-AMBI – multivariate AMBI (Muxica et al., 2005), proposed for the WFD and MSFD.

WFD Ecological state	S	H'	AMBI	M-AMBI	MSFD Ecological state
High	S ≥ 30	H`>4	**0.0 < AMBI ≤ 1.2**	M-AMBI ≥ 0.85	Good
Good	**30> S ≥ 25**	3 < H`≤ 4	1.2 < AMBI ≤ 3.3	**0.85 > M-AMBI ≥ 0.55**	
Moderate	25 > S ≥ 15	**2 < H`≤ 3**	3.3 < AMBI ≤ 4.3	0.55 > M-AMBI ≥ 0.39	Not good
Poor	15> S ≥ 10	1 < H`≤ 2	4.3 < AMBI ≤ 5.5	0.39 > M-AMBI ≥ 0.20	
Bad	S < 10	H`≤ 1	5.5 < AMBI ≤ 6.0	0.20 > M-AMBI	

Using the above classification systems, the results for the diversity and biotic indices presented in Table II.3.38 indicate good status for the station M15 (101 m depth), they do meet the MSFD requirements for achieving good environmental status with respect to the macrozoobenthos.

Table II.3.38. Ecological state of macrozoobenthos in the circalittoral shelly muds with *Modiolula* at transect Igneada, the Turkish Black Sea according to average diversity and biotic indices.

Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
M15 (101m)	27	2.67	0.79	0.73	Good

Meiobenthic Ecological Status

The significance of differences between samples collected on the three transects analyzed were tested using non-parametric test Kruskal – Wallis. The results showed no significant differences between the majority of samples (H<Hcrit; p = 0.04090; alpha < 0.05), so the null hypothesis has been rejected. Further on, testing the Bray-Curtis similarity on fourth root transformed abundance data indicated three main groups with a similarity of about 60 % (Fig. II.3.7).

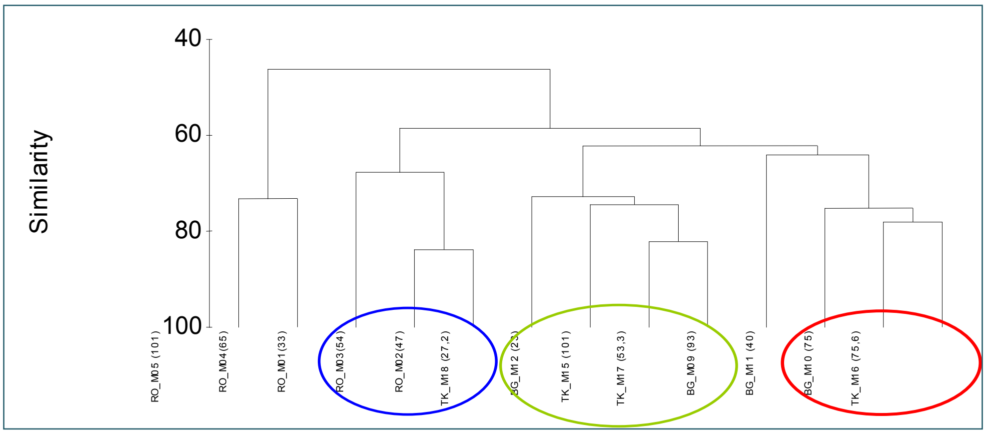


Figure II.3.7. Bray-Curtis similarity between samples based on fourth root transformed abundances.

A high similarity (about 90%) was found between samples situated within the **Upper circalittoral mud with *Mytilus galloprovincialis* beds on mud and sandy mud** habitat from Constanta transect, situated at 47 and 54 m depth, respectively. On the contrary, the meiobenthic community found on Igneada transect at 53.3 m depth is pretty similar to that found within the **Circalittoral shelly mud with *Modiolula*** corresponding to 101 m on the same transect. Within the **Lower circalittoral mud with *Terrebelides*** habitat, the greatest similarity was found between samples situated at approximately 75 m on Bulgarian and Turkish transects (Fig. II.3.7).

The highest density and biomass have been recorded on Bulgarian transect, at 40 and 75 m, respectively (Fig. II.3.8).

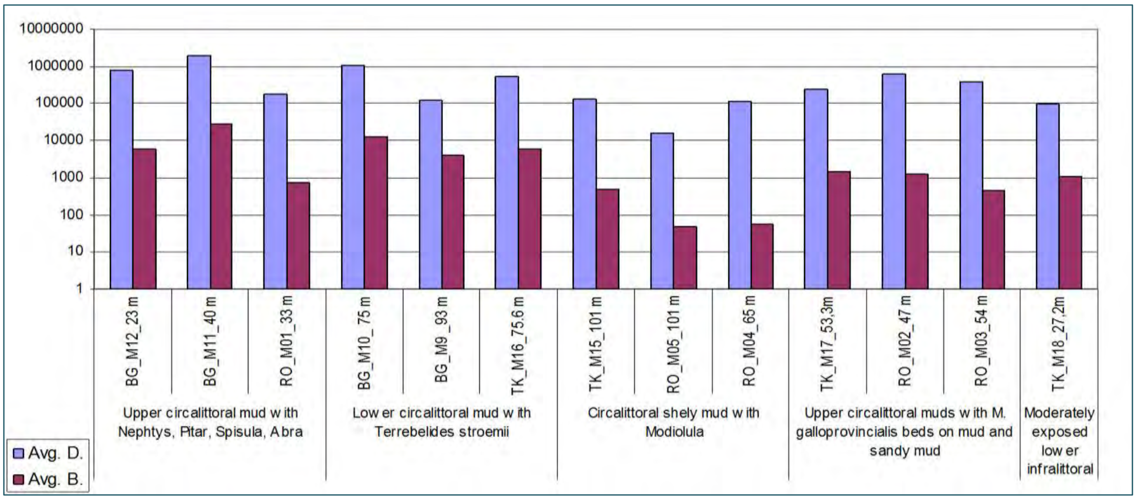


Figure II.3.8. Distribution of average density (ind·m-2) and biomass (mg·m-2) of meiobenthic community in the samples analysed.

The Bray – Curtis analysis based on presence-absence transformed data shows an overall similarity of 40% between samples, 3 major groups being distinguished (Fig. II.3.9). The best similarity was set between samples situated within the **Upper circalittoral mud with *Nephtys, Pitar, Spisula, Abra*** on the Galata transect at 40 m and 23 m depth, respectively. The sample situated at 33 m within the same habitat but on the Constanta transect is clearly demarked due to much less diverse meiobenthic community found on the latter.

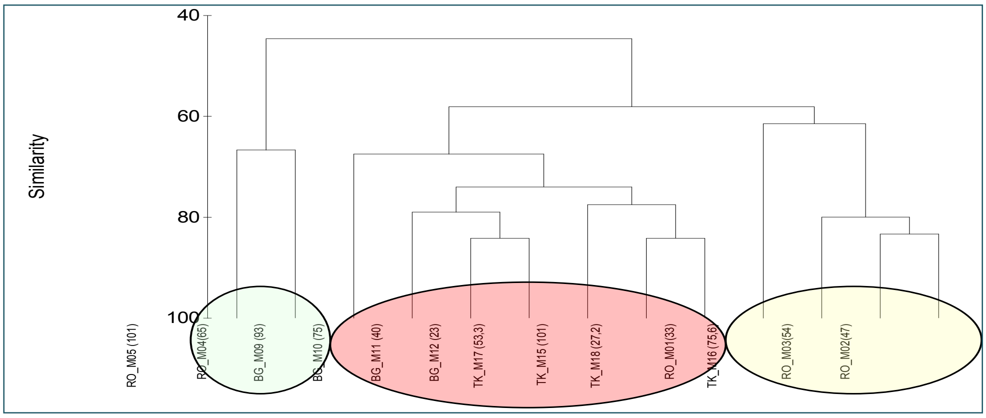


Figure II.3.9. Bray-Curtis similarity between samples based on presence/absence transformed data.

The Kruskal – Wallis pairwise comparison of meiobenthic taxa revealed significant differences between group associations (H>Hcrit; df = 15; alpha = 0.05). Nematoda showed significant differences (p<0.05) compared to all the other groups. The correlation between groups was analyzed using the Spearman correlation. A moderate correlation was found between

Nematoda – Harpacticoida (Rs = 0.59; p = 0.033), Nematoda –Turbellaria (Rs=0.60; p = 0.029), and Cilliophora – Gastrotricha (0.63; p = 0.022). The strongest correlation was observed between Harpacticoida – Turbellaria (0.83; p = 0.00039).

According to the analysis of Bray- Curtis similarities based on fourth root transformed abundance data, it shows a similarity of about 90% between two taxonomic groups: Polychaeta – Harpacticoida and Foraminifera Hard Shells – Ostracoda, respectively (Fig. II.3.10).

Figure II.3.10. Bray-Curtis Similarity based on forth-root transformed abundance data between taxa.

Taking into account the presence – absence transformed data to analyze Bray – Curtis similarity (Fig. II.3.11) a similarity of about 90% is observed between Foraminifera_HS and Ostracoda, on the one hand, and of about 85% between Harpacticoida – Nematoda, and Polychaeta, on the other hand.

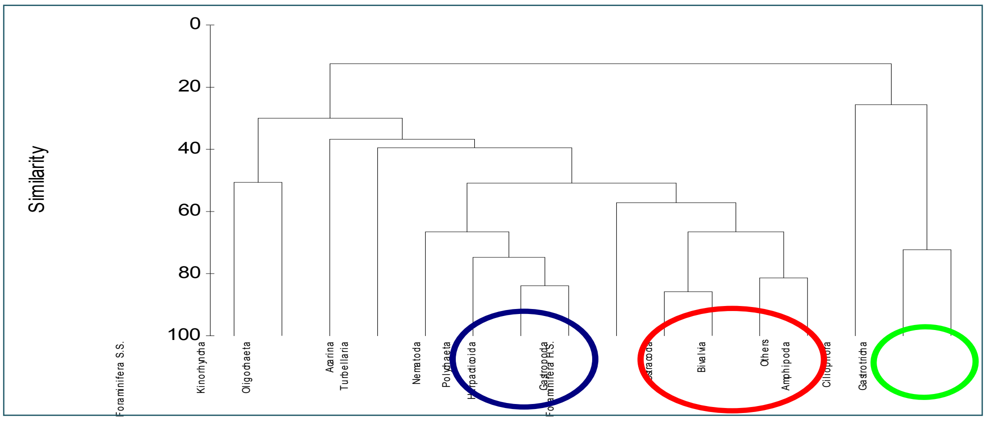


Figure II.3.11. Bray-Curtis similarity between taxa based on presence/absence transformed data.

Assessment of ecological status based on meiobenthic community

1. Moderately exposed lower infralittoral sand with *Ch. gallina and L. divaricata*

Descriptor 1

1.6. Habitat condition
1.6.1 Species state and communities
This habitat is characterized by the dominance of Nematodes followed by juvenile polychaetes as density, while the polychaetes dominate in terms of biomass (Table II.3.39). Representatives of 9 taxa were found. It is a complex habitat modeled by suspensivores (bivalves) and detritivores (polychaetes and crustaceans). The coarse sand favors the selection of epidetritivore nematodes and represents also a good habitat for the harpacticoides.

Descriptor 6

6.2 Condition of benthic community

In order to assess the state of environment (GES) we choose to use indices based on most frequent and abundant meiobenthic organisms and lately more and more used in biomonitoring studies (as proxy for the trophic state of the environment and pollution). Due to the presence of coarse substrata, we did not expect a high density of nematodes, but rather a balanced proportion between forams and harpacticoides. However, the presence of polychaetes which dominate in terms of biomass, show us a habitat prone to organic loading. Thus, the proportion of nematodes exceeds few times the proportion of harpacticoids and foraminiferans. Based on the assumption that nematodes and foraminiferans, as the main representatives of meiobenthos in the Black Sea, are suitable to indicate the state of environment in normal oxic conditions, we proposed to use the following indexes in our study:

- Dominance after Density of Nematoda % (DDN%) not to exceed 50% of all meiobenthic community and the dominance after density of foraminiferans (DDF%) > 5 (in littoral zones) or DDF% > 30 (in deep waters) – good ecological status
- 50 > DDN% < 75; DDF% < 30 – moderate ecological status
- 75 > DDN% < 95; DDF% < 30 – bad ecological status

According to this index, the habitat is in **good status** (DDN% = 48.99 and DDF% = 7.6), the density recorded for the main groups of meiobenthic taxa being in accordance with densities recorded in the littoral zones in sandy habitats (Table II.3.39)

Table II.3.39. The dominance after density (DD %) and biomass (BD %) of meiobenthic groups.

Moderately exposed lower infralittoral sand with *Ch. gallina* and *L. divaricata*		
Meiobenthos	**TK_M18_27.2m**	
	DD%	**BD%**
Nematoda	49.00	1.72
Harpacticoida	4.02	2.23
Polychaeta	18.88	40.20
Foraminifera H.S.	7.63	9.18
Bivalvia	5.22	33.84
Ostracoda	2.01	7.06
Gastrotricha	0.40	3.09
Turbellaria	3.21	0.30
Ciliophora	1.61	0.15
Others	8.03	2.23

2. Upper circalittoral mud with *Nephtys, Pitar, Spisula, Abra*

Descriptor 1

1.6. Habitat condition

1.6.1 Species state and communities

The meiobenthic community of lower limit of circalittoral is characterized by predominance of nematodes and foraminiferans. The associated macrobenthic community is mainly composed of detritivore polychaetes, and a rich community of mollusks, which enhance the accumulation, transformation and transfer of assimilable food to the lower trophic levels. The substrate type is favorable for a diverse functionally and taxonomically community of nematodes as well as to a wide range of other meiobenthic groups such as: Turbellarians, Ostracods, and Kynorhyncha etc. More than 10 meiobenthic groups have been found in this habitat. At 40 m depth in the same habitat situated on the Galata transect, the meiobenthic community is more selective, being dominated by foraminiferans and ostracods.

Descriptor 6

6.2 Condition of benthic community

Two of the samples situated within the lower limits of this habitat are characterized by dominance of nematodes. According to the indices proposed (DDN% and DDF %), on the Galata (BG_M12_23 m) and Constanta transect (RO_M01_33m) the results indicate the conditions for moderate status of the environment (DDN % > 50; DDF% < 30). The station located in deeper waters (BG_M11_40 m) is by far dominated by foraminiferans, which may indicate either a very specialized niche at this depth or the implication of „hazard" when the samples have been collected (due to patchy distribution of forams and nematodes) (Table II.3.40). The ecological status of the station BG_M11_40 m is considered good, in accordance with the DDN and DDF (DDN% <50; DDF %> 30).

Upper circalittoral mud with *Nephtys, Pitar, Spisula, Abra*						
Meiobenthic taxa	**BG_M11_40 m**		**BG_M12_23 m**		**RO_M01_33 m**	
	DD%	**BD%**	**DD%**	**BD%**	**DD%**	**BD%**
Nematoda	2.23	0.06	56.37	2.84	73.46	6.53
Harpacticoida	0.02	0.01	4.01	3.19	0.00	0.00
Polychaeta	0.02	0.03	6.35	19.36		
Foraminifera H.S.	92.14	84.40	22.30	38.42	20.59	62.67
Turbellaria			0.41	0.05		
Bivalvia	0.34	1.69	0.10	0.94		
Gastropoda	0.04	0.18				
Ostracoda	5.07	13.57	5.59	28.14	1.60	14.25
Acarina	0.02	0.03	1.93	5.88		
Kinorhyncha			1.93	0.77	1.60	1.12
Others	0.12	0.03	1.02	0.40		
Oligochaeta					2.75	15.43

Table II.3.40. The dominance after density (DD %) and biomass (BD %) of meiobenthic groups.

3. Upper circalittoral muds with *M. galloprovincialis* beds on mud and sandy mud

Descriptor 1

1.6. Habitat condition

1.6.1 Species state and communities

The nematodes and foraminiferans formed the dominant community in all samples analyzed within this habitat. A total of 8 higher taxonomic groups (Table II.3.41) were found. Besides the above mentioned ones, the harpacticoides have been recorded at all stations although not in high abundances. This may suggest a mesotrophic environment, proper to a diverse community of detritivores organisms.

Descriptor 6

6.2 Condition of benthic community

According to the indices proposed, the sample TK_M17_53.3 m from the Turkish transect is in moderate (almost good) ecological status (DDN < 75; DDF < 30), while the samples from the Romanian transect (RO_M03_54 m and RO-M02_47 m) are in bad ecological status (DDN % > 75; FFN% < 30). However, the previous studies carried out at the Romanian coast attest a higher diversity of nematodes within the *Mytilus* community, an average of 60 species being encountered in the central part of this community. The higher diversity and abundance of communities are not necessary indicators of disturbed conditions but of availability of trophic and habitat niches. However, comparing with the littoral zone the number of opportunistic species of nematodes is smaller and usually the number of forams at the Romanian coast increases with depth. So, in respect with these considerations, above the calculated indices showing a predominant bad ecological status, we incline to consider the samples from the Romanian transect falling in the area of distribution of this habitat in a moderate ecological status.

Table II.3.41. The dominance after density (DD %) and biomass (DB %) of meiobenthic groups.

Upper circalittoral muds with *M. galloprovincialis* beds on mud and sandy mud						
Meiobenthic taxa	TK_M17_53.3m		RO_M03_54 m		RO_M02_47 m	
	DD%	BD%	DD%	BD%	DD%	BD%
Nematoda	64.81	3.93	93.71	29.68	84.86	15.19
Polychaeta	4.23	15.51	0.81	15.55		
Harpacticoida	0.51	0.49	0.41	2.03	0.39	1.11
Foraminifera H.S.	27.07	56.12	4.46	48.35	12.27	75.15
Ostracoda	2.71	16.40			0.26	4.67
Bivalvia	0.68	7.55				
Turbellaria			0.41	0.34	1.96	0.92
Oligochaeta			0.20	4.06	0.26	2.95

4. Lower circalittoral mud with *Terrebelides stroemi*

Descriptor 1

1.6. Habitat condition

1.6.1 Species state and communities

Given the depth range of this habitat, the natural factors limit the distribution of many species found up to these depths. The meiobenthic communities (as the macrobenthic ones) become poorer as diversity and abundance. However, some meiobenthic species still form constant and viable populations. A certain part of them could even increase in abundance compared with the shallower depths. In the Black Sea, the selection of species and groups at these depths is naturally done by the oxygen saturation and the distribution of the trophic resources. The diversity in case of nematodes and forams is usually higher and abundances are lower as the depth increases (between 50 – 90 m). An overall of 14 taxonomic groups was found within this habitat.

Descriptor 6

6.2 Condition of benthic community

According to indices proposed and the above remarks, the **ecological status of this habitat on all transects could be considered good.** It should be highlighted that there is an increased numerical contribution of ostracodes in the sample collected at 93 m compared with the other samples from 75 m, where the foraminiferans are dominant (Table II.3.42).

Lower circalittoral mud with *T.stroemii*						
Meiobenthic taxa	**BG_M9 _93 m**		**BG_M10_ 75 m**		**TK_M16_75.6 m**	
	DD%	**BD%**	**DD%**	**BD%**	**DD%**	**BD%**
Nematoda	7.69	0.09	23.51	0.75	18.61	0.63
Harpacticoida			0.15	0.07	0.15	0.08
Polychaeta			0.07	0.14	0.31	0.64
Foraminifera H.S.	18.27	7.56	67.17	73.04	78.69	91.57
Foraminifera S.S.			0.19	0.04	0.15	0.01
Bivalvia			0.82	4.78		
Gastropoda			0.07	0.39	0.08	0.44
Ostracoda	69.55	84.14	6.35	20.18	1.85	6.30
Acarina					0.08	0.16
Turbellaria			0.07	0.01		
Kinorhyncha			1.23	0.31	0.08	0.17
Oligochaeta			0.11	0.22		
Bivalvia	1.92	4.29				
Gastropoda	1.92	3.86				
Others	0.64	0.06	0.26	0.07		

Table II.2.42. The dominance after density (DD %) and biomass (DB %) of meiobenthic groups.

5. Circalittoral shelly mud with *Modiolula phaseolina*

Descriptor 1

1.6. Habitat condition

1.6.1 Species state and communities

About 10 taxonomic groups were present within this habitat. It could be seen that the majority of groups are distributed on the Igneada transect, although except the nematodes and foraminiferans the others have been found in very low densities. However, the nematodes at these depths, in majority composed of species capable of high mobility, r-strategists and high tolerant to oxygen deficiency (e.g., *Sabatieria* species) are numerically prevalent against the foraminiferans. Work by Slitter (1971), however, does suggest active predation on forams in muddy bottom communities by free-living, marine nematodes. This could be one of the explanations invoked to explain in some cases the higher abundances of nematodes to forams' detriment.

Descriptor 6

6.2 Condition of benthic community

According to the indices proposed, which would indicate a bad ecological status of the environment (Table II.3.43), the special natural conditions found here make them quite inappropriate to be used in the recommended way. I suggest for future studies a possible **indicator to be used: the presence of foraminiferans in more than 50% of the samples analyzed and presence of at least 4 representatives of meiobenthic groups (Ostracoda, Turbellaria, Foraminifera, Nematoda), especially for the habitats situated between 150 – 180 m.**

Table II.3.43. The dominance after density (DD %) and biomass (DB %) of meiobenthic groups.

Circalittoral shely mud with *Modiolula phaseolina*						
Meiobenthic taxa	**RO_M05_101 m**		**RO_M04_65 m**		**TK_M15_101 m**	
	DD%	**BD%**	**DD%**	**BD%**	**DD%**	**BD%**
Nematoda	84.62	10.76	97.10	72.83	79.39	8.14
Polychaeta	10.26	78.94			1.52	9.41
Harpacticoida	5.13	10.30	2.17	25.74	0.91	1.47
Foraminifera H.S.					14.24	49.98
Ostracoda					1.21	12.43
Bivalvia					0.91	17.18
Amphipoda					0.30	0.33
Turbellaria			0.72	1.43		
Ciliophora					0.30	0.08
Others					1.21	0.98

CONCLUSIONS

In general, the ecological state of macozoobenthic population has degraded in the northwestern Black Sea part since 1980. Nevertheless, at present, the human pressures on the ecosystem have apparently not exceeded the resilience of macrobenthic communities, and the investigated area achieved a good environmental status in proportion of 56%, remaining 44% is not good over the study period (ANNEX 2).
The variation in the ecological state results between teams underlines the necessity for intercalibration of the taxonomic expertise and the methodological standards in order to achieve comparable results between the countries in the Black Sea region under MSFD reporting in the future.

The assessment of good ecological status (GES) based on meiobenthic community has been proved a reliable tool especially in case of anthropogenic influenced habitats. In case of our study, the results of assessment are similar to those provided by analysis performed for the macrobenthic community.

However, the indices proposed seem to be feasible but the distribution dictated by the natural conditions should not be ignored.
In order to validate the indices proposed these should be tested against more data and much important to be confronted with all range of physico-chemical parameters and dynamic processes governing the life cycle of meiobenthic communities (e.g., seasonal changes). The interrelation with the condition of macrobenthic community should not also be disregarded in spite of the fact that sometimes a negative correlation could be met in report with the meiobenthic diversity or abundance.

For future development of the indices related to meiobenthic community, the studies should be focused on species in order to detect the indicator species and moreover to be promoted the molecular techniques, bioassay and toxicological studies.
Per ensemble, our results show that the ecological status of habitats analyzed is good to moderate, 2 habitats (*Moderately exposed lower infralittoral sand with Ch. gallina and L. divaricata* and *Lower circalittoral mud with Terrebelides stroemi)* from the Turkish transect met the conditions (according to indices analyzed) for good status, while the Romanian and Bulgarian habitats assessment showed a moderate ecological status.
The integration of all assessment levels (habitat, different eco-system components) into a single score indicating status and performance of an ecosystem is another major challenge, especially in the case of the MSFD.

GAPS

- Data is scarce, disperse and heterogeneous.
- No specific MSFD indicators developed at regional level.
- Needs for habitats monitoring programs with standardized spatial and temporal data.
- Lack of reference/baseline conditions.
- Lack of knowledge of benthic habitats in Turkish waters.
- Lack of knowledge on habitats modeling, size distribution.
- A need of determination of common thresholds.
- Evolution of climate changes - Insufficient knowledge concerning the consequences of global changes in the Black Sea
- Scarcity of long series of data concerning ecological pressures

RECCOMMENDATIONS

- The variation in the ecological state results between teams underlines the necessity for intercalibration of the taxonomic expertise and the methodological standards in order to achieve comparable results between the countries in the Black Sea region under MSFD reporting in the future.
- The integration of all assessment levels (habitat, different eco-system components) into a single score indicating status and performance of an ecosystem is another major challenge, especially in the case of the MSFD.

UNCERTAINTIES

The cooperative regional efforts of the states in the Black Sea Basin are seen as a guarantee that the Black Sea ecosystem will be sustained and rehabilitated and that the strategic and intermediate targets developed jointly by the EU, Black Sea Commission, ICPDR and other organizations by the political will, scientific cooperation, stakeholder understanding and social welfare should be achieved.

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ANNEX 1. List of macrozoobenthic species

Crt. nr.	Species	Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata*	Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys*, etc		*Mytilus galloprovincialis* beds on mud and sandy mud		Lower circalittoral mud with *Terebellides stroemi*		Circalittoral shelly silt with *Modiolula phaseolina*	
		TR	**RO**	**BG**	**RO**	**TR**	**BG**	**TR**	**RO**	**TR**
1	*Haliclona (Reniera) cinerea* (Grant, 1826)			+						
2	*Suberites carnosus* (Johnston, 1842)						+		+	
3	*Sycon ciliatum* (Fabricius, 1780)								+	
4	Porifera indet.					+				
5	*Amphinema dinema* (Péron & Lesueur, 1810)				+		+			
6	*Clytia hemisphaerica* (Linnaeus, 1767)			+					+	
7	*Gonothyraea loveni* (Allman, 1859)			+						
8	*Obelia longissima* (Pallas, 1766)		+	+			+			
9	*Podocoryna carnea* M. Sars, 1846			+						
10	*Actinothoe clavata* (Ilmoni, 1830)		+	+		+	+			
11	*Pachycerianthus solitarius* (Rapp, 1829)								+	
12	Anthozoa indet.					+				+
13	*Amphiporus bioculatus* McIntosh, 1874	+			+				+	
14	*Carinina heterosoma* Müller, 1965	+		+						
15	*Micrura fasciolata* Ehrenberg, 1828				+				+	
16	Nemertini indet.	+	+	+	+	+	+	+	+	+
17	Plathelminthes indet.	+								
18	*Alitta succinea* (Leuckart, 1847)		+	+						
19	*Aricia sp.*	+					+	+		
20	*Aricidea catherinae* Laubier, 1967	+								
21	*Aricidea claudiae* Laubier, 1967	+		+	+		+			
22	*Aricidea pseudoarticulata* Laubier, 1967					+		+		
23	*Aonides paucibranchiata* Southern, 1914	+							+	+
24	*Capitella capitata* (Fabricius, 1780)	+		+			+		+	
25	*Capitomastus minima* (Langerhans, 1880)									+
26	*Caulleriella bioculata* (Keferstein, 1862)	+								
27	*Chaetozone caputesocis* (Saint-Joseph, 1894)	+								
28	*Dipolydora quadrilobata* (Jacobi, 1883)			+	+				+	
29	*Erinaceusyllis erinacea* (Claparède, 1863)							+		+
30	*Eunice vittata* (Delle Chiaje, 1829)	+								
31	*Exogone (Exogone) naidina* Örsted, 1845	+							+	
32	*Glycera sp.*	+								
33	*Harmothoe imbricata* (Linnaeus, 1767)			+						
34	*Harmothoe impar* (Johnston, 1839)		+	+				+	+	
35	*Heteromastus filiformis* (Claparède, 1864)	+	+	+	+	+	+	+	+	+
36	*Lagis koreni* Malmgren, 1866	+		+						
37	*Lagis sp.*	+								
38	*Leiochone leiopygos* (Grube, 1860)	+								
39	*Magelona mirabilis* (Johnston, 1865)	+								
40	*Melinna palmata* Grube, 1870	+	+	+					+	
41	*Microphthalmus similis* Bobretzky, 1870	+								
42	*Mysta picta* (Quatrefages, 1865)									+
43	*Neanthes caudata* (Delle Chiaje, 1828)					+				
44	*Nephtys hystricis* McIntosh, 1900						+			
45	*Nephtys (cf) paradoxa* Malm, 1874	+				+	+	+		+
46	*Nephtys hombergii* Savigny in Lamarck, 1818	+	+	+	+		+		+	
47	*Nephtys sp.*	+					+			
48	*Nereiphylla rubiginosa* (Saint-Joseph, 1888)			+						
49	*Oriopsis armandi* (Claparède, 1864)				+		+		+	
50	*Pholoe inornata* Johnston, 1839			+						+
51	*Phylo sp.*	+								
52	*Phyllodoce mucosa* Örsted, 1843	+	+		+		+	+	+	+
53	*Polycirrus jubatus* Bobretzky, 1869				+		+		+	
54	*Polydora cornuta* Bosc, 1802	+		+						

Crt. nr.	Species	Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata*	Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys*, etc		*Mytilus gallopro vincialis* beds on mud and sandy mud		Lower circalittoral mud with *Terebellides stroemi*		Circalittoral shelly silt with *Modiolula phaseolina*	
		TR	**RO**	**BG**	**RO**	**TR**	**BG**	**TR**	**RO**	**TR**
55	*Prionospio cirrifera* Wirén, 1883	+	+	+	+		+		+	
56	*Pygospio elegans* (Claparede, 1863)	+								
57	*Sphaerosyllis bulbosa* Southern, 1914						+			
58	*Spio decoratus* Bobretzky, 1870	+								
59	*Syllides fulvus* (Marion & Bobretzky, 1875)							+		+
60	*Syllides longocirratus* (Örsted, 1845)								+	
61	Spionidae indet.	+					+			
62	*Terebellides stroemii* Sars, 1835			+	+	+	+	+	+	+
63	Oligochaeta		+	+	+	+	+	+	+	+
64	*Bela nebula* (Montagu,1803)	+								
65	*Bittium reticulatum* (da Costa,1778)	+					+			
66	*Caecum trachea* (Montagu,1803)	+								
67	*Calyptraea chinensis* (Linnaeus, 1758)	+		+						
68	*Chrysallida interstincta* (J.Adams,1797)	+								
69	*Cyclope neritea* (Linnaeus, 1758)	+		+		+				
70	*Ecrobia ventrosa* (Montagu,1803)	+								+
71	*Epitonium commune* (Lamarck, 1822)	+								
72	*Gibulla sp.*	+								
73	*Lepidochitona caprearum* (Scacchi, 1836)									
74	*Mangelia coarctata* (Forbes,1840)	+								
75	*Nassarius reticulatus* (Linnaeus, 1758)	+		+			+			
76	*Pusillina lineolata* (Michaud, 1830)	+		+						+
77	*Retusa truncatula* (Bruguière, 1792)	+		+	+		+	+		
78	*Rissoa splendida* (Eichwald,1830)	+								
79	*Tricolia pullus* (Linnaeus, 1758)	+								
80	*Trophonopsis breviata* (Jeffreys, 1882)						+	+		+
81	*Turbonilla pusilla* (Philippi,1844)	+								
82	*Abra sp.*						+			
83	*Abra ovata* (Philippi, 1836)						+			
84	*Abra alba* (W. Wood, 1802)	+	+	+	+		+		+	
85	*Abra prismatica* (Montagu, 1808)		+	+	+		+			
86	*Acanthocardia paucicostata* (G. B. Sowerby II, 1834)	+	+	+			+			
87	*Anadara kagoshimensis* (Tokunaga, 1906)			+						
88	*Anadara transversa* (Say, 1822)	+								
89	*Cerastoderma glaucum* (Bruguière, 1789)	+				+	+	+		+
90	*Chamelea gallina* (Linnaeus, 1758)	+		+						
91	*Gouldia minima* (Montagu, 1803)	+		+						
92	*Lucinella divaricata* (Linnaeus, 1758)	+								
93	*Modiolula phaseolina* (Philippi, 1844)				+		+		+	+
94	*Mya arenaria* Linnaeus, 1758		+							
95	*Mytilaster lineatus* (Gmelin, 1791)	+		+						
96	*Mytilus galloprovincialis* Lamarck, 1819	+	+	+	+	+	+			
97	*Papillicardium papillosum* (Poli, 1791)	+								
98	*Parvicardium exiguum* (Gmelin, 1791)	+		+						
99	*Parvicardium simile* (Milaschewisch, 1909)			+			+		+	
100	*Pitar rudis* (Poli, 1795)	+	+	+		+	+	+		
101	*Spisula sp.*	+								
102	*Spisula solida* (Linnaeus, 1758)	+								
103	*Spisula subtruncata* (da Costa, 1778)		+	+		+	+			+
104	*Tellina tenuis* da Costa, 1778	+						+		
105	*Thracia phaseolina* (Lamarck, 1818)	+								
106	*Phoronis euxinicola* Selys-Longchamps, 1907	+	+	+	+		+	+	+	
107	*Amphibalanus improvisus* (Darwin, 1854)	+		+						
108	*Ampelisca sarsi* Chevreux, 1888	+		+						+
109	*Ampelisca pseudospinimana* Bellan-Santini & Kaim-Malka, 1977									+
110	*Caprella acanthifera* Leach, 1814				+					

Crt. nr.	Species	Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata*	Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys*, etc		*Mytilus gallopro vincialis* beds on mud and sandy mud		Lower circalittoral mud with *Terebellides stroemi*		Circalittoral shelly silt with *Modiolula phaseolina*	
		TR	RO	BG	RO	TR	BG	TR	RO	TR
111	*Corophium sp.*	+								
112	*Medicorophium runcicorne* (Della Valle, 1893)		+	+						
113	*Megaluropus massiliensis* Ledoyer, 1976	+								
114	*Megamphopus cornutus* Norman, 1869				+					
115	*Microdeutopus gryllotalpa* Costa, 1853		+							
116	*Microdeutopus versiculatus* (Bate, 1856)	+								+
117	*Orchomene similis* (Chevreux, 1912)									+
118	*Perioculodes longimanus* (Bate & Westwood, 1868)	+		+	+		+	+		+
119	*Phtisica marina* Slabber, 1769		+	+			+			
120	*Synchelidium maculatum* Stebbing, 1906			+				+		
121	*Eudorella truncatula* (Bate, 1856)						+	+	+	
122	*Iphinoe elisae* Băcescu, 1950	+	+	+		+	+	+		
123	*Iphinoe maeotica* Sowinskyi, 1893	+								
124	*Iphinoe tenella* Sars, 1878	+								
125	*Pseudocuma longicorne* (Bate, 1858)	+								
126	*Apseudopsis ostroumovi* Bacescu & Carausu, 1947	+							+	+
127	*Diogenes pugilator* (Roux, 1829)	+								
128	*Liocarcinus navigator* (Herbst, 1794)			+						
129	*Upogebia pusilla* (Petagna, 1792)	+	+	+			+			
130	*Amphiura stepanovi* Chernyavskii, 1861			+	+	+	+	+	+	+
131	*Molgula appendiculata* Heller, 1877								+	
132	*Leptosynapta inhaerens* (O.F. Müller, 1776)				+				+	
133	*Stereoderma kirchsbergii* (Heller, 1868) Panning, 1949							+		+
134	Holothuroidea indet.							+		
135	*Leptinogaster histrio* (Pelseneer, 1929)	+								
TOTAL species per habitat and transect		78	24	52	25	17	43	24	31	27
TOTAL species per habitat		78	55		36		51		41	

ANNEX 2.
Ecological state of macrozoobenthos after MSFD

Habitat	Transect	Stations	S	H'	AMBI	M-AMBI	MSFD Ecological state
Moderately exposed lower infralittoral sand with *Chamelea gallina* and *Lucinella divaricata*	Igneada	M18 (27m)_GEM	49	3.63	1.60	0.91	Good
		M18 (27m)_SINOP	46	2.49	0.07	0.87	Good
Upper circalittoral mud with *Abra, Spisula, Pitar, Cardiidae, Nephtys*	Constanta	M01 (33m)	24	2.20	4.38	0.61	Moderate (Not good)
	Galata	M12 (23m)_GEM	37	2.75	3.51	0.68	Good
	Galata	M11 (40m)_GEM	25	2.40	4.38	0.46	Moderate (Not good)
***Mytilus galloprovincialis* beds on mud and sandy mud**	Constanta	M02 (47m)	13	2.36	3.56	0.56	Moderate (Not good)
	Constanta	M03 (54m)	18	3.07	3.42	0.71	Moderate (Not good)
	Igneada	M17 (53m)	17	2.88	1.57	0.64	Good
Lower circalittoral mud with *Terebelides stroemii*	Galata	M10(76m)_GEM	23	2.43	4.23	0.49	Moderate (Not good)
	Galata	M10(76m)_TUR	15	2.21	2.26	0.51	Moderate (Not good)
	Galata	M10(76m)_NIMRD	18	3.10	1.93	0.74	Good
	Galata	M09(93m)_GEM	16	2.39	2.74	0.52	Moderate (Not good)
	Igneada	M16 (76m)	24	2.93	2.11	0.64	Good
Circalittoral shelly silt with *Modiolula phaseolina*	Constanta	M04 (65m)	25	3.60	2.47	0.92	Good
	Constanta	M05 (101m)	18	3.16	1.21	0.86	Good
	Igneada	M15 (101m)	27	2.67	0.79	0.73	Good

III. DESCRIPTOR 2: Non-indigenous species

Completed by:
Kremena Stefanova, Snejana Moncheva, Radka Mavrodieva, Florin Timofte, Laura Boicenco, Funda Üstün, Fatih Sahin

GES: Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems

Criteria:

2.1.1 *Trends in abundance, temporal occurrence and spatial distribution in the wild of non-indigenous species, particularly invasive non indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species*
2.2.1 *Ratio between invasive non-indigenous species and native species in some well-studied taxonomic groups (e.g. fish, macroalgae, molluscs) that may provide a measure of change in species composition (e.g. further to the displacement of native species)*
2.2.2 *Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible*

List of figures

INTRODUCTION

The introduction of non-native species respectively invasive species into oceanic and especially coastal waters is among the four highest risks for the marine environment, exaggerated further in relation to the global climatic changes projections and caused extremely severe environmental, economic and public health impacts. The non-indigenous invaders induced considerable changes in the structure and dynamics of marine ecosystems and the food web by outcompeting original inhabitants. There is mounting experimental and empirical evidence that invasions have striking impacts on the proportions of native species and the ecosystem resilience and health (HELCOM, 2012). An issue for the non-indigenous species management is that, once a marine organism has been introduced to its new environment, it is nearly impossible to eradicate the unwanted organism, if it has established to the area. The presence of NIS in itself indicates some degree of deviation from the pristine ecological status. The consequence is that assessing a status of an area as “bad” means that the area will stay in the bad status without the possibility to return to the past condition (HELCOM, 2012).

The impact invasive NIS have on the environment to which they have been introduced (described as ‘biological pollution”) can be categorised at various levels:

- Individual (internal biological pollution by parasites or pathogens);
- Population (by genetic change);
- Community (structural shift);
- Habitat (modification of physical-chemical conditions);
- Ecosystem (alteration of energy and organic material flow

As many as 217 introduced species have been recorded in the Black Sea until 2006. Nearly 10 % of the established alien species in the Black Sea and coastal aquatic habitats are considered to be highly invasive and another 16 % as moderately invasive. Two highly invasive as *Rapana venosa* and *Mnemiopsis leidyi* have become central to the Black Sea with their notorious detrimental effect on the ecosystem (TDA, 2007). Only in the decade 1996-2005 a total of 48 non-indigenous species were listed, the most numerous in the plankton community: phytoplankton (16) and zooplankton (8) (TDA, 2007). The trend is increasing, i.e. the process and the risk of bioinvasions are still high. It is expected the numbers of non-indigenous to increase, as there are already indications of this trend consequently, an early detection of invasive species is important.

During 2006-2011 a total of 21 new alien species were registered along the Bulgarian coast, 14 phytoplankton (cysts are not included), two zooplankton, one zoobenthic, three fish and one macrophyts (IAR-BG, 2013).

MATERIAL and METHODS

Criteria and indicators of status

In the prioritisation process relating to the evaluation of this descriptor, emphasis is placed on state assessment, which is a precondition for evaluating the extent of an impact. Some of alien species are identified as invasive and their abundance or biomass, trends in population, temporal occurrence and spatial distribution are an important indicator to determine the status of the species and respectively the probability to achieve good status. Proposed indicators to criteria 2.1.1 are:

Indicator: *Mnemiopsis leidyi* biomass [$g \cdot m^{-3}$] (based on Initial Assessment report of Bulgaria)
Gelatinous predator, which consumes less than 10% of the zooplankton biomass per day, cannot reduce their abundance and biomass (Burrell & Van Engel, 1976; Larson, 1979; Purcell, 1994 in Finenko et al., 2009). However, higher consumption rates (more than 20 % of zooplankton biomass per day) result in a sharp reduction of the prey abundance (Deason, 1982; Matsakis & Conover, 1991; Shiganova et al., 2008). On the base of calculated critical biomass of ctenophore *M.leidyi* that does not affect mesozooplankton abundance it was identified **4** $\mathbf{g \cdot m^{-3}}$ or **120** $\mathbf{g \cdot m^{-2}}$ (Vinogradov et al. 2005) as a threshold for GES.

Indicator: Stable decreasing trend of *M.leidyi* biomass (more than 5 years) or maintenance (minimum for 5 years) of the biomass in lower value than the threshold (if regular data available)

Criteria and indicators of pressure and impact

Proposed indicators to this criteria is:

Indicator: Ratio between non-native species and native species (based on Initial Assessment report of Bulgaria)
The indicator is applied to the components of the pelagic ecosystem phytoplankton and zooplankton. In the Bulgarian coast since last century, more than 70 non-native species of all ecological groups were identified, the majority of them belong to the group of phytoplankton (43%) and 11-12% of the zooplankton. The proposed GES threshold in the proportion of abundance (or biomass where it is appropriate) of alien species to native is <10% (according to expert judgment).

RESULTS and DISCUSSIONS

Indicator *Mnemiopsis leidyi* biomass [g·m^{-3}]

Invasion of the North Atlantic ctenophore *Mnemiopsis leidyi* to the Black Sea, via ballast water, in the early 1980s had serious consequences for the ecosystem. It started to control the abundance of zooplankton, its biological diversity, the structure of the community, and the regularities of the functioning of the pelagic ecosystem (Konsulov & Kamburska, 1998, Shiganova et al., 2001; Kideys & Romanova, 2001, Moncheva & Kambusrka, 2003; Kamburska, 2004; Mihneva , 2011). As a key controlling factor for the mesozooplankton, *M. leidyi* becomes a reliable indicator of the pelagic ecosystem dynamic and food web functioning. *M.leidyi* mass development induces trophic cascades, as it affects (direct impact) the population size and composition of the zooplankton (secondary consumers) and indirectly the primary producers in the food chains (Daskalov, 2002; Daskalov et al., 2007). In the present decade, *Mnemiopsis* population was controlled by *Beroe ovata*. The *Mnemiopsis leidyi* impact on trophic zooplankton structure was reduced to two months (July-August) of the year instead of 6-8 months before *B.ovata* arrival.

As a result of the mosaic distribution (Fig. III.1) usually observed in gelatinous species, Mnemiopsis total biomass varied from bellow 1 to 193 g·m^{-3} (mean 30.28 g·m^{-3} ± 48.057). The *M.leidyi* biomass was reduced approximately 4 folds from north (Romania) to south (Bulgaria) and slightly increased to Turkey. The maximum biomass was noted in Romania shelf area (st. M04 – shelf habitat) - 193 g·m^{-3} (Fig. III.2) where the largest individuals used to concentrate, while the ctenophore abundance varied to a significantly smaller degree (0.5 – 2 ind·m^{-3}). Follow the same water masses the highest biomass was registered (31.993 g·m^{-3}) in Bulgarian shelf (Fig. III.2). Huge variability in biomass of *M.leidyi* was demonstrated in the coastal and shelf area. Open sea maintained low biomass values in three transects (Fig. III.3). Obviously, recorded concentrations exceeded the GEnS threshold almost in all study area, except Romania open sea stations - M06 and M07.

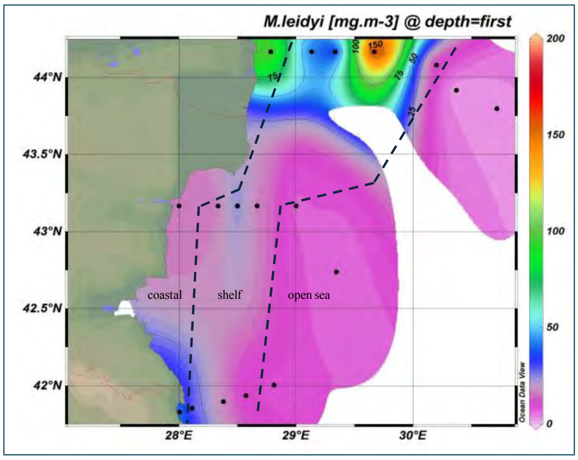
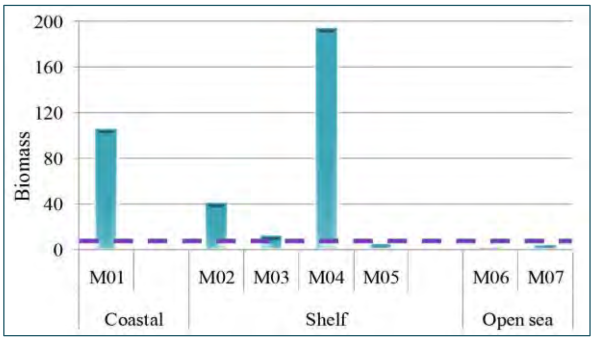
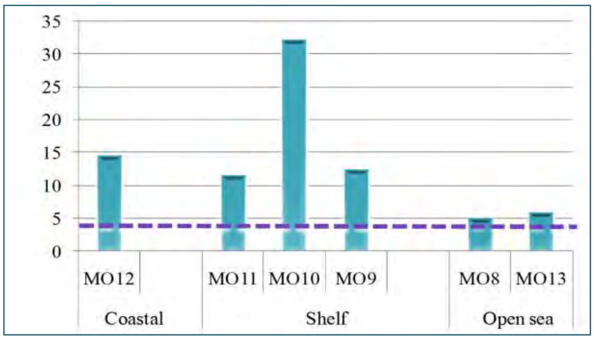


Figure III.1. ***M.leidyi*** **biomass distribution along the Constanta, c. Galata and Igneada transects.**





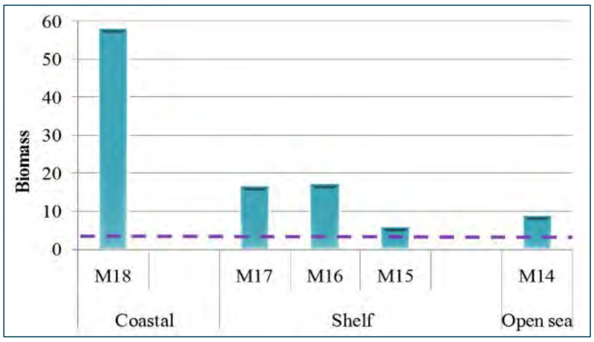


Figure III.2. Biomass of Mnemiopsis leidyi along the three transects
(a) Constanta,
(b) c. Galata,
(c) Igneada.

Purple line shows GEnS threshold (4g·m-3).

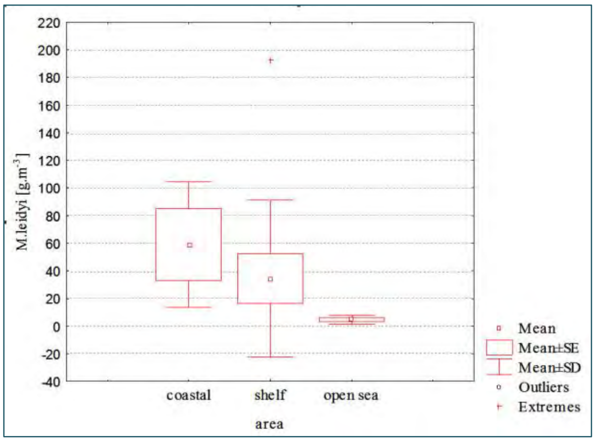


Figure III.3. Boxplots of *M.leidyi* biomass (±SD) averaged by coastal, shelf and open sea habitats.

Indicator Stable decreasing trend of *M.leidyi* biomass (more than 5 years) or maintenance (minimum for 5 years) of the biomass in lower value than the threshold (if regular data available)

Coastal and shelf area off the Bulgarian Black Sea presented large biomass fluctuations and higher values in comparison with the open sea. Despite the irregular monitoring and missing data identified trend is decreasing especially at coast. After 2003 *M.leidyi* biomass decreased with range from 0.1 to 24 $g \cdot m^{-3}$ (Fig. III.4). The summer population during the period 1998-2014 (Fig. III.4) has never reached a size as elevated as in the end of 1980s-middle 1990s. The areas of large aggregates of *M. leidyi* ("hot spots") were the adjacent areas to Cape Kaliakra and Galata, Varna and Burgas Bay (Western Black Sea). The magnitude of the summer pulse of the population correlated strongly with the physical forcing, the plankton fauna components and grazing by *B. ovata* (Kamburska, 2004).

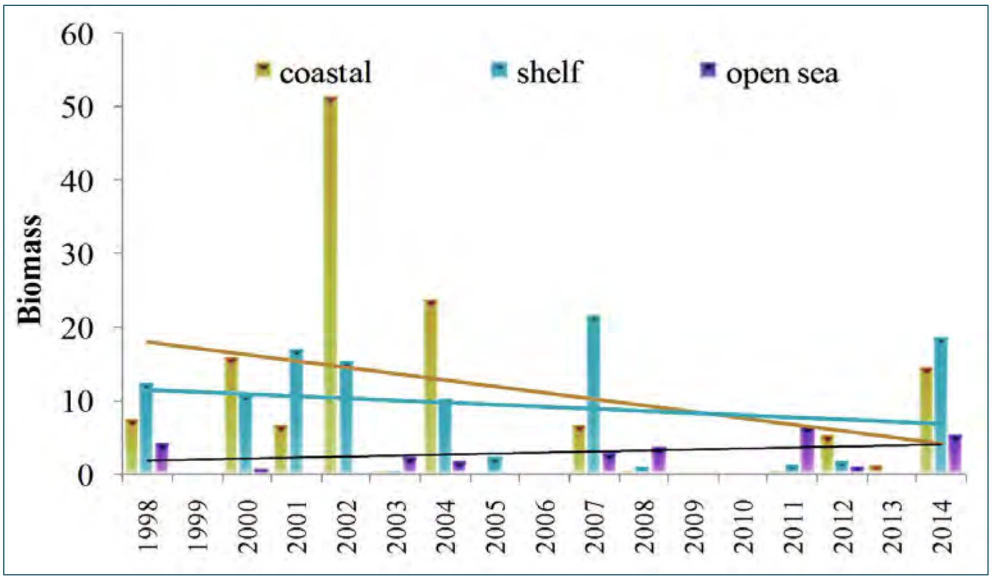


Figure III.4. Biomass of *M.leidyi* [g·m-3] and trendlines in c. Galata stransect during 1998-2014 in summer.

Ratio non-native/native species

Phytoplankton (Coastal, shelf and open sea)

Non-native phytoplankton species list for the Black Sea (still a draft produced by the CBD AG to the Black Sea Commission), is the most complete for the moment (listed 52 non-native phytoplankton species). During (MISIS Joint Cruise) 6 phytoplankton non-native species were identified out of which 2 species in Romanian Black Sea waters (*Lessardia elongata, Peridinium quinquecorne*) and 5 species in Bulgarian Black Sea waters (*Lessardia* *elongata, Gymnodinium nanum, Gymnodinium pulchrum, Gyrodinium cochlea, Gyrodinium flagellare*) all from class Dinophyceae. Both the abundance and biomass were low with a maximum abundance measured at RO shelf station (M02) of *L. elongata* (0.51% of the total phytoplankton abundance) and in the open sea station (M13) in BG Black Sea waters (*L. elongata* and *G. nanum* 0.2% of the total phytoplankton abundance) (Fig. III.5). *L. elongata* was the most frequent species observed over the entire RO and BG pelagic habitats. First published in 2003 (Saldarriaga et al., 2003) *L. elongata* was confirmed in Romanian Black Sea waters in 2004 (Ediger & Velikova, 2009) and there after found as common species in the phytoplankton assembly (Moncheva et al., 2012).

An increase in the abundance from coastal to open sea was a pattern of non-natives spatial distribution observed only at the BG transect, contrasting to the general decrease of total abundance. Highest non-native species biomass was found in the shelf, while lowest - in the coastal area for both transect (Fig. III.6). Overall the biomass and abundance of the non-native species in RO and BG pelagic habitats were similar low and did not exceed 1%.

The results show that abundance and biomass of the non-native phytoplankton along the RO and BG transects were much lower than the GES threshold (proportion of abundance and biomass of non-native species to native <10% (proposed in the BG IAR and GES Report, 2012). - (Fig. III. 6).

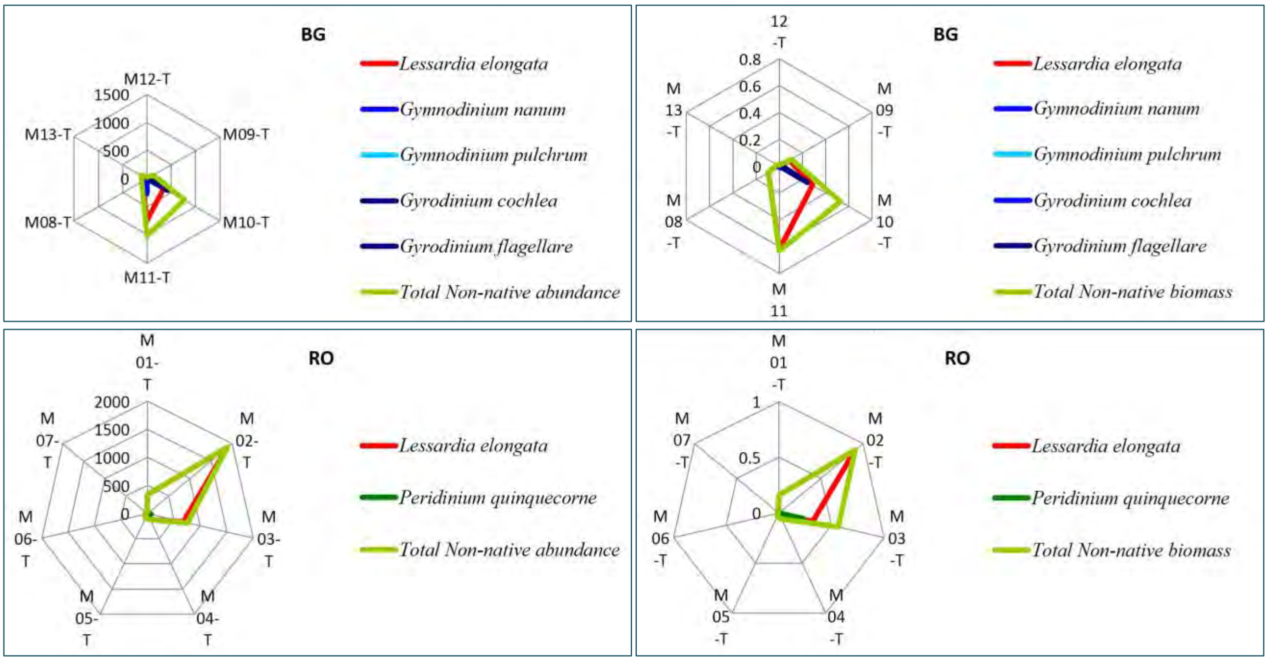


Figure III.5. Non-native species in phytoplankton community by abundance [cells·L-1] (left panel) and biomass [mg·m-3] (right panel) in BG and RO transects.

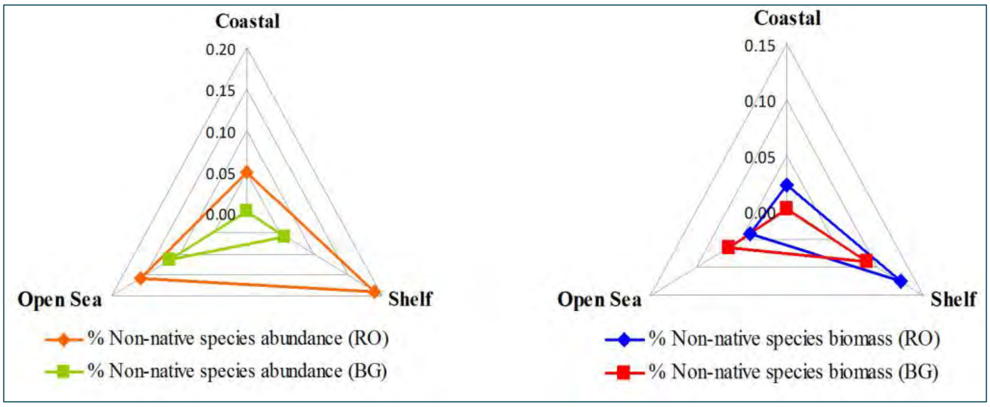
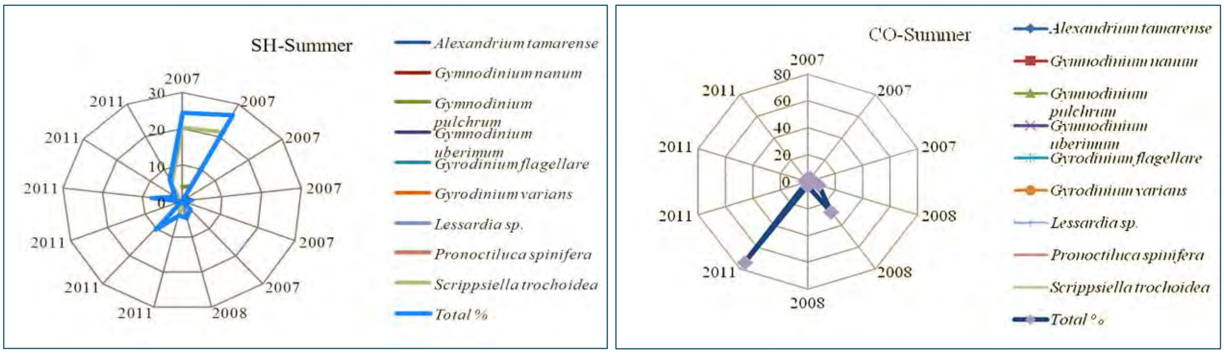


Figure III.6. Proportion of non-native/native species in phytoplankton community in % from total abundance and biomass in BG and RO transects by habitats (coastal, shelf and open sea).

The assessment based on data for the period 2007-2011 in the BG pelagic habitats provide evidence of a number of cases when the proportion of non-native species in the phytoplankton community structure contributed to more than 60% in the total summer biomass (Fig. III.7).

During an extensive survey of phytoplankton cysts Robino et al. (2010) found out viable cysts of 8 new for the Black Sea species, not listed in the Black Sea phytoplankton check-list, with a relatively high density of cysts accumulation identified not only in the coastal but also in the shelf bottom sediments, altogether suggesting that the problem should not be neglected.



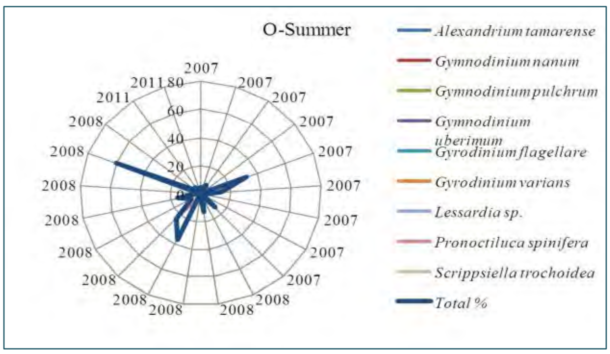


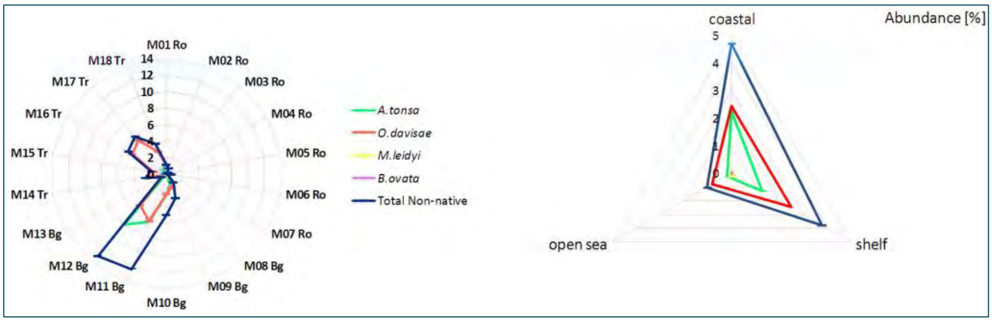
Figure III.7. Proportion (%) of non-native phytoplankton species in the summer phytoplankton community total biomass (2007-2011) along BG Galata transect habitats (Co-coastal, SH-shelf, O-open sea).

Zooplankton (Coastal, shelf and open sea)

Copepods - *A.tonsa* and *O.davisae* and cthenophors - *M.leidyi* and *B.ovata* are non-native species identified in plankton fauna community during the cruise survey. Only in two stations in front of Bulgaria abundance of alien (mainly copepod) species exceed the threshold of 10% (12-13%) (Fig. III.8a). Ratio of non-native species biomass to native in 39% of observations is over than the indicator threshold as a consequence of *M.leidyi* dominance. Zooplankton community structure at coastal and shelf areas was characterized with comb jelly prevalence in the biomass (Fig. III.8b).

Results suggested importance of two alien copepods in zooplankton community. *Acartia tonsa* and *Oithona davisae* became established in the Black Sea ecosystem in the 1970s and 2000s, respectively. The success of their establishment was determined by biological features of the species and vulnerability of the native copepod community to invasions (Gubanova, 2000; Gubanova et al., 2014). *A. tonsa* is considered as an opportunistic species, resistant to pollution and eutrophication, and is usually confined to coastal waters with high food concentrations and relatively high temperatures (Conover, 1956, Paffenhöfer & Stearns, 1988, Lawrence et al., 2004, Gubanova, 2003). After the first appearance of *O. davisae* in Sevastopol Bay in 2000 (Zagorodnyaya, 2002), specimens were found again in 2005. The invader has been expanding along the Black Sea coast since 2009 (Altukhov, 2010; Mihneva & Stefanova, 2011; Selifonova, 2011). *A. tonsa* and *O. davisae* have survived in their new Black Sea environment, reproduced there and established self-sustaining populations (Gubanova et al., 2014). The current plankton community shift in the Black Sea associated with small flagellate development may be a significant driving force contributing to the proliferation of the *O. davisae* population, especially in the eutrophic inlets (Mihneva & Stefanova, 2013). The successful introduction and later establishment of a species depend both on the biology of the alien species and the recipient community conditions. Usually, the invasion of a new species is followed by a number of changes to the ecosystem (Alimov et al., 2004).The low resilience of the native zooplankton community to invasions of new copepods in the Black Sea has been preconditioned by changes in the ecosystem caused by eutrophication, pollution, overfishing (1970s–1980s) and invasions of the predatory ctenophores (1990s; 2000s) (Gubanova et al., 2014). It appears that *O. davisae* and *A. tonsa* occupy a niche in the shallow coastal waters especially during the warm months (Mihneva & Stefanova, 2013).

a)



b)

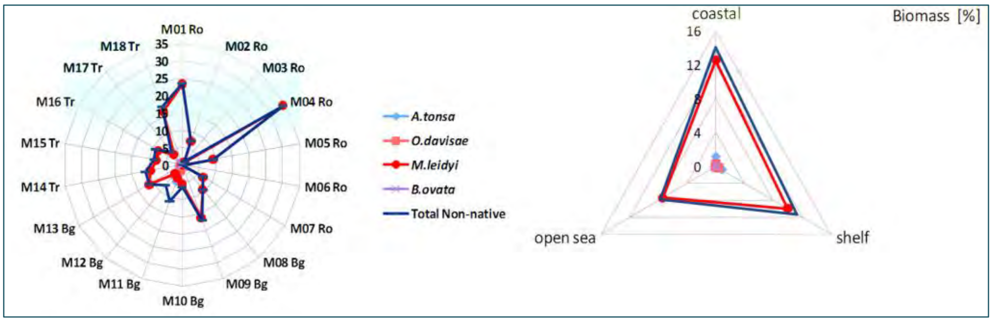


Figure III.8. Proportion of non-native/native species (in percent) in zooplankton community abundance (a) and biomass (b).

CONCLUSIONS

Although the proportion of the non-native zooplankton species assemblage was relatively low (<35% of occasions) and corresponded to GEnS the NIS impact could not be ignored. The alteration of *M.leidyi* population trough the years is irregular and its dynamic still manipulated zooplankton standing stock and community structure.
Although the abundance and biomass of the non-native phytoplankton along the RO and BG transects were much lower than the GES threshold , the recent history of bioinvasions in this particular environment, calls for further investigations of cysts /non-natives biodiversity as an imperative for the sustainable management of the Black Sea ecosystem.

GAPS and RECOMMENDATIONS

- lack of specific monitoring strategy for NIS with relevant frequency and the degree of deficiency of scientific knowledge
- regular monitoring of abundance, biomass, temporal occurrence, impact of non-native species which are already established
- development of appropriate indicators for benthic community

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IV. DESCRIPTOR 5: Eutrophication

Completed by:
Luminita Lazar, Laura Boicenco, Dan Vasiliu, Galina Shtereva, Colpan Beken, Snejana Moncheva, Boryana Dzhurova

“Human induced eutrophication is minimised, especially the adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom water”

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INTRODUCTION

The Black Sea has special natural features and a catchment area five times higher than its surface. Therefore, it has been vulnerable to anthropogenic pressures and pollution sources (BSC, 2008). Until the '60s, the Western part of the Black Sea was known as one of the most productive seas, with luxuriant pelagic fauna, being an example of natural eutrophic ecosystem due to the permanent Danube's nutrients input (Gomoiu, 1981). Further, with anthropogenic activities enhancement, increased use of fertilizers, wastewater discharges, detergents, etc., the nutrients regime has undergone significant changes. These changes were related to the river nutrients input that increased significantly (Mee & Topping, 1999; Cociaşu et al., 2008) and led to alterations in the North-Western Black Sea ecosystem. Thus, at the beginning of '80s, phytoplankton has developed excessive and intense blooms became annual and extended in time and frequency. During 1983-1988, more than 20 blooms were observed, produced by 8 species, of which 5 (*Prorocentrum minimum, Skeletonema costatum, S. subsalsum, Eutreptia lanowii, Emiliania huxleyi*) were found in maximal densities known until then at the Romanian coastal area. At Constanta, the average phytoplankton biomass was 4,777 mg/m^3, 10 times higher than in 1959-1963. The algae species with abundance more than 100,000 cells/L abundance increased from 34, in 1960-1970, to 54, in 1983-1988 (Bodeanu et al., 2004; Gomoiu, 1992). The eutrophication effects appeared soon: the transparency decreasing, higher quantities of organic matter decomposition and oxygen depletion (Gomoiu, 1992) and bottom waters became seasonally hypoxic or even anoxic (ICPDR – ICBS, 1999) transforming the North Western part of the Black Sea into a highly eutrophic one (Zaitsev in Mee, 1999). In the early 90s, have found decreasing nutrients input resulted in the first recovery signs (decreasing of phytoplankton blooms, improvement of bottom oxygen regime, increasing of benthic macro fauna (Gomoiu, 1992). Thereby, in 2005, the North Western part of the Black Sea seems to have a strong altered ecosystem, but relative functional. Malfunction symptoms like incapacity of recycling high organic matter input from river or biological activity or dominant monospecific phytoplankton blooms were still evident. The Black Sea coastal and shelf waters have been still predominant eutrophic (BSC, 2008). Recently, based on the Romania's Initial Assessment, the emphasized spatial and seasonal variability and the extreme phenomena from the NW Black Sea coast makes the current eutrophication state definable as a moderate - good, equivalent to an eutrophic - mesotrophic state which, under the action of climatic factors and human impact more pronounced in the coastal zone, can easily pass to extreme states like unsatisfactory (hypertrophic) or very good (oligotrophic), conditions occasionally encountered in the waters of the NW Black Sea, often seasonally (Lazar et al., 2013).

The eutrophication reduction (EcoQ3) is subject of both Black Sea Strategic Action Plan (2009) implemented by the riparian and the Marine Strategy Framework Directive through the Descriptor 5. The latter consider that the Good Environmental Status – GES has been achieved when the biological community remains well-balanced and retains all necessary functions in the absence of undesirable disturbance associated with eutrophication (e.g. excessive algal blooms, low dissolved oxygen, declines in sea grasses, kills of benthic organisms and/or fish) and/or where there are no nutrient-related impacts on sustainable use of ecosystem goods and services (Borja, 2013). Subsequently, the European Commission has selected a set of indicators which could be taken into account in the eutrophication status assessment (Cardoso et al., 2010; European Commission, 2010):

· Criterion 5.1. Nutrient concentrations do not lead to an undesirable disturbance to the balance of organisms present in the water or to the quality of the water concerned resulting from accelerated growth of algae; and

· Criterion 5.2. The direct effects of nutrient enrichment associated with algal growth do not constitute or contribute to an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned ; and

· Criterion 5.3. Indirect effects of nutrient enrichment associated with growth of macroalgae, sea grasses, and reductions of oxygen concentrations do not constitute an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned.

In this context, besides of the riparian EU countries obligations (Romania and Bulgaria) to meet the requirements of the EU Directives (particularly WFD and MSFD) there is a strong need to have powerful tools to assess the Black Sea eutrophication at regional level. Therefore, this assessment aims to a harmonized approach of the eutrophication assessment at the (sub) regional level – the Western Black Sea.

Description of the land-based sources

The description of the main land-based sources of the area was done based on each country assessment on a geographical basis, from North to South:

Romanian littoral

The main sources of the nutrients enrichment of the Romanian littoral are generally represented by the point sources. As diffuse source, on the Romanian Black Sea coastal zone the agriculture is limited, being negligible for the marine ecosystem state (RO-IAR, 2012). On the other hand, even if there are measurements from the major cities of the coastal zone, the atmospheric deposition was not quantified.

Currently, the main point sources, Hot Spots (HS) at the Romanian littoral are urban agglomerations (four municipal waste water treatment plants – Constanta Nord, Constanta Sud, Eforie and Mangalia), industrial units (Rompetrol – Midia and Constanta Port) and the Danube River with its three arms (Chilia, Sulina and Sf.Gheorghe) (Fig.IV.1).

The sampled transect is approximatively situated in the middle of the shore, near the Constanta area. However, due to the main current North-South and extended influence of the sources it will be given a short description of the entire littoral.

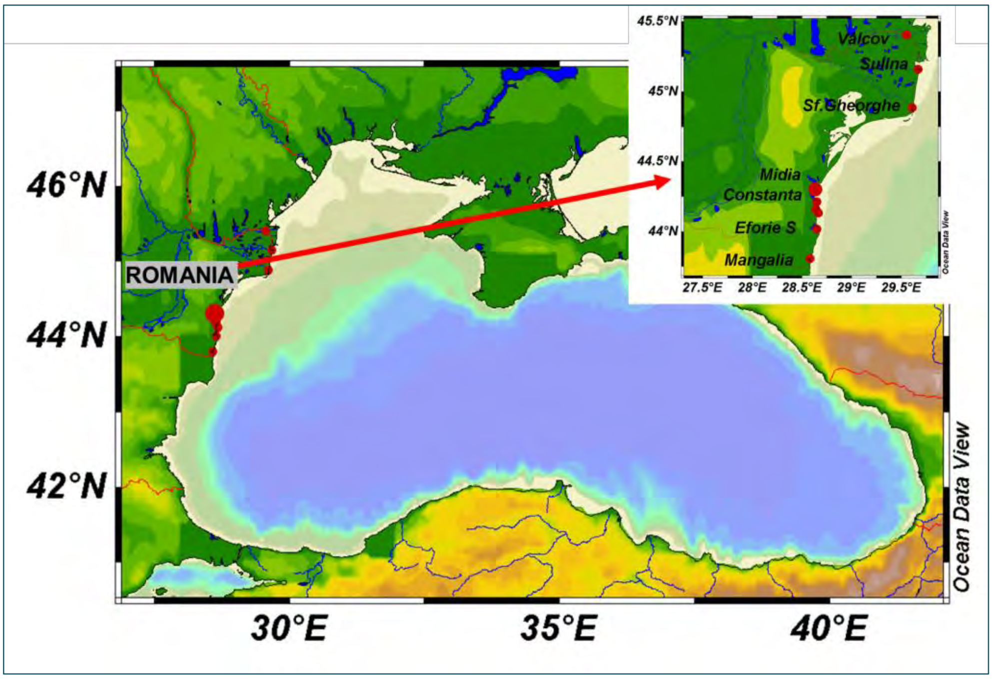


Figure IV.1. Hot Spots at the Romanian Black Sea littoral.

Excepting the Danube, the northern part of the shore has no point sources, being an area devoid of urban agglomerations and declared as Danube Delta Biosphere Reserve and RAMSAR site.
The main land-based sources are concentrate on the southern littoral where the industrial, human and tourism activities are better developed (Fig. IV.1). The biggest urban agglomeration is situated around the major city of the coast, Constanta and it has two WWTPs directly discharging into the Black Sea: Constanta North and Constanta South. By far, the second is the most important, being the biggest WWTP at the Romania littoral, designed for mechanical-biological treatment (conventional biological treatment with activated sludge) and mesophilic anaerobic stabilization of separated sludge from wastewater treatment process. It was rehabilitated during 1999 – 2001 and has a capacity of 3200 l/s industrial and domestic wastewaters (Figs. IV.2 and IV.3).

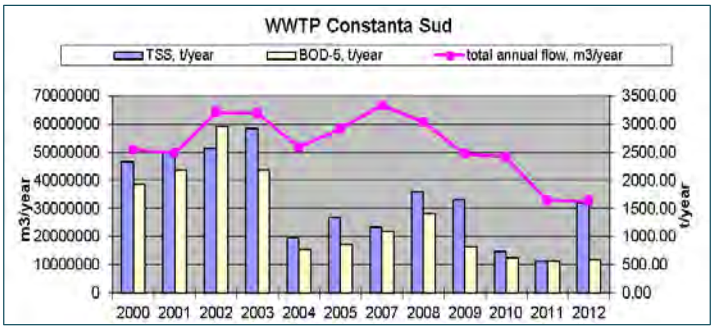


Figure IV.2. Total suspended solids and BOD5 discharge WWTP Constanta Sud.

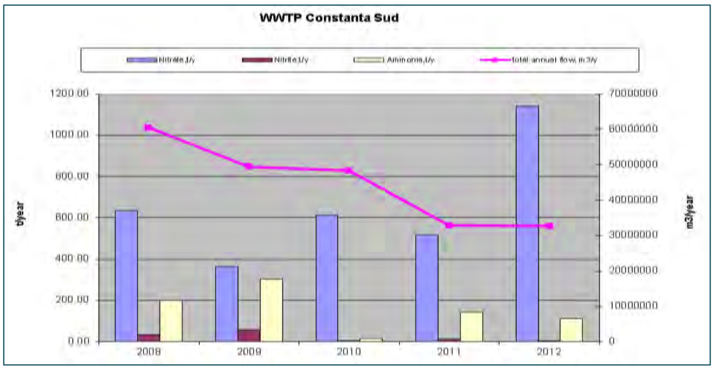


Figure IV.3. Inorganic nitrogen discharge WWTP Constanta Sud.

The main industrial pollution sources are found also in the Constanta's area: Petromidia refinery and Constanta Port, both with insignificant nutrients discharges compared with WWTPs and rivers.

Danube

The Danube is the main land-based sources of nutrients and organic matter from the Romanian Black Sea littoral through the direct discharges of its three arms Chilia, Sulina and Sf. Gheorghe. The Danube's nutrients load for

2007-2011 outlined the dominance of the nitrate form in the inorganic nitrogen pool (Table IV.1):

Table IV.1. Danube's nutrients input (2007-2011) – RO IA, 2012.

Parameter	2007 (t/y)	2008 (t/y)	2009 (t/y)	2010 (t/y)	2011 (t/y)
NH_4	35565,87	35983,35	36213,23	40887,22	22979,87
NO_2	18346,48	17469,28	15904,93	26328,41	16928,56
NO_3	952221,15	1142323,23	1235117,21	1905304,65	1043114,35
PO_4	29803,40	12346,36	15848,19	32735,53	21613,83

For the same interval, 2007-2011, the highest input was discharged through Chilia arm (the largest one) (Fig. IV.4).

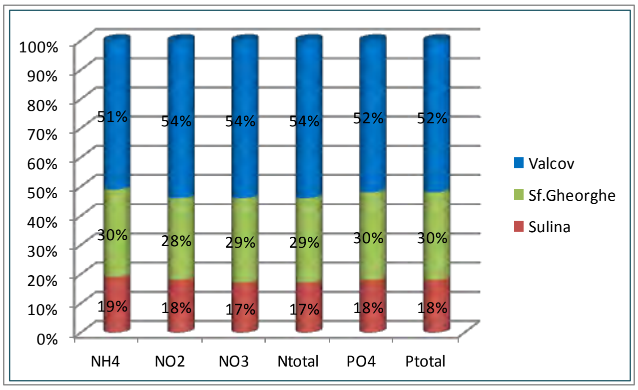


Figure IV.4. Percentage distribution of nutrient loadings for each Danube's arm.

Conclusions

Even if, qualitative, the Danube's waters are not exceeding the allowable limits according to the legislation, the marine environment is seasonally excessive enriched with nutrients. This input is particularly linked to the changes in its flow regime. Apart from the local Romanian land-based sources, Ukrainian rivers discharge and input of nutrients to the Northern part of the Black Sea should be taken into account when assessing eutrophication status along the Romanian coast.

Bulgarian littoral

The investigation of Land Based Sources (LBS) nutrients input was undertaken in the frame of the Initial Assessment of the Marine Environment in the Bulgarian Black Sea part (prepared by the Institute of Oceanology) in implementation of the European Marine Strategy Framework Directive 2008/56/EC (MSFD).

Overview of pressures and impacts was undertaken using data provided by the Black Sea Basin Directorate (BSBD) on human activities along the Bulgarian Black Sea coast and on discharges (WWTPs and rivers) into the sea or adjacent water bodies in 2006 -2011. The data allowed to be compared the contributions of various sources in the total load of nutrients to the Black Sea and reveals trends in nutrients input.

Main human activities in the area

The main drivers along the Bulgarian coast impacting on the marine environment are: industry, tourism, navigation, port activity, urbanization and agriculture. The main types of **industry** contributing to the amount of industrial waste waters (WW) are chemical industry, energy production (thermal power stations), machine industry, food industry, shipbuilding and ship repair. The WWs from big industrial complexes mainly discharge into the coastal lakes. The amount of industrial WWs is not a significant part in generated total WWs. The main points LBSs impacting the marine environment are the rivers and municipal WWTP discharge.

Varna Bay is the second largest bay along the Bulgarian Black Sea coast. Varna city is the third largest economic centre in Bulgaria. The marine environment in the Varna area (Varna Bay – c.Galata) is impacted by the same drivers as was mentioned above. The Varna Bay area is also the biggest tourist area along the Bulgarian Black sea coast. The main types of industry contributing to the amount of industrial waste waters in the Varna region are chemical industry, energy production (thermal power stations), shipbuilding and ship repair. Several of the largest chemical plants of the country - "Solvey Sodi" JSC, "Agropolychim" JSC, “Devnya Cement” JSC are located on the territory of the Varna district (municipality of Devnya). The Chemical Industrial complex plays a major role in emission of pollutants in the Varna Bay -lake system and it is responsible for WQ of two rivers discharging into the lakes – Provadijska and Devnenska.

Urbanization. The population of the entire Black Sea basin (Bulgaria) is 1,507,724 inhabitants. People are mainly concentrated in two large municipalities - Varna and Burgas. Respectively, these cities are with maximal population density and generate the largest share of municipal WWs. The share of untreated WWs as a part of total WWs discharge decreased from

2005 to 2010 (BG-IAR, 2013). The population in the Varna municipality is in range 320,000 - 330,000 inhabitants in 2004-2010 (Table IV.2).

Table IV.2. Number of inhabitants and visitors in Varna municipality (NSI data).

	2004	2005	2006	2007	2008	2009	2010
Inhabitants	319,979	319,800	319,495	322,144	326,528	329,173	330,001
Visitors		471,734	800,637	876,816	853,946	789,681	736,001

Waste waters from 11 municipal WWTPs along the Bulgarian coast directly discharge into the sea. Additionally, 5 sewages discharge untreated waste water into the sea. A few WWTPs discharge into the coastal lakes connected to the sea. Their indirect impact on the water quality of the sea is predominated by the large pollutants load they bring to the environment. Large are the nitrogen and phosphorus loads of the WWTPs of the cities of Varna and Burgas, which discharge indirectly into the sea. Varna Bay is located in the northern part of the Bulgarian Black Sea coast, locked between c. Galata and c. St. George (Fig. IV.5). It is the second largest bay along the Bulgarian Black Sea coast after Burgas Bay. On the west it is artificially linked by two canals to Varna Lake, which has a major impact on it. The bay length is 4.5 km and width of 7.5 km, with a surface area of about 20 km^2. WWTPs located in North region in Bulgaria, including Varna Bay, discharge (directly or indirectly) significant nitrogen and phosphorus load into the sea (Figs. IV.6, IV.7 and IV.8).

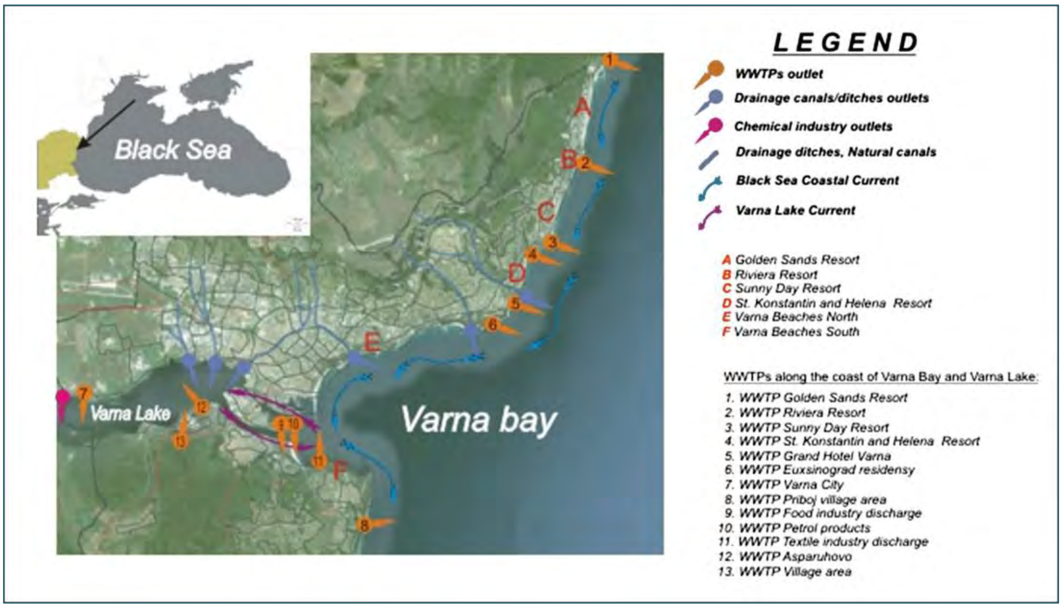


Figure IV.5. Map of Varna Bay-Varna Lake system-environmental pressures and their pathways: Varna Lake Current, Black Sea Coastal Current, Waste Water Treatment Plants (WWTPs) (Moncheva et al., 2012).

Varna Bay is characterized by specific hydrological and hydro physical regime due to geographical features as shoaled bottom, unlimited water exchange with the sea and connections *via* two artificial canals with Varna Lake. The geographic position of Varna Bay predetermines the strong influence of Varna Lake. The lake system provided freshwater inflow through the canal forming an area of lower salinity in the western part of Varna Bay. Three drainage ditches/canals discharge into the bay and another three canals - in the eastern part of Varna Lake, respectively (Fig. IV.5). Totally, thirteen WWTPs, with different capacities and technologies discharging into the Varna lakes –Varna bay system and adjacent area represent the major point sources of nutrients and organic enrichment. Despite reconstruction of the existing WWTPs and construction of new WWTPs in the seaside, the nutrients loads of municipal WWs are much more significant than those of industrial WWs. The nutrient loads from these lakes (Beloslav and Varna Lakes) into the Black Sea are large and pose a serious threat (Shtereva & Dzhurova, 2006).

Tourism. The tourism is another major driver responsible for human impact on the marine WQ. During the last two decades, it is among the fast growing sectors of the local economy. Tourist activity is concentrated in and around the cities and some seaside resorts and vacation villages exist along the Bulgarian coast (Fig. IV.8).

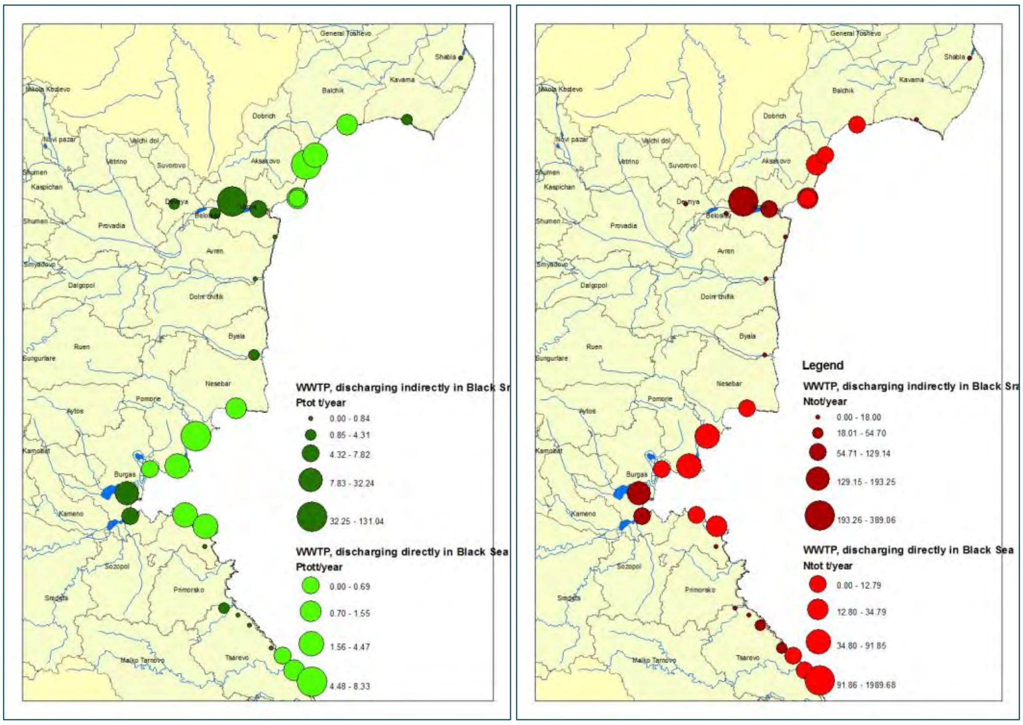


Figure IV.6. Nitrogen and Phosphorus loads from WWTPs along Bulgarian coast.

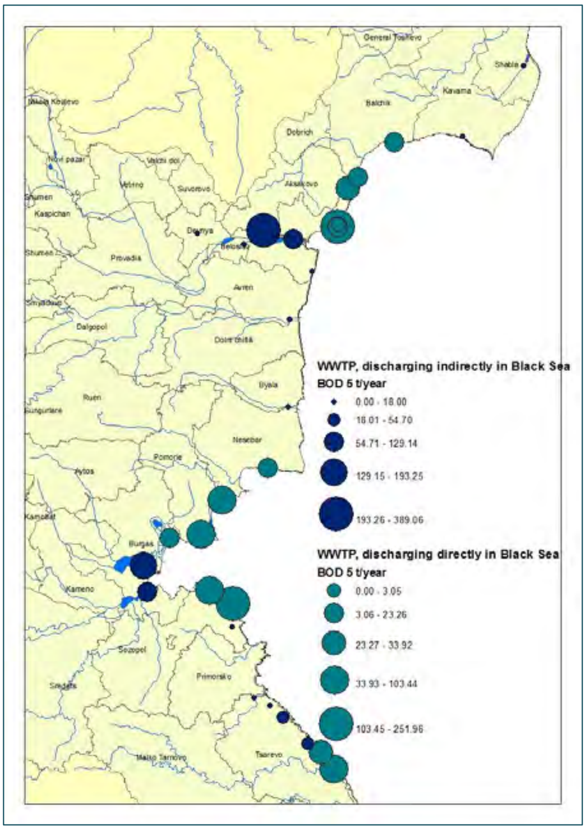


Figure IV.7. BOD load of WWTPs.

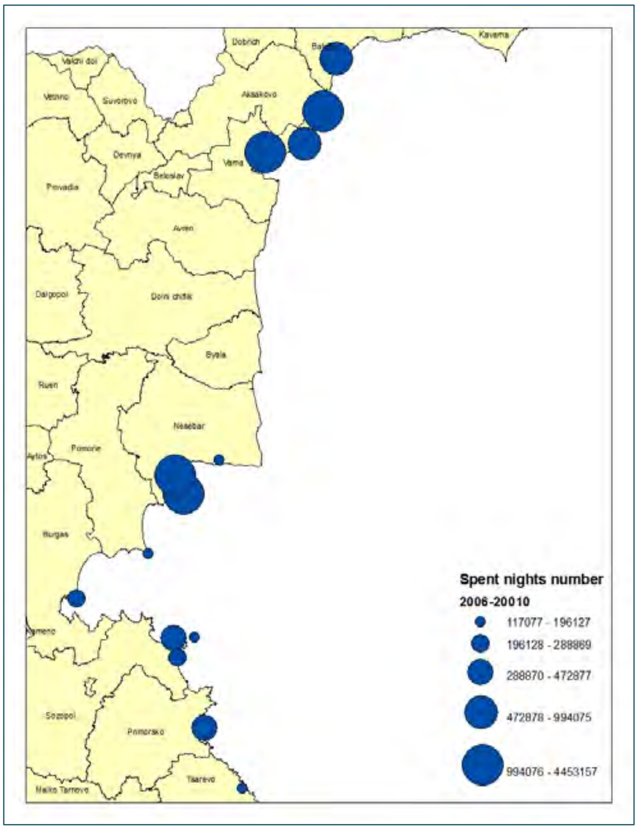


Figure IV.8. Spent nights number along the coast.

An increase in nights spent in hotels in Varna area resorts within 2005 -2007 period was established (Table IV.3).

Table IV.3. Overnights in Varna city, Golden sands and St. "Konstantin and Helena" resorts in the period 2004-2010.

Overnights	2004	2005	2006	2007	2008	2009	2010
Varna	4203691	4915665	5005937	4927229	4793207	3879451	4010898
Golden sands		3233960	3327189	2977462	2377462	2377100	2335516
St. Konstantin & Elena		1043926	991915	1053624	1048754	946484	929596

A number of new touristic facilities in the resorts are not connected to the sewage system. Consequently, the nutrients input into the sea increase as untreated WWs stem to coastal waters. Besides, some WWTPs have insufficient capacity to treat the increased amount of wastewaters delivered in summer (during touristic season the population along the coast usually doubles). These WWTPs often discharge insufficiently treated or untreated waters to the Black Sea which results in elevated nutrients level in the coastal waters. Thus, in summer this has especially negative effects such as algal blooms, pathogenic bacteria development, etc. and, in general, the coastal zone loses its aesthetic view. Varna city and the resorts on north seaside have a significant contribution to the tourism development in the Varna district.

One of the main municipal WWTPs "Varna" (city of Varna), with standard discharge of $24.42 \cdot 10^6$ m^3/s, deposits into the Varna Lake-Varna Bay system on the average 650t nitrogen and 130 t phosphorus per year, as estimated for the period 2005-2010. WWTP Varna has been technologically upgraded in 2010; it generates sludge of 8879 t/y. A slight decreasing trend in nutrients and organic matter (BOD, COD) was established during the last years (BG-IAR, 2013).

Thus, the tourist resorts on the coast, built and expanded greatly since 1990, are a factor exerting additional pressure on the marine ecosystem along the Bulgarian coast (Moncheva et al., 2012). Since 2008 a decreasing trend in touristic activity was observed (BG-IAR, 2013). However, having in mind the status of WWTPs and their insignificant capacity, especially during the high touristic season, the pressures in terms of nutrient loads remain significant. WWTP's loads for nutrients and organic matter (BOD) in North coastal area are presented in Fig. IV.9.

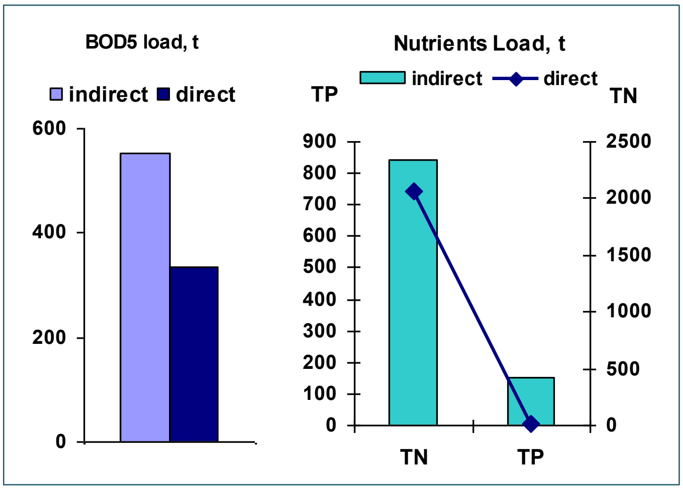


Figure IV.9. Nutrients and BOD loads from WWTP located in North Black Sea area.

Five bigest WWTPs ("Varna", "Asparuhovo", Golden sands", Grand Hotel Varna", Priboj") in theVarna area discharging into the sea or Varna lake (total Q=28,293,302 m^3/y) enter 144.7t TP, 734t TN, 552t BOD and 2116t COD per year within 2006 -2010 period.

Agriculture. Agricultural inputs reach coastal waters, principally, through the rivers discharge as point LBS and diffuse sources. Riverine nutrient input is also responsible for excessive enrichment of sea waters with nitrogen, phosphorus and organic substances. The Black Sea basin of Bulgaria is characterized with catchment area of 16,570 km^2 which is differentiated in 9 river's sub basins. The largest river along the Bulgarian coast is Kamchia, and it is also the most significant source of nutrients (nitrate, nitrite, ammonia nitrogen; phosphate phosphorus) and Total organic carbon (TOC) for the Bulgarian Black Sea waters (Table IV.4). The Kamchia River length is 245 km with catchment area of 5,358 km^2. The role of the Kamchia River and its influence on the coastal area ecological status is identified in the aquatorium located south and east-south from its mouth (Shtereva et al., 2006).

Table IV.4. Nutrients loads.

Rivers	NO3-N	NO2-N	NH4-N	DIN	PO4-P	TOC
	ktons	ktons	ktons	ktons	ktons	ktons
Kamchia River	1.44	0.06	0.24	1.74	0.49	2.19
Other rivers	0.18	0.01	0.06	0.25	0.09	2.13
Total	1.62	0.07	0.1	1.99	0.58	4.32

Similar to the Danube River (Berlinsky et al., 2006), the loads of the Bulgarian rivers, directly discharging into the Black Sea, increased toward 2010 in parallel with the rise in their flows (BG-IAR, 2013). Locally, there is no direct riverine discharge over the Varna bay water system. Devnenska and Provadijska Rivers flowing into the Beloslav lake affect the area indirectly through the current Varna lake-Varna bay that is the main source of nutrients and pollution loads. In 2011 Provadijska R. enters in the lakes ~257t inorganic nitrogen and 22t phosphorus.

Diffuse sources of pollutants can be landfills which do not meet the European requirements (unregulated, landfills without isolation) settlements without sewerage system and wastewater treatment plants, as well as a result of agricultural activity. Use of fertilizers and the development of animal husbandry contribute directly to contamination of coastal water bodies by nitrogen and phosphorus or indirectly through canals from coastal lakes. Agricultural inputs reach coastal waters principally through diffuse sources, either carried out by surface water and, in some areas, possibly groundwater or, for N, *via* the atmosphere. The total area used for agriculture activity is 1,008,385 ha. The available information on fertilizers use in the municipalities of Varna district along the coast, gives grounds to assume that two municipalities - Aksakovo and Varna are the areas of most intensive diffuse input of nutrients to the Black Sea (BG-IAR, 2013).

In the coastal municipalities 11,142 livestock farms were registered in 2011 (National statistical institute's data). The total number varied from 11,080 (2008) to 13,000 (2009). There is no accessible data on the amount and level of pollution of wastewaters generated by the animal husbandry in the Bulgarian Black Sea basin. Information on the level of treatment of these waters and places of their discharge is also missing, therefore, the share of livestock farms in the nutrient load stemming to the Black Sea cannot be quantitatively assessed.

Yet, using data on the number of farmed animals by species and proposed by the World Health Organization (WHO) factors (WHO, 1993; Valiela et al.,1997), the released amount of phosphorus and nitrogen by the animal husbandry business was calculated. Such estimated diffuse nutrient loads, produced by various human activities (not only for farms empirical equations can be used), may not give the factual amount released, however, the estimates give a qualitative idea that the diffuse sources along the Bulgarian coast are just as important as the point. For example, the coastal erosion contributes by ~20 % of the total nitrogen load and about 1 % of the load for total phosphorus. Livestock farms nitrogen load amounts to 18% of the total, and phosphorus - 50%.

Apart from the gap in data on nutrients loads from livestock and agriculture, the loads stemming through ground waters to the sea are also unknown. The ground waters impact on WQ of the coastal area cannot be assessed as the ground water discharges are not measured. The level of pollution of ground waters is known (BG-IAR, 2013). Thus, except for very rough estimates, it is impossible to properly evaluate the contribution of the diffuse sources along the Bulgarian coast to the excessive nutrient enrichment of Black Sea coastal waters.

The data of Institute of Hydrology and Meteorology (NIMH) reveals lower NO_3-N load in the North region (including the Varna area), than in the south part of the Bulgarian Black Sea coast (Fig. IV.10).

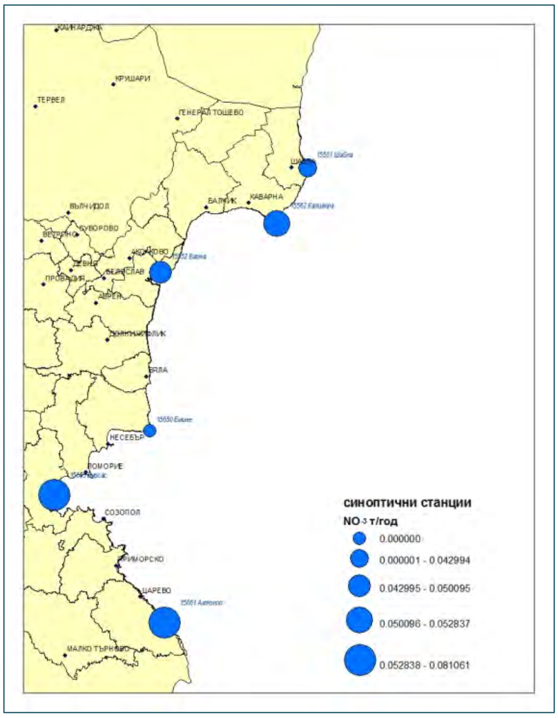


Figure IV.10. Nitrate loads along Bulgarian coast.

Existing information on dredging activities is incomplete. It specifies the location of dumping of dredged spoils, without specifying in what intervals is performed dredging, duration, and the composition of the deposited material, as well as the level of its pollution. The available data do not allow to evaluate whether dumping in the sea poses a serious contributor to pollution and nutrients enrichment.

Conclusions

Riverine nutrient input and municipal waste waters treatment plants discharges are responsible for excessive enrichment of sea waters with nitrogen, phosphorus and organic substances. The analysis of available data reveals two sites as areas with high anthropogenic impact -Varna Bay and Burgas Bay, owing to the direct or indirect influence of industrial and municipal runoff, port operations and tourism development and also inputs of nutrients from diffuse sources. Another area impacted by eutrophication and pollution area is the Black Sea coast where the Kamchia River enters the sea bringing significant loads of nitrogen and phosphorus. It is impossible to evaluate properly the contribution of the diffuse sources along the Bulgarian coast due to the gap in data for nutrients input through ground waters to the sea and nutrients loads from livestock and agriculture.

Turkish littoral

In the Black Sea, some cities use the sewerage system directly disposing with deep marine outfalls but most of the small settlement areas used septic tanks or package biological treatment. On the other hand, present sewerage systems show also variety such as combined or separate system. Ordu, Giresun city centres have separate sewerage systems whereas Sinop, Trabzon and Zonguldak have combined systems but only the Samsun city centre have both combined and separate sewerage system (Bakan et al., 1996).

Figure IV.11. Black Sea coastal towns and major rivers location points (Bat et al., 2009).

Many industrial untreated industrial and agricultural wastes drain into the sea. Ersoy et al. (2007) pointed out that approximately 450-500 tons of solid waste is disposed of each day in the Black Sea Region of Turkey.

Domestic discharge is the greatest source of organic matter discharged into coasts. In Turkey, many towns and cities situated on the coast, however, sewage is discharged usually with primary treatment only followed with deep marine outfalls. Organic matter is an important nutrient, as it is a source of food for many benthic invertebrates in the marine ecosystem.

Turkey is a developing country where the industrial and urban developments mostly occur in coastal areas through increased input of wastes impose a further stress on the Turkish coasts of the Black Sea. The application of the agreements requires that each country which has a coast to the Black Sea, concerned creates an environmental policy. Harmonization of legislation and standards, preparation of effluent discharge inventories and mapping of major pollution sources and establishment of water monitoring programmes have been ongoing processes and the river basin management plans are also being prepared since Turkey committed to implement WFD.These components are stated in the activities of the Black Sea Environmental Programme but the legislative frame for their realization still does not exist in all countries in the region (Bat *et al*., 2009).

Conclusions

The İğneada region is, on the other hand, an area much less effected by land based sources of pollution since there are no rivers and having less than 2000 inhabitants where package biological treatment for waste water management is used (Fig. IV.11). The area is part of the Yıldız Mountains Biosphere Reserve having protection status. The relevant administration unit where İğneada is located (Kırklareli city) has three protected areas, almost no industrial facilities and less inhabitants, hence, it is the less impacted unit in the Marmara Basin (River Basin Protection Plans of Turkey, 2011).

MATERIAL and METHODS

The network sampling consisted of three transects: Romania (stations 1-7), Bulgaria (stations 8-12), Turkey (stations 14-18) and an intercalibration one (station 13) covering coastal (stations 1, 12 and 18) shelf (stations 2, 3, 4, 5, 9, 10, 11, 15, 16 and 17) and open waters (stations 6, 7, 8, 14 and 13) (Cruise report, 2013) (Fig.1).

A core set indicators was chosen for the eutrophication assessment of the western Black Sea in respect with the descriptor's 5 criteria and the intercalibration exercise grouped as follows:

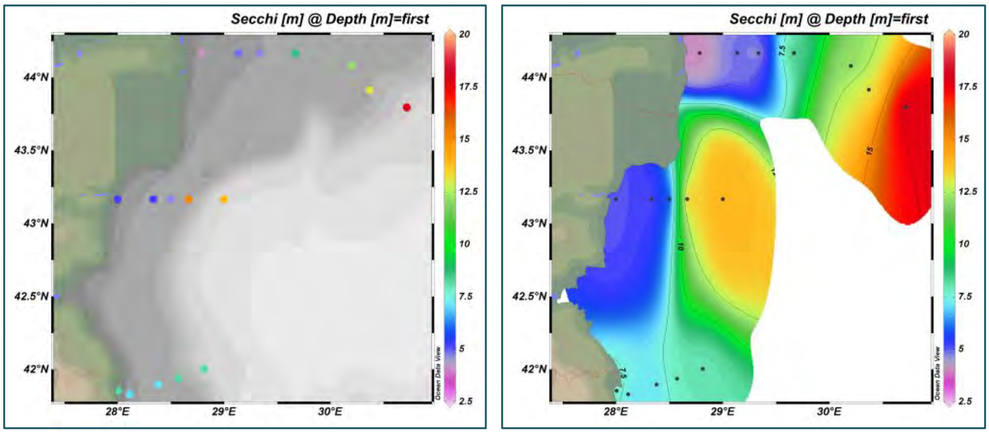
- causes of eutrophication – nutrients levels – concentrations of phosphate, silicate, nitrogen oxidised forms, ammonium in water column (Grasshoff et al., 1999)
- direct effects of eutrophication – chlorophyll *a* concentrations in water column (Jeffrey & Humphrey, 1975)
- indirect effects of the eutrophication – water dissolved oxygen content (Winkler method, onboard – Grasshoff et al., 1999) and transparency (in-situ measurements, Secchi disk).

Based on the data achieved in the cruise, on the reference values and acceptable deviations of these parameters (specific for each country) it was tested the integrative tool for the eutrophication assessment proposed by the Baltic2Black project (with BSC partner), BEAST (**B**lack Sea **E**utrophication **AS**sessment **T**ool).

Despite some differences in terms of methodological aspects, the results of the intercalibration exercise between the cruise participants showed generally satisfactory agreements for nutrients and chlorophyll *a* measurements. Thus, the nutrients and chlorophyll *a* concentrations taken into account in the current report are as follows:

- for the Romanian waters, the concentrations gathered by NIMRD (nutrients) and GEOECOMAR (chlorophyll a) were considered;
- for the Bulgarian waters, the concentrations gathered by IO-BAS were considered;
- for the Turkish waters, the concentrations gathered by TUBITAK were considered.

All maps were made with Ocean Data View (ODV) software version 4.5.6.
ODV is a computer program for the interactive exploration that displays data in two basic ways: (1) either by showing the original data at the data locations as colored dots of user-defined size or by projecting the original data onto equidistant or variable resolution rectangular grids and then displaying the gridded fields. Method 1 produces the most elementary and *honest* views of the data, instantly revealing occasional bad data values and regions of poor sampling. In contrast, method 2 produces *nicer* plots and avoids the overlapping of the colored dots that occurs with method 1 (Fig.IV.12), especially for large dot-sizes. Users should note, however, that the gridded fields of method 2 (Fig.IV.12) are actually data products and that small scale or extreme features in the data may be modified or lost as a consequence of the gridding procedure (weighted-average gridding) (Schlitzer, 2014).
All ODV representations done within the scope of this assessment have used method 2 with sampling stations marked as black dots (Fig.IV.12).



(a) (b)

Figure IV.12. Examples of ODV maps done by method 1 - original data (a) and method 2 – data products by weighted average gridding.

RESULTS and DISCUSSIONS

A. Causes of eutrophication - Nutrients levels

Due to the natural variability, the influence of the nutritional factor in the temperate zone is generally based on the following facts: maximum nutrients concentrations are found at the end of the winter and early spring, shortly before phytoplankton blooms, then sharp decrease of the nutrient concentrations after spring blooms which persisted often until autumn; changes into nutrients ratio are similar with those from phytoplankton populations. Thus, the biogenic elements reservoir controls directly the phytoplankton development and the Liebig Law (of the minimum) permits us to state that this development is directly controlled by that nutrient with minimum concentrations. A normal nutrition requires a stable ratio (Redfield ratio) within the main elements, C:N:P=106:16:1. Additionally, diatoms need, among other nutrients, silicic acid to create biogenic silica for their frustules. As a result of this, the Redfield-Brzezinski nutrient ratio was proposed for diatoms and stated to be C:Si:N:P = 106:15:16:1 (Brzezinski, 1985). If this ratio is deeply impaired (mainly due to the anthropogenic influence) the photosynthetic activity is altered. Usually, the nutrients concentrations of the phytoplankton are higher than those of the seawater, thus it is outlined the role of the biological regeneration, nutrients input form the water masses circulation, resuspension from sediments, etc.

In the attempt to find a distinction between natural variability and anthropogenic impact the assessment of the nutrients level in the western Black Sea took into consideration phosphate, silicate and inorganic nitrogen forms at the surface (horizontal distribution) and within water column (vertical distribution).

A.1. Horizontal (surface) distributions

The nutrients content of the surface layer, well oxygenated, is mainly influenced by the anthropogenic input (from land based sources including rivers and atmospheric deposition) and the winds and currents regime. In the area, all the rivers input has a significant influence on the shallow waters, less saline and nutrients enriched waters from the northern western Black Sea being pushed towards the southern shelf by the wind driven surface currents and the regional circulation oriented north to south down to the western Black Sea (shelf waters) confirmed by the mean fields of the sea surface currents (2.5m) one day prior sampling (22 July 2013) (MyOcean Black Sea Physics Analysis and Forecast - *http://www.myocean.eu/web/69-myocean-interactive-catalogue.php/*) and salinity distribution (Fig.IV.13 and IV.14).

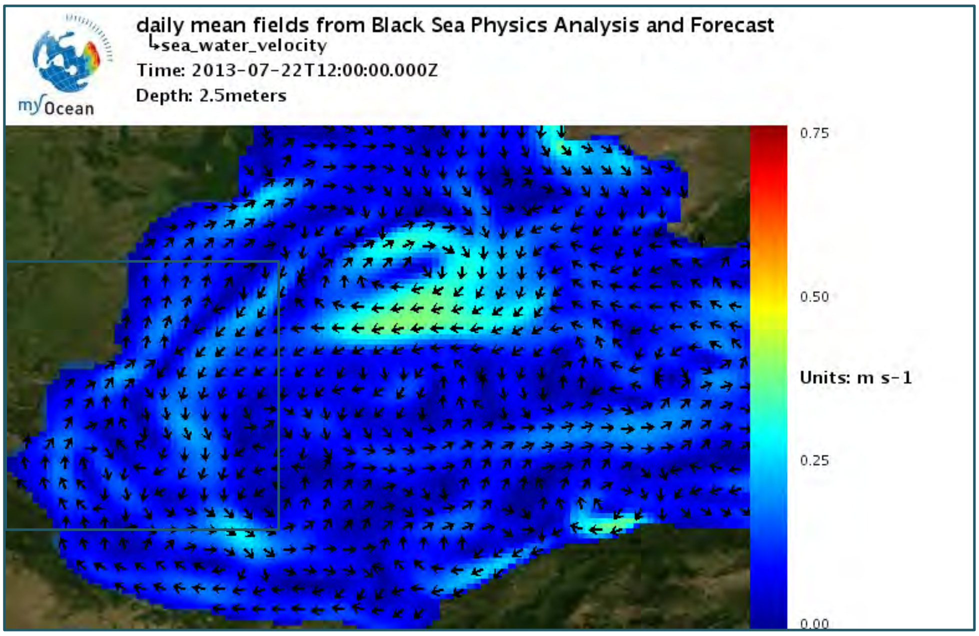


Figure IV.13. Mean fields of the sea surface currents (2.5m) one day prior sampling (22 July 2013) (MyOcean Black Sea Physics Analysis and Forecast - *http://www.myocean.eu/web/69-myocean-interactive-catalogue.php/*) – red square delimiting approx. the sampling area.

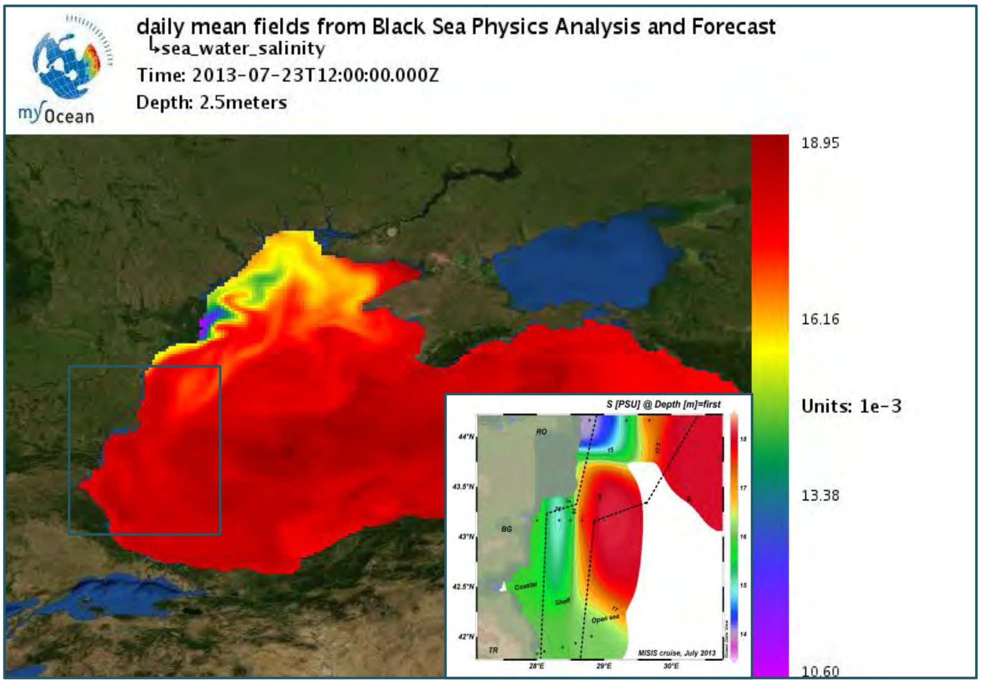


Figure IV.14. Salinity – Daily mean fields one day prior sampling (22 July 2013) (MyOcean Black Sea Physics Analysis and Forecast
http://www.myocean.eu/web/69-myocean-interactive-catalogue.php/) – black square delimiting approx. the sampling area **and Horizontal distribution, MISIS cruise, 23 July 2013.**

In this context, at surface, the highest values of the phosphate and silicate concentrations were found in the Romanian shelf waters, located closest to the NW rivers influence area. For phosphate, the 0.10µM isoline delimits two significant different areas (t-test, p=0.0251) with average concentrations of 0.45µM respectively 0.07µM. The main difference between this areas consists of the highest variability of the values from the northern part (Fig.IV.15).

In case of silicate, the 2.0µM isoline delineates the same two areas (t-test, p=0.0928) with average concentrations of 6.7µM respectively 1.0µM (Fig.IV.16).

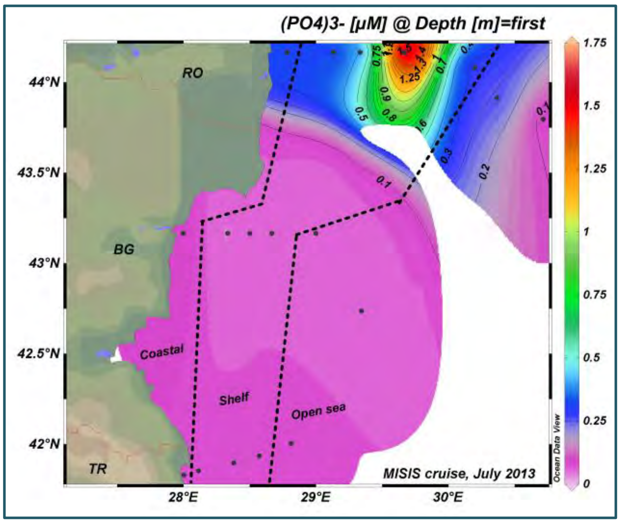


Figure IV.15. Phosphate concentrations - Horizontal distribution.

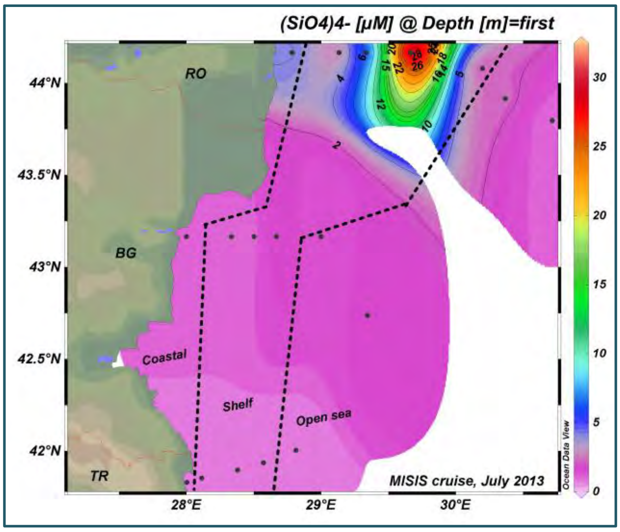


Figure IV.16. Silicate concentrations - Horizontal distribution.

Phosphate and silicate have had similar horizontal distributions, with lowest concentrations in the southern coastal area and highest in the shelf waters influenced by the currents regime and rivers input. Phosphate concentrations ranged within 0.04 – 1.63 µM (mean of 0.22µM, std.dev. 0.37µM). The general statistics of the different marine areas are included in the Table IV.5.

Table IV.5. Main statistics of the phosphate concentrations in the marine areas – Western Black Sea, July 2013.

Water body	N	Min. (µM)	Station	Max. (µM)	Station	Mean (µM)	Std. Dev. (µM)
Coastal	3	**0.08**	MO 18	**0.31**	MO 1	0.16	0.13
Shelf	10	**0.04**	MO 11	**1.63**	MO 4	0.29	0.48
Open sea	5	**0.05**	MO 8	**0.25**	MO 6	0.10	0.08

Silicate concentrations oscillated from 0.61µM to 30.90µM (mean 3.20µM and std.dev. 7.03µM) with the same pattern as phosphate, being significantly correlated each other (r^2=0.97) (Fig.IV.17).

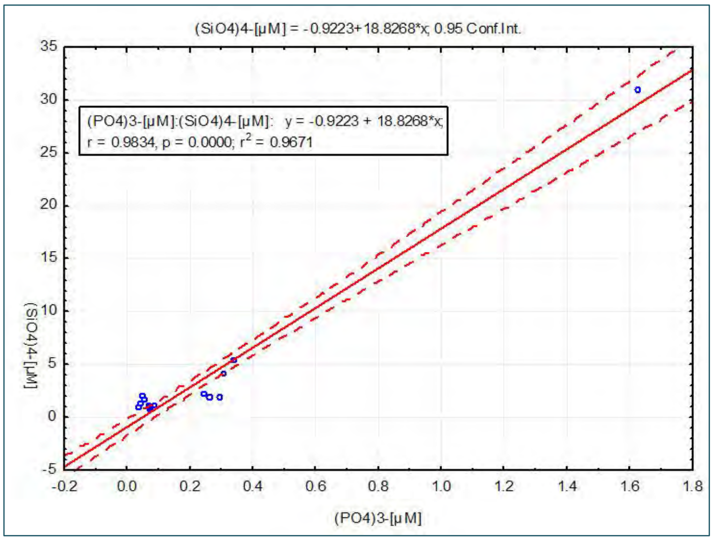


Figure IV.17. Scatterplot of silicate concentrations against phosphate concentrations

The main statistics of the different marine areas are included in the Table IV.6.

Table IV.6. Main statistics of the silicate concentrations in the marine areas – Western Black Sea, July 2013.

Water body	N	Min. (µM)	Station	Max. (µM)	Station	Mean (µM)	Std. Dev. (µM)
Coastal	3	**0.61**	MO 17, 18	**4.10**	MO 1	1.92	1.90
Shelf	10	**0.61**	MO 15, 16	**30.90**	MO 4	4.55	9.37
Open sea	5	**0.61**	MO 14	**2.20**	MO 6	1.29	0.64

In both cases, even if the phosphates and silicates concentrations were higher in the coastal areas, there were no significant differences between coastal, shelf and open seawaters (test F, p=0.6357 for phosphate and p=0.6847 for silicate) (Fig.IV.18 and IV.19).

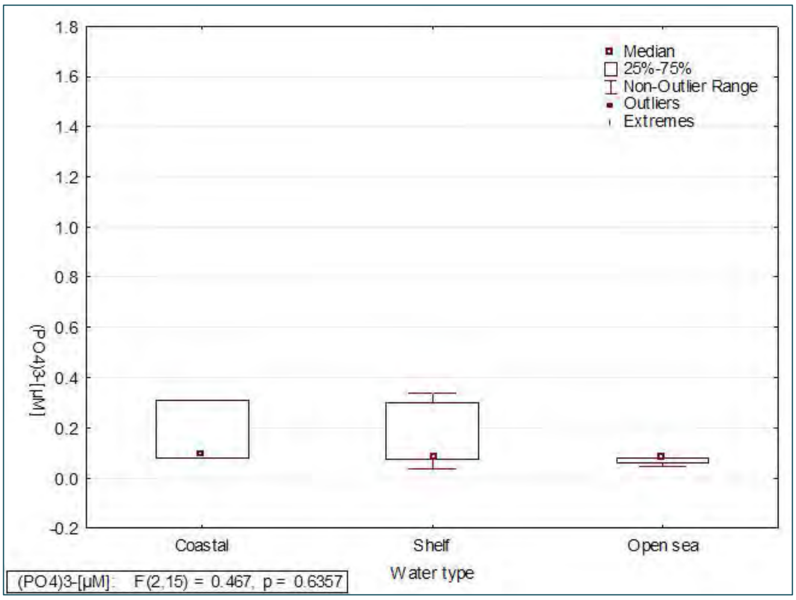


Figure IV.18. Boxplot of phosphate grouped by water type.

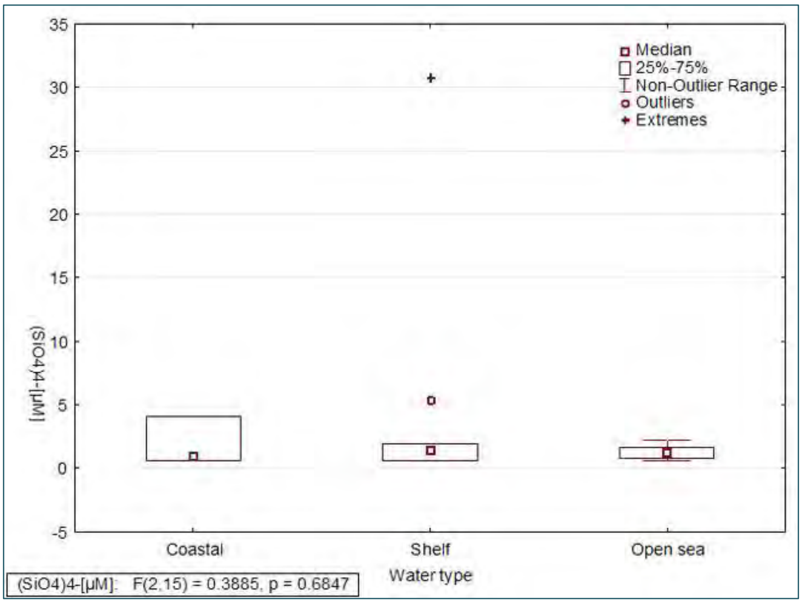


Figure IV.19. Boxplot of silicate grouped by water type.

Long-term data (1964-2013, surface, summer) for the Romanian shelf waters (East Constanta transect) highlighted decreasing trends for both phosphate (Fig.IV.20) and silicate (Fig.IV.21) concentrations. It is to note that data from MISIS cruise fitted well with the historical data.

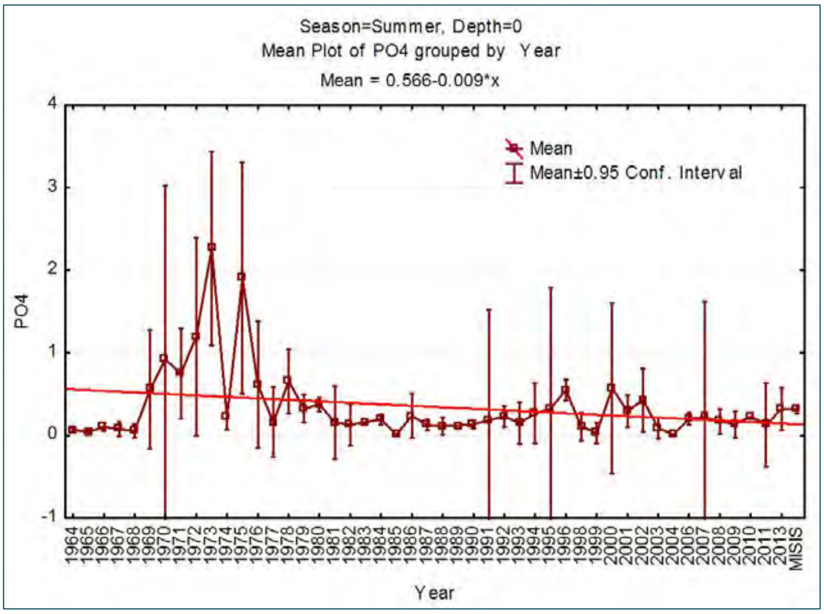


Figure IV.20. Long-term (1964-2013) compared with MISIS cruise data (2013) - mean phosphate concentrations, Romanian shelf waters.

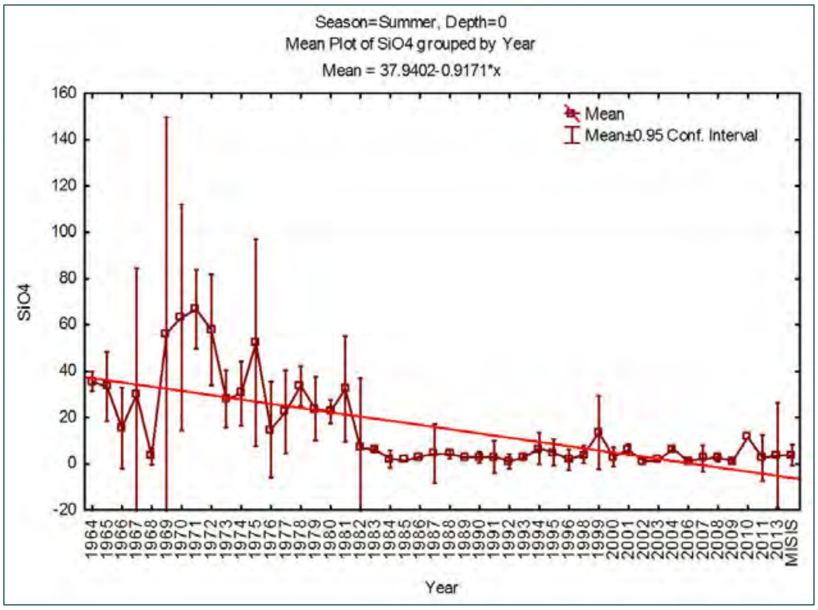
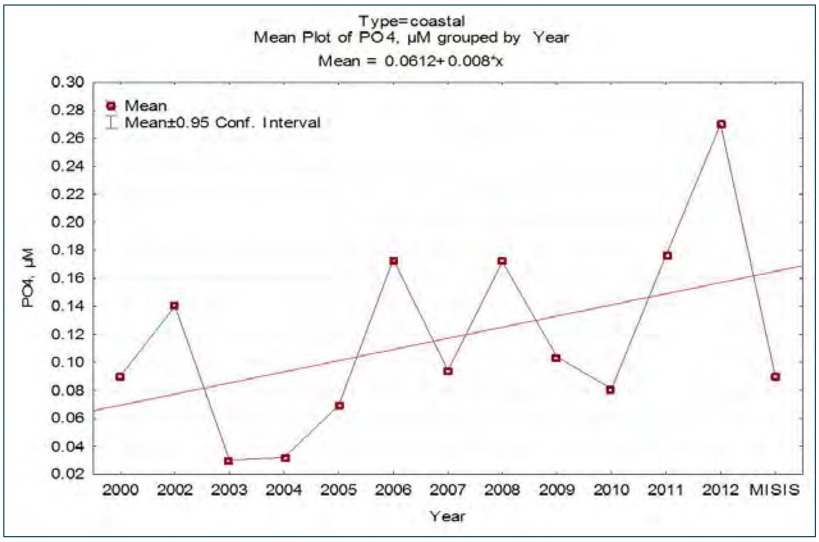


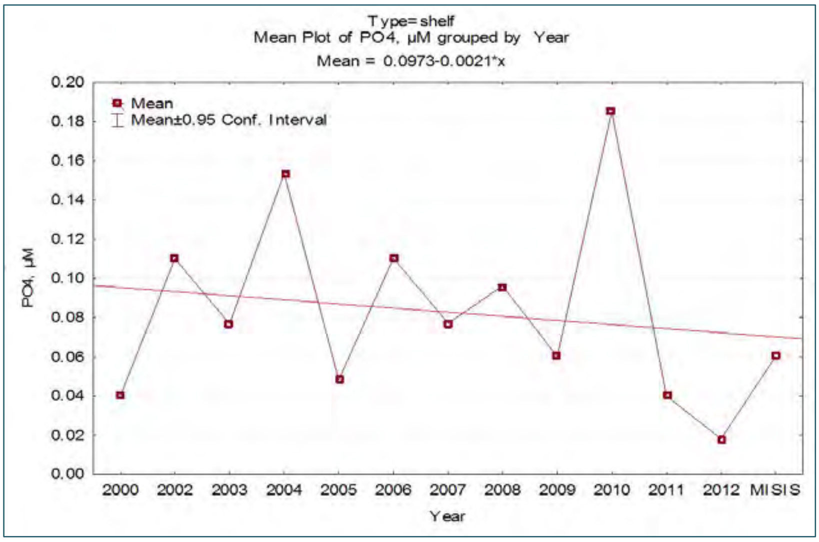
Figure IV.21. Long-term (1964-2013) compared with MISIS cruise data - mean silicate concentrations, Romanian shelf waters.

Long-term data (2000-2012, surface, summer) for the Bulgarian coastal, shelf and open waters compared with data from MISIS cruise highlighted generally low phosphate concentrations and different trends for coastal (increasing) and shelf and open waters (decreasing)(Fig.IV.22).

a)



b)



c)

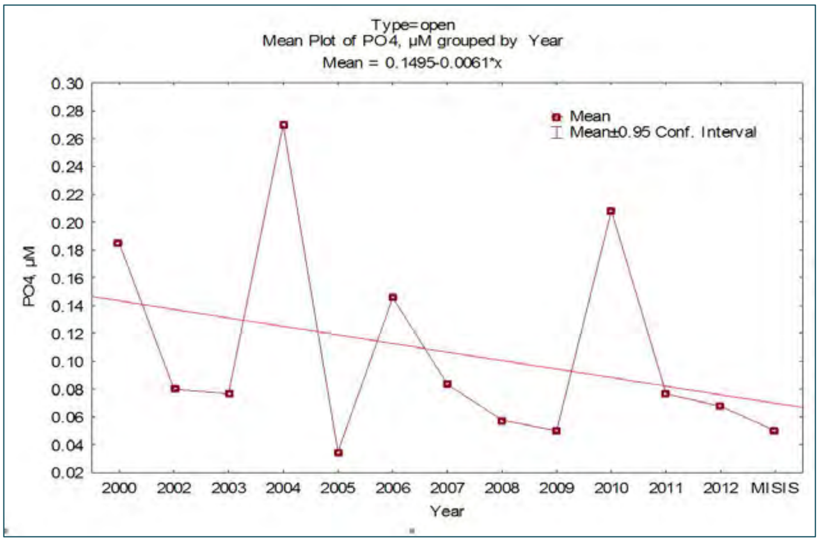


Figure IV.22. Long-term (2000-2012) compared with MISIS cruise data - mean phosphate concentrations, Bulgarian coastal (a), shelf (b) and open (c) waters

The comparisons with the existing data from the Turkish transect revealed higher variability in the coastal waters, where phosphates concentrations decreased values after 2005. On shelf, was observed less inter-annual variability with MISIS cruise values fitting well into the series (Fig.IV.23).

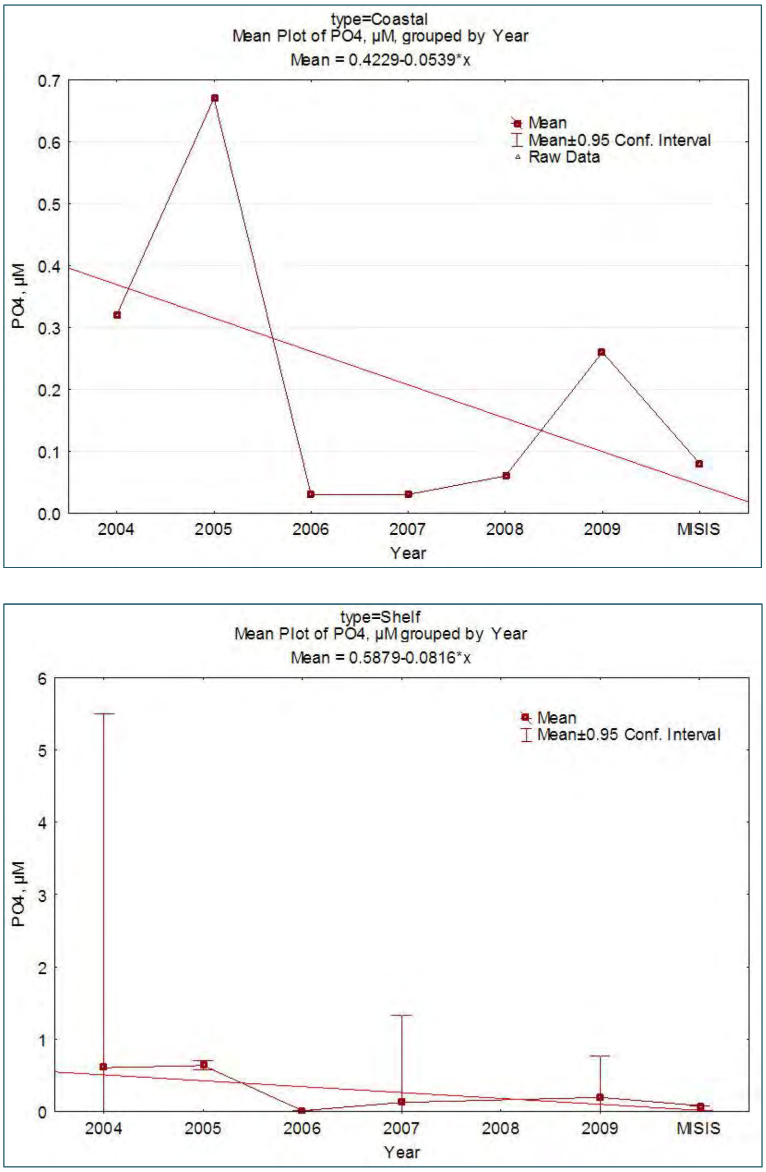


Figure IV.23. Existing data (2004-2012) compared with MISIS cruise data - mean phosphate concentrations, Turkish coastal and shelf waters

The inorganic forms of nitrogen (oxidized forms, TNOx, as sum of nitrite and nitrate and ammonium, NH_4^+) had different distributions, linked to their sources. Thus, for TNOx, besides the riverine loads which delineates through 1.00µM isoline the northern and southern areas (significantly different, t-test, $p<0.0001$), it is highlighted the anthropogenic influence of the Constanta's area, both port and WWTP. Along the coast it is also outlined the influence of the Varna's area, the second largest bay along the Bulgarian Black Sea coast (Fig.IV.24).

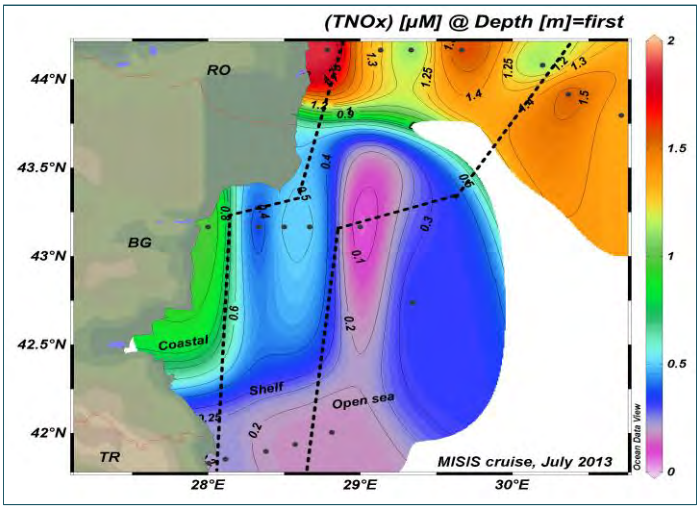


Figure IV.24. TNOx concentrations - Horizontal distribution, MISIS cruise, July 2013.

For ammonium, it seems that the main pressure, usually higher in the summer, driving to the maximum concentrations found in coastal area, is the neighbouring of the major cities of the littoral, Constanta and Varna (Fig.IV.25). Actually, ammonium is the dominant form of the inorganic nitrogen only in the coastal waters (Fig.IV.26).

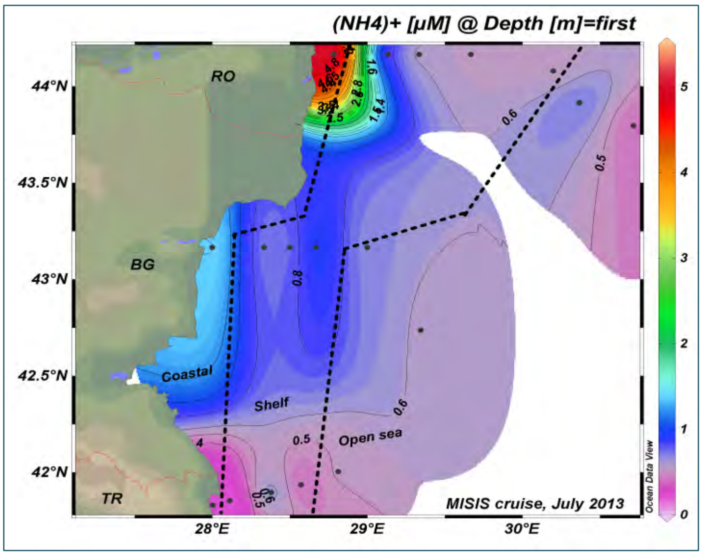


Figure IV.25. Ammonium concentrations - Horizontal distribution, MISIS cruise, July 2013.

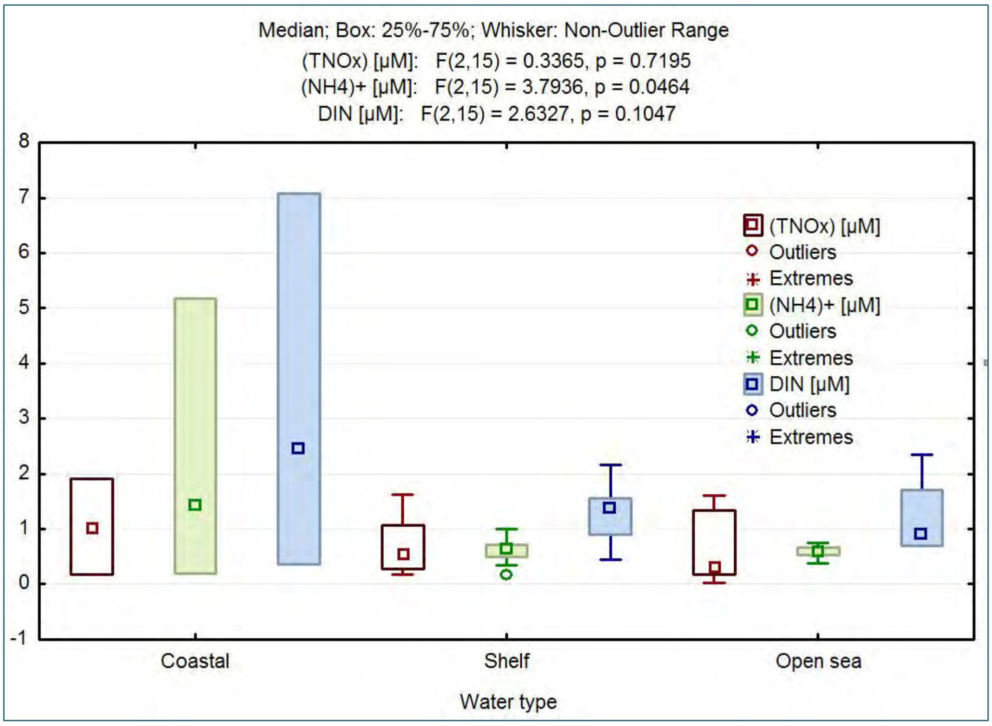


Figure IV.26. Box plot of multiple variables (TNOx, NH4, and DIN) grouped by water type - MISIS cruise, July 2013.

The dissolved inorganic nitrogen, DIN, followed the same pattern as its components, emphasizing their synergic effect. So, apart from the coastal area influence, it was observed a slight gradient from north to south (as the main current) that delimitates the areas through the 2.0μM isoline (Fig.IV.27).

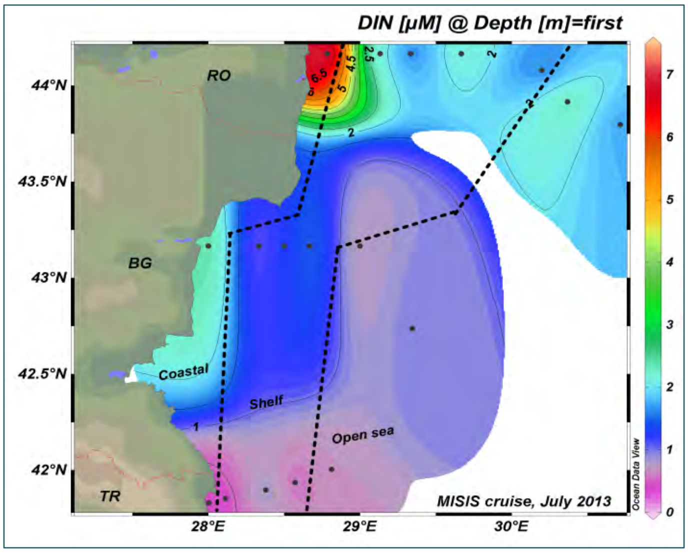


Figure IV.27. Dissolved inorganic nitrogen concentrations - Horizontal distribution, MISIS cruise, July 2013.

The general statistics of the different marine areas are included in Table IV.7.

Table IV.7. Main statistics of the inorganic nitrogen concentrations, DIN (sum of nitrite, nitrate and ammonium) in the marine areas – Western Black Sea, July 2013

Water body	N	Min. (µM)	Station	Max. (µM)	Station	Mean (µM)	Std. Dev. (µM)
Coastal	3	**0.37**	MO 18	**7.08**	MO 1	3.29	3.44
Shelf	10	**0.45**	MO 17	**2.17**	MO 4	1.29	0.58
Open sea	5	**0.69**	MO 14	**2.35**	MO 6	1.28	0.73

Long-term data (1980-2013, surface, summer) for the Romanian shelf waters (East Constanta transect) highlighted decreasing trend for inorganic nitrogen (DIN as sum of nitrate, nitrite and ammonium) concentrations (Fig.IV.28). It is to note that data from MISIS cruise fitted well with the historical data and trend.

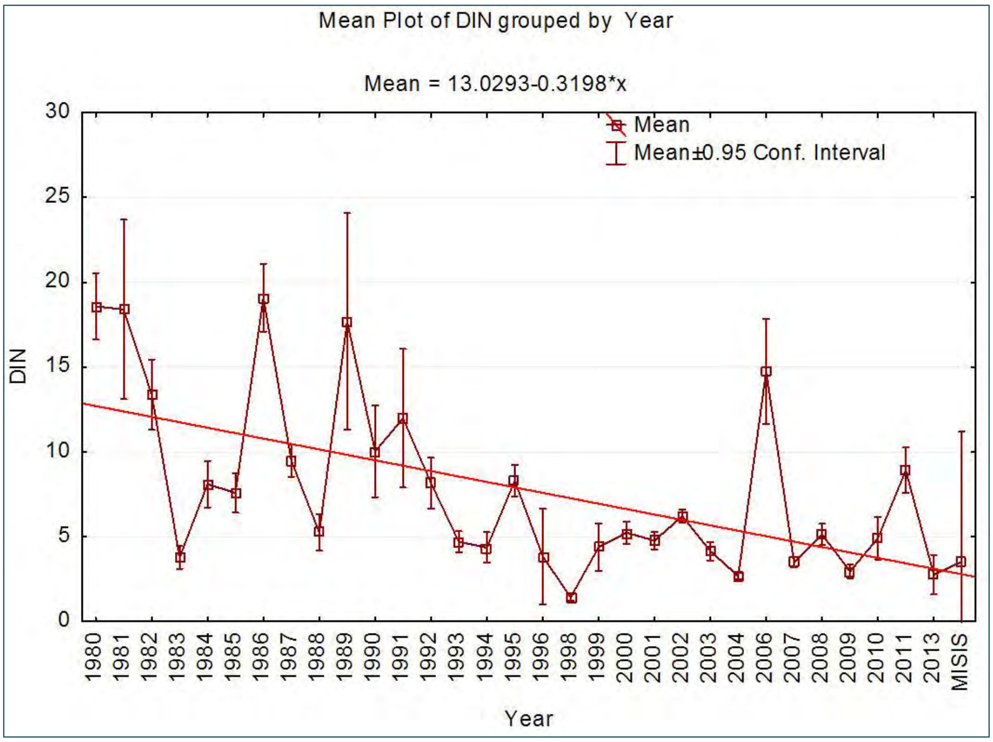
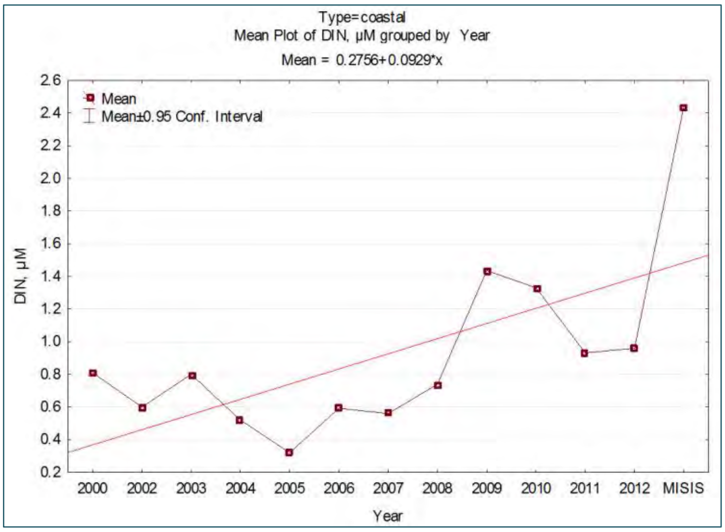
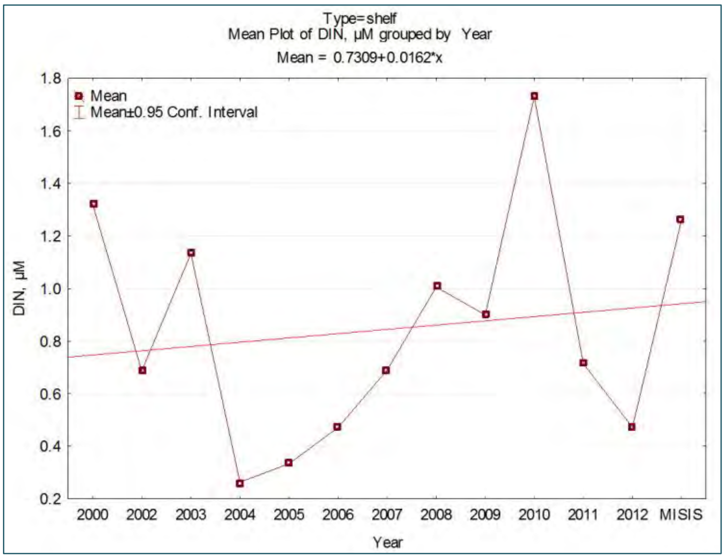


Figure IV.28. Long-term (1980-2013) compared with MISIS cruise data (2013) - mean Dissolved Inorganic Nitrogen DIN (µM) concentrations, Romanian shelf waters.

Long-term data (2000-2012, surface, summer) for the Bulgarian coastal, shelf and open waters compared with data from MISIS cruise highlighted generally low DIN concentrations and an increasing trend for coastal and shelf waters while the concentrations are not defining yet a trend in the open waters (Fig.IV.29).



a)



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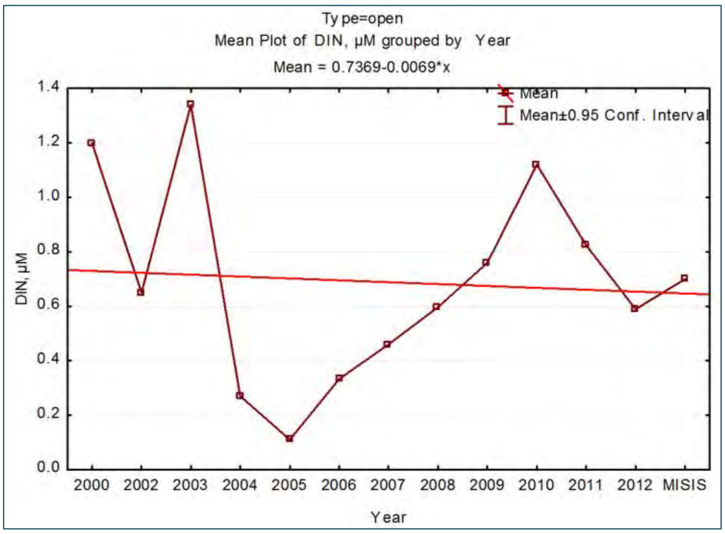
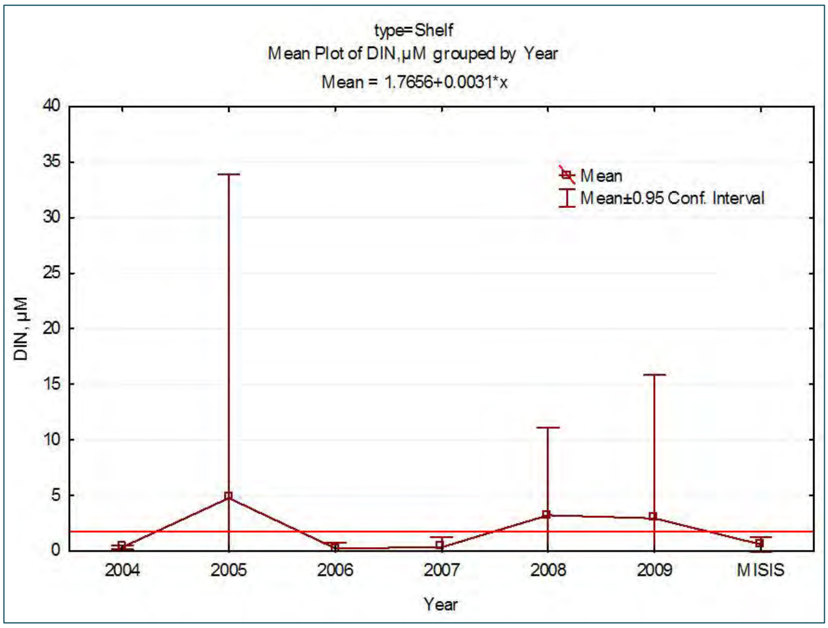


c)

Figure IV.29. Long-term (2000-2012) compared with MISIS cruise data - mean Dissolved inorganic Nitrogen, DIN, concentrations, Bulgarian coastal (a), shelf (b) and open (c) waters

Existing data (2004-2009, surface, end of summer) for the Turkish coastal and shelf waters compared with data from MISIS cruise showed the highest variability in the coastal zone. However the values from 2013 (MISIS) interrupted the four years increasing concentrations series. On the shelf values were homogenous (Fig.IV.30).



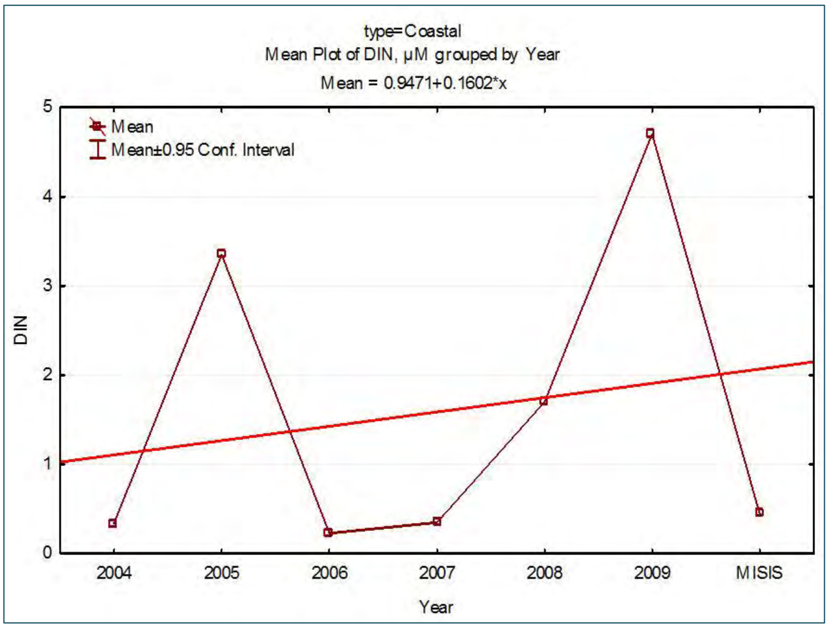


Figure IV.30. Existing data (2004-2012) compared with MISIS cruise data - Dissolved inorganic Nitrogen, DIN, concentrations, Turkish coastal and shelf waters.

A.2. Water column distributions

The seasonal variations of the nutrients concentrations mainly appear in the surface layer as biological activity result. In spring, the intensity and the duration daylight causes the phytoplankton's blooms, which results in a sharply decrease of the nutrients from the photic zone. Zooplankton and fish consume a large part of phytoplankton and the nutrients are returned to the sea as metabolic products. In the same time, in spring, the vertical mixing contributes to the replenishment by bringing the nutrients rich water from the bottom in the upper zone. However, early summer, the sun heating causes the development of the thermocline (at 20-40m) that inhibits the vertical mixing. Subsequently, the upper thermocline layer is nutrients depleted and the thermocline is acting as a barrier.

Phosphates

In the coastal waters from the temperate zone, the seasonal variations occur both in phosphates and organic phosphorus compounds. In the winter, most of the phosphorus is present as phosphates. Their concentrations are sharply decreased in early spring, being used in the phytoplankton proliferation. The feeding with phytoplankton of zooplankton and fish returns the phosphorus from their excretions to seawater both as phosphates and organic compounds. The latter become dominant in May-June. After blooms, the phosphates regeneration is quickly done from detritus (Armstrong, 1965; Peres, 1961).

In the open waters the concentrations from the surface layer depend on water masses exchange from deeper layers and become higher in the upwelling areas. There is a remarkable correlation between phosphate concentrations in the surface layers and zooplankton content. The latter represents an indirect measure between phytoplankton availability as food and productivity as well. The phosphates concentrations sharply increase near thermocline as result of the detritus oxidation and zooplankton activity (Dafner, 2007). The maximum concentrations are usually found nearby oxygen minimum or carbon dioxide maximum. In the deeper layers (below maximum phosphate depth) the vertical diffusion, where available, makes the distribution more or less uniform.

The phosphate content of the shallow water column, in the coastal areas, was quite homogenous with slightly increased values in the stations under the rivers influence (Table IV.8). For shelf and open waters there is also a trend of slightly increased values in the Romanian transect where is the longest shelf, under the influence of direct river discharges and winds and currents regime (Fig.IV.32).

Table IV.8. Main statistics for phosphate concentrations – water column, MISIS cruise, July 2013

Parameter	Area	N	Min. (µM)	Depth (m)	Sig-T	Max. (µM)	Depth (m)	Sig-T	Mean (µM)	Std. Dev. (µM)
Phosphate $(PO_4)^{3-}$	**Coastal**	**9**	**0.01**	12	10.6	**0.88**	14	10.6	0.22	0.28
	Shelf	43	**0.04**	15	8.8	**4.15**	96	14.5	0.54	0.75
	Open sea	39	**0.04**	12	12.3	**3.28**	165	16.2	0.72	0.81

Generally, the phosphate concentrations reached two peaks: a first maximum near thermocline, at 15.4-15.7 Sig T (85-110 m depth) (0.63 – 1.97µM) and the second at 16.0-16.2 Sig.T (180-200m) (1.10 – 3.28µM) in the minimum oxygen layer. The minimum concentrations were found in the upper mixed layer above the oxic/anoxic interface, 9.0 – 14.0 Sig T (0-50m) (Figs.IV.31 and IV.32).

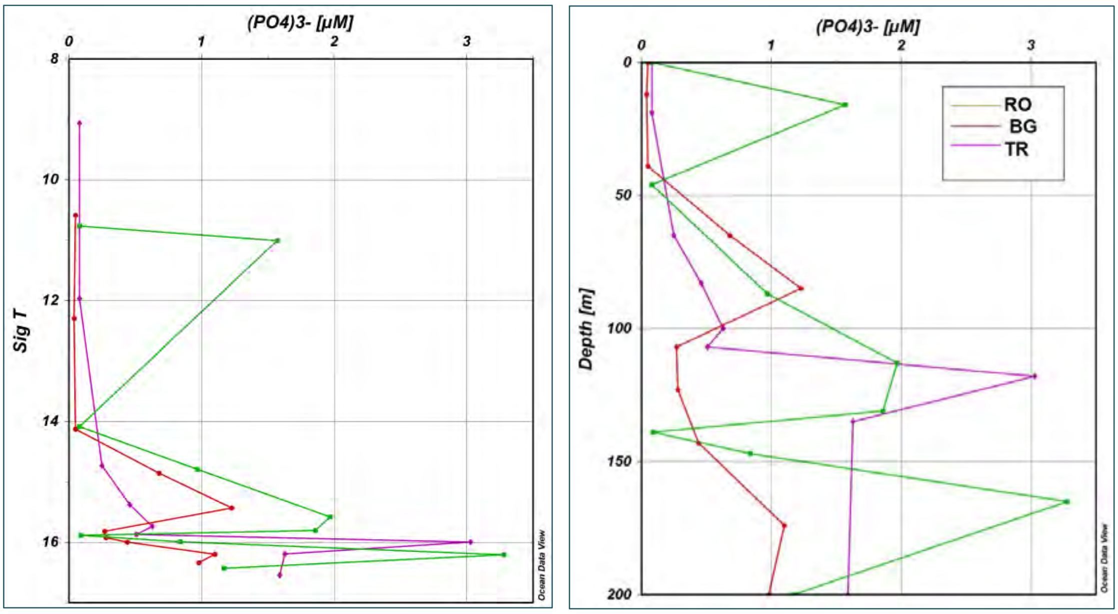


Figure IV.31. Phosphate concentrations versus SigT (L) and depth (R) in the water column – open waters, Western Black Sea, MISIS cruise, July 2013.

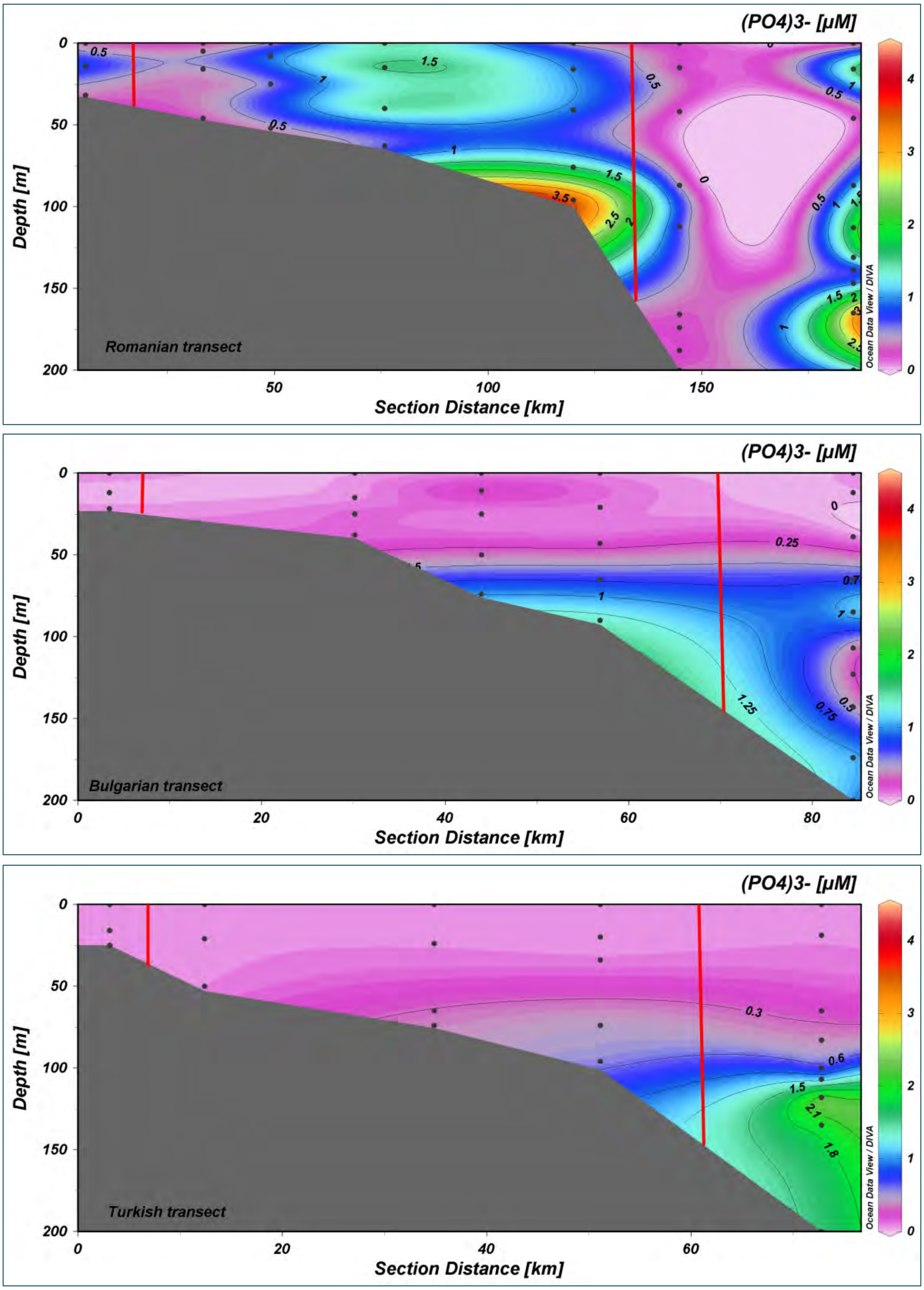


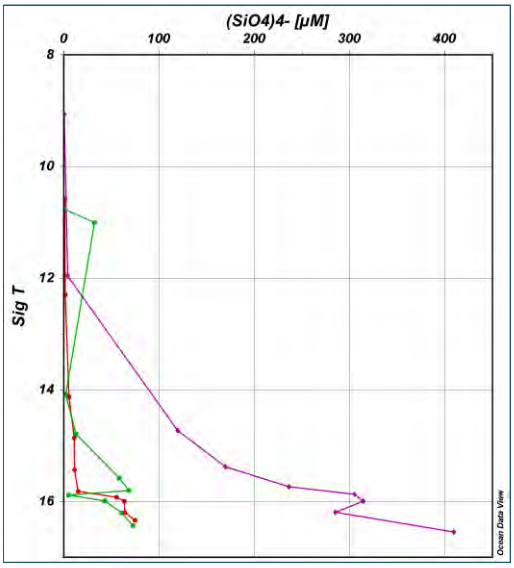
Figure IV.32. Phosphate distribution in the water column – coastal, shelf and open waters, Western Black Sea, MiSIS cruise, July 2013.

Silicates

The silicate content of the coastal waters is generally comparable and rose due to the terrigenous input. Due to the phytoplankton blooms, particularly diatoms, the seasonal variations are similar with the phosphates: sharp decreases in spring and starting of the regeneration in summer up to highest values from winter, sometimes interrupted by the autumnal bloom (Riley, 1971). In the open waters, the concentrations are usually low, excepting the upwelling events. Generally, they go up with the depth (Table IV.9 and Fig.IV.33). Thus, in the absence of rivers input, the lowest values were found at the surface nearby the Turkish transect and the highest at maximum sampling depth (Fig.IV.33). It is to note that, in the same area, in the water column the silicate concentrations were considerably higher overall areas. Since the intercalibration results were the poorest for silicates, could be also a methodological difference between analyses, rising in different results (Fig.IV.33). The silicate and phosphate concentration were best correlated into the open sea waters (r=0.48) than into shelf (r=0.28) and coastal waters (r=0.01) highlighting the same driving forces (water masses mixing) and, possibly, the anthropogenic disturbances.

Table IV.9. Main statistics for silicate concentrations – water column, MISIS cruise, July 2013.

Parameter	Area	N	Min. (µM)	Depth (m)	Sig-T	Max. (µM)	Depth (m)	Sig-T	Mean (µM)	Std. Dev. (µM)
Silicate $(SiO_4)^{4-}$	Coastal	9	0.61	0	9.1	59.30	25	13.8	11.35	18.81
	Shelf	43	0.61	0	8.7	175.18	96	15.3	23.92	36.88
	Open sea	39	0.61	0	9.1	409.42	200	16.3	67.15	104.88



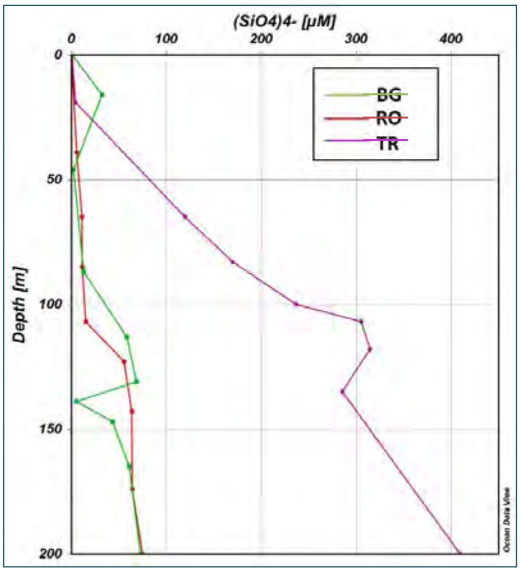


Figure IV.33. Silicate concentrations versus SigT (L) and depth (R) in the water column – open waters, Western Black Sea, MISIS cruise, July 2013.

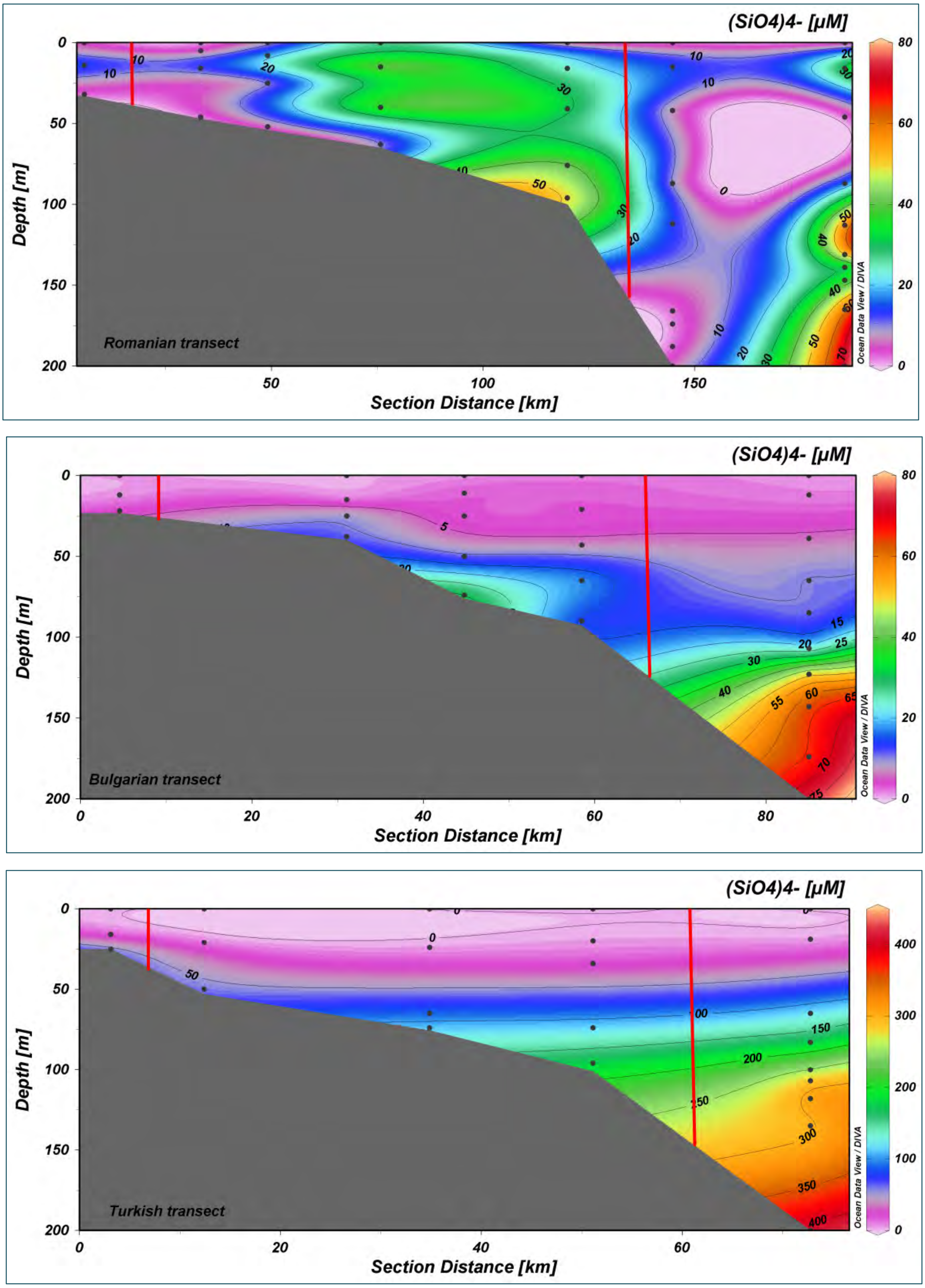


Figure IV.34. Silicate distribution in the water column – coastal, shelf and open waters, Western Black Sea, MISIS cruise, July 2013.

Nitrogen forms

The seasonal variations of the nitrate, nitrite and ammonium concentrations occur in the surface layer as a result of the biological activity. In spring, the phytoplankton proliferation into the coastal waters results in the sharp decrease of the inorganic nitrogen concentrations from the euphotic zone. The phytoplankton is consumed by zooplankton and fish and the nitrogen is returned as excretion products (e.g. ammonium and urea). The vertical mixing contributes as well to the replenishment of the inorganic nitrogen stock. In the summer, due to the presence of the thermocline, the vertical mixing is inhibited and the upper mixed layer is depleted of inorganic nitrogen. Usually, at this time the main form is ammonium from excretions and rapidly assimilated by phytoplankton again driving to the constant regeneration of nitrate. Nitrification is complete up to January when the thermocline breaking allows the homogenous nitrate distribution into the water column (Peres, 1961; Riley, 1971; Horne, 1969; Jensen, 2009).

Table IV.10. Main statistics for oxidized nitrogen forms (sum of nitrite and nitrate) and ammonium concentrations – water column, MISIS cruise, July 2013.

Parameter	Area	N	Min. (µM)	Depth (m)	Sig-T	Max. (µM)	Depth (m)	Sig-T	Mean (µM)	Std. Dev. (µM)
TNOx	Coastal	9	0.07	0	9.1	2.48	25	12.6	1.34	0.93
	Shelf	43	0.17	20	12.8	4.54	38	14.0	1.33	0.99
	Open sea	39	0.02	200	16.3	4.87	85	15.4	1.20	1.00

The oxidized nitrogen forms (sum of nitrite and nitrate), TNOx, generally showed quite homogeneous concentrations in the upper mixed layer (slight increased values on surface – coastal station, Romanian transect). Due to better stratified layers in the Bulgarian and Turkish transect it was observed the maximum (2.51-4.87µM) at 15.4 Sig T, above the oxycline, where the nitrification processes are prevailing and the nitrate is regenerated. Then, once the oxygen depletion started the concentrations are continuously went down due to denitrification dominance in the suboxic conditions (Fig.IV.35 and IV.36).

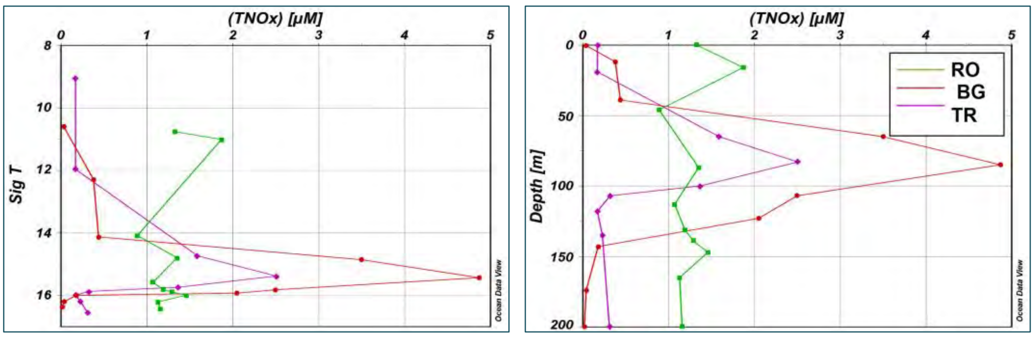


Figure IV.35. TNOx concentrations versus SigT (L) and depth (R) in the water column – open waters, Western Black Sea, MISIS cruise, July 2013.

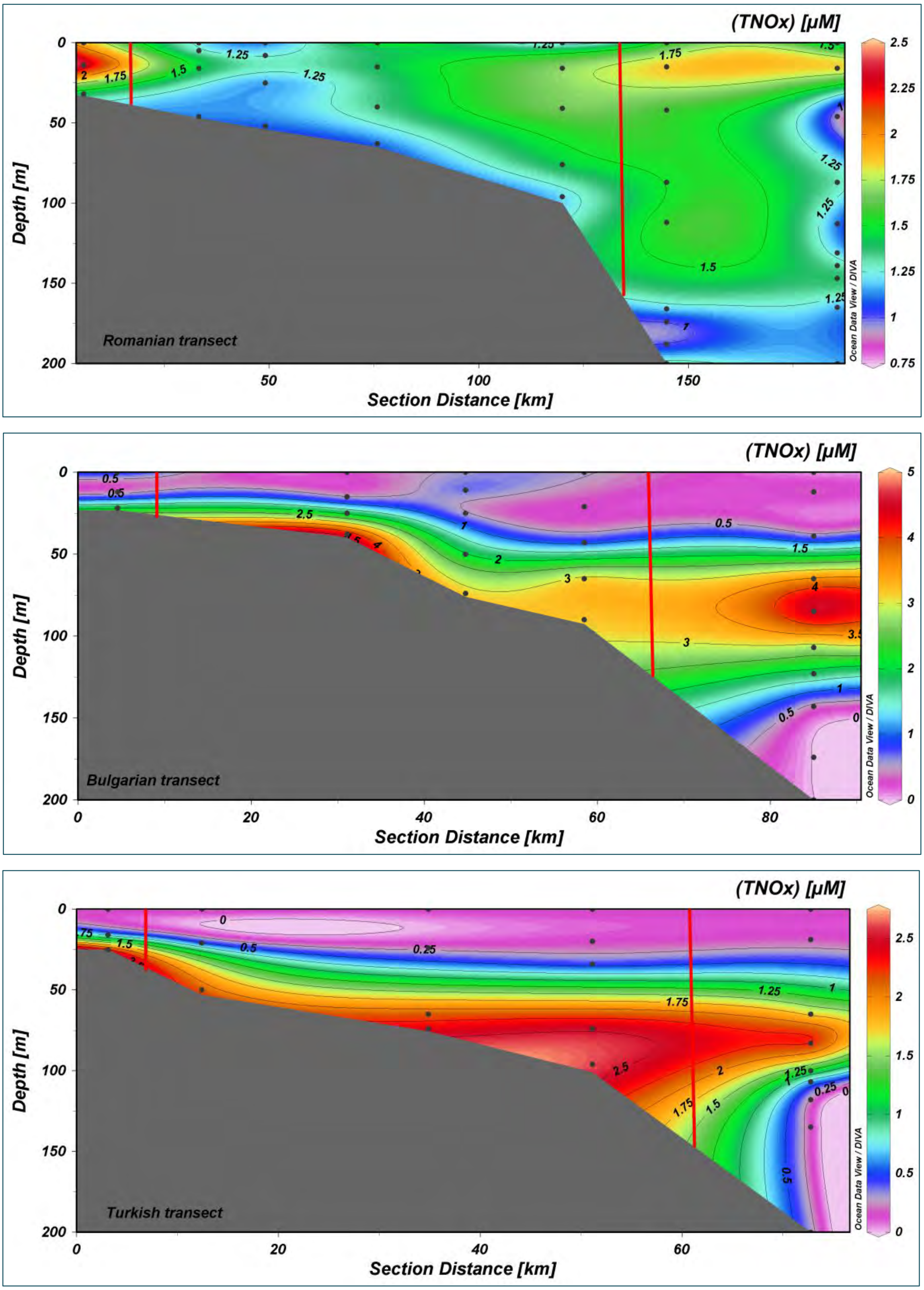


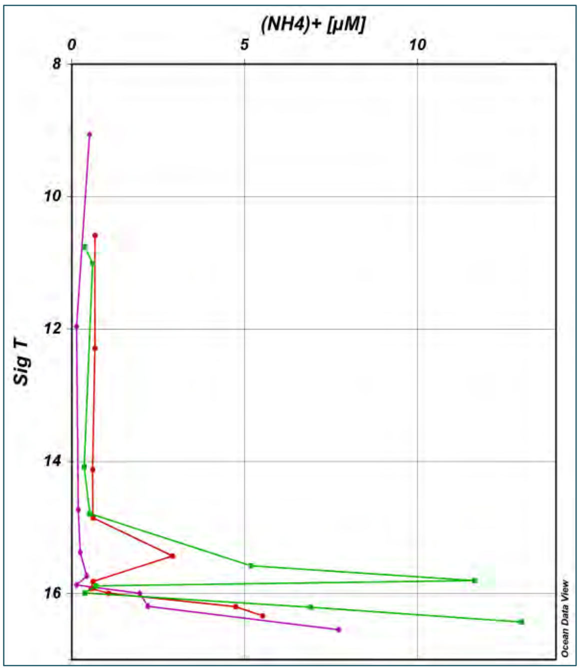
Figure IV.36. TNOx distribution in the water column – coastal, shelf and open waters, Western Black Sea, MISIS cruise, July 2013.

Ammonium

Ammonium generally showed homogenous and low concentrations in the oxic layer. The peak was observed below oxycline (15.4-15.8SigT). Then, once the oxygen depletion started, the ammonium concentrations were continuously went up due to remineralization processes and reached maximum values 5.51-13.01µM (Fig.IV.37 and IV.38).

Table IV.11. Main statistics for ammonium concentrations – water column, MISIS cruise, July 2013.

Parameter	Area	N	Min. (µM)	Depth (m)	Sig-T	Max. (µM)	Depth (m)	Sig-T	Mean (µM)	Std. Dev. (µM)
Ammonium $(NH_4)^+$	Coastal	9	0.20	0	9.1	5.18	0	8.0	1.55	1.55
	Shelf	43	0.14	50	13.8	2.53	96	14.4	0.72	0.43
	Open sea	39	0.14	19	12.0	13.01	200	16.4	2.09	3.11



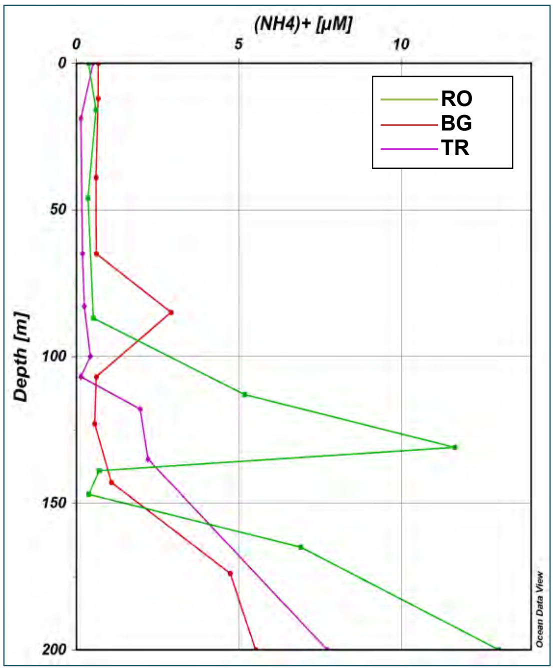


Figure IV.37. Ammonium concentrations versus SigT (L) and depth (R) in the water column – open waters, Western Black Sea, MISIS cruise, July 2013.

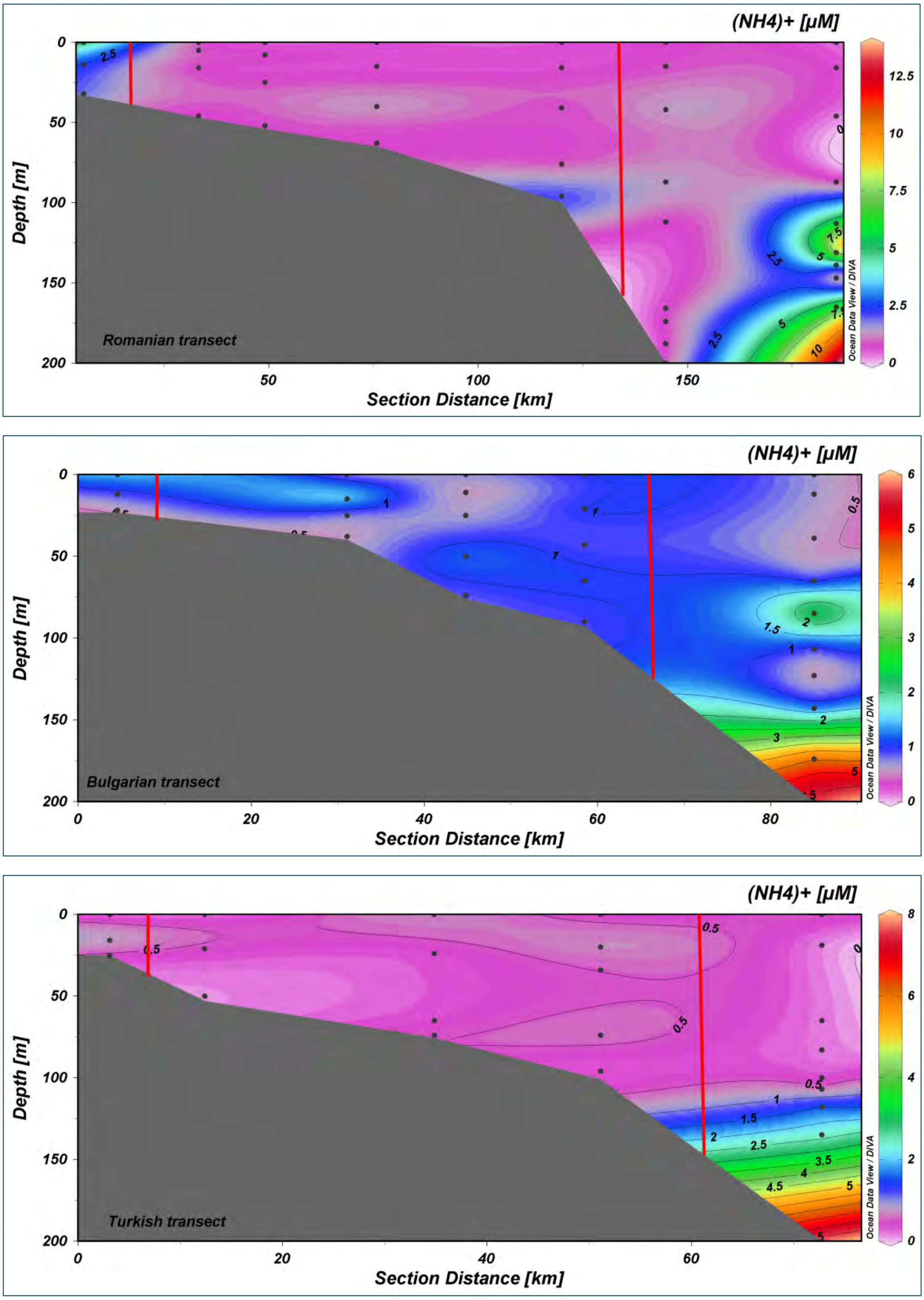


Figure IV.38. Ammonium distribution in the water column – coastal, shelf and open waters, Western Black Sea, MISIS cruise, July 2013.

A.3. Ratios

The optimal N/P ratio for phytoplankton growth is 16:1 (based on molar concentrations) and is called the Redfield ratio. Significant deviations from 16 at low N/P-ratios (below 10) might indicate potential nitrogen limitation and at high N/P ratio (above 17) potential phosphorus limitation of phytoplankton growth. Deviations in the range 10-16 indicate that either of the nutrients may be limiting (Ekhlom, 2008). This might affect the biological state of the ecosystem, in particular the phytoplankton biomass, species composition and eventually food web dynamics [1]. Anthropogenic eutrophication in coastal environment results from increased delivery of land-based nutrients considerably enriched in nitrogen (N) and phosphorus (P) compared to silicon (Si). These nutrient inputs strongly modify the nutrient balance N:P:Si in the coastal waters with respect to phytoplankton stoechiometry, *i.e.* N:P=16 for marine phytoplankton (Redfield et al., 1963) and N:Si=1 for coastal diatoms (Bzrezinski, 1985). This in turn modifies the composition of the phytoplankton community characterized by a dominance of opportunistic non-siliceous species (Officer & Ryther, 1980; Billen et al.,1991).

In contrast to N and P riverine fluxes (which have been strongly modified in the past 50 years), silica fluxes (which originate essentially from the weathering of rocks) has remained rather constant or even decreased, due to eutrophication and/or trapping in reservoirs. Therefore silica has become a limiting factor for river diatoms in the main branch of the large rivers resulting in lower Si/N and Si/P ratios in estuaries and coastal regions. Whereas increased N, P deliveries to the coastal zone are recognized as a major threat to the ecological functioning of near shore coastal ecosystems, less attention has been paid to their imbalance in regard to silica (Officer & Ryther, 1980; Conley et al., 1993; Turner & Rabalais, 1994; Justic et al.,1995a; Justic et al., 1995b; Billen & Garnier, 1997; Turner et al., 1998; Conley, 1999; Humborg et al., 2000; Cugier et al., 2005; Billen & Garnier, 2007; Humborg et al., 2008). However, Si/P and Si/N ratios determine the phytoplankton community structure, especially the shift from diatoms to non-diatoms and these changes may have major impacts on water quality in the proximal, i.e. nearshore part of the coastal zone (Turner et al., 2003; Cugier et al., 2005; Howarth & Marino, 2006) [2].

Ratios were calculated based on inorganic nitrogen concentrations (sum of oxidized forms and ammonium) as N, phosphate concentrations as P and silicate concentrations as Si (Table IV.12).

[1] www2.dmu.dk/1_Viden/2_Miljoetilstand/3_vand/4_eutrophication/nutrient.asp

[2] www.nine-esf.org/sites/nine-esf.org/files/ena_doc/ENA_pdfs/ENA_c8.pdf

Table IV.12. Main statistics for nutrients ratios – surface, MISIS cruise, July 2013.

Ratio	Area	Min.	Max.	Mean	Deviation	Std. Dev.
N/P	Coastal	4.6	26.4	18.0	P limited	11.7
	Shelf	1.3	27.7	11.3	No trend	9.5
	Open sea	8.7	21.4	14.0	No trend	5.2
Si/P	Coastal	7.6	13.2	10.7	Si limited	2.8
	Shelf	6.0	35.0	14.3	No trend	9.5
	Open sea	7.6	26.8	16.0	No trend	0.6
Si/N	Coastal	0.4	1.7	0.9	No trend	0.7
	Shelf	0.7	14.2	2.6	N limited	4.2
	Open sea	0.5	1.8	1.2	No trend	0.6

Coastal waters from the anthropogenic influenced areas (Romanian and Bulgarian transects) showed both phosphorus and silicon limitations and high variability (expressed as standard deviation) which is important in term of phytoplankton species shift floristic composition. Meanwhile, for the Turkish transect it was observed a nitrogen limitation overall. Generally, the open waters, controlled by the biological activity and water masses mixing are the most balanced (Fig. IV.39).

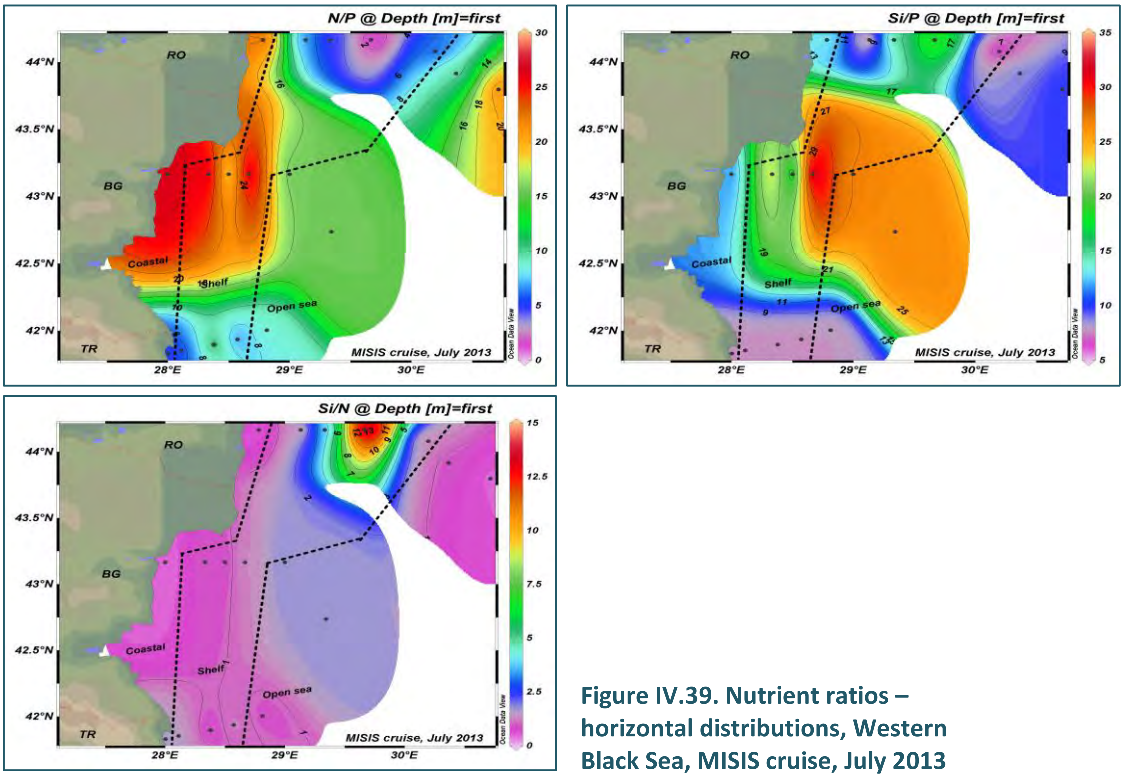


Figure IV.39. Nutrient ratios – horizontal distributions, Western Black Sea, MISIS cruise, July 2013

Nutrients ratios in the water column were the most variable in the coastal waters for N/P and Si/P and in the open waters for Si/N (Fig.IV.40).

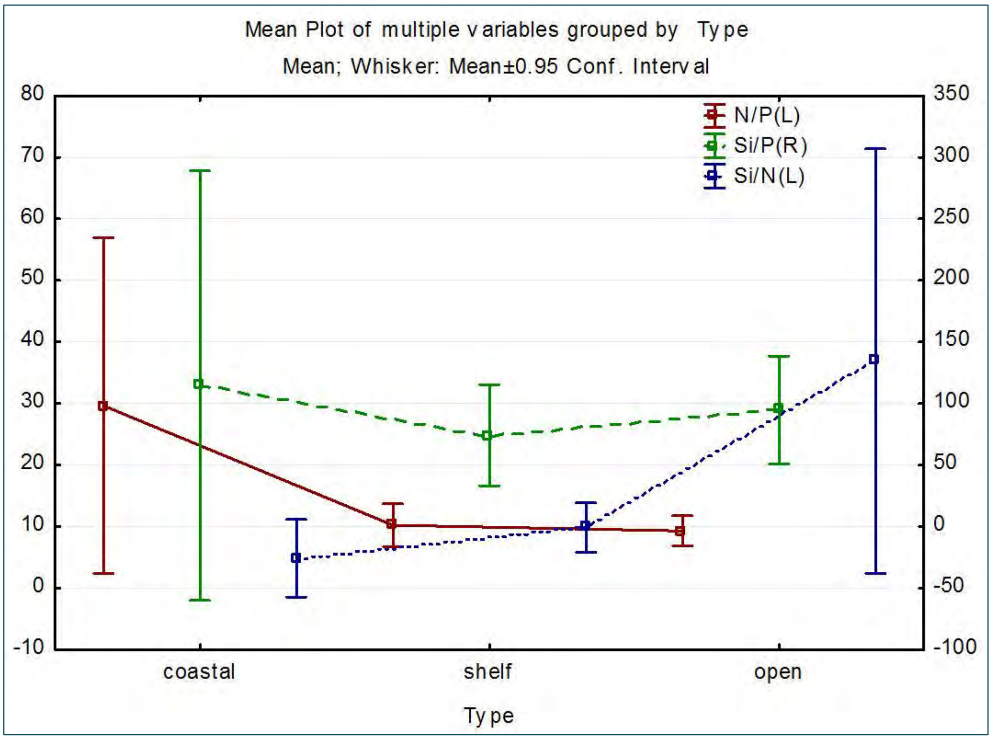


Figure IV.40. Nutrient ratios in the water column – coastal, shelf and open waters, Western Black Sea, MISIS cruise, July 2013.

B. Direct effects

B.1. Chlorophyll a

Spatial distributions

The surface chlorophyll *a* showed concentrations, ranging between 0.12 and 3.43 µg/L. Statistically significant correlation was found between chlorophyll *a* and salinity ($r = -0.75$, $p<0.0001$, $n=69$), thus suggesting the influence of the Danube's discharges on the surface chlorophyll *a* regime. The highest values were observed in the areas influenced by the Danube's discharges, as well as anthropogenic pressures (the Romanian coastal waters; 3.07–3.44 µg/L), while the lowest, in the open waters (0.12–0.35 µg/L), except the TR open waters, where the surface chlorophyll a was found significantly higher (0.35 µg/L) (Fig. IV. 41) The Danube's influence on the spatial variability of surface chlorophyll *a* was also observed in the Bulgarian shelf waters (stations M11 and M10 showed relatively high concentrations closely linked to lower surface salinities).

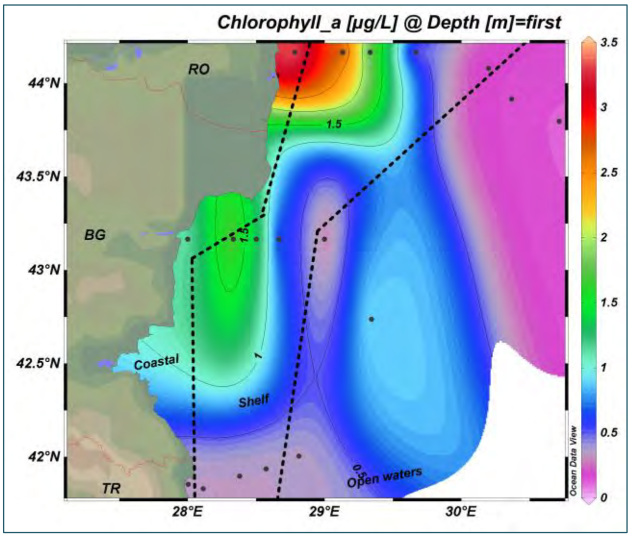


Figure IV.41. Surface Chl a distribution in the Western Black Sea (MISIS Joint cruise, July 2013).

The non-parametric test Kruskal-Wallis (K-W) was applied to test the differences between water bodies for the variable chlorophyll *a*, because of the non-normal distribution of the variable considered. The results (p=0.008) showed significant differences between coastal, shelf, and open waters in terms of surface chlorophyll *a*.

Further, the Mann-Whitney (M-W) significance test was applied to analyze the differences between every pair of water bodies and the results showed significant higher surface chlorophyll *a* in the coastal waters (2.13 ± 1.28 µg/L) as compared with shelf and open waters (0.70 ± 0.71 µg/L and 0.19 ± 0.09 µg/L, respectively) and no significant differences between shelf and open waters in terms of variable surface chlorophyll *a* (Table IV.13).

Anyway, it is worth to mention that the significant differences between water bodies are due to the surface chlorophyll *a* spatial variability observed on the Romanian and Bulgarian transects (1.39 ±1.40 µg/L and 0.86 ± 0.83 µg/L, respectively); in the Turkish waters, the surface chlorophyll *a* showed homogeneous distribution along the studied transect (0.34 ± 0.01 µg/L).

Table IV.13. Mann-Whitney significance tests between water bodies in terms of chlorophyll a.

		Coastal waters	Shelf waters	Open waters
Coastal waters	Chl *a*		*0.030*	*0.011*
Shelf Waters	Chl a	>		
Open waters	Chl *a*	>		

Upper right values represent the level of significance (p); the absence of p values shows not significant differences between water bodies
Lower left symbols compare water bodies in the first column with water bodies in the first row.

Vertical distributions

The vertical distribution of chlorophyll *a* was normal for the summer season, with a Deep Chlorophyll Maximum (DCM) layer at depths between 39- 46 m. In the Romanian waters, the DCM layer was not observed in the coastal stations, the highest chlorophyll *a* concentrations were found in the surface layer (3.07–3.44 µg/L), most probably due to the Danube's influence, suggested by the lower surface salinities (between 13.83 and 14.82 PSU). Unlike the Romanian waters, the Bulgarian and Turkish coastal waters showed chlorophyll maxima at depths of 12-15 m (1.17 – 1.44 µg/L as compared with 0.32 – 1.05 µg/L in the surface layer), associated to the thermocline location, conforming to the typical summer vertical profile.
The Turkish shelf and open waters showed a pronounced DCM (the highest subsurface chlorophyll *a* in the open waters – 2.14µg/L), located between depths of 16 and 23 m (Fig.IV.41). A deeper chlorophyll maximum layer was observed in the Romanian shelf and coastal waters, at depths of 40 – 45 m, except for the shallowest shelf station (M03) which showed a strong subsurface maximum at 9 m depth (Fig.IV.41), where also the salinity was lower (15.74 PSU). In the Bulgarian waters, the shallower shelf stations (M10 and M11) the chlorophyll maxima were located at depths of 12 – 15 m (Fig.IV.42), where also the salinities recorded low values (15.02 – 15.69 PSU). The significant difference of vertical depth maxima could be related to the phytoplankton community structure and its adaptation to the light regime; the K-W test does not reveal significant differences in terms of the nutrient regimes at those depths.

The K-W test does not show statistically significant differences between the surface homogenious layers (SHL) magnitude on the investigated transects. However, slight higher chlorophyll *a* concentrations in the DCM were found in the Bulgarian waters (1.68 ± 1.17 µg/L) as compared with the Romanian and Turkish waters (1.12 ± 0.63 µg/L and 1.2 ± 0.61 µg/L, respectively).

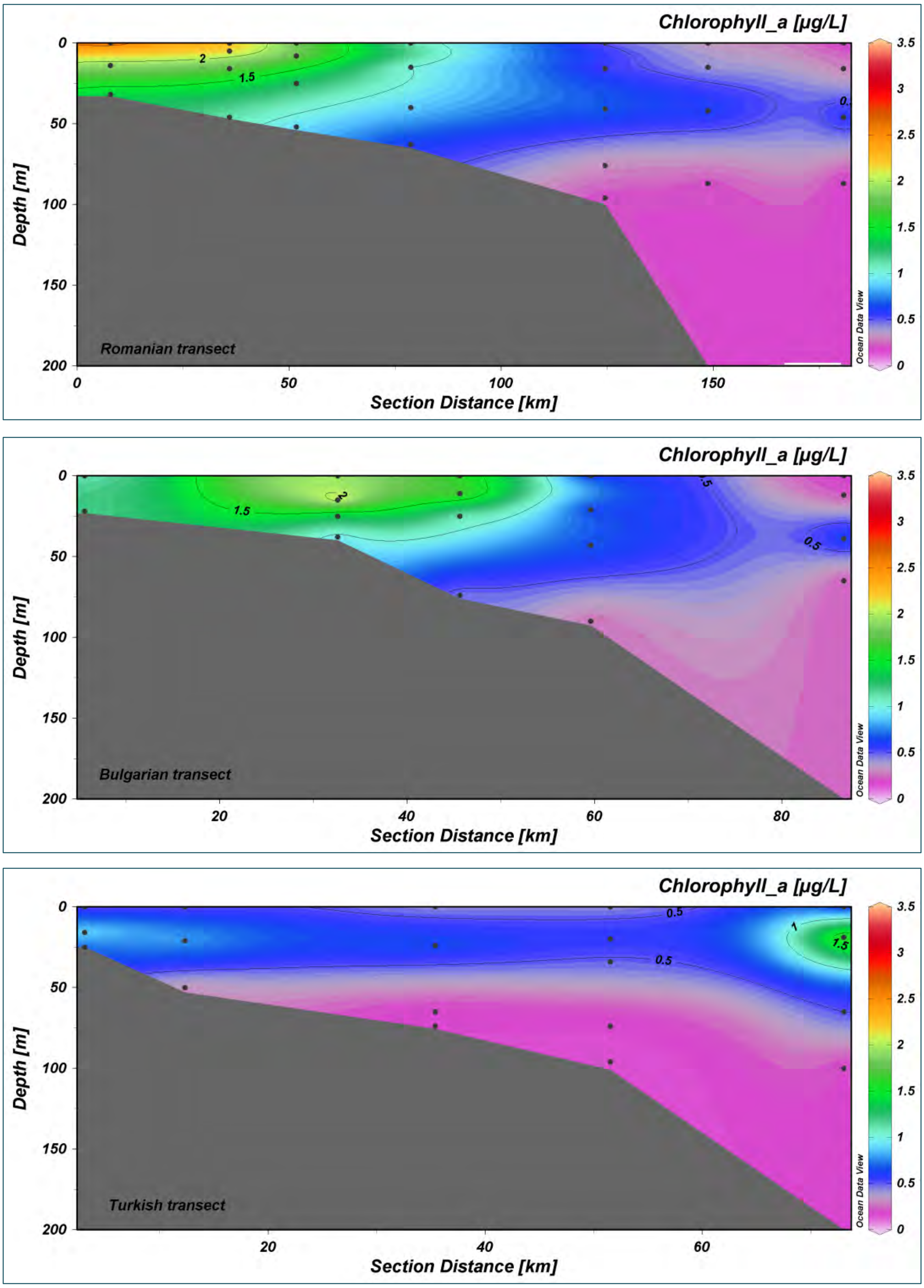


Figure IV.42. Chlorophyll a distribution in the water column – coastal, shelf and open waters, Western Black Sea, MISIS cruise, July 2013.

According to the Articles 9 and 10 of MSFD, RO (RO-IAR, 2012) and BG (GES Report, 2013) have set environmental targets for the Chl a (indicator 5.2.1). Even if   assessment weather the indicator Chl a was in GES or not based only on a single sampling survey could not be preclusive in the case of RO, the 75th percentile surface Chl a (2.75 µg/l) was found lower than the target value established for coastal and marine waters (3.08 µg/l), thus suggesting that the indicator was in GES. In case of BG, the integrated surface Chl a down to the thermocline measured in the open (0.2 ± 0.08 µg/l) and in the coastal waters (1.2 µg/l) was close or at the threshold boundary of the target values asigned for the summer season (0.15 – 0.2 µg/l on the open sea and 1.2 for the coastal waters), while Chl a measured in the shelf waters (1.7 ± 1.3 µg/l) was out of the range of the target values (0.5 – 0.7 µg/l), e.g the indicator was not in GES. The MISIS data were quite in conformity with the long-term trends (90-2012) found over BG pelagic habitats. A pronounced general trend of reduction of chlorophyll a was observed in the coastal and shelf habitats but at sustained high year to year and within the season variability around or above the thresholds, while at the open sea in majority of measurements the concentrations were above the threshold – Fig. IV.43.

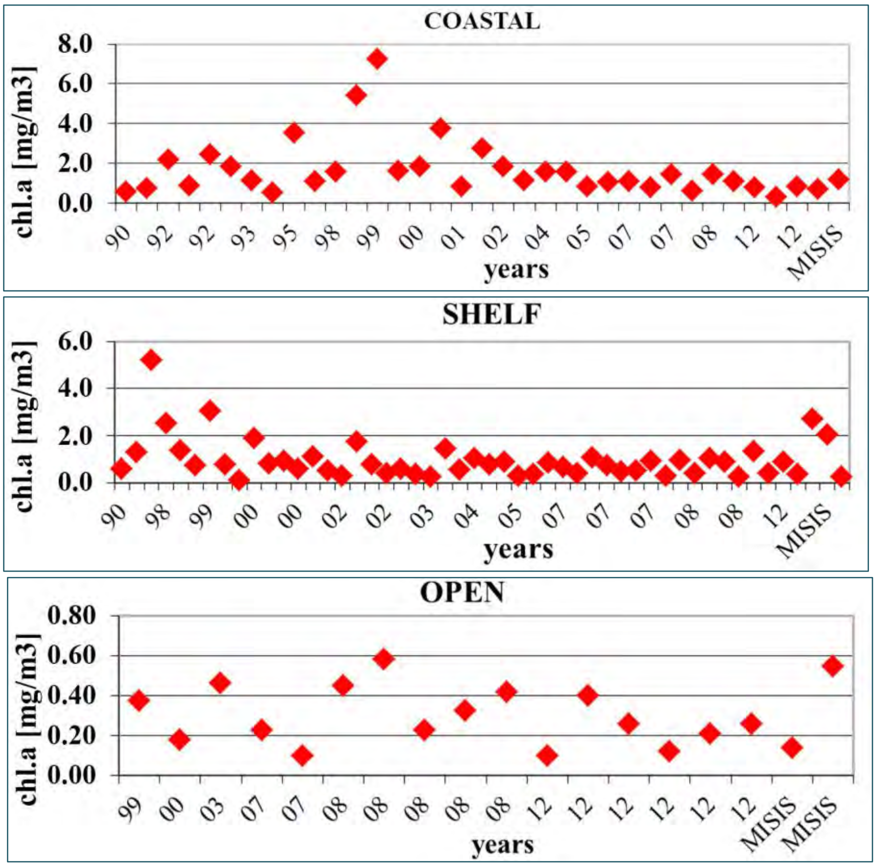


Figure IV.43. Long-term (1990-2012 including MISIS data) variation of chlorophyll a [mg/m3] along the BG coastal, shelf and open sea habitats (Galata transect).

Conclusions

The surface chlorophyll *a* concentrations showed a quite large variability in the western Black Sea, strongly influenced by the Danube's discharges and anthropogenic pressures in the coastal areas.
The water column distribution of chlorophyll *a* showed a pronounced DCM layer, except the Romanian coastal waters, strongly affected by the Danube's discharges. The location of the DCM layer varies largely (between 9 and 46 m depth), the deeper chlorophyll maxima being observed in the Romanian and Bulgarian open waters (39 – 46 m depth). No statistically significant differences were found between the magnitudes of DCM layers on the three transects investigated.

C. Indirect effects of eutrophication
C.1. Dissolved Oxygen

The dissolved oxygen regime variability depends on several antagonistic factors. Thus, the contributing factors to the seawater oxygen enrichment are: currents and winds regime, atmospheric contact and photosynthetic processes. While, there are more numerous and varied factors contributing to the depletion of seawater oxygen content: the suprasaturated water masses contact with the atmosphere, which may sometimes benefit from the contribution of oxygen to maintain the balance at the air – water interface, respiration, biological and chemical oxidation processes (of reducing agents (e.g. H_2S, FeS), dissolved and particulate organic matter, sediments, enzymatic processes, bacterial oxidation, etc.), water masses stratification (Riley, 1971; Horne, 1969; Peres, 1961; Best, 2007).

Horizontal distributions

The oxygen content of the surface seawater was homogenous and summer specific (240.8 – 319.0µM) with highest values into the Bulgarian shelf waters (Fig.IV.44).

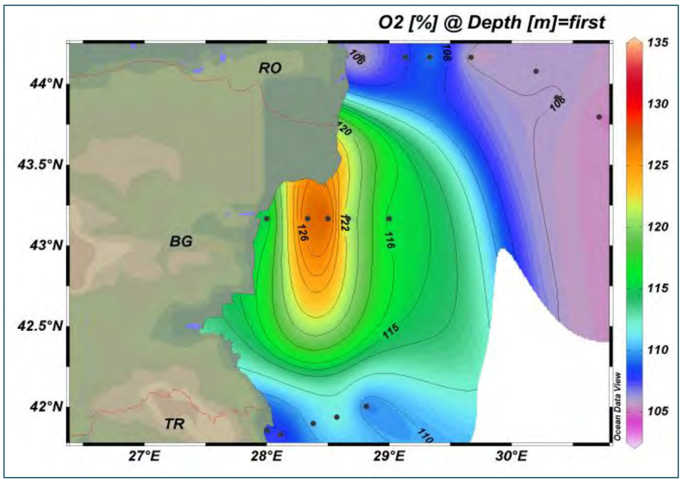


Figure IV.44. Dissolved oxygen (%) content of the surface waters - MISIS cruise, July 2013.

The oxygen saturation in surface BG waters during the summer (2006-2011) varies in range 87-105% in coastal area, 95-106% in shelf and 97-107% in open area (Initial Assessm. Report, 2013). Oxygen depletion significantly affects the water quality and ecosystem function. Absence of oxygen impairs the oxic ecosystem both directly and indirectly the dissolved oxygen content (µM) and saturation (%) in bottom waters vary within 190-240µM and saturation, respectively 59.8-101% (minimum in Romanian coastal area). Only in Bulgarian coastal waters (22m) the saturation exceeds 100% at the bottom. A similar situation in the same area (D=22-40m) in Bulgarian waters during the summer 2012-2013 was observed (187-223µM, 98-107%). The dissolved oxygen content <200µM in bottom waters of BG coastal area was measured in 2009-2010 period corresponding to OS<70% (Initial Assessment Report, 2013). The range of both parameters in bottom shelf waters with depth 40 -74m was as follows: 94-250µM/l DO and 44-76% OS (MISIS cruise). Higher values in Romanian shelf were measured.

Water column distributions

It is well known that the Black Sea is a strong stratified system. The upper layer biogeochemistry, below the permanent anoxic involves four distinct layers (BSC, 2008, Sorokin, 2002, Konovalov, 2000) identified as well based on MISIS cruise data (Fig.IV.45).

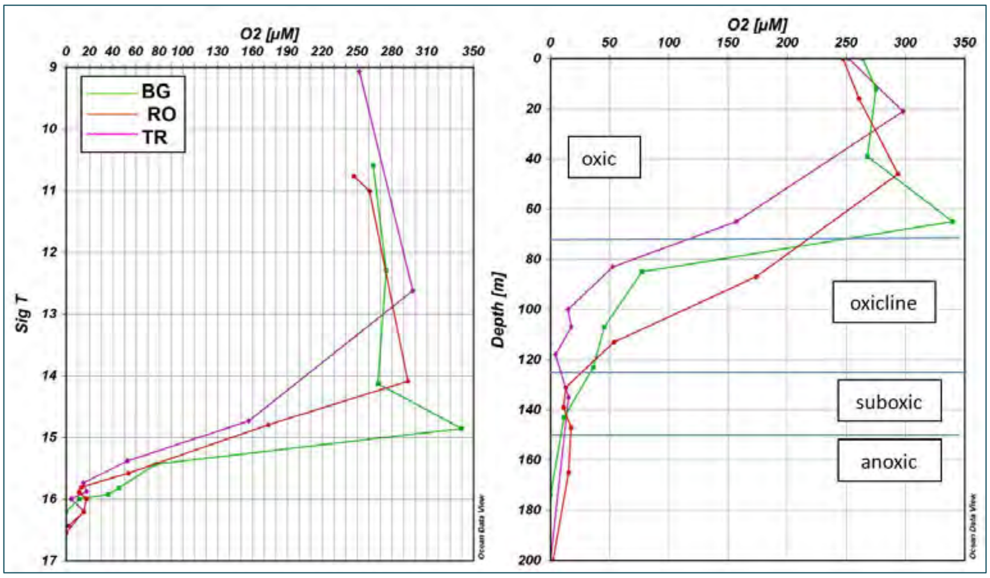


Figure IV.45. Dissolved oxygen water column content – Western Black Sea MISIS cruise, July 201.

Oxic layer – situated approx. in the range 0-65m (9.1-14.9 Sig.T), is characterized by biological processes, with high dissolved oxygen levels (246.6-339.8µM, average 280.9 µM) and quite low nutrients concentrations. In this layer, below thermocline, the nutrients concentrations started to increase due to the regeneration. Thus, the highest values of the oxygen concentrations were found there, due to the phytoplankton enhanced activity sustained by nitrates high concentrations. High DO content corresponds to DCM or to Chl maxima, resp. to Fluorescence maxima in thermocline layer. The thickness of the oxic zone varies between 70-80m in peripheral areas (St 07, 08, 14) and about 60m in area of western central cyclonic gyre (St. M13) with elevated isopycnal surface (Fig.IV.44).

Oxycline –(part of the oxic layer) the oxygen concentrations started to decrease (22.7 – 173.7µM, average 81.4µM) was situated approx. in the range 65-174m (14.7-16.0 Sig.T) and includes (65-90m) the cold intermediate layer (CIL).
In oxicline layer the dissolved oxygen content reduces to ~10 µM, whereas the nitrate concentration increases to 4.8µM. Oxygen distribution below the oxycline is characterized by small vertical gradients (Yakushev et al., 2008)

Suboxic layer – is located between the oxic surface layer and anoxic deep layer. The suboxic zone is defined as the layer below depth, where oxygen decreases to near zero (O2 < 10 µM) (Murray et al.,1995).The lowest oxygen content in range 11.5 -15.5µM/l was measured in July 2013 at depths corresponding to SigT 15.8-15.9.
This poor oxygen layer (11.0-17.6µM, average 14.7µM) located at depth 100-166m (15.7-16.2 SigT) being known that in the anticyclones the upper limit of the suboxic layer is located at increased depths (~15,8 Sig T) and it is narrower (10-20m)(BSC, 2008). Many important redox reactions (with Fe, Mn, N,and other redox elements) pass in the suboxic layer. Suboxic zone is characterized by high bacterial numbers and enhanced microbial production primarily through chemosynthesis (Pimenov, 2000).
Its structure is spatial and temporal variable due to the blooms magnitude. Thus, „Knorr" expedition (May - June2001) showed in the Western Black Sea, during a phytoplankton bloom that the suboxic superior limit was found at 15.15 SigT (BSC, 2008). The suboxic layer is followed by deep anoxic layer charaterized by large H_2S and ammonium pools.

Anoxic layer the oxygen was absent at depths within 174-200m in Romanian and BG parts and below 135m in Turkish area. Hydrogen sulfide in concentration >0.1µM was found at 167m in Romanian waters (St.M07), at 174m in Bulgarian waters (St. M08) and 135m in Turkish waters(St. MO4). The comparison with Intercalibation station M13 (D=2000m) reveals an upper depth of H2S appearance (110m) there due to the specifics of hydrology. Anoxic zone bellow SigT=16.2 was established in the entire investigated area. In conformity to hydrological specifics in different three areas (deep stations location) the results reveal different vertical distribution of chemical parameters (different location of extremes) (Fig.IV. 31, IV 34, and IV 41).
The layer is defined by a particular chemistry with three main characteristics: low TNOx concentrations consumed through denitrification (anaerobic phaenomena) – identified in the cruise data (Fig.IV.45 and 46), sulphate reduction and sulphide occurrence, decreasing of the redox potential (Horne, 1969).

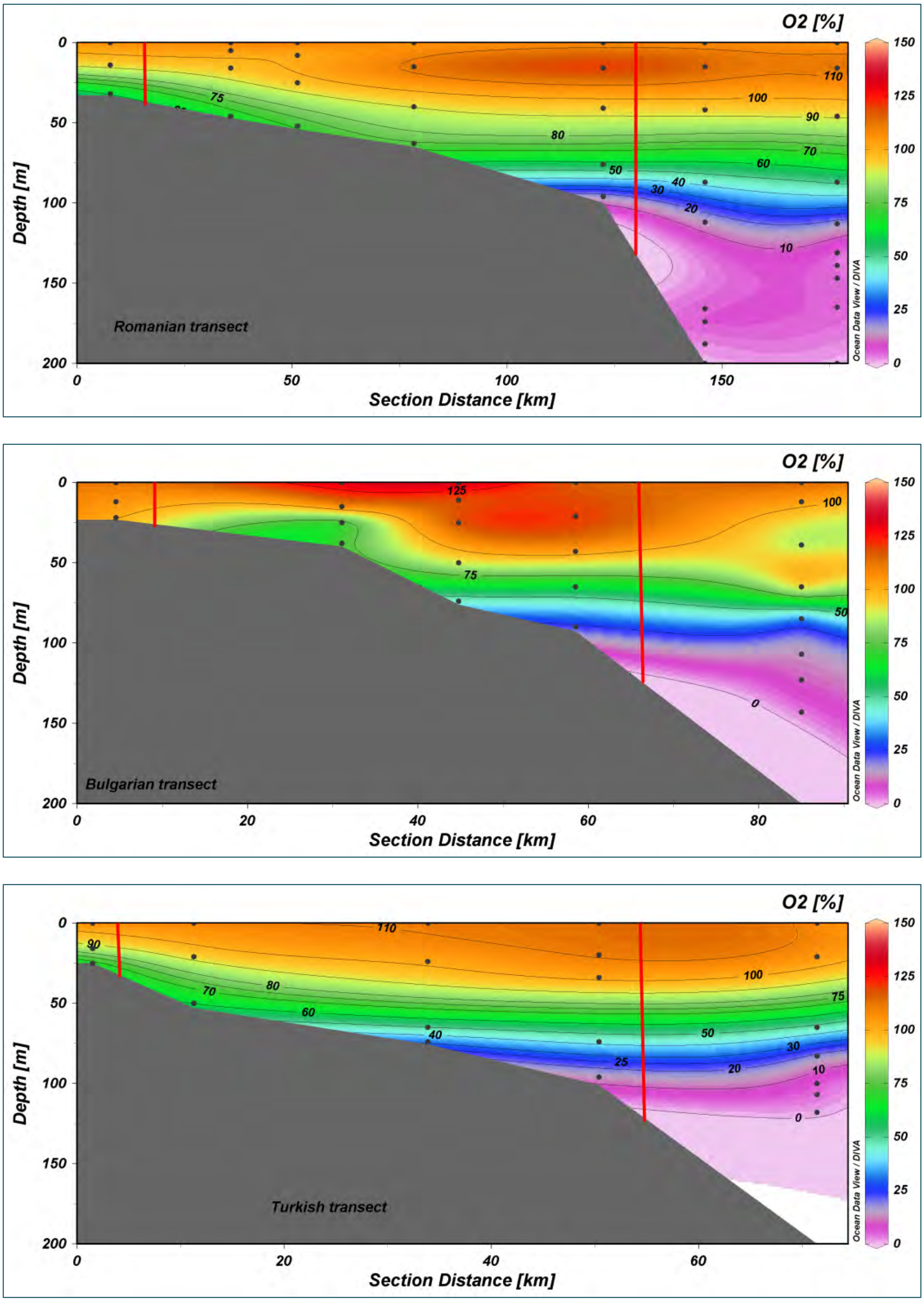


Figure IV.46. Oxygen saturation distribution in the water column – coastal, shelf and open waters, Western Black Sea, MISIS cruise, July 2013.

C.2. Transparency

Transparency is a measure of the clarity of the water showing its attenuation of light penetration into the water column. It is influenced by the properties of absorption and water diffusion, in turn, dependent on the existing amount of particulate matter and dissolved substances. Generally, into the seawater is found alive or dead organic particles (e.g. phytoplankton), small particles and inorganic coloured solutes (e.g. humic acids). Thus, transparency integrates many of the concrete effects of eutrophication, such as the disappearance of perennials and flowering or algal blooms intensification.
The transparency (N=17) ranged from 4.0m to 18.0m with minimum values (4.0-5.0m) in the Romanian and Bulgarian coastal and nearby shelf areas and highest (13.0 -18.0m) in the open waters (Fig.IV.47)

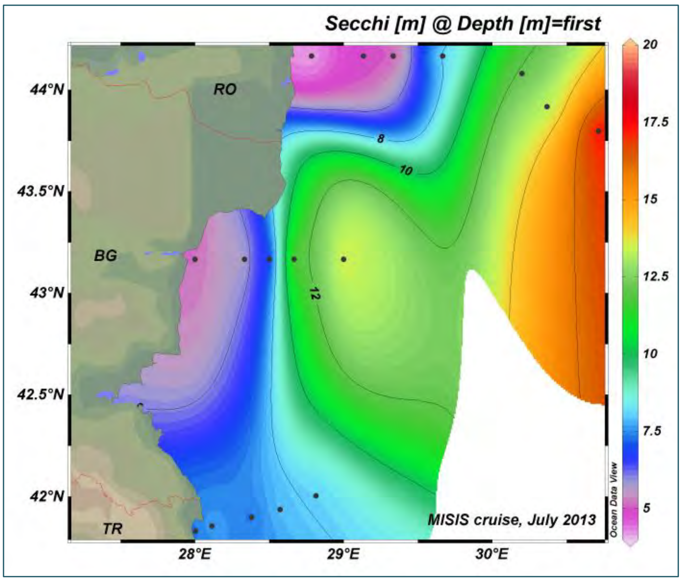


Figure IV.47. Seawater transparency - MISIS cruise, July 2013.

Significantly correlated with salinity (0.90) and chlorophyll *a* concentrations (-0.67), the transparency is gradually increasing from coastal and shelf to open waters, particularly in the Romanian and Bulgarian transects far from anthropogenic influences (mainly freshwater and nutrients discharges), where reaches the maximum values (Fig.IV.48).

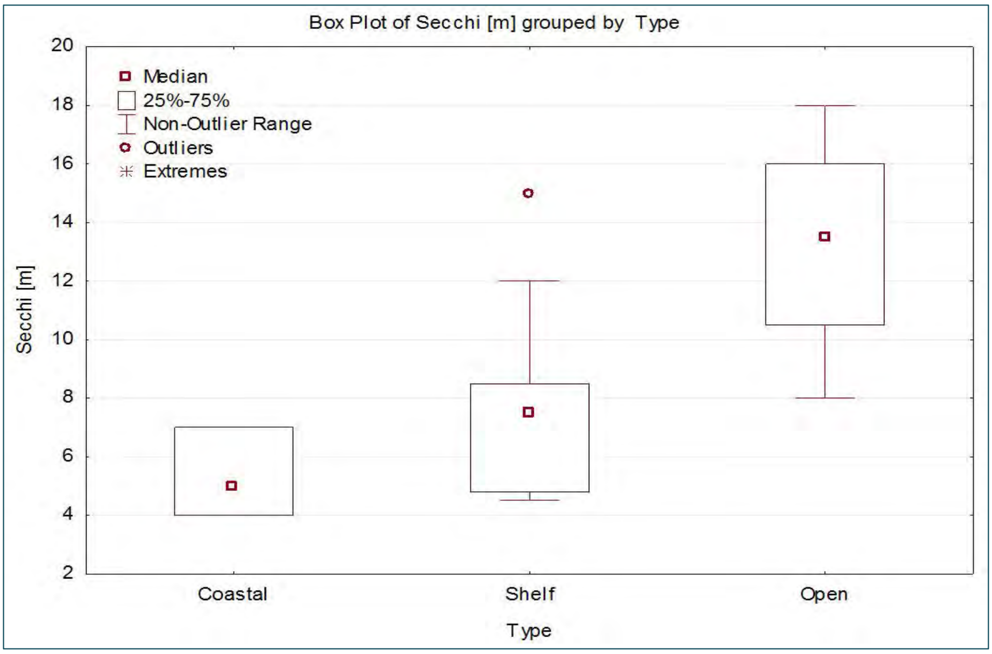


Figure IV.48. Boxplot of transparency grouped by water type.

Long-term data (1964-2011, surface, summer) for the Romanian shelf waters (East Constanta transect) highlighted no trend for transparency, and it is to note that data from MISIS cruise fitted well with the historical data (average 6,2m, standard deviation 4,2m) (Fig.IV.49).

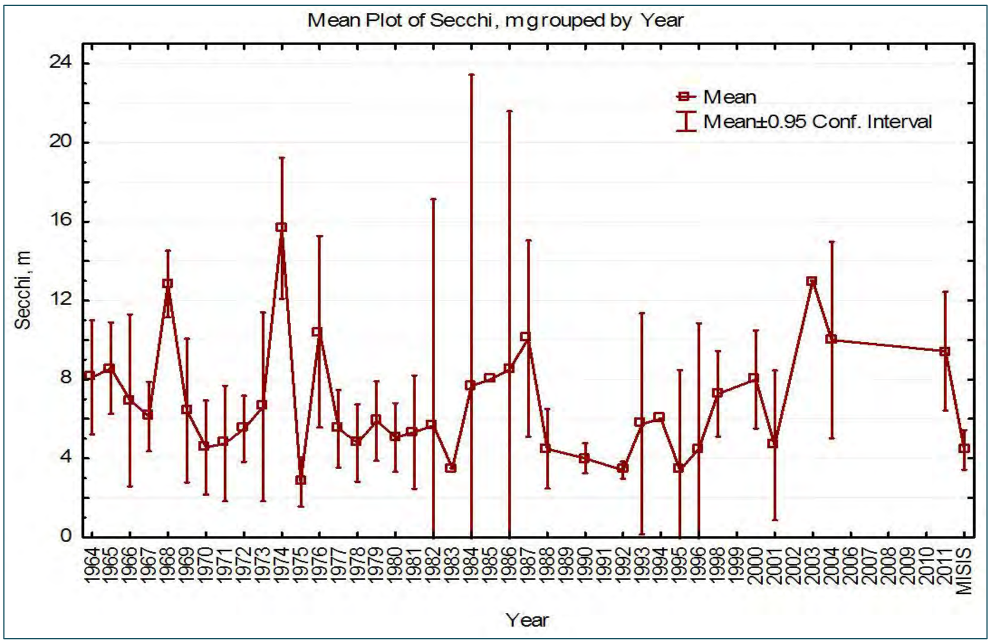


Figure IV.49. Long-term (1964-2011) compared with MISIS cruise data (2013) – transparency [m], Romanian shelf waters – summer.

D. Western Black Sea eutrophication integrated assessment – BEAST tool

Description of the BEAST tool

BEAST (Black Sea Eutrophication assessment Tool) was developed in the frame of Baltic2Black project based on the HELCOM Eutrophication Assessment Tool (HEAT 2.0). HEAT 2.0 it was developed based on the OSPAR “Common Procedure” and taking the requirements of the MSFD Commission Decision into consideration.

Thus, BEAST categories are divided into three criteria: C1 - causes of eutrophication, C2 - direct effects and C3 - indirect effects. Each criterion could have a set of indicators (based on availability and expert choice). The result of each indicator status is done by EUT_Ratio and it is included, according to its own weight (chose by expert), into a qualitative response: high, good, moderate, poor and bad (Table IV.14 and IV.15).

Table IV.14. Category 1 – Nutrients levels – BEAST.

C1: Nutrient levels	RefCon	AcDev	EUT Target	Unit	Resp	EUT T-score			EUT status	EUT S-score			EUT Ratio	Ind Conf	Weight	C1 EUT sum	C1 EUT status	C1 conf	C1 Weight
DIP	0.10	50%	0.15	µM	+	H	M	L	0.06	H	M	L	0.400		50%				
TNOx	1.00	50%	1.50	µM	+	H	M	L	0.44	H	M	L	0.293		50%				
Add new indicator																			
															100%	0.347	HIGH		

Table IV.15. Glossary of terms used in BEAST.

RefCon	Reference conditions
AcDev	Acceptable deviation (from reference conditions)
EUT_Target	Eutrophication target (or calculated from RefCon ± AcDecv
Resp.	Numerical response to nutrient enrichment (+ or ÷)
EUT_T-score	Confidence score assigned to the eutrophication target (H = high; M = moderate; L = low)
EUT_status	Eutrophication states (based on monitoring data from a given period/year)
EUT_S-score	Confidence score assigned to the eutrophication status (H = high; M = moderate; L = low)
EUT_Ratio	Eutrophication Ration (calculated from: EUT_status/EUT_Target)
Ind_conf	Indicator confidence (calculated from EUT_T-score and EUT_S-score)
C1_EUT_sum	Eutrophication Sum for Criteria 1 (the sum of individual EUT_ratio's)
C1_EUT_status	Eutrophication Status for Criteria 1 (five classes: High, Good, Moderate, Poor and Bad)
C1_conf	Confidence (weighted) for Criteria 1
C1_Weight	Weight factor assigned to Criteria 1 (100; 50 or 33%; pending the number of criteria covered)

Within the categories, BEAST is averaging the parameters or taking a weighted mean (according to the significance of the parameter or the data quality) while, 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 another qualitative response, the “Final eutrophication status”: high, good, moderate, poor and bad.

For the Western Black Sea eutrophication assessment, based on one summer cruise (MISIS, July 2013), it was used a core set indicators (due to their availability, reference conditions availability and relevance) as follows:

- C1 - causative factors – nutrients (DIP - ortophosphate, TNOx – sum of nitrate and nitrite) weighted as 50% each.
- C2 - direct effects – phytoplankton blooms – chlorophyll *a* (as an estimate of the Total biomass)
- 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)

The reference conditions used were provided by each country project partner expert and for the EU MS (Romania and Bulgaria) were acquired as a MSFD obligation while for Turkey represented the results of a national project.

By applying BEAST, we had 17 qualitative results (for each station of the network) grouped in high, good, moderate, poor and bad eutrophication status. In order to assess the GES it was chose the threshold between Good-Moderate status as GES boundary (Table IV.16 and Fig.IV.50).

Table IV.16. BEAST results – MISIS cruise, July 2013.

Transect	Station	Type	Qualitative status	Assigned value	GES
Romanian	MO1	Costal	Good	2	GES
	MO2	Shelf	Good	2	GES
	MO3	Shelf	Good	2	GES
	MO4	Shelf	Poor	4	Non-GES
	MO5	Shelf	Good	2	GES
	MO6	Open	Good	2	GES
	MO7	Open	High	1	GES
Bulgarian	MO12	Coastal	High	1	GES
	MO11	Shelf	Good	2	GES
	MO10	Shelf	High	1	GES
	MO9	Shelf	High	1	GES
	MO8	Open	High	1	GES
Turkish	MO18	Coastal	Good	2	GES
	MO17	Shelf	Good	2	GES
	MO16	Shelf	Good	2	GES
	MO15	Shelf	Good	2	GES
	MO14	Open	Good	2	GES

Based on the BEAST results the eutrophication status of the Western Black Sea in summer 2013 was High-Good for coastal and open waters and Poor – High for shelf waters. The only one "poor" status responsible for not achieving the GES is the shelf station from Romanian transect where the phosphate and silicate concentrations where highest (Fig.IV.50). Actually it was found the significant correlation ($p<0.05$) of their values with BEAST (phosphate, $r=0.82$, silicate, $r=0.78$) and correspondingly for the ratios (N/P, $r=-0.63$, Si/N, $r=0.78$).

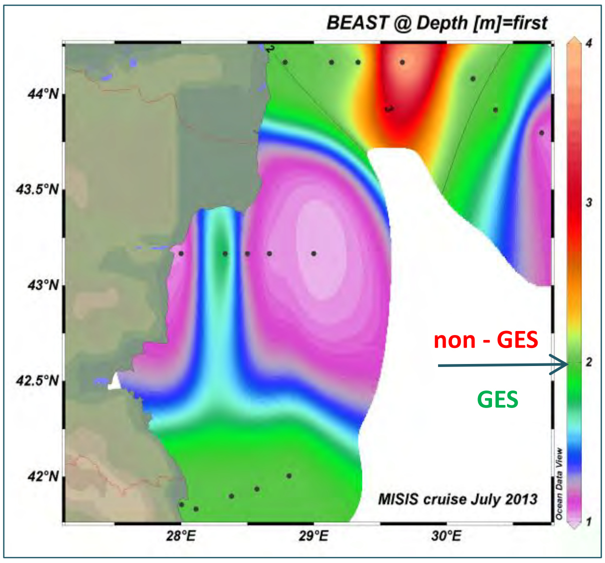


Figure IV.50. BEAST surface distribution – MISIS cruise, July 2013.

Due to no correlation of BEAST with salinity (interpreted as no influence of the river discharge) and in absence of any other quantified anthropogenic influences it is to note that the poor eutrophication status is mainly influenced by the currents and winds regime and the water mixing phaenomena. Considering the name of the descriptor 5 "*Human induced eutrophication is minimised, especially the adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters*" involves a strong need to better understand/define the threshold between natural variability (including climate change) and anthropogenic impact.

CONCLUSIONS

The actual assessment of the eutrophication state of the Western Black Sea waters confirms the phenomenon complexity. The evaluation took into consideration a core set indicators (mainly based on their availability) and contributed to define the actual state as good which, under the climate factors and the anthropogenic impact more pronounced in coastal and shelf waters, could easily passes to one extreme state (poor or high).

The climate changes manifested through the alteration of the rivers hydrological regime, seawater temperature increase, intensification of the water masses stratification, winds and currents regime etc., are important influencing factors of the current eutrophication state of the Western Black Sea waters.

Generally, the eutrophication state is anthropogenic influenced by two main sources – the NW rivers input and the anthropogenic input of the major cities from the coastal zone, Constanta and Varna.

GAPS and RECOMMENDATIONS

The gaps and recommendations on the eutrophication assessment of the Western Black Sea emerged as particular needs for the present report (V.1) generalized into G&R for the regional level (V.2).

A. MISIS Cruise findings

In order to have comparable results there is need to establish the core set indicators to assess eutrophication at (sub) regional level based on their relevance not availability. It is very important to have also the capability to obtain the data.

The use of integrative tool (ex. BEAST) was done based on reference values established by each country and core set parameters as were identified due to each laboratory capability. No common decision on the integration of different indicators into weights for BEAST. Considering that BEAST principle works on reference values and accepted deviations it will be difficult to introduce in the integrated assessment biological parameters like “species shift in floristic composition” or others.

No atmospheric deposition of nutrients was quantified.

The assessment was based on one cruise (summer), which does not allow us to make a real assessment of the eutrophication state.
Need to better understand/define the threshold between natural variability (including seasonality and climate change) and the anthropogenic impact, which is a "must" for the descriptor 5.

B. General recommendations for eutrophication assessment at the (sub)regional level (Western Black Sea)

GAPS	RECOMMENDATIONS
No/few data on open sea waters	Systematic use of additional tools such as remote sensing of surface chlorophyll, ferry boxes, and smart buoys is recommended if data are validated with in-situ data. Develop reliable algorithms (more research needed related the CDOM, seawater optical properties, etc.) for satellite derived Chlorophyll *a* in the shelf waters.
GAPS	**RECOMMENDATIONS**
No/few data on atmospheric deposition of nutrients	Monitoring of atmospheric deposition of nutrients. Coupled atmosphere-river-coastal sea models need to be developed at the regional scale for the estimate of critical nutrient loads from terrestrial sources, in relation to transitional/ coastal retention, and chemical and biological target indicators
No info on the threshold between natural variability (including climate change) and anthropogenic impact, which is a must in the descriptor 5.	Research on natural background nutrient enrichment (e.g. import by upwelling; import from pristine/ good status rivers) for determination of pristine state and separation of natural productive status from anthropogenic impacted eutrophic status; climate change impacts on availability and transformation of nutrients and organic matter from land to the sea.
The link to land-based inputs is not well established.	Identification of critical nutrient loading thresholds beyond which the whole system is changing into an alternative steady state;
No indicators/parameters considered for assessing the impact of human induced eutrophication on the vertical distribution of nutrients, DO, chlorophyll a, etc.	More research is needed for developing indicators/parameters that considered the effects of eutrophication on nutrients, DO, chlorophyll distribution within the water column (with special emphasis on open waters). Data/information from literature, past and recent cruises should be taken into consideration for assessing the temporal variability of the position and magnitude of suboxic layer, nutricline, DCM, etc. (models to be developed).

Need to distinguish between natural range and increase of spatial extension of anoxic sediments due to anthropogenic organic loading.	Research on factors that govern the occurrence and extension of hypoxic/ anoxic sediment surface. Additional continuous monitoring tools (benthic observatories, etc.) to be used for hypoxic/anoxic events study (and factors governing) in the sensitive areas.
No assessment tools that account for shifts in species composition and frequency of blooms in the scoring	Development of phytoplankton assessment tools that account for shifts in species composition and frequency of blooms in the scoring Development of monitoring tools that account for rapid changes in algal communities, allowing detection of bloom peaks (continuous measurements, ships-of-opportunity, remote sensing tools, algorithm development, real-time monitoring, etc.).
Non integrative tool to assess eutrophication.	BEAST (or other tool) must be robust, integrated, sufficiently sensitive, comparable, and with recognized scientific merit.
Need for Quality Assurance guidelines for the descriptor - an essential requirement for successful monitoring, allowing for appropriate intercalibration and comparative assessment.	The procedures aim to ensure that monitoring results meet the required levels of precision and confidence. Those procedures can take the form of standardizing sampling and analytical methods, replicate analyses and laboratory testing schemes.

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V. DESCRIPTOR 8: Contaminants

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“Contaminants are at a level not giving rise to pollution effects.”

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INTRODUCTION

Contaminants are defined in the European legislation as:

“substances (i.e. chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bio-accumulate and other substances or groups of substances which give rise to an equivalent level of concern” (Water Framework Directive, Article 2(29)).

Preventing and reducing inputs to the marine environment, with a view to phasing out pollution, is clearly stated as one of the main objectives of the **Marine Strategy Framework Directive**, in line with international commitments at global and regional level. Pollution by contaminants is one form of pollution of the marine environment and the aim of Descriptor 8 is to ensure that the levels of contaminants in the marine environment do not to give rise to pollution effects. Contaminants can arise from numerous anthropogenic sources such as land-based industrial activity, pollution by ships, atmospheric deposition, oil, gas and mineral exploration and exploitation and riverine inputs. It should be noted, however, that natural oceanographic and geological factors, including geothermal activity, can sometimes be responsible for elevated levels of some contaminants (such as heavy metals).

The assessment of achievement of Good Environmental Status (GES) under the Marine Strategy Framework Directive 2008/56/EC (MSFD) Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution effects" should be based upon monitoring programmes covering the concentrations of chemical contaminants and also biological measurements relating to the effects of pollutants on marine organisms. The combination of conventional and newer, effect based, methodologies, with the assessment of environmental concentrations of contaminants provides a powerful and comprehensive approach. As the occurrence of adverse effects at various levels of organization (organism, population, community, and ecosystem) needs to be avoided, monitoring schemes should also indicate the approaching of critical values as early warning.

Therefore, for the purpose of implementing Descriptor 8 under the MSFD, three core elements of data assessment are recommended (JRC, 2010):

- Concentrations of contaminants in water, sediment and/or biota are below environmental target levels identified on the basis of ecotoxicological data.

- Levels of pollution effects are below environmental target levels representing harm at organism, population, community and ecosystem levels.

- Concentrations of contaminants in water, sediment and/or biota, and the occurrence and severity of pollution effects, should not be increasing.

The assessment following MISIS Joint cruise with respect to contaminants followed the criterion 8.1. Concentrations of contaminants, indicator 8.1.1. MSFD criteria and indicators, D8:

Criterion	Indicator	Parameter	Assessment criteria
8.1. Concentrations of contaminants	8.1.1 Concentrations of contaminants measured in relevant matrix (such as biota, sediment and water) in a way that ensure comparability with the assessments under Directive 2000/60/EC	Heavy metals and persistent organic pollutants in seawater	The Environmental Quality Standard Directive (Directive 2008/105/EC, updated by Directive 2013/39/EC) establishes requirements for the chemical status of surface waters including marine waters defining an Environmental Quality Standard (EQS);
		Heavy metals and persistent organic pollutants in sediments	Environmental Assessment Concentrations (EAC, OSPAR); Effect Range Low values (ERL,US EPA);
8.2. Effects of contaminants	8.2.1 Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored	-	-
	8.2.2 Occurrence, origin (where possible), extent of significant acute pollution events (e.g. slicks from oil and oil products) and their impact on biota physically affected by this pollution	-	-

Available information on contaminants in the survey area

Romanian Black Sea waters

State of the Romanian Black Sea marine ecosystem in terms of hazardous substances is assessed in the framework of the national monitoring programme by NIMRD on the basis of the following indicators:

- the presence of dangerous chemicals in surface seawater: total petroleum hydrocarbons (TPHs), heavy metals (HM), organo-chlorinated pesticides (OCPs), polyaromatic hydrocarbons (PAHs);

- contamination of marine sediments with hazardous chemicals: total petroleum hydrocarbons (TPHs), heavy metals (HM), organo-chlorinated pesticides (OCPs), polyaromatic hydrocarbons (PAHs);

- bioaccumulation of hazardous chemicals (HMs, OCPs) in marine molluscs.

Information on contamination is included in the report on the Initial assessment of the Romanian Black Sea waters (NIMRD, 2012), based on monitoring data generated between 2006-2011 from a network of 40 stations located on territorial waters, up to 30 nm distance from the shore, with a sampling frequency of 2 - 4 times / year, as follows:

Heavy metals

Concentrations of heavy metals in the marine waters recorded the following average values: copper 10,02 µg/L; cadmium 0,99 µg/L; lead 3,78 µg/L; nickel 3,65 µg/L; chromium 3,84 µg/L. (Fig. V.1). In relation to environmental quality standards in the field of water recommended by national legislation (Order 161/2006), most of the values didn't exceed the proposed limits. Differences in spatial distribution of heavy metal in marine waters highlight in some cases Danube input or contribution of terrestrial sources of pollution. In comparison, the values observed along the East Constanta transect that goes up to 30 nm far from the shore are often reduced.

Accumulation of heavy metals in marine sediments was characterized by the following average values: copper 33,05 µg/g ; cadmium 1,03 µg/g; lead 26,71 µg/g; nickel 36,54 µg/g; chromium 44,58 µg/g. The presence of heavy metals in sediments from different geographical areas is characterized by a high degree of variability, depending on the element, sediment type, distance from shore and the influence of anthropogenic sources. Most metals have increased accumulation in marine area in front of Danube mouths and also in the vicinity of harbours and WWTP discharges, while the other areas are generally characterized by moderate values (Fig. V.2).

The evolution of heavy metal concentrations in marine ecosystem components in the last decade shows different behaviours depending on the element or matrix investigated. In general, the annual values fall within specific areas of multiannual variability, with some trends of stabilization in recent years.

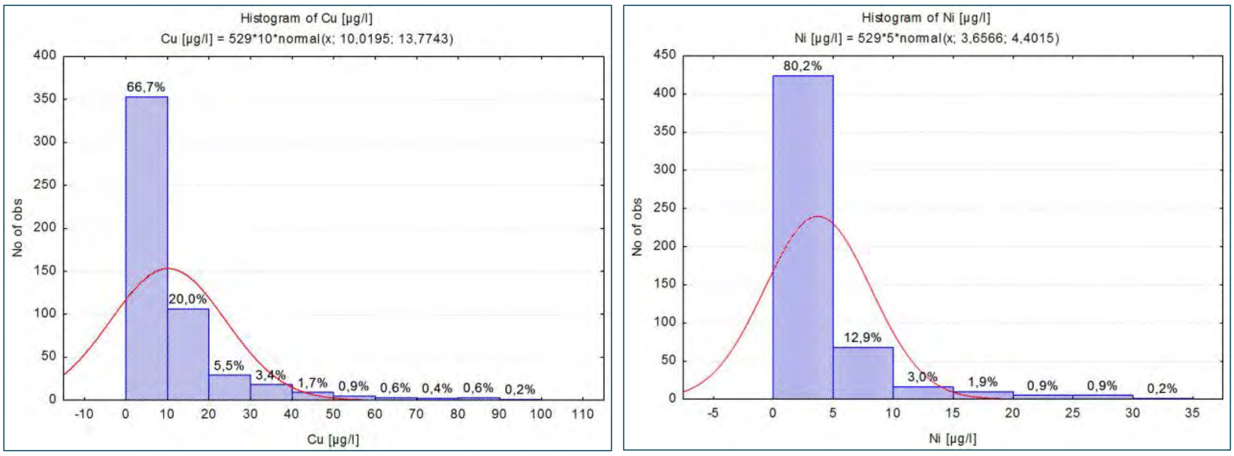


Figure V.1. Distribution of metals concentration in Romanian marine waters during 2006-2011.

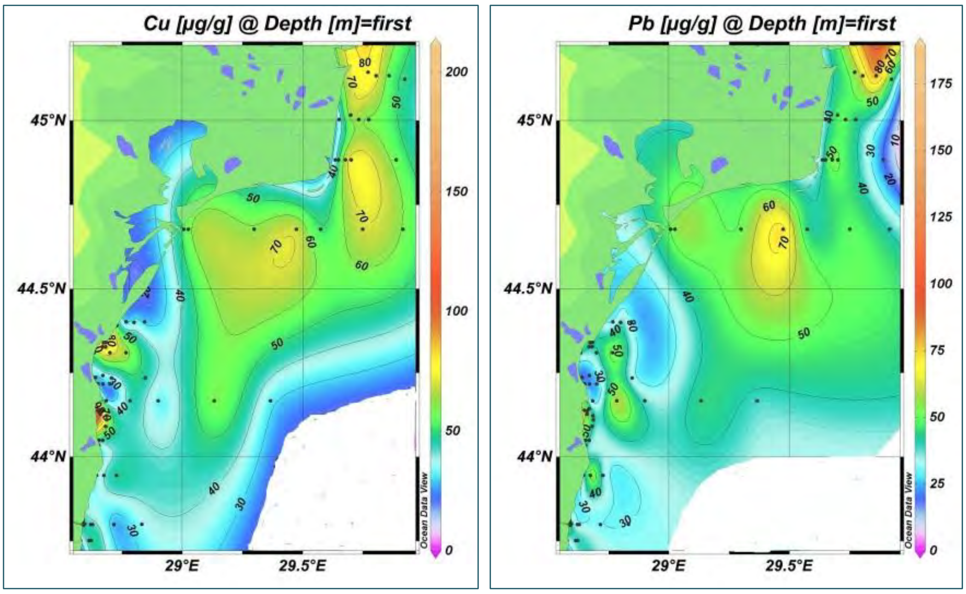


Figure V.2. Distribution of heavy metals in marine sediments along the Romanian coastal zone (2006 - 2011)

Organochlorine pesticides

Dominant compounds in water and sediment during 2006 – 2011 were HCB, lindane, heptachlor and aldrin. The levels of these compounds vary, in sediments, between detection limit and 0.07 µg / g dry sediment and, in water, between detection limit and 0.35 µg / L. The other organochlorine compounds investigated (dieldrin, endrin, DDE, DDD and DDT) varies, in sediments, between detection limit and 0.005 µg / g dry sediment and, in water, between detection limit and 0.02 µg / L. There is no significant variation of pesticides values in water and sediments from different studied areas, although, in water, extreme values are more numerous at sampling points located closer to shore.

In the last years (2009 - 2011), oganochlorine pesticides values appear to have a significant decrease comparing to 2006 - 2008 for lindane, aldrin and DDT, both in water and sediments. For the other investigated compounds concentrations remain within the same range of variation (Fig. V.3).

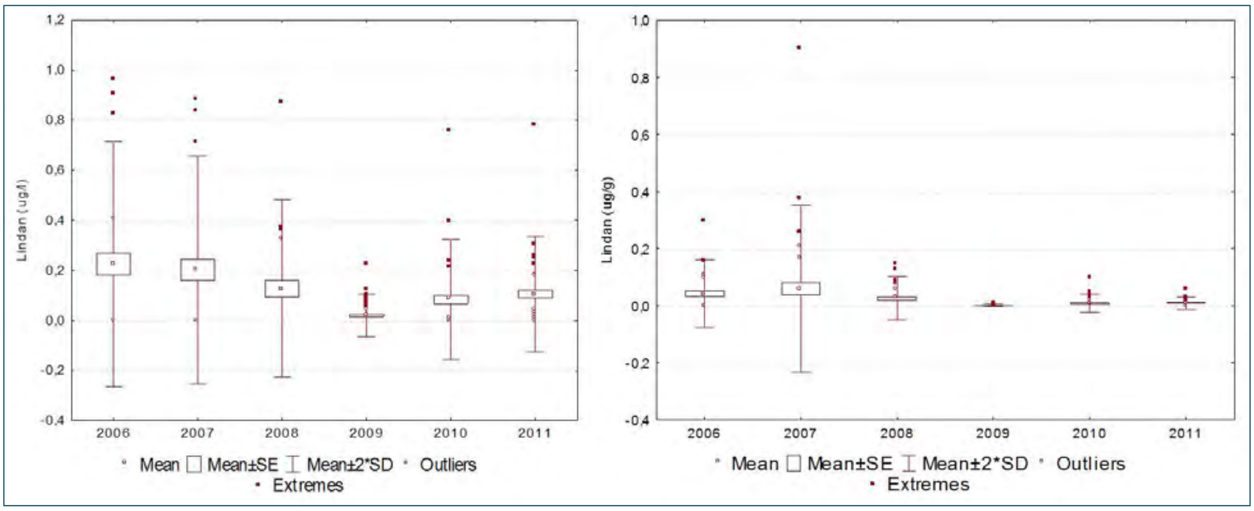


Figure V.3. Lindane variation in water (left) and sediment (right) between 2006 -2011 at the Romanian Black Sea sector.

Total Petroleum Hydrocarbons (TPHs)

The total petroleum hydrocarbons content in water varied in the range of 10.9 – 3592.0 (µg/L) with an average of 379.4 (µg/L). Low values, below maximum permitted levels under national legislation (Order 161/2006) were recorded in 44% of samples. Extreme values of TPH in the range of 1.0 – 3.6 mg/L are more frequent at sampling points located closer to shore. Compared to 2001-2005 period, in 2006-2011 the level of water pollution is diminished (Fig. V.4). TPHs content in sediments falls within the range 4.2 - 6770.0 µg/g with an average of 273.3 µg/g. 48% of samples have showed a low load in total petroleum hydrocarbons, while a higher pollution level was recorded in the vicinity of harbours and wastewater treatment plants.

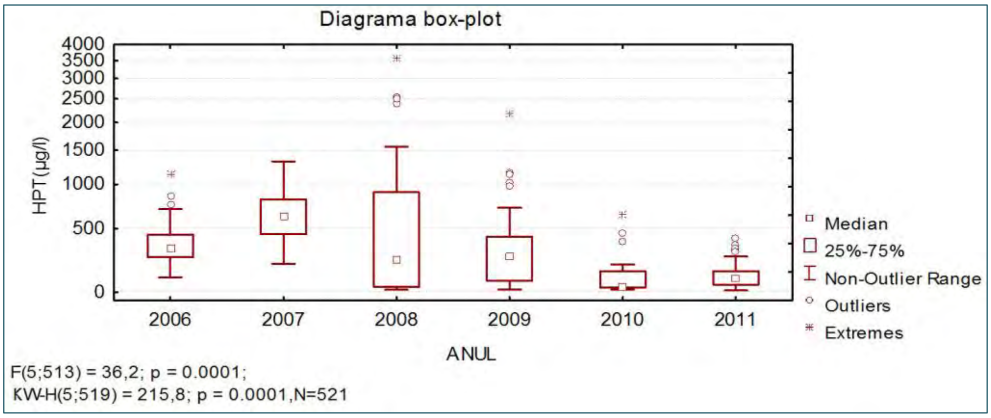


Figure V.4. Trends of TPHs (µg/l) in water from the Romanian Black Sea sector in 2006-2011.

Polycyclic Aromatic Hydrocarbons (PAHs)

Total content of polynuclear aromatic hydrocarbons - ΣPAH_{16} (µg/L) in water ranged from 0.01 to 16.543 (µg/L) with an average of 2.369 (µg/L). Dominants compounds were represented by anthracene, phenanthrene and benzo[a]anthracene. Total content of polynuclear aromatic hydrocarbons - Σ PAH_{16} (µg/g) in sediment ranged from 0.0026 to 16.425 with an average of 1.5313 (µg/g). In recent years (2008-2011), the levels of ΣPAH concentrations indicate their downward trend compared with previous years (Fig. V.5).

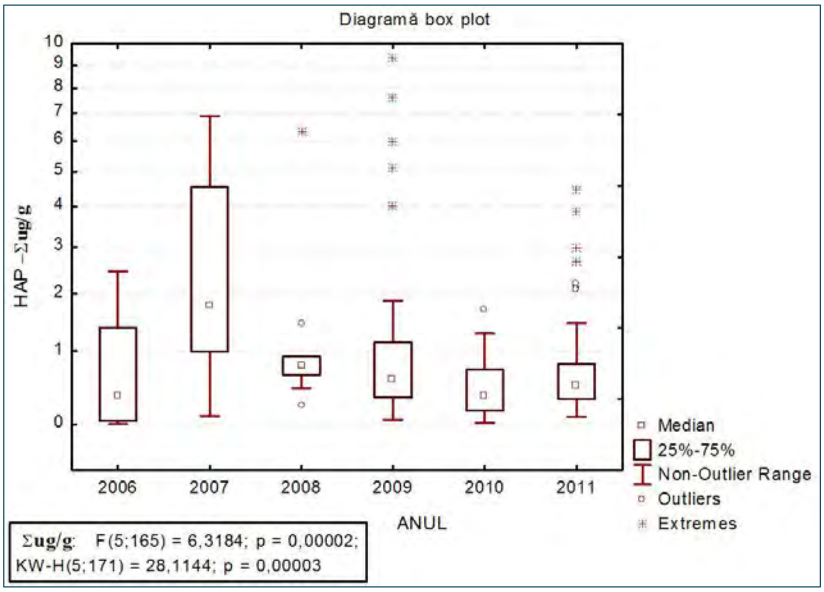


Figure V.5. Trends of ΣPAHs (µg/g) in sediments from the Romanian Black Sea sector in 2006-2011.

Bulgarian Black Sea waters

The information about sea water and sediment pollution is poor. Monitoring of pollutants was carried out in Bulgarian Black Sea area irregularly during last decade. Until now a chemical monitoring (for priority and specific substances) of coastal waters in context of EU Water Framework Directive 2000/60/EC (WFD) has not been carried out. The presented data (Table V.1) was collected in the frame of scientific projects or screening in regard to WFD implementation. Most of published data in literature is for heavy metals (Andreev & Simeonov, 1990; Andreev et al., 1994; Iordanova et al., 1999; BSBD Annual reports, 2006, 2007, 2009, 2010, 2011).

Table V.1. Range of metals concentrations in sea water.

μg/L	Pb	Ni	Cd	Cu	Hg	Zn	Co
Max	0.87	0.82	0.026	0.85	0.010	1.65	0.026
Min	0.40	0.61	0.018	0.60	0.005	0.80	0.019

The screening of coastal waters was carried out in 2006 organized by Black Sea Basin Directorate (BSBD), Ministry of Environment and Waters. The water and sediments samples were analysed in EU accredited Laboratory (Tables V.2 and V.3). Metals content (Pb, Ni, Cd) corresponded to the Bulgarian Water Quality standards.

Table V.2. Metals in sea water (BSBD Report, 2006).

Area	Ni, μg/L	Pb, μg/L	Cd, μg/L	Al, μg/L	Li, μg/L
Krapets (north area)	3.8		0.97	37	**57**
Varna Bay	5.9	1.7	0.84	65.2	61

The screening of the sediments along the coast in 2006 reveals higher metals concentrations in Varna area (Table V.3).

Table V.3. Metals in marine sediments (2006).

Area	Ni, mg/kg	Pb, mg/kg	Hg, mg/kg	Al, mg/kg	Li, mg/kg
Krapets (north area)	48.3	36.4	0.34		
Varna Bay	30.8	44.1	0.52	16.6	4.99

Another source of information about contaminants in sediments is the BSERP Project Report (Table V.4).

Table V.4. Metals in sediments in the area between c. Kaliakra and c. Galata
(sampling campaign 2003, BSERP Project).

μg/g	Pb	Ni	Cd	Cu	Hg	Zn	Co
Max	33	41	0.22	70	0.15	72	16
Min	15	16	0.13	5	0.02	35	6

The survey results for pollutants are presented in Table V.5. Most of organic pollutants concentrations are <DL (Detection limit of the measurement). All species of pesticides (α-, β-, γ-hexacyclohexane, DDD, DDE, DDT) concentrations are <DL (1 ng/L). Total petroleum hydrocarbons (TPH) in range between DL and 360 µg/l were established. Xylene isomers were found in Varna Bay waters. Ethylbenzene and three Xylenes isomers were registered in coastal water in front of Kamchia River mouth.

Table V.5. Organic pollutants in waters (BSBD Report, 2006).

Area	DEHP ng/L	Xylene µg/L	Ethylbenzene
Krapets (north area)	3800	0.9	0.97
Varna Bay	1022	yes	-

The measurements in the next years provided by EEA laboratory in coastal area of Varna region show that this area (Annual reports) is not polluted with organic pollutants (Table V.6).

Table V.6. Organic pollutants content in waters.

Pollutants	Content, µg/L
α HCH	<0.004
Lindane	<0.005
Heptachlor	<0.004
Aldrine	<0.007
Dieldrin	<0.008
Endrin	<0.01
o,p DDE	<0.008
p,p DDE	<0.011
o,p DDD	<0.011
p,p DDD	<0.01
o,p DDT	<0.009
p,p DDT	<0.009
Benzo(a)pyrene	<0.001
Benzo(a)anthracene	<0.001
Benzo(b)fluoranthene	<0.001
Benzo(k)fluoranthene	<0.001
Benzo(g,h,i)perylene	<0.001
Indeno(1,2,3-c,d)pyrene	<0.001

Pesticides (4,4'-DDT, 4,4'-DDD, 4,4'-DDE) and pollutants with industrial origin (Pentachlorobenzene, HCB, 4-tert.-Octylphenol, DEHP, Bispehnol A) were established in sediments of Varna Bay. PCB 153 and all species PAHs were found in sediments, but the content is not presented in the report.

One study reveals that the concentrations of each of the 13 PCB congeners in sediments of Varna Bay and navy canals were below the detection limit (DL) (Shtereva et al., 2004). For most of them DL is 0.015 µg/kg and only for

tetrachlorobiphenyl – 0.020 µg/kg. The results reveal pesticides content below DL, as follows: for DDT, DDD, DDE, Methoxychlor – 0.009µg/kg; for endrin, aldrin and dieldrin – 0,006 µg/kg, for hexachlorcyclohexanes (HCHs) –0.004 µg/kg, α-HCH and β-HCH were not detected. At all stations γ-HCH was the dominant isomer and Lindane content of 60 µg/kg was measured only in Canal between Varna Lake and Varna Bay (Shtereva et al., 2004). The recent data (2009 -2010) for TPH content in Varna Bay sediments indicate spatial variability in the range between 0.4 and 31.27µg/g in average 10.67µg/g, depending on the bottom substrate (Shtereva, 2010). High TPH content was associated with dominating pelite composition and fine sediment fractions.
Measurements of priority substances and specific substances in water were carried out twice in 2011 in coastal area. The results do not reveal a pollution of sea water (Annual report of BSBD, 2011) since all concentrations are bellow DL (Table V.7). Only for Cd, Hg, Pentachlorbenzene, Benzo(b)fluoranthen, Benzo(g,h,i)perylen, Ideno(1,2,3-c,d) pyren and some PCBs DL is below the standard (Regulation for Quality standards, 2010).

Table V.7. Monitoring results for priority and specific substances in water (2011).

Contaminants	Concentration
Hg, µg.dm^3	<1
Pb, µg.dm^3	<3
Ni, µg.dm^3	<3
Zn, µg.dm^3	<3
Cu, µg.dm^3	<3
Cd, µg.dm^3	<1
Li, µg.dm^3	<3
Co, µg.dm^3	<3
As, µg.dm^3	<3
Al, µg.dm^3	<20
4-tert-octilphenol, µg/dm^3	<0.01
Naphtalen µg.dm^3	<0.05
Antracen µg.dm^3	<0.05
Fluoranthen, µg.dm^3	<0.05
Benzo(b)fluoranthen, µg.dm^3	<0.05
Benzo(k)fluoranthen, µg.dm^3	<0.05
Benzo(a)pyren µg.dm^3	<0.05
Ideno(1,2,3-c,d)pyren, µg.dm^3	<0.05
Benzo(g,h)perylen, µg.dm^3	<0.05
Pentachlorbenzene, µg/dm^3	<0.010
Hexachlorbenzene, µg.dm^3	<0.010
2,4' DDE µg.dm^3	<0.010
2,4' DDD µg.dm^3	<0.010
4,4' DDD µg.dm^3	<0.010
2,4' DDT µg.dm^3	<0.010
Terbutrin, µg.dm^3	<0.010
Ethilbenzen, µg.dm^3	<1
m-Xylene, µg.dm^3	<1

o- Xylene, μg.dm³	<1
p- Xylene, μg.dm³	<1
2,4,4-trichlorobiphenil (PCB 28), μg.dm³	<0.010
2,2,5,5-tetrachlorobiphenil(PCB 52), μg.dm³	<0.010
2,2,4,5,5-pentachlorobiphenil(PCB 101), μg.dm³	<0.010
2,3,3,4,4-pentachlorobiphenil(PCB 105), μg.dm³	<0.010
2,3,3,4,4,5-pentachlorobiphenil(PCB 118), μg.dm³	<0.010
2,2,4,4,5-хексахлорбифенил(PCB 138), μg.dm³	<0.010
2,2,4,4,5,5-hexachlorobiphenil(PCB 153), μg.dm³	<0.010
2,3,3,4,4,5-hexachlorobiphenil(PCB 156), μg.dm³	<0.010
2,2,4,4,5,5-hexachlorobiphenil(PCB 180), μg.dm³	<0.010

Turkish Black Sea waters

Heavy metals

Altaş and Büyügüngör (2007) investigated the impact of marine activities on heavy metal pollution in shore and offshore stations of the Sinop, Samsun and Ordu coasts located in the middle Black Sea region between May 2000 and October 2001. Cd, Pb, Zn, Ni and Cu concentrations in seawater did not exceed, with two exceptions, the Marine General Quality Criteria given in Turkish Environmental Regulation. Whereas, the same heavy metals from the same location mostly exceeded the criterion in 2010-2011 (Büyügüngör et al., 2014).

Yemencioglu et al. (2006) measured dissolved forms of the redox-sensitive elements Mn, and Fe in the oxic/anoxic transition zone, or suboxic zone, of the Black Sea. The maximum dissolved iron (Fe^{+2}) concentrations was 16.754 µg/L which was smaller than those of dissolved manganese (439.504 µg/L). Coban et al. (2009) measured heavy metals in seawater of Zonguldak coast and found 1.686 µg/L for Cd, 5.824 µg/L for Cr, 39.281 µg/L for Mn, 7.753 µg/L for Cu, 8. 334 µg/L for Ni, 8.081 µg/L for Pb and 54.535 µg/L for Zn.

The content of some metals in the Black Sea water is, on the whole, negligible. Still, it does not indicate that there exists no such problem as pollution of the Black Sea with toxic metals. Many of heavy metals in the Black Sea water exceeded the criterion. As the metal dissolve in seawater to a limited extent, they are present on suspended particles and deposit, after coming down the rivers and with discharges, in the bottom sediments where they gradually accumulate.

Organic contaminants

The monitoring programme of Turkey has not been designed to cover contaminants measurements in sea water; therefore, the data is very scarce. However a snapshot example in terms of spatial distribution do exist where total PAH (polyaromatic petroleum hydrocarbons), individual PAH, BTEX, and organochlorine pesticides, were measured at surface and 10 m depths at 17 stations selected with 50 nautical-mile intervals and 1 mile off the coast of the Turkish Emergency Response borders in the Black Sea in June, 2007, within the scope of the project titled "Establishing Emergency Response Centers and Status Evaluation of Our Seas" carried out by the TUBITAK MRC Environmental Institute.

According to the results of the measurements, it has been observed that many individual PAHs are at the level of 0,001 µg/L (lowest measurement limit) but the concentrations of fluoranthene off the coasts of Eregli and Kastamonu and acenaphtene off the coasts of Sile and between Ordu and Giresun are high. Total PAH values measured with spectrofluorometer were between 0,2 µg/L and 1,1 µg/L. Benzene, toluene, E-benzene and xysilen of BTEX group parameters were measured lower than 0,5 µg/L, the lowest value to be measured along the whole coastal line of the Black Sea. Organochlorine pesticides were also measured lower than 0, 01 µg/L which is the lowest detectable value.

Sediment contamination

In a recent work to support the future implementation of WFD and MSFD in the Turkish coastal and marine waters (TUBITAK-MRC and MoEU-GDEM, 2014), a supporting feasibility and assessment study has been conducted considering the GES descriptors of MSFD and the ongoing monitoring activities. Within the scope of D8 the below evaluations on sediments were made.

According to the results of the Black Sea monitoring project carried out by the Institute of Marine Sciences and Management of the Istanbul University in 2010, it has been observed that pollution still exists in areas with the same river inputs as the past years. Total organic carbon values on surface sediment in sea bottom are relatively high at stations in front of Igneada, Zonguldak and Sinop. It was measured low at stations between Samsun and Ordu (Sur et al., 2012). It has been observed that industrial/domestic contaminants were transported by rivers especially to areas close to river mouths and metals were accumulated on the bottom sediment with anthropogenic or natural contributions.

Oil pollution detected in Black Sea sediment is extremely high in samples taken from the waters of Zonguldak, Samsun, Bartin and Trabzon. Zonguldak area was emphasized as to be the most important one among others in terms of oil pollution (Sur et al., 2010). According to status evaluations on surface sediments carried out by Sur et al. (2012) within the scope of the same project, the pollution level for Al, Cd, Cu, Pb, V and Hg was measured extremely high at one station, very high at nine stations, medium high at fifteen stations and low at one station. This study helps us to determine whether the contaminants are anthropogenic or not. Maximum and minimum metal concentration ranges are found as for aluminium: 14,52-2,30 (%); cadmium: 1,04-0,03 (µg/g); copper: 407,93-2,87 (µg/g); lead: 79,78-2,51 (µg/g); mercury: 2,86-0,47 (µg/g); vanadium: 1215,81-10,82 (µg/g). The maps below (Figs. V.6 and V.7) was created under the light of the data regarding the organic contaminants and metals in sediment obtained from the project (Black Sea monitoring report dated 2010 by Institute of Marine Sciences and Management of Istanbul University).

Assessment of state of contamination

Since the sediment quality criteria have not been identified for the TR coastal/marine waters and the specific contaminants have not been agreed upon yet, ERL: Effects Ranges Low (Long & Morgan, 1990; Long et al., 1995; Mac. Donald et al., 1996) method was used for to evaluate the level of contamination. However, the values presented with ERL where used to make the traffic light assessment in Fig. 8 should not be used as the threshold between good and not good status which might lead to unreliable results (Occonor, 2004) but rather to be understood as the possible critical values that may damage biota.

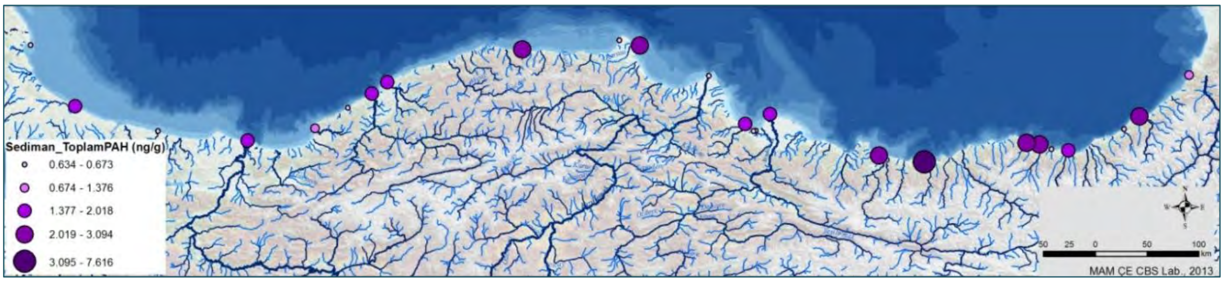


Figure V.6. Map of Black Sea Sediment Pollution Status (2010, Polycyclic Aromatic Hydrocarbons).

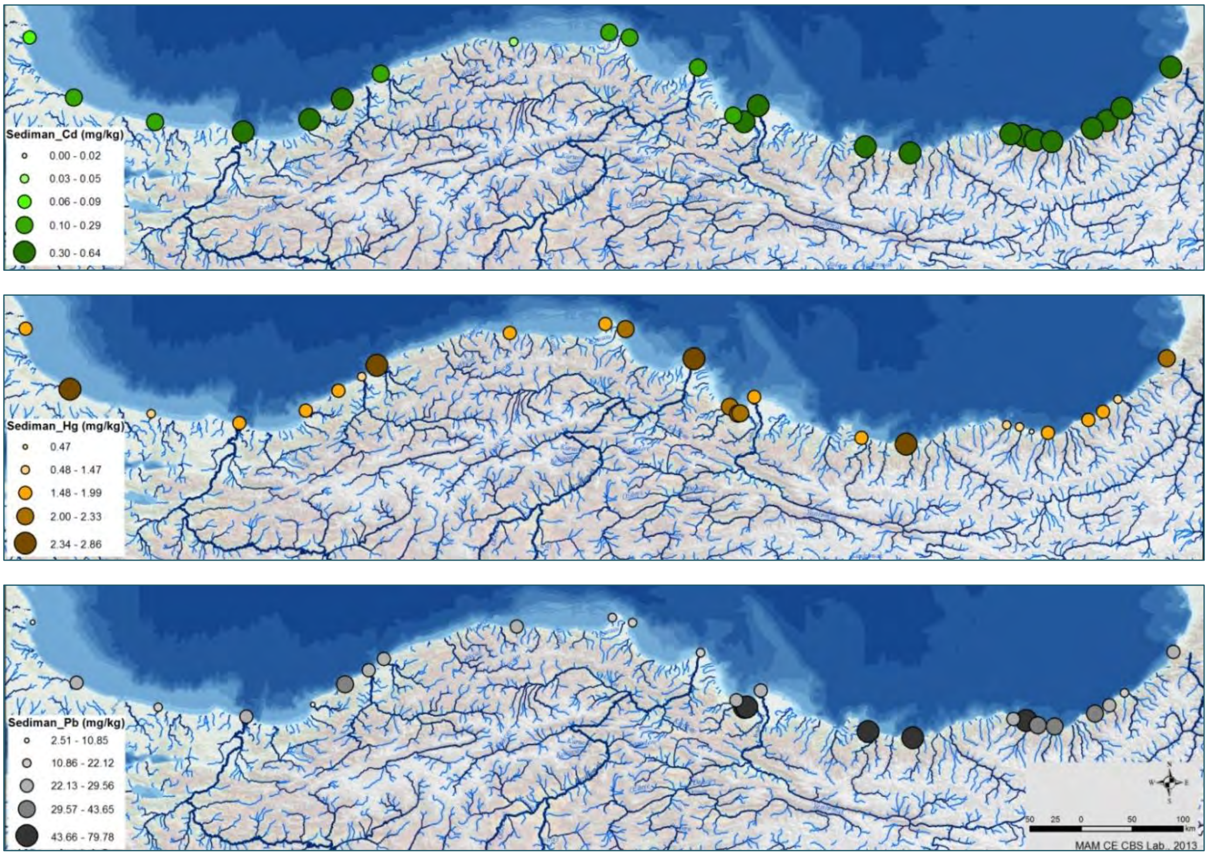


Figure V.7. Map of Black Sea Sediment Pollution Status (2010, Heavy Metals).

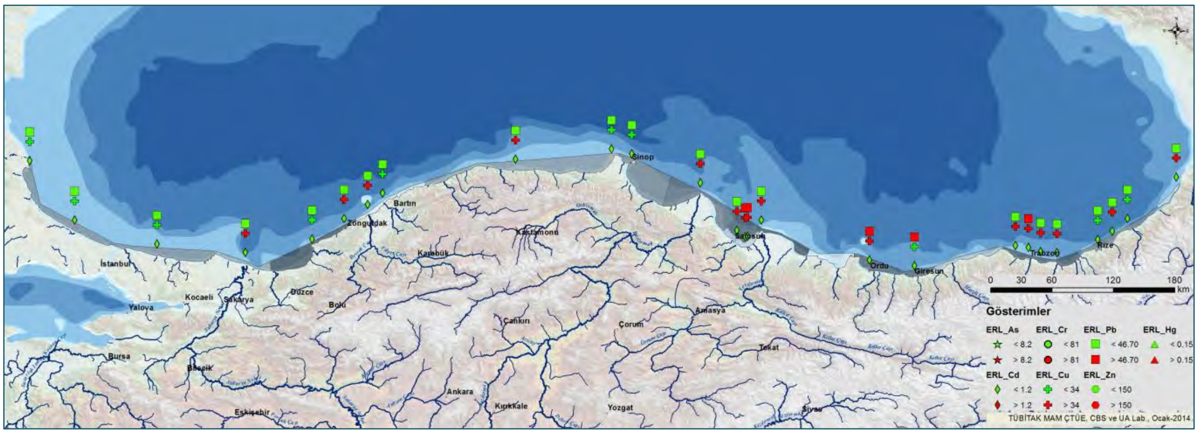


Figure V.8. Traffic light assessment of sediment metal contamination and toxicity with ERL.

Heavy metals in sediments

Heavy metal pollution in sediments of the Black Sea has attracted considerable research attention since last 20 years. Sources of heavy metals in the Black Sea environment can be mainly attributed to terrestrially derived wastewater discharges, agricultural and industrial run-off, river run-off atmospheric deposition of combustion residues and shipping activities (Boran and Altınok, 2010). Heavy metal levels in sediment from the Black Sea were investigated by many researchers (Table V.8) (modified Bat *et al*. submitted for publishing).

Table V.8. Heavy metals (µg/g dry wt) in sediments from Turkish coast of the Black Sea

Region	Zn	Ni	Cu	Mn	Pb	Cd	Cr	Co	Hg	Reference
South West Black sea 1993 1995	-	-	-	-	2.9-42.0	-	-	-	-	Ünsal, 2001
Kızılırmak	-	-	11.80-356.56	-	6.22-11.32	-	-	-	0.86-4.0	Ünsal *et al.,* 1995
Yeşilırmak	-	-	528.12 ± 357.0	-	38.74 ± 39.5	-	-	-	0.29-2.02	Ünsal *et al.,* 1995
Whole Black Sea 1989	50-108	38-130	29-68	355-751	14-35	-	32-171	7-37	-	Kıratlı and Ergin, 1996
South Black sea 1988-1989	24-138	11-202	15-87	112-1064	12-66	-	13-224	< 20	-	Yücesoy and Ergin, 1992
South West Black sea 1999	50-111	34-88	20-47	312-995	19-51	-	51-135	9-19	-	Ergin *et al.,* 2003
Black Sea 1997-1998	57-127	2.2-69.1	23-75	354-902	11-30	0.6-0.9	22-122	5.2-17.2	-	Topçuoğlu, 2000
Sinop 1993	-	-	-	-	-	-	218.1 ±20.1	-	-	Topçuoğlu *et al.,* 1998
Rize 2001	484.2 ± 1.1	-	506.5 ± 1.5	647.5 ± 1.9	39.2± 4.3	<0.02	-	-	-	Topçuoğlu *et al.,* 2003b
İğneada 1998	119.3 ± 0.7	31.57 ± 0.51	13.57 ± 0.08	519.1 ± 4.5	< 0.05	< 0.02	74.7 ± 1.4	21.45 ± 4.44	-	Topçuoğlu *et al.,* 2002
Amasra 1997	92.6 ± 0.37	33.50 ± 0.77	27.60 ± 0.24	338.2 ± 0.3	21.4 ± 5.6	0.73 ± 0.08	58.5 ± 0.4	8.28 ± 0.51	-	Topçuoğlu *et al.,* 2002

Region	Zn	Ni	Cu	Mn	Pb	Cd	Cr	Co	Hg	Reference
Sinop 1997	91.5 ± 0.45	65.20 ± 0.71	37.3 ± 0.14	424.3 ± 1.3	15.1 ± 2.9	0.89 ± 0.11	115.5 ± 0.5	13.40 ± 0.71	-	Topçuoğlu *et al.*, 2002
Perşembe 1997	82.9 ± 0.16	18.50 ± 0.22	69.9 ± 0.20	514.1 ± 1.1	31.1 ± 2.0	0.93 ± 0.04	21.8 ± 0.1	16.8 ± 0.97	-	Topçuoğlu *et al.*, 2002
Samsun (2002-2003)	109.55-261.65	7.9-49.25	32.9-64.85	441.55-668.75	12.13-223.7	<0.02	53.05-99.3		-	Bakan *et al.*, 2007
Yeşilırmak outer 1996, 2001,2002	325.3	128.1	59.9± 1.0	2915± 5	<0.01	<0.02	1276.5	63.7± 2.3	-	Balkıs *et al.*, 2007
Yeşilırmak inner 1996, 2001,2002	119.8	129.9 ±1	43.7± 2.1	864±3	<0.01	<0.02	370.8 ±1	33.6± 1.9	-	Balkıs *et al.*, 2007
Kızılırmak outer 1996, 2001,2002	91.4± 0.5	104.6 ±1	23.0± 0.3	989±4	<0.01	<0.02	231.9 ±1	22.7± 1.2	-	Balkıs *et al.*, 2007
Kızılırmak inner 1996, 2001,2002	119.5	120.0 ±2	27.6± 0.7	1206± 1	<0.01	<0.02	720.6 ±1	27.4± 1.2	-	Balkıs *et al.*, 2007
Yomra 2002-2003 (0-2 cm)	182 ± 17	26.53 ± 0.79	56.86 ± 0.34	672.8 ± 3.0	<0.1	<0.02	74.24 ±7.00	23.90 ± 1.40	-	Ergül *et al.*, 2008
Yomra 2002-2003 (0-8 cm)	169 ± 16	23.61 ± 2.19	52.03 ± 0.61	651.9 ± 2.27	<0.1	<0.02	70.02 ± 7.04	22.60 ± 1.30	-	Ergül *et al.*, 2008
Trabzon	56.5-286.3	10.6-29.2	13.68-315.99	-	12.34-83.78	-	-	-	-	Öşeker and Erüz, 2011
West Black Sea 2008	82.8-183.9	56.9-93.3	39.8-72.46	416-763	20.29-37.76	0.18-0.53	73-117	12.9-21	0.03-0.13	Ozkan and Buyukisik, 2012
East Black Sea 2008	83.9-124.2	49.5-140.6	57.83-71.83	383-755	18.13-44.33	0.24-0.45	54-147	19.7-28.9	0.04-0.07	Ozkan and Buyukisik, 2012

MATERIAL and METHODS

The study area for contamination state was the Western Black Sea, three transects from the Romanian, Bulgarian and Turkey waters. Map of sampling stations and stations coordinates and depths are presented on Table 1 and Fig. 1.

Water samples for pollutants were collected from the surface layer (more precise, 1 m below the surface) from the 5 l Niskin bottles of the Rosette System. About 1 liter seawater was transferred into glass bottles, which were stored at refrigerator temperature until their subsequent analysis in laboratory. NIMRD analysed the pollutants in water at all stations. Sediment samples for pollutants were taken using a Van Veen sampler grab, from the surface undisturbed layer. GEOECOMAR collected sediment samples from all transects, for granulometry and heavy metals, and NIMRD collected samples from all transects for persistent organic pollutants (POPs). The samples were stored frozen (at – 20 ÷ - 24 °C) and analysed subsequently in laboratory (Fig. V.9). Details on specific seawater and sediment pollutants analysed, methods and responsible institutions are presented in the Table V.9.

Figure V.9.Seawater and sediment samples collection.

Table V.9.Specific pollutants analysed in seawater and sediment sampled in July 2013 during MISIS cruise, analytical methods and responsible institutions.

SEAWATER POLLUTANTS							
Parameters	**Heavy metals (HM)**	**Total petroleum hydrocarbons (TPH)**	**Poly-aromatic hydrocarbons (PAH)**	**Organo-chlorine pesticides (OCP)**	**Poly-chlorinated biphenyls (PCB)**	**Total organic carbon (TOC)**	
Responsible:							
NIMRD	**X** (RO, BG, TR transects)	**X** (RO, BG, TR transects)	**X** (RO, BG, TR transects)	**X** (RO, BG, TR transects)	**X** (RO, BG, TR transects)	**X** (RO, TR transect)	
GEOECOMAR	-	-	-	-	-	-	
TUBITAK	-	-	-	-	-	-	
Methods	GF-AAS	Fluorescence method	GC-MS	GC-ECD	GC-ECD		
SEDIMENT POLLUTANTS							
Parameters	**Heavy metals (HM)**	**Total petroleum hydrocarbons (TPH)**	**Poly-aromatic hydrocarbons (PAH)**	**Organo-chlorine pesticides (OCP)**	**Poly-chlorinated biphenyls (PCB)**	**Total organic carbon (TOC)**	**Grain size**
Responsible:							
NIMRD	X (inter-calibration samples)	X (inter-calibration samples; RO, BG, TR transects)	X (inter-calibration samples; RO, BG, TR transects)	X (inter-calibration samples; RO, BG, TR transects)	X (inter-calibration samples; RO, BG, TR transects)		
GEOECOMAR	X (inter-calibration samples; RO, BG, TR transects)					X (RO, BG, TR transects)	X (RO, BG, TR transects)
TUBITAK	X (inter-calibration samples; TR transect)		X (inter-calibration samples)				
Methods	GF-AAS Flame-AAS WDXRF ICP-MS	Fluorescence method	GC-MS	GC-ECD	GC-ECD		

RESULTS and DISCUSSIONS

1. SEAWATER

Heavy metals

Besides natural sources (erosion of rocks, volcanic emissions), heavy metals (arsenic, cadmium, copper, chromium, mercury, lead, nickel, tin, zinc) are released into the environment in large quantities from activities associated with mining, metallurgy, manufacture, fossil fuel or waste incineration. It is considered that the lack of adequate control measures in the riparian countries, especially in the period before the 90', when industries recorded the maximum development, was a major cause of pollution of the Black Sea. Of course, in addition to the direct contribution of coastal activities (domestic and industrial wastewater, storm water, etc.), should not be neglected pollutants generated in hydrological basins of major rivers (Danube, Dnieper, Dniester, Bug, Cuban, Don) which flows into the sea (Mee & Topping, 1998). Along with land-based activities, shipping, oil and gas exploitation and dumping of dredged material represent potential sources of pollution to the marine environment. Atmospheric transport of heavy metals is another major pathway by which these contaminants end up in the marine environment (Hacisalihoglu et al., 1991; UNEP, 2002 and 2006).

Although they are normal constituents of the marine environment, when anthropogenic sources introduce additional quantities, metals enter in the biogeochemical cycles and, as a result of toxic potential, may interfere with the normal functioning of ecosystems (OSPAR, 1992). Metals in sea water are often associated with particulate matter and accumulate in sediments, where may remain for long periods. Through complex interactions they can be fixed, re-suspended or up-taken marine organisms. Heavy metals are persistent pollutants of the environment and even in the hypothetical situation of reducing anthropogenic contributions; sedimentary reserves of metals accumulated over time continue to threaten the health of the marine ecosystem.

During MISIS cruise, water samples from surface layer were collected for metals analysis. Total metals (dissolved and suspended forms) have been determined in unfiltered seawater samples, acidified up to pH=2 with Ultrapure HNO_3. Metals were analysed by graphite furnace – atomic absorption spectrometry (GF – AAS).

Metals concentrations in surface seawater collected during July 2013 from all transects were found to be rather low, varying within the following ranges: 0.10 - 2.99 µg/L Cu; 0.05 - 0.76 µg/L Cd; 1.16 - 3.70 µg/L Pb; 0.14 - 12.38

µg/L Ni; 1.14 - 6.06 µg/L Cr. (Table V.10, Fig. V.10). Data obtained during MISIS cruise for heavy metals in surface seawater are comparable and included within typical ranges reported for Black Sea coastal or open waters (Table V.11).

Generally, a slight decreasing gradient from coastal to open sea was noticed for most analysed metals, with the exception of lead and chromium, but with no statistically significant differences (Fig. V.11).

These measurements from July 2013 indicated a low level trace metal pollution of marine waters, concentrations of cadmium, lead and nickel being much below recommended EQS from European Legislation (Directive 2013/39/EU).

According to literature data from other marine regions, concentrations of cadmium in seawater are normally situated below 0.10 µg/L (IPCS, 1992), and nickel between 0.20 and 0.70 µg/L (Alzieu, 1999). Copper was reported from 2 µg/L in open sea waters to 15 µg/L in estuarine areas, with variation ranges of 1–5 µg/L in coastal areas from Baltic and Mediterranean seas. Dissolved chromium in oceans ranges within 0.12 µg/L at surface, up to 0.35 µg/L in deeper waters, this element being strongly represented by suspended form, rather than dissolved. Background dissolved lead in coastal and marine waters varies between 0.10 – 0.45 µg/L, this element being also rapid adsorbed by suspended matter (Alzieu, 1999).

Table V.10. Concentrations of metals (µg/L) in surface water samples from the Romanian, Bulgarian and Turkish area, July 2013.

Element	Mean	StdDv	Max	Min	MAC-EQS* Directive 2013/39/EU
Cu (µg/L)	0.65	0.76	2.99	0.10	-
Cd (µg/L)	0.21	0.17	0.76	0.05	1.50
Pb (µg/L)	2.45	0.71	3.70	1.16	14.00
Ni (µg/L)	4.38	3.67	12.38	0.14	34.00
Cr (µg/L)	2.54	1.42	6.06	1.14	-

** dissolved fraction*

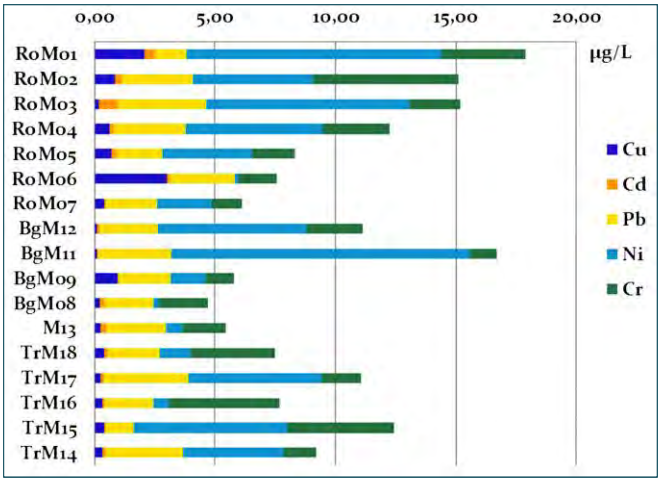


Figure V.10. Heavy metals levels (µg/L) in surface water samples from the Romanian, Bulgarian and Turkish area, July 2013.

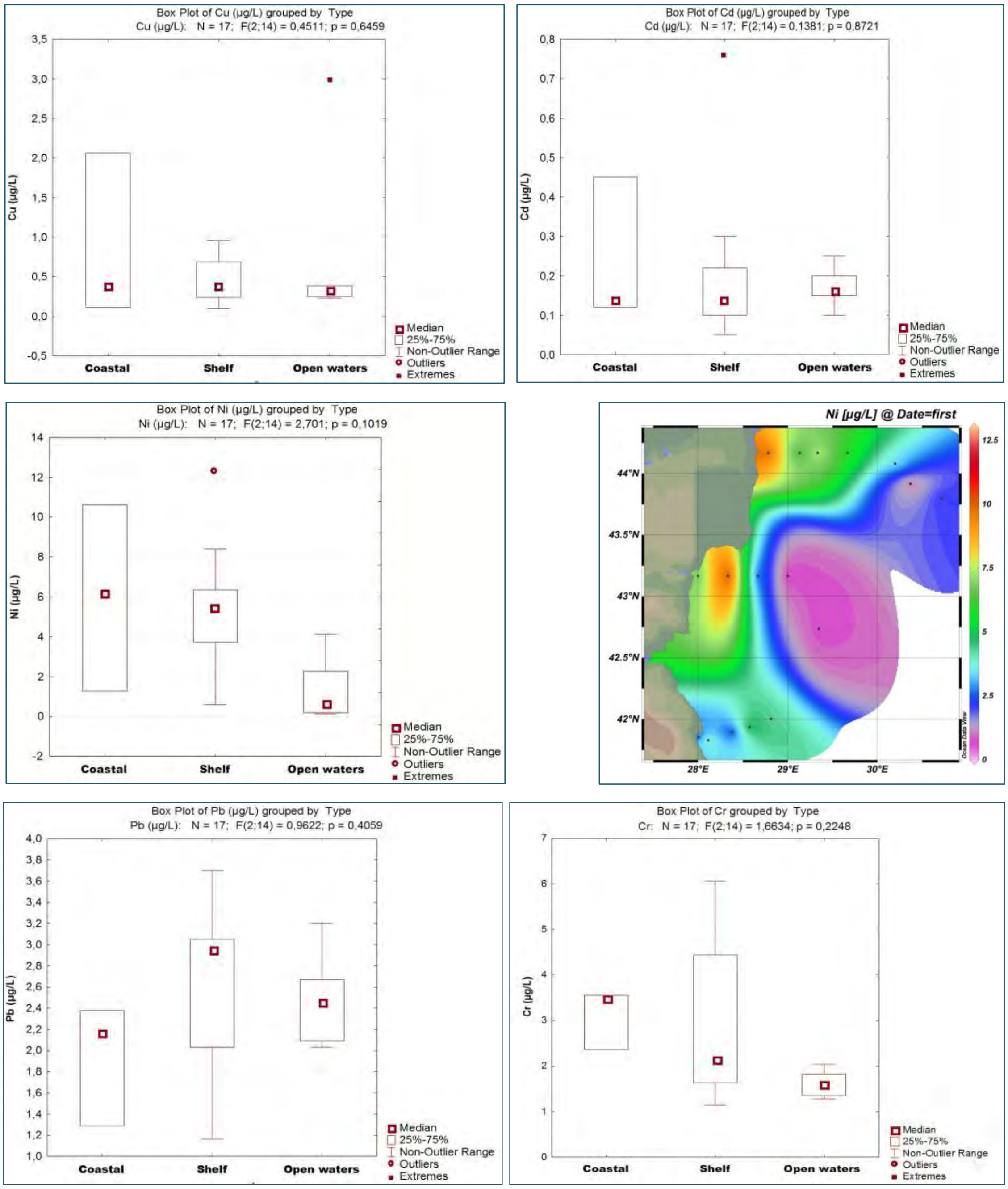


Figure V.11. Distribution of metals (µg/L) in coastal, shelf and open sea waters from the Romanian, Bulgarian and Turkish area, July 2013.

Table V.11. Comparative data on heavy metals in seawater of the Black Sea region.

	Cu (µg/L)	Cd (µg/L)	Pb (µg/L)	Ni (µg/L)	Cr (µg/L)
MISIS cruise, July 2013 (RO, BG, TR transects) (total metals)	0.65 (0.10-2.99)	0.20 (0.05 - 0.76)	2.45 (1.16-3.70)	4.38 (0.14-12.38)	2.54 (1.14-6.06)
IA RO Transitional, Coastal and Marine Waters (2006-2011) (total metals) (NIMRD, 2012)	10,02	0,99	3,78	3,65	3,84
RO Coastal and Marine waters (2013 Monitoring data) (total metals)	1,14 (0,37 -4,14)	0,56 (0,16 - 2,87)	2,40 (0,04 - 7,04)	1,42 (0,24 - 8,13)	2,45 (0,37-10,34)
Ukrainian coastal Black Sea waters (total metals) (BSC, 2008)	0.40 - 3.00	0.05 - 0.15	1.00 - 9.30		
BG coastal Black Sea waters (Andreev *et al.*, 1994)	0.60 - 0.85	0.018 - 0.026	0.40 - 0.87	0.61 - 0.82	
BG coastal Black Sea waters (BSBD Report, 2006)		0.84 - 0.97	1.70	3.8 - 5.9	
TR coastal Black Sea waters (total metals) (Coban *et al.*, 2009)	7.75	1.68	8.08	8. 33	5.82
W Black Sea (dissolved metals) (Tankere *et al.*, 1996)	1.78	0.016	0.037	0.94	
NW Black Sea (dissolved metals) (Zeri *et al.*, 2000)	1.19 – 218.11	0.15–1.59		0.23 – 11.15	

Conclusions

Metals concentrations in surface seawater from July 2013 indicated a low level trace metal pollution, concentrations of cadmium, lead and nickel being much below recommended EQS from European Legislation (Directive 2013/39/EU).

Generally, a slight decreasing gradient from coastal to open sea was noticed for most analysed metals.

Organochlorine Pesticides and Polychlorinated Biphenyls

The organochlorine pesticides (OCP) and polychlorinated biphenyls (PCB) concentrations determined in water samples are presented in Table V.12 and V.13, where ∑OCPs (μg/L) is the concentration of the nine individual compounds (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, p,p'DDE, p,p'DDD and p,p'DDT) and ∑PCBs (μg/L) is the concentration of the seven individual compounds (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180).

Table V.12. Concentrations of individual and total OCPs (∑OCPs) (μg/L) in water samples from the Romanian, Bulgarian and Turkish area, July 2013.

Stations	HCB	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p' DDT	∑ OCPs
MO1(RO32m)	0.028	0.039	<0.003	<0.003	<0.002	0.010	<0.002	<0.002	<0.002	0.091
MO2(RO47m)	0.027	0.051	<0.003	<0.003	<0.002	0.008	<0.002	<0.002	<0.002	0.100
MO3(RO52m)	0.015	0.028	<0.003	<0.003	<0.002	0.009	<0.002	<0.002	<0.002	0.066
MO4(RO65m)	0.037	0.030	<0.003	<0.003	<0.002	0.007	<0.002	<0.002	<0.002	0.088
MO5(RO100m)	0.050	0.020	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002	0.087
MO6(RO496m)	0.047	0.058	0.037	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002	0.156
MO7(RO1000m)	0.063	0.049	<0.003	<0.003	<0.002	0.013	<0.002	<0.002	<0.002	0.139
MO8(BG1167m)	0.042	0.044	<0.003	<0.003	<0.002	0.003	<0.002	<0.002	<0.002	0.103
MO9(BG92m)	0.051	0.134	<0.003	<0.003	0.010	0.012	<0.002	<0.002	<0.002	0.218
M10(BG77m)	0.028	0.032	<0.003	<0.003	<0.002	0.009	<0.002	<0.002	<0.002	0.082
M11(BG40m)	0.111	0.169	0.075	<0.003	<0.002	0.026	<0.002	0.007	0.038	0.434
M12(BG23m)	0.009	<0.003	<0.003	<0.003	<0.002	0.007	<0.002	<0.002	<0.002	0.033
M13(BG1000m)	0.007	<0.003	<0.003	<0.003	<0.002	0.008	<0.002	<0.002	<0.002	0.032
M14(TR1118m)	0.019	0.023	<0.003	<0.003	<0.002	0.011	<0.002	<0.002	<0.002	0.066
M15(TR101m)	0.021	0.034	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002	0.072
M16(TR75.6m)	0.057	0.127	0.281	<0.003	0.009	<0.003	0.006	0.007	0.010	0.502
M17(TR54m)	0.079	0.284	<0.003	<0.003	0.092	<0.003	0.020	0.017	0.096	0.596
M18(TR27m)	0.023	0.065	0.185	<0.003	0.021	0.011	0.099	<0.002	<0.002	0.411

Total organochlorine pesticides concentrations varied from 0.032 to 0.596 μg/L. Total polychlorinated biphenyls concentrations varied from 0.039 to 0.726 μg/L. There is an obvious difference between open sea area and waters closer to shore where the anthropic influence is stronger (Figs. V.12 and V.14).

The concentrations of OCPs and PCBs were higher in coastal and shelf waters sampled from M15 - M18 stations (Figs. V.14 and V.15).

The major OCPs compounds were HCB, lindane, and heptachlor. The highest values measured were: 0.284 μg/L for lindane, 0.281 μg/L for heptachlor and 0.111 μg/L HCB. More than 75% values for aldrin, dieldrin, p,p' DDE, p,p' DDD and p,p' DDT were under detection limit.

Table V.13. Concentrations of individual and total PCBs (∑PCBs) (µg/L) in water samples from the Romanian, Bulgarian and Turkish area, July 2013.

Stations	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑ PCBs
MO1(RO32m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO2(RO47m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO3(RO52m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO4(RO65m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO5(RO100m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO6(RO496m)	<0.004	0.007	<0.006	<0.004	<0.006	<0.007	<0.003	0.040
MO7(RO1000m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO8(BG1167m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
MO9(BG92m)	0.006	0.160	<0.006	<0.004	<0.006	<0.007	<0.003	0.195
M10(BG77m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
M11(BG40m)	<0.004	0.042	<0.006	<0.004	<0.006	<0.007	<0.003	0.075
M12(BG23m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
M13(BG1000m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
M14(TR1118m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
M15(TR101m)	<0.004	<0.006	<0.006	<0.004	<0.006	<0.007	<0.003	0.039
M16(TR75.6m)	<0.004	0.498	<0.006	<0.004	<0.006	<0.007	<0.003	0.531
M17(TR54m)	0.009	0.555	<0.006	0.113	0.014	<0.007	0.023	0.727
M18(TR27m)	<0.004	0.319	<0.006	<0.004	<0.006	<0.007	<0.003	0.352

The major PCB compound was PCB 52. The highest values measured, 0.555 µg/L and 0.498 µg/L were recorded in shelf waters from M16 and M17 stations. More than 85% values for the other PCBs compounds were under detection limit.

For Bulgarian transect, the results for organochlorine contaminants are comparable with BSBD results (Annual reports, BSBD, 2006-2011), except for HCB, lindane and PCB 153 for which were measured higher concentrations. For Turkey area, the results for organochlorine pesticides levels are comparable with previous results reported in Black Sea Turkish waters (Ozkoc, et al., 2007), except for lindane for which were measured higher concentrations. The concentrations of organochlorine pesticides are comparable with those reported in the previous period in Romanian waters (NIMRD, 2012).

Comparison to Assessment Criteria

The results of the water analyses were compared to Directive 2013/39/EU amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

There is no threshold value set as EQS available for PCBs in water. Some concentrations of regulated pesticides in water exceeded the threshold values set out by Directive 2013/39/EU as MAC/AA, especially for lindane and Sum of Cyclodiene (Table V.14). For heptachlor is hard to say if the concentrations exceeded the threshold value since the detection limit is much higher than the EQS. Same remark applies to the value 0.008 for "Sum of Cyclodiene" which represents the sum of detection limits for individual compounds (aldrin, dieldrin and endrin).

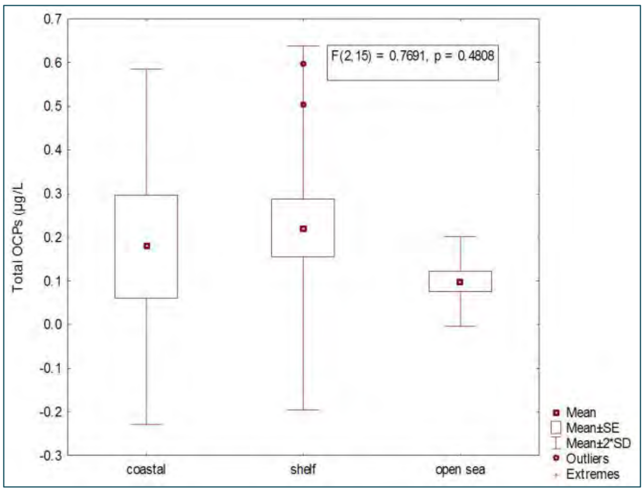


Figure V.12. Box plot of ∑OCPs (µg/L) in coastal, shelf and open sea waters from the Romanian, Bulgarian and Turkish area, July 2013.

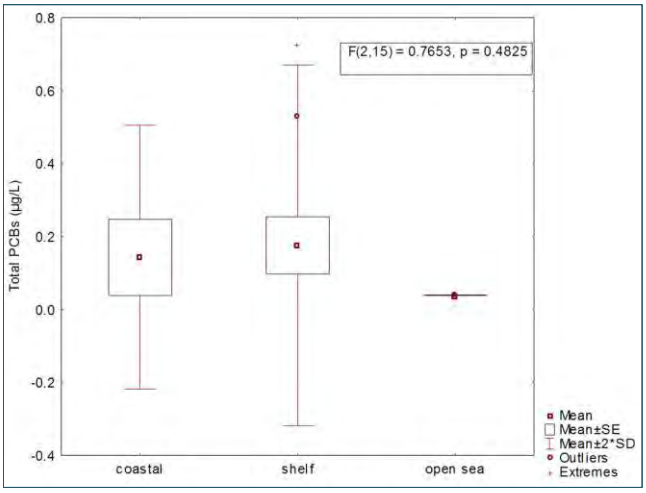


Figure V.13. Box plot of ∑PCBs (µg/L) in coastal, shelf and open sea waters from the Romanian, Bulgarian and Turkish area, July 2013.

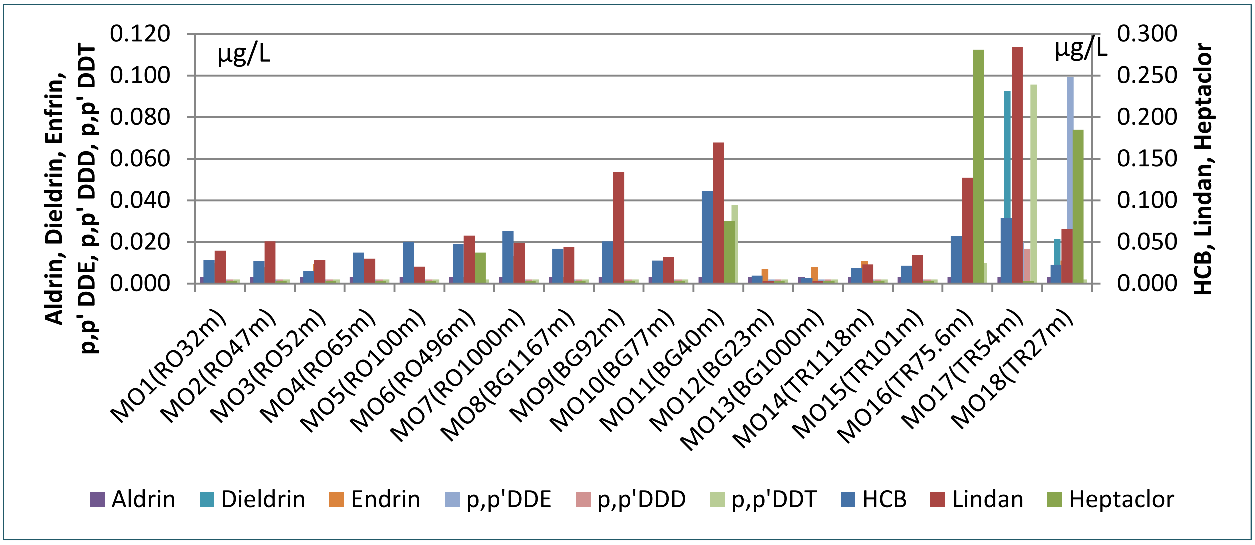


Figure V.14. Individual OCPs levels (µg/L) in water samples from the Romanian, Bulgarian and Turkish area, July 2013.

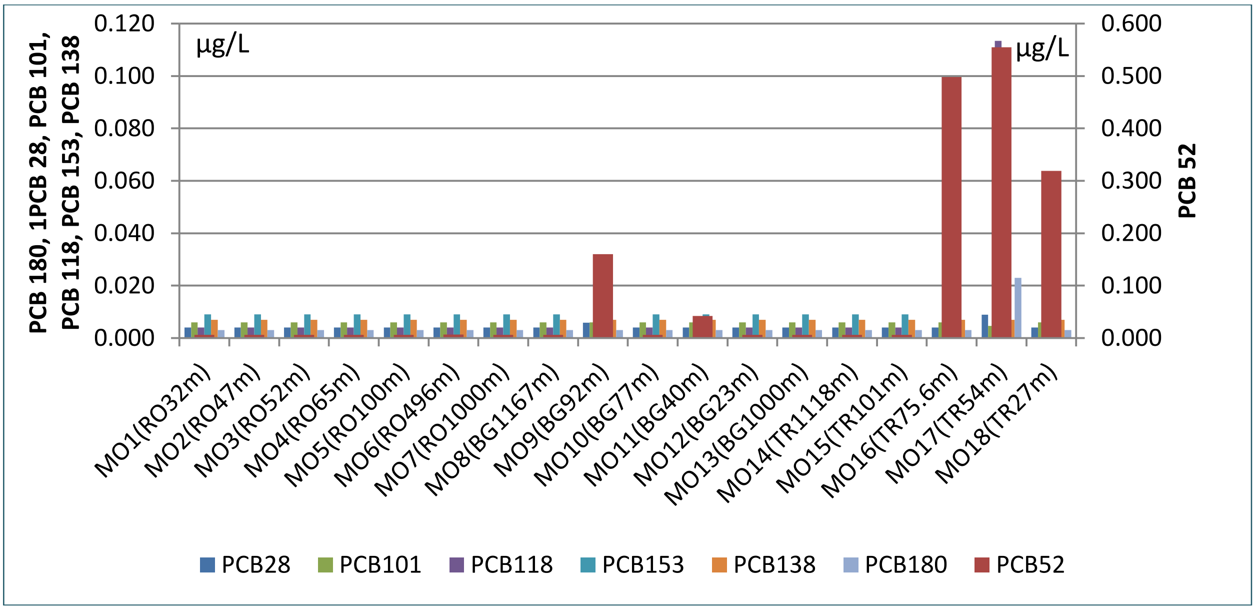


Figure V.15. Individual PCBs levels (µg/L) in water samples from the Romanian, Bulgarian and Turkish area, July 2013.

Table V.14. Concentrations of OCPs (µg/L) by comparison with EQS (Directive2013/39/EU) in water samples from the Romanian, Bulgarian and Turkish area, July 2013.

Stations	HCB[a]	Lindane[a]	Heptachlor[a]	Sum Cyclodiene[b]	p,p'DDT[b]	Sum DDT[b]
MO1(RO32m)	0.028	0.039	<0.003	0.015	<0.002	<0.006
MO2(RO47m)	0.027	0.051	<0.003	0.013	<0.002	<0.006
MO3(RO52m)	0.015	0.028	<0.003	0.014	<0.002	<0.006
MO4(RO65m)	0.037	0.030	<0.003	0.012	<0.002	<0.006
MO5(RO100m)	0.050	0.020	<0.003	<0.008	<0.002	<0.006
MO6(RO496m)	0.047	0.058	0.037	<0.008	<0.002	<0.006
MO7(RO1000m)	0.063	0.049	<0.003	0.018	<0.002	<0.006
MO8(BG1167m)	0.042	0.044	<0.003	0.008	<0.002	<0.006
MO9(BG92m)	0.051	0.134	<0.003	0.025	<0.002	<0.006
MO10(BG77m)	0.028	0.032	<0.003	0.014	<0.002	<0.006
MO11(BG40m)	0.111	0.169	0.075	0.031	0.038	0.047
MO12(BG23m)	0.009	<0.003	<0.003	0.012	<0.002	<0.006
MO13(BG1000m)	0.007	<0.003	<0.003	0.013	<0.002	<0.006
MO14(TR1118m)	0.019	0.023	<0.003	0.016	<0.002	<0.006
MO15(TR101m)	0.021	0.034	<0.003	<0.008	<0.002	<0.006
MO16(TR75.6m)	0.057	0.127	0.281	0.015	0.010	0.023
MO17(TR54m)	0.079	0.284	<0.003	0.098	0.096	0.132
MO18(TR27m)	0.023	0.065	0.185	0.035	<0.002	0.103
EQS (Directive**2013/39/EU**)	0.050	0.020*	0.00003**	0.005	0.01	0.025

[a] refers to MAC-EQS; [b] refers to AA-EQS; * the MAC value in the Directive 2013_39_EU refers toHCH, not to gamma HCH; ** the MAC value in Directive2013_39_EU refers to heptachlor and heptachlor epoxide

Conclusions

Concentrations of organochlorine compounds in water are higher or comparable with those reported in the Black Sea region in previous expedition.

The BS waters were dominated by the presence of lindane and cyclodiene, which often exceeded the threshold values set out by Directive 2013/39EU.

Except PCB 52, the values measured for other PCBs compounds were low or under detection limit.

Total petroleum hydrocarbons

As shown in Fig. V.16, the concentrations of petroleum hydrocarbons in MISIS water samples ranged between 82.5 and 221.6 (µg/L) with an average of 147.4 (µg/L). The maximum concentration was reported at station M09 and the minimum at station M11, both of them in shelf waters. Among the 18 seawater samples tested, there were 83% samples with low TPH values <200 µg/L. The distribution of concentrations in coastal, shelf and open waters did not point out statistically significant differences between the means (Fig. V.17).

The average total TPH concentration (µg/L) calculated as 147.4 ± 40.5 is comparable with the mean value of 103 ± 64.0 reported for the Black Sea waters in 1992 - 2006 (BSC, 2008). Generally, the total hydrocarbon concentration in seawater which can induce harmful effect on the aquatic organisms is about 50 (µg/L). Most of the countries use this value as seawater quality standard (Maximum Allowed Concentration - MAC). Our results showed that in all water samples, the TPHs content exceeded this value up to four times.

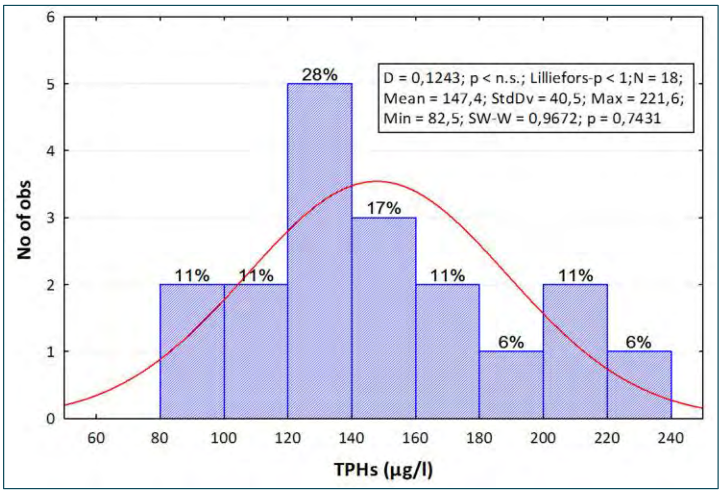


Figure V.16. Histogram of TPHs (µg/L) in waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect, July 2013.

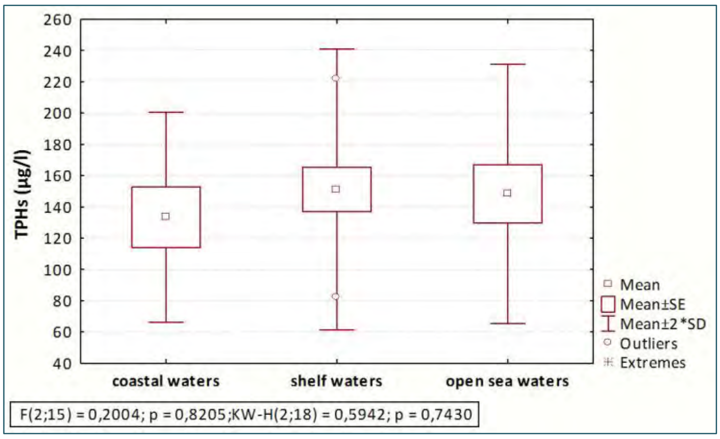


Figure V.17. Distribution of petroleum hydrocarbon concentrations (µg/L) in coastal, shelf and open sea waters including the Romanian, Bulgarian and Turkish transect, July 2013.

Polycyclic aromatic hydrocarbons (PAHs) levels and composition

The total polycyclic aromatic hydrocarbons - Σ_{16}PAH (µg/L) content in water samples (n=18) ranged from 0.339 to 2.107 with a mean of 0.690 ± 0.400 (Fig. V.18). Table V.15 showed the concentration values of the individual PAH compounds in all of MISIS water samples. Of the total PAHs, the 2-3-ring PAHs contributed to about 75% while 4-6-ring PAHs accounted for 25%. Naphthalene and phenanthrene were found as the most dominant compounds with average distribution of 34% and 18 % of the total PAHs in the Western Black Sea waters.

The statistical analysis of data shows significant differences ($p < 0.05$) between the mean value of the total Σ_{16}PAH (µg/L) in coastal waters (1.290 ± 0.707) and shelf (0.586 ± 0.138), and open sea (0.520 ± 0.146) waters (Fig.V.19). Highest abundance of total PAHs compound in coastal waters were dominated by 2-3 rings PAHs, characteristic for discharges, oil and oil product spill (Fig. V.20). Both pyrolytic (4-6 aromatic rings) and petroleum (2-3 rings) PAHs are present in shelf and open sea waters (Fig. V.21 and V.22).

The polycyclic aromatic hydrocarbon level caused by anthropogenic impact is estimated as the ratio between the concentrations of 4-6-aromatic ring pyrolitic PAHs and the 2-3-aromatic ring PAHs formed under natural conditions, characteristic for oil and oil products. High molecular mass PAHs (HMW)- with 4-6 aromatic rings {fluoranthene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo(g,h,i)perylene, dibenzo (a,h)anthracene, indeno(1,2,3-c,d)pyrene} are generated as a follow-up of the fossil fuel (hydrocarbons, coal, oil or natural gases) incomplete combustion at high temperatures - pyrolysis. These pyrolytic PAHs are often determined in the atmosphere dust of urban areas, unlike low molecular mass PAHs (LMW) - with 2-3-aromatic rings (naphtalene, acenaphtene, acenaphtylene, fluorene, phenantrene, anthracene), characteristic for discharges, oil and oil product spills.
A subunitary LMW/HMW ratio usually indicates a pyrolytic pollution, while in case of values >1 it indicates the abundance of low molecular mass PAHs, characteristic for oil and oil products.

As shown in Table V.15, the value of LMW/HMW index at all stations of sampling ranged from 0.89 to 11 with high values in coastal waters. The data indicate that 89% of samples are petroleum origin. The LMW/HMW > 1 ratio calculated for the Western Black Sea waters indicates pollution with oil products, which is not confirmed by the low total petroleum hydrocarbon content (TPH <200 µg/l) recorded in Romanian, Bulgarian and Turkish Black Sea waters during the MISIS Joint Cruise. In these cases, the sources of PAHs emission may be different, random and irregular The values of the

LMW/HMW ratio >1 only accounts for the high concentrations of naphthalene and phenanthrene (2-3 aromatic rings) dominant compounds not only in July 2013, but also confirmed by 2006 - 2011 monitoring data of Romanian Black Sea waters.

The same petroleum profile was reported by Nagy et al. (2002), for the Hungarian upper section of the Danube River in the period of 2007 - 2010. Naphthalene and phenanthrene were found as the most dominant compounds with average distribution of 21 and 26% of the total PAHs in the river water.

Comparison to Assessment Criteria

High concentrations (µg/L) were determined for benzo[k]fluoranthene (0.026 ± 0.006), benzo[a]pyrene (0.028 ± 0.012) and anthracene (0.073 ± 0.079), their values exceeding the maximum allowable concentration - MAC by DIRECTIVE 39/2013/EU Annex I/Part A: Environmental Quality Standards (Table V.15).

Conclusions

Naphthalene and phenanthrene were found as the most dominant compounds in coastal waters of the Western Black Sea.
Possible sources of polycyclic aromatic hydrocarbons showed that the PAHs were from both the pyrogenic and petrogenic origin in shelf and open sea waters.

Table V.15. Concentrations of individual, total (∑16PAHs) PAHs, CPAHs% - the carcinogenic PAHs percentage and LMW/HMW index in waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect, July 2013.

Station M*	Nap**	Acl	Ac	Fl	Phe	An	Fa	Py	B[a]a	Chry	B[b]fl	B[k]fl	B [a] pyr	B (g,h,i) p	D (a,h) a	Ip	*$\sum_{16}$PAHs*	***CPAH %***	LMW/ HMW
MO1 RO	0.723	0.041	0.036	0.244	0.466	0.348	0.029	0.049	0.030	0.014	0.004	0.022	0.051	0.009	0.021	0.019	**2.107**	*11.8*	7.44
MO2 RO	0.191	0.011	0.021	0.016	0.204	0.030	0.022	0.023	0.004	0.011	0.002	0.025	0.033	0.015	0.015	0.009	**0.631**	*25.1*	2.98
MO3 RO	0.092	0.018	0.040	0.049	0.068	0.106	0.039	0.023	0.008	0.011	0.005	0.025	0.045	0.017	0.018	0.019	**0.584**	*36.1*	1.77
MO4 RO	0.065	0.013	0.016	0.036	0.077	0.030	0.024	0.025	0.005	0.014	0.003	0.027	0.022	0.018	0.020	0.014	**0.410**	*42.3*	1.36
MO5 RO	0.226	0.015	0.020	0.058	0.146	0.131	0.018	0.028	0.009	0.008	0.008	0.023	0.030	0.012	0.011	0.009	**0.750**	*20.6*	3.85
MO6 RO	0.190	0.024	0.025	0.025	0.159	0.088	0.032	0.032	0.006	0.018	0.013	0.036	0.021	0.023	0.024	0.013	**0.729**	*30.0*	2.33
MO7 RO	0.061	0.019	0.021	0.026	0.021	0.011	0.024	0.025	0.006	0.013	0.004	0.033	0.023	0.018	0.022	0.012	**0.340**	*53.0*	0.89
M08 BG	0.034	0.013	0.024	0.024	0.062	0.047	0.036	0.036	0.003	0.021	0.002	0.038	0.016	0.025	0.023	0.014	**0.415**	*51.1*	0.96
M09 BG	0.182	0.012	0.018	0.020	0.117	0.003	0.023	0.034	0.001	0.011	0.003	0.029	0.048	0.018	0.017	0.010	**0.545**	*35.5*	1.82
M10 BG	0.448	0.011	0.021	0.070	0.132	0.047	0.010	0.010	0.002	0.005	0.001	0.011	0.008	0.007	0.007	0.004	**0.794**	*8.2*	11.23
M11 BG	0.128	0.020	0.024	0.020	0.043	0.021	0.024	0.021	0.001	0.011	0.008	0.026	0.025	0.016	0.015	0.012	**0.414**	*38.3*	1.61
M12 BG	0.502	0.014	0.025	0.039	0.088	0.069	0.025	0.022	0.002	0.011	0.013	0.021	0.042	0.015	0.017	0.009	**0.914**	*19.3*	4.19
M13 BG	0.213	0.023	0.024	0.049	0.122	0.029	0.022	0.027	0.004	0.019	0.009	0.025	0.021	0.017	0.026	0.011	**0.642**	*28.2*	2.55
M14 TR	0.143	0.015	0.029	0.057	0.059	0.031	0.018	0.019	0.004	0.012	0.002	0.024	0.021	0.015	0.019	0.007	**0.467**	*30.1*	2.37
M15 TR	0.174	0.020	0.024	0.030	0.086	0.098	0.020	0.020	0.010	0.012	0.011	0.023	0.017	0.015	0.013	0.008	**0.581**	*25.7*	2.90
M16 TR	0.127	0.019	0.022	0.033	0.084	0.084	0.027	0.028	0.008	0.014	0.007	0.032	0.026	0.020	0.019	0.016	**0.566**	*34.8*	1.87
M18 TR	0.368	0.018	0.018	0.035	0.204	0.080	0.018	0.019	0.001	0.008	0.001	0.020	0.027	0.013	0.015	0.008	**0.851**	*15.2*	5.59
M17 TR* **	0.280	0.021	0.020	0.112	15.997	0.185	4.435	4.267	0.967	0.631	0.020	0.030	0.038	0.023	0.024	0.025	**27.074**	*38.6*	1.59
MAC****	130					0.1	0.12				0.017	0.017	0.027						

*18 samples of: 7 from the Romanian transect (M 01- coastal, M02-M 05 shelf and M06-M07 open sea waters), 6 from the Bulgarian transect (M12 coastal, M09-M11) shelf and M08 open sea waters), and 5 from the Turkish transect (M 18 coastal , M17– M 15 shelf and M14 open sea waters);

** Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene: Chry, Benzo[b]fluoranthene: B[b]fl, Benzo[k]fluoranthene: B[k]fl, Benzo[a]pyrene: B[a]pyr, Benzo (g,h,i)perylene: B(g,h,i)p, Dibenzo(a,h)anthracene: D(a,h)a, Indeno(1,2,3-c,d)pyrene :Ip;

M17 TR*** The outlier value of *$\sum_{16}$PAHs* 27,074 (µg/L), determined in M17 station, is not included in the statistical analysis of the data.

MAC****maximum allowable concentration/ANNEX I/PART A : Environmental Quality Standards (EQS)/DIRECTIVE 39/2013

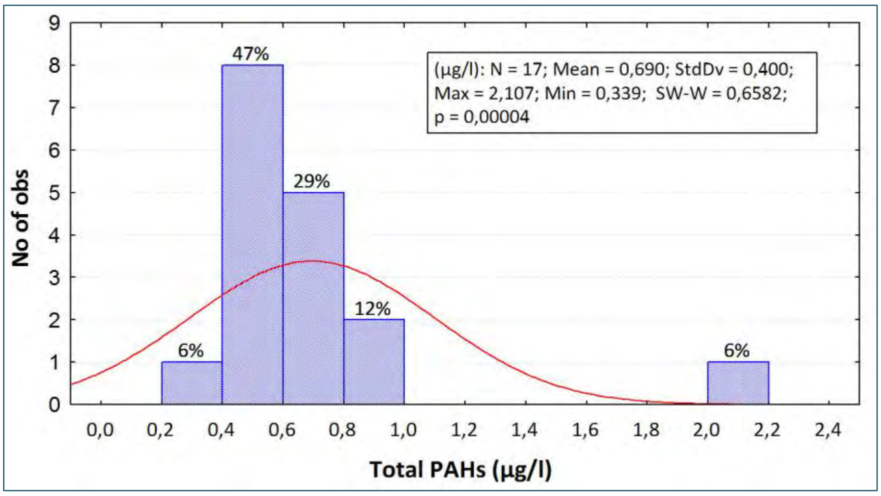


Figure V.18. Histogram of ∑16PAHs (µg/L) in waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect, July 2013.

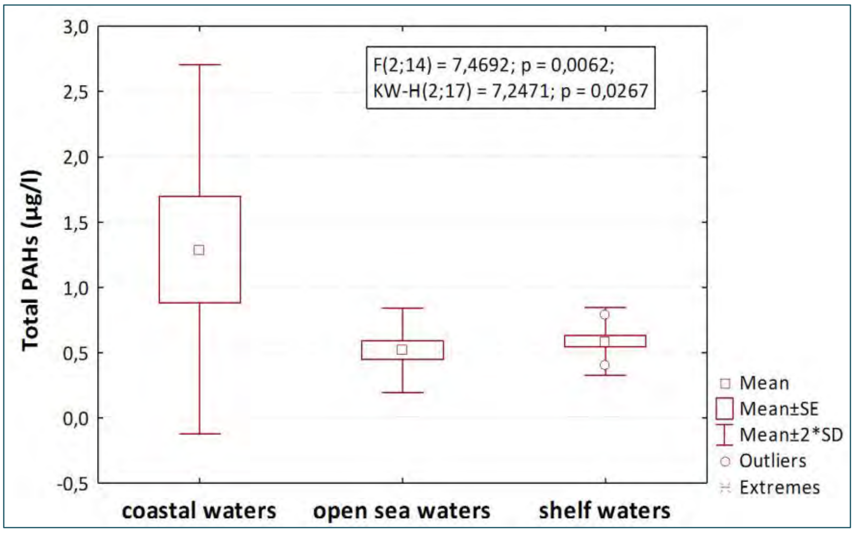
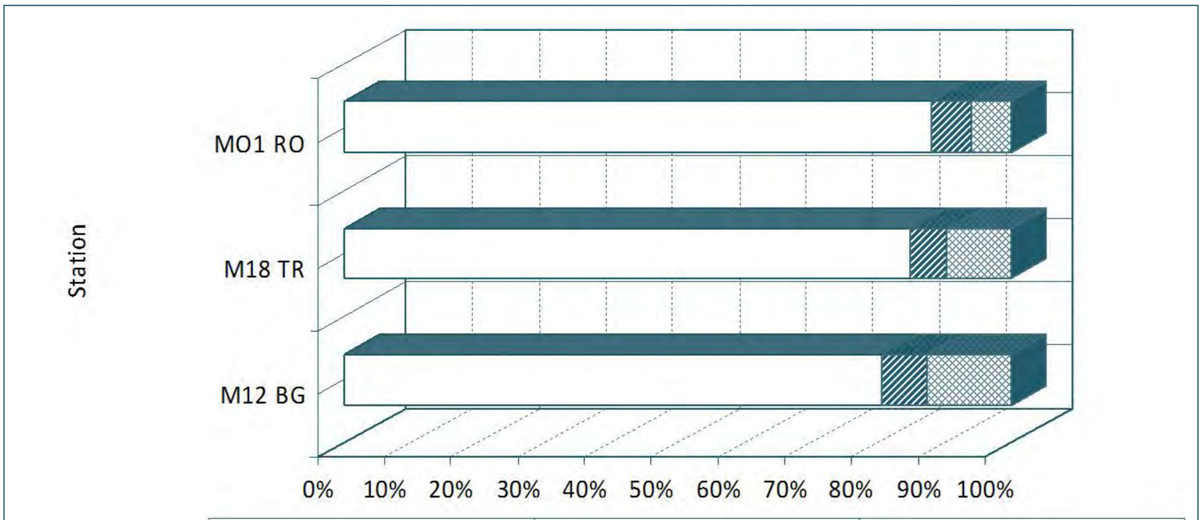
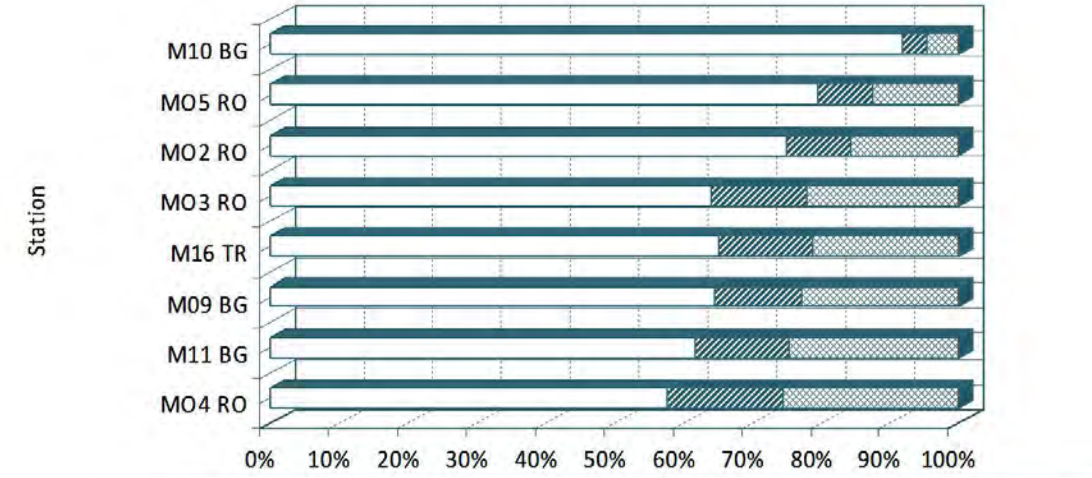


Figure V.19. Distribution of the polycyclic aromatic hydrocarbons - Σ_{16}PAH (µg/L) in coastal, shelf and open sea waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect, July 2013.



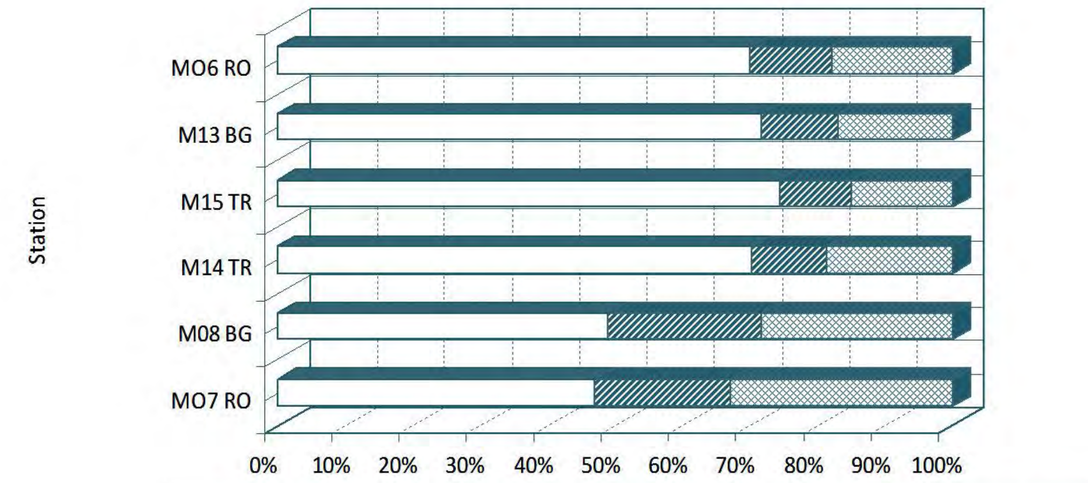
	M12 BG	M18 TR	MO1 RO
PAH(5-6 rings)	0,117	0,082	0,127
PAH(4 -rings)	0,060	0,047	0,123
PAH(2-3 rings)	0,737	0,722	1,858

Figure V.20. Distribution of PAHs in coastal waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect according to the number of aromatic rings.



	MO4 RO	M11 BG	M09 BG	M16 TR	MO3 RO	MO2 RO	MO5 RO	M10 BG
PAH(5-6 rings)	0,104	0,102	0,124	0,120	0,130	0,099	0,093	0,037
PAH(4 -rings)	0,069	0,057	0,069	0,077	0,081	0,059	0,061	0,028
PAH(2-3 rings)	0,237	0,255	0,351	0,369	0,374	0,473	0,595	0,730

Figure V.21. Distribution of PAHs in shelf waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect according to the number of aromatic rings.



	MO7 RO	M08 BG	M14 TR	M15 TR	M13 BG	MO6 RO
PAH(5-6 rings)	0,112	0,118	0,088	0,087	0,109	0,131
PAH(4 -rings)	0,068	0,095	0,053	0,062	0,072	0,088
PAH(2-3 rings)	0,160	0,203	0,333	0,432	0,461	0,510

Figure V.22. Distribution of PAHs in open sea waters from the Western Black Sea, including the Romanian, Bulgarian and Turkish transect according to the number of aromatic rings.

2. SEDIMENTS

Characteristics of surface sediments

The characterization of the surface sediments encountered during the MISIS cruise of the R/V Akademic is based on grab sediment samples collected in 13 stations at water depths shallower than app. 100 m (deepest stations M05 and M15 – 101.0 m water depth). The sampling stations were distributed as follows: five stations (M01-M05) on the Constanta transect; four stations (M09-M12) on the Galata transect; four stations (M15-M18) on the Igneada transect.

In each station primary sediment samples were recovered with a Van Veen grab with a sampling surface of 0.135 m^2. As soon as the sediment sample was on deck the grab was carefully opened to ensure a minimum disturbance of the sediment pack. Each sample was photographed and macroscopically described (Annex I). The temperature of the sediment pack was measured with a Digi-Sense® ThermoLogR thermistor thermometer.

After the completion of these operations sediment samples for laboratory analyses were collected by several teams from the 0 to 2 centimetres layer of surface sediment. Samples for inorganic analyses from all three transects were collected by GeoEcoMar, the following characterization being based on these results.

Macroscopic description and grain size composition of surface sediments

With a single exception – station M18 from the Igneada transect, the collected grab sediment samples belonged to the "m*ud*" category (mixed silt and clay sediments). In station M18 the recovered sediment was a terrigenous-biogenenous sand.

Nevertheless, the sediment samples visual analysis identified several well defined mud types. Typical *Mytilus* muds were identified in stations M01, M02, M11 and M12, at water depths <50 m, while typical "*white*" *Modiolus* muds appeared in stations M04, M05 and M15, at water depths greater than 65 m.

Intermediate water depths were characterized by the presence of transition muds, with *Mytilus* and *Modiolus* – stations M03 and possibly M10.

The sediments in stations M16 and M17 from the Igneada transect were mostly terrigenous muds with very few shells

H_2S smell was usually absent, being signalled only in station M15.

The most interesting sedimentary sequence was identified in station M09 from the Galata transect. Here typical *Modiolus* mud, characteristic for the station depth, was overlaid by 10 cm of terrigenous mud, soft, sticky, greasy, gelatinous, elastic, light grey at the top (1-2 cm), with 4-5 mm of fluid yellowish brown mud, dark grey for the rest, without shells (see photo, Annex I). This indicates an abrupt change of the sedimentary regime while the uniformity of the superficial sediment layer seems to indicate its deposition in a very short time (possibly a sediment slide).

In all stations the sediments were characterized by the presence of an oxidized layer of variable thickness, from a few millimetres to 1.5-2 cm, followed by reduced sediment.

In station M05 small manganese nodules were identified on the sediment surface (Fig. V.23). They formed through post-depositional mobilization of manganese, its migration to the sediment surface and re-precipitation of the favourable surfaces of small *Modiolus* and/or *Mytilus* shells.

Figure V.23. Fe-Mn nodules recovered from sediments in station M05.

Temperature of the sediment usually varied between 7.93°C and 8.67°C, with higher temperatures in the shallower water sediments (water depths <35 m). The highest sediment temperature (14.52°C), was recorded in the shallowest station (M12 – water depth=23.2 m).

Detailed grain size analyses were performed in GeoEcoMar sedimentology laboratory (Table V.16). After elimination of the eventual vegetal remains, the sediment sample were first separated in two fractions, >1 mm and <1 mm by sieving on a 1.00 mm mesh sieve. The fraction >1 mm was analysed by the sieving method while the fraction <1 mm was analysed by laser difractometry on a laser difractometer Malvern Mastersizer 2000E Ver.5.20, after dispersion and homogenization by 10 minutes ultrasonication.

The dimensional scale Udden-Wentworth, with sand/silt limit at 0.063 mm and silt/clay limit at 0.004 mm was used to separate grain size fractions. The grain size composition of the samples was calculated by cumulating the results of the sieving and laser difractometry analyses. The cumulated percentages of sandy, silty and clayey fractions were used to sedimentologically classify the sediments according to Sheppard (Fig. V.24, Table V.17).

The results evinced the clear dominance of silty sediments, present in all stations with the exception of station M18, characterized by silty sand having in composition more than 50% fine and very fine sand. From the other stations, the sediments from M02, M09 and M17 were pure silts, with >75% silt; in station M12 the sediment was a sandy silt, with ca. 16% fine and very fine sand and app. 25% coarse silt. The remaining stations were characterized by the presence of clayey silts.

With the exception of the coarser sediments from stations M12 and M18 all the rest were characterized by the dominance of the fine fraction (from fine silt to fine clay), usually representing ≈60% from the sediment composition, with a maximum of 85% in station M05 The dominance of the fine clayey-siltic sediments, confirmed by the median diameter values (Table V.18), varying between 6.45 ϕ and 7.45 ϕ, indicates as the main sedimentary process the deposition of suspensions.

Table V.16. Detailed grain size composition and Sheppard classification of sediment samples collected during the R/V Akademic MISIS cruise.

Sample	Sand, %					Silt, %				Clay, %		
	Very coarse	Coarse	Medium	Fine	Very fine	Coarse	Medium	Fine	Very fine	Coarse	Medium	Fine
	1.00-2.00	0.500-1.00	0.250-0.500	0.125-0.250	0.063-0.125	0.03-0.063	0.016-0.031	0.008-0.016	0.004-0.008	0.002-0.004	0.001-0.002	<0.001
M 01	0.09	1.00	1.56	1.90	2.85	9.38	21.20	21.95	19.40	11.79	5.40	3.48
M 02	0.00	0.00	0.00	0.00	0.47	11.11	28.55	21.65	17.38	11.85	5.42	3.59
M 03	0.00	0.00	1.25	4.60	4.25	6.47	18.99	20.76	19.72	13.34	6.28	4.35
M 04	0.00	0.00	0.13	2.89	4.45	8.24	22.14	21.04	18.28	12.64	5.98	4.22
M 05	0.00	0.00	0.00	0.32	0.50	1.30	12.72	24.27	26.89	19.44	8.86	5.69
M 09	0.00	0.00	0.00	0.00	0.00	6.99	25.94	23.91	19.81	13.27	6.06	4.02
M 11	0.00	0.00	0.00	0.41	4.50	13.24	22.87	21.16	17.65	11.22	5.39	3.56
M 12	0.00	0.00	0.00	1.02	15.08	25.61	18.13	13.94	12.45	7.40	3.75	2.63
M 15	0.00	0.00	0.11	3.28	6.90	8.69	16.39	20.19	20.71	13.43	6.25	4.05
M 16	0.00	0.00	0.00	0.59	6.37	13.67	20.48	20.34	18.28	11.46	5.39	3.42
M 17	0.00	0.00	0.02	0.21	2.95	11.77	24.45	23.78	19.18	10.69	4.32	2.64
M 18	1.98	9.72	12.43	27.35	22.46	5.78	5.59	5.04	4.52	2.90	1.27	0.95

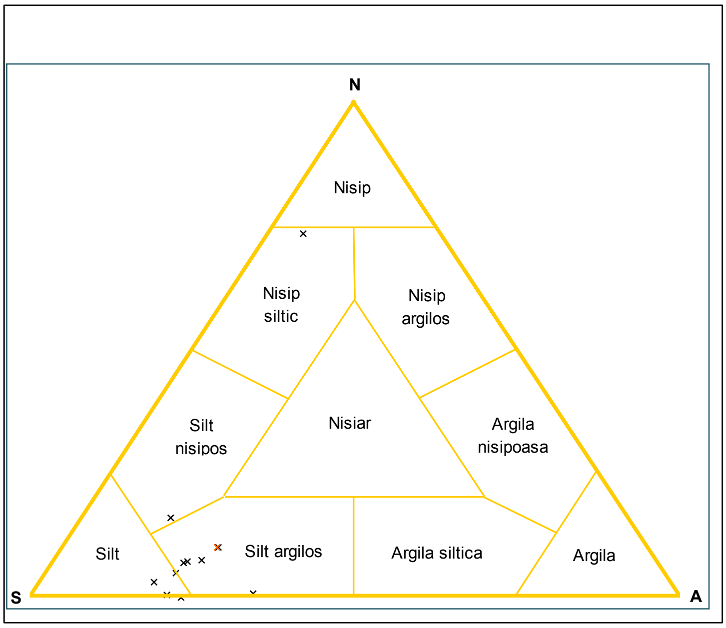


Figure V.24. Sheppard's diagram for sediments from the MISIS cruise.

Table V.17. Sediment composition in terms of sand-silt-clay and Sheppard's classification.

Sample	Grain size composition			Shepard class
	Sand %	Silt%	Clay %	
M 01	7.40	71.94	20.66	Clayey silt
M 02	0.47	78.68	20.85	Silt
M 03	10.09	65.94	23.97	Clayey silt
M 04	7.48	69.69	22.84	Clayey silt
M 05	0.82	65.19	33.99	Clayey silt
M 09	0.00	76.65	23.35	Silt
M 11	4.91	74.92	20.17	Clayey silt
M 12	16.10	70.12	13.78	Sandy silt
M 15	10.29	65.98	23.73	Clayey silt
M 16	6.96	72.77	20.27	Clayey silt
M 17	3.19	79.17	17.64	Silt
M 18	73.94	20.94	5.12	Silty sand

Table V.18. Statistical parameters of grain size distributions for MISIS sediments.

Md. (ϕ)	Mz (ϕ)	σ	Sk	K
6.55	6.60	1.80	0.00	1.18
6.43	6.65	1.54	0.24	0.95
6.68	6.71	1.94	-0.04	1.22
6.56	6.68	1.82	0.06	1.13
7.35	7.44	1.39	0.14	1.00
6.68	6.86	1.49	0.22	0.96
6.43	6.53	1.70	0.11	1.02
5.44	5.73	1.83	0.27	0.88
6.72	6.63	1.93	-0.07	1.11
6.45	6.48	1.77	0.05	0.99
6.45	6.53	1.52	0.11	1.03
2.95	3.40	2.26	0.32	1.52

Md – median diameter; Mz – mean graphical diameter;
σ – standard deviation (sorting); Sk – skewness; K – kurtosis

For the sediments with higher sand percentages (stations M12 and M18), the higher values of the median diameter (5.44 ϕ and 2.95 ϕ) indicate a more energetic environment.

The values of the standard deviation, a numerical measure of the sediment sorting, varying in the range 1.39 – 1.94, with an exceptional value of 2.26 (station 18), indicate a poor sorting. These values are normal for clayey-siltic sediments, the particles agglomeration process preventing the selective deposition.

The positive skewness (asymmetry) values usually indicate depositional processes while the negative ones are indicators of erosive processes. The actual values, oscillating around 0 apparently indicate equilibrium between deposition and erosion.

Chemical characterization of sediments

The chemical characterization of the sediments from sampling stations done during the R/V Akademic MISIS cruise is based on the inorganic analyses of a set of 13 samples from the 0 to 2 cm sediment layer, the same set used for grain size analyses.

After preliminary treatment, consisting of air drying, grinding and sieving through a 0·063-mm mesh sieve, the sediment samples were analysed for major components (exerting major controls on the distributions of the trace elements), minor and trace elements (Table V.19).

Titration methods were used for analysing $CaCO_3$ (Black, 1965) and total organic carbon (Gaudette et al., 1974).

Co, Ni, Cu, Pb and Zn were analysed by FAAS and Cd by ETAAS on a Pye Unicam SOLAAR 939E double-beam absorption spectrophotometer with deuterium-lamp background correction. A wet digestion technique, consisting of boiling with concentrated nitric acid (Jickells & Knapp, 1984), was used to dissolve the trace elements. After drying, the residue was dissolved in diluted hydrochloric acid and brought to 50 ml. The system was calibrated with a series of standard solutions prepared from pure metals.

Fe_2O_3 (total), TiO_2, MnO, Rb, Ba, Sr, Cr, Zr and V were directly analysed from compacted powders by X-ray fluorescence spectroscopy on a VRA–30 XRF sequential spectrometer, fitted with an X-ray tube with a tungsten anode. An analyser crystal LiF 200 was used to select the characteristic wavelengths, and measurements were made with a Na(Tl)I scintillation detector. Calibration was carried out with the help of a series of international standards kindly provided by the U.S. Geological Survey. The National Institute of Standards and Technology, U.S.A., the National Research Council, Canada and IAEA-MEL – Monaco.

From the beginning it has to be stressed out that the statistical sample of results was too small for the wide investigated area to allow an accurate characterization. Nevertheless, some general inferences on the chemical composition of the sediments and the degree of inorganic pollution may be done.

Both the analytical results (Table V.19) and their synthesis through the main statistical parameters of the component distributions (Table V.20) show a rather high compositional variability.

As expected, higher coefficients of variation (>50%) characterized the biogenic components (calcium carbonate, TOC, strontium), highly redox sensitive elements (V – C_v=55% and Mn – C_v=41%) as well as some technophyllic metals (Cu, Pb, Cd – coefficients of variation around 40%).

However, the highest coefficients of variation was recorded for TiO_2, mostly as a result of the very low concentrations, close to the detection limit (0.03% TiO_2), recorded in the Romanian stations with *Modiolus* muds, situated on the so-called "*sediment starving continental shelf*" (Panin et al., 1999). Another significant contribution to the total variability of the TiO_2 concentrations has the unusually high concentration recorded in station M12, characterized by sandy silt sediment. Possibly favourable hydro-dynamic conditions determined here enrichment in heavy minerals including rutile and possible other titanium minerals, a hypothesis sustained by the highest Zr concentration also recorded here.

The remaining chemical components have lower coefficients of variation, ranging from a minimum of 23.3% – Ba to a maximum of 38.6% – Rb.

Table V.19. Results of chemical analyses of sediment samples from the R/V Akademic MISIS cruise.

Station	$CaCO_3$ %	TOC %	Fe_2O_3 %	TiO_2 %	Mn, µg/g	Rb, µg/g	Zr, µg/g	Ba, µg/g	Sr, µg/g	Ni, µg/g	Co, µg/g	Cr, µg/g	V, µg/g	Cu, µg/g	Pb, µg/g	Zn, µg/g	Cd, µg/g
M01	17.79	1.56	5.09	0.29	782	101	148	338	306	35.1	7.7	76	34	28.2	23.1	57.0	0.247
M02	15.66	2.78	5.84	0.31	999	141	121	510	349	56.5	11.1	88	43	50.3	44.3	85.6	0.386
M03	53.60	1.14	2.65	0.03	558	57	124	443	729	25.5	5.8	55	32	17.8	21.0	35.9	0.164
M04	52.81	1.80	2.32	0.03	937	55	142	398	1028	29.4	5.9	44	5	24.9	23.6	36.5	0.202
M05	45.09	2.37	2.71	0.03	1162	69	148	589	841	54.4	8.2	43	33	48.5	30.8	51.4	0.299
M09	19.88	2.57	5.40	0.22	1425	122	128	423	448	57.8	12.5	69	72	48.8	45.2	82.3	0.273
M10	14.17	1.93	5.64	0.57	614	126	126	431	305	46.4	10.5	83	51	40.2	31.1	73.0	0.342
M11	12.39	1.73	6.23	0.60	674	127	155	461	227	50.2	12.8	57	38	39.0	31.7	74.4	0.304
M12	14.37	0.82	4.33	0.84	457	96	327	370	244	31.3	8.1	40	39	18.4	15.0	45.3	0.159
M15	51.48	0.78	2.27	0.03	349	31	133	291	946	15.7	4.4	38	17	13.8	16.0	26.1	0.107
M16	11.22	1.23	5.74	0.55	643	125	195	310	215	50.3	10.9	87	89	27.6	27.7	64.6	0.229
M17	14.45	1.24	5.50	0.63	511	113	190	250	316	41.6	9.7	81	91	31.3	23.6	61.9	0.294
M18	22.37	0.11	4.65	0.45	583	53	177	365	729	17.4	8.6	66	54	14.3	9.2	30.0	0.057

Table V.20. Main statistical parameters of the distribution of the inorganic chemical components of sediment in MISIS stations.

Component	C_{mediu}	C_{median}	**s**	**K**	**Sk**	C_{Min}	C_{Max}	C_v, %	**n**
$CaCO_3$, %	26.56	17.79	17.145	-1.218	0.887	11.22	53.60	64.55	13
TOC, %	1.55	1.56	0.766	-0.375	-0.058	0.11	2.78	49.54	13
Fe_2O_3, %	4.49	5.09	1.477	-1.423	-0.606	2.27	6.23	32.90	13
TiO_2, %	0.35	0.31	0.276	-1.215	0.163	0.03	0.84	78.21	13
Mn, µg/g	745.7	642.8	306.53	0.534	1.007	349	1425	41.11	13
Rb, µg/g	93.5	101	36.14	-1.369	-0.400	31	141	38.64	13
Zr, µg/g	162.6	148	55.19	7.083	2.482	121	327	33.93	13
Ba, µg/g	398.4	398	92.78	0.147	0.396	250	589	23.29	13
Sr, µg/g	514.1	349	296.17	-1.303	0.646	215	1028	57.61	13
Ni, µg/g	39.36	41.63	14.63	-1.277	-0.328	15.7	57.8	37.17	13
Co, µg/g	8.94	8.58	2.608	-0.817	-0.183	4.371	12.82	29.16	13
Cr, µg/g	63.6	66.3	18.57	-1.634	-0.087	38	88	29.19	13
V, µg/g	46.0	39	25.49	-0.139	0.520	5	91	55.38	13
Cu, µg/g	30.99	28.15	13.259	-1.396	0.214	13.76	50.31	42.78	13
Pb, µg/g	26.32	23.57	10.538	-0.052	0.425	9.21	45.18	40.04	13
Zn, µg/g	55.69	57.03	19.99	-1.313	-0.009	26.1	85.6	35.89	13
Cd, µg/g	0.236	0.247	0.0950	-0.452	-0.374	0.057	0.386	40.29	13

– C – concentration; – s – standard deviation; – K – kurtosis;
– Sk – skewness; C_v – coefficient of variation

The parameters K (Kurtosis) and Sk (Skewness) define the aspect of the frequency distribution curve of the component concentration, with K defining the peakedness and Sk the symmetry of the curve. For a normal (gaussian) distribution the curve is mesokurtic (K=0) and symmetric (Sk=0). Although most of the distribution curves are slightly platikurtic, it is probably as a result of too few data and slightly asymmetric, with either left or right asymmetry. For the absolute majority of the components the distribution may be accepted as normal. Zr, due to the already signalled high

concentration in station M12, has an anomalous distribution.
It is interesting that the distribution of all the technophyllic metals may be accepted as normal, which might be an indication of little to no metal pollution.

One of the most important chemical component of the sediments, mostly biogenic, is the calcium carbonate. Its concentrations in the analysed samples vary from 11.22% (station M16) to 53.6% (station M03), with a mean concentration of 26.6% $CaCO_3$ (Table V.19). The $CaCO_3$ concentrations increase with depth (Fig. V.25), although the linear correlation between depth and carbonate concentration is significant only at a confidence level of 80-85%.

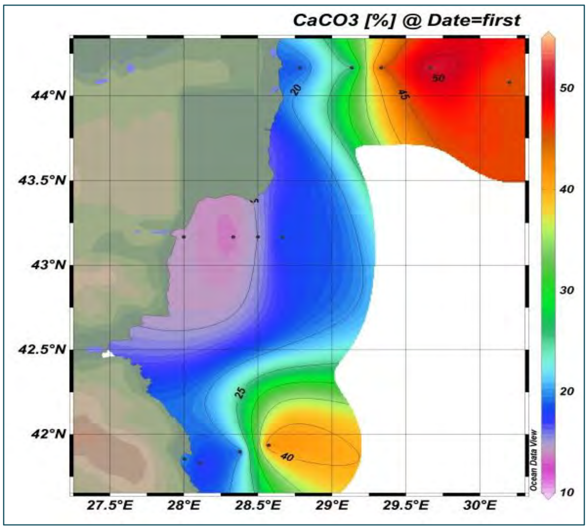


Figure V.25. Distribution of CaCO3 content (%) in sediments from the MISIS cruise.

The sediments encountered during the MISIS cruise may be classified in terms of $CaCO_3$ concentration in:

- low calcareous sediments – 10%<$CaCO_3$ conc.<30% – sediments from stations M01, M02, M09-M12, M16-M18, representing ≈70% from the total number of samples,
- calcareous sediments – 30%< $CaCO_3$ conc.<50% – present in just one station – M05,
- carbonated sediments – 50%< $CaCO_3$ conc.<70% – present in three stations (M03, M04 and M15).

Another important component of the sediments is the organic matter, represented here by the TOC (total organic carbon) concentrations. In the constitution of the MISIS sediments TOC varies from 0.11% (station M18) to 2.78% (station M02), with a mean concentration of 1.55%. As a result of a higher biological productivity of a deeper water column, the TOC concentrations increase with the water depth, rather similar with the carbonate concentrations. This relation may be altered by high local productivities, such as in station M02, where the highest TOC concentration was recorded.

The TOC concentration in sediments is also controlled by their grain size composition (Secrieru & Oaie, 2009), a higher percentage of fine fraction (<16 µm) allowing a better preservation of the buried organic matter. This explains why the lowest TOC concentration was recorded in the one station where the sediments were represented by sand (station M18) (Fig. V.26).

All the other investigated components are of terrigenous origin and are introduced into the Black Sea mainly through the North-Western Rivers, in both dissolved and particulate forms, with the Danube being the main contributor. Diffuse discharge and atmospheric input play probably a significant part in determining the sediment total concentrations for some heavy metals (Cd, Pb, Ni).

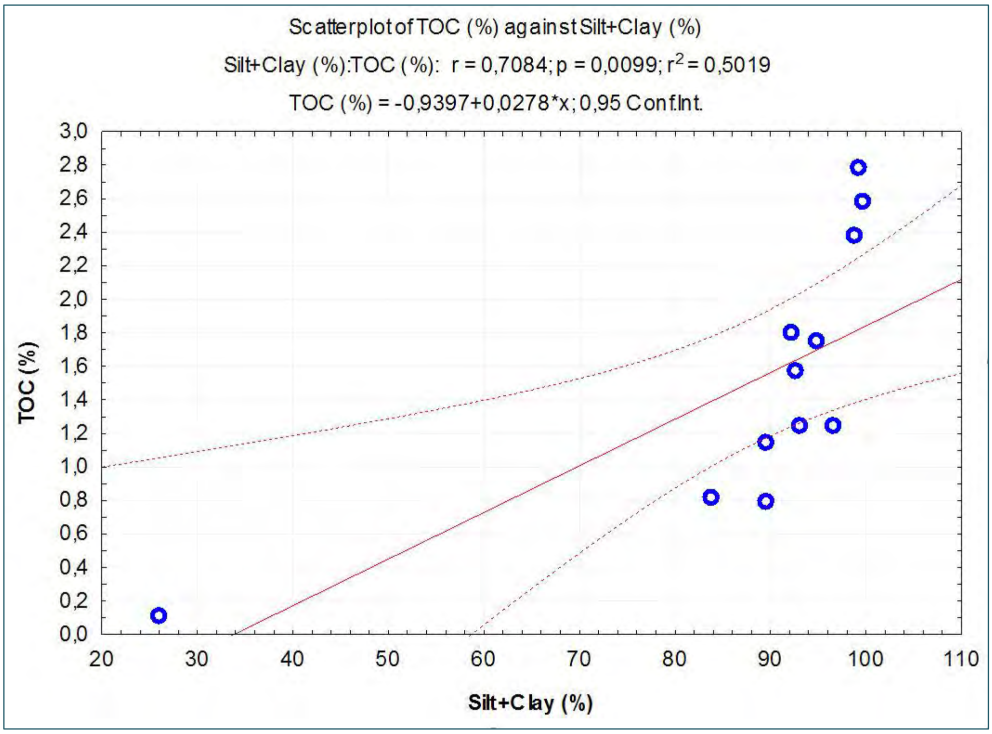


Figure V.26. Correlation of TOC (%) against fine fraction (silt+clay) content (%) in sediments from the MISIS cruise.

The paired inter-component relations were examined by means of the linear correlation coefficients (Table V.21). Even a cursory examination of the correlation coefficients matrix immediately reveals the dilatant role played by calcium carbonate for the terrigenous components. $CaCO_3$ is negatively correlated, either statistically significant or insignificant, with the majority of the terrigenous components. From all the analysed components only Sr, Ba and Mn have positive correlations with calcium carbonate, Sr being the only statistically positive correlated with $CaCO_3$.The highly significant positive correlation of Sr is explained by its isomorphic substitution for Ca in the crystalline lattice of calcium carbonates, especially aragonite.

More interesting are the statistically positive correlations of TOC with many terrigenous components (Ba, Rb, Zn, Ni, Mn, Pb, Cu, Cd) (Fig. V.27), despite the biogenic marine origin of the organic matter. These strong associations (Ba, Ni, Pb, Cu, Cd) are probably explained by a combination of factors – biogenic inputs of metals, the organic matter being a good concentrator, the dependence of TOC concentration of the fine fraction of sediment, the main source of terrigenous components and, possibly, the dependence of manganese nodules formation on a highly reducing environment in the sediment below the uppermost layer.

On the other hand, considering the very small statistical sample, insufficient for general conclusions, another possible explanation might be pure coincidence among the highest and smallest TOC concentrations and the highest and smallest concentrations of metals.

A statistically significant, positive correlation relates Fe_2O_3, the main analysed representative of the terrigenous fraction and Rb, a very good proxy for aluminium (another major component of the terrigenous fraction, not analysed), both being also correlated with most of the other terrigenous components. Fe_2O_3 is better correlated with Cr, V and Co while Rb is better correlated with Zn, Ni, Pb, Cu and Cd.

These relations are extremely important as both components are frequently used for normalization of the environmentally important metal concentrations.

Table V.21. Matrix of linear correlation coefficients for the analyzed components of MISIS sediments.

Component	**$CaCO_3$, %**	**TOC, %**	**Fe_2O_3, %**	**TiO_2, %**	**Zr, µg/g**	**Ba, µg/g**	**Sr, µg/g**	**Rb, µg/g**	**Zn, µg/g**	**Ni, µg/g**	**Mn, µg/g**	**Cr, µg/g**	**V, µg/g**	**Co, µg/g**	**Pb, µg/g**	**Cu, µg/g**	**Cd, µg/g**
$CaCO_3$, %	1																
TOC, %	-0.079	1															
Fe_2O_3, %	**-0.955**	0.220	1														
TiO_2, %	**-0.851**	-0.265	**0.720**	1													
Zr, µg/g	-0.363	-0.474	0.102	**0.691**	1												
Ba, µg/g	0.186	**0.644**	-0.097	-0.320	-0.320	1											
Sr, µg/g	**0.923**	-0.141	**-0.894**	**-0.809**	-0.361	0.150	1										
Rb, µg/g	**-0.839**	**0.543**	**0.898**	**0.588**	0.054	0.118	**-0.880**	1									
Zn, µg/g	**-0.688**	**0.742**	**0.802**	0.348	-0.187	0.296	**-0.717**	**0.948**	1								
Ni, g/g	-0.487	**0.830**	**0.582**	0.176	-0.169	0.490	-0.527	**0.814**	**0.907**	1							
Mn, µg/g	0.022	**0.804**	0.080	-0.393	-0.393	**0.600**	0.089	0.280	0.502	**0.661**	1						
Cr, µg/g	**-0.684**	0.256	**0.786**	0.362	-0.231	-0.160	**-0.640**	**0.731**	**0.669**	0.480	0.112	1					
V, µg/g	**-0.678**	-0.018	**0.682**	0.548	0.196	-0.346	**-0.623**	**0.588**	0.494	0.439	0.032	**0.700**	1				
Co, µg/g	**-0.801**	0.469	**0.886**	0.541	-0.004	0.228	**-0.735**	**0.882**	**0.886**	**0.806**	0.402	**0.613**	0.636	1			
Pb, µg/g	-0.275	**0.936**	0.444	-0.112	-0.460	**0.516**	-0.323	**0.699**	**0.876**	**0.891**	**0.754**	0.439	0.234	**0.673**	1		
Cu, µg/g	-0.324	**0.924**	0.448	-0.026	-0.374	**0.631**	-0.318	**0.676**	**0.850**	**0.932**	**0.761**	0.388	0.234	**0.697**	**0.921**	1	
Cd, µg/g	-0.412	**0.854**	0.534	0.138	-0.311	0.450	-0.493	**0.789**	**0.875**	**0.878**	0.480	0.522	0.242	**0.639**	**0.840**	**0.896**	1

($r_{13;\,0.05;\,95}$ = 0.553)

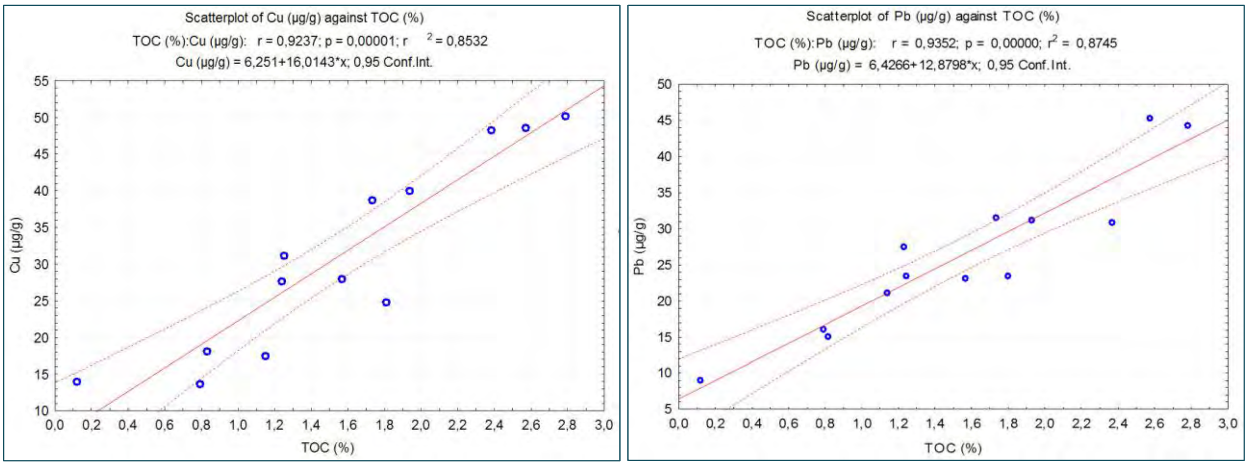


Figure V.27. Correlation of TOC (%) against Cu and Pb content (µg/g) in sediments from the MISIS cruise.

Assessment of heavy metals contamination state

The assessment of heavy metal contamination in the marine sediments, especially those from the Western and North-Western Black Sea is rather difficult, due to the high natural sedimentary variability. For the present study a supplementary difficulty resides in the too small number of sediment samples available for the assessment of a very large area.

The simplest way to get an idea of the sediment quality is to compare the actual data with existing environmental quality standards for sediments, which are rather scarce, and/or sediment quality guidelines (Table V.22). In Romania quality criteria for sediments are established through the Order 161/2006 of the Ministry of Environment. Unfortunately these are dealing with sediments in general and are not specific for marine sediments.

Quality criteria specific for marine sediments, established through the Order 1888/2007 of the Ministry of Environment are in power only for the areas used for marine farming.

Table V.22. National quality criteria for metals in sediments, actual metal concentrations and other quality guidelines (μg/g).

Metal	Ord. 161/2006	Ord. 1888/2007	Actual range	Actual mean	ERL[1]	PEL[1]	Upper Crust[2]
Cadmium	0,8	20	0.06 - 0.39	0.24	1.2	4.21	0.1
Chromium	100	100	38 - 88	63.6	81	160	69
Copper	40	150	13.8 - 50.3	31.0	34	108	39
Lead	85	100	9.2 - 45.2	26.3	46.7	112	17
Zinc	150	150	26.1 - 85.6	55.7	150	271	67
Nickel	35	100	15.7 - 57.8	39.4	20.9	42.8	55

ERL – effects range low; PEL – probable effects level
[1] – Data after Buchman (2008); [2] – Data after Li (2000)

An analysis of the Table V.22 data indicates that Cd, Pb and Zn do not exceed any of the quality limits, Cr exceeds ERL (the concentration below which adverse effects are unlikely) in four stations (M02, M10, M16, M17) but is below all the others criteria.

Cu and Ni are the only two heavy metals frequently exceeding both the national criteria and the ERL values. In fact Ni exceeds the ERL in the vast majority of the analysed samples (app. 85%), in two samples exceeding even PEL, the concentration above which adverse effects are expected to occur frequently.
The immediate conclusion of these results could be that the investigated sediments are contaminated with Cu and Ni. However, all these limits have been established by means of laboratory experiments based on biological criteria (adverse effects on biota). This is clearly evident if the ERL and PEL for Ni (20.9 μg/g and respectively 42.8 μg/g) are compared with its abundance in the upper crust of the Earth – 55 μg/g, greater than both quality guidelines.

In such cases a better idea on the contamination may be obtained by using the relations of the contaminating metals with non-technophyllic components.
The analysis of the linear correlation coefficients (Table V.21) indicated Rb as the most appropriate element for normalizing the concentrations of both

nickel and copper. A subsequent linear regression analysis identified the following relations relating Ni and Cu concentrations to those of Rb:

Ni (µg/g) = 0.3296*Rb (µg/g) + 8.5350, r^2 = 0.663 (1), and

Cu (µg/g) = 0.2482*Rb (µg/g) + 7.7805, r^2 = 0.458 (2)

both statistically significant at a confidence level >95%.

The scatter diagrams of Rb - Ni (Fig. V.28) and Rb - Cu (Fig. V.29) concentrations showing also the prediction intervals at a 95% confidence level for relations (1) and (2), i.e. the interval where 95% from Ni and Cu concentrations predicted on the basis of Rb concentrations should be, evidenced a single sample (Station M05) exceeding the upper limit of the respective prediction intervals.

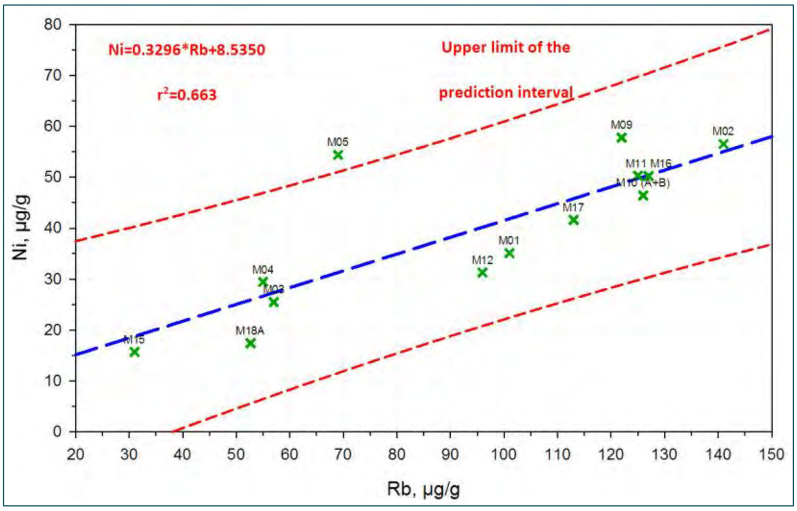


Figure V.28. Scatter diagram of Rb and Ni concentrations.

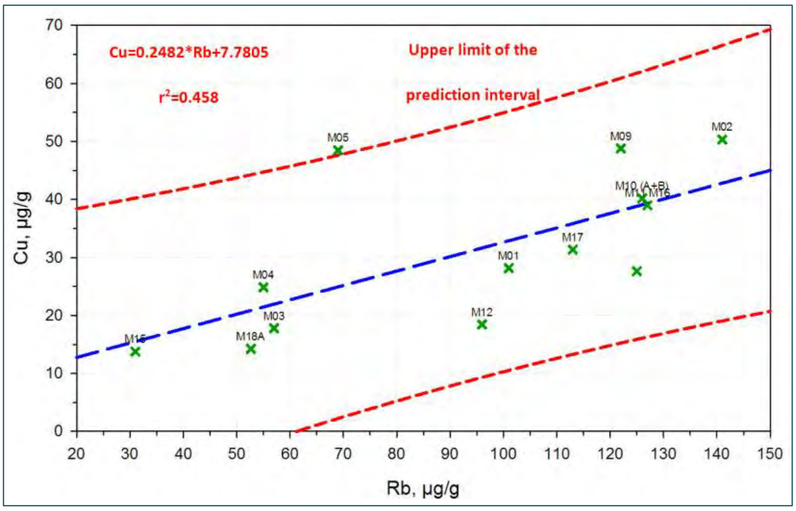


Figure V.29. Scatter diagram of Rb and Cu concentrations.

These exceeding are a clear indication of Ni and Cu excesses in the Station M05 sediment sample. Question is “are these excesses anthropogenic?”

Again the matrix of linear correlation coefficients provides the answer. Both Ni and Cu are statistically significant correlated with Mn. At the same time station M05 was the only station where the presence of manganese nodules was signalled and it is well known that manganese nodules concentrates an entire series of heavy metals – Ni, Co, Ba, Cu, Zn, Pb, etc. (Cronan, 1976; Calvert, 1976).
The conclusion is immediately evident: the Ni and Cu excesses in Station M05 sediment are the result of their concentration in manganese nodules, a natural process. The hypothesis is further sustained by the highest Ba concentration, another metal the manganese nodules have a high affinity for, also in station M05.

Conclusions

From a sedimentological point of view the sediments encountered during the R/V Akademic MISIS cruise are rather homogenous, belonging, with a single exception; station M18, to the category of siltic sediments – silts, clayey silts and in station M12 sandy silt. The sediment in station M18 was fine sand.
The chemical variability was greater, the sediments varying from calcareous to carbonate. Accordingly, the coefficients of variations for heavy metals of environmental interest were usually high, making difficult the assessment of metal contamination. Further difficulties for the assessment were raised by the reduced number of available samples.
However, a comparison with available environmental quality standards and sediment quality guidelines showed that the concentrations of most metals were below the established limits.
Frequent exceeding of the limits were identified for Ni and Cu but a further analysis using Rb as a normalizing element demonstrated that this is mostly the result of a higher natural background.
The excessive enrichments of Ni and Cu in station M05 were demonstrated as being the result of metal concentration in shallow water manganese nodules.
Based on these result it may be affirmed that metal contamination of sediments does not represent a problem for the investigated area. However, again due to the limited number of samples, this affirmation is rather uncertain and needs further confirmation.

Organic pollution of sediments

Organochlorine Pesticides and Polychlorinated Biphenyls

Organochlorine pesticides (OCP) and polychlorinated biphenyls (PCB) concentrations determined in sediment samples analysed in NIMRD pollution laboratory are presented in Table V.23 and V.24, where ∑OCPs (µg/kg dry weight) is the concentration of the nine individual compounds (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, p,p'DDE, p,p'DDD and p,p'DDT) and ∑PCBs (µg/kg dry weight) is the concentration of the seven individual compounds (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180).

Table V.23. Concentrations of individual and total OCPs (∑OCPs) (µg/kg dry weight) in sediment samples from the Romanian, Bulgarian and Turkish area, July 2013.

Stations	HCB	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p' DDT	∑ OCPs
MO1(RO32m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	0.711	<0.200	<0.200	2.611
MO2(RO47m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	1.727	<0.200	<0.200	3.627
MO3(RO52m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	0.509	<0.200	<0.200	2.409
MO4(RO65m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	2.214	<0.200	<0.200	4.114
MO5(RO100m)	2.504	4.868	1.421	<0.200	0.494	<0.300	11.438	0.985	0.583	22.793
MO9(BG92m)	<0.300	<0.300	0.200	<0.200	0.200	<0.300	1.786	<0.200	<0.200	3.686
MO10(BG77m)	4.349	4.661	2.577	0.287	2.401	0.397	7.401	2.533	15.679	40.285
MO11(BG40m)	<0.300	<0.300	<0.200	2.854	<0.200	<0.300	0.984	<0.200	<0.200	5.539
MO12(BG23m)	3.056	<0.300	<0.200	<0.200	<0.200	<0.300	2.678	<0.200	<0.200	7.334
MO15(TR101m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	0.641	<0.200	<0.200	2.541
MO16(TR75.6m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	1.961	<0.200	<0.200	3.861
MO17(TR54m)	<0.300	<0.300	<0.200	<0.200	<0.200	<0.300	0.738	<0.200	<0.200	2.638
MO18(TR27m)	<0.300	<0.300	0.506	0.526	0.903	1.544	0.788	3.170	1.854	9.890
ERL*(µg/kg)	20.00	3.000	-	-	2.00	-	2.20	-	-	-

** **ERL** – Effects Range Low were developed by the United States Environmental Protection Agency for assessing the ecological significance of sediment concentrations. Concentrations below the ERL rarely cause adverse effects in marine organisms (OSPAR Commission 2008)*

Total concentrations of the organochlorine pesticides varied from 2.40 to 40.28 µg/kg dry weight. Total concentrations of the polychlorinated biphenyls varied from 3.40 to 142.87 µg/kg dry weight. Higher OCPs and PCBs concentrations are associated with sediment composition, most of the samples belonging to clayey silt sediment type (Fig. V.30 and V.31). The concentration of contaminant in sediment depends largely on the retaining capacity of the sediments; sediments with large amounts of clay minerals can retain larger amounts of pesticides residues than the sandy-clay or sandy silt sediments (Ahmed El Nemr et al., 2012).

Table V.24. Concentrations of individual and total PCBs (∑PCBs) (μg/kg dry weight) in sediment samples from the Romanian, Bulgarian and Turkish area, July 2013.

Stations	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑ PCBs
MO1(RO32m)	2.74	0.63	7.06	0.56	0.61	<0.70	1.92	14.23
MO2(RO47m)	1.42	28.16	10.63	11.46	5.08	14.06	3.47	74.29
MO3(RO52m)	24.80	59.03	24.43	1.07	0.68	14.86	6.07	130.95
MO4(RO65m)	1.28	7.55	1.52	0.09	<0.60	<0.70	<0.30	12.03
MO5(RO100m)	<0.40	139.87	<0.60	<0.40	<0.60	<0.70	<0.30	142.87
MO9(BG92m)	13.58	30.40	3.66	<0.40	<0.60	<0.70	0.94	50.28
M10(BG77m)	<0.40	1.62	1.09	0.60	0.80	4.79	2.49	11.79
M11(BG40m)	<0.40	5.08	1.21	<0.40	<0.60	<0.70	1.65	10.05
M12(BG23m)	<0.40	82.98	1.48	1.05	<0.60	5.49	1.01	93.02
M15(TR101m)	<0.40	<0.30	2.56	7.26	3.00	<0.70	<0.30	14.52
M16(TR75.6m)	<0.40	<0.30	0.71	<0.40	<0.60	<0.70	<0.30	3.41
M17(TR54m)	<0.40	12.79	1.98	<0.40	<0.60	<0.70	0.49	17.35
M18(TR27m)	<0.40	1.77	<0.60	<0.40	<0.60	<0.70	0.45	4.92
EAC*(μg/kg)	1.7000	2.7000	3.0000	0.6000	40.0000	7.9000	12.0000	-

**EAC*- *Environmental Assessment Criteria represent the contaminant concentration in the environment below which it can be assumed that no chronic effects will occur in marine species, including the most sensitive species (OSPAR Commission 2008)*

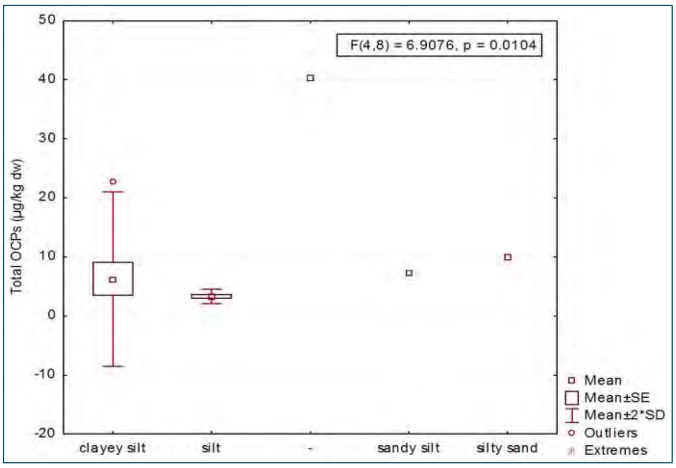


Figure V.30. Box plot of ∑OCPs (μg/kg dry weight) in different types of sediment from the Romanian, Bulgarian and Turkish area, July 2013.

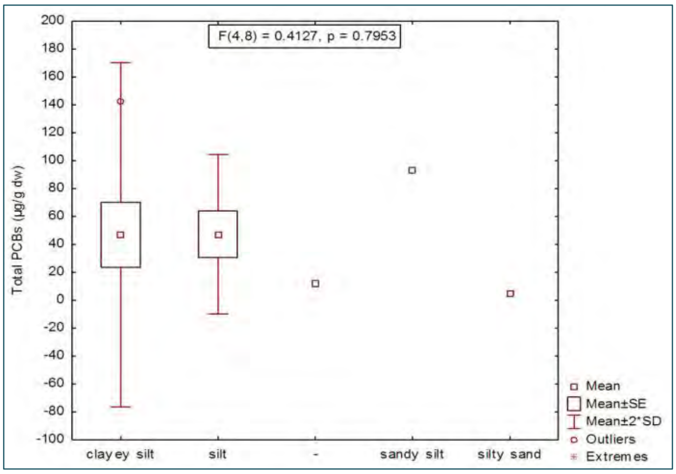


Figure V.31. Box plot of ∑PCBs (μg/kg dry weight) in different types of sediment from the Romanian, Bulgarian and Turkish area, July 2013.

The highest values for OCPs were measured for p, p' DDT (15.67 µg/kg dry weight) and HCB (4.34 µg/kg dry weight) in station M10 (77m) and p, p' DDE (7.4 µg/kg dry weight) and lindane (4.86 µg/kg dry weight) in station MO5 (100m) (Fig. 32). The highest values for PCBs were measured for PCB 52 (139.87 µg/kg dry weight) in station MO5 (100m) and PCB 28 (24.79 µg/kg dry weight), PCB 101 (24.43 µg/kg dry weight) and PCB 138 (14.85 µg/kg dry weight) in station MO3 (52m) (Fig. V.33).
Except for some extreme values obtained in the case of PCBs, the other chlorinated contaminants were comparable with previous data obtained in the Black Sea region (Fillmann et al., 2002; Ozkoc et al., 2007).

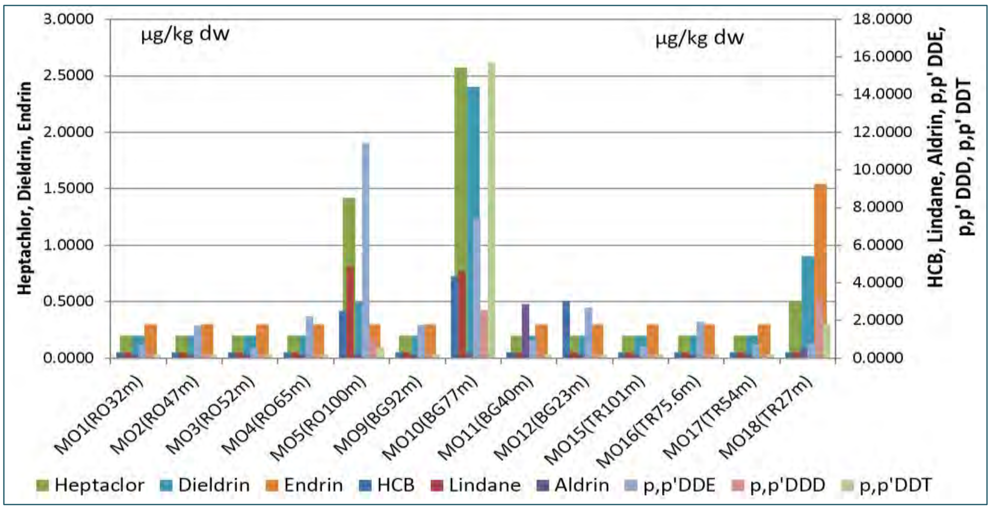


Figure V.32. Individual OCPs levels (µg/kg dry weight) in sediment samples from the Romanian, Bulgarian and Turkish area, July 2013.

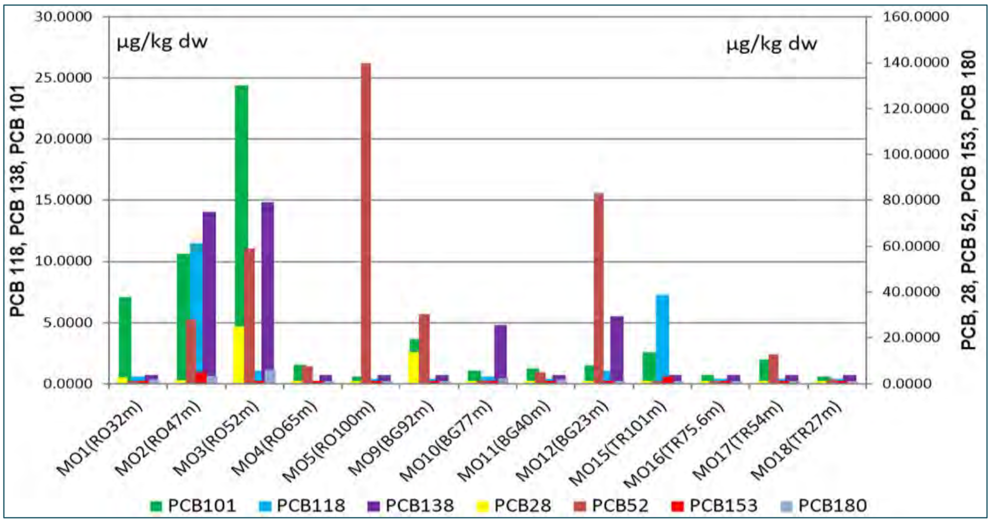


Figure V.33. Individual PCBs levels (µg/kg dry weight) in sediment samples from the Romanian, Bulgarian and Turkish area, July 2013.

Comparison to Assessment Criteria

The results of the sediment analyses were compared to Environmental Assessment Criteria (EACs) proposed by OSPAR as a means for assessing the significance of concentrations of hazardous substances in the marine environment and if EACs were not available with Effects Range Low (ERLs) developed by the United States Environmental Protection Agency for assessing the ecological significance of sediment concentrations (OSPAR Commission, 2008). EACs (lower) are concentrations below which it is reasonable to expect that there will be an acceptable level of protection of marine species from chronic effects from specific hazardous substances. Concentrations below the ERL rarely cause adverse effects in marine organisms (Table V.23 and V.24).

There are no threshold value (EACs or ERLs) available for heptachlor, aldrin, endrin, p, p' DDD and p, p' DDT in sediments. p, p' DDE and lindane exceeded the ERLs value in 3 samples. The concentrations of HCB and dieldrin were below the ERLs values in all samples.
Except PCB 153 and PCB 180, the others PCBs exceeded EACs values in some samples.

Conclusions

Concentrations of organochlorine compounds in sediments are higher or comparable with those reported in the Black Sea region in previous expedition.
Highest concentrations of PCBs recorded for the Romanian area is probably the influence of discharges from the River Danube.

Total petroleum hydrocarbons (TPHs)

TPH contents (µg/g dry weight) in sediments varied between 74.5 and 327.9 with a mean of 163.8. The data indicate that 69 % of samples fall in the range of 100 - 200 (µg/g dw) (Fig. V.34). The lowest values were measured at stations M17 and M15, whereas the highest values were found at stations M01-M02 and M10. The statistical analysis of data did not point out significant differences between the mean values in sediment samples from the Romanian, Bulgarian and Turkish transects (Fig. V.35).

The average values obtained for TPH are above the expected threshold for unpolluted sediments which are considered either 10 µg/g (UNEP, 1991; Volkman et al., 1992) or 50 µg/g, which is permissible concentration (PC) in the frame of the routine monitoring system in some countries (BSC, 2011). Bouloubassi and Saliot (1993) reported that hydrocarbon concentration may reach values as high as 100 µg/g in unpolluted sediments with high organic content due to contributions from biogenic sources. Values higher than this, such as those recorded at stations on the Romanian, Bulgarian and Turkish transects (189.6 ± 86.1, 168 ± 59.0 and 126.7 ± 51.1 µg/g dw) are indicative of petroleum inputs (Volkman et al., 1992). Wakeham (1996) has reported similar levels of hydrocarbons (total aliphatic 10–153 µg/g dry wt) in sediments from the Black Sea. This author, however, worked with off-shore samples but also recorded maximum contamination associated with Danube inputs (Readman et al., 2002).

Analyses of MISIS sediments indicate that TPH levels of the sediments were comparable to levels encountered in the Mediterranean Sea, Northern Black Sea, North Western Black Sea and Southern Black Sea (Bouloubassi & Saliot 1993; Readman et al., 2002; Balkıs et al., 2012). In contrast, the highest TPH values were much lower than areas which are chronically contaminated by oil, such as the Arabian Gulf and New York Bight (Farrington & Tripp, 1977; Readman et al., 1996) (Table V.25).

Conclusions

The Western Black Sea area is only moderately contaminated by petroleum hydrocarbons, oil inputs are shown to be low and comparable to relatively uncontaminated areas on a worldwide basis. A major contribution to the Western Black Sea is shown to be associated with inputs through the River Danube.

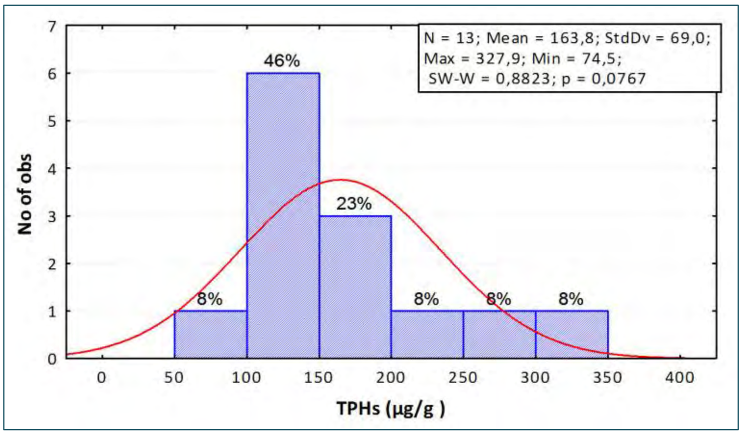


Figure V.34. Histogram of TPHs (µg/g) in sediments from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

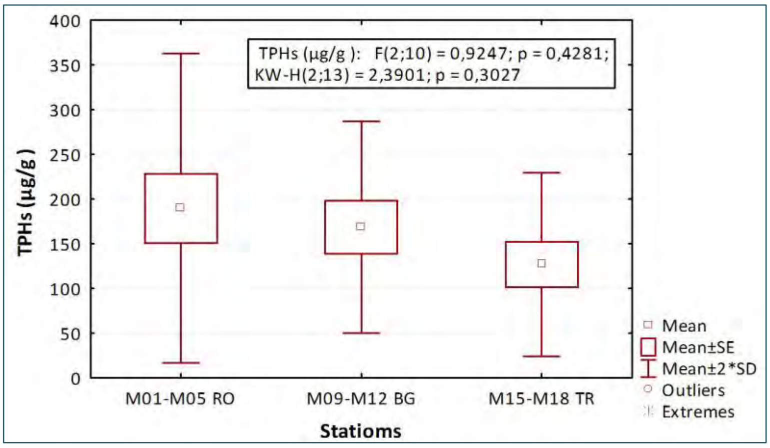


Figure V.35. Distribution of petroleum hydrocarbon concentrations (µg/g) in sediments from the Romanian, Bulgarian and Turkish waters, July 2013.

Table V.25. Worldwide concentrations of total hydrocarbons in sediments (µg/g dry weight).

Area	TPHs (µg/g d w)	References
Antarctica (pristine)	< 0.5	Lenihan *et al.* (1990)
Rhone River, France, Mediterranean Sea	25–170	Bouloubassi and Saliot (1993)
Oman Gulf	6–22	Fowler *et al.* (1993)
Saudi Arabia, Gulf	19–671	Fowler *et al.* (1993)
Victoria Harbour, Hong Kong	60–646	Hong *et al.* (1995)
Kuwait, Gulf	40–240	Readman *et al.* (1996)
Saudi Arabia, Gulf	11–6900	Readman *et al.* (1996)
Black Sea	7–153a	Wakeham (1996)
New York Bight, USA	35–2900	Farrington and Tripp (1977)
Bosphorus, Black Sea, Turkey	12–76	Readman *et al.* (2002)
Sochi, Black Sea, Russia	7.6–170	Readman *et al.* (2002)
Odessa, Black Sea, Ukraine	110–310	Readman *et al.* (2002)
Coastline, Black Sea, Ukraine	2.1–6.6	Readman *et al.* (2002)
Danube Coastline, Black Sea, Ukraine	49–220	Readman *et al.* (2002)
Southern Black Sea Shelf, Turkey	0.3–363	Balkis *et al.* (2012)
Western Black Sea	74.5- 328	In this study (2013)

Polycyclic aromatic hydrocarbons (PAHs) levels and composition

Results of total of polycyclic aromatic hydrocarbon analyses are shown in Table V.26, where $\sum_{16}$PAHs (µg/kg dry weight) is the sum of the sixteen determined PAHs, LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings), CPAHs% - the carcinogenic PAHs percentage to the total PAHs and B(a)Peqv - the total equivalent of toxicity by benzo(a) pyrene (µg/kg dw).

The total polycyclic aromatic hydrocarbon (Σ_{16}PAH) content of the sediment samples (n=13) collected during the MISIS cruise of the R/V Akademic has ranged from 26.4 to 1841.0 (µg/kg dw) with the highest value at MO2 station. The average total PAH concentration in MISIS sediments calculated as 463.3 (µg/kg dw) is higher than the mean value (138.3 µg/kg dw) reported for the Black Sea basin by Readman (2002) (Table V.27). Of the areas sampled in this study, highest concentrations (mean of 775.1 ± 633.4 µg/kg dw) were observed at sites influenced by the Danube. This is explained by the fact that the river drains extensive urbanised inland areas (Equipe Cousteau, 1993 and Wakeham, 1996). According to Traven et al. (2008), the marine bottom sediments can be classified into three categories, depending on the total content of PAHs: slightly polluted (ΣPAHs<250 µg/kg dw), polluted (ΣPAHs from 250 to 500 µg/kg dw), highly polluted (ΣPAHs >500 µg/kg dw). Our results allow the classification of sediments as slightly polluted (54%) and highly polluted (46%) and comparable to different coastal areas of the Black Sea (Table V.27).

Compositions of PAHs (µg/kg dw) in sediments from the Black Sea indicate primarily a petrogenic origin at stations M01-M05 on the Romanian transect except M02 station (Fig. V.36). The PAHs in sediments indicate a high pollution degree, with significant concentrations ranging between 169.7 - 214.0 and 81.5 - 442.8 (µg/kg dw) for naphthalene and phenanthrene. This dominance of low molecular weight two and three ringed structures is typical of petroleum (petrogenic) sources of PAHs. The same petroleum profile was reported by Readman et al. (2002), for sites along the Danube coastline.

A pyrolytic profile is observed at stations M09-M10 on the Bulgarian transect (PAHs with 4-6 aromatic rings are dominant) and a mixture of pyrolytic and petrogenic PAHs in the sediments on the Turkish transect, Igneada, with a slightly petroleum predominance (Fig. V.37 and V.38).

Identification of PAHs sources

Possible sources of the PAHs emission into environment can be found through the use of indices, which are the ratio of concentrations of some PAHs in the sample (Yunker et al., 2002; Soclo et al., 2000). The abundance ratio of two- and three ring hydrocarbons (LMW) to four- to six-ring hydrocarbon (HMW) PAHs is commonly used ratio to help in distinguishing the petroleum and pyrolytic sources. Values below 1 are considered as combustion sources and values above 1 are considered for petroleum contributions. As shown in Table V.26, the value of LMW/HMW index at all stations of sampling ranges from 0.2 to 11.

The data indicate that the most significant petroleum PAHs pollution (ΣPAHs > 500 µg/kg dw and LMW/HMW index > 1) was at stations: M01, M03 and M04.

Stations with greatest pyrolytic inputs (ΣPAHs > 500 µg/kg dw and LMW/HMW index < 1) include M10, M09, and M02 stations (Fig. V.39). The following individual compounds with 4-aromatic rings are dominant at these stations: pyrene (from 110.7 to 254.7 µg/kg dw), fluoranthene (from 124 to 253.1 µg/kg dw), chrysene (from 86.4 to 151.9 µg/kg dw) and benzo[a]anthracene (from 30.1 to 132.4 µg/kg dw).

Assessment of PAHs' total toxicity by the total equivalent of toxicity by benzo(a) pyrene

The amount of potentially carcinogenic PAHs in sediments varied from 6.5 to 1114.2 (µg/kg dw), representing 8% and 85 % of the total concentration of polycyclic aromatic hydrocarbons. The largest percentage of carcinogenic PAHs was in sediments from stations M02 and M10 (61% and 85%, respectively).

Benzo(a)pyrene is the only PAH out of the sixteen determined PAHs for which toxicological data for the calculation of the carcinogenicity factor are available. The US EPA (1993) approach uses benzo(a)pyrene (BaP) as the index chemical (i.e., having a relative potency of 1.0) and includes TE (toxicity equivalent) values for seven carcinogenic PAHs. These PAHs include benzo(a)anthracene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, indeno(1,2,3-cd) pyrene and TE for those PAHs are 0.1, 0.1, 0.1, 1, 0.01, 1.0 and 0.1 respectively. Therefore, to assess PAHs' total toxicity, the total B(a)Peqv was calculated as total equivalent concentration as benzo(a)pyrene), using the toxicity equivalent (TE) for each PAH according to the following equation:

$$\text{Total B(a)Peqv} = \Sigma_i C_i \times TE_i \ (\mu g/kg)$$

where:

C_i – concentration of the respective PAHs (µg/kg);

TE_i – the toxicity equivalent of the corresponding PAHs

The total B(a)Peqv concentrations calculated for all samples investigated varied from 1.4 to 64.3 µg/kg dw. The largest values were found at stations M10 and M02 (64.3 - 51.5 µg/kg dw) and indicating the presence of local pollution sources (Fig. V.39). Slightly polluted sediments (ΣPAHs<250 µg/kg dw) showed a low level of toxicity with the total B(a) Peqv (µg/kg dw) < 10 (Fig. V.40).

Comparison to Assessment Criteria

In this study, the PAH results of the sediment analyses were compared to the ERL -Effects Range Low values for assessing the ecological significance of sediment concentrations. Effects Range (ER) values were established by the US National Oceanic and Atmospheric Administration (NOAA) as sediment quality guidelines. The guidelines can be used to predict adverse biological effects on organisms. The ER-Low (ERL) value is the lower tenth percentile of the effect concentration and is defined by the US EPA as the concentration of a contaminant above which harmful effects may be expected to occur. Adverse effects on organisms are rarely observed when concentrations fall below the ERL value (Table V.26). Most of the individual PAH concentrations do not exceed the ERL values in samples, except naphthalene and phenanthrene concentrations in some stations.

According to the total content of PAHs, the total equivalent of toxicity by benzo(a) pyrene- B(a) Peqv and ERL values in the sediments from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters in July 2013 can be classified as:

- slightly polluted (54%) with ΣPAHs < 250 µg/kg dw and low level of toxicity - the total B(a) Peqv (µg/kg dw) < 10; individual PAH concentrations do not exceed the ERL values, concentrations below the ERL rarely cause adverse effects in marine organisms;
- highly polluted (46%) with ΣPAHs > 500 µg/kg dw, with naphthalene and phenanthrene concentrations that exceed the ERL values at some stations, indicating a petroleum pollution; high level of toxicity the total B(a) Peqv (µg/kg dw) > 10, but do not exceed ERL values for individual carcinogenic PAHs.

Conclusions

Both pyrolytic and petrogenic PAHs are present in MISIS sediments. The data indicate that petroleum origin PAHs pollution was observed at stations on the Romanian transect, whereas pyrolitic origin PAHs pollution was dominant at stations on the Bulgarian transect.

Table V.26. Concentrations of individual, total (∑16PAHs)PAHs, equivalent of toxicity by benzo(a) pyrene - B(a)Peqv (µg/kg dry weight) , CPAHs% - the carcinogenic PAHs percentage and LMW/HMW index - in sediments from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Sampling station -M*	Nap**	Acl	Ac	Fl	Phe	An	Fa	Py	B[a]a	Chry	B[b]fl	B[k]fl	B[a]pyr	B(g,h,i)p	D(a,h)a	Ip	$\sum_{16}PAHs$	CPAH %	B(a)P_{eqv}	LMW/ HMW
M18 TR	4.5	2.3	3.0	3.2	3.8	3.1	1.0	1.6	0.4	0.4	<1.3	0.9	0.6	0.6	0.6	0.4	**26.4**	25	1.40	3.1
M12 BG	1.9	1.8	3.5	4.4	25.0	11.2	10.7	17.1	8.0	4.6	4.7	5.3	5.6	3.4	3.3	2.1	**112.7**	57	10.95	0.7
M15 TR	16.9	2.6	3.9	4.1	47.2	15.8	8.4	11.3	2.1	2.2	<1.3	4.2	2.5	2.8	2.7	1.6	**128.1**	29	6.00	2.4
M16 TR	31.1	2.4	4.5	7.4	47.8	7.3	6.8	13.8	0.8	1.7	2.3	4.2	1.9	2.4	2.4	1.5	**138.3**	27	5.20	2.7
MO5 RO	6.0	1.5	2.7	4.7	81.5	24.5	4.4	11.5	0.4	3.7	5.1	6.9	2.7	3.8	3.5	2.4	**165.4**	27	7.77	2.7
M17 TR	14.2	3.1	3.6	9.1	43.0	10.4	25.6	28.9	14.3	9.4	6.6	2.2	3.4	1.7	1.4	0.8	**177.8**	53	7.34	0.9
M11 BG	18.0	1.9	2.3	4.8	91.4	12.4	18.0	38.0	26.8	6.2	1.3	5.2	2.9	3.1	3.0	2.1	**237.6**	45	9.45	1.2
MO4 RO	211.4	4.3	5.9	20.5	177.3	49.8	4.8	12.7	4.3	3.2	1.4	4.3	5.0	2.6	2.7	1.8	**512.0**	8	8.86	11.0
M09 BG	13.3	1.6	2.4	6.4	90.7	13.3	124.8	158.8	32.3	90.7	29.9	13.4	4.5	2.3	2.7	1.3	**588.4**	78	15.73	0.3
MO3 RO	183.2	3.3	5.8	16.0	262.4	34.0	16.7	20.9	17.6	6.9	20.9	12.4	6.0	<0.6	3.3	2.6	**611.9**	18	14.65	4.7
M10 BG	10.6	5.7	3.0	8.4	74.1	7.3	124.9	110.7	30.1	86.4	139.9	21.0	40.6	72.2	3.8	<0.4	**738.7**	85	64.31	0.2
MO1 RO	169.7	3.0	5.8	11.8	293.4	57.8	29.8	60.0	30.8	39.2	21.8	9.2	4.0	3.0	3.6	2.2	**745.1**	27	14.37	2.7
MO2 RO	214.0	5.1	8.9	26.5	442.8	29.5	253.1	254.7	132.4	151.9	252.4	59.7	2.3	1.9	3.1	2.7	**1841.0**	61	51.57	0.7
ERL ***	**160**				**240**	**85**	**600**	**665**	**261**	**384**			**430**	**85**		**240**				

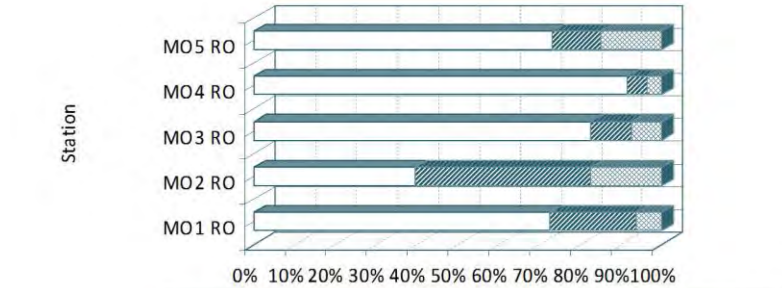
*13 samples of sediments: 5 from the Romanian transect (M 01-M 05), 4 from the Bulgarian transect (M12-M 09), and 4 from the Turkish transect (M 18 – M 15).

** Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene: Chry, Benzo[b]fluoranthene: B[b]fl, Benzo[k]fluoranthene: B[k]fl, Benzo[a]pyrene: B[a]pyr, Benzo (g,h,i)perylene: B(g,h,i)p, Dibenzo(a,h)anthracene: D(a,h)a, Indeno(1,2,3-c,d)pyrene :Ip.

*** **ERL** (µg/kg dry weight) -The ER-Low (ERL) value is the lower tenth percentile of the effect concentration and is defined by the US EPA as the concentration of a contaminant above which harmful effects may be expected to occuriv. Adverse effects on organisms are rarely observed when concentrations fall below the ERL value.

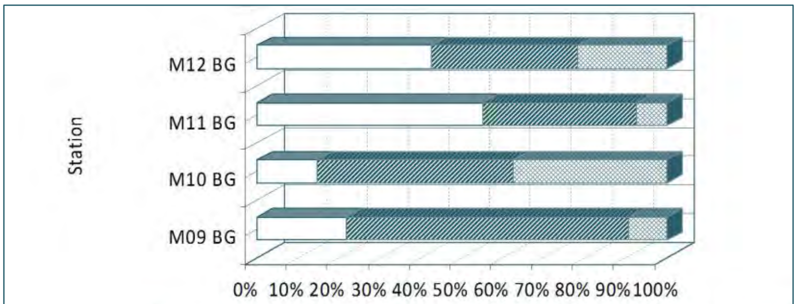
Table V.27. Comparison of PAH concentrations in sediments (µg/kg dry weight) from different coastal areas of Black Sea.

Area Survey	Total PAHs	References
Abyssal, Black Sea	200–1200 (Σ28 PAHs)	Wakeham (1996)
Danube River mouth, Black Sea	2400 (Σ28 PAHs)	Wakeham (1996)
Danube River	10–3700 (Σ4 PAHs)	Equipe Cousteau (1993)
Bosphorus, Black Sea, Turkey	13.8–531 (Σ17 PAHs)	Readman *et al.* (2002)
Sochi, Black Sea, Russia	61.2–368 (Σ17 PAHs)	Readman *et al.* (2002)
Odessa, Black Sea, Ukraine	66.9–635 (Σ17 PAHs)	Readman *et al.* (2002)
Coastline, Black Sea, Ukraine	7.2–126 (Σ17 PAHs)	Readman *et al.* (2002)
Danube Coastline, Black Sea, Ukraine	30.5–608 (Σ17 PAHs)	Readman *et al.* (2002)
Istanbul Strait, Turkey	2.1-3152 (Σ16 PAHs)	Karacık(2008)
Marmara Sea	144 (Σ16 PAHs)	Karacık(2008)
Istanbul Strait, Turkey	0.4–1703 (Σ8PAHs)	Taşkın (2010)
Southern Black Sea Shelf, Turkey	13–2342 (Σ15 PAHs)	Balkıs (2011)
The estuarine coast of Danube, Ukraine	329-1093 (Σ16 PAHs)	Tsymbalyuk (2010)
Western Black Sea	26 -1841 (Σ16 PAHs)	In this study (2014)



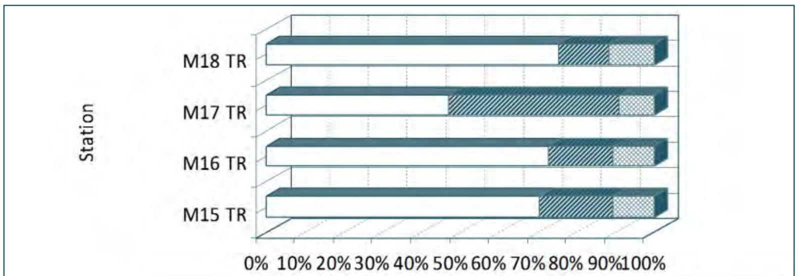
	MO1 RO	MO2 RO	MO3 RO	MO4 RO	MO5 RO
PAH(5-6rings)	43,8	322,1	45,1	17,7	24,5
PAH(4 rings)	159,7	792,1	62,1	25,0	20,0
PAH(2-3 rings)	541,5	726,8	504,8	469,3	120,9

Figure V.36. Percentage composition of Σ PAHs (µg/kg dw) in sediments from the Black Sea according to the number of aromatic rings: a) stations (M01-M05) on the Romanian transect, Constanta-East.



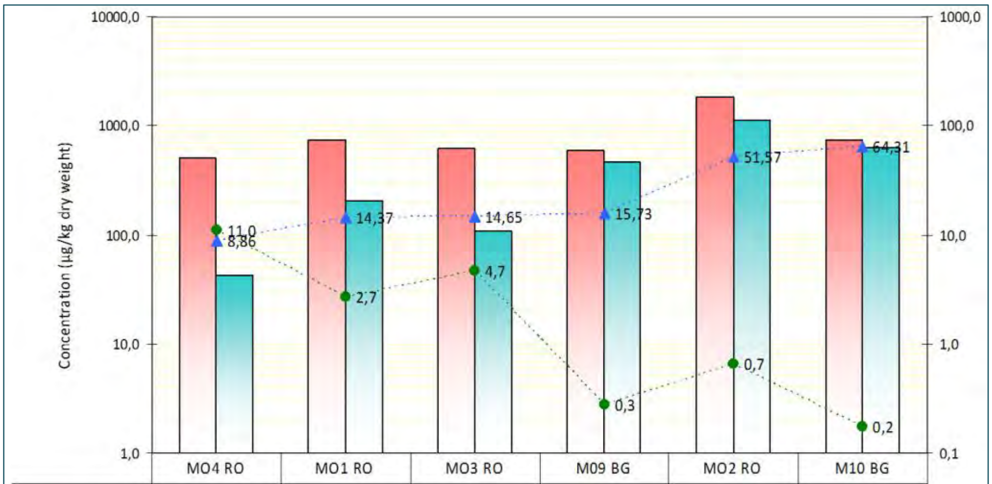
	M09 BG	M10 BG	M11 BG	M12 BG
PAH(5-6rings)	54,0	277,5	17,6	24,4
PAH(4 rings)	406,6	352,2	89,1	40,3
PAH(2-3 rings)	127,7	109,1	130,8	47,9

Figure V.37. Percentage composition of ΣPAHs (µg/kg dw) in sediments from the Black Sea according to the number of aromatic rings: b) stations (M09-M12) on the Bulgarian transect - Galata transect.



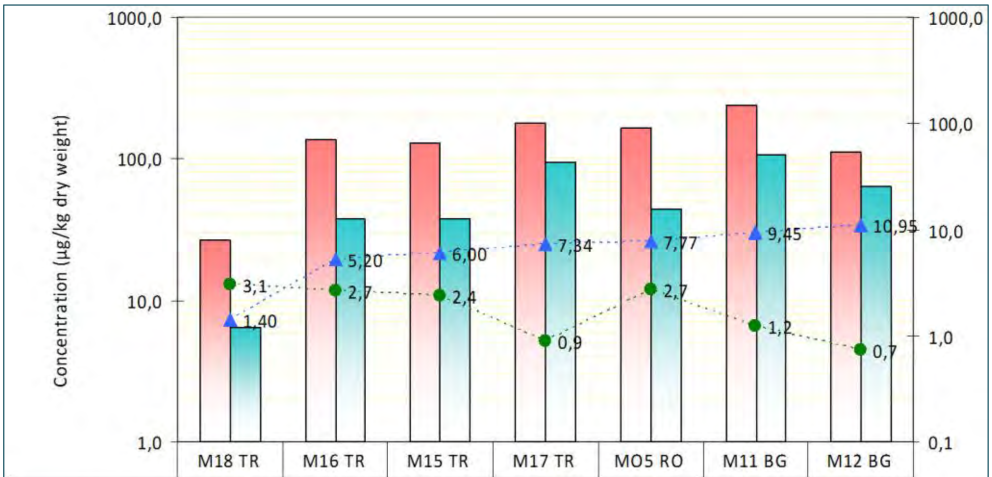
	M15 TR	M16 TR	M17 TR	M18 TR
PAH(5-6rings)	13,8	14,7	16,1	3,1
PAH(4 rings)	24,0	23,0	78,3	3,4
PAH(2-3 rings)	90,4	100,5	83,5	19,9

Figure V.38. Percentage composition of ΣPAHs (µg/kg dw) in sediments from the Black Sea according to the number of aromatic rings: c) stations (M15-M18) on the Turkish transect, Igneada.



	M04 RO	M01 RO	M03 RO	M09 BG	M02 RO	M10 BG
∑16PAHs	512,0	745,1	611,9	588,4	1841,0	738,7
CPAH	42,7	203,6	107,1	460,6	1114,2	629,6
B(a)Peqv	8,86	14,37	14,65	15,73	51,57	64,31
LMW/HMW	11,0	2,7	4,7	0,3	0,7	0,2

Figure V.39. High level pollutions in the MISIS sediments from the stations M01-M04 and M09-M10, the sum of 16 PAHs, by 7 carcinogenic PAHs, B(a)Peqv (µg/kg dw) and LMW/HMW.



	M18 TR	M16 TR	M15 TR	M17 TR	M05 RO	M11 BG	M12 BG
∑16PAHs	26,4	138,3	128,1	177,8	165,4	237,6	112,7
CPAH	6,5	37,8	37,8	94,3	44,5	106,7	64,8
B(a)Peqv	1,40	5,20	6,00	7,34	7,77	9,45	10,95
LMW/HMW	3,1	2,7	2,4	0,9	2,7	1,2	0,7

Figure V.40. Slightly polluted sediments at stations on the Romanian, Bulgarian and Turkish transect, the sum of 16 PAHs, by 7 carcinogenic PAHs, B(a)Peqv (µg/kg dw) and LMW/HMW.

CONCLUSIONS

New data on a wide range of contaminants (HM, TPH, PAH, OCP, PCB) in seawater and sediments from the Western Black Sea were obtained following MISIS Joint Cruise, July 2013, thus contributing to further integrated assessments of the Black Sea state of environment.

Seawater

-Metals concentrations in surface seawater indicated a low level trace metal pollution, concentrations of cadmium, lead and nickel being much below recommended EQS from European legislation (Directive 2013/39/EU). Generally, a slight decreasing gradient from coastal to open sea was noticed for most analysed metals.

- Concentrations of organochlorine compounds in water were higher or comparable with those reported in the Black Sea region in previous expeditions. Seawater samples were dominated by the presence of lindane and cyclodiene, which often exceeded the threshold values set out by Directive 2013/39/EU. Except PCB 52, the values measured for other PCBs compounds were low or under detection limit.

-Naphthalene and phenanthrene were found as the most dominant compounds in coastal waters of the Western Black Sea. Investigation on possible sources of polycyclic aromatic hydrocarbons evinced both pyrogenic and petrogenic origin in shelf and open sea waters.

Sediments

- A comparison with sediment quality guidelines showed that the concentrations of most metals were below the proposed limits. Frequent exceeding of the limits was identified for Ni and Cu, but a further analysis demonstrated that this is mostly the result of a higher natural background. Based on these result it may be affirmed that metal contamination of sediments does not represent a problem for the investigated area. However, due to the limited number of samples, this affirmation is rather uncertain and needs further confirmation.

- Concentrations of organochlorine compounds in sediments were higher or comparable with those reported in the Black Sea region in previous expeditions. Highest concentrations of PCBs recorded along the Romanian transect could be probably the influence of river discharges.

-The Western Black Sea area is only moderately contaminated by petroleum hydrocarbons; values were comparable to relatively uncontaminated areas on a worldwide basis.

- Both pyrolytic and petrogenic PAHs were present in MISIS sediments. The results indicated that petroleum origin PAHs pollution was observed at stations on the Romanian transect, whereas pyrolitic origin PAHs pollution was dominant at stations on the Bulgarian transect.

With respect to MSFD/ Descriptors 8, results from the MISIS Joint Cruise will promote further work toward common understanding of good environmental status and will contribute to the development of Black Sea environmental targets in a harmonised approach.

GAPS and RECOMMENDATIONS

The purpose of assessments under Descriptor 8 is to determine whether this aspect of GES is being achieved within assessment region. The approach involved (measurements of contaminant concentrations and effects, followed by comparisons against targets) needs additional research, for a better understanding of the underlying fundamental principles and for the further development of monitoring approaches (JRC, 2010).

MSFD GES target setting implies understanding of the processes affecting contaminant cycling and availability, the responses of marine organisms to contaminants, the identification of sources and the availability of appropriate monitoring tools. Scientific knowledge of the functional relationships between pressures and impacts, and the consequent responses contains significant gaps. The implementation of measures to ensure the Good Environmental Status as described under Descriptor 8 requires a combination of several assessment tools which need to be developed.

A number of gaps in knowledge were identified, as well as issues that need addressing in various research areas, as follows:

Understanding of the ecosystem responses to pollution

Research could contribute to a better understanding of the relationship between pressures, their effects and impacts on the marine environment. Hazardous substances, especially synthetic chemicals, occur in the environment as mixtures. The mixtures and their combined effect on organisms and the ecosystem are currently unknown, but this should be subject to ongoing work and research for understanding of causal relationships and of processes between contaminants and their effects on biota and to quantify the effect and impact of contaminants at the population level and higher levels of biological organization. Especially important is to assess the effects of complex mixtures of inorganic and organic pollutants upon organisms and ecosystems. For example, assessment of sediment toxicity based on the total concentration of potentially carcinogenic PAHs (CPAH) express as the benzo[a]pyrene toxicity equivalents.

Levels of pollution effects on the ecosystem components, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship is established should be monitored. Implementing of biological effects techniques used in environmental health assessment, like assays for specific inhibition of enzymes, induction of proteins, pollutant metabolites, DNA microarrays, immunotoxicity, physiological responses and pathology, is needed.

Introducing an ecotoxicology monitoring programme will allow integration of chemical and biological effects measurements. Combination of biological effects and chemical measurements will provide an improved assessment due to the ability to address effects that are potentially caused by a wide range of contaminants as well as those that are more clearly linked to specific compounds or groups of compounds.

Linking sources, pathways and environmental status: biogeochemistry of substances

Little is known on the relationship between the mechanisms of entry of pollutants (riverine, atmospheric, etc.) into marine waters and their availability and potential effects on organisms and ecosystems. Research is need on long time series that relate pollutant exposure and cycling to effects to organisms and ecosystem functioning.

Data for better quantification of contaminants fluxes and inputs into marine environment and their sea/air and water/sediments interfaces exchanges is lacking. Monitoring programmes would need to be designed to allow tracing back chemicals from the environment via their pathways to the sources in order to allow the appropriate development of programmes of measures to achieve good environmental status and assess progress being made (for instance, identification of possible sources of PAHs compounds, biogenic or anthropogenic (pyrolitic or petrogenic) based on their molecular indices (LMW/HMW)).

Monitoring programme should allow the combination of the data covering waterborne and atmospheric inputs, environmental concentrations and biological effects of hazardous substances.

Climate change

Warming of the atmosphere in response to climate change may increase the tendency for atmospheric transport of certain substances, more rain and floods can result in higher run-off from land and increased storminess may lead to additional remobilization of contaminants from marine sediments. Change in sea water temperature and other possible biological impacts of climate change add to the stress on organisms and coupled with pollution effects may make marine organisms more vulnerable to chemical contamination. An improved understanding of these processes may lead to the need for a regular review of assessment criteria.

Knowledge on the marine food webs with regard to contaminants

The transfer of contaminants through the food chain needs to be better understood, and also the possibility of additive, synergistic and antagonistic effects. The toxic effects of chemical contaminants on marine organisms are dependent on bioavailability and persistence, the ability of organisms to accumulate and metabolize contaminants, their interference with specific metabolic or ecological processes. Little is known about contaminant uptake in the first trophic levels (plankton), and how different biogeochemical statuses of marine ecosystems favour the bioaccumulation and cycling of contaminants.

Aggregation of information on substances

There is a multitude of chemicals (and effects of them) in the environment and methods for a sound aggregation of information from monitoring should be addressed in an integrated assessment framework for contaminants and biological effects.

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VI. Contaminants in Biota

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INTRODUCTION

Within the scope of the Marine Strategy Framework Directive, presence of hazardous substances in biota represent relevant criteria and indicators under Descriptor 8 (“Concentrations of contaminants are at levels not giving rise to pollution effects”) and 9 (“Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards”).

In this regard Member States are required to take into account relevant existing environmental targets.

Thus limits have been considered based on OSPAR methodology and EU legislation concerning concentrations in relevant foodstuffs: (EC) no. 1881/2006 updated by Commission Regulation (EC) no. 629/2008, setting maximum levels for certain contaminants in foodstuffs - only applicable to a few substances relevant for this indicator: cadmium, lead and mercury and (EC) no. 1259/2011 amending Regulation (EC) no. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs.

In order to allow comparison with assessment criteria (Table VI.1), it is necessary to choose the bases on which all concentrations must be expressed in the scope of each descriptor: dry weights for organochlorines and PAHs in soft body tissues in the scope of Descriptor 8 and wet weights for metals, organochlorines and PAHs in soft body tissues in the scope of Descriptor 9.

Available information on contaminants in biota in the area

For Bulgaria, available contaminants data in biota are mainly in fishes. Stancheva *et al*. (2012) and Rizov (2012) measured metal content (Cd, Mn, Fe, Cu and Pb) and PCBs in muscle tissue of different fishes (bluefish, goby, sprat) in Varna region and adjacent area.

There are some data regarding heavy metal accumulation in different species. Spanos *et al*. (2008) reported the presence of moderately to seriously polluted coastal zones in Varna area and different accumulation of heavy metal corresponding to different species: e.g. P*olychaeta* accumulated preferably Co, Cr, Cu, and Pb; C*rustacea* - As, Cd, and Ni; *Mollusca – Zn.* Data obtained for heavy metal in 2010-2011 period revealed that the concentrations correspond to the Bulgarian standards (Regulation 31, 2004). Only for As the measured concentrations exceed the maximum admissible level. The highest heavy metals content was measured in mussels (*Mytilus galloprovincialis*) (Fig. VI.1). Also, some data on PCBs content in *Mytilus galloprovincialis* were reported by Rizov in 2012.

In Romania there is available data on heavy metals and organochlorine pesticides in molluscs (*Mytilus galloprovincialis, Rapana venosa* and *Scapharca inequivalvis*) in the frame of the monitoring programme.

Most mussel samples investigated during 2006 - 2011 have values entered accumulation of heavy metals in the normal range of variability, such as: copper 95% values <5 µg/g; 93% cadmium values <1 µg/g; lead 78% values <1.5 µg/g; 94% nickel values <2 µg/g; 93% chromium values <2 µg/g (NIMRD, 2012).

Table VI.1. Assessment criteria in biota with respect to Descriptor 8 and Descriptor 9.

Criterion	Indicator	Parameter	Assessment criteria
DESCRIPTOR 8: CONTAMINANTS AND POLLUTION EFFECTS			
8.1. Concentrations of contaminants	8.1.1. Concentrations of contaminants measured in relevant matrix (such as biota, sediment and water) in a way that ensure comparability with the assessments under Directive 2000/60/EC	Heavy metals and persistent organic pollutants in biota (molluscs)	Commission Regulation (EC) no. 1881/2006, updated by Commission Regulation (EC) no 629/2008, setting maximum levels for certain contaminants in foodstuffs; Environmental Assessment Concentrations (EAC, OSPAR)
8.2. Effects of contaminants	8.2.1. Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored	-	-
	8.2.2. Occurrence, origin (where possible), extent of significant acute pollution events (e.g. slicks from oil and oil products)and their impact on biota physically affected by this pollution	-	-
DESCRIPTOR 9: CONTAMINANTS IN FISH AND OTHER SEAFOOD			
9.1. Levels, number and frequency of contaminants	9.1.1 Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels	Heavy metals and persistent organic pollutants in biota (molluscs)	Commission Regulation (EC) no. 1881/2006, updated by Commission Regulation (EC) no 629/2008, setting maximum levels for certain contaminants in foodstuffs and amended by Commission Regulation (EC) no. 1259/2011, as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs
	9.1.2 Frequency of regulatory levels being exceeded	-	-

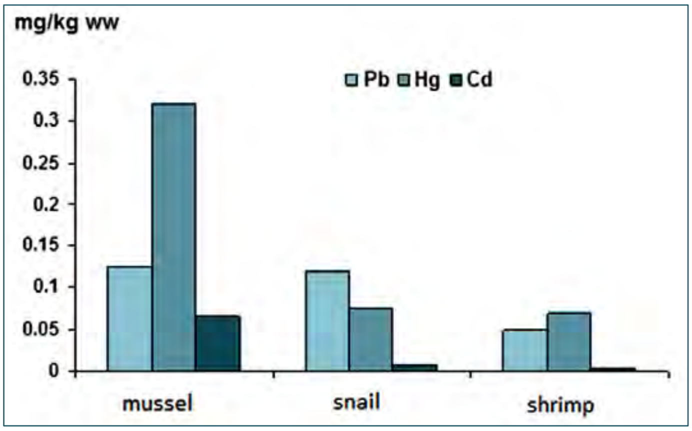
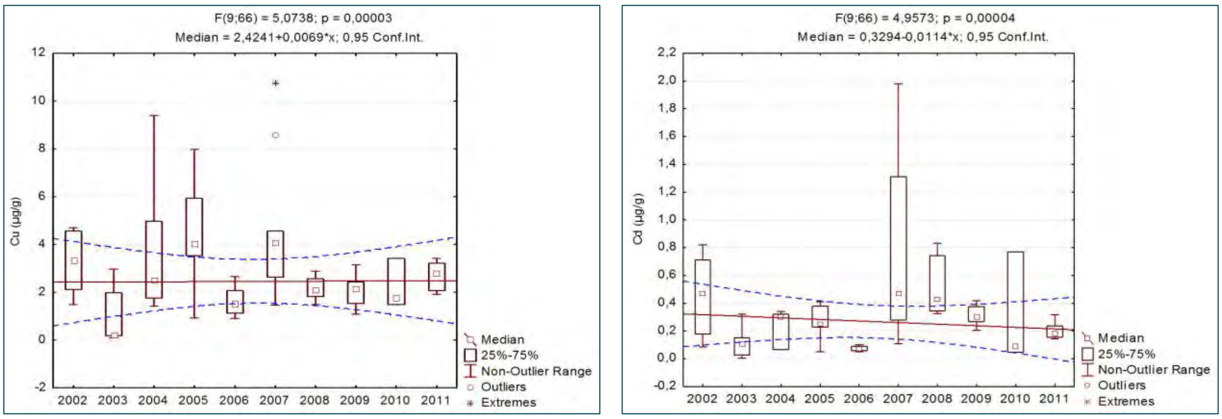


Figure VI.1. Heavy metals concentrations in biota collected in Varna region (2010-2011).

Bioaccumulation of cadmium in mussels registered a slight decrease in recent years, copper, nickel and chromium were enrolled between limits multiannual trends stabilization, while lead was characterized by a pronounced variability (Fig. VI.2).



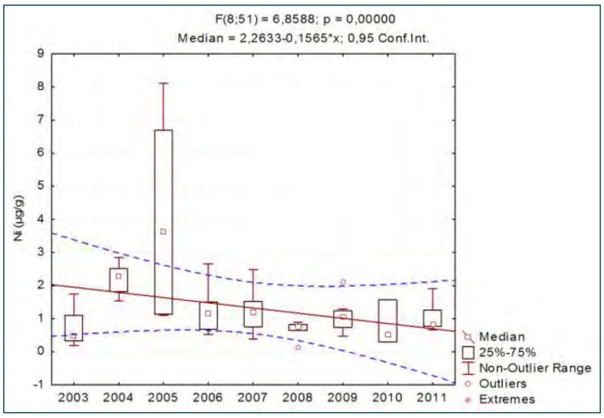


Figure VI.2. Trends in heavy metal concentrations in *Mytilus galloprovincialis* in the last decade.

Dominant organochlorine pesticides in molluscs are HCB, lindane, heptachlor and DDT. The range of these compounds varied between detection limit and 0.35 µg/g dry tissues for lindane and DDT and between detection limit and

0.15 µg/g dry tissue HCB and heptachlor. Concentrations of other compounds investigated (aldrin, dieldrin, endrin, DDE and DDD) is varying between detection limit and 0.07 µg/g dry tissue.

In the last years (2009 - 2011) it was noticed the significant depression of organochlorine pesticides, in particular the values for lindane, aldrin, heptachlor and DDT (Fig. VI.3). The other investigated compounds varied in the same range of variation as previous period (NIMRD, 2012).

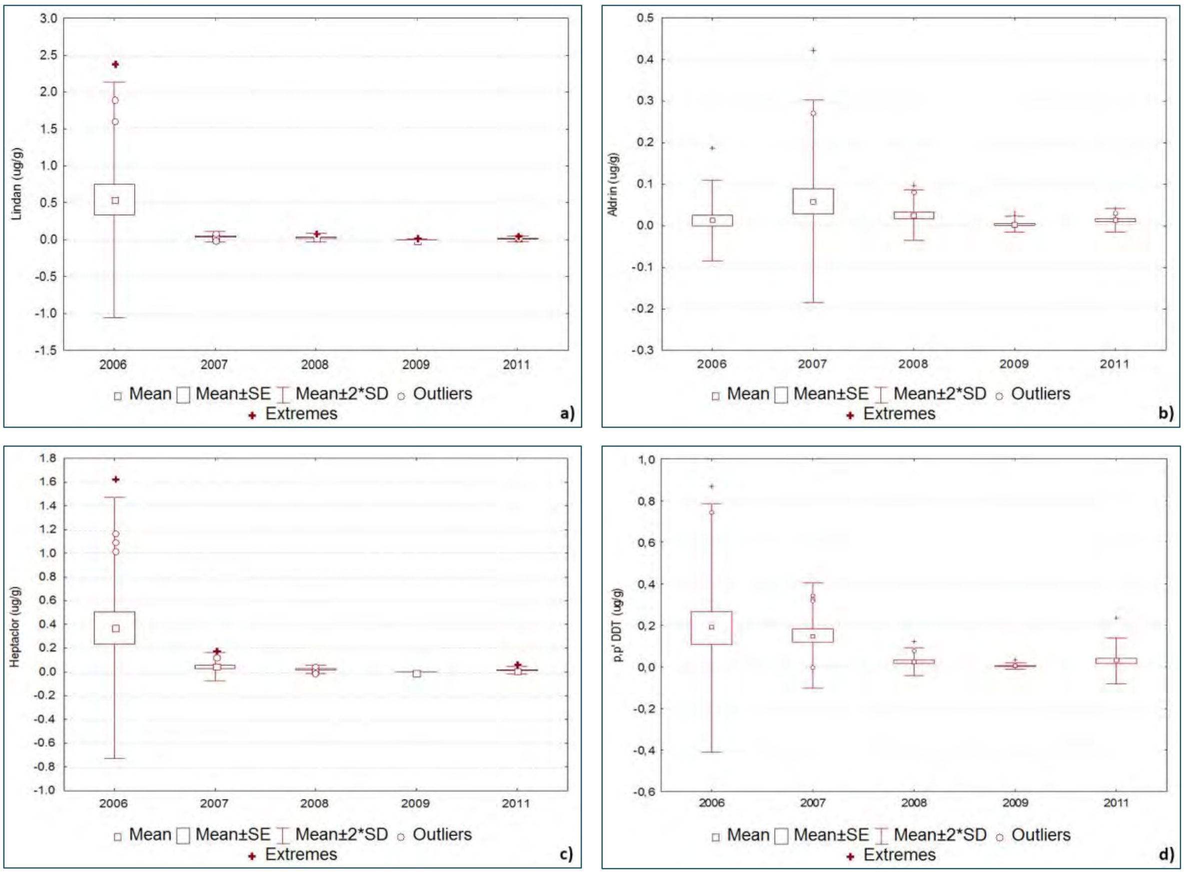


Figure VI.3. The variation of lindane (a), aldrin (b), heptachlor (c) and DDT (d) concentrations in biota between 2006 -2011.

In Turkey, because of the lack of regular data collection in the subsequent years at the same stations a temporal trend analyses couldn't be carried out on biota samples. Since, monitoring target specie(s) have not been identified; different species were sampled at different monitoring occasions which failed to produce systematic data sets for trend analysis.

Recently, Bat (2014) reviewed the results of the selected heavy metals (Fe, Zn, Ni, Cu, Mn, Pb, Cd and Co) in marine biota collected from the Black Sea coasts of Turkey:

- **Zooplankton** are key links in the transfer of carbon and play an important role in the biogeochemical cycling of metals through marine food webs. The highest Cu and Pb concentrations were found in Trabzon and Samsun coast, respectively;
- Metal concentrations in all studied **macroalgae**, green algae, brown algae and red algae decrease in the order: Fe>Mn>Zn>Pb>Ni>Cu>Co>Cd, Fe>Mn>Ni >Pb> Zn> Cu>Co> Cd and Fe>Mn> Zn> Ni> Pb> Cu>Co>Cd, respectively. The highest heavy metals measured in different algal divisions were: Fe and Zn in green algae; Ni, Cu, Mn and Pb in brown algae; Cd and Co in red algae;
- Among the **molluscs** the mussel, *Mytilus galloprovincialis* is commonly used as biomonitors of heavy metal pollution in coastal waters, followed by *Rapana venosa* and *Patella caerulea* (Bat, 2014). The order of bioaccumulation of metals in the sense of decreasing values was: Fe> Zn > Cu> Pb >Mn> Ni > Cd > Co.
- Very few data are available on heavy metal levels in **crustaceans** species of economic interest from the Turkish Black Sea coast;
- In **fish** the metal concentrations decrease in the order Zn>Fe>Cu>Mn>Pb>Ni>Co>Cd. Fe, Cu and Co accumulated in higher concentrations in liver and less in muscle.

In terms of metal pollution, İğneada, Trabzon, Bartin and Samsun areas were more polluted than other locations of Turkish coast of Black Sea (Bat, 2014).

MATERIAL and METHODS

NIMRD collected 13 samples of molluscs – *Mytilus galloprovincialis, Rapana venosa* and *Scapharca inequivalvis* (4 from the Romanian transect, 6 from the Bulgarian transect, and 3 from the Turkish transect) (Table VI.2). The dredge was launched in order to collect biota samples for pollutants. One sample consisted of 10 – 15 individuals from the same species, shell length measured, whole soft tissue being separated on board in clean conditions, wrapped, frozen and subsequently analysed in the NIMRD laboratory for heavy metals and POPs.

Table VI.2. Biota sampling for pollutants (Cruise report, 2013).

Station	Cast	Day	Start			End			Dredge length (M)
			Latitude (° ')	Longitude (° ')	Time (hh:mm)	Latitude (° ')	Longitude (° ')	Time (hh:mm)	
M01-M02		23/07/13	44°10.125'N	028°48.973'E	09:16	44°10.121'N	028°49.349'E	09:21	500
M04-M05	A	24/07/13	44°10.103'N	029°40.399'E	07:22	44°10.099'N	029°40.756'E	07:27	490
M04-M05	B	24/07/13	44°10.096'N	029°41.903'E	07:51	44°10.101'N	029°42.918'E	08:06	1350
M12-M11	A	26/07/13	43°09.999'N	028°10.026'E	07:24	43°09.999'N	028°10.026'E	07:29	440
M12-M11	B	26/07/13	43°10.005'N	028°10.922'E	07:43	43°10.013'N	028°11.892'E	07:58	1300
M12-M11	C	26/07/13	43°10.058'N	028°16.684'E	08:46	43°10.074'N	028°17.349'E	08:56	895
M11-M10		26/07/13	43°15.329'N	028°18.425'E	15:09	43°15.470'N	028°18.771'E	15:14	535
M10-M09		27/07/13	43°08.790'N	028°27.147'E	08:00	43°09.066'N	028°27.110'E	08:05	512
M18	A	30/07/13	41°50.763'N	028°01.808'E	12:35	41°50.558'N	028°02.046'E	12:40	500
M18	B	30/07/13	41°49.810'N	028°02.540'E	13:27	41°50.234'N	028°02.191'E	13:37	920
M17		30/07/13	41°52.495'N	028°07.455'E	17:46	41°52.999'N	028°07.689'E	17:56	980

For heavy metals, the biota samples were freeze-dried, digested with nitric acid in Teflon vessels on hot plate, and metals were analysed by GF-AAS. GC-ECD method was used for OCPs and PCBs, and GC-MS method for PAHs.

RESULTS

PAHs levels and composition

The PAH concentrations determined in the tissues of the three collected species are presented in Table VI.3, where $\sum_{16}$PAHs (µg/kg dry weight tissue) is the concentration of the sixteen determined PAHs, CPAHs% - the carcinogenic PAHs percentage to the total PAHs and LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings).

The PAH concentrations determined in the tissues of the two species with relevance for human consumption (*Mytilus galloprovincialis* and *Rapana venosa)* expressed as µg/kg wet weight tissue are presented in Table VI.4, where $\sum_{16}$PAHs (µg/kg wet weight) is the concentration of the sixteen determined PAHs, CPAHs % - the carcinogenic PAHs percentage to the total PAHs and B(a)P_{eqv} (µg/kg wet weight) - the total equivalent of toxicity by benzo(a) pyrene.

Total concentrations of the priority PAHs (sum of the 16 EPA priority pollutants) in the molluscs varied from 308.7 to 3756.9 (µg/kg dw), respectively 61.7 to 641.5 (µg/kg ww). This results are comparable to that obtained by Karacık *et al.* (Karacık et al. 2009) in the mussels of Istanbul Strait (215-3005 µg/kg dw, respectively 43.0 – 601.0 µg/kg ww) (Table VI.5).

According to Varanasi *et al.* (1993), the seafood can be classified into four categories, depending on the total content of PAHs: not contaminated (ΣPAHs < 50 µg/kg dw), minimally contaminated (ΣPAHs from 50 to 495 µg/kg), moderately contaminated (ΣPAHs from 500 to 5000 µg/kg dw) and highly contaminated (Σ PAHs > 5000 µg/kg dw). The results (Table VI.3) allow the classification of the biota samples as minimally (31%) and moderately contaminated (69 %) with high levels in *Scapharca inequivalvis* (Fig. VI.4). There was a significant difference ($p < 0.05$) between the total PAH concentrations determined in the two species sampled of *Mytilus galloprovincialis* and *Scapharca inequivalvis.*

The following individual compounds are dominant in the three molluscs species: phenanthrene, naphthalene, fluorene, benzo[a]pyrene, benzo[a]anthracene and dibenzo(a,h)anthracene. Petroleum PAHs (LMW) have been observed to be more readily accumulated by organisms than PAHs generated by combustion of organic matter (HMW) (Farrington et al., 1983).

The results are in agreement with previous findings which show that the mollusks are enriched in low molecular weight PAHs relative to higher molecular weight PAHs (Table VI.3). Scatterplot of phenanthren against the total $\sum_{16}$PAHs (µg/kg dw) in bivalve shows a good correlation (Fig. VI.5). LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings) were calculated for all samples. The value of LMW/HMW index in all samples is above 1 and it indicates petroleum pollution. The molluscs sample**,** dominated by the 3-ring PAH compounds (>50%), with a high proportion of naphthalene (19%) is consistent with a composition profile following an acute petrogenic (petroleum) exposure (Topping *et al.*, 1997). Of the thirteen samples analysed, five (38%) have showed this pattern. The lower molecular weight (LMW) percentages of PAHs vary between 59 and 97 with an average value of 84 ± 14.

Table VI.3. Concentrations of individual, total (∑16PAHs)PAHs (µg/kg dry weight tissue) , CPAHs% - the carcinogenic PAHs percentage and LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings), in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Species/ Sampling	Nap**	Acl	Ac	Fl	Phe	An	Fa	Py	B[a]a	Chry	B[b]fl	B[k]fl	B[a]pyr	B(g,h,i)p	D(a,h)a	Ip	$\sum_{16}$PAHs	CPAHs	LMW/ HMW
station -M*																		%	
Mytilus BG(11-10)	48.6	6.4	8.3	34.8	85.9	26.4	11.8	9.7	7.7	5.9	2.4	8.7	16.8	8.1	8.5	18.8	**308.7**	*31.9*	2.14
Mytilus RO(01-02)	13.9	6.7	8.7	30	144.4	19	5.1	8.2	15.1	4.2	4.8	5.4	36.7	6.2	13.6	2.8	**325.0**	*31.5*	2.18
Rapana RO(01-02)	24.9	5.4	6	31.1	102.6	24.2	8.2	13.8	11.4	7.4	1.1	7.1	33	14.2	22	15.7	**328.0**	*40.8*	1.45
Rapana TR(18-17)	14.8	5.2	7.3	13.5	241.4	15.7	7.6	8.7	14.9	10	7.2	14.1	32.9	21.7	40.5	19.9	**475.4**	*37.3*	1.68
Mytilus BG(12-11)	62.2	5.7	6.1	36.3	446.4	23.1	6.8	12.5	5	3.6	4.4	6.4	7.2	8.9	6.6	8.5	**649.6**	*10.8*	8.29
Mytilus BG(10-09)	107.4	5.1	11.5	62.9	687.9	37.3	4.6	9.6	8.9	3.1	5.4	8.3	11.3	5.7	16.5	3.6	**989.1**	*7.8*	11.86
Mytilus TR(17)	50.2	5	10.5	54.1	1165.1	42.3	14.3	11.4	15.3	11.2	4.9	13.2	71.0	15.6	24.1	10.2	**1518.7**	*12.6*	6.93
Scapharca RO(01-02)	90.8	8.3	11.3	160.4	1297.9	28.4	19.7	21.7	16.2	5.6	16.7	17	35.2	8.8	27.3	12.6	**1778.1**	*10.2*	8.83
Rapana BG(11-10)	707.6	18.3	21.6	222.1	1137.5	15.1	10	10.6	9.2	3.4	2.3	4.9	4.8	7.4	5.1	4.9	**2184.5**	*2.9*	33.99
Scapharca BG(12-11)	497.4	12.6	10.3	167.5	1751.4	39.3	18.3	18.7	5.3	3.3	4.6	7.9	9.5	4.5	4.8	5.9	**2561.2**	*3.2*	29.99
Mytilus RO(04-05)	633.9	23.8	27.6	279.4	1532.0		9.1	14.5	5	5.2	6.3	13.3	17.8	6.3	15.6	12.6	**2602.4**	*4.1*	23.63
Rapana BG(12-11)	896.5	18.4	13.7	257.6	1905.4	26.9	17.5	13.4	7.1	4.4	2.2	7.1	6.0	7.1	20.1	4.2	**3207.8**	*2.8*	34.96
Scapharca TR(18-17)	788.0	11	15.7	119.4	2363.9	8.8	18.2	17.9	92.6	9.3	9.1	21.5	222.9	16.6	21.6	20.3	**3756.9**	*12*	7.35
EAC***(µg/kg)	340				1700	290	110	100	80				**600**	110					

*13 samples of molluscs - *Mytilus, Rapana and Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 – M 17).

** Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene: Chry, Benzo[b]fluoranthene: B[b]fl, Benzo[k]fluoranthene: B[k]fl, Benzo[a]pyrene: B[a]pyr, Benzo (g,h,i)perylene: B(g,h,i)p, Dibenzo(a,h)anthracene: D(a,h)a, Indeno(1,2,3-c,d)pyrene

*** **EAC-** Environmental Assessment Criteria represent the contaminant concentration in the environment below which it can be assumed that no chronic effects will occur in marine species, including the most sensitive species (OSPAR Commission, 2008)

Table VI.4. Concentrations of individual, total (∑16PAHs)PAHs, CPAHs% - the carcinogenic PAHs percentage and the total equivalent of toxicity by benzo(a) pyrene-B(a)Peqv (µg/kg wet weight), in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Species/ Sampling station -M*	Nap**	Acl	Ac	Fl	Phe	An	Fa	Py	B[a]a	Chry	B[b]fl	B[k]fl	B[a]pyr	B(g,h,i)p	D(a,h)a	Ip	$\sum_{16}$PAHs	CPAHs %	B(a)P_{eqv}
Mytilus BG(11-10)	9.7	1.3	1.7	70	17.2	5.3	2.4	1.9	1.5	1.2	0.5	1.7	3.4	1.6	1.7	3.8	**61.7**	31.9	5.83
Mytilus RO(1-2)	2.8	1.3	1.7	6.0	28.9	3.8	1.0	1.6	3.0	0.8	1.0	1.1	7.3	1.2	2.7	0.6	**65.0**	31.5	10.64
Rapana RO(1-2)	5.0	1.1	1.2	6.2	20.5	4.8	1.6	2.8	2.3	1.5	0.2	1.4	6.6	2.8	4.4	3.1	**65.6**	40.8	11.72
Rapana TR(18-17)	3.0	1.0	1.5	2.7	48.3	3.1	1.5	1.7	3.0	2.0	1.4	2.8	6.6	4.3	8.1	4.0	**95.1**	37.3	15.83
Mytilus BG(12-11)	12.4	1.1	1.2	7.3	89.3	4.6	1.4	2.5	1.0	0.7	0.9	1.3	1.4	1.8	1.3	1.7	**129.9**	10.8	3.25
Mytilus BG(10-9)	21.5	1.0	2.3	12.6	137.6	7.5	0.9	1.9	1.8	0.6	1.1	1.7	2.3	1.1	3.3	0.7	**197.8**	7.8	6.09
Mytilus TR(17-16))	10.0	1.0	2.1	10.8	233.0	8.5	2.9	2.3	3.1	2.2	1.0	2.6	14.2	3.1	4.8	2.0	**303.7**	12.6	19.92
Rapana BG(11-10)	141.5	3.7	4.3	44.4	227.5	3.0	2.0	2.1	1.8	0.7	0.5	1.0	1.0	1.5	1.0	1.0	**436.9**	2.9	2.40
Mytilus RO(4-5)	126.8	4.8	5.5	55.9	306.4	0.0	1.8	2.9	1.0	1.0	1.3	2.7	3.6	1.3	3.1	2.5	**520.5**	4.1	7.44
Rapana BG(12-11)	179.3	3.7	2.7	51.5	381.1	5.4	3.5	2.7	1.4	0.9	0.4	1.4	1.2	1.4	4.0	0.8	**641.6**	2.8	5.65

*13 samples of molluscs - *Mytilus, Rapana and Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 – M 17).
** Naphtalene: Nap, Acenaphthylene: Acl, Acenaphthene: Ac, Fluorene: Fl, Phenanthrene: Phe, Anthracene: An, Fluoranthene: Fa, Pyrene: Py, Benzo[a]anthracene :B[a]a, Crysene: Chry, Benzo[b]fluoranthene: B[b]fl, Benzo[k]fluoranthene: B[k]fl, Benzo[a]pyrene: B[a]pyr, Benzo (g,h,i)perylene: B(g,h,i)p, Dibenzo(a,h)anthracene: D(a,h)a, Indeno(1,2,3-c,d)pyrene

Table VI.5. PAH concentrations (µg/kg dry weight tissue) in bivalve mollusks from worldwide.

Sites	Range (∑PAHs µg/kg dw)	References
The Western Black Sea,	308 - 3756	In this study (2013)
İstanbul Strait, Turkey, Black Sea	215-3005	Karacık *et al.* (2009)
Ligurian Coast/Italy	129-2638	Piccardo *et al.* (2001)
Venice Lagoon/Italy	67-2434	Moschino *et al.* (2011)
Saronikus Gulf/Greece	184-2454	Vlahogianni *et al.* (2008)
The Galician coast, NW Spain	50-200	Soriano *et al.* (2006)
Ria de Vigo/Spain	59-739	Ruiz *et al.* (2011)
Mediterranean Coast	25-80	Baumard *et al.* (1999)
Scottish inshore /offshore	150-2255	McIntosh *et al.* (2004)
Scottish Coastal Waters	62-755	Webster *et al.* (2008)
Arachon bay, France	280-480	Baumard *et al.* 1998)
San Fransisco estuary,California USA	25-580	Oros *et al.* (2005)
Boston Harbor /Massachusetts Bay/USA	44-3333	Hunt and Slone (2010)
San Francisco Bay/USA	21-1093	Oros *et al.* (2007)
South and Southeast Asia	11-1133	Isobe *et al.* (2007)
Victoria Harbor/Hong Kong	600-22,858	Fang *et al.* (2009)
Bahia Blanca Estuary/Argentina	349-1597	Arias *et al.* (2009)
Ilha Grande Bay-RJ, Brazil	4.88-584	Renato *et al.* (2012)
Guanabara Bay-RJ, Brazil	60-6271	Renato *et al.* (2012)
São Sebastião Channel-SP, Brazil	180-1630	Renato *et al.* (2012)
Mundau-Manguaba Lagoons-PB, Brazil	41.4-525	Renato *et al.* (2012)

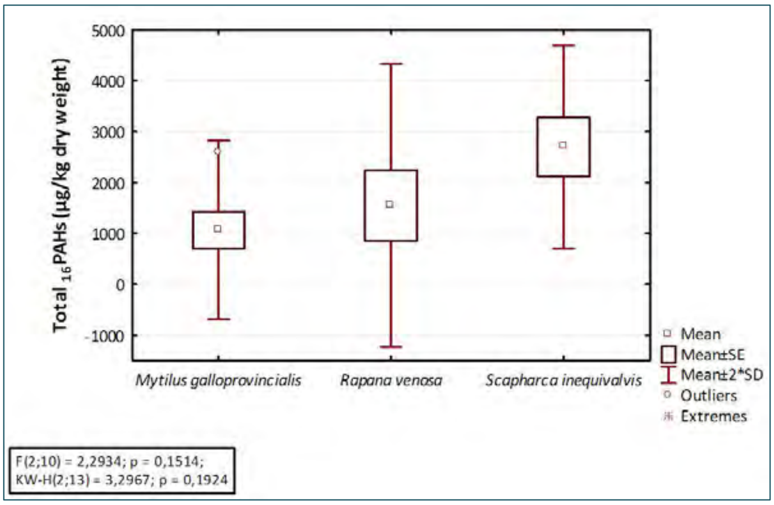


Figure VI.4. Box plot of ∑16PAHs (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013.

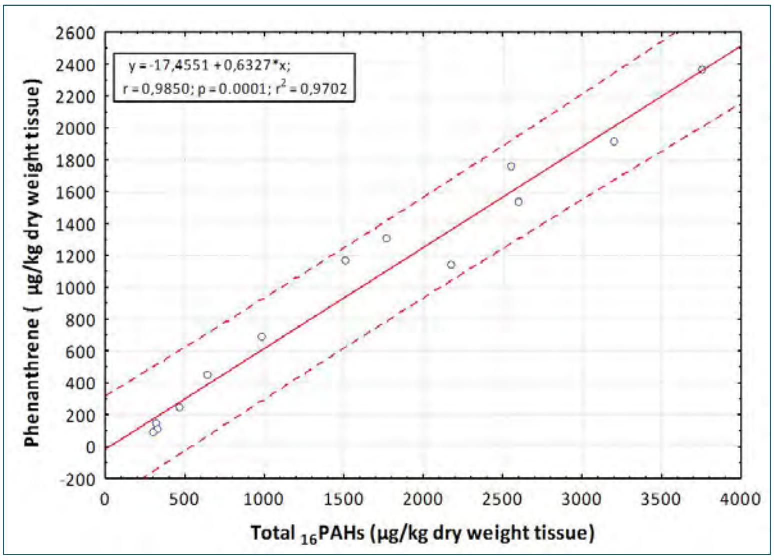


Figure VI.5. Scatterplot of phenanthren against Total ∑16PAHs (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013.

According to Webster (Webster *et al.* 1997) PAH concentrations >250 µg/kg ww tissue are likely to be indicative of a more severe (e.g. emergency) or long term chronic exposure. Mussel tissue PAH concentrations in the range 150–250 µg/kg ww may indicate an acute exposure or low-level chronic exposure to PAH contaminants. PAH concentrations between 50 and 150 µg/kg ww can be considered as background for pre-spawning mussels and concentrations < 50 µg/kg ww is considered as typical background/reference values for post-spawning mussels *(*McIntosh et al. 2004).

Of the ten samples analysed, four showed $\sum_{16}$PAHs greater than 250 µg/kg ww and six samples contained between 50 and 150 µg/kg ww PAH. There was no significant difference (p>0.05) between the means of the total PAH concentrations (µg/kg ww) determined in the two species sampled of *Mytilus galloprovincialis* (213 ± 175.9) and *Rapana venosa* (309.8 ± 278.0).

The mollusc sample**,** dominated by the 3-ring PAH compounds (>50%), with a high proportion of naphthalene (19%) is consistent with a composition profile following an acute petroleum exposure (Topping et al., 1997). Of the ten samples analysed, four-have showed this pattern.

Organochlorine Pesticides and Polychlorinated Biphenyls

The organochlorine pesticides and polychlorinated biphenyls concentrations determined in the tissues of the three collected species are presented in Table VI.6 and Table VI.7, where ∑OCPs (µg/kg dry weight tissue) is the concentration of the nine individual compounds (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, p,p'DDE, p,p' DDD and p,p' DDT) and ∑PCBs (µg/kg dry weight tissue) is the concentration of the seven individual compounds (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180).

The organochlorine pesticides and polychlorinated biphenyls concentrations determined in the tissues of the two species with relevance for human consumption (*Mytilus galloprovincialis* and *Rapana venosa)* are presented in Table VI.8 and Table VI.9, where ∑OCPs (µg/kg ww tissue) is the concentration of the nine individual compounds (HCB, lindane, heptachlor, aldrin, dieldrin, endrin, p,p'DDE, p,p' DDD and p,p' DDT) and ∑PCBs (µg/kg wet weight tissue) is the concentration of the seven individual compounds (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180).

Total concentrations of the organochlorine pesticides varied from 4.87 to 52.91 µg/kg dw. Total concentrations of the polychlorinated biphenyls varied from 2.86 to 61.08 µg/kg dw. *Mytilus galloprovincialis* and *Scapharca ineqiuvalvis* accumulated the higher quantity of OCPs and PCBs, probably because of their filter-feeding system (Fig. VI.6 and VI.7).

The major OCPs compounds are p, p' DDE, p, p' DDD and p, p' DDT. The other investigated pesticides were under detection limit (Fig. VI.8). The concentration detected for p, p' DDT and its metabolites are comparable with other results obtained for Black Sea mussels and previous data obtained in the Romanian Black Sea waters (Kurt & Ozkoc, 2004; Ozkoc et al., 2007; Okay et al., 2011; NIMRD, 2012; NIMRD, 2013). Except PCB 28 and PCB 101 that were under detection limit in all samples, the other PCBs were detected in concentrations between detection limits and 48.40 µg/kg dw. Higher levels were measured for PCB 138, PCB 153 and PCB 180 and especially PCB 52 in some samples (Fig. VI.9).

Table VI.6. Concentrations of individual and total OCPs (∑OCPs) (µg/kg dry weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Species/ Sampling station -M*	HCB	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p' DDT	Total OCPs
Rapana RO(01-02)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	10.14	6.11	7.00	25.44
Rapana BG(10-11)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	10.01	0.20	3.10	15.51
Rapana BG(11-12)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	<0.20	<0.20	2.60	5.20
Rapana TR(17-18)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	2.17	0.20	<0.30	4.87
Mytilus RO(01-02)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	7.26	43.15	<0.30	52.91
Mytilus RO(04-05)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	17.84	7.79	7.60	35.43
Mytilus BG(09-10)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	9.57	<0.20	4.66	16.63
Mytilus BG(10-11)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	<0.20	15.82	9.76	27.98
Mytilus BG(11-12)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	22.28	0.20	5.55	30.23
Mytilus TR(16 -17)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	7.75	4.64	<0.30	14.89
Scapharca RO(01-02)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	13.77	<0.20	14.21	30.38
Scapharca BG(11-12)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	14.72	<0.20	<0.30	17.42
Scapharca TR(17-18)	<0.50	<0.40	<0.30	<0.30	<0.30	<0.40	12.52	<0.20	<0.30	15.22

*13 samples of molluscs - *Mytilus, Rapana* and *Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 – M 17).

Table VI.7. Concentrations of individual and total PCBs (∑PCBs) (µg/kg dry weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Species/ Sampling station -M*	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	Total PCBs
Rapana RO(01-02)	<0.40	<0.30	<0.60	0.15	2.98	5.28	0.95	10.66
Rapana BG(10-11)	<0.40	<0.30	<0.60	<0.40	3.38	1.21	<0.30	6.59
Rapana BG(11-12)	<0.40	<0.30	<0.60	<0.40	2.98	1.01	16.49	22.18
Rapana TR(17-18)	<0.40	<0.30	<0.60	<0.40	0.16	<0.70	<0.30	2.86
Mytilus RO(01-02)	<0.40	9.74	<0.60	1.23	4.97	<0.70	<0.30	17.95
Mytilus RO(04-05)	<0.40	27.56	<0.60	1.34	2.28	5.03	<0.30	37.51
Mytilus BG(09-10)	<0.40	<0.30	<0.60	<0.40	0.73	1.82	<0.30	4.55
Mytilus BG(10-11)	<0.40	<0.30	<0.60	<0.40	0.89	3.80	<0.30	6.69
Mytilus BG(11-12)	<0.40	<0.30	<0.60	<0.40	1.74	2.16	<0.30	5.91
Mytilus TR(16 -17)	<0.40	<0.30	<0.60	<0.40	<0.60	<0.70	<0.30	3.30
Scapharca RO(01-02)	<0.40	48.40	<0.60	1.93	0.72	5.53	3.49	61.08
Scapharca BG(11-12)	<0.40	4.38	<0.60	<0.40	<0.60	<0.70	<0.30	7.38
Scapharca TR(17-18)	<0.40	31.52	<0.60	<0.40	<0.60	<0.70	<0.30	34.52
EAC**(µg/kg)	1.7	2.7	3	0.6	40	7.9	24	

*13 samples of molluscs - *Mytilus, Rapana and Scapharca*: 4 from the Romanian transect (M 01-M 05), 6 from the Bulgarian transect (M12-M 09), and 3 from the Turkish transect (M 18 – M 17).
** **EAC-** Environmental Assessment Criteria represent the contaminant concentration in the environment below which it can be assumed that no chronic effects will occur in marine species, including the most sensitive species (OSPAR Commission 2008)

Table VI.8. Concentrations of individual and total OCPs (∑OCPs) (µg/kg wet weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Species/ Sampling station -M*	HCB	Lindane	Heptachlor	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p' DDT	Total OCPs
Rapana RO(01-02)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	2.535	1.528	1.750	6.360
Rapana BG(10-11)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	2.503	<0.050	0.775	3.878
Rapana BG(11-12)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	<0.050	<0.050	0.650	1.300
Rapana TR(17-18)	<0.125	<0.100	<0.075	<0.075	<0.075	<0.100	0.543	0.050	<0.075	1.218
Mytilus RO(01-02)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	1.090	6.479	<0.045	7.944
Mytilus RO(04-05)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	2.679	1.170	1.141	5.320
Mytilus BG(09-10)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	1.437	<0.030	0.700	2.497
Mytilus BG(10-11)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	0.030	2.375	1.465	4.201
Mytilus BG(11-12)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	3.345	0.030	0.833	4.539
Mytilus TR(16 -17)	<0.075	<0.060	<0.045	<0.045	<0.045	<0.060	1.164	0.697	<0.045	2.236

*10 samples of mollusks – *Mytilus and Rapana*: 3 from the Romanian transect (M 01-M 05), 5 from the Bulgarian transect (M12-M 09), and 2 from the Turkish transect (M 18 – M 17)

Table VI.9. Concentrations of individual and total PCBs (∑PCBs) (µg/kg wet weight tissue) in biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters, July 2013.

Species/ Sampling station -M*	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	Total PCBs
Rapana RO(01-02)	<0.100	<0.075	<0.150	0.038	0.745	1.320	0.238	2.628
Rapana BG(10-11)	<0.100	<0.075	<0.150	<0.100	0.845	0.303	<0.075	1.548
Rapana BG(11-12)	<0.100	<0.075	<0.150	<0.100	0.745	0.253	4.123	5.445
Rapana TR(17-18)	<0.100	<0.075	<0.150	<0.100	0.040	<0.175	<0.075	0.615
Mytilus RO(01-02)	<0.060	1.462	<0.090	0.308	0.746	<0.105	<0.045	2.509
Mytilus RO(04-05)	<0.060	4.138	<0.090	0.335	0.342	0.755	<0.045	5.431
Mytilus BG(09-10)	<0.060	<0.045	<0.090	<0.060	0.110	0.273	<0.045	0.623
Mytilus BG(10-11)	<0.060	<0.045	<0.090	<0.060	0.134	0.571	<0.045	0.944
Mytilus BG(11-12)	<0.060	<0.045	<0.090	<0.060	0.261	0.324	<0.045	0.826
Mytilus TR(16 -17)	<0.060	<0.045	<0.090	<0.060	<0.090	<0.105	<0.045	0.435

*10 samples of mollusks – *Mytilus and Rapana*: 3 from the Romanian transect (M 01-M 05), 5 from the Bulgarian transect (M12-M 09), and 2 from the Turkish transect (M 18 – M 17)

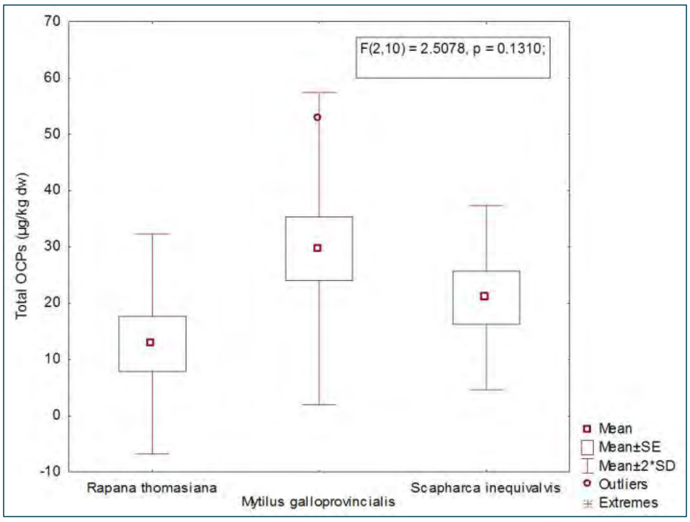


Figure VI.6. Box plot of ∑OCPs (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013.

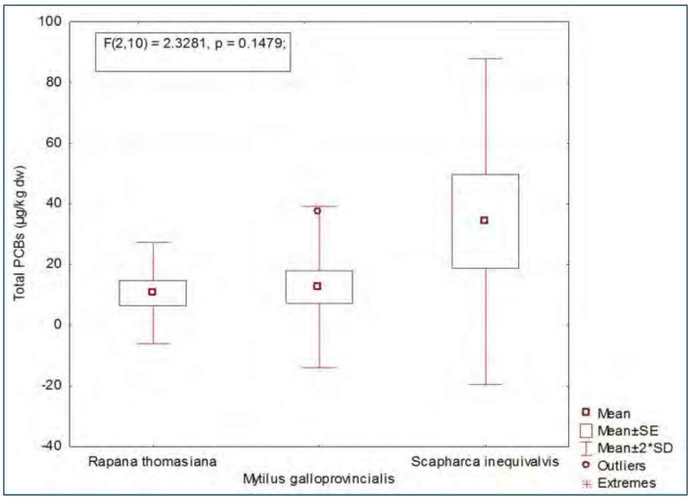


Figure VI.7. Box plot of ∑PCBs (µg/kg dry weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013.

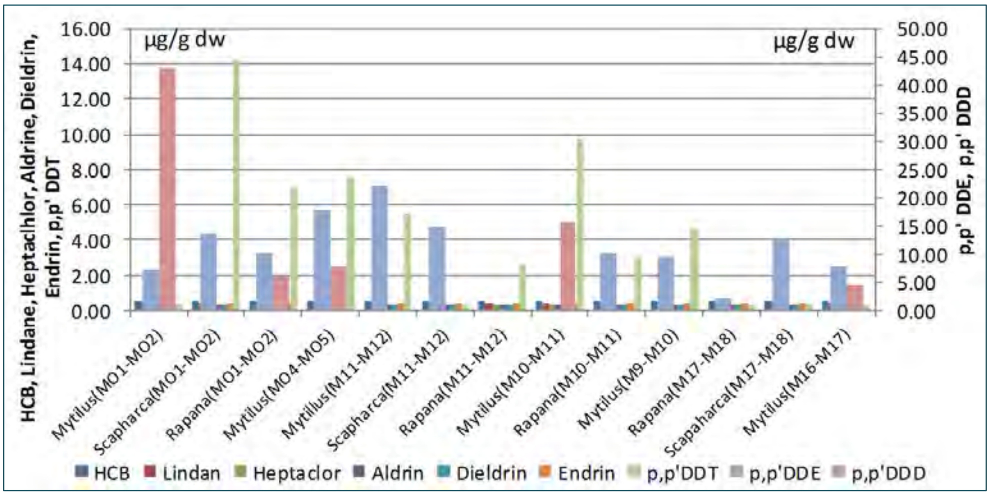


Figure VI.8. Individual OCPs levels (µg/kg dry weight tissue) in mollusks from the Romanian, Bulgarian and Turkish waters, July 2013.

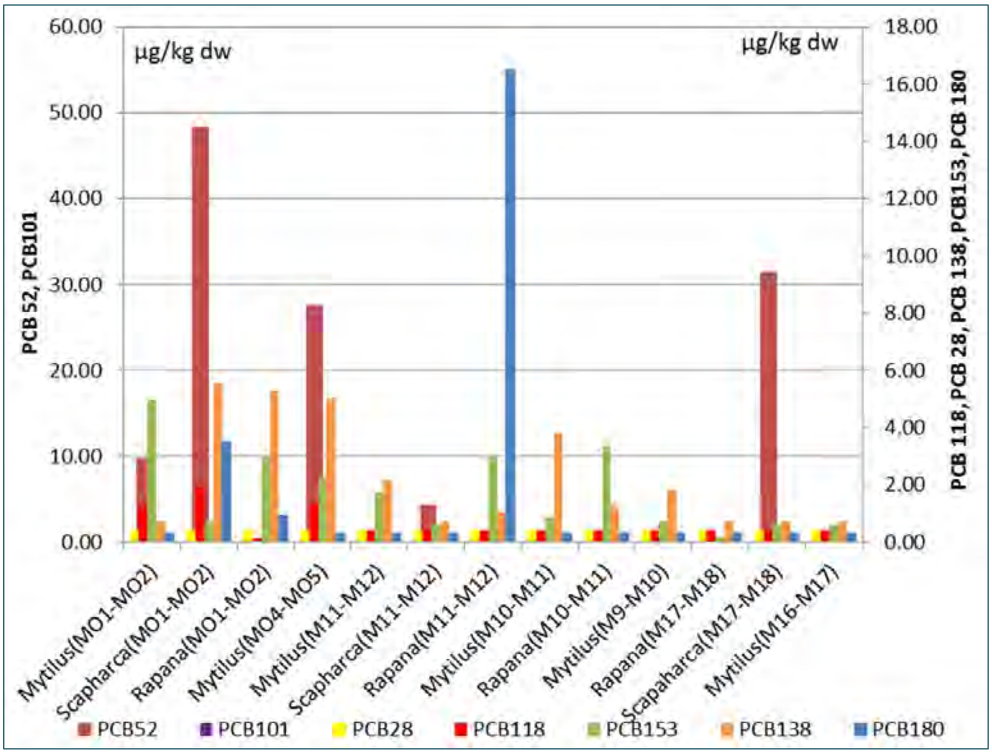


Figure VI.9. Individual PCBs levels (µg/kg dry weight tissue) in mollusks from the Romanian, Bulgarian and Turkish waters, July 2013.

Heavy metals

Marine organisms are continuously exposed to varying concentrations of metals in their environment. There is certain selectivity in metal accumulation, so there must be a distinction between essential and non-essential metals. Essential metals like copper, zinc, manganese, iron and cobalt are essential components of many enzymes and respiratory pigments. Therefore, marine organisms must provide sufficient metals for tissues to sustain metabolic and respiratory needs. Deficiency of these metals, but equally accumulation over certain levels might have harmful effects (White & Rainbow, 1985). Non-essential metals (lead, arsenic, mercury, cadmium) are highly toxic, even at very low levels, especially if accumulated in the metabolically active sites. Body is required to restrict non-essential metal accumulation or to pass them into the non-toxic forms (Depledge & Rainbow, 1990).

Thus, although metals are essential components of life, they become harmful when present in excess. Increasing levels of bioavailable metals in the marine environment is a problem for human health and marine ecosystems. Given the great diversity of biotic and abiotic factors governing metal bioaccumulation, like availability of food, hydro-chemical conditions, genetic differences, physiological status, etc., discrimination between changes in the level of accumulation in response to environmental contamination and those caused by natural variation of the physiology of the organism is sometimes difficult (UNEP, 1993).

Heavy metals concentrations determined in the whole soft tissue of the molluscs species investigated in July 2013 registered the following averages and variation ranges (reported as wet weight tissue) (Table VI.10):

- *Mytilus galloprovincialis*: 2.04 (1.13 - 4.48) µg/g Cu; 0.68 (0.21 - 1.12) µg/g Cd; 0.104 (0.002 - 0.216) µg/g Pb; 2.48 (0.97 - 6.00) µg/g Ni; 0.34 (0.07 - 1.31) µg/g Cr;
- *Rapana venosa*: 7.32 (4.76-9.35) µg/g Cu; 4.10 (1.23 - 5.42) µg/g Cd; 0.124 (0.019 - 0.254) µg/g Pb; 0.64 (0.33 - 0.92) µg/g Ni; 0.30 (0.14 - 0.57) µg/g Cr;
- *Scapharca inequivalvis*: 2.03 (1.93 - 2.15) µg/g Cu; 2.51 (2.20 - 2.86) µg/g Cd; 0.024 (0.004 - 0.050) µg/g Pb; 2.47 (1.10 - 4.47) µg/g Ni; 0.29 (0.19 - 0.47) µg/g Cr.

With respect to interspecific differences, *Rapana* samples had a higher bioaccumulation capacity for copper and cadmium, whereas nickel levels were diminished, in comparison with the other mollusks (Fig. VI.10).

For mussels (*Mytilus edulis*) from North Sea and Baltic Sea the following threshold values, corresponding to normal background for metals, were proposed: Cu 2.0 µg/g ww; Cd 0.4-0.8 µg/g ww; Pb 0.4-1.0 µg/g ww; Ni 0.8-1.0 µg/g ww; Cr 0.4-0.6 µg/g ww (EPA, 2002). In reference to these values, concentrations of heavy metals in mussels investigated in July 2013 were included in their variation range, with the exception of nickel.

Data obtained during MISIS cruise, July 2013, concerning heavy metals in molluscs are comparable with other data reported for the Black Sea or other marine regions, even showing diminished levels, as in case of lead (Table VI.11).

Table VI.10. Concentration of heavy metals in three species of marine molluscs from the Western Black Sea, July 2013.

Species	Station	UM	Cu (µg/g ww)	Cd (µg/g ww)	Pb (µg/g ww)	Ni (µg/g ww)	Cr (µg/g ww)
Mytilus galloprovincialis	RO/M01-M02	µg/g ww	2.20	0.37	0.216	1.95	0.14
Mytilus galloprovincialis	RO/M04-M05	µg/g ww	1.75	0.45	0.072	0.97	0.07
Mytilus galloprovincialis	BG/M12-M11	µg/g ww	4.48	0.84	0.092	1.00	0.17
Mytilus galloprovincialis	BG/M11-M10	µg/g ww	1.17	0.21	0.072	2.69	1.31
Mytilus galloprovincialis	BG/M10-M09	µg/g ww	1.51	1.05	0.172	6.00	0.15
Mytilus galloprovincialis	TR/M17-M16	µg/g ww	1.13	1.12	0.002	2.30	0.22
Rapana venosa	RO/M01-M02	µg/g ww	7.48	1.23	0.138	0.33	0.23
Rapana venosa	BG/M12-M11	µg/g ww	4.76	5.40	0.086	0.65	0.24
Rapana venosa	BG/M11-M10	µg/g ww	7.68	4.36	0.254	0.92	0.57
Rapana venosa	TR/M18-M17	µg/g ww	9.35	5.42	0.019	0.66	0.14
Scapharca inequivalvis	RO/M01-M02	µg/g ww	2.15	2.20	0.018	1.85	0.22
Scapharca inequivalvis	BG/M12-M11	µg/g ww	2.03	2.47	0.050	4.47	0.47
Scapharca inequivalvis	TR/M18-M17	µg/g ww	1.93	2.86	0.004	1.10	0.19

Table VI.11. Heavy metal concentrations (µg/kg wet weight tissue) in bivalve molluscs from worldwide.

	Cu (µg/g ww)	Cd (µg/g ww)	Pb (µg/g ww)	Ni (µg/g ww)	Cr (µg/g ww)
MISIS Cruise, July 2013, Black Sea (RO, BG, TR)	2.04 (1.13 - 4.48)	0.68 (0.21 - 1.12)	0.104 (0.002 - 0.216)	2.48 (0.97- 6.00)	0.34 (0.07- 1.31)
IA RO Black Sea (2006 – 2011) (NIMRD, 2012)	2,61 (0,91 – 10,77)	0,36 (0,05 – 1,98)	0,98 (0,02 – 10,29)	1,05 (0,11 –2,66)	0,97 (0,01 – 6,07)
TR Black Sea coast (Bat, 2014) (2002 – 2012)	0.48 - 38.00	0.05 - 1.28	0.21 - 4.20	0.40 - 8.76	
BG Black Sea (Stancheva *et al.*, 2012)		0.04 – 0.09	0.11 – 0.18		
Mediterranean Sea (Conti and Cecchetti, 2003)	0.85 - 1.77	0.05 - 0.08	0.26 - 0.38		0.08 - 0.20
North Atlantic Ocean (Besada, 2002)	0.88 - 1.93	0.07 - 0.57	0.10 - 1.64		
North Atlantic Ocean (Ugur, 2002)	2.41 – 5.83		0.77 – 6.02		
Sea of Japan (Shulkin, 2003)	0.78 – 23.80	0.28 – 5.20	0.20 – 56.60	0.14 – 0.80	

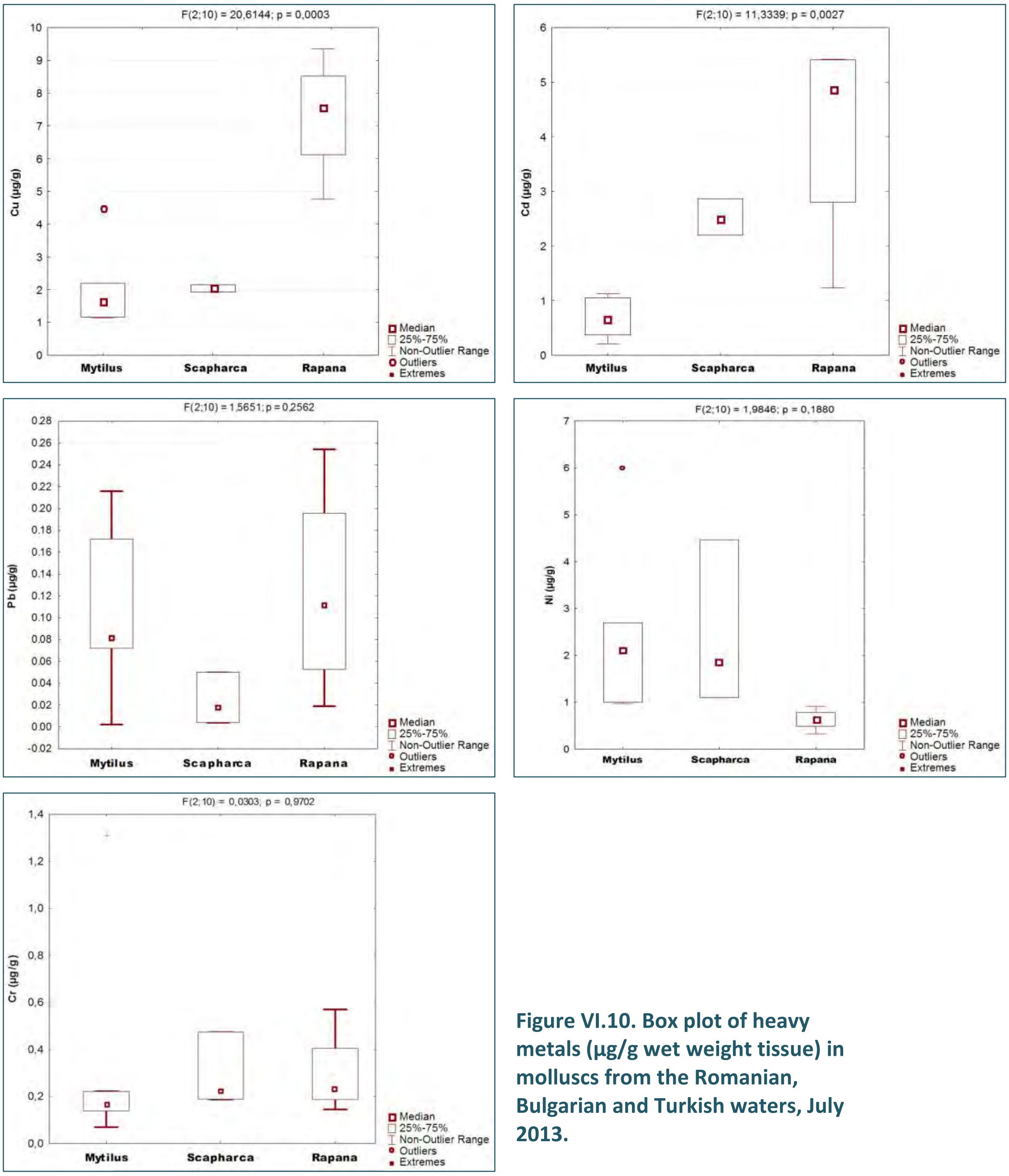


Figure VI.10. Box plot of heavy metals (µg/g wet weight tissue) in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013.

Comparison to Assessment Criteria

In the scope of Descriptor 8 the results of the biota analyses were compared to Environmental Assessment Criteria (EACs) proposed by OSPAR as a means for assessing the significance of concentrations of hazardous substances in the marine environment. EACs (lower) are concentrations below which it is reasonable to expect that there will be an acceptable level of protection of marine species from chronic effects from specific hazardous substances (Table VI.3 and VI.7).

Most of the individual PAH concentrations do not exceed the EAC values in samples except naphthalene (38%,) phenanthrene (23%) and benzo[a]anthracene (8%) . According to EACs proposed by OSPAR and the total content of PAHs the biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters in July 2013 can be classified as:

- minimally contaminated (31%) with ΣPAHs from 308.7 to 475.4 µg/kg dw found in *Mytilus* galloprovincialis and *Rapana thomasiana;*
- moderately contaminated (31 %) with ΣPAHs from 649.6 to 1778.1 µg/kg dw found in *Mytilus* and *Scapharca;*
- highly contaminated (38%) with ΣPAHs from 2184.5 to 3756.9 µg/kg dw found in *Mytilus, Rapana* and *Scapharca* with long-term biological effects (e.g. impaired growth, reproduction and survival) and acute biological effects (survival).

There are no EACs values available for OCPs in molluscs. As regard to PCBs, only PCB 52 and PCB 118 exceeded the EAC values in 30% and respectively 15% of samples.

The EU Marine Strategy Framework Directive – Descriptor 9 requires that "*contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards*".

At this moment the EU legislation that establish maximum admissible levels of contaminants *in fish and other seafood for human consumption are* EC no. 1881/2006 - only applicable to a few substances relevant for this indicator: benzo[*a*]pyrene, cadmium, lead, and mercury and EC no. 1259/2011 amending Regulation (EC) no. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs.

Benzo[*a*]pyrene can be used as a marker for the occurrence and effects of carcinogenic PAHs in food. The Commission Regulation (EC) no. 1881/2006 sets a maximum concentration of 10 µg/kg wet weight for benzo[*a*]pyrene in bivalve molluscs.

Only one sample of *Mytilus galloprovincialis* returned a benzo[*a*]pyrene concentration (14.2 µg/kg wet weight) greater than 10 µg/kg wet weight.

Benzo(a)pyrene is the only PAH out of the sixteen determined PAHs for which toxicological data for the calculation of the carcinogenicity factor are available. The EPA (1993) approach uses benzo(a)pyrene (BaP) as the index chemical (i.e., having a relative potency of 1.0) and includes TE (toxicity equivalent) values for seven carcinogenic PAHs (US EPA., 1993). These PAHs include benzo(a)anthracene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, indeno(1,2,3-cd) pyrene and TE for those PAHs are 0.1, 0.1, 0.1, 1, 0.01, 1.0 and 0.1 respectively. Therefore, to assess PAHs' total toxicity, the total $B(a)P_{eqv}$ was calculated as total equivalent concentration as benzo(a)pyrene), using the toxicity equivalent (TE) for each PAH according to the following equation:

$$\text{Total } B(a)P_{eqv} = \Sigma i \; Ci \; X \; TEi \; (\mu g/kg \; ww)$$

where:

Ci – concentration of the respective PAHs (µg/kg ww);
TEi – the toxicity equivalent of the corresponding PAHs

Benzo[*a*]pyrene equivalents can be used to assess the combined risk to humans from consuming mussels containing PAHs. Data from risk based consumption limits models suggest that for an acceptable risk of 10^{-5}, benzo[*a*]pyrene equivalents values of < 10 µg /kg wet weight would permit consumption of up to two 114 g meals per month (McIntosh et al., 2004; Webster L. et al., 2008.)

The calculated total $B(a)P_{eqv}$ at sampling stations was within the range 2.4 to 19.9 (µg/kg ww). Seven bivalve samples had $B(a)P_{eqv}$ values < 10 µg /kg wet weight, but one sample of *Mytilus galloprovincialis* (19.9 µg /kg ww) and two of *Rapana venosa* (11.7and 15.8 µg /kg ww) have showed concentrations with unacceptable risk (Fig. VI.11 and VI.12).

According to European Regulation 208/2005/EC, the total benzo[*a*]pyrene equivalents and the total PAH concentrations the biota from the Western Black Sea, including the Romanian, Bulgarian and Turkish waters in July 2013 can be described as:

- benzo (a) pyrene - B [a] P present in all samples exceed the maximum allowable concentrations established by European Regulation no. 208/2005 (10 µg/kg wet weight) in 10% of bivalves

- the total benzo[*a*]pyrene equivalents showed risk of adverse health effects in 30% of samples.

- dominants compounds in biota were represented by a low molecular weight PAHs with 2-3 aromatic rings (phenanthrene and naphthalene), characteristic for petroleum; 40% of samples indicate a low-level chronic exposure to PAH contaminants ($\sum_{16}$PAHs < 250 µg/kg wet weight)

EU legislation doesn't refer to OCPs. The Commission Regulation (EC) no. 1259/2011 sets a maximum concentration of 75 ng/g wet weight for sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 in muscle meat of fish and fishery products and products thereof. None of the samples exceeded the regulated level for PCBs (Table VI.8).

For the time being, there are no recommended Environmental Assessment Concentrations (EACs) for metals in fish and shellfish, with respect to descriptor 8. The alternative approach proposed by Webster et al. (2008), which have been adopted by OSPAR (2009), is to assess the contaminant concentrations in marine biota with respect to their human health risk. The Commission Regulation (EC) no. 1881/2006 (and subsequent additions and amendments) sets maximum concentrations for contaminants in foodstuffs to protect public health, i.e. to ensure that contaminant concentrations are toxicologically acceptable. This regulation includes maximum levels for Pb, Hg and Cd in bivalve molluscs and fish muscle, and these are the values that can be selected for the assessment. It is recognized that this approach is not fully satisfactory in the context of an assessment addressing environmental risk, but their use an interim solution for addressing the need for criteria until a more appropriate approach and values can be define and agreed (OSPAR, 2009).

Thus, in comparison with EC regulatory value for cadmium in bivalve molluscs (1 µg/g ww), all *Mytilus* samples were below the limit, whereas *Scapharca* and *Rapana* from all transects presented higher bioaccumulation level. We should mention that *Rapana* was also analysed as whole soft tissue, i.e. including viscera, where metals have the tendency to accumulate. In case of lead, all three species of molluscs were much below regulatory value (1.5 µg/g ww) (Fig. VI.13).

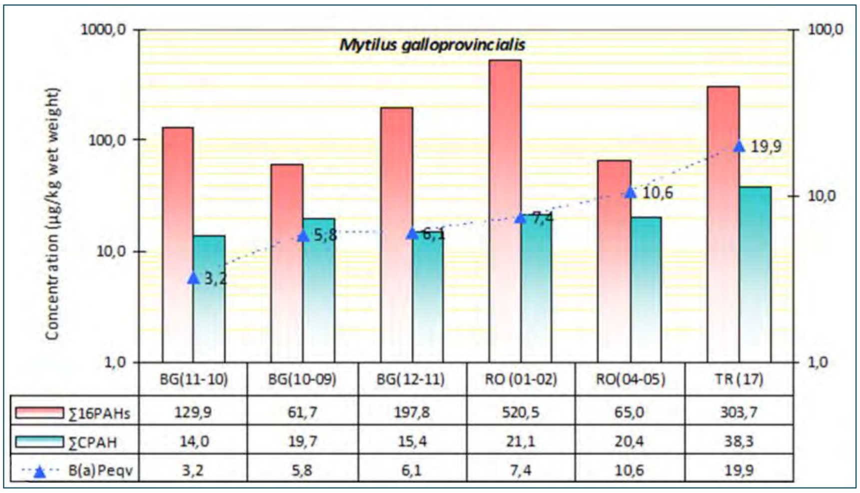


Figure VI.11. Levels of *Mytilus galloprovincialis* pollutions from the Romanian, Bulgarian and Turkish waters, the sum of 16 PAHs, by 7 carcinogenic PAHs and total B(a)Peqv (µg/kg ww), July 2013.

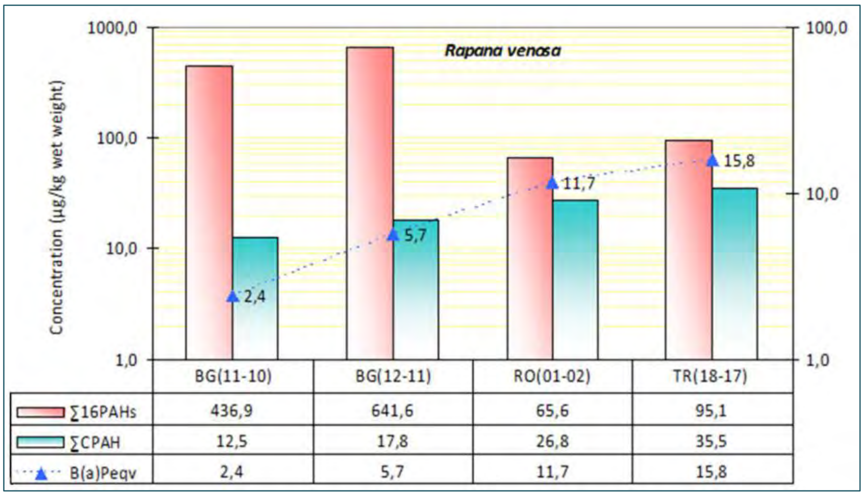


Figure VI.12. Levels of *Rapana venosa* pollutions from the Romanian, Bulgarian and Turkish waters, the sum of 16 PAHs, by 7 carcinogenic PAHs and total B(a)Peqv (µg/kg ww), July 2013.

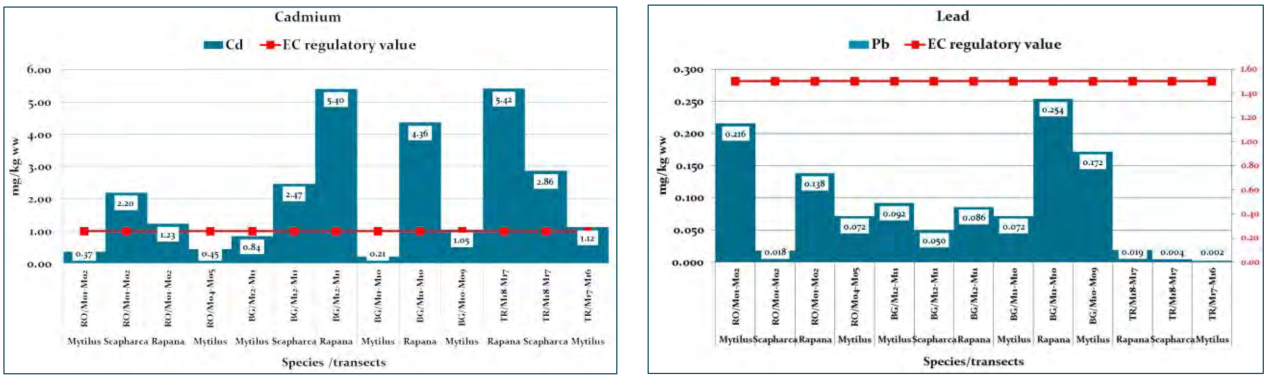


Figure VI.13. Cadmium and lead concentrations in molluscs from the Romanian, Bulgarian and Turkish waters, July 2013, in comparison with EC regulatory values.

CONCLUSIONS

The polycyclic aromatic hydrocarbon source for the three mollusc species (*Mytilus galloprovincialis, Rapana venosa and Scapharca inequivalvis)* may be mostly an acute petroleum exposure.

Benzo[*a*]pyrene, total equivalent of toxicity by benzo(a) pyrene concentrations and total content of PAHs not present a risk of adverse health effects for 40 % of samples (*Mytilus galloprovincialis)*, the remainder of 60% indicate a significant carcinogenic health risks associated with the consumption of these bivalves.

In the absence of maximum admissible levels for OCPs in fish and other seafood for human consumption and EACs values proposed for OCPs it's hard to assume the ecological status from this point of view.

None of the samples exceeded the regulated level for PCBs, so there is no risk for human health in respect with this class of compounds. The occasional PCB levels which exceed EACs may not pose a significant risk for the ecosystem.

Since for the moment there are no recommended Environmental Assessment Concentrations (EACs) for metals in fish and shellfish, the contaminant concentrations were assessed with respect to their human health risk. Thus, in comparison with EC regulatory values for cadmium and lead, *Mytilus galloprovincialis* samples were below the maximum admissible limits.

GAPS and RECOMMENDATIONS

There are no threshold values established as EACs for some contaminants like OCPs and heavy metals. On the other hand, adoption of OSPAR environmental assessment concentrations is not fully satisfactory, as there are different oceanographic conditions in the OSPAR and Black Sea region and different contaminant inputs. Adaptation to such conditions may result in different responses of species to contaminants. In respect with these considerations, Black Sea countries should introduce an ecotoxicology monitoring programme, which will allow integration of chemical and biological effects measurements. Combination of biological effects and chemical measurements will provide an improved assessment due to the ability to address effects that are potentially caused by a wide range of contaminants as well as those that are more clearly linked to specific compounds or groups of compounds. For this purpose, it is necessary to train the stuff in respect with analytical and biological assessment methodologies that allow for the detection of anthropogenically induced changes to individual compartments of marine ecosystem.

As a result of MISIS cruise, but also at Black Sea level, there is few data on contaminants in fish and other seafood for human consumption. To assess the ecological status in respect to Descriptor 9, (especially, *9.1.2 Frequency of regulatory levels being exceeded)*, it is necessary to develop a proper database for contaminants levels in fish and other seafood for human consumption. On the other hand, EU legislation doesn't refer to OCPs as regard to maximum admissible levels in fish and other seafood for human consumption. To assess the ecological status in respect to Descriptor 9, the Black Sea countries must refer to national legislation. It is necessary to harmonize legislation at EU level.

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VII. DESCRIPTOR 10: Marine litter

Quantification in the Black Sea - a pilot assessment

Completed by:
Snejana Moncheva, Kremena Stefanova, Anton Krastev, Anatoli Apostolov

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List of figures and tables

INTRODUCTION

According to "Marine Litter (ML) analytical overview" (UNEP, 2005) about 6.4 million tons of debris are disposed in the ocean each year, weighing about the same as a million elephants, some 8 million items are dumped every day, approximately 5 million of which (solid waste) are thrown overboard or lost from ships, over 13 000 pieces of plastic litter are floating on every km^2. The annual International Coastal Clean-up (ICC) 10 program found out the importance of land-based sources, including shoreline-recreational activities altogether accounting for around 90 % of marine litter (UNEP, 2009). Various programs and organizations (IMO, UNEP, IOC-UNESCO, FAO) and recently the EU MSFD (Descriptor 10) recognized marine litter as an issue of global threat from environmental, economic, human health and safety, and aesthetic aspect (STAP 2011). At the same time Commission Staff Briefing Paper (EC SWD(2012) 365) concluded "a lack of knowledge on the amounts, sources pathways and distribution trends and impacts of marine litter, due to limited systematic regional measurements". In the proposal for a new "General Union Environment Action Programme to 2020: Living well, within the limits of our planet" the Commission states that in order to protect, conserve and enhance the EU's natural capital, the programme shall ensure that by 2020 "an EU-wide quantitative reduction target for marine litter is established". Studies of ML in the Black Sea region are very scarce and fragmented, limited to the Regional Activity on Marine Litter, supported by UNEP, launched in 2005, UNEP Report (2009) "Marine Litter: A Global Challenge" and recent NGO campaigns focusing mainly on beach/shoreline assessments. The UNEP Reports "Marine Litter in the Black Sea Region: A Review of the Problem" and a "Draft Strategic Action Plan for Management and Abatement of Marine Litter in the Black Sea Region" (BSC, 2009) evaluated existing data, policies, activities, and institutional arrangements and proposed several actions to deal with the problem, which were included in the BS SAP 2009. According to UNEP (2009) vessel-based transect surveys estimated between 6.6 and 65.7 items/km^2 of floating plastic litter, and beach surveys along the Turkish Black Sea coast recorded between 58 and 1395 kg litter per km. By material glass was the most abundant (31 %), followed by plastic (25 %) and metal (21 %), while data from the beaches of Crimea, Ukraine indicated a predominance of plastics (80 – 98%). Some reports (BSC 2009, UNEP 2009, Topcu et al. 2012) identified municipal waste/sewage and landfills as the most important sources of ML, along with marine transport, ports and recreational activities, while ARCADIS Report (2013) top-ranked recreational and tourism activities (recreational fishing contributing to 45 %), considering shipping/ports to represent only a minor source (8 %). Illegal, unreported and unregulated (IUU) fishing in the Black and Azov Seas is also considered an important source of ML due to discarded and abandoned nets (“ghost” fishing), although comprehensive specific studies are very limited (Birkun, 2002, Radu et al., 2003).

Thus, there is an urgent need for elaboration of strategy for assessment and monitoring of ML in the Black Sea and to develop a set of indicators to be included in the Black Sea Integrated Monitoring and Assessment Program (BSIMAP) 2013 – 2018. Among the different issues application of harmonized methodological approaches for quantification of ML is of crucial importance. In line with the objectives of FP7 Project MISIS (Contract 07.020400/2012/616044/SUB/D2) to provide “MSFD Guiding Improvements in the Black Sea Integrated Monitoring System” a joint survey was conducted in August 2013 in the North Western Black Sea. Investigation of seabed ML was one of the tasks among the multidisciplinary measurements in the cruise program.

The aim of the present study is a pilot quantitative assessment of bottom ML in the Black Sea coastal and shelf areas and testing the applicability of Remote Operational Vehicle (ROV) available at IO-BAS, applying the methodological MSFD GES Guidance (2013) elaborated by the Task Scientific Group on ML.

MATERIAL and METHODS

Study area

The pilot survey of bottom ML during the MISIS Project Joint Black Sea Cruise (22 – 31 July, 2013) was conducted along 3 transects in the North-Western Black Sea (Romania, Bulgaria and Turkey) at 6 polygons (Fig. VII.1, Table VII.1).

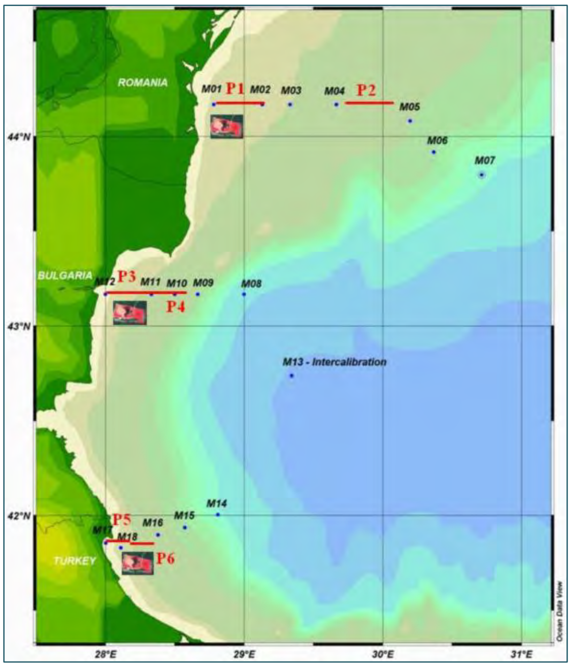

Figure VII.1. **Map of sampling polygons.**

Table VII.1. **Coordinates of sampling polygons and trawled area.**

Polygon	Depth [m]	Start		End		Dredged area [m^2]
		Latitude	Longitude	Latitude	Longitude	
P1-RO	33.0	44°10.125'N	028°48.973'E	44°10.121'N	028°49.349'E	1250
P2-RO	65.0	44°10.103'N	029°40.399'E	44°10.101'N	029°42.918'E	4600
P3-BG	24.0	43°09.999'N	028°10.026'E	43°10.074'N	028°18.771'E	7925
P4-BG	69.0	43°08.790'N	028°27.147'E	43°09.066'N	028°27.110'E	1280
P5-TR	38.0	41°50.763'N	028°01.808'E	41°50.234'N	028°02.191'E	3550
P6-TR	67.0	41°52.495'N	028°07.455'E	44°10.121'N	028°49.349'E	1250

The sites were selected to ensure that they comprise areas with uniform substrate, representative for generating/accumulating litter as recommended by UNEP (Cheshire et al., 2009).

Methods

The methodology was in compliance to MSFD GES TSG-ML Monitoring Guidance (2013) for large scale evaluation and monitoring of sea-floor ML. ML was collected by towing a beam trawl (2.5 m width and mesh size aperture 5 cm) at a speed of 2.5 – 3 knots. The hauls were positioned following a depth stratified scheme in the coastal (23 – 35 m) and shelf bottom (depths within 40 – 69 m) (Table VII.1). Collected items were measured on board, sorted by type of the material and size and the results integrated for each polygon (from 3 – 4 tows) per km^2, corresponding to the classification system in the Monitoring Guidance (2013). The trawled area at each polygon is presented in Table VII.1.

In addition to trawling, in the coastal bed (depth of ~ 40 m), the ROV (Remote Operating Vehicle) “Diablo” (Mariscope) was deployed in order to test its applicability to quantify marine litter (full details of the vehicle are provided below).

ROV Specification:

Model: .... Mariscope FO 200
Length: .... 1300 mm
Weight: .... 65 kg
Highest horizontal velocity: .... 4 kn
Operating horizontal velocity: .... 2 kn
Maximal depth: .... 300 m
Maximal operating depth: .... 150 m
Image resolution: .... 500 × 582 px
Camera resolution: .... 330 lines
Camera focus: .... 3.5 mm
Camera horizontal viewing angle: .... 52° (in water)
Navigation: .... sonar, compass

RESULTS and DISCUSSIONS

Trawl survey

Polygons surveyed area ranged from 1250 to 7925 m^2, covering a total of 19 855 m^2 (~ 0.02 km^{-2}). The abundance and distribution of ML showed considerable spatial variability. Marine debris were found on all transects with densities ranging from 304 to 20 000 items/km^2 in average 6359 items/km^2 (SE = 2015). The number of items decreased from north to south with maximum in front of Romanian coast (Fig. VII.2), and were approximately 3 times less in front of Bulgaria (9598 items/km^2) and Turkey (7956 items/km^2). In coastal areas (< 40 m depth), the abundance of ML was generally much higher than on the continental shelf. In the three coastal polygons, activities related to fishing and tourism significantly contribute to littering of the seafloor with notable temporal, particularly seasonal, variations. The marine debris at the coastal area (9234 items/km^2) exceeded about two times shelf density (5603 items/km^2) with exception of the observed area in front of Bulgaria (Fig. VII.2).

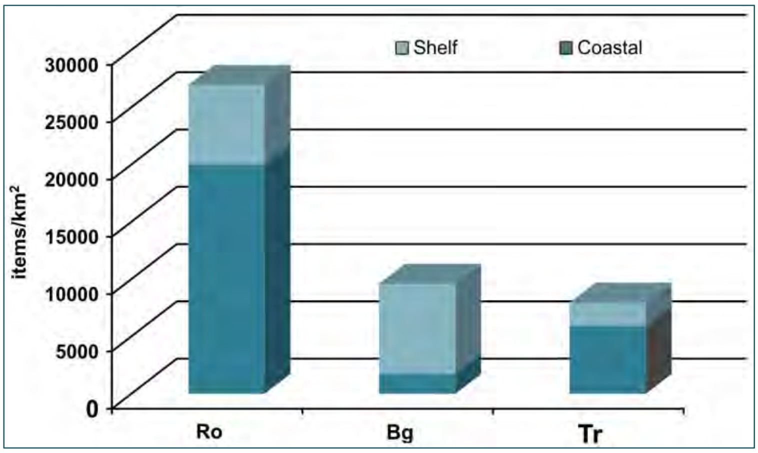

Figure VII.2. Coastal–shelf distribution of marine litter integrated by transects in the NW Black Sea.

Among the categories by material the most frequent and abundant debris was plastic constituting 68 % (Fig. VII.3). Variety of marine litter was accumulated in P2 (natural products – 50 %, metals – 9 %, miscellaneous – 16 %), while metal accounted for more than 90 % in Polygon 1 (Fig. VII.3). The nature of the ML suggests mainly shipping/fishing origin (Fig. VII.4).

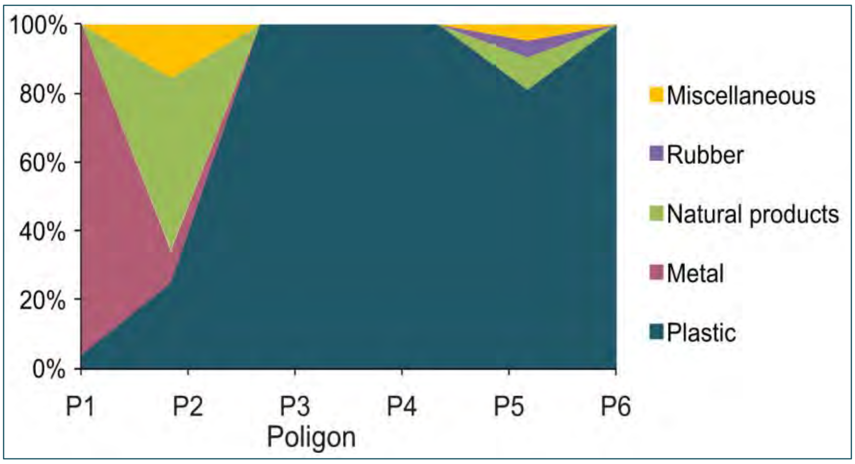

Figure VII.3. Composition of marine debris found on sea-floor (24 – 69 m) surveys off Romania-Bulgaria and Turkey by polygons (P).

Figure VII.4. Litter sorting.

“B” size (100 cm^2) fragments of marine debris were the most common size fraction (67 %) found in the study area. Plastics dominated in all categories between A-D, while the large (E) size class was represented by metal (Fig. VII.5).

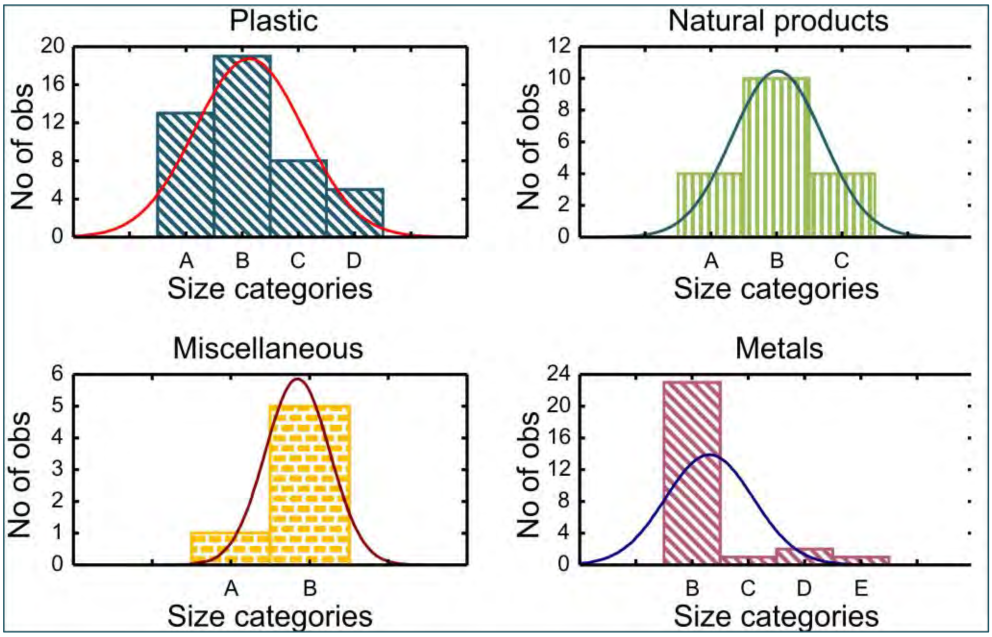

Figure VII.5. Histograms of ML size distribution by material A: < 5 * 5 cm = 25 cm2, B: < 10 * 10 cm = 100 cm2, C: < 20 * 20 cm = 400 cm2, D: < 50 * 50 cm = 2500 cm2, E: < 100 * 100 cm = 10 000 cm2 = 1 m2, F: > 100 * 100 cm = 10 000 cm2= 1 m2.

Remote Operating Vehicle (ROV) test

As one of the critical elements in application of ROV is the adequacy of observation regarding the size of the object the following equation was theoretically derived:

$$l = k_h \times l_h \times h \times tg\left(\frac{\pi}{2} - \alpha + k_v \times l_v\right) \quad (1)$$

where:
l – dimensions of an object on a horizontal surface detected on a ROV camera image [units of h];
k_h – image horizontal angular coefficient (0.0091 rad/%);
l_h – horizontal object partition in the image [% of image width];
h – vertical elevation of the camera from the horizontal surface;
α – angle of camera tilt [rad];
k_v – image vertical angular coefficient (0.0067 rad/%);
l_v – object vertical distance from the image centre [% of image height].
The equation was calibrated over standard objects and tested in working conditions: depth 23 m, at horizontal visibility 4 m and underwater current – 0.5 – 1 kn (S).

The rate of bottom area surveyed was estimated to be 280 sq.m per minute. Five test objects were selected as representative (Fig. VII.6): a grab trace with known size – width 36 cm (used for formula calibration), a pile of sea grass with calculated length of 23 cm, a goby fish with calculated length of 17 cm, a *Rapana* snail with calculated length of 12 cm and a metal frame with calculated width of 8 cm. Vertical proportions marked on Fig. 5 are used as l_v and horizontal ones – as l_h in equation (1). The angle of the camera tilt – α, and the vertical elevation – h is controlled by the operator. The other two parameters – k_h and v_h, are derived by the camera specifications and tested practically.

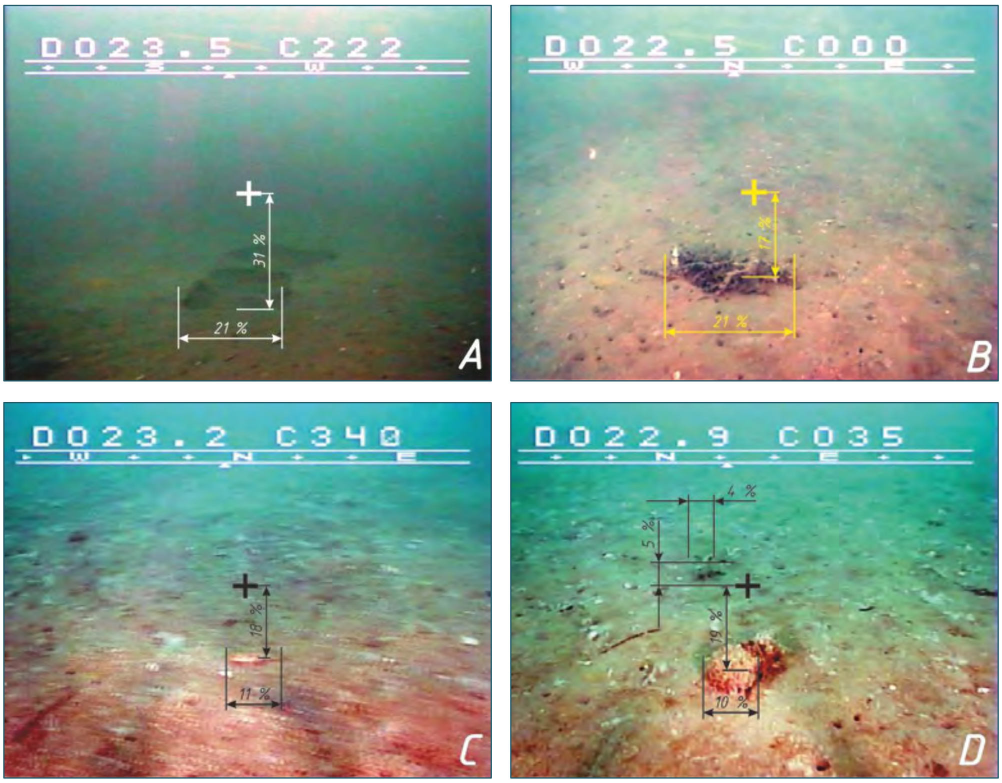

Figure VII.6. A. Grab trace (lights are off in order to eliminate turbidity); B. Pile of sea grass; C. Goby; D. *Rapana* snail (in front) and a metal frame.

Limitations of the ROV application

The image resolution is sufficient to eliminate the interference on the measurement error, thus the principal limitation factor becomes object identification. The method is efficient at object size over 5 cm, water current less than 1 kn, depth less than 100 m at optimal carrying vessel tonnage 10 BRT.

ROV survey pros

There is a variety of ROV survey advantages. First, the ROV operational time is virtually unlimited. Next, the deep water surveys (under air-breathing diving limit of 50 m) are possible. The operational cost is low relative to other methods such as diving surveys. The direct operating control allows detailed observations and prolonged expositions if necessary which is not possible using a dragged sledge. The method is not destructive to the observed objects and the sea bottom environment. Also, although ROV survey is a visual method, it is possible to estimate objects dimensions.

ROV survey cons

Due to its specifics ROV surveying has its setbacks. First, its maintenance is expensive, complex and delicate. Next, ROV is power source dependent and non-autonomous. It requires a complex infrastructure of control devices and a qualified operator. Distance limitation due to the cable connection prevents surveying large areas continuously. ROV operation is strongly dependent of water currents and is applicable in relatively still conditions only. And last, as a visual method ROV surveying is turbidity dependent.

Discussion

Marine litter originates from various land- and sea-based sources. It is a complex and challenging problem, which stems from the prevailing production and consumption patterns and severe impacts of human activities superimposed on the natural complexity of the marine environment on the one hand and the adequacy and efficiency of management policies on the other. Escalating concern about marine debris has been driven by the rapid and widespread accumulation of persistent plastics over the last several decades, representing between 75 % – 83 % of all items found globally and in Europe, while all other categories (textiles, paper, metal, wood) constitute the remaining 25 – 17 % (Barnes et al., 2009; Gregory, 2009; Moore, 2008, UNEP/MAP, 2009). Plastic was the most common debris material found in our study (68 %) which is quite in line with the global findings. The average quantity of ML found in the NW Black Sea during this survey (6359 ± 2015 items/km^2) was an order of magnitude higher than the amount (126 ± 82 items/km^2) reported for example for the western Baltic Sea (Galgani et al. 2000), although significant ambiguity can be introduced due to differences in methods used for sampling and analysis. The marine debris at the coastal area were found to exceed about two times shelf density related most likely to the proximity to land-based sources, active human activities in the coastal marine domain and accumulation on the bottom due to weaker currents (Barnes et al. 2009; Katsanevakis, 2009; Katsanevakis & Katsarou, 2004). The high ML density observed in the Bulgarian shelf polygon could be associated to the intensive fishing and shipping in this particular area (IAR-BG, 2013). In addition Barnes et al. (2009) discussed existing accumulation zones offshore with very high debris densities despite being far from coasts, related to a number of factors such as prevailing wind, currents, eddy circulation patterns and convergence zone of seabed sediment movements. Thus natural dispersal of floating and suspended ML by wind and sea currents represents a transboundary problem that needs basin scale concerted management strategies of different sectoral activities.

Solid waste management is one of the major environmental problems in the Black Sea region (Celik, 2002) and is a likely source of marine litter especially in the shallow areas. Berkun et al. (2005) reported municipal and industrial solid wastes are dumped on nearby lowlands or directly to the sea on the southern coast of the Black Sea. Although no special research on abandoned nets has been conducted in the Black Sea the few literature sources are more than alarming for 'ghost' fishing as a serious hazard for cetaceans, not mentioning the economic loss for fishery, and the environmental costs. Thus for example 194 dead dolphins and harbour porpoises (*Phocoena phocoena*), 18 424 turbot (*Psetta maeotica*), 143 sturgeon (*Acipenser* spp.), 401 spiny dogfish (*Squalus acanthias*) and 1359 rays (*Raja clavata* and *Dasyatis pastinaca*) were found entangled in bottom-set gillnets in Ukrainian waters in a survey conducted in 1991 (Birkun, 2002). Radu et al. (2003) documented 35 harbour porpoises as by-catch in the abandoned illegal gill and trammel nets in the Romanian Exclusive Economic Zone in 2002. Tonay et al (2010) reported 294 *P. phocoena* stranded individuals in the period 2007 – 2009 and suggested by-catch and illegal turbot fishing as the most likely cause.

The ML management is further complicated by the lack of consistent monitoring and data at regional and global scale.

CONCLUSIONS

Even if limited in time and spatial coverage the results represent a pilot effort for quantitative estimates of ML in the Black Sea bottom. The test of ROV offer useful practical information which altogether can be used to refine the design of monitoring programmes and suggest options for "statistical power introducing criteria". The study implies the TSG-ML Guidelines methodology for collection, classification and quantification of litter, data integration, analysis and reporting as a tool for ML assessment in a harmonised way.

However an accurate and meaningful estimation of ML distribution and density could be achieved only in the context of a broader regional management framework ensuring a large-scale integrated monitoring across countries and environments (beaches, water column and sea floor) complemented by adequate understanding of the hydrodynamic features and bottomscape of the marine environment. To this end monitoring programmes for demersal fish stock assessment combined with on-going monitoring of benthic communities if using an harmonized protocol may provide consistent support for monitoring litter at the Black Sea basin-wide scale on a regular basis and within the MSFD framework at relatively low cost. The value of ML quantification can be empowered only by the identification of the main sources of pollution and further research on the potential harm on biota and the marine environment to enable a more target-orientated implementation of measures.

GAPS

- Scarce and fragmented investigations of ML in the Black Sea region, limited to the Regional Activity on Marine Litter (BSC, 2009, UNEP, 2009) and campaigns focusing mainly on beach/shoreline assessments.
- Information on sources of marine litter.

RECOMMENDATIONS

- Improve other components of litter monitoring (microplastic, effects of litter on biota etc.).
- Estimation of ML distribution and density to be considered in the context of a broader regional management framework ensuring a large-scale integrated monitoring across countries and environments (beaches, water column and sea floor) complemented by adequate understanding of the hydrodynamic and sea bottom features of the marine environment.
- Monitoring programmes for demersal fish stock assessment combined with on-going monitoring of benthic communities if using a harmonized protocol may provide consistent support for monitoring litter at the Black Sea basin-wide scale on a regular basis and within the MSFD framework.
- The value of ML quantification can be empowered only by the identification of the main sources of pollution and further research on the potential harm on biota and the marine environment to enable a more target-orientated implementation of measures.

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VIII. GAPS and RECOMMENDATIONS

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GAPS and RECOMMENDATIONS of MISIS to support MSFD implementation at regional/subregional levels

The analysis of gap and recommendations were prepared by a number of Black Sea experts who have implemented MISIS basing on their long lasting experience in the region and as well using the findings of the MISIS joint survey and the stemmed SoER produced for the MISIS area (BG, RO and TR waters of the Black Sea).

In this study, some of the GES descriptors were considered. They are water column habitats in relation to D1 and D2, sea bottom habitats in relation to D1, D6 and D2, eutrophication for D5, contaminants and effects for D8 and marine litter for D10.

1. Analysis of specific gaps & recommendations given by the experts for certain descriptors considered in MISIS

Descriptor 1 - Water column habitats

Phytoplankton and zooplankton communities were studied during the joint MISIS cruise as well as nutrients. An intercalibration exercise on phytoplankton was organised and all findings were discussed in the SoER.

The results of the intercalibration exercise call for more frequent ring tests and intercalibration campaigns in order to improve the comparability of generated data as an important prerequisite for adequate ecological state assessment at basin-wide scale.
Another general and key recommendation would be the integration of pressure data (fluxes of nutrients from various sources including non-point sources) and relevant meteorological information that is crucial for the adequacy of the assessments.

Below specific gaps and recommendations raised with this study are presented.

	GAPS	RECOMMENDATIONS
1	In the context of global climate change it is now recognized that a partition of the marine autotrophic pool into a suite of phytoplankton functional types, would increase our understanding of the role of phytoplankton in the global carbon cycle and biogeochemistry imposing the need of further research including experimental studies, in order to fill in the gaps in our knowledge.	The results give ground to suggest that as phytoplankton functional types (PFTs) are relevant proxies of ecosystem functioning, incorporation of PFTs into the monitoring programs and biogeochemical models may improve our predictive capabilities and the capacity for a better ecosystem management.
2	There is a lack of adequate phytoplankton time-spatial monitoring resulting in insufficient knowledge of its natural variability and the associated key drivers and pressures.	Infrastructure improvements and effective introduction of less applied approaches such as remote sensing and Continuous Plankton Recorders should be considered as overarching and critical issues for implementation of the MSFD.

		Satellite-borne remote sensing is crucial in providing the necessary spatio-temporal scales of observation required to proper measure and understand phytoplankton time-spatial patterns of distribution and variability and overcome scale mismatches, options that should be more efficiently exploited and employed in the Black Sea regional monitoring. However the importance of *in situ* data could not be ignored as they may be essential for validation and thus the advance of the new emerging technologies.
3	There is insufficient knowledge of the capacity of harmful algae to produce toxins. Lack of official statistics on the phycotoxin poisonings and targeted research and monitoring of potentially toxic species.	Although limited to a single survey only, the data on potentially toxic phytoplankton species in the Black Sea demonstrate the need for establishing a system of monitoring as the basis of environmental safety control of biological resources. Poor identification of potentially toxic species, poor knowledge of the toxicity of species and lack of standards for the density of toxic species allowed should also be considered.
4	Despite the Black Sea regional initiatives such as Black Sea GOOS (ARGO floats and other drifters in the Black Sea), the bio-ecological operational oceanography is still poorly developed and the results inefficiently explored.	
5	There is a lack of advanced genetic and genomic methodologies for taxonomic revisions of organisms including phytoplankton.	
6	Lack of an integrated monitoring strategy with relevant frequency and parameters of pelagic system and the pressures; irregular data.	Regular monitoring of zooplankton community with relevant frequency (seasonal), including all components of plankton fauna – micro-, meso- and macrozooplankton. Sustain monitoring of transects with long-terms observations.
7	Lack of reliable indicators of zooplankton along with the validation of proposed reference levels and thresholds.	Harmonize analytical techniques, indicators/reporting formats and assessment methodologies at regional level.
8	Quality control and assurance.	Further development of guidelines of identification and application of inter-comparison exercises in taxonomy.

Descriptor 1 and 6 - Sea bottom (benthic) habitats

	GAPS	RECOMMENDATIONS
1	Benthic habitats data is scarce and heterogeneous. Needs for habitats monitoring programs with standardized spatial and temporal data.	Organization of habitats monitoring programmes; highly recommended for Turkey.
2	Further development of MSFD relevant benthic indicators. Lack of reference/baseline conditions. Need for common determination of thresholds.	The integration of all assessment levels (habitat, different eco-system components) into a single score indicating status and performance of an ecosystem is another major challenge, especially in the case of the MSFD.
3	Comparability of data.	Inter-calibration of the taxonomic expertise, development of human capacity and the mobility of experts among the institutes in order to achieve comparable results between the countries in the Black Sea region.

Descriptor 2 - Non-native species

D2 is monitored mostly in relation to biodiversity components considered in D1 where the associated gaps and recommendations are mostly valid for the monitoring of non-native species too.

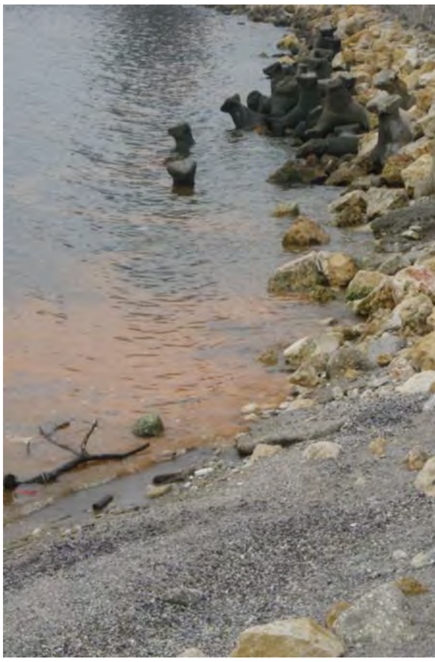
Still specific gaps exist such as the lack of specific monitoring strategy for NIS with relevant frequency, the regular monitoring of abundance, biomass, temporal occurrence and the impact of already established non-native species and the need of development of appropriate indicators for benthic community.

Descriptor 5 - Human induced eutrophication

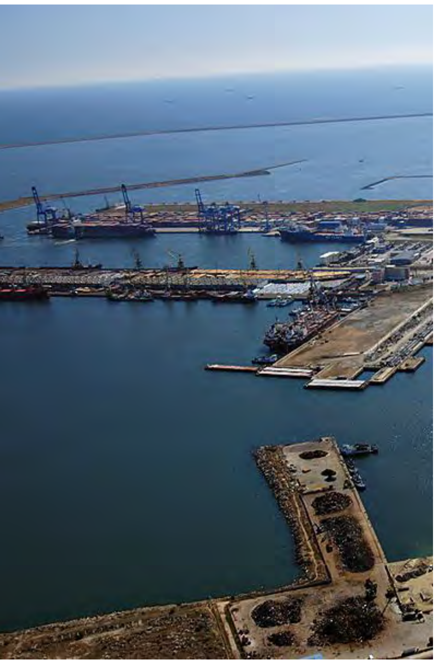
The assessment made in MISIS was based only on one cruise (summer) which does not allow us to make a real assessment of the eutrophication state.

Below general recommendations for eutrophication assessment at the (sub) regional level (Western Black Sea) are provided.

	GAPS	RECOMMENDATIONS
1.	Lack of coordination of monitoring efforts between organizations involved (at national and sub-regional scale).	Better coordination of monitoring efforts either at national or sub-regional scale through avoiding overlappings, infrastructure sharing, adjust the schedule of cruises so that the different cruises covering the sub-region to be planned in the same period of the year, etc. This type of coordination has to be recognized by the Ministries of each country
2.	No/few data on marine waters (open sea).	Systematic use of additional tools such as remote sensing of surface chlorophyll, ferry boxes, and smart buoys is recommended if data are validated with in-situ data. Develop reliable algorithms (more research needed related the CDOM (Colored dissolved organic matter), seawater optical properties, etc) for satellite derived Chl *a* in the shelf waters.
3.	No/few data on atmospheric deposition of nutrients.	Monitoring of atmospheric deposition of nutrients. Coupled atmosphere-river-coastal sea models need to be developed at the regional scale for the estimation of the source of critical nutrient loads and the relative importance of terrestrial sources compare to transitional/ coastal retention.
4.	No info on the threshold between natural variability (including climate change) and anthropogenic impact which is a must in the descriptor 5.	Research on natural background nutrient enrichment (e.g. import by upwelling; import from pristine/ good status rivers) for determination of unimpacted state and separation of naturally productive status from anthropogenically eutrophic status; climate change impacts on availability and transformation of nutrients and organic matter from land to the sea.
5.	The link to land-based inputs is not well established.	Identification of critical nutrient loading thresholds beyond which the whole system is changing into an alternative steady state;
6.	No indicators/parameters considered for assessing the impact of human induced eutrophication on the vertical distribution of nutrients, DO, chlorophyll a, etc.	More research are needed for developing indicators/parameters that considered the effects of eutrophication on nutrients, DO, chlorophyll distribution within the water column (with special emphasis on open waters). Data/information from literature, past and recent cruises should be taken into consideration for assessing the temporal variability of the position and magnitude of suboxic layer, nutricline, DCM (Deep Clorophyll maximum) etc.
7.	Need to distinguish between natural range and increase of spatial extension of anoxic sediments due to anthropogenic organic loading.	Research on factors that govern the occurrence and extension of hypoxic/ anoxic sediment surface. Additional continuous monitoring tools (benthic observatories, etc – see HYPOX) to be used for hypoxic/anoxic events study (and factors governing) in the sensitive areas.



8.	No assessment tools that account for shifts in species composition and frequency of blooms.	Development of phytoplankton assessment tools that account for shifts in species composition and frequency of blooms. Development of monitoring tools that account for rapid changes in algal communities, allowing detection of bloom peaks (continuous measurements, ships-of-opportunity, remote sensing tools, algorithm development, real-time monitoring, etc.).
9.	Lack of well tested integrated assessment tools.	BEAST (or other tool) must be robust, integrated, sufficiently sensitive, comparable, and with recognized scientific merit.
10.	Need for Quality Assurance/Control guidelines for the descriptor - an essential requirement for successful monitoring, allowing for appropriate intercalibration and comparative assessment.	The procedures aim to ensure that monitoring results meet the required levels of precision and confidence. Those procedures can take the form of standardizing sampling and analytical methods, replicate analyses and laboratory testing schemes.



Descriptor 8. Contaminants and effects

The purpose of assessments under Descriptor 8 is to determine whether this aspect of GES is being achieved within assessment region. The approach involved (measurements of contaminant concentrations and effects, followed by comparisons against targets) needs additional research, for a better understanding of the underlying fundamental principles of pollution effects and for the further development of monitoring approaches (JRC, 2010).

MSFD GES target setting implies understanding of the processes affecting contaminant cycling and availability, the responses of marine organisms to contaminants, the identification of sources and the availability of appropriate monitoring tools. Scientific knowledge of the functional relationships between pressures and impacts, and the consequent responses contains significant gaps. The implementation of measures to ensure the GES requires a combination of several assessment tools which need to be developed.

A number of gaps in knowledge were identified, as well as issues that need addressing in various research areas, as follows:

	GAPS	RECOMMENDATIONS
1.	The mixtures and their combined effect on organisms and the ecosystem are unknown.	Need to establish integrated research for understanding of causal relationships and of processes between contaminants and their effects on biota and to quantify the effect and impact of contaminants at population level and higher levels of biological organization.
2.	Ecotoxicology monitoring programme.	Develop monitoring activities beyond the detection of pollution levels only.
3.	Little is known on the relationship between the mechanisms of entry of pollutants (riverine, atmospheric, etc.) into marine waters and their availability and potential effects on organisms and ecosystems.	Research is needed on long time series data that relate pollutant exposure and cycling to effects to organisms and ecosystem functioning. Monitoring programme should allow the collection of the integrated data covering waterborne and atmospheric inputs, environmental concentrations and biological effects of hazardous substances
4.	Understanding the combined effects of climate change and pollution on organisms.	An improved understanding of these processes may lead to the need for a regular review of assessment criteria.
5.	The transfer of contaminants through the food chain needs to be better understood, and also the possibility of additive, synergistic and antagonistic effects.	Advance way of organizing new research projects
6.	A sound aggregation of information from monitoring.	Development of an integrated assessment tool for contaminants and biological effects at the national/regional levels.

Understanding of the ecosystem responses to pollution

Research could contribute to a better understanding of the relationship between pressures and their effects on the marine environment. Hazardous substances, especially synthetic chemicals, occur in the environment as mixtures. The mixtures and their combined effect on organisms and the ecosystem are currently unknown, but this should be subject to ongoing work and research for understanding of causal relationships and of processes between contaminants and their effects on biota and to quantify the impact of contaminants at the population level and higher levels of biological organization. Especially important is to assess the effects of complex mixtures of inorganic and organic pollutants upon organisms and ecosystems. For example, assessment of sediment toxicity based on the total concentration of potentially carcinogenic PAHs (CPAH) express as the benzo[a]pyrene toxicity equivalents.

Levels of pollution effects on the ecosystem components, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship is established, should be monitored. Implementing of biological effects techniques used in environmental health assessment, like assays for specific inhibition of enzymes, induction of proteins, pollutant metabolites, DNA microarrays, immunotoxicity, physiological responses and pathology, is needed.

Introducing an ecotoxicology monitoring programme will allow integration of chemical and biological effects measurements. Combination of biological effects and chemical measurements will provide an improved assessment due to the ability to address effects that are potentially caused by a wide range of contaminants as well as those effects that are more clearly linked to specific compounds or groups of compounds.

Linking sources, pathways and environmental status: biogeochemistry of substances

Little is known on the relationship between the mechanisms of entry of pollutants (riverine, atmospheric, etc.) into marine waters and their availability and potential effects on organisms and ecosystems.

Data for better quantification of contaminants fluxes and inputs into marine environment and their sea/air and water/sediments interfaces exchanges is lacking. Monitoring programmes would need to be designed to allow tracing back chemicals from the environment via their pathways to the sources in order to allow the appropriate development of programmes of measures to achieve good environmental status and assess progress being made. For instance, identification of possible sources of PAHs compounds, biogenic or anthropogenic (pyrolitic or petrogenic) based on their molecular indices (LMW/HMW).

Climate change

Warming of the atmosphere in response to climate change may increase the tendency for atmospheric transport of certain substances, more rain and floods can result in higher run-off from land and increased storminess may lead to additional remobilization of contaminants from marine sediments. Change in sea water temperature and other possible biological impacts of climate change add to the stress on organisms and coupled with pollution effects may make marine organisms more vulnerable to chemical contamination. An improved understanding of these processes may lead to the need for a regular review of assessment criteria.

Knowledge on the marine foodwebs with regard to contaminants

The transfer of contaminants through the food chain needs to be better understood, and also the possibility of additive, synergistic and antagonistic effects. The toxic effects of chemical contaminants on marine organisms are dependent on bioavailability and persistence, the ability of organisms to accumulate and metabolize contaminants, their interference with specific metabolic or ecological processes. Little is known about contaminant uptake in the first trophic levels (plankton), and how different biogeochemical statuses of marine ecosystems favour the bioaccumulation and cycling of contaminants.

Aggregation of information on substances

There is a multitude of chemicals (and effects of them) in the environment and methods for a sound aggregation of information from monitoring should be addressed in an integrated assessment tool for contaminants and biological effects.

Descriptor 10 / Marine litter

	GAPS	RECOMMENDATIONS
1	Scarce and fragmented investigations of ML in the Black Sea region, limited to the Regional Activity on Marine Litter (BSC, 2009, UNEP, 2009) and campaigns focusing mainly on beach/shoreline assessments.	Coordinated regional cooperation. Improve other components of litter monitoring (microplastic, effects of litter on biota etc.). Estimation of ML distribution and density to be considered in the context of a broader regional management framework ensuring a large-scale integrated monitoring across countries and environments (beaches, water column and sea floor) complemented by adequate understanding of the hydrodynamic and sea bottom features of the marine environment. Monitoring programmes for demersal fish stock assessment combined with on-going monitoring of benthic communities if using a harmonized protocol may provide consistent support for monitoring litter at the Black Sea basin-wide scale on a regular basis and within the MSFD framework.
2	Information on sources of marine litter.	The value of ML quantification can be empowered only by the identification of the main sources of pollution and further research on the potential harm on biota and the marine environment to enable a more target-orientated implementation of measures.

2. The analysis of gaps & recommendations for overall implementation needs

Besides the specific issues raised for each descriptor practiced in MISIS, some key and common (crosscutting) needs are summarized below:

Expansion of COVERAGE of monitoring programmes:

Re-scaling of monitoring programmes which is critical especially for water column habitats is required. This might be in general assumed as increasing the sampling frequencies and the spatial coverage (Higher resolutions in time and space).

Monitoring the open water habitats besides the coastal and shelf areas are needed besides the need of increasing the areal coverage of sea bottom monitoring of habitats, litter, etc.

Monitoring of water column and sea bottom habitats at a pressure gradient from source to non-impact area (usually being the open waters) might be crucial for some cases to assess the human induced impacts.

Integration of advanced TECHNIQUES to the monitoring programmes is necessary to achieve the needs of expanding programmes and to fill in some information gaps mentioned in GES-descriptor based gap analysis above.

These are widely accepted applications named as new, advanced or innovative methods which still requires a lot of infrastructure and human capacity building.

Operational monitoring and observations including remote sensing techniques will improve the monitoring capacity in time and space with an increasing number of parameters (from physics to chemistry and biology).

New generation identification methods of species and barcoding of them will obviously change the speed and sensitivity of species identification work as well as will help to understand much efficiently the ecosystem functions.

Joint programming of national monitoring activities through a better NETWORKING of activities of different responsible organizations is an obligation at this age. To keep the track, first it is extremely important to sustain what is already achieved in the projects and further on to manage expensive and specific new infrastructures with a common strategy is necessary.

To connect different operational monitoring facilities, multi-purpose use of buoys and to start planning these tools as integrative part of the ongoing routine monitoring programmes should be the way forward.

Obviously, this new concept of monitoring based on networking and integration must be supported with the share of experiences and strategies of human capacity building among different competant and new and responsible institutions.

INTEGRATION of more information and data on pressures-state-impacts should be aimed with new monitoring programmes. This is critically important for almost all descriptors.

For example, a feasible list of contaminants /chemicals established basing on priority and specific pollutants to be monitored in different matrices and their biological effects need to be handled in an integrated way. This approach is also needed for D1/D2/D6, D5 and D10.

OPTIMIZATION of programmes is needed because expansion of the scope of monitoring activities and challenging coordination efforts might fail if the optimization of the programme is not done properly. Therefore, a risk-based monitoring might be targetted which requires a comprehensive pressure analysis first.

Integration of sub-monitoring programmes created for different descriptors would also be a rationalistic approach (like common planning of D8 and D9 and D3 with D10).

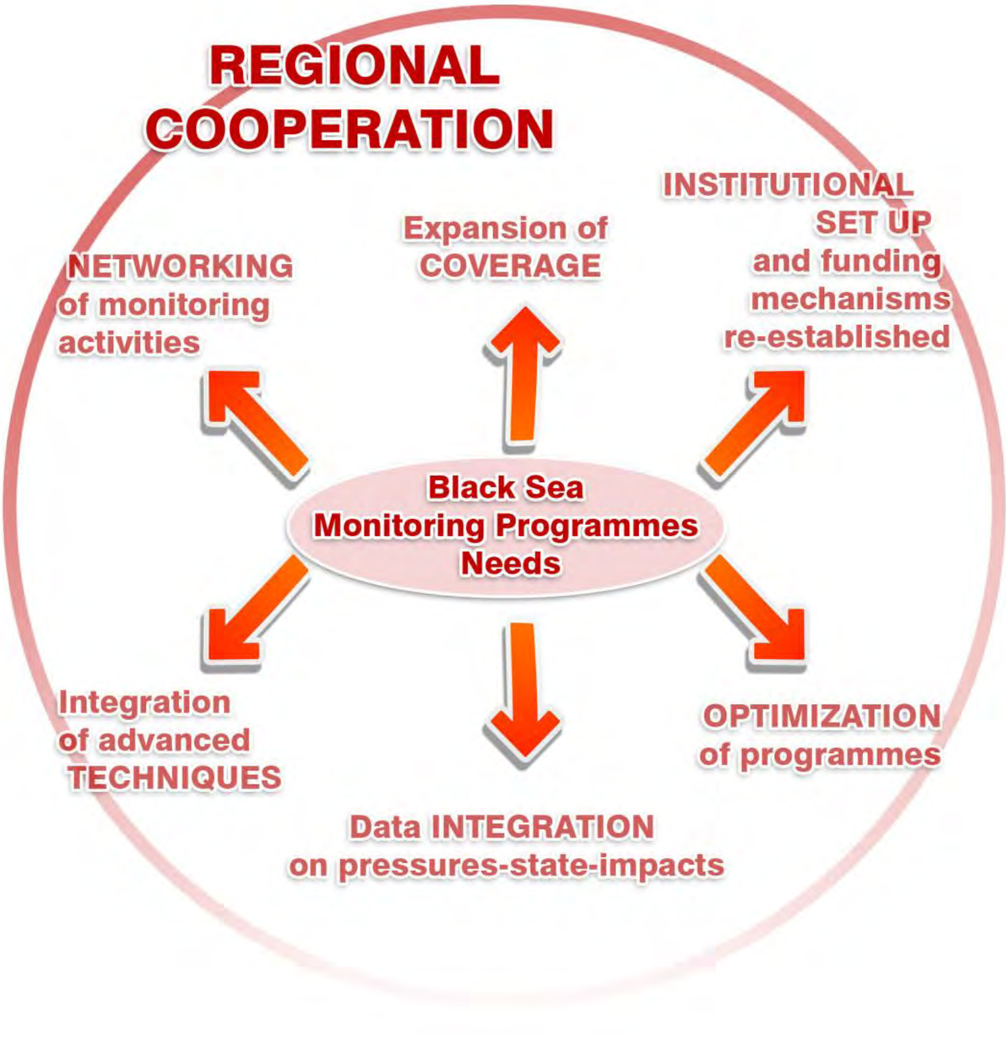
Finally, duplication of cruises, infrastructure and databases must be avoided.

INSTITUTIONAL SET UP and funding mechanisms need to be re-established at the national level for efficient implementation of the Directive and more broadly the ecosytem based management.

The re-establishing monitoring activities and the revision of the fundamental mechanisms require a higher level integration of activities of environment, fisheries, transport and other sectorial administrations. This asks for the coordination by a responible organization and cooperation of competant institutes.

Development of required policy tools might be necessary for the regulation of responsibilities and also technical matters.

Increased funding besides its continuity is necessary and need to be planned at least for 3 (preferably 5-6 years) years programmes.
Feasibility studies also need to be conducted which would support the optimization of programmes.



New dimensions for ongoing *REGIONAL COOPERATION*

Besides, the crucial role of the high level adoption tools of Black Sea Commision regional cooperation might be better advanced with joint national efforts and the continuous support of the EU.

Joint Programming of national monitoring efforts among the countries for the region or a sub-region with multi-lateral agreements would be extremely helpful and feasible. By this way, joint surveys -as practices in MISIS- in open waters and sharing of infrastructure would be possible.

A new Activity Center or Advisory Group for the use of existing regional expertise on remote sensing could be establihed in the frames of Black Sea Commission. However, this can be replaced with other efforts of competent organizations like the IOC initiative to support the development of Black Sea regional cooperation in operational oceanography. As a first step the BSC may try to cooperate with IOC and promote the idea operational monitoring to become an integral part of national monitoring programmes.

State of the Environment

Report of the Western Black Sea based on Joint MISIS cruise

MSFD Guiding Improvements in the Black Sea Integrated Monitoring System

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