ESTABLISHING THE ECOLOGICAL QUALITY STATUS USING BENTHIC INVERTEBRATES AS BIO-INDICATORS IN MARINE MONITORING

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Abstract

Marine environmental quality is assessed usually by means of different monitoring parameters in water and sediment. The biological criteria are considered important components of water quality because they are direct measures of condition of the biota. New European rules emphasize the importance of biological indicators, in order to establish the ecological quality status of marine environment. Benthic invertebrates are used as bio-indicators of marine monitoring, because these organisms are adapted for life on or in particular bottom types, are more stable than planktonic organisms and respond relatively rapidly to anthropic and natural stress.

The present paper analyses the structure and the quantitative development of zoobenthic communities from Romanian shallow waters between 2002 and 2006 when a slight recovery tendency, due to the diminution ecological pressure by eutrophication/pollution has been noted.

The main parameters characterizing the structure and the populations in the communities, the specific composition, abundance and weight dominance of species and groups in communities have evinced the tendencies of marine zoobenthic evolution and its present potential productivity.

Key words: Black Sea, macrozoobenthos, marine environment, ecological quality status

Introduction

Macrozoobenthic species have been the focus of monitoring programmes for many reasons: they are sedentary or sessile and integrators of environmental quality. They are relatively easy to collect, to identify and their ecology and habitat requirements are generally well known. They are responsive in a predictive manner to changes in sediment quality and water, and therefore good environmental indicators.

Sampling methodology and methodologies of data analysis

The results are based on the processing of 120 qualitative and quantitative zoobenthic samples, systematically collected during research cruises, carried out on the monitoring sampling network of the Romanian Black Sea coastal waters (Sulina to Vama Veche), at 5m to 20 m depths between 2002 and 2006. (Fig.1)

The procedures of collection onboard and laboratory processing of samples were accomplished according to the standard methods for sampling and treatment of soft bottom macrozoobenthos samples (Todorova and Konsulova, 2005). The samples were gently sieved through metal gauze sieves with mesh size 1.0 x 1.0 mm and 0.5 x 0.5 mm. The collected samples were fixed with 40 % buffered formaldehyde and the containers were appropriately labeled for further identification of the sample. Sorting, taxonomic identification, abundance and biomass determination have been performed in the Marine Ecology Department's laboratory. AMBI 4.0 (AZTI-Tecnalia) and Shannon - Wiener index (H') were employed for the statistical analyses of data to establish the water ecological status.

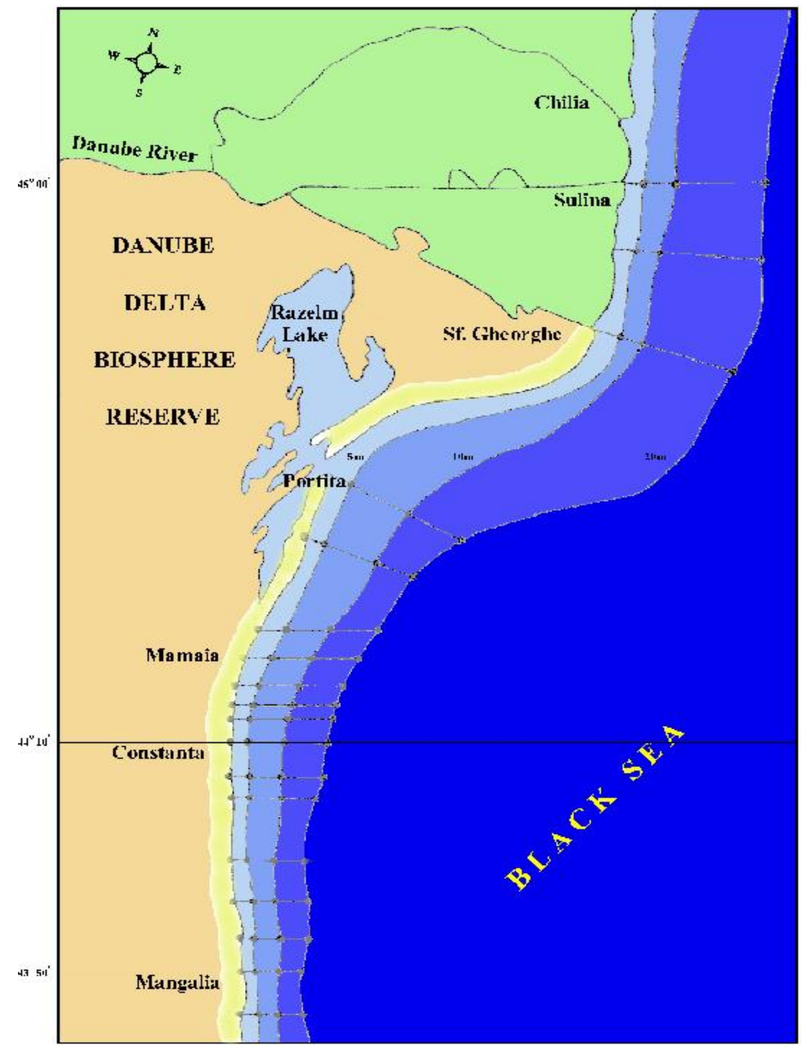


Fig.1 – Monitoring sampling network of the Romanian Black Sea coastal waters

RESULTS AND DISCUSSION

The coastal waters zones, very sensible to strong terrestrial influences also besides high ecological factors fluctuation, register firstly both structural and functional modifications which can appear in the ecosystem under the anthropogenic pressure impact.

The biological elements taken as good ecological quality status indicators are the benthic invertebrates because these organisms live on different types of substratum, are more stable than the planktonic organisms and have a quick –acting response to natural or anthropic stress. This ecological research aimed mainly at the aspects concerning the macrobenthic fauna from the Romanian littoral zones with soft sediments (5m – 20 m depths):

- knowledge of the qualitative state of zoobenthic fauna, as for the *species diversity* (Table 1),
- knowledge of the quantitative structure of zoobenthos (densities and biomasses);
- establishing of ecological quality status using benthic invertebrates as bio-indicators in marine monitoring.

1. Qualitative and quantitative state of benthic communities in marine shallow waters

The life of benthic invertebrates, depending on the substratum and depth, is organized on communities with structural and functional patterns. The biocoenosis of fine sands from the infralittoral zone, in which the characteristic species is the bivalvia *Lentidium mediterraneum*, covers the vast region north of Constantza and southern beaches with bottoms deeper than 4 m.

Beginning with the northern part (Sulina – Gura Buhaz) to the central and southern marine sectors (Cazino Mamaia – Vama Veche) the difference regarding the specific diversity, densities and biomasses values but, also, the direct anthropogenic pressure were registered.

Therefore, of the Danube mouths the analysis of the specific composition of benthic fauna conducted for the entire zone to the identification of 34 macrozoobenthic species distributed as follows: 15 worms, 8 molluscs, 9 crustaceans and two species belonging to other groups (Table 1).

Beginning with the central but mostly, the southern marine sectors the number of species continually increased, 38 macrozoobenthic species being identified (Cazino – Mamaia), 67 species in south (Eforie – Vama Veche) respectively (Table 1; Fig.2).

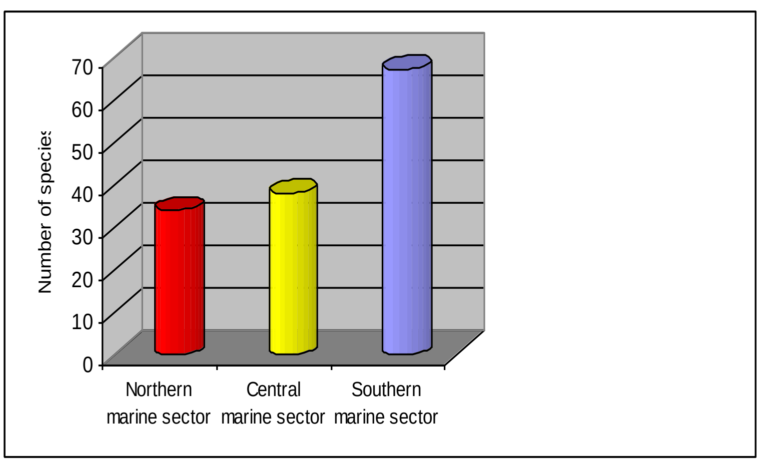


Fig. 2- Evolution of total number of macrozoobenthic species in the Romanian shallow waters between 2002 and 2006

Analyzing the qualitative structure of benthos on sandy bottoms from northern to southern littoral zone of the Romanian marine waters it can be noticed that the dominant groups were polychaetes worms and crustaceans - 24 species, 23 species representing 31% from total number of species identified, follow by mollusks – 17 species (23%) and other groups – 11 species (15%), respectively (Fig. 3).

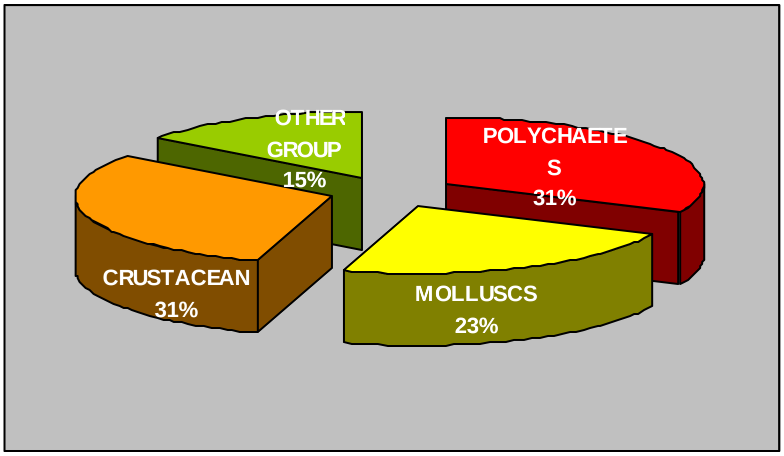


Fig. 3 – The repartition on main groups of benthic invertebrates in sandy bottoms, in biocoenoses with *Lentidium mediterraneum*

Table 1 - The benthic fauna identified in marine shallow waters between 2002 and 2006 (areas Sulina –Vama Veche) at 5 to 20 m depths

Benthic species	**Northern sector**	**Central sector**	**Southern sector**
POLYCHAETA GROUP			
Harmothoe reticulata	+	+	+
Phyllodoce maculata		+	+
Mysta picta	+	+	+
Pectinaria koreni	+	+	+
Nephthys hombergii	+	+	+
Nerine cirratulus	+	+	+
Nereis diversicolor	+	+	
Nereis zonata	+	+	+
Platinereis dumerili			+
Perinereis cultrifera			+
Neanthes succinea	+	+	+
Prionospio cirrifera	+	+	+
Spio filicornis	+	+	+
Pygospio elegans		+	+
Polydora cornuta	+	+	+
Sphaerosylis bulbosa			+
Sillys gracilis		+	+

Exogone gemmifera	+	+	+
Heteromastus filicornis	+	+	
Capitella capitata		+	+
Capitomastus minimus	+		+
Pomatoceros triqueter			+
Melinna palmata	+	+	+
Spionidae varia	+	+	+
MOLLUSK GROUP			
Lentidium mediterraneum	+	+	+
Abra fragilis			+
Middendorfia caprearum			+
Venus gallina		+	+
Cardium edule	+		+
Anadara inaequivalvis	+	+	+
Mya arenaria	+	+	+
Spisula subtruncata	+		+
Telina tenuis			+
Mytilus galloprovincialis	+	+	+
Mytilaster lineatus			+
Rapana venosa			+
Ciclope neritea		+	+
Odostomia rissoides	+		
Hydrobia ventrosa	+	+	+
Retusa truncatula			+
Nassarius reticulatus			+
CRUSTACEANS GROUP			
Balanus improvisus	+	+	+
Iphinoe elisae	+		+
Iphinoe tenella			+
Iphinoe maeotica		+	+
Cumella limicola			+
Dexamine spinosa			+
Melita palmata			+
Corophium runcicorne	+	+	+
Microdeutopus gryllotalpa	+	+	+
Stenothoe monoculoide	+		
Perioculodes longimanus			+
Ampelisca diadema	+	+	+
Phthysica marina	+		+
Diogene pugillator			+
Crangon crangon	+	+	
Upogebia pusilla			+
Pilumnus hirtellus			+
Rhytropanopeus harisii tridentatus	+		+
Liocarcinus holsatus		+	
Carcinus mediterraneus			+

Athanas nitescens			+
Caprella acanthifera			+
OTHERS GROUP			
Protodryllus flavocapitatus	+		+
Tetrastema bacescui			+
Leptoplana tremellaris	+		+
Amphiporus bioculatus		+	+
Actinia equina			+
Pontolineus		+	+
Dysidea fragilis			+
Cerianthus solitarius			+
Micrura fasciolata			+
Clunio marinus		+	
Chironomidae larvae			+

Mollusks represent the most important group in the benthos of sandy bottoms. Besides *Lentidium mediterraneum*, between 2002 and 2006 the most important species at 5 to 20 m depths were: *Cardium edule, Venus gallina, Mya arenaria, Anadara inaequivalvis*. The last two species are the new-opportunistic, self-acclimatized benthic species, appeared and spread extensively through the highly eutrophicated marine environment. They settled successfully in sandy littoral shallow water zones and became mass species which, quantitatively, came to dominate other mollusks.

Regarding the presence of *L. mediterraneum*, the characteristic species of the community on sandy bottoms it can be noted that the species presented a relatively high frequency in the area of interest. The populations consisted of individuals with a length between 1 and 6 mm, ranging between 20 to 3,800 specimens per square meter. By the presence of the high percentage of young specimens (with the length between 1 to 3 mm), which settled on the substratum, about 44% of the total stock, at 5 m depths, it can be considered that this community is recovering following an improvement of environmental conditions.

Worms ranked second in terms of groups with the greatest trophic value inside the *Lentidium* biocoenoses (Bacescu, 1965). *Spio filicornis* was the most common polychaete, followed by : *Eteone picta, Nephthys hombergi, Pygospio elegans, Pektinaria korenii.* As like *L. mediterraneum* the worm *Spio filicornis*, characteristic polychaet species in the community on sandy bottoms, less tolerant to alteration of environmental conditions is set down as a good indicator for the biocoenoses state. In the last years the evolution of *Spio* reflects a rehabilitation of its populations in the community being registered almost steadily in all stations, at 5 m depths. Also, it should be noted that throughout the entire investigated zone the population of some opportunistic species have proliferated and become dominant, such as *Neanthes succinea* and *Polydora cornuta*.

Crustacean ranked third trophic importance in the *Lentidium's* biocoenosis. The most common species was *Perioculodes longimanus*, followed by *Corophium crassicorne, Mycrodeutopus gryllotalpa* and the cosmopolitan species *Ampelisca diadema*.

Decapods crustaceans were also present in this biocoenosis but their great mobility enabled them to avoid the collectors. However, in the southern part of the littoral *Crangon crangon, Diogenes pugilator, Rhitropanopeus harisii tridentatus, Carcinus sp., Pilumnus hirtellus* were found. The presence of these benthic species, although in small number (2-3 ind./sample) constitute a positive signal for the biodiversity conservation state and, also for this littoral zone less affected by the anthropogenic factors.

Concerning the quantitative structure of the whole studied zone, the folowing aspects can be pointed out: a general look at the *densities of populations* on the shallow biotic bottoms leads to the remark that the values of this specific parameter were more higher in the central and southern marine zones of the littoral, namely 43.679 ind./sqm (Cazino-Mamaia) and 47.812 ind/sqm (Eforie Sud – Vama Veche) than 23.373 ind/sq.m in the northern part (Sulina – Sf. Gheorghe)(Fig.4)

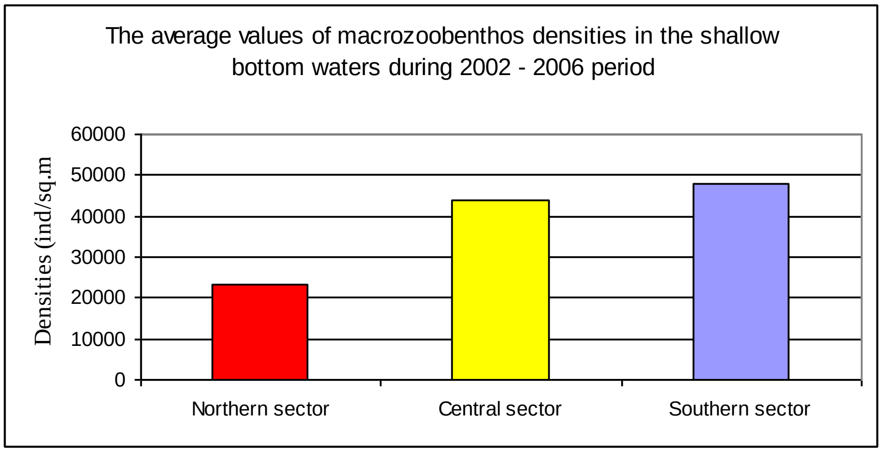


Fig. 4- The average values of macrozoobenthos densities in the shallow bottom waters between 2002 and 2006

According to the quantitative data of biomasses obtained 2002 and 2006 there were important differences between these marine zones. Thus, in the northern part of the littoral (Sulina – Sf. Gheorghe) an average value of 3,730 g/square.m was estimated. In this zone molluscans group dominanted, mostly *Mytilus galloprovincialis,* whose presence became a constant factor in the sandy bottoms from the northern part due to the modifications of the substratum such as, the extension of mud fraction favourable for mussels development. This situation was observed beginning with 2003 and maintained up to now, the small size specimens dominating the existing populations.

In the central marine sector, Cazino- Mamaia, the biomasses was estimated as about 2,125g/square.m, the molluscs being well represented by their populations in this area. Within the molluscs group, two species *Mya arenaria* and *Scapharca inaequivalvis* are the weight dominant; these are new-opportunistic, self-acclimatized benthic species, appeared and spread extensively throughout the highly eutrophicated marine environment.

In the southern part of the littoral (Eforie Sud – Vama Veche) the highest biomasses were registered - 10,409 g/square.m. Dense colonies of mussels which reached biomasses of about thwice and respectively, five times more than in both northern and central marine sectors were found (Fig.5).

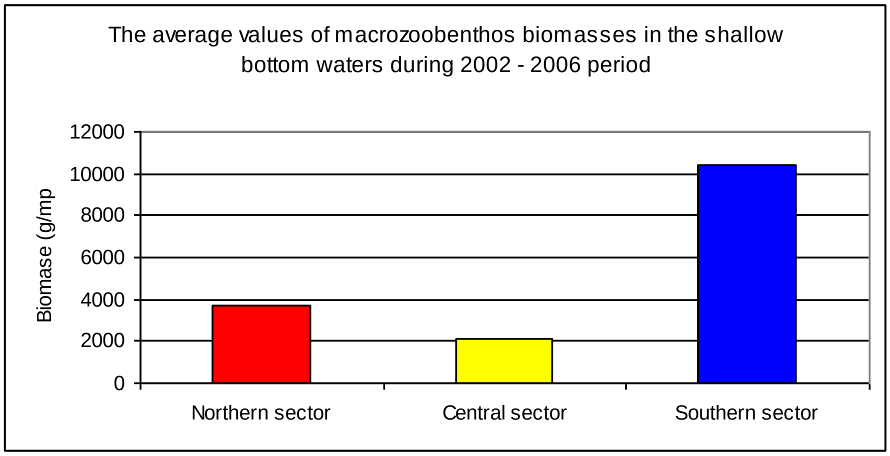


Fig. 5 - Average values of macrozoobenthos biomass in the shallow bottom waters between 2002 and 2006

2. Establishing the ecological quality status using benthic invertebrates as bio-indicators in marine monitoring

By means of the determination of some biological data, such as, specific composition (number of species) and quantitative structure (densities and biomasses) of macrozoobenthos have been calculated some relevants indices to establish the ecological state of the studied marine areas.For statistical analyses of data the biotic and diversity indices were used (AMBI, M-AMBI, Shannon – Wiener (H’).

2.1. AZTI Marine Biotic Index (AMBI) (www.azti.es)

AMBI was created by Borja et al.(2000) and has been applied to different European geographical areas, experiencing various human impact (Borja et al., 2003). The AMBI offers a “pollution classification” of a particular site, representing the benthic community “health” (sensu Grall and Glemarec, 1997). For the soft-bottom macrofauna the species could be classified into five ecological groups, according to their sensitivity/tolerance to an increasing stress gradient:

Group I – species very sensitive to organic enrichment and present under unpolluted conditions (initial state). They include the specialist carnivores and some deposit feeding tubiculous polychaetes;

Group II – species indifferent to enrichment, always present in low densities with non- significant variations with time. This includes suspension feeders, less sensitive carnivores and scavengers;

Groups III – species tolerant to excess organic matter enrichment. These species may occur under normal conditions, but their populations are stimulated by organic richment. They are surface deposit – feeding species, as tubiculous spionids;

Group IV – Second order opportunistic species. Mainly small sized polychaetes ; subsurface deposit feeders, such as cirratulids;

Group V – First order opportunistic species. These are deposit feeders, which proliferate in reduced sediments;

The AMBI has been applied in the assessment of the "Ecological Status", under the European Water Framework Directive (WFD) (Borja et al., 2003). In this particular case the authors recommend the use of AMBI only as a part of a set of measures and indices (a multimetric approach), such as diversity and richness, in order to minimize misclassification problems in the assessment of the "Ecological Status" (Borja et al., 2004). This index is useful, in order to study the evolution of a site after an impact. The values of the AMBI index classified by Borja (2005) are given in Table 2.

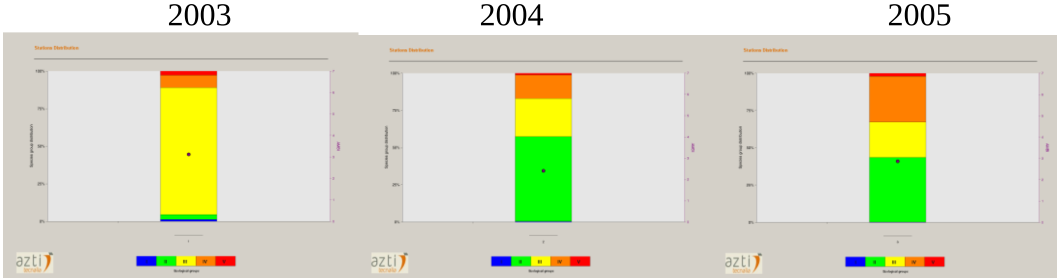
Table 2– The values of the index AMBI supposed to show various levels of benthic disturbance (*Borja at all*, 2005)

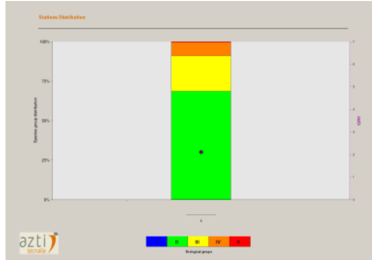
Ecological status	AMBI values
High	0.2<AMBI ≤ 1.2
Good	1.2<AMBI ≤ 3.3
Moderate	3.3<AMBI ≤ 4.3
Poor	4.3<AMBI ≤ 5.0 5.0<AMBI ≤ 5.5
Bad	5.5<AMBI ≤ 6.0 Azoic zone (7.0)

To establish the ecological status of water body types from the northern to the southern part of the Romanian coastal waters macrozoobenthos samples collected from sandy and mixed sediments have been analyzed; the studied areas were located between Sulina and Vama Veche. The graphics represents the classification of species in one of the five ecological groups according to their sensitivity/tolerance to an increasing stress gradient (Grall and Glemarec, 1997) and their repartition on ecological groups, in each studied area (a, b, c).

a) Northern marine sector (Sulina –Sf.Gheorghe)

2003 2004 2005

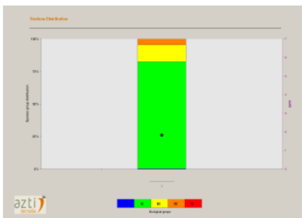




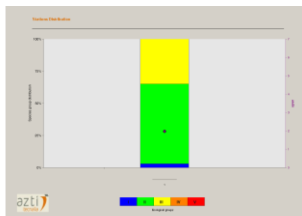
2006

b) Central marine sector (Cazino – Mamaia)

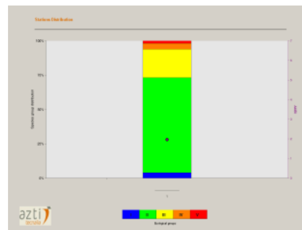
2002



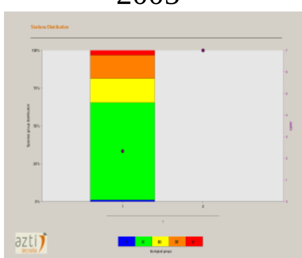
2003



2004



2005



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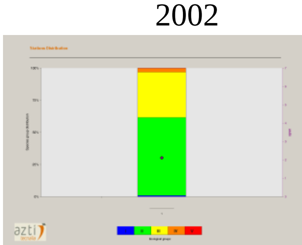
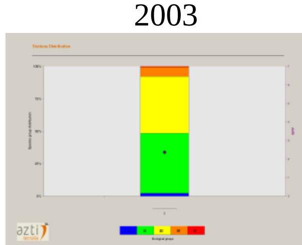
c) Southern marine sector (Eforie Sud-Vama Veche)

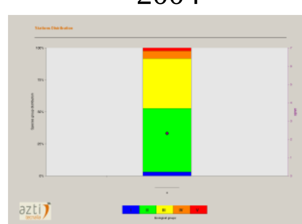
2002



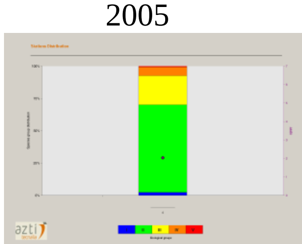
2003



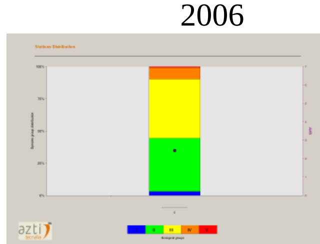
2004



2005



2006



The repartition on ecological groups of present species on the entire studied areas relieved the dominance of the species indifferent to enrichment (second group), 62.3 %, following by species tolerant to excess organic matter enrichment (third group), 31.5 %, second order opportunistic species (IV-V groups), 5% and species very sensitive to organic enrichment and present under unpolluted conditions (initial state), 1.2% (I – group).

According with the values of AMBI in each studied location the results were (Table 3):

Table 3 - Values of index AMBI obtained in each studied area:

Period	**Northern sector**	**Central sector**	**Southern sector**
2002	-	1.79	2.11
2003	3.12	2.06	2.36
2004	2.40	2.05	2.32
2005	2.86	2.36	2.03
2006	2.11	2.51	2.44
Average	**2.65**	**2.15**	**2.25**

The average values resulted using the biotic index AMBI for each marine sector inscribed in the limits which indicate a good ecological status (**2.15 – 2.65)** according with the limits rated for the sandy bottoms zones: **1.2<AMBI ≤ 3.3 (**Borja si Muxica -2005) (Table 2). The good quality status situation is characterized by a slight deviation from the reference conditions and the modifications which can appear in this stage are gradual and reversible.

2.2. Shannon-Wiener– Diversity index (H') – the one of the most used index for determination the species diversity into biocoenosis.

The diversity index (H') represented values ranging between :

1.95 - 2.73 (northern sector) – average values 2.41
1.73 - 2.70 (central sector) – average values 2.39
2.42 - 3.35 (southern sector) – average values 2.95

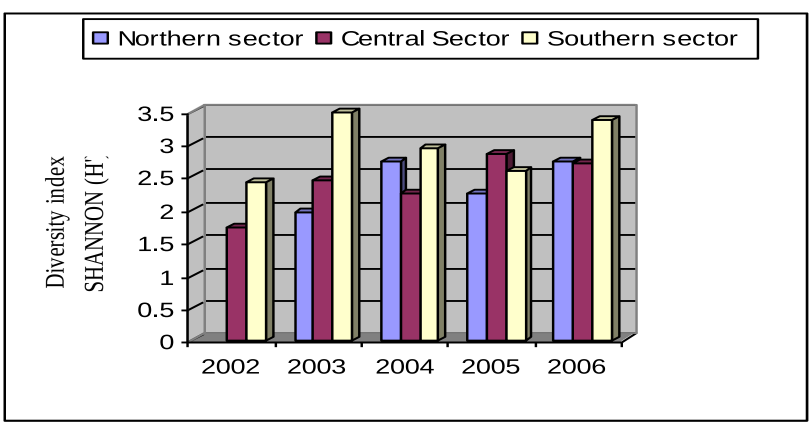


Fig. 6– Diversity index (H’) variation in studied areas between 2002 and 2006

The average values resulted using the diversity index (H’) for each marine sector define a moderate ecological state in northern and central marine sector with a slight tendency to a good status in those zones less influenced by pollution/eutrophication from the southern part of the littoral. The obtained values inscribed in the limits which indicate a moderate ecological status : **3.1>H’ ≥2.2** according with the limits rated for the sandy bottoms zones **(**Borja and Muxica , 2005).

The moderate quality status is a “transitory class “ characterized by a moderate deviation from the reference conditions and the modifications which can appear in this stage are, yet, gradual and reversible.

2.3. Multivariate AMBI (M-AMBI)

For getting a correct evaluation of the ecological status of benthic communities can be used another method of calculation such as M-AMBI index (Multivariate AMBI), also. In the process of M-AMBI calculation, it takes into account the values of AMBI, richness (number of species) and Shannon’s diversity (H’). The values of the M- AMBI index classified by Borja (2005) are given in Table 4.

Table 4 – The values of the index supposed to show the ecological quality (Borja and all, 2005)

Ecological status	M-AMBI
High	M-AMBI ≥0.85
Good	0.85>M-AMBI ≥0.55
Moderate	0.55>M-AMBI ≥0.39
Poor	0.39>M-AMBI ≥0.20
Bad	> 0.2

The average values resulted using the M-AMBI index for each marine sector presented slight variations ranging between 0.81 (north) and 0.85 (south) inscribed in the limits which indicate a good ecological status according with the limits rated for the sandy bottoms zones: 0.85>M-AMBI ≥0.55 (Borja et all -2006) (Table 5).

Table 5 - Values of index AMBI obtained in each studied area:

Period	**Northern sector**	**Central sector**	**Southern sector**
2002	-	0.73	0.80
2003	0.59	0.77	0.88
2004	0.87	0.84	0.85
2005	0.93	0.89	0.89
2006	0.84	0.92	0.87
Average	**0.81**	**0.83**	**0.85**

In the analysed period (2002-2006) in the given environmental conditions both AMBI and M- AMBI revealed a tendency under ecological status improvement from North to South.(Table 6). The metrics based on community diversity and percentage share of ecological groups are able to distinguish the changes in ecological status.

Table 6 – Classification of the ecological status of Romanian coastal waters according to the values of diversity index H', AMBI and M-AMBI, between 2002 and 2006

Zones	AMBI	H'	M-AMBI
Northen sector (Sulina – Sf. Gheorghe	2.65	2.41	0.81
Central sector Cazino – Mamaia	2.15	2.29	0.83
Southern sector Eforie Sud – Vama-Veche	2.25	2.95	0.85
Classification of the ecological status	**Good**	**Moderate**	**Good**

CONCLUSION

- In the current period (2002-2006) the evolution of macrozoobenthos from the shallow waters relieved a slight improvement, mostly qualitative, manifested through increased specific diversity in the entire marine sector: 34 species in the predanubian sector, 38 species in central sector, 67 species in the southern part of the littoral, respectively;
- From the quantitative point of view, the benthic communities redressing needs a long period with ameliorated environmental conditions, taking into account that some species, very sensitive to organic enrichment, recuperate more difficultly when the intensity of different environmental factors is to high;
- The results obtained using the different metric indices (AMBI, H' and M-AMBI) characterized a moderate quality status of the investigated water bodies type with a slight tendency to a good status, mostly in those marine areas which are less influenced by pollution/eutrophication namely, in the southern part of the Romanian littoral.

SELECTIVE REFERENCES

Borja, A., J. Franco & V. Pérez, 2000. - *A marine biotic index to establish the ecological quality of soft bottom benthos within European estuarine and coastal environments*, Marine Pollution Bulletin, 40(12): 1100-1114.

Borja, A., Franco, J., Muxika, I., 2003. - *Classification tools for marine ecological quality assessment: the usefulness of macrobenthic communities in an area affected by a submarine outfall.* ICES CM 2003/Session J-02, Tallinn, Estonia, 24–28 September.

Borja, A., A. B. Josefson, A. Miles, I. Muxika, F. Olsgard, G. Phillips, J. G. Rodríguez and B. Rygg, 2006 (in press). *An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive.* Marine Pollution Bulletin..

Todorova Valentina, 2005 – *Benthic metrics and suitability for the assessment of the ecological status of coastal and transitional water* – paper prepared and presented for Macrozoobenthos Workshop (Varna, 2005).