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SOME MACROPHYTE INDICATORS FOR ASSESSMENT OF ECOLOGICAL STATUS IN SOUTH AND NORTH PARTS OF BULGARIAN BLACK SEA COAST IN COMPLIANCE WITH WFD AND MSFD

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Abstract: *Macroalgae are important biological element in WFD. Some indexes and indicators which are used for ecological status assessment are also applied in MSFD for different descriptors. Macrophytobenthos communities are good indicators of ecological status because directly penetrate nutrients with their surface from sea water. The main goal of this paper is to propose and to apply some indexes for assessment of coastal ecosystems ecological status for the aims of WFD and MFSD. Examples were given to demonstrate the use of indexes. From the final results, obtained from indexes, a high status in South part of Bulgarian coast was established and in north part in Varna bay (Galata, Buna and Trakata), bad status was revealed because of influence of high contaminants concentrations. Strong correlation was established between different indexes compared and between them and Secchi dept as indirect indicator for nutrient enrichment.*

Key words*: Sea biology, macrophytobenthic communities, ecological indexes, WFD, MSFD.*

Introduction.

The EU Marine Strategy Framework Directive (2008/56/EC; MSFD)[16] which includes Water Framework Directive, provides a framework within which each Member State shall take the necessary measures to achieve or maintain a "good environmental status" in the marine environment by the year 2020. For achieving the aims of the two directives MSFD and WFD, ecological indicators were proposed. Benthic macrophytes, especially seagrasses and perennial macroalgae, are sensitive to anthropogenic stress [21; 23;14;6], mainly linked with changes in nutrients input and light transparency leading to eutrophication [4; 5]. In this context, the emphasis is given to anthropogenic eutrophication as a threat to coastal and transitional waters. Macrophytes as primary producers, are one of the main biological elements, because they penetrate nutrients with their surface and thus are good indicators of ecological status. The European Marine Strategy Framework Directive aims at good environmental status (GES) in marine waters, following an ecosystem-based approach, focused on 11 descriptors related to ecosystem features, human drivers and pressures. We propose alternative indicators for use in the Marine Strategy Framework Directive (MSFD). Following Commission Decision 2010/477, we have applied four different indicators. We tested their efficiency. Two of these indicators, had already been applied during the WFD. Another indicators, are proposed here as a new monitoring tool for the MSFD in Bulgarian Black Sea waters. The Ecological Indexes were designed to estimate the habitat-based ecological status of rocky coastal waters using benthic macrophyte communities as bioindicators [21].

The aim of this paper is to propose another index EI p.c. (Ecoogical index - projected cover), which is on the same principal as EI and estimated on the same way, but uses vertical projected cover proportions, not biomass and lower depth distribution limit of Cystoseira. Different indexes - EEIc [21], EI (biomass), EI (percent cover) and lower depth distribution limit of Cystoseira were used and verified and compared with some data. Also numerical threshold values were set. An examples will be provided in order to review the application of different indexes as a tool for estimating the ecological status of coastal waters of Bulgarian Black Sea under the requirements of the WFD and MSFD. More detailed information with more data will be provided in another paper.

Materials and Methods.

Sampling was carried out with method of squares (19) and hydrobotanical transects with help of scuba diving technique [2; 9; 13; 18; 24]. Minimum 12 random samples were collected from every transect with help of metal frame – 20 x 20 cm at 0.3 to 3m depth. Detailed photo and video records were made for documentation of substrate and cover of macrophyte communities. Thus, as a sampling site, an area of minimum 15x15m (15m width and length depends on slope of the transect) was considered. Most of sites along the Bulgarian Back Sea coast were sampled once annually - in the summer season and some of them in autumn. Each collected sample was placed in a plastic bag with label on which number, date, site and depth was and all materials were transported to laboratory in a cool box for the consequent processing and analysis. Samples were preserved in a freezer (-20°C), or in formalin (4%). Species identification of macroalgae was carried out in the laboratory by using a microscope for identification of species. Samples were well rinsed under running water through a sieve to remove sand and animals. Then were sorted and identified to species, with help of stereomicroscope. Species identification was realized according to Zinova, [31], Konaklieva [10], 2000, Temniskova et al.,[29]. Taxonomy was standardized, as used Algae base [11]. Samples were dried on filter paper and weighed on scales with accuracy to second sign. Wet weight was multiplied by coefficient to obtain values in $g.m^{-2}$. [17].

In order to estimate percentage coverage, a transparent double bottom square PVC container, filled with sea water and having at its bottom a square 20 x 20 cm matrix divided in 100 squares was used. The surface covered by each sorted taxon in vertical projection floating in sea water was quantified as percentage of coverage (2 cm^2 = 1% sampling surface). The percentage coverage of epiphytes on macrophyte surface was roughly assessed. The total coverage often exceeded 100% due to the presence of different layers in the vegetation, i.e. mainly canopy and understorey layers, epiphytes. For species present with insignificant abundance a coverage value of 0.01% was allocated. The sorted taxa then were dried for a while on filter paper and weighed (wet biomass) and finally were dried to constant weight in an oven (70 grades _C) and weighed (dry biomass) [20].

For implementation of WFD and MSFD some indexes and indicators of ecoogical status were elaborated. One of them was Ecological index [9] which was approved by the European commission and Bugarian Ministry of Environment and have been used for the aims of WFD in Bulgaria and Romania[9;16]. Ecological status of coastal waters was assessed on the base of initially developed ecological index (EI) and its classification system [6], revised and filled out in process of intercalibration - second phase in frames of GIG Black Sea [7;8] and approved with regulation 4/14.09.2012 [25]. Different sensitivity groups of macrophytes were proposed, taking into consideration the peculiarities of Back Sea and weight coefficients were proposed for ecological state groups.

ESGIA-coef =1, ESGIB-coef =0.8, ESGIC-coef=0.6, ESGIIA-coef=0.6, ESGIIB-coef=0.8, ESGIIC-coef=1

The index is described in details in Dencheva, Doncheva, 2014[9]. In the frames of last phase of intercaibration, referent value was revised [3] and in result of this, the following borders of the ecological state classes of the EI (biomass) index were calculated:

Table 1. Biomass proportions of sensitive (ESGI) and tolerant species (ESGII), EI (biomass) values and EI-EQR of macrophytobenthic communities for different status classes .

Biomass proportion of more sensitive species	**EI**	**Ecoogical status**	**EI-EQR**
> 78 – 100 % ESGI or 0.78 - 1	> 7.8 - 10	High	> 0.84 - 1
> 60 - 78% ESGI or 0.6 - 0.78	> 6 - 7.8	Good	> 0.64 - 0.84
> 40 - 60% ESGI or 0.4 - 0.6	> 4 - 6	Moderate	> 0.43 - 0.64
0 - 40% ESGI or 0 - 0.4	> 2 - 4	Poor	> 0.21 - 0.43
0 - 100% ESGII(A+B) or 0-1 0 - 100% ESGIICa or 0 - 1	> 1 - 2 0 - 1	Bad	> 0.11 - 2 0 - 0.11

To calculate the value of EI we apply the following rules and formulas:
When ESGI = 0 - Bad status , we take the most sensitive group left from ESGII.
In case when ESGI = 0, ESGII (A+B) = 0 and ESGIICa has biomass proportion from 0 - 100%, EI takes values of 0 – 1, and is calculated with the following formulae:
EI- bad (0-1) = [ESGIICa/ESGII], when ESGI=0, ESGII(A +B) = 0.
When ESGI= 0 and 0%>ESGII(A+B)≤ 100% we have EI with bad status (1-2). In this case the index is expressed as the biomass proportion of the most sensitive subgroup selected from ESGII. The following formula is used:
EI-bad1-2)=[(ESGIIA/(ESGIIA+ESGIIB+ESGIIC))*0.6+(ESGIIB/(ESGIIA+ESGIIB+ESGIIC)*0.8]+1,
When the proportion of sensitive species (ESGI) is between 0 - 40%, EI takes values between 2 - 4 and we have a poor status. The following formula is applied:
EI-poor(2-4)=[(ESGIA/(ESGI+ESGII))*1+(ESGIB/(ESGI+ESGII))*0.8+(ESGIC/(ESGI+ESGII))*0.6]*5)+2
When the proportion of sensitive species is between 40-60%, EI is between 4-6. At 60-80% biomass proportion, EI is between 6 - 8 and at 80 - 100% , EI is between 8 and 10. In these three cases EI is calculated following this formula:
EI high, good, moderate (4-10) =[(ESGIA/(ESGI+ESGII))*1+(ESGIB/(ESGI+ESGII))*0.8+(ESGIC/(ESGI+ESGII))*0.6]*10,
Referent value is RC = **9.32**
EI-EQR high-good-moderate = [10* (ESGI/(ESGI+ESGII)]/ref value;
EI-EQR poor = [5*(ESGI/(ESGI+ESGII)+2]//ref.value;
EI-EQR bad (1-2) = [(ESGII(A+B)/ESGII)+1]/ref.value, when ESGI=0;
EI-EQR bad (0-1) = (ESGIICa/ESGII)/ref. value, when ESGI=0, ESGII(A +B) = 0.
For example, if EI bad (0-1) = 0,27, then EI-EQR = EI/ref value = [ESGIICa/ESGII]/ref. value = 0,27/9,32=0,029**.**In cases when EI-EQR> 1, we equate the value to 1. For example if EI = 9,7 then EI-EQR = [10* (ESGI/ESGI+ESGII)]/9,32 , or EI-EQR = 9,7/9,32 = 1,04 , hence we equate EI-EQR = 1
The new index EI (vertical projected coverage), which is proposed in this paper differs from EI (biomass) with referent value and borders for ecological state classes in the classification system, namely (Table 2):

Table 2. Vertical coverage percentage proportions of sensitive (ESGI) and tolerant species (ESGII), EI (vertical percent coverage) values and EI(p.c.) - EQR of macrophytobenthic communities for different status classes:

Projected coverage proportion of more sensitive species	**EI (p.c.)**	**Ecoogical status**	**EI-EQR**
> 66 – 98 % ESGI or 0.78 – 0.98	> 6.6 - 9.8	High	> 0.73 - 1
> 48 – 66 % ESGI or 0.6 - 0.78	> 4.8 - 6.6	Good	> 0.53 - 0.73
> 28 – 48 % ESGI or 0.4 - 0.6	> 2.8 – 4.8	Moderate	> 0.31 - 0.73
0 – 28 % ESGI or 0 - 0.4	> 1 – 2.8	Poor	> 0.11 - 0.31
0 – 100 % ESGII(A+B) or 0 - 1 0 – 100 % ESGIICa or 0 - 1	> 0.5 - 1 0 – 0.5	Bad	> 0.06 – 0.11 0 - 0.055

In construction of ecological index was taken into account the EEIc index [19; 20; 21; 22]
Another difference is that , for this EI (P.C.) index we have the following coefficients:

ESGIA-coef = 1, ESGIB-coef = 0.8, ESGIC-coef=0.6, ESGIIA-coef=0.8, ESGIIB-coef=0.8 ESGIIC-coef=1

To calculate the value of EI, the following rules and formulas are applied:

EI (p.c.) high, good, moderate (3-9.8) = [(ESGIA/(ESGI+ESGII(A+B) +ESGIIC))*1+((ESGIB/(ESGI+ESGII(A+B+ESGIIC))*0.8+(ESGIC/(ESGI+ESGII(A+B) +ESGIIC))*0.6]*10 = [(ESGIA/ESG)*1+(ESGIB/ESG)*0.8+(ESGIC/ESG)*0.6]*10
EI (p.c.) poor (1-3) = 7.1429*[((ESGIA/(ESGI+ESGII(A+B) +ESGIIC))*1+(ESGIB/(ESGI+ESGII (A+B) +ESGIIC)*0.8+(ESGIC/(ESGI+ESGII(A+B) +ESGIIC)*0.6]+1 = 7.1429*[(ESGIA/ESG)*1+(ESGIB/ESG)*0.8+(ESGIC/ESG)*0.6] + 1
EI (p.c.) - bad (0.5-1) = 0.5*[(ESGIIA/(ESGIIA+ESGIIB+ESGIIC))*0.8+((ESGIIB/(ESGIIA+ESGIIB+ESGIIC))*0.8] +0.5 = 0.5*[(ESGIIA/ESGII)*0.8 + (ESGIIB/ESGII)*0.8]+0.5
EI (p.c.) bad (0-0.5) = 0.5*(ESGIICa/ESGII), when ESGI=0, ESGII(A+B) = 0

Referent value is equal to **9**.

EI (p.c.) - EQR is equal to EI (p.c.) divided by referent value 9. The referent value is obtained from data series from investigations from 2012 to 2016 years referent sites.
Ecological Evaluation index (EEI c) is calculated also for comparison of the three indexes. Original methodology with vertical percent coverage was used. [21]. Only ecological state groups of species which were established for the Black Sea coast are applied in this assessment, for more comparable results.

Another indicator is proposed which is lower depth distribution of Cystoseira perennial species (valid for minimum coverage value of 10%).This indicator lower depth distribution limit of perennial species is core indicator in HELCOM and follows the depth distribution limits (last specimen or minimum coverage value of 10%) of specific sensitive species, which are important for habitat structure (key-species), perennials with a persistent biomass for a certain time scale adapted to stable conditions (K-Strategy).The indicator describes the condition and abundance of a habitat - forming macrophyte species and indirectly the condition for all the associated flora and fauna. The deeper the macrophyte extends, the larger is the volume of the habitat, thus, supporting more viable populations, having more diverse species assemblages, offering feeding grounds for predators and ensuring enough spawning grounds for pelagic fishes [12].

We propose the threshold value between good and no good status of this indicator to be 3 m depth. The grounds for this proposal are some data from depth limit distribution of Cystoseira. Despite these data we need more detailed investigations and more data to verify this threshold. In this paper small part of data obtained is presented. More details will be prepared in another publication. The lower depth limit of Cystoseira - 10 m is approved also from historical data [32]. In our opinion it is even little lower. Here we have in mind not single case in one point, but some average value. In the north part of Back Sea coast, limits of Cystoseira distribution are lower, than in south part. It is in conformity with the fact that in south part exist very good conditions such as light availability and continuous hard rocks, which favor growth of macroalgal communities. In north also coastline is more smooth and very exposed.

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Table 3. Lower depth limit distribution of Cystoseira borders.

Lower depth limit distribution of Cystoseira borders	Ecological status
6-10m	HIGH
3-6m	GOOD
1.5-3m	MODERATE
0-1m	POOR
0m	BAD

Results and discussion

In 2012 year some polygons were investigated from national monitoring for assessment of ecological status of macrophytobentic communities according to Water Framework Directive and also monitoring of project MISIS. More than 200 samples were processed. On figure 1 are presented results from some polygons investigated as an example for application of different indicators. More detailed information will be given in another publication. Polygons are selected to present different ecological state classes.

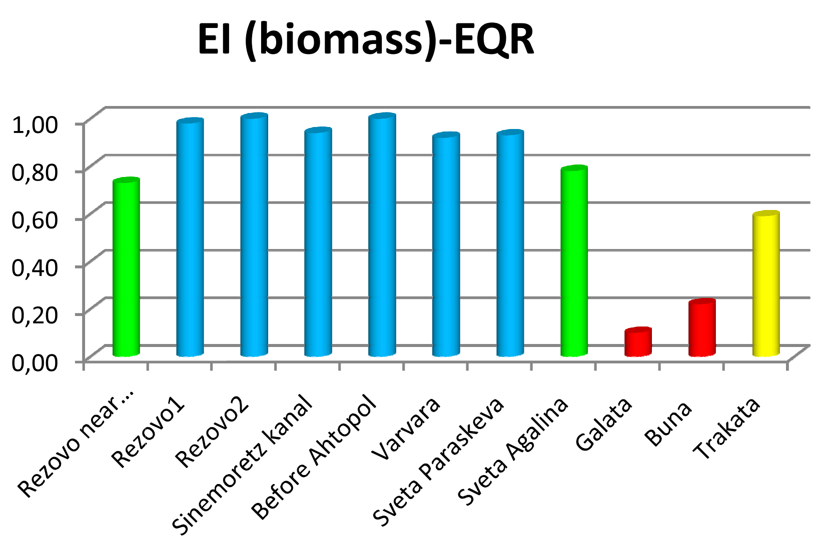

Fig. 1. Ecological Quality Ratio of Ecological Index (biomass) and ecological state classes. Green-good, blue-high, yellow-moderarate, red-bad.

Rezovo, Sinemorets, Before Ahtopol, Varvara, Sveta Paraskeva, Sveta Agalina are situated in south part of Bulgarian Black Sea coast and were characterized as good and high conditions – small or negligible amount of contaminants, higher intensity of light penetration. Some of these polygons were referent sites (Rezovo, Sinemorets, Sveta Paraskeva, Before Ahtopol). They are situated in Strandja Marine protected area. Other polygons Trakata, Galata, Buna are part of Varna bay which is one of most polluted areas in Bulgarian Back Sea coast and the bay is water body at risk. The ecological status in Varna bay polygons is bad and in Trakata polygon it is moderate, because it is at higher distance from channels connected Varna bay with Varna lake. The highest concentrations of nutrients and other pollutants enter the bay from these channels [26; 27; 28]

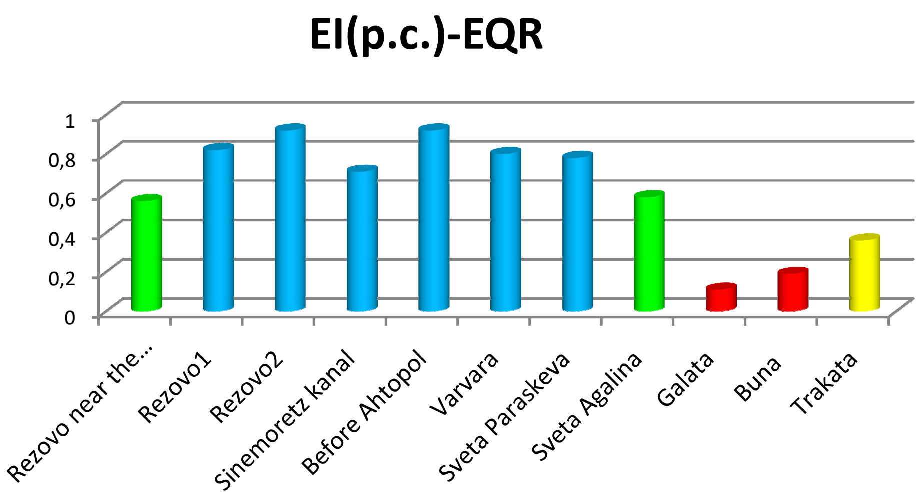

Fig.2. Ecological Quality Ratio of Ecological Index (vertical projected coverage) and ecological state classes. Green-good, blue-high, yellow-moderate, red-bad.

Simmilar results was revealed when calculating Ecological index (projected cover). Again Rezovo 1, Rezovo, 2, Sinemorets kanal, Varvara, Sveta Paraskeva, Near Ahtopol were characterized with high ecological status and Sveta Agalina and Rezovo near the border were in good ecological status. Galata, Buna were in bad status and Trakata - moderate status. The comparison between two indexes shows similar results and the two ecological quality ratios establish equal ecological state classes.

The third index EEI c – EQR was calculated also and similar results revealed. (Fig.3).

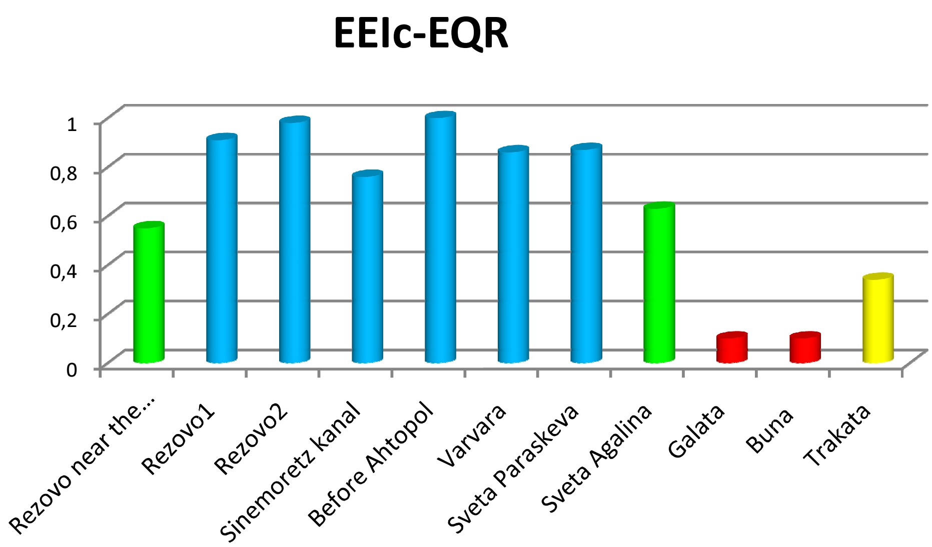

Fig.3. Ecological Quality Ratio of Ecological Evaluation Index (EEIc) original method (vertical projected coverage) and ecological state classes. Green-good, blue-high, yellow - moderate, red-bad.

Rezovo 1, Rezovo, 2, Sinemorets kanal, Varvara, Sveta Paraskeva, Near Ahtopol were characterized with high ecological status and Sveta Agalina and Rezovo near the border were in good ecological status. Galata, Buna are in bad status and Trakata,- moderate status. The results from EEIc were in conformity with these of other two indexes. There was a strong linear correlation between the three indexes compared (Fig. 4, 5 and 6).

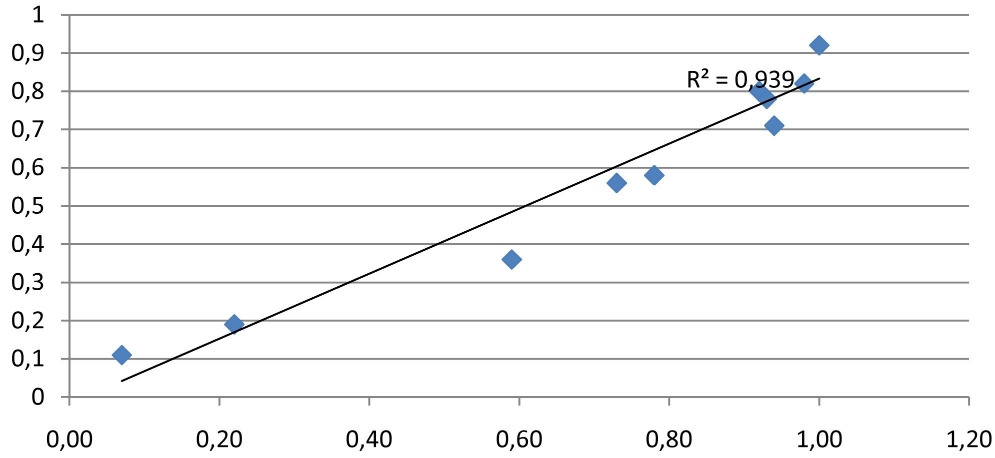

Fig.4. Linear correlation between EI (biomass) and EI (vertical projected coverage).

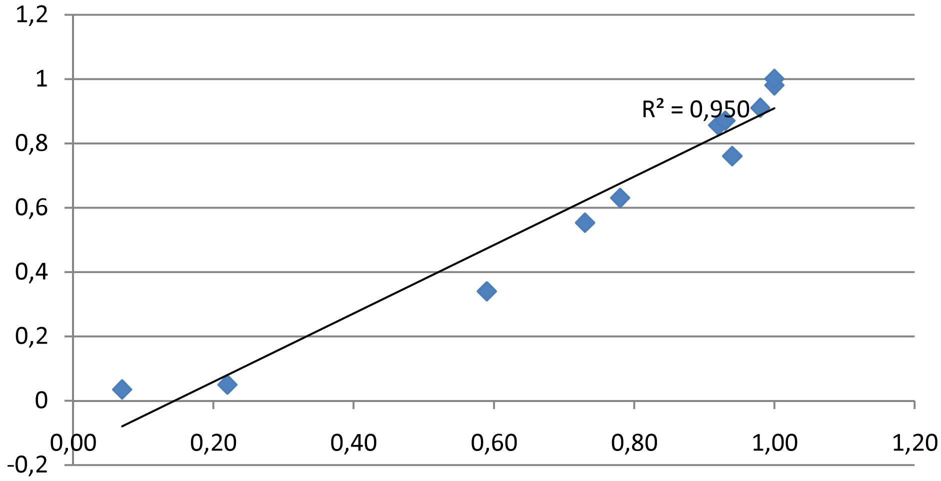

Fig.5. Linear correlation between EI (biomass) and EEI c (vertical projected coverage).

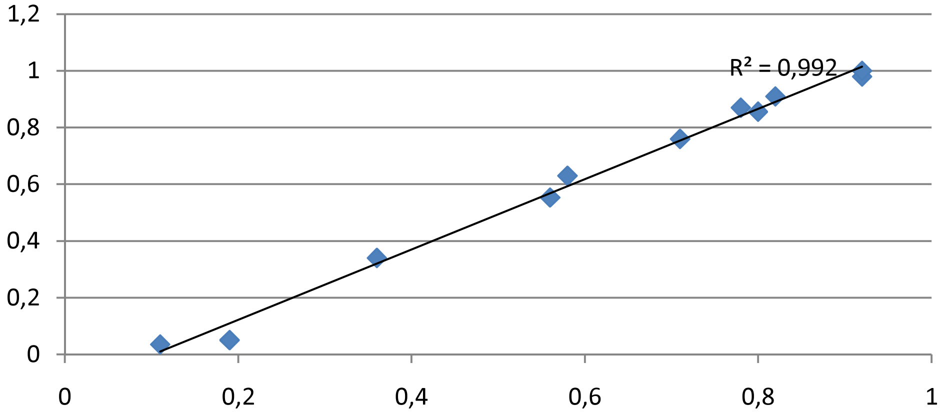

Fig.6.Linear correlation between EI (percent coverage) and EEIC.

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The highest correlation was between EI (percent coverage) and EEI c (R^2 = 0.99). The lowest one was between EI (biomass) and EI (vertical projected coverage).

There is still necessity of more data and calculations of the indexes in other polygons to obtain more reliable data series for these correlations. But this is an example which shows that really there is strong correlation between these indexes.

The European Marine Strategy Framework Directive aims at good environmental status (GES) in marine waters, following an ecosystem-based approach, focused on 11 descriptors related to ecosystem features, human drivers and pressures.

In this aspect we tried to find connection between Secchi disk depth, measuring transparency as proxy of nutrient concentration availability and Ecological indexes used.

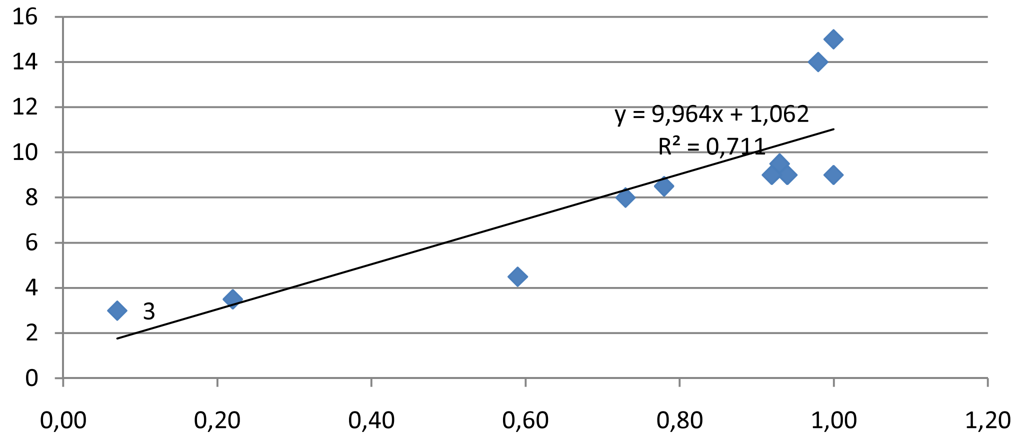

Fig.7. Linear correlation between Secchi dept (m) and EI (biomass).

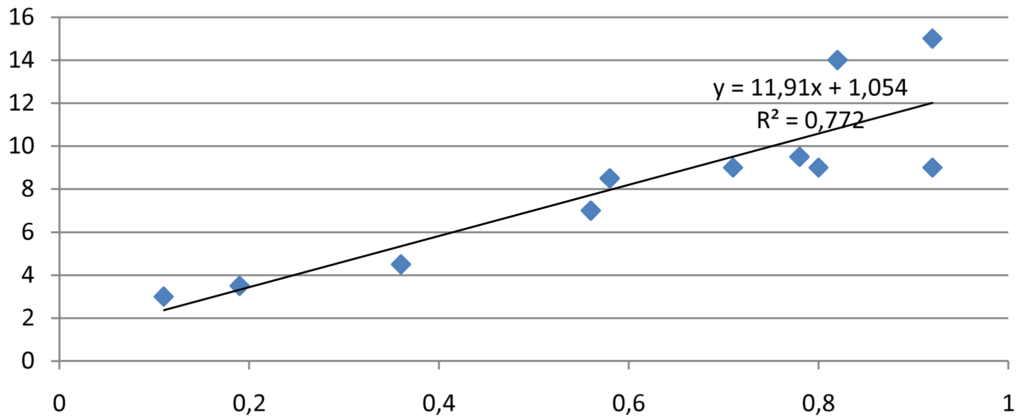

Fig.8. Linear correlation between Secchi depth (m) and EI (percent coverage).

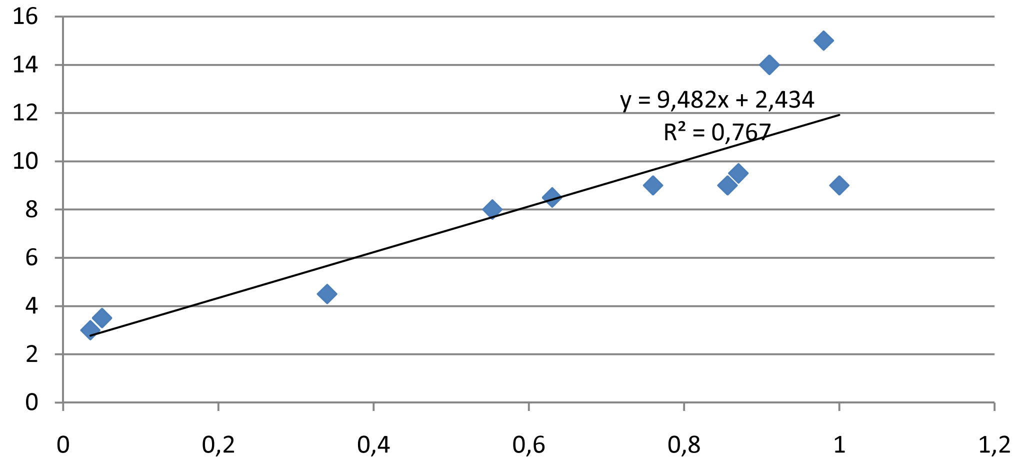

Fig.9. Linear correlation between Secchi depth (m) and EEI c (vertical percent coverage).

Good linear correlation was determined between Secchi depth and Ecological indexes. The highest correlation was established between Secchi depth (m) and EI (vertical projected coverage) – R^2 =.0.774 and the lowest one was between EI (biomass) and Secchi depth - R^2 = 0.71. (Fig.7, 8, 9).
Finally another indicator of ecological status is lower depth limit of Cystoseira, used in HELCOM countries in compliance with WFD and MSFD.
The lower depth limit decreases with increase of eutrophication and decrease of light availability. In Trakata low value (3.2 m depth) was registered and in Buna and Galata it was equal to ”0” m, because Cystoseira disappeared in this area in the strong eutrophication period. In other polygons, it was with higher values. In Rezovo near the borther where good ecological status was determined, the lower depth limit was little lower than in Rezovo, Sinemorets, Varvara, Sveta Paraskeva, where the water quality was high.

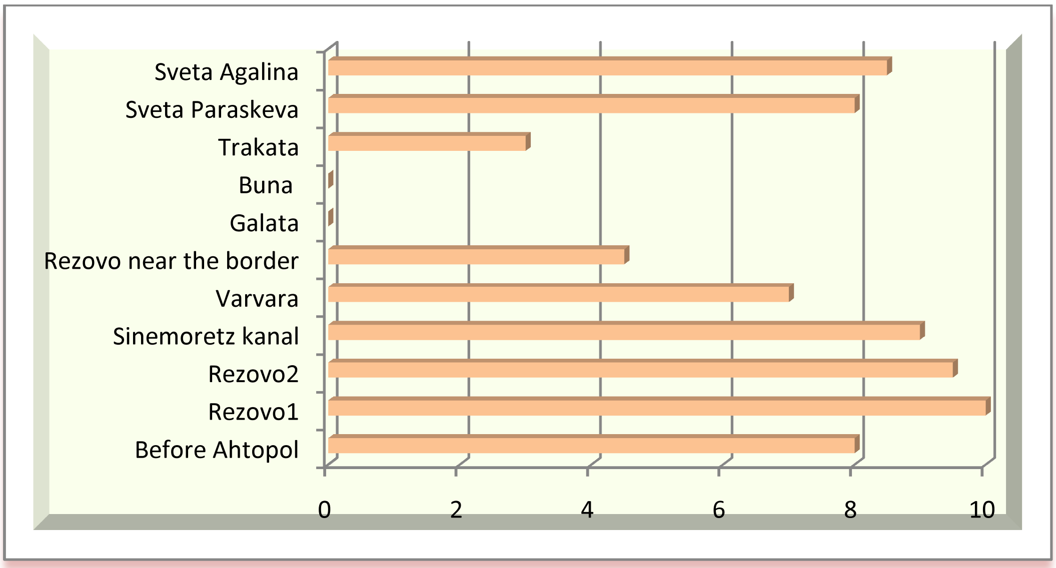

Fig.10. Lower depth distribution limit of Cystoseira barbata community from the investigated transects.

There was a strong correlation between Secchi depth measured water transparency and lower depth limit of Cystoseira community. Very well is proved in literature the dependence of dept distribution of olygothrophyc photophilic macrophyte communities from light penetration [1; 30].

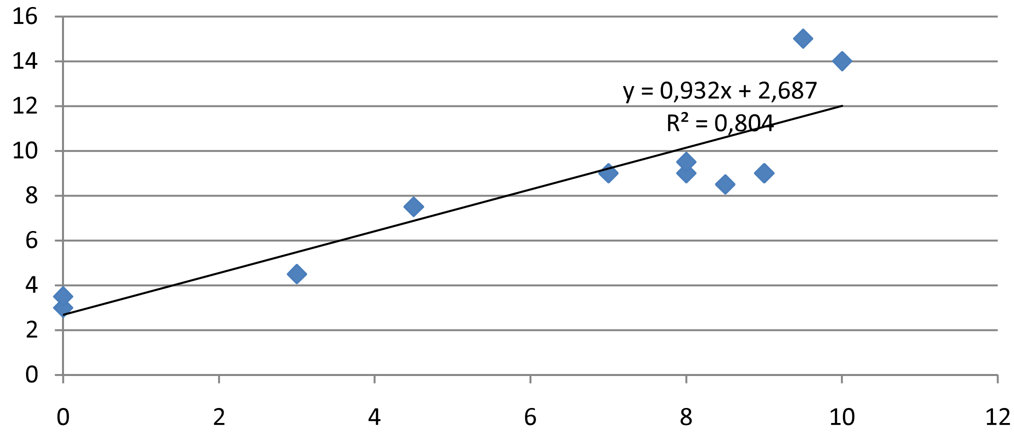

Fig.11. Linear correlation between Secchi depth and lower depth limit of Cystoseira community in the investigated polygons (2012 year).

The light transparency lowers with nutrient and organic enrichment. It indirectly indicates the eutrophication level.

Conclusions.

Strong correlation was found between ecological indexes, also between them and Secchi depth – indirect indicator for nutrient enrichment.

The Ecological indexes classification scheme can be applied in vegetated coastal and transitional water habitats where benthic macrophyte growth is not limited by hard substratum absence and by low salinity, respectively. To avoid any natural environmental gradient bias such as light attenuation down to water column sampling should be realized in shallow waters. Ecological indexes take into consideration species' functional attributes in an attempt to understand response mechanisms and to predict how communities are affected by human-induced stress. (Orfanidis, 2011). The initial monitoring results will help decision makers in undertaking relevant action for the improvement of ecological conditions. The Ecological indexes and indicators are designed to (1) cover the prerequisites of the European WFD and MSFD which are the operational tool for setting the objectives for water protection in Europe , and (2) to offer water managers a tool for comparing, ranking and setting management priorities at different spatial levels. Conceptually, it is more appropriate for assessing the impact of chronic pressures such as eutrophication, sedimentation, aquatic habitat destruction, pollution by organic matter, and general degradation. (Orfanidis et al., 2011).

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