Red Algae (Rhodophyta) in Biomonitoring of Coastal Ecosystems

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Abstract— *The formation, species composition and functional role of red algae were studied in the Black Sea's coastal zone. In sublittoral plant communities, red algae are dominant both by their number of species in the phytocenosis and by their active thallus - the one that ensures their high metabolism. Algae with a large specific surface area of thallus can accumulate heavy metals in higher concentrations. In the paper, we discuss possibilities of using red algae as biomarkers of marine pollution, and as bio filters in the processes of water natural.*

Keywords— biological monitoring, biomarkers, heavy metals, red algae (Rhodophyta), sublittoral zone.

I. INTRODUCTION

Biomonitoring as a system of long-term observations and measures of control over marine environment goes down to two key components: diagnosis and prognosis. When carrying out diagnostic monitoring, we study the "here and now" condition of the ecosystem polluted by the most widespread and hazardous marine contaminants. At that, such monitoring is focused both on environmental factors and on various biological responses that testify to derating of a biotic community's parameters. Methodology that underlies prognostic monitoring is active experimenting in situ that allows us to cross-relate - within a well-planned study - the results of forecasting environmental implications of existing impacts upon the ecosystem [8].

Recently, the use of biomarkers has taken on special significance for assessing the implications of pollutant effects upon water ecosystems. In seashore ecosystems, algae has a leading role in autotrophic synthesis of organics, as it is precisely algae that determine the biological efficiency level and take part in determining the quality of natural sea water. In this connection, we see that algae's role is more than logical as monitoring indicators for seashore ecosystems pollution by the most hazardous substances - heavy metals, i.e. pollutants that can get into sea water by many a way. Algae response to heavy metals presence in water is very often the key informant in assessing the marine community condition, if compared to other types of hydrobionts. [4].

It was algae that were there first indicator organisms in qualitative assessments of marine environmental conditions: presence or absence of certain species is a testimony to the environmental status of a water basin. Algae can also be used as biological markers of long-term toxic effects, while their response is what allows us to both forecast changes in the composition of a biotic community and provide well-founded guidance for an active intervention by way of bioremediation and recovery of the affected biocenotic communities before irreversible processes begin in the ecosystem that suffers from anthropogenic impacts [6].

With this in mind, we have carried out studies aimed at providing rationale to the idea of using red algae (with different lifecycles) as biomarkers, when doing biological monitoring of the coastal zone.

The key goal of this study was as follows: to investigate the formation of sublittoral phytocenoses and to determine the specific surface area of red algae's thallus. Special care was taken for heavy metals distribution in the abiotic component of the ecosystem and for accumulation of the metals in algae of the coastal zone's biocenosis. Our findings paved the way for shaping the species composition and subsequent reconstruction of a benthic community on an artificial reef.

II. MATERIAL AND METHOD

The study was done in the littoral area of the Black Sea's north-western district, using lightweight diving equipment. Algae samples were taken in the zone spanning from the water edge down to 30 m depths, using area frames 0.1 and 0.25 sq/m, and differentiated by species and size; later the species abundance and dry/wet weight of each species' biomass were determined [7 ; 5].

When determining specific thallus area of red algae, we chose 10 specimen, dissected them singling out the basal part, medpart and apical part of each plant. Then clipping of thallus was done, their parameters measured, and subsequently squared by known formulae [9] and using computer-aided systems of image analysis: «Image analysis system», «Video

TesT-Morphologia» and «MaxSoft 3.0». The weight of thallus clippings was measured on electronic scales to within 0.001 g. The specific surface area of a plant was expressed as a thallus square per weight unit (S/W)[10].

When analyzing the epiphytic complex of diatoms, algae's thallus suspension was concentrated by filtering through membranes, and the sediments fixed by 2.5 per cent glutaric aldehyde. The diatoms' species composition was determined in vivo and on permanent preparations prepared in the following way. The algae were washed free of the fixative and placed on a cover-glass with a tiny splash of H_2O_2, and scorched in open flames through an asbestos blanket. The cover-glass with plants was placed onto the object plate with Canada balsam [1].

Water samples taken by a plastic sampler were then filtered through a membrane and fixed by HNO_3 up to 0.1 per cent by volume. Extraction of metals from the water was done using a 0.01 M methylisobutyl ketone solution of in an acetyl-acetate hexamethyl dithiocarbamate hexa methylammonium buffer at pH 6.0. Sediment samples were taken by a Teflon tube stratameter, dried up and brought to constant weight at 105^{o}C. Metals from bottom sediments containing fractions of oxidized, reduced and helate compounds were recovered by the method of sequential extraction [3].

Metal accumulation in algae thallus was determined using an atomic absorption spectrophotometer. Samples were prepared by incineration using the «Digester system». A weighed quantity was treated by the mixture of HNO_3+H_2SO_4+$HClO_4$ according to the pattern as follows: 20 minutes at 30^{0}C, 5 hours at 75^{0}C and 30 minutes at 150^{0}C. Then the samples were filtered out and diluted to volume of 10 ml. Subsequently the solution was cooled off, pH brought to 3.0, and analyzed using «Hitachi» and «Perkin-Elmer» equipment, relying on calibration standards CRM «Sea lettuce» [2].

Artificial reefs up to 20 sq/m were erected at depths down to 10 m, chessboard, on mud-sand bottom, no other algae around; the structures were made of shell stone covered with a mesh.

The findings were finally processed in software packages «Microsoft Exel» and «STATISTICA 5.0 for Windows».

III. RESULTS AND DISCUSSION

In the north-western area of the sea, red algae either form independent communities or belong to phytocenoses whose edificator species may be brown or green algae. Plant formations where edificators are *Cystoseira crinita (Desf.) Bory*, *Phyllophora nervosa (DC) Grev.* or *Ulva rigida Ag.* do belong to the most widespread communities in the sublittoral sea zone.

When studying multilayer plant formations made up by brown algae *C.crinita* having projective cover of 80 per cent, we identified 20 species of brown and red algae. The basic stratum of them was formed by *Cystoseira crinita*, in the second one were found *Ph. nervosa* and *Laurencia obtusa*, in the third layer - *Corallina mediterranea* and *Gelidium latifolium*. Among the aerial plants on the thallus of large algae having the biggest biomass, *Ceramium rubrum*, *Polysiphonia subulifera* and *Laurencia coronopus* (Table 1) were found.

TABLE 1
ALGAE BIOMASS AND THALLUS SURFACE AREA IN PLANT FORMATION *CYSTOSEIRA CRINITA*

Depth, m	*Cystoseira crinita*		Lower layer algae		Epiphytic algae	
	biomass, g/m²	S of thallus, m²	biomass, g/m²	S of thallus, m²	biomass, g/m²	S of thallus, m²
1 m	1209-6920 4148±48	11.5-65.7 39.4	754-1753 1364±21	7.5-45.5 25.4	0-311 169±10	0-31.8 15.9
3 m	3950-8163 6696±71	37.5-77.5 63.5	609-3102 1433±18	35.2-136.9 57.4	205-2032 1194±21	16.0-30.6 40.0
5 m	1830-8609 5373±42	17.4-81.7 51.0	330-1085 809±15	12.8-54.1 24.5	165-3109 1709±24	13.1-77.3 47.9
7 m	1519-2845 2322±35	14.4-27.0 22.0	165-1400 799±17	7.9-10.3 9.1	129-4211 2214±30	11.7-81.5 49.2
9 m	712-1250 989±21	6.8-11.8 9.3	500-1480 835±17	8.3-14.3 9.9	115-1552 867±17	11.7-64.6 38.8

In another multi-layer phytocenosis growing on a stony substrate at the depth of 10 m, red algae were also dominant. The upper level with projective cover of < 20 per cent was formed by *C.crinita*; at the same time the basis of the plant formation was made up by perennial photophilous red sea-grass: *Ph.nervosa, G.latifolium, Laurencia pinnatifida*. In the composition of the phytocenosis, we found certain forms of sciaphilic red algae *Apoglossum ruscifolium* and *Polysiphonia elongata*, along with filiform and lamellate epiphytic red algae growing on *Cystoseira* and *Phyllophora*, including *Rhodochorton purpureum*.

In a two-layer phytocenosis (3 m deep) with green algae *Ulva rigida* as edificator, red algae also occupied over 70 per cent of the formation, many of them (*Callithamnion corymbosum, C.rubrum, P.subulifera, Ph.nervosa*) having 100 per cent occurrence.

The specific thallus area (S/W) of algae is a very important indicator that determines the level and rate of metabolism in aquatic organisms. With very few exceptions, perennial species-edificators had relatively small specific thallus area: *Cystoseira crinita* – 95 cm^2/g, *Phyllophora nervosa* – 136 cm^2/g, *Ulva rigida* – 355 cm^2/g. At the same time, seasonal and annual algae usually had a slightly higher thallus S/W. Among perennial algae, certain sciaphilic forms and organisms living in the lower sublittoral (*A. ruscifolium, Gracilaria verrucosa, Gelidium crinale*) also had a larger thallus area (Table 2).

TABLE 2
SPECIFIC THALLUS AREA OF DOMINANT RED ALGAE SPECIES IN THE BLACK SEA (cm^2/g)

Algae species	Specific surface area sm^2/g
Apoglossum ruscifolium (Turn.) J. Ag.	630±39
Callithamnion corymbosum (J.E.Smith) Lyngb.	1815±123
Ceramium ciliatum (Ell.) Ducl.	321±24
Ceramium rubrum (Huds.) Ag.	262±10
Ceramium strictum Grev. et Harv.	418±33
Chondria tenuissima (Good et Wood) Ag.	259±16
Corallina mediterranea Aresch.	667±50
Corallina officinalis L.	429±43
Gelidium crinale (Turn.) Lamour.	712±58
Gelidium latifolium (Grev.) Born. et Thur.	171±39
Gracilaria verrucosa (Huds.) Papenf.	606±64
Laurencia coronopus J. Ag.	68±7
Laurencia obtusa (Huds.) Lamour.	66±10
Phyllophora nervosa (DC.) Grev.	136±14
Polysiphonia denudata (Dillw.) Kütz.	598±47
Polysiphonia elongata (Huds.) Harv.	405±27
Polysiphonia subulifera Harv.	273±12

It was established that the surface area of algae populations in phytocenoses undergoes significant change in different seasons subject to the vegetation depth. Maximum values of thallus surface in sublittoral plant formations are characteristic of late summer when the weight of summer and annual algae reaches its top.

Thus, in plant formations of the investigated sea area, by range of species - and in deeper waters also by biomass - prevailing are red algae with divided and filiform thallus that are structural and functional dominants in these plant communities.

When determining heavy metal contents in water and in bottom sediments in the area, we found that heavy metal concentrations in the abiotic substance are by and large typical for slightly polluted sea zones. The content of dissolved metal forms in water was slightly above the average concentrations in coastal waters. In bottom sediments, metal concentrations were by a huge ratio higher than in water per se. At that, about half the metals found in the sediments were bound in strong complexes that can be extracted only by an EDTA solution and, apparently, were not available to many an osmotrophic aquatic organism. At the same time, the oxidized and reduced metal forms we revealed can travel to the environment thus enriching bottom waters. It was found out that metal concentrations in water vs. sediments are slightly different, including such pairs of elements close to each other in their chemistry as Zn: Cu and Zn : Cd, which is a sign that some biological way may exist of removing them from the solution, since high metal concentrations in bottom sediments can be hardly accounted for solely by absorption processes (Table 3).

TABLE 3
HEAVY METAL CONTENT IN WATER AND BOTTOM SEDIMENTS

Metal	Water, µg /l	Bottom sediments, µg /g of dry weight	Metal content in bottom sediment fractions, %		
			I	II	III
Cu	4.9±0.8	35.5±7.2	46.0	14.6	39.4
Zn	15.4±3.0	33.8±4.6	45.2	-*	54.8
Cd	0.7±0.2	3.2±0.5	41.2	-*	58.8
Ni	3.4±0.4	24.8±5.6	42.9	13.4	43.7
Pb	1.9±0.3	9.4±2.3	40.1	12.4	47.5

Note: -*- not found F I – extraction: 25% NH^3OHCI + 35% CH_3COOH
F II- extraction 30% H_2O_2, F III – extraction 0.1 M EDTA at 70^0 C

Analysis of algae belonging to different taxonomic ranks in a plant community revealed differences in heavy metal content: red algae generally accumulate more Zn, Cu, Ni, Cd and Pb than brown and green algae. At that, metal content differences were revealed not only between different algae groups, but also orders, families and geni. Thus, the content of some metals in algae tissue cells of genus *Laurencia (L. coronopus, L.obtusa)* sampled for analysis in one of the bays differed considerably. A similar way of accumulating certain heavy metals was also observed in algae having a thallus surface area close to the specific one.

Analysis of the dynamic pattern of some heavy metals content in water and algae showed that high concentrations of copper, cadmium and lead in water do not always go hand in hand with maximum accumulation of these in algae's thallus. It seems to be obvious that in the absence of local pollution, algae preferentially accumulate the metals that are necessary for their metabolism. The top content of copper in algae was observed in April and September, whereas that in water occurred in July and November. The content of cadmium in algae thallus went up during summer time and considerably dropped in autumn. Maximum content of lead in algae fell on June-July when the man-induced impact of transportation in coastal ecosystems goes up radically.

Of agar-producing algae (*Ph.nervosa, G.verrucosa* , *G.latifolium*) sampled for analysis in different areas, it is characteristic to have a rise in metal content from January till September [2]. A similar pattern in metal accumulation is characteristic of red algae of the genus *Polysiphonia* (*P.sanguinea* и *P.subulifera*): during the period from April till September the rate of metals in thallus tissues perceptibly increased. To theses algae is an also peculiar species difference in metal accumulation, which is first and foremost associated with their formation: the specific thallus surface area of *P.sanguinea* is considerably larger than that of *P.subulifera*.

On the basis of measurements concerning metal content in algae, we could identify species with similar patterns of metal bioaccumulation - of the metals belonging to a high-priority list of potentially hazardous chemical substances. Relying on cluster analysis, we can count the following species as similar in metal accumulation: *C.corymbosum, C.strictum, P.sanguinea* and *Gelidium crinale*. At that, to the same cluster belong the algae having highly divided thallus, i.e. the ones with large specific surface area of thallus and S/W ratio. At the same time, mineral composition of the algae varied in quite a wide range: ash content of the dry weight made up from 17.9% in *G.latifolium* and 24.6% in *C.strictum* to 80.7% in *C. mediterranea* (Table 4).

TABLE 4
HEAVY METALS IN THE THALLUS OF DOMINANT RED ALGAE SPECIES

Algae	Zn	Cu	Ni	Cd	Pb
Ceramium corymbosum	83.4±12.0	27.0±3.3	19.3±2.6	3.4±0.7	4.9±1.3
Ceramium rubrum	66.5±11.6	19.4±2.0	8.3±1.5	2.2±0.3	3.8±0.5
Ceramium strictum	77.8±7.0	18.8±2.9	13.6±1.2	2.6±0.5	5.8±1.0
Gelidium crinale	73.3±10.2	23.5±1.7	17.1±1.4	2.9±0.4	4.0±0.6
Gelidium latifolium	58.1±9.6	15.0±1.8	10.1±1.0	2.0±0.5	6.1±1.9
Gracilaria verrucosa	83.4±8.2	11.5±2.1	7.7±1.8	1.9±0.3	2.3±0.6
Laurencia coronopus	27.6±2.0	7.8±1.3	6.9±1.2	1.8±0.4	2.6±0.3
Laurencia obtusa	16.6±1.9	5.9±1.2	5.6±1.0	1.3±0.3	2.0±0.4
Polysiphonia elongata	60.8±8.1	20.1±3.0	18.8±3.1	3.1±0.6	3.0±0.4
Polysiphonia sanguinea	75.8±3.9	25.8±2.3	26.3±3.0	4.7±0.3	3.5±0.8
Polysiphonia subulifera	53.7±5.0	18.7±2.0	8.3±1.6	0.9±0.2	2.2±0.4
Phyllophora nervosa	63.2±8.2	21.4±7.0	19.2±3.9	1.9±0.3	3.0±1.0
Cystoseira crinita	50.0±6.3	11.9±1.5	3.3±0.5	1.1±0.2	1.7±0.3
Ulva rigida	61.8±6.9	12.6±1.9	4.1±0.8	1.2±0.2	1.0±0.2

IV. CONCLUSION

The established facts about metal accumulation in sea grass - linked to thallus formation, along with species- and environmentally specific peculiarities of the latter - turn out to be determinative for choosing monitor plants in different taxons and ecological groups in the context of sea ecosystems pollution. This is particularly important for coastal ecosystems that are most susceptible to human impact.

In the sublittoral zone of the sea, red algae belonging to various bottom plant formations play the role of functional dominating species of the biocenosis. High biodiversity of red algae occupying different biotopes in the coastal area is what allows us to use them as indicator/monitor/biomarker-plants when assessing the ecological state of the community when water is contaminated by most dangerous substances. Red algae with a large specific surface area of thallus are capable of accumulating high concentrations of hazardous pollutants including heavy metals. It is exactly this peculiarity that allows to successfully use the algae - along with other types of sea organisms - in bio-filters involved in the processes of self-purification of natural water, relying at that both on the group-specific and selective pattern of heavy metal accumulation [6].

The above statement is based on the following established facts: First, algae provide us with a comprehensive picture of the ecosystem state, which is particularly important when the latter's pollution demonstrates a discreet pattern. Second, algae have an explicit capability of accumulating heavy metals, which both contributes to the accuracy of chemical examinations and indicates that bio-available forms of the former are present in the environment. Third, using algae as monitor plants opens up a promising perspective for developing practical guidance on how to remove heavy metals from polluted water basins via the use of bio-filters implanted in artificial reefs.

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