J. Black Sea/Mediterranean Environment
Vol 12: 129-153 (2006)

Biogenic Properties of Deep Waters from the Black Sea Reduction (Hydrogen Sulphide) Zone for Marine Algae

Gennady G. Polikarpov, Galina E. Lazorenko and Natalya N. Tereschenko

The A.O. Kovalevsky Institute of Biology of the Southern Seas, National Academy of Sciences of Ukraine.
Pr. Nakhimova, 2, Sevastopol 99011, Ukraine.

Abstract

Generalized data of biogenic properties investigations of the Black Sea deep waters from its reduction zone for marine algae are presented. It is shown on board and in laboratory that after pre-oxidation of hydrogen sulphide by intensive aeration of the deep waters lifted to the surface of the sea, they are ready to be used for cultivation of the Black Sea unicellular, planktonic, and multicellular, benthic, algae instead of artificial medium. Naturally balanced micro- and macroelements structure and enriched content of nutrients are characterized the Black Sea deep waters. As a matter of fact after full oxidation of hydrogen sulphide they become the high-quality nutrient medium comparable with and even exceeding of the best man-made cultural medium.

Keywords: algae, Black Sea, deep waters

Introduction

The life of marine hydrobionts, their populations and communities depends directly on environment quality.

e-mail: g_polikarpov@mail.ru

Hydrochemoecological investigations assess both the chemical structure of environment and the state of selected test-objects: marine unicellular algae, macrophytes and animals. The study of quality of waters from the Black Sea reduction zone for oxibionts is of great special interest in relation to existence of the huge thickness of anoxic waters in this reservoir contained the natural xenobiotic (in relation to oxibionts) - hydrogen sulphide. Moreover, in these waters the composition of many chemical elements and their concentrations differ from those in the oxygen zone of the Black Sea (Skopintsev, 1975). Hydrological, windy, seasonal and climatic factors on the Black Sea, intensive shipping, regulated rivers flowing in this deep reservoir, an increase of anthropogenic press on its ecosystem can change the upper level of the hydrogen sulphide zone. Sometimes such level can reach the horizon 150 m and even closed to the surface (Skopintsev, 1975; Sorokin, 2002). The appearance of hydrogen sulphide on these horizons and possible invasion of it into oxygen zone can produce the negative consequences of xenobiotic impact on hydrobionts inhabited in oxygen area of the Black Sea.

The first investigations on influence of the Black Sea deep anoxic waters from the reduced zone upon marine plants were initiated by research programs of the Radiation and chemical biology Department of the A.O. Kovalevsky Institute of Biology of the Southern Seas, National Academy of Sciences of Ukraine, in 1984 on board the R/V “Professor Vodyanitsky”. The deep waters from the Black Sea reduced zone were studied from different points of view. First of all, it was necessary to assess the negative effect of such medium contained original maximum concentrations of hydrogen sulphide on selected test-objects immediately after lifting of deep waters aboard of the ship laboratory. Second variant was the study of the deep waters quality exposed to the atmosphere after full oxidation of hydrogen sulphide in them on marine organisms (Polikarpov et al., 1985; 1986 a,b,c, 1987, 1988, 1990, 2004; Lazorenko and Gulin, 1987; Lazorenko et al., 1992; Lazorenko, 1998; Tereshchenko, 1986).

It was shown that different hydrobionts have their own sensitivity to natural concentrations of hydrogen sulphide in the Black Sea deep waters. The most sensitive among them are the Black Sea amphipods who withstand the hydrogen sulphide concentration up to 1.5 $mg \cdot L^{-1}$ (Polikarpov et al., 1985). The great set of results on influence of waters from the Black Sea reduced zone had been received by the authors on marine algae (Polikarpov et al., 1986 a,b,c; 1987, 1988, 1990, 2004; Lazorenko and Gulin, 1987; Lazorenko et all., 1992; Lazorenko, 1998; Tereshchenko, 1986).

The purpose of this work was to carry out the complex analysis of the investigation of the Black Sea reduced zone natural waters biological quality for 7 main species of phytoplankton algae and for one green seaweed *Ulva rigida* Ag.

Material and methods

Our experiments with algae were carried out both on the board of the RV «Professor Vodyanitsky» and in the IBSS laboratory condition. The choice of objects was connected with their role in the Black Sea ecosystem. Marine phytoplankton, as the first level of trophic nets, produces the primary organic matter in the seas and oceans. Therefore subsistence of higher trophic levels in ecosystems depends on the reaction of phytoplankton to the quality of the environment. In our experiments with the Black Sea phytoplankton the algologically pure cultures of Pyrophites (*Prorocentrum micans* Ehr., *Peridinium trochoideum* (Stein) Lammermann, *Gymnodinium kovalevskii* Pitz., *Gymnodinium sp*.) and Bacillariophyta (*Ditylum brightwellii* (West), *Coscinodiscus granii*, *Sceletonema costatum)* were used. The choice of *U. rigida* was done because this seaweed is widely spread in coastal areas of the Black Sea. It is well known that this macroalga is an indicator of different kinds of environment pollution (Güven and Topçuoğlu, 1994). Belonging to mesosaprobic hydrobionts *U. rigida* is considered as perspective species for mariculture.

In our experiments with the Black Sea microphytes the main indicators of their state were the rate of cells division and cells number. The Black Sea waters collected from the depths of 1000-2000 m were the mostly investigated media. The concentrations of major biogenic elements in deep waters from the reduced zone of the Black Sea and in its surface layers are very different (Skopintsev, 1975; Sorokin, 2002). First of all it is concerned to phosphorus (Skopintsev, 1975). The important role of this macroelement in bioenergetic and biosenthetic processes is well known. Therefore we collected and studied waters from different depths of the reduced zone with different concentrations of phosporus. For determination of phosporus and hydrogen sulphide in comparable media (surface and deep waters) the standard hydrochemical techniques were used (Methods, 1978). Temperature of used media was 14-15^{o}C during all experiments. In case of phytoplankton algae were conducted in three replications. In each glass with the investigated medium the same initial number of cells was added. During 17 days the aliquote volume of cells suspension, equaled to 0.1 mL, was picked out for the definition of the cells quantity. The scheme of experiments with the Black Sea microphytes is given in Fig. 1.

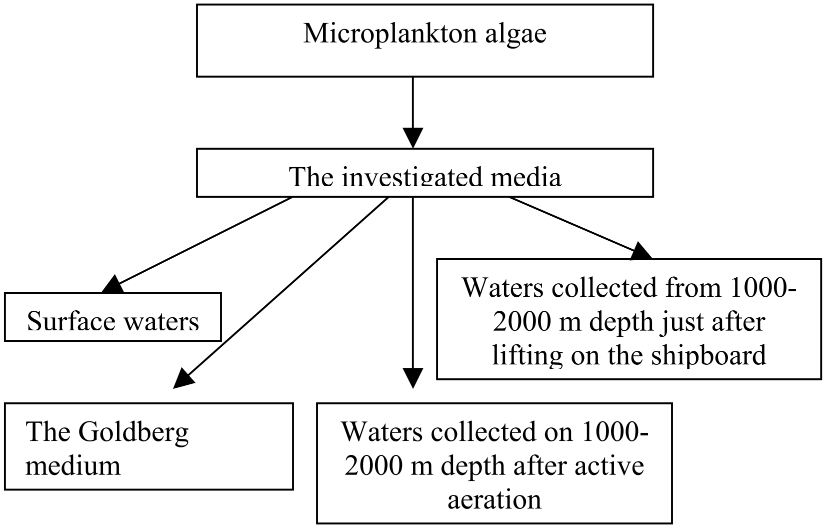

Fig. 1. The scheme of experiments with the Black Sea microphytes

The seaweed *U. rigida* was used for assessment of possible influence of deep waters after their aeration on its kinetics of phosphorus metabolism. The investigation of phosphorus metabolism between the seaweed and the medium was carried out by the technique of traced atoms with utilization of ^{32}P. High sensitivity of this method and rapid testing with its help, the absence of side effects of the radioactive indicator for an organism in combination with the technique of fragments give the possibility to carry out of analysis on the same samples of the seaweed which is necessary for reliable definition of metabolism kinetics's parameters. They are: the concentration factor (CF) ^{32}P, the rate of mineral phosphorus accumulation by the seaweed from the medium (V, $\mu P \cdot L^{-1} \cdot h^{-1}$), the part of ^{32}P derived from *U. rigida* in water without any amount of the radioactive indicator. Deep waters were collected from 1000 m. Before the beginning of experiments these waters were actively aerated for 6 hours. The scheme of the experiments with *U. rigida* is given in Fig. 2.

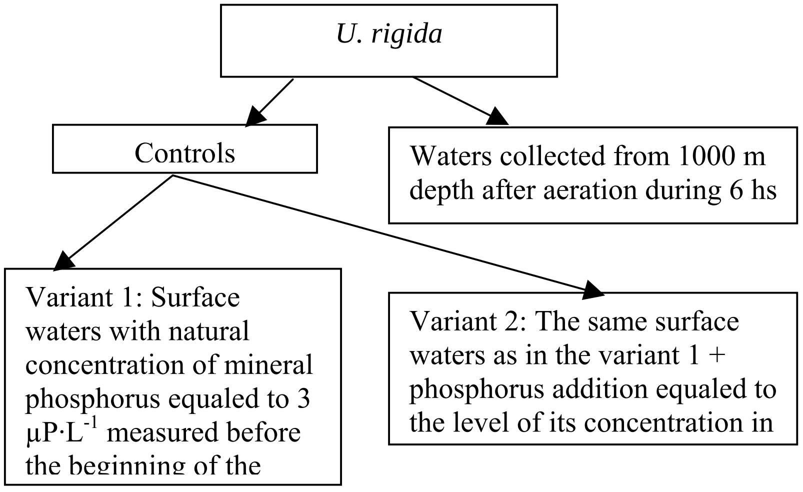

Fig. 2. The scheme of the experiments with *U. rigida*

The selection of two variants of the control was caused by dependence of ^{32}P accumulation by this seaweed on the concentration of mineral phosphorus in waters (Egorov and Tereschenko, 1985). In the experiments with this seaweed its fragments with 15 mm in diameter were incised from central part of *U. rigida* thallus. The initial biomass of such fragments was 10-12 mg wet weight. Ten fragments were added to the experimental media with volume 1-2 L. In each temporal point 8-10 fragments and 3-5 samples of waters were analyzed. All experiments were carried out in three replications. The temperature of waters in aquaria was 14-15^{o}C. The average mean was calculated. Its confidence interval was defined with 95% reliability.

Results and Discussion

Concentrations of hydrogen sulphide and phosphorus in waters collected from depths of 1000 and 2000 m in the reduced zone of the Black Sea for our experiments with algae (Table 1) were in agreement with known data for the same horizons of the sea (Skopintsev, 1975; Sorokin, 2002).

Table 1. Initial concentrations of hydrogen sulphide and phosphorus in waters collected in the reduced zone of the Black Sea for the experiments with algae

№ station	Horizon, m	Hydrogen sulphide, $mgH_2S{\cdot}L^{-1}$	Phosphorus, $\mu P{\cdot}L^{-1}$
1	2000	9.98	300
2	1000 1900	9.72 11.95	246 336
3	1000 2000	10.15 12.20	223 264
4	1000 2000	10,50 11.56	345 340
5	1000 2000	9.81 11.76	250 280

Various concentrations of hydrogen sulphide in experimental media are obtained by aeration of deep waters during different time. The dependence of hydrogen sulphide concentrations in waters collected from horizon 2000 m on the time of their aeration is presented as an example in Fig. 3.

The Black Sea microphytes

The experiments with one of investigated the Black Sea microalgae *P. micans* were carried out in two series (Table 2). The change of temporary dependencies of *P. micans* cell numbers is given in Fig. 4.

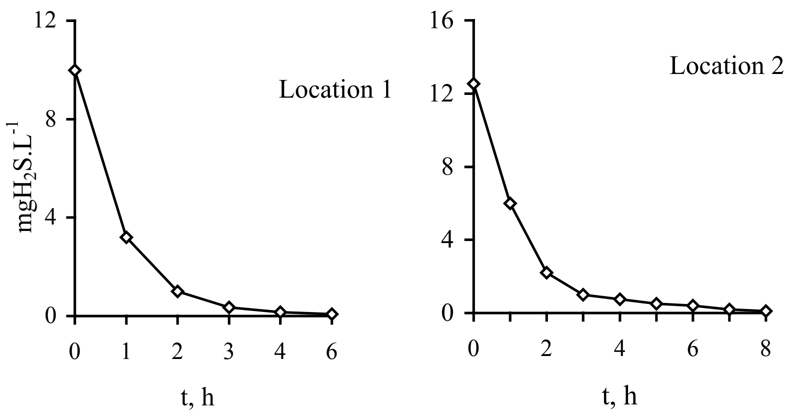

Fig. 3. The change of hydrogen sulphide concentrations in waters collected
from the depth 2000 m during its active aeration

Variant of experiment	The depth of sampling, m	Hydrogen sulphide, $mgH_2S \cdot L^{-1}$	Phosphorus, $\mu P \cdot L^{-1}$	Coefficients		R^2
				a	b	
Series I						
1	0	0	3			
2	1000	9,72	246	165	0.158	0,90
3	-/-	1,34	-/-	168	0.169	0,91
4	-/-	0,57	-/-	196	0.178	0,90
5	-/-	0,4	-/-	203	0.196	0,91
Series II						
1	0	0	9			
2	2000	12,2	264	173	0.172	0,98
3	-/-	9,49	-/-	183	0.178	0,99
4	-/-	7,71	-/-	206	0.179	0,98
5	-/-	4,57	-/-	214	0.181	0.98

Table 2. Initial concentrations of hydrogen sulphide, mineral phosphorus and values of coefficients
in the equation and the approximation reliability of exponential curves in Fig. 4

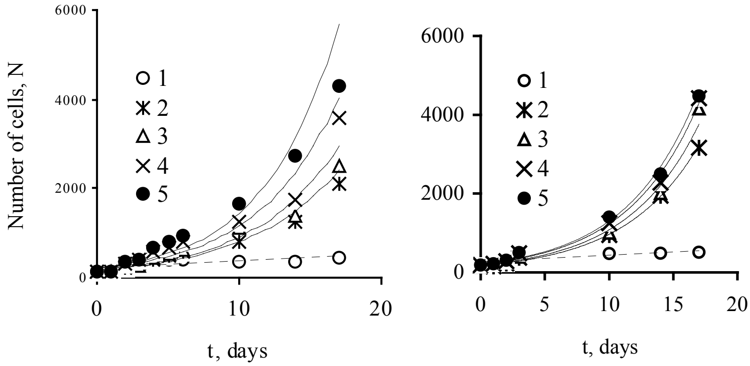

Fig. 4. The time dependence of *P. micans* cells number: in surface waters (I), in deep waters with different natural hydrogen sulphide (2-5) and phosphorus concentrations
(246 μP·l^{-1}, series I and 264 μP·l^{-1}, series II)

The curves 2-5 in Fig. 4 are exponential and described by the equation (1):

$$\mathbf{N = a \cdot e^{bt}} \qquad (1),$$

where: **N** – *P. micans* cells number,

t – the time of its cultivation in the medium,

a and **b** – the coefficients.

The values of approximation reliability (R^2) (Table 2) of the curves 2-5 in Fig.4 were high. They were ranged from 0,9 to 0,98. The coefficient ”a” in equation (1) corresponds to the number of cells of the microphyte (*P. micans),* which is necessary for its population at the beginning of the experiment to get the comparable level of survival under different initial concentrations of hydrogen sulphide in the medium. The coefficient ”b”, determining the curves slope to the ordinate axis (number of cells

N) can be estimated as an average rate of cells division (the average level of cells number change in a unit of time) at exponential time dependence of this index of population function. This coefficient most likely represents the effect of initial hydrogen sulphide concentrations in media on the division rate of *P. micans* cells. They were increased from 0,158 (9,72 $mgH_2S·L^{-1}$) to 0,196 (0,4 $mgH_2S·L^{-1}$) in experiments of the series I. So, the difference made up to 24%. In the series II coefficient "b" was changed substantially less (about 4%). First of all this distinction can be caused by differences of hydrogen sulphide initial concentrations in compared media varying from 9,72 to 0,4 $mgH_2S·L^{-1}$ (series I) and from 12,2 to 4,57 $mgH_2S·L^{-1}$ (series II). In deep waters used in experiments with *P. micans* the difference of mineral phosphorus concentrations was 18 $\mu P·L^{-1}$ (Table 2). Perhaps mineral phosphorus is the second factor affecting numerical value of coefficient "b". Directly unaccounted factors influencing this coefficient value are others, available in deep waters, micro- and macroelements or organic matter needed for optimal development of *P. micans.*

The curves 1 (Fig. 4) representing the results of experiments with *P. micans* in surface waters with values of approximation reliability (R^2) 0.51 and 0.65 accordingly are linear corresponding to the dependence of the following type:

$$\mathbf{N = cx + d} \qquad (2),$$

where: **N** – the number of population cells,

c – coefficient determining the curves slope to the ordinate axis,

d – the initial cells number. It was equal to 234 (series I) and 273 (series II).

The results of the study of the Black Sea diatoms *G. kovalevskii* and *D. brightwellii* are plotted in Fig.5. Analogically to *P. micans,* the equations described the change of cells number in investigated diatoms with time of the observation have the exponential type as well.

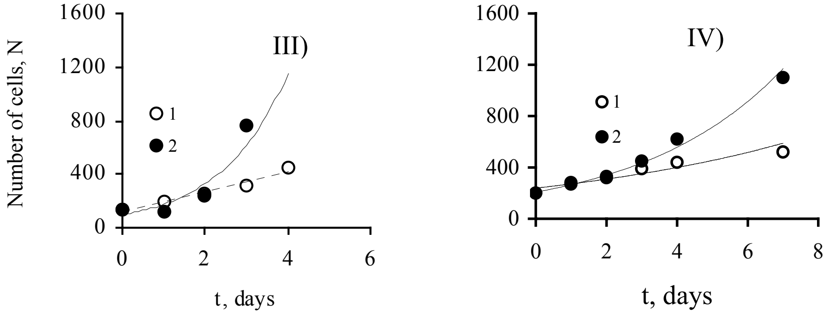

Fig. 5. The dependence of cells number of *G. kovalevskii* (III) and *D. brightwellii* (IV)
on the experiment's time in surface (1) and deep waters (2) of the Black Sea

In the study with deep waters (R^2=0,9) the kind of the equation described the change of cells number of *G. kovalevskii* with time was: $N = 94e^{0.624t}$. The number of cells this microphyte in surface waters also followed the exponential law. It was described by equation $N=140e^{0.29t}$ under R^2 = 0,98. Analogically for *D. brightwellii* the kinds of the equations were: $N = 207e^{0.247t}$ (R^2 = 0,98 for deep waters) and $N = 239e^{0.129t}$ (R^2 = 0,88 for surface waters). So, the coefficients "a" and "b" in equations for *G. kovalevskii* and *D.brightwellii* represent the same concept loads as for *P. micans.*

One can see that the adaptation period for the Black Sea investigated microphytes depends on their species belonging, initial hydrogen sulphide concentration and time oxidation of this natural xenobiotic in the medium. The relation of rates division among investigated the Black Sea microalgae in deep and surface waters (Fig. 6) shows that, in spite of maximal high initial concentrations of hydrogen sulphide in the deep waters, there was no fatal toxic effect on the phytoplankton.

The surface waters of the Black Sea do not content of biogenic elements in quantities sufficient for more then 17 days of the cultivation of microphytes (Skopintsev, 1975; Lanskaya, 1967) First of all it is concerned of phosphorus which concentrations in samples collected in different areas of the Black Sea for the experiments with investigated microalgae were ranged from 3 to 9 $\mu P \cdot L^{-1}$.

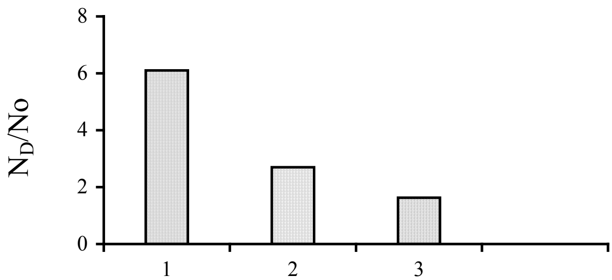

Fig.6. The relation of cells number of the Black Sea pyrophytes in deep waters (N_D) with initial concentration of hydrogen sulphide equaled to 12.2 $mgH_2S \cdot L^{-1}$ and in surface waters (No) in 17 days from the beginning of the experiments: 1 – *P. micans*, 2 – *G. kovalevskii*, 3 – *Gymnodinium sp.*

At the same time in deep waters collected from depths of 1000-2000 m, phosphorus concentrations were in 30-130 times higher.

Similar to phosphorus, other elements can also affect a cells number of unicellular algae in the medium of their cultivation (Lanskaya, 1967). Therefore in another series of experiments the quality of deep oxidized waters from the reduce zone of the Black Sea was compared to the traditionally used of Goldberg's medium in laboratory for cultivation. As known the base of this medium are surface waters of open part of the seas. The content of microelements in the nutritions Goldberg's medium is higher (Lanskaya, 1967) then in deep waters (Skopintsev, 1975; Sorokin, 2002) except for manganese (Table 3). Our experiments have shown that the rate of division and cells number of the Black Sea microalgae (after full oxidation of hydrogen sulphide in investigated deep waters) were reliably higher then in the Goldberg's medium (Fig. 7).

Perhaps as we think it is caused by properties of the salts composition including microelements content (Skopintsev, 1975). Other words, the favorable action of the Black Sea deep waters on the microphytes is obviously caused by naturaly equilibriated composition of chemical elements, their forms and other biologically active matter which are in total more available for these algae.

Table 3. Concentrations of important chemical elements (mg-atom·L^{-1}) in the Black Sea deep waters and the Goldberg's medium

Chemical element	Water from the depth of 1000 m (Skopintsev, 1975)	The Goldberg's medium (Lanskaya, 1967)
N	82	2014
Co	0,1	102
P	7	20
Fe	0,5	1
Mn	5,3	0,8

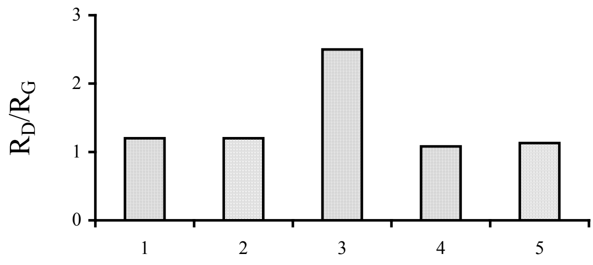

Fig. 7. The ration of division rate of microalgae in aerated waters collected
from the depth of 2000 m (R_D), and in the Goldberg's medium (R_G):
1 – *D. brighthwellii*,2 – *C. granii*, 3 – *P. micans*, 4 - *S. costatum*, 5 – *P. trochoideum*

Thus the waters from the reduced zone of the Black Sea even high initial concentrations of hydrogen sulphide do not produce fatal action on the Black Sea various phytoplankton species. Moreover after full oxidation of hydrogen sulphide the biological quality of deep waters is higher then in cultural nutritious Goldberg's medium. This conclusion was also confirmed by another methods of definition investigated by us the microphytes reaction on the Black Sea deep waters (Lazorenko and Gulin, 1987; Lazorenko et al., 1992). Some results of ^{14}C kinetic accumulation by diatom *D. brightwellii* in deep waters after their full oxidation without hydrogen sulphide, in the surface waters and in Goldberg's medium were obtained (Lazorenko and Gulin, 1987). In our investigation fulfilled in collaboration with Moscow Engineering-Physical Institute we obtained using the method of delayed fluorescence that deep waters do not exert a negative influence upon plankton algae. Moreover, as demonstrated above, such waters are favorable medium for their cultivation (Lazorenko et al., 1992).

The Black Sea macrophyte

The ^{32}P kinetics of accumulation by the Black Sea green macroalga *Ulva rigida* studied in aerated deep waters in comparison with surface waters with phosphorus concentration equaled to 3 $\mu P \cdot L^{-1}$. It was shown that ^{32}P accumulation by fragments of this the sea lettuce thallus occurs more intensive in deep waters with mineral phosphorus concentration 230 $\mu P \cdot L^{-1}$ (Fig. 8).

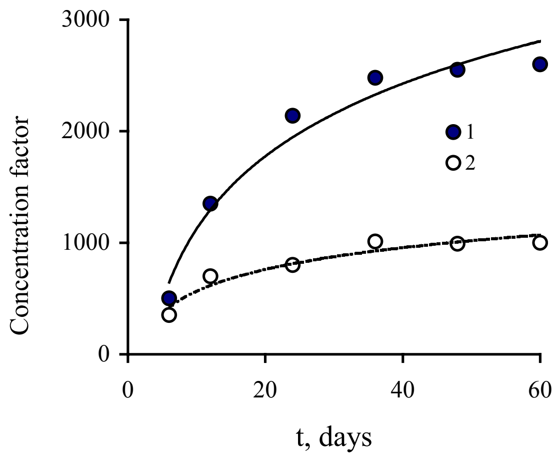

Fig. 8. The dependence of ^{32}P concentration factor change in the sea lettuce on the experiment time

Concentration factor (CF) of ^{32}P in the sea lettuce in the first 2.5 days of the experiment is described by the equation:

$$\mathbf{CF = a \ln t - b} \qquad (3),$$

where: **a** and **b** – coefficients.

In particular to the experiment with deep waters ($R^2 = 0.96$) (curve 1) the equation was:

$$CF = 939.7 \ln t - 1039.5.$$

For the case of surface waters the curve 2 was described by the equation:

$$CF = 281.5 \ln t - 83.2 \ (R^2 = 0.93).$$

As one can see the differences between values "a" and "b" in the equations described by curves 1 and 2 in Fig. 8 are significant. For the coefficient "a" it was 3.3 times and for coefficient "b" was 12.5 times. To the end of the experiment (Fig. 8), CF of ^{32}P by the sea lettuce in deep waters was in 2.6 times higher then in surface waters. The different phosphorus content in the comparable media is one of the reason. For the definition of possible factors regulated the phosphorus metabolism of *U. rigida* the influence of different additions of mineral phosphorus to surface waters on the rate of ^{32}P accumulation by this seaweed was studied. Before the beginning of the experiments fragments of alga were kept in the deep and surface waters during 72 hs for the formation of intracellular content different levels of this element in the experimental samples and for the assessment of possible influence of its adaptation to different phosphorus concentrations in the media. The scheme of these experiments is given in Fig. 9.

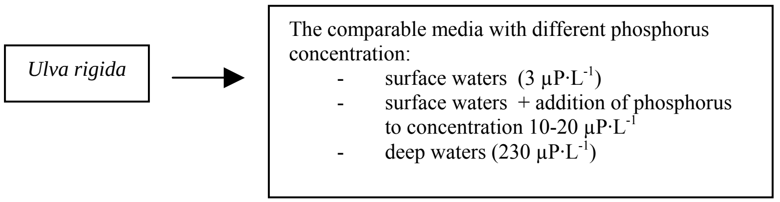

Fig. 9. The scheme of the different media phosphorus concentration experiment

The results of the sea lettuce study are illustrated in Fig. 10. They show that with addition of about 10-20 μP·L^{-1} of the mineral phosphorus to surface waters the rate of ^{32}P accumulation by this alga practically did not depend on the condition of its preliminary adaptation. With increasing of the concentration of mineral phosphorus added to the surface waters the ^{32}P rate accumulation by the sea lettuce in such media was decreased in 3.6 times for alga preliminary adapted to the aerated deep waters with mineral phosphorus concentration equaled to 230 μP·L^{-1} (curve 1) in comparison to the alga cultivated before the start of the experiment in surface waters (curve 2). When the adaptation of alga had been in the aerated deep waters (Fig. 10, curve 1) the kind of ^{32}P rate of accumulation by the sea lettuce (V) depended on the mineral phosphorus concentration in waters (C) with the approximation reliability value 0.94 was logarithmic: $V = 61.6\ln C - 106.4$. In case of alga's preliminary adaptation to surface waters (Fig. 10, curve 2) the dependence was described by the same kind of equation: $V = 15 \ln C - 17$. The value of the approximation of reliability was equaled to 0.98.

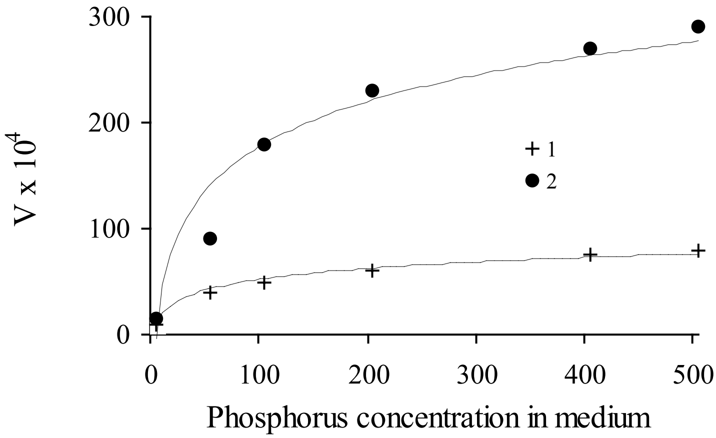

Fig. 10. The dependence of ^{32}P rate accumulation by *U. rigida* (V, μP·L^{-1}·h^{-1}) on the phosphorus concentrations (μP·L^{-1}) in medium after preliminary alga adaptation for 72 hs
in aerated deep waters (1) and surface waters (2)

So, after preliminary adaptation in the medium, some intracellular phosphorus concentration influencing on the process of ^{32}P accumulation by alga was formed. It was decided to use the different media to research such phenomenon: aerated deep waters (1), initial surface waters (2) and the same surface waters with addition of mineral phosphorus (3) as the comparable media. Phosphorus concentrations in them were equaled to 230 μP·L^{-1} (medium 1), 3 μP·L^{-1} (medium 2) and 230 μP·L^{-1} (medium 3 as the sum with additional phosphorus up to 227 μP·L^{-1}). ^{32}P accumulation by the sea lettuce was studied in the comparable media. ^{32}P concentration factors were taken as the parameter of this process. The results of the experiments are given in Fig. 11.

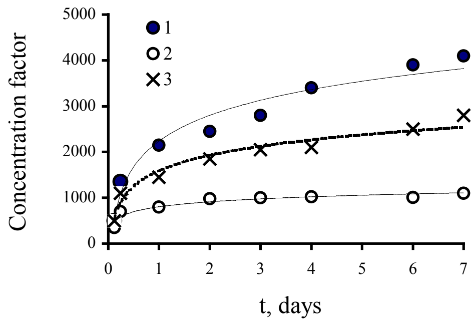

Fig. 11. The time dependence of ^{32}P concentration factors for *U. rigida* cultivated
in aerated deep waters (1), surface waters (2) and surface waters with phosphorus addition (3)

On the 7th day of the experiments the ^{32}P concentration factors were equaled to 4000 (aerated deep waters), 1080 (surface waters) and 2900 (surface waters with addition of phosphorus). So, phosphorus content in medium is one of the regulating factor of ^{32}P accumulation by the sea lettuce. But it is not the only one parameter determinates this process intensity. The influence of phosphorus content in the media on its metabolism in the sea lettuce is confirmed in the study of ^{32}P loss by this alga. The scheme of such experiment is given in Fig. 11.

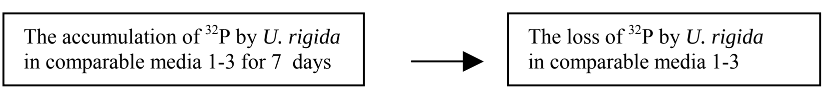

Fig. 11. The scheme of experiment of ^{32}P loss by *U. rigida*

The analysis of ^{32}P loss by the sea lettuce (Fig. 12) showed that this isotope accumulated by alga in the media with phosphorus concentration equaled to 230 μP·L^{-1} (curves 1, 3) reflects its preferential intake into non-exchangeable funds of phosphorus which *U. rigida* uses for the growth and production in the conditions with high mineral phosphorus concentrations in the medium.

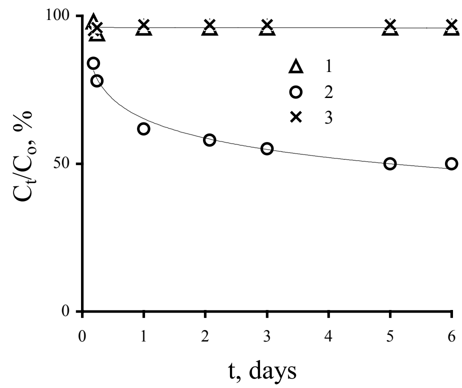

Fig. 12. The time dependence of ^{32}P loss by the sea lettuce in the compaable media:

1 – surface waters with phosphorus addition,
2 – natural surface waters,
3 – aerated deep waters, Co and C_t - ^{32}P concentration in alga on 7th day of its accumulation and in time of the loss

The kinetics of ^{32}P loss in surface waters (curve 2) and deep waters (curve 3) (Fig. 12) are reliably different. The losses of ^{32}P in deep waters and surface waters with phosphorus addition up to the same concentration of this element in the medium were the same. So, the phosphorus concentrations in the medium play the leading role in the process of ^{32}P loss by *U. rigida*. An initial level of phosphorus content in alga, its quantity in waters and other hydrochemical components which are in oxidated deep waters from reduced zone of the Black Sea are components of the regulation of the metabolism process between *U. rigida* and medium of its cultivation. Moreover, phosphorus is not a unique favorable factor effecting on ^{32}P accumulation by green alga *U.* r*igida.* The investigation of the Black Sea deep waters showed that the influence aeration of this medium on this process was not negative.

Conclusion.

The results of the study of the biogenic properties of waters from the Black Sea reduced zone showed that action of these deep waters were not negative for investigated species of the Black Sea phytoplankton algae even with initial maximal hydrogen sulphide concentrations.

After full oxidation of this xenobiotic the Black Sea deep waters can be used as nutritious medium for cultivation of unicellular plankton and multicellular benthic algae in laboratory conditions and in a mariculture (closed, semi-natural as well as in open sea).

In case of rapid lifting/invasion of deep hydrogen sulphide waters into oxygenic zone (including the reason of natural cataclysm or large-scaled anthropogenic industrial or military activities), the different intensity of “blooming” of the proper parts of the sea have to be expected. It is required to take it into consideration of ecosystems protection in the sphere of nature-conservative measures and ecological prognosis.

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Received: 10.04.2005

Accepted: 20.06.2005