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Recently Discovered New Biospheric Pelocontour Function in the Black Sea Reductive Bathyal Zone

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Abstract

Two remarkable scientific events happened recently (Zaitsev, Polikarpov, Egorov et al., 2007, 2008) in the field of the newly developed deep-sea ecology and molismology of the hydrogen sulphide pelocontour in the Black Sea bathyal zone:

- discovery (in 2007) of *viable spores of oxybionts* in this aggressive zone against openly living oxybionts – those organisms which normally inhabit all three biocycles of the biosphere – the land, the fresh waters and the marine environment as well as
- Determination (in 2008) of the *safe sojourn duration of the oxybionts' spores* by radioisotope dating in layers of the studied pelocontour sediment.

Generalization and discussion of these events are presented.

Key words: Biosphere, the Black Sea, pelocontour, hydrogen sulphide bathyal zone (HSBZ), oxybionts, anoxybionts, living spores of oxybionts from the land, fresh waters and the sea, radioisotope dating, time of spores survival in the HSBZ.

Introduction

Discovery of hydrogen sulphide in the deep layers of the Black Sea in 1890 by the well-known oceanographers and geologists N.I. Andrusov and I.B. Shpindler (Andrusov 1890) was a crucial event in the knowledge of its ecosystem.

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Further investigations showed that about 87 % of its water column (pelagial) and about 75 % of its bottom (benthal) are naturally contaminated by this toxic substance and are inhabited only by anoxic sulphur reducing bacteria (Leonov 1960, Muromtsev 1978, Constantinov 1986, Zenkevich 1963) and, as shown later, also by *Archaea* (Lam et al. 2007, cited by Gasol, Pinhassi, Alonso-Sáez, Ducklow, Herndl, Koblížek, Labrenz, Luo, Morán, Reinthaler, Simon 2008).

An unanimous opinion among biologists and ecologists was formed: the originality of the Black Sea in the World Ocean is due *inter alia* to the fact that its water column and bottom are divided into two unequal parts – a small upper “living” zone, rich in oxygen and inhabited by aerobic (oxybiotic) organisms, and a great lower “dead zone”, inhabited by anoxic bacteria). The lower zone from 200 m up to the maximum depth (2212 m) was called an azoic (without life) area (Băcescu et al. 1971). ‘No life, except anaerobic bacteria, exists bellow 200 m in the Black Sea’ was emphasised in the Ocean World Encyclopedia (Groves and Hunt 1980).

Following the methane seeps discovery in 1989 in the Black Sea bathyal (Polikarpov and Egorov 1989), it became clear that coral-like carbonate (aragonite) ‘dwellings’ and the total great fields of their “reefs” are obliged to activity of the most ancient anaerobic archeomicrobes *Archaea* on the basis of methane seeps. The age of such ‘dwellings’, comparable with the age of all stages of the Black Sea evolution after its last connection to the Mediterranean, was measured with the radiogeochronology method (Gulin, Polikarpov, Egorov et al. 2002, Egorov, Polikarpov, Gulin et al. 2003, Polikarpov, Egorov, Gulin et al. 2003).

The absence of living oxybiotic organisms in the lower, “dead”, zone of the Black Sea depends on the absence of molecular oxygen in it – that was the biologists conclusion since XIX century until now. Some inanimate remains of some oxybionts - plants and invertebrates, including morphologically rather well-preserved, founded in the hydrogen sulphide zone, are not proofs that they (“hosts” of the remains) live *in situ* or inhabit the “dead” zone. Their sedimentations areas are determined by processes of “dead bodies rain” and by marine currents.

Material Sources

Founded on scientific ideas of V. I. Vernadsky (1967, 1987), who is the creator of biogeochemistry and biospherology, regarding to the exceptional environmental role of the outside interfaces of the water column, one of the authors of this article (Zaitsev 1961) discovered the marine neuston in the surface microlayer of the Black Sea. This community of organisms proved to be a global (in the World Ocean) biocoenose (Zaitsev 1971). And both authors (Zaitsev, Polikarpov 1964) revealed its key role in the ecological processes, occurred in the water column and at the bottom (Polikarpov, Zaitsev 1969). As a result of development of these researches, the conception of contour biotopes and communities of the sea was proposed for its external boundaries. There are the boundaries between the sea and the atmosphere (aerocontour), as well as sandy beaches (psammocontour), rocky shores (lithocontour), muddy bottom (pelocontour) and river waters (potamocontour) (Zaitsev 1986). The most intensive physical-chemical processes having important biological and ecological consequences occur in all of these contour biotopes. As a result, biocoenoses were formed with a high biological diversity and significant biomasses in these biotopes during the organic evolution. In the second half of the XXth century ecologically critical zones ("hot spots") appeared (Zaitsev, Polikarpov 2007) and started to determine an ecological status of all marine ecosystems.

On the other hand, it was discovered that marine waters lifted from the Black Sea anoxic depths and contacted with the atmosphere are highly fertile ones for marine algae (Polikarpov, Lazorenko, Tereshchenko et al. 1986; Polikarpov, Lazorenko, Tereshchenko 2006). Before the cited above study it seemed "*a priori*" for many marine biologists that such deepwaters should be a xenophobic factor for sea plants because of their different composition and concentrations of chemical substances in comparison to the oxygenated water layer.

In this context it was naturally to concentrate our attention on the pelocontour of the Black Sea bathyal zone, which had been, as mentioned above, considered as dead, hostile, for oxybiotic organisms. The fact that the spores of bacteria and fungi can be found at the bottom of seas and oceans was well-known from the times of V. I. Vernadsky: "… a latent life of spores concentrated in marine muds and sediments …" (Vernadsky 1965, p. 156). But before our teams' researches (Zaitsev, Polikarpov, Egorov et al. 2007, 2008) there were no data, as far as we know, in science on the presence of viable spores of oxybiotic organisms and therefore also on terms of lifeability of viable spores of oxybionts in bottom sediments of the Black Sea bathyal zone.

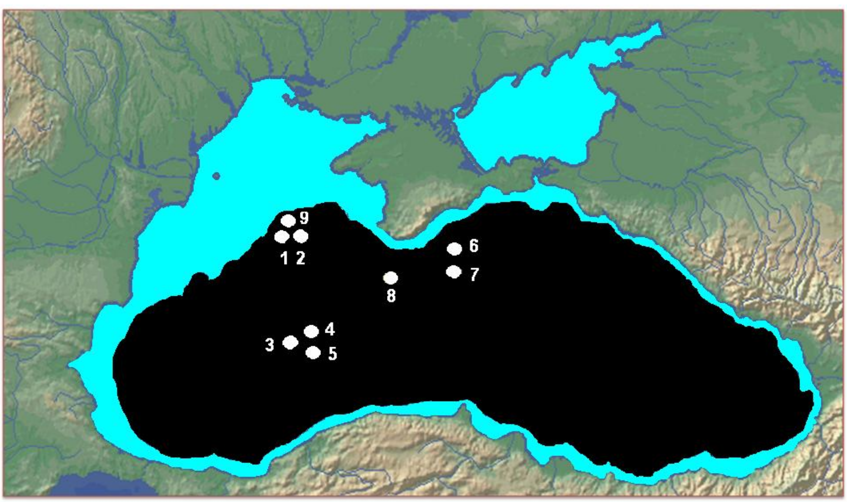
The specialists of the Department of radiation and chemical biology, the Institute of Biology of Southern Seas (IBSS), collected samples of sediments in the Black Sea hydrogen sulphide bathyal at the depths from 730 to 2104 m using column samplers (Zaitsev, Polikarpov, Egorov et al. 2007, 2008) with all possible means of maintenance of sterility (Fig. 1).

Part of these samples was studied at the laboratories of the Department of ecology of contour communities, the Odessa Branch of IBSS, under a 800^x power microscope and the other part was incubated at the same laboratories in presence of oxygen and in anoxic conditions.

Living cultures of aerobic bacteria, unicellular algae and fungi were obtained from spores after different periods of incubation time (in some hours for bacteries, in a week for algae, and in one-two months for fungi).

No living micro- and macroalgae as well as invertebrates were found in our deep sea samples in the Black Sea.

Figure 1. Position of bottom sampling stations (1-9) in the Black Sea hydrogen sulphide bathyal zone at depths from 730 to 2104 m. Black colour – the projection of the hydrogen sulphide zone (on the base of data by Zaitsev, Polikarpov, Egorov et al. 2007, 2008).
*[Published for the first time].



Results and Discussions

Taking into consideration the high scientific novelty and important ecological meaning of the discovered data, the authors' team repeated samplings and each time the same results were obtained: there are dormant stages of oxybiotic organisms in the Black Sea pelocontour of the hydrogen sulphide bathyal zone, which species normally inhabit the upper aerobic zone of the sea, and some of them inhabit the fresh waters and even the terrestrial biotopes (Fig. 2).

Another quintessence of the works presented above was the first determination of viable state periods of oxybionts spores in the Black Sea bathyal (Table 1) fulfilled with the help of radioisotope dating (Gulin, Poikarpov, Egorov, Korotkov 2000, Gulin, Egorov, Stokozov, Mirzoeva 2008).

Table1. Assessment of safe sojourn of oxybionts spores in the Black Sea pelocontour in a latent state up to their germination in laboratory conditions (Zaitsev, Polikarpov, Egorov et al. 2008)

Thickness of studied layers of pelocontour in the point with coordinates ($44^o42.060'$ and $32^o08.831'$), cm	Maximal assessment of these layers age with saved lifeability spores of oxybionts, years
0-1.5	15
1.5-5.0	50

It is clear from the Table 2, that living spores of oxybionts "slept" up to a half of a century before "waking up" in the laboratory under favorable oxygen and temperature conditions.

The following organisms were cultivated from bathyal samples (Table 2, Figures 3, 4).

Figure 2. Conjectural transfer ways of oxybionts' dormant stages into the Black Sea bathyal zone (1-5) and theirs hypothetical partial return (rise 6-6').

I O_2 – aerobic (oxygenous) zone;

II H_2S – anaerobic (hydrogen sulphide, reductive) zone.

1 – from upper aerobic zone of the sea;

2 – from mud slides through submarine canyons;

3 – from atmospheric precipitations;

4 – from river waters;

5 – from terrestrial aeolian deposits;

6 – hypothetical return (upwelling) of dormant stages of oxybionts into the upper oxygenated layer and their further tranfer via foodwebs (plankton/neuston-nekton-aquatic birds) to the native lands and freshwaters (6'). *[Published for the first time].

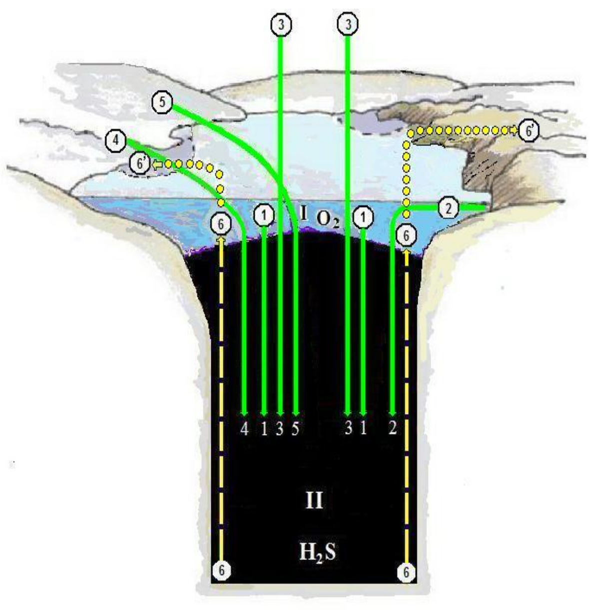


Table 2. Living oxybiotic organisms, grown from dormant stages collected in the hydrogen sulphide pelocontour of the Black Sea bathyal zone (from Zaitsev, Polikarpov, Egorov et al. 2007, 2008)

HIGHER TAXA	Genera	Species
BACTERIA		
	Bacillus	*B. sp. sp.*
	Lactobacillus	*L. sp. sp.*
	Listeria	*L. sp. sp.*
	Oscillospira	*O. sp.*
	Coliform bacteria	
UNICELLULAR ALGAE		
Bacillariophyta	*Skeletonema*	*S. costatum*
	Chaetoceros	*C. sp.*
Dinophyta	*Peridinium*	*P. sp.*
Cyanophyta	*Anabaena*	*A. spiroides*
	Gleocapsa	*G. sp.*
	Oscillatoria	*O. sp.*
	Aphanotece	*A. sp.*
Chlorophyta	*Pterosperma*	*P. cristatum*
	Monoraphidium	*M. arcuatum*
Chrysophyta	*Emiliana*	*E. huxleyi*
	Pseudopedinella	*P. pyriforme*
Silicoflagellata	*Distephanus*	*D. speculum*
FUNGI		
	Arenariomyces	*A. trifurcates*
	Corollospora	*C. maritima*
	Acremonium	*A. sp.*
	Chaetimium	*C. globosum*
		C. murorum
	Schizothecium	*S. sp.*
	Sporormiella	*S. cylindrospora*
	Sordaria	*S. fimicola*
	Aspergillus	*A. niger*
		A. sp.
	Alternaria	*A. alternate*
		A. chlamydospora
		A. consortiale
		A. radicina
		A. tenuissima
	Hunicola	*H. sp.*
	Penicillium	*P. citrinum*
		P. sp.
	Stachybotrys	*S. chartarum*
	Ulocladium	*U. chartarum*
	Candida	*C. sp.*

The following oxybiotic (aerobic) organisms were found *in 10^3 cells in one gramme* of mud (wet weight) from the hydrogen sulphide pelocontour of the Black Sea bathyal zone:

Heterotrophic bacteria – from 310 to 11,250,
Coliform bacteria – from 15 to 50,
Propagule (spores and hypha of fungi) – from 20 to 3.755,
unicellular alga *Emiliania huxley* (spores) – 14.0,
cysts of Dinophyta - from 16 to 104.

Anaerobic organisms in the same mass of mud were represented by heterotrophic anaerobic bacteria quantities *of 10^3 cells:* from 410 to 5,250.

So, in the presumed "azoic" zone of the Black Sea the aerobic organisms are much more large in number than the anaerobic ones.

Some of the first living aerobic organisms raised from dormant spores collected in the sediments of the hydrogen sulphide bathyal Black Sea zone at the depths from 730 to 2104 m are represented in the Figures 3 and 4. A drawing scale of 10 µm in both figures is shown.

Figure 3. Fungi from the Black Sea hydrogen sulphide bathyal zone: marine origin species,

Arenariomyces trifurcatus Hohnk, E. B.G. Jones, ascospores (1), *Corollospora maritima*, Werdermann, ascospores (2); terrestrial and fresh water origin species, *Sporormiella cylindrospora* S.I. Ahmed & Cain, ascospores (3), *Sordaria fimicola* (Roberge ex Desm.), ascospores (4); *Stachybotrys chartarum* (Ehrenb.), conidiophores and conidia (5), *Alternaria tenuissima* (Fr.) Wiltshire, conidia (6) (photo by N. I. Kopytina).

*[Published for the first time].

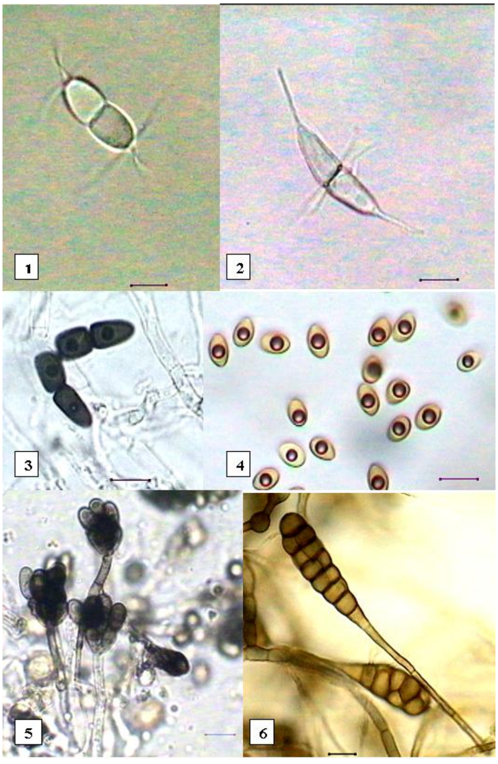


Figure 4. Unicellular algae from the Black Sea hydrogen sulphide bathyal zone:

Anabaena sp., fresh waters (7), *Emiliania huxley* (Lohm.) Hay & Mohler, marine waters (8), *Chaetoceros* sp., marine waters (9), *Oscillatoria* sp., fresh waters (10), *Pseudopedinella pyriforme* N. Carter, marine waters (11), *Skeletonema costatum* (Grev), marine and brackish waters (12) (photo by A. V. Kurilov and L. M. Terenko).

*[Published for the first time].

Conclusion

I. The new received data prove that the prevailing definitions of the hydrogen sulphide area of the Black Sea as “dead” or “azoic” zone according to scientific literature are not correct because first of all they ignore free-living anoxic organisms, and secondly they do not take in consideration the “latent life” of spores of oxybiontic organisms shown in our works (Tables 1 & 2). In fact, together with different free-living anaerobic bacteria, there are also many aerobic species of bacteria, algae and fungi recently discovered by our teams in the form of dormant stages are able to regenerate under favorable conditions for germination and vegetative growth.

Discovered spores of oxybionts-inhabitants fresh waters and dry land (first of all, among fungi) together with spores of marine species of oxybionts are the material for examination of the ways of penetration of spores of these all species into the Black Sea bathyal pelocontour.

The main contribution to the process of sedimentation of suspended organic particles, including the abioseston and tripton is due to the fall-out of dead organisms and living spores from the upper layers of the Black Sea. Along with them, microscopic organisms, including spores and cysts, transfer into the Black Sea bathyal with atmospheric fall-outs, river waters and sewage waters. Besides, large portions of deep shelf mud with living organisms and spores slide down the slopes of the submarine canyons. Caused by turbidity currents, slumping, and gravitational sliding were and are important processes in the geological history of the Black Sea (Ross et al. 1974).

II. As for the living creatures (1) inhabiting their “own” anoxic environment or (2) those turned out to be in hostile hydrogen sulphide media (and which have freshly deceased before the time of sampling), they theoretically can be distinguished into: the first category of organisms passing all their normal life cycle in the anoxic media – anoxybionts (e.g. species of the genus *Microspira*), and the second category - oxybionts – spores of aerobic bacteria (among these there are species belonging to widely distributed genera *Bacillus*, *Lactobacillus*, *Listeria*, and colifirm bacteria), spores of algae (species belonging to the divisions Bacillariophyta, Dinophyta, Cyanophyta, Chlorophyta, Chrysophyta, Silicoflagellata), spores of fungi pertaining to species of marine, fresh water and terrestrial origin, and eggs of some invertebrata. According to published scientific information and to our data, there are no convincing proofs about the presence of living plants (except their spores) and animals in the Black Sea hydrogen sulphide pelocontour in capacity of representatives of the first (1) category. As for the second (2) category, theoretically it is not excluded that not only dead and decomposing, but also dying specimens of primitive invertebrates like, for example, water bears (Tardigrada) might be expected to be found in the hydrogen sulphide pelocontour of the Black Sea. Tardigrada are true cosmopolitan species. There is experimental data in which tardigrades in anhydrobiosis (dormancy induced by low humidity or by desiccation) were exposed in the open space to cosmic radiation, vacuum, and temperatures close to absolute zero and have been revived in normal conditions. These invertebrates belonging to the size category of meiobenthos, inhabit the Black Sea shelf and can fall into the bathyal zone simply sliding down the slopes of submarine canyons. If oxybionts (including the mentioned above Tardigrada) are able to live and to breed in the Black Sea hydrogen sulphide pelocontour and to form populations, biocoenoses, biogeocoenoses, they would be discovered by the deep-sea marine biologists and ecologists. But such convincing evidences deserving credit are not known. This constatation means that, speaking by the strict and irreproacchable scientific language of V. I. Vernadsky, at present there is absence *of an empiric generalization, that is a logical conclusion from accurate data of a scientific observation* (Vernadsky 1965).

III. New information (Tables 1-3) about the life of the Black Sea bathyal raises some questions which require special investigations. Our teams have assessed the term of imprisonment of the studied species – living spores – in the bathyal pelocontour which is at least 50 years (Table 2). A question arises: how long the spores of oxybiontic species can stay in the hydrogen sulphide and in absence of oxygen retaining their capacity to live? Are there physiological and biochemical differences between organisms in the bathyal pelocontour and those permanently living in the upper oxygenated layer of the sea? Are the oxybiontic organisms from the hydrogen sulphide pelocontour able to return into the aerobic zone of the Black Sea, with what probability, when and in what ways?

Probably, such a possibility is bigger for the inhabitants of the continental slope, where the vertical mixing of water layers is more intensive than over the abyssal plain. Some influence on this process can exert submarine volcanic activity and strong tectonic events.

The question of returning of oxybiontic spores into oxybiosphere from the anoxybiospheric reserve (the bank of spores in the bathyal pelocontour) has a purely theoretical meaning now. First of all – for the hypothetical case of loss of the respective genotype in the oxybiosphere up to the moment of the replenishment of this genotype by the flux of returned identical spores from the anoxic bathyal bank. Nevertheless it looks rather realistically according to our first assessments of the very impressive density of living oxybiotic bacteries and spores in the reduction bathyal sediments. If a large stock of them is available and is permanently re-stocked in the Black Sea bathyal why not a permanent flux of returning survived spores exists from the bathyal up to sea surface and then out of the sea to freswaters and the land (Figure 2)

Thus, the generalization and discussion of recent joint investigations of the IBSS and the Odessa Branch of IBSS, the National Academy of Sciences of Ukraine (Zaitsev, Polikarpov, Egorov et al. 2007, 2008) show perspectivity of the new line of research in the field of the *deep sea ecology and molismology of the pelocontour of the Black Sea bathyal*. This line of research becomes especially scientifically interesting and practically of current importance because of considerable increasing of man-made impact on the vast ecosystem of the Black Sea bathyal pelocontour, namely: during the construction of transboundary, transcontinental and national gas pipelines, oil pipelines, communications and other large-scale engineering, geological and prospecting works.

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