The hypothesis of virus circulation between the land and the hydrosphere

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Abstract

The researchers all over the world observe contamination of hydrosphere by viruses from a land. The opportunity of viruses from land to master a hydrobionts (as a new hosts) is experimentally proved. That can result in evolution of viruses and in occurrence of "new" viruses potentially dangerous for flora and fauna of our Planet.

So, in our experiments for the first time the viruses from the land (bacteriophages) have been adapted to the marine environment and to a new host - microalgae. The fact, finding by us, – the presence of algal viruses in clinical material suggest a previously unknown ways of marine viruses circulation and about their possibility role in pathology of land organisms, including people.

The hypothesis of virus circulation between the land and the hydrosphere was substantiated and announced.

Key words: viruses from a land, Black Sea algal viruses, hypothesis of virus circulation between the land and the hydrosphere

Introduction and short Review of Literature

Viruses from a land (human viruses and viruses of animals, plants and bacteria) are brought in a hydrosphere (including World ocean, continental and underground waters) of the Earth. The viruses from a land are alien (allochthoneus) for aquatic ecosystem. The basic way of their hit to hydrosphere is a drift through waste water. This process is promoted by the natural phenomena, including and cataclysms, accompanying by floods of the rivers, landslips, floods, hurricanes, tsunami et al. Thus contamination by land viruses can occur not only in hydrosphere, but also pollution of the land by viruses of hydrosphere can occur. But this aspect in circulation of viruses practically is not investigated, though is extremely actual (Степанова, 2010).

The viruses brought from a land in aquatic environment are exposed to influence of various factors that can cause their destruction or preservation (survival). Different particles of microplankton can absorb the land viruses and that facilitates their transportation to marine sediment, to shell of crawfishes, mollusks and also increases an opportunity of hit in filtering organisms. Viruses from a land, including pathogenic human viruses, as a result of a survival and accumulation are usually defined in samples of hydrosphere and often they are the reason of infection of the people. Currently we have a necessity to estimate comprehensively the ways of viruses from a land in hydrosphere in connection with occurrence of facts about changes of viral properties and virulence, of their transition from one ecosystem in others and with mastering of other ecological niches and new hosts (Степанова, 2004; 2010; Munn, 2006; Sano Daisuke et al., 2006). Therefore the contaminated reservoirs are estimated by the researchers as "natural laboratories of creation of new viruses".

New knowledge about the biology and ecology of the hydrosphere viruses, received worldwide in recent decades, are the basis for evaluating the stability of hydroecosystems and increasing the predictability of the impact of global change on biogeochemical processes in all waters of the world (Proposal for SCOR WG ..., 2005). Algal viruses are of great interest for researchers because they have a key position in the regulation of the number of micro-algae that are of great importance to our Planet.

By the end of the 20th century, scientists had discovered viruses or virus-like particles for 44 taxa of eukaryotic algae, included in 10 out of 14 known classes (types) of algae (Van-Etten et al., 1991). At the present stage, according to the literature, up to 65 separate taxa of algal viruses with significantly different morphology have already been identified (Coy et al., 2018; Short et al., 2020; Viruses of Microorganisms ..., 2018). Virions of algal viruses have the shape of an icosahedron and range in diameter from 20 to 210 nm. They are characterized by the presence of a supercapsid. Infecting a wide range of their hosts (microalgae), algal viruses, as a rule, are lytic viruses and exhibit a narrow species and sometimes strain specificity (Short et al., 2020). Through dedicated research efforts, algal viruses have been recognized as diverse, dynamic and ecologically important members of the biosphere.

The presented message is devoted to the analysis of own results of study of Black Sea environment contamination by viruses from a land and possible consequences of their development in marine environment and hydrobionts with a review of the literary data in this direction.

Materials and Methods

For study of viral contamination of Black sea environment we used the samples of marine sediments, mussels *Mytilus galloprovincialis* and gills from different species of fishes. We collected this material in 1994-2001 in different bays of Sevastopol. The data of sanitary and epidemic station of monitoring human viruses in samples of marine water in bays of Sevastopol in 1990-1994 were analysed also. Collection and processing of a material, the used virological methods, dates and the places of selection of samples are in detail described in (Степанова, 2004). Study of possible adaptation of viruses from a land to hydrobionts were carried out in experiment, with using bacteriophages of phytopathogenic bacteria received in department of virology of the Kiev National University by Taras Shevchenko and also with using liquid cultures of algae *Tetraselmis viridis* and *Phaeodactylum tricornutum* received from a collection of microseaweeds of department of ecological physiology of algae of Institute of Biology of the Southern Seas of NAS of Ukraine. The method used in experiment is based on (Степанова, Спосіб…, 2004 – Pat.65864A UA, №200306549) and is in detail described in (Степанова, 2004).

Experiment of mastering of hydrobionts (algae - the cells of eukaryotes) by viruses that entered into the hydrosphere from the land (bacteriophages - viruses of prokaryotic cells). In this experiment marine algae (*T.viridis* and *P.tricornutum*) were used as the hydrobionts-eukaryotes. Viruses from the land (viruses of prokaryotes) were represented by bacteriophages of phytopathogenic bacteria *Xanthomonas axonopodis pv. beticola* (strain 7325) - isolates 7325-1/1, 7325-10/1, 7325-17/1, 7325-4; *Erwinia carotovora* (strain 216) - isolate 216V2 and *Pseudamonas syringae pv. atrofacies* (strain 1025) - isolate 1025/2.Viruses were obtained at the Department of Virology of the Taras Shevchenko Kyiv National University. The titers of bacteriophages were in the range of 10^7-10^8 pfu. In experiments on the adaptation and mastering of bacteriophages to new host - *T.viridis*- viral isolates 7325-1/1, 7325-10/1, 7325-17/1, 7325-4, 216V2 and 1025/2 were used. In adapting to algae *P.tricornutum* the same viral isolates, except viral isolate 1025/2, were used. Experiments were performed on a basis of author's method of algal virus isolation (Степанова, Спосіб…, 2004). Adapting of viruses to the algae *T.viridis* was performed in three ways, but to *P.tricornutum* - only one way. Description of these methods will be presented in the "Results and discussion". Before each of the three methods the initial inoculation (in an equal volume 2.0 ml) of algae was conducted by viral suspensions that was in 10 times dilution by sterile seawater. The first two ways were carried out in small volumes in bacteriological test tubes, and a third way was carried out in large volumes with using the flask.

Search of marine viruses in clinical material. As clinical material we used cervico-vaginal secretion of 182 women which were on Black sea beaches 2-5 months before beginning of their diseases. Samples were collected at the clinic of the Central City Hospital Donetsk, Ukraine in

the autumn-winter period in 2007 and 2008. Samples of cervico-vaginal secretion were collected in volume about 0.2-0.5 ml in women with three different gynecological diagnoses: 36 samples with a diagnosis of "coleitis", 63 samples with a diagnosis of "uterine fibroids", and 83 samples with a diagnosis of "cervical erosion". Samples were stored and transported to the place of their study (Sevastopol) in frozen (-18 degrees Celsius) state. After thawing, the clinical material was pooled in 41 combined (united, composite) samples with the same diagnoses: 11 composite samples from women with a diagnosis of "coleitis", 14 - diagnosed with "uterine fibroids" and 16 - with a diagnosis of "cervical erosion". So, each of 41 samples consisted of clinical materials of 2-5 women with the same diagnosis. Isolation of algae viruses from clinical samples was performed according to the author's method with using culture of *T.viridis*, *P.tricornutum* and *D.viridis*.

Results and Discussion

Results of study the contamination of samples of Black Sea environment by viruses from land - human pathogenic viruses (adeno-, reo-, rota- or enteroviruses, including virus of hepatite A) and bacteriophages of phytopathogenic bacteria - are submitted in the Table 1, that shows contamination from 2,1 % up to 74 % from number of investigated samples.

Table 1

Contamination of samples of Black Sea environment by viruses from a land - human pathogenic viruses (adeno-, reo-, rota- or enteroviruses, including virus of hepatite A - VHA) and bacteriophages of phytopathogenic bacteria

Studied material (samples)	Number of studied samples	Percent of samples, contaminated by viruses from a land
Marine water	1135 (method of isolation of viruses); 102 (serological method)	2,1% (24 variants of entero- and adenoviruses); 9,8% (antigens of VHA)
Marine sediments	17	11,8% (2 antigens of rota- and reovirus)
mussels *Mytilus galloprovincialis* (more than 1300 mussels)	108 from 54 pools	24% (1 enterovirus and 12 antigens of rota-, reo-, adenoviruses and VHA
Marine fishes (86 species of fishes)	17 (8 from not ecologically good M. bay and 9 from relatively better S. bay)	74% (bacteriophages of phytopathogenic bacteria were in 6 samples from M. bay) 11% (1 contaminated sample from S. bay)

The data of the foreign researchers about contamination by human pathogenic viruses of samples of marine water and water from rivers, internal reservoirs and of marine sediments show 8,7-84,5 % of contaminated samples. In samples from mollusks, fishes and others hydrobionts the human viruses are defined in 8,4-87,0 % from number of investigated samples. Virus contamination of water and hydrobionts was observed mainly in areas of dump of sewer waters and in places dense residing of the population. The review of information from published papers about results of study of hydrosphere contamination by viruses from a land and the methods, used for it, was described in (Степанова, 2004; 2010). Our data (see in the Table 1) testify that virus contamination of samples from Black Sea environment do not exceed the top border of parameters of virus contamination revealed by the foreign colleagues. Our results also specified,

that the pollution by viruses from a land more often was observed in closed bays with dump of waste water. The filtering mollusks were offered by the foreign colleagues as indicators-organisms for search of viruses from a land in hydrosphere. The experience of our researches showed a role as the bioindicator in search of viruses from a land in Black Sea environment not only mollusks, but also fishes.

Established contamination of Black Sea organisms by viruses from a land gave us suggestion about an opportunity of their adaptation (with mastering) to the new hosts - hydrobionts. For the benefit of this assumption some data and statements of the researchers were described (Малюта, 1984; Степанова, 2004; Munn, 2006). This suggestion was supported also by the first fact established by us about of presence in blood of the ill dolphin of antibodies to a virus of hepatite C, and according to history of disease the infecting of an animal was connected to use for injection the syringes, before second-hand at the people (Stepanova, 2006). Experiment carried out in laboratory conditions and directed on adaptation by three different ways bacteriophages of phytopathogenic bacteria (viruses of procariotic cells) to *Tetraselmis viridis* and *Phaeodactylum tricornutum* algae (eucariotic cells) essentially has confirmed an opportunity of mastering by viruses from a land of marine environment and hydrobionts (Соловьев et al, 2008: Степанова, 2004; Stepanova, 2004). The conditions of experiment reflected a usual situation in a nature at possible coincidence of mass hit of viruses from a land in hydrosphere that is real in case of dump of waste water, and seasonal blooms or increase of number of the phytoplankton representatives. Thus, for the first time it was proved, that the contamination by viruses of a land of hydrosphere has potential danger not only for the people (in cases of their infecting through marine food, swimming, recreation and melioration, etc.), but also for hydrobionts. On the basis of own results and literary data we offered the hypothesis about circulation of viruses between a land and hydrosphere (or about exchange of viruses between a land and hydrosphere). This virus exchange in a nature results in occurrence of “new viruses” and they may be potentially dangerous for organisms of a land and hydrobionts. The essence of a hypothesis is reflected in the submitted Figure 1.

Exchange of viruses between a land and hydrosphere - process, caused by the natural phenomena (drifts by a wind of different particles from a land, flood, landslips, tsunami etc.). However the anthropogenous factor (such as constant dump in hydrosphere of waster water, use of water and hydrobionts for various needs etc.) has an important role in this process also. Having high ecological plasticity, the viruses, at hit in new conditions, are capable to overcoming of barriers of species, and the occurring changes in their ecology are connected to use by them of other conditions and opportunities (Львов, 1975).

The evolution of viruses is of interest first of all not as the mechanism of occurrence of completely "new" viruses, but faster as the way of the existing or slightly modified variants of viruses to the new hosts. The adaptation of viruses of procaryotic hosts (bacteriophages of phytopathogenic bacteria) to the new eucaryotic hosts (algae) can be estimated as manifestation of "uneven (spasmodic) evolution" (Воронцов, 2004).

The occurrence of "new" viruses with unknown properties in hydrosphere can result in destruction of the certain species of hydrobionts, can lead to infringements in trophodynamics and in composition of marine food webs, to ecological accidents connected to changes of circulation of organic carbon, oxygen and carbonic gas not only in hydrosphere, but also in an atmosphere of the Earth (our Planet).

So the sharp decrease of number of the phytoplankton representatives, that caused by lysis by "new" algae virus, can cause infringements in the basis of trophic pyramid of hydrosphere and in circulation of carbonic gas - oxygen of biosphere of our Planet.

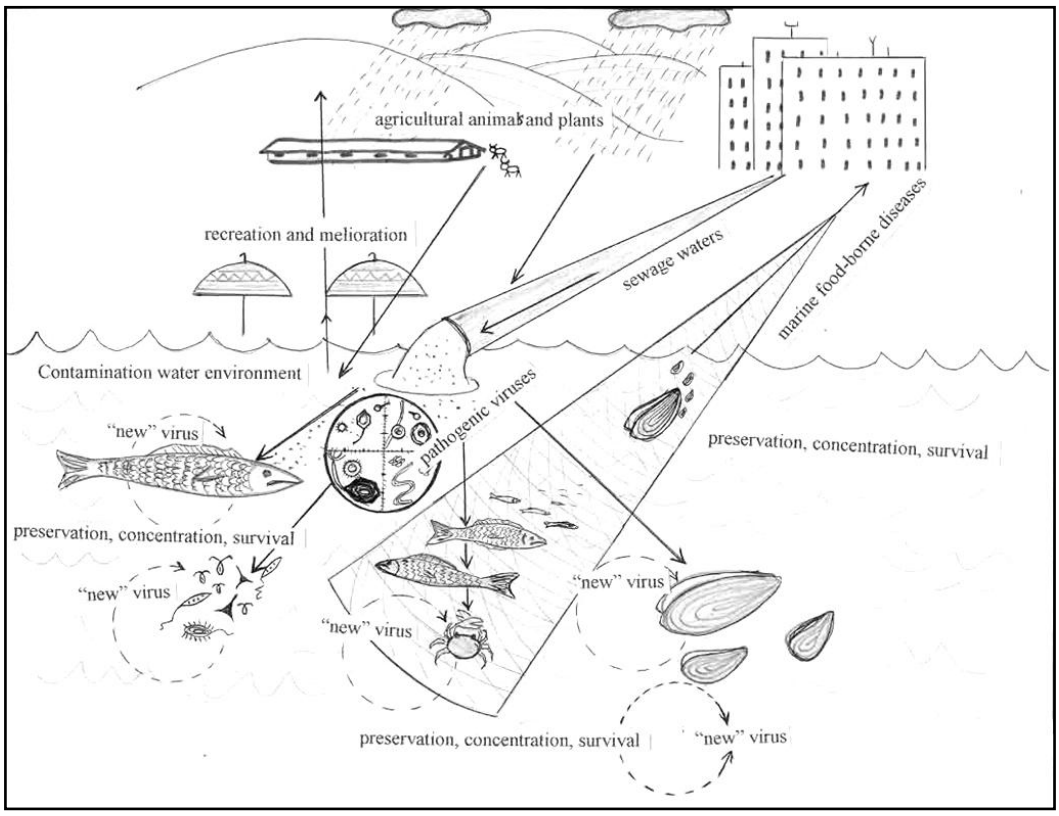

Figure 1. Contamination of hydrosphere by viruses from a land and probable mastering by them of new ecological niches and hosts - hydrobionts (Степанова, 2004; Stepanova, 2004)

Experiment of mastering of hydrobionts (algae - the cells of eukaryotes) by viruses that have been entered into the hydrosphere from the land (bacteriophages - viruses of prokaryotic cells)

Our earlier results showed the contamination to 74% of the number of samples of water, sediment and aquatic organisms in the Black Sea near Sevastopol by viruses from the land (Степанова, 2004). We primarily indicated the possibility of infection and the involvement in the epidemic process of specific hosts of these viruses - the inhabitants of the land. Questions about destiny and circulation of viruses from the land in the hydrosphere, their preservation, survival and accumulation in the hydrobionts, their possible ways and mastering of marine environment are little studied. Viruses from a land are dangerous for the hydrobionts? It is logical to assume that in solution of these questions can and should engage the hydrobiologists who involved in the course of their researches in microbiology and virology.

It is known that viruses have a high ecological plasticity. This allows them to adapt to new environmental conditions and to mastering new hosts. In the late 20th century it was experimentally established that enteroviruses (as pollutants of hydrosphere), not only persist but can replicate in protozoa (Feras, Kesa, 1990). Some observations and statements, described by others researches, indicate about such a possibility (Малюта, 1984; Munn, 2006). Analysis of the available data in this area is represented in the literature review in our book (Степанова, 2004). Fact of contamination of hydrobionts of Black Sea by viruses from the land suggests the possibility of the mastering of these viruses (as marine pollutants) the new hosts - aquatic organisms. We pointed out and for the first time established fact of the presence in the blood of a sick dolphin the antibodies to hepatitis–C. In the course of the epidemiological investigation, it was determined that the infection of animal has been associated with the use of injection syringes which have already been used for humans (Степанова, 2004; Stepanova, 2006).

What can complete the contact of allochthonous viruses (pollutants of hydrosphere) with hydrobionts in the aquatic environment? Can the viruses from a land (bacteriophages of

phytopathogenic bacteria) master the aquatic organisms - algae (eukaryotic) as the new host? Is it possible? For this purpose we carried out experimental research.

Initial inoculation by bacteriophages of phytopathogenic bacteria in the algae cultures for the duration of up to 30 days usually resulted in visual differences with the control of growth of these non infected algae cultures. Infected cultures had more turbidity and color intensity, indicating their more active growth and development. Thus, during the first interaction between bacteriophages and algae culture effect of potentiating algae growth was observed. What is the reason for this effect of potentiating growth of non-specific hosts by alien (non-specific) viruses? This may be due to presence of nutrients in viral suspensions that can be the main cause of potentiation of the growth and development of algae cultures. It is not possible to imagine anything else. However, after 2-3 "blind" inoculations these phenomena of potentiation of growth and development of algae culture disappeared.

In any of the three ways (methods) of adaptation of alien virus in case of oppression of microalgae culture for establishing stability of the latent period further inoculations are carried out. In the first method, after a month of contact between the virus and the non-specific host (algae), 3-5 blind inoculations every 7-10 days until the inhibitory effect on algae was appeared were carried out. In experiments with using *T.viridis* inoculation were continued up to incubation period was stable within 20-24 h with the lysis during following 3-5 days, when with using *P.tricornutum* - within 2-4 days with the lysis during following 5-7 days.

In second method of adaptation a second subsequent inoculation was made for appearance of inhibitory effect in algae at least one of used viral isolates. After this all the infected samples the third inoculation was carried out. It was a blind inoculation for samples that showed no inhibitory effect. Effect of suppression was recorded through subsequent inoculations to establishing a stable latent period.

In the third method of adaptation to the initially infected algae culture (in the flask) the stabilizing medium of Goldberg in an amount of 10-50 ml (periodically, after 10-15 days) was added. In other words, when the latter method was carried out for a long time (about 2 months) it was as culturing the infected algae culture.

Results of the mastering (adaptation to) of hydrobionts by viruses from a land are shown on the example of bacteriophages of phytopathogenic bacteria *Xanthomonas axanopodis pv. beticola* (strain 7325) - viral isolates 7325-1/1 , 7325-10/1 , 7325-17/1 , 7325-4 and *T.viridis* algae culture (Table 2).

Table 2.
Adaptation of bacteriophages *Xanthomonas axanopodis pv. beticola*
to marine microalgae *Tetraselmis viridis*

Ways of adaptation	Bacteriophage isolates, appearance of the bacteriophage's inhibitory effect on algae			
	7325-1/1	7325-10/1	7325-17/1	7325-4
“blind” inoculations in a fresh algae culture in 7-10 days	after 4 inoculations	Did not study	after 3 passages	after 5 passages
long-term exposure (to 50 days) of bacteriophages and algae after second inoculation	after 2 inoculations	after 3 inoculations	after 3 inoculations	after 3 inoculations
long-term cultivation of infected algae culture	Did not study	after 90 days	Did not study	after 90 or more days

The adaptation (mastering) was performed simultaneously by all three methods described. The most rapid result of inhibition by bacteriophages algae culture growth and development was obtained in a series of “blind” inoculations, i.e. in the first way (method). After third inoculation of the first way of adaptation the inhibition of algae culture after 20-24 h with followed lysis in 3-5 days with one of the viral isolates (7325-17/1) was observed. Viral isolates (7325-4 and 7325-1/1) cause depression *T.viridis* algae culture after fourth and fifth inoculations, respectively.

The second way - long-term observation after the second inoculation of viruses in the culture of microalgae *T.viridis* - revealed after 50 days the inhibitory effect of one of the viral isolates (7325-1/1). However, during the third inoculation the effect of oppression of algae culture has already been observed for all the isolates. In subsequent inoculations the incubation period was reduced to 20-24 h with the manifestation of lysis in following 3-5 days.

The third way of adaptation of the bacteriophages to the marine microalgae - long-term cultivation of initially infected *T.viridis* was made for only two isolates - 7325-10/1 and 7325-4. However, after 90 days, the effect of oppression of algae culture, the volume of which had already reached more than 200 ml, was observed only in the presence of a viral isolate 7325-10/1. Oppression manifested in the increase of transparency of the culture and the appearance of sediment on the bottom of the flask, indicating a violation of the motor functions of cells. The effect of oppression is not turned into a lysis of *T.viridis*. Duration of observed inhibition did not exceed 3-4 days, during which the effect of inhibiting with following cell lysis was recorded (fixed) through subsequent inoculations of supernatant to a fresh (young) *T.viridis* algae culture in volumes of 2.0 ml and the use the test tubes in accordance with the author's technique.

Only first way of adaptation bacteriophages *Xanthomonas axanopodis pv. beticola* (strain 7325) to a new host - marine microalgae *T.viridis* and *P.tricornutum*, is the closest to natural conditions in the marine environment. This method is to hold the “blind” inoculations in a fresh algae culture. Bacteriophages *Erwinia.carotovora* (viral isolate 216V2) and *Pseudamonas syringae pv. atrofacies* (viral isolate 1025/2) caused inhibition of *T.viridis* algae culture only after 4 - 6 inoculations. The first effects of oppression *P.tricornutum* by all used bacteriophages, except viral isolate 1025/2, not used in the experiment with *P.tricornutum*, observed only after 7-8 'blind' inoculations.

Such high concentrations of virus and microalgal cells, which were used in performed experimental studies, not found in nature. However, lysis of the algae with adapted viruses we observed with their prior dilution from 10 in 5 to 10 in 10. This suggests that if there is getting in a sea of similar viruses or bacteriophages (by washing with soil with sewage or landslides), then in contact with the cells of algae the infection is possible, especially during the phytoplankton bloom. In other words, with coincidence of certain conditions the viruses brought from the land in the open water ponds, are able to transcend the species barrier and adapt to the new hosts (inhabitants of hydrosphere). Two other used methods of adapting of bacteriophages to a new host also confirm the ability of viruses from a land to overcome species-specific barriers, as there are many observations and facts described in (Малюта, 1984).

Adapted to aquatic organisms bacteriophages of phytopathogenic bacteria have been partially explored (as viral suspensions). The results of this study show that infectious titer of viral suspensions was 10 in 9 -10 in 10 infectious units/ml and there was their insensitivity to chloroform. The inoculation of adapted in our experiment viral isolates to his former master (host) - *Xanthomonas axanopodis pv. beticola* (strain 7325), conducted by the classical method of double-layer agar (Практикум…, 2000), did not reveal their ability to cause lysis of bacterial cell culture of the former owner (host). Perhaps there have been significant changes in the properties of viruses that were adapted to the new hosts. In other words, as a result of the experimental work "new" viruses as the “new" algal viruses were obtained. The cause of appearance of these "new" viruses were other living conditions to which they have had to adapt and they took advantage of these conditions in order to survive according with demands of evolution.

Ways of penetration of viruses in cells of prokaryotes and eukaryotes are different. However, the essence of the process of transport of nucleic acids of bacteriophage in bacteria and mechanism of viral penetration in eukaryotic cells is still far from its final determination. It can be assumed that the process of mastering (development and adaptation) appeared with followed by infection of cells of eukaryotes (algae) through natural openings, which serve to absorb from the environment of nutrients or release of metabolic products of microorganisms. The subsequent course of events is not known. It is possible that the algal enzymes dissolved viral capsid, which contributed to the exit of DNA from the bacteriophage. Process of bacteriophages in getting a new host (eukaryotic cell) with further viral replication could be accompanied by changes in viral genetic, biochemical, and hence the enzymatic properties. This in turn could result in the loss of infectivity in relation to its former host - phytopathogenic bacteria. To confirm our assumptions, we need the further extensive research. However, the authors feel the need to report not only on results but also assumptions about those moments that may underlie the mechanisms and processes that support the adaptation of allochthonous viruses in hydrosphere and viral mastering of new hosts - an aquatic organisms.

Adaptation of viruses of prokaryotic (bacteriophages of phytopathogenic bacteria) at new hosts - eukaryotes (microalgae) - can be viewed as a manifestation of "jump evolution" (Воронцов, 2004), leading to the emergence of "new" viruses. The emergence of "new" viruses with unknown properties in the hydrosphere can lead to the death of certain species of aquatic organisms, to breaks and loss of links in the food chain, to environmental disasters related to changes in the circulation of organic carbon, oxygen, and carbon dioxide, not only in the hydrosphere, but also in Earth's atmosphere. So the sharp decline in the number of members of the phytoplankton due to lysis by the "new" algal viruses can lead to disturbances in the base of the food pyramid in hydrosphere and in the circulation of carbon dioxide - the oxygen of the biosphere of our Planet.

The results obtained in the course of the experiments have led to certain conclusions based on empirical facts:

1. Initial contact of prokaryotic viruses (bacteriophages of phytopathogenic bacteria) with eukaryotes (unicellular algae) was accompanied by the potentiating effect on the growth and development of the algae cultures
2. "Blind" inoculations of infected by bacteriophages algae led to the effect of their oppression, that was as indicate on the viral replication in cells and confirmed by further inoculations.
3. The adaptation of bacteriophages to a new host (microalgae) was accompanied by a loss of ability (property) to cause active infection for the former host - the bacteria.

Further studies using highly sensitive techniques can explain the mechanisms that underlie the observed and described cases of the adaptation of virus of prokaryotes to eukaryotes, which is new to science fact.

The presented results were described for the first time in 2004 (Степанова, 2004; Stepanova, 2004) and in view of our own results about contamination of the Black Sea by viruses from land, as well as published data are the basis for hypothesis about the circulation of viruses between the land and the hydrosphere (Степанова, 2007) .

So, on an example of study a contamination of Black Sea environment by viruses from a land *in vivo* and *in vitro* and literature data was established, that the pollution by viruses from a land of hydrosphere often connected with anthropogenous activity, can result in occurrence of "new" viruses potentially dangerous for flora and fauna of our planet. Such pollution can be accompanied by infringements in links of marine trophic webs, in dynamics of consumption of carbonic gas and circulation of oxygen. With the purposes of study and all-round estimation of ways of viruses from a land in hydrosphere with the further realization of preventive measures directed on decrease and restriction of virus contamination of aquatic environment the following is offered:

1. To take into account reservoirs of the Earth, as "natural laboratories of creation of new viruses" in conditions of anthropogenous pollution.

2. To do monitoring of background contamination of hydrosphereby by viruses from a land with an execution in case of necessity a blocking and quarantine measures directed on protection of flora and fauna from virus infecting.

3. To attract attention of the scientists, public and representatives of authority to growing danger connected with contamination of hydrosphere by viruses from a lan

Search results of marine viruses in clinical material, as further evidence in favor of the author's hypothesis about circulation of viruses between the land and the hydrosphere.

It was the first search of marine viruses in human clinical material and it was our research. We were looking for viruses of marine microalgae (*T.viridis, D.viridis* and *P.tricornutum*) in clinical material (cervico-vaginal secretions) from the women which were at the Black Sea beaches 2-5 months before their gynecological diseases were manifested. The received results are shown in the Table 3.

During this study after 7-10 days of contact of material with algae we did the "blind" inoculations (sowings) in cases of absent oppression (lysis) of algae cultures in first, second, third and next inoculation (from 1 to 10 inoculation). However we observed the oppression of algae culture growth with subsequent lysis only using for infection (inoculation) studied material of *P.tricornutum* algae culture.

The first inoculation of *P.tricornutum* by a studied material resulted in display of an oppression of growth of culture with further lysis in 7-10 days. Further inoculations (sowings) with inoculations material have determined a stable latent period (or incubation period) about 2-4 days, as earlier was defined for different variants PtV, which were isolated from samples of water and mussels of Black sea environment (Степанова, 2004).

Table 3.

Inoculation of fluid algae cultures by clinical material (cervico-vaginal secretion) from women which were on Black sea beaches 2-5 months before of beginning their diseases

<table>
<tr><th rowspan="2">Diagnosis (primary number of samples)</th><th rowspan="2">Number of united samples</th><th rowspan="2">No of inocula-tion</th><th colspan="3">Reaction of algae cultures on inoculation</th></tr>
<tr><th>T.viridis</th><th>D.viridis</th><th>P. tricornutum</th></tr>
<tr><td rowspan="5">colpitis (36)

uterus fibroids (63)

erosion of uterus cervix (83)</td><td>11c</td><td>1</td><td rowspan="5">Growth</td><td rowspan="5">Growth</td><td rowspan="5">Oppression</td></tr>
<tr><td></td><td>2</td></tr>
<tr><td>14f</td><td>3</td></tr>
<tr><td></td><td>4</td></tr>
<tr><td>16e</td><td>5-10</td></tr>
<tr><td colspan="3">Isolation of algae viruses from 41 united samples</td><td>No virus</td><td>No virus</td><td>16 PtV[1)]</td></tr>
</table>

[1)]-16 PtV specimens were isolated: 6 among 11c; 5 among 14f and 5 among 16e

In a result the study of 41 united samples has allowed to isolate 16 variants of *P.tricornutum* virus (PtV) and no any virus variant to *T.viridis* and *D.viridis*. Thus, about 40% of the investigated united clinical samples were contaminated by marine virus - PtV - algal virus of *P.tricornutum*.

For the time being we don't know about the role which this virus has in gynecological diseases, but we think that this fact of discovery of algae viruses in clinical material of women is very interesting and it is an evidence of a new unknown ways in ecology of marine viruses.

Thus, our hypothesis of an exchange of viruses between a land and hydrosphere (Степанова, 2007) has received one more confirmation - fact of infecting (inoculating) of marine viruses in organism of the inhabitants of a land with possible (probable) mastering of a new ecological niche (women's vagina) and new host - human, that is reflected in a Fig.2 (Stepanova et al., 2011).

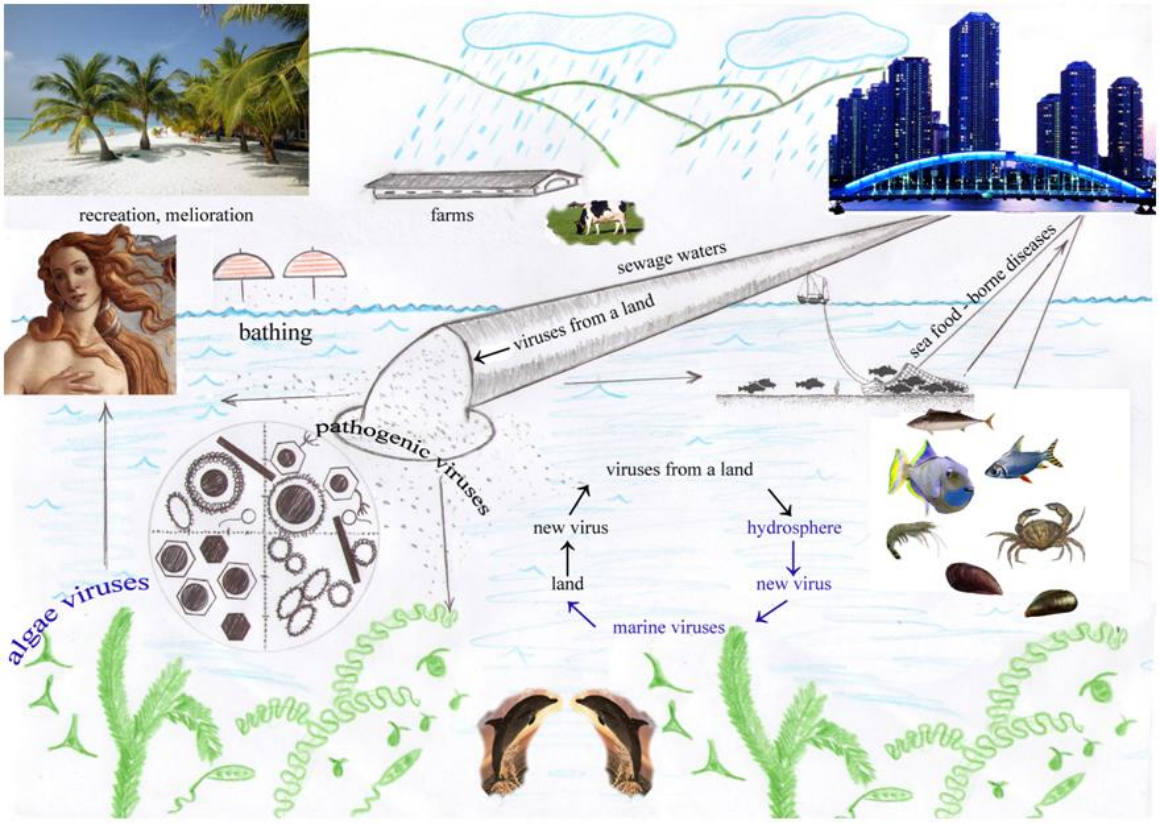

Figure 2. Contamination of hydrosphere by viruses from land and contamination of land by aquatic viruses, and this reflects the author's hypothesis of virus sharing between the land and the hydrosphere (Stepanova et al., 2011)

Conclusion

Experimentally the ability to adapt of viruses from a land (bacteriophages of phytopathogenic bacteria) to the marine environment and aquatic organisms (microalgae) was detected. And it was the first observed fact of adaptation of viruses of cells-prokaryotes to cells-eukaryotes.

The fact of isolation of the marine viruses (algal viruses) from human clinical material was the first noted by us. This fact demonstrated new ways in the ecology of marine viruses.

On the basis of our own results and published data about the circulation of viruses between the land and the hydrosphere was hypothesized. And new viruses can appear in resulting of this circulation.

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