BIOGEOCHEMICAL FACTORS OF WATER QUALITY FORMATION IN THE AMUR LIMAN

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1. INTRODUCTION

Complex factors such as hydrological regime, salinity, river runoff and discharge determine water quality in the Amur Liman as well as in the estuaries of many other rivers. The following aspects of water chemical composition formation seem most urgent to be studied: origin and a long-distant migration of stable organic substances of natural and anthropogenic character contained in suspended matter; sedimentation and accumulation of these substances at the bottom under the changes of the hydrological regime and salinity level in the zones of river and sea water mixing. The unity of organic matter production and destruction processes means the presence of common limiting factors. Taking into account that river discharge content includes organic substances of different structure, we may suppose that the intensity of their transformation and destruction affects the supply of biogenic elements and carbonic gas for primary producers. That is why the formation of water quality in the Amur Liman depends on the complex of biogeochemical processes in the water depths, in the zone of river discharge and in bottom sediments.

The Amur-Okhotsk Project studies of the Amur River impact on the primary productivity of the Okhotsk Sea are focused on iron and view iron ions as the main limiting-productivity factor (Narita et al., 2004; Kuma, 2004; Terashima, Nagao, 2007). Assessments of the influence of the salinity regime and iron ions on phytoplankton conditions serve theory justification. Although processes of destruction of organic matter of natural and anthropogenic origin are closed related to production processes, they are still weakly studied, in particular in relation to the forms of iron migration. Some authors in their phytoplankton condition studies assess nitrate concentrations without taking into consideration their microbiological origin from nitrogen-containing organic substances. In discussions of iron availability they only point out the role of biotransformation in the transformation of non-dissolved iron, contained in humic complexes, into the dissolved state (Saitoh et al., 1996; Suzuki et al., 2002; Terashima and Nagao, 2007 a).

Recent data show that some hydrophobic organic substances (DDT, PAHs, fullerene) have the direct contact with natural polymers, and with humic substances (HS) in particular, which play the role of sorbents and facilitate organic matter migration (Terashima and Nagao, 2007 b; Hong et al., 2010). PAHs may have higher concentrations in oligotrophic waters than expected, due to the prevalence of small-size phytoplankton in this environment. The results of experiments showed that natural phytoplankton was less resistant to PAHs (pyrene and phenanthrene) than phytoplankton species growing in cultures (Echeveste et al., 2010). Natural concentrations of PAHs can increase significantly due to special episodes of air transfer or oil spills, which may cause phytoplankton cell death in oligotrophic waters.

30 polycyclic aromatic hydrocarbons were analyzed in seawater along the continental shelf of Spain after the oil spill from the tanker «Prestige» in November 2002 (Grueiro-Noche et al., 2010). Naphthalene and methyl-naphthalene had the highest concentrations of 0.22 microg/L and 0.13 microg/L, respectively. Dimethyl-naphthalene in very high concentrations (0.36 microg/L) was found in bottom sediment samples in December 2002 and February 2003, whereas in September 2004 naphthalene concentrations decreased to about 0.08 microg/L and methyl-naphthalene to about 0.06 microg/L.

Microbial complexes that participate in the destruction of easily-decomposed organic substances are the decisive factor that determines biogenic matter supplies for primary producers. Oxygen in this case is the limiting factor. The deficit of oxygen, specific to deep water and bottom sediments, significantly change the mechanisms of organic matter transformation and destruction. Processes of transformation and destruction of stable organic substances in bottom sediments still remain weakly studied.

Further aggravation of the ecological situation in the Far Eastern seas, and in the Okhotsk and Japan Seas in particular, may be caused by the Amur River discharge of dissolved and suspended organic substances of different resistance to biodegradation. Objective assessment of factors, which control the Okhotsk Sea productivity, should be based on complex biogeochemical studies of the Amur-Okhotsk mega-ecosystem, including those that would reveal direct and indirect relations between processes of organic matter production and destruction.

2. MATERIALS AND METHODS

Water and bottom sediments were sampled in the Amur Liman during the complex expedition of the Pacific Oceanological Institute, the Institute of Marine Biology and the Institute of Water and Ecological problems FEB RAS in June 2006 (*Figure 1*). Intensity of production processes was estimated based on distribution of chlorophyll *a,* nutrients, oxygen and partial CO_2 pressure in the Amur estuary. Most parameters (salinity, oxygen, nutrients, chlorophyll *a*) were measured with standard techniques in the Pacific Oceanological Institute (Kondratyeva et al., 2008; 2009).

Assessment of microbiological activity was based on the results of selective cultivation of plankton and benthos microbial complex (MC) on different sources of carbon: phenol and stable polycyclic aromatic hydrocarbons (PAH). The method of initiated communities was used to assess potential capacity of microbial complexes to transform PAH (Kondratyeva, Stukova, 2008). MC activity of different substrates was revealed by the change of optical density at 490 nm. Plankton and benthos MC response reactions to water pollution were estimated based on reactions to toxic phenols (0.1%) at different salinity regimes (1-3 % NaCl) and stable PAH (naphthalene, phenanthrene – 100 mg/L).

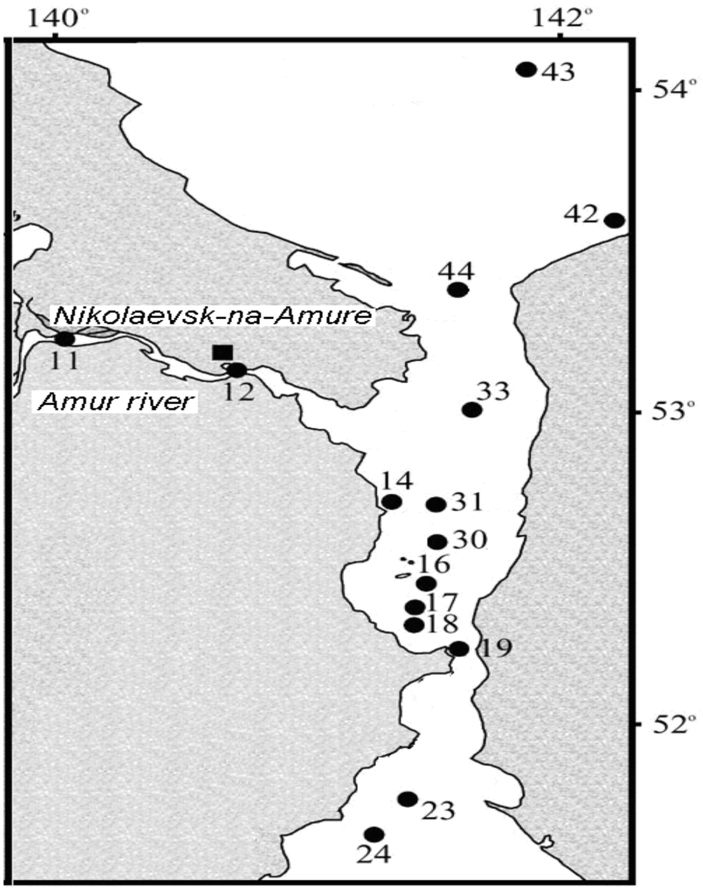

Figure 1. Sampling Stations in the Amur River Estuary and the Amur Liman in June 2006

3. RESULTS AND DISCUSSION

Simultaneous assessment of organic matter (OM) and biogenic elements discharged from the Amur River into the Amur Liman and their involvement in microbiological processes was undertaken in 1997 based on such parameters as chlorophyll *a* concentrations and the structure of microbial complexes. These parameters reflect specifics of production and destruction processes in water ecosystems and dynamics of substance circulation as well as indicate stability of their functioning.

Thus, chlorophyll *a* concentrations in water gradually decreased downstream from Komsomolsk-on-Amur to the Amur lower reaches (8.67 – 6.19 mg/dm^3). In the Amur Liman along the northern waterway near the Ozerpah village chlorophyll *a* concentration in phytoplankton was 4.08 mg/dm^3. The total number of heterotrophic microorganisms (OM destructors) in water samples from the observation stations there also gradually decreased (Kondratyeva, 2001). Dynamics of these two parameters indicated the decrease of intensity of production-destruction processes along the main Amur water flow in the Amur Liman.

Analysis of bottom sediments, collected in various parts of the Amur Liman, revealed the most complicated correlation between production and destruction processes. For example, benthos microbial cenoses that participate in OM destruction and utilization were found to increase in number in macrophyte growths. High numbers of cellulose-decomposing and sulphate–reducing microorganisms were observed in the structure of bacteriobenthos. Participation of benthos microbial complexes in the transformation and destruction of aromatic hydrocarbons of different origin was proved by studies of activity of bacterial enzymes polyphynoloxidase and peroxydase. In the zone of suspended matter discharged into the liman, polyphynoloxidase activity in the bottom sediments was 2.6 times higher that in the Amur lower reaches. The obtained data showed that enzymatic activity of benthos microbial complexes is accelerated in the zone of sedimentation of OM of different genesis. As the result of these processes different volatile substances, dissolved OM and biogenic elements appear in water and affect water quality and primary products (Microorganisms …, 2000).

Chlorophyll *a* concentrations serve as a criterion of interrelated processes of production and destruction as biogenic elements and result from destruction of easily-available organic substances irrespective of their origin. At the bottom destruction processes involve allochthonous substances, including stable substances, which come with river discharge, and autochthonous organic components, which sediment due to seasonal die-off of water vegetation and phytoplankton.

Sediment organic mater (SOM) is a highly complex and heterogeneous composite initially resulting from the physical, chemical, and microbial evolution of biopolymer materials. These biopolymers are further decomposed to fulvic and humic acids, kerogen, and even black carbonaceous materials under various degrees of diagenetic processes. SOM is believed to serve as a principal geo-sorbent for hydrophobic organic contaminants and to influence the mobility, reactivity, bioavailability PAHs in water ecosystems. SOM characteristics can be altered on account of microbial processes based on different types of final electron acceptors in aerobic, anoxic, and obligate anaerobic conditions (Xing, 2001; Hong et al., 2010).

Our studies in 2004 revealed that qualitative content of OM changes and stable toxic substance concentrations increase downstream the Amur to its lower reaches. Water samples from the Amur lower reaches most often contained such high-molecular polyaromatic hydrocarbons as benzapyrene, dibenzoanthracene and benzopelylene, whereas water samples from the liman contained low-molecular hydrocarbons, including naphthalene and phenanthrene (Kondratyeva et al., 2007).

Further chromatographical and microbiological studies showed that river-discharged aromatic compounds spread along the northern waterway in the direction of the Okhotsk Sea and undergo transformation. It is proved with activity of plankton and benthos microbial complexes. Although MC that grow in the shallow southern part of the Amur Liman were less active than naphthalene destructors, they turned out to be able to transform phenanthrene, which gets into water ecosystems from natural and anthropogenic sources (Kondratyeva, Stukova, 2008).

The role of microbiological processes in Amur Liman water quality formation is undoubted, especially taking into account intensive river-discharged amount of allochthonous dissolved and suspended substances, which, while decomposing, supply biogenic elements to producers. In the zone of sea and fresh water mixing sedimentation processes are not just accelerated, but coupled with increased rate of OM destruction. That is why, special studies should be focused on OM destruction mechanisms in conditions of a changing salinity regime and the impact of destruction-resulting products (nitrates, nitrites, carbonic gas) and toxic intermediates on the growth of autotrophic organisms.

Important information on phytoplankton conditions and activity of microbial complexes was obtained in 2006 during the complex expedition of the FEB RAS research institutes in the Amur Liman (Kondratyeva et al., 2008). Expedition data analysis allows viewing together processes of production and microbiological destruction in various parts of the Amur River estuary and identifying prevailing factors, which affect these processes. Experimental data show (Table 1), that salinity changes affect the utilization of carbohydrates and nitrogen-containing OM, and therefore the rate of production of carbonic gas, nitrates and nitrites from easily decomposed OM.

Table 1. Activity of Amur Liman Microbioceonoses in Peptone, Glucose and Phenol of Different Salinity (optical density at 490 nm)

Sampling Sites	Peptone		Glucose		Phenol	
	1 % NaCl	3 % NaCl	1 % NaCl	3 % NaCl	1 % NaCl	3 % NaCl
Amur River mouth	0.95	0.6	1.35	0.75	0.285	0.075
Central part	1.4	1.0	1.75	1.5	0.345	0.10
Northern part	1.25	0.7	0.57	0.59	0.12	0
Southern part	0.90	0.63	1.0	0.95	0	0

Microbiological studies show that bacterioplankton activity is affected by such factors as salinity, biogenic elements (phosphate ions), pH and toxic substances. The potential rates of peptone and glucose utilization decrease when salinity increases. Maximal bacterioplankton activities are registered at the salinity level up to 3%. The studies of pH impact show that glucose is most effectively utilized in the pH range of 7.2-7.6. The increase of pH in water media (over 7.8) is coupled with the decrease of potential activity of easily available sources of carbon. MC activity dynamics changes significantly in the presence of toxic phenol (0.1%). Inhibition of easily available organic substances is evident in spite of abiotic factor dynamics. The central part of the Amur Liman is heavily polluted with labile biochemical organic substances. Pollution affects the capacity of MC to utilize peptone and glucose in a wide range of salinity. Maximal activity of MC at high salinity (3 % NaCl) was registered at stations 16 and 19, where fresh water influence in summer time was the least as compared to stations 12 and 14. Stations in the central part of the estuary (31 and 33) should be singled out, as high rates of peptone and glucose utilization were recorded there. Maximal concentration of dissolved carbon (DIC = 1.626 mmol/kg) was also registered at station 31 (Kondratyeva et al., 2008). It might be explained with the intensive microbiological destruction of organic substances, discharged by the Amur River.

Special research revealed local zones, where MCs were highly resistant to phenol. Our experiments showed that surface MC at stations, situated in the zone of Amur discharge influence (the internal part of the estuary), could utilize phenol. In some areas phenol utilization rate depended on a salinity regime. The inhibition effect increased with salinity increase. High microbial activity correlated with phenols at salinity range 1-3 %. NaCl was registered in the zone of river water discharge (station 11). Zones of low MC activity under phenol pollution were also identified. These zones were at the maximum distance from the Amur discharge impact (stations 42, 43, 44).

The correlation analysis showed that a factor of salinity did not play an important role in chlorophyll *a* synthesis in the Amur Liman (Table 2). Primary productivity at stations located along the Amur River flow in the direction of the Okhotsk Sea depended much on concentrations of nitrates ($R^2 = 0.975$) and carbonic gas ($R^2 = 0.72$) in water. Oxygen was a limiting factor mostly in the Amur estuary zone where salt concentrations were rather low. It is quite understandable keeping in mind oxygen demand for organic matter oxidation due to active bacterial destruction.

In different Amur Liman parts autotrophic assimilation of CO_2 varied in a wide range. In the Tatar Strait the coefficient of correlation between primary production and CO_2 concentrations was negative ($R^2 = -0.59$). Heterotrophic assimilation of CO_2 with MC participation might be specific to this particular part. Such CO_2 assimilation is typical for eutrophic water ecosystems. In the Amur Liman south no close correlations were revealed between chlorophyll *a* concentrations and hydrochemical parameters that characterize destruction rate of nitrogen-containing OM in the nitrogen cycle (NO^{-2}, NO^{-3}).

It should be noted that negative correlation between nitrates and chlorophyll *a* synthesis was observed at the stations located along the north water way ($R^2 = -0.970$) and in the Amur lower reaches ($R^2 = -0.628$). This fact can be used to explain the inhibiting role of nitrites, which might be discharged with Amur water. That is why, discharge of nitrites or their accumulation due to nitrogen cycle disturbance caused by toxic substances may have the dominant impact on primary production.

Table 2. Correlation between Hydrochemical Parameters and Chlorophyll a Concentrations in the Amur Liman

Northern part		Southern part	
Parameters	Correlation coefficient (R^2)	Parameters	Correlation coefficient (R^2)
Chl / NO_3	$R^2 = 0.97$	Chl / NO_3	$R^2 = 0.28$
Chl / NO_2	$R^2 = -0.97$	Chl / NO_2	$R^2 = -0.11$
Chl / CO_2	$R^2 = 0.72$	Chl / CO_2	$R^2 = -0.59$
Chl / O_2	$R^2 = 0.29$	Chl / O_2	$R^2 = 0.57$
Chl / Sal	$R^2 = 0.156$	Chl / Sal	$R^2 = -0.24$

Microbial complexes of water ecosystems participate in various biochemical processes in water surface, depth and bottom sediments. They play an important role in supporting the efficiency of interrelated processes of production and destruction of organic matter and mineralization of different organic compounds. Due to fermenting systems MC take an active part in self refining of marine and fresh water ecosystems if they are polluted with substances of different origin (natural and anthropogenic). They participate in the cycle of substances, including such important elements as carbon, nitrogen, iron, serum and phosphorus.

Positive correlations were observed between the total number of bacterioplankton (TNB), the number of phenol-resistant bacteria (PhRb) and salinity (Sal) only in the northern part of the Amur Liman (Table 3). In the southern part of the Amur Liman positive correlation was only found between phenol-resistant bacteria and salinity. Microbial complexes showed a negative reaction to the presence of hydrocarbons (Hydr) in water irrespective of their habitat area.

Table 3. Correlation between Abiotic Parameters (Sal - salinity, Hydr - hydrocarbons) and Microbiological Index (PhRb – phenol resistant bacteria, TNB – total number of bacteria) in the Amur Liman

Northern part		Southern part	
Parameters	Correlation coefficient (R^2)	Parameters	Correlation coefficient (R)
TNB / Sal	$R^2 = 0.92$	TNB / Sal	$R^2 = -0.71$
TNB / Hydr	$R^2 = -0.73$	TNB / Hydr	$R^2 = -0.65$
PhRb / Sal	$R^2 = 0.99$	PhRb / Sal	$R^2 = 0.99$
PhRb / Naph	$R^2 = 0.93$	PhRb / Naph	$R^2 = 0.52$
PhRb / Hydr	$R^2 = -0.99$	PhRb / Hydr	$R^2 = 0.08$

A high number of PhRb, grown on naphthalene (Naph), was coupled with a high bacterioplankton activity, especially in the northern part of the Amur Liman (R = 0.93).

Activity of microbiological destruction of Amur-discharged nitrogen-containing OM at the observation stations located in the direction of the Okhotsk Sea was proved with high coefficients of correlation between concentrations of nitrites, nitrates and CO_2 ($R^2 = 0.702$ – 0.856). Intensive destruction of nitrogen-containing OM in desalinated waters is also reflected in positive correlation between concentrations of nitrates and carbonic gas ($R^2 = 0.826$).

Biogeochemical processes in the northern and southern parts of the Amur Liman were found to be noticeable different. In the Liman south correlation between total bacterioplankton number and its ability to grow on glucose (R^2 = 0. 83) was high. Once again the obtained estimates proved that an active growth on glucose is typical for microbial complexes of those habitat areas, where intensive growth of autotrophic organisms is observed. In the northern waterway similarly high correlation was found between the number of bacterioplankton and utilization of nitrogen-containing organic substances.

The research undertaken in 2004 showed that PAH transformation mechanisms depend of PAH composition and origin. The correlation between the growth of bacterioplankton in naphthalene and its transformation product pyrocatechol (R^2 = 0, 92) was found to be typical in the northern part of the Amur Liman (Kondratyeva, Stukova, 2008a). Pyrocatechol and salicylic acid may result from the destruction of aromatic compounds of autochthonic origin and of PAHs that belong to suspended matter, accumulated in bottom sediments.

In 2006 low growth activity on PAH characterized nearly all MC of the Amur liman. Increased activity in regard to naphthalene was registered at station 23 at the entrance to the Tatar Strait. We assume that at this part of the Amur Liman water surface is polluted with hydrocarbons of anthropogenic origin, including products of their transformation, i.e. naphthalene.

Microbiological activity under three-cyclical phenanthrene was higher compared to bicyclical naphthalene. It is evident in regard to the microbial growth and formation of colored products of transformation at the stations of the central part of the Amur Liman and in waters in the direction of the Tatar Strait and the Japan Sea (stations 30, 33, 17 and 23).

According to our data MC at station 23 showed high activity under naphthalene and phenanthrene that can be explained by the chronic pollution of the southern part of the Amur liman with stable hydrocarbons of different genesis. In water masses spreading towards the Japan Sea the ecological situation seems vulnerable. The risks of hydrobionts accumulation of PAH and formation of toxic intermediate products of hydrocarbon destruction is very high here.

Bicyclical naphthalene was most actively decomposed in bottom sediments at stations lower the Amur Estuary and in the central part of the Amur Liman (Kondratyeva., Stukova, 2008b). Here, at the biogeochemical barrier active sedimentation and further transformation of PAH of different genesis take place under the changing salinity regime. At the stations of the maximal distance from the Amur Estuary (station 42, 43, 44) naphthalene was decomposed slowly and benthos MC developed weakly (Figure 2). We can assume that there is no pollution of bottom sediments with PAH at these stations. When salinity increased up to 3% benthos MC did not utilize mono-aromatic hydrocarbons produced with PAH transformation.

Maximal activity of benthos MC under the three-cyclical phenanthrene at many research stations (the Amur Estuary, Amur Liman south, coastal waters at the north-west of the Sakhalin Island) may be associated with its different origin. In the zone of the Amur River impact phenanthrene and its derivatives may be both of natural and of anthropogenic origin. In bottom sediments of shallow waters and in the southern part of the Amur Liman transformation of autochthonous PAH, which are the part of water vegetation, may take place. This was confirmed with a high activity of benthos MC under phenol (stations 18, 19).

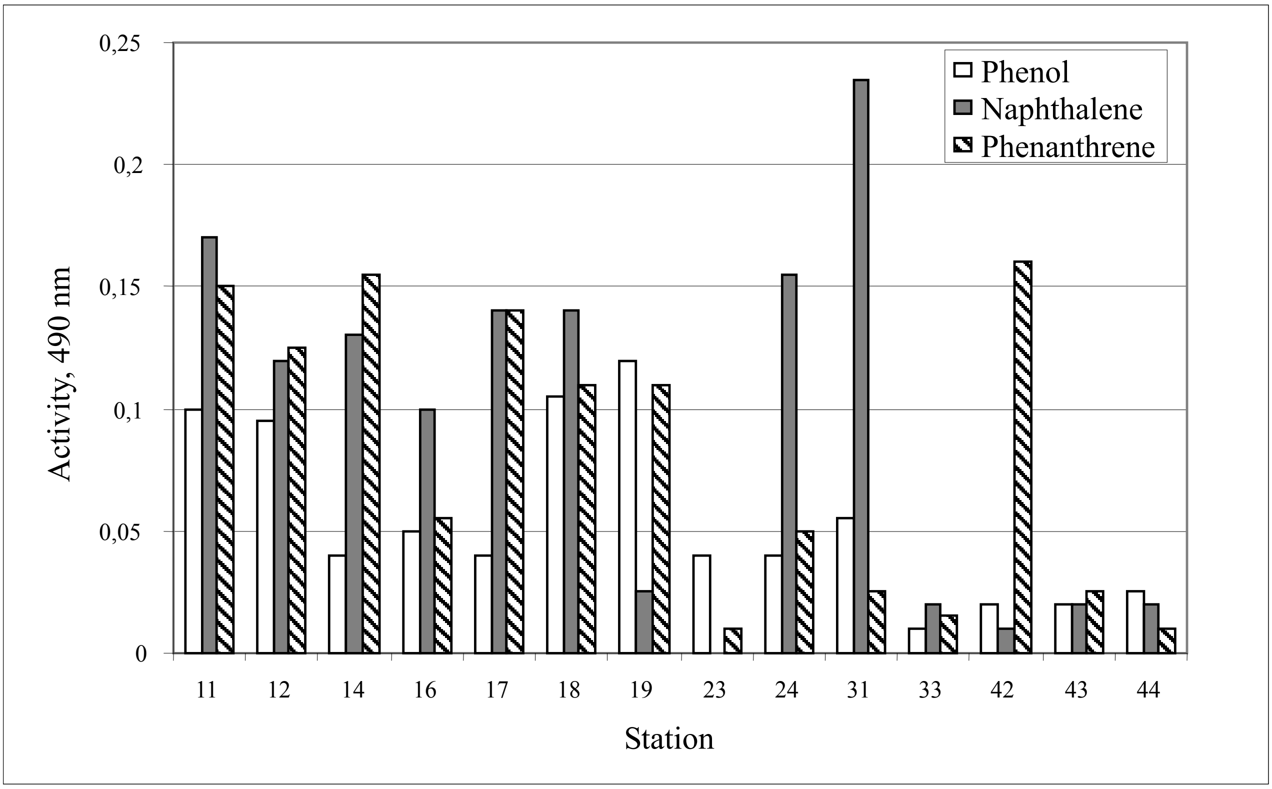

Figure 2. Activity of Benthos Microbioceonoses in Aromatic Hydrocarbons

PAH accumulation in bottom sediments is related to changes in direction of microbiological processes of OM destruction and formation of toxic intermediates. For example, benthos community destruction of PAH revealed volatile benzene derivatives, which cause secondary pollution of close-to-bottom water layers with dissolved substances. Under the lack of oxygen benzene molecules can transform into more toxic methylated forms (toluol, xylenes) and act as electron donors (Figure 3).

In natural conditions after oxygen exhaustion OM oxidation continues supported by secondary anaerobes and use of other electron acceptors: Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, CO_2. In anaerobic conditions the following chain of reducing processes is observed: nitrate reduction, Mn^{4+} reduction, Fe^{3+} reduction, methane formation, sulfate reduction (hydrogen sulfide formation). Reduction of elements – acceptors causes changes not only in their dissolubility, but also in their toxicity.

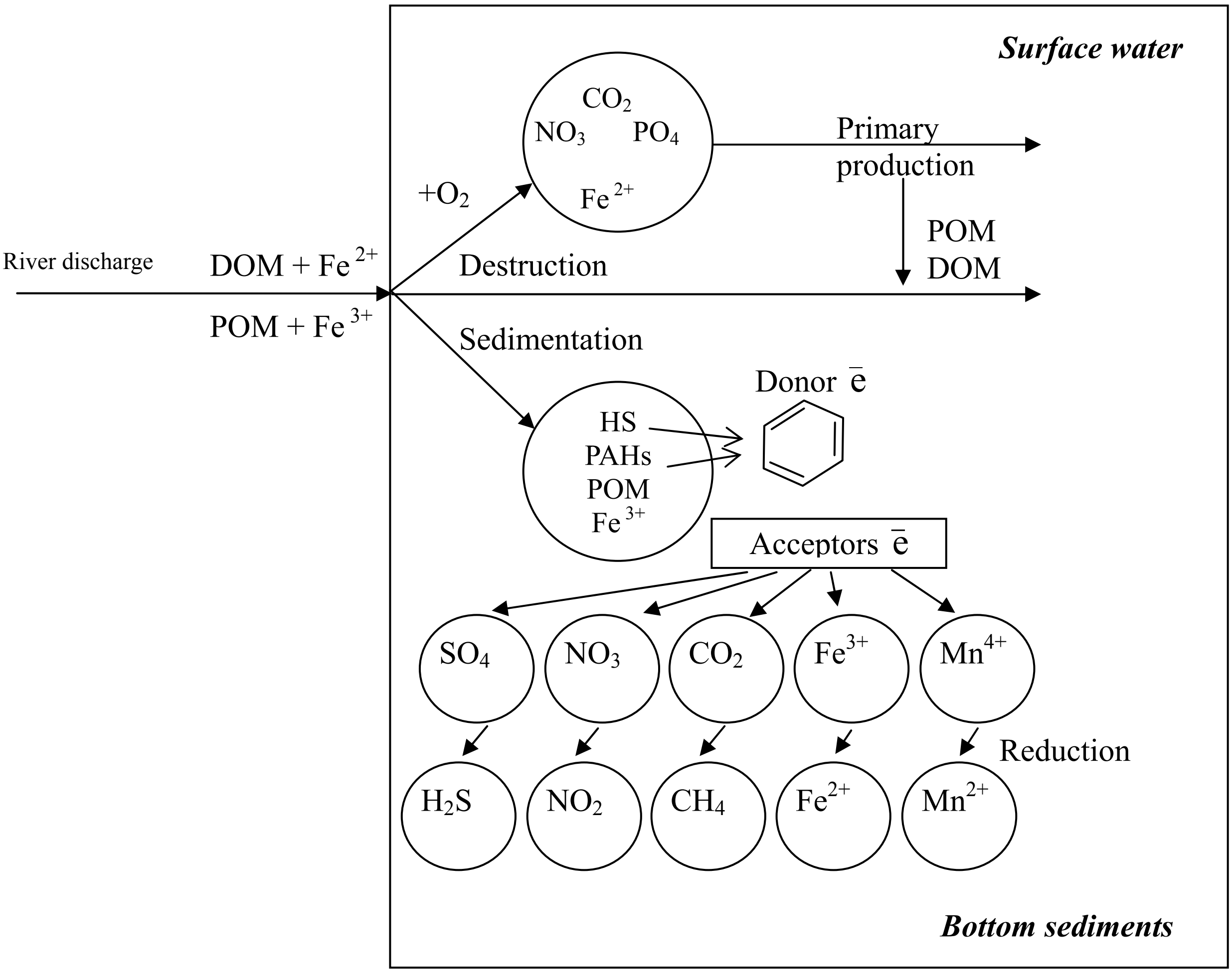

Figure 3. Scheme of Destruction of Dissolved Organic Mater (DOM) in Surface Water and Transformation of Particular Organic Mater (POM), Humic Substances (HS) in Bottom Sediments in Different Oxidation-Reduction Conditions

4. CONCLUSION

Dynamics of biogeochemical processes in the zone of sea and fresh water mixing in various parts of the Amur Liman differs significantly and depends on the input of the river discharge, which in its turn depends on river hydrologic regime and the content of discharged organic matter. In the southern and northern parts of the Amur Liman production and destruction processes are limited with different factors. At present, due to the lack of actual data it seems rather difficult to assess a separate effect of each component of biogeochemical processes, which happen in the Amur Liman. That is why it is also difficult to identify a common factor in the Amur Liman that regulates the interrelation between production and destruction processes. Primary productivity of the Far Eastern seas depends on a great number of abiotic and biotic factors. An important role is played by toxic substances, discharged with river water, and products of microbiological destruction of organic substances of different genesis, formed in the bottom sediments.

When coastal waters are polluted with aromatic hydrocarbons and in case of oxygen deficit, nitrates, sulfates and insoluble forms of iron can play the role of electron acceptors.

Under the lack of oxygen PAH transformations may cause transformations of insoluble forms of iron into the dissolved state. In this case iron is no more a limiting factor for primary production. Accumulation of nitrites, methylated benzene derivatives, hydrogen sulfide and methane in close-to-bottom water layers crucially worsens the ecological situation in the ecosystem. That is why the role of iron in productivity of coastal marine ecosystems should be considered based on total carbon circulation, which is effected both in water and bottom sediments by complicated biogeochemical processes.

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