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ENVIRONMENTAL ASSESSMENT THE COASTAL AQUATORY OF KUIALNIK ESTUARY: MICROPHYTES, ZOOBENTHOS, DIVERSITY OF MICROBIOTA IN THE WATER AND PELOIDS

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We determined the concentration of macro- and microelements, such as: sodium, potassium, calcium, phosphorus, magnesium, iron, chlorides. We have also measured the concentration of hydrogen ions (pH) and nitrogen of urea in samples of water and peloids which were taken from different places of Kuialnik estuary including territories around of such villages: Kotovka, Iliinka, Kovalevka, Novo Kovalevka, Belyayevsky district of Odessa region and such villages as: Korsuntsev, Krasnosilka, Kominternivskiy district of Odessa region.

It was found that concentration of sodium and chlorides was high and increased from southern area to the northern part of Kuialnik estuary where volume of water is lesser. Hydrochemical investigation showed that concentration of macro- and microelements in the deep soils (peloids) was lesser than in water samples and soils taken from the bottom of estuary. Investigation of tissue homogenates of brine shrimp (Artemia salina) showed high enzyme activity of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase. The same biochemical parameters tested in biomass of blue-green algae (cyanoprokaryota), unicellular algae Microcystis sp. where high activity of alkaline phosphatase and high concentration of calcium, potassium and iron were registered. We studied the following microbiota: ferro-oxidizing bacteria (destructors of iron), manganese-oxidizing bacteria (destructors of manganese), sulfate-reducing bacteria, mycobacterium, thiobacteria and representatives from some other microbial groups in the water and peloid samples. Such integrated environmental investigation allowed to assess the environmental situation in different plots of the Kuialnik estuary more properly and complete.

Key words: microphytes, zoobenthos, microbiota, enzymes, macro- and microelements.

В.М. Байрактар, ¹ Л. А. Полукарова² ЕКОЛОГІЧНА ОЦІНКА ПРИБЕРЕЖНИХ АКВАТОРІЙ КУЯЛЬНИЦЬКОГО ЛИМАНУ: МІКРОФІТИ, ЗООБЕНТОС, МІКРОБНА РІЗНОМАНІТНІСТЬ У ВОДІ І ПЕЛОЇДАХ

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У цьому дослідженні ми визначили концентрації макро- і мікроелементів, таких як: натрій, калій, кальцій, фосфор, магній, залізо, хлориди. Було встановлено, що концентрація натрію і хлоридів була високою і збільшувалася з південної частини до північної частини Куяльницького лиману де об'єм води значно менше. Гідрохімічні дослідження показали, що концентрації макро- і мікроелементів в глибинної грязі (пелоїдів) набагато менше, ніж у зразках грязі (пелоїдів), які взяті з придонних шарів лиману. Дослідження гомогенатів тканин у такого представника зообентосу як зяброногі рачки (Artemia salina) показали високу активність ферментів лактатдегідрогенази, аланінамінотрансферази, аспартатамінотрансферази, лужної фосфатази. Ті ж біохімічні показники були досліджені у біомасі сіньозеленої водорості (ціанопрокаріоти) одноклітинних водоростей Місгосузtія, де відзначалася висока активність ферменту лужна фосфатаза і висока концентрація кальцію, калію і заліза.

Ключові слова: мікрофіти, зообентос, мікробіота, ферменти, мікро- і макроелементи.



В. Н. Байрактар, ¹ Л. А. Полукарова² ЭКОЛОГИЧЕСКАЯ ОЦЕНКА ПРИБРЕЖНЫХ АКВАТОРИЙ КУЯЛЬНИЦКОГО ЛИМАНА: МИКРОФИТЫ, ЗООБЕНТОС, МИКРОБНОЕ РАЗНООБРАЗИЕ В ВОДЕ И ПЕЛОИДАХ

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В этом исследовании мы определили концентрации макро- и микроэлементов, таких как: натрий, калий, кальций, фосфор, магний, железо, хлориды. Также измеряли концентрации ионов водорода (рН) и азота мочевины в пробах воды и пелоидах, которые были взяты из разных мест Куяльницкого лимана, в том числе на территориях вокруг таких сел как Котовка, Ильинка, Ковалевка, Ново-Ковалевка Беляевского района Одесской области и сел Корсунцы, Красноселка Коминтерновского района Одесской области. Было установлено, что концентрация натрия и хлоридов была высокой и увеличивалась от южной к северной части Куяльницкого лимана где объем воды значительно меньше. Гидрохимические исследования показали, что концентрации макро- и микроэлементов в глубинной грязи (пелоидах) гораздо меньше, чем в образцах грязи (пелоидах), которые взяты из придонных своев лимана. Исследование гомогенатов тканей у такого представителя зообентоса как жаброногие рачки (Artemia salina) показали высокую активность лактатдегидрогеназы, аланинаминотрансферазы, аспартатаминотрансферазы, фосфатазы. Те же биохимические показатели были исследованы в биомассе сине-зеленых водорослей (цианопрокариотов) одноклеточных водорослей Microcystis, где отмечалась высокая активность фермента щелочная фосфатаза и высокая концентрация кальция, калия и железа.

Ключевые слова: микрофиты, зообентос, микробиота, ферменты, микро- и макроэлементы.

Investigation of ecological situation in the Kuialnik estuary plays an important role for biocenosis support and biodiversity condition (TACIS regional programme, 2004).

The southern part of Kuialnik estuary is placed around Odessa city, other northern part is located close to villages Iliinka, Kovalevka and New Kovalevka. Lately Kuialnik estuary has catastrophic ecological situation, because in the coastal territory exists illegal sandpit where sand is taken for construction industry and it blocks off inflow of water to replenish the water reserves of Kuialnik estuary. Such situation lasts already during ten years (Bayraktar, 2011a). Despite the absence of flow to replenish the water in the Kuialnik estuary, it is replenished only by rain water. Therefore in the Kuialnik estuary salt concentration of sodium chloride in waters and peloids very high. Furthermore in the Kuialnik estuary exist from microphytes representative exists only unicellular algae Microcystis sp. However, we still can see the representatives of zoobenthos, such as brine shrimp (Artemia salina), for which increasing of water salinity is acceptable. There are many different urban and regional programs, but no one works in reality for Kuialnik's benefit.

The hydrochemical parameters of waters and peloids which were taken from different places of Kuialnik estuary were investigated. The research program included the following parameters: concentration of macro- and microelements including: sodium, potassium, calcium, phosphorus, magnesium, iron, chlorides. Except of hydrochemical parameters it was investigated biochemical parameters of unicellular algae Microcystis sp. and filtrated homogenates from tissues of brine shrimp (Artemia salina). Also we determined microbiological content of waters and peloids of Kuialnik estuary. The microbiological



investigation was included such bacteria's as Enterobacteriaceae group with opportunistic infections of Escherichia coli, Klebsiella pneumonie, Enterococcus faecalis, Proteus, Cirobacter, Enterobacter, and other species. The group of saprophytic bacteria was investigated in water and peloids, namely Myxobacteries, Ferro-oxidizing bacteria (destructors of iron), Manganese-oxidizing bacteria (destructors of manganese), and Sulfatereducing bacteria.

Microbiota condition is informative, because it gives such possibilities to assess ecological condition and biocenosis in generally and to specify that microbial loading in reality exists in the estuaries' water and peloids samples of which were taken near to bottom and from deep of soil estuary (Palmer, 1986; Polukarova, 2011). The aim of research was to specify ecological condition in Kuialnik estuary using hydrochemical parameters, biochemical parameters of zoobenthos (Artemia salina) and biodiversity of microbiota.

For achievement of aim few tasks were formulated. We planned to investigate biochemical parameters of water and peloids; to investigate biochemical parameters of the filtered homogenate of tissues in collected samples of brine shrimp (Artemia salina), and to determine in the water and peloids the microbiota diversity, including bacteria, yeast, and fungi.

MATERIALS AND METHODS

It was revealed that the hydrochemical parameters of waters and peloids in the Kuialnik estuary including macro- and microelements. All tested hydrochemical parameters are based on a principle of spectrophotometric analysis and were made using biochemical analyzer Cobas, (Hoffman La Roche Company, Switzerland). All tests were conducted using specific test kits for each studied parameter. The kits were made by the BioSystems Company S.A. (Costa Brava, Spain). Such electrolytes concentration as sodium and potassium was determined by ion-selective electrode measurement analyzer (Instrumentation Laboratory Company, Bedford, IL, USA). Determination of calcium (method of color changing by methylthymol blue in alkaline media), phosphorus (method of formation phosphomolybdate complex in acidic media), magnesium (calmagite in alkaline media forms a stable chromogenix), iron (ferrozine method), chlorides (mercury thiocyanate in acid media react with trivalent ferric ions and form a red complex).

As a representative of zoobenthos it was investigated filtered homogenate of tissues from collected samples of brine shrimp – Artemia salina, Macro- and microelements concentration included following parameters: sodium, potassium, calcium, phosphorus, magnesium, iron, chlorides. In the investigation it was included some parameters of metabolism: protein (total) (biuret method), glucose (glucose oxidase/peroxidase), triglycerides (method of glycerol phosphate oxidase/peroxidase), and nitrogen of urea (urease/glutamate dehydrogenase).

In filtered homogenates it was determined the enzyme activity: Lactate dehydrogenase (recovery of pyruvate by NADH with forming lactate and NAD+), phosphatase (conversion of phosphate groups with 4-nitrophenyl phosphate in 2-amini-2-methyl-1-propanol), amylase (catalytic concentration was determined by the formation of 4-nitrophenol), some aminotransferases by alanine and aspartate amino acids, was determined as well as (catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm by means of the malate dehydrogenase coupled reaction).

For isolation and identification of microbiota from water and peloid samples which were taken from Kuialnik estuary special selective medias were used such as: Enterococcosel Agar, Pseudosel Agar, Endo Agar, Inhybitory Mold Agar (IMA), Staphylococcosel Agar,



Corynebac Agar, AGV Agar. All selective Agars made by the (Becton Dickinson Company, USA). For isolation of myxobacterias, ferro oxidizing bacteria (destructors of iron), manganese-oxidizing bacteria (destructors of manganese), sulfate-reducing bacteria we used special selective medias collected by the recipe of (HiMedia Laboratories, Mumbai, India), sulphate reducing medium, manganese Agar Base, medium for isolation of Iron bacteria.

Statistical significance was evaluated by Student's t-test with P – value: P<0.1; P; <0.05; P; <0.01.

RESULTS AND DISCUSSION

In waters which are closer to the coastal aquatory of Kuialnik estuary the common species of microphyte is the unicellular algae Microcystis sp. which placed on the bottom of water. Such representative of zoobenthos as brine shrimp (Artemia salina) plays an important role in ecological support and self-purification of Kuialnik estuary; its contamination is more than 70-120 items per square meter at the end of May. Concentration of sodium and potassium is higher in the waters at the bottom of estuary and lesser in the surface layer waters and deep soils. On the contrary, the concentration of calcium and phosphorus is higher in the waters and lesser in the soil from waters bottom and deep soil of estuary. Chlorides content is lesser in the deep soil (up to 200%); in the surface layer waters it about of 760 mmol/L.

Total mineralization of waters in estuary is high but Artemia salina living at such mineralization. By chemical type of water has sodium-chloride group and contain in the summer (July): sodium concentration from 430 to 540 mmol/L and chlorides from 758 to 768 mmol/L in the autumn close to Illinka village (September) chlorides concentration 647 mmol/L., around the coast of village Korsuntsev, chlorides concentration 635 mmol/L and around the coast of Krasnosilka village 632 mmol/L. The water of the estuary is characterized stiffness, related mainly to the presence of magnesium cations, the alkaline reaction of water (pH), high organic content presence of sufficient amounts of nutrients for photosynthesis. The composition of the ions and the total mineralization of water are continuously changing under the influence of meteorological conditions. These changes are mostly seasonal than climatic. Typically, when the seasonal changes in the smallest concentration of salts observed in spring (during the melting of snow and ice) and the largest - at the end of summer or winter. During the growing season, which begins for the brine shrimps (Artemia salina) in the spring - in the second part of April, during heating the natural brine to 4-5 degrees of Celsius, and until the end of October – early November (when temperature cooled to 4-5 degrees of Celsius) in the shallow the Kuialnik estuary. If the temperature is unfavorable for the brine shrimps (Artemia salina) their populations exist in the form of cysts, which can help to save Artemia population.

Seasonal investigations of the concentration of macro- and microelements in Artemia salina during summer (the end of July) showed more high parameters in comparison with the same parameters at the beginning of autumn (middle of September) are generalized in Table 3. During the summer the concentration of sodium in the tissues of brine shrimps (Artemia salina) concentration of calcium, iron, chlorides and nitrogen of urea is increasing in comparison with autumn period. However concentration of potassium, phosphorus, magnesium decreased. Contrary in the autumn concentration of potassium, phosphorus, magnesium increasing in the homogenates of tissues and concentration of sodium, calcium, iron, chlorides, nitrogen of urea, decreasing.

Macrophytes in the Kuialnik estuary do not exist, except of unicellular algae Microcystis sp. which is possibly to meet in the coastal waters close to the bottom. The



reason for such situation is very high salinity where most of macrophytes can not exist. For the complex assessment and supporting positive biocenosis on the Kuialnik estuary it is necessary to make a lot of measures, including seasonal monitoring of environmental condition. To follow necessary measures which will improve and support in the Kuialnik estuary waters reserve area to specify bioindication of coastal area of estuary. Basic biochemical properties of microphytes, zoobenthos and hydrochemical parameters of water and peloids are generalized in Tables 1-3.

Table 1 Hydrochemical parameters of waters and peloids and parameters of microphytes and zoobenthos in the Kuialnik estuary.

Macro- and Microelements	Unit	Waters	Peloids from soil surface	Peloids from deep soil	Microcystis sp. (homogenates of unicellular algae)	Artemia salina (tissue homogenates)
Sodium	mmol/ L	439.1±4.1	549.7±7.5	304.1±9.9	603.2±6.8	218.9±3.2
Potassium	mmol/ L	53.77±2.6	59.98±2.9	4.14±0.4	64.27±1.7	2.25±0.07
Calcium	mmol/ L	*53.6±0.9	44.3±1.5	11.7±0.5	50.0±2.6	9.84±0.7
Phosphoru s	mmol/ L	**0.3±0.1	***0.06±0.01	***0.01±0.00	***0.02±0.00	**0.13±0.0 2
Magnesiu m	mmol/ L	*2.23±0.1	**2.32±0.04	*1.70±0.1	2.33±0.1	1.61±0.2
Iron	μmol/L	1.0±0.2	4.0±0.3	2.0±0.2	39.0±1.3	14.0±0.2
Chlorides	mmol/ L	766.3±12.2	764.9±13.3	280.4±3.5	758.0±10.5	218.4±6.7
Nitrogen of urea	mmol/ L	***0.01±0.00	***0.01±0.00 6	***0.05±0.01	***0.01±0.00	0.65±0.08

Standard deviation was calculated, statistical significance of difference was evaluated by Students t-test. Note: P-value * P≤0.1; ** P≤0.05; *** P≤0.01.

Table 2 Biochemical parameters of brine shrimps (Artemia salina) in filtered homogenates of tissue and same parameters in unicellular algae Microcystis sp.

Enzyme activity	Unit	Artemia salina	Microcystis sp.	
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Alanineaminotransferase	μmol/min x 10 ⁻² g	11.6±0.4	4.9±0.2	
Amylase	μmol/min x 10 ⁻² g	8.4±0.2	**0.6±0.05	
Aspartateaminotransferase	µmol/min x 10 ⁻² g	33.2±0.9	11.6±0.5	
Cholinesterase	μmol/min x 10 ⁻² g	*3.2±0.1	*1.6±0.1	
Lactate dehydrogenase	μmol/min x 10 ⁻² g	14.9±1.1	8.3±0.4	
Lypase	μmol/min x 10 ⁻² g	*3.2±0.1	3.2±0.2	
Phosphatase (alkaline)	μmol/min x 10 ⁻² g	96.2±1.4	76.36±1.7	

Standard deviation was calculated, statistical significance of difference was evaluated by Students t-test. * $P \le 0.1$; ** $P \le 0.05$.

Microbiota in waters and peloids (soils) is represented with Thiobacillus thiooxidans which is autotrophic, sulfur-oxidizing bacterium, oxidized elemental sulfur to sulfite,



consumes sulfur and produces sulfuric acid. Thiobacillus thiooxidans is a chemolithotrophic acidophilic bacterium that grows on elemental sulphur as energy source and is important in the microbial catalysis of sulphide oxidation. It oxidizes both elemental sulphur and sulphide to sulphuric acid. Thiobacillus thiooxidans was obtained from peloid samples from the bottom of Kuialnik estuary (Odessa, Ukraine).

Table 3
Seasonal concentration of macro- and microelements in Artemia salina during summer (end of July)
and autumn (midst of September)

Macro- and microelements and parameters of metabolism	Unit	Artemia salina (July)	Artemia salina (September)
Sodium	mmol/L	218.9±4.1	174.2±3.65
Potassium	mmol/L	2.25±0.2	3.86±0.2
Calcium	mmol/L	*9.84±0.1	4.15±0.2
Phosphorus	mmol/L	**0.13±0.02	*1.6±0.1
Magnesium	mmol/L	1.61±0.06	*2.07±0.1
Iron	μmol/L	13.0±0.4	6.0±0.2
Chlorides	mmol/L	218.4±2.5	160.2±2.4
Nitrogen of urea	mmol/L	**0.65±0.05	**0.19±0.05
Glucose	mmol/L	***0.03±0.002	***0.02±0.003
Triglycerides	mmol/L	**0.09±0.02	***0.04±0.006
Protein (total)	g/L	*1.7±0.1	**0.2±0.02

Standard deviation was calculated, statistical significance of difference was evaluated by Students t-

Except for hydrochemical parameters and microbiota content, the quantitative analysis of brine shrimps (Artemia salina) is very informative in terms of peloids' formation and self-purification of Kuialnik estuary (Polukarova, 2012) - see Fig.1.

Optimal growth medium (Thiobacillus thiooxidans OGM) compositions: KH2PO4 - 1.0 g/L., (NH4)2SO4 - 2.54 g/L., MnSO4 - 0.02 g/L., MgSO4 - 0.1 g/L., CaCl2 - 0.03 g/L., FeCl3 - 0.02 g/L., powdered S-2 - 2.0 g/L., Nystatin - 0.1 g/L., pH- 4.0. The inoculated culture was then incubated in thermostat at 30 degrees of Celsius.

Thiobacillus ferrooxidans - is a gram negative, highly acidophilic (pH 1.5 to 2.5), obligatory autotrophic and aerobic Proteobacteria (Tsuyoshi, 1994). These bacteria are motile and possess polar flagella. Thiobacillus ferrooxidans is also thermophilic, preferring temperatures of 45 to 50 degrees of Celsius. Thiobacillus ferrooxidans derives energy from oxidation of ferrous iron to ferric iron, and reduced-sulfur compounds to sulfuric acid. Fine sulfur deposits may accumulate in the cell wall of the bacteria. Other byproducts of metabolism (sulfuric acid) are sometimes associated with the oxidative corrosion of concrete and pipes. For the continuous growth of the bacterium, Thiobacillus ferrooxidans requires approximately 14 mg phosphorus, 2 mg magnesium, 5 mg potassium and 100 mg nitrogen. These measures correspond to per gram of dry biomass amount.

^{*} P\le 0.1; ** P\le 0.05; *** P\le 0.01.





Fig.1. Brine shrimps (Artemia salina) from the Kuialnik estuary.

The colonies of Thiobacillus ferrooxidans showed in the Fig.2. Although Thiobacillus ferrooxidans is classified as an aerobic organism, requiring oxygen to grow and survive, it can multiply under anaerobic conditions as well. Anaerobic oxidation has been demonstrated using elemental sulfur with ferric sulfate. However, elemental sulfur or ferric iron must be present in order for the bacterium to grow.



Fig.2. The colonies of Thiobacillus ferrooxidans growth in selective medium.



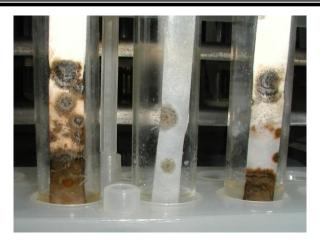


Fig. 3. Myxobacterial growth in test tubes on the stripes of filter paper.

The mycobacterium are a group of bacteria that predominantly live in soil. They are common in animal dung and organic-rich soils of neutral or alkaline pH. Some of them grow by utilizing cellulose, but many of them feed by secreting antibiotics to kill other bacteria and then produce enzymes to lyse the cells of their prey (Gaylarde, 1990; Voordouw, 1992).

The vegetative cells of all mycobacterium are aerobic, Gram-negative, elongated rods with either rounded or tapered ends. They glide in water films across solid surfaces, secreting slime (polysaccharide) tracks in which many cells migrate to produce feathery extensions at the colony margin (Bayraktar, 2011b). At the onset of nutrient depletion the cells migrate back along the slime tracks, aggregating by chemotaxis, to form large concentrations of cells. These aggregates then develop into fruiting bodies which are raised above the agar surface and typically develop a bright yellow, red or brown pigmentation – see Fig.4.



Fig. 4. Mycobacterium growth on the filter paper (yellow and brown colonies).



The sulfate-reducing bacteria isolation was carried out in Postgate medium C (sPGC) (Postgate 1984). The medium consists of the following: NaCl - 6.9 g/L, MgCl2 x 6 H2O - 1.0 g/L, KH2PO4 - 0.5 g/L, NH4Cl - 0.1 g/L., Na2SO4 - 4.5 g/L, CaCl2 x 2H2O - 0.5 g/L, MgSO4 x 7H2O - 1.0 g/L, FeSO4 x 7H2O - 0.005 g/L, trisodium citrate - 0.01 g/L, lactatic acid 20% - 5.5 ml/L, yeast extract - 1 g/L, agar - 20 g/L, distilled water - 1.0 L (Voordouw, 1992). The pH was finally set at 7.2.

The plates were incubated at 30 degrees of Celsius for 10 days under anaerobic conditions filled with carbon dioxide. The preparation and inoculation of plates were carried out inside of environmental chamber which contained a mixture of gases (nitrogen 80%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment (Gaylarde, 1990). Preparation and inoculation of plates were carried out inside of anaerobic controlled environmental chamber. After weekly incubating, several colonies of SRB were observed. The different bacteria were isolated and allowed to grow on separate plates and were found as the same type as Desulfovibrio desulfuricans that were identified by Bergey's Manual of Systematic Bacteriology.



Fig. 5. Sulfate-reducing bacterial growth of Desulfovibrio desulfuricans in test tubes with selective medium.

Sulfate-reducing bacteria (SRB) form one group of sulfate reducing prokaryotes; they are a large group of anaerobic organisms that play an important role in many biogeochemical processes. The main genus is Desulfovibrio that includes Desulfovibrio desulfuricans; these bacteria are nonpathogenic (see Fig. 4). Desulfovibrio desulfuricans is the typical anaerobic, nonpathogenic bacteria which convert the sulfate ions, SO4-2, into S-2 and H2S-.

CONCLUSIONS

We determined the biochemical parameters of unicellular algae Microcystis sp. We have also studied the biochemical parameters of homogenates of brine shrimps (Artemia salina) tissue in seasonal aspect. We investigated the hydrochemical parameters of water and peloids from the surface layer and deep soils samples of the Kuialnik estuary and specified the



condition of microbiota. We were managed to investigate and isolate from water and peloids different bacteria's of Enterobacteriaceae group, Pseudomonas, Candida group of yeast cultures, and group of Streptomycetes.

We determined saprophytic bacteria from the water and peloid samples of Kuialnik estuary, such bacterial species as: Myxobacterias, Ferro oxidizing bacteria – Thiobacillus ferrooxidans (destructors of iron), the group of lithotrophic iron-oxidizing bacteria which involved in the oxidation of iron in aquatic ecosystems, manganese oxidizing bacteria (destructors of manganese), sulfate reducing bacteria (Desulfovibrio desulfuricus), Thiobacteria (Thiobacillus thiooxidans), Myxobacteria.

We proved that the complete assessment of ecological condition on the Kuialnik estuary would be possible if it is conducted by wide investigation including unicellular microphyte content, zoobenthos and microbiota diversity. We specified the ecological condition in the coastal aquatory of Kuialnik estuary and testified its as negative for the growth of macrophytes due to high salinity level; there exist only unicellular microphyte Microcystis sp. From the other side waters and peloids contain microbial diversity most of them are saprophytes.

The quality of the peloids is extremely important for medicinal purposes and could be constantly monitored for the presence of pathogenic microorganisms.

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