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THE ENVIRONMENTAL ASSESSMENT OF COASTAL AQUATORY THE TILIGUL ESTUARY BY BIOINDICATORS (MACROPHYTES AND MICROBIOTA)

We have investigated the Tiligul estuary algae species including red, green and brown macrophytes. It has been determined what is the level of enzymatic activity, proteinic, carbohydrate, lipidic, nitrogenous metabolism and concentration of macro- and microelements. Apart from macrophytes we have analysed filtering capabilities of tissues and compared it to the biochemical parameters of collected samples containing of mollusca - *Mitilaster lineatus* and brine shrimp – *Artemia salina*. For indication of anthropogenic load to the environment, investigated microbiota content in the coastal aquatory of water was done.

We have found that macrophytes content during summer is rich and has a good enzymatic activity. Another finding is the fact that mollusca - *Mitilaster lineatus* and brine shrimp – *Artemia salina* take an active part in self-purification of Tiligul estuary. It was determined that microbiota has antropogenic load to the environment in amount which could be acceptable by nature for self-elimination coliform bacteria which are conditional pathogenic.

In conclusion, our investigation has confirmed a clear correlation between isolated bacteries, yeast and fungi. The investigation included parmeters of enzyme activity, concentrations of macro- and micrioelements and metabolism for each specie of microbiota.

Keywords: macrophytes, algae, mollusca, brain shrimp, enzymes, macroelements, microbiota.

Исследовались некоторые виды водорослей Тилигульского лимана, включая красные, зеленые и бурые. Определялась ферментативная активность, белковый, углеводный, жировой, азотный обмен, концентрация макро- и микроэлементов. Кроме макрофитов, были исследованы фильтраты тканей по биохимическим показателям, полученных от моллюсков *Mitilaster lineatus* и жаброногих рачков *Artemia salina*.

Для определения антропогенной нагрузки на окружающую среду было изучено содержание микробиоты в прибрежных водах Тилигульского лимана. Установлено, что содержание макрофитов в летний период довольно обильно и многообразно с высокой активностью ферментов.

Установлено, что моллюски *Mitilaster lineatus* и жаброногие рачки *Artemia salina* играют активную роль в самоочистке Тилигульского лимана. Было установлено, что большое разнообразие микробиоты является антропогенной нагрузкой на окружающую среду в количестве, которое может быть подвергнуто природной элиминации колиформных бактерий, являющихся условно патогенными.

Результат проведенных исследований показал, что существует четкая граница между выделенными бактериями, дрожжами и грибками. Были исследованы показатели ферментативной активности, концентрация макро- и микроэлементов, показатели метаболизма для каждой выделенной культуры.

Ключевые слова: макрофиты, водоросли, моллюски, жаброногие рачки, ферменты, макроэлементы, микробиота.

Досліджувалися деякі види водоростей Тилігульського лиману, включаючи червоні, зелені та бурі. Визначалася ферментативна активність, білковий, вуглеводний, жировий, азотний обмін, концентрація макро- і мікроелементів. Крім макрофітів, були досліджені фільтрати тканин за біохімічними показниками отриманих від молюсків Mitilaster lineatus і жаброногих рачків Artemia salina.

Для визначення антропогенного навантаження на навколишнє середовище було вивчено вміст мікробіоти в прибережних водах Тилігульського лиману. Встановлено, що вміст макрофітів в літній період досить таки численний і різноманітний з високою активністю ферментів.

Встановлено, що молюски Mitilaster lineatus і жаброногі рачки Artemia salina грають активну роль в самоочищенні Тилігульського лиману. Було встановлено, що велика різноманітність мікробіоти є антропогенним навантаженням на навколишнє середовище в кількості, яка може бути піддана природній елімінації коліформних бактерій які є умовно патогенними.

Результат проведених досліджень показав, що існує чітка межа між виділеними бактеріями, дріжджами і грибками. Були досліджені показники ферментативної активності, концентрація макро- і мікроелементів, показники метаболізму для кожної виділеної культури.

Ключові слова: макрофіті, водорості, молюски, жаброногі рачки, ферменти, макроелементи, мікробіота.

Increasing antropogen load from agriculture to water systems make necessary to find effective methods the monitoring of their ecological condition.

Investigation of ecological condition the Tiligul estuary play important role for biocenosis support and biodiversity.

Part of Tiligul estuary is placed in the Koblevo village, Nikolaev`s region of Ukraine, and connected with the Black sea by water channel.

It the Tiligul estuary inhabit around 63 species of macrophytes including red, green and brown species [3].

The Tiligul estuary was investigated by the TACIS regional programme, it concerned only biodiversity of wild birds, invertebrates, fishes, macro- and micro algae [4]. However such biochemical characteristics of algae as concentration of macro- and microelemens content in the water, near to bottom soil and deep soil wasn`t investigated. The investigation also didn`t cover activity of enzymes in macrophytes and zoobenthos, which abundantly grow in estuary during sammer period. It didn`t investigated microbiota including bacteries in the water, near to bottom soil and deep soil. It wasn`t investigated microbiota including bacteries, yeast, fungi, which are on the surface of macrophytes. Integrated research allow to more complete to assess due to bioindication and seasonal monitoring ecological situation and antropogenic loading to the estuary.

Microbiota parameters is also informative, because it gives possibilities to assess ecological condition and biocenosis generally and to specify which intensity of microbial load could exist in estuarie`s water, soil which is near to bottom and deep soil and determine ability of Tiligul estuary to self-purification [2].

The aim of research was to establish ecological condition in Tiligul estuary by investigationg biodiversity of macrophytes, zoobenthos, microbiota and their bioche-

mical parameters.

To achieve the objective of this investigation, several tasks have been formulated. Firstly, to investigate the biodiversity of macrophytes in the Ttiligul estuary. Secondly, to investigate biochemical parameters from collected samples of macrophytes. Thirdly, to investigate biochemical parameters the homogenate filtrates of tissues from collected samples of mollusca - *Mitilaster lineatus* and brine shrimp – *Artemia salina*. To investigate in the water, soil which are near to bottom and from deep soil microbiota, including bacteries, yeast and fungi [1].

Materials and Methods:

We have investigated macrophytes from the samples received from Tiligul estuary including green, red and brown species of algae. For identification of macrophytes it was made external objective examination and microscopy of native material.

Biochemical investigation for each macrophyte was made, it included testing for enzymatic activity, concentration of macro- and microelements and parameters of nitric, proteinic, carbohydrate and lipidic metabolism in macrophyte filtrated homogenates.

From the members of zoobenthos it was investigated mollusca - *Mitilaster lineatus* and brine shrimp (*Artemia salina*) samples of which collected from coastal aquatory of water the Tiligul estuary. It was tested enzyme activity in homegenate filtrate of mollusca's and brine shrimp`s tissues, such enzymes as: Lactate dehydrogenase, Phosphatase (alcaline), Transferases, Cholinesterase, Alpha-amylase, Lypase. Furthermore, it was tested concentation of macro- and microelements which included: sodium, potassium, calcium, phosphorus, magnesium, iron, chlorides. In the investigation it was included parameters of metabolism such as: protein (total), glucose, triglycerides, urea.

All the above listed parameters are based on a principle of spectrophotometric analysis and made using biochemical analyzer Cobas, which produce (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). For Chlorides and Cholinesterase it was used the kits made by the (Pliva, Lachema Diagnostika, Brno, Czech Republic). The investigation of concentration for such electrolytes as sodium and potassium made using ionometric determination by biochemical analyzer ILyte Na/K with ionselective block (Instrumentation Laboratory Inc., Bedford, MA, USA). Most valuable for investigation cationes it is: sodium, potassium, calcium, magnesium, iron. Most valuable for investigation anions it is chlorides and phosphorus. Each sample sample was tested for biochemical parameters thrice.

For isolation and identification of microbiota from samples which were taken from Tiligul estuary we have used classical methods of microbiological isolation bacteries, yeasts and fungi on special selective medias such as: fluid Thioglycollate medium, Enterococcosel Agar, Pseudosel Agar, Endo Agar, Inhybitory Mold Agar, Staphylococcosel Agar, Corynebac Agar, AGV Agar. All selective Agars made by the (Becton Dickinson Company, USA).

Statisitical deviation and significance of difference was evaluated by Student`s t-test with coefficient ($P<0.05$).

Results and discussion:

Important role in the Tiligul estuary for self-purification play the following macrophytes which were identified: *Chondria tenuissima, Cladophora albida, Enteromorpha clathrata, Enteromorpha compressa, Enteromorpha flexuosa, Enteromorpha intestinalis, Polysiphonia elongata, Rhizoclonium tortuosum, Scytosiphon simplicissimus, Ulva rigida, Potamogeton pectinatus, Ceratophyllum demersum* and others some of which presented in Fig.1.

Fig. 1. Biodiversity of algae in the Tiligul estuary: 1 – Enteromorpha flexuosa; 2 – Polysiphonia elongate; 3 – Chondria tenuissima

The biochemical parameters investigated in some macrophytes presented in the Tab.1.

Apart from macrophytes very informative is quantity of mollusca and brain shrimps, which are allow to self-purification for waters of Tiligul estuary.

Hydrological investigation of water, near to bottom soil of estuary and deeper soil, showed, that in the Tiligul estuary salty water concentration of Sodium was 201.8±36.73 mmol/L, Potassium 4.3±1.24 mmol/L, Calcium 7.41±6.08 mmol/L, Phosphorus 0.02±0.006 mmol/L, Magnesium 1.64±0.015 mmol/L, Iron 4.0± 4.58 mmol/L, Chlorides 208.5±43.28 mmol/L.

The water which is closer in channel to the Black See has less parameters of Calcium, Potassium, Sodium, Chlorides compare with the same in Tiligul estuary. As far from the channel which connect Tiligul estuary with Black See, parameters of Calcium, Potassium, Sodium and Chlorides increase. This channel play important role especially when water in the Tiligul estuary come down, so in this case water via channel appear from the Black See in the estuary and replenish water resources.

Antropogenic load by microbiota is dominant during summer time for Tiligul estuary during summer vacation period when people take a vacation around the Black Sea which by the channel connected with the Tiligul estuary and with sea current water appear through channel into Tiligul estuary. However antropogenic load to environment is in such amount, when nature has ability for self-purification. Bacteria isolated from the samples of water includes: Enterococcus faecalis, Escherichia coli and

Table 1

Enzymatic activity, concentration of macro- and microelements, parameters of metabolism in algae homogenates from samples collected from Tiligul estuary

No	Macro- and microelements	Unit	Enteromorpha clathrata	Enteromorpha compressa	Polysiphonia elongata	Chondria tenuissima
1	Lactate dehydrogenase	μmol/min x 10^{-2}g	36.52±0.36	87.9±2.80	169.32±5.68	109.56±0.60
2	Transferases	μmol/min x 10^{-2}g	858.2±7.45	590.96±2.48	416.66±4.02	190.9±2.36
3	Phosphatase (alkaline)	μmol/min x 10^{-2}g	6.64±0.18	59.76±0.68	1.68±0.15	1.62±0.02
4	Amylase	μmol/min x 10^{-2}g	31.54±0.29	46.97±1.13	94.28±0.45	152.38±2.64
5	Lypase	μmol/min x 10^{-2}g	8.3±2.20	11.62±0.48	16.6±0.73	19.92±0.50
6	Cholinesterase	μmol/min x 10^{-2}g	3.32±0.05	10.66±0.22	1.64±0.02	3.35±0.04
7	Sodium	mmol/L	49.3±0.55	122.4±0.8	103.2±0.79	136.0±0.98
8	Potassium	mmol/L	38.3±0.25	5.91±0.54	7.95±0.05	14.01±0.20
9	Calcium	mmol/L	3.01±0.06	4.39±0.06	3.84±0.29	7.70±0.45
10	Phosphorus	mmol/L	0.38±0.01	0.23±0.02	0.45±0.02	0.56±0.04
11	Magnesium	mmol/L	1.36±0.03	1.61±0.18	1.62±0.07	3.61±0.06
12	Iron	μmol/L	6.0±0.20	84.0±2.08	43.0±1.56	45.0±1.65
13	Chlorides	mmol/L	67.6±1.10	140.8±1.22	133.4±3.11	183.1±1.87
14	Glucose	mmol/L	0.01±0.003	0.01±0.005	0.016±0.003	0.01±0.003
15	Triglycerides	mmol/L	0.25±0.02	0.33±0.02	0.34±0.03	1.42±0.02
16	Urea	mmol/L	0.36±0.02	0.11±0.02	0.81±0.04	1.44±0.03

Standard deviation was calculated and statistical significance of difference was evaluated by Student`s t-test (P<0.05)

some other species directly indicate antropogenic influence to microbiocenosis.

Resume:

1. It was specified ecological condition in water from channel which connect Tiligul estuary with the Black See and the coastal aquatory of Tiligul estuary and claimed as positive ecological condition.

2. For the complex assessment and supporting positive biocenosis on the Tiligul estuary it is necessary to make seasonal monitoring of ecological condition. To follow necessary measures which will improve and support Tiligul water reserve area to specify bioindication of coastal area of Tiligul estuary.

3. It was tested biochemical parameters for samples of macrophytes. From the zoobenthos representatives it was also studied biochemical parameters of mollusca (*Mitilaster lineatus)* and brine shrimp (*Artemia salina)*

4. Investigated hydrochemical parameters of water samples, soils on the bottom and taken from the deep of soil samples from Tiligul estuary to specify condition of microbiota.

5. It was isolated from samples of water, peloides on the bottom and deep of soil different bacteries including: Enterococcus faecalis, Pseudomonas fluorescens, Esherichia coli, Staphylococcus aureus which directly indicate anropogenic load to environment.

6. Isolated yeast cultures Candida tropicalis, Pichia guilliermondii, Saccharomyces cerevisiae also give some imagination for the antropogenic load to the Tiligul estuary.

7. This article show, that complete assessment of ecological condition the Tiligul estuary is possibly when conducted wide investigation including macrohytes content, zoobenthos and microbiota diversity.

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ДОСЛІДЖЕННЯ І ПІДБІР НАТУРАЛЬНИХ ІНГРЕДІЄНТІВ ДЛЯ ПРИГОТУВАННЯ БЕЗАЛКОГОЛЬНИХ НАПОЇВ

Досліджені натуральні ароматизатори, екстракт білого чаю, соки та їх дози для виробництва напоїв. Розроблені рецептури двох нових напоїв на основі вищевказаної сировини.

Ключові слова: екстракт, ароматизатор, дегустаційна оцінка, сік.

Natural flavours, extract of white tea, juices and their doses, are neat for the production of drinks. Compounding of two new drinks is developed on the basis of the higher indicated raw material.

Keywords: extract white tea, a flavour, tasting estimation, juice.

Виробництво і споживання безалкогольних напоїв у світі з кожним роком зростає. Обсяги споживання безалкогольних напоїв в Україні за період з 1980 до 1990 р. зросли з 19,9 до 29,5 дм³/рік на особу, а в наступні роки скоротилися до 19 дм³/рік , в той