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ҚАЗАҚСТАН РЕСПУБЛИКАСЫ ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ

## БАЯНДАМАЛАРЫ

# **ДОКЛАДЫ**

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК РЕСПУБЛИКИ КАЗАХСТАН

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# BIODIVERSITY OF THE MICROFLORA OF THE SALT LAKES DZHAKSY-KLYCH AND BUGA-DZHAILY

**Abstract.** The article illustrates the results of microbiological and chemical studies of samples of salt lakes in the south of Kazakhstan, such as Dzhaksy-Klych and Buga-Dzhaily. It was found that the samples from the bottom of the dried up Small Aral Sea are dominated by ions of magnesium, potassium, calcium, iron and copper. Moreover, the presence of pesticides was revealed: acetamiprid, fenhexamide, folpet-like compounds, tetrahydrophtolamide, which are used in the agro-industry. Samples of Lake Dzhaksy-Klych were isolated from different layers of halite, magnesium, and sulfate salts. Salt-containing raw material of Lake Buga-Dzhaily contains  $96.0 \pm 8.9$  wt.% NaCL, the content of magnesium ions ranges from  $0.17 \pm 0.01$  - $4.01 \pm 0.0$  wt.%, The presence of sulfur is within  $0.26 \pm 0.02\%$ .  $1.71 \pm 0.12$  wt%. The amount of heavy metals is below the permissible concentration. It was found that the increased content of magnesium in the samples proves the presence of mixed sodium-magnesium layers. he number of heterotrophic microorganisms in the samples of salt lakes is approximately 103-104 CFU/g, enterobacteria -103 CFU / g, micromycetes in the amount of  $(6.0 \pm 0.5)$  x 103 CFU/g g were found only in the sample of sulfate salts and silt sampled at a distance of 1.0 m from the coastline. The distribution and density of microflora on the coastline indicate an increased anthropogenic factor due to salt extraction.

**Key words:** salt lakes, salt-containing raw materials, microflora, heterotrophic microorganisms, micromycetes, pesticides.

**Introduction.** Continental salt water bodies differ markedly in chemical and microbiological composition, while the seas are similar in this composition. The composition of these waters directly depends on the geological, climatic and biogeochemical conditions and can be neutral, alkaline and acidic. Salt water bodies are poor in the presence of microorganisms. Moreover, the variety of these organisms is limited. Continental salt water bodies are often confined to areas with an arid climate, for example, in the south of Kazakhstan.

Numerous studies show a low level of biodiversity of microorganisms, which are widespread in the water itself and the coastal area. Most organisms, adapting to extreme conditions, begin to adapt at the cellular and functional levels [1,2]. Such changes have been investigated for further industrial application [3].

According to the chemical composition of some sodium salts, salinization of water can be sulfate, chloride, soda or mixed, with the most harmful effects of Na + and Cl- ions. The salinity of water is the main factor in the adaptation of aquatic organisms to environmental conditions. Adaptive mechanisms are based on the regulation of the concentration of ions in the intracellular fluid and its osmotic pressure [4,5]. The concentration of inorganic ions in the aquatic environment can vary over a very wide range, which leads to an increase in the ionic gradient between the body and the environment.

The maintenance of osmotic pressure inside the cell under conditions of different salinity of the aquatic environment is carried out by two mechanisms: a change in the concentration of organic osmotically active substances in the cytoplasm and a change in the content of inorganic ions in it [6,7]. The molecular aspects of adaptation of prokaryotes to changes in the salinity of the aquatic environment have been established [8]. According to [9, 10], NaCl in concentrations higher or lower than optimal, but still within acceptable limits, can play the role of a stress factor that causes morphophysiological and behavioral changes in organisms. The influence of salinization of water bodies on the stability and change in the structure of the population of organisms of aquatic organisms has been established [11,12,13].

On the other hand, in studies by Loukas et al. shows that the salinity level of the reservoir is a less significant limiting factor than expected. The formation of biocenoses of salt water bodies is influenced by a combination of factors such as pH of the environment, oxygen content and ionic composition of water, anthropogenic impact, hydrological and geographical characteristics of the reservoir, and paleoclimatic parameters [14,15,16, 17]. The influence of climatic conditions on the landscape is shown in the work on the basis of a model of biological and ecological productivity of landscape systems of the South Kazakhstan region, including Almaty, Zhambyl, Turkestan and Kyzylorda regions using long-term data from 32 meteorological stations located in the region [18].

In this regard, the purpose of the study was to study the effect of saline raw materials on the biodiversity of microflora in the Dzhaksy-Klych and Buga-Dzhaily lakes located in the south of Kazakhstan.

**Objects and methods of research.** As a research objects were used salt-containing raw from lakes Dzhaksy-Klych and Buga-Dzhaily: halite, halite-sulfate, sulphate, sulphate-magnesium salt, brine, surface brine (halite layer), brine (sulfate formation) etc.

Lake Dzhaksy-Klych –the biggest salt lake of the Caspian lowlands located in the Aral sea region. The lake is of marine origin and consists of two parts with an area of 18 and 58 km. The thickness of the salt deposit is about 2 m.

Lake Buga-Dzhayly is one of the largest lakes in the system of salt lakes located in the Suzak district of Turkestan region. Every year, up to 150 thousand tons of table salt is extracted by open method.

Sampling was carried out in accordance with GOST 33770-2016. Samples were taken from the Dzhaksy-Klych Deposit by "ONYX-R" LLP and the authors of the article; from the Buga-Dzhaily Deposit-by "As-Dinar" LLP.

Microscopic studies were performed using "Tauda" and "Mikmed-5" microscopes, light microscopy methods.

Microorganisms were isolated and cultured on the appropriate nutrient media: heterotrophs - on MPA, micromycetes - on Chapek medium, enterobacteria-on Endo-Ploskirev medium. Haloresistance of isolated cultures of microorganisms was determined by their ability to grow at various concentrations of NaCl from 0 to 20%.

In the study of sulfur-containing raw materials, a mass spectrometer with inductively coupled plasma was used, the elements were determined in accordance with ST RK ISO 17294-2-2006. Chemical analysis was carried out according to GOST 13685-84.Research conditions: temperature-250C; humidity -83.0%; pressure-714 mm Hg.

Statistical processing of the results was performed by calculating the arithmetic mean and the standard deviation. All determinations were carried out in 3-and 5-fold repetitions. The data was processed using an IBM Pentium personal computer based on Excel application software packages.

Research results and discussion. To use salt-containing raw materials from two salt lakes in the South of Kazakhstan as bases for cosmetology products, it was necessary to make sure that they were harmless to human health. In this regard, physical, chemical and microbiological studies were carried out. In previous studies (Aladin et al., 2008), it was found that the content of sulfates in the Aral sea salts exceeds 31% (of the total amount of salts), and sodium chloride is only 54%. The ionic composition of the salts is as follows: sodium - from 2.83 to 13.73%; sulfate-ion-7.5-30.14; calcium - up to 1.08; magnesium-3.03; potassium-0.93; carbonate-ion-0.18; chlorine-2.09; water - up to 55.23%. The analysis of the elemental composition of salt samples taken during expedition trips in 2019 from the dried bottom of the Small Aral sea revealed the predominance of magnesium, potassium, calcium, iron and copper ions in them (table 1). The chemical composition of the Aral salts belongs to the chloride group, although in some cases there are also deposits of sulfide compounds, while the proportion of sodium chloride in samples from 15 sampling points is 89.98%, and sulfates-no more than 2.0%.

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Elements Mas. % mkg/l 0.001039747 593,7200 В Mg 0.046351029 63073,6600 0,003912761 78,0350 A1 0.005430454 203,2210 P K 0,018169232 11514,1000 Ca 0,236304506 67786,4576 Ti 0,000126723 5,2341 0,025324563 1263,2342 Fe 0.002123332 Cu 2132,5411 Zn 0.002343253 3.1234 0,000045364 2,2534 Aφ 0,000012432 0,3243 Co 0,0006243001 7,2364 Ba 0,0034524321 Cr 32,4323 0.000254364 4.6300 Ni 0.0000354231 15,6534 Mo Na<sub>2</sub>SO<sub>4</sub> 1,89 1,98 g/l NaCl 89.98 1188,98 g/l

Table 1 – Chemical composition of salts of the bottom of the Small Aral sea (main components)

Due to the fact that the Aral sea region is characterized as an agro-industrial region where a variety of pesticides are widely used for agricultural production, analyses were conducted for the content of pesticides in samples of salt-containing samples. In the analyzed samples was not detected biphenyl, diphenylamine, but is revealed acetamiprid, phenhexamid, pholpet-like compositions, tetrahydrofolate. In total, about 400 active substances were searched on LC-MS/MS and GC-MS/MS. Almost all compounds were found on GC-MS/MS, acetamiprid on LC-MS/MS. Comparing the detector responses (peak size), by far the largest residue was found for phengexamide (a fungicide) used to protect against the gray mold *Botrytis cinerea* in crops including strawberries, raspberries, or blueberries. Other substances are found only in trace amounts.

In the average samples of lake Buga-Dzhaily salt, the pH of the salt solution did not exceed 6.34±0.5. No lead, arsenic, zinc, or iron were found in the samples. Chemical analysis of averaged samples of salt-containing raw materials showed that the NaCL content in the feed salt ranges from 96.68-98.06 wt.%; in the food salt-92.88 – 93.94 wt.%; in the brine -26.13±0.01 wt.%; in the sedimentary silt -11.02 wt.%. Elemental analysis showed the presence of magnesium ions, the content of which in the feed salt is in the limit, wt.% -0.17±0.01; in the food salt-1.87±0.11; brine -4.01±0.0; sludge -0.79±0.0. In addition, all analyzed samples revealed the presence of sulfur, the amount of which in the samples of feed salt was in the range, wt.% -0.26±0.02; in brine-1.71±0.12; in silt sediment -0.49±0.0; in food salt, an increase in the sulfur content to 0.98±0.05 was noted. The content of other elements, including heavy metal ions, is below the safety limit. Analysis of the elemental composition of sample salt samples showed that in addition to sodium chloride and sulfate (0.71-1.05% wt.) and insoluble residue (0.07-0.28% wt.) in decimal and hundredths of % wt. boron, magnesium, potassium, and calcium are noted. The prevalence of magnesium in these samples shows the probability of mixed sodium-magnesium layers in the Buga-Dzhaily deposit.

Microscopy of samples of surface brine and brine taken from the sulfate layer of lake Dzhaksy-Klych showed the absence of living objects. Crystals of various sizes and cuboidal shapes were found. In the brine taken from the halite formation from a depth of 0.3 m, in addition to cuboidal forms of salt crystals, amorphous formations and a few amoeboid structures were found. The microflora is represented along with small coccoid and rod-shaped motile bacteria and clusters of small convoluted motile microorganisms noted earlier in the studies of E. G. Dobrynin(1984) of the Eupatoria solprom garden brine.

As a result of microbiological studies, it was found that the number of heterotrophic microorganisms in the samples taken from lake Dzhaksy-Klych ranges from 10<sup>4</sup> CFU/g. However, the number of heterotrophic microorganisms increases to 10<sup>5</sup> CFU/g in samples of halite salt samples taken at a distance of 3-5 m from the coastline. The smallest number of microorganisms was found in samples of sulfate salts,

where the number of heterotrophic microorganisms was within the range of  $(4.2\pm0.3)$  x  $10^3$  CFU/g. The number of enterobacteria in all samples ranges from  $10^3$  CFU/g. Micromycetes in the amount of  $(6.0\pm0.5)$  x  $10^3$  CFU / g were found only in a sample of sulfate salts and silt taken at a distance of 1.0 m from the coastline.

In samples of salt-containing raw materials of lake Buga - Dzhaily, the number of heterotrophic microorganisms was in the range of 10<sup>4</sup> CFU/g, enterobacteria-10<sup>3</sup> CFU/g. In almost all samples, micromycetes of 10<sup>3</sup> CFU/g were detected, with the exception of samples of deep silt and halite salt taken from a depth of 1.0 m from the center of the lake. From the studied samples, taking into account the morphological and cultural properties of microorganisms, 10 cultures of microorganisms were isolated, the dominant part of which consisted of representatives of the genera-Micrococcus, Bacillus, Pseudomonas. The genus Micrococcus is represented by three dominant species-M.luteus, M. roseus, Micrococcus sp. Enterobacteria are represented by the species – Enterobacter sp. Micromycetes are represented by the genera Aspergillus, Mucor, Penicillium, Fusarium. The presence of these taxonomic groups of microorganisms and the nature of the distribution of microflora indicates the contamination of the surface of salt-containing raw materials as a result of human activity.

Haloresistance of isolated cultures of microorganisms was determined by their ability to grow at various concentrations of NaCl from 0 to 20%. As follows from the presented data, it was found that M. roseus grows intensively on a medium with 3-10% NaCl, good growth is observed on a medium with 13% NaCl, and there is no growth on a medium with 17-25% NaCl. Micrococcus sp. it grows intensively on medium with 3-5% NaCl, on medium with 10-17% NaCl it grows well, and on medium with 20-25% NaCl there is no growth. M. luteus and Bacillus sp. they grow intensively on a medium with 0-17% NaCl, on a medium with 20% NaCl, good growth is observed, with an increase in the percentage of NaCl to 25%, there is no growth. Cultures of *Pseudomonas sp.* and *Enterobacter sp.* grow intensively on medium with 3-10% NaCl, with an increase in the concentration of NaCl to13-17% culture Pseudomonas sp. it grows well, with a further increase in the salt concentration to 20% or higher, the growth of this culture stops, and the growth of *Enterobacter sp.* it is suppressed already on the medium with 17-25% NaCl. Aspergillus sp. it grows intensively on a medium with 3-13% NaCl, on a medium with 17-20% NaCl there is good growth, and on a medium with 25% NaCl there is no growth. Mucor sp. on the medium with 3-5% NaCl, good growth is observed, in other variants, the growth of this culture is absent. On a medium with 3-10% NaCl Penicillium sp. it grows intensively, and good growth is observed in the environment with 13-17% NaCl. Fusarium sp. it grows intensively on medium with 3-5% NaCl, with an increase in the percentage of NaCl by 10-13%, there is a good growth, and with an increase from 17% to 25%, the growth is completely absent.

**Conclusion.** Thus, the results of physico-chemical and microbiological studies showed that the salt-containing raw materials of the Dzhaksy-Klych and Buga-Dzhaily deposits contain  $96.0 \pm 8.9$  wt.% NaCL, the content of magnesium ions ranges from  $0.17\pm0.01$  - $4.01\pm0.0$  wt.%, the presence of sulfur is noted in the range of  $0.26\pm0.02$  - $1.71\pm0.12$  wt.%. the Content of other elements, including heavy metal ions, is below the safety level, in decimal and hundredths of % wt. boron, potassium and calcium are noted.

It was found that the number of heterotrophic microorganisms ranges from  $10^3$ - $10^4$  CFU/g, enterobacteria - $10^3$  CFU/g, and micromycetes were found only in samples of salt-containing raw materials from the Buga-Dzhaily deposit. The nature of the distribution and abundance of microflora indicates the anthropogenic presence in the area of salt production. It was found that the cultures of *M. luteus, Bacillus sp., Aspergillus sp., Penicillium sp.* and *Fusarium sp.* isolated from salt-containing substrates are resistant to 17-20% NaCl.

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### ЖАҚСЫ ҚЫЛЫШ ЖӘНЕ БҰҒА-ЖАЙЛЫ ТҰЗДЫ КӨЛ МИКРОФЛОРАСЫНЫҢ БИОЛОГИЯЛЫҚ ӘРТҮРЛІЛІГІ

**Аннотация.** Мақалада Қазақстанның оңтүстігіндегі Жақсы қылыш және Бұға-жайлы сынды тұзды көл үлгілерін микробиологиялық және химиялық зерттеу нәтижелері көрсетілген.

Континентальды тұзды су айдындары химиялық және микробиологиялық құрамы бойынша айтарлықтай ерекшеленеді, ал теңіздер де құрамы бойынша ұқсас келеді. Судың құрамы тікелей геологиялық, климаттық және биогеохимиялық жағдайға байланысты бейтарап, сілтілі және қышқыл болуы мүмкін. Тұзды су объектілерінде микроорганизмдер популяциясы өте төмен және таралуы тұздың концентрациясына тікелей қатысты.

Адаптивті механизмдер жасушаішілік сұйықтықтағы ион концентрациясын және оның осмостық қысымын реттеуге негізделген. Су ортасындағы бейорганикалық ион концентрациясы кең ауқымда өзгеруі мүмкін, бұл организм мен қоршаған орта арасындағы ионды градиентті жоғарылатады. Қазақстанның оңтүстігіндегі екі тұзды көлден алынған тұзды шикізатты косметикаға негіз ретінде пайдалану үшін оның адам денсаулығына зиянсыз екендігіне көз жеткізу мәселесін алға қойдық. Осыған байланысты физикалық, химиялық және микробиологиялық зерттеулер жүргізілді.

Құрғаған Арал теңізінің түбінен алынған сынамаларда магний, калий, кальций, темір және мыс иондары басым екендігі анықталды. Сонымен қатар, пестицидтердің: агроөнеркәсіпте қолданылатын ацетамиприд, фенгексамид, фолпет тәрізді қосылыстар, тетрагидрофтоламид бар екендігі айқындалды. Жақсы қылыш көлінің сынамалары галит, магний және сульфат тұзының түрлі қабаттарынан оқшауланған. Бұға-жайлы көліндегі тұзды шикізат құрамында  $96.0 \pm 8.9$  масс% NaCL, магний иондарының мөлшері  $0.17 \pm 0.01$  - $4.01 \pm 0.0$ % құрайды, күкірттің мөлшері  $0.26 \pm 0.02$ % аралығында кездеседі.  $1.71 \pm 0.12$  мас.%. Ауыр металл мөлшері қажетті концентрациядан төмен. Сынамалардағы магний құрамының жоғарылауы натрий-магний аралас қабаттарының бар екендігін дәлелдейтіні анықталды. Бұл тұзды көлдегі гетеротрофты микроорганизмдер саны шамамен 103-104 КҚБ/г құрайды, энтеробактериялар 103 КФБ/г,  $(6.0 \pm 0.5)$  х 103 СҒU/гг мөлшеріндегі микромицеттер тек сульфат үлгісінде анықталған тұз бен лай жағалау сызығынан 1.0 м қашықтықта сыналды. Жағалау сызығындағы микрофлораның таралуы мен тығыздығы тұз шығару әсерінен антропогендік фактордың жоғарылағанын көрсетеді.

**Түйін сөздер:** тұзды көл, құрамында тұзы бар шикізат, микрофлора, гетеротрофты микроорганизмдер, микромицеттер, пестицидтер.

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#### БИОРАЗНООБРАЗИЕ МИКРОФЛОРЫ СОЛЕНЫХ ОЗЕР ДЖАКСЫ-КЛЫЧ И БУГАЖАЙЛЫ

**Аннотация.** В статье представлены результаты микробиологических и химических исследований образцов соленых озер юга Казахстана, таких как Джаксы-Клыч и Буга-Джайлы.

Континентальные соленые водоемы существенно различаются по химическому и микробиологическому составу, а моря похожи по составу. Состав этих вод напрямую зависит от геологических, климатических и биогеохимических условий и может быть нейтральным, щелочным и кислым. Популяция микроорганизмов в соленых водоемах очень мала, и их распространение зависит от концентрации соли.

Адаптивные механизмы основаны на регуляции концентрации ионов во внутриклеточной жидкости и ее осмотического давления. Концентрация неорганических ионов в водной среде может широко варьироваться, что приводит к увеличению ионного градиента между организмом и окружающей средой. Для применения солесодержащего сырья из двух соленых озер на юге Казахстана в качестве основы для косметических средств, необходимо было убедиться, что оно безвредно для здоровья человека. В связи с этим были проведены физико-химические и микробиологические исследования.

Установлено, что в пробах со дна высохшего Малого Аральского моря преобладают ионы магния, калия, кальция, железа и меди. Кроме того, выявлено наличие пестицидов: ацетамиприда, фенгексамида, фолпетоподобных соединений, тетрагидрофтоламида, которые используются в агропромышленном комплексе. Пробы озера Джаксы-Клыч были выделены из разных слоев галита, магния и сульфатных солей. Солесодержащее сырье озера Буга-Джайлы содержит  $96.0 \pm 8.9$  мас.% NaCL, содержание ионов магния колеблется от  $0.17 \pm 0.01$  до  $4.01 \pm 0.0$  мас.%,

Содержание серы находится в пределах  $0.26 \pm 0.02\%$ .  $1.71 \pm 0.12$  мас.%. Количество тяжелых металлов ниже допустимой концентрации. Установлено, что повышенное содержание магния в образцах свидетельствует о наличии смешанных натрий-магниевых слоев. Количество гетеротрофных микроорганизмов в образцах соленых озер составляет примерно 103-104 КОЕ/г, энтеробактерий -103 КОЕ/г, микромицеты в количестве  $(6.0 \pm 0.5)$  х 103 КОЕ/г обнаружены только в образце сульфатных солей и илов, отобранных на расстоянии 1.0 м от береговой линии. Распределение и плотность микрофлоры на береговой линии указывают на повышенный антропогенный фактор из-за добычи соли.

**Ключевые слова:** соленые озера, солесодержащее сырье, микрофлора, гетеротрофные микроор-ганизмы, микромицеты, пестициды.

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