

## ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (PhD)  
in the educational program 8D05301 – «Chemistry»

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**Research of the composition and properties  
of therapeutic muds of the North-Eastern region of Kazakhstan  
and extraction of lipid biologically active components**

### **General overview of the work**

The dissertation is devoted to a comprehensive study of natural therapeutic muds (peloids) from the saline lakes of Northeastern Kazakhstan, with a focus on their lipid components. The work includes physicochemical and microbiological analyses of the peloids, as well as the development of a two-stage method for selective lipid extraction. The obtained compounds were structurally identified using UHPLC-HRMS techniques, followed by a preliminary assessment of their antioxidant and anti-inflammatory activities and subsequent testing as part of a cream formulation.

### **Relevance of the research topic**

Kazakhstan's therapeutic muds have long been used in spa and balneological practice; however, their modern scientific investigation remains limited. Studies of the chemical composition and therapeutic properties of peloids were previously conducted by a group of domestic scientists led by E.A. Tokpanov and K.M. Akhmedenov, who investigated the muds of lakes in Southern and Western Kazakhstan, focusing primarily on chemical-ecological and geological aspects. The least explored component of peloids is the organic fraction, which is rich in lipid compounds capable of exhibiting anti-inflammatory, antimicrobial, and regenerative effects. The northeastern region of Kazakhstan is characterized by highly mineralized lakes and significant accumulation of organo-mineral sediments. Nevertheless, data on the chemical composition of lipids in these peloids are fragmentary. This creates a discrepancy between their widespread practical use and the lack of a scientifically validated mechanism of action. Studying the lipid components opens opportunities for the development of innovative pharmaceutical and cosmeceutical products, as well as for the rational and resource-efficient utilization of natural therapeutic muds.

**The aim of the dissertation** is a comprehensive study of the composition and properties of therapeutic muds of the region with the isolation and identification of lipid components. To achieve this aim, the following objectives were addressed:

1. To perform a comprehensive analysis of the natural therapeutic muds of Northeastern Kazakhstan using modern analytical methods to determine their elemental composition and main physicochemical parameters;
2. To develop a method for extracting biologically active lipid components from the natural therapeutic muds of Northeastern Kazakhstan, including the selection of effective solvent systems and optimal extraction conditions, followed by lipid identification using the UPLC-PDA-HRMS technique;

3. To evaluate the antioxidant activity of the lipid extracts using the ABTS assay, establish correlations with their chemical composition, and develop effective cream formulations based on these extracts.

### **Key provisions for defense**

1. The peloids of the Arasan, Maraldy, Moyldy, and Tuzkala lakes in Northeastern Kazakhstan comply with sanitary and balneological standards in terms of pH (8,7–9,0), moisture content (28–57%), concentration of toxic elements (As, Pb, Cd, U — within permissible limits), and microbiological safety. Considering their total organic carbon content (TOC 3,32–5,28%, TOC/N up to 18,6) and formation characteristics, these peloids can be classified as priority natural sources for the extraction of biologically active lipid compounds.

2. A differentiated two-stage method for extracting lipid complexes from natural peloids has been developed using a binary solvent system of isopropanol–acetonitrile (1:1, v/v), which ensures a balanced lipid class profile (GL — 48,46%, SP — 29,39%, FA — 11,07%, ST — 9,49%). In the first stage, amphiphilic lipids (SP, GP, PR, GL, ST) were extracted from dry raw material by shaking for 45 minutes at a raw material-to-solvent ratio of 1:30; in the second stage, nonpolar and moderately polar fractions (GL, FA, ST) were obtained from raw peloid samples demineralized using 2 M HCl by shaking for 15 minutes at a ratio of 1:20.

3. The lipid extract obtained from the natural mud of Lake Moyldy using the developed two-stage isopropanol–acetonitrile (1:1, v/v) method exhibited high antioxidant potential (ABTS – 0,93) and was employed to formulate a physicochemically stable cream composition. The optimal extract concentration of 7% in the cream ensures a physiological pH level (5.83), stable structural–rheological properties (spreadability – 5,21 cm, retention time – 16,5 s), and pronounced anti-inflammatory activity, which promotes accelerated epithelialization of skin lesions by the 10th day.

**The scientific novelty** of the dissertation lies in the fact that, for the first time:

- a comprehensive analysis of the peloids from saline lakes of Northeastern Kazakhstan was carried out, including physicochemical, microbiological, geochemical characteristics and lipid profiling. Criteria for sample prioritization for the extraction of biologically active lipids were determined based on total organic carbon content and IR spectroscopy data;

- a scientifically substantiated two-stage lipid extraction method using an environmentally friendly isopropanol–acetonitrile (1:1) system was developed. The method ensures selective recovery of polar and nonpolar fractions under optimized extraction conditions;

- structural identification of a wide spectrum of lipids was performed using UPLC-PDA-HRMS. Biologically active compounds such as ceramides, sulfo- and glycosphingolipids, acylsterol glycosides, and N-acyl taurines were identified, expanding understanding of the lipid diversity of the region's natural therapeutic muds;

- the influence of extraction parameters on the lipid composition and antioxidant activity was established. It was shown that demineralization promotes enrichment with neutral lipids, while longer extraction time enhances the yield of

amphiphilic components. A positive correlation between the content of sterol lipids and antioxidant activity was observed.

**The object of the research** is natural therapeutic muds (peloids) from the saline lakes of the North-Eastern region of Kazakhstan.

**The subject of the research** is the physico-chemical characteristics of peloids, their lipid compounds, methods of their extraction, structure, and presumed biological activity.

### **Research Methods**

To achieve the objectives of the study, a set of modern analytical and instrumental methods ensuring the reliability and reproducibility of the results was applied. The macro- and microelement composition of the peloids was determined using ICP-MS, flame photometry, and potentiometry; mobile forms of elements (N, P, K, S) were analyzed according to standardized GOST and ISO procedures. The granulometric composition was studied by the pipette method, the mineral composition by XRD, and the surface microstructure by SEM. Organic matter, including carbon of humic and fulvic acids, was analyzed by the pyrophosphate method with subsequent titrimetric determination; the elemental composition (TOC, TIC, TN, TS) was determined using a VarioMax elemental analyzer. The chemical bonds and functional groups of organo-mineral components were examined by Fourier-transform infrared spectroscopy (FTIR). For the development of the lipid extraction method, various solvent systems (MTBE:CH<sub>3</sub>OH, CHCl<sub>3</sub>:CH<sub>3</sub>OH, IPA, ACN) were tested. Structural identification of the obtained compounds was performed using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) on a Q-Exactive Orbitrap mass analyzer, which ensured high accuracy and sensitivity of determination. The antioxidant activity of the lipid extracts was evaluated using the ABTS method. The anti-inflammatory properties of the developed cream compositions were studied *in vivo* on laboratory rats.

**The practical significance** of the research lies in the following:

- A scientifically substantiated two-stage method for extracting lipid complexes from the natural peloids of saline lakes in Northeastern Kazakhstan has been developed. This method ensures selective isolation of polar and nonpolar fractions and enhances the efficiency of utilizing organo-mineral raw materials. The obtained results can be applied in analytical and chemical-technological practice to standardize the methods of lipid extraction and analysis from complex organo-mineral matrices.

- Based on the obtained extracts, formulations of cream compositions were developed and tested; these compositions are characterized by physicochemical stability and reproducible structural-rheological properties. The optimal concentration of the lipid extract in the cream is 7%. The developed compositions can be used in the pharmaceutical and cosmeceutical industries for creating formulations and skincare products based on natural lipid complexes.

### **Relation of work with research programs**

The results presented in this dissertation were obtained within the framework of the grant funding project of the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan on the topic "Development of a

technology for obtaining new cosmeceutical products based on domestic natural raw materials" for 2024–2026 (AP23488960).

**The validity and reliability of the results** are ensured by the accuracy and systematic nature of the analytical and experimental studies carried out. The main results were obtained using direct, well-established, and modern experimental methods. The use of certified reference materials (CRM), multiple repetitions of experiments and high-precision analytical equipment ensures the reproducibility and reliability of the obtained results.

### **Approbation of the results of the dissertation work**

The main statements and results of the dissertation were presented at 10 international and national scientific conferences: Polish-Kazakh Meeting: Relationship Between Chemistry and Biology (Poland, Kazakhstan, 2023, 2024, 2025); "Химические технологии функциональных материалов" (Russia, 2023); "Проблемы теоретической и экспериментальной химии" (Russia, 2024); "Science and industry - challenges and opportunities" (Poland, 2024); IX Congreso Iberoamericano de Peloides (Spain, 2025); "Современные достижения и тенденции химии и химической технологии в XXI веке" (Kazakhstan, 2023); "ФАРАБИ ӨЛЕМІ" (Kazakhstan, 2024); "GYLYM JÁNE BILIM - 2024" (Kazakhstan, 2024).

**Publications.** Based on the results presented in the dissertation, 3 research articles and 1 review have been published in international journals indexed in the Scopus database, with percentiles of 31 (Q3), 85 (Q1), and 89 (Q1). In addition, 2 articles were published in peer-reviewed scientific journals of the Republic of Kazakhstan recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education (CQASSHE), and 1 patent for a utility model was obtained.

### **Description of the doctoral student's contribution to each publication**

Sabitova A., Akimzhanova Kh., Mussabayeva B.K., Bayakhmetova B.B., Orazzhanova L., Klivenko A., Nurgaliyev N., Yermoldina E. Physico-chemical Characteristics of Natural Mud of Salt Lakes of North-East Kazakhstan // Engineered Science. – 2023. – Vol. 25. – Art. 930. – DOI: 10.30919/es930: research, methodology, validation, visualization, writing – original draft, editing.

Akimzhanova K. G., Sabitova A. N., Mussabayeva B. K., Bayakhmetova B. B. Inorganic Composition and Physico-Chemical Properties of the Peloid of the Salt Lake Moiyldy (Kazakhstan) As a Natural Source of Biologically Active Substances // Chemical Engineering Transactions. – 2023. – №103. – P. 433–438. – DOI: 10.3303/CET23103073: research, methodology, validation, visualization, writing – original draft, editing.

Akimzhanova K., Sabitova A., Mussabayeva B., Kairbekov Zh., Bayakhmetova B., Proch J. Chemical composition and physicochemical properties of natural therapeutic mud of Kazakhstan salt lakes: a review // Environmental Geochemistry and Health. – 2024. – Vol. 46. – P. 43. – DOI: 10.1007/s10653-023-01813-3: research, methodology, validation, visualization, writing – original draft, editing.

Mussabayeva B., Akimzhanova Kh., Sabitova A., Ydyrys A., Kruszka D.,

Cerazy-Waliszewska J., Łęska B., Tabisz Ł. Extraction of lipids from natural saline mud and their interpretation from point of biological activity in the context of salt lakes of North-East Kazakhstan // Engineered Science. – 2025. – Vol. 36. – Art. 1628. – DOI: 10.30919/es1628: research, methodology, validation, visualization, writing – original draft, editing.

Akimzhanova Kh., Sabitova A., Mussabayeva B., Bayakhmetova B. Химическая и минералогическая характеристика природных грязей соленых озер Мойылды и Тузкала как потенциального природного ресурса // Доклады НАН РК. – 2023. – №346, Vol. 2. – Р. 58–74. – DOI: 10.32014/2023.2518-1483.210: research, methodology, validation, visualization, writing – original draft, editing.

Akimzhanova Kh., Sabitova A., Kairbekov Zh., Mussabayeva B., Bayahmetova B. Chemical characteristic of the black and white mud of the Shoshkaly lake // Известия НАН РК. – 2023. – №457, Vol. 4. – Р. 31–43. – DOI: 10.32014/2023.2518-1491.190: research, methodology, validation, visualization, writing – original draft, editing.

### **The structure and scope of the dissertation**

The work consists of an introduction, five chapters, a conclusion, a list of references, and 7 appendices. The dissertation comprises 134 pages, including 18 figures and 16 tables. The list of references contains 241 titles.