

**V.Kh. Qasimova**<sup>1</sup>, Doctor of Biotechnological Sciences, teacher  
ORCID ID: <https://orcid.org/0009-0000-3305-8450> **e-mail:** [vafaxal@outlook.com](mailto:vafaxal@outlook.com)

**F.V. Hasanli**<sup>2</sup>, graduate student, teacher  
ORCID ID: <https://orcid.org/0009-0008-7929-4326> **e-mail:** [feridahasanli@gmail.com](mailto:feridahasanli@gmail.com)

**L.R. Karimova**<sup>1</sup>, Candidate of Biological Sciences, teacher  
ORCID ID: <https://orcid.org/0009-0005-5950-9543> [leylikarimova@bsu.edu.az](mailto:leylikarimova@bsu.edu.az)

**T.A. Kholina**<sup>1</sup>, Candidate of Biological Sciences, teacher  
ORCID ID: <https://orcid.org/0009-0003-1098-9369> **e-mail:** [tatyanaxolina2302@gmail.com](mailto:tatyanaxolina2302@gmail.com)

<sup>1</sup> Baku State University, Baku, Azerbaijan

<sup>2</sup> Minqecevir State University, Minqecevir, Azerbaijan

## **SIGNIFICANCE OF POLYENE ANTIBIOTICS IN INCREASING OF MEMBRANE PERMEABILITY AND IN TREATMENT ANIMAL AND PLANT INFECTION**

**Abstract.** *It is shown that using polyene antibiotics (PA) in combination with dimethyl sulfoxide sharply increase ionic permeability membranes and biological activity of antibiotics. The comparative physical and chemical characteristics of dimethyl sulfoxide and PA is consideration. The effects of a complex interaction and PA examined by the bilayer lipid membranes (BLM). By the method of determine the parameters biological activity of PA it was show that off all the most studied PA promises more effective were amphotericin B and levorin. Results are started according to BLM conduction of cholesterol in the membranes. On the basis of PA developed an ecological model of environmental protection by establishing membrane-active concentration of PA against viral, fungal and bacterial infection for animals and plants. Using polyene antibiotics (PAs) in combination with dimethyl sulfoxide (DMSO) was shown to increase ion permeability of membranes and biological activity of antibiotics sharply. The comparative physical and chemical characteristics of dimethyl sulfoxide and PAs were determined. The effects of a complex relation between PAs and the bilayer lipid membranes (BLM) were studied. The parameters of biological activity of PAs and BLM were determined. It was shown that among all the studied PAs, amphotericin B and levorin were the most effective. Ion permeability of BLM was shown to depend on the concentrations of amphotericin B, levorin and cholesterol. On the basis of PAs, biological active preparations were developed against viral, bacterial and fungal diseases. It has been shown that the used preparation can completely prevent the growth of the Tobacco mosaic virus. It should be especially noted that after treatment with the "Infanvir" preparation the infected plants are not only cured but also regenerated in full. Moreover, the vegetable plants treated with such preparation had 2 times more harvest than the reference ones. It is supposed that the antiviral and antifungal effect of the "Infanvir" preparation is related to their interaction with lipid components of cytoplasmic membranes, causing destruction of the structure of pathogenic microorganism cells.*

**Keywords:** *polyene antibiotics, dimethyl sulfoxide, amphotericin B, levorin, lipid membranes, membrane permeability, animal and plant infection.*

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## Introduction

Polyene antibiotics are one of the most effective compounds in the fight against fungal infections [Borowski, 2000, Gray et al; 2012]. The main representatives of the PA are amphotericin B, nystatin, mycoheptin and levorin, the chemical structure of which is shown in fig. 1. Molecules PA contain in its composition lactone ring, conjugated system of double bonds and hydrophilic chain consisting of the hydroxyl and carbohydrate groups. Biological activity PA is dependent on the presence of sterol in the cell membranes of defined sterol structure [Recamier et al., 2010]. Polyenes are more sensitive to the membranes, in which structure contains ergosterol [Ciesielski et al., 2016]. Thanks to this aspect of the polyenes used in medicine for therapeutic purposes. Currently for the treatment of the systemic fungal infections are mainly used, amphotericin B and nystatin. Comparative analysis of the biological activity of amphotericin B and nystatin shows that the amphotericin B is approximately 6 fold more effective against the majority of fungi than nystatin [Azaltos et al., 1985]. On BLM amphotericin B shown that, the conductivity of the channel about B 10 times higher than nystatin channel [Kasumov, 2009]. Amphotericin B and nystatin are very close to each other in their chemical structure, but with membrane cholesterol amphotericin B more sensitive than to nystatin. Studies have shown that the presence of a certain number of double bonds in the chromophore PA is an important factor that determines their biological activity [Samedova et al., 2018]. Amphotericin B and Nystatin differ by the number of double bonds in the polyene structure chromophore molecules. Nystatin double bonds is less than the Amphotericin B and nystatin however biological activity markedly. PA selection as the object of the study was not accidental. The peculiarity of the PA is that it is only in the nature of a class of compounds that form in cell membranes and lipid structural channels of molecular dimensions, that selectively permeable to ions and organic compounds [Ibragimova et al., 2006; Kasumov 2009; Recamier et al., 2010; Cohen, 2010].

Studies of the molecular mechanism of interaction of PA with membranes showed that polyenes in combination with sterols create in the membrane channels of particular structure [Kasumov, 2009]. However, despite the presence of large amounts of PA and their derivatives, none of them on the effectiveness of its action can't be compared with amphotericin B and levorin in the treatment of systemic fungal infection. In recent years, the efforts of scientists aimed at getting new dosage forms PA and development of new ways their delivery to the affected organs and tissue. Studies have shown that the presence of a certain number of double bonds in the chromophore PA is an important factor that determines their biological activity. Amphotericin B and Nystatin differ by the number of double bonds in the polyene structure chromophore molecules. Nystatin double bonds is less than the Amphotericin B and nystatin however biological activity markedly. PA selection as the object of the study was not accidental. The peculiarity of the PA is that it is only in the nature of a class of compounds that form in cell membranes and lipid structural channels of molecular dimensions, that selectively permeable to ions and organic compounds [Ibragimova et al., 2006; Kasumov 2009; Recamier et al., 2010; Cohen, 2010].

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## **Materials and methods**

PA is highly soluble in DMSO. Polyenes have amphoteric properties. They form a cation in an acidic medium and in an alkaline-anion [Yu, Quinn, 1994]. In conjunction with DMSO are liquid polyenes dark yellow color, bitter taste, and odor. In the preparation of the active form of PA antibiotic first converting the substance from a crystal to the molecular form. After thorough mixing, the composition of the PA is maintained with DMSO overnight at room temperature. The liquid is then filtered and stored in a dark, cool place. The result is a mother of PA solution, ready for use. Application of the PA in this combination of components is highly efficient.

The biological activity is determined by the PA bilayer lipid membrane (BLM) [Ibragimova et al., 2006]. BLM were prepared from total phospholipids isolated from cells by applying a drop of phospholipids per hole in Teflon cell. Total cholesterol was purified phospholipids and neutral lipids other washing acetone and kept at 0°C at a concentration of 20 mg/ml in chloroform-methanol solution at a volume ratio (2:1).

Integral membrane conductivity was studied as a function of concentration of antibiotic reached maximum conductivity of the membrane, which is taken as an active component of the PA. Understanding the mechanism of functioning of the PA in the membranes were prepared by BLM method [15]. Combined measurement of the electrical characteristics of lipid membranes in current clamp mode, and the membrane voltage. The measurement of the membrane potential ( $E_m$ ) in current clamp mode. Aqueous solutions surrounding the membrane, mixed with a magnetic stirrer. The method is based on the ability of the PA dramatically increases the permeability of the lipid membrane to the corresponding ions by detecting changes in the electrical conductivity of the membrane. Research and measurement integral conductivity of the membrane potential were current using electrometric Keithley-301 (USA).

## Results and its discussion

Dimethyl Sulfoxide ( $(\text{CH}_3)_2\text{SO}$ ) obtained by the oxidation of the dimethyl sulfide ( $(\text{CH}_3)_2\text{S}$ ) with nitric acid (Vaisman, Berkowitz, 1992.) Currently as the oxidant for oxidant for this purpose take hydrogen peroxide  $\text{H}_2\text{O}_2$ . Dimethyl sulfoxide is the first member of the homologous series sulfoxide  $\text{R}_2\text{SO}$ . In their further oxidizing a sulfone  $\text{R}_2\text{SO}_2$ . The chemical structure of DMSO is clear, colorless, slightly bitter-tasting liquid from its own odor, highly soluble in water [Yu Quinn, 1994]. Organic sulfoxides have a pyramidal structure with the sulfur atom at the apex. The sulfoxides ( $\text{RR}'\text{SO}$ ) radicals R and R' differ from each other and exist in two optically active forms. Amphiphilic molecule DMSO and high level. The negative pole of the dipole is on the oxygen atom. DMSO has a streamlined structure which follows from the temperature dependence of refractive index, density, viscosity and other characteristics. Protophilic DMSO solvent, and therefore its associates are easily destroyed by the addition of substances that are proton donors. Studies of the absorption spectra of DMSO in the wavelength range of 350 nm-2200 nm show that the spectrum of DMSO a number of organic compounds usually used study their physico-chemical characteristics and molecular structures [Yu, Quinn, 1994]. Table 1 shows some physical characteristics and DMSO and waters. Relatively high boiling point and large latent heat of vaporization (53J/M at 25 C) indicate that DMSO B molecules are aligned with each other, forming a polymeric chain due to oxygen bonds [Vaisman, Berkowitz, 1992]. DMSO inherent properties such as amphiphility, polarity, high resorption.

Studies of the biological activity of polyene antibiotics PA indicate that these compounds interact specifically with sterols antibiotic sensitive organisms such as fungi and elementary [Gray et al., 2012]. Researches molecular mechanism of interaction of PA with membranes that create in membrane polyenes channels through which cells from the outer environment may diffuse ions and intracellular components leading to cell lysis [Cohen, 2010]. It has been suggested that the biological activity of (PA) may depend on the nature of molecular interactions between the charged groups of phospholipid molecules and antibiotics. It expects that the membrane occurs as a result of hydrogen bonding between the (PA) and phosphate groups of phospholipid molecules. Comparative analysis of the biological activity of amphotericin B and nystatin shows that the amphotericin B is more effective against fungi than nystatin [Aszalos et al., 1985].

Comparative data show that at the polyene chain of amphotericin and nystatin A1 and A is the same as studies have shown antifungal activity of these two antibiotics identical to each other. These data suggest that the presence of definite number of double bonds in the chromophore of (PA) is an important factor in determining their sensitivity to the membranes. There is a direct correlation between the number of double bonds in the chromophore and biological activity of antibiotics. The higher the number of double bonds in the chromophore, (PA) the higher biological activity [Kasumov, 2009].

Levorin antibiotic have higher selectivity of action on the membrane and different from the other antibiotics polyene increased water solubility. The structure of the lipid bilayer, as well as the structure itself penetrating molecules is an important factor that contributes to the permeability of solubility in aqueous compounds. The molecules DMSO have a high degree of resorption by the fact that the value of the permeability DMSO located between water and fat. Table 1. It is suggest that DMSO

increases the permeability of the large number of drug compounds across biological membranes, and promotes their penetration deep enough into the cell. The molecules DMSO increases and promotes membrane penetration substrates deep enough into the cell.

Table 1. Physical properties of DMSO and water

Physical properties	DMSO	Water
Molecular weight	78.13	18.02
Density at 20°C	1.1014	18.02
Melting point 20°C	18.4	0.00
Boiling point	189.0	100.00
Surface tension at 20°C (10 <sup>-3</sup> x Pa x c)	46.2	72.75
Viscosity at 20°C (10 <sup>-3</sup> x Pa x c)	2.20	1.002
Dielectric constant at 20°C	48.9	80.20

First physic-chemical properties of amphotericin B were studied and levorin in combination with DMSO and mixed in different ratios. The dependence of the biomolecular membrane conductivity on the concentration of amphotericin B and levorin. Amphotericin B dramatically increases membrane permeability for ions and water, and non-electrolyte organic compounds. The dependence of the membrane on the concentration of amphotericin B increases the proportionality eighth to the tenth degree and extent of this depends on the structure of molecules PA. The sharp dependence of membrane conductivity on the concentration of amphotericin B allowed suggested that the ion permeability is associated with the formation of channels in the membranes polyene oligomeric structure.

By increasing DMSO concentration aqueous solution increases polyene channel assembly efficiency and enhanced stability of the channel in the conducting state. Amphotericin B at a concentration 1x10<sup>-6</sup> M in 10<sup>5</sup>-10<sup>6</sup> again reduces the native resistivity membrane (1-5x 10<sup>-8</sup>Om/sm<sup>2</sup>), prepared from total phospholipids. Shows the dependence of the conductivity on the concentration of biomolecular membranes of the amphotericin B at different concentration of cholesterol in membranes. Biomolecular membrane conductivity depending on the concentration of amphotericin B. The membranes of phospholipids with cholesterol in a weight ratio 20:1. Membranes were formed in solution 10 ml KCL, pH = 6, 5, t = 22C. The addition of cholesterol to phospholipids increases permeability membranes in the presence of amphotericin B for selective one valence anions.

However, in the study of aromatic antibiotic it was found that, unlike amphotericin B levorin is not selective permeability to anions and cations of alkali metals. Levorin differ from antibiotic nystatin, amphotericin B and mycoheptine molecules by the presence of additional aromatic groups – p-aminoacetophenone, which contains a positively charged nitrogen. With increasing concentrations of the antibiotic membrane conductivity increases in proportion to the 4<sup>th</sup> degree of concentration levorin. Depending on the study on the concentration of membrane conductance levorin led to speculation about the presence in the membranes of channels of molecular dimensions, inducing ion permeability. One might think that selective permeability to cations associated with the formation of negatively charged membrane pore. Rather, transfer of cations through the membrane though the border of the channel hydrophilic. Important information on the mechanism of membrane

permeability in the presence of aromatic antibiotic can be recovered from the data transfer through the membrane of small ions, such as guanidine and hydrazine. Levorin in the presence of these ions, permeate through the membrane, rather than the ions K and Na.

The presence of the same number of double bonds in the chromophore levorin and amphotericin B is an important factor determining their high sensitivity to membranes. Studies have shown that the most effective of the studied PA are amphotericin B and levorin. A special role in the formation of conductive channels of levorin and amphotericin within the membrane belongs dimethyl sulfoxide DMSO. DMSO has the ability to greatly enhance the biological activity of the PA and to induce in the membranes of selective permeability to ions and organic compounds. Give the result of the experiments suggest that the selective action mechanism is based on specific molecular interactions with membranes antibiotics.

Studies conducted showed that the chromophores PA molecules interact with phospholipids, form a channel in a stoichiometric ratio of 1:1. Stoichiometric coefficient assembly of single channels for different PA may differ from each other and to be equal to between 3 and 17A (Ibragimova et al., 2006). Computer analysis showed that the formation of ion channels in presence of an amide derivative of amphotericin B ionizing group of molecules can be addressed both inside and outside the channel, i.e. polar groups may be in two conformational forms mycosamine through rotation around the glycoside linkages. There is an assumption that the biological activity of the PA may depend on the nature of intermolecular interactions between charged groups molecules antibiotics into the membrane occurs due to the formation of hydrogen bonds between PA and phospholipid molecules.

### **Practical significance of the work**

Widely used antibiotics in livestock crop received after became apparent adverse effects on the suppression of phytopathogenic microflora send useful species of birds and animals that feed on plant pollination. Antibiotics possess a number of valuable benefits to combat phytopathogenic microorganisms as compared to other substances. Antibiotics have a selective effect and inhibiting the development of pathogenic bacteria and fungi, virtually harmless to plants and animals [Lewis, Papavizas, 1987; Ibragimova et al., 2014]. When selected an antibiotic necessary condition is the absence of toxicity. For example, PA used in low concentrations. ( $10^{-6}$ - $10^{-4}$ M), are not toxicity for plants and animals. Studies shown that most used antibiotic well into absorbed in animal and plant tissue concentration of antibiotic. Required for suppression of pathogenic organisms in the tissues of animals and plants depends on the properties of the antibiotic and the external conditions. As a basis for development of effective antiviral, antibacterial and antifungal drugs PA. Based on the obtained data, calculated minimum concentration of the maximum of its biological activity. Studies have revealed a group of PA new compounds which has the ability to effectively and selectively inhibit pathogenic infection. It was established that preparation able to inhibit viral and fungal infections of plants. Processing plants infected with viral or fungal infection, by spraying a solution leads to the effective disposal of plant infections. As a result of laboratory tests of soil samples, which are grown vegetables, it was found that in the soil contains a small amount of nitrogen, high phosphorus and a small amount potassium pH of the soil sample is weakly alkaline. In Tab. 2 shows the composition of the soil on the mineral elements on the basis of soil gradation.

Table 2. Soil composition of mineral elements on the basis of soil gradation

Sample name	pH	The degree to which soil is provided with mineral elements on the basis of soil gradation			Conductivity on particle size distribution of the soil (mS)	NaCl (mg/kg) standard 150-300	KCl (mg/kg) standard 350-700
		nitrogen 40-120 mg/kg	phosphorus 15-60 mg/kg	potassium 300-600 mg/kg			
		Index of mineral sample provision					
		nitrogen N NH <sub>3</sub> mg/kg	phosphorus P <sub>2</sub> O <sub>2</sub> mg/kg	potassium K <sub>2</sub> O mg/kg			
SOIL	7,55	7,76	133,32	212,08	1,18	520	516

Despite the missing mineral elements in the soil aphids grown culture studies have shown the high efficiency of the drug on the pathogens of vegetables. It should be noted that preparation has the ability to completely inhibit the growth of the tobacco mosaic virus (Tobacco mosaic virus) (Ibragimova, et al., 2014). Infected plants after preparation treatment not only recover, but regeneration occurs wilted plants from infection. It assumed that anti-virus and anti-fungal effect preparation shown by binding of the antibiotic to the membranes with subsequent formation of the complex in them, which is a channel formation of the drug inhibitory as reproduction of viruses and fungal cells. The proposed facility is non-toxic, harmless drug that can be used by environmental pollution and promote sustainable use of its agriculture with the cultivation of vegetable and fruit crops. In the course of basic research was the first to develop a new preparation against viral, bacterial and fungal infections. It was studied the action of the preparation formed by microorganisms *Streptomyces*, *Staphylococcus* a number of pathogens *Escherichia* *Candida albicans*, and opportunistic bacterial and viral Coxsackie A, ECHO and herpes simplex virus type I and II antimicrobial activity preparation studied in various assay systems. Established that preparation at low concentrations (10<sup>-7</sup>-10<sup>-6</sup> M) has antibacterial and antifungal effects on the culture *Salmonella typhimuium*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* gram-positive culture *Staphylococcus aureus* and fungi *Candida albicans* cells, as well as antiviral activity lain on Coxsackie A.20, ECHO 9 and herpes simplex type I and II. For both preparation obtained Eurasian patents.

**Conclusions**

It was shown that membrane-active compounds of “Infanvir” have the ability at low concentration (10<sup>-7</sup>-10<sup>-6</sup> M) to annihilate the pathogenic microorganisms. The characteristic molecule of “Infanvir” is that simultaneously and highly selective may suppress virology, purulent and fungi infections. One of main pedologic and ecologic issues is to search for preparations protecting vegetable culture against pathogenic microorganisms. The current protective agents cannot completely prevent spreading viral and fungal infections. To find a biologically active preparation against pathogenic microorganisms of vegetable cultures, a range of experiments with one

of polyene antibiotics has taken place in hothouse facilities and open soils of vegetable farms located within the territory of the Apsheron peninsula, where vegetable cultures such as cucumber, tomato, eggplant and pepper are cultivated. "Infanvir", the biologically active preparation developed in the pedology faculty of Baku State University (the preparation content is not disclosed due to the patent consideration) against virus and fungal infections of vegetable cultures has been tested for the first time.

1. The "Infanvir" preparation has been developed on the basis of membrane-active macrolide antibiotics. The mechanism of action of such compounds is based on structural channels of molecular size generated by them in cell membranes and which are selectively permeable for ions and organic compounds.

2. The investigations carried out in hothouse facilities as well as in open soils have demonstrated high efficiency of the "Infanvir" preparation with respect to pathogenic microorganisms.

3. The treatment of plants and soil itself affected by virus (the Tobacco mosaic virus) and fungal infections by spraying the affected areas subject to calculation 100 ml initial solution dissolved in 10 l water at [22].

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**В.Х. Касимова, Ф.В. Гасанли, Л.Р. Керімова, Т.А. Холіна**  
**ЗНАЧЕННЯ ПОЛІСНОВИХ АНТИБІОТИКІВ У ПІДВИЩЕННІ**  
**ПРОНИКНОСТІ МЕМБРАНТА В ЛІКУВАННІ ІНФЕКЦІЙ ТВАРИН І РОСЛИН**

**Анотація.** Показано, що використання полієнових антибіотиків (ПА) у поєднанні з диметилсульфоксидом різко підвищує іонну проникність мембран та біологічну активність антибіотиків. Розглянуто порівняльні фізичні та хімічні характеристики диметилсульфоксиду та ПА. Досліджено вплив комплексної взаємодії ПА на двошарові ліпідні мембрани (БЛМ). Методом визначення параметрів біологічної активності ПА було показано, що з усіх найбільш вивчених ПА найефективнішими виявилися амфотерицин В та леворин. Результати розпочато відповідно до провідності холестерину в мембранах за допомогою БЛМ. На основі ПА розроблено екологічну модель захисту навколишнього середовища шляхом встановлення мембранно-активної концентрації ПА проти вірусних, грибкових та бактеріальних інфекцій для тварин та рослин. Було показано, що використання полієнових антибіотиків (ПА) у поєднанні з диметилсульфоксидом (ДМСО) різко підвищує іонну проникність мембран та біологічну активність антибіотиків. Визначено порівняльні фізичні та хімічні характеристики диметилсульфоксиду та ПА. Досліджено вплив комплексного зв'язку між ПА та двошаровими ліпідними мембранами (БЛМ). Визначено параметри біологічної активності ПА та БЛМ. Було показано, що серед усіх досліджених ПА найефективнішими були амфотерицин В та леворин. Показано, що іонна проникність ВЛМ залежить від концентрацій амфотерицину В, леворину та холестерину. На основі ПА розроблено біологічно активні препарати проти вірусних, бактеріальних та грибкових захворювань. Було показано, що використаний препарат може повністю запобігати росту вірусу тютюнової мозаїки. Слід особливо зазначити, що після

обробки препаратом «Інфанвір» уражені рослини не тільки виліковуються, а й повністю регенеруються. Більше того, овочеві рослини, оброблені таким препаратом, мали в 2 рази більший урожай, ніж контрольні. Вважається, що антивірусний та протигрибковий ефект препарату «Інфанвір» пов'язаний з їх взаємодією з ліпідними компонентами цитоплазматичних мембран, що викликає руйнування структури клітин патогенних мікроорганізмів.

**Ключові слова:** полієнові антибіотики, диметилсульфоксид, амфотерицин В, леворин, ліпідні мембрани, проникність мембран, інфекції тварин та рослин.

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**Вафа Халіл Касимова**

доктор біотехнологічних наук, викладач, Бакинський державний університет

**Адреса робоча:** З. Халілова, 23, Баку, Азербайджан AZ1148

ORCID ID: <https://orcid.org/0009-0000-3305-8450> **e-mail:** [vafaxal@outlook.com](mailto:vafaxal@outlook.com)

**Фаріда Вакіф Гасанли**

аспірант, викладач, Мінгачевірський державний університет, Мінгачевір, Азербайджан

**Адреса робоча:** Д. Алієва, 21, Мінгачевір, Азербайджан AZ-4500

ORCID ID: <https://orcid.org/0009-0008-7929-4326> **e-mail:** [feridahasanli@gmail.com](mailto:feridahasanli@gmail.com)

**Лейлі Рашид Керімова**

канд. біол. наук, викладач, Бакинський державний університет

**Адреса робоча:** З. Халілова, 23, Баку, Азербайджан AZ1148

ORCID ID: <https://orcid.org/0009-0005-5950-9543> **e-mail:** [leylikarimova@bsu.edu.az](mailto:leylikarimova@bsu.edu.az)

**Тетяна Олександрівна Холіна**

канд. біол. наук, викладач, Бакинський державний університет

**Адреса робоча:** З. Халілова, 23, Баку, Азербайджан AZ1148

ORCID ID: <https://orcid.org/0009-0003-1098-9369> **e-mail:** [tatyanaxolina2302@gmail.com](mailto:tatyanaxolina2302@gmail.com)