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Biotesting of *Chlorella* sp algae for certain medicinal drugs

In recent years, the study of drugs as pollutants in the country is gaining momentum. This is because the country does not use special wastewater treatment devices for pharmaceuticals. They enter the environment in different ways. Therefore, research on biotesting drugs in living organisms is underway. The article presents the results of experiments conducted to assess the effect of paracetamol, azithromycin, suprastin on the number and biomass of *Chlorella* sp algae. *Chlorella* sp algae are a widely used organism for biotesting. Among the above drugs in the study, paracetamol had a negative effect on the number and biomass of algae. The minimum harmful concentration of paracetamol for chlorella was 5 mg/L, the minimum harmful concentration of azithromycin for chlorella was 200 mg/L, the minimum harmful concentration of chlorella suprastin was 8 mg/L. When comparing the effect of paracetamol, azithromycin, suprastin on the number of green algae *Chlorella* sp, it was observed that paracetamol had the highest effect. Concentration of paracetamol 10 mg/L brought the number of chlorella close to 0. High concentrations of paracetamol and suprastin in the biomass of chlorella showed contamination of the environment.

Keywords: *Chlorella* sp, medicinal drug, biotesting, biomass.

Introduction

Recently, there is a growing scientific interest in pharmaceuticals as chemical agent that pollutes the environment [1]. After the use of pharmaceuticals, it is absorbed into the body and undergoes metabolic processes such as hydroxylation, glucuronidation, and decomposition. Drugs and their metabolites enter the environment in different ways. Expired or incompletely used drugs are found in landfills, as people dispose of them with chemical waste. However, most drugs are excreted in human feces and feces as a result of physiological processes and then released into treatment facilities. Cleansers remove organic components, proteins, carbohydrates, and lipids. Despite this, cleansers do not have the power to eliminate drug metabolites; they are biologically active elements in the human body. Pharmaceuticals can be stable in the environment and are not completely broken down or absorbed by the body. In recent years, the release of pharmaceutical ingredients into the aquatic environment has become relevant for research.

The relationship between pharmaceuticals and the natural environment and living organisms is poorly understood. In many cases, drugs are widely used as dangerous pollutants, so they can penetrate the membrane and resist. In some cases, the environmental impact of pharmaceuticals may be greater than that of agricultural pollutants [2, 3].

Currently, the pharmaceutical industry in Kazakhstan is developing rapidly. More than 7,000 medicines are registered in the State Register of the Republic of Kazakhstan. However, in our country, drugs are not considered harmful to the environment and are not controlled by environmental authorities. For them, the waste of the pharmaceutical industry is toxic, and it is necessary to look for ways to treat this wastewater [4]. Therefore, the harmful effects of pharmaceuticals in Kazakhstan on the environment may be higher than in other countries.

Chlorella is a division of *Chlorophyta*, single-celled green algae belonging to the class of protococci. They are often found in sewage, lakes, seas, and soils. *Chlorella* is used to obtain oxygen in closed ecosystems. In terms of productivity, it is equal to meat and exceeds the productivity of wheat. If wheat contains 12% protein, *chlorella* contains more than 50% protein. It contains 15 different vitamins. However, all the products of *chlorella* are coated with a special coating, which cannot be broken down by digestive enzymes of taste. Nonetheless, modern scientists have found a solution to this problem. Now *chlorella* cells break down at high pressure, compacted the resulting mass in the form of tablets or leaving it in powder form. Another method of disintegrating *chlorella* cells is to dry them at 50° C for 4 hours by actively stirring. In both cases, the addition of water is sufficient, as *chlorella* cells can decompose in the human body [4].

Experimental

The object of study was a culture of single-celled green microalgae *Chlorella* sp. Paracetamol, azithromycin, and suprastin were obtained as toxic substances. Concentrations of drugs are based on the maximum allowable concentration of each drug in an aqueous medium.

The number of chlorella microalgae initially calculated in the study was 52.95 million/ml. The algae were divided into 5 groups and poured into a 50 ml flask. 75 studies were performed in each experiment when counting cells; and 75 studies were performed when measuring biomass. A total of 450 studies were conducted, including all experimental studies. Table 1 describes the concentration of algae and the concentration of toxicants.

Table 1
Concentrations of experimental groups and doses included in them

Group number	Administered drug	Dose concentrations mg/l	Frequency of observations, days
1 (control group)	Distilled water	-	1, 5, 7, 14, 21
2	Paracetamol	2,5	
	Azithromycin	100	1, 5, 7, 14, 21
	Suprastin	2	
	Paracetamol	5	
3	Azithromycin	200	
	Suprastin	4	
	Paracetamol	7,5	
4	Azithromycin	300	
	Suprastin	6	
	Paracetamol	10	
5	Azithromycin	400	
	Suprastin	8	

Paracetamol is a drug with analgesic and antipyretic effects belonging to the group of anilides. Acetaminophen (APAP) is widely used in Western countries. Chemical formula is $C_8H_9NO_2$. Molecular Mass: 151.165. MAC in an aqueous medium — 2.5 mg/L. Paracetamol is a common non-narcotic analgesic in use and has a slight anti-inflammatory effect. It can also cause liver, circulatory, and renal failure if used in large doses [5]. Diseases of these organs increase when the drug is taken with alcohol.

The mechanism of action and safety profile of paracetamol are well studied, as well as its effectiveness has been approved, and it is one of the most important pharmaceutical drugs of the World Health Organization.

In addition, paracetamol is a chemically similar metabolite. When phenacetin is ingested, an analgesic effect occurs immediately. In terms of pain relief, paracetamol is no less than phenacetin and also has a slight anti-inflammatory effect. When paracetamol enters the body, it does not cause the formation of methemoglobin. In addition, this drug has side effects, especially nephrotoxic and hepatotoxic, when used for a long time or in overdose. At the same time, paracetamol is classified as a safe, economical drug and ibuprofen.

Paracetamol blocks both forms of the cyclooxygenase enzyme, thereby slowing down the synthesis of prostaglandins. The drug acts through the central nervous system, thereby affecting the center of pain and participating in thermoregulation. Cellular peroxidases in peripheral tissues reduce the effect of paracetamol on cyclooxygenase, so it can be seen that it has no anti-inflammatory effect. In the absence of a factor that stops the synthesis of prostaglandins, it shows that the drug does not adversely affect the gastrointestinal tract and water and salt metabolism.

Scientists predict that paracetamol blocks the third form of cyclooxygenase, which is located only in the two central nervous systems and does not affect the second and third types of enzymes located in other tissues. This explains the ability of the drug to relieve heat and the lack of anti-inflammatory effect [6].

As for the pharmacokinetics of paracetamol, its absorption capacity is high, the maximum concentration (C_{max}) is 5–20 $\mu\text{g/ml}$; the time to reach the maximum concentration (TC_{max}) is 0.5–2 hours. The level of binding to plasma proteins is 15%. It enters the body through the blood-brain barrier. That is, it enters

through the barrier between the central nervous system and the cardiovascular system. 1% of the drug is excreted in breast milk. The effective plasma concentration of paracetamol reaches 10-15 mg/kg at the time of administration.

97% of the drug is metabolized in the liver: 80% are conjugated with glucuronic acid and sulfates to form inactive metabolites (glucuronide and paracetamol sulfate), 17% are exposed to methylation and hydroxylated to the process of hydroxylation. In case of insufficient glutathione, these metabolites can destroy the enzyme systems of hepatocytes and cause necrosis.

Withdrawal of the drug from the body by half is 1–4 hours. Excreted by the kidneys in the form of metabolites, and 3% of paracetamol is excreted unchanged. In older people, the time of excretion increases [7].

Toxicity of paracetamol is caused by a decrease in glutathione reserves in the body, so its active metabolites begin to accumulate in the body. Their hepatotoxic effect is high. Excessive accumulation of paracetamol metabolites in the body leads to the development of hepatic necrosis through the binding of liver proteins [8].

Azithromycin is a semi-synthetic antibiotic, the first member of the class of azalides, which differs slightly in structure from the classic macrolides. Chemical name 9-Deoxy-9a-aza-9a-methyl-9a-homoerythromycin A. Chemical formula — $C_{38}H_{72}N_2O_{12}$.

MAC in the aquatic environment — 100 mg/L. 14-part macrolides are obtained in a lactone ring by modifying the mixing of nitrogen atoms between 9 and 10 carbon atoms, thus turning the ring into a 15-part macrolide. By taking azithromycin in this way, it is possible to increase the acid resistance by 300 times compared to erythromycin.

Azithromycin is a widely used antibacterial drug, which has a bacteriostatic effect due to its class of azalides. Together with the 50S-subunit of ribose, it breaks down peptide translocase during translation, stops protein synthesis, inhibits bacterial growth and proliferation, and produces high concentrations of bactericides. It affects against external and intracellular pathogens.

Azithromycin gram is active against many microorganisms: *Streptococcus* spp., *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus viridans*, *Staphylococcus epidermidis*, *Staphylococcus aureus*; affects gram-negative bacteria: *Moraxella catarrhalis*, *Bordetella pertussis*, *Bordetella parapertussis*, *Legionella pneumophila*, *Haemophilus ducreyi*, *Campylobacter jejuni*, *Neisseria gonorrhoeae* and *Gardnerella vaginalis*; also has an effect on some anaerobic microorganisms: *Bacteroides bivius*, *Clostridium perfringens*, *Peptostreptococcus* spp; and chlamydia *trachomatis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Mycobacterium avium* complex, *Ureaplasma urealyticum*, *Treponema pallidum*, *Borrelia burgdorferi*.

Azithromycin has no effect on erythromycin-resistant gram-negative bacteria.

Antacids (containing Al_3^+ and Mg_2^+ ions), ethanol and food slow down the absorption of azithromycin. No prothrombotic time deviations were found with concomitant use of warfarin and azithromycin, however, given the interaction of macrolides and warfarin, which enhances the anticoagulant effect, patients are contraindicated. It has the potential to increase the concentration of digoxin, as it slows down the inactivation of intestinal flora. Toxicity of erythromycin increases when taken in combination with ergotamine and dihydroergotamine. When used in combination with triazolam, the excretion of azithromycin slows down and the pharmacological action of triazolam increases. In addition, azithromycin increases the plasma concentrations of cycloserine, indirect anticoagulants, methylprednisolone, felodipine, microsomal oxidizing drugs and increases their toxicity. Lincosamides slow down the action of azithromycin, and increase the effectiveness of tetracycline and chloramphenicol. Pharmaceutically incompatible with heparin.

Azithromycin is rapidly absorbed and dispersed in plasma and tissues when taken orally. With a single dose of 500 mg of azithromycin, 37% of the drug is absorbed in plasma at a dose of 0.41 μ g/ml within 2-3 hours. Food inhibits the effect of azithromycin, so it should be taken after meals or within 1-2 hours before meals. The drug is distributed throughout the body in a short time; high concentrations of azithromycin in plasma exceeded 50 times and are found in tissues. Depending on the body and tissues, the concentration of the drug is between 7-9 μ g/ml.

The volume of distribution in the body is 31 L/kg. The therapeutic dose of azithromycin can be found in the tissues within 5-7 days after oral administration.

It enters the phagocytes, which accumulate at the site of inflammation, thereby forming a therapeutic concentration of the drug. It increases the maximum use of oxygen by pathogens. Concentrations of azithromycin are more common in infected tissues than in non-infected tissues. It has the property of not being excreted from the body for a long time, on average 2-4 days. Excretion from the body through the bileduct is the

main route of excretion. On average, 50% is excreted unchanged in the bile. The remaining 50% is excreted in the form of 10 different metabolites. Metabolites are formed by the process of demethylation of nitrogen and oxygen, by the decomposition of the cladinose conjugate during the process of hydroxylation of desosamine and aglycone rings. Metabolites have no antibacterial activity.

An average of 6% of the administered dose of the drug is excreted in the urine. The time of excretion from the body is slower for the elderly. *Chloropyramine* (“Suprastin”) is an antihistamine drug that is a blocker of the histamine receptor H1. Chemical formula: $C_{16}H_{20}ClN_3$. Molecular mass: 289.81. It is also a chemical aromatic waste and organochlorine compounds. The maximum concentration of chemical compounds in this group in the aqueous medium is 2 mg/L.

Chloropyramine is one of the essential drugs for Russian life. However, chloropyramine does not have such a list in Kazakhstan. It is not used by the US FDA. It competitively eliminates the pharmacological action of histamine, which occurs with the help of histamine receptor H1. The drug also has a central effect (stimulating and sedative, i.e., prevents the transmission of allergic reactions and shocks) and peripheral effects such as atropine. Chloropyramine is a chlorinated analogue of tripeleamine (pyribenzamine) — a classic antihistamine drug; ethylenediamine belongs to the group of antihistamines. It has antihistamine and m-cholinoblocking, antiemetic effect, and antispasmodic and peripheral cholinoblocking activity.

In the case of oral administration, the ornament is completely removed from the gastrointestinal tract. The therapeutic effect of chloropyramine develops between 15-30 minutes after application, the maximum concentration in the body occurs in 3-6 hours. Distributed well in the body and central nervous system.

The liver is actively metabolized. Most often excreted by the kidneys. Excretion in children is faster than in adults.

90-95% of it is excreted by the kidneys. The remaining 5-10% is mixed with bile and excreted in the feces. Accumulation of the drug in the body due to hepatic or renal insufficiency due to metabolism by the liver, as well as excessive accumulation of the dose may be accompanied by side effects [9].

Concentrations of drugs were obtained based on the maximum allowable concentration of each drug in the aqueous medium.

The microalgae culture was grown for 7 days in a 250 ml flask under 2000-2500 lux light bulbs at a temperature of 25–28° C and continuous aeration.

In the laboratory, chlorella is grown in liquid and agar media. After sterilization, the liquid medium is cooled and poured into flasks under sterile conditions. Cultivation of microalgae is prepared in 250-300 ml flasks or special cultivators. The inoculators are administered at a dose of 106 chlorella cells per 1 ml. After inoculation, the flasks are closed with tight cotton and gauze stoppers and covered with sterile paper covers. When growing algae, luminescent or special racks were constantly aerated with daylight bulbs with an intensity of 200-2500 lux.

For storage of algae in the laboratory, bleached tami or 04 nutrient media are used. They should be collected in test tubes or smeared on Petri dishes. Standard microbiological methods are used to grow algae in agar medium: inoculation with bacteriological loops as a stroke or applying the suspension to the agar medium with a spatula. 04 nutrient media were used in the experimental work. The culture medium is prepared in distilled water. Ingredients: K_2HPO_4 – 0.1; KH_2PO_4 – 0.1; $CaCl_2$ – 0.04; NH_4NO_3 – 0.3.

Methods based on the control of algae viability, quantity, test reactions to the amount of chlorophyll in the algae during cultivation in the nutrient medium are used to bio test the quality of the aquatic environment by chlorella.

In determining the quality of the aquatic environment monitors changes in the number of unicellular algae, the number of living and dead cells, their morphological characteristics, the composition of photosynthetic pigments. These parameters allow estimating the reduction of the quantitative spas of wastewater and the factors that affect them. Identification of living and dead cells is based on morphological changes caused by the contaminant in the water. Characteristics of dead cells include albinization, ugly forms, and more. Cell count is calculated by magnifying 320 times under a microscope in Goryaev’s chamber [10].

The principle of operation of the Goryaev chamber. Horizontal and vertical lines in the camera create 15 rows and 15 columns, that is, 225 cells are displayed in the camera. The size of the cells is determined by the following formula:

$$N = \frac{a \cdot 4000 \cdot b}{c}$$

where n — the number of cells in the medium of 1 µl; a — the number of cells counted in the chamber; b — the large number of cells in the cell; c — the size of the growing cell.

A solution of the drug in distilled water was introduced into medium 04 at different concentrations. The drugs were administered to each group within the maximum concentration, in concentrations 2, 3, 4 times higher. The obtained concentrations were used to determine the limit of resistance of microalgae to the drug. No drug was used in the control group (group I). In the experimental variants, 3 repetitions were used.

The study of the experimental groups was conducted for 21 days. Cell counting was performed on days 5, 7, 14, 21. Micromed-3 microscope and Goryaev's camera were used to count living cells.

As a result of the development and growth of organisms in the aquatic environment, continuous biomass growth develops. This process is called biological productivity, and recycled biomass is called biological product. Biological productivity is formed by the appearance of primary and secondary products, i.e., due to the growth of biomass of autotrophs and heterotrophs. Biological productivity is the biomass produced by a population or community per unit of area per unit of time, which is divided into total or gross primary product, and productivity also includes energy and volatile substances.

The phenomenon of biological productivity itself is considered in two aspects: the nature of the population and the population in the aquatic environment, which considers the biological productivity of reservoirs. Biological productivity of a population is determined by its species characteristics and habitat conditions. The biological productivity of reservoirs determines their ability to provide a certain species in the aquatic environment. Therefore, the productivity of grain in the cultivation of certain species in agriculture depends not only on soil fertility, but also on the biological productivity of water. Thus, population and aquatic productivity are different aspects of the same phenomenon.

Photocolorimetry was used to measure biomass. Transfer the suspension from the control group flasks into a 1 cm thick photocolorimeter cuvette. To determine the concentration of chlorophyll is carried out by determining the optical density in the region of the red maximum absorption (663-665 nm). To determine the concentration of chlorophyll, take three dimensions in a photocolorimeter and use the arithmetic mean as the final result [11].

To determine the chlorophyll a pigment in the suspension, the relationship between its concentration in µg / ml and the optical density D₆₆₅, measured in a 1 cm thick cuvette at 665 nm, is used:

$$\text{Chl}_a = 1/1.9 * D_{665}$$

where Chl_a — concentration of chlorophyll a pigment corresponding to the wavelength of 665 nm, µg / ml; 11.9 — calculation coefficient; D₆₆₅ — optical density.

The photocolorimetry of the extract should also be measured at a wavelength of 750 nm. It is necessary to record non-specific absorption caused by the encounter of other parts in the extract. The amount of non-specific absorption must be measured before measuring at 665 nm.

We calculate the concentration of chlorophyll by the following formula:

$$\text{Chl} = 11.9 * (D_{665} - D_{750})$$

where Chl_a — concentration of chlorophyll a pigment corresponding to the wavelength of 665 nm, µg / ml; 11.9 — calculation coefficient; D₆₆₅, D₇₅₀ — optical density.

Based on the above chlorophyll a concentrations, it is possible to calculate the estimated amount of phytoplankton biomass (Tab. 2).

Table 2

A scale that determines the level of pollution of a natural reservoir by the amount of phytoplankton biomass

Indicator	Water purity classes				
	Clean in size	Clean	Less polluted	Polluted	Extremely polluted
Phytoplankton biomass mg/L	<0,1	0,1-1,0	1,5-5,0	5,1-50,0	<50

The concentration of chlorophyll takes up 2.5% of the dry biomass, or 6.75% of the organic carbon fraction. Thus, for the transition from the concentration of chlorophyll a to biomass, we can take the number 15 as a calculation factor. Thus, the following formula is used to measure the biomass of phytoplankton:

$$B_c = 15 * Chl_a$$

where B_c – phytoplankton biomass; 15 – calculation coefficient; Chl_a – the concentration of chlorophyll a pigment.

The amount of biomass can be used to determine water pollution by looking at the following 2 tables.

Determination of phytoplankton biomass is carried out by the method of aggregation of populations of individual species. To do this, it is necessary to determine the average mass of algae cells that make up the population of the sample. To determine the biomass, at least 30 specimens of each alga are taken from each sample, with an average value for the population of each species. Multiply the volume found in each volume (μm^3) by its quantity (thousand cells per liter) to obtain the value of water biomass in mg/L or g/m^3 [12].

Results and Discussion

The effect of paracetamol on the abundance and biomass of Chlorella sp algae

Paracetamol was administered in a culture medium inoculated with chlorella culture 04 dissolved in distilled water at the following concentrations: Group II — 2.5 mg/L; Group III — 5.0 mg/L; Group IV — 7.5 mg/L and group V — 10.0 mg/L (maximum MPC in the aquatic environment is 2.5 mg/L). The selected concentrations were used to determine the limit of resistance of microalgae to the active substance paracetamol. Paracetamol-free culture medium was used in the control group. On day 1 of the experiment, the number of chlorella cells in all groups was pre-measured and the number of cells was around 1 million/ml (Fig. 1).

On the fifth day the number of cells in the control group (group I) increased by about 2.14 million/L. The number of group II cells increased from 1 million/L on the first day of the study to 1.32 million/L on the fifth day. However, it is 39% less than the control group ($p < 0.01$). The number of chlorella cells in the flasks of group III was 1.1 million/mL ($p < 0.01$), while the number of cells in group IV was 1.02 million/ml ($p < 0.001$). Group V chlorella cells contained 1.14 million/ml ($p < 0.001$). That is, on the fifth day of the study, there was a significant change in the number of algae cells. In addition, the color of the culture medium in the paracetamol-containing flasks began to change to brown.

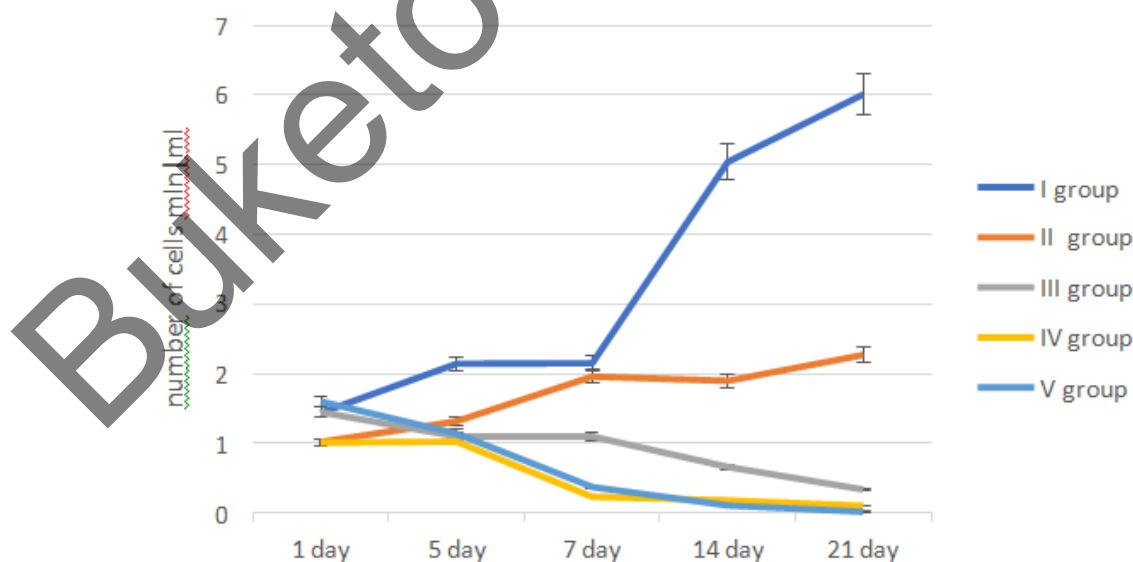


Figure 1. Effect of different concentrations of paracetamol on the number of cultures of microalgae *Chlorella sp*

On the seventh day of the study, the number of chlorella cells in the control group and the group with the maximum allowable concentration (group II) began to increase. In group I, the number of cells increased

from 2.15 million/ml, in group II — to 1.96 million/ml. In group III, the number of cells remained unchanged (1.1 million/ml). In group IV, the number of cells decreased sharply by 0.23 million/ml. In group V, the number of cells decreased by 0.38 million/ml ($p < 0.001$). On the 14th day of the study, the number of cells in the control group increased by an average of 5.04 million/ml, and the number of cells in group II reached 1.9 million/ml. Although the MPC volume remained in group II, it can be seen that the number of cells remained unchanged. The number of cells in group III decreased by 0.67 million/ml ($p < 0.01$). The number of group IV cells decreased to 0.18 million/ml. The number of cells in group V decreased to 0.1 million/ml ($p < 0.01$). Due to the high concentration of paracetamol in the groups, it can be seen that the number of chlorella cells in these groups is much lower than in the control group.

During the last 21 days of the experiment, the number of chlorella cells in the control group increased by 6 million/ml. Group II cells were 63 less than in the control group ($p < 0.001$). In group III a concentration of 2 times higher than the maximum allowable concentration decreased by 0.34 million/ml ($p < 0.001$). In group IV, the number of cells was 99% lower, in group V it was 100% destroyed ($p < 0.001$). In addition, as mentioned above, in groups other than paracetamol concentrations, the color of the culture medium changed to dark brown. From the discoloration of the medium, it can be seen that paracetamol is harmful to chlorella cells.

Thus, it can be seen that the concentration of paracetamol 5.0, 7.5, 10 mg/L is significantly more harmful than the concentration of the maximum allowable concentration. However, it can be observed that the concentration of paracetamol 2.5 mg/L slows down the growth of chlorella cells.

In addition, the effect of different concentrations of paracetamol on the growth of chlorella biomass was studied during the above scientific experiment (Fig. 2). On the first day of the experiment, the biomass of chlorella was calculated in all experimental groups before the introduction of paracetamol. First, the concentration of chlorophyll a pigment in the suspension was determined, and the amount of biomass was calculated using a special formula. That is, on the first day of the study, the biomass content of all groups was approximately 0.5355 mg/L. This indicates that the water is unpolluted.

On the fifth day of the study, the biomass of chlorella in the control group was 2.6375 mg/L, and the biomass of groups II and III was slightly lower than in the control group. That is, the biomass of group II chlorella was 1.785 mg/L, while the biomass of group III was 1.334 mg/L. In group IV, the amount of biomass increased slightly (4.1595 mg/L). No significant changes were observed in group V compared to the control group, the biomass was 2.365 mg/L ($p < 0.05$).

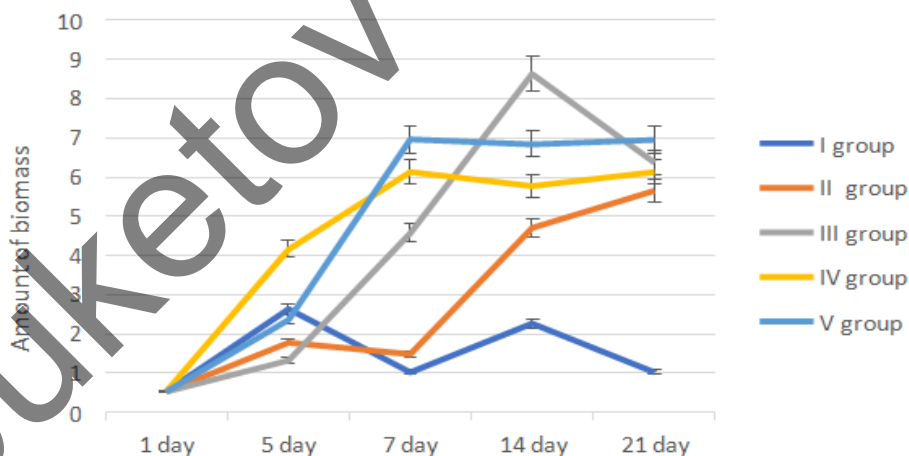


Figure 2. Effect of different concentrations of paracetamol on the biomass of *Chlorella* sp microalgae culture

Significant changes were observed on day 7 of the study of the effect of paracetamol on the biomass of chlorella algae. The biomass of chlorella in the control group (group I) is 1.0265 mg/L. However, no significant changes were observed in the group with the maximum allowable concentration, the biomass was 1.4875 mg/L. However, in group III, the amount of biomass is significantly increased, 4.5815 mg/L ($p < 0.001$), thus, the medium is less polluted on the scale of determining the purity of water by biomass. Therefore, even in the study of the seventh day, group III is moderately polluted. That is, in group IV the biomass is 6.1285 mg/L, in group V — 6.9615 mg/L ($p < 0.001$).

On the 14th day of the experiment, the control group showed a biomass content of 2.261 mg/L, and in group II — 4.7005 mg/L. In group II, the figure is higher than before. That is, the amount of biomass is 8.6275

mg/L. In the group, it was slightly lower, 5.7715 mg/L and 6.8425 mg/L ($p < 0.001$). However, in these groups, the biomass index indicates that the water is polluted.

On the last 21 days of the study, the biomass of chlorella in the control group was 1.0225 mg/L. In group II, the amount of biomass increased by 5.6525 mg/L, in group III the environment is dirty ($p < 0.001$), although the indicator is slightly lower (6.3665 mg / l). High concentrations of paracetamol were used at 6.1285 mg/L in group IV and 6.9615 mg/L in group V ($p < 0.001$).

Thus, as can be seen from Figure 2, paracetamol 2.5; 5; 7.5; at concentrations of 10 mg/L on the last day of the study, the amount of biomass increased and the environment became polluted.

The effect of azithromycin on the number and biomass of Chlorella sp algae

Azithromycin is a semi-synthetic antibiotic, the first member of the class of azalides, which differs slightly in structure from the classic macrolides.

Azithromycin was administered in a culture medium inoculated with chlorella culture 04 in a solution dissolved in distilled water at the following concentrations: Group II — 100 mg/L; Group III — 200 mg/L; Group IV — 300 mg/L and group V — 400 mg/L (EC in the aquatic environment — 100 mg/L). The selected concentrations were used to determine the limit of resistance of microalgae to the active substance azithromycin. Azithromycin was not used in the control group. On day 1 of the experiment, the number of chlorella cells in all groups was pre-measured and the number of cells was around 1.0-1.3 million/ml (Fig. 3).

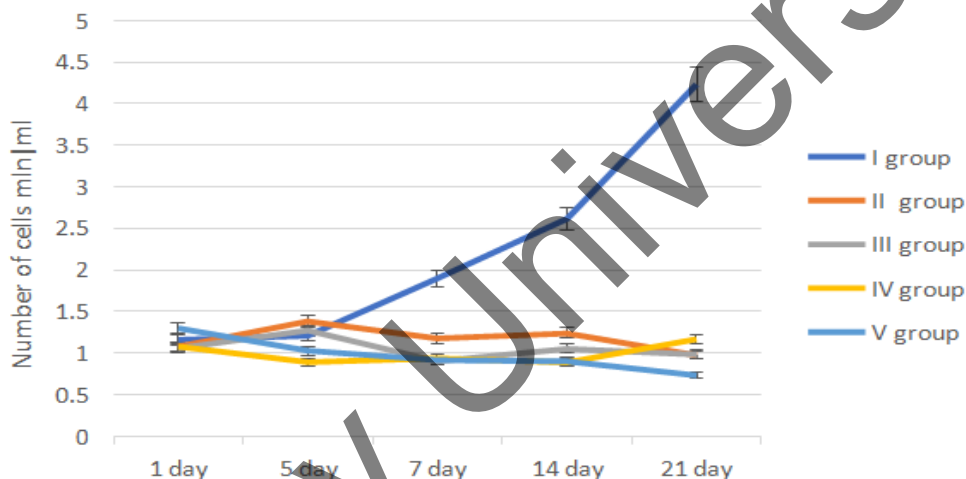


Figure 3. The effect of different concentrations of azithromycin on the number of cultures of microalgae *Chlorella sp*

On the fifth day of the experiment, the number of chlorella in all groups did not change significantly. In the control group it was 1.2 million/ml, in the second group — 1.3 million/ml, in the third group — 1.27 million/ml. In group IV, the number of chlorella cells was slightly reduced (0.8 million/ml). In group V, the number of cells was 1.02 million/ml ($p < 0.05$). In addition, the study revealed that parts of azithromycin in the culture medium began to develop in the medium, which developed in the form of its own bacterial colonies, displacing chlorella cells.

On the seventh day of the study, the number of cells in the control group began to increase gradually, reaching 1.9 million/ml. In group II, the number of cells decreased by 1.18 million/ml ($p < 0.01$). In group III, the number of algae cells was about 0.9 million / ml ($p < 0.001$).

When determining the effect of azithromycin on the number of chlorella algae, on the 14th day of the study, the number of cells in group I increased to 2.61 million/ml. In the remaining groups, a significant decrease in the number of chlorella cells can be observed. The number of cells in group II was 1.2 million/ml, in group III — 1.05 million/ml, in group IV — 0.8 million/ml, in group V — 0.9 million/ml ($p < 0.001$).

On the last day of the study, the number of group I cells increased to 4.02 million/ml. In other groups, the number of chlorella cells is significantly reduced, which reduces the chances of future development. In groups II and III the number of cells was 0.9 million/ml, in group IV — 1 million/ml, in group V the number of cells decreased by 0.7 million/ml ($p < 0.001$).

Thus, the effect of azithromycin on the number of chlorella cells was found to slow down cell growth in all groups except the control group. On day 21 of the study, the number of cells in group V decreased by 84% compared to the control group.

When assessing the effect of different concentrations of azithromycin on the biomass of *Chlorella* sp microalgae cultures, no high levels of water pollution were observed, but low levels of environmental pollution were recorded in all groups (Fig. 4). On the first day of control, the baseline biomass in all groups was 0.5355 mg/L.

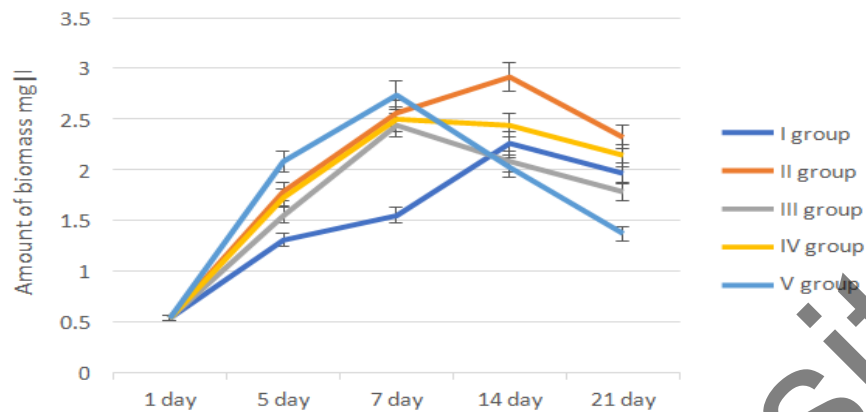


Figure 4. Effect of different concentrations of azithromycin on the biomass of *Chlorella* sp microalgae culture

On the fifth day of the study, the biomass level in group I was 1,309 mg/L, in group II — 1,785 mg/L; in group III — 1.547 mg/L, and in group IV — 1.7255 mg/L ($p < 0.01$). In group V, the change was significant, the biomass increased by 2.0825 mg/L ($p < 0.01$). This change indicates that the environment has a low level of pollution. On the seventh day of the experiment, the biomass of chlorella in the control group was 1.547 mg/L; in group II was 2.5585 mg/L; in group III was 2.4395 mg/L, in group IV was 2.499 mg/L, in group V was 2.7375 mg/L ($p < 0.05$). On the 14th day of the study, the biomass of the control group was 2.261 mg/L. There were no significant differences in groups using different concentrations of azithromycin. However, the amount of biomass indicates that the water is less polluted. The level of biomass in group II was 2.91 mg/L, in group III — 2.08 mg/L, in group IV — 2.43 mg/L, in group V 2.023 mg/L ($p < 0.05$). On the last 21 days of the experiment, the biomass level in the control group was 1.96 mg/L. The biomass of algae in group II was 2.32 mg/L, in group III — 1.785 mg/L, in group IV — 2.142 mg/L, in group V — 1.368 mg/L ($p < 0.05$).

Thus, the antibiotic azithromycin did not cause a sharp increase in the biomass of chlorella algae. However, the latest research shows a low level of pollution.

The effect of suprastin on the number and biomass of Chlorella sp algae

Chloropyramine (“Suprastin”) is an antihistamine drug that is a blocker of the histamine receptor H1. It is also a chemical aromatic compound and organochlorine compound. The maximum concentration of chemical compounds in this group in the aqueous medium is 2 mg/L. Suprastin was introduced into the culture medium inoculated with chlorella culture 04 dissolved in distilled water at the following concentrations: Group II — 2 mg/L; Group III — 2 mg/L; Group IV — 2 mg/L and group V — 2 mg/L. On the first day of the experiment, the number of chlorella cells in all groups was pre-measured and the number of cells was around 1.0-1.2 million/ml (Fig. 5).

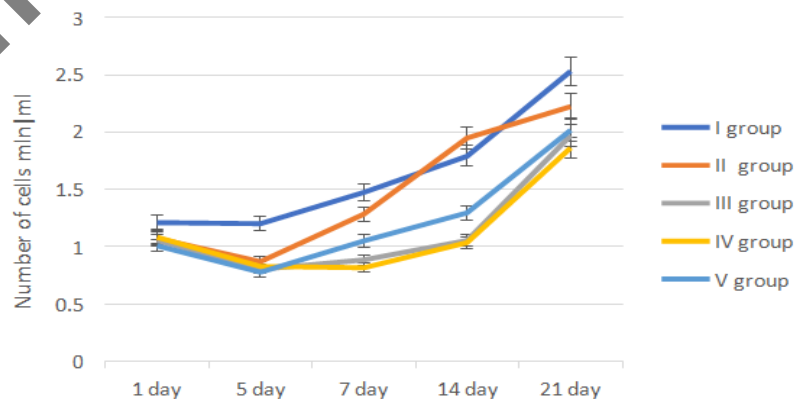


Figure 5. Effect of different concentrations of suprastin on the number of cultures of microalgae *Chlorella* sp

On the fifth day of the study, there were changes in the number of chlorella cells. The number of cells in the control group was 1.2 million / ml, while in group II the number of cells decreased to 0.87 million/ml ($p < 0.01$). The number of chlorella cells in group III was 0.8 million/ml, in group IV — 0.87 million/ml, in group V — 0.77 million/ml ($p < 0.001$). That is, any concentration of suprastin on the fifth day has a detrimental effect and inhibits the growth of chlorella cells.

On the seventh day of the study, the number of cells in the control group reached 1.47 ml/ml. In group II, the number of cells increased till 1.28 million/ml. There was no change in group III and group IV; and in group V there was an increase in the number of cells by 1 million/ml ($p < 0.05$).

On day 14 of the study, the number of chlorella cells in the control group increased by 1.79 million/ml. In group II, as in the control group, there is an increase in the number of cells — 1.28 million/ml. In groups III, IV, V, the number of cells increased by the same amount to 1 million/ml.

On the last day of the study, the growth groups in the study environment began to recover. The number of cells of chlorella algae in the control group was 2.53 million/ml, in group II — 2.2 million/ml, in group III — 1.97 million/ml, in group IV — 1.86 million/ml, in group V — 2 million/ml arrived.

In the study of the effect of the antihistamine suprastin on the growth of biomass of chlorella culture, a detrimental effect was observed on the last day in group V (Fig. 6). In the study, the initial amount of biomass in all groups was about 0.0238 mg/L.

On the fifth day of the study, there were no significant differences between all groups. That is, there is no evidence of environmental pollution. Biomass in all groups was approximately 1.01 mg/L ($p < 0.05$). On the seventh day of the census, the pollution was not observed. The amount of biomass in group I reached 1.039 mg/L, in group II — 0.85 mg/L, in group III — 1.3 mg/L, in group IV — 1.54 mg/L, in group V — 1.7 mg/L ($p < 0.05$).

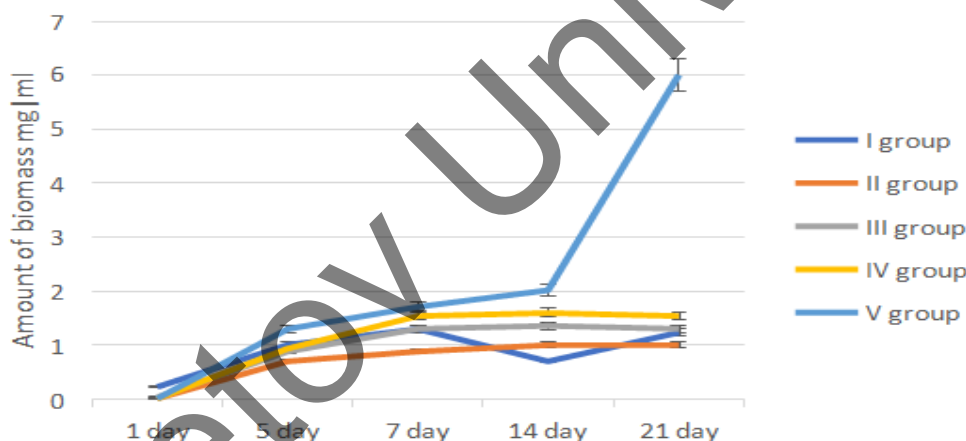


Figure 6. Effect of different concentrations of suprastin on the biomass of *Chlorella* sp microalgae culture

On the 14th day of the experiment, the biomass of chlorella in the control group was 0.71 mg/L, in group II — 1.01 mg/L, in group III — 1.3 mg/L. In group IV, the biomass content reached 1.6 mg/L, in group V — 2 mg/L ($p < 0.01$). The amount of biomass in group IV and group V indicates a low level of pollution.

On the last day of the study, except for group V, the biomass level was close to normal. In group V, the biomass level reached 6.0 mg/L ($p < 0.001$), so it can be seen that there is a level of water pollution.

Different concentrations of paracetamol, azithromycin, suprastin have been studied to determine the limits of resistance of algae to the active substances of these drugs. The study found that the minimum concentration of paracetamol harmful to chlorella was 5 mg/L, the minimum concentration of azithromycin harmful to chlorella was 200 mg/L, and the minimum concentration of suprastin harmful to chlorella was 8 mg/L.

Conclusions

Doses of paracetamol above 5.0 mg/L significantly reduced the number of *Chlorella* sp microalgae. Algae with a maximum concentration of 2.5 mg/L decreased growth compared to the control group. At a concentration of 10 mg/L, the number of algae approached 0. At concentrations of paracetamol 2.5 mg/L; 5 mg/L, 7.5 mg/L, 10 mg/L, chlorella biomass exceeds 5 mg/L and is considered moderately contaminated. Azithromycin is an antibiotic *Chlorella* sp had a high effect on the number of microalgae. At concentrations

of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, there is no increase in the number of algae compared to the control group. The effect of azithromycin on algae biomass was considered moderately polluted because the concentrations other than the control group were higher than the biomass above 1.5 mg/L. Suprastin, an anti-histamine, did not significantly affect the number of *Chlorella* sp microalgae. Concentrations other than the control group fell below 1 million/ml on the fifth day of the experiment. The concentration of suprastin 8 mg/L brought the biomass of chlorella to 6.0095 mg/L. It means that the environment is polluted. When comparing the effects of paracetamol, azithromycin, suprastin on the number of green algae *Chlorella* sp, it was observed that paracetamol had the highest effect. Concentration of paracetamol 10 mg/L brought the number of chlorella close to 0. High concentrations of paracetamol and suprastin in the biomass of chlorella showed contamination of the environment. The minimum concentration of paracetamol harmful to chlorella was 5 mg / l, the minimum concentration of azithromycin harmful to chlorella was 200 mg/L, the minimum concentration of suprastin harmful to chlorella was 8 mg/L.

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А.М. Задағали, А.К. Жаманғара

Chlorella sp балдырын кейбір дәрілік препараттарға биотестілеу

Елімізде соңғы жылдары дәрілік препараттарды қоршаған ортаны ластаушы ретінде зерттеу жұмыстары қарқын алып келеді. Себебі елімізде фармацевтикалық препараттарды арнайы ақаба сулардан тазартатын құрылғылар қолданылмайды. Олар қоршаған ортаға әртүрлі жолмен енеді. Сондықтан дәрілік препараттарды тірі организмдерге биотестілеу зерттеулері жүріп жатыр. Мақалада парацетамол, азитромицин, супрастин дәрілерінің *Chlorella* sp балдырларының саны мен биомассасына әсерін бағалау үшін жүргізілген эксперименттердің нәтижелері берілген. *Chlorella* sp балдыры кең қолданылатын биотест ағза болып табылады. Зерттеу кезінде жоғарыда аталған препараттардың ішінде парацетамол балдырлардың саны мен биомассасына кері әсер етті. Хлореллаға парацетамолдың зиянды мінималды концентрациясы 5 мг/л, азитромициннің хлореллаға зиянды ең төменгі концентрациясы 200 мг/л, хлореллаға супрастиннің зиянды ең төменгі концентрациясы 8 мг/л болды. Парацетамол, азитромицин, супрастин препараттары *Chlorella* sp жасыл балдырларының санына әсерін салыстыру барысында парацетамол препараты ең жоғары әсер еткені байқалды. Парацетамолдың 10 мг/л концентрациясы хлорелла санын 0-ге жақындатты. Ал хлорелла биомассасына парацетамол мен супрастин препаратының жоғары концентрациясы ортаның ластағандығын көрсетті.

Кілт сөздер: *Chlorella* sp, дәрілік препараттар, биотестілеу, биомасса.

А.М. Задагали, А.К. Жамангара

Биотестирование водорослей *Chlorella sp* на некоторые лекарственные препараты

В последние годы изучение лекарственных препаратов как загрязнителей в стране набирает обороты. Это связано с тем, что в стране не используются специальные устройства для очистки сточных вод для фармацевтических препаратов. Они попадают в окружающую среду разными путями. Поэтому ведутся исследования по биотестированию лекарств на живых организмах. В статье представлены результаты экспериментов, проведенных по оценке влияния парацетамола, азитромицина, супрастина на численность и биомассу водорослей *Chlorella sp*. Водоросли *Chlorella sp* являются широко используемыми организмами для биотестирования. Среди перечисленных выше препаратов в исследовании негативное влияние на численность и биомассу водорослей оказывал парацетамол. Минимальная вредная концентрация парацетамола для хлореллы составила 5 мг/л, минимальная вредная концентрация азитромицина для хлореллы — 200 мг/л, минимальная вредная концентрация супрастина — 8 мг/л. При сравнении действия парацетамола, азитромицина, супрастина на численность зеленых водорослей *Chlorella sp* отмечено, что наиболее высокий эффект оказывал парацетамол. Концентрация парацетамола 10 мг/л приблизила численность хлореллы к нулю. Высокие концентрации парацетамола и супрастина в биомассе хлорелл показали загрязнение окружающей среды.

Ключевые слова: *Chlorella sp*, лекарственные препараты, биотестирование, биомасса, вредная концентрация.

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