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Toxic Effects of Aquatic Pharmaceuticals on *Chlorella sp.* in Kazakhstan

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ABSTRACT

Pharmaceuticals are crucial for human health, but their release into the environment through various means can contaminate groundwater, surface waters, soil, and microorganisms. The contamination of water by pharmaceuticals in Kazakhstan is not well-studied. Understanding the influence of pharmaceutical ingredients on the ecosystem and public health is a key area of ecological research. Globally, researchers are investigating the risks posed by pharmaceuticals in water sources and their environmental effects. This study uses *Chlorella sp.* to test the impacts of pharmaceuticals on aquatic biota, examining growth rate and growth inhibition. The study followed OECD Research Method 201. Pharmaceuticals with high pollution potential in Kazakhstan's water resources, including ketoconazole, terbinafine, drotaverine hydrochloride, telmisartan, benzylpenicillin, and azithromycin, were studied. Azithromycin was found to be the most toxic to *Chlorella sp.* (0.33 ± 0.05 mg/L), while amoxicillin had the least toxic effect (853.54 ±0.27mg/L). Azithromycin has significant effect to *Chlorella sp.* resistance, especially in smallest experimental concentrations. At 0.2 mg/L, azithromycin nearly halved the growth rate compared to the control, growth inhibition was over 87% at 0.15 mg/L (r^2=0.89). *Chlorella sp.* showed minimal sensitivity to high concentrations of amoxicillin, with slight decrease of growth (2% at 1 mg/L, 57% at 1000 mg/L).

1. INTRODUCTION

Increased availability of medicines, development of healthcare systems, and the use of medicines in medical facilities lead to higher pharmaceutical waste in the environment. Water pollution by pharmaceuticals and their derivatives is an important research area in environmental toxicology. Pharmaceutical detection in the water ecosystem documented in many developed countries. The concentration of pharmaceuticals in water depends on usage rates, absorption in patients, decomposition in wastewater systems, and distribution in water or precipitation [1, 2]. Previous research in Kazakhstan focused on defining active pharmaceutical ingredients in water [3, 4]. Recent studies, such as those published in "Environmental Science & Technology", revealed pharmaceuticals in Shanghai's Huangpu River, including antibiotics and cardiovascular drugs. This research conducted by Chen et al. [5] found a wide range of pharmaceuticals along the Huangpu River, including antihypertensive drugs, antiarrhythmic drugs and lipid-lowering drugs. Our research identified pharmaceutical pollutants in the Akmola region's and Astana city's wastewater [4, 6].

The ongoing release of antibiotics into the environment has consistently subjected aquatic and soil organisms to prolonged exposure to these substances [7, 8]. Even at low concentrations, antibiotics show pronounced toxic effects and can act synergistically with pharmaceuticals and ecotoxicants [9]. Antibiotics have a significantly strong impact on algae and aquatic plants [10, 11], with studies showing these compounds

inhibit photosynthesis and increase oxidative stress [12]. Microorganisms and fungi can develop resistance to antibiotics after prolonged low-concentration exposure [13, 14]. While invertebrates like *Hydra attenuate* and crustaceans like *Daphnia magna, Ceriodaphnia dubia* and *Artemia salina* show low levels of acute toxicity [15, 16], fish experience acute toxicity just at high concentrations, though non-toxic concentrations have been reported [11, 17].

Algae are vital to ecosystems, serving as primary producers for most aquatic species [18]. Their roles in the food cycle, oxygen production, and food supply are critical. Pharmaceuticals' adverse effects on algae can have cascading consequences for higher trophic levels [19]. Algae are sensitive indicators of ecosystem changes, useful for early detection and prevention of undesirable effects. They are also beneficial as test subjects due to their quick response times [18]. Chlorella has a spherical shape with a diameter of 2-10 microns and contains chlorophyll a and b [20]. Previous studies indicated that pharmaceutical mixtures negatively impact aquatic biota, causing growth inhibition. Research has shown several active pharmaceutical ingredients can harm surface water biota in Kazakhstan and Russia, including amoxicillin, azithromycin, oxytetracycline, ketoconazole, terbinafine, benzylpenicillin, and telmisartan [21-23].

In early studies conducted in Kazakhstan, a list of 237 APIs was derived from 7,713 pharmaceutical products containing 1,684 APIs for prioritization [22]. The highest exposures in surface waters were observed for benzylpenicillin, metronidazole, sulbactam, ceftriaxone, and sulfamethoxazole, while the highest exposures in fish plasma were noted for lisinopril, orlistat, telmisartan, drotaverine, and terbinafine. The highest concentrations in waters were showed for metronidazole, benzylpenicillin, ceftriaxone, sulbactam, and sulfamethoxazole, while fish plasma showed the highest levels for lisinopril, orlistat, telmisartan, drotaverine, and terbinafine. Ecotoxicity data for aquatic species like daphnia, fish, and algae were available for 154 of the 237 active pharmaceutical ingredients (APIs), and therapeutic concentration data for human plasma were available for 201 of these. Compounds with the highest ecotoxicological risk included amoxicillin, clarithromycin, azithromycin, ketoconazole, and benzylpenicillin, while non-apical assessments ranked lisinopril, orlistat, estradiol valerate, drotaverine, and estradiol highest. The prioritization was based on prior studies but relied on product availability data in Kazakhstan due to the lack of local ecotoxicity data, as more commonly available drugs are likely to have wider usage. APIs found in many products are likely to be used more widely than those in only a few products. The results identified amoxicillin, clarithromycin, azithromycin, ketoconazole, benzylpenicillin, terbinafine, drotaverine, diclofenac, benzathine benzylpenicillin, and telmisartan as posing the highest environmental risks.

Although the ranking method differed from previous studies, some of the highly ranked compounds in this study, like amoxicillin, clarithromycin, diclofenac, and azithromycin, also scored high in prior studies. For example, amoxicillin was found to be a significant hazard to aquatic organisms in the UK, France, Italy, Iran, Korea, and Spain [24, 25]. In the U.S., Cooper et al. identified sulfamethoxazole, diclofenac, and clarithromycin as high-risk pharmaceuticals, while Roos et al. highlighted ketoconazole in Swiss waters [26]. Lisinopril, orlistat, estradiol valerate, cinnarizine, drotaverine, estradiol, and clotrimazole were noted as having minor effects on fish, with estradiol identified by Guo et al. [24] as a potential concern.

Most of the drugs ranked highly in Kazakhstan are used to treat infectious and parasitic diseases, many of them being antibiotics, which are among the most well-studied pharmaceuticals regarding their acute toxicity to aquatic organisms [27]. However, there is limited data on their chronic effects in aquatic ecosystems. Most ecotoxicity studies focus on the acute toxicity of antibiotics to algae, with EC_{50} values ranging from 0.002 mg/L to 1,283 mg/L [28].

Many of the pharmaceuticals on the list have been detected in monitoring studies globally, supporting the validity of the approach. For example, amoxicillin was detected at 28 μg/L and 82.7 μg/L in German hospital wastewater. Clarithromycin and azithromycin were found in wastewater at 647 ng/L and 260 ng/L, respectively [29].

Several substances are toxic to aquatic life, such as clotrimazole, which has been shown to affect the larval stage of *Xenopus tropicalis* at 0.1 μg/L [30]. Porsbring et al. demonstrated that clotrimazole inhibits the growth of algal communities and affects their physiology, while ketoconazole disrupts fish cytochrome P450 enzyme activity [31]. Hegelund et al. in 2004 investigated the response of fish to ketoconazole, finding that it affected rainbow trout and killifish at 12 and 100 mg/kg by inhibiting the activity of the cytochrome P450 enzyme in fish [32]. Benzylpenicillin was found toxic to *Microcystis aeruginosa* with an EC_{50} of 0.005 mg/L [33]. The environmental risk of clarithromycin has also been widely studied, with high bioconcentration factors and its detection in surface waters. For example, Oguz and Mihciokur [34] studied the environmental risks of drugs in Turkey and concluded that clarithromycin may pose a potential hazard to living organisms due to its high bioconcentration factor. Additionally, the substance was detected in surface waters, with the highest concentration of clarithromycin in rivers observed in Italy at a concentration of 0.02 μg/L [35]. A significant amount of literature has been published in recent decades on the toxicity and prevalence of diclofenac. Diclofenac has been extensively researched, with 142 articles covering 38 countries reporting its ecotoxicological effects [36]. This assessment identified human prescription APIs most likely to appear in Kazakhstan's surface waters, posing significant risks to aquatic life.

For Kazakhstan this study is a crucial issue, because of the lack of knowledge about pharmaceutical pollutants in this country. Especially the impact of the pharmaceuticals to the water ecosystem and aquatic biota is one of the actual problems, despite of the previous studies of the researchers. The data on priority pharmaceuticals and their effective doses for aquatic biota have led to the consideration and development of treatment methods in the water supply system in Kazakhstan.

The purpose of this research is assessment of the toxicity of active pharmaceutical ingredients in surface waters to the green algae species *Chlorella sp.* The research objectives include evaluating the effects of pharmaceutical ingredients (ketoconazole, terbinafine, drotaverine hydrochloride, telmisartan, benzylpenicillin sodium salt) on algae amount and biomass.

2. MATERIALS AND METHODS

The research followed OECD Method 201: Test for Inhibition of Freshwater Algae and Cyanobacteria [37]. This

method was chosen because of it is a standard freshwater alga and cyanobacteria Growth inhibition test. This method can assess the effects a substance in a short test duration. The single-celled algae *Chlorella sp.* was used in the study. Algae are good indicators of adverse chemical effects due to their sensitivity to environmental changes [29]. Pharmaceuticals studied as ecotoxicants included amoxicillin, azithromycin, oxytetracycline, ketoconazole, terbinafine, benzylpenicillin, and telmisartan (Table 1). Solvents used were 96% ethyl alcohol, dimethyl sulfoxide (DMSO), and acetone.

Table 1. Active pharmaceutical ingredients (API) and their concentrations for the experiments with *Chlorella sp.*

API	Concentrations, mg/L				
Group	1	2	3	4	5
Amoxicillin	0	250	500	750	1000
Azithromycin	0	0.05	0.10	0.15	0.20
Oxytetracycline hydrochloride	0	2	4	6	8
Ketoconazole	0	20	50	100	-
Terbinafine	0	20	50	100	-
Telmisartan	0	5	10	15	-
Benzylpenicillin	0	5	25	50	

Experiments used different concentrations of prior pharmaceuticals, potentially dangerous levels. Experiential data were conducted in triplicate. The active pharmaceutical ingredients' concentrations were chosen according to their dose of application for human health and their elimination rate. The present toxicity assessment of pharmaceuticals to aquatic biota used glass made equipment. All glassware for the test were cleaned with detergent to prevent any pollution from the environment, then all of them was sterilized. Sterilization was performed in autoclaves "Panasonic MLS-3781L" and "SPVA-75-I-HH". The SIS growth media was prepared in 2 L of Duran bottles. Stock solution of tested pharmaceuticals was prepared in 50 mL of volumetric flasks. All reagents were scaled in analytical scale "Sartorius CPA 623S". The toxicity test on *Chlorella sp.* cultured in 250 ml Erlenmeyer flasks. Algae were grown shaking with 100 cycles/min condition in incubated shaker "SI-300 Lab companion". *Chlorella sp.* cell number was counted in Goryav chamber "Minimed" through microscope "Olympus CX41" and in order to define their biomass we used photometer "KFK 3" (at 720 nm wavelength). Algae amount and biomass in each flask were measured twice (the first and last test day) [38]. Typically, 1/5 of the test solution was solved with DMSO and acetone. Optical density was measured using a photometer. DMSO and acetone were used as background for photometry. During the experiment, all solutions were stored in an illuminated light chamber. Rate of the algae biomass growth was calculated using the Eq. (1) [37]:

$$\mu_{i-j} = \frac{\ln Xj - \ln Xi}{t} \quad (1)$$

where, μ_{i-j} is the average growth rate from i to j; Xi and Xj are the biomass of algae in control and test containers at times i and j; and t is the time interval from i to j.

Inhibition percentage was calculated using Eq. (2):

$$\%Ir = \frac{(\mu c - \mu t)}{\mu c} * 100 \quad (2)$$

where, %Ir is the inhibition percentage of the *Chlorella sp.*, μ is the inhibition percentage at the average growth rate; μc is the average biomass growth rate μ in the control group; μt is the average growth rate μ in the test group [37].

Chlorella was grown on Tamiya nutrient medium (Table 2), with potassium nitrate as the nitrogen source. Nitrogen is essential for microalgae biomass synthesis, and pH changes in the nutrient solution depend on the nitrogen source. The Tamiya medium has an excess of potassium ions, leading to pH increases during growth. Since potassium nitrate is an alkaline salt, the growth of microalgae on the Tamiya medium is accompanied by a rise in the pH of the sample and the accumulation of carbonate and bicarbonate ions in it. A rise in pH result increase Phosphorus and Magnesium [25]. Cultivation on Tamiya medium leads to significant changes in the initial ion ratios with a lack of some elements and an excess of others. When a part of the biomass is removed and new parts of the medium are added to the background solution, this imbalance is aggravated, which significantly suppresses the growth of algae during prolonged cultivation.

Table 2. Contents of Tamiya growth medium

Chemical Compounds	Formula
Nitrogen source:	
Potassium nitrate	KNO_3
Magnesium sulfate heptahydrate	$MgSO_4*7H_2O$
Potassium dihydrogen phosphate	KH_2PO_4
Iron (II) sulfate heptahydrate	$FeSO_4*7H_2O$
Ethylenediaminetetraacetic acid	$C_{10}H_{16}N_2O_8$
Micronutrient source:	
Manganese chloride	$MnCl_2*4H_2O$
Zinc sulfate heptahydrate	$ZnSO_4*7H_2O$
Molybdenum trioxide	MoO_3
Ammonium metavanadate	NH_4VO_3

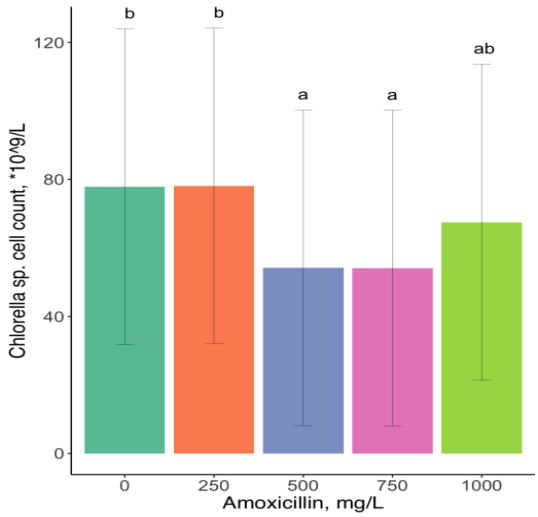
Experimental parameters such as *Chlorella sp.* cell amount and biomass were measured after 72 hours of application with pharmaceuticals. Test objects were growth in this period under 20°C temperature, constant shaking and light intensity 76 $\mu E*m^2s^{-1}$. Chemical component data were analyzed using a hierarchical experimental design structure with several types of experimental units. Results are a means ± SE. During the statistical analysis was used one-way ANOVA, both equal and unequal variance t-tests in R Studio software. Significance tests at a probability level of $p<0.05$ were carried out on all data.

3. RESULTS

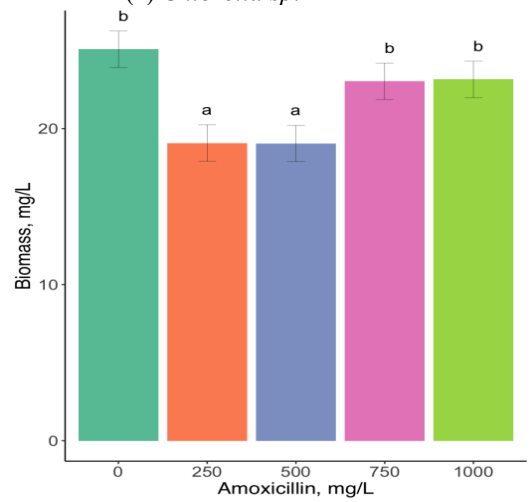
The results of using control solvents such as deionized water and dimethyl sulfoxide (DMSO) showed low inhibition at high concentrations tested. 8 mg/L concentration of deionized water, 9.41 ± 2.7% inhibition of the growth of the tested *Chlorella species* was counted, whereas at a DMSO 15 mg/L concentration, 8.12 ± 3.3% deceleration of vegetative growth was observed. Therefore, the claim that the inhibition of algae biomass was caused by their solvents can be ignored and it can be assumed that antibiotics caused a significant decrease in growth. In general, it has been observed that *Chlorella sp.* was sensible to macrolides compared to other drugs.

Experimental results showed changes in biomass and cell numbers of *Chlorella sp.* under the influence of various

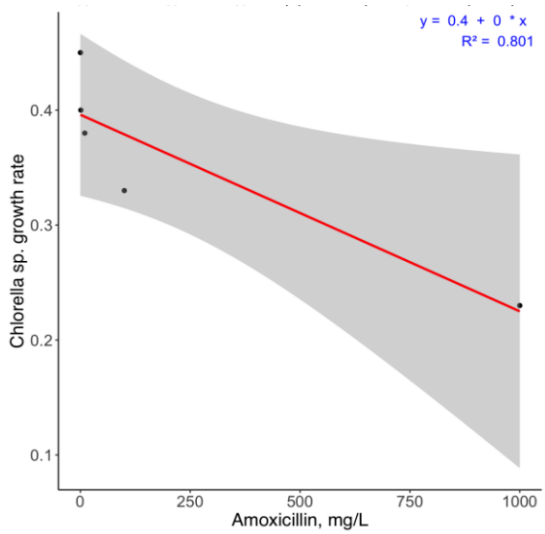
pharmaceuticals. For instance, amoxicillin significantly affected biomass at concentrations of 1-10 mg/L but did not significantly affect cell count until concentrations of 10-100-1000 mg/L, which decreased cell count ($p<0.05$). At high concentrations (1000 mg/L), cell count changed moderately ($p<0.05$) (Figures 1(a)). Biomass of *Chlorella sp.* decreased under influence of 1-10 mg/L of amoxicillin concentration significantly ($p<0.05$) (Figure 1(b)). Amoxicillin logarithmically decreased *Chlorella sp.* growth rate ($r^2=0.80$). At 1000 mg/L, the growth rate halved compared to the control ($0.26 \pm 0.02*10^{-1}$). Although these results practically do not differ from the previous study [39], where 72-hour amoxicillin exposure to *Pseudokirchneriella subcapitata* demonstrated less than 10% decrease at a 1500 mg/L concentration and was detected as a non-toxic substance to this species. This discrepancy can be according to different standardized approaches and the alga for evaluating an antibiotics' effects on this species. Despite differing standardized approaches and species for evaluating antibiotic effects on algae, our results, along with foreign researchers [9], detected amoxicillin as harmless to algae. *Chlorella sp.* showed minimal sensitivity to high concentrations of amoxicillin, with slight inhibition of biomass growth at 1 mg/L (2%), 57% at 1000 mg/L (Figures 1(c), 1(d)). Compared to other substances, the EC_{50} value indicates lower toxicity for amoxicillin due to its rapid degradation and low bioavailability [25]. Thus, amoxicillin can be considered non-toxic for algae, since the EC_{50} value exceeds 100 mg/L.



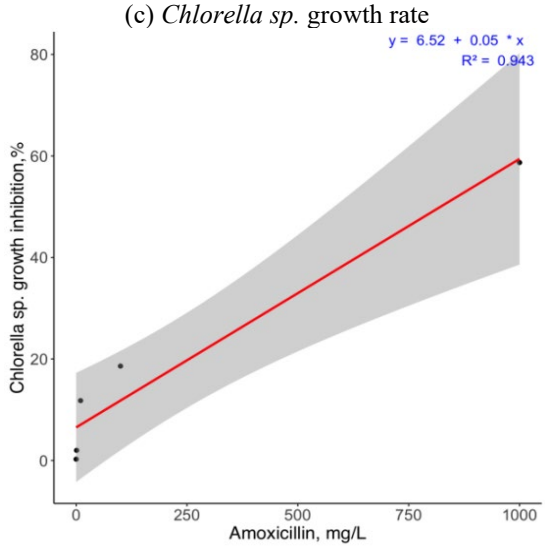
(a) *Chlorella sp.* cell count



(b) *Chlorella sp.* biomass

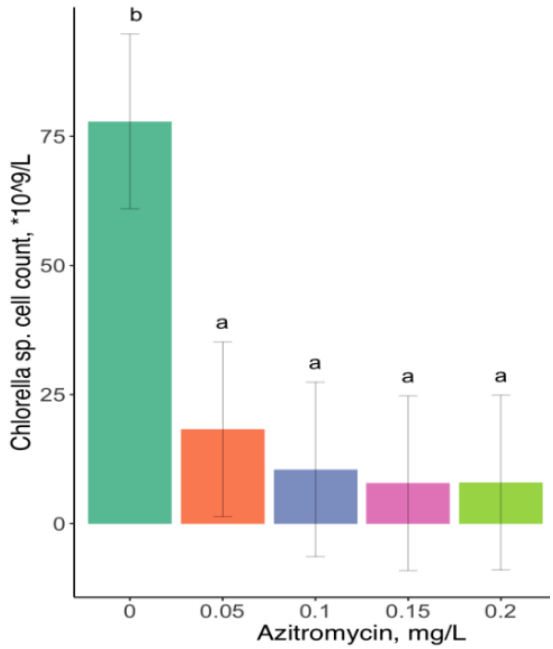


(c) *Chlorella sp.* growth rate

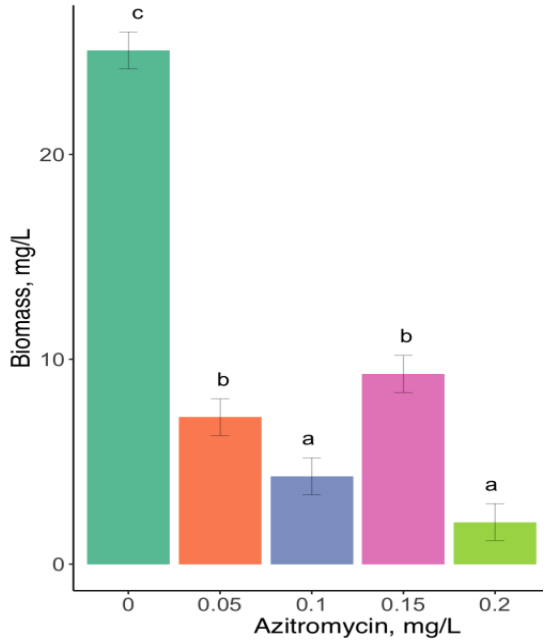


(d) *Chlorella sp.* growth inhibition

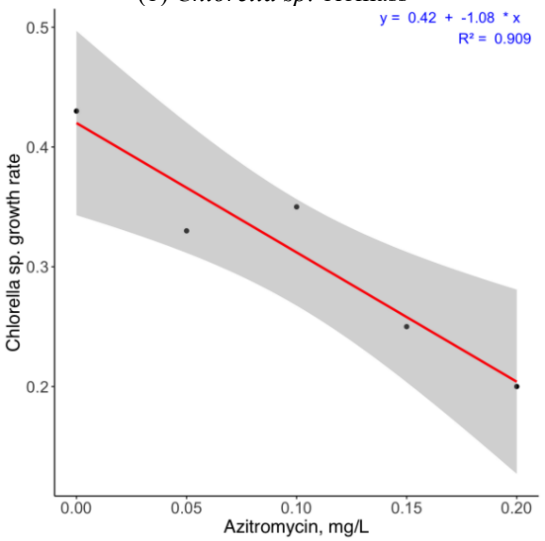
Figure 1. *Chlorella sp.* cell count and biomass, growth rate and growth inhibition under the influence of amoxicillin at 72 hours exposure ($p<0.05$)



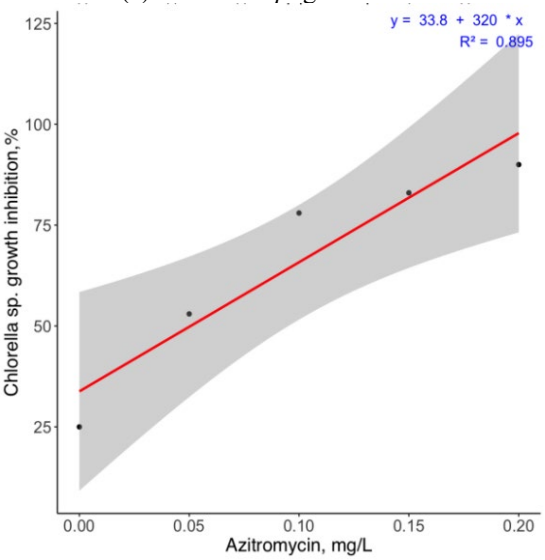
(a) *Chlorella sp.* cell count



(b) *Chlorella sp.* biomass



(c) *Chlorella sp.* growth rate



(d) *Chlorella sp.* growth inhibition

Figure 2. *Chlorella sp.* cell count and biomass, growth rate and growth inhibition under the influence of azithromycin at 72 hours exposure ($p<0.05$)

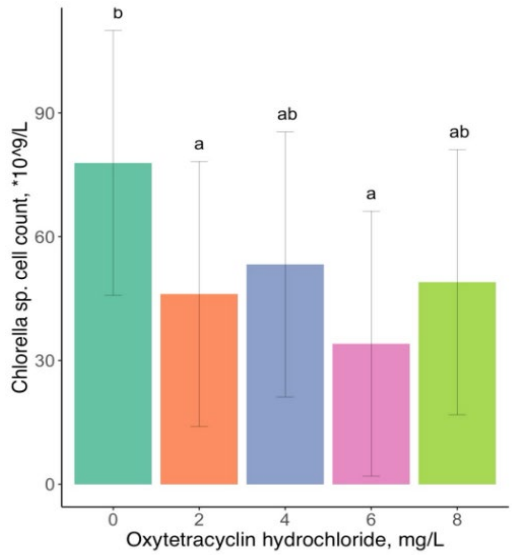
Azithromycin significantly affected cell count at all concentrations (0.05-0.2 mg/L) compared to the control ($78*10^9 \pm 0.01$/L) ($p<0.05$), reducing cell count in experimental 2-5-th groups to 10 ± 0.01 and $8*10^9 \pm 0.03$/L (Figure 2(a)). Algae biomass was also significantly reduced from 25 ± 0.08 in control to 7.5 ± 0.05 - 2.7 ± 0.001 under the influence of experimental groups' azithromycin data in compare with the control data ($p<0.05$) (Figure 2(b)). During the experiment the high sensitivity of algae was determined to low concentrations of Azithromycin (Figure 2(a)). At 0.2 mg/L, azithromycin nearly halved the rate of biomass growth compared to the control, with growth inhibition reaching over 87% at 0.15 mg/L (r^2=0.89) (Figure 2(c), 2(d)). These results align with other studies, indicating macrolides' high toxicity to cyanobacteria and algae due to interference with protein synthesis in gram-positive bacteria. Limited studies on azithromycin's toxicity to algae exist, but Zhou et al. [40] determined an EC_{50} of 0.026 mg/L for azithromycin, classifying it as a toxicant with high level effect to the aquatic ecosystem. This is consistent with our study's EC_{50} of 0.33 ± 0.05 mg/L. Moreover, as during the case of the macrolide (clarithromycin) previously tested in our study, the excretion of azithromycin in wastewater cleaning is 0%. The macrolide antibiotics' concentration in the several areas in China was 17 ng/L [25]. The study by Osorio et al. [41] demonstrated that azithromycin was widespread and accumulated in the basins of Iberian rivers in Spain and Portugal.

Under oxytetracycline exposure, cell numbers and biomass were significantly reduced at 2 mg/L and 6 mg/L, with cell numbers dropping from $80 \pm 0.5*10^9$/L to $46 \pm 0.9*10^9$/L ($p<0.05$) (Figure 3(a)). Biomass decreased significantly from 25 ± 0.7 to 1.7 ± 0.03 in 2 mg/L oxytetracycline, with further reductions at higher concentrations, in 4-8 mg/L oxytetracycline till 6 ± 0.8 ($p<0.05$) (Figure 3(b)).

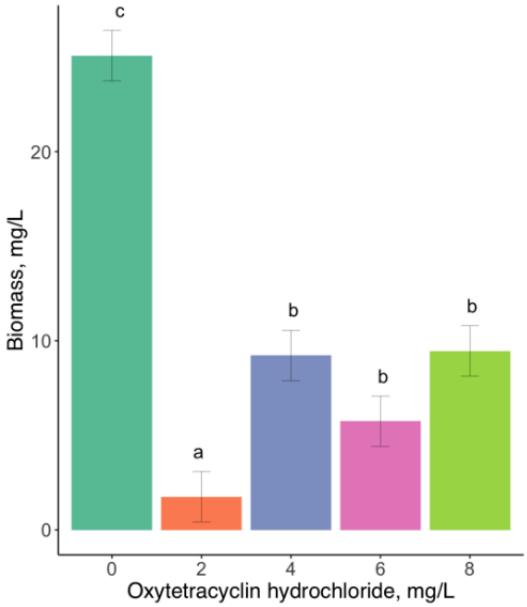
The growth rate at 8 mg/L was 0.2 ± 0.03/d, which is 2 times slower compared to the control. Inhibition of algae growth in the highest tested concentration was more than 94% (Figure 3(c)). Figure 3(d) illustrates decreasing rate of the algae biomass growth rate the oxytetracycline hydrochloride action at concentrations of 2-8 mg/L. In the control, the growth rate was 0.45 ± 0.006/d. However, higher concentrations reduced the rate during the test. The results of the test with oxytetracycline hydrochloride coincide with the results of previous studies. In Kolar et al.'s [42] study, the oxytetracycline effect on *Pseudokirchneriella subcapitata* was not manifested in lower concentrations, in which 72-hour 50% growth inhibition (EC_{50}) was 1.04 mg/L. Lutzhoft et al. [43] conducted a study of a 72-hour inhibition test of oxytetracycline hydrochloride on green algae *Selenastrum capricornutum*. His result (EC_{50} = 4.5 mg/L) was close to our results in terms of effect concentration (3.56 ± 0.35 mg/L).

Ketoconazole significantly reduced Chlorella *sp.* cell numbers from 26.8 ± 0.5 to 21.2 ± 0.2 in 20 mg/L, 22.6 ± 0.5 in 50 mg/L, and 14.1 ± 0.6 in 100 mg/L (Figure 4(a)), with biomass moderately higher than control at 50-100 mg/L ($p<0.05$) (Figure 4(b)). From the beginning of the test, ketoconazole affected to the biomass growth of algae cells. The algae biomass growth rate in the control was 0.4 ± 0.01, at 20mg/L concentration was 0.1 ± 0.02, at 50 mg/L concentration was 0.05 ± 0.004, at 100 mg/L concentration was 0.009 ± 0.06 (Figure 4(c)). According to our results, a significant decrease in the number of *Chlorella sp.* cells under ketoconazole impact and reduced biomass growth rate. Growth rate was decreased 62.91 ± 0.004% at 20 mg/L, 87.61

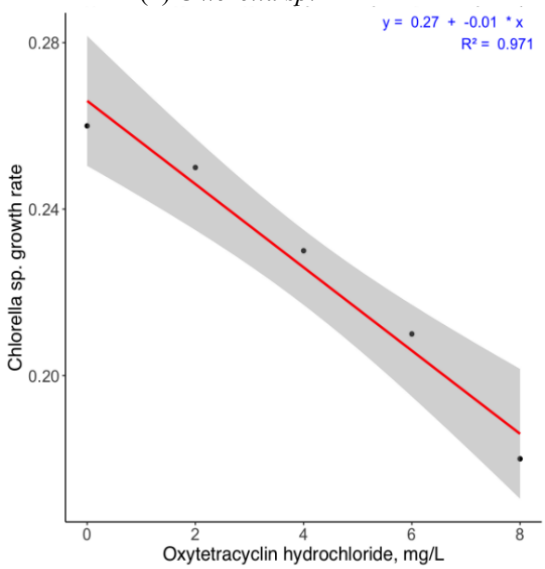
± 0.01% at 50 mg/L, and 97.7 ± 0.004% at 100 mg/L ($p<0.05$) (Figures 4(d)), with an EC_{50} of ketoconazole 28.53 ± 0,53 mg/L (r^2=0.83).



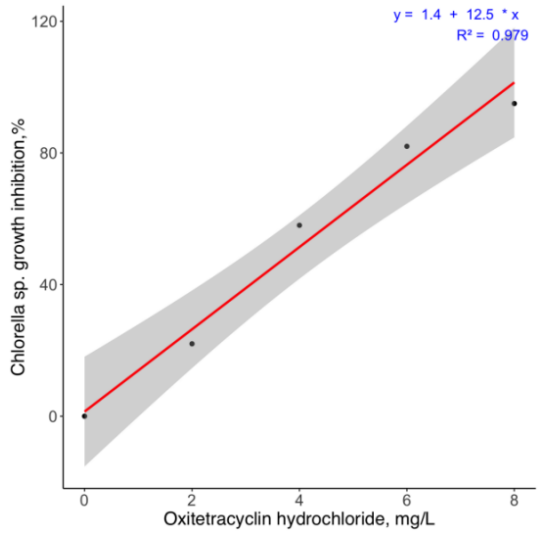
(a) *Chlorella sp.* cell count



(b) *Chlorella sp.* biomass

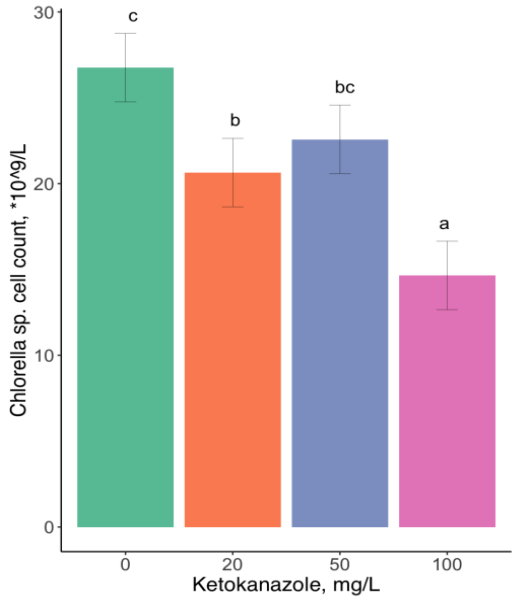


(c) *Chlorella sp.* growth rate

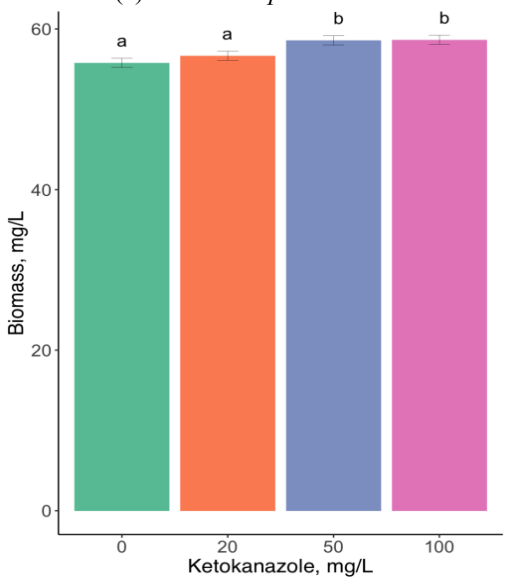


(d) *Chlorella sp.* growth inhibition

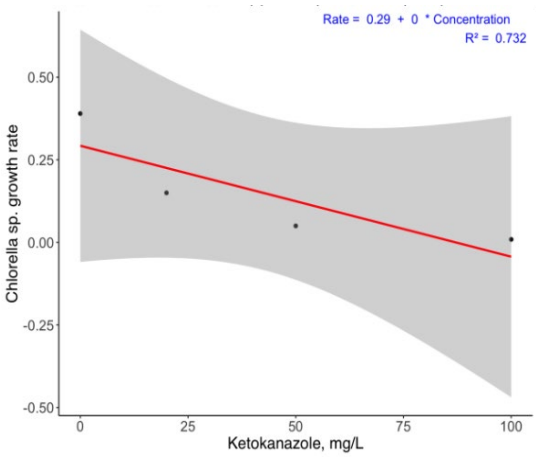
Figure 3. *Chlorella sp.* cell count and biomass, growth rate and growth inhibition under the influence of oxytetracycline hydrochloride at 72 hours exposure ($p<0.05$)



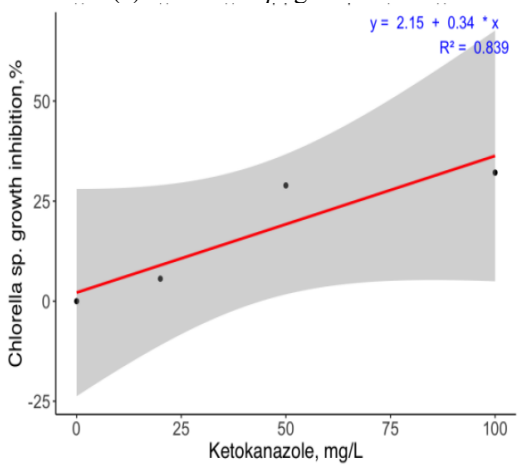
(a) *Chlorella sp.* cell count



(b) *Chlorella sp.* biomass



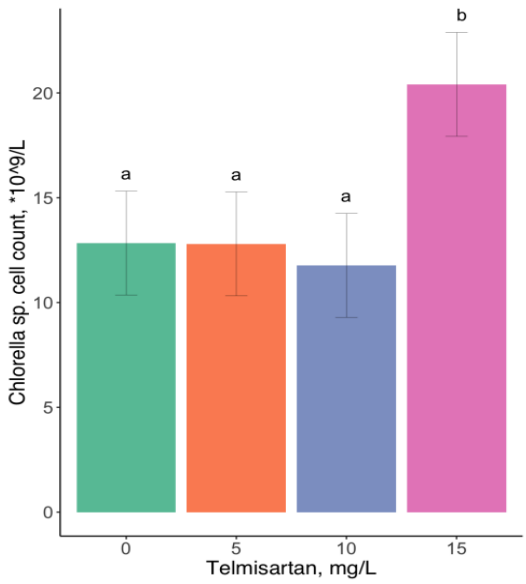
(c) *Chlorella sp.* growth rate



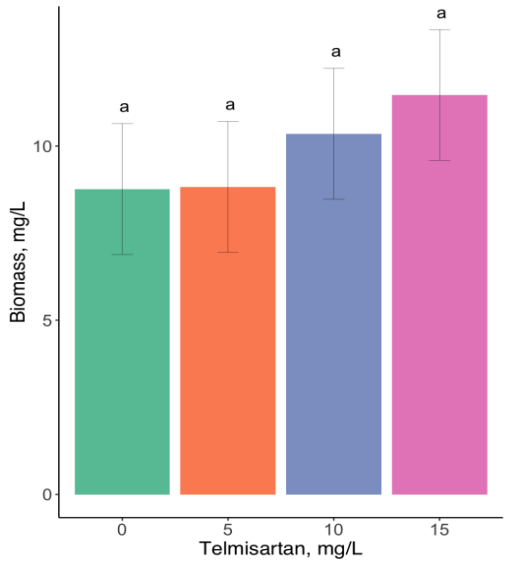
(d) *Chlorella sp.* growth inhibition

Figure 4. *Chlorella sp.* cell count and biomass, growth rate and growth inhibition under the influence of ketoconazole at 72 hours exposure ($p<0.05$)

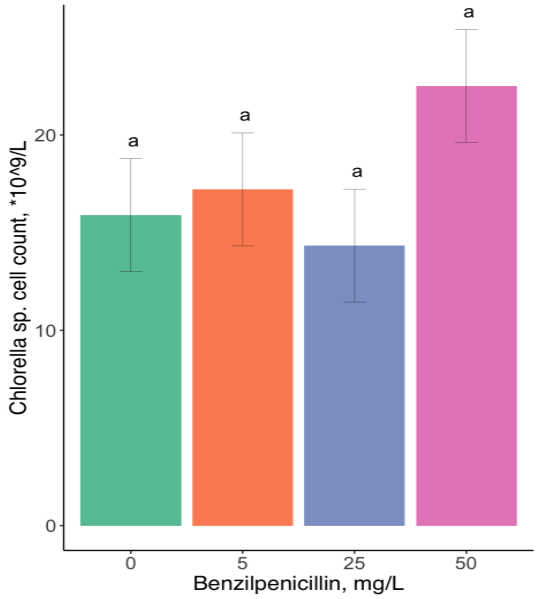
Telmisartan at concentrations 15 mg/L increased the number of algae to 0.05 ± 0.02/d (Figure 5(a)), with biomass like the control (Figure 5(b)). This suggests telmisartan is not harmful to *Chlorella sp.* Similarly, benzylpenicillin increased biomass significantly without changing cell numbers, indicating its non-toxicity at tested concentrations ($p<0.05$) (Figure 5(c), 5(d)).



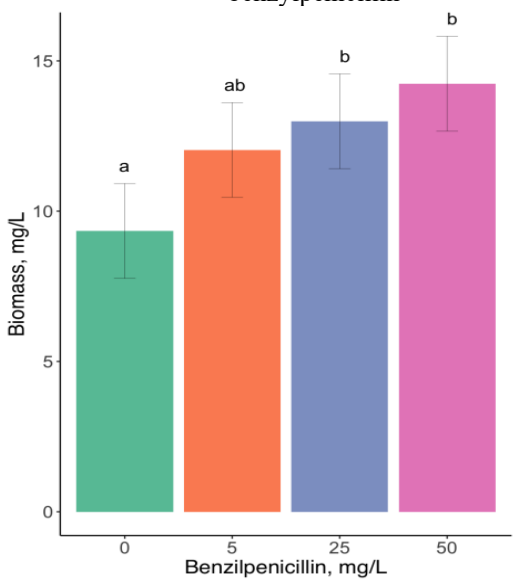
(a) *Chlorella sp.* cell count, under influence telmisartan



(b) *Chlorella sp.* biomass, under influence telmisartan



(c) *Chlorella sp.* cell count, under influence benzylpenicillin



(d) *Chlorella sp.* biomass, under influence benzylpenicillin

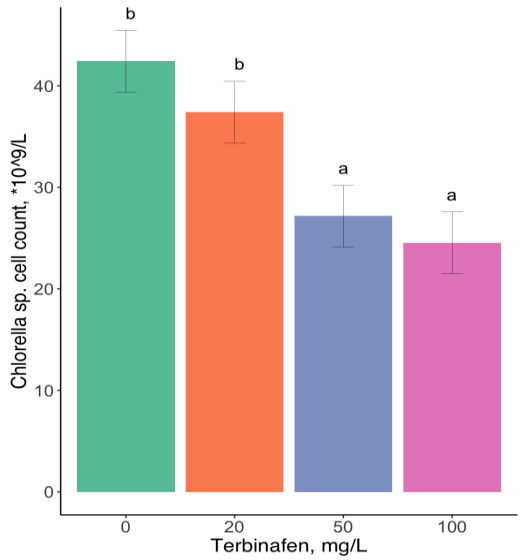
Figure 5. *Chlorella sp.* cell count and biomass, growth rate and growth inhibition under the influence of telmisartan and benzylpenicillin at 72 hours exposure ($p<0.05$)

Terbinafine significantly reduced *Chlorella sp.* growth from the first day of exposure. The algae biomass growth rate in the control data was 0.6 ± 0.002, dropping to 0.08 ± 0.003 at 20 mg/L, 0.02 ± 0.01 at 50 mg/L, and 0.04 ± 0.01 at 100 mg/L (Figure 6(a)). The number of *Chlorella sp.* cells decreased sharply in terbinafine growing concentrations; biomass remained stable (Figure 6(b)). The bioGrowth inhibition was 86.14 ± 0.05% in the first group, 96.19 ± 0.007% in the second, and 94.01 ± 0.01% in the third group, with an EC_{50} of 51.6 ± 0.09 mg/L (Figure 6(c), 6(d)).

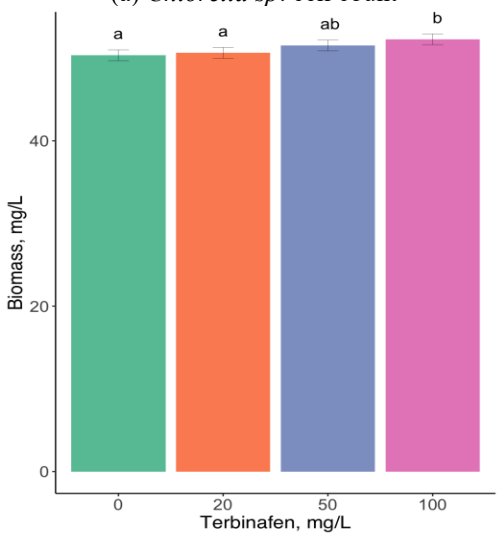
Summarizing the study results (Table 3), azithromycin has the highest toxic effect to *Chlorella sp.*, in an EC_{50} of 0.33± 0.05 mg/L, while amoxicillin was the least toxic, with an EC_{50} of 853.54 ± 0.27 mg/L.

Table 3. Comparison of half of the maximum effective concentration (EC_{50}) parameters of the tested active pharmaceutical ingredients (API) with *Chlorella sp.*

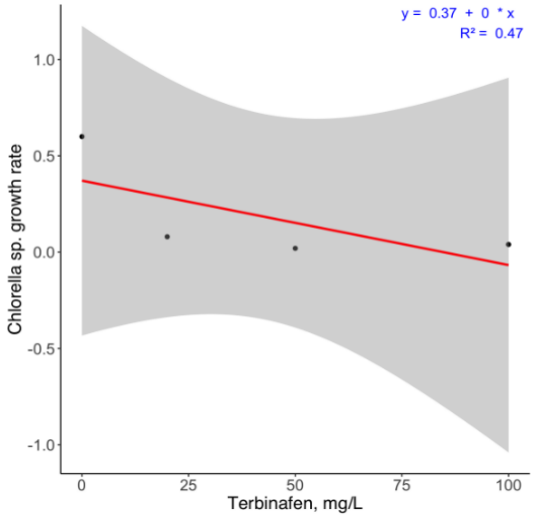
API	Concentrations, mg/L	EC_{50}, mg/L
Amoxicillin	1-1000	853.54 ±0,27
Azithromycin	0.01-0.15	0.33 ±0,05
Oxytetracycline hydrochloride	2-8	3.56 ±0,35
Ketoconazole	20-100	28.53±0,53
Terbinafine	20-100	51.60±0,09



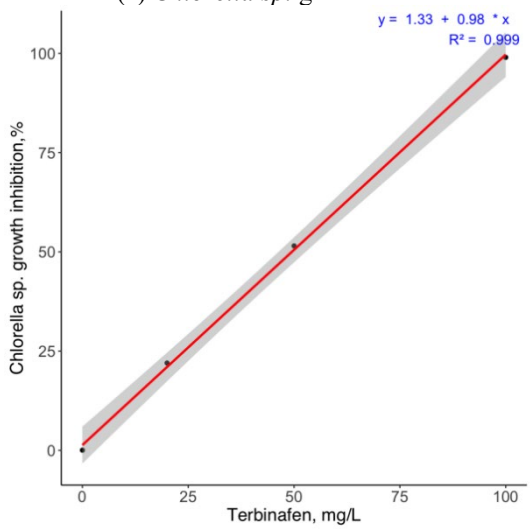
(a) *Chlorella sp.* cell count



(b) *Chlorella sp.* biomass



(c) *Chlorella sp.* growth rate



(d) *Chlorella sp.* growth inhibition

Figure 6. *Chlorella sp.* cell count and biomass, growth rate and growth inhibition under the influence of terbinafine at 72 hours exposure ($p<0.05$)

4. DISCUSSION

Pharmaceuticals (ketoconazole, terbinafine, drotaverine hydrochloride, telmisartan, benzylpenicillin sodium salt) with high pollution potential in Kazakhstan were assessed. Previous research on the effects of antibiotics on the protozoa of chlorella has shown a valuable effect on the population density of this species. In this study, 2,4-dichlorophenol and ciprofloxacin (with concentrations of 10.75 mg/L and 29.09 mg/L, respectively) were identified as the most toxic substances, as noted by Backhaus [44]. Additionally, research was conducted on the toxicity of antibiotics for *Chlorella species*, revealing that *Chlorella sp.* is particularly sensitive to macrolides like azithromycin, with an EC_{50} of 0.33 mg/L. Daughton [45] explored the effects of the synthetic antifungal ketoconazole on duckweed (*Lemna minor*), a freshwater plant often used in phytotoxicity studies. *L. minor* exhibited the highest sensitivity, with EC_{50} values ranging from 0.08 to 0.16 mg/L [29].

Palomaki [46] examined the toxicity of terbinafine through experiments on the green algae *Pseudokirchneriella subcapitata*, finding that terbinafine was highly toxic to the

algae. The EC_{50}, based on growth rate, was calculated at 90 nM, with a biomass of 50 nM. The research indicated that terbinafine affects *P. subcapitata* similarly to fungi by causing cell death due to high squalene levels. Due to its toxicity to *P. subcapitata* and *Charophyceae* algae, terbinafine was classified as a "highly toxic pharmaceutical ingredient for aquatic organisms," as confirmed by Backhaus [44]. The common antibiotic amoxicillin turned out to be the least toxic for algae of the *Chlorella sp.,* since its EC_{50} value exceeded 800 mg/L. Algae species are very sensitive to macrolide antibiotics. The studied macrolides azithromycin showed the greatest toxicity against *Chlorella sp.* Their EC_{50} value was less than 1 mg/L.

The problem of pharmaceutical pollutants in Kazakhstan is the causes of the aquatic ecosystem's pollution since wastewater from domestic sewage, not purified from pharmaceuticals, enters surface waters. Currently, wastewater treatment does not provide for selective or general purification methods, although such methods are used in developed countries.

Foreign studies have consistently shown significant impacts of pharmaceutical pollutants on aquatic organisms. Addressing pharmaceutical contamination in surface waters is critical for environmental sustainability and public health. Risk assessments and control measures, such as regulatory limits for residual substances and water resource monitoring, are essential. Control can be carried out through the foundation of regulatory limits for the content of chemical substances in water and monitoring of water resources. It should be noted that the developement of an environmental passport can also mitigate the impact of pharmaceutical ingredients by regulating the consumption and disposal of pharmaceuticals.

5. CONCLUSIONS

Telmisartan and benzylpenicillin at observed concentrations were not toxic to *Chlorella sp.* The EC_{50} values for the remaining pharmaceuticals were azithromycin 0.33 ±0.05 mg/L, oxytetracycline hydrochloride 3.56 ± 0.35 mg/L, ketoconazole 28.53 ± 0.53 mg/L, terbinafine 51.6 ± 0.09 mg/L, and amoxicillin 853.54 ± 0.27 mg/L. Azithromycin was the most toxic to *Chlorella sp.*, while amoxicillin had the least toxic effect. The data obtained from our studies provide a basis for the development of a pharmaceutical passport. Effective doses of these pollutants can be used for environmental regulations and to regulate the use of the most toxic drugs. These results will also be a prerequisite for the certification of these harmful substances. The next stage requires studies that will show the level of pollution in surface waters, as well as study methods for cleaning wastewater from pharmaceutical pollutants.

REFERENCES

[1] Mendes, G.D., Hamamoto, D., Ilha J., Pereira, A.D., De Nucci, G. (2007). Anastrozole quantification in human plasma by high-performance liquid chromatography coupled to photospray tandem mass spectrometry applied to pharmacokinetic studies. Journal of Chromatography B, 850(1-2): 553-559. https://doi.org/10.1016/j.jchromb.2006.11.044

[2] Burkhanova, G.S., Roshana, N.R., Gorbunova, S.V., Kas'yanova, V.S., Kuterbekov, K.A., Bekmyrza, K.Zh., et al. (2021). Operational stability of the Pd–6 wt % in–0.5 wt % Ru–1 wt % Co membrane during its cyclic operation in manufacturing high-purity hydrogen. Russian Metallurgy (Metally), 3: 313-319.

[3] Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Teta Cet al. (2022). Pharmaceutical pollution of the world's rivers. Proceedings of the National Academy of Sciences of the United States of Americathis link is disabled, 119(8).

[4] Tulegenova, S., Zhantokov, B., Beisenova, R., Shyngysbayeva, Zh., Dukenbayeva, A., Rakhymzhan, Zh. (2024). Monitoring and Evaluation of Pharmaceutical Eco-Pollutants in Wastewater in Kazakhstan Cities. Journal of Ecological Engineering, 25(7): 359-370.

[5] Chen, C.E., Zhang, H., Ying, G.G., Jones, K.C. (2013). Evidence and recommendations to support the use of a novel passive water sampler to quantify antibiotics in wastewaters. Environmental Science and Technology, 47(23): 13587-13593. https://doi.org/10.1021/es402662g

[6] Beisenova, R., Tulegenova, S., Tazitdinova, R., Orkeyeva, A., Beisenbekova, Z. (2022). The problem of water resources pollution with active pharmaceutical substances and the possibility of its solving. Journal of Environmental Management and Tourism, 13(5): 1353-1360.

[7] Gothwal, R., Shashidhar, T. (2015). Antibiotic pollution in the environment: A review. CLEAN – Soil Air Water, 43: 479-489.

[8] Bengtsson-Palme, J., Larsson, D.G.J. (2016). Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International, 86: 140-149. https://doi.org/10.1016/j.envint.2015.10.015

[9] González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I., Leganés, F., Rosal, R., Boltes, K., et al. (2013). Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment. Water Research, 47: 2050-2064.

[10] Brain, R.A., Hanson, M.L., Solomon, K.R., Brooks, B.W. (2008). Aquatic plants exposed to pharmaceuticals: effects and risks. Reviews of Environmental Contamination and Toxicology, 192: 67-115. https://doi.org/10.1007/978-0-387-71724-1_3

[11] Brausch, J.M., Connors, K.A., Brooks, B.W., Rand, G.M. (2012). Human pharmaceuticals in the aquatic environment: A review of recent toxicological studies and considerations for toxicity testing. Reviews of Environmental Contamination and Toxicology, 218: 1-99. https://doi.org/10.1007/978-1-4614-3137-4_1

[12] Nie, X.P., Liu, B.Y., Yu, H.J., Liu, W.Q., Yang, Y.F. (2013). Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole exposure to the antioxidant system in Pseudokirchneriella subcapitata. Environmental Pollution, 172: 23-32. https://doi.org/10.1016/j.envpol.2012.08.013

[13] Kollef, M.H., Bassetti, M., Francois, B., Burnham, J., Dimopoulos, G., Garnacho-Montero, J., et al. (2017). The intensive care medicine research agenda on multidrug-resistant bacteria, antibiotics, and stewardship. Intensive Care Medicine, 43: 1187-1197.

[14] Willyard, C. (2017). The drug-resistant bacteria that pose

the greatest health threats. Nature, 543: 15.
[15] Wollenberger, L., Halling-Sørensen, B., Kusk, K.O. (2011). Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere, 40: 723-730. https://doi.org/10.1016/S0045-6535(99)00443-9
[16] Kołodziejska, M., Maszkowska, J., Białk-Bielińska, A., Steudte, S., Kumirska, J., Stepnowski, P., et al. (2013). Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. Chemosphere, 92: 1253-1259. https://doi.org/10.1016/j.chemosphere.2013.04.057
[17] Santos, L.H.M.L., Araújo, A.N., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M.C. (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. Journal of Hazardous Materials, 175: 45-95. https://doi.org/10.1016/j.jhazmat.2009.10.100
[18] Wise, R. (2002). Antimicrobal resistance: Priorities for action. Journal of Antimicrobial Chemotherapy, 49(4): 585-586. https://doi.org/10.1093/jac/49.4.585
[19] Hirsch, R., Ternes, T., Haberer, K., Kratz, K.L. (1999). Occurrence of antibiotics in the aquatic environment. Science of The Total Environment, 1-2(225): 109-118. https://doi.org/10.1016/S0048-9697(98)00337-4
[20] Baumann, M., Weiss, K., Maletzki, D., Schussler, W., Schudoma, D., Kopf, W., Kuhnen, U. (2015). Aquatic toxicity of the macrolide antibiotic clarithromycin and its metabolites. Chemosphere, 120: 192-198. https://doi.org/10.1016/j.chemosphere.2014.05.089
[21] Beisenova, R., Tulegenova, S., Tazitdinova, R., Kovalenko, O., Turlybekova, G. (2020). Purification by Ketoconazole Adsorption from Sewage. Systematic Review Pharmacy, 11(6): 550-554. https://doi.org/10.31838/srp.2020.6.84
[22] Aubakirova, B.N. (2017). The effect of pharmaceutical ingredients on representatives of aquatic biota [Ph.D. Dissertation]. Astana: Gumilyov Eurasian National University.
[23] Aubakirova, B.N., Beisenova, R., Boxall, A. 2017. Prioritisation of pharmaceuticals based on risks to aquatic environments in Kazakhstan, Integrated Environmental Assessment and Management, 13(5): 832-839. https://doi.org/10.1002/ieam.1895
[24] Guo, J.H., Sinclair, C.J., Selby, K., Boxall, A.B.A. (2016). Toxicological and ecotoxicological risk-based prioritization of pharmaceuticals in the natural environment. Environmental Toxicology and Chemistry, 35(6): 1550-1559. https://doi.org/10.1002/etc.3319
[25] Cooper, E., Siewicki, T., Phillips, K. (2008). Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment. Science of The Total Environment, 398(1-3): 26-33. https://doi.org/10.1016/j.scitotenv.2008.02.061
[26] Roos, V., Gunnarsson, L., Fick, J., Larsson, D.G.J., Ruden, C. (2012). Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Science of The Total Environment, 421-422: 102-110. https://doi.org/10.1016/j.scitotenv.2012.01.039
[27] Kummerer, K. (2001). Emission and biodegradability of pharmaceuticals, contrast media, disinfectants and AOX from hospitals. In: Pharmaceuticals in the Environment, pp. 29-41. https://doi.org/10.1007/978-3-662-04634-0_4
[28] Yasojima, M., Nakada, N., Komori, K., Suzuki, Y., Tanaka, H. (2006). Occurrence of levofloxacin, clarithromycin and azithromycin in wastewater treatment plant in Japan. Water Science & Technology, 53(11): 227-233. https://doi.org/10.2166/wst.2006.357
[29] Kummerer, K. (2009). Antibiotics in the aquatic environment - A review - Part I. Chemosphere, 75(4): 417-434. https://doi.org/10.1016/j.chemosphere.2008.11.086
[30] Shi, H., Sun, Z., Liu, Z., Xue, Y. (2012). Effects of clotrimazole and amiodarone on early development of amphibian (Xenopus tropicalis). Toxicological & Environmental Chemistry, 94(1): 128-135. https://doi.org/10.1080/02772248.2011.634643
[31] Porsbring, T., Blanck, H., Tjellström, H., Backhaus, T. (2009). Toxicity of the pharmaceutical clotrimazole to marine microalgal communities. Aquatic Toxicology, 91(3): 203-211. https://doi.org/10.1016/j.aquatox.2008.11.003
[32] Hegelund, T., Ottosson, K., Rådinger, M., Tomberg, P., Celander, M.C. (2004). Effects of the antifungal imidazole ketoconazole on Cyp1A and Cyp3A in rainbow trout and killifish. Environmental Toxicology and Chemistry, 23(5): 1326-1334. https://doi.org/10.1897/03-155
[33] Halling-Sorensen, B. (2000). Algal toxicity of antibacterial agents used in intensive farming. Chemosphere, 40(7): 731-739. https://doi.org/10.1016/S0045-6535(99)00445-2
[34] Oguz, M., Mihciokur, H. (2014). Environmental risk assessment of selected pharmaceuticals in Turkey. Environmental Toxicology and Pharmacology, 38(1): 79-83. https://doi.org/10.1016/j.etap.2014.05.012
[35] Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R., Fanelli, R. (2003). Strategic survey of therapeutic drugs in the rivers po and lambro in northern Italy. Environmental Science & Technology, 37(7): 1241-1248. https://doi.org/10.1021/es020158e
[36] Acuna, V., Ginebreda, A., Mor, J.R., Petrovic, M., Sabater, S., Sumpter, J., Barcelo, D. (2015). Balancing the health benefits and environmental risks of pharmaceuticals: Diclofenac as an example. Environment International, 85: 327-333. https://doi.org/10.1016/j.envint.2015.09.023
[37] OECD. (2016). Guidelines for the testing of chemicals freshwater alga and cyanobacteria, growth inhibition test No 201. Paris: Organisation for Economic Co-operation and Development.
[38] Ebert, I., Bachmann, J., Kuhnen, U., Kuster, A., Kussatz, C., Maletzki, D., Schlüter C. (2011). Toxicity of the fluoroquinolone antibiotics enrofloxacin and ciprofloxacin to photoautotrophic aquatic organisms. Environmental Toxicology and Chemistry, 30(12): 2786-2792. https://doi.org/10.1002/etc.678
[39] Sangion, A, Gramatica, P. (2016). PBT. assessment and prioritization of contaminants of emerging concern: Pharmaceuticals. Environmental Research, 147: 207-306. https://doi.org/10.1016/j.envres.2016.02.021
[40] Zhou, H., Ying, T., Wang, X., Liu, J. (2016). Occurrence and preliminarily environmental risk assessment of selected pharmaceuticals in the urban rivers, China. Scientific Reports, 6(1): 1-10. https://doi.org/10.1038/srep34928
[41] Osorio, V., Larranaga, A., Acena, J., Perez, S., Barcelo, D. (2016). Concentration and risk of pharmaceuticals in

freshwater systems are related to the population density and the livestock units in Iberian Rivers. Science of The Total Environment, 540: 267-277. https://doi.org/10.1016/j.scitotenv.2015.06.143

[42] Kolar, B., Arnus, L., Jeretin, B., Gutmaher, A., Drobne, D., Durjava, M.K. (2014). The toxic effect of oxytetracycline and trimethoprim in the aquatic environment. Chemosphere, 115: 75-80. https://doi.org/10.1016/j.chemosphere.2014.02.049

[43] Lutzhoft, H.C.H., Halling-Sørensen, B., Jørgensen, S.E. (1999). Algal toxicity of antibacterial agents applied in Danish fish farming. Archives of Environmental Contamination and Toxicology, 36(1): 1-6. https://doi.org/10.1007/s002449900435

[44] Backhaus, T. (2014). Medicines, shaken and stirred: A critical review on the ecotoxicology of pharmaceutical mixtures. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1656): 20130585. https://doi.org/10.1098/rstb.2013.0585

[45] Daughton, C.G. (2003). Pollution from the combined activities, actions, and behaviors of the public: Pharmaceuticals and personal care products. NorCal SETAC News, 14(1): 5-15.

[46] Palomaki, A. (2010). Toxicity and mode of action of the pharmaceutical fungicide's fluconazole and terbinafine to freshwater algae. Goteborg: Gothenburg University.

NOMENCLATURE

API	Active Pharmaceutical Ingredients
DMSO	Dimethyl sulfoxide
mg/L	Concentration, mg/L
SE	Standard error
EC_{50}	Half of the maximum effective concentration, mg/L