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THE EFFECT OF FIVE ANTIBIOTICS TO THE GROWTH OF CHLORELLA SPECIES

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There is a risk of pollution of the aquatic environment with the removal and disposal of active pharmaceutical ingredients with animal toxicity and its metabolites [1]. In comparison with other chemicals, pharmaceuticals do not occur in specific region, they spread over the world [2]. The physico-chemical properties of drugs are common with xenobiotics. Pharmaceuticals are persistent to aquatic environment. Human pharmaceuticals have been named as “psedo-persistent” [3].

Drugs enter to the environment via wastewater when patients excrete them after consumption. Unused drugs and dispose of in drains [1].

Currently, around 160 of various APIs were detected in the aquatic environment. The presence of them were detected in landfills, effluents from hospitals, WWTPs, surface water, seawater, groundwater and drinking water [4].

There are not enough studies have been performed on effects of pharmaceuticals to environmental species. The ecotoxicological data on acute study of various pharmaceuticals to aquatic and terrestrial organisms are available. However, these information is not enough. There is still deficit on chronic studies of many drugs [5].

Many studies were conducted on ecotoxicological effects of pharmaceuticals to the environmental species [6-9]. It was found that, the range of wastewater concentration of diclofenac was enough to have adverse impact on aquatic organisms, while maximally measured STP effluent concentration of propranolol and fluoxetine had effect on zooplankton and benthic organisms [4]. Another harmful effect of pharmaceuticals is endocrine disruption. It effects to the function of hormones and can be permanent even at low level [10]. Also, estrogens that were detected in aquatic environment had an adverse impact on fish reproduction and could lead to the population decline [4].

According to Brausch et al. study, antibiotics in comparison with other class of drugs are the most acutely toxic compounds [3]. Antibiotics impact to biological system is different from other drugs, because initially they are established to resist to microbes and bacteria. Due to the huge consumption of antibiotics in the last 50

years, the gene pool of most bacteria has changed and they become resistant to compounds [10].

Photoautotrophs as algae play an important role in total biomass in the aquatic environment. In addition, algae are the major carbon sources for the aquatic system. They are widely used as pollution indicators in surface water. It is significant to consider of performing toxicity studies of pharmaceuticals to algae species [6].

The aim of the following study was to assess the toxicity of 5 antibiotics as amoxicillin, clarithromycin, azithromycin, sulfamethoxazole, oxytetracycline hydrochloride to the growth of algae Chlorella species (Chlorella sp.).

In order to perform assessment five antibiotics were purchased from Sigma-Aldrich UK. Chlorella species were kindly presented fromthe “Applied Ecology” Laboratory of L.N.Gumilyov Eurasian National University. The study was conducted according to the OECD 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test [11].

Table 1 provides data of EC_{50} of five tested antibiotics to algae Chlorella species. The present table shows that azithromycin is the most toxic to Chlorella in comparison with other substances. EC_{50} of this compound is 0,33±0,05 mg/L. It can be noted that the following concentration can be detected in surface water.

Table 1 – The comparison of EC_{50} parameters of five tested pharmaceuticals *to Chlorella species* ($p<0,05$). APIs – active pharmaceutical ingredients; EC_{50} – half maximal effective concentration

APIs	**Tested concentrations, mg/L**	**EC_{50}, mg/L**
Amoxicillin	1-1000	853,54±0,27
Azithromycin	0,01-0,15	0,33±0,05
Clarithromycin	0,01-0,15	0,59±0,004
Sulfamethoxazole	1-15	3,3±0,51
Oxytetracycline hydrochloride	2-8	3,56±0,35

The results of the performed oxytetracycline hydrochloride test are persistent with previous with results of previous studied papers. In 1999 Holten Lutzhoft et al. [12] conducted the study 72 h of oxytetracycline hydrochloride inhibition test to green algae Selenastrum capricornutum. His finding (EC_{50}=4,5 mg/L) wasclose to our effect concentration results. Chlorella sp. did not show a sensitivity to amoxicillin in high concentrations.There was a slight growth inhibition (2%) of Chlorella sp. to this antibiotic in concentration 1 mg/L, while in 1000 mg/L was reached only 57%. These results hardly differ from previous study of Gonzalez-

Pleiter et al., where 72 h of exposure with amoxicillin to green algae *Pseudokirchneriella subcapitata* showed less 10% of inhibition in concentration 1500 mg/L and was considered as not toxic to algae [13]. This inconsistency may be due to comparable different standardized approaches and species for the assessment of the antibiotic to algae. Clarithromycin showed above 94% of inhibition of algae biomass in concentration 0,15 mg/L after 96 h of exposure. The growth rate decreased in 3 times (0,16±0,08 d^{-1}) in comparison with controls (0,37±0,04 d^{-1}). These results are in agreement with Baumann et al. results where 10% of effect concentration (EC_{10}) values ranged of 23-28 µg/L for clarithromycin and its metabolite for *Desmodesmus subspicatus*, while this value for *Anabaena flos-aquae* was 1.1 µg/L [9].

The results of sulfamethoxazole in our study is encouraging to compare this figure with that found by Eguchi et al. who found that EC_{50} of sulfamethoxazole was 1,53 mg/L to *Selenastrum capricornutum*. This value is lower than our half maximal effect concentration (3,3±0,51 mg/L). However, *Selenastrum capricornutum* is more sensitive in comparison with *Chlorella species* [14].

The high sensitivity of Chlorella to azithromycin was seen in low concentration during the test. In concentration 0,2 mg/L the growth pace decreased in almost 4 times in comparison with controls. The growth inhibition reached more than 87% even in concentration 0,15 mg/L. These results are consistent with those of other studies and suggest that macrolides are very toxic to cyanobacteria and algae, as it has impacts on the growth of Gram-positive bacteria by hindering with the protein synthesis [9].

Overall, it was found that algae are sensitive to macrolides in comparison with other pharmaceutical groups. Amoxicillin can be considered as non-toxic to algae, as EC_{50} value exceed 100 mg/L. Macrolide substances as azithromycin and clarithromycin are the most toxic compounds to algae as their half maximal concentration was lower than 1 mg/L. The following results can be recommended for the addition of the material safety data sheet for pharmaceuticals.

To sum up,it can be noted that due to population growth the consumption of antibiotics is increasing in Asian and European countries and Kazakhstan. Therefore, it is recommended to conduct further studies on toxicity of other antibiotics to aquatic environment.

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