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EFFICIENCY OF WASTEWATER TREATMENT FROM CHLORAMPHENICOL USING *Lemna minor*

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The article studies the effectiveness of wastewater treatment contaminated with chloramphenicol, a broad-spectrum antibiotic often found in the wastewater of pharmaceutical enterprises and healthcare facilities.

The aim of the study was to determine the efficiency of chloramphenicol removal from model solutions using the biological agent *Lemna minor* depending on the initial concentration of the antibiotic and the treatment time.

Model solutions with initial chloramphenicol concentrations of 2, 5, 10, and 20 mg/L were used. The treatment time ranged from 1 to 72 hours.

Methods. The chloramphenicol content in the model solutions was determined using high-performance liquid chromatography.

Results. Lemna minor effectively reduces the concentration of chloramphenicol, with a maximum reduction of 33.0% achieved at an initial concentration of 10 mg/L and 29.5% for 20 mg/L after 72 hours of treatment. The duckweed biomass was 0.04 g/mL. At 2 and 5 mg/L concentrations, the cleaning efficiency gradually increased for the first 24 hours, reaching a maximum of 23.2% and 26.8%, respectively, to 72 hours. This indicates that *Lemna minor* can effectively reduce antibiotic content in water but a long contact time is required to achieve maximum efficiency.

In the control experiments where *Lemna minor* was not used, the chloramphenicol concentration remained unchanged over 72 hours, confirming the absence of natural decomposition or change in antibiotic content without a biological agent.

Conclusions. The studies confirm the effectiveness of *Lemna minor* as a biological agent for reducing chloramphenicol concentrations in wastewater by up to 33%. The use of duckweed helps reduce the environmental impact of the antibiotic and contributes to lowering the risk of antibiotic resistance development.

Key words: wastewater, treatment, biological method, duckweed, antibiotics.

Every year, pharmaceutical enterprises discharge millions of cubic meters of wastewater containing residues of active pharmaceutical ingredients into water bodies. For example, in the European Union countries, the volume of such discharges exceeds 2 million m^3 annually [1]. Antibiotics entering aquatic ecosystems can cause serious environmental problems, including the development of antibiotic-resistant microorganisms, changes in the structure and functioning of aquatic ecosystems, and adverse effects on the flora and fauna of natural water bodies [2].

Many antibiotics in the environment do not decompose naturally and can accumulate in water, soil, and even living organisms, posing severe threats to human health and biodiversity [3].

Today, physical, chemical, physical, chemical, and biological methods are used to treat wastewater from pharmaceutical enterprises. Physical methods such as sedimentation and filtration are designed for preliminary wastewater treatment from insoluble particles [4–6]. Among chemical methods, oxidation using oxidizers such as ozone, chlorine-containing reagents, and

hydrogen peroxide is commonly used to treat wastewater from antibiotics by breaking down their chemical structures. However, this can lead to toxic by-products that require additional treatment for neutralization [7]. The photocatalysis method uses ultraviolet light and catalysts to accelerate chemical reactions, allowing antibiotic molecule breakdown. However, its disadvantages include the need for significant energy consumption and expensive complex equipment. Adsorption on activated carbon effectively removes organic pollutants, including antibiotics, at low concentrations and is used in post-treatment wastewater. However, the drawback of the adsorption method is the need to regenerate the adsorbent, which requires significant material and financial costs [9]. The ion exchange method is effective but expensive and complex [10]. High levels of wastewater treatment from antibiotics and other pollutants can be achieved with membrane methods such as ultrafiltration and hyperfiltration. However, these methods are costly due to the need for membrane regeneration [11]. The coagulation method using mineral coagulants effectively removes organic pollutants but is not sufficiently effective against antibiotics [12]. Aerobic biological treatment is ineffective for removing pharmaceutical substances and their metabolites from wastewater due to their high resistance to biodegradation by activated sludge microorganisms [13]. Some antibiotics exhibit toxicity towards the bacterial component of activated sludge. In contrast, others, such as tetracycline [14], can adsorb onto activated sludge flocs without changing their structure, leading to reduced wastewater treatment efficiency.

Recently, scientists have paid significant attention to the method of biological wastewater treatment from pollutants such as heavy metal ions, nitrogen and phosphorus compounds, and organic substances, including antibiotics, using higher aquatic plants. In particular, several researchers are studying the possibility and effectiveness of using higher plants such as duckweed (*Lemna aoukikusa* [15], *Lemna minor* [16], *Spirodela polyrhiza* [17], *Lemna aequinoctialis* [18]) and vetiver grass (*Chrysopogon zizanioides* [19]) for efficient wastewater treatment from antibiotics.

For instance, the potential of using vetiver (*Chrysopogon zizanioides*), a perennial grass that grows quickly and can be cultivated hydroponically, has been analyzed [20]. Vetiver effectively removed over 90% of ciprofloxacin and tetracycline from wastewater. The plants reach up to 1.5 meters in height with vertical roots up to 4 meters long, requiring significant land areas for cultivation and wastewater treatment processes. The need for and complexity of processing the used plants should also be noted.

In contrast, the use of *Lemna aequinoctialis* duckweed allows for the effective removal of streptomycin from water, reducing its concentration by 72–82% [19], while *Lemna minor* effectively removes amoxicillin, enrofloxacin, and oxytetracycline with an efficiency of 89–92% [17]. Moreover, duckweed is easily cultivated, is a renewable resource, and can be used to produce alternative energy sources. However, specific parameters of the wastewater treatment process using duckweed, such as the concentration of antibiotics in treated water, time, and biomass quantity, have yet to be studied.

Therefore, this work aims to establish the effectiveness of pharmaceutical wastewater treatment from chloramphenicol using *Lemna minor* depending on the antibiotic's initial concentration and the treatment time.

Materials and Methods

Solutions for determining the content of chloramphenicol, an antibiotic from the amphenicol group, were prepared for the study using water for chromatography in which powdered chloramphenicol was dissolved to achieve concentrations of 2, 5, 10, and 20 mg/L.

The chloramphenicol content in the solutions after treatment for a particular duration was determined using high-performance liquid chromatography (HPLC) and a calibration curve.

Chromatography of the samples was performed on an Agilent 1260 Infinity II liquid chromatograph, and the results were processed using Agilent OpenLab software.

Peaks in chromatography are displayed as graphs of detector voltage versus time. The peak areas on the graph are calculated from the obtained chromatograms and are measured in mV×sec. This value is used to quantify the concentration of chloramphenicol because it is proportional to its amount in the sample.

A mobile phase consisting of methanol and Solution A in a ratio of 32:68 was used to separate solution components, along with a stationary phase of octadecylsilane end-capped deactivated silica gel for chromatography.

Peaks were detected at a wavelength of 277 nm with an injection volume of 10 µL.

Solution A was prepared, as follows: 2.0 g of sodium heptanesulfonate was dissolved in 900 mL of water. Then, 6.8 g of potassium dihydrogen phosphate and 5 mL of triethylamine were added. The pH was adjusted to 2.5 using phosphoric acid, and the volume was brought to 1000 mL with water.

Lemna minor samples were collected from a pond in the Stavyshche, Zhytomyr region. For adaptation to indoor conditions, the duckweed samples were placed in a bioreactor filled with 2.5 L of settled tap water. To maintain a water temperature of 20–25 °C, an AquaEL Platinum Heater with an electronic thermostat was used. The natural lighting duration was 12-16 hours per day. A Collar aPUMP aquarium compressor provided water aeration with a capacity of 100 L/hr.

Model solutions of chloramphenicol were prepared for the study using settled tap water in which powdered chloramphenicol was dissolved to achieve concentrations of 2, 5, 10, and 20 mg/L.

Eight polypropylene bioreactors with a volume of 125 cm^3 (dimensions 5×5×5 cm) were filled with model solutions. The water depth in the containers was 3 cm.

A total of 2.5 g of wet *Lemna minor* mass was evenly distributed across the water surface in four bioreactors, with a duckweed layer thickness of 0.5 cm. Four bioreactors were filled with model solutions without adding duckweed for control purposes.

The purification process was studied in static mode for 1, 2, 4, 6, 21, 24, 48, and 72 hours at a constant temperature of 22 °C.

Water samples were taken from the middle layer of the bioreactors and filtered through a Phenex-RC syringe filter with a pore diameter of 0.45 µm. The filtrate was dissolved in the mobile phase in a volume ratio 1:1, and the resulting solutions were chromatographed.

To calculate the removal efficiency of chloramphenicol (E, %) from the pharmaceutical wastewater, the following formula was used:

$$E = \frac{C_0 - C_t}{C_0} \times 100,$$

where: C_0 is the initial concentration of chloramphenicol (mg/L); C_t is the concentration of chloramphenicol in the treated model solutions for t hours (mg/L); 100 is for converting the result into percentage.

Results and Discussion

The calibration curve based on chromatographing solutions' results for determining chloramphenicol's content is shown in Fig. 1.

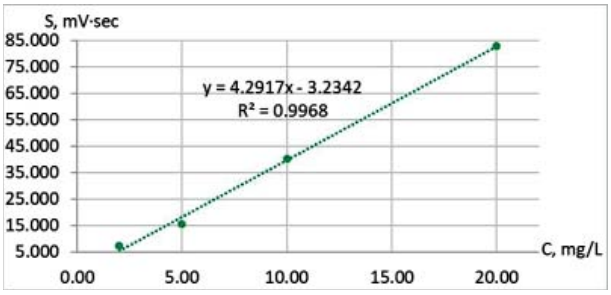

Fig. 1. **The dependence of the peak area of chloramphenicol on its content in solutions**

The results of chromatographic determination of the chloramphenicol content in purified model solutions according to the time of their treatment with *L. minor* are presented in Table 1.

The table shows that increasing the treatment time of the model solutions using duckweed reduces chloramphenicol content in the solutions for all initial concentrations of the antibiotic. For example, at an initial concentration of 20 mg/L, after 72 hours of treatment, the antibiotic content decreased to 14.34 mg/L.

After 48 hours of purification, the solution's antibiotic concentrations become practically unchanged. The higher the initial concentration of chloramphenicol, the greater the amount remaining in the treated solution, but the percentage reduction is the same for all concentrations.

During the purification of model solutions, yellowing of *Lemna minor* leaves was observed, which indicates a disruption of photosynthesis and damage to chloroplasts under the action of the antibiotic.

The results of chromatographic determination of the chloramphenicol content in purified model solutions according to the time of their treatment without *L. minor* are presented in Table 2.

Chloramphenicol does not undergo degradation or removal in the model solutions without *Lemna minor*, as evidenced by the unchanged concentration values in the model solutions over the study period. These data serve as a control indicator for assessing the effectiveness of duckweed in the treatment process, as shown in Table 1. Clearly, without adding duckweed, there is no reduction in chloramphenicol concentration.

Table 1

Chloramphenicol content in model solutions depending on the time of their treatment using *Lemna minor*

τ, hr	Initial chloramphenicol content in model solutions C_0, mg/L			
	2	5	10	20
0	2.47	4.39	10.13	20.01
1	2.47	4.39	10.12	20.00
2	2.47	4.39	10.07	19.86
4	2.47	4.38	9.95	19.36
6	2.47	4.36	9.67	18.95
21	2.29	3.99	9.13	17.53
24	2.17	3.80	7.98	16.04
48	2.08	3.45	7.38	14.38
72	2.08	3.43	7.04	14.34

Table 2

Chloramphenicol content in model solutions depending on the time of treatment without the use of *Lemna minor*

τ, hr	Initial chloramphenicol content in model solutions C_0, mg/L			
	2	5	10	20
0	2.47	4.39	10.13	20.01
1	2.47	4.39	10.12	20.01
2	2.47	4.39	10.13	20.01
4	2.47	4.39	10.13	20.01
6	2.47	4.39	10.13	20.00
21	2.47	4.39	10.12	20.00
24	2.46	4.39	10.12	20.00
48	2.46	4.39	10.12	20.00
72	2.46	4.39	10.12	20.00

Thus, the results indicate the importance of *Lemna minor* as a biological agent in reducing chloramphenicol content in solutions, as without duckweed, its content remains unchanged over 72 hours.

The change in chloramphenicol content in model solutions with concentrations of 2 and 5 mg/L depending on the treatment time in bioreactors with *L. minor* is shown in Fig. 2.

A decrease in chloramphenicol content was observed in the solutions with duckweed for both tested concentrations (2 and 5 mg/L).

In the control solutions (without duckweed), the chloramphenicol concentration remains unchanged over 72 hours. This confirms that the reduction in chloramphenicol content in the experimental samples occurs specifically due to the action of duckweed.

The most significant reduction in antibiotic content was observed within the first 24–48 hours, after which the process slowed down, and the content remained practically unchanged during the 72-hour treatment period.

Obviously, a decrease in the content of the antibiotic involves the oxidation of the chloramphenicol molecule with the help of enzymes such as cytochrome P450 or other oxidoreductases. These enzymes can change the structure of chloramphenicol making it easier to break down [21].

The change in chloramphenicol content in model solutions with concentrations of 10 and 20 mg/L depending on the treatment time in bioreactors with *L. minor* is shown in Fig. 3.

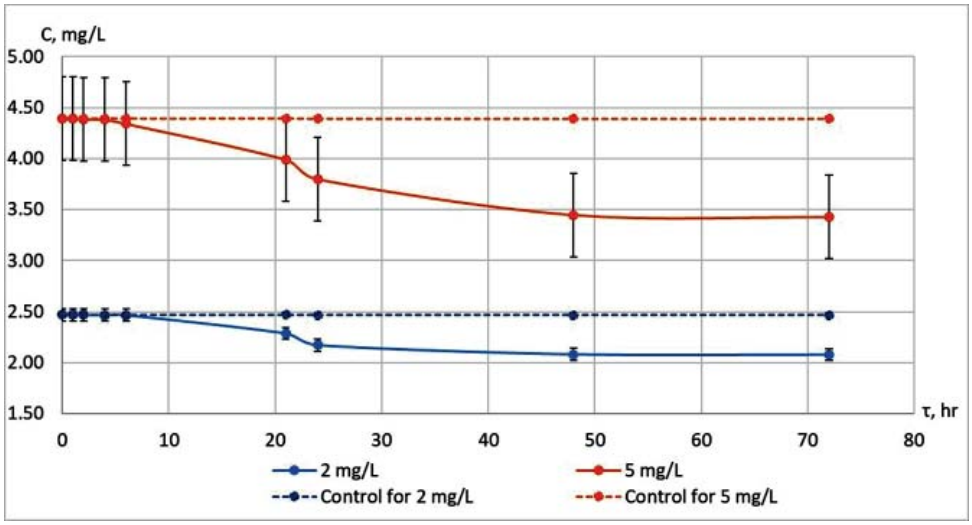

Fig. 2. **The dependence of chloramphenicol content in model solutions 2 and 5 mg/L with *Lemna minor* and in control solutions on the time of treatment**

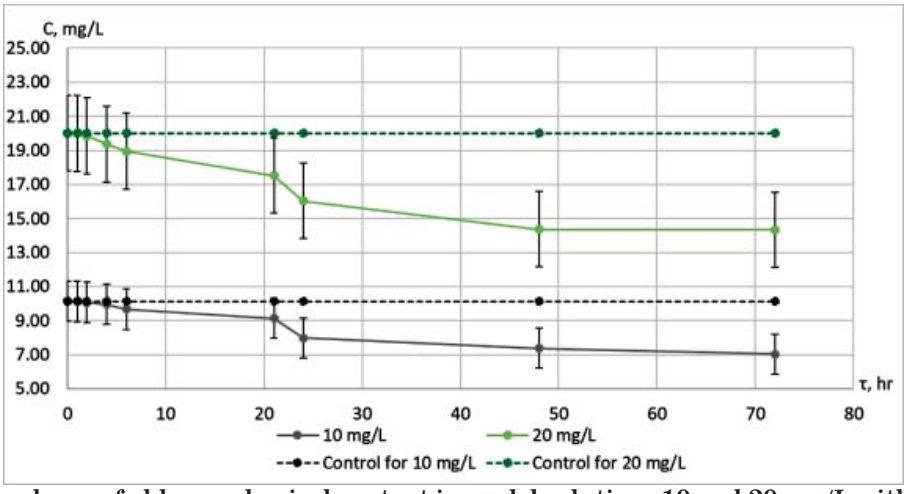

Fig. 3. **The dependence of chloramphenicol content in model solutions 10 and 20 mg/L with *Lemna minor* and in control solutions on the time of treatment**

The dependencies of chloramphenicol content over 72 hours for initial antibiotic concentrations in model solutions of 2 and 5 mg/L and 10 and 20 mg/L show almost no difference.

The efficiency of chloramphenicol removal from solutions using *L. minor* over time is shown in Table 3.

The results presented in Table 3 indicate that using *Lemna minor* effectively removes chloramphenicol from solutions. The efficiency depends on the treatment time of the model solutions and the antibiotic's initial concentration.

At lower concentrations (2 and 5 mg/L), the efficiency gradually increases during the first 24 hours and reaches a maximum of 23.2% and 26.8%, respectively, after 72 hours. At higher concentrations (10 and 20 mg/L), a gradual increase in efficiency was also observed, reaching a maximum of 33.0% at a chloramphenicol concentration of 10 mg/L and 29.5% at 20 mg/L after 72 hours.

This suggests that *Lemna minor* can effectively reduce antibiotic concentrations, but a long time of the biological treatment process is required to achieve maximum efficiency when using duckweed for solution purification.

Conclusions

It has been established that increasing the treatment time of model solutions using *Lemna minor* reduces chloramphenicol content in model solutions with initial concentrations of 2–20 mg/L. For example, the initial antibiotic concentration of 10 mg/L decreases

Table 3

The efficiency of chloramphenicol removal from solutions using *Lemna minor* depending on the time of treatment

τ, hr	Initial chloramphenicol content in model solutions C_0, mg/L			
	2	5	10	20
	E, %			
1	0.0	0.0	0.1	0.1
2	0.0	0.1	0.6	0.8
4	0.1	0.2	1.9	3.3
6	0.2	0.7	4.8	5.5
21	10.8	11.1	10.6	12.9
24	17.6	16.4	23.0	20.6
48	23.0	26.2	29.4	29.3
72	23.2	26.8	33.0	29.5

to 7.04 mg/L after 72 hours of treatment, corresponding to a removal efficiency of 33%.

The dependencies of chloramphenicol content on treatment time for initial antibiotic concentrations in model solutions of 2, 5, 10, and 20 mg/L show little difference. The reduction in antibiotic content in the first 21 hours is 11-13% compared to the initial chloramphenicol content. After 48 hours, the reduction reaches 23-29%, and the chloramphenicol content remains unchanged with further treatment. The chloramphenicol content in bioreactors without *Lemna minor* remained unchanged during the treatment of model solutions.

Based on these dependencies a rational treatment time of 48 hours was determined.

Thus, using *Lemna minor* for chloramphenicol removal is a practical and feasible method to achieve up to 33% antibiotic removal from pharmaceutical wastewater and can be recommended for implementation in pharmaceutical wastewater treatment systems.

Author Contributions

Larysa Sablii: definition of the research direction, setting the aim and tasks of scientific researches, discussion of the results and formulation of conclusions.

Liubov Kika: development of the research method, experimental work, data collection and processing, article writing.

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Conflict of Interest

There were no conflicts of interest.

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ЕФЕКТИВНІСТЬ ОЧИЩЕННЯ СТІЧНИХ ВОД ВІД ХЛОРАМФЕНІКОЛУ З ВИКОРИСТАННЯМ *Lemna minor*

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Статтю присвячено дослідженню ефективності очищення стічних вод від хлорамфеніколу, антибіотика широкого спектра дії, який часто присутній у стічних водах фармацевтичних підприємств і лікувальних закладів.

Метою роботи було визначити ефективність видалення хлорамфеніколу з модельних розчинів за допомогою біологічного агента *Lemna minor* залежно від початкової концентрації антибіотика та тривалості очищення.

Використовували модельні розчини із початковими концентраціями хлорамфеніколу 2, 5, 10 і 20 мг/дм^3. Тривалість очищення розчинів приймали 1–72 год.

Методи. Вміст хлорамфеніколу у модельних розчинах було визначено за допомогою високоефективної рідинної хроматографії.

Результати. Ряска ефективно знижувала концентрацію хлорамфеніколу, зокрема, досягнуто максимального зниження концентрації на 33,0% за початкової концентрації 10 мг/дм^3 та на 29,5% для 20 мг/дм^3 за тривалості очищення — 72 години. Біомаса ряски становила 0,04 г/см^3. За концентрацій 2 і 5 мг/дм^3 ефективність очищення поступово зростала протягом перших 24 годин і досягла максимуму 23,2% та 26,8%, відповідно, через 72 години. Це свідчить про те, що ряска може ефективно знижувати вміст у воді антибіотиків, однак для досягнення максимального ефекту потрібен тривалий час контакту.

У дослідах з контрольними зразками, де не використовували ряску, концентрація хлорамфеніколу залишалася незмінною уродовж 72 годин, що підтверджує відсутність природного розкладу або зміни вмісту антибіотика без біологічного агенту.

Висновки. Проведені дослідження підтверджують ефективність використання *Lemna minor* як біологічного агента для зниження концентрації хлорамфеніколу в стічних водах — до 33%. Використання ряскових дозволяє зменшити вплив антибіотика на навколишнє середовище і сприяє зменшенню ризику розвитку антибіотикорезистентності.

Ключові слова: стічні води, очистка, біологічний метод, ряска, антибіотики.