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# Management of Macrolide Antibiotics (Erythromycin, Clarithromycin and Azithromycin) in the Environment: A Case Study of Environmental Pollution in Lithuania

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**Abstract:** Of all the antibiotics used today for human treatment in the world, macrolide antibiotics—erythromycin, clarithromycin and azithromycin—stand out the most for misuse, and they were included as high-risk substances in the monitoring Watch List of Regulation 2018/840/EU. The aim of the present research was to investigate the level of target human pharmaceuticals' prevalence in the environment by the substance flow analysis (SFA) approach and to determine the potential risks of the antibiotics for the environment. The target for the environmental investigations was Lithuania. For SFA, 2021 consumption input data were used, and such key processes as the consumption rate, disposal and distribution of pharmaceuticals in the environment were analyzed. The analysis revealed that the largest part of pharmaceutical contaminants (80.1%) enters wastewater treatment plants. The risk quotient approach was based on the determination of predicted environmental concentrations (PECs), measured environmental concentrations (MECs) and their ratio to the predicted no-effect environmental concentrations (PNECs). The analysis revealed that clarithromycin causes a high potential risk for the aquatic environment in effluents from the wastewater treatment plants (WWTP); PEC/PNEC > 7. For azithromycin and erythromycin, the estimated PEC values were between 0.1 and 1. Clarithromycin concentration in the effluents of two target WWTPs showed a significant risk quotient (MEC/PNEC) of higher than 7. Recommendations on how to reduce the release of pharmaceutical residues into the environment have been proposed in the framework of the environmental management system.

**Keywords:** erythromycin; clarithromycin; azithromycin; substance flow analysis; environmental risk assessment; pharmaceutical substances management



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## 1. Introduction

Macrolides are ranked by the World Health Organization as some of the most important antibiotics in the world for human medicine. Antibiotics are most commonly known as medicines for the treatment of infectious diseases in humans and animals, or as livestock growth promoters used in agriculture and aquaculture [1]. Excessive and increasing use of antibiotics and abuse of these pharmaceuticals trigger public concern [2]. The fact that their occurrence may accelerate the spread of antibiotic resistance of the natural environment is of greatest concern [3,4] and is a pressing issue for humans. As much as 90% of pharmaceutical substances enter the environment in their original form [5]. Antibiotics, however, are excreted as metabolites or parent compounds, which, in case of incomplete metabolism, are not fully broken down in the intestine, and after passing through the sewage collection and treatment networks, enter the aquatic environment and ecosystems [6]. Their residues enter the aquatic and terrestrial environment in a variety of ways, but the most common path is their distribution via urban treatment plants [7]. It is crucial to mention that antibiotics residues are difficult to degrade, and even though it has already been known for more than a decade that conventional wastewater treatment methods are not designed for their proper treatment, it is still a major vexing issue [8]. As already mentioned, antibiotic residues in water bodies are of great interest due to their continuous occurrence, pseudo-persistence and ecotoxicological effects on human health and the natural environment. Therefore, it is particularly important to continuously identify, monitor and control the release of pharmaceutical substances into the environment.

There are reliable and publicly available databases that provide significant drug consumption data. After proper data processing, they could be used in different research approaches. In this research, input data for material flow analysis was used from the two main databases available online:

- The ESAC-Net, which provides European reference data on antimicrobial consumption both in the community and the hospital sector. For the expression of antibiotic consumption, the system uses the number of Defined Daily Doses (DDDs) per 1000 inhabitants per day. All antibiotics are grouped according to the Anatomical Therapeutic Chemical (ATC) classification system.
- The State Medicines Control Agency of Lithuania (VVKT), which provides all consumed drugs (classified in ATC system) registered in Lithuania. The medicine consumption is expressed as DDD per 1000 inhabitants per day [9].

Based on the latest data collected by ESAC-Net concerning the consumption of antibacterials for systematic use, Greece was the leading country of drugs consumed, with the average value of 29 DDD/1000 inhabitant/day in 2018–2020. The Netherlands came in last place with the average value of 9.2, while Lithuania averaged 15.5 (Figure 1).

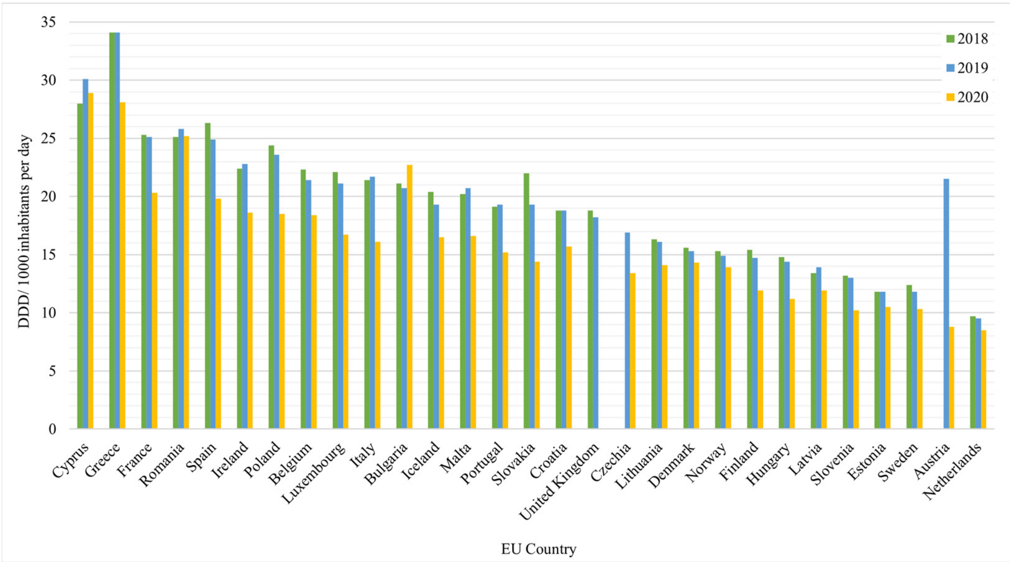


**Figure 1.** Consumption rate of antibacterials for systemic use (ATC group J01) in the community and hospital sector in Europe, reporting year 2018–2020.

Based on the data from VVKT for 2017–2021, a significant decrease in the consumption of antibiotics was observed in Lithuania (Figure 2). In 2017–2018, the consumption of antibiotics was stable, but it dropped by 19.3% in 2019 and continued to decrease in the following years—10.2% and 5.1%, respectively.

According to the data collected by VVKT, the total consumption of target antibiotics in Lithuania in 2017–2021 reveals a slightly different trend. Considering the consumption of only three target antibiotics, a drop is visible only in the year 2020 (20%), and the highest peak of consumption can be observed in 2019 (Figure 3). Based on statistics, the macrolides, as a group of test substances, take 13% in terms of the consumption of antibiotics in Lithuania [9]. Considering the test substances alone, consumption of clarithromycin stood out from the consumption of other antibiotics (Figure 3).

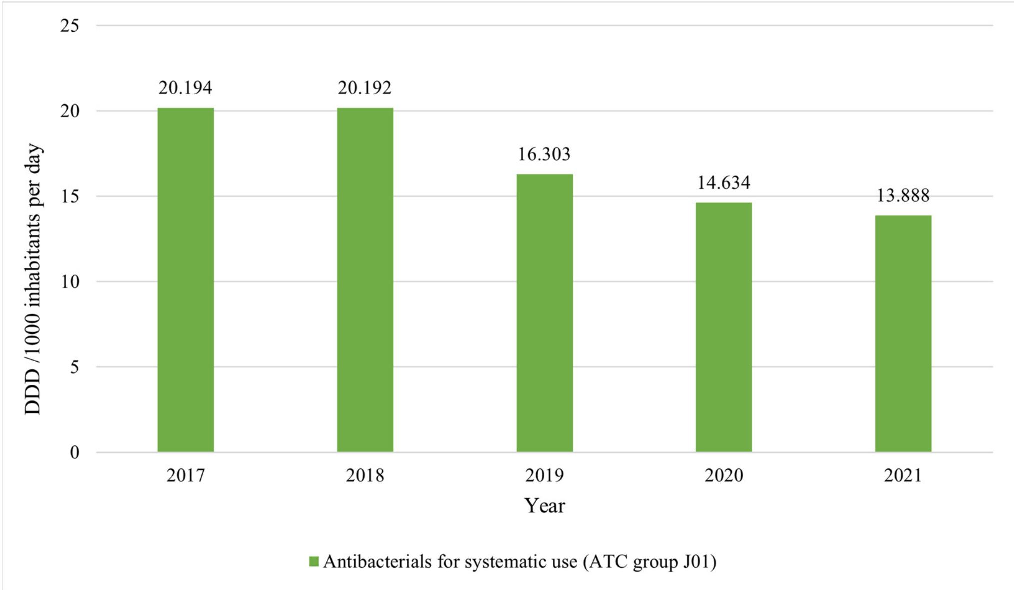


**Figure 2.** Consumption rate of antibacterials for systemic use (ATC group J01) in the community and hospital sector in Lithuania, reporting years 2017–2021.

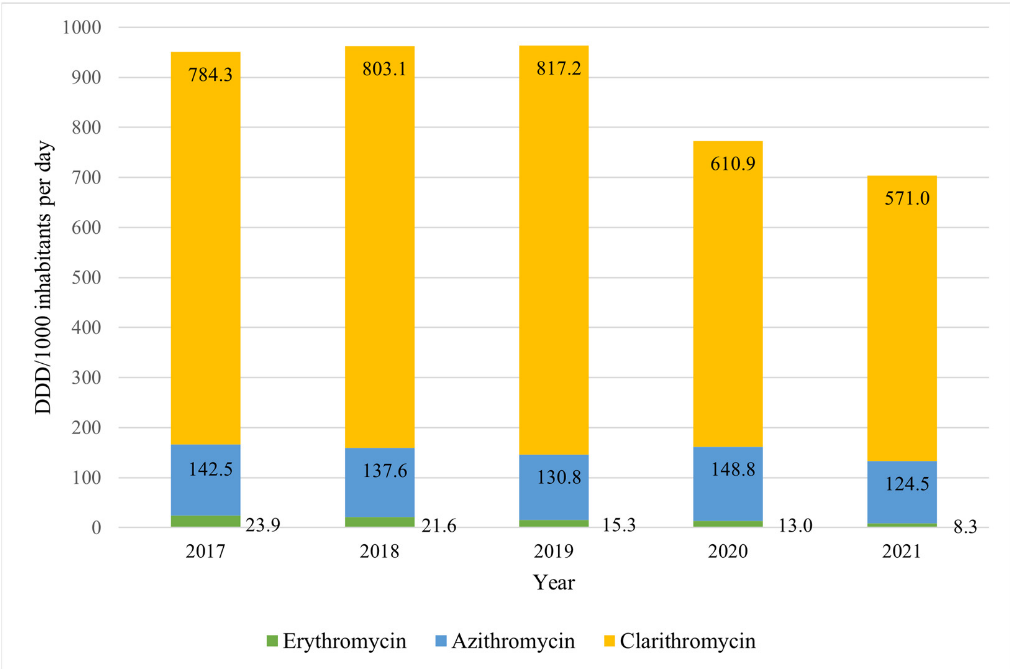


**Figure 3.** Consumption rate of target macrolide antibiotics in Lithuania, reporting years 2017–2021.

Figure 3 shows the antibiotics consumption recalculated from the number of drugs sold in packages to mass units (Section 2.1.).

Antibiotics consumption in Lithuania is very similar to the average of European countries. This allows us to assume that a particularly high consumption of antibiotics and their incorrect disposal are still observed in the country. Lithuania is one of the European countries that contributes to the pollution of the Baltic Sea. An average of 1800 tons of pharmaceutical products (e.g., macrolide antibiotics, clofibric acid and central nervous system agents, primidone and carbamazepine, etc.) per year enter the sea from the surrounding countries [10,11]. This fact strongly motivated an investigation into the sources, discharges and related risks for the environment of the pharmaceutical substances erythromycin, clarithromycin and azithromycin consumed in the Baltic Sea region of

Lithuania. The main objective of this research was to perform a substance flow analysis at the level of the whole country and evaluate the environmental impact risk assessment to show the impact on the Baltic Sea environment, and also to provide recommendations on how to accurately reduce pollution and leakage of these investigated substances.

## 2. Materials and Methods

### *2.1. Macrolide Antibiotics Substance Flow Analysis*

The Mathematical Material Flow Analysis (MMFA) method was used to perform material flow analysis. This method is designed to describe and simulate material flows in mathematical models in order to understand the system and investigate the possible causes of material flows, as well as to design sustainable, environmentally friendly measures for flow management [7,12].

The input data do not consider pharmaceutical production statistics, as target medicines are only imported in Lithuania. All input data variables are defined by mass units (kg) for the MMFA and were estimated from the data on the consumption of azithromycin, clarithromycin and erythromycin, publicly available on the VVKT (National Medicines Control Agency of Lithuania) database. VVKT provides data mainly in two different ways:

1. As data presented in the international anatomic therapeutic chemical classification (ATC) system and the daily defined dose (DDD) methodology [13];
2. As data in monthly reports on the quantity of drugs sold in packages.

The present research used 'b'-type data. Reports give comprehensive information about the sold drugs: generic and trade names of drugs, pharmaceutical form (solid, liquid, etc.), strength in mg, quantity of doses (in pieces) per package and number of packages sold per month.

Then, the amount of the drug sold/consumed (kg) was calculated by using three constituents only:

$$Q(drug\ sold, j) = \frac{S \times N \times M}{1000000} \tag{1}$$

$Q_{(drug\ sold,j)}$—amount of drug sold, kg;
$S$—drug strength, showing the quantity of substance in the pharmaceutical, mg;
$N$—quantity of doses in package, pieces;
$M$—number of packages sold, pieces.

The drug excretion rate was taken from the official and approved data of drugs provided by the electronic medicines compendium [14] available online (Table 1). It is crucial to mention that the rate, which can be found within the database, varies depending on the dose exposed by the drug, which differs depending on the available ones in the market. For the SFA evaluation, the highest rates from the database were used.

**Table 1.** Macrolide antibiotics excretion rate.

| Macrolide Antibiotic | Excreted in Active Form, % |
|---|---|
| Erythromycin | 15 |
| Azithromycin | 12 |
| Clarithromycin | 40 |

Note(s): MMFA results are defined in a Sankey diagram by the program e!Sankey 5. The loads of antibiotics given for 2021 are in kg/year.

The certain substance flow analysis of pharmaceuticals in the environment involves several different input–output data variables, e.g., those which were used in the evaluation of the household's block. This one was too complex to rephrase in text, so they were defined by the schema showed in Figure 5, e.g., share between medicines consumed by humans and expired medicines or unused medicines incineration at home, bringing them to the pharmacies, disposal as municipal waste, continuing using them or discharging into the sewer systems. These variables strongly depend on customer consciousness and environmental awareness, level of education of the consumer, doctors and pharmacists, wastewater treatment actions that are held and maintained regionally, etc. All these aspects must be revised and updated as frequently as possible so that input and output data can be evaluated properly. For example, it could be predicted that the share of expired medicines will decrease in the future due to improved communication between patient and doctors or the quantity of pharmaceuticals recommended to use for the treatment of illness.

Within every block mapped in SFA schema, the totality of the received pollution load (in kg) is shown, and at least one output is given a certain value, so the other ones could be easily calculated using a proportion.

### 2.2. Environmental Risk Assessment

The guidelines on the environmental risk assessment of medicinal products for human use, published by the European Medicines Agency [15], were used for this research.

This research is based on theoretical and real environmental risk assessment in Lithuania. Based on the methodology of C. Karlsson, the measured environmental concentration (MEC), predicted environmental concentration (PEC) and predicted no-effect concentration (PNEC) of the test substances in the environment are assessed in the research.

The $A_{(location,\,j)}$ value is directly proportional to $A_{(Lithuania,\,j)}$ value, which depends on $P_{(Lithuania)}$ [16]:

$$A(location, j) = \frac{A(Lithuania,\ \ j)}{P(Lithuania)} \times P(location) \quad (2)$$

where:

$A_{(location,\,j)}$—amount of substance sold ($j$ = 1,2,3,4 (Klaipeda, Palanga, Kretinga, Nida)), kg.
$A_{(Lithuania,\,j)}$—pharmaceutical substances sold in total, kg.
$P_{(Lithuania)}$—population.

For the calculations, it was assumed that the amount of medicine consumed depends only on the size of the population in the geographic area [16–19].

#### 2.2.1. Study Area

Klaipeda County is one of 10 counties in Lithuania. It is the only county that borders the coastline. The coordinates are as follows: 55°43 north latitude, 21°07 east longitude. The county covers the area of 5222 km$^2$ and is the home of 320,014 residents. There are seven municipalities in Klaipeda County: Klaipeda city, Kretinga, Neringa, Palanga, Silute and Skuodas.

Four municipalities were selected for the analysis of the test substances: Klaipeda, Palanga, Kretinga and Neringa (Nida). Under the Water Framework Directive, all of the four wastewater treatment plants are located in the Lithuanian coastal river basin district assigned to the Nemunas river basin district (RBD), covering an area of 1077 km$^2$. This makes up 2.3% of the total Nemunas RBD area [20]. The population of Lithuania amounts to approximately 2,808,900; 170,000 residents live in Klaipeda, 13,000 in Palanga, 18,127 in Kretinga and 1700 in Nida. The target cities' wastewater treatment plants (WWTP) influent/effluent concentrations were used according to MORPHEUS project output data [21]. See Figure 4 for the location of WWTPs in Lithuania.

In all of the four wastewater treatment plants, wastewater is treated mechanically and biologically; they meet the standards and have sufficient capacity for efficient wastewater treatment. Table 2 summarizes the cleaning processes used in the treatment plants.

Pursuant to the Wastewater Management Regulation prepared by the order of the Minister of Environment of the Republic of Lithuania, wastewater at these wastewater treatment plants is treated as required [22].

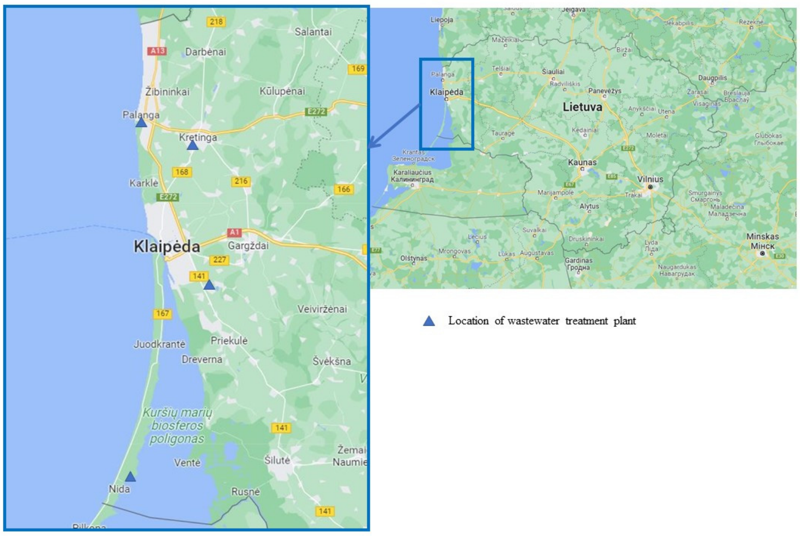


**Figure 4.** Locations of the target sampling points of Lithuanian wastewater treatment plants (WWTP).

**Table 2.** Technological treatment processes that are applied in the examined wastewater treatment plants (WWTP).

| Treatment Section in WWTP | Klaipeda | Palanga | Kretinga | Nida |
|---|---|---|---|---|
| **Mechanical processing (lattice, sand traps, settlers, etc.)** | applicable | | | |
| **Primary sedimentation** | applicable | | | |
| **Biological processing** | Four aerotanks with nitrogen and phosphorus removal. | Two aerotanks with nitrogen and phosphorus removal; denitrification basin; anaerobic, anoxic and oxy phases; the effluent stream entering the biological processing is divided into denitrification and dephosphation. | Two aeration tanks are used for nitrogen removal by activated sludge technology. | For the nitrogen removal, activated sludge technology is used. |
| **Sedimentation after biological treatment** | Part of the sludge is returned back to the biological step.Excess sludge removal. | | | applicable |
| **Chemical processing** | Organic carbon is sometimes used to support denitrification. | Chemical treatment is sometimes performedusing flocculantsAl2O3 and BrentapilusVP1. | NA | NA |
| **Sedimentation** | Sedimentation and sludge removal. | | | |

Note(s): Comments: NA—not applicable.

### 2.2.2. Predicted Environmental Concentration, Adapted to Local Conditions of Antibiotics in WWTP Effluent and Surface Waters

Risk quotients (RQs) were used to evaluate the environmental risk that the occurrence of antibiotics could produce in the organisms after a discharge into the environment.

The calculation of RQs was carried out by comparing the PEC and MEC of the target pharmaceutical with the PNEC.

The predicted environmental concentration ($PEC_{j,k}$, ng/L) from a wastewater treatment plant is calculated for each drug ($j$) using the formula specified below:

$$PEC_{j,k} = \frac{A_{location,\, j} \times 10^{12} \times E_j \times (1 - R_j)}{Q_{person} \times P_{location} \times 365 \times D} \quad (3)$$

where:

$E_j$—excretion coefficient, %.
$R_{,j}$—wastewater treatment coefficient, %.
$A_{location}$—a total sale data for the drug per year (kg).
$Q_{person}$—the value set by the European Medicines Agency is 200 l/day. This is the estimated human water consumption in the survey location [15,23].
$D$—factor of wastewater dilution by surface water flow. When calculating $PEC_{j,eff}$, it was assumed that $D$ = 0. In estimating $PEC_{j,sw}$, the dilution factor was 0 [15].

2.2.3. Environmental Management System

The environmental management system was based on the mathematical environmental system theory according to Staniškis et al., 2010 [24]. Theoretically, the main task of the management system is to find such a control effect ($U_{(t)}$) and factors appropriate for it that would allow the setting of objectives for the management system ($X_{in}(t)$) to be achieved. The main task of the management system is to reduce the negative impact on the environment by such pollution prevention measures that would be the most cost-effective.

The function presented in the Results section is defined by the main constituents below:

U(t)—management effect which compensates for the deviation of the outputs. This parameter helps to determine the change in the concentration of the test substances that occurs in wastewater;

D(t)—interference that affects the object;

$X_{iš}(t)$—regulated process outputs.

For the system to work effectively, and the conditions not to change, the deviation should be equal to 0 ($\Delta$ = 0).

## 3. Results and Discussion

### *3.1. Analysis of the Macrolide Antibiotics Substance Flows (ASF) in Lithuania*

#### 3.1.1. System Definition

Flows of the test substances—azithromycin (AZI), clarithromycin (CLA) and erythromycin (ERY)—are based on VVKT data of sold pharmaceuticals in 2021 in the territory of Lithuania. The analysis examines the processes, sources of generation of pharmaceutical substances by hospitals and households, and the reasons why pollutants enter the natural environment. The research area covered three main blocks: consumption of pharmaceutical substances, their disposal and their distribution in the environment. The total annual consumption of the three test substances is the functional unit examined. See Table 3 for the main input data for the SFA.

**Table 3.** Quantity of macrolide antibiotics sold in Lithuania in 2021.

| Target Substance | Year | Quantity of Drug Sold, kg/a | Share, % |
|---|---|---|---|
| Erythromycin | 2021 | 8.3 | 1.2% |
| Azithromycin | | 124.5 | 17.7% |
| Clarithromycin | | 571.0 | 81.1% |
| | **Total** | **703.8** | |

### 3.1.2. Consumption

- *Health institution sector and hospitals*. According to the VVKT data, 26.74 kg (i.e., 3.8% of totally consumed macrolides) of macrolide antibiotics was sold directly for the health institution sector. According to the latest available data within the country, only a small portion of medicines remain unused in hospitals [25]. It was assumed that only 5% of medicines were exposed to hazardous waste management equipment due to expired date or improper storage of medicines, while the remaining 95% were consumed by patients. A total of 33% of the total target macrolide antibiotics that entered the health institution sector were released in an active form by excretion in faeces and urine and ended up in sewage collection networks (see Section 2.1. for the excretion rates) [26].
- *Public institutions and households.* All the establishments that are not directly related to therapeutic activities can be attributed to the source of public institutions and households. The effluence of the pharmaceutical substances analyzed may occur in places such as retirement homes for the elderly, boarding houses or prisons. For this research, it was assumed that unused medicines makes up approximately 8% [27]. The remaining 92% was assumed to be consumed and excreted through urine and feaces. See Figure 5 for the matrix of the loads estimated in households. The matrix defines only the output data from public institutions and households. Based on the literature analysis [25,28], consumers dispose of expired medicines in a few typical ways: they incinerate them at home, bring them to the pharmacies, dispose of them as municipal waste, continue using them or discharge them into the sewer systems; 21.6% were unaware of how to treat them. All of the sections in Figure 5 make up 100% (defined by the orange square). The schema shows that in the group of the 21.6% who were unaware of how to dispose of the waste, it is assumed that 75% of the unused medicines were disposed of as municipal waste and 25% flushed away into the sewage system. Moreover, all discharges to the wastewaters based on most typical wastewater treatments in Lithuania [28] were separated into urban WWTPs, individual treatment in wastewater pits and septic tanks, or direct effluents to surface waters. These three different types of treatments were used as the main approach for the expired and consumed medicine load calculations.

| | | Type of pharmaceuticals waste treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Households & public institutions** | **Incineration** | **Landfill** | **Urban WWTPs** | **Individual treatment in wastewater pits & septic tanks** | **Direct effluent of untreated sewages to surface waters** | **Unware how to use** | **Comments** |
| Expired medicines | 8% - [1]Mehtonen et al., 2020 | 19.1% - incinerated at home | 26.1% - disposed of medicines in garbage containers | 15.4% - discharged unused drugs into the sewer | | | | According to ref. 6.2% of expired drugs were continued to use. This part was estimated using excretion rates. Rates' values discussed in section 2.1. |
| | | 11.6% - brought unused medicines to the pharmacy | | 6.2% - continued using | | | 21.6% | Reference to survey reasearch - [2]Latožienė R & Patašienė D, 2017. |
| | | *NA* | 75% - disposed unused medicines as municipal waste | 25% - flushed to the sewages | | | | It was assumed that pharmaceutical waste which humans declared as unknown how to treat has ended up in landfills or was flushed with the sewages. |
| Consumed by human (unexpired medicines) | 92% | *NA* | *NA* | 100.0% | | | | Excretion rate estimated separately. Rates' values discussed in section 2.1. |
| | | | | 76.5% | 21.0% | 2.5% | | Pharmaceutical loads share between effluent was estimated for the all 4 steps above. Ref. for the provided share between wastewater treatments in Lithuania - [3]State Audit report VAE-12, 2020. |

**Figure 5.** Medicine consumption matrix for output data estimation from the public institutions and household flows. References: [1] [27]; [2] [25]; [3] [28].

- *Production of pharmaceutical substances*. During the research, it was identified that target substances are not manufactured in Lithuania but are used in laboratories only for the scientific purposes or verification of complying with quality requirements. Even though the generated part of wastewater only from laboratories may be insignificant or irrelevant for the assessment of the balance in this specific case for Lithuania, it is still a source of pollution with pharmaceutical substances that might be crucial in a country having macrolide antibiotics-manufacturing facilities. In this case, it was assumed that only 0.01% of all consumed antibiotics were used in the SFA-block manufacture of pharmaceuticals. Such sources account for about 10% of hazardous wastewater that will be treated as hazardous, and the rest will be disposed of in a shared sewerage system [18].

#### 3.1.3. Discharge and Disposal

- *Wastewater treatment plants.* In assessing the effluents of pharmaceutical residues to surface waters via wastewater treatment plants (WWTPs), a wastewater treatment efficiency of 78.6% for AZI, 53.3% for ERY and 54% for CLA was accepted. The average rates were estimated on the scientific research base of target antibiotics already performed at WWTPs [22,29–32]. The target macrolide antibiotics part that was removed from the effluents is considered in sludge. In 2014–2019, the Department of Environmental Protection carried out 3028 inspections in household units handling municipal sewage centrally, and 8% of municipal wastewater treatment offenses were determined. A total of 70% of them were released into surface waters incompletely treated, 16% were untreated and 14% revealed other handling breaches, such as clogged sewage collection systems, improper equipment installation or exploitation and others. According to the produced report, this accounted for 1% of the total number of respondents [28]. Thus, it is assumed that due to wastewater collection systems, which are not fully repaired or exploitable, effluents from wastewater collection networks to surface waters will account for 1%. It defines only the effluent leakage into the soil.
- *Untreated wastewater.* According to the annual national reports of wastewater management and treatment, 24.2% of centrally untreated or insufficiently treated municipal wastewater is discharged into the environment every year. For SFA, it was assumed that 24.2% of water effluents from the WWTPs are not treated. This block involves only the input from WWTPs. It was assumed that improper exploitation of wastewater collection networks causes losses of 15% of discharge leakage into the soil, while the remaining 85% reach surface waters.
- *Individual wastewater treatment*. A total of 21% of the Lithuanian population individually treats wastewater generated by the residents themselves [28]. The residents treat wastewater in several ways: by draining wastewater into tanks and collecting it there, collecting wastewater in septic tanks or using individual treatment plants, which, in most cases, are low-capacity biological wastewater treatment plants [28]. The analysis of the water supply and wastewater treatment in Lithuania showed that, in most cases, tanks and septic tanks are unsuitable for use because they are old, leaking and improperly exploited. In most cases, constructions are not sealed, with an open bottom, which creates excellent conditions for pollutants to sink into soil and groundwater. It was assumed that discharge from individual wastewater treatment split into 60% of pollutants leakage into soil from septic tanks and wastewater pits, 30% to WWTPs from septic tanks and 10% to surface waters from small-capacity individual treatment plants.
- *Incineration.* All unused drugs collected from residents in pharmacies, as well as from health facilities or laboratories, are transferred to the hazardous waste managers and dispatched for combustion. The analyzed literature revealed that there is no evidence of concentrations of antibiotic residues in the air [33]; thus, it is assumed that active antibiotics fully decompose by incineration.
- *Landfills*. Landfills still pose a risk because leachates may pollute groundwater and surface water, even though they are commonly redirected to a municipal WWTP [33–35]. The Review of Regional Waste Management Centres (RWMC) provided information that revealed that 11 of the currently exploited landfills are fully equipped with

leachate collection systems. Nine of them collect wastewater in tanks and return them for treatment in urban wastewater treatment plants, two of them treat wastewater in biological wastewater treatment plant and by the reverse osmosis method on site, and one treats wastewater in a tank, partly using a reverse osmosis system, and partly returns the water to central wastewater treatment networks (approximately 60%). Based on the proportion of municipal waste generated regionally, the part that is discharged back to urban WWTPs comprises 77%, and 23% is treated on site to surface waters. The determined antibiotics removal rates in biological and reverse-osmosis wastewater treatments used on landfill sites were 37% and 79%, respectively [36]. Moreover, in these two flows, leakage to groundwaters must be considered. Scientists all around the world detect antibiotic residues in the soil and groundwaters of landfills [37–39]. Even though such research has not been carried out in Lithuania yet, according to the RWMC's annual reports about groundwater monitoring on landfill sites, it is systematically detected that chloride, ammonium and other controlled parameters (which differ between regions) exceed the limit values of specified concentrations according to the Surface Wastewater Management Regulation. Reports declare that higher values do not pose a potential risk to the environment and must be monitored; however, this shows a potential pollution pathway to the environment. It was cautiously assumed that 5% of antibiotic residues are discharged to soil.

- *Sludge.* Sewage sludge is a by-product of wastewater treatment. According to the Environmental Protection Agency of Lithuania's summary of treated waste quantities in 2020, 38% of urban sewage treatment sludge was recycled (14.6% was used for biogas extraction), 31.6% was incinerated, 26.8% was processed in land and 3.6% was disposed of in other ways. Overall, it is assumed that 68.4% of the target substances are discharged into soil, and the remaining 31.6% are disposed of by incineration.

#### 3.1.4. Distribution in Environment

- *Soil and surface waters.* Pharmaceutical residues that have penetrated into aquaculture with sediment will enter the soil by filtration, and due to water circulation, will eventually enter the food chain through drinking water [40–42]. However, pharmaceutical substances that flow between water bodies have not been analyzed in SFA. Discharges end up in soil and surface waters. It was assumed that pharmaceuticals migrate, as after being affected and absorbed by aquacultures, they precipitate and enter the groundwater.

#### 3.1.5. Results of ASF Analysis

A total of 39.6% of the total sold macrolide antibiotics are discharged to individual WWTs, municipal WWTPs, incineration and landfills; the rest is disintegrated by digestion. The highest amount of pharmaceutical substances' discharges is from households (96.3%). The share of macrolide antibiotic flows after the consumption stage are distributed accordingly:

- Treated in individual wastewater treatment—17.3%.
- Collected via central sewage networks and treated in WWTPs—66.0%.
- Treated by incineration—6.4%.
- Disposed of in landfills—8.2%.
- Directly entered surface waters from households—2.0%.
- The main macrolide antibiotic streams to the environment are via water bodies. Discharges that affect the natural environment are distributed as presented below:
- Soil—40.8%.
- Surface waters—41.4%.
- Totally incinerated and assumed that no active compound will leave after this combustion—16.7%.
- Accumulate in sludge of leachate treated in landfills—1.1%, and it might be assumed that it will end up in soil.

ASF analysis results are specifically defined in Figure 6. Material flow analysis does not emphasize groundwater and air contamination, and the fate of target substances in the natural environment is not within the scope of this research; therefore, the flow of pollutants ends at the soil and surface waters systems.

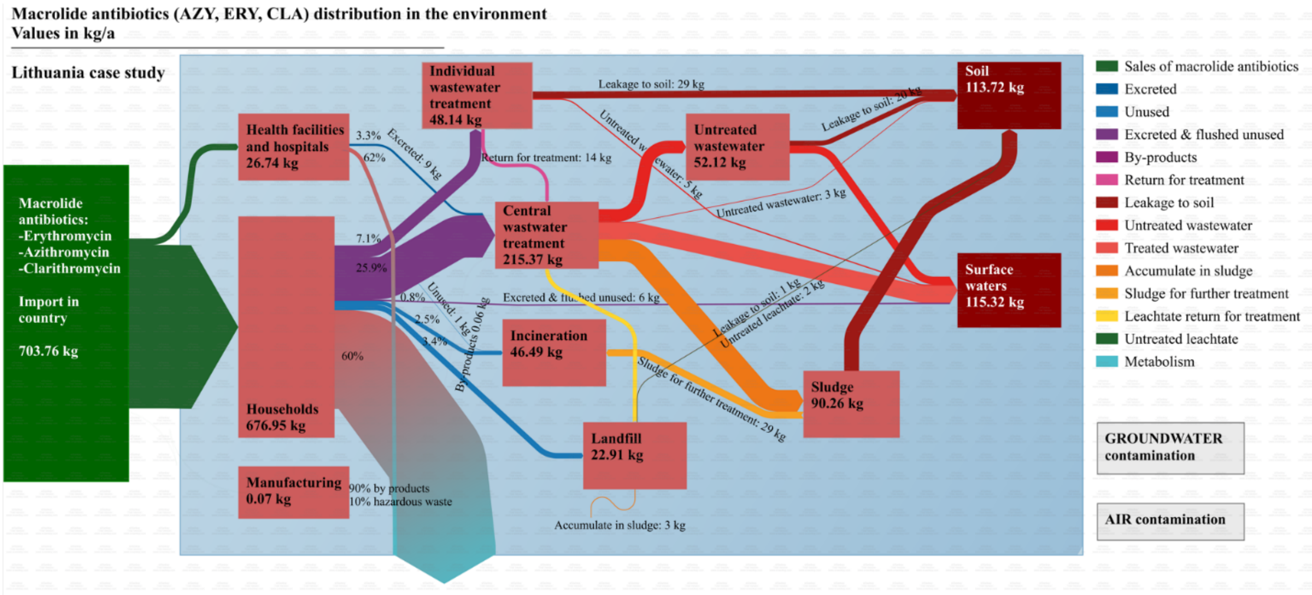


**Figure 6.** Macrolide antibiotics (erythromycin, clarithromycin and azithromycin) flow in Lithuania in 2021.

## *3.2. Environmental Risk Assessment*

### 3.2.1. Predicted Concentrations of Selected Antibiotics in Surface Waters

The removal ($R_j$), excretion ($E_j$) factors and predicted no-effect concentration (PNEC) values were obtained from the literature and reported in Table 4.

**Table 4.** Consumption of the test substances (kg/a) in the study area, estimated predicted environmental concentration ($PEC_{(j,eff)}$) in effluents, $PEC_{(j,sw)}$ in surface waters, $E_j$ and $R_j$ fractions, PNEC.

| Target Pharmaceutical Substance | Pharmaceutical Sales in Klaipeda, Palanga, Kretinga and Nida, kg/a | $PEC_{j,eff}$, ng/L | $PEC_{j,sw}$, ng/L | [1] $E_j$, % | [2] $R_j$, % | [3] PNEC, ng/L |
|---|---|---|---|---|---|---|
| Azithromycin | 11.92 | 15.58 | 1.56 | 0.12 | 0.786 | 20 |
| Clarithromycin | 54.67 | 512.04 | 51.20 | 0.4 | 0.54 | 70 |
| Erythromycin | 0.79 | 2.83 | 0.28 | 0.15 | 0.533 | 20 |

Note(s): References: [1] [14]; [2] [22,29–32]; [3] [19,43,44].

PNEC values were selected as 1000-times lower than the most sensitive species assayed in acute toxicity tests, following [19,43]. RQs ($PEC_{eff}$/PNEC) for clarithromycin had the highest value in surface waters but were still lower than 1, and in the effluents, they were higher than 7, which shows that they pose a threat to the natural environment. $PEC_{eff}$/PNEC and $PEC_{sw}$/PNEC results are presented in Figure 7.

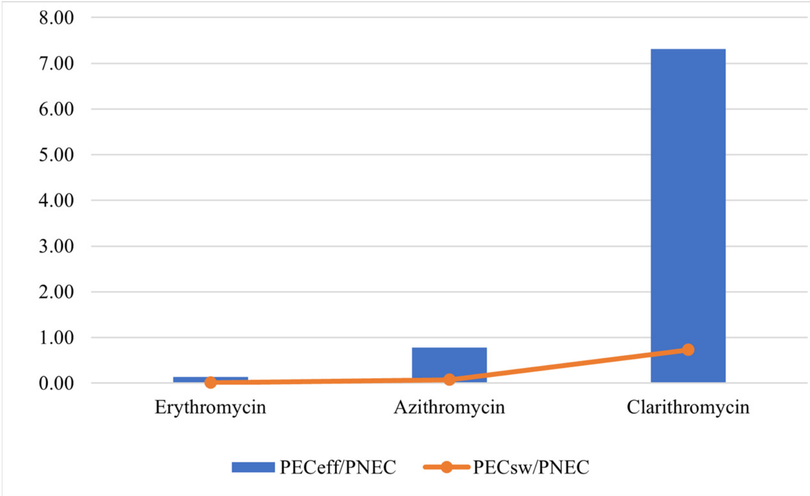


**Figure 7.** Ratios of macrolide antibiotic predicted environmental concentrations (PECs) in wastewater treatment plant (WWTP) effluents and surface waters in Klaipeda district and predicted no-effect concentrations (PNECs).

### 3.2.2. Discussion of Selected Measured Environmental Concentrations Values in Target Area

The test substances azithromycin (AZI), clarithromycin (CLA) and erythromycin (ERY) were detected in the influent of Klaipeda, Kretinga, Palanga and Nida wastewater treatment plants. The highest concentrations of the test substances before treatment were detected in Kretinga in winter of 2018: AZI—593.8 ng/L, CLA—4113.9 ng/L, ERY—147.5 ng/L. The lowest concentrations of the test substances before treatment were detected in Nida in summer of 2017: AZI—11.9 ng/L, ERY—2.5 ng/L and CLA—46.7 ng/L (Table 5, [22]).

**Table 5.** Concentration of pharmaceutical substances [22] and removal efficiency in the selected wastewater treatment plants. Meaning of abbreviations used in table: azithromycin (AZI); clarithromycin (CLA); erythromycin (ERY); influent (In); effluent (Eff); removal efficiency (RE); not detected (n.d.); not applicable (NA).

| | | ***2017 Summer Season*** | | | | ***2018 Winter Season*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Klaipeda City** | **Palanga City** | **Kretinga City** | **Nida City** | **Klaipėda City** | **Palanga City** | **Kretinga City** | **Nida City** |
| **AZI** | **In (ng/L)** | 37 | 76.4 | 182.4 | 11.9 | 582.6 | 205.4 | 593.8 | 14.3 |
| | **Eff (ng/L)** | 13.4 | 19.9 | 12.1 | 11 | 127.6 | 52.5 | 36.3 | 12.7 |
| | ***RE (%)*** | *64* | *74* | *93* | *8* | *78* | *74* | *94* | *11* |
| **CLA** | **In (ng/L)** | 126.5 | 474.8 | 1326.7 | 243.6 | 2871.2 | 662.3 | 4113.9 | 46.7 |
| | **Eff (ng/L)** | 229.3 | 150.2 | 73.7 | 62 | 1297.7 | 532.8 | 507.8 | 197.4 |
| | ***RE (%)*** | *−81* | *68* | *94* | *75* | *55* | *20* | *88* | *−323* |
| **ERY** | **In (ng/L)** | 95.5 | 49.9 | 359.2 | 2.5 | 76.1 | 10.1 | 147.5 | n.d. |
| | **Eff (ng/L)** | 75.2 | 29.3 | 33.0 | 3.9 | 85.2 | 20.2 | 57.4 | 0.6 |
| | ***RE (%)*** | *21* | *41* | *91* | *−56* | *−12* | *−100* | *61* | *NA* |

The highest concentrations of the test substances after treatment were detected in Palanga in winter of 2018: AZI—127.6 ng/L, CLA—1297.7 ng/L and ERY—147.5 ng/L. The lowest concentrations of the test substances after treatment were detected in Nida, at 0.6 ng/L (Table 5, [22]). In all four cities, the highest concentrations of the test substances after treatment were those of clarithromycin. Detection of pharmaceutical substances in surface water bodies in both summer and winter periods was more than 50%. The total average chemical load of the test pharmaceutical substances found and tested in 2017 and

2018, which enter the four coastal wastewater treatment plants, was 47.35 kg/a. The total annual load of the three pharmaceutical substances in the effluents (after treatment) was 14.49 kg/a. The highest load was in the Klaipeda city WWTP, and the lowest was in the Nida WWTP.

The study performed by Luczkiewicz et al. (2018) shows a strong influence of seasonality on the removal of AZI and CLA antibiotic residues from wastewater. An efficiency of only about 22% was obtained in evaluating the overall efficiency of the removal of the test antibiotics during the analyzed season. However, after the elimination of minus values, the average efficiency of 62% can be seen. In summer, the overall efficiency of removal of antibiotics ranges from 51 to 79%.

3.2.3. Results of Risk Quotients (RQs)

For risk quotation (MEC/PNEC), measured target pharmaceutical concentrations in influents and effluents detected in winter season were used. MECs were significantly higher, probably due to higher antibiotics consumption.

Figure 8 presents the ratio of MECs in influents and effluents in the target WWTPs and the predicted noneffect concentrations (PNECs) of the test substances. PNECs were used following [19,43–45]. In calculating $PEC_j$ in the influents of WWTPs, it was assumed that Rj = 0 for each substance since no treatment occurred yet.

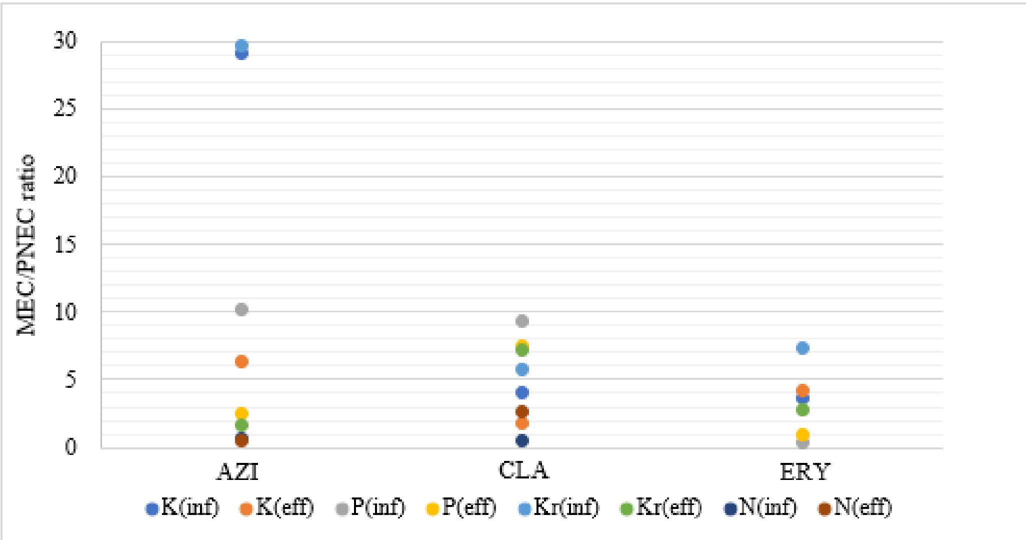


**Figure 8.** Ratios of macrolide antibiotic measured environmental concentrations (MECs) in WWTP effluents and surface waters in Klaipeda district and PNECs. Meanings of abbreviations used in the figure: K—Klaipeda; P—Palanga; Kr—Kretinga; N—Nida; inf—influent; eff—effluent.

Overall, all three target macrolide antibiotics show potential risk to the aquatic environment, as ratios in almost all cases exceed 1. The highest ratios were detected in the WWTPs of Klaipėda, Kretinga, with values close to 30 in influents, and, in effluents, values were higher than 1 in Kretinga and 6 in Klaipėda.

Reliability of the risk assessment of the measured and predicted concentrations of the test substances is assessed according to the Coetsier set of criteria. The calculated PECs may be acceptable ($0.1 < PEC/MEC < 1$), acceptable with minor overestimation ($1 < PEC/MEC < 4$), significantly overestimated ($4 < PEC/MEC < 8$) or severely overestimated ($PEC/MEC > 8$) [46]. Table 6 presents the results of the ratio of $PEC_{j,k}$ to MEC. The reliability of the PEC of AZI, CLA and ERY are acceptable or acceptable with minor overestimation in three of the four wastewater treatment plants, whereas PECs for the Nida WWTP are acceptable with minor overestimation in effluents for CLA and AZI, with a significantly overestimated ERY result. See Table 6.

**Table 6.** PEC (acceptable, overestimated and underestimated) reliability values based on the ratio of PEC to MEC (Coetsier set of criteria). Meaning of abbreviations used in table: azithromycin (AZI), clarithromycin (CLA) and erythromycin (ERY).

| | | Klaipeda | | Palanga | | Kretinga | | Nida | |
|---|---|---|---|---|---|---|---|---|---|
| | | K(inf) | K(eff) | P(inf) | P(eff) | Kr(inf) | Kr(eff) | N(inf) | N(eff) |
| PECj,k/MEC | AZI | 0.12 | 0.12 | 0.36 | 0.29 | 0.12 | 0.43 | 5.20 | 1.20 |
| | CLA | 3.88 | 3.94 | 1.68 | 0.96 | 2.71 | 1.01 | 23.68 | 2.60 |
| | ERY | 0.08 | 0.03 | 0.61 | 0.14 | 0.04 | 0.05 | - | 4.72 |

After proper evaluation of the deviations in the results and PEC remodeling, this methodology could be used when it is not possible to measure concentrations of pharmaceutical substances or other pollutants.

### *3.3. Application of the Environmental Management System Theory to Manage the Concentrations of Pharmaceutical Substances and Antibiotics in Wastewater*

The environmental management system is aimed at reducing the pollution of the pharmaceutical substances azithromycin, clarithromycin and erythromycin in the aquatic environment.

Wastewater containing azithromycin, clarithromycin and erythromycin constitutes the object of the research. The concentration of the test substances (ng/L) and the quantity of the substances consumed in Lithuania per year (kg per year) are the variables of the state of the system. The concentration of wastewater containing antibiotics (ng/L) is the object managed. The objective of the system: $X_{in}(t) = 0$. The strategy on how to gradually reduce pharmaceutical contamination up to complete control and leak termination is developed. To achieve results, gradual reduction of the system objective $X_{out}$ (t) is proposed:

up to 2025 $X_{in} \rightarrow 1/4X_{out}$
up to 2030 $X_{in} \rightarrow 1/2X_{out}$
up to 2035 $X_{in} \rightarrow 0X_{out}$

The management system is presented in Figure 9.

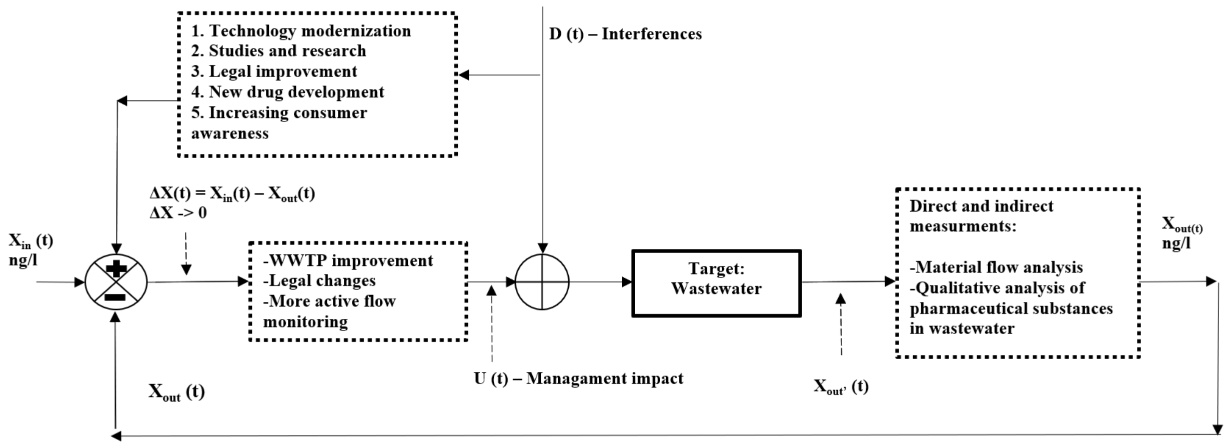


**Figure 9.** Environmental management system for wastewater containing pharmaceutical substances azithromycin, clarithromycin and erythromycin.

In order to achieve the objective, it is recommended to take into account economic and environmental aspects. The following basic system controls are proposed:

(1) Integration of efficient, more-modern management processes in the water and wastewater treatment plants of Lithuanian cities.

(2) Legal changes in connection with the removal of pharmaceutical substances from wastewater.
(3) More active monitoring of the flows of pharmaceutical substances.

Mechanical and biological treatment processes, and mainly the activated sludge technology, are used in Lithuanian wastewater treatment plants for wastewater treatment. In Lithuania, wastewater is treated quite well and meets regulatory requirements. However, there is very little information and very little existing research on how to remove pharmaceutical substances from wastewater and achieve a reduction in their pollution. The fourth-stage treatment technology is suggested to be implemented to supplement the existing system with ozonation or activated carbon technologies [22,47]. After modernization of wastewater treatment plants, theoretically, the direct load of pollutants should decrease by 60–70% [47]. In addition, new advanced wastewater treatment technologies, such as electrochemical treatment and the use of enzymes, fenton, fungi, coagulation or flocculation, are being developed. Other advanced oxidation technologies can also be applied. For better cleaning efficiency, connecting ozone and filter systems has been suggested as well.

Following the ASF analysis, it has been observed that the highest consumption of pharmaceutical substances is in households, where it is quite difficult to control wastewater. Separation of domestic wastewater flows from other generated flows is a possible solution for broader analysis and monitoring in the environment and for the assessment of which flows contain the highest pollutant loads after domestic wastewater. After separating the flows, it would be possible to monitor other flows more actively and find new solutions to control them. For example, after separating flows of hospitals and the outpatient sector, it would be possible to treat wastewater before handing it over to urban wastewater treatment plants (WWTP) [48]. Moreover, after assessing the efficiency of wastewater facilities, it would be possible to make more efficient and easy-to-implement solutions for further wastewater treatment.

There is still no legal basis in Lithuania for the removal of pharmaceutical substances from wastewater. More detailed data on the concentrations of pharmaceutical substances in surface water in Lithuania were obtained from the implemented MORPHEUS project [49].

## 4. Conclusions

Material flow analysis based on the 2017–2021 data revealed the two most significant factors which should be controlled in order to prevent surface waters from pollution by emerging substances or reduce the contamination as much as possible:

- Households as the main source of pollution by the pharmaceutical residues (96.3% of total discharge);
- Treatment plants as the main source of pollution release into the environment (83.3% of total discharge).

Both worldwide scientific literature and research conducted in Lithuania suggest that that sewage treatment plants are not able to efficiently abate pharmaceutical residues. Therefore, attention should be paid to the current status of WWT facilities and capability of wastewater treatment.

A significant proportion of the active pharmaceuticals enter the environment together with untreated wastewater from the urban wastewater treatment plants. In Lithuania, it took 24% of all municipal wastewater amounts collected by the central sewage systems and by the terms of material flow analysis, which is 18.7% of total target macrolide antibiotics discharge.

Risk assessment for the macrolide antibiotics azithromycin, clarithromycin and erythromycin revealed that the predicted concentrations in effluents might pose a risk for the aquatic environment. For the clarithromycin (CLA), a high level of risk was revealed, with a result higher than 7. The same risk quotient result (RQ > 7) for CLA was obtained in two out of four analyzed target wastewater treatment plants (WWTP).

Based on the research carried out, the environmental management system of the test pharmaceutical substances was proposed for the mitigation of environmental consequences and to improve the abatement strategies. A management system was set up providing recommendations related to the modernization of technologies, updating analyses and research, improving legal acts and financing of projects, developing new medicines and raising consumer awareness. Upon the implementation of preventive and technological management measures, the expected efficiency should be up to 80%.

There are many signs that new legal requirements for the management of wastewater contaminated with pharmaceutical substances will soon be developed in Lithuania, as there are numerous initiatives regarding micropollutants and pharmaceuticals in water cycles and wastes containing medicines. Particular attention is expected be paid to the qualitative parameters of:

- Pharmaceutical usage and consumption in general;
- Prescribing minimized doses of medications to patients only when necessary;
- Advanced and high-quality pharmaceutical residues treatment from healthcare facilities and WWTPs.

Consumers should be better informed not only about the benefits of antibiotics but also about the harm they cause to the environment and to humans.

As it is crucial to continuously improve abatement strategies, this type of research is particularly important in the development of river basin management plans and might contribute to the development of water management policies at local, regional and national levels.

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