

RESEARCH ARTICLE

Migration of antibiotics residual quantities in aquatic ecosystems

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The uncontrolled antimicrobial agents use leads to the spread of antibiotic-resistant pathogenic strains. The ingress of antibacterial drugs into surface water through sewage from livestock premises, people's houses, hospitals and during medical and agronomic activities exacerbates this problem. Antimicrobials in surface waters have several ways of developing the process: lethal interaction of antibiotics with hydrobionts, elimination of sensitive taxa and alteration of trophic chains; neutralization of antimicrobials (self-destruction of the molecule, hydrolysis, sorption, abiotic and biotic transformation); non-lethal interaction of antibiotics and hydrobionts with the acquisition of antibiotic resistance signs). There are following indicators proposed to predict the antimicrobial agents impact on the aquatic ecosystem: accumulation factor; the amount of substance per unit mass of plant or animal in one cubic meter of water (mg or mcg per 1m³); the maximum amount of a substance that can be contained in an ecosystem without disturbing its basic trophic properties. The basic principles of a dynamic chamber model construction for studying the effect of antibiotics on the ecosystem are based on the following basic statements: 1) the trophic chain is divided into the chambers in which substance is instantaneously mixed in all parts of the chamber in the same way in any direction; 2) the transfer of the substance from one chamber to another occurs according to the laws of first order kinetics, which is described by the system of differential equations. In this case, transition coefficients of a substance between the chambers are constant. It is advisable to use a dynamic chamber model to analyze the migration pathways of substances in a freshwater non-flowing reservoir. For instance, its simplified version consists of the 3 chambers, namely: water – sediments (silt) – biota. It is advisable to use a stationary chamber model for analysing the pathways of antibiotics which enter into the sea with the river flows.

Key words: Antibiotics; Migration; Surface water; Chamber model.

Introduction

Surface water biodiversity is one of the most important environmental indicators. It subjects to some seasonal changes throughout the year, but an increasing anthropogenic burden on the environment and climate change brings its own significant adjustments. This is primarily due to the fish, birds and micro-organisms migration and the entry of field washes on the reservoirs surface, production wastes, raw sewage, human and animal life, etc. (Hobson et al., 2019; Pruden et al., 2013; Nellums et al., 2018; Sun et al., 2019; Fang et al., 2018).

Pesticides, including those with antimicrobial properties, should only be transported by specialized land and water vehicles. Although, the threat of their entry into the aquatic environment remains high. Moreover, it always exists when pesticides are used. Thus, the sanitary rupture zone from surface water objects must be not less than 500 m, with barbell and rainfall – 300 m during fan spraying with pesticides with antimicrobial action. Pesticides are also widely used for dressing seed and planting material, coating, water repellency, encapsulation, toxication, etc. (Xu et al., 2019; Alonso-Díaz et al., 2019; Patyka et al., 2016).

The classification of antibiotics and antimicrobial substances of different origin (natural and artificial) is the result of human thought for a better understanding of the process and its systematization. The active substances or their decay products manifest their complex effects on aquatic biota in surface waters, regardless of whether it is the latest generation antibiotic from wastewater of a medical institution or a previously created antimicrobial effect pesticide or an antibacterial substance of natural origin. (Grenni, et al., 2018; Feng et al., 2018).

The main objective of this work is to analyze the main routes of entry of compounds with antimicrobial activity into surface waters and offer a convenient model for assessing the migration of aquatic antibiotics in an ecosystem.

Materials and Methods

We used the methods of system analysis and mathematical modeling in this work.

Surface water is the main route of antimicrobial agents migration in the environment. Aqueous trophic chains undergo significant changes under the load of antibiotics, which manifested in the pathogenic microorganisms's antibiotic resistance development, diseases of aquatic biota and a decrease in its productivity. This problem is inherent in all countries, therefore the World Health Organization (WHO) is coordinating the states efforts to combat the antibiotics spreading in ecosystems and the acquisition of antibiotic resistance by pathogenic microorganisms (Sharma et al., 2018; Ushkalov & Danchuk, 2017).

Antibiotics (Greek. Anti – against and bios – life) are organic compounds of microbial, plant or animal origin and products of chemical modification of these substances. As well as they can be synthetic products, which can selectively inhibit the growth and development of bacteria and other microorganisms. We can classify and distinguish the antibiotics according to chemical structure, polypeptide, polyenoic, macrolide and other (Isaenko et al., 2005).

A number of countries, in particular EU, USA, and China in varying degrees, show antimicrobials residues in their surface waters. As is expected, antimicrobials residues concentration is much higher near the pollution sources especially. (Schafhauser et al., 2018; Gelband et al., 2015). The vast majority of synthesized antimicrobials are not biocumulative. However, their constant entry into the aquatic environment causes an effect similar to persistent pollutants. The ways of entries of antimicrobial agents in surface water are quite diverse, as shown in Figure 1.

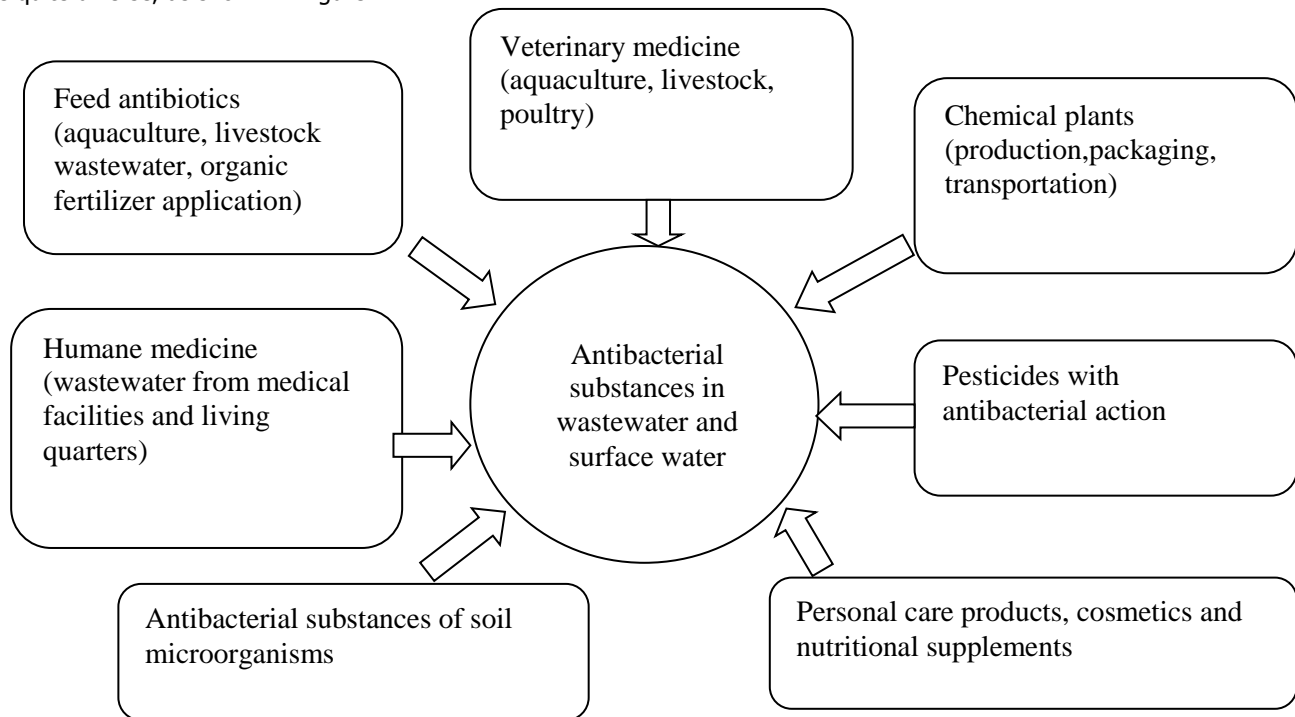


Figure 1. The main ways of the antimicrobial agents entries in surface water.

The ingress of antibacterial substances synthesized by terrestrial soil biota into the aquatic environment is an absolutely normal phenomenon, to which surface water microorganisms are able to adapt. Although, the issue of combined action with synthetic antibiotics is completely unclear.

Results and Discussion

Most of the threat to aquatic ecosystems are drains from hospitals and various veterinary facilities, almost evenly distributed throughout the state. Waste of Chemical Pharm Plant and packing plants exhibit a predominantly local impact on the ecosystem at their location. There is also the possibility of the antimicrobial agents ingestion into surface waters upon the application of organic fertilizers and the treatment of antimicrobial pesticidal preparations of agricultural crops. The use of feed antibiotics in aquaculture to stimulate the productivity of fish, crustaceans and mollusks, or for therapeutic and preventive purposes also contributes to the spread of antibiotics in the aquatic environment (Schafhauser et al., 2018).

Accordingly to cosmetics and personal care products (creams, lotions, lipsticks, shadows, toothpastes, soaps, shampoos, etc.), it is commonly believed that they do not significantly affect the antibiotic-resistant pathogenic strains spreading in the aquatic environment, but this is not always the case. For example, triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) was previously used as a pesticide. It is currently used as a supplement for personal care products (toothpaste, soap, deodorant, etc.), with the bacteria already becoming resistant to this compound (Dhillon et al., 2015; Li et al., 2018; Lee et al., 2018).

Regarding the antibiotics and food additives should be noted that they are limited to a subtherapeutic dose to prevent food spoilage (meat, fish, vegetables, etc.), ie as preservatives. The main ways to use them is to store food on the ice that contains antibiotics; immersion of the food product into the antibiotic solution for a certain time; treatment of the surface of the food product with antibiotic solution by irrigation. In most countries of the world, only two antibiotics-nutritional supplements are allowed – nisin (food supplement E234) and pimaricin (E235), and in some countries it is still restricted to chlortetracycline (another name bioomycin) in ice for catching freshwater fish and nystatin usually for irrigation of meat (carcasses) together with chlortetracycline.

The latest technologies in the production of agricultural products aim to remove antibiotics and introduce the use of disinfectants in the manufacturing processes (Danchuk et al., 2019).

The presence of an active antibiotic is prohibited in finished foods and goods, but there is waste and wastewater from long-term storage of raw materials for foodstuffs where antibiotics and food additives have been used (Vlasenko & Vlasenko, 2016; Bogdanova & Frolov, 2016; Voitsitskiy et al., 2018).

The main ways of antibiotics interaction in the surface waters of ecosystems are shown in Figure 2.

It should be noted that each group of antimicrobials has a biological effect: some disrupt the nucleic acids synthesis, protein and microbial cell envelope, enzyme activity and peptidoglycan polymerization; others – the functioning of cell membranes and the course of redox reactions (breathing, etc.). In addition, each chemical compound has its own chemical activity, and it is impossible to reject the effect of both antimicrobial agents and their degradation products on the aquatic biota.

The microbial contamination of reservoirs is the main source of contamination of the aquatic biota by pathogenic antibiotic-resistant microorganisms. Therefore, the discharge into the surface water of industrial runoff and especially household wastewater from medical institutions causes contamination of the surface of the body of fish, crustaceans and molluscs, which are always inhabited by microorganisms released from water and sediments (Gelband et al., 2015).

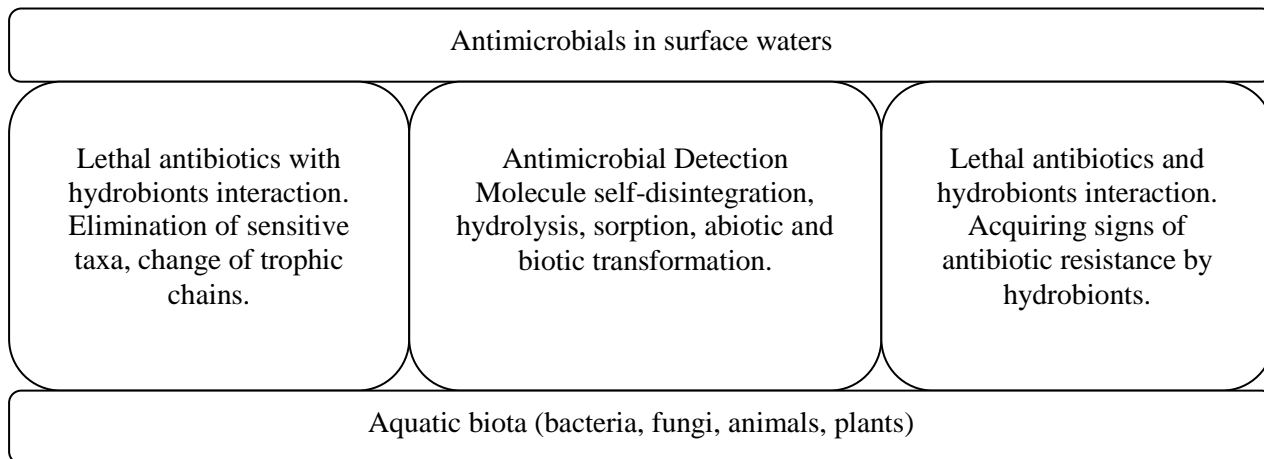


Figure 2. The antimicrobials and water biota interaction.

Considering the above, water contaminated by faeces, animal carcasses and plant debris is especially dangerous. On the other hand, the presence in the aquatic environment of minor amounts of antibiotics contributes to the development of antibiotic resistance in pathogenic and conditionally pathogenic microorganisms (Nulty et al., 2016).

It is advisable to evaluate the substances migration capacity by trophic chains of both aquatic and terrestrial ecosystems. They are very persistent in classification (decay time is more than 2 years), stable (within 0.5–2 years), moderately persistent (1–6 months). Regarding the unstable substances (time of disintegration within 1 month or even several weeks or days) it does not need to evaluate their migration ability. This fully applies to antibiotics.

The migration routes study of the antibiotics effects on organisms of biocenoses, in particularly aquatic ones, is important for organizing the necessary measures to protect the environment and to evaluate the antibiotic resistance in pathogenic and conditionally pathogenic microorganisms.

The accumulation coefficient (K_n) it is an important complex indicator that can be used to characterize the effect of an antibiotic / pesticide with an antimicrobial effect on an object. It represents the ratio between the content of a substance in the body (as a rule, mg μ g per 1 kg of mass) to its concentration in the medium (mg μ g per 1 kg of mass):

$$n = \frac{C_1}{C_2},$$

where K_n – the coefficient of accumulation of the substance; C_1 – for algae is the amount of a substance per unit mass of a plant, and for the animal world – per unit mass of tissues or organs (for example, muscle tissue in fish) or aquaculture production (for example, caviar) C_2 – the content of this substance for algae in the growth medium, and for fish and other species of aquatic wildlife – in their habitat and migration, taking into account trophic chains.

Another indicator that determines the accumulation of a substance from a reservoir to plants and animals is the indicator calculated as the ratio of the amount of a substance per unit mass of a plant or animal (usually mg μ g per 1 kg of mass) in one cubic meter (m^3) of water (mg or μ g on 1 m^3):

$$n = \frac{C_1}{C_2},$$

where K_n – the transition coefficient of the substance; C_1 – is the concentration of a substance in 1 kg of a plant (animals); C_2 – is the concentration of a substance in 1 m^3 of water

The capacity of ecosystems is also an important indicator for a particular antibiotic. The maximum amount of this substance that can be contained in an ecosystem without disturbing its basic trophic properties, etc. (Kutlakhmedov et al., 2018).

When antibacterial substances enter in freshwater bodies of water, they are distributed between water, bottom sediments (silt) and biota with the possibility of mutual transition. Water in reservoirs plays the role of a link in the chain of migration of antibiotic residues in bottom sediments and organisms living in it. Antibacterial substances that have fallen into water bodies can be in water soluble form or in the form of suspensions.

A convenient model for assessing an antibiotics migrations in an aquatic ecosystem may be the chamber models method, which have shown its high efficiency in radioecology (Kutlakhmedov et al., 2011) and in studying the migration paths of resistant to pesticide decay (Khyzhnyak et al., 2018). This method is used to describe the substances migrations by trophic chains, which are divided into chambers (English – box), and the transition between the chambers is due to transition factors. According to the

methods of interaction between the cameras, the models are divided into stationary and dynamic. In fig. 3. simple stationary (a) and dynamic (b) 3-chamber models are presented.

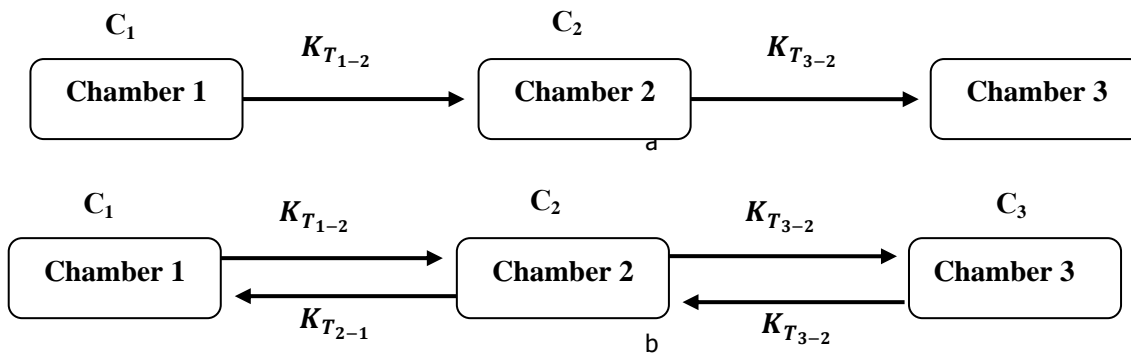


Figure 3. The simplest stationary (a) and dynamic (b) chamber models; hereinafter: **1, 2, 3** – the number of the camera model; **C₁, C₂ i C₃** – the concentration of substances in the respective chambers; $K_{T_{1-2}}, K_{T_{2-3}}$ – are direct coefficients of substance transfer to the corresponding chambers. $K_{T_{2-1}}, K_{T_{3-2}}$ – are the inverse coefficients of the transition of a substance to the corresponding chambers. **Note:** Two digits with a dash between them as an index of the transition coefficient indicate the numbers of the chambers between which the substance is redistributed.

The stationary chamber model is based on the postulate on the presence of stable statistical equilibrium in the chamber system, while the concentration of the test substance in each chamber is distributed evenly. It is also believed that the transfer of matter between the chambers is described by a system of simple differential equations. According to these postulates, a system of differential equations for the concentration of a substance in 3 chambers of the stationary model (Figure 3a) has the form:

$$\begin{aligned} \frac{dC_1}{dt} &= -K_{T_{1-2}} \cdot C_1; \\ \frac{dC_2}{dt} &= K_{T_{1-2}} \cdot C_1 - K_{T_{2-3}} \cdot C_2 + K_{T_{3-2}} \cdot C_3; \\ \frac{dC_3}{dt} &= K_{T_{2-3}} \cdot C_2 - K_{T_{3-2}} \cdot C_3; \end{aligned}$$

where here and then: $d(dC_1, dC_2, dC_3 \text{ and } dt)$ – differentiation symbol.

The dynamic chamber model (Figure 3b) is based on the following basic statements: 1) the trophic chain is divided into chambers in which matter is instantly mixed in all parts of the chamber equally in any direction; 2) the transfer of matter from one chamber to another occurs according to the laws of the first-order kinetics, described by a system of differential equations. In this case, the transfer coefficients of the substance between the chambers are constant values. According to these postulates, the system of differential equations for the accumulation of matter in the 3-chamber dynamic model (Figure 3b) is:

$$\begin{aligned} \frac{dC_1}{dt} &= K_{T_{2-3}} \cdot C_2 - K_{T_{1-2}} \cdot C_1; \\ \frac{dC_2}{dt} &= K_{T_{1-2}} \cdot C_1 - K_{T_{2-3}} \cdot C_2 - K_{T_{2-1}} \cdot C_2 + K_{T_{3-2}} \cdot C_3; \\ \frac{dC_3}{dt} &= K_{T_{2-3}} \cdot C_2 - K_{T_{3-2}} \cdot C_3 \end{aligned}$$

Practically for any complex system of trophic chains, a system of differential equations can be solved (using programs, for example, MAPLE 6) and the obtained values of substance concentration (C) over time for any values of transition coefficients (K_n) when a substance enters chamber 1. If there is a constant source of pollution or there is a constant flow of a substance into chamber 1, the following system of differential equations is added:

$$\frac{dC_0}{dt} = K_{n_0} C_0$$

where C_0 – is the concentration of a substance at the source at the time of the beginning of its migration; K_{n_0} – coefficient of substance transition from the source to the chamber 1. In a freshwater non-flowing reservoir, it is advisable to use a dynamic chamber model to analyze the migration paths of substances trapped to it, in particular, a simplified version consists of 3 chambers, namely: water - bottom sediments (sludge) – biota, which is shown in Figure 4.

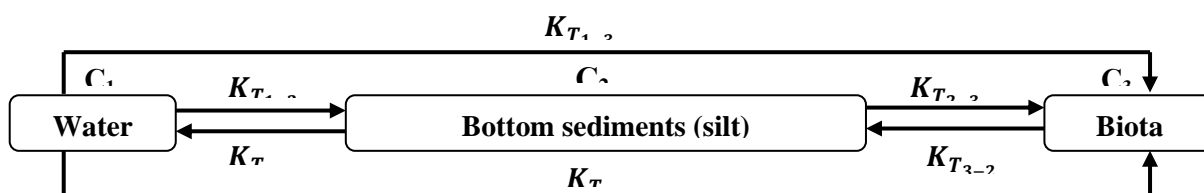


Figure 4. The dynamic chamber model of a streamless freshwater ecosystem.

In ordinary non-flowing water bodies with sapropelic bottom sediments (consisting mainly of organic substances – residues of aquatic organisms) such as substances that have fallen to them, they are concentrated, as a rule, in the upper layer of bottom sediments with a thickness of 10–20 cm. The large number of organic substances and the substances in a highly dispersed colloidal state cause the high absorption capacity of this bottom sediments layer. Precisely because of this, bottom sediments in the reservoir play the role of a depot, in which substances that fall into it.

The concentration equilibrium between water and sediments may shift in one direction or another. The degree of mineralization and the pH value (hydrogen index) of the water of the reservoir is one of the most important factors influencing this process. The biota (hydrofauna and flora) plays an extremely important role in the distribution of substances in reservoirs. The bulk of living organisms in natural reservoirs are plankton (a collection of organisms that inhabit the water column and are passively transferred by water currents) and microbenthos (a set of microorganisms that inhabit the soil of the reservoir). Substances contained in detritus (suspended in water organic matter consisting of animal body particles and plant fragments), along with it passes into the bottom sediments. Part of the water-soluble substances accumulates as plants and animals from water, and the other – comes to these organisms from the bottom sediments.

The state of the reservoir antibiotics contamination under conditions of a constant equilibrium between water and bottom sediments can be described by the following formula (in a simplified form, under the conditions of receipt of one substance) (Kutlakhmedov et al., 2011):

$$A = C \cdot S(H + k \cdot h),$$

where **A** – concentration of a substance in a reservoir; **C** – volume concentration of a substance in water; **S** – the surface area of the reservoir, which is approximately equal to the area of its bottom; **H** – the depth of the reservoir; **k** – the rate of accumulation of matter by a layer of bottom sediments; **h** – the thickness of the sediment layer.

Based on the above formula, it is possible to determine what proportion of substances that have entered the reservoir is contained in bottom sediments:

$$F = \frac{k \cdot h}{H + k \cdot h},$$

where **H** is the depth of the reservoir; **k** is the rate of accumulation of a substance by a layer of bottom sediments; **h** is the thickness of the sediment layer.

Based on this, the proportion of substance contained in water is 1 – F. The indicator F is called the factor (factor) of the reservoir capacity.

The total amount of substance in the biota of the reservoir (**A_b**) is calculated by the formula:

$$A_b = P \cdot K \cdot S \cdot H,$$

where, **P** is the amount of biota per unit volume of water (kg/m³), **K** – is the average coefficient of accumulation of the substance, **S** – is the surface area of the reservoir (km²); **H** – depth of the reservoir (m).

The capacity factor of the biotic component of the reservoir (**F_b**) is calculated by the formula:

$$F_b = P \cdot K \cdot H / (H + k \cdot h + P \cdot K \cdot H).$$

The reservoir factor of the whole reservoir (**F**) taking into account the biota is calculated by the formula:

$$F = \frac{k \cdot h + P \cdot K \cdot H}{H + k \cdot h + P \cdot K \cdot H}$$

Antibiotics that enter the water bodies, which are mostly highly soluble. This contributes to the fact that the overwhelming amount of them from the water accumulates in aquatic organisms, enters the bottom sediments as a result of sorption by the soil and the absorption of benthos from the water, as well as precipitated dendrites. A certain part of these substances is accumulated by plants attached to the soil.

In the case of a cascade of reservoirs, the greater their number, the higher the factor of its capacity. Thus, the capacity of the entire cascade (**F_k**) with **n** reservoirs is calculated by the formula:

$$F = 1 - \prod_{i=1}^n (1 - F_i),$$

where **i** – the number of the reservoir; **F_i** – capacity **i** reservoir.

The high activity of biota in the reservoirs of the cascade allows you to dramatically increase their capacity and bring the cascade's capacity factor to almost 1 when water comes out of the cascade of reservoirs with an insignificant content of substances trapped in it. On this property of cascades a system of reservoirs-deactivators is built.

Fundamental to the migration of substances, including antibiotics, in the rivers are both their quantity (those that come from surface runoff, groundwater, etc.) and their physicochemical properties and water. Also an important factor is the rate of flow of water, the bottom topography, the presence of stretches, shoals, hydraulic structures, the possibility of formation of bottom sediments and the like. These factors are decisive in the mechanism of mixing substances with water in rivers, which is called turbulent diffusion. In conditions of small rivers of the plain type, complete mixing may end at a distance of 200–500 m below the discharge of wastewater. In medium and large rivers of the plain type, complete mixing takes place, as a rule, at a distance of several tens of kilometers from the place where the sewage flows into the river, and the duration of this process is several days.

The distribution of polluted water jets in the flow of river water is affected by the difference between the density of wastewater and river water (due to the difference in temperature or the degree of salinity). Warm wastewater moves in the surface layer of river

water, and highly mineralized water sinks to the lower layers, creating an outwardly impression of well-being in this section of the river.

It should be noted that the consumption of drinking water containing antibiotics, trapped to it in places of withdrawal from reservoirs and rivers, with surface and groundwater, is extremely dangerous for human health. First of all, when they enter the body, its normal microflora is disturbed.

To the seas and oceans antibiotics come mainly from rivers. They focus primarily in the places of their flow. The main depot of concentration of antibiotics, as well as other substances that got into the sea, is the biota of the continental shelf with its high biological productivity. In this regard, it is advisable to divide the marine ecosystem into two subsystems – the continental shelf and the open sea.

To analyze the migration paths of substances, including antibiotics, entering the sea with river runoff, it is advisable to use a stationary chamber model, since the return path of substance transfer from the shelf to the river runoff is almost absent and ebb, in the river with a fish that spawns in them, in the spawning grounds). A simplified chamber model of the marine ecosystem is shown in Figure 5.

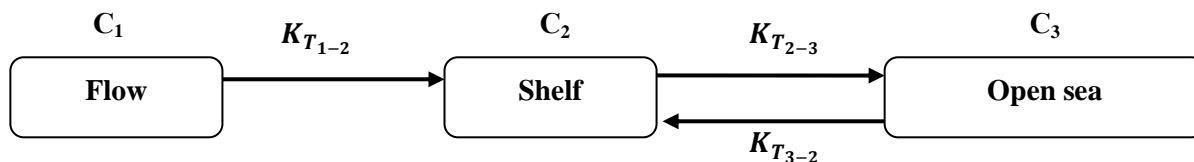


Figure 5. Stationary chamber model of the marine ecosystem when substances are released into the sea with river flows.

The calculation of the capacity of the main components (blocks) of the marine ecosystem, in particular, the shelf (**F_s**) and open sea (**F_m**) can be carried out using formula:

$$F_s = \frac{C_2}{C_1}, \quad F_m = \frac{C_3}{C_1}$$

It should be noted that in real conditions the flow of substances (including antibiotics) that have fallen into the water of the rivers, occurs not on the entire sea shelf, but on its relatively small area. For small sections of the shelf, the reserve capacity is even smaller. In the open ocean, biota insignificantly accumulates substances (including antibiotics) that got into it due to their very low concentration in a huge amount of water, as well as a small amount of biota compared to the one that lives on the shelf. The migration of antibiotics by trophic chains of ecosystems is a rather complicated process, which depends on many factors. These are, in particular, for aquatic ecosystems, the physicochemical properties of the substances themselves and of water, the effect of abiotic (temperature, water salinity, etc.) and biotic (aquatic microorganisms and other organisms that can accumulate or transform antibiotics) substances to organisms, inclusion to metabolism and much more (Kutlakhmedov et al., 2018). In order to forecast this process in ecosystems, in particular, in water, a promising method is the chamber model, based on determining the transition coefficients between the chambers (blocks) of the ecosystem.

Conclusion

Uncontrolled use of antimicrobial agents leads to the spread of antibiotic-resistant pathogenic strains. The ingress of antibacterial drugs into surface water through wastewater from livestock buildings, people's rooms, hospitals, during medical and agronomic events significantly exacerbate this problem. Antimicrobial agents in surface waters have several ways of developing the process: the lethal interaction of antibiotics with hydro-Biont and the elimination of sensitive taxa, the alteration of trophic chains; neutralization of antimicrobial drugs (molecule self-decomposition, hydrolysis, sorption, abiotic and biotic transformation) non-lethal interaction of antibiotics and aquatic organisms with the acquisition of signs of antibiotic resistance). In order to simulate the effects of antimicrobial agents on the aquatic ecosystem, it is proposed to use stationary and dynamic chamber models.

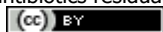
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