UDC 579.6

METAL-RESISTANT MICROORGANISMS OF TAP WATER: THEORETICAL JUSTIFICATION AND BIOTECHNOLOGICAL APPLICATION

O.A. HAVRYLIUK[1], I.O. BIDA[1], V.M. HOVORUKHA[1], Y. P. DANKO[2], G.V. GLADKA[1], A.V. SACHKO[3], L.S. YASTREMSKA[2], O.B. TASHYREV[1], PH.V. MUCHNYK[1]

[1]D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv

[2]National Aviation University, Kyiv

[3]Yuriy Fedkovych Chernivtsi National University, Chernivtsi

Water is one of the most essential resources for all living things. The issue of drinking water quality is acute both in Ukraine and in other countries due to the development of industry, as well as the constant growth of the world's population. After purification and disinfection of water at water treatment plants and then in the water supply system, water quality is not controlled. An important indicator that determines the quality of water is its microbial composition.

Microorganisms get into water mains from water intake sites and live and multiply in outdated and modern systems of drinking water distribution. In the sample of tap water, which was taken from the water supply system of Kyiv, we determined a significant content of microorganisms – 2.45×10^3 CFU/mL. The number of chemoorganotrophic microorganisms in the filler, which was removed from the filter cartridge for water purification, was also high – 1.6×10^5 CFU/g. In addition, the filler for water filtration contained thousands and tens of thousands of metal-resistant microorganisms. Thus, the maximum permissible concentration (MPC) of Cu(II) (ionic form) for the microbiome of the filter was 125 ppm, as well as Cr(VI) – 350 ppm. The concentration of living microorganisms in the medium was 2.91×10^5 CFU/g of filter at the presence of MPC of Cu(II). The number of *microorganisms was 9.7×10^2 CFU/g at the presence of Cr(VI) (in form of CrO_4^{2-}) in the nutrient medium. The resistance of the microbiome of the water filter to Cu(II) chelated by citrate was investigated, becouse heavy oxidizing metal compounds are able to precipitate in biological media. It was found that the resistance of microorganisms to Cu(II) citrate was 28 times higher than to the ionic unchelated form and amounted to 3500 ppm. The isolated microbiome is polyresistant and has a high adaptive potential to action of toxic metal compounds.*

Key words: *filter microbiome, tap water, toxic compounds of chromium and copper, chromium- and copper-resistant microorganisms, environmental biotechnologies*

Topicality. Water is one of the most essential resources for all living organisms. The problem of drinking water quality is acute both in Ukraine and in other countries due to the development of industry, as well as the constant growth of the world's population.

The quality of drinking water is determined by physicochemical and microbiological parameters. Physicochemical parameters include organoleptic properties, pH, redox potential (Eh), hardness and mineralization of water.

Another indicator that determines water quality is the microbial composition. Microorganisms get into water pipes from places of water intake and live and multiply in outdated drinking water distribution systems [1, 2]. Even after water purification at water treatment plants, organic substances are present in the water, which leads to the formation of microbial communities. The microorganisms that are part of them are representatives of a wide range of physiological and taxonomic groups. Most often, the microbiome of water includes representatives *Pseudomonas* spp. [2–4], *Bacillus* spp. [4–6], *Staphylococcus* spp. [4, 6], *Enterobacter* spp. [2, 7], *Salmonella* spp. [7, 8], *Escherichia coli* [1, 2, 8].

Existing methods of water disinfection (chlorination, ozonation) do not lead to the complete death of microorganisms, and therefore they get into the water supply system. Despite the means used for maximum water purification, water is a nutrient medium for microorganisms. Thus, water contains soluble organic compounds (dominantly organic acids) and mineral components (cations K^+, Mg^{2+}, etc., as well as anions SO_4^{2-}, PO_4^{3-}, NH^{4+}), ie all the components necessary for the growth of chemoorganotrophic microorganisms in water pipes. Microorganisms form biofilms, which are a diversified microbial communities. Thus, the mobilization of metals and the diffusion of their ions into aqueous solutions, as well as the presence of living microorganisms in the pipelines create the preconditions for the selection of metal-resistant cultures of microorganisms. With prolonged contact of microorganisms with toxic compounds, they are able to adapt at the physiological and molecular-biological level, ie acquire resistance to their action. On the one hand, the presence of such microorganisms is an indicator of the quality of water supply systems, which indicates the contamination of tap water by heavy metals due to corrosion and erosion. On the other hand, polluted tap water is a source of environmentally promising metal-resistant microorganisms that can be used in the latest environmental biotechnology to purify industrial wastewater and contaminated groundwater from heavy metal compounds.

In this regard, the aim of the work was to determine the number of microorganisms in tap water, as well as to investigate the quantitative patterns of the resistance of the microbiome of the filter (for drinking water purification) to heavy metal compounds (on the example of copper and chromium).

Materials and methods of the research. To study the patterns of resistance of microorganisms to copper and chromium was selected microbiome of filler, which was removed from the used cartridge from the filter for purification of drinking water (Fig. 1).

Fig. 1. Filter-jug system for drinking water purification

To prepare 250 ml of a solution of Cu(II) citrate with a concentration of 30 000 ppm, 29.4 g of $CuSO_4 \times 5H_2O$ crystals were dissolved in 100 ml of distilled water (pH = 3.4). Then 60 g of dry trisubstituted sodium citrate ($Na_3C_6H_5O_7$) was added and stirred until complete dissolution. The pH of the solution was 5.5 and therefore it was neutralized by $NaHCO_3$ to pH=6,5. The obtained solution was added to a 250 ml Mora flask and made up to volume with distilled water.

To prepare 250 ml of a 30 000 ppm of copper sulphate solution, 29.4 g of $CuSO_4 \times 5H_2O$ crystals were dissolved in 100 mL of distilled water, added to a Mora flask (250 ml) and made up to volume with distilled water. The solution had a pH = 3.4. The solutions were sterilized by boiling in a water bath for 30 minutes in a sealed vial.

To prepare 250 ml of chromate solution with a concentration of Cr(VI) - 30 000 ppm, 28 g of K_2CrO_4 salt was added to a measuring beaker and dissolved in 200 mL of distilled water. After complete dissolution, the solution was poured into a Mora flask (250 ml) and made up to 250 ml with distilled water. The solution was poured into a sterile vial and sealed. Usually, chromate solutions do not require additional sterilization.

The iron concentration was determined photocolorimetrically by qualitative reaction with o-phenanthroline.

To prepare 300 ml of NA (nutrient agar) with a 2% agar concentration, 6 g of agar, 3.9 g of NB powder (HiMedia Laboratories Pvt. Ltd., India) and 290.1 ml of distilled water were added to a 500 ml vial. Stirred and autoclaved at 1.5 atm for 30 minutes.

Chemoorganotrophic microorganisms were isolated from ten-fold dilutions of the precipitate removed from the cartridge in sterile saline (NaCl, 0.85%) by the Koch method. The method is based on the Koch principle, according to which each colony is the offspring of one cell of microorganisms. Determination of the number of microorganisms by this method consists of three stages: preparation of ten-fold dilutions of microorganisms, sowing on agar medium in Petri dishes and counting the colonies that have grown.

Inoculation of microorganisms was performed by the surface plate method (the streak plate method) using a Drygalsky spatula. After that, Petri dishes with nutrient medium were kept for 4-5 days at room temperature, 0.2 ml of each dilution of microorganisms was added to their surface by a sterile pipette and distributed with a sterile Drygalsky glass spatula on the surface of the medium. A new sterile spatula was used for each dilution. Sowing was performed in triplicate. Microorganisms were cultured for 7 days at a temperature of 28 °C.

The number of cells of microorganisms (CFU - colony-forming units) in 1 ml of the test suspension of microorganisms was calculated by the formula:

$$M = \frac{a \times 10^n}{V},$$

where *M* – the number of cells in 1 ml; a – the average number of colonies (from 3 to 300); 10^n – dilution factor; *V* – volume of the inoculum, mL.

The number of microorganisms in the samples was counted in the total number of microorganisms per 1 g of absolutely dry sample according to the formula:

$$X = a \times K,$$

where: *X* – the number of cells in 1 g of dry sample (CFU/g); *a* – the number of cells in 1 ml of suspension of the studied microorganisms (CFU/mL); *K* – the coefficient of the humidity of the sample. The humidity coefficient was determined by the formula:

$$K = \frac{100}{100 - A},$$

where: *A* – humidity of the sample.

The moisture content (*A*) in the samples was determined by weight. The samples were dried in an oven (t = 105 ºC) to constant weight. Humidity was determined by the formula:

$$A = \frac{B}{(P - B)} \times 100\%,$$

where: *B* – the weight of evaporated moisture; P – is the weight of the sample.

Isolation and quantitative accounting of copper-resistant and chromium-resistant microorganisms in the cartridge filler from the used filter for tap water purification.

Copper-resistant microorganisms were isolated under sterile conditions on nutrient agar (NA, HiMedia) in the presence of Cu^{2+} in two modifications. The first modification contained the Cu^{2+} cation chelated with Na-trisubstituted sodium citrate. In the second modification, Copper(II) was added in the form of $CuSO_4$ sulfate without the addition of chelators (preparation methods see above). A concentration gradient of Cu^{2+} was created in Petri dishes in the agar medium. For the variant of the experiment with the first modification of copper (Cu^{2+} cation), a concentration gradient from 25 to 250 ppm was created with the step of 50 ppm. In the second variant (cation Cu^{2+} chelated with citrate) the concentration gradient was 100…5500 ppm Cu^{2+} with the step of 500 ppm. Chromium-resistant microorganisms were isolated under sterile conditions on nutrient agar (NA, HiMedia) with a concentration gradient of 50…1000 ppm of Cr(VI) with the step of 100 ppm. Metal-free medium was used as a control. The solutions of metals and 25.0 ml of the medium were added to the plates and kept for 4 days at 20 ºC to check sterility and drying. The microorganisms were cultured at 28 ºC for 7 days. Sowing was performed in triplicate.

Results and their discussion. The quality of drinking water is reduced by outdated water supply systems made of cast iron and steel pipes. Once water enters the water supply system, its quality cannot be controlled. Pipelines corrode by complex conjugate redox reactions. Initially, metallic iron, Fe^o, is oxidized by protons and formed molecular hydrogen and iron(II):

$Fe^o + 2H+ 2e = Fe^{2+} + H_2$.

Then, Fe^{2+} is oxidized by dissolved oxygen to form iron(III) compounds in both insoluble and soluble state (Fe_3O_4 and [$Fe^{3+\cdot}$ ligand] respectively):

$3Fe^{2+} - e = Fe^{3+}$

$3Fe^{2+} + 4O_2 = Fe_3O_4$

$Fe_3O_4 + CH_3COOH = [Fe^{3+}(CH_3COO^-)_2]^+ + 2H^+$.

Ligands that chelate (convert to a soluble state) insoluble iron(III) oxide are organic acids - microbial exametabolites (acetate, propionate, etc.). Iron(0) of pipelines are the low-potential compound with a standard potential $E_o' = - 500$ mV. In contrast, iron(III) compounds are high-potential oxidants, the Eh of Fe^{3+} = + 771 mV. Therefore, the oxidation reaction of Fe(0) by Fe^{3+} occurs with the formation of Fe^{2+}:

$$Fe^{o} + Fe^{3+} + e = Fe^{2+}.$$

After that, Fe^{2+} is re-oxidized with water-dissolved O_2 to Fe(III) above named compounds, and the redox cycle is closed. Molecular hydrogen, formed by the reduction of protons by metallic iron, is used by microorganisms as a source of energy for growth. The biomass of hydrogen-oxidizing microorganisms is further hydrolyzed with the formation of organic acids, which accelerate the corrosion processes due to the chelation of insoluble iron(III) compounds.

According to the theoretical model presented by us, a complex system of active conjugated chemical and biological corrosion of pipelines and microbial water pollution functions in the pipelines. This corrosion is carried out by a diversified microbial communities. Theoretically, we assume that it consists of aerobic, facultative anaerobic and strict anaerobic microorganisms, which form both planktonic forms and biofilms of fouling on the inner surface of the pipes. The zone of growing of aerobic microorganisms is water containing soluble O_2 of air, and facultative anaerobic - the boundary between water and biofilms. Despite the presence of oxygen in tap water, the section of phases "iron - biofilm" creates ideal conditions for the growth of obligate anaerobic microorganisms. Thus, a potential of -500 mV is created on the surface of iron, and in addition, Fe^{2+} (oxidation product of Fe^{o}) is also a low-potential reducing agent (Eh = -150… -200 mV) and this compound diffuses into the water layer adjacent to the biofilm. Finally, H_2, which is formed during corrosion of Fe^{o}, is an electron donor for strict anaerobic microorganisms (acetogens, sulfate reducers, etc.). The stability of the microbiome should be ensured by a continuous supply of soluble organic and mineral compounds (water flow in the pipes) as well as H_2, Fe^{2+} and Fe^{3+} due to the incessant corrosion of Fe^{o}. Thus, despite the existing modern methods of pre-treatment of water, inside the pipes are created all the necessary and sufficient conditions for the development of diversified microbial groups. It should be noted that all iron compounds, such as Fe^{o} Fe^{2+}та Fe^{3+} belong to the representative metals. According to our definition, the main types of negative (damaging) action of all metals of the periodic table (a total of 48 metals) are oxidation of organic compounds and enzymes, substitution of metals in structural cell components and active enzyme centers, as well as simultaneous combined negative action as oxidation. and substitution. Therefore, among all 48 metals, we have chosen the so-called "representative metals", which typically show these negative effects of metals on microorganisms. For example, Cd^{2+}, Ni^{2+} are typical substitute metals. They cannot be reduced by microorganisms due to thermodynamic inhibition, but they actively replace metals in the active centers of enzymes and in structural cellular components due to their stereochemical analogy with Ca^{2+}, Mg^{2+} and others. Oxidizing metals include, for example, $V^{5+}O_4^-$ with high redox potential (+ 450 mV), but it is not able to replace metals in enzymes and structural components of microorganism cells. A typical representative of the metals of combined action is Hg^{2+}. This cation is a powerful oxidant ($E_{o\ (pH=3.0)}$ = +800 mV) and an active substituent (Ca^{2+} is stereochemical analogue). Compounds Fe^{2+} and Fe^{3+} together are also representative metals. Thus, Fe^{3+} is a stereochemical analogue of Mg^{2+}, as well as an oxidizing metal (E_o = +71 mV), Fe^{2+} is an active substitute (analogue of Mg^{2+}). We have previously shown that representative metals of combined action cause in microorganisms linked resistance to other representative metals. Hence, it has been suggested that tap water microorganisms adapted to iron compounds may also be resistant to other toxic metals of combined action that are absent in the tap. We have selected such metals of combined action such as CrO_4^{2-} та Cu^{2+}. Anion of CrO_4^{2-} is an oxidant (E_o' = +555) and a substitute (stereochemical analogue SO_4^{2-} with the size of the ion 0.3 nm), and Cu^{2+} is also an oxidant ($E_{o\ (pH=4.5)}$ = +500 mV) and a substitute (stereochemical analogue of Mg^{2+} with the size of the ion 0.078 nm).

Therefore, we theoretically substantiated that a significant number of microorganisms that are resistant to toxic metals (that are absent in water pipes) should be in tap water.

In this regard, the aim of the work was to determine the number of microorganisms in tap water, as well as to investigate the quantitative patterns of the resistance of the microbiome of the filter (for drinking water purification) to heavy metal compounds (on the example of copper and chromium).

It is well known that the microbial composition is an important indicator of drinking water quality. In Ukraine, the quality of tap water is regulated by State sanitary rules and regulations 2.2.4-171-10 [12]. According to these rules, the total microbial count at t = 37 °C for 95% of the water samples taken from the water supply network and investigated during the year should not exceed 100 CFU/ml. Also in the tap water for 98% of water samples, taken from the water network during the year, the microorganisms of the *E. coli* group, enterococci, pathogenic enterobacteria, enteroviruses, adenoviruses, and pathogenic intestinal protozoa should be absence. An increased microbial count (2.45×10^3 CFU/mL) was detected in the sample of tap water taken from the Kyiv water supply system (Fig. 2).

Fig. 2. Microorganisms isolated from tap water on the Nutrient agar medium (first ten-fold dilution)

This value exceeds the sanitary permissible norms by 24.5 times (norm is 100 CFU/mL). It can be assumed that the excess microbial count is caused by contamination of tap water with organic compounds that are substrates (sources of carbon and energy) for microorganisms. At a certain even low concentration of such substances, the growth of the chemoorganotrophic microorganisms is possible. Another reason may be the leaching of microorganisms from damaged pipes containing a concentrate of various types of microorganisms. According to the results of our research, the concentration of organic compounds in the studied tap water was

12 ppm. The number of chemoorganotrophic microorganisms in the filler, which was removed from the filter cartridge for water purification, was also high and amounted to 1.6×10^5 CFU/g. The resistance of microorganisms to metals was investigated using three variants of the experiment. In the first variant, chromium was used, the source of which was a solution K_2CrO_4. In the second variant of the experiment the copper sulfate ($CuSO_4$×5H_2O) solution was used as a source of copper. In the third variant of the experiment used a solution of copper ($CuSO_4$×5H_2O), which was chelated by citrate. In addition, the filler for water filtration contained thousands and tens of thousands of metal-resistant microorganisms (Fig. 3). Thus, the maximum permissible concentration (MPC) of Cu^{2+} (ionic form) and Cr(VI) in the form CrO_4^{2-} for the filter microbiome was 125 ppm and 350 ppm respectively (Fig. 3). At the presence of MPC of Cu^{2+} in the medium, the concentration of living cells of microorganisms was also high and amounted to 2.91×10^5 CFU/g of filler. At the presence of MPC of Cr^{6+}, the number of microorganisms was 9.7×10^2 CFU/g (Fig. 3).

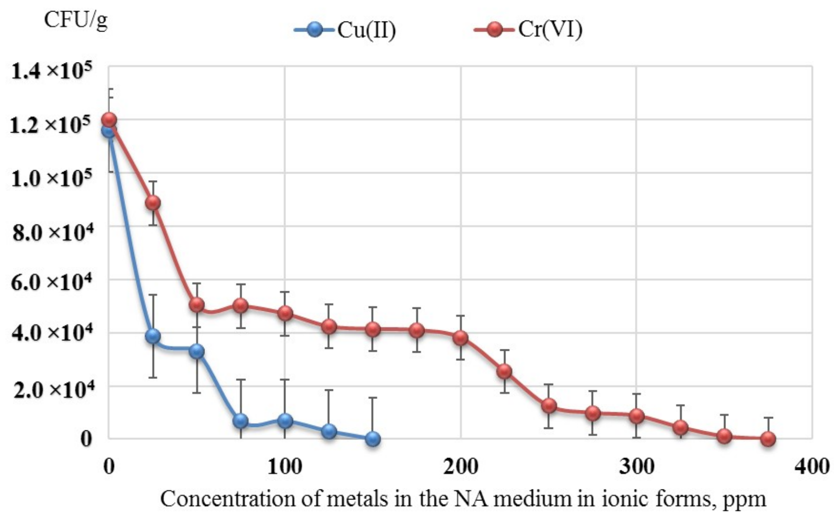

Fig. 3. The resistance of the microbiome of the water filter to the action of compounds of Cu(II) and Cr(VI)

Despite the significant resistance of the filter microbiome to toxic metal compounds, increasing their concentration even to 50 ppm in the culture medium led to the catastrophic reduction of the number of living microorganisms by an order of magnitude (Fig. 3). Quantitative patterns of resistance of microorganisms were hyperbolic. That is, the decrease in the concentration of living cells of microorganisms occurred in the form of a hyperbolic curve, on the "right shoulder" of which there is a disappearing number of microorganisms that are resistant to metals in high concentrations (Fig. 3, Fig. 4).

Given that in biological media, toxic oxidizing metal compounds can precipitate, the resistance of the microbiome of the water filter to Cu^{2+} chelated by citrate was investigated. It was found that the resistance of microorganisms to Cu^{2+} citrate was 28 times higher than to the ionic non-chelated form. Thus, the MPC of Cu^{2+} in the form of citrate was 3500 ppm (Fig. 4).

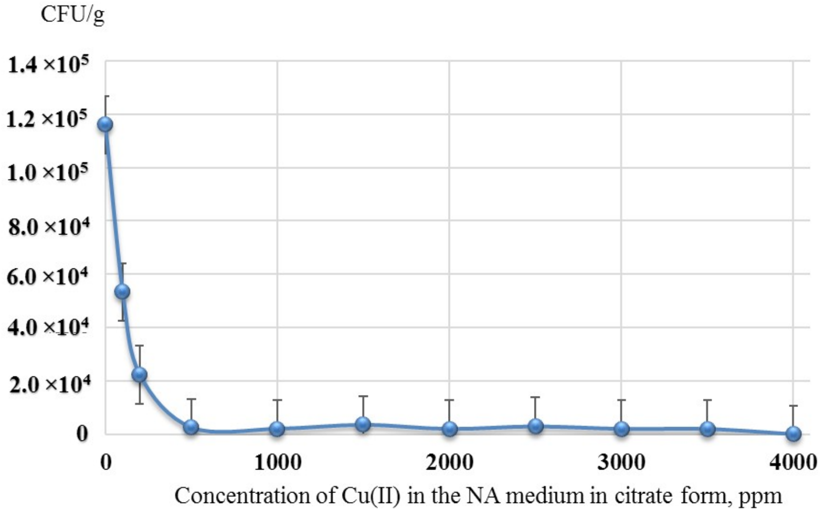

Fig. 4. Resistance of the microbiome of the water filter to Cu^{2+} chelated with citrate

At the MPC of Cu^{2+} in the form of citrate, the number of living cells of microorganisms was also high and amounted to 1.94×10^3 CFU/g at concentration 3500 ppm of Cu^{2+}.

The obtained results indicate that the water supply systems of Kyiv are obsolete and probably affected by the processes of corrosion and erosion of pipelines. This is evidenced by the presence of a large number of metal-resistant microorganisms that were isolated from the filler for the filter, which was used to purify tap water. However, tap water and cartridges for water filter cartridges are a source of industrially promising metal-resistant microorganisms that can be used to develop environmental biotechnologies for the treatment of industrial wastewater from metal compounds.

Many studies provide bacteriological characteristics of drinking water obtained from filter analysis. Thus, the authors (Vandevre-les-Nancy, France) [6] tested Point-of-use (POU) water filters in order to compare the quality of the filters during 31 and 62 days of use. 140 water samples were analyzed. The taxonomic position of microorganisms isolated from filters was established belonging to the species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Penicillium* spp., *Acinetobacter heemoliticus*, *Brevundimonas vesicularis*, *Bacillus* spp., *Micrococcus* spp., *Aspergillus fumigatus* and *Pseudomonas aeruginosa*.

A wide range of microorganisms were isolated from water filters in Oslo (Norway) including opportunistic pathogens, for example (*Methylobacterium*) and endospores (*Paenibacillus*). Three types of agar colonies were obtained from drinking water samples: flat translucent colonies with an uneven edge (*Commamonadaceae*, можливо, *Rhodoferax*), off-white (*Afipia*) and pink colonies (*Methylobacterium*). Different pink colonies were observed in parallel replicates (*Methylobacterium; Methylorubrum*), flat grayish colonies (*Curvibacter*) and a large number of small gray colonies (*Burkholderiales*, можливо, *Polaromonas*) [9].

Similar studies were performed with tap water samples in Shanghai (China). The species of *Pseudomonas*, *Aeromonas*, *Acinetobacter*, *Corynebacterium*, *Flavobacterium*, *Sulphatobacteria* were isolated [3].

Microbial biodiversity was studied in drinking water samples collected from different points of the water supply system in Wroclaw, Poland. Determination of the taxonomic position of the isolates was performed by sequencing of 16S rRNA genes and MALDI-TOF mass spectrometry. It was found that in drinking water in Wroclaw there are microorganisms of the following genera and species: *Bacillus* spp., *Brevundimonas* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Microbacterium testaceum*, *Micrococcus* spp., *Delftia* spp., *Advenella* spp [10].

Samples from tap water in the city of Al-Sadr (Baghdad) [11] were examined for the presence of pathogenic microorganisms, including *Giardia lamblia*. The results showed that 3.47% of the samples contained cysts – *G. lamblia*, 2,08% – *Entamoeba histolytica* and *E. dispar*, *Cryptosporidium* spp. and oocysts *Cyclospora* *cayetanensis* was observed in 1,38% samples. Free-living amoebae, ciliates and flagella were also found in all samples. This result indicates the potential role of drinking water in the infection of these intestinal parasites in the study area.

The microbiome, which was dominated by three bacterial cultures, was isolated from drinking water and water samples taken at different stages of its purification at the water treatment plant in Kiev [5]. The species composition of bacterial isolates was analyzed by analysis of 16S rRNA gene sequences. The following types of bacteria have been identified: *Bacillus nanhaiensis, Brevibacterium frigoritolerans* and *Lysinibacillus fusiformis*.

The results of monitoring the quality of surface water in the places of water intakes of drinking water pipelines in Ukraine indicate that at present the concentrations of priority hazardous chemicals are already approaching the maximum available concentration (MAC), and in some cases even exceed them. In this situation, the possibility of obtaining high-quality drinking water becomes sharply more complicated, since the existing water treatment facilities practically do not provide a barrier function in relation to man-made chemicals, they enter the drinking water in transit.

According to the results of atomic absorption analysis, the content of Cu in tap water did not exceed 0.05 ppm, and Ag and Mn were not detected. However, trace amounts of Pb were detected. The concentrations of Fe(II) was 10 ppm.

Based on our theoretical concept, a high resistance of the microbiome of tap water to oxidizing metals - Cu(II) and Cr(VI) was found. The presence of metal-resistant microorganisms in tap water, even in small quantities, indicates the presence of favorable conditions for their development in water supply systems.

CONCLUSIONS

We have experimentally confirmed the generalized theoretical concept of the existence of a stable diversified microbiome in water supply networks, which is polyresistant to toxic metals of multiple negative effects. The constant inflow of mineral and organic compounds into the network and the inevitable corrosion of iron pipelines leads to the formation of a microbiome that is resistant not only to iron compounds such as Fe(II) and Fe(III), but also to highly toxic metals of combined damaging action – Cu(II) and Cr(VI). Obviously, to improve water quality, it is necessary to ensure maximum purification of water from organic compounds at water treatment plants. The inevitable activation of the microbiome metabolism due to the accumulation of corrosion products in water determines the need to use inert materials (plastic, etc.) in water mains. The polyresistance of the studied microbiome to highly toxic metals indicates an extremely high adaptation potential of "ordinary" microorganisms to foreign extreme factors.

The obtained results indicate that the studied water supply systems are obsolete and probably affected by the processes of corrosion and erosion of pipelines. This is evidenced by the presence of a large number of metal-resistant microorganisms that were isolated from the filler of the filter, which was used to purify tap water. The isolated microbiome is polyresistant and has a high adaptive potential to toxic metal compounds.

REFERENCES

1. Mujahid T.Y. Bacteriological Quality Analysis of Tap Water of Karachi, Pakistan International Journal of Advanced Research / T.Y. Mujahid, K. Siddiqui, R.Z. Ahmed [et al.] // International Journal of Advanced Research – 2015. – 3(2). – P. 573–578 http://www.journalijar.com

2. Botsaris G. Microbial quality and molecular identification of cultivable microorganisms isolated from an urban drinking water distribution system (Limassol, Cyprus) / G. Botsaris, L. Kanetis, M. Slany [et al.] // Environ Monit Assess – 2015. – 187 (739) http://dx.doi.org/10.1007/s10661-015-4957-9

3. Li M. Antibacterial Performance of a Cu-bearing Stainless Steel against Microorganisms in Tap Water / M. Li, L. Nan, D. Xu [et al.] // Journal of Materials Science Technology – 2015. – 31. – P. 243-251 doi: http://dx.doi.org/10.1016/j.jmst.2014.11.016

4. Atuanya E.I. Effects of Storage/Biofilm Formation on Physico-chemical and Bacteriological Qualities of Potable Water Supply in Benin City Nigerian / Atuanya E.I, Seidu, R.I., Orjiakor P. // Society for Experimental Biology Journal – 2018. – 14(2) https://doi.org//10.5812/semj.68911.

5. Roi I.Yu. Species Identification of Water Microorganisms Resistant to Chlorine Compounds / I.Yu. Roi, N.A. Klimenko, G.M. Zdorovenko, V.V. Goncharuk // Journal of Water Chemistry and Technology – 2015. – 37(3) . – pp. 145–150 http://dx.doi.org/10.3103/S1063455X1503008X

6. Arnaud Florentin MD Water and surface microbiologic quality of point-of-use water filters: A comparative study / Arnaud Florentin MD, MSc, Julie Lizon PharmD [et al.] // American Journal of Infection Control – 2016. – 44(9). – P. 1061–1062 http://dx.doi.org/10.1016/j.ajic.2016.02.028

7. Khan M.A. Assessment of microbial quality in household water tanks in Dubai, United Arab Emirates / Khan M.A. [et al.] // Environmental Engineering Research – 2017. – 22(1). – P. 55–60 https://doi.org/10.4491/eer.2016.051

8. Sadik N.J. Quantification of multiple waterborne pathogens in drinking water, drainage channels, and surface water in Kampala, Uganda, during seasonal variation / N.J. Sadik, S. Uprety, A. Nalweyiso [et al.] // GeoHealth – 2017. – 1(6). – P. 258–269 https://doi.org//10.1002/2017GH000081.

9. Charnock C. Diversion and phylogenetic relatedness of filterable bacteria from Norwegian tap and bottled waters / C. Charnock, T.L. Vo, T.N.-T. Nguyen, R.X. Hagen // Journal of Water and Health – 2019. – 17(2) https://doi.org/10.2166/wh.2019.284

10. Leginowicz M. Biodiversity and antibiotic resistance of bacteria isolated from tap water in Wrocław, Poland / M. Leginowicz, A. Siedlecka, K. Piekarska // Environment Protection Engineering – 2018. – 44(4) https://doi.org/10.5277/epe180406

11. Al-Sagur I.M. Investigation of Giardia lamblia and other parasites in tap water as a potential source of transmission in some regions of Baghdad / I.M. A l-

Sagur, S.H. Mahmood, H.M.A. Al-Obaidi // Iraqi Journal of Science – 2015. – 56(1). – P. 337-344

12. Державні санітарні норми і правила ДСанПіН 2.2.4-171-10 «Гігієнічні вимоги до води питної, призначеної для споживання людиною»

МЕТАЛ-РЕЗИСТЕНТНІ МІКРООРГАНІЗМИ ВОДОПРОВІДНОЇ ВОДИ: ТЕОРЕТИЧНЕ ОБҐРУНТУВАННЯ ТА ЗАСТОСУВАННЯ У БІОТЕХНОЛОГІЯХ

О.А. ГАВРИЛЮК[1], І.О. БІДА[1], В.М. ГОВОРУХА[1], Я.П. ДАНЬКО[2], Г.В. ГЛАДКА[1], А.В. САЧКО[3], Л.С. ЯСТРЕМСЬКА[2], О.Б. ТАШИРЕВ[1], Ф.В. МУЧНИК[1]

[1]Інститут мікробіології та вірусології ім. Д.К. Заболотного НАН України, м. Київ

[2]Національний Авіаційний Університет, м. Київ

[3]Чернівецький національний університет ім. Ю. Федьковича, м. Чернівці

Вода є одним з найнеобхідніших ресурсів для всього живого. Проблема якості питної води гостро постає як в Україні, так і в інших країнах світу у зв'язку з розвитком промисловості, а також невпинним зростанням населення планети. Після очищення та дезінфекції води на станціях водопідготовки і, у подальшому, у водопроводі, якість води не контролюється. Важливим показником, що визначає якість води, є її мікробний склад.

Мікроорганізми потрапляють у водопроводи з місць забору води і живуть та розмножуються у застарілих та сучасних системах розподілу питної води. У зразку водопровідної води, що була відібрана з системи водопостачання м. Київ, нами був визначений значний вміст мікроорганізмів – 2.45×10^3 КУО/мл. Кількість хемоорганотрофних мікроорганізмів у наповнювачі, що був вилучений із картриджа фільтра для очищення води, також була високою і становила 1.6×10^5 КУО/г. Крім того, наповнювач для фільтрування води містив тисячі і десятки тисяч металрзистентних мікроорганізмів. Так, максимально-допустима концентрація Cu(II) в іонній формі для мікробіому фільтра становила 125 мг/л, а Cr(VI) – 350 мг/л. За наявності Cu(II) за максимально-допустимої концентрації у середовищі кількість живих клітин мікроорганізмів також була високою і становила 2.91×10^5 КУО/г наповнювача. За наявності Cr(VI) (у вигляді CrO_4^{2-}) у поживному середовищі, кількість мікроорганізмів становила 9.7×10^2 КУО/г. Оскільки, у біологічних середовищах сполуки важких металів-окисників здатні випадати у осад, було досліджено стійкість мікробіому фільтру для води до цитрату Cu(II). Було встановлено, що стійкість мікроорганізмів до цитрату Cu(II) була у 28 разів вищою, ніж до іонної нехелатованої форми і становила 3500 мг/л. Ізольований мікробіом є полірезистнтним і має високий адаптивний потенціал до дії сполук токсичних металів.

***Ключові слова*:** мікробіом фільтра, водопровідна вода, токсичні сполуки хрому і купруму, хром- та мідьрезистентні мікроорганізми.

МЕТАЛ-РЕЗИСТЕНТНЫЕ МИКРООРГАНИЗМЫ ВОДОПРОВОДНОЙ ВОДЫ: ТЕОРЕТИЧЕСКОЕ ОБОСНОВАНИЕ И ИСПОЛЬЗОВАНИЕ В БИОТЕХНОЛОГИЯХ

О.А. ГАВРИЛЮК[1], И.О. БЕДА[1], В.М. ГОВОРУХА[1], Я.П. ДАНЬКО[2], Г.В. ГЛАДКА[1], А.В. САЧКО[3], Л.С. ЯСТРЕМСКАЯ[2], А.Б. ТАШИРЕВ[1], Ф.В. МУЧНИК[1]

[1]Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, г. Киев

[2]Национальный Авиационный Университет, г. Киев

[3]Черновицкий национальный университет им. Ю. Федьковича, г. Черновцы

Вода является одним из самых необходимых ресурсов для всего живого. Проблема качества питьевой воды остро стоит как в Украине, так и в других странах мира в связи с развитием промышленности, а также непрерывным ростом населения планеты. После очистки и дезинфекции воды на станциях водоподготовки и в дальнейшем, в водопроводе, качество воды не контролируется. Важным показателем, определяющим качество воды, является ее микробный состав. Микроорганизмы попадают в водопроводы из мест забора воды и живут и размножаються в устаревших и современных системах распределения питьевой воды. В образце водопроводной воды, которая была отобрана из системы водоснабжения г.Киев, нами было определено значительное содержание микроорганизмов – 2.45×10^3 КОЕ/мл. Количество хемоорганотрофних микроорганизмов в наполнителе, который был удален с картриджа фильтра для очистки воды, также было высоким и составляло1.6×10^5 КОЕ/г. Кроме того, наполнитель для фильтрации воды содержал тысячи и десятки тысяч металрзистентних микроорганизмов. Так, максимально допустимая концентрация (МДК) Cu(II) в ионной форме для микробиома фильтра составляла 125 мг/л, а Cr(VI) - 350 мг/л. При наличии Cu(II) по МДК в среде количество живых клеток микроорганизмов также было высоким и составиляло 2.91 × 105 КОЕ/г наполнителя. При наличии Cr(VI) (в виде CrO_4^{2-}) в питательной среде, количество микроорганизмов составляло 9.7×10^2 КОЕ/г. Поскольку в биологических средах соединения тяжелых металлов-окислителей способны выпадать в осадок, была исследована устойчивость микробиома фильтра для воды до Cu(II) хелатированной цитратом. Было установлено, что устойчивость микроорганизмов к цитрату Cu(II) была в 28 раз выше, чем к ионной нехелатованой форме и составляла 3500 мг/л. Изолированный микробиом полирезистентен и обладает высоким адаптивным потенциалом к соединениям токсичных металлов.

***Ключові слова*:** микробиом фильтра, водопроводная вода, токсичные соединения хрома и меди, хром и медьрезистентние микроорганизмы.