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CYTOGENETIC EVALUATION OF THE DRINKING WATER TOXICITY

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Abstract

There was considered the use of biotesting for the assessment of the quality of drinking water from the different water supply sources (artesian, packaged and water-pipe one). The method consists in determination of the toxicants action on the specially selected organisms in the standard conditions with registration of changes at behavior, physiological, cellular and subcellular level using hematological indices of fish, frogs, rats and the lymphocyte cultures of the peripheral human blood. Physical-chemical methods determine only the presence and number of chemical elements in the tested water samples because of the very large number of possible combinations of chemical compounds in water solutions (more than 75 million combinations), including behavior of anthropogenic compounds and the natural vulnerability of the water ecosystems to the combined effects from its toxic influence.

As the optimal set of determination of the some structural and functional changes of cell genome as the result of the toxic influence of combination of the chemical compounds in the water solutions was offered the micronuclear test and leukocytic formula of the fish, frog and rat blood as biomarker. The reaction of fish, frog, rat test-organisms on the toxic irritation is presented in the change of qualitative content of the cells of peripheral blood. There were demonstrated the prospects of the use of hematological indices of the following test-organisms: *Danio rerio* fishes, *Xenopus* clawed frogs, *Wistar* rats and also the lymphocytes cultures of the human peripheral blood. The special attention was paid to the assessment of the risk for human health of the toxic substances in drinking water, genotoxicity and cytotoxicity that are revealed using hematological indices of animal cells. The universality of cellular organization opens the wide possibilities for toxicological studies using peripheral blood of the different groups of animals (fish, frogs, rats), human lymphocyte cultures and allow assume the following possibility of extrapolation of the received results on human organism.

Keywords: cytotoxicity, genotoxicity, micronuclear test, leukocytic blood formula, drinking water.

1. Introduction

Methodological possibilities for the study of toxicity of the different substances on test-organisms essentially increased during the last twenty years. The assessment of drinking waters quality by the biotesting methods became especially topical because of the swift growth of the number of potentially dangerous chemical compounds that pollute the natural sources of drinking water supply. Biological methods give the integral assessment of toxicity caused by the total action of all complex of polluting substances that are contained in the water medium taking into account its synergetic and antagonistic interaction.

Biotesting is the method of biological control that presuppose the purposeful use of the standard test-organisms and methods for determination of the toxicity degree of the water medium, based on the measurement of test-reaction of organism, its separate function or system [1, 2]. The use of ecotoxicological biotests (vegetable and animal test-organisms) and its cellular biomarkers is very important for the objective and complex control on the increasing number of xenobiotics, polluting the water medium, most of which are not properly normalized by the existing standards. The regular use of drinking water even with maximum allowable contents (MAC) of toxic chemical elements can cause the disorder of lungs, kidneys and other organs functions. For example, according to SSanRN 2.2.4-171-10 the cadmium MAC in drinking waters is 0,001 mg/dm^3. As far as for the normal human life activity it is necessary to take 1,5–2 liters of water per day, human organism can get with water, correspondent to the SSanRN standards, 0,0015–0,002 mg of cadmium. The cadmium surplus can case malignant tumors and the other toxic, cytotoxic, genotoxic effects. In SSsanRN 2.2.4-171-10 standard are cited the MAC of

the other chemical elements besides cadmium, so human runs risks regularly taking the drinking water with concentration of the different chemical substances even within MAC norm. The system of monitoring of drinking water quality in Ukraine like in the most other countries of the world gives only assessment of the exceeding of chemical elements MAC (mainly toxicants) for the water objects. The MAC values by SSanRN (state sanitary rules and norms) practically do not take into account the specificity of formation of water quality, including the behavior of anthropogenic compounds and natural vulnerability of the water ecosystems to the pollution action and its combined effects. It is also unknown how dangerous these effects are for the living organisms and human life activity. Standard only ascertains the quantitative factors of presence of particular chemical substances and compounds but it does not assess the general qualitative influence of water solution on the living organisms.

Universality of the cellular organization opens the wide possibilities for toxicological studies using the different groups of animals and plants with the further extrapolation of the received results on human blood cells and organism [3].

The problems of biotesting of the water samples (artesian, surface) at cellular level are elucidated in scientific works [4, 5] in details. There are described researches carried out at subcellular and molecular levels, which experiments are laborious and expensive, so, its wide practical use is limited.

The aim of this work – comparative assessment of the toxicity of drinking water from the different water supply sources (artesian, packaged, water-pipe) on the blood cells of test-organisms from the different systematic groups and trophic levels: on water organisms – *Danio rerio* fishes and *Xenopus* clawed frogs; on endothermal animals – *Wistar* rats; and on the lymphocytes culture of the human peripheral blood.

In literature [6, 8, 9] were cited the data about water influence on hematological indices of living organisms that is indicator of not only the physiological state of organism but also one of the main criteria of revelation of drinking waters pollution. The system of test-organisms blood is very sensitive to the action of potentially dangerous exogenous factors. The cells of test-organisms blood are one of the first that face with substances and compounds that enter into organism. This fact causes the necessity of compulsory assessment of test-organisms peripheral blood state under condition of determination of its toxic properties.

Such studies are necessary because there are more than 75 million of chemical compounds in environment. Biotesting has the certain advantages comparing with physical-chemical analysis that does not allow reveal the unstable compounds or quantitatively determine the ultralow concentrations of ecotoxicants. Biotesting gives the possibility to receive the integral assessment of toxicity fast.

For analysis of the influence of toxic substances in the water samples on the living organisms and its blood cells and on the lymphocytes culture of the human peripheral blood we selected the following set of test-objects: vertebral animals – *Danio rerio* fishes, *Xenopus* clawed frogs, *Wistar* rats. Toxic substances in the water samples (artesian, packaged and water-pipe) demonstrated the toxic and cytotoxic influence on the different blood cells (erythrocytes, lymphocytes) of the selected set of test-objects that is caused by the different degree of organization of test-organisms cells and consequently by its place in phylogenetic range. In this connection because of specific peculiarities of sensitivity to pollutants there must be used the set of objects, presenting the different taxonomic groups and complex approach to the testing of water samples.

In our studies the complex approach is consists in the fact, that the general toxicity and cytotoxicity are studied at both organismic and cellular levels. As the result we receive the complex assessment of toxic effect. Especially at organismic level we can analyze reactions of representatives of the different systematic groups and trophic levels (acute and chronic toxicity), at the cellular level – structural and functional changes of genome (geno- and cytotoxicity).

The set of cellular criteria: genotoxicity indices – micronuclear test contains the cells with microkernels and double kernels (structural disorders in the hereditary cellular apparatus). Cy-

totoxicity indices – leukocytic blood formula contains quantitative characteristics of leukocytes of test-organisms peripheral blood (reflect functional changes). In the cells of vegetable root the genotoxicity indices are also determined using micronuclear test and cytotoxicity index is a mitotic index (calculation of the dividing cells in studied samples). The methods are described in scientific works [6, 7, 14].

In this work are cited the received results of determination of the toxicants influence in the different types of drinking waters (water-pipe, packaged, artesian) on erythrocytes and lymphocytes of peripheral blood of the water organisms (*Danio rerio* fishes, *Xenopus* clawed frogs) and also on lymphocytes of peripheral blood of endothermal animals (*Wistar* rats) and on the lymphocyte culture of the human peripheral blood **(Table 1)**.

Table 1
Comparative assessment of the studied waters on the peripheral blood of Danio rerio fishes, Xenopus frogs, Wistar rats and on the lymphocyte culture of the human peripheral blood

Studied water samples		Control water	Artesian water	Packaged water	Water-pipe water
Erythrocytes of the fish peripheral blood, ‰	mC	0	0	1,67±0,63	3,63±0,86*
	2K	0	0	3±0,79*	4±1,24*
Erythrocytes of the frog peripheral blood, ‰	mK	0	0	1,33±0,52	3,33±0,74*
	2K	0	0	2,33±0,69*	3,66±0,82*
Lymphocytes of the fish peripheral blood %		86,7±2,62	82,2±2,46	80,8±2,66	68,4±1,96*
Lymphocytes of the frog peripheral blood, %		78,4±2,54	76,7±2,68	75,2±2,52	60,2±2,19*
Lymphocytes of the rat peripheral blood, %		45,4±1,89	25,5±3,03*	41,8±2,82	35,4±1,63*
Lymphocyte culture of the human peripheral blood **, %		42,6±2,32	40,8±2,14	38,2±1,76	30,8±1,50*

*Note: MK – erythrocytes with microkernels; 2K – erythrocytes with double kernels; * – p<0,05 comparing with the control group; ** – lymphocyte number at the general blood analysis is 40 %*

2. Materials and methods

In the work were used artesian, packaged and water-pipe waters. The control water was prepared in laboratory conditions according to SSTU 4174:2003 recommendations that corresponded to the SSanRN 2.2.4-171-10 [10]. Biotesting on the water test-organisms was carried out on 40 individuals of *Danio* rerio fishes and on 40 individuals of *Xenopus* clawed frogs, cultivated in laboratory conditions. Test-organisms were divided into 4 groups in 10 individuals. Every group was placed in certain aquarium: № 1 control water, № 2 water-pipe water, № 3 artesian water and № 4 packaged water. In 96 hours after exposure the blood was taken from the caudal vein of every fish. In 192 hours blood was taken from the hind leg of every frog. The preparation and analysis of cytological preparations from peripheral blood of fishes and frogs was carried out by the standard method [2, 6]. At the same time was carried out an experiment on 40 white rats of *Wistar* line. Rats were also divided into 4 groups (in 10 animals in each one). All animals were on the certain water diet during two months, rats of the group № 1 drunk the control water; of the group № 2 – water-pipe water. Rats from the group № 3 drunk artesian water and animals of the

group № 4 – packaged water. For determination of hematological indices the blood was taken from the caudal vein. The general blood analysis with calculation of leukocyte formula was carried out by the standard method [11].

For experiment on the lymphocyte culture of the human peripheral blood was used the lymphocyte culture taken from the practically healthy donor, women of 40 years old, who did not take medical preparations and did not undergo X-ray examination during the year. Before separation of lymphocytes from the whole blood there was carried out the general analysis of peripheral blood, where the number of lymphocytes was 40 % from the general number of form elements in the human blood. The blood was taken by disposable syringe from the ulnar vein, poured out in 1 ml into sterile heparinized (5 units in 0,1 ml of physiological solution – 0,9 % NaCl) rotary test tubes. From the whole blood were separated lymphocytes as following: the contents of test tube (1,0 ml) preliminarily mixed by pipetting were layered in test tube, contained the solution for separation of lymphocytes (ficoll-urografin), the liquids were not allowed to mix, then the test tube cover was closed. Then test tube was centrifuged at 4000 turns/minute during 20 minutes, supernatant liquid was eliminated without touching the sediment. Then was added the 500 mcl of sterile physiological solution, test tube coved was closed and test tube was centrifuged at 4000 turns/minute during 10 minutes. Supernatant liquid was eliminated without touching the erythrocyte suspension. The received suspension of peripheral blood was divided into 4 groups. Then in test tubes with blood in sterile conditions were added the culture medium and water samples (control, artesian, packaged and water-pipe water) in 0,1 ml. The equal amounts of 0,1 % water eosin solution on the Ringer medium and 0,1 % trypan blue solution on distilled water are mixed before the calculation of the human lymphocytes number. Then 1–2 drops of the received solution was added to the drop of suspension of lymphocytes cells. Then the received mixture with cells of human lymphocytes is carried to the Goryaev chamber where the colored cells are calculated by 100 cells in the field of view. The calculation of lymphocytes of peripheral human blood was carried out by the standard method [11]. Cytogenetic tests in vitro are directed on the demonstration of the chromosomal disorders induction in cultivated cells, in this case – lymphocytes of peripheral blood that are evenly distributed and are in the same phase of cellular cycle (G_0) [8, 16].

In experiment for cytotoxic analysis was taken into account only calculation of lymphocytes number in the blood of fishes, frogs, rats and the lymphocytes culture of the human peripheral blood. For genotoxic analysis was carried out the calculation of the number of erythrocytes with microkernels and double kernels on the peripheral blood of fishes and frogs. The statistical processing of the received results was carried out using Microsoft Excel programs of statistical analysis. There was calculated the arithmetical mean, mean deviation, arithmetical mean error, toxic effect is considered as the valid at statistically reliable difference with the control [12] **(Table 1)**.

3. Experiments

There was carried out biotesting and cytological analysis of the studied water samples (artesian, packaged and water-pipe water).

Morphological changes of erythrocytes of the blood of fishes and frogs at the moment of toxicants effect were revealed using microscopy at the general increase x1000. On every preparation were studied 300 cells. The calculation of the number of received microkernels and double kernels in erythrocytes were expressed in promilles – ‰.

As the result of research were received the data on the content in peripheral blood of fishes and frogs the erythrocytes with microkernels and double kernels in the studied water samples (artesian, packaged, water-pipe) **(Table 1)**. The number of microkernels and double kernels reliably ($p<0,05$) increased in erythrocytes of fish blood in packaged water from 1,67 to 3 ‰ and in water-pipe water from 3,63 to 4 ‰ comparing with the control water. Analogously reacted erythrocytes in frog blood – the number of microkernels and double kernels of erythrocytes of frog blood in packaged water reliably ($p<0,05$) increased from 1,33 to 2,33 ‰ and in water-pipe water from

3,33 to 3,66 ‰ comparing with the control water. The received data of the study of artesian water corresponded to the control water data.

Cytological analysis was carried out on lymphocytes of peripheral blood of fishes, frogs, rats and on the lymphocyte culture of the human blood for the study of water samples (artesian, packaged and water-pipe). The blood preparations were analyzed under the light microscope at general increase x1000. On every preparation in different sectors were studied 250 cells, the calculation of lymphocyte number was expressed in percentage – %.

As the results of experiment were received data that represented the decrease of lymphocytes number in the blood of fishes, frogs, rats and in the lymphocyte culture of the human blood in all water samples (artesian, packaged, water-pipe). In fish blood the lymphocyte number in artesian water decrease insignificantly by 4,5 %, in packaged water by 5,9 %. And in the water-pipe water the decrease of lymphocyte number was reliable ($p<0,05$) 18,3 %, comparing with the control water. The quantitative index of lymphocytes in the frog blood in artesian and packaged water samples decreased insignificantly by 1,7 % and 3,2 % respectively and in the water-pipe sample the lymphocytes number in blood reliably ($p<0,05$) decreased by 18,2 %. In the peripheral blood of endothermal animals (rats) the reliable ($p<0,05$) decrease of lymphocytes number was in artesian 19,2 % and water-pipe 10 % samples, and in the packaged water the lymphocytes number decreased insignificantly by 3,6 % comparing with control.

In experiment on the lymphocyte culture of the human peripheral blood in artesian water sample the lymphocytes number decreased by1,8 %, in packaged water by 4,4 %. The reliable ($p<0,05$) decrease of lymphocytes number was in the water-pipe water – by 11,8 % comparing with the control water. The received results can indicate the development of inflammatory processes as the test-organisms reaction on the toxicants effect in the water samples.

4. Results and its discussion

Cyto- genotoxic assessment of the different types of water (artesian, packaged, water-pipe) on the blood cells of test-organisms and lymphocyte culture of the peripheral human blood is presented at the **(Table 1)**.

On the received data we can see that at microkernel erythrocytes test and at the lymphocytes quantitative characteristics the fish peripheral blood reacts similarly to the frog, rat blood and the human lymphocyte culture. That is why it is recommended to use fishes for screening of the substances potentially dangerous for human, that cause pathology and cancer diseases and also the presence and influence of genotoxic substances entering into the drinking water. In the scientific work [13] are cited the results that are proved by the coefficient of correlation between indices in fishes and in lymphocytic culture of the peripheral human blood. The received values of coefficients of the linear correlation [13] also testify to the interconnection between almost all indices determined on fishes and the number of injured aberrant metaphases with metabolic activation.

The fishes are the good test-organism, it can be used for express-analysis of drinking waters, it can be easily cultivated in laboratory conditions, the duration of experiment is only 96 hours. At the use of fishes as test-organisms the expensive reagents are not used and the most important fact, that fishes react similarly with the other animals and the received data indicate the correlation with the higher organisms.

The formation of microkernels in erythrocytes of fish and frog blood, the fragmentation of chromosomes often appear at the process of cancer disease development, at viral infection, bacterial infection and also at the influence of ionizing irradiation and different mutagens on cells. The strong correlation of the number of injured aberrant metaphases and metabolic activation was detected between the indices of determined lymphocytes culture of the human peripheral blood and the indices of cells of onion roots, that was described in the scientific works [13, 14] in details.

At determination of the cytotoxicity of the different types of drinking water (artesian, packaged, water-pipe) as the biomarker were used the form element of the fish blood – lymphocytes.

There was assessed the toxicity of water medium by determination of the lymphocytes number and its relation to the other elements of fish blood – monocytes, neutrophils, eosinophils, basocytes in comparison with control and experimental water medium samples. The calculation of the form blood elements was described in the work [6] in details, SSTU7387:2013.

Lymphocytes play the protective role in organism. The growth of its number in blood is the response of animal and human organism on the influences of viral infections, inflammatory processes. The decrease of lymphocyte number is typical for the initial stage of the infectious-toxic process and is connected with its migration of the nidi of inflammation [15].

In the present work were received results of the decrease of lymphocytes number in test-organisms blood. These data can indicate the toxic effects of the studied water samples on test-organisms, the received data are presented at the **(Table 1)**.

The cellular organization of test-objects gives the possibility for ecotoxicological studies with the further extrapolation of the received results on the human organism [16, 17]. For characterization of the structural and quantitative changes of the most important components of the cellular kernel (chromosomes and gens) that are the carriers of genetic information is used cytogenetic method – micronuclear test [6, 17]. The question of the role of microkernels formation in carcinogenesis stills open for today. In any case microkernels indicate the genomic instability [18].

At determination of the quality of drinking waters by the biotesting methods appears the range of important questions related to extrapolation of the received results on the human organism. For example, is the data about water samples toxicity received on animal and vegetable test-organisms the signal of dander for human? There were carried out the numerous works with test-organisms including the ones at the cellular level. There were used the examples of the works by the native and foreign scientists that give a possibility to correlate the results received at the cellular level on the higher organizational levels correctly.

The most available for extrapolation on the human organism are methods that assess mutagenicity, geno- and cytotoxicity that is (sub)cellular effects. This conclusion is based on the results of such international programs as Gene-Tox, International Program on Chemical Safety – IPCS, realized in 90-ies [14, 19].

In the European list are registered more than 100000 chemical substances (EINECS). Among it only the 30–40 ones are regularly tested on presence and concentration in the most important ecosystems of European countries [20]. The significant part of the toxic substances cannot be determined in the natural and waste waters because of the absence of correspondent analytic methods or high cost of such analysis.

5. Conclusions

1. Biomonitoring of the natural and drinking waters is a topical problem at the modern stage of social development that is solved by the scientific associations in many countries. The result of research of assessment of drinking waters (artesian, packaged, water-pipe) toxicity allow reveal the changes in hematological indices of fishes, frogs, rats and lymphocyte culture of the human peripheral blood that can be offered for biotesting.

2. As the result of micronuclear test carried out on the erythrocytes of frog and fish blood the studied water samples demonstrated the genotoxic effect, the number of erythrocytes with microkernels and double kernels in the fish blood reliably ($p<0,05$) increased from 1,67 to 4 ‰, in the frog blood from 1,33 to 2,33 ‰ respectively, comparing with the control water. The received data of the study of artesian water corresponded to the control water data.

3. At cytological analysis of the studied water samples (artesian, packaged and water-pipe) lymphocytes of the test-organisms peripheral blood and lymphocyte culture of the peripheral blood reacted almost similarly. In all water samples was observed the decrease of lymphocytes number of the test-organisms peripheral blood but in the water-pipe water was reliable ($p<0,05$) decrease of lymphocytes number in the fish blood by 18,3 %, in frogs by 18,2 %, in rats by 10 % and in lymphocytes culture of the human peripheral blood by 11,8 % comparing with the control water.

In artesian water sample by 19,2 % reliable ($p<0,05$) decrease of lymphocytes number was only in the rat blood.

4. Test-organism reaction on irritation of the toxic substance in the drinking waters was demonstrated in the formation of microkernels and double kernels in erythrocytes of the fish and frog blood and also in the change of quantitative content of erythrocytes of fish, frog and rat peripheral blood and of the lymphocyte culture of the human peripheral blood. As the result of experiment we can give a positive assessment of the only artesian water quality. Packaged and water-pipe water is not good for taking.

5. The assessment of drinking water toxicity using fishes, frogs, rats and the lymphocytes culture of the human peripheral blood corresponds to the modern standards of the researches of water samples quality. The results of presented work prove the scientists' data about the prospects of using test-organisms in biotesting of the drinking water.

References

[1] Arkhipchuk, V. V., Goncharuk, V. V. (2004). Problemy kachestva pit'evyh butylirovannyh vod. Himija i tehnologija vody, 26 (4), 403–414.

[2] Goncharuk, V. V., Vergolyas, M. R. (2012). Pat. 97199 MPK: G01N 33/18. Sposib pidgotovky biologichnogo ob'jekta dlja vyznachennja toksychnosti vody. a201012982; zajavl. 01.11.2010; opubl. 10.01.2012; bjul. № 1.

[3] Boltina, I. V., Vergolyas, M. R., Povjakelj, L. I., Zlacjkyj, I. A., Zavaljna, V. V., Kovalenko, O. V., Makarov, O. O., Zajecj, Je. R., Semenova, A. Ju. (2012). Materialy IX zj'ida ukrajinskogo tovarystva genetykiv selekcioneriv im. M. I. Vavylova. Kyiv: LOGOS, 4, 249–250.

[4] Albertini, R. J., Anderson, D., Douglas, G. R., Hagmar, L., Hemminki, K., Merlo, F. et. al (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutation Research/Reviews in Mutation Research, 463 (2), 111–172. doi: 10.1016/s1383-5742(00)00049-1

[5] Pavanello, S., Clonfero, E. (2000). Biological indicators of genotoxic risk and metabolic polymorphisms. Mutation Research/Reviews in Mutation Research, 463 (3), 285–308. doi: 10.1016/s1383-5742(00)00051-x

[6] Goncharuk, V. V., Vergolyas, M. R. (2013). DSTU 7387:2013. Jakistj vody. Metod vyznachennja cyto- ta ghenotoksychnosti vody i vodnykh rozchyniv na klitynakh krovi prisnovodnoji ryby Danio rerio (Brachydanio rerio Hamilton-Buchanan).

[7] Vergolyas, M. R., Lutsenko, T. V., Goncharuk, V. V. (2013). Cytotoxic effect of chlorophenols on cells of the root meristem of welsh onion (Allium fistulosum L.) seeds. Cytology and Genetics, 47 (1), 34–38. doi: 10.3103/s0095452713010118

[8] Boltina, I. V. (2008). A. s. (Svidoctvo pro derzhavnu rejestraciju prav avtora na tvir) Modyfikacija metodu vyvchennja mutagennoi' aktyvnosti rechovyn (metafaznogo analizu aberacij hromosomv kul'turi limfocytiv peryferychnoi' krovi ljudyny) in vitro z metabolichnoju aktyvacijeju. № 23794; zajav. 21.12.2007; opubl. 05.03.2008.

[9] Trakhtenbergh, Y. M., Tymofyevskaja, L. A., Kvjatkovskaja, Y. Ja. (1987). Metody yzuchenyja khronycheskogho dejstvyja khymycheskykh y byologhycheskykh zaghrjaznytelej. Riga: Zynatys, 172.

[10] Derzhavni sanitarni normy ta pravyla. Ghighijenichni vymoghy do vody pytnoji, pryznachenoji dlja spozhyvannja ljudynoju (DSanPiN 2.2.4-171-10).

[11] Balakhovskyj, Y. S.; Menjshykov, V. V. (Ed.) (1982). Rukovodstvo po klynycheskoj laboratornoj dyaghnostyke. Moscow: Medycyna, 235.

[12] Antomonov, M. Ju. (2006). Matematycheskaja obrabotka y analyz medyko-byologhycheskykh dannykh. Kyiv: FMD, 558.

[13] Goncharuk, V. V., Vergoljas, M. R., Boltyna, Y. V. (2013). Yssledovanye mutagennosty y genotoksychnosty pyt'evoj vodyy. Hymyja y tehnologyja vodyy, 35 (5), 426–435.

[14] Fiskesjö, G. (2008). The Allium test as a standard in environmental monitoring. Hereditas, 102 (1), 99–112. doi: 10.1111/j.1601-5223.1985.tb00471.x

[15] Goncharuk, V. V., Vergoljas, M. R. (2014). Toksycheskoe vlyjanye bakteryj Escherichia coli v zavysymosty ot yh soderzhanyja v vode na test-organyzmyy. Hymyja y tehnologyja vodyy, 36 (1), 83–91.

[16] Goncharuk, V. V., Boltina, I. V., Vergolyas, M. R. (2011). Pat. 93964 MPK: G01N 33/18. Sposib vyznachennja mutagennoi' aktyvnosti pytnoi' vody i kul'tural'ne seredovyshhe dlja jogo realizacii'. a201000606; zajavl. 22.01.2010; jpubl. 25.03.2011; Bjul. № 6.

[17] Ghoncharuk, V. V., Vergolyas, M. R., Boltina, I. V. (2011). Pat. 95717 MPK G 01 33/18. Sposib vyznachennja genotoksychnosti vodnogo seredovyshha. a201004569; zajavl. 19.04.2010; opubl. 25.08.2011.; Bjul. № 16.

[18] Inoue, A., Yokomori, K., Tanabe, H., Mizusawa, H., Sofuni, T., Hayashi, Y. et. al (1997). Extensive genetic heterogeneity in the neuroblastoma cell line NB(TU)1. International Journal of Cance, 76 (6), 1070–1077. doi: 10.1002/(sici)1097-0215(19970917)72:6<1070::aid-ijc23>3.0.co;2-7

[19] Arhipchuk, V. V., Goncharuk, V. V. (2001). Biotestirovanie kachestva vody na kletochnom urovne. Himija i tehnologija vody, 23 (5), 531–544.

[20] Postel, S. (1987). State of the world. New York: W.W. Norton, 169–173.