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Bioindication Assessment of Drinking Water Toxicity in Large Cities of Ukraine

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***Abstract*:** Intense anthropogenic impact on drinking water sources contributes to the load on aquifers, which leads to deterioration of groundwater quality. Modern practice of water economy proves that the use of traditional methods of assessing the ecological status of waters does not fully reflect the real state of the biological properties of drinking water. In present study modern method of biotesting using test organisms was proposed and proved efficiency for assessing the quality of drinking water. Bioassays are toxic, mutagenic and carcinogenic properties of the aquatic environment, differentiating sources of drinking water in accordance with the requirements of water consumption. Comprehensive toxic and environmental assessment of drinking water was calculated as an average arithmetic toxicity index, determined by daphnia and onions. The drinking water quality in the city of Kherson (Ukraine) was allocated 7 functional zones with different degrees of anthropogenic load. According to the analysis it was observed that inadequate quality drinking water was observed at the industrial zone, toxic properties drinking water at residential area of multi-storey, individual buildings and transport load area and the best drinking water were in residential area of mixed development and residential area of multi-storey buildings with a local water supply system. Use of ecological and toxicological test objects in bioindication system with various levels of organization allowed assessing the ecological status of underground sources water in accordance with the requirements of drinking water supply. According to the bioindication classification, drinking water pollution scale determined an extremely high degree of anthropogenic load within the historic center test site.

***Keywords*:** Drinking water quality, Groundwater, Test sites, Biotesting

In the context of intensive development of society, the problem of providing quality drinking water is one of the main environmental and socio-economic issues of sustainable development around the world. High-quality water supply is complicated by the uneven distribution of water resources on the continental parts of the globe, their intensive pollution by wastewater from agriculture (Pichura et al 2015, Zelenskaya et al 2018), industry and the utilities sector (Breus et al 2018, Breus et al 2019). Water scarcity is observed in 30% of North and South America, 33% in Europe, 60% in Asia, and 99% in Australia and Africa (Stadnyk 2010).In most residential areas of the world, the main source of drinking water supply is surface water, which due to intense anthropogenic impact does not meet the established quality requirements (Pichura et al 2020a, Haroon et al 2021). In this regard, to improve the quality of water, groundwater is used (by 2/3 of Europe's population), which is more protected from external negative influences and is characterized by a stable chemical composition. However, the main feature of their use for the purposes of the national economy is low development: only 8% of the projected groundwater reserves and 13% of the approved operational. The number of these reserves decreases every year due to the violation of their production regimes, which leads to depletion and pollution of aquifers, the restoration of which to its natural state is work-consuming and time-consuming. Given the intense anthropogenic pressure on all components of the environment and global climate change, issues related to environmental protection, including the provision of quality drinking water to the population have been raised in international forums, such as Aqua Ukraine, The United Nations Environmental Forum, Indo-European Water Forum in New Delhi etc., And for most countries of the world this problem has become one of the main priorities of public health policy to achieve their sustainable development (Pichura et al 2020b, Ondieki et al 2021). For high-quality water supply, there is a growing need to use an improved system for assessing the toxic and ecological status of drinking water using bioindication methods based on the specific response of test organisms to the content of toxic substances in the aquatic environment. For a long time, bioindication methods were used only to determine the quality of natural and wastewater, but with the adoption of European and national standards for human health protection from adverse effects of water pollution, the need for their use in assessing groundwater quality (Manickavasagam 2019, Larionova et al 2017). The aim of the article is to study the quality of drinking water and determine its toxicity using the method of bioindication assessment on the example of the city of Kherson (Ukraine).

MATERIAL AND METHODS

Studies of the toxicity of drinking water from underground sources in the city of Kherson (Ukraine) were conducted by biotesting using Daphnia *Magna straus* and onion *Allium cepa* L. (Vergolyas 2016). Drinking water samples were taken according to DSTU ISO 5667-11: 2005. Water sampling points for research were carried out in areas of the city that differ in relief, hydrological regime of underground layers, the level of destruction of the territory and depending on the depth of wells at different test sites (Fig. 1).

I test site - residential area of multi-storey buildings;

II test site - industrial zone;

III test site - residential area of mixed development;

IV test site - transport load area;

V test site - the area of the historic center;

VI test site - a residential area of multi-storey buildings with a local water supply system;

VII test site - a residential area of individual buildings.

To establish a toxico-ecological assessment of drinking water using daphnia, 8 water samples were taken from each landfill. In a container of water was placed 10 daphnia, the experiment was repeated three times. The number of live individuals was recorded after 24, 36, 48, 72, 92 hours. The criterion for the toxicity of drinking water was the number of dead daphnia in relation to the control.

For each investigated landfill and drinking water sampling site, the toxicity index was calculated according to the following formula:

$I = 100 (I_1 - I_0)/I_1$

I_1-I_0 - the number of live daphnia in the control and experiment at a fixed time of exposure to the test sample of drinking water with the test object.

Fig. 1. Map-scheme of spatial distribution of test sites in Kherson

When conducting experiments to determine the toxicity of selected samples of drinking water, all exposure factors were unchanged. The following levels of drinking water toxicity were taken into account:

It <20 - the allowable degree of toxicity;

It = 21- 50 - toxic water;

It> 51 - highly toxic water.

Water classes were determined according to the gradation scale of toxicity and bioindication classification of the level of drinking water pollution (Table 1).

Statistical analysis of the study results was to establish correlations between the time of toxic effects of drinking water and the survival of daphnia. For the reliability of the results of the study to determine the integrated toxicity of drinking water on the studied landfills, onions were used. For this the bulb was placed in a test tube with water. The number of test tubes with bulbs was 45 pieces, 5 pieces for each test sample of drinking water, taking into account the control sample. After 96 hours, measurements of the average length and number of roots in onion bunches were performed for each variant of the experiment. The criterion for drinking water toxicity was inhibition of onion root growth compared to control.

The comprehensive toxic-environmental assessment of drinking water was calculated as the arithmetic mean of the toxicity index, for the two selected test items. When conducting experiments to determine the toxicity of selected drinking water samples, all abiotic factors of possible influence on biotesting were unchanged.

RESULTS AND DISCUSSION

The main source of drinking water supply in the city of Kherson (Ukraine) is the groundwater of the Upper Sarmatian aquifer at a depth of 80-100 m. Their formation in the urban area is under the influence of natural and anthropogenic factors. Natural factors influencing the quality composition of groundwater in the urban area of Kherson (Ukraine) are the infiltration of precipitation and surface water within the Dnieper River basin, the inflow of groundwater from the Ukrainian Crystal Shield. However, in the conditions

Table 1. Bioindication classification scale for assessing water sources by pollution classes

Water quality class	Characteristics of the level of pollution
I	Weak
II	Moderate
III	High
IV	Highly dangerous
V	Dangerous

of unbalanced land use in the southern regions of Ukraine, irrational use of water resources in all spheres of the economy, the main factors influencing the quality of groundwater are anthropogenic (Breus et al 2020, Breus et al 2021). At the same time, the quality of drinking water becomes a limiting factor that affects the health of the population. The progressive growth of the level of anthropogenic pressure on the aquatic environment, the systematic inflow of pollutants into water supply sources, requires a rapid assessment of the ecological condition of water sources with low time consumption and little financial resources. In this regard, the suitability of water from underground sources for drinking purposes, determining the level of their toxicity within the urban system of the city of Kherson (Ukraine) was carried out on the basis of biotesting using invertebrates and plant bioassays. Biological testing was performed for three days, which allowed determining the level of acute toxicity of drinking water. During the experiment, the assessment of drinking water toxicity was performed on the basis of recording the number of live daphnia (Table 2).

Samples of drinking water of the VII test site in terms of survival of daphnia were 80%, and the level of toxicity of drinking water is acceptable. No significant morphological changes in the body were observed in dead individuals. Live daphnia actively moved in the water column during the entire exposure of the test. The amount of daphnia in drinking water samples of the III test site at the end of the experiment was 70%. The bodies of the dead were light. After 96 hours, the appearance of new daphnia was observed, which indicated the presence of phytoestrogens in drinking water samples, which determined the fertility of females. According to the toxicity index, water is assessed as toxic. The magnitude of the deviation of daphnia survival in all test samples of drinking water from the control sample was different for all studied landfills. .According to the biotesting, it was observed that the average time of death of 50% daphnia in test water samples at the V test site was 40 hours, II test site - 65 hours, IV test site - 85 hours. Therefore, according to the calculations of the toxicity index and reactions of daphnia to the content of pollutants in drinking water, low drinking water quality was observed at the II and V test sites. The biotesting of drinking water using the test object of daphnia, the intra-territorial zoning of the studied urban system was carried out (Fig. 2). It is proved that with increasing testing time the number of live daphnia decreases in all studied samples of drinking water. The rate of variation in mortality of test individuals is equal to 92% and depends on the time of toxic action of drinking water. Bioindication assessment of drinking water quality using daphnia allows to establish the level of toxicity of waters with different content of pollutants. However, the reliability of the result of such biotesting is only 35%. The single bioassay is not a universal and unified method in assessing the suitability of water in accordance with the requirements of drinking water supply. Therefore, it was expedient to establish a reliable assessment of the state of drinking water in Kherson (Ukraine) by means of biotesting using onions *Allium cepa* L (Table 3). According to the testing of drinking water using the test object *Allium cepa* L., was observed that the highest index of drinking water toxicity at the I test site. Germination of onion roots in all tested water samples throughout the experiment had inhibited growth. The length of onions in drinking water at the V test site had low values relative to the control sample. Only one sample out of five had a clear germination of onion roots. Drinking water is rated as highly toxic.

Progressive germination of onion roots was observed in the tested samples of drinking water at the VI test site, and green shoots appeared on the first day of the experiment. According to the toxicity index, water is toxic in the samples of

Table 2. Assessment of the level of drinking water toxicity by daphnia

Test site	Arithmetic mean survival rates of daphnia, hours							I_t	Level of toxicity
	1	6	24	36	48	72	96		
I test site residential area - multi-storey buildings	10	10	10	10	9	9	9	10	Permissible degree of toxicity
II test site - industrial zone	9	9	6	5	4	4	3	70	Strongly toxic
III test site - residential area mixed development	10	9	9	9	8	8	7	30	Toxic
IV test site – transport loaded area	10	10	10	9	9	7	6	40	Toxic
V test site - area of the historic center	9	8	8	3	3	3	1	90	Highly Toxic
VI test site - industrial zone with local water supply system	10	10	9	9	9	9	9	10	Permissible degree of toxicity
VII test site - area of individual construction	10	10	9	8	8	8	8	20	Permissible degree of toxicity
Control sample	10	10	10	10	10	10	10	0	Non-Toxic

the IV test site, inhibition of root growth of the test object was observed. One in five had the growth of green shoots. Three test samples of drinking water had the same root germination, but they had a tortuous shape. In this test site, the toxicity index of drinking water ranges from 20-50, so the water is rated from toxic to highly toxic. Three test drinking samples at the III test site had a clear and uniform germination of onion roots. Germination of green shoots was observed in four samples. Water from underground sources of this landfill according to the results of biotesting on onions is high quality.

Slow and inhomogeneous germination of onion roots was observed in drinking water samples of the II test site. One sample showed the germination of green shoots 2.5 cm long. According to the toxicity index, drinking water was assessed as toxic. In VII test site, four samples of onions had a clear germination of roots. The appearance of green shoots with a length of 9, 5, 9, 8, 6 (cm) was observed. The obtained value of the length of the onion roots is close to the control sample of drinking water. Water is rated on the toxicity index as non-toxic. Homogeneous and clear root germination was noted in the control sample. In four samples there was growth of the green shoots, 7 cm long, and 5 pieces. Drinking water is of excellent quality. According to the results of the study, the intra-territorial zoning of the studied urban area according to the index of drinking water toxicity was carried out (Fig. 3).

The ambiguity of the obtained results of drinking water quality assessment by the values of daphnia survival and germination of onion roots showed that living organisms, depending on the level of their organization, react differently to the content of pollutants in drinking water. Therefore, when establishing an integrated environmental assessment of drinking water, the reactions of various test objects are comprehensively taken into account (Table 4). The low-quality drinking water was observed at I, IV, V test sites, toxic properties were characterized by drinking water at II, VII test sites, quality drinking water by biotesting - at III and VI test sites. Based on the test results using both biological indicators, the zoning system of the city of Kherson (Ukraine) was zoned according to the generalized index of drinking water toxicity. The account of the basic features of adaptation processes, level of sensitivity, and resistance of test organisms at carrying out biotesting should be carried out taking into account the established terms and time of testing.

At the same time there is an expediency of carrying out the general assessment of toxicity of waters on the content of pollutants. In this case, taking into account the changes in the

Table 3. Biotesting of drinking water in Kherson (Ukraine) by test object *Allium cepa L.*

Test Site	The total number of roots, pcs.	Average length of roots, cm (M)	Relation to control (%)	Toxic index I_t
I test site - residential area multi-storey buildings	40	0,36	14	85,6
II test site - industrial zone	57	1,70	68	32,0
III test site - residential area- mixed development	77	2,20	88	12,0
IV test site - transport loaded area	38	1,20	48	52,0
	78	2,00	8	20,0
V test site - historic center area	41	0,66	26	73,6
VI test site - an industrial zone with a local water supply system	124	2,40	96	4,0
VII test site - a zone of individual construction	92	1,90	76	24,0
Control Sample	72	2,50	-	0

Table 4. Comprehensive toxic and ecological assessment of drinking water in Kherson (Ukraine)

Test site	I_t (by *Daphnia magna straus*)	I_t (by *Allium cepa L.*)	I_t (Average)	Toxico-ecological assessment of drinking water
I test site – a residential area of multi-storey buildings	10	85	48	Toxic
II test site – industrial zone	70	32	51	Highly Toxic
III test site – residential area of mixed development	30	4	17	Non-Toxic
IV test site – transport loaded area	60	20	40	Toxic
V test site – historic center area	90	73	80	Highly Toxic
VI test site – an industrial zone with a local water supply system	10	4	7	Non-Toxic
VII test site – a zone of individual construction	20	24	22	Toxic
Control Sample	0	0	0	Non-Toxic

Table 5. Bioindication classification scale for assessing water sources by pollution classes

Test site	Toxicity Index, I_t	Toxicity rating	Water quality class	Characteristics of the level of pollution
Residential area with multi-storey buildings	48	Toxic	III	High
Industrial area	51	Highly Toxic	III	High
Residential area with mixed buildings	17	Non-Toxic	I	Weak
Transport loaded area	40	Toxic	II	Moderate
City area and historical center	80	Highly Toxic	IV	Highly dangerous
Industrial zone with local water supply system	7	Non-toxic	I	Weak
Residential area with a standalone buildings	22	Toxic	II	Moderate

reaction of daphnia and onions in drinking water samples, the level of toxicity of drinking water at certain territorial landfills was determined. Due to the use of several test objects in the bioindication system for determining the quality of drinking water, the issue of developing a single scale for biological assessment of aquatic toxicity is relevant. According to the gradation scale of toxicity and drinking water quality classes, the level of drinking water pollution was set for each test site (Table 5).

The variability of toxic-ecological assessment according to the reactions of test organisms, bioindication differentiation of pollution level was established, which characterizes the ecological state of groundwater sources and their compliance with the established requirements for household and drinking needs. Thus, the transport load zone (IV test site) is a transitional zone between moderate and high levels of drinking water pollution. While drinking water taken from the residential area of individual buildings (VII test site) is in the range of pollution from moderate to weak. Extremely dangerous level of pollution is characteristic of the functional zone of the city and historical center (V test site). Intensive anthropogenic pressure on the territory, location of administrative institutions, the presence of dense multi-storey buildings, and the development of transport infrastructure led to unsatisfactory environmental conditions, which create conditions for the formation of poor-quality drinking water of the test site. Bioindication assessment of drinking water allows us to determine the consequences of systematic and volley pollution of water sources by the reaction of test objects of the average "pollution effect" over time. In addition, biological methods provide an assessment of the intensity of the processes of self-healing of groundwater after the action of the pollutant.

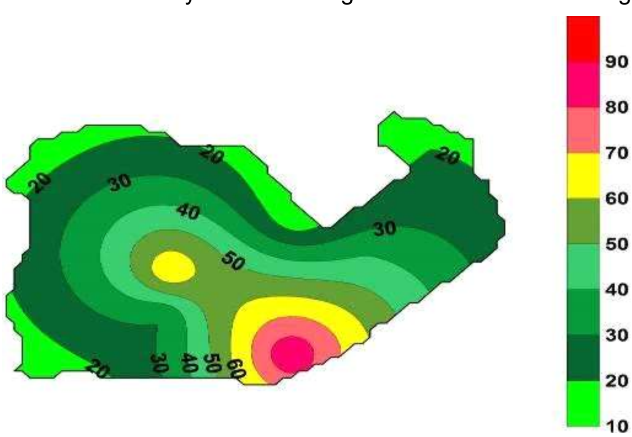

Fig. 2. Zoning of the urban system of the city of Kherson (Ukraine) according to the toxicity index (test object Daphnia magna straus)

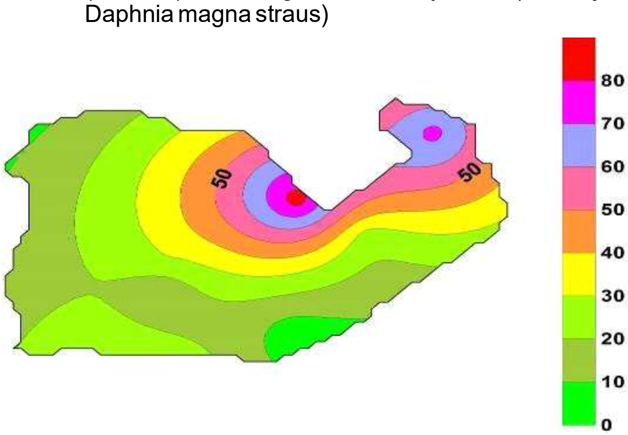

Fig. 3. Zoning of the urban system of the city of Kherson (Ukraine) according to the index of drinking water toxicity (test object *Allium cepa* L)

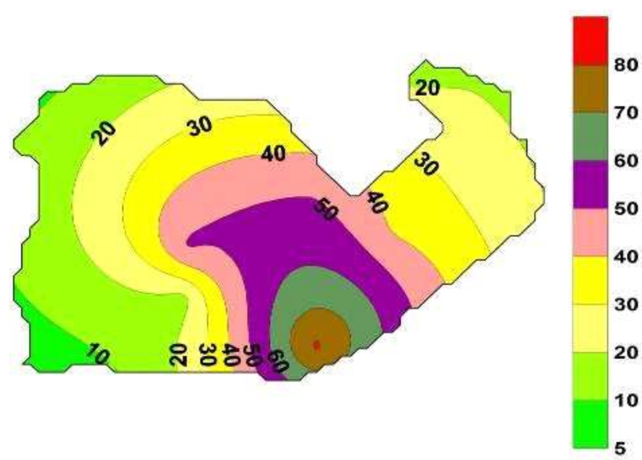

Fig. 4. Zoning of the urban system of the city of Kherson (Ukraine) according to the generalized index of drinking water toxicity

CONCLUSIONS

The biotesting is a fast and reliable method of assessing the quality of drinking water, which should be used for systematic environmental control over the quality of water supply sources. Assessment of drinking water toxicity was made based on morphological changes, survival rates of daphnia and biometric indicators of germinated onions. It was established that low-quality drinking water was present at II, V test sites, toxic properties of drinking water at I, IV, VII test sites, quality drinking water according to the results of biotesting was found - at III and VI test sites.The general index of drinking water toxicity for all studied test sites was calculated, according to which the intra-territorial zoning of the studied urban area was carried out. According to the conducted ecotoxicological and bioindication assessment of the state of drinking water sources, the level of drinking water pollution was determined at all test sites in the city of Kherson (Ukraine). The use of bioindication research will contribute to the development of scientifically sound measures to improve the quality of drinking water within the studied urban area.

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