UDC 577.34:574.64:504.062

Toxicity assessment of waters of surface sources of water service on Allium cepa L. with the use of digital-photo-chromium-analysis

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The purpose. To justify expediency of use of factor of chlorophyll pigment (FCP), determined in onion leaves (A. cepa) by means of digital-photo-chromium-analysis (DPCA), for biotesting toxicity of waters of surface sources of water service. **Methods.** Biological testing (Allium-test), technique of DPCA by FCP, correlation and regression analysis. **Results.** Modification of Allium-test due to determination of FCP in leaves of bulbs instead of measurement of length of their little roots for assessment of quality of waters has been entirely justified and has confirmed presence of reliable rectilinear link between these indexes, and also the values of indexes of toxicity calculated on their basis (higher, than 50%). **Conclusions.** In the researches the opportunity is confirmed of biotesting surface sources of water service with the use of FCP, determined on the 8th day using DPCA technique in leaves of onion (A. cepa) which has appeared in conditions of CE «Zhytomyrvodokanal» more sensitive (index of toxicity in group D1 — 55,96, D2 — 53,18%) to pollution in short-term chronic experiment in comparison to index of length of rooted bunch (54,72 and 52,07% accordingly). In spite of the fact that use of DPCA demands significant material inputs, the offered technique is more informative (on the average on 4,12%) and less labor-consuming (on the average on 44,86%) than measurement of little roots at each bulb with much longer processing the gained data. To determine FCP in leaves of onion it is possible not only for assessment of quality of water in conditions of water canals and sewage disposal plants, but also for optimization of water treatment with application of disinfectants and other reagents.

Key words: *biotesting, Allium-test, index of toxicity, length of rooted bunch, factor of chlorophyll pigment, chronic toxic effect.*

https://doi.org/10.31073/agrovisnyk201807-10

Water pollution of surface water sources from year to year becoming more dangerous due to climate change, maintaining the concentration of toxic substances in the water with the increase in average temperature, and with the progressive increase in anthropogenic load on water bodies, particularly drains unauthorized [1]. These water quality problems that its purpose must be sufficiently clean, occur in Ukraine against the background of obsolete regulatory indicators, which allow for uncomfortable conditions for the existence of many species of aquatic and do not meet EU standards [1-2]. Therefore, along with analytical methods for the determination of the state designated waters relevance becomes use of biological testing, biomonitoring method that allows you to quickly and efficiently establish the toxicity level of the water in relation to the biota [3-7].

As shown by many researchers conducted an analysis of indicators used in biomonitoring research, special attention concerning the quality of surface water organisms deserve biochemical reactions that are among the most sensitive to the action of pollutants [7-9]. For general toxicity bioassay water on plant organisms most common biochemical test reaction is intensity or accumulation of chlorophyll content in chloroplasts. For such bioassay, lower (phytoplankton) and higher (daisy) aquatic plants are used. In addition, the technique of biotesting on the content of chlorophyll can be improved due to its definition in the sheets of higher terrestrial plants, which traditionally conducted testing of waters of different origins [8, 9]. As test objects, they are much more convenient and cheaper than aquatic plants, do not require cultivation costs, etc. These advantages are the usual onion *Allium cepa* L., which bulbs can be stored at appropriate temperature, humidity and light conditions for laboratory studies throughout the year.

To apply a test reaction to the accumulation of chlorophyll in the leaves of the onion for the detection of water toxicity, it is necessary to modify the well-known Allium test [10], obtaining for biotesting not only the formed root system, but also sprouted leaves of the bulbs. That is, to conduct an experiment in which the difference between the accumulation of chlorophyll in the leaves of the bulbs exposed during the exposure in contaminated and pure water will be detected. In addition, the chlorophyll content can be determined by applying an alternative approach - using digital-photo-chrome analysis on the chlorophyll coefficient (C*, %) [9], which will reduce the labor intensity and time of the experiment compared with analytical methods.

Taking into account the foregoing, the **purpose** of the research was to substantiate the feasibility of using the chlorophyll coefficient defined in the leaves of common bulb *(A. cepa)* using digital-photo-chroma-analysis (DPCA) for the biotesting of water toxicity of surface water sources.

Materials and methods of research. Experimental data concerning the water toxicity of surface water sources in Zhytomyr were obtained by methods of bioassay on the onion of the standard Stuttgart Rizen variety, including those proposed by us [3-5, 8-10]. The bulbs used in the study were similar in size (d = 1.5±0.5 cm). To determine the quality of water, three groups were formed - control and two experimental (n=20). Samples of water were taken in September 2014 in the amount of 1 dm 3 per group once a day using generally accepted techniques [3]. The bulbs were immersed in the test tube with water for 8 days. Normal onion growth rates were measured by measuring the length of the root and adult leaves, and the accumulation of chlorophyll on the leaves of onion was determined using a digital-photo-chrome-analysis (DPCA) [9] upon completion of the studies. Testing of water samples in triple repetition was carried out with daily replacement of used water to water of appropriate quality.

The research was carried out according to the following scheme:

- *Controlling group* – samples of dechlorized (24 h) water.
- *Researched group D1*: samples of water – from Denyshy reservoir.
- *Researched group D 2:* samples of water – from Vidsichne reservoir.

Biotesting – by the chlorophyll coefficient (C*, %) in onion leaves on the 8th day of research.

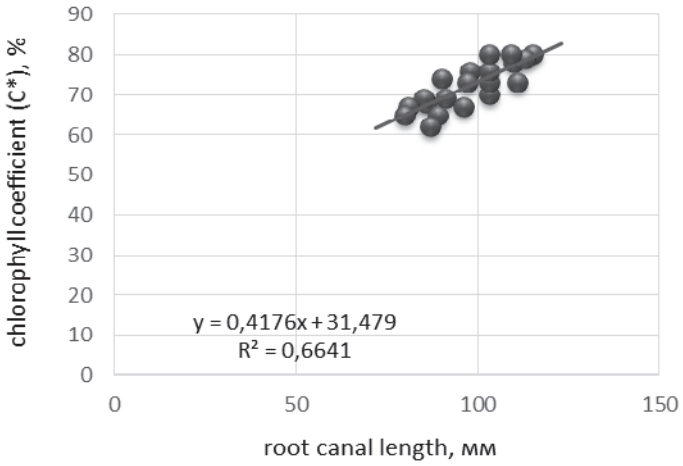
Test-objects: onion common bulbs *(A. cepa)***.**

The water toxicity index, which should not exceed 50%, was calculated based on the results of the test, using the generally accepted formula [6].

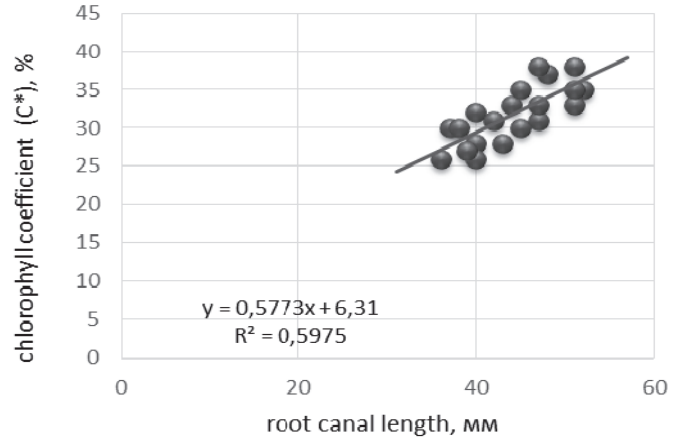
Results of research. To characterize the chlorophyll content in chloroplasts of cells, the chlorophyll coefficient (CC) is a special value, which is used to determine the degree of absorption of the plant surface of the blue and red components of the light spectrum [7]. The definition of the CC with the help of DPCA allows to avoid mistakes in the subjective perception of color by the human eye for uneven lighting, and to quickly evaluate the individual parts of plants (leaf, stem, shield, etc.) and plant groupings in a certain territory or water area [9].

Detection of the difference in the coloration of plants using the technology of DPCA can be used to compare contaminated and pure water on the common onion *(A. cepa)*. Thus, in waters of superficial water sources in Zhytomyr, some pollutants were in concentrations that inhibited photosynthetic processes in the onion leaves, resulting in lower chlorophyll coefficients in the experimental groups than in the control (Fig.1).

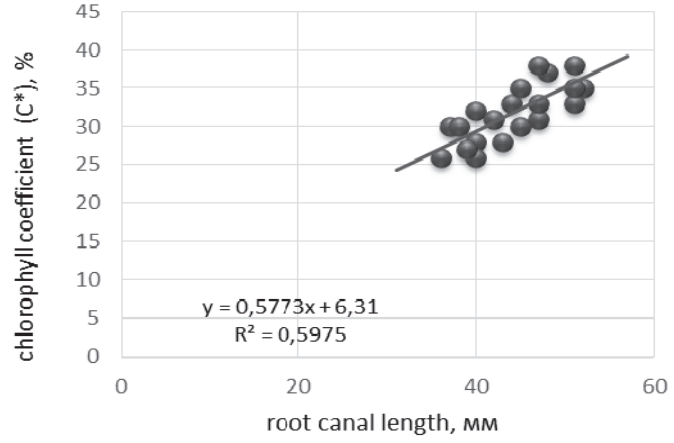
a



b



C



The equation of rectilinear regression between the length of the onion root bowl and the chlorophyll coefficient in its leaves on the 8th day of research on biotesting of water samples in the following groups: a - control, b - from Denyshy reservoir, c - from Vidsichne reservoir of Teteriv River.

In order to determine the appropriateness of the use of CC instead of the test-response of root growth, which was fixed in the onion, common for the biotesting of water quality, regression and correlation analyzes

were conducted in which a straightforward regression and a high probable correlation between the indicated parameters were found. That is, it has been shown that the value of the chlorophyll coefficient in the leaves of the bulb significantly depends on the length of its root beam (the greatest strength of influence we found in the control - 66.41, in the experimental groups D1 - 59.75 and D2 - 59.98%).

Consequently, according to the data obtained, the faster the growth of the root system of the bulbs occurred, the more chlorophyll was synthesized in the growing leaves from the moment they appeared and until the end of the research on the 8th day. In addition, the toxicity indexes of the experimental waters determined for CC, though slightly, however, were higher than the indices determined by the indicator of the length of the root beam, as shown in Table:

Water toxicity indicators of surface water sources in Zhytomyr and the correlation between the length of the onion root with the chlorophyll coefficient in its leaves (n=20)

Indexes	Researched groups		
	K (settled tap water)	Д-1 (Denyshy reservoir)	Д-2 (Vidsichne reservoir)
IT_{lrb} , % DDI, h	- 6,0	54,72 6,0	52,07 5,8
IT_{cc} , % DDI, h	- 3,1	55,96 3,5	53,18 3,2
Correlation coefficient (r)	0,8149 ± 0,1366**	0,7730 ± 0,2470*	0,7745 ± 0,2327*

Notes:

IT – toxicity index; DDI – duration of determination of index; lrb – length of the root bow of onion; CC – chlorophyll coefficient in the leaves of onion;

probability of correlation connection: ** – $p \leq 0,001$; * – $p \leq 0,01$

The obtained data testify in favor of application in the biological testing of water toxicity by the chlorophyll coefficient determined in the onion leaves, which is an indicator more sensitive to the action of pollutants than the length of the roots. Although DPCA requires somewhat higher material costs, since the price of the software is more than 60 thousand UAH. and tends to increase in contrast to the measurement using the ruler of the onion roots. However, the technology used compared to the existing one is more informative and less labor-intensive, does not require long processing of the received data, and expenses for it are one-time.

Consequently, the modification of the Allium test by calculating the chlorophyll coefficient instead of measuring the length of the roots to assess the quality of the low contaminated water is fully justified and confirmed by the presence of a reliable straightforward link between these indicators, as well as the values of the toxicity indexes of the experimental waters that were found on their basis more than 50%.

Conclusions

The conducted researches confirmed the possibility of biotesting of water from surface water sources using the chlorophyll coefficient determined on the 8th day by the technology of DPCA in the leaves of common bulb *(A. cepa)* which was more sensitive in the conditions of Municipal Enterprise Zhytomyrvodokanal" (toxicity index 55.96 U group D1 and 53.18% in group D2) to contaminations in the short-term chronic test compared with the index of root canal length (54.72 and 52.07% respectively). Despite the fact that the use of DPCA requires significant material costs, the technology used is more informative (an average of 4,115%) and less labor-intensive (44,86% on average) than the measurement of the roots of each bulb with the long processing of the data received.

Determination of the chlorophyll coefficient in the onion leaves can be used not only for assessing the quality of water in the conditions of water and wastewater treatment plants, but also for optimizing water treatment using disinfectants and other reagents.

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