S. M. Kvaternyuk, Cand. Sc. (Eng.); Assistant Professor

MULTISPECTRAL MEASUREMENTS OF THE PIGMENTAL PARAMETERS OF THE PHYTOPLANKTON IN AQUEOUS MEDIA

The increase of the indirect measurement accuracy of phytoplankton pigment parameters in natural aquatic habitats is necessary for the solution of the problems of ecological monitoring of water objects, their ecotoxicological control as well as multiparametric control of water quality. Applying the technique of the mathematical modeling of light scattering in small angle approximation in multilayer nonuniform media, the direct problem of determination the spectral characteristics of natural aquatic habitats on changes of the pigment parameters of phytoplankton is solved. In the given research, the process of the in direct measurement of the phytoplankton pigment parameters in aquatic habitats by means of multispectral method is studied, the regression equations are obtained, enabling to determine the relation between the chlorophyll **a** *and general chlorophyll, as well as the relation between the carotinoids and general chlorophyll. For this purpose, the procedure of multiregression with step-by-step inclusion of the independent variables is used. The analysis of the methodical and instrumental errors of the phytoplankton pigment parameters measurements in aquatic habitats is performed, using in multispectral device of ecological measuring control light emitting diodes, laser diodes and monochromator as the radiation source. Optimal variants of the realization of the means of multispectral ecological control of pigment parameters of the phytoplankton in the aquatic habitats is chosen, depending on the cost of their realization and general measurement error. In the process of measurement the relation between the chlorophyll* ***a*** *and general chlorophyll of the phytoplankton in the aquatic habitat, the least general error of 0.381% was obtained for the 6-channel device of multispectral measuring control with the width of spectral range in each channel of 20 nm. In the process of measurement the relation between carotinoids and general chlorophyll of the phytoplankton in the aquatic habitat 5 channel device with the width of spectral range in each channel 20 nm was chosen to be an optimal variant of the device for multispectral ecological control, it enables to obtain general measurement error of not more than 0.486%.*

Key words: *multispectral method, aqueous media, spectral characteristics, phytoplankton, pigments.*

Introduction

Improved multispectral method of ecological control of natural aquatic habitats comprises the analysis of the digital images of the aqueous objects, obtained in several spectral ranges [1 – 5]. The given method can be used for the indirect measurement of structural and pigment parameters of the phytoplankton. In the process of studying the array of multispectral images the images of the objects of control in the reflected light as well as fluorescent images can be used [4]. Study of phytoplankton concentration in aqueous objects is widely used in satellite monitoring facilities [5], which enable to monitor rapidly the changes in large water bodies. Besides multispectral cameras multiwave lidars are also used for this purpose [6]. The importance of the subject is stipulated by the necessity to study the impact of toxic and biogenic substances on the state of phytoplankton in naturalaquatic habitats, constant increase of the accuracy of the ecological control of the dangerous waste impact onwater bodies, by means of bioindetification by the phytoplankton for the substantiation of the corresponding measures of ecological safety. One of the important factors, negatively influencing the quality of the surface water is their cultural euthrophication, that unlike natural one is the results of human activity and consists in rapid increase of the water body trophicity due to the arrival of the biogenic elements and organic substances, amount of which greatly exceeds the normal natural levels.

The importance of the problem is stipulated by the necessity to improve the accuracy of the indirect measurements of the phytoplankton pigment parameters in natural aquatic habitats for the solution of the problems dealing with the ecological monitoring of the water objects, their ecotoxicological control and multiparametric control of water quality.

The aim of the research is the improvement of the multispectral method of the indirect measurement of phytoplankton pigment parameters in natural aquatic habitats.

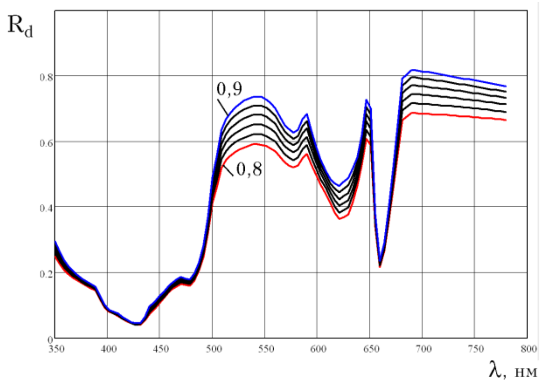
Study of the process of the indirect measurement of the phytoplankton pigment parameters in aquatic habitats by means of multispectral method

Applying the technique of the mathematical modeling of light scattering in small-angle approximation in multilayer non-uniform media [7, 8], we will solve the direct problem of the determination of natural aquatic habitats spectral characteristics with the known parameters of the phytoplankton for two cases of the phytoplankton pigment parameters change, namely:

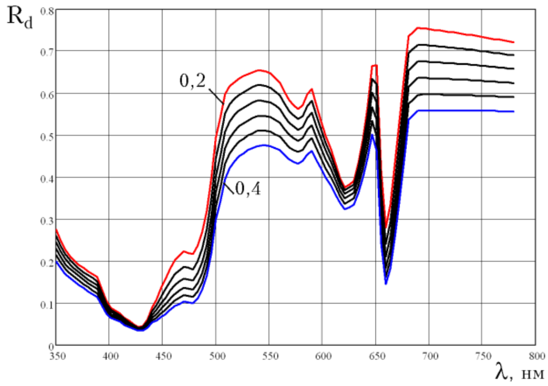
a) relation between chlorophyll ***a*** and general chlorophyll of the phytoplankton in aquatic habitats varies from 0.8 to 0.9; relation between carotinoids and general chlorophyll remains unchanged 0.27;

b) relation between chlorophyll ***a*** and general chlorophyll of the phytoplankton in aquatic habitat remains unchanged 0.8; relation between carotinoids and general chlorophyll varies from 0.2 to 0.4.

In each of the examples the biomass of the phytoplankton in aqueous medium is 17.7 mg/l, the content of the chlorophyll ***a*** in the green weight of the phytoplankton we assume to be 0.5%. Spectral characteristics of the absorption factor, scattering factor and anisotropy factor for aqueous medium with out the phytoplankton are introduced in the model on the base of the approximation by the results of the experimental research. Spectral characteristics of the absorption factors of the main pigments of the phytoplankton are introduces in the mathematical model, using linear interpolation in Matchcad 13.0 on the basis of the table of the experimental data of measurements [9]. Fig. 1 shows the calculated spectral characteristics of the diffuse reflection factor on the surface of the aquatic habitats if the pigment parameters of the phytoplankton change.



a)

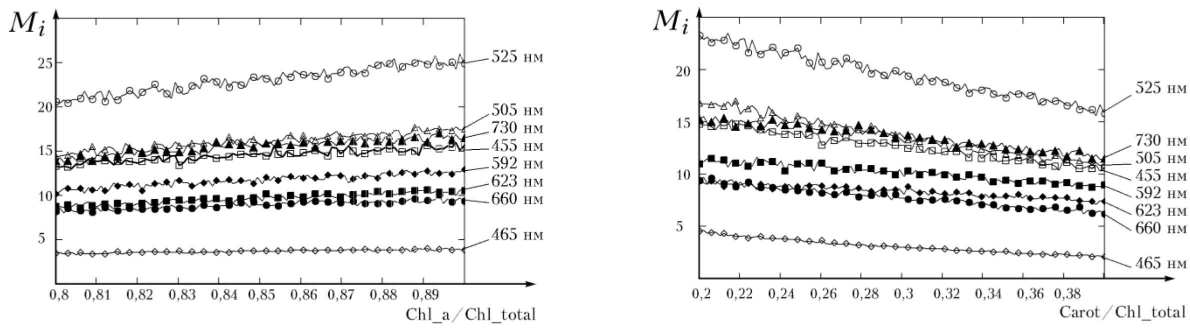


b)

Fig. 1. Spectral characteristics of the diffuse reflection factor on the surface of the aquatic habitaton the change of the phytoplankton pigment parameters: a) relation between the chlorophyll ***a*** and general chlorophyll from 0.8 to 0.9; b) relation between the carotinoids and general chlorophyll from 0.2 to 0.4

When multispectral method of measurement with narrowband sources of radiation and wideband CID camera in each pixel of the image is used, then multispectral parameters, determined by spectral characteristics of the radiation source, CID camera sensitivity and diffuse reflection factor of the investigated object will be obtained [10].

The line of the light emitting diodes, line of laser diodes and tunable monochromator with a slot, providing the bandwidth of 5, 10 and 20 nm are used as the radiation sources. As CID camera MDC140BW with such parameters will be used: separating power 1.3 Mp, spectral range 350 – 1000 nm, dynamic range 66 dB. Spectral characteristics of the radiation sources and digital camera are given in the previous work [10]. The results of the calculation of multispectral parameters by the known spectral characteristics on the change of the pigment parameters of phytoplankton and usage of the radiation source on the base of light emitting diodes line are given in Fig. 2.



a) **b)**

Fig. 2. Results of the calculation of multispectral parameters by the known spectral characteristics on the change of the phytoplankton pigment parameters and usage of the radiation source on the base of the line of light emitting diodes: a) relation between the chlorophyll ***a*** and general chlorophyll from 0.8 to 0.9; b) relation between the carotinoids and general chlorophyll from 0.2 to 0.4

Determination of the phytoplankton pigment parameters in natural aquatic habitats, based on multispectral measurements is performed by means of multiple regression. Output data for performing multiple regression is the table where one of phytoplankton pigment parameters in natural aquatic habitat is the output (dependent) variable that change in certain range with the preset step in the process of mathematical modelling of spectral characteristics (solution of the direct optical problem) and multispectral parameters, obtained at the stage of modelling of multispectral telemeasurements are independent variables. The out put data for the determination of the relation between the chlorophyll ***a*** and general chlorophyll are shown in Table 1.

Table 1

Example of the output data for the indirect measurement of the relation between the chlorophyll *a* and general chlorophyll of the phytoplankton in aquatic habitat by multispectral parameters, applying multiple regression

Chl a/Chl	M_{455}	M_{465}	M_{505}	M_{525}	M_{592}	M_{623}	M_{660}	M_{730}
0.8000	0.8451	0.8423	0.8299	0.7707	0.8405	0.7347	0.8306	0.8152
0.8010	0.8240	0.8168	0.7785	0.7801	0.7911	0.7902	0.8426	0.8088
0.8020	0.8560	0.8509	0.7916	0.7950	0.7970	0.7682	0.8260	0.8501
0.8030	0.8094	0.8343	0.8065	0.8001	0.8373	0.7928	0.8412	0.8739
0.8040	0.8783	0.8163	0.7905	0.7886	0.8039	0.8156	0.8482	0.8515
0.8051	0.8546	0.8391	0.7973	0.8261	0.8136	0.8095	0.8530	0.8475
0.8061	0.8547	0.8604	0.7675	0.7916	0.8331	0.7931	0.8322	0.8068
…	…	…	…	…	…	…	…	…
0.8990	0.9852	0.9937	0.9546	0.9660	0.9474	0.9622	0.9702	0.9937
0.9000	0.9656	0.9338	1.0000	0.9645	0.9819	0.9567	0.9750	0.9983

Applying step-by-step regression we will analyze the independent variables, which enable to determine, in the most accurate way, the dependent variables– pigment parameters. Multiple regression procedure with step-by-step inclusion of the independent variables is used, the given procedure performs the selection of the independent variables at each step, adding or eliminating them from the model, taking into account the criterion, set by the user [12, 13]. On the basis of the data from the Table 1, the program performs the step-by-step multiple regression during 8 steps, gradually adding variables, taking into account their contribution into the accuracy of the determination of the preset parameter. The results of the calculations at each step of multiple regression are given in Table 2.

Table 2

Results of the calculation of multiple regression for the indirect measurement of the relation between the chlorophyll *a* and general chlorophyll of the phytoplankton in aquatic habitat with step-by-step adding of variables

N	λ, nm	F	Δ	R
1	623	917.5048	0.9149797	0.95052421
2	623; 505	809.8696	0.7037207	0.97133801
3	623; 505; 592	713.5499	0.6165290	0.97830397
4	623; 505; 592; 455	662.5289	0.5565090	0.98254419
5	623; 505; 592; 455; 525	609.5619	0.5201907	0.98492687
6	623; 505; 592; 455; 525; 660	569.3224	0.4922263	0.98665912
7	623; 505; 592; 455; 525; 660; 465	538.3920	0.4692637	0.98801336
8	623; 505; 592; 455; 525; 660; 465; 730	489.7288	0.4605149	0.98858490

In the process of multiple regression for the indirect measurement of the relation between the chlorophyll ***a*** and general chlorophyll of the phytoplankton in aquatic habitat for different variants of radiation sources such regression equations are obtained:

$$Chla/Chl_1 = 0{,}29787 + 0{,}16039M_{623} + 0{,}06336M_{505} + 0{,}19368M_{592} + \\ + 0{,}14549M_{455} + 0{,}17271M_{525} + 0{,}12526M_{660} + 0{,}10238M_{465} + 0{,}08851M_{730}, \quad (1)$$

$$Chla/Chl_2 = 0{,}33111 + 0{,}22585\mathrm{M}_{530} + 0{,}17719\mathrm{M}_{515} + 0{,}14727\mathrm{M}_{650} + \\ + 0{,}14142\mathrm{M}_{638} + 0{,}15618\mathrm{M}_{520} + 0{,}09231\mathrm{M}_{790} + 0{,}07662\mathrm{M}_{450} + 0{,}03137\mathrm{M}_{405}, \quad (2)$$

$$Chla/Chl_3 = 0{,}84395 + 0{,}81649\mathrm{M}_{525} - 0{,}45051\mathrm{M}_{660} - 0{,}20284\mathrm{M}_{420} + \\ + 0{,}72046\mathrm{M}_{680} - 0{,}3271\mathrm{M}_{490} + 0{,}36594\mathrm{M}_{355} - 0{,}81482\mathrm{M}_{740} + 0{,}82842\mathrm{M}_{690} - \\ - 0{,}13667\mathrm{M}_{350} - 0{,}61912\mathrm{M}_{775} + 0{,}51734\mathrm{M}_{760} + 0{,}17554\mathrm{M}_{570}, \quad (3)$$

$$Chla/Chl_4 = 0{,}83276 + 0{,}09019M_{630} - 0{,}21874M_{420} + 0{,}64834M_{510} - \\ - 0{,}48688M_{660} + 1{,}4328M_{680} - 0{,}10277M_{390} - 0{,}27049M_{430} - 1{,}12618M_{740} + \\ + 1{,}21003M_{700} - 0{,}23555M_{460} - 0{,}20729M_{370} + 0{,}17691M_{360}, \quad (4)$$

$$Chla/Chl_5 = 0{,}84915 + 1{,}68577\mathrm{M}_{530} - 0{,}82097\mathrm{M}_{670} - 0{,}5809\mathrm{M}_{430} - \\ - 0{,}17872\mathrm{M}_{410} + 1{,}0752\mathrm{M}_{510} - 0{,}26958\mathrm{M}_{470}, \quad (5)$$

where $Chla/Chl_1$ – is the relation between the chlorophyll ***a*** and general chlorophyll when light emitting diodes are used as the radiation source in multispectral device of measuring control; $Chla/Chl_2$ –are laser diodes; $Chla/Chl_3$ – monochromator, 5 nm; $Chla/Chl_4$ – monochromatora, 10 nm; $Chla/Chl_5$ – monochromator, 20 nm.

In the process of multiple regression for the indirect measurement of the relation between carotinoids and general chlorophyll of the phytoplankton in the aquatic habitat for different variants of radiation sources such regression equations are obtained:

$$Carot/Chl_1 = 0{,}86138 - 0{,}09823\mathrm{M}_{505} - 0{,}11656\mathrm{M}_{660} - 0{,}10233\mathrm{M}_{592} - \\ - 0{,}12034\mathrm{M}_{525} - 0{,}16144\mathrm{M}_{455} - 0{,}13776\mathrm{M}_{730} - 0{,}19058\mathrm{M}_{465} - 0{,}09215\mathrm{M}_{623}. \quad (6)$$

$$Carot/Chl_2 = 0{,}85369 - 0{,}22387\mathrm{M}_{450} - 0{,}2105\mathrm{M}_{515} - 0{,}117\mathrm{M}_{405} - \\ - 0{,}13637\mathrm{M}_{530} - 0{,}12124\mathrm{M}_{520} - 0{,}06657\mathrm{M}_{638} - 0{,}0938\mathrm{M}_{790} - 0{,}04778\mathrm{M}_{650}, \quad (7)$$

$$\begin{aligned}
&Carot/Chl_3 = 0{,}27766 - 1{,}55723M_{495} + 0{,}43216M_{435} - 0{,}90252M_{540} + \\
&+ 0{,}42295M_{630} + 0{,}63618M_{470} + 0{,}40105M_{615} - 0{,}68175M_{695} - 0{,}47237M_{385} - \\
&- 0{,}50621M_{395} + 0{,}4372M_{610} - 0{,}5911M_{535} - 1{,}05293M_{680} + 0{,}50642M_{780} + \\
&+ 1{,}34478M_{450} + 0{,}63184M_{415} + 0{,}84613M_{600} - 0{,}76279M_{575} + 1{,}0374M_{755} - \\
&- 0{,}55564M_{530} - 0{,}75484M_{725} + 0{,}2128M_{350},
\end{aligned} \tag{8}$$

$$\begin{aligned}
&Carot/Chl_4 = 0{,}31698 - 1{,}86827\mathrm{M}_{500} + 1{,}06961\mathrm{M}_{430} + 2{,}16797\mathrm{M}_{470} + \\
&+ 0{,}75255\mathrm{M}_{620} - 1{,}66055\mathrm{M}_{5500} - 0{,}68378\mathrm{M}_{390} - 1{,}54955\mathrm{M}_{670} + 0{,}83739\mathrm{M}_{780},
\end{aligned} \tag{9}$$

$$\begin{aligned}
&Carot/Chl_5 = 0{,}30716 + 1{,}06509\mathrm{M}_{490} + 1{,}8995\mathrm{M}_{430} - 3{,}44399\mathrm{M}_{510} + \\
&+ 1{,}37479\mathrm{M}_{630} - 1{,}82412\mathrm{M}_{690},
\end{aligned} \tag{10}$$

where $Carot/Chl_1$ – is the relation between the carotinoids and general chlorophyll, when light emitting diodes are used in multispectral device of measuring control as the radiation source; $Carot/Chl_2$ – are laser diodes; $Carot/Chl_3$ – is monochromator, 5 nm; $Carot/Chl_4$ – monochromator, 10 nm; $Carot/Chl_5$ – is monochromator, 20 nm.

Analysis of the methodical and instrument errors of pigment parameters of the phytoplankton measurement in natural water bodies

Methodical error of pigment parameters measurement δ_m of the phytoplankton in natural water bodies, applying multiple regression is determined by the degree of accuracy the obtained regressive equation reflects the dependence between the dependent variable – pigment parameters of the phytoplankton in the aquatic habitat (in the given research by the relation between the chlorophyll ***a*** and general chlorophyll and the relation between the carotinoids and general chlorophyll) and the results of multispectral measurements. Values of the methodical error of measurement in the process of the indirect measurement of pigment parameters, obtained in the process of multiple regression and used in multispectral device of measurement control as the radiation source light emitting diodes, laser diodes and monochromator are written in Table 3.

We will analyze the instrumental component of the measurement error of the phytoplankton pigment parameters in natural water bodies, using CID camera of MDC140BW type on the base of Sony ICX285AL photomatrix with the digit capacity of 12 bit and signal/noise ratio of 66 dB. The error component, stipulated by available noise and random crosstalks in CID camera is [13]:

$$D_{s/n} = 20\,lg\left(\frac{A_{signal}}{A_{noise}}\right), \qquad \delta_{noise\,ccd} = 100\% / \left(10^{D_{s/n}/20}\right), \tag{11}$$

where $D_{s/n}$ – is signal/noise ratio, A_{signal} – is root mean square value of signal amplitude, A_{noise} – is root mean square value of noise and random crosstalks amplitude in CID camera.

Quantizing error $\delta_{ADC\,ccd}$ in case of a large number of digits may be described by the equi-partition law, that corresponds to the equal density of the quantizing error probability within the limits of $\pm h_k/2$, where h_k – is the quantizing step [14]:

$$\Delta_{ADC\,ccd} = \pm\frac{u_{ref}}{2^n - 1}, \qquad \delta_{ADC\,ccd} = \frac{\Delta_{ADC\,ccd}}{u_{ref}} \cdot 100\%, \tag{12}$$

where u_{ref} – is the reference voltage, n – is digit capacity of ADC.

Root-mean-square value of the quantizing error is [14]:

$$\delta_{SD\,ADC\,ccd} = \frac{\delta_{ADC\,ccd}}{2\sqrt{3}}. \tag{13}$$

We will determine the random component of the instrumental error of measurement on condition that the quantizing errors and errors, stipulated by random crosstalks are statistically independent:

$$\delta_{rand.Mi} = \sqrt{\delta^2_{noise.ccd} + \delta^2_{SD\,ADC\,ccd}}. \tag{14}$$

Random component of the instrumental error for the indirect measurement of the phytoplankton pigment parameters is determined by random components of measurement error in each of the spectral channels that make part of the general regression equation, as well as components, that take into account correlation connection between multispectral parameters [15, 16]:

$$\delta_{\text{instr.}} = \sqrt{\sum_{i=1}^{N} \delta^2_{rand.Mi} + 2\sum_{i=1}^{N}\sum_{j<i} R_{ij}\delta_{rand.Mi}\delta_{rand.Mj}}, \tag{15}$$

where $\delta_{rand.Mi}$, $\delta_{rand.Mj}$ – is random component of the error in i^{th} and j^{th} channel; R_{ij} – is the correlation factor between multispectral parameters, obtained after multiple regression; N – is total number of channels.

When CID camera of MDC140BW type, based on Sony ICX285AL photomatrix, with digit capacity of 12 bits and signal/noise ratio of 66 dB is used, the error component, stipulated by available noise and random crosstalks in CID camera is not larger than $\delta_{rand.Mi} = 0.0502\%$ [16].

Total measurement error of the phytoplankton pigment parameters of aquatic habitat will be determined by the sum of the instrumental and methodical errors:

$$\delta_{genN} = \delta_{\text{instr.}} + \delta_m. \tag{16}$$

The results of the instrumental and total errors calculation during the indirect measurement of the pigment parameters of the phytoplankton of the aquatic habitat are written in Table 3.

Table 3

Results of the errors calculation in the process of indirect measurement of the pigment parameters of the phytoplankton in aquatic habitat

Radiation source	Number of spectral channels	Methodical error, %	Instrumental error, %	Total error, %
Relation between chlorophyll **a** and general chlorophyll of the phytoplankton in the aquatic habitat				
Light-emitting diodes	8	0,461	0,4	0,861
Laser diodes	8	0,365	0,4	0,765
Monochromator, 5 nm	12	0,115	0,6	0,715
Monochromator, 10 nm	12	0,110	0,6	0,71
Monochromator, 20 nm	6	0,126	0,255	0,381
Relation between the carotinoids and general chlorophyll of the phytoplankton in aquatic habitat				
Light-emitting diodes	8	0,403	0,353	0,756
Laser diodes	8	0,414	0,4	0,814
Monochromator, 5 nm	21	0,226	0,261	0,487
Monochromator, 10 nm	8	0,293	0,187	0,48
Monochromator, 20 nm	5	0,320	0,166	0,486

Conclusions

As a result of studying the process of the indirect measurement of phytoplankton pigment parameters in aquatic habitats regressive equations were obtained applying multispectral method, they enable to determine chlorophyll ***a*** and general chlorophyll ratio as well as carotinoids and general chlorophyll of the phytoplankton ratio in aquatic. In the process of step-by-step multiple regression the value of the methodical error of the phytoplankton pigment parameters measurements and the amount of the spectral channels of multispectral measuring control devices, depending on the type of radiation sources are obtained. On the base of the obtained number of channels and instrumental error in each channel the values of instrumental and general errors of the phytoplankton pigment parameters measurements are obtained. Variants of the realization of multispectral measuring control will differ greatly by the cost, depending on the number of spectral channels and ways they are created. For instance, in the process of measurement of thechlorophyll**a**andgeneralchlorophyllofthephytoplanktonratiointheaquatic habitat the least general error of 0.381% is obtained for the 6-channel device of multispectral measuring control with the width of spectral range in each channel of 20 nm. When 12-channel device with the width of spectral range in each channel of 5 nm is used the value of the methodical error decreases, however, as a result of the increase of the total number of channels the instrumental error increases, that gives total error of 0.715%, that is considerably greater than the value for the 6-channel device. It is obvious, that the cost of 6-channel device with the width of the spectral range in each channel of 20 nm will be far less than the cost of 12-channel device with the width of the spectral range of 5 nm. In the process of measurement of the carotinoids and general chlorophyll of the phytoplankton ratio in aquatic habitat the general error of measurements does not practically change, if 21-channel device with the width of the spectral range in each channel of 5 nm is used – 0.487%, 8-channel device with the width of spectral range of 10 nm – 0.48% and 5 channel device with the width of the spectral range of 20 nm – 0.486%. That is why, optimal variant for the realization of the device for multispectral ecological control of the relation between carotinoids and general chlorophyll of the phytoplankton in aquatic habitats will be5 channel device with the width of the spectral range in each channel of 20nm.

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Kvaternyuk Sergiy – Candidate of Science (Engineering), Assistant Professor with the Department of Ecology and Ecological Safety.
Vinnytsia National Technical University.