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MULTISPECTRAL CONTROL OF PESTICIDE CONCENTRATIONS IN AQUATIC ENVIRONMENTS USING BIOINDICATION ON PHYTOPLANKTON

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Abstract. The aim of the work is to improve the method and means of multispectral measurement of the content of unsuitable chemical plant protection products in aqueous media using bioindication on phytoplankton. The article analyzes the errors of indirect measurements and the reliability of the control of the concentration of pesticides using the developed means of environmental monitoring of integral parameters of pollution of water bodies.

Key words: ecological monitoring, multispectral measurements, water, phytoplankton, bioindication.

Introduction

The quality of surface water is an important problem of environmental safety in Ukraine. As a result of the activities of agricultural enterprises, a significant number of plant protection chemicals get into water bodies with surface runoff. Ecological control of water bodies pollution is an actual task that can be solved both by methods of physicochemical analysis of samples and by methods of biotesting. Methods of physicochemical analysis of environmental samples, allow component-wise determination of the concentration of each pollutant, but require large material and financial costs for their implementation. At the same time they do not allow to take into account the complex, synergetic and antagonistic effect of the influence of pollutants on the aquatic ecosystem. In contrast, with the help of bioindication on various aquatic organisms, it is possible to evaluate the complex effect on aquatic ecosystems and to carry out integrated monitoring of water pollution [1]. In particular, monitoring of the state of natural water bodies can be carried out on the basis of bioindication on phytoplankton. An automated system for studying phytoplankton parameters based on the recognition of phytoplankton particles in a flow measuring cell, based on an analysis of the geometric parameters of these particles, was proposed in [2]. In [3] the multispectral method is used for remote satellite environmental monitoring of phytoplankton content in water bodies, which allowed analyzing the spatial distribution of phytoplankton concentration in water bodies with high resolution. The choice of characteristic wavelengths for the study of phytoplankton samples of water bodies is determined by the spectral dependences of the relative efficiency of phytoplankton pigment absorption [4]. To investigate the contamination of water bodies with hazardous waste components, as well as assess the toxicity of water, specialized biosensors have been developed to detect heavy metal ions, unsuitable pesticides and other toxic substances in aqueous media [5–7].

Materials and methods

Systems of multispectral ecological monitoring of parameters of aqueous media basically have general principles of scattering and absorption of light by inhomogeneous biological media. Particles of phytoplankton have certain features of the shape, structure and spectral properties of pigments, which allows to calculate the resulting optical characteristics of reflection and transmission of light radiation, affecting the formation of multispectral images. When solving the inverse problem, the parameters of phytoplankton (for example, the volume concentration of particles) in the near-surface layer of the aquatic medium are determined by means of multiple regression on the basis of the obtained array of multispectral images of the surface of the water body.

The method of multispectral television measuring control of the ecological state of water bodies is known based on phytoplankton parameters, which consists in sampling phytoplankton, determining the qualitative and quantitative composition of microalgae cells, comparing the obtained data with the normalized values. When sampling and determining the qualitative and quantitative composition of microalgae, a flow-through multispectral television measurement assay of continuous-acting phytoplankton particles is used, in which the image of particles in a flow measuring cuvette is obtained from the characteristic wavelengths of phytoplankton pigments by means of a microscope and a television CCD camera with images from the database particles of phytoplankton of certain types in a specialized processor in real time with the use of the optimal Bayesian classifier with a solving function based on the Mahalanobis distance, the absolute and relative number of phytoplankton particles of each species that are present in the sample are determined, and the Simpson and Shannon indices are calculated and submitted to the indicator [8]. The disadvantage of this method is its focus on solving a highly specialized task of ecological monitoring of the state of water bodies in terms of phytoplankton parameters. In this case, the size and shape of the particles are used to automatically recognize the phytoplankton species.

The research is based on the task of improving the method of multispectral television control of parameters of inhomogeneous biological media, in which, due to the introduction of new operations and their sequence, the measurement accuracy ncreases. The objective is achieved by the fact that the method of multispectral television control of the parameters of inhomogeneous biological media, which includes sampling, determination of the qualitative and quantitative composition of suspended particles by means of flow-through multispectral television measurement analysis of continuous-action particles, comparison of the obtained data with normalized values, is supplemented by the fact that they form multispectral images in the passage of radiation through aqueous media with dispersing particles phytoplankton at characteristic wavelengths from 300 to 1100 nm using a wideband CCD camera, a switchable drum narrowband optical filter, and a source of illumination with a uniform radiation spectrum from 270 to 1200 nm, and on the basis of obtained data using a computer, multispectral images are analyzed and mediated measure the necessary parameters in each image fragment using the regression equation that binds this parameter to the results of the multispectral measurements. Fig. 1 shows the structural scheme of the control, which implements the proposed method.

The means of control contains an illumination source 1 with a uniform emission spectrum from 270 to 1200 nm. Position 2 denotes the water sample under study with scattering phytoplankton particles. The switchable narrowband optical filter 3 is connected to a reducer 4 which is connected to a stepper motor 5. A wide-band CCD camera 6 is optically coupled to a switchable narrow-band optical filter 3. The output of the CCD camera 6 is connected to a computer 7. The stepper motor is connected to a microprocessor control unit 8 which is connected to a computer 7.

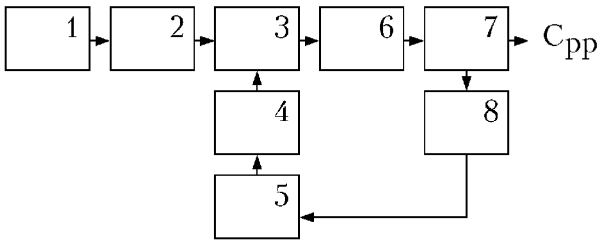

Fig. 1. Means of multispectral television measurement control

The method is implemented in this way.

1. Samples of the test aqueous medium containing dissolved toxic contaminants, in particular pesticides. In the sample it is necessary to add a phytoplankton culture.

2. The surface of the medium is illuminated by a source with a uniform emission spectrum from 270 to 1200 nm.

3. Multispectral images of the surface of the medium at characteristic wavelengths are formed using a wideband CCD camera and a switchable narrowband optical filter, the optical filter being switched by a stepper motor using a microprocessor control unit.

4. Analyze the resulting multispectral images using a personal computer, which allows you to indirectly measure the parameters of the environment in each image fragment using a regression equation that relates these parameters to the results of multispectral measurements

$$\tilde{N}_{pp} = a_0 + a_1 M(l_1) + a_2 M(l_2) + ... + a_n M(l_n), \quad (1)$$

where $\tilde{N}_{pp}$ – concentration of unsuitable pesticide preparations – chemical plant protection products; a_i – coefficients of the regression equation; $M(l_i)$ – results of multispectral measurements.

Experimental studies of the control of the content of pesticide preparations – unsuitable chemical plant protection products were carried out using pesticide preparations Roundup (manufacturer, MONSANTO Europe S.A.). The preparation contains in its composition isopropylamine salt of glyphosate (41.5 %), surface active substance (15.5 %) and water (43 %). In accordance with Directive 1999/45/EC, a safety data sheet has been developed for this preparation [9], which indicates the following information pertaining to lethal concentrations for certain test objects, as well as data on bioaccumulation and rate of degradation in the environment.

In particular, for rainbow trout (Oncorhynchus mykiss) LC50 = 8.2 mg/l in the study of acute toxicity for 96 hours in running water. For daphnia (Daphnia magna) LC50 = 11 mg/l in the study of acute toxicity for 96 hours in non-flow water. For green algae algae (Selenastrum capricornutum), LC50 = 8.0 mg/l in the study of acute toxicity for 96 hours in non-flow water. For duckweed (Lemna minor), LC50 = 6.0 mg/l for acute toxicity studies for 7 days in non-flow water.

With regard to the rate of decomposition of components of pesticidal preparations in the environment, the safety data sheet indicates that the half-life of glyphosate in the aquatic environment is up to 7 days, the half-life of the surfactant in the formulation is up to 1 month. Bioaccumulation of components of pesticidal preparations in aquatic organisms is absent.

Experimental part

The method of biotesting is based on determining the change in the intensity of algae multiplication when exposed to toxic substances contained in the test water as compared to the control. An indicator of the intensity of reproduction is the growth factor of the number of algal cells. Short-term biotesting – 96 h – which allows to determine the presence of acute toxic effects on algae, and long-term biotesting – 14 days – the presence of chronic toxic effects. The criterion of toxicity is a reliable decrease in the growth rate of the number of cells in the test water in comparison with the control. Chlorella vulgaris Beijer was used as a test object. Algae is grown on an artificial nutrient medium Uspensky № 1. The algae culture is introduced into a sterile flask with nutrient medium in an amount giving a light green color. After inoculation, the flask is closed with a sterile wadded-gauze stopper and a parchment paper cap. Cultivated algae with round-the-clock illumination by fluorescent lights placed at a distance of 30–40 cm from the surface of the culture, illumination 2000–3000 lux. The algae culture is periodically mixed, shaking 1–2 times a day. Optimum temperature for growing algae is 18–20 °C [10]. To investigate the chronic toxic effect of pesticide products on the algae, testing has been carried out for 14 days. The results of multispectral measurements are given in Table 1.

Table 1

Results of multispectral measurements of pesticide concentrations

Sample number	C_{pp}, mg / l	Wavelength, nm							
		315	364	400	440	490	540	670	750
Control	0	5	6	10	16	17	22	21	23
1	2.353	0.1	0.2	1	1.1	2	4	5	5.2
2	4.706	4	6	8	9	12	15	12	16
3	9.412	1	1.1	3	3.1	5.2	6	7.5	7.6
4	18.824	5	9	2	2.1	2.3	1.8	2.8	3
…									
62	75.294	0.4	1	3	3.1	4.9	4	5.1	5.9

Multiple regression is performed in the program STATISTICA 6.0. The general purpose of multiple regression is to analyze the relationship between several independent variables and a dependent variable. In this case, the degree of dependence of independent variables and the dependent variable is expressed using the multiple regression coefficient R. Using step-by-step regression, we analyze independent variables (in this case, multispectral parameters) that allow the most accurate determination of dependent variables – pesticide concentrations. At the beginning, it is necessary to determine which multispectral parameters corresponding to certain wavelengths allow the most accurate measurement of the concentration. We use the procedure of multiple regression with incremental inclusion of independent variables, which selects independent variables at each step, adding or removing them from the model based on the user-defined criterion [11, 12]. We accept the value of Fisher's criterion F in the STATISTICA program by default, namely, an independent variable is included in the model, if $F>1$, and is excluded from the model if $F = 0$. Based on the data in Table 1, the program performed a stepwise multiple regression in 6 steps, gradually adding the variables taking into account their contribution to the accuracy of the determination of concentration. The results of calculations at each step of multiple regression are given in Table 2.

Table 2

Multiple regression calculation results for mediated measurement of pesticide preparations with incremental addition of variables

N	Λ, nm	δ_m, %	R
1	540	26.124	0.477
2	540; 490	24.666	0.670
3	540; 490; 315	23.972	0.781
4	540; 490; 315; 750	8.430	0.920
5	540; 490; 315; 750; 440	1.780	0.963
6	540; 490; 315; 750; 440; 670	0.895	0.999

We also check the independent variables that are used in performing the regression for the presence of a correlation relationship between each other, that is, we can verify the presence of multicollinearity. Since the correlation coefficient is less than 0.7 between multispectral parameters, the multicollinearity does not arise.

At known concentrations of pesticides in the samples after performing the multiple regression the following regression equation was obtained, which relates multispectral measurement results and concentration of pesticides

$$C_{pp} = 90.9295\text{-}5.993\cdot T(540)+11.4789\cdot T(490)-\\ -15.4314\cdot T(315)-8.6162\cdot T(750)+\\ +25.8531\cdot T(440)-13.9306\cdot T(670). \quad (2)$$

Let's analyze the instrumental component of the error of multispectral measurements using a CCD camera of LT365R type based on the Sony ICX674 photo matrix with a resolution of 14-bit and a signal-to-noise ratio of 68.6 dB [13]. In this case, the analog-to-digital conversion error arises from a finite number of allowed signal levels in the level-quantization $d_{ADC\,ccd}$ and instrumental error due to the presence of noise and random interference in the CCD camera $d_{noise\,ccd}$. The emergence of instrumental error is due to many factors and its law of distribution of its probability density is close to normal. We calculate its value based on the signal-to-noise ratio for the CCD camera $D_{S/N} = 68.6$ dB

$$d_{noise\,ccd} = 100\% / \left(10^{D_{s/n}/20}\right) = \\ = 100\% / \left(10^{68.6/20}\right) = 0.037\ \%. \quad (3)$$

The quantization error $d_{ADC\,ccd}$ at a large number of discharges can be described by the rectangular law of probability density distribution, which corresponds to the equal probability density of the quantization error in the range $\pm h_k/2$, where h_k – is the quantization step.Taking into account the maximum and minimum signal levels on the elements of the matrix:

$$d_{ADC\,ccd} = \frac{F_H}{2\cdot F_X\cdot 2^n}\cdot 100\ \%; \quad (4)$$

$$d_{ADC\,ccd} = \frac{F_H}{2\cdot F_{X\max}\cdot 2^n}\cdot 100\ \% = \frac{1}{2\cdot 2^{14}}\cdot 100\ \% = 0.003\ \%.;$$

Mean square error of quantization [14]

$$d_{SD\,ADC\,ccd} = \frac{d_{ADC\,ccd}}{\sqrt{12}} = 0.00088\%. \quad (5)$$

We calculate the random component of the measurement error of each coordinate in the n-dimensional multispectral space based on the root-mean-square values of the components:

$$d_{rand.Mi} = \sqrt{d^2_{noise\,ccd} + d^2_{ADC\,ccd}} = \\ = \sqrt{0.037^2 + 0.00088^2} \approx 0.037\%. \quad (6)$$

The random component of the measurement error of each coordinate in the n-dimensional multispectral space is determined by the random components of the measurement error in each of the spectral channels, so the total random component of the error of the indirect measurements will be determined by the random errors of the corresponding multispectral parameters that fall into the general regression equation

$$d_{\text{instr.}} = d_{rand.f} = \sqrt{d^2_{rand.M1} + d^2_{rand.M2} + d^2_{rand.M3} + d^2_{rand.M4} + d^2_{rand.M5} + d^2_{rand.M6}} = 0.091\%. \quad (7)$$

The total error in measuring the concentration of pesticides will be determined by the sum of the instrumental and methodological errors:

$$d_{gen} = d_{\text{instr.}} + d_m = 0.091 + 0.895\ \% = 0.986\ \%. \quad (8)$$

The reliability of the measurement control reflects the degree of objectivity of the results obtained in comparison with the true value of the measured quantity. The reliability of control consists of instrumental and methodical reliability

$$D = D_m \cdot D_{instr.}, \quad (9)$$

where D_m – methodical reliability; $D_{instr.}$ – instrumental reliability.

Consider the instrumental reliability of control:

$$D_{instr.} = 1 - a - b, \quad (10)$$

where α – instrumental error of the first kind; β – instrumental error of the second kind.

Controlled parameters in this paper are the concentrations of pesticides. These measurements are carried out with a certain error Δ. Accordingly, the errors of the first and second kind are determined as follows:

$$a = \int_{C_A}^{C_B} f(C)\left[\int_{C_A-\Delta}^{C_A} j(\Delta)\,d\Delta + \int_{C_B}^{C_B+\Delta} j(\Delta)\,d\Delta\right]dC, \quad (11)$$

$$b = \int_{C_B}^{C_B+\Delta} f(C)\left[\int_{C_B-\Delta}^{C_B} j\left(\Delta\right)d\Delta\right]dC + \\ + \int_{c_A-\Delta}^{C_A} f\left(R\right)\left[\int_{C_A}^{C_A+\Delta} j\left(\Delta\right)d\Delta\right]dC, \quad (12)$$

where f(C) – is the probability distribution density of the concentrations of pesticide preparations; $\varphi(\Delta)$ – is the density of the probability distribution of measurement errors; C_A, C_B – concentration tolerance limits.

The value of pesticide concentrations and the values of the measurement errors are distributed according to the normal law of probability density. The tolerance limits are set within 10 % of the deviation from the concentration value, which corresponds to the reliable determination of the monitored parameter. As a result, we get the value α=0.033, β=0.029. Reliability of control $D_{instr.}$ =0.938 .

Methodical reliability is defined as

$$D_m = \frac{K}{K_{\sum}}, \quad (13)$$

where K – is the number of parameters considered; $K_{\sum}$ – the number of parameters influencing decision making.

Thus, when controlling pesticide concentrations, the value of methodical reliability can be taken as 1 and the reliability of the measurement control is completely determined by instrumental reliability.

Results and discussion

When using pesticides in agriculture, they get into water bodies with surface runoff causing changes in aquatic ecosystems. Since chemical plant protection products contain many chemical ingredients, it is only possible to assess their integrated effects on aquatic ecosystems through bioindication. The method of multispectral television measurement control used in this work in combination with a bioindication of phytoplankton made it possible to measure the concentration of pesticide preparations in an aquatic environment with high accuracy. The used modern CCD camera type LT365R has a rather high resolution and a signal-to-noise ratio of 68.6 dB, which allowed obtaining an instrumental error to 0.091 %. At the same time, the methodical error in measuring the concentration of pesticides is quite high and amounts to 0.895 % and almost completely determines the overall measurement error of 0.986 %. Since the law of distribution of the probability density of the total error of concentration measurements is determined by the convolution of the distribution laws for all components of the error, while the main part of the total error is the methodical component of the error distributed according to the normal law, we can assume that the law of distribution of the probability density of the total measurement error also corresponds to normal law. In this case, the errors of the first and second kind and the reliability of the control were calculated, which were, respectively, α = 0.033, β = 0.029 and D = 0.938. The received value of reliability of the control confirms the possibility of using the developed means in specialized laboratories of environmental institutions.

Conclusions

The method of multispectral measuring control of the content of unsuitable chemical plant protection products in aqueous media using bioindication on phytoplankton was improved, which allowed to increase the reliability of the control up to 0.938 by the example of controlling the concentration of the pesticide Roundup in aqueous media. A multispectral television metering control tool, that uses the transmission of radiation through a thin layer of the aqueous medium under study, is developed. The tool allows you to quickly measure the concentration of phytoplankton particles in the aquatic environment, which can be a bio-indicator of its pollution by toxic substances, in this case, pesticide preparations. The proposed method and means of environmental control can be used to control other parameters of inhomogeneous natural media.

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