The 5th IEEE International Symposium on Smart and Wireless Systems within the International Conferences on Intelligent Data Acquisition and Advanced Computing Systems
17-18 September, 2020, Dortmund, Germany

Multispectral Control of Ecotoxicity of Waters Using Duckweed (Lemna Minor)

Roman Kochan [1], Volodymyr Pohrebennyk[2], Serhii Kvaterniuk[3], Roman Petruk[4], Olena Kvaterniuk[5], Marcin Bernas[6], Rafal Szklarczyk[7], Ruslana Ziubina[8]
[1, 6, 7, 8] University of Bielsko-Biala, 2 Willowa, Bielsko-Biala, Poland, 43-309, e-mail: rkochan@ad.ath.bielsko.pl, mbernas@ath.bielsko.pl, rszklarczyk@ath.bielsko.pl, ruslana.zubina@gmail.com, http://www.eng.ath.bielsko.pl
[2] Lviv Polytechnic National University, 12 Bandera St., Lviv, Ukraine, 79013, e-mail: vpohreb@gmail.com, https://lpnu.ua
[3, 4] Vinnytsia National Technical University, 95 Khmelnytske Shose, Vinnytsia, Ukraine, 21021, e-mail: serg.kvaternuk@gmail.com, https://vntu.edu.ua
[5] Vinnytsia Mykhailo Kotsiubynskyi State Pedagogical University, 32 Ostrozhskyi St., Vinnytsia, Ukraine, 21100, e-mail: lenakvt@gmail.com, https://vspu.edu.ua

***Abstract*—With an improved method and means of control multispectral studied the combined effect of industrial waste on the ecological condition of water bodies. The proposed method consisted in examining the surface of a sample of the aquatic environment covered with floating macrophytes (small duckweed (Lemna minor L.).), which were used for biotesting. At the same time, by studying the array of multispectral images of the object, the object surface was segmented into zones corresponding to damaged and undamaged duckweed leaves. The paper gives an example of testing an aquatic environment contaminated with wastes of galvanic production and determined its composition using X-ray fluorescence spectroscopy. The proposed method and tool made it possible to assess the ecological state of water bodies, taking into account the synergistic interaction of chemicals.**

Keywords—Biological Indicators; Ecotoxicological Control; Multispectral Control Of Water

I. Introduction

To assess the impact of technogenic pollution on the ecological state of a water body, it is necessary, first of all, to take into account its effect on such phytoplankton parameters as the specific concentration of phytoplankton pigments in the aquatic environment (in particular, chlorophyll a, carotenoids, etc.) and the parameters of species biodiversity. The traditional approach to determining the ecological state of water bodies by physical and chemical parameters is imperfect, since the parameters of aquatic organisms are not taken into account. In addition, most of the pollutants entering the aquatic environment have a synergistic interaction with each other, which complicates the assessment of their impact on the ecological state of water bodies [1], [27]. Thus, the response of aquatic organisms to pollutants depends not only on their concentrations, but also on synergistic interactions, both between the chemicals themselves and between pollutants and aquatic organisms. An integrated assessment of the biological usefulness of water as a habitat for biota is carried out using bioindication and biotesting, which was used in environmental practices in many countries, when society realized the danger to human health toxic water pollution. In [1] – [9], [27] the theory of environmental safety management of environmental objects is proposed, taking into account the synergetic interaction between natural and man-made components..

Ecotoxicological control is carried out in order to regularly monitor compliance with environmental quality standards and prevent the ingress of toxic substances into water bodies. At the same time, toxicity is the degree of manifestation of the toxic effect of various chemical compounds and their mixtures, one of the important factors determining the quality of water and giving an idea of the danger when it is used. Toxic control is a necessary part of a comprehensive water quality control system. Determination of toxicity by biotesting, is to conduct analyzes using live test organisms. The results promptly signal the dangerous effects of chemical contamination on the vital activity of organisms, and not by individual components, but by their mixtures often enough of unknown origin. Toxic effects, registered by methods of biotesting, include complex synergistic, antagonistic and additional effects of all chemical, physical and biological components present in the test water, adversely affecting the physiological, biochemical and genetic functions of test organisms. Toxicity, which is established by methods of biotesting, is an integral indicator of environmental pollution [10], [11].

Toxic substances can enter the environment from natural and anthropogenic sources. Anthropogenic pollution may be primary and secondary. Primary pollution due to pollutants from anthropogenic sources. Secondary pollution is caused by the appearance in the environment of an excess amount of waste products and the remains of organisms associated with the disruption of natural ecological relationships as a result of primary pollution. Both can cause toxic effects. Toxicity, which is established by methods of biotesting, is an integral indicator of environmental pollution. Like all integral indicators, it has the disadvantage that it does not reveal the pollutants present in the sample, so the results of biotesting may in some cases not coincide with the findings of water pollution obtained on the basis of hydrochemical analyzes.

In order to ensure the environmental safety of wastewater, it is necessary to create a system to control their toxicity, which will allow evaluating the effectiveness of the treatment facilities and justifying wastewater treatment methods, determining maximum allowable discharges for industrial facilities, assessing the ecological state of natural waters, assessing the toxicity of chemical materials, assessing the effectiveness of environmental protection events in the industrial facility.

The toxicity criterion is the quantitative value of the test parameter, on the basis of which it is concluded that water is toxic. Among the test parameters, survival, fertility, suppression of the enzymatic and metabolic activity of organisms are most often used. A test reaction is a change in any biochemical, morphological, behavioral, or functional indicator in a test object under the influence of toxicants or their mixtures. Processing of toxicity measurement results is performed by calculating the arithmetic mean value of the toxicity index for a series of investigated samples. At the same time, at least three measurements are made for each test sample in a short time compared with the exposure time. The degree of toxicity should be expressed by the three maximum levels of the toxicity index: the permissible level (toxicity index $T < 20\%$); average level (toxicity index $20\% \leq T < 50\%$); high level (toxicity index $T \geq 50\%$). The average effective concentration (EC50) is the concentration of a toxic substance that causes a change in the test reaction by 50% under the established conditions of exposure for a given period of observation. If the test reaction implies the death of 50% of the test objects, then this concentration of the toxic substance will correspond to the average lethal concentration (LC50). In addition, the concentration of a toxic substance that causes a change in the test reaction by 20% corresponds to EC20, and in case of the death of 20% of the test objects – LC20.

Environmental risks are addressed in EU directives [10]. There are two approaches to assess the environmental risk of hazardous substances: probabilistic, which is fully consistent with the classical definition of the concept of risk, and deterministic. The probabilistic approach allows to take into account the variability of distribution of hazardous substances in the environment and uncertainties due to the limited number of test species of organisms. To assess the probabilistic risk, the distribution of environmental indicators is used (for example, concentrations of hazardous substances in water and its toxicity to aquatic organisms), which cover their entire possible range. The result of the assessment of this risk is the calculated probability of the onset of adverse effects if hazardous substances enter the aquatic environment, for example, death or suppression of the development of aquatic organisms. A significant drawback of the probabilistic risk assessment is the large amount of experimental data needed, which limits the application of this approach in the practice of regulating pesticide circulation. The EU has begun the development of a system for assessing the probable risk of pesticides. According to European experts, probabilistic methods should be introduced gradually, first of all (when they are most needed) to help in making decisions on environmental safety. When assessing the deterministic environmental risk of exposure to pollutants, fixed values of toxicity and concentration of pollutants in the aquatic environment are used, which simplifies the assessment of the technogenic impact of pollutants on water bodies. An indicator of deterministic risk is the ratio of toxicity and concentration (TER – Toxicity Exposure Ratio). Moreover, the EU directives indicate safe concentrations of pollutants, correspond to the permissible TER value for certain test organisms, in particular, TER> 100 for daphnia and fish, and also TER>10 for phytoplankton or higher aquatic plants [11], [12].

II. Methods

Multispectral methods make it possible to control the parameters of the content of pigments in the aquatic environment, the area of coverage of the water surface by higher aquatic plants, as well as the indices of species biodiversity, both for phytoplankton and for macrophytes [15] – [22], [27]. The method and tool for multispectral control has been improved, which can be used to determine ecotoxicity as samples of water from natural water bodies, domestic and industrial wastewater, as well as aqueous solutions of chemicals. The method consists in determining the relative dimensions of the surface of a water sample covered with macrophytes, which have morphological changes, based on an analysis of an array of digital images obtained using a broadband CMOS camera, when illuminating a sample of an aqueous medium at characteristic wavelengths. In this case, duckweed plants (Lemna minor L.) are used and, by processing an array of digital images obtained at selected wavelengths, parts of the image surface area are determined that correspond to macrophytes without chlorosis and leaf necrosis (A), macrophytes, the surface of which has changed its spectral characteristics under the influence of pollutants (B) and the surface of a sample of an aquatic environment without macrophytes (C).

In Fig. 1 shows the structure of the means for multispectral monitoring of ecotoxicity of water samples [14]. The tool contains a sample of contaminated water 1 in a cuvette 2; floating layer of the test object duckweed small 3; integrating sphere 4, screens 5 and substrate 6 covered with a diffuse reflective coating based on barium sulfate, a set of LEDs or laser diodes 7, digital camera 8, objective 9, thermocouple 10, thermostat 11, computer for processing an array of digital images obtained at selected lengths waves 12.

The method is carried out in this way:

Duckweed plants (3) in an amount of 100-200 pieces are placed in a sample of a contaminated aquatic environment (1) in a beaker (2) with a volume of 200 ml, the surface of which is uniformly illuminated by radiation scattered by a spherical surface (4) and a horizontal surface (6). The luminous flux of the selected LEDs or laser diodes (7) should provide an illumination level sufficient for the normal operation of the selected digital camera with a known sensitivity at a given wavelength. Thermocouple (10) and thermostat (11) are designed to ensure the specified experimental conditions in the study of ecotoxicity with an accuracy of ± 1 °C. Screen 5 prevents the passage of radiation directly from the LEDs to the digital camera or beaker [28]. In this case, the water sample reaches the surface only after diffuse scattering.

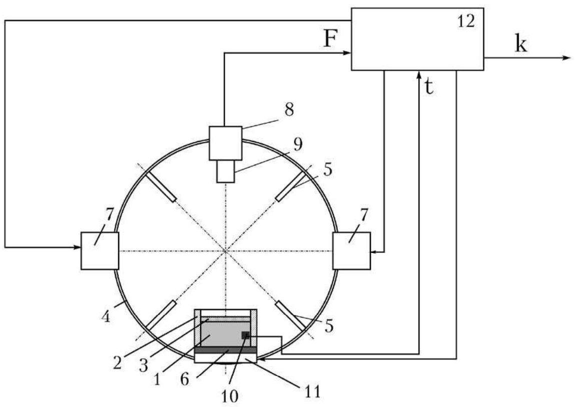

Figure 1. The structure of the means of multispectral control of ecotoxicity of water samples.

Samples of contaminated water with macrophytes-biotests are kept in a thermoluminostat for 7 days. The thermoluminostat control circuit provides lighting/ dimming operation in a ratio of 18/6 hours per day.

Every day, water samples are taken from the thermoluminostat and examined in a multispectral ecotoxicity monitor to calculate the portion of the image surface area corresponding to macrophytes without chlorosis and leaf necrosis (A), macrophytes whose surface has changed its spectral characteristics under the influence of pollutants (B) and the sample surface aquatic environment without macrophytes (C). An array of digital images of the surface of a sample of a polluted aquatic environment with macrophytes is obtained by illuminating the surface using a set of LEDs or laser diodes (7) at selected wavelengths obtained in the course of solving the inverse optical problem of determining the pigment content of higher aquatic plants by spectral characteristics. In this case, a broadband digital camera 8 with an objective 9 is used, tuned to obtain a clear image of floating macrophytes on the surface of a sample of a polluted water environment. For each pixel of the digital image array at selected wavelengths, the content of the main pigments was calculated and the image was segmented into parts corresponding to macrophytes without chlorosis and leaf necrosis, macrophytes, the surface of which changed its spectral characteristics under the influence of pollutants and the surface of a sample of the aquatic environment without macrophytes [28]. After calculating the areas of the indicated segments of the multispectral image, a coefficient is calculated that determines the ecotoxicity

$$k_{t,c} = \frac{B_{t,c}}{A_{t,c} + B_{t,c}} 100\%,$$

for the investigated aquatic environment k_t and control sample k_c.

Next, the dependence of ecotoxicity on the content of pollutants in a water sample was investigated for the results of studies carried out within 14 days of a sample of polluted (k_t) and clean (k_c) water. A graph of the dependence of the ecotoxicity coefficient on the concentration of pollutants (C) was built and the corresponding regression equations were obtained. The obtained dependencies make it possible to determine the concentration of pollutants in a sample of the aquatic environment and assess the complex impact of several pollutants. To obtain reliable values of the ecotoxicity of pollutants in aquatic environments when using duckweed as a test object, the duration of research (T_i) should be at least 7 days.

Thus, the control of ecotoxicity of samples of polluted aquatic environments with one or more pollutants is reduced to the segmentation of the image of the surface of the sample of the aquatic environment. In this case, an array of images of the surface of the aquatic environment obtained at the characteristic wavelengths of pigments is used, which makes it possible to separate the part of the image of the sample surface covered with intact duckweed leaves, leaves with chlorosis and necrosis, which have changed their spectral characteristics and the surface of the water sample without macrophytes. With an increase in the concentration of pollutants in the aquatic environment, the relative area of damaged duckweed plants increases and the ecotoxicity coefficient k_t increases to 100%.

III. Results

As a result of the activities of the enterprises of the electronic industry, in particular, OJSC "Plant "Terminal"" (Vinnitsa, Ukraine), industrial waste has accumulated on their territory. After going through the bankruptcy procedure, wastes were stored for a long time in storage facilities. According to Table I, shows the composition of two samples of the galvanic deposit obtained using the method of X-ray fluorescence spectroscopy.

TABLE I. THE RESULTS OF THE ANALYSIS OF ELECTROPLATING SLUDGE PRODUCTION BY X-RAY FLUORESCENCE SPECTROSCOPY

Sample №1		Sample №2	
Element	*Mass fraction, %*	*Element*	*Mass fraction, %*
14Si	0,734±0,084	14Si	1,823±0,287
16S	3,003±0,101	16S	5,035±0,292
17Cl	22,981±0,163	20Ca	70,699±0,346
20Ca	0,909±0,111	22Ti	0,443±0,065
22Ti	2,940±0,123	24Cr	8,734±0,129
23V	0,754±0,075	25Mn	0,129±0,060
26Fe	1,268±0,028	26Fe	8,135±0,096
28Ni	1,059±0,023	28Ni	3,883±0,051
29Cu	55,251±0,207	29Cu	0,355±0,016
30Zn	0,140±0,020	30Zn	0,270±0,011
38Sr	0,031±0,004	38Sr	0,118±0,005
44Ru	0,021±0,007	44Ru	0,015±0,004
45Rh	0,015±0,005	45Rh	0,021±0,005
46Pd	0,028±0,006	46Pd	0,026±0,006
47Ag	0,024±0,006	47Ag	0,026±0,006
48Cd	0,045±0,011	48Cd	0,044±0,011
50Sn	1,055±0,023	50Sn	0,055±0,011
56Ba	9,272±0,179	78Pt	0,028±0,006
60Nd	0,330±0,053	79Au	0,020±0,006
82Pb	0,141±0,010	80Hg	0,017±0,004
		82Pb	0,106±0,008

Sample No. 1 of industrial waste of a light green color is a contaminated copper oxychloride, belongs to plant protection products and falls under the 3rd hazard class. Sample No. 2 industrial waste yellow-brown color is a sludge electroplating, neutralized using calcium oxide. Contains oxides and hydroxides of heavy metals (Zn, Cr, Ni) and falls under the 3rd hazard class. The results of the study of the ecotoxicity of water samples contaminated with industrial waste using the example of galvanic sludge are shown in Fig. 2.

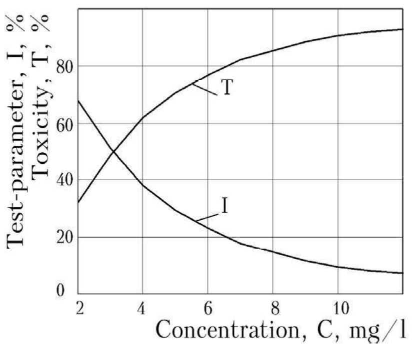

Figure 2. The dependence of the test parameter and the toxicity of water samples with the presence of hazardous components of electroplating sludge biotesting using a test object of duckweed (Lemna minor L.).

IV. CONCLUSION

The method and means of multispectral monitoring of ecotoxicity of surface waters has been improved. The essence of this method is to determine the relative sizes of water surface segments with higher aquatic plants that have morphological changes based on the results of multispectral analysis images taken with a broadband digital camera when illuminated and aquatic environments narrowband radiation sources. Scientifically grounded design solutions and prototypes of advanced technical means for multispectral monitoring of aquatic toxicity based on indirect measurements of parameters of higher aquatic plants. Developed software Multispectral devices 1.029 for technical means of multispectral control of toxicity of aquatic environments, managing technical means of control, as well as segmentation and filtering of multispectral images and their processing using regression equations, neural network and neuro-fuzzy network [23] – [26]. Scientific and methodological recommendations have been developed for the implementation of the proposed scientific bases for the use of multispectral methods and technical means, which take into account the influence of their characteristics and parameters on the effectiveness of the process of controlling the toxicity of aquatic environments and assessing the ecological status of water bodies in the system of managing their environmental safety.

ACKNOWLEDGMENT

The research for this paper was financially supported by the University of Bielsko-Biala, Poland.

REFERENCES

[1] S. Azarov, O. Zadunaj, “Analysis of the stability of water bodies to the action of destabilizing factors,” *Environmental safety and environmental management*, 2018, vol. 26, issue 2, pp. 34–42.

[2] S. L. Wang S. L., X. L. Jin, Z. L. Huang, G. Q. Cai, “Break-out of dynamic balance of nonlinear ecosystems using first passage failure theory,” *Nonlinear Dynamics*, vol. 80, issue 3, 2015, pp. 1403–1411.

[3] V. Maystruk, K. Abdella, “Modelling the Effects of Pollution on a Population and a Resource in a Polluted Environment,” *Applied Mathematics*, vol. 2011, 2011, pp. 1–31.

[4] D. Tian at al., “Nonlinear responses of ecosystem carbon fluxes and water-use efficiency to nitrogen addition in Inner Mongolia grassland, ” *Functional Ecology*, vol. 30, issue 3, 2015, pp. 490–499.

[5] Y. Destania, Jaharuddin, P. Sianturi, “Stability Analysis of Plankton Ecosystem Model: Affected by Oxygen Deficit,” *Applied Mathematical Sciences*, vol. 9, issue 81, 2015, pp. 4043–4052.

[6] M. Karpinski, V. Pohrebennyk, N. Bernatska, J. Ganczarczyk, O. Shevchenko, “Simulation of artificial neural networks for assessing the ecological state of surface water,” *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*, 18 (2.1), 2018, pp. 693–700.

[7] V. Pohrebennyk, A. Petryk, “The degree of pollution with heavy metals of fallow soils in rural administrative units of Psary and Płoki in Poland.” *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management*, SGEM, 17 (52), 2017, pp. 967–974.

View publication stats

[8] Mitryasova, O., Pohrebennyk, V. 2017. "Integrated Environmental Assessment of the Surface Waters Pollution: Regional Aspect, "*17th International Multidisciplinary Scientific GeoConference SGEM,* 2017, Vienna, Austria, 33(17), pp. 235–242.

[9] Mitryasova O., Pohrebennyk V., Kardasz P. "Hydrochemical Aspects of Surface Water Quality Assessment," *18th International Multidisciplinary Scientific Geoconference SGEM,* 2018, 5.2 (18), pp. 513–520.

[10] H. Frear, J. Boyd, "Use of Daphnia magna for microbioassay of pesticides," *J. Econom. Entomol.*, Issue 60, 1967, pp. 1228–1236.

[11] V. Tonkopii, A. Zagrebin, I. Iofina, "Bioidentification of xenobiotics as a basis of water management," *Sustainable use and development of watersheds*, 2008, pp. 349–353.

[12] European Commission, "Directive 2001/688/EC: Commission Decision of 28 August 2001 establishing ecological criteria for the award of the Community eco-label to soil improvers and growing media (notified under document number C (2001) 2597)," Official Journal of the European Communities, EC, L. 242, 12.9.2001, pp. 17–22.

[13] European Commission, "Working Document Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC". Sanco/3268/2001 rev.4 (final), EC, 2002, 62 p.

[14] M. Foudoulakis, "Ecotoxicological risk assessment for plant protection products in Europe," in *Ecotoxicology, Ecological Risk Assessment and Multiple Stressors* Eds. G. Arapis, N. Goncharova, Ph. Baveye, NATO Security through Science Series – C: Environmental Security, Series IV: Earth and Environmental Series, Vol. 6, 2006, pp. 137–154.

[15] P. Symvoulidis et al., "Serial sectioning and multispectral imaging system for versatile biomedical applications," *IEEE International Symposium on Biomedical Imaging*, 2014, pp. 890–893.

[16] R. M. Kudela et al., "Application of hyperspectral remote sensing to cyanobacterial blooms in inland waters," *Remote Sensing of Environment*, issue 2, 2015, pp. 1–10.

[17] S. Shi et al., "Improving Backscatter Intensity Calibration for Multispectral LiDAR," *IEEE Geoscience and Remote Sensing Letters*, vol.12/issue 7, 2015, pp. 1421–1425.

[18] V. Starovoitov et al., "Multispectral image enhancement based on fusion and super-resolution," *15th European Signal Processing Conference*, 2007, pp. 2174–2178.

[19] V. Martsenyuk, V. Petruk, S. Kvaternyuk, V. Pohrebennyk, R. Petruk, A Klos-Witkowska. "Multispectral control of water bodies for biological diversity with the index of phytoplankton," *16th International Conference on Control, Automation and Systems,* 2016, pp. 988–993.

[20] R. Petruk, V. Pohrebennyk, S. Kvaternyuk, O. Bondarchuk, M. Cygnar. "Multispectral television monitoring of contamination of water objects by using macrophyte-based bioindication," *16th International Multidisciplinary Geoconference SGEM*, 2016, pp. 597–602.

[21] S. Kvaternyuk, V. Pohrebennyk, R. Petruk, A. Kochanek, O. Kvaternyuk, "Multispectral television measurements of parameters of natural biological media," *17th International Multidisciplinary Scientific GeoConference SGEM*, 2017, pp. 689–696.

[22] V. Ishchenko, V. Pohrebennyk, Y. Kozak, A. Kochanek, R. Politylo, "Assessment of batteries influence on living organisms by bioindication method," *16th International Multidisciplinary Geoconference SGEM*, 2016, pp. 85–92.

[23] H. Rakytyanska, "Optimization of fuzzy classification knowledge bases using improving transformations," *East.-Eur. J. of Enterp. Technol.*, Vol. 2, Issue 5 (86), 2017, pp. 24–31.

[24] H. Rakytyanska, "Fuzzy classification knowledge base construction based on trend rules and inverse inference," *East.-Eur. J. of Enterp. Technol.*, Vol. 1, Issue 3 (73), 2015, pp. 25–32.

[25] V. Martsenyuk, Z. Mayhruk, M. Karpinski, N. Milian, I. Andrushchak, O. Veselska, "On implementation of decision tree induction in cloud platforms," *2019 Advances in Science and Engineering Technology International Conferences (ASET),* 2019, pp. 1–6.

[26] A. Sachenko, V. Kochan, V. Turchenko, V. Tymchyshyn, N. Vasylkiv, "Intelligent nodes for distributed sensor network," *Proceedings of the IEEE Instrumentation and Measurement Technology Conference*, 1999, pp. 1479–1484.

[27] S. Kvaterniuk, V. Pohrebennyk, R. Petruk, V. Petruk, A. Kochanek, "Assessment of the complex effects of hazardous waste components in aquatic ecosystems," *Geolinks. International conference of geosciences*, Book 3, Vol. 1 , 2019, pp. 19–26.

[28] S. Kvaternyuk, "Multispectral television measuring control of integral parameters of pollution using higher aquatic plants in a complex for sewage treatment," *Environmental problems*, Vol. 2, No. 3, 2017, pp. 121–126.