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THE APPLICATION OF THE COMPUTER IMAGE
ANALYSIS METHODS IN ENVIRONMENTAL
BIOMONITORINGZASTOSOWANIE METOD KOMPUTEROWEJ ANALIZY
OBRAZU W BIOMONITORINGU ŚRODOWISKA

Abstract

Intensive development of computer techniques in the area of optics, optoelectronics and computer image analysis methods make scientific premises for the implementation of these solutions in other scientific disciplines. The application of image analysis in image diagnostics in medicine is at the moment on a high level of technological development and crucial in a proper medical diagnosis. The methods of image processing and analysis are developed, among others in: photogrammetry, geomatics, remote sensing, in GIS systems, in the analysis of the spectra of scanners, sonars, echo-sonars, photo-radars, in studying structures of materials, ground, etc. [1–3]. Unfortunately, there are still very few applications for the methods in the environmental protection and biomonitoring mentioned above. Most studies, apart from chemical analytics, are connected with the application of classic research methods based on visual observation [4, 5]. Thus it is so important to develop co-operation and studies on the possibility of the implementation of automatic methods based on computer image analysis in this field.

Keywords: computer image analysis methods, image processing, automation

Streszczenie

Intensywny rozwój technik komputerowych w dziedzinie optyki, optoelektroniki oraz metod komputerowej analizy obrazu stwarza przesłanki naukowe dla wdrażania tych rozwiązań w innych dziedzinach nauki. Zastosowanie analizy obrazu w diagnostyce obrazowej z zakresu medycyny znajdują się obecnie na wysokim stopniu rozwoju technologicznego i są wręcz nie zbędne dla prawidłowej diagnozy prowadzonej przez pracowników medycznych. Metody obróbki i przetwarzania obrazu są rozwijane np. w zakresie: Fotogrametrii, Geomatyki, Teledetekcji w systemach GIS, w analizie widm skanerów, sonarów, echosond, fotoradarów, w badaniu struktury materiałów, podłoża, itd. [1–3]. Niestety nadal bardzo mało jest zastosowań dla ww. metod w zakresie ochrony środowiska oraz w biomonitoringu. Większość badań poza analityką chemiczną wiąże się tu z wykorzystaniem metod badawczych klasycznych opartych na obserwacji wizualnej [4, 5]. Dlatego tak ważne jest rozwijanie współpracy oraz badań nad możliwością wdrożeń metod automatycznych opartych o komputerową analizę obrazu w tej dziedzinie.

Słowa kluczowe: metody komputerowej analizy obrazu, przetwarzanie obrazu, automatyzacja

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1. Introduction

Recently, an intensive development of biological environmental monitoring methods has been observed [6]. The assessment of toxicity of samples taken from the environment requires a proper selection of a bioindicator [7], i.e. the organism particularly sensitive to specific pollutants and a toxic effect should include unambiguously interpretable symptoms [8]. Most biotests are based on classic methods involving visual observation. The researcher records and interprets the symptoms resulting from a toxic impact of pollutants on the indicator organisms. The development of new methods is mainly based on looking for new bioindicators or sensitive biological material (enzymes, physiological reactions etc.) [9, 10]. There are very few studies in the automation of classic methods to eliminate errors resulting from subjective records. Particular scientific premises are given here by the possibility of application of computer image analysis methods [11]. In many cases this would make it possible to objectively assess the analysis of toxicity of the studied sample. The attempts to make engineering projects, e.g. construct instruments for biotests are only possible if proper methods of image analysis are implemented to correctly assess toxic influence on the used bioindicator organisms. Just the assessment of proper image analysis procedures of the studied biological material makes the modified method semi-automatic [12]. The computer records the changes and marks the character of these changes; both qualitative and quantitative. Such solutions allow the quantified and objective assessment of the state of the studied material.

The examples of such applications of computer analysis in biotests can be macroprograms in the Aphelion environment [11]:

1. Following the changes of the withdrawal of tentacles in *Hydra vulgaris*. In this method the compactness coefficient was applied as the measure in defining the toxic influence of the examined pollutants on these coelenterates.

The selection of a coefficient describing the changes:

$$\text{COMPACTNESS} = \frac{16S}{L^2} \quad (1)$$

where:

- L – the object perimeter in the image points,
- S – the number of points belonging to the object.

This coefficient is included in the interval $(0.1 > \text{value} > 0)$ and takes value 1 for the ideal square. Such a definition was applied due to the fact that discrete geometry is used in the program. The constructed macro is extremely useful in on-line monitoring and gives satisfactory effects in defining toxic concentration of the studied pollutants.

1. The algorithm was prepared in two versions:
 - the version for the analysis of a single image,
 - the version for the automatic analysis of a series of images [11].
2. The measurement of the area of the common duckweed *Lemna minor* leaves. Two versions of the algorithm for the macroprogram were prepared:
 - the version for the analysis of a single image,
 - the version for the automatic analysis of a series of images.

The created tool using techniques of computer image analysis meets all the established requirements:

- it returns a repetitive result – the measurement error does not exceed 4.4% of the measured value,
 - it is simple in use,
 - it is relatively inexpensive [11].
3. Studies of changes of the mean velocity in the test population of *Daphnia magna* [12] exposed to the selected toxin.
 4. Detection of the movement of embryos in cocoons of *Lymnaea stagnalis* exposed to specific toxins [4, 5].
 5. Recognition of individuals in the population of *Daphnia magna* in the fluotox test and detection of individuals showing fluorescence at the exposure to the UV.

The comparative assessment of the influence of the Brij group detergent on *Lemna minor* was made. The macroprogram written earlier for the measurement of the duckweed area was adjusted to experimental conditions. One should notice that measurement of the area by a standard (planimetric) method is very time- and work-consuming, and generates errors due to inadequacy of manual measurements). The studies carried out by means of the method of image analysis gave very accurate results, and did not require additional time- and work-consuming measurements.

1.1. Methods

1. Different dilutions of the Brij 58 detergent solutions were made in a geometric sequence with coefficient $q = 1.25$.
2. Populations of *Lemna minor* were introduced into every tray with the solution test, 10 individuals per each tray. Every test group was represented in 3 repetitions.
3. The images for each test group of *Lemna minor* were made (preserving constant, and established camera and distance options).
4. Every image was analysed in the Aphelion program and the obtained results made the mean area of the whole test population given in pixels (the macroprogram version for automatic analysis of the series of images was applied).
5. The program returns the results in the calculation sheet Excel.
6. The exposure to the effect of the toxicant in defined concentrations lasted 3 weeks, the images for each test group were taken in 5 measurement series.
7. The obtained results were compared within the groups, between the groups and with the control.

The experiment was carried out in strictly controlled conditions, with the regime of the same parameters during photographing of each *Lemna minor* group. The possibility of evaporation of water from the solutions was prevented by covering all the trays with a glass sheet, not limiting access to the light, needed for vegetation.

1.2. Results

Data in Table 1 present mean values of the area of the whole test population calculated for each test group represented in 3 repetitions. The result is given in pixels, thus it was very important to keep the same camera parameters of taking the images.

Table 1

The measurement results (in pixels) of the mean area of the test *Lemna minor* population (3 repetitions), exposed to different concentrations of Brij 58

	Concentration mg/l	Series 1	Series 2	Series 3	Series 4	Series 5
A	44.00	5859.11	6068.33	6580.78	6644.22	7337.89
B	55.00	7711.29	8272.56	8102.56	8834.33	9633.11
C	68.75	6803.78	7467.00	7871.78	8067.89	8959.56
D	85.94	5981.00	5435.44	6582.44	6489.78	7169.67
E	107.42	4766.22	5277.56	5664.33	5686.33	6220.36
F	134.28	5011.67	5232.78	5557.78	5946.00	6496.50
G	167.85	5986.44	5456.33	5633.67	5839.22	6443.47
H	209.81	4901.22	4920.00	5224.44	5410.33	6208.11
I	262.26	5493.11	5587.22	6029.28	6734.00	7368.44
J	327.83	5663.39	5967.56	6287.00	7270.67	8065.89
K	409.78	6241.39	6691.33	7064.67	7539.22	8070.11
Control	0	5718.28	6154.56	6356.69	7058.33	7741.16

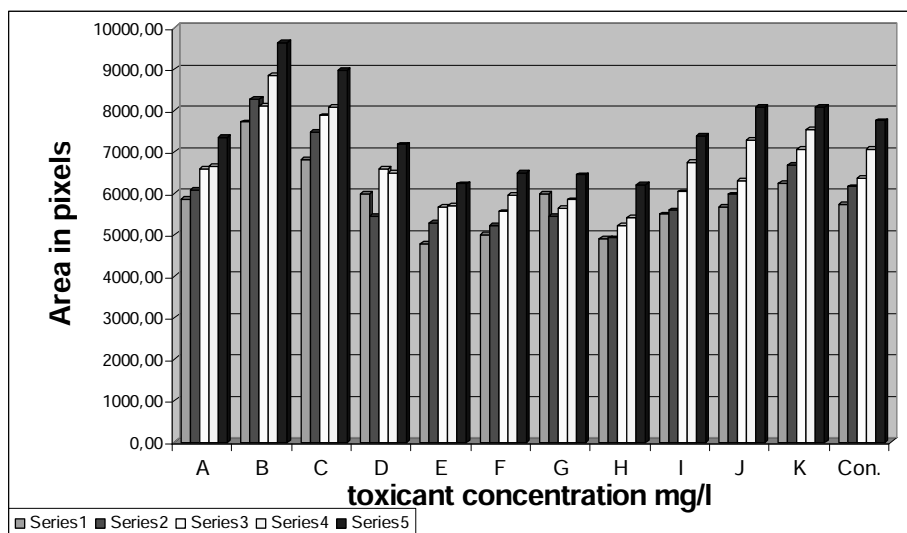


Fig. 1. The graph: the results of the mean area for all studied groups in 5 measurement series

Rys. 1. Wykres: zestawienie wyników średniego pola powierzchni dla wszystkich badanych grup w 5 seriach pomiarowych

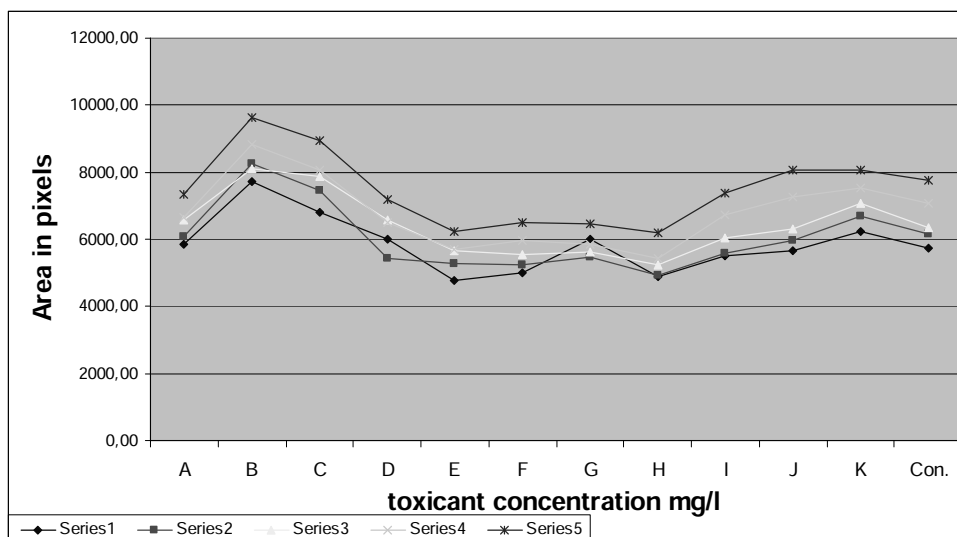


Fig. 2. The cumulated graph of: mean values of the area in each measurement series

Rys. 2. Skumulowany wykres: średniej wartości pola powierzchni w każdej serii pomiarowej

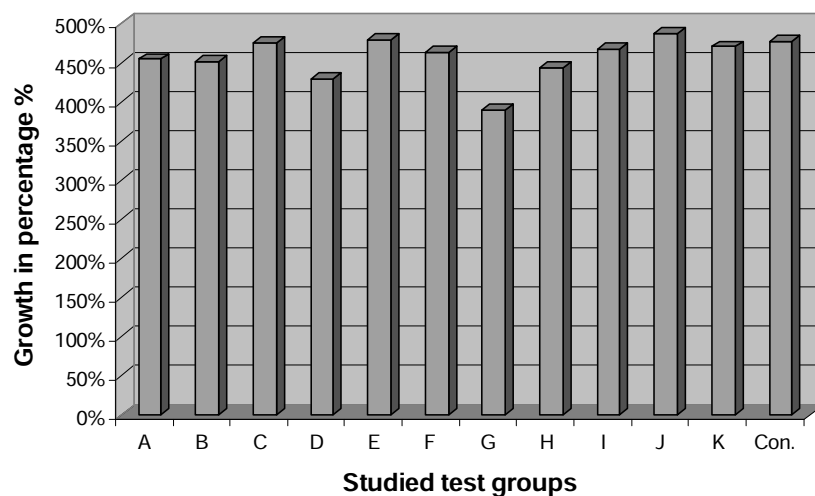


Fig. 3. Dynamics of the growth in each test group within 5 measurement series

Rys. 3. Dynamika przyrostu w każdej grupie testowej w obrębie 5 serii pomiarowych

1.3. Discussion of the Results

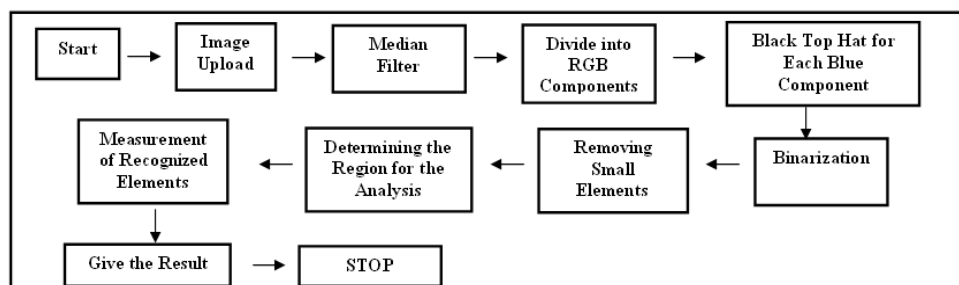
The analysis of measurement results within all the groups shows the growth of the surface area in each group. This is caused by the fact that applied concentrations of the Brij 58 detergent are not toxic for *Lemna minor*. Compared to the control there is a lack of

significant differences in growth dynamics for each test group. Each test population is characterized by the growth in time. One can plan subsequent experiments connected with the increase of concentration of the studied surfactant to determine a toxic concentration for *Lemna minor*, analysing dynamics of the growth of the studied water populations.

The experiment carried out proves that the applied measurement tool correctly recognizes objects of *Lemna minor*. The result is the area for each test group. The macroprogram analyses the series of images from the defined catalogue, the obtained data are returned in an Excel calculation sheet. This makes a rich database, used in further statistic analyses referring to the studied objects.

Because the difference between a „single” and „serial” version is only such that the images contained in one catalogue are cyclically processed by the algorithm for a single image, and the results are put in the calculation sheet – only a „single” algorithm, as presenting the main idea of the analysis will be presented in detail. Below, in Fig. 4, the block scheme was presented for the created algorithm [11].

The algorithm starts from uploading the analysed image. Then a median filter is applied to reduce the noise. The next stage is transformation of the coloured image into a monochromatic one. The image is divided into RGB components. For further analysis a blue component was applied, because it gave the greatest contrast of the duckweed. In the next step the black top hat filter is applied to contrast the objects better. Then binarization is made (corrected entropy threshold) and small elements (much smaller than the duckweed plants, treated as noise) are removed – image erosion + image reconstruction. The next phase is determining the region where the duckweed is. At this stage all the stuff prepared during the image acquisition has been used. The duckweed is in a round breaker whose edge is marked with a black lining (Fig. 5).



Rys. 4. Schemat blokowy algorytmu detekcji rzęsy

Fig. 4. The block scheme of the duckweed detection algorithm

Namely, this edge is used for determination of the circle within which the studied objects are found. After determining the region interesting to researchers, the surface area and the containing elements are calculated. The next action is making the outline of the objects recognized by the program to verify the correctness of their recognition by the user and giving the result [11].

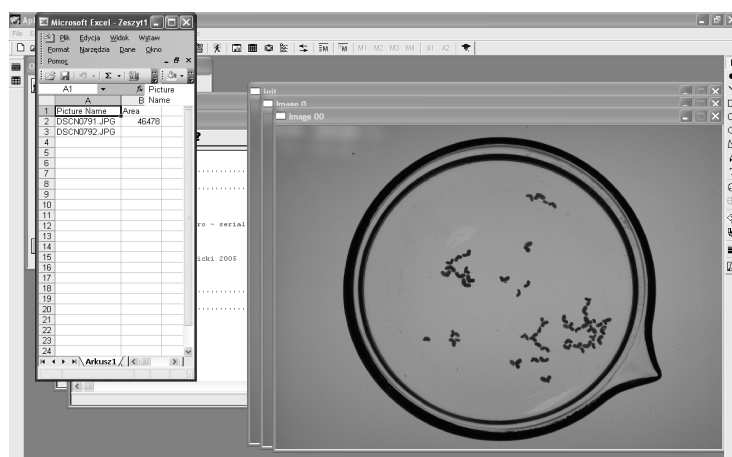


Fig. 5. The image of the analysed object (tray with the *Lemna minor* population, the edge is marked black)

Rys. 5. Zdjęcie analizowanego obiektu (szalka z populacją *Lemna minor* z zaczernionym brzegiem)

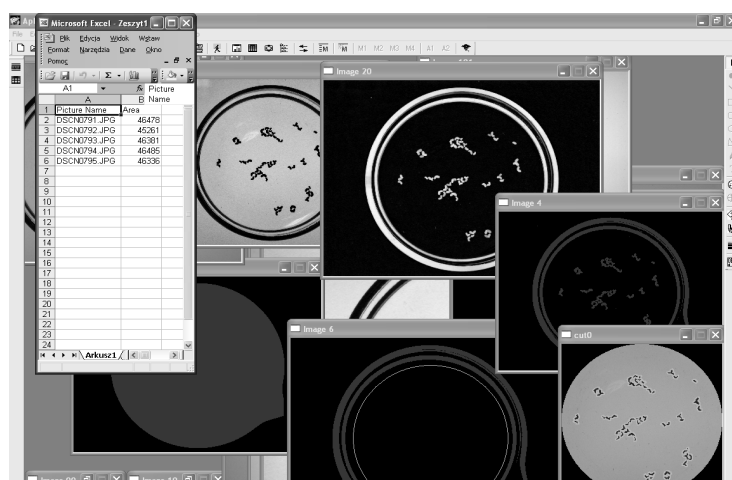


Fig. 6. Analysis process, according to the detection algorithm

Rys. 6. Proces analizy zgodnie z algorytmem detekcji

2. Conclusions

- The new method gives promising results.
- It significantly increases the speed and accuracy of the analysis carried out.
- The modified biotest with the application of the computer image analysis is a semi-automatic method and makes it possible to make fully automatic instruments.

- The repetitiveness of the results was on the level of 4.4%. In biological test the accepted error is in the confidence level of 0.05.

As has been presented, the application of computer image analysis brings new measurement opportunities in the biotests applied so far. The application of computer image analysis methods supplements the biological tests with a possibility of studying parameters that used to be difficult to measure. Thus computer image analysis is a scientific discipline whose application in biotests can be a powerful tool to increase sensitivity and objectivity of these methods in water quality assessment.

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