APPLICATION OF BIOSENSORS FOR PLANTS MONITORING

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Abstract: *Current methods of diagnostics of plant state need to conduct expensive and long-time physical-chemical and microbial analyses of soil and plant samples. The chlorophyll fluorescence induction method allows determining the functional state of plant in express mode without plant damage and it gives an opportunity to estimate the influence of stress factors on the plant state. In recent decades a number of researches of the chlorophyll fluorescence induction were significantly increased because of appearance of relatively inexpensive portable fluorometers. This paper represents results of testing biosensors developed at the V.M. Glushkov institute of Cybernetics of NAS of Ukraine on base of chlorophyll fluorescence induction method. It were developed appropriate software to facilitate data acquisition and processing. Analysis of some experimental results by means of neural networks is discussed.*

Keywords: *fluorometer, biosensor, wireless sensor network, chlorophyll fluorescence induction, neural network, information technology.*

ACM Classification Keywords: *H.4 Information system application*

Introduction

Over the past decade portable devices of "Floratest" family were developed and manufactured in V.M. Glushkov Institute of Cybernetics of NAS of Ukraine. The researchers of chlorophyll fluorescence induction (CFI) effect encounter the problem to gain sufficient amount of data by means of autonomous fluorometers. Besides, the time of a measurement of chlorophyll fluorescence induction varies from several minutes to one hour, depending on environmental conditions, species of plants and experiment specificity. The temperature and humidity of air and soil, illuminance can vary, that can influence on reliability of measuring data. All this has to be taken into account during ecological and agro-ecological monitoring. So, to overcome above-mentioned disadvantages, it was designed wireless biosensors that are combined in wireless sensor network together with special network coordinators, and concentrator [Palagin at al, 2017]. The biosensors were tested in laboratory and field conditions. This paper represents some important results of that testing and data analysis.

Work objectives

Work objectives are testing of developed biosensors and developing database, software and methods to facilitate acquisition and processing of measured data.

Measurement of CFI and its parameters

The technique of laboratory or field experiment includes next:

1. Selecting plants. Planning and choosing testing plants. Goal of an experiment has to be taken into consideration when experiment is planned and plants are selected. A chosen plant-indicator has to be sensitive to stress factor [Guo and Tan, 2015].

2. Plants are grown in identical conditions in pots or on field with identical soil.

3. The grown plants are divided into few groups – control and experimental.

4. Experimental plants are put on influence of stressful factors of different degree in accordance with testing program.

5. Network of biosensors measures chlorophyll fluorescence induction (CFI) of control and experimental plants in accordance with testing program for type of stress and its degree in scheduled terms.

The using of few fluorometers or the developed network of wireless biosensors allows reducing the time needed for measurements and it can provide data that are more adequate. The time can be calculated according to formula:

$$t_e = \frac{\sum_i^N (t_{ad} + t_m + t_{pr})}{N_s},$$

where t_e is a time to get experimental data; N is an amount of measurements; t_{ad} is a time of dark adaptation of leaf; t_{pr} is a time needed to prepare the next measurement; t_m is a time of measurement of chlorophyll fluorescence induction curve, N_s is a number of sensors.

If the sensors are placed on a leaf under sunlight then the dark adaptation has to be not less than 20 minutes. If the plant (or its leaf) is placed during long period in a shadow then 5 minutes is enough for the dark adaptation.

6. Measuring data of chlorophyll fluorescence induction, acquires by biosensors from control and experimental plants.

7. It is useful to record the air and soil temperature and humidity during a measurement of chlorophyll fluorescence induction. In addition, chemical and biological analysis of soil can be used for specific

biological researches. It is allows to take into consideration climatic effect as additional stress factor on parameters of chlorophyll fluorescence induction.

8. The results of measurements are processed by means of graphical, statistical and correlation analysis and machine learning technique. Before analysis, the measured data can be normalized.

9. The finish result of testing is detecting the sensibility of biosensors to influence of different stresses.

Typical curve of chlorophyll fluorescence induction is shown on figure 1. For analysis of measured curves the researchers typically analyze special parameters of CFI curves such as: F_o (initial level of chlorophyll fluorescence); F_m (maximum level of chlorophyll fluorescence); F_{st} (stationary level of chlorophyll fluorescence); $F_v = F_m - F_o$ (variable fluorescence); Fv/Fm ratio, Area (the area above the fluorescence curve between F_o and F_m), F_j (fluorescence value at point J, t ≈ 2 ms); F_i (fluorescence value at I, t ≈ 30 ms) and so on. Also the machine learning method is getting popular recent years [Kalaji at al, 2017].

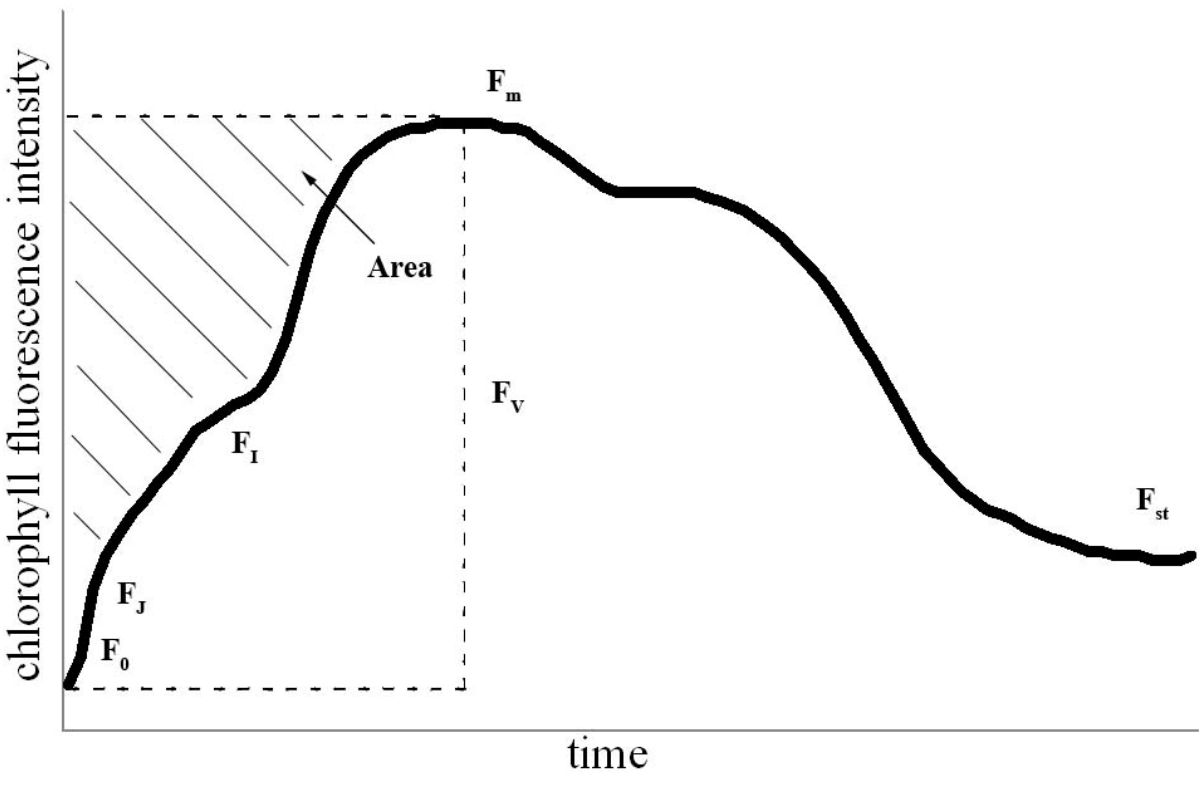

Figure 1. The typical curve of chlorophyll fluorescence induction

Development of software and database for work with chlorophyll fluorescence induction curves

Several activities have to be repeated during processing chlorophyll fluorescence induction curves (CFI) by means of personal computer: opening file with measuring results, graph building for previous visual estimation of dynamics of CFI curve, grouping different measurements, calculation of curves parameters and so on. It gets a lot of time. The special software FAnalyzer was developed to simplify the processing of chlorophyll fluorescence induction curves.

Functions of the developed software are the following:

1) receiving measuring data from biosensors and further data output in form of a graph.

2) storing the received measuring data on hard disk and opening in form of graphs;

3) opening and storing several curves of CFI in one file. The file can be opened later and processed by means of program packages such as R, Excel, Matlab and so on.

4) calculation and storing CFI curves parameters, that are frequently used to analyze the measuring results (F_m, F_o, F_{st}, Rfd, Area, F_i, F_j and other), and main statistical indicators for that parameters.

The graphical user interface of program is shown on figure 2.

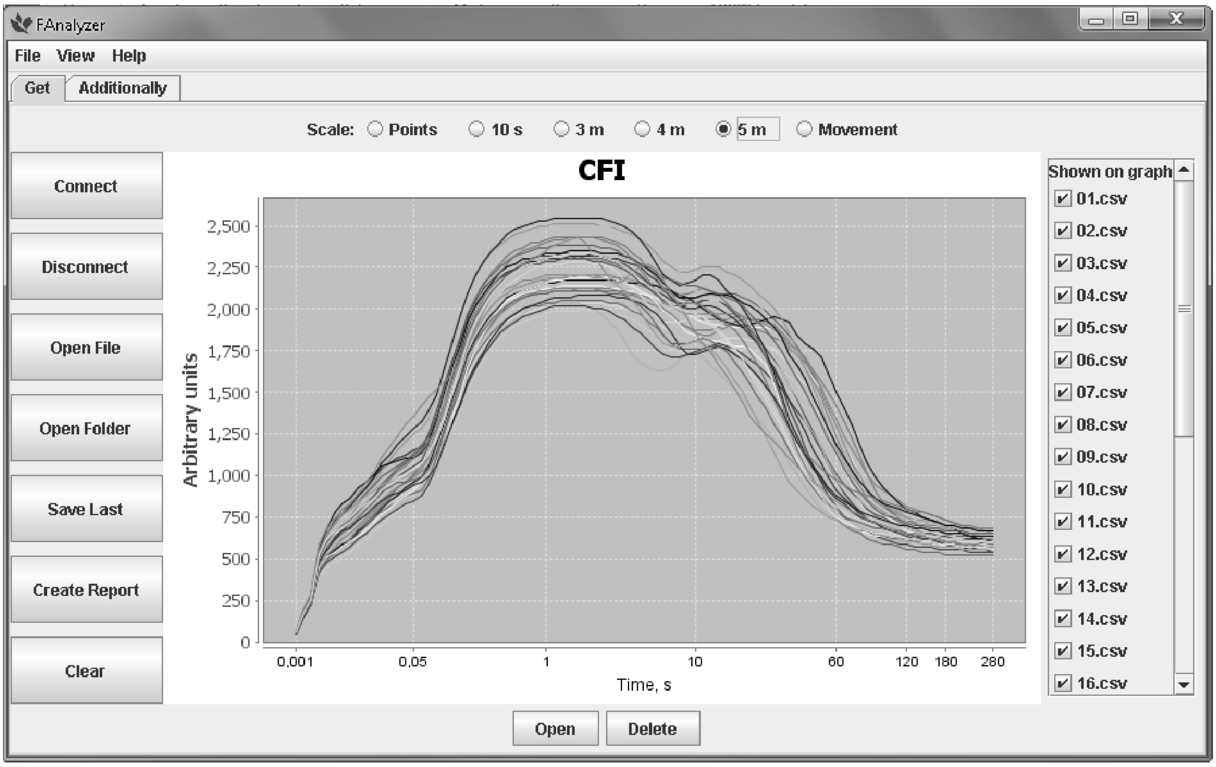

Figure 2. Graphical user interface

The suggested software allows to reduce time for preparing data to analysis, to calculate main parameters of CFI and proper statistical indicators. The calculation can be used for comparative analysis of plant states in conditions of influence of stress factors and in normal conditions.

During using multiple biosensors simultaneously it is necessary to store, process and visualize a large amount of measuring data. For convenience of users, the database and proper graphical user interface were developed. They allow storing a large amount of measurements in one place for further data analysis of measuring data by means of tools and methods, selected by user. During the database development, a set of entities was defined to represent in the database. The last ones contain information about: plant information; type of monitoring of plant state; measured curve of chlorophyll fluorescence induction; information about soil, air and parts of plant (in case of chemical-biological analysis);information about devices and sensors, used for measurements; weather information; information about a person, conducting measurements; information about an organization and a location, where measurement was conducted.

A database management system MySQL was used for database implementation. The database diagram is shown on figure 3.

Importing data to the database can be carried by means of special software.

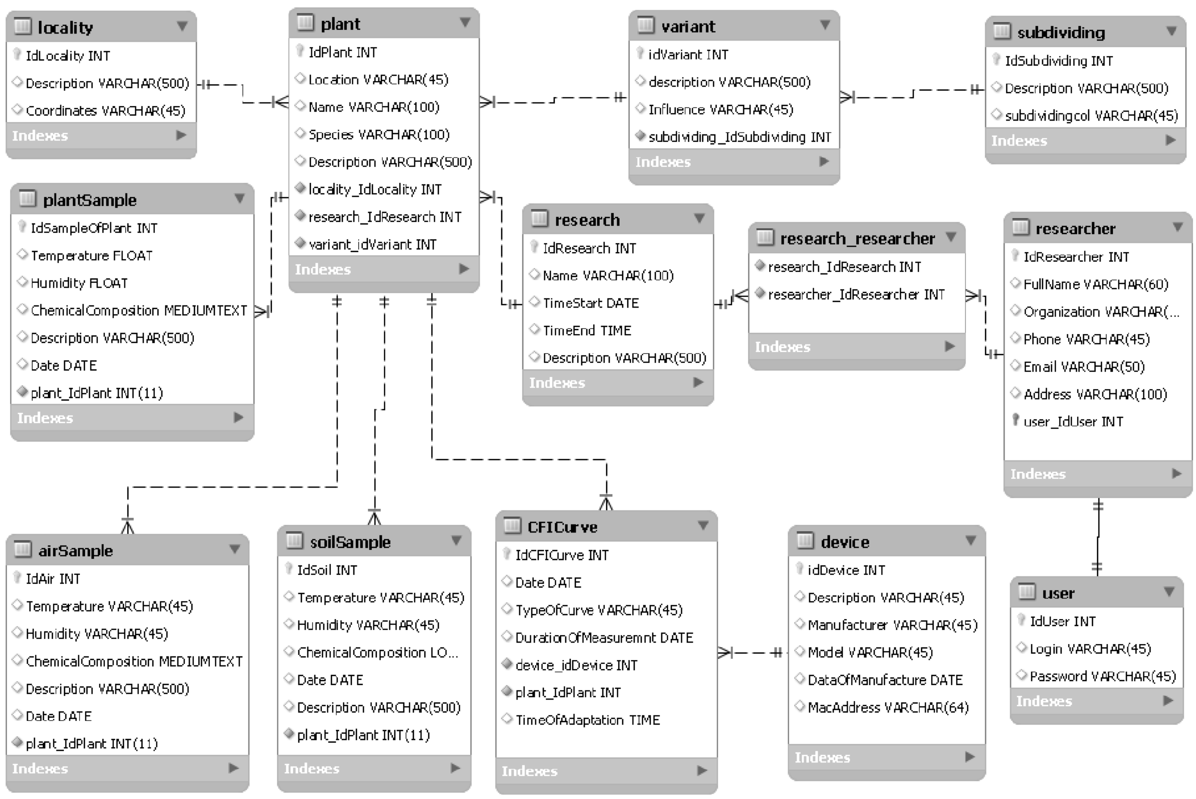

Figure 3. The database diagram

Research of change of chlorophyll fluorescence induction under influence of copper

To research the influence of heavy metals on plants it is reasonable to select the goose-foot plant. Goose-foot has a wide natural habitat, grows in a different environmental conditions. It was studied the influence of different doses of toxicant, copper sulphate ($CuSO_4$), on the test plants Plants were cultivated in 12 pots, three-four plants per pot. The plants were divided into 4 groups. Different concentration of $CuSO_4$ were dissolved in water and brought into the soil of these four groups.

Group 1 (V1) – control group without $CuSO_4$.

Group 2 (V2) – 1 g of CuSO4 / 1 kg of soil.

Group 3 (V3) – 3 g of CuSO4 / 1 kg of soil.

Group 4 (V4) – 6 g of $CuSO_4$ / 1 kg of soil.

The experiment was conducted during 13 days. At the beginning of the experiment the chlorophyll fluorescence induction was measured in all groups of plants (figure 4). The same day the water solution of CuSO4 was brought into soil of test plants.

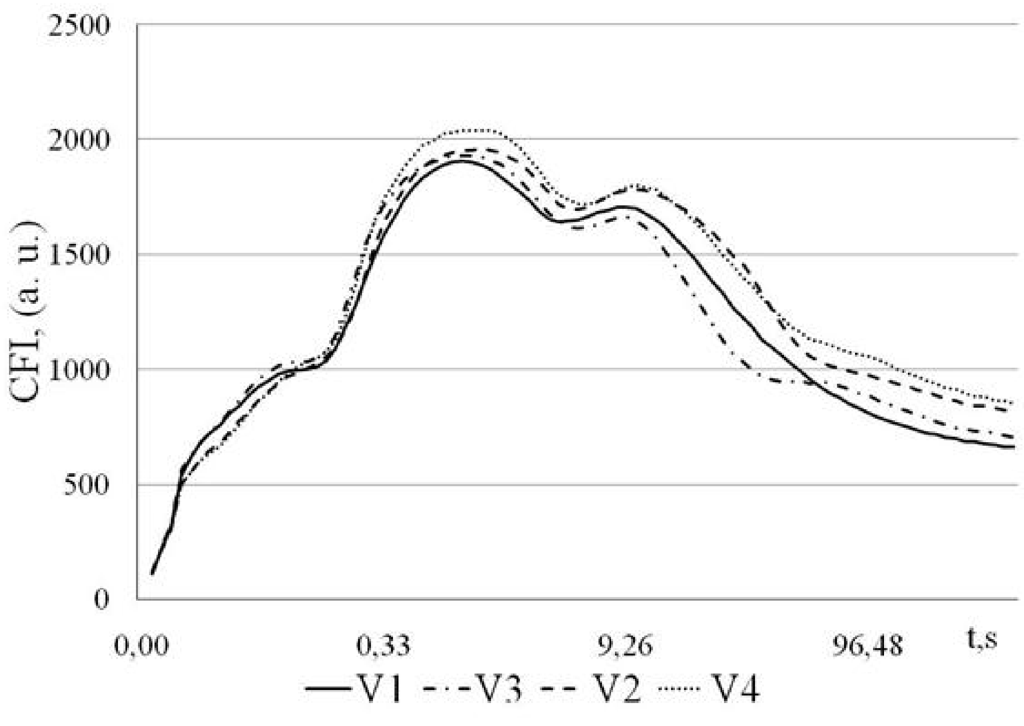

Figure 4. The intensity of chlorophyll fluorescence of goose-foot plant before of toxicant bringing in

The chlorophyll fluorescence intensity of test plants changed under the influence of toxicant. Figures 5 and 6 show graphs of the chlorophyll fluorescence on the second and third days of the impact of copper sulphate. It can be easily seen, that on the third day the maximum level of the chlorophyll fluorescence induction parameters (F_{st}, F_m) considerably decreased for plants that had been treated by toxicant.

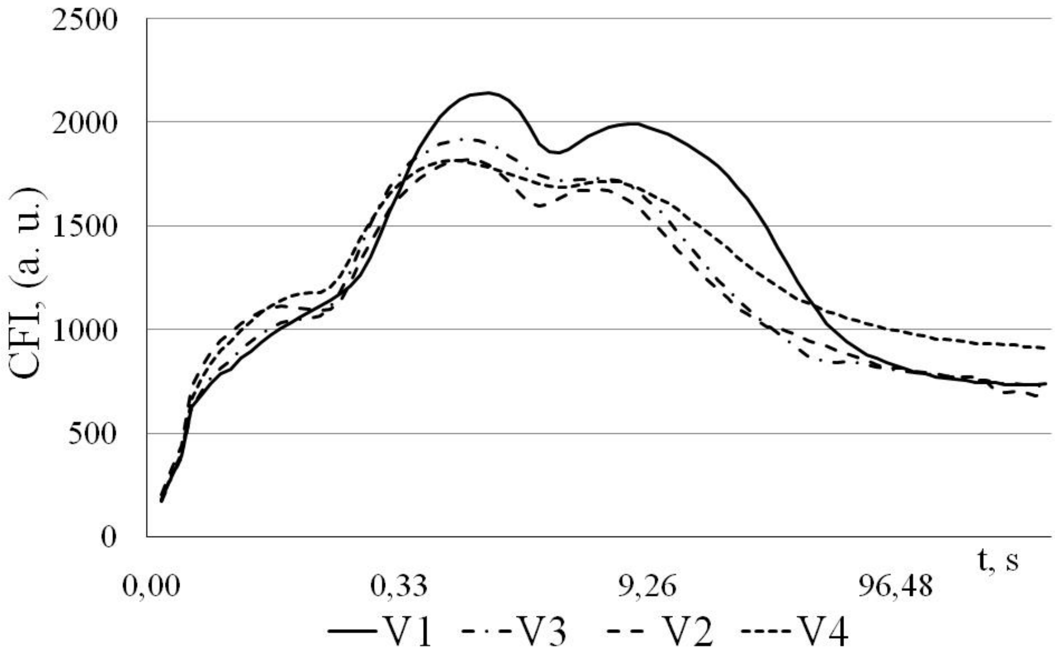

Figure 5. The intensity of chlorophyll fluorescence of goose-foot plant on the second day of toxicant influence

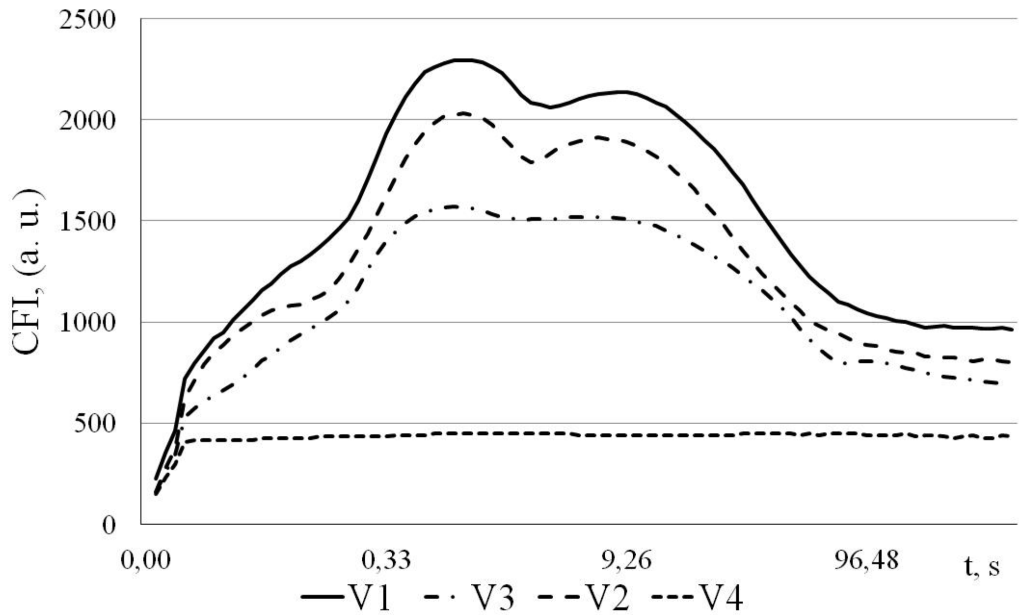

Figure 6. The intensity of chlorophyll fluorescence of goose-foot plant on the third day of toxicant influence

It should be noted, that on the sixth day of toxicant influence only one group of plants V2 remained, two other groups of plants V3 and V4 perished.

Analysis of parameter F_v/F_m provides information about the photochemical reactions, which are most sensitive to environmental factors. The maximum difference of the parameter F_v/F_m between the control group and the group V4, which received the maximum dose of copper sulphate, equals 38 %. At the beginning of the experiment this parameter had almost the same value in the three groups V1, V2, V3, V4 – 0,906 on the average. In the control group parameter F_v/F_m decreased by 5,8 % in comparison with the first day of measurement. In the group of plants V2 on the fifth day of toxicant influence the parameter F_v/F_m decreased by 5 % and the overall decrease equaled 4,6 % in comparison with the first day of experiment. The value of parameter in the group V3 decreased on the six day of the influence of copper sulphate. In the group V4 this parameter decreased by 39 % on the third day of the influence of toxicant. Figure 7 shows changes of the parameter during experiment.

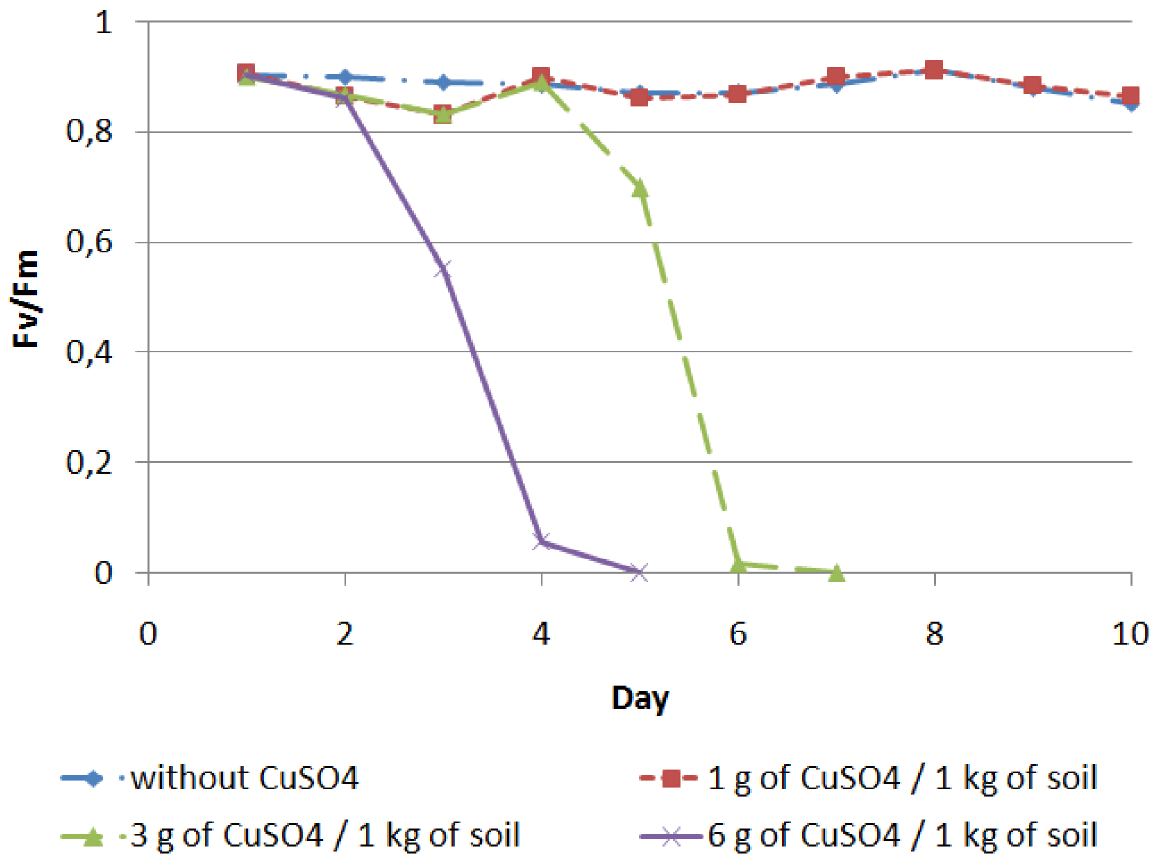

Figure 7. Changes of the parameter F_v/F_m during the testing

Analysis of results shows, that different doses of copper sulphate influenced on the photosynthetic apparatus of plants in different ways. Thus, the dose of 6 grams of copper sulphate was critical for plant of group V4. Also, the dose of 3 g of copper sulphate is critical for plants of group V3 and causes irreversible changes in the plants. Photosynthetic apparatus of plants, treated by 3 g of $CuSO_4$, stops to function on eighth day of toxicant influence. The dose of 6 grams breaks the photosynthetic processes in plants on the third day. However, it should be noted, that the dose of 1 gram of $CuSO_4$ does not cause any serious changes in plants and also does not break the photosynthesis of plants.

For developing methodical support for wireless biosensors the experiment was conducted to research the influence of heavy metals on plants. It allowed to estimate the dose of copper sulphate, that is critical for plants, and to determine informative parameters of chlorophyll fluorescence induction curves. During application of industrial methods the obtained results will be used to detect the presence of heavy metals in plants and estimate their impact on ecological state of certain territories.

Using neural networks for determination of plants under stress

Nowadays neural networks and widely used for the analysis of biological and agricultural data in viral diseases of plants, pest determination, water consumption estimation, plant quality estimation etc. [Samborska at al.].

Researches of influence of herbicide on chlorophyll fluorescence were conducted at the V.M. Glushkov Institute of Cybernetics and the enough amounts of data were gained for using neural networks. Herbicide Roundup (glyphosate) was used for experiments. Roundup is a broad-spectrum systemic herbicide. The plants of Datura stramonium (weed) were divided into three groups. One, control group was not treated and two others were sprayed with different doses of herbicide.

Two-layer feed-forward network was chosen for classification of curves. Neural network has 89 inputs and 3 outputs (every measured curve consist of 89 points). Second, output layer consist of three neurons (three variants of curves). The required number of neurons of hidden layer was determined by conducting series of experiments. The performance (P) of the training was evaluated using means square error.

There were trained neural networks with different number of hidden layer neurons (from 1 to 364). The training of every network was repeated 30 times and the results were averaged and combined in vector P_{mean} (figure 8). Thus, the neural network works most efficiently with not more than 70 neurons in the hidden layer. A neural network with 25 neurons in the hidden layer was chosen for further use. The network uses the sigmoid transfer function for hidden neurons and the softmax function for output neurons.

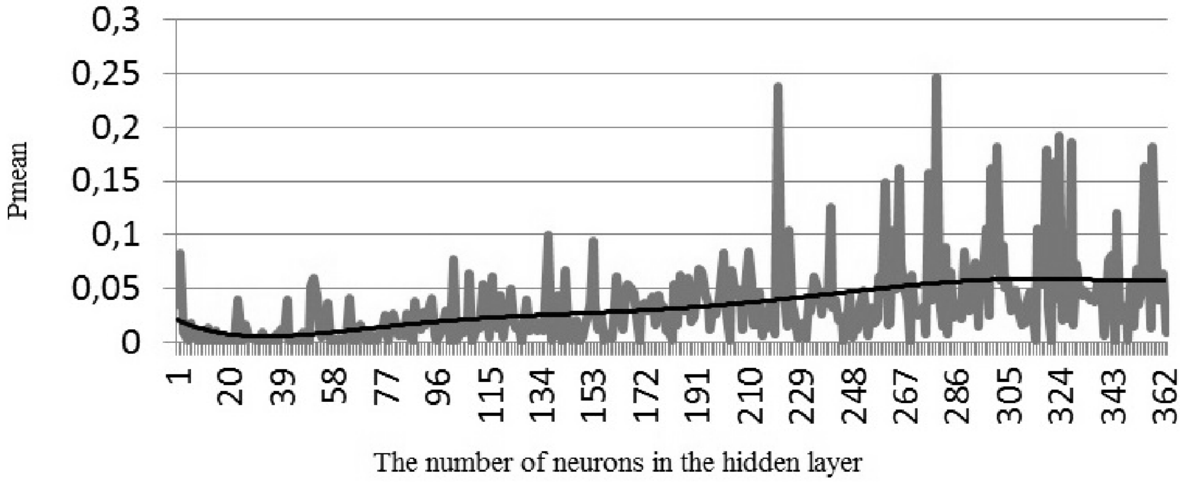

Figure 8. . Dependence of the mean square error of the neural network training on the number of neurons

The neural network was trained on data measured in different days. The data measured in different days were used separately for training of the network. The results of the training are presented in table 1.

As seen from table 1, the smallest errors of recognition were received with data in 7 and 11 days. It is known that Roundup breaks the synthesis of the amino acids on 5-6 day and plants wade and discolor after two weeks. But after two weeks the curves of chlorophyll fluorescence of the treated leaves had serious difference even on one plant, therefore the neural network recognition is unsatisfactory. On the contrary the Student's test confirmed the difference between curves of plants of different groups at the end of second week.

Thus we showed that neural networks can be trained for stress recognition of plants using curves measured by sensors developed at V.M. Glushkov Institute of Cybernetics of NAS of Ukraine.

It is useful to use neural network for creating methods for evaluation of the state of plants in the city and the farm. It can be used at the stage of making decision (start watering, give fertilizer, etc.). Neural network training needs a representative set of data to make valid managerial decision, so the wireless biosensor networks allow to receive enough number of fluorescence induction curves.

Table 1 Results of the neural network training using data measured in different days, where E is an error of training, Ev is an error of validation, Et is an error of testing, Em is a mean calculated from three previous errors.

The number of curves	E, %	Ev,%	Et,%	Em,%	Notes
40	64,3	66,7	33,3	60,0	Before treatment of the herbicide
43	16,1	33,3	66,7	25,6	Before treatment of the herbicide
41	0	33,3	50,0	12,2	The third day after treatment
43	80,6	66,7	83,3	79,1	The fifth day
43	0	0	33.3	4.7	The seventh day
30	0	0	20	3.2	The eleventh day
43	19,4	16,7	66,7	25,7	The thirteen day
21	3,2	0	66,7	11,6	The twentieth day

Using neural networks for determination of plants under stress

CFI curves of different plant species have some significant difference, thus they can be used for determination of specie of plant that are shown in [Kirova at al, 2009] by means of OJIP curve (CFI curve received during nearly 10 seconds) and neural network. With aim of testing the developed sensors for this task, a set of plants was measured. The set includes 176 curves from 6 species. The curves were measured during 5 minutes (full curve of CFI) and 10 seconds (OJIP curve) for next plants: soybean, goosefoot, ficus elastic, ficus benjamina, euphorbia, zinnia.

Two-layer feed-forward network with 89 inputs and 25 neurons in the hidden layer was chosen. The network uses the sigmoid transfer function for hidden neurons and the softmax function for output neurons as in previous experiment. The output layer consists of 6 neurons.

The results of testing of the neural network present in table 2. The neural network was trained 100 times and errors of testing were averaged after.

Table 2. The results of determination of plant species

Duration of measurement of CFI	5 minutes	10 seconds
minimal testing error, %	0	0
mean testing error,%	6,52	9,80

So, the curves of developed sensors can be used for taxonomic determination of plants. The curves measured during 5 minutes are more appropriate for this task. There are raised the issue of determination of plants with large amount of curves of very close species. The approach to solve it is described in [Kirova at al, 2009].

Conclusion

It was conducted the series of experiments for the testing biosensors developed at the V.M Glushkov Institute of Cybernetics of NAS of Ukraine to determine the sensitivity of biosensors to influence of stressful factors of different nature on experimental plants. The suitable software and database were developed to facilitate data processing. As result of using neural network, it can be concluded that neural network can recognize the different dose of fertilizer before changing of leaves appears and a 5 minutes measurement of CFI is more informative for determination of plant species then 10 seconds measurement.

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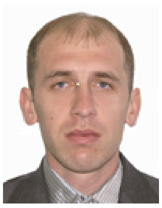

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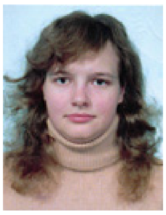

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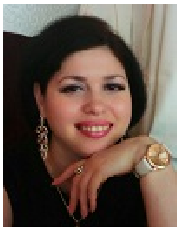

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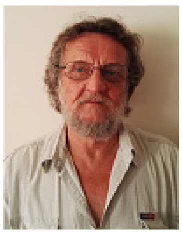

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