Technology to provide continuous monitoring services of inland and coastal waters with the use of bioindication organisms

Jakub DELIKAT
Bionitec Sp. z o.o., Toruń, Poland

jakub.delikat@globedata.pl

Mariusz CZERWIŃSKI
Bionitec Sp. z o.o., Toruń, Poland

mariusz.czerwinski@globedata.pl

Abstract

As part of the implemented project, a new technology was developed that allows for the provision of continuous monitoring services of inland and coastal waters with the use of bioindication organisms. The use of organisms as bioindicators is not a new topic, but many of the systems on offer are either very simple or require frequent maintenance. The proposed technology allows for the construction of cheap, floating, fully automatic water biomonitoring stations using various species of mussels, both fresh and saltwater. Contamination of water used for food purposes and on farms is a major contributor to increased mortality, growth retardation and disturbance and physiological functions, changes in DNA (genotoxicity), changes in tissues (cytotoxicity) and organs of individuals exposed to chemical compounds. One of the most dangerous classes of toxins affecting animals and humans in contact with contaminated water are cyanotoxins released by dying cyanobacteria. They contribute to serious medical conditions as well as fatal events. This type of toxin is relatively difficult to detect due to the seasonal variability of blooming. One of the effective and automatic methods of detecting water contamination in continuous mode is biomonitoring with the use of Dreissena polymorpha mussels.

Keywords: *water biomonitoring, inland and coastal waters, mussels, Dreissena polymorpha.*

1. Introduction

Research conducted by many research groups over the last thirty years shows more and more clearly how important is the quality of water, not only that used for food, but also used on farms (e.g. for watering animals) or naturally circulating in the environment. The impact of pollutants on living organisms, including humans, has been shown to result from the interaction between the contamination and the organism, population or biocenosis. It manifests itself, inter alia, in increased mortality, inhibition and disturbances in growth and physiological functions, changes in DNA (genotoxicity), changes in tissues (cytotoxicity) and organs of individuals exposed to chemical compounds [1]. Since in Poland (as in an increasing number of other countries) the principle of sustainable development has gained a constitutional rank, the method of socio-economic development that integrates political, social and economic activities should be carried out in keeping with the natural balance and durability of basic natural processes. According to the report of the Energy for Europe Foundation, Poland is classified as a country with relatively small water resources. In the international program Population and Environment carried out in 1993, Polish water resources were ranked 72nd out of 100 analyzed countries of the world. Among European countries, Poland is the country most threatened by water deficit. Calculated per capita, Polish water resources amount to approx. 1,580m^3/year, and currently the threshold of renewable water resources in the world is equal to 1,700m^3/year per person. The average level in Europe is 4,500m^3/year per person. In the period of the greatest droughts, water resources in Poland can drop below 1000m^3/year per person.

The level of increased interest in the state of the environment, and especially in its potential threats, may be demonstrated by the systematically growing financial outlays allocated, inter alia, to for activities identifying them and counteracting these threats. According to a report prepared by researchers from the Department of Chemistry and Biochemistry, Bureau of Technology Commercialization at the University of Texas in Austin, USA, the market for detecting and protecting the environment against biological substances and chemicals in 2002 reached the value of USD 2.3 billion, and in 2007 it was estimated at USD 4 billion. In turn, the market for detecting environmental threats in 2003 was valued at USD 3.4 billion and continues to grow. The above data shows how crucial the task is to find effective methods of detecting and preventing environmental threats. On the other hand, as already mentioned, many of them are not used on a large scale, as they lack the appropriate economic justification. For example, it is difficult to imagine water surveys in all water intakes in the country performed at least once every hour (only "large" intakes with an annual consumption of more than 800,000 cubic meters are 258 in Poland, and there are around 5.5 thousand inventoried water intakes - on the basis of the Guidebook of the State Hydrogeological Service [2]). The situation is quite dramatic in the case of Polish lakes as well as of many European ones.

According to the study by prof. Adam Choiński, Poland has 7,081 lakes with an area of more than 1ha, therefore Poland belongs to the zone with a small lake area [3]. Hence, the loss of each of them (eutrophication, overgrowth, overgrowth and transformation into marshes) is an irreparable and irreversible loss of national and social property, especially in the face of future generations of citizens. It has an impact on the country's climate, water management, species balance, domestic tourism and the general health condition of the society. The scale of the phenomenon is reflected by the fact that in just less than 40 years (1954-1992) as many as 2215 lakes disappeared, which accounts for 11.22% of the lake area at that time (data from the catalogs of Polish lakes in 1954 and 1992 by Prof. A. Choiński). The process of disappearance of small lakes with an area of up to 5ha, which in Poland account for about 44% of the total number of lakes, is particularly dynamic. It should be emphasized that lakes are a significant link in the circulation and retention of water in the balance catchments, and in the system of changes taking place at the present time, water pollution manifested by eutrophication is the most noticeable. We are dealing here with inflows of both water nutrients and toxic agents. Accumulation of pollutants is a complex and long-lasting process that may go unnoticed for years. It is mainly related to the concentration of pollutants in bottom sediments and deeper parts of lake basins, and their intensity and importance (as a result of progressive eutrophication) is most often evidenced by the disappearance of many fish species. Restoring the state of equilibrium is a time-consuming and costly process, but it is nevertheless necessary if future generations are to inherit the natural environment in good condition and subjected to anthropogenic pressure as little as possible. It should also be noted that, as is well known, the costs associated with prevention are many times lower than those associated with reversing undesirable phenomena. Therefore, such an important role is played by effective and common methods of monitoring the state of the environment, including flowing and stagnant waters. These methods undoubtedly include systems based on biosensors, which are cost-effective, have a wide range of applications and can be deployed continuously in real time.

2. Occurrence and determination of impurities

The impact of pollutants can be divided into two categories depending on the extent of their impact. Point pollutants are relatively easy to identify and examine their nature (type of pollutant emitted, its concentration, total amount released into the environment over a period of time, etc.). They usually come from industrial and municipal wastewater discharges, outflows from industrial, municipal and hazardous waste landfills, sometimes they appear only temporarily (e.g. as a result of an accident, failure of transport lines, deliberate discharge of harmful substances to the environment). On the other hand, diffuse pollution, i.e. pollution covering large areas or water bodies, is very difficult to identify, characterize and then to counteract (e.g. neutralization, protection). They are mainly caused by runoff from agricultural crops, contaminated soils and urban areas, as well as bottom sediments, dry and wet deposition from the atmosphere, etc. They are characterized by the fact that in many cases diffuse pollution is an unknown mixture of substances with difficult to determine toxicity (due to differences in the proportions of quantitative components and the amount of emissions difficult to determine). Moreover, many diffuse pollutants (as opposed to the most intensive point pollutants) cause the transfer to the environment of substances with concentrations that become dangerous only after prolonged exposure to living organisms, and temporarily do not clearly mark their presence. In addition, their distribution over a larger area causes a decrease in point detection with possible long accumulation in the bodies of organisms. Hence, diffuse pollution is the main cause of diseases of individual organs, especially among mature animals. They also lead to genotoxicity, which manifests itself in mutations often transferred to the next generations, leading to significant phenotypic changes in a sufficiently long time. Mutations refer to genetic changes in somatic or germ cells. Mutations in somatic cells can contribute to a variety of defects up to the formation of cancer, while mutations in germ cells can cause genetic diseases in the next generation. While the relationship between exposure to individual chemicals and carcinogenesis in humans is well researched, similar relationships for hereditary defects are difficult to prove [1]. Nevertheless, exposure of living organisms to long-term effects of various substances is not only toxic, but often teratogenic and genotoxic, because the scope of contamination and their persistence may be so large that their effect is chronic and its effects appear after a sufficiently long time [4]. One of the greatest threats is cyanobacterial blooming and the cyanobacterial toxins released by them in eutrophic water reservoirs (lakes, ponds, dam reservoirs) and coastal waters, which are dangerous to human health and life. Among cyanobacteria toxins, we can distinguish, among others neurotoxins (e.g. anatoxin-a, anatoxin-a(s), saxitoxin and neosaxitoxin), causing cancer (e.g. microcystins, lipopolysaccharides), dermatotoxins (e.g. lyngbyatoxin-a, aplysiatoxin and lipopolysaccharides, nodrocularins, and nodroculars cylindrospermopsin) [5]. The most common toxin is a hepatotoxin called microcystin. Currently, more than 70 different structures of these compounds are known. Hepatotoxins, which include microcystins and nodularins, are responsible for poisoning animals and humans that come into contact with toxic blooming. Due to their chemical structure, they are characterized by high durability in the water environment. Moreover, neurotoxins (anatoxins and saxitoxins), due to the low value of the lethal dose, are among the strongest natural toxins. The presence of the neurotoxic amino acid BMAA (ß-N-methylamino-L-alanine) may be closely related to cases of Alzheimer's disease registered in Canada [6].

Determination of water components by chemical or physical methods, apart from the significant cost-consumption, has several other features that significantly limit their use as elements of constant environmental supervision. One of them is the need to conduct most of the tests in a laboratory equipped with appropriate reagents and research equipment. Another is related to the time that elapses between the moment the sample is taken and the moment the results are obtained (this also includes the time needed to collect samples and transport them). Nevertheless, one of the most important features is the need to determine in advance what risk is to be related to a specific study. Is it e.g. a marking of the presence of a specific species of bacteria, an element or a toxin? There is a very high probability that for a particular series of tests, factors that have not been thought of before and then ignored in selecting a test method will go unnoticed. This property is practically absent in the case of using live bioindicators with an appropriate sensitivity spectrum. If any substance or biotic factor adversely affects the selected test organism, an appropriate response to the adverse factors (including death) will occur, whether or not the researcher predicted certain stimuli (e.g. poisons). Hence, biomonitoring methods are much more versatile than laboratory physico-chemical approaches (although the latter are much more precise and are necessary in the processes of accurate hazard identification). Particularly noteworthy is the fact that the high seasonal variability of phytoplankton, which is a good indicator of early eutrophication, makes the assessment of reservoir fertility difficult [7]. Phytoplankton, like phytobenthos, absorb nutrients directly from the water. Then part of it is filtered through filterers, which also include mussels. Hence, the indicative methods based on these molluscs can reliably inform about the risk of early eutrophication.

3. Biomonitoring

The main idea of the use of mussels of the species Dreissena polymorpha, together with the classification of patterns observed in the behavior of molluscs, comes from Prof Ryszard Wiśniewski, a hydrobiologist known in Poland and abroad, a long-term employee of the Nicolaus Copernicus University in Toruń. The mere fact of using living organisms, including mussels, to monitor the surrounding environment is not a new idea. Many solutions were created decades ago. Most of them, however, stopped at the level of technical advancement of that time and still offer only the basic functionality. It consists in measuring the angle of the shell and inferring the state of the environment on the basis of the results of this measurement. In the simplest version, the measuring system consists of a group of mussles that are permanently attached to the prepared base. The zebra mussel belongs to sedentary species which are not disturbed by the phenomenon of permanent immobilization. Often, these mollusks settle on their kinsmen, creating extensive colonies. Organisms form such a dense system that most of them are practically forced to stay in one place. The mussle eats by taking water up through the inlet siphon, trapping food particles, and then removing the water through the drain siphon. The matter that the mollusk finds valuable is digested and absorbed, and the unfit particles are clumped into larger clumps (pseudofecal matter) and removed from the body along with the discarded water. A side effect of this process is the filtration of water from suspensions, the particles of which are small enough to remain in the water for a long time. The phenomenon of floating matter is particularly visible in the so-called re-suspension layer, i.e. the area of highly hydrated sediments directly adjacent to the bottom of the eutrophic reservoir. Due to the action of filtering organisms, the particles of the suspension are bound into larger lumps, which more easily fall to the bottom and clump into the soil of greater density than the resuspension layer. In the case of mussels, the filtration process is accompanied by contractions of the shell sphincters, which are manifested in the movements of both flaps.

In biomonitoring, a system measuring the opening angle is placed on the one flap or both shell flaps, thanks to which it is possible to register changes and dynamics of dilation over time. In older designs, a system of metal rods was used to change the resistance of the active element. Today, the magnet Hall effect sensor is used, which can measure changes in field strength with high accuracy and resolution over a wide range. Moreover, this type of construction is resistant to the factors prevailing in water reservoirs and has a relatively low temperature dependence (most systems independently perform temperature compensation). By collecting the matter floating in the water, the mussel reacts to its chemical composition. Since Dreissena is a eurytopic species, it can survive very unfavorable conditions, including significant contamination of the aquatic environment. On the other hand, it is sensitive to the composition of the absorbed food, which allows its reactions to be used in biomonitoring. Both of these facts make Dreissena a very useful and quite universal bioindicator. Eurytopicity is ensured by a wide range of concentrations and harmful substances detected by molluscs. Food sensitivity, in turn, enables a reaction to even low concentrations of harmful substances. It has one more important meaning. Due to the characteristic reactions of the mussel, it is possible to detect which class of substances the contamination belongs to and in which concentration class it occurred. Obviously, the clam is not a physico-chemical sensor and as such does not provide a response comparable even to a small degree with a laboratory or even field test. Nevertheless, biomonitoring has two main advantages. First, it is universal. If a physico-chemical sensor is not designed to detect a specific substance, it will not respond to it during operation. A living organism was designed by nature and thus reacts to anything that is harmful to it. As a rule, what harms mussles is also dangerous to humans, and the concentrations detected by mollusks are so low that mussels reactions can be interpreted as a potential hazard rather than contamination. Secondly, the mussels can function continuously and are cheap to obtain. A set of physico-chemical sensors, which would have a similar versatility and sensitivity and could work in a continuous mode, is incomparably more expensive, not to mention the necessity of servicing and the risk of theft (mussels, unlike measuring equipment, are rather not an object of desire even for scrap collectors, and the biomonitoring station can only be damaged by the robber’s ignorance). Importantly, mussels are such a complex organism (e.g. compared to bacteria) that they have a whole range of specific reactions, which allows for labeling the classes of substances and their concentrations, and on the other hand, simple enough (e.g. in comparison with fish) that they can function practically without human care.

4. Technology

As already mentioned, using the mussels reaction to infer the purity of the water is not a new idea. They are used in selected water intake stations, treatment plants, rivers and lakes. Nevertheless, the vast majority of these solutions work basically in two states, i.e. they either indicate the absence of a threat or its presence. In the first case, individual specimens have their shells open to varying degrees and move them, which is characteristic of their life activity. However, when all or almost all shells are closed, this situation is interpreted as a threat (stress occurs in mussels). The worst condition is the complete opening of the shells and the absence of any movement for most or all individuals. Such a situation means the death of mollusks, possibly caused by high and deadly water contamination. The creators of biomonitoring solutions sometimes add mixed states, i.e. they inform about a potential, unspecified threat if most of the mussels close their shells. The problem is that mussels can succumb to a variety of stress factors, not necessarily chemical ones. One example would be noise from a motor vehicle, human activity or oars hitting the water surface. Mussels also react to rapid changes in lighting, temperature, fluctuations in pH, and many other stimuli. Hence, the functioning of biomonitoring in a two-state manner is quite ambiguous and often erroneous (false alarms).

The idea of the founders of Bionitec differs from the approach described above. The first distinguishing feature is the use of a sufficiently large colony of mussels so that their reactions are statistically significant. Besides, there is always a certain proportion of individuals that may die, which should not be of great importance, as long as this proportion is sufficiently small compared to the entire group. In many previous solutions 8-10 individuals were used. For comparison, Bionitec devices contain at least 50 of them. The second distinguishing feature is the approach to recorded data. First, in an appropriately equipped laboratory, research is carried out on a large group of individuals (approx. 1000), how mollusks react to particular chemical stimuli, other physical stimuli and how they behave in the absence of these stimuli. Recorded time series are then subjected to filtering and advanced data analysis. Among others, methods based on DWT (Discrete Wavelet Transform) and classifiers k-NN (k-Nearest Neighbors), SRDA (Spectral Regression Discriminant Analysis) and FDA (Fisher Discriminant Analysis) are used. This approach was successfully used for the first time by the team of R. Wiśniewski, P. Przymus and K. Rykaczewski [8] [9]. Due to the large amount of recorded data, the solution uses techniques for processing big data in real time, utilizing the capabilities of specialized GPGPU computing processors (architecture used, among others, in the world's strongest supercomputers). The detected patterns are indicators determining the most probable type of contamination and the class of its intensity (details are determined later as a result of a toxicological laboratory test). If a threat is detected, an appropriate message or alert is sent to authorized persons or bodies. The solution can also make autonomous decisions, e.g. to temporarily shut off the drinking water intake.

The project also resulted in the development of a new biomonitoring station with easy assembly and disassembly of bioindicators. Hall sensors installed in bioindicator mounting bars record the strength of the magnetic field from a small magnet attached to the mussel shell. Bioindicators are attached with one shell to the bioindicator plate, which is then placed in milled slots on the bioindicator mounting bar. A small magnet is attached to the second, free shell. During the free movement of the shell, the magnetic field recorded by the Hall sensor changes. From the recorded time series, machine learning algorithms (e.g. discriminant classifiers) identify markers related to the presence of pollutants in the water that are dangerous for humans and animals (e.g. farms), as well as the processes of over-fertilization of the reservoir. Recorded waveforms are sent by a GSM transmitter (modem) to analytical servers. On the other hand, servers can automatically notify appropriate services or activate specific devices after detecting disturbing tags. The simplicity and modularity of the station's construction allows for the implementation and maintenance of water monitoring points with relatively low costs and low overhead. The station can also be serviced by people without specialist technical qualifications, immediately after undergoing a short on-line training.

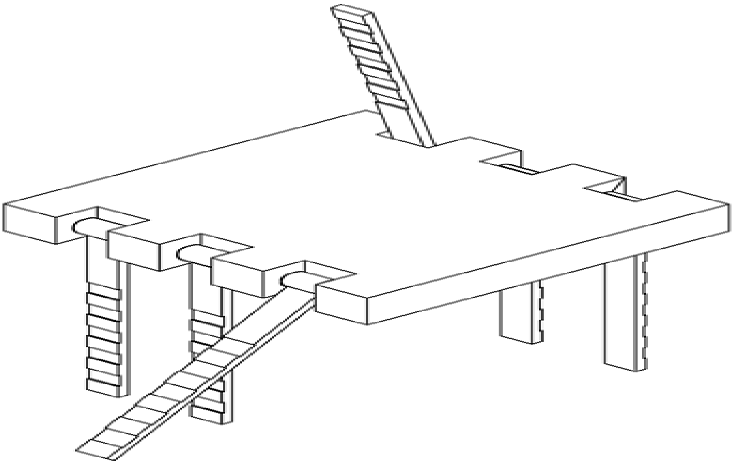

Figure 1. The biomonitoring station with rotationally movable mounting bars.
Source: *own materials.*

Figure 2. Mounting method of bioindicating plates with mussels on station mounting bars.
Source: *own materials.*

During the project the following technological domains have been developed and implemented:

- multidimensional spaces of measurement data and the characteristics of their areas,
- designating similar and self-similar areas in a multidimensional space,
- visualization of the results of advanced data analysis,
- classification machine learning,
- probabilistic classification,
- regression models and SVM in the construction of classifiers,
- optimal classifiers and techniques of results representation.

5. Test results

The new technology developed as a result of the project allows for early warning against contamination of water reservoirs with substances that are harmful and toxic to both humans and animals, e.g. farms. It can also be used in public health systems, systems of counteracting terrorist attacks, as well as in military systems protecting bases against chemical and biological attacks. The diagrams below show examples of bioindicators' reactions (patterns detected) in a monitoring station to contamination of utility waters with the chemical compound $C_3H_8NO_5P$ known as glyphosate. It is an organic chemical compound from the phosphonate group and it is often used as an active ingredient in non-selective herbicides utilized in agriculture. In 2017, the European Chemicals Agency sustained reports that glyphosate causes serious eye damage and is dangerous to aquatic organisms [10]. On the other hand, the International Agency for Research on Cancer (MABR) has classified glyphosate as a possibly carcinogenic substance to humans (group 2A) due to limited evidence increasing the risk of non-Hodgkin's lymphoma [11].

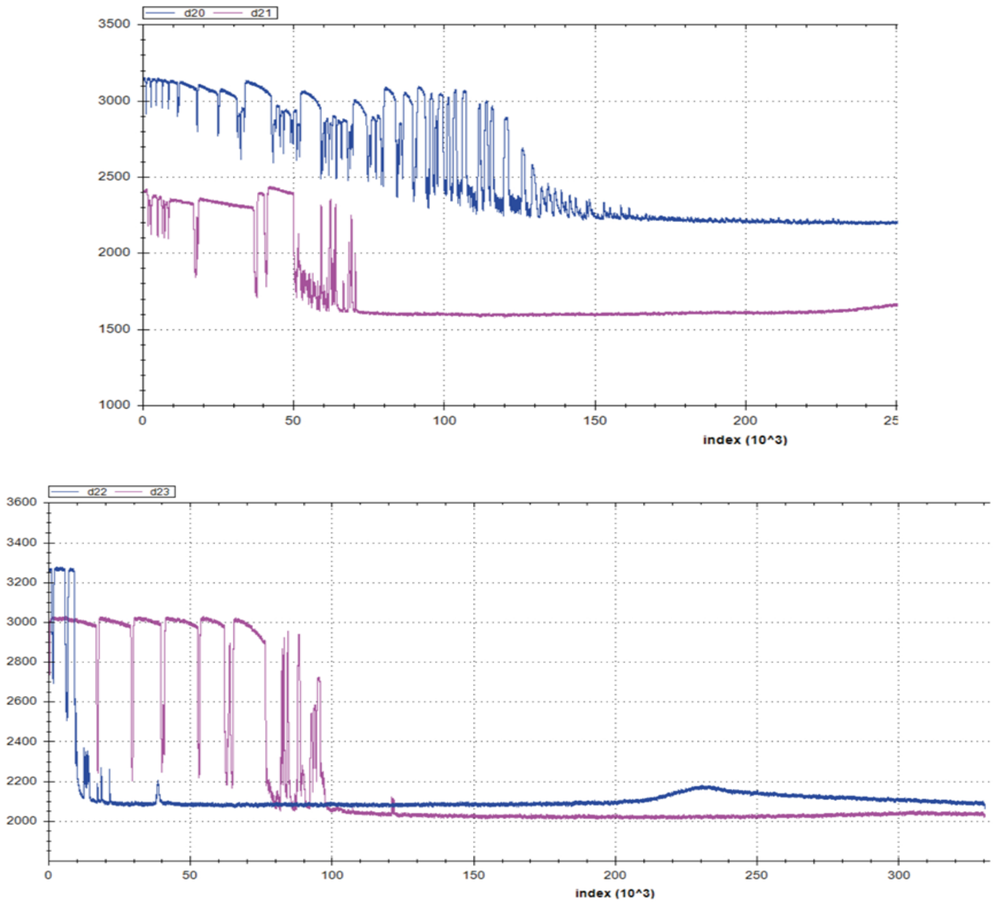

Figure 3. Recorded movements of the mussel shells in response to a toxin. There can be noticed a repetitive pattern of movements of each individual.
Source: *own materials.*

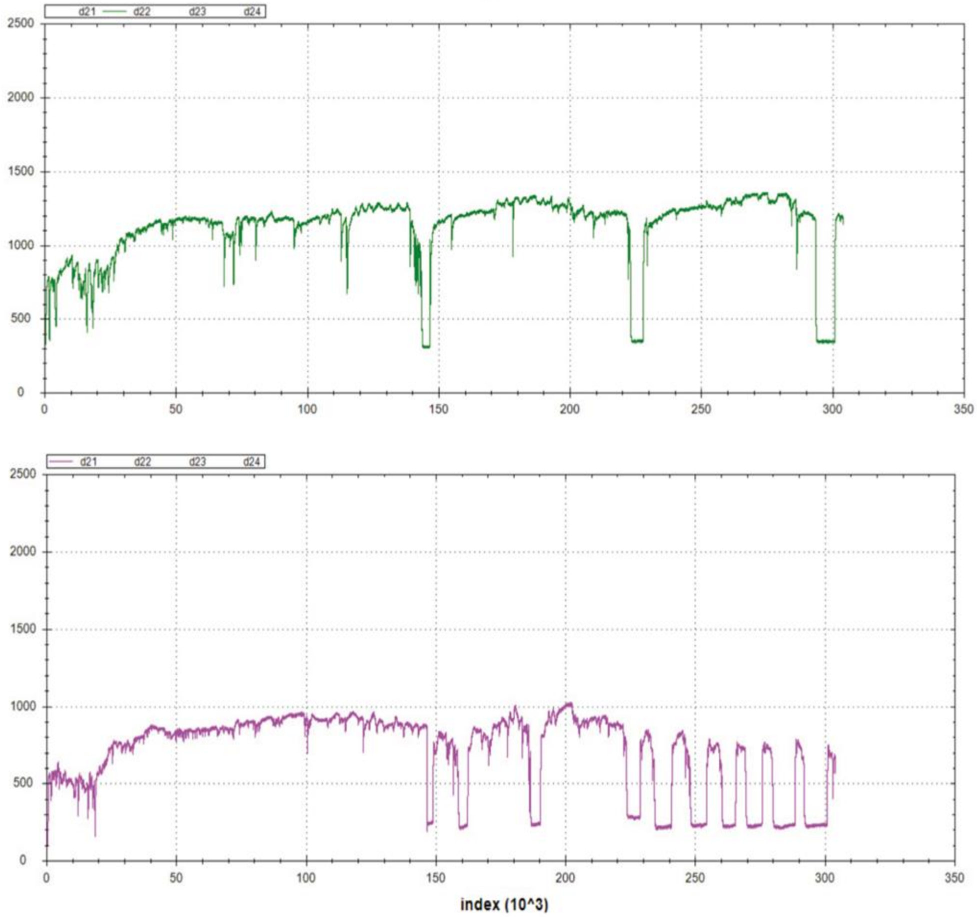

Figure 4. Recorded movements of mussel shells in the control group. A significant difference in the movements of shells compared to the previous, toxin treated group can be seen.
Source: *own materials.*

Acknowledgements

The project was co-financed by the European Regional Development Fund under the Regional Operational Program of the Kujawsko-Pomorskie Voivodeship for 2014-2020. Project title: "Implementation of research works aimed at generating new knowledge in the form of technology for the provision of services of continuous monitoring of inland and coastal waters with the use of bioindication organisms".

(PL: Projekt współfinansowany ze środków Europejskiego Funduszu Rozwoju Regionalnego w ramach Regionalnego Programu Operacyjnego Województwa Kujawsko-Pomorskiego na lata 2014-2020. Tytuł projektu: "Realizacja prac badawczych mających na celu wytworzenie nowej wiedzy w postaci technologii do świadczenia usług ciągłego monitoringu wód śródlądowych i przybrzeżnych z wykorzystaniem organizmów bioindykacyjnych".)

References

[1] Traczewska TM. (2011), *Biologiczne metody oceny skażenia środowiska.* Wyd. 1 Wrocław: OWPW: 19-21, 15-17, ISBN 978-83-7493-597-5.

[2] Frankowski Z, Gałkowski P, Mitręga J. (2009) *Struktura poboru wód podziemnych w Polsce.* Wyd. 1 Warszawa: PIG: ss.162; ISBN 978-83-7538-450-5.

[3] Choiński A. (2006) *Katalog jezior Polski*. Wyd. 1 Poznań: WN UAM; ISBN 83-232-1732-7.

[4] Miller WE, Petreson SA, Greene JC, Callahan CA. (1985), *Comparative texicology laboratory organism for assessing hazardous waste sites*. Environ Quality; 14(4):569-574.

[5] Ilnicki P. (2002) *Przyczyny, źródła i przebieg eutrofizacji wód powierzchniowych. Przegląd Komunalny*; 2:35-49.

[6] Błaszczyk A, Mazur-Marzec H. (2006) *BMAA i inne neurotoksyny cyjanobakterii*. Polish Hyperbaric Research; 4(17):7-14.

[7] Soszka H. (2009) *Problemy metodyczne związane z oceną stopnia eutrofizacji jezior na potrzeby wyznaczania stref wrażliwych na azotany.* Woda-Środowisko-Obszary Wiejskie; t. 9 z. 1 (25):151-159.

[8] Przymus P, Rykaczewski K., Wiśniewski R. (2014) *Zebra mussels' behaviour detection, extraction and classification using wavelets and kernel methods*. Future Generation Computer Systems; 33:81-89.

[9] Przymus P, Rykaczewski K, Wiśniewski R. (2011) *Application of Wavelets and Kernel Methods to Detection and Extraction of Behaviours of Freshwater Mussels. Future Generation Information Technology*. Third International Conference, FGIT 2011 in Conjunction with GDC 2011 Jeju Island, Korea, December 8-10, 2011 Proceedings. 1th edition Berlin Heidelberg: Springer-Verlag: 43-54 ISBN: 978-3-642-27141-0.

[10] *Glyphosate not classified as a carcinogen by ECHA – All news – ECHA (https://echa.europa.eu/pl/-/glyphosate-not-classified-as-a-carcinogen-by-echa*) [access 2020-09-25].

[11] Guyton, Kathryn Z, Loomis, Dana, Grosse, Yann, El Ghissassi, Fatiha. (2015) *Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate*. The Lancet Oncology. 16 (5), s. 490–491. DOI: 10.1016/S1470-2045(15)70134-8. PMID: 25801782.