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LIVING ORGANISMS IN WATER QUALITY BIOMONITORING SYSTEM

ORGANIZMY ŻYWE W SYSTEMIE BIOMONITORINGU JAKOŚCI WODY

Abstract: The typical living organisms used for biological monitoring of surface waters and their use in applications are presented in the paper. The main focus is on the characterisation and use of freshwater mussels. The paper discusses the role of biomonitoring in surface water supply systems. Three-stage biomonitoring system which has been functioning for 20 years in Water and Wastewater Company (ZWiK Sp. z o.o.) in Lodz is presented as an example. The system employs living organisms such as mussels, perch and luminescent bacteria.

Keywords: bioindicators, water quality biomonitoring, mussels

Introduction

Bioindication is used to assess the quality of lake and river surface water, groundwater and, more and more often, treated water. The degree of water contamination is determined using short-term cultures (bioassays) or through long-term observation, *ie* continuous recording of the condition of aquatic organisms (biomonitoring). Behavioural changes observed in bioindicators are a sign of stress resulting from adverse or harmful effect of external factors.

Bioindication allows for detection of total toxicity of all hazardous substances, which is particularly important for substances with synergistic toxic effect. It is thus a measure for evaluation of total toxicity of the controlled system and a valuable addition to periodic laboratory tests of water.

The usefulness of species or groups of species whose behaviour may serve as a biological indicator of the change in the quality of ecosystems and environment is

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being analysed [1]. Response of indicator organisms may differ in terms of sensitivity, from behavioural and physiological changes to morphological changes and mortality. The paper [2] discusses the role of bioindication in water quality analysis, aquatic indicator organisms useful in bioindication, active and passive bioindication approaches and measures of restoration of water resources. The organisms most frequently used as bioindicators include crustaceans (including bivalves), fish, protozoa and algae [3–5]. Numerous publications [6–12] provide information on sorption properties of marine and freshwater algae. They discuss kinetics and sorption equilibrium of heavy metals in algae-solution system, influence of abiotic factors on the process of sorption and desorption of analytes from biomass. The results of laboratory tests conducted on different species and types of algae to assess their usefulness as natural sorbents are provided. Studies conducted on algae take into consideration, among others, their chemical composition, biology, taxonomy, physiography and their application for the assessment of water pollution with heavy metals, pesticides and radionuclides. In another study [13] water quality was assessed in five oxbow lakes of Lyna. Apart from physico-chemical indicators, typical biological indicators were used. Water quality was assessed based on the assumption that healthy aqueous ecosystem is inhabited by abundant invertebrate communities. Several groups of invertebrates were analysed. Dietera, Oligochaeta and Gastropoda larvae were shown to be the most abundant. The structure of invertebrate communities is not only a source of information on water quality but it may also help to develop measures for the protection of water resources. Biomonitoring plays a particularly important role in surface water intake systems. Surface waters are characterised by high variability of quality and chemical composition related to, among others, surface runoff, discharge of untreated wastewater, inflow of leachates from landfills, contamination from industrial accidents, increased use of chemicals in agriculture and internal factors such as algal bloom. Furthermore, despite establishment of safeguard zones, surface waters may become contaminated as a result of ecological disasters or terrorist attacks.

The criteria for selection of organisms to be used in biomonitoring of water in water supply systems (and for other purposes) are strict: such organisms must react quickly and reliably to environmental changes, responses should be unambiguous and easily interpretable, their maintenance in laboratory conditions should be easy, living conditions and habits should allow automated and continuous monitoring of their behaviour. There are two approaches that may be adopted in biomonitoring. One approach involves working with the selected groups of living organisms which satisfy the above criteria and the other is observing the behaviour of typical groups of organisms in their natural habitats in ecosystem, as mentioned before. The former approach is usually applied in laboratory conditions, particularly in inspection of the quality of drinking water.

Taking into consideration the criteria applicable to specific groups of organisms useful in biomonitoring, the paper discusses freshwater mussels used most frequently as bioindicators of negative changes in their living environment (both in laboratory and natural conditions). Other organisms which may serve as bioindicators are discussed. The paper presents three-stage water quality control system at Water and Wastewater

Company [Zaklady Wodociągow i Kanalizacji – ZWiK Sp. z o.o.] in Lodz as an example of biomonitoring used for the surveillance of the quality of drinking water with the use of mussels and other organisms.

Characterisation and use of bivalves in biomonitoring

Bivalves (*Bivalvia*) belong to a class encompassing 8000 species, the majority of which lead a fairly sedentary life, moving to other sites only when they are in danger [14]. 38 species of gastropods and 6 species of bivalves are protected pursuant to the Polish law (Regulation of the Minister of Environment as of 28th September 2004 on the species of wild animals under protection; Journal of Laws No. 20). These are only benthos aquatic animals (known already in the Cambrian) of laterally compressed bodies enclosed by a shell consisting of two valves. Bivalve body is bilaterally symmetrical, usually elongated, laterally compressed and enclosed between two, usually symmetrical, halves of shell. Both halves (right and left) are joined together at the top with a conchiolin ligament. In some species it is an arrangement of calcareous plates and various type of teeth that form the hinge. Hinge plates are an important element in bivalve structure as they prevent the shell from becoming disarticulated when the shell opens and closes. Bivalves vary greatly in size and weight from several millimetres of freshwater *Pisidium* up to 250 kg of the giant clam (*Tridacna gigas*). Bivalve shell is composed of three layers: the outermost layer made of conchiolin, middle layer made of calcite and the innermost layer made of nacre. Nacreous layer is composed of tiny aragonite platelets [15]. 34 species of bivalves have been observed in Poland's inland waters and 9 species have been observed in the Baltic Sea. Bivalves feed mainly on detritus, algae, protozoa and bacteria. They are excellent filters. Within one hour an individual is able to filter as much as 1.5 litre of water. Freshwater mussels have strong sense of touch. Numerous sensory receptors are located on labial palps, foot surface and along the edges of the siphons. Bivalves protect themselves against hostile environment. The following may be interpreted as a potential threat: change of water temperature, sudden water flow or increased concentration of pollution. Upon the inflow of substances which are harmful to their metabolism they close the valves and siphons abruptly thereby reducing their physiological activity. They are also very sensitive to increased concentrations of coarse-grained suspensions in water. They have an excellent chemical sense, which makes them even more sensitive to the presence of chemical substances in water. This feature has been used for bioindication purposes in water bodies. The crustacean *Balanus improvisus* and the mussel *Mytilus trossulus* were used as biomonitors of the trace metals Cu, Zn, Cd, Fe, Pb, Mn and Ni at five selected sites of the Gdansk Bay [16]. Biomonitoring run as a pilot of the future programme was to establish local contamination of water with metals the loads of which might vary according to what flows into the bay with waters of the Vistula river. The study showed correlations between accumulated concentrations of metals in the two bioindicators. Similar study was described in the paper [17]. Native and caged mussels were used for the monitoring of biological pollution in the Basque coast. After 3 weeks the mussels were collected from each site and analysed (from molecular to organism level) to determine chemical contaminant concentrations (metals, PAHs, PCBs, phthalates and nonylphenol). The authors concluded that the use of native mussels is an appropriate and cost-effective approach to monitoring the effects of pollution as it allows for adaptation to the changing site conditions. On the other hand, caged mussels remain particularly useful to determine the effects of large and transient pollution as they provide a highly sensitive and rapid response. Since 1983, the Ontario Ministry of the Environment has run its comprehensive 26-year Niagara River biomonitoring program using mussels (*Elliptio complanata*) [18, 19]. The objective of the program was to determine the presence and fluctuation of pollution at selected sites: several sites in Canada and five in the USA. Over the years no significant contamination was detected and in some cases pollution concentrations in tissues of the analysed mussels were found to have decreased. Optimistic results concerning the decreased concentrations of hexachlorobenzene, chlorobenzene, pesticides and industrial chemicals were reported.

Biomonitoring System at ZWiK Sp. z o.o. in Lodz

Mussels as bioindicators

Mussels may be useful also outside their natural environment. Placed in flow-through aquariums they form an important element of water quality control system in water supply company. The Lodz's water supply company introduced its first bioindicator system in mid-1990s. At that time perch fry was used to determine the quality of surface water drawn from Sulejowski Reservoir. Later on, mussels were introduced for biomonitoring at that intake. The same bioindication system was implemented at another Lodz's surface intake, *ie* the Pilica intake in Tomaszów Mazowiecki. The originator and founder of the entire biomonitoring system at Water and Wastewater Company (ZWiK Sp. z o.o.) in Lodz was its Chief Technology Officer – Bogumił Rzerzycha.

At present, biomonitoring at ZWiK Sp. z o.o. in Lodz is based on a comprehensive water quality system located throughout entire water supply network providing water to points of consumption in Lodz, Tomaszów Mazowiecki, Rokiciny and villages along the road connecting Tomaszów Mazowiecki and Lodz. Perch (*Perca fluviatilis*) and bivalves – swollen river mussels (*Unio tumidus*) are used as indicator species. Such bioindicator systems were installed: at surface water intakes and groundwater wells, after each water treatment stages and at outlets of the treatment plant. Furthermore, in 2011, rapid toxicity tests employing bioluminescent bacteria (*Vibrio fischeri*) were put into place [20].

SYMBIO system employing mussels comprises three elements (Fig. 1):

– Flow-through aquarium – to which the tested water is fed. 8 specimens attached to plastic plates are placed inside. The occurrence of harmful substance in water induces stress in mussels and they respond by immediate closure of shell;

– System controller – receiving signals from the probes. System controller analyses data, converts it into digital format and triggers the alarm system in case of water pollution. The controller also sends the processed data to a higher level system – a PC;

– PC – it provides data visualisation, stores data and generates reports. An alarm message is displayed on the monitor screen and a sound is emitted through PC's loudspeakers.

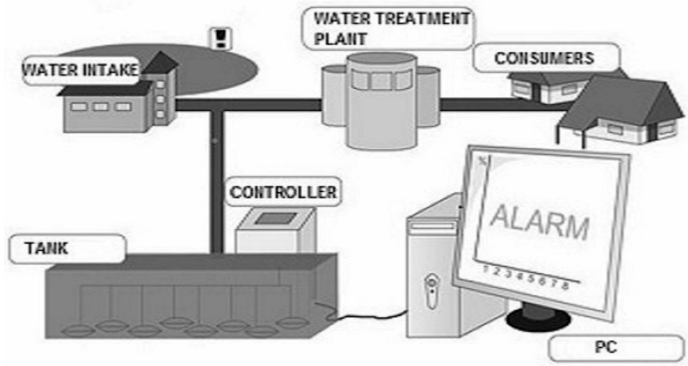

Fig. 1. Water quality monitoring using mussels

Bioindicators used to control water quality at ZWiK Sp. z o.o. in Lodz comply with the requirements for indicator species. Mussels – swollen river mussels are collected from lakes in the vicinity of Poznań selected as water bodies subject to minimal anthropogenic pressures, *ie* without direct point sources of pollution or runoff from agricultural catchment. Size, age and condition of mussels are taken into consideration during selection of specimens. Similarly sized mussels (with larger specimens being preferred) that do not differ much in terms of morphology from other population specimens are selected. The mussels are then transported in lake water in a special, thermally insulated container. Acclimatisation is required to minimize mussel stress resulting from change in environmental conditions. The selected specimens spend two weeks in special aquariums with temperature gradually adjusted to that of the target tank. The target tank which contains 8 mussels at a time is made of stainless steel (Photo 1). Mussels are placed in the tank for 3 months. This period is optimal for their functioning in the system as they do not require additional feeding. After 3 months, the mussels are replaced with other specimens, and the ones that were taken out of the aquarium are returned to their natural environment. The tank is designed to eliminate or minimise external factors that may adversely affect mussel activity. It provides darkness and isolation from noise, it also cushions ground vibrations. Installed in the tank is an air-operated filter that ensures proper oxygen saturation of water. Measuring probes connected to a magnet are attached to the shell of each mussel and record their activity (Photo 2). Mussels at different activity levels (different state of opening/closure of the shell) are selected for the tank. Integrated with the tank is a system controller that processes the data and sends them to the PC. PC software provides data visualisation and data storage as well as report generation and storage. This allows for up-to-date evaluation of system operation and following the history of mussel activity.

Data represented as bar graphs shows current degree of valve opening for each mussel in percent (sampling is done every 1s). Line graphs allow for following the

Photo 1. Aquarium with mussels

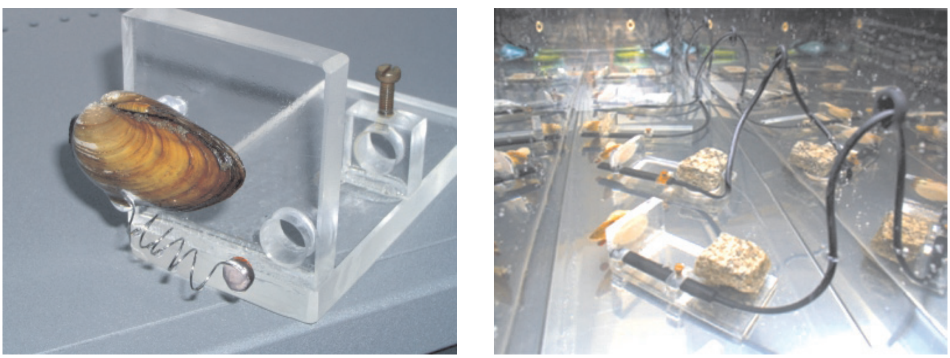

Photo 2. Mussels with measurement probes

history of mussel activity. Under natural conditions, the biorhythm of each mussel comprises cyclical periods of increased and decreased activity. During its decreased activity the valves may be partly or entirely closed for a period of several up to a dozen or so hours. Closure of the valves to only a few percent or its gradual closure may not be a sign of stress. Only a sudden, and what is important, simultaneous closure observed in a group of mussels may be considered a reaction to stress.

Upon a sudden change of water quality, mussels abruptly close their valves and the system generates the alarm. An alarm message is displayed on the computer screen, a sound is emitted through the PC's loudspeakers and a signalling lamp is lit.

Perch fry as a bioindicator

The other biological indicator species is perch (*Perca fluviatilis*). Aquariums with the fish and the tanks containing the mussels are installed in parallel arrangement. Perch, similarly to mussels, are collected at proper age (1–2 years), in proper condition and with the desired set of behavioural characteristics (no or poorly developed predatory features). The specimens are collected and replaced by an ichthyologist. The necessary reserves of fish for emergency replacement are located throughout ZWiK Sp. z o.o.'s sites in Lodz. Replacement dates are suited to natural biological cycle of perch (size, condition, habitat, population in water bodies): spring (March–April) – date depends on disappearance of ice cover; summer (June–July) – when perch reach the length of at least 4 cm; autumn (October–November) – before drop of temperature of surface water below 4 ^{o}C and icing. When no longer used as biomonitors, the fish are returned to natural habitat by the ichthyologist responsible for fish replacement. Observation of perch is done on a systematic basis by the employees of ZWiK Sp. z o.o. in Lodz. The alarm generated by mussel activity monitoring system and observation of intoxication of fish will result in implementation of actions indicated in the instruction prepared for this purpose.

Ecotoxicological tests

Apart from the internationally used methods employing crustaceans and fish (due to their vital role in the food chain), ecotoxicological bioassays with living organisms at different level of organisation and development, such as bacteria, are receiving increased use. Luminescent bacteria (*Vibrio fischeri*) are the third species used by Lodz's ZWiK biomonitoring system. Under normal conditions a great part of the bacteria's metabolism is dedicated to emitting light (luminescence). Bioluminescence occurs as a result of chemical reactions, oxygenation of luciferin in the presence of oxygen and enzymes. Luminescent bacteria emit visible light as a part of their normal metabolic processes. Any change in these processes through exposure to factors affecting metabolism results in decreased luminescence intensity. DeltaTox analyser measures the amount of light emitted by bacteria. Change in luminescence intensity occurs very rapidly, results may be available after 5–15 minutes of incubation of the bacteria-containing sample. Although it is a popular rapid solution, it may not be used as a sole indicator for water quality [21]. The paper [22] presents a group of specific tests called Toxkits which employ cryptobiotic stages of the test organisms. Cysts, resting eggs of crustaceans or algal cells, stored in special protective tubes, are dormant and have a shelf-life of up to several months. The most popular toxkits include: Microtox (with luminescent bacteria *Vibrio fischeri*); Spirotox, Protoxkit (using protozoa); Rotoxkit F (with rotifier *Brachionus calyciflorus*); Daphtoxikit F, Thamnotoxkit (using crustaceans).

Summary and conclusions

Bioindicators are any animal or plant species having a narrow range of tolerance for variation in environmental conditions (so-called stenotopic species). They are usually used to assess the degree of environmental degradation or determine changes occurring in a biocenosis or an ecosystem. Bioindicators may include organisms at various levels of organisation and development. Different species of mussels, gastropods and algae are among the most widely used. The requirement for early detection of water quality changes, including toxicity, necessitated the introduction of ecotoxicological tests to analytical science as they provide practically immediate responses based on reactions of simple organisms such as bacteria or protozoa. Responses of living organisms to unfavourable habitat conditions are used mainly for surveillance of water quality in rivers, freshwater and saltwater bodies and water supply networks. The three-stage biomonitoring system used at ZWiK Sp. z o.o. in Lodz comprising a flow-through aquarium with mussels, an aquarium with perch and ecotoxicological tests provides continuous surveillance of water quality both at production and distribution stages ensuring safety of water consumers. It is also a valuable addition to chemical analyses run periodically by water control laboratories.

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Abstrakt: Przedstawiono typowe organizmy żywe służące do biomonitoringu wód powierzchniowych oraz ich wykorzystanie w prezentowanych w pracy aplikacjach. Główną uwagę poświęcono charakterystyce i wykorzystaniu małży słodkowodnych. Uwzględniono rolę jaką odgrywa biomonitoring w wodociągowych systemach ujmowania wód powierzchniowych. Za przykład posłużył trójstopniowy system biomonitoringu funkcjonujący od 20 lat w Zakładzie Wodociągów i Kanalizacji Sp. z o.o. w Łodzi, wykorzystujący małże, narybek okonia i bakterie luminescencyjne.

Słowa kluczowe: bioindykatory, biomonitoring jakości wody, małże