

Biological methods used to assess surface water quality

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Received – 22 May 2015/Accepted – 24 November 2015. Published online: 31 December 2015; ©Inland Fisheries Institute in Olsztyn, Poland
Citation: Szczerbińska N., Gałczyńska M. 2015 – Biological methods used to assess surface water quality – Arch. Pol. Fish. 24: 185-196.

Abstract. In accordance with the guidelines of the Water Framework Directive 2000/60 (WFD), both ecological and chemical statuses determine the assessment of surface waters. The profile of ecological status is based on the analysis of various biological components, and physicochemical and hydromorphological indicators complement this assessment. The aim of this article is to present the biological methods used in the assessment of water status with a special focus on bioassay, as well as to provide a review of methods of monitoring water status. Biological test methods include both biomonitoring and bioanalytics. Water biomonitoring is used to assess and forecast the status of water. These studies aim to collect data on water pollution and forecast its impact. Biomonitoring uses organisms which are characterized by particular vulnerability to contaminants. Bioindicator organisms are algae, fungi, bacteria, larval invertebrates, cyanobacteria, macroinvertebrates, and fish. Bioanalytics is based on the receptors of contaminants that can be biologically active substances. In bioanalytics, biosensors such as viruses, bacteria, antibodies, enzymes, and biotests are used to assess degrees of pollution.

Keywords: bioanalytics, bioindication, biomonitoring, biotest

Introduction

Apart from chemical methods, biological methods are being used increasingly often for assessing surface water quality. Thanks to the possibility of omitting the initial stage of sample preparation, analyses conducted using such methods are often less expensive and less time-consuming. Biological test methods include both biomonitoring and bioanalytics. Biomonitoring aims to assess the state of the natural environment and levels of pollution. This method uses plants and animals that serve as bioindicators. Bioanalytics is based on using receptors of pollution with biologically active substances. Biosensors such as viruses, bacteria, enzymes, and antibodies or bioassays can be used to assess levels of environmental pollution (Nałęcz-Jawecki 2003, Traczewska 2011). The aim of this article is to present the biological methods used in the assessment of water status with a special focus on bioassay, as well as to review methods for monitoring water status.

Biomonitoring

Biomonitoring is one of the three elements of environmental monitoring. The International Organization for Standardization (ISO) defines water monitoring as a planned process of sampling, measuring, and analysing different water features, and they are often designed to test compliance with

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respective standards. Biomonitoring is divided into passive and active. Passive biomonitoring is the use of organisms, organism associations, and parts of organisms which are a natural component of the ecosystem and appear there spontaneously. Active biomonitoring includes all methods which insert organisms under controlled conditions into the site to be monitored.

Biological monitoring relies on two types of organisms: so-called biological indicators characterized by a determined tolerance level to environmental factors, and monitoring organisms capable of accumulating chemical elements or compounds. The selection of the group of organisms used depends on the type of observation (Traczewska 2011).

Methods adopted for assessing surface water quality based on biological indicators have been undergoing development for many years and are still being improved upon; they can be divided into two groups: the saprobic system – planktonic organisms and periphyton (Europe) and the system based on macroinvertebrates (USA). Fish and aquatic plants can also be used as indicator organisms.

Indices based on selected species or groups of organisms

The saprobic system developed by Kolwitz and Marsson (1909) is the oldest biological method used to assess the quality of surface waters. The relationships between the amount of oxygen dissolved in water and CO₂, and the level of organic pollution of water as well as species diversity and abundance of organisms are of crucial importance to this method (Bonada et al. 2006). Originally, the method included categorizing water into three classes: polysaprobic water (high levels of pollution), mesosaprobic water (average levels of contamination), and oligosaprobic water (no pollution). The system was developed further and the number of indicator species was expanded and water classes were supplemented with six additional classes. Nine classes of water quality were designated based on the

saprobic index: ksenosaprobic water, oligosaprobic water, β -mesosaprobic water, α -mesosaprobic water, polysaprobic water, isosaprobic water, metasaprobic water, hypersaprobic water, and ultrasaprobic water. Over the years, the system has been modified many times and various versions of the system have been developed (Klimaszyk and Trawiński 2007). Nowadays, in many countries this system has been replaced by biotic indices, point-based systems, and observations of changes in population in individual ecological groups.

The biotic system combines the diversity of defined systematic groups into one index or point scoring. When calculating the biotic index, the abundance of organisms in a sample is not taken into consideration, whereas in the scoring system the parameter is included in the calculation. The general biotic index is used in the assessment of water quality and relies on the analysis of benthic fauna and thanks to the analysis of small vertebrates it allows assessing pollution introduced to flowing waters (Lavado et al. 2006). A profile of selected biotic systems is presented in Table 1 (Woodiwiss 1964, Chandler 1970, Armitage et al. 1983, Cota et al. 2003, Scardi et al. 2006, Klimaszyk and Trawiński 2007, Traczewska 2011, Królak et al. 2011).

The BMWP Score has been modified for use in many countries, e.g., in Poland, this system is called the BMWP-PL. It is a combination of the biodiversity index and the Polish biotic index. In this method, taxa present in a given area of a river are identified and assigned a specific number of points. Then the index values are calculated. Margalef's equation is used to calculate the biodiversity index (Hering et al. 2006, Klimaszyk and Trawiński 2007, Królak et al. 2011).

Another index developed to facilitate observations of changes in water quality is the Index of Biotic Integrity (IBI) which is based on the use of fish. The quality of the aquatic environment of a given group of fish was assessed in comparison to undisturbed environments corresponding to the environment under analysis. This index takes into account the composition of species, diversity, trophic relationships, size, and condition of fish. Water is classified according to

Table 1
An overview on selected biotic indices, based on macroinvertebrates

Biotic system	General information	Selected examples of indicator organisms	Selected advantages	Selected disadvantages
Trent Biotic Index (TBI)	It is based on the number of defined taxa of benthic invertebrates in relation to the presence of six key organisms found in the fauna of the sample site This index serves as basis for developing, for example, the Extended Biotic Index (EBI), Belgian Biotic Index (BBI), and Danish Fauna Index (Viborg index)	Type: <i>Platyhelminthes</i> , <i>Annelida</i> , <i>Mollusca</i> Subtype: <i>Crustacea</i> Order: <i>Plecoptera</i> , <i>Ephemeroptera</i> , <i>Trichoptera</i> <i>Neuroptera</i> , <i>Coleoptera</i> Family: <i>Chironomidae</i> , <i>Simuliidae</i> , <i>Elmidae</i>	Identification of organisms to the family, genus, or species. It is easily understood by non-biologists	The presence of drift organisms will affect the index score. It is not responsive to inorganic pollution
Chandler Biotic Index	Macroinvertebrates are identified and counted and each individual group is given its own score	Order: <i>Ephemeroptera</i> , <i>Tricladida</i> , <i>Diptera</i> Family: <i>Taeniopterygidae</i> , <i>Pentidae</i> , <i>Perlodidae</i> , <i>Isopenitidae</i> , <i>Chloropertidae</i> , <i>Leuctridae</i> , <i>Capniidae</i> , <i>Nemouridae</i> , <i>Glossiphoniidae</i> , <i>Simuliidae</i>	Good correlation scores of variables associated with organic compounds. It does not require rigorous sampling techniques	More time is required for sorting, identifying and counting the organisms. It is not responsive to inorganic pollution
Biological Monitoring Working Party Score (BMWP Score)	Benthic macroinvertebrates are identified to the family and then each family is allocated a score between one and ten. The index is calculated by summing the scores for each family represented in the sample	Family: <i>Planariidae</i> , <i>Neritidae</i> , <i>Pisicollidae</i> , <i>Astacidae</i> , <i>Siphonuridae</i> , <i>Peltidae</i> , <i>Calopterygidae</i> , <i>Pleidae</i> , <i>Scirtidae</i> , <i>Sialidae</i> , <i>Psychomyiidae</i> , <i>Simuliidae</i> , <i>Chironomidae</i> , <i>Oligochaeta</i>	It gives a reasonable correspondence between chemical classification and biological scores and it can measure the effect of pollution over a period	It is based on the European benthic macroinvertebrate families only of streams

three classes (Klimaszyk and Trawiński 2007). Bioindicators most sensitive to water pollution are brown trout *Salmo trutta* L., roach, *Rutilus rutilus* (L.), and pikeperch *Sander lucioperca* (L.). Slightly less sensitive species used as bioindicators are carp, *Cyprinus carpio* L., bream, *Abramis brama* (L.), and perch, *Perca fluviatilis* L. Fish can serve as indicators of accumulation or sensitivity. The European Fish Index (EFI) was developed based on the IBI in 2004. Because of its limitations (oversimplified database), it was further developed into the new European Fish Index (EFI+) based on a database compiled from more than 14,000 stations located in 2,700 rivers in 15 European countries (EFI+ Manual 2009, Adamczyk et al. 2013).

Many aquatic species show specific bioaccumulative abilities. These include:

- *C. carpio*: heavy metals, polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCB), dioxins (Oikari 2006, Klobučar et al. 2010);
- *S. trutta*: non-steroidal anti-inflammatory drugs, PAHs, pesticides, PCB, dioxins (Scardi et al. 2006, Fent 2008);
- *Dreissena* sp. and *Mutilluss* sp.: cadmium, PCB (Traczewska 2011);
- *Myriophyllum propinquum* A. Cunn.: arsenic (Robinson et al. 2006);
- *Centella asiatica* L.: copper (Mokhtar et al. 2011);
- *Eichhornia crassipes* (Mart.) Solms: zinc and chromium (Zayed and Terr 2003, Aisien et al. 2010);
- *Lemna minor* L.: lead (Prasad et al. 2001);
- *Pitia stratiotes* L.: chrome (Odjegba and Fasidi 2004);
- *Salvinia minima* Baker: cadmium (Olguín et al. 2002).

Community structure indices

The description of a population's response to environmental disturbance is based on diversity coefficients. Three structural population parameters are used in calculations: its size, abundance, and

uniformity. Three equations are also used for to assess diversity: Shannon-Wiener, Simpson, and Margalef. The coefficients of diversity are independent of the size of the sample as they are quantitative and dimensionless. The disadvantage lies in the fact that the values change depending on the equation used and in that tolerance, sensitivity, and methodology of identification of the organisms are not taken into consideration. In order to facilitate the assessment of biocenosis, this method was modified by the introduction of the Sequential Comparative Index (SCI). This system includes the random selection of organisms from the analysed sample and is used to determine the number of series on the grounds of morphological similarity of the organisms. SCI is the quotient of the number of series divided by the number of selected organisms. The values of the index range from 0.1 to 1.0 and the higher the values, the greater the diversity (Traczewska 2011).

Macrophytes as indicators of trophic status

In European Union countries, the Water Framework Directive (WFD) is the key document that sets forth the rules for the conservation of inland surface, transitional, coastal, and underground waters. The requirements in the document must be met by individual types of waters. Waters of moderate quality are characterized by moderate disturbances in the values of biological elements, whereas waters of poor or bad ecological quality have values below moderate levels (Hutorowicz and Napiórkowska-Krzebietke 2014). In the WFD, macrophytes are recognized as important for determining the ecological status of water bodies (EC 2000). Species indicator values can vary significantly depending on river or lake type and are therefore unsuitable for detecting differences in ecological tolerances of species across Europe. Many macrophyte methods are used to assess the trophic states of water bodies in Europe: the Macrophyte Index (MI, lake) and the Trophic Index of Macrophytes (TIM, river) in Germany, the Mean Trophic Rank

(MTR, river) in the UK, *Indice biologique macrophytique en rivière* (IBMR, river) in France, the Ecological State Macrophyte Index (ESMI, lake) and Macrophyte River Index (MRI, river) in Poland (Szozkiewicz et al. 2009).

The MI (Melzer 1999) is applicable in calcareous lakes in the Alps and the pre-alpine region. A total of 45 species of submerged macrophytes is included in a catalog of nine indicator groups. The mean MI of a lake correlates with its total phosphorus concentration during circulation time. The ESMI was developed in 2006 for two types of lakes (charophyte-colonized stratified and non-stratified lakes). The ESMI evaluates two aspects of the macrophyte community, namely taxonomic composition and abundance, which are combined into one multimetric index. ESMI is calculated by examining plants along designated lake transects 20–30 m in width. The number of transects depends on the shoreline and the area of the lake. The ESMI values range from 0 to 1, where 1 denotes pristine conditions and 0 highly degraded habitats (Ciecierska and Kolada 2014).

The MTR is a method focused on the impacts caused by phosphate enrichment (Dawson et al. 1999, Holmes et al. 1999). Each of 129 aquatic plant species is allocated a Species Trophic Rank (STR) score according to its response to eutrophication. A low STR indicates that the plant is either tolerant of eutrophication or alternatively has no preference and is termed “cosmopolitan”. The MTR of a sampling site is expressed by integrating the STRs of the species present at a site as a mean value, weighted according to the relative percentage cover of the individual species. In TIM (Schneider and Melzer 2003) macrophyte indicator values are given for a total of 49 species of submerged macrophytes on a scale from 1 to 4 (1 indicating oligotrophic and 4 polytrophic conditions). The IBMR is applicable to natural and artificial running waters. Each of 207 taxa is allocated a *cote spécifique* according to its response to eutrophication. IBMR species values range from 0 to 20 with 0 indicating hypertrophic and 20 indicating oligotrophic conditions. The Macrophyte River Assessment Method (MMOR) has been used in Poland

since 2007. This method employs the quantitative and qualitative characteristics of macrophytes in the section of river under analysis. This method allows establishing the level of river degradation and determining its trophy using the Macrophyte Index for Rivers (MIR) (Szozkiewicz et al. 2009).

Biotests

Biotests are based on using living organisms that react in a specific way to the pollution of water with metal, organic (e.g.: PAHs, PCB, pesticides, pharmaceuticals), and biogenic compounds. The analyses are conducted in laboratories rather than in the field. Different publications adopt various criteria for distinguishing the analyses based on tests, yet the most popular criterion is the type of organism used in the analysis. Biotests use both animal and plant organisms. Detecting viruses and bacteria that pollute water is possible using biotests (Markert et al. 2012, Sadowska 2012). Biotests allow identifying toxic, mutagenic, or carcinogenic substances in an analysed sample of water and facilitate the assessment of the effect these substances have on organisms. For this purpose, the values of indices are calculated based on the dose-response relationship. The indices determining toxicity are Effective Concentration (EC₂₅, EC₅₀) and Effective Dose (ED₂₅, ED₅₀). The parameters describe the concentration of a given toxic substance in an environment or its dose which produces (25 or 50%) a given biological effect to a previously specified extent. It is also possible to determine the level of a toxic factor present in the environment which results in weakening or hindering a particular process, which is known as Inhibition Concentration (IC₅₀). In the case of acute toxicity, it is the Lethal Dose (LD₅₀) and Lethal Concentration (LC₅₀) that is the lethal dose and concentration which results in death after a specified time in half of the organisms under study. When determining the dose and exposition time to a given toxic substance, the concentration values or dose limit are established:

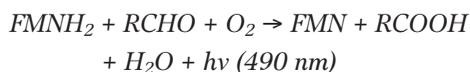
- No Observed Effect Level/Concentration (NOEL, NOEC);

- Lowest Observed Effect Level/Concentration (LOEL, LOEC);
- No Observed Adverse Effect Level (NOAEL);
- Lowest Observed Adverse Effect Level (LOAEL).

These are measured as mg or µg of a given substance per 1 kg of body mass of an organism in a 24-hour period (Napiórkowski et al. 2008).

Bacterial tests

The most commonly used bacteria in biological tests are the species *Vibrio fischeri* and *Vibrio harveyi*, which are capable of luminescence. They are Gram-negative, curved-rod shaped, facultatively aerobic bacteria with polar flagella. Salt water is their natural habitat (Girotti et al. 2002, Nałęcz-Jawecki 2003, Danyluk et al. 2007, Traczewska 2011). Bacteria of this species can emit green-blue light (wavelength 490 nm) thanks to a specific set of genes the so-called lux operon. In bacteria characterized by luminescence, luciferin is oxidized by the enzyme luciferase. Luciferase is bound through oxygen with a reduced flavin mononucleotide which acts as a cofactor. As a result, luciferase transforms aldehyde into fatty acid and attains higher energy levels. The result of this is oxyluciferin. The particles of oxyluciferin move from an excited state to a ground state and cause luminescence, and the cofactor is oxidized to flavin mononucleotide. This process can be represented with the following equation (Pogorzelec and Piekarska 2013):



Many factors affect bacterial luminescence. Bacteria emit light under optimal conditions using 10% of the energy coming from metabolism. When harmful compounds affect bacteria their metabolism is disturbed, thus their ability to luminescence is inhibited. The loss of luminescent abilities is proportional to the amount of harmful compounds affecting the bacteria (Steliga et al. 2009, Pasternak et al. 2010). Microorganisms are used in the screening tests such

as Microtox, LUMISTox, ToxAlert 10, and ToxAlert 100. They are characterized by good sensitivity and reproducibility, and relatively quick result times (Dewhurst et al. 2002, Danyluk et al. 2007, Traczewska 2011).

Microtox systems are based on the decreasing bacterial luminescence of *V. fischeri* in the presence of a given substance over a specified period of time (5 or 15 min) depending on the test selected. The test is conducted according to the procedures specified by the manufacturer, and the change in luminescence is measured with a photometer (Guzzella 1998, Pasternak et al. 2010, Arendarczyk et al. 2011). Lyophilized bacteria, which can be stored for one year at a temperature of - 20°C, are used for the test (Steliga et al. 2009). Prior to the test, the bacteria are suspended in deionized water. Having read the results, the calculations should be carried out using software recommended by the manufacturer (Pasternak et al. 2010). Another species used in biotests is *Salmonella typhimurium*, which a Gram-negative, facultatively anaerobic bacteria. The bacteria are used in the Ames test to detect, among other things, mutagenic substances (DeLuca et al. 1983). This procedure permits determining primary mutations in many *Salmonella typhimurium* LT2 strains (Kołwzan 2009). The strains used in the test are incapable of histidine synthesis, yet when affected by mutagenic substances the mutations are reversed and the strains are capable of histidine synthesis (Kołwzan and Traczewska 1994). This test can be conducted with classical methods (plate counting method) or biosensors. The sensor, which is placed in a culture devoid of histidine, reacts with the decrease in current intensity when in the presence of a mutagen. During Mutatests and Vitotoxs, additional genes responsible for luminescence are introduced to the strains. As a result of the action of a substance damaging DNA, luciferin synthesis is restored and light is emitted (Sun and Stahr 1993, Verschaeve et al. 1999, Węgrzyn and Czyż 2003, Kołwzan 2009, Steliga et al. 2009). *Bacillus subtilis*, a common Gram-positive bacteria, have the ability to decompose organic substances of plant origin. Mutant *B. subtilis* are used in repair tests aimed at

analysing the effect of mutagenic compounds on the increased mortality of the analysed cells. The mutant bacteria are incapable of recombinant repair rec-assay, i.e. they do not utilize one of the DNA repair pathways of a cell. Damage to this pathway affects other pathways as well. The cells become more susceptible to DNA damage and even with the slightest damage cell growth can be inhibited. In this test mutagenic compounds result in DNA degradation and cell growth inhibition, yet they do not cause mutation (Karube and Tamiya 1987, Kołwzan 2009).

Escherichia coli are facultatively anaerobic, Gram-negative bacteria naturally present in the large intestines of humans and warm-blooded animals. The strains of the bacteria are used in SOS-Chromotest to detect compounds which may affect DNA leading to its destruction. The test is based on the induction of a *sfiA* gene function as a result of the action of chemical compounds. The level of expression of induced genes is measured with a colorimeter as β -galactosidase activity. The process occurs through the fusion of gene operons: *sfiA* (part of the SOS repair system) and *lacZ* (responsible for galactosidase synthesis) (Quillardet et al. 1982, Mankiewicz et al. 2002, Płaza et al. 2005). SOS is an induction system which activates at the risk of cell death. In a cell with improved DNA it is repressed by a *lexA* protein. As a result of DNA damage, the level of the *recA*⁺ gene product increases leading to the modification of DNA III polymerase by the protein. The polymerase continually replicates DNA joining random alkali against fragments of damaged DNA. As a result, the number of mutations in a cell increases, yet the cell survives. Additionally, induction leads to increases in the proteolytic activity of the *recA* protein, which results in *lexA* protein degradation and the derepression of the SOS system (Quillardet et al. 1982).

E. coli are used in the ColitagTM and Colilert tests conducted with the aim of assessing water quality in terms of microbiological pollution. The ColitagTM test is based on the identification of β -glucuronidase, an enzyme characteristic of *E. coli*, and β -galactosidase, from coliform bacteria. Ortho-nitrophenyl- β -D-galactopyranoside (ONPG)

chromogenic substrate is used to determine the amount of coliform bacteria. The substrate shows a yellow coloration in the presence of the bacteria following hydrolysis by β -galactosidase in a sample of water. However, in order to detect *E. coli* bacteria in a sample of water, a fluorogenic substrate characteristic of β -glucocuronidase is used. The side effect of the reaction is fluorescence. The results of the test are obtained in approximately 24 hours (Trepeta and Edberg 1984, Covert et al. 1989, 1992, Edberg et al. 1989, 1990, Bej et al. 1991, Rompré et al. 2002, Kołwzan 2009, Nikaeen et al. 2009). In turn, the Colilert test relies on using O-nitrophenyl- β -D-glucuronate (characteristic for coliform bacteria) and 4-methylumbelliferyl- β -D-glucuronide (MUG) (characteristic for *E. coli*) (Chang et al. 1989, Olson 1991).

Enterolert is used to determine fecal contamination in water. This test is also based on MUG and fluorescence and operates on similar principles as the two tests described above (Abbott et al. 1998, Eckner 1998, Maheux et al. 2009).

In the analysis of a water sample, test packages based on different strains of bacteria are used; for example, the MARA test (Microbial Assay for Risk Assessment) which, apart from bacteria, uses yeast. The indicator employed in this test is the change of color of bacteria strains and yeast because of inhibited metabolism. The characteristic equation of metabolism inhibition allows for the approximate determination of the type of pollution. Bacterial luminescence is recorded using a luminometer, and then it is computer-processed (Gabrielson et al. 2003, Wadhia et al. 2007).

Biological tests using animals

In biological tests of water quality conducted on animals, the most commonly used species is *Daphnia magna*, an invertebrate with a well-developed digestive tract and a relatively small size (female approx. 2-6 mm, male approx. 2.2-3.5 mm). *Daphnia magna* is naturally present both in permanent and seasonal

water reservoirs. Crustaceans are used, among others, in the IQ-ToxTM test, which is intended to detect toxic substances present in drinking water. The test consists of observing the feeding of the analysed organisms with galactose-a substrate labelled with a fluorogenic marker. If there are chemical pollutants present in the water, the processes of sugar decomposition and the release of fluorogenic markers are disturbed, thus disturbing the organism's ability to luminesce. It takes 75 minutes to assess water quality with this test (Kühn et al. 1989, Kołwzan 2009). *Daphnia magna* is also used in a test for acute toxicity (determinations of the share of organisms showing lethal effects) and chronic toxicity (decrease in reproduction) (Nikitin 2014).

Biological tests are also conducted using mussels from the genus *Anodonta* (length 10 cm) which live in stagnant and flowing waters and filter feed on phytoplankton and zooplankton. Water treatment plants use short-term mussel farming. Mussels close their shells when the level of pollution is water is high. Therefore, observations of mussels permits assessing changes in water quality as the mussels are sensitive to pollution and live in very pure waters (Markert et al. 2012). Mussels of the genus *Anodonta* are particularly sensitive to increased levels of Fe, N-NH₄, or Cl in water. They also react if the water is polluted with Cd, Cu, Hg, plant protection products, or formaldehyde (Couillard et al. 1993).

Artemia salina is a stenothermal crustacean present only in salt water. The length of an adult form can reach 15 mm. This crustacean is resistant to high concentrations of chlorides in water. *Artemia* larvae are used in a test which consists of determining the number of organisms in lethal stage in a given water sample. The test is carried out in salt water for 24 hours. Dead organisms are counted using a magnifier (Napiórkowski et al. 2008).

Hydra attenuata is a species of Hydra measuring from 5 to 22 mm in length. This hydra is found in stagnant or slow-moving fresh waters. Its cylindrical body is white-pink in color and has a radial symmetry. It is highly sensitive to water pollution, and it is used for testing. The adult, non-budding forms of normal morphology are placed in water for 96 hours

and observed using a magnifier. In case of water pollution, morphological changes will be observed in the organisms. Five stages of such changes have been identified: organisms without any morphological changes; organisms with thickened tentacle ends; organisms with shortened tentacles; organisms in lethal stage caused by the so-called tulip stage; disintegration of the organism (Trottier et al. 1997, Napiórkowski et al. 2008).

The water quality test packages available on the market use a number of indicator organisms. Toxkit is an example of a test which includes exposure of organisms to water samples for a period of 24 to 74 hours. The number and length of live organisms is determined in each plate well, thus the survival and growth rates are identified (Dewhurst et al. 2002, Wolska et al. 2008, Steliga et al. 2009, Arendarczyk et al. 2011). Tests such as Toxkit are user-friendly and provide good sensitivity, repeatability, and relatively short time of analysis. These tests are in line with guidelines set forth by the OECD, the ISO, and the USEPA (Nałęcz-Jawecki 2003) (Table 2).

Biological tests using plants

Tests with plant organisms can be used to assess the condition of surface waters. For example, the freshwater algae growth inhibition test uses chlorophyta: *Pseudokirchneriella subcapitata* – microalgae with a sickle-shaped body 8-14 µm in size, and *Desmodesmus subspicatus* – oval-shaped algae 7-15 µm in size. The algae are sensitive to harmful substances in water, including trace metals. The organisms are incubated with harmful substances in a static culture at a temperature of 21-24°C for 72 hours, then the increase in biomass or the inhibition of biomass increase is measured (OECD 2011).

Lemna minor, a freshwater plant, is one of the world's smallest vascular plants (2-7 mm wide). Thanks to high adaptability, it has a cosmopolitan distribution, yet it is also sensitive to pollution. As a floating plant, *Lemna minor* is at risk from the toxic action of surfactants or hydrophobic substances

Table 2
Comparison of toxkit package test

Indicator organism	Test	Realization time	Result	Norm
<i>Brachionus calyciflorus</i>	Acute rotoxkit F	24 hours	Death rate percent	ASTM E1440-91
<i>Brachionus calyciflorus</i>	Short-chronic Rotoxkit F	48 hours	Inhibition of growth	AFNOR T90-377, ISO 20666
<i>Brachionus plicatilis</i>	Rotoxkit M	24-48 hours	Death rate	ASTM E1440-91
Larvae of the saltwater genus Artemia	Artoxkit M	24-48 hours	Death rate	ASTM E1440-91
<i>Daphnia magna</i> , <i>Daphnia pulex</i>	Daphtoxkit F	48 hours	Immobilization, death rate percent	OECD Guideline 202, ISO 6341
<i>Ceriodaphnia dubia</i>	Ceriodaphtoxkit F	24 hours	Death rate percent	OECD Guideline 202, ISO 6341
<i>Thamnocephalus platyurus</i>	Thamnotoxkit F	24-48 hours	Death rate percent	ISO 14380
<i>Thamnocephalus platyurus</i>	Rapidtoxkit F	30-60 minutes	Reduction or no food	ISO 14380
<i>Tetrahymena thermophila</i>	Prottoxkit F	24 hours	Inhibition of growth	OECD Guideline 202
<i>Selenastrum capricornutum</i>	Algalttoxkit F	72 hours	Inhibition of growth	ISO 8692, OECD Guideline 201

present on the surfaces of water as well as pollution with trace metals. The test that uses this plant is conducted according to standard ISO or OECD procedures. After 7 days, the influence of the analysed compounds on plant growth is determined. Toxic compounds can induce changes in the number and morphology of roots, the number and surface of fronds, and the number of plants in dry and fresh biomass (Orzechowski 2005, Bielińska and Nałęcz-Jawecki 2009).

Bioanalysis can also be based on using biosensors, which are a combination of classical analysis and modern technology. Biosensors are small in size, highly sensitive, selective, and not susceptible to interference (Filipiak et al. 1996, Matejczyk and Suchowierska 2011). They can be used for a long period of time and therefore are applied in many analytic and diagnostic techniques. The sensor comprises a biologically active material and a transducing element that detects the activity and concentration of a given chemical substance in the sample (Pogorzelec and Piekarska 2013). The biosensor uses a biological detection system (e.g. microorganism, an antibody, an enzyme, or DNA) and a transducer (detector), which converts biological

processes into an electrical signal. They can be categorized as catalyst-based and receptor-based biosensors depending on the biological material used for detection. The detectors can also be classified according to the type of phenomenon used in the detection process: electrochemical, potentiometric, conductometric, amperometric, piezoelectric, optical, or thermal. The application of biosensors based on a combination of biological and electronic components is a quick, precise, sensitive method of detecting even the smallest amounts of chemical compounds, toxins, or microorganisms (Kołwzan 2009).

Conclusions

Increasingly more chemical and biological substances that have either direct or indirect effects on aquatic ecosystems and human health are introduced to surface waters. Therefore, monitoring water quality is crucial. The aim of water quality analysis is to monitor concentrations of substances introduced to waters by anthropogenic pollution. Over the years, biological methods of water quality assessment have been

developed substantially. Such methods are increasingly incorporated into technological solutions, i.e., the use of biosensors or biologically active deposits in water purification. The advantage of biological methods is that water quality assessment is conducted in the same way as a living organism would react to pollution. The reactions of living organisms to pollution are manifested in physiological, morphological, and behavioral changes. Moreover, it is possible to forecast changes occurring in aquatic environments. Research based on the use of biological methods is often less expensive and time-consuming since some stages of sample preparation can be omitted. The advantage of such methods is that the analysis can be conducted in a laboratory as well as in the natural habitat of the organisms used for testing (Nałęcz-Jawecki 2003, Traczewska 2011). Biotests can also be used to supplement standard analyses since different methods can be applied to conduct environmental tests. Particular tests are characterized by different sensitivity to various compounds; therefore, the use of multiple testing methods is recommended in order to obtain more reliable results (Codina et al. 1994).

Author contribution. N.S. and M.G. conceived of and designed the study, conducted literature review and wrote the paper.

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