Review

Some Considerations About Bioindicators in Environmental Monitoring

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Abstract

Toxic chemicals introduced into the environment can penetrate ecosystems and can be found in the whole biosphere. Chemical contamination may affect ecosystems, causing changes in the functions of particular organisms. Adverse effects of xenobiotics and their metabolites on living organisms can be observed. In the last few years investigations have focused on searching for bioindicators (both plant and animal organisms) that accumulate toxic substances. The aim of the present study was to discuss selected methods of environmental quality assessment based on living organisms used as bioindicators, paying special attention to water ecosystems.

Keywords: biomonitoring, bioindicators, xenobiotics, environment

Introduction

Growing social concern about environmental quality could be observed in recent years, both on a global and local scale. This is connected with more and more convincing evidence that environmental pollution results in degradation of particular ecosystems. Emission of harmful substances has negative effects on the natural environment, human health and agricultural production efficiency. When the consequences of environmental pollution become visible, it is often too late to prevent them. Chronic toxic effects, impossible to notice at the initial stage of the process, may manifest themselves after many years [1].

Toxic chemical substances introduced into the environment may be transported by the air, water and living organisms (Figure 1). These substances can be found in the whole biosphere. They become a part of the natural biogeochemical cycle and accumulate in the food chain. They also affect humans, causing (directly or indirectly) various poisonings, toxicoses, and even neoplastic diseases. Water constitutes the "trouble spot" of all ecosystems, as many pollutants are waterborne [2]. It also plays an important role as a solvent of various substances, and as a medium in the cycle: air-soil-plants-animals.

Due to constant technological progress the natural environment undergoes numerous changes, deteriorating its quality, which often results in negative interactions between particular ecosystem components. During the biological evolution living organisms needed complex defense and adaptation mechanisms to survive under changing environmental conditions. Most of them managed to adapt to specific environments, but when their adaptability threshold is crossed they die [3].

Environmental toxicology deals with toxic substances, their adverse effects on living organisms, and environmental pollution assessment. Chemical contamination may affect ecosystems, causing changes in the functions of particular organisms or modifying the physical properties of the environment. The relationships between the xenobi-

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otic, environment and organism may, under certain conditions, result in the degradation of toxic compounds through their modification, inducing changes in the environment and producing a negative effect on living organisms [3].

Xenobiotics may penetrate into the organisms via air, water, soil, dust and food, through the skin, respiratory system and alimentary tract. Some chemical substances showing strong toxic properties may cause local cellular damage, but in the majority of cases their effects can be observed when they penetrate into the circulatory system, undergo metabolism and accumulate in various organs (some of their metabolites may be excreted). The number of xenobiotics released into the environment is still growing, which is very dangerous as they can modify the functions of the endocrine, reproductive, nervous and immune system. New compounds often undergo changes, and their metabolism is very slow due to the lack of previous contacts of the organism with such substances. The degree of exposure depends, among other things, on their concentration in a given ecosystem, stability, rate of migration and potential bioaccumulation. The information on the effects of these substances on human health is scant, so it is difficult to estimate the degree of risk they pose. Only about 10% of commercial chemical compounds have been tested for their carconogenesis, mutagenesis and reproduction-related toxicity [4]. It is estimated that since the beginning of mankind about six million chemical compounds have been produced, most of them in the 20th century, and still over a thousand are introduced each year [1].

Environmental pollution constitutes a serious threat to the existence of ecosystems. It follows that environmental monitoring must become a constant element of pollution control and prediction on a local scale. Environmental monitoring is a an integral part of international projects implemented within the 6th Framework Program financed by the European Union. Particular attention is paid to the identification of xenobiotics and their metabolites. Another aim of the projects is to determine the changes they may undergo in the environment, thus posing a threat to the functioning of living organisms, and especially to human health and life.

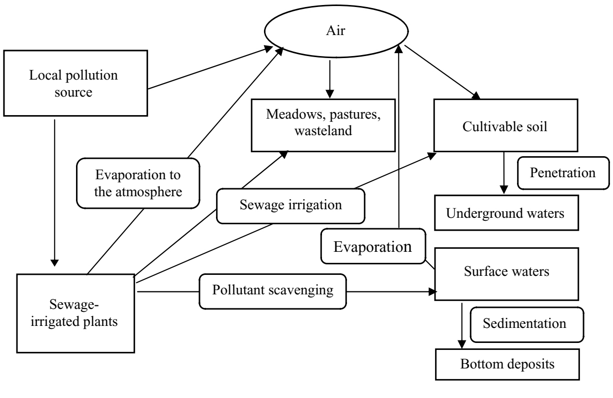

Fig. 1. Circulation of xenobiotics in the environment [3].

The aim of the present study was to discuss selected methods of environmental quality assessment based on living organisms used as bioindicators, paying special attention to water ecosystems.

General Characteristics of Xenobiotics

Humans are more and more frequently exposed to the effects of exogenous compounds – xenobiotics (gr. xenos – alien), e.g. food preservatives or environmental pollutants. Most xenobiotics undergo changes in the human organism, mainly in the liver (very seldom are they excreted in an unchanged form). These reactions may be divided into two phases. The main reaction in the first phase (equation 1) is hydroxylation catalyzed by one of the enzymes classified as monooxygenases or cytochromes P-450.

$$RH + O_2 + NADPH + H^+ \rightarrow R - OH + H_2O + NADP \quad (1)$$

where: RH – xenobiotic.

Reduction and hydrolysis may also take place in this phase. The compounds formed in the next phase are transformed to various metabolites by specific enzymes. Typical reactions are: a coupling reaction (e.g. with glucuronic acid, sulfuric acid, acetic acid, glutathione or amino acids) or methylation [5]. The main aim of both phases of xenobiotic transformation is to increase their water solubility (polarity), which makes it easier to excrete them from the organism. Getting to know the mechanism of action of such compounds at the cellular level provides the basis for preventing a chemical attack against living organisms [6, 7].

Much attention has been paid recently to the problem of environmental pollution with chemical compounds characterized by estrogenic properties, present in lakes, rivers, oceans, crops and food products of animal origin. Exposure to these compounds, especially at early stages of intrauterine life, may produce permanent and irreversible effects. Estrogenic activity is typical of polychlorinated biphenyls (PCBs), dioxins, plant protection chemicals, e.g. DDT (dichlorophenyl-trichloroethane), drugs administered in heart diseases, nephropathy, hepatopathy and inflammation of reproductive organs [8-10]. Some of them may also show mutagenic and carcinogenic effects [11].

Many animal populations have already experienced the effects of xenoestrogens, which manifested themselves in reduced fertility of birds, fish, crustaceans and mammals. Some bird species inhabiting the Great Lakes (North America) lose their reproductive power as a result of fish contamination (their main source of nourishment). Also, fertility disturbances, testiculoma, prostatic hypertrophy, sexual development disorders, disturbances of thyroid and hypophysis functions and reduced immunity, observed in recent years in men, are connected with the presence of xenoestrogens in the natural environment.

However, specialists differ in their opinions on the effects of xenoestrogens – some authors even think that the problem of environmental estrogens does not exist [12].

An example may be polychlorinated biphenyls (PCBs), which due to their specific physicochemical properties have been widely applied to heat engineering, hydraulics, and the plastics industry. However, due to their high chemical stability, they are present in the environment. A natural consequence of their affinity for fats is the accumulation of these substances in the organism, resulting from their active uptake from the environment (e.g. water, air, food), combined with biological concentration increase in the trophic pyramid (Table 1). Their toxic effects result from disturbances in the endocrine system in humans and animals. Hormonally active xenobiotics can disturb the endocrine functions of the male gonad, because they affect hormone synthesis, storage, secretion, transportation and release, as well as binding to the receptor. The mechanism of action of PCBs is based on the stimulation of the so-called Ah receptor (cellular protein), which results in the transcription of genes of enzymes metabolizing drugs and xenobiotics (e.g. molecular forms of cytochrome P-450). The Ah receptor also affects the expression of genes regulating the growth and differentiation of cells. Its activation manifests itself, among others, in the inhibition of estrogenic receptor synthesis. The main source of PCBs are food products, and their accumulation in fatty tissue starts as early as during intrauterine life, and continues in infancy (when their source is mother's milk). This is especially dangerous due to the fact that the detoxication mechanisms of young organisms in the period of fast growth are not fully developed [13-15].

Dioxins (polychlorinated dibenzodioxins and dibenzofurans - PCDD and PCDF) are also toxic to living organisms [15,16]. Their presence in the air, water and soil is directly connected with industrial activities as they are formed as by-products in the chemical, pharmaceutical, and pulp and paper industries. Even trace amounts of these substances in the environment may increase the risk of cancerogenesis [17,18]. The biological reaction depends on the binding of dioxins and their analogues to the Ah receptor, through which they penetrate into the cell nucleus, where they fix DNA. This process is similar to the carcinogenic activity of polynuclear aromatic hydrocarbons, and causes changes in the gene sequence. This in turn initiates various biochemical mechanisms resulting in different toxic effects, including hepatocellular damage, fetal damage and neoplastic diseases [19].

Polynuclear aromatic hydrocarbons (PAHs) differ in their mutagenic and cancerogenic effects. They are formed during incomplete combustion of organic compounds. Their main sources are the petro- and carbochemical industry, thermal-electric power stations and domestic furnaces, car exhausts and cigarette smoking. PAHs penetrate into the organism through the respiratory system, alimentary system and skin, in case of direct contact. Their metabolism, similar to the metabolism of other xenobiotics, is connected with the presence of monooxygenases and transferases. The enzymes responsible for metabolic activation of procancerogens are usually certain kinds of cytochrome P-450. A specific monooxygenase participating in their metabolism is referred to as cytochrome P-448 or aromatic hydrocarbon hydroxylase. The activity of metabolizing enzymes depends on numerous factors, such as the species, genetic predispositions, age and sex. Chemical cancerogens (or their metabolites) given to animals or introduced into cell cultures usually bind covalently to cell macromolecules, including DNA, RNA and proteins, which may lead to irreversible damage and changes in the genetic material [20-23].

Table 1. Biological PCB increase in the food chain [4].

Object	Concentration [ppm]	Level of biological increase
Phytoplankton	0.0025	1
Zooplankton	0.123	49.2
European smelt	1.04	416
Lake trout	4.83	1932
Herring gull's eggs	124	49600

Another group of compounds that are the center of attention are nitroso-amines, formed as a result of reactions of secondary amines with nitrates. These reactions occur first of all in food products stored at room temperature, and in the digestive tract, after consumption of vegetables containing excessive amounts of nitrates. In laboratory animals nitroso-amines cause hepatocellular damage and produce teratogenic, mutagenic and cancerogenic effects [3].

Living organisms are used more and more often to determine the level of environmental pollution. Being components of ecosystems, they can provide valuable information on the degree of environmental degradation (especially as regards aquatic ecosystems). Bioindicators used in environmental monitoring, as well as improvement of assessment methods, allow us to explain the mechanisms of action of e.g. xenobiotics or other harmful substances, and to determine their toxic effects on living organisms [24,25].

Biomonitoring in Ecoanalytics

Fast development of an interdisciplinary technique known as ecoanalytics makes it possible to detect and determine even trace amounts of ecotoxins present in the natural environment. Due to high costs of complex chemical analyses, and complicated and time-consuming procedures of sample preparation, analysts search for quicker and more specific methods, including bioindicatory systems enabling determination of changes taking place in ecosystems and particular organisms. The use of biological material, combined with analytical techniques, allows improvement of the sensitivity and accuracy of traditional chemical methods. A wide range of specific and selective biological reactions enables direct analyte determination in complex matrices (Figure 2) [26].

The biological methods employed in environmental analysis may be divide into two groups:

- bioanalytics (the use of biological matter for environmental analyses; biosensors, biotests),
- biomonitoring (the use of biota in classical chemical analysis – early warning system; bioindicators) [27].

Special attention should be paid to biosensors, defined as a subgroup of chemical sensors in which biological mechanisms are used for chemical compound detection. An active biological layer here may be enzymes, microorganisms, antibodies, nucleic acids or hormonal receptors, as well as plant and animal tissues. Combined with a properly selected transducer, designed for detection of chemical substances or determination of their activity, they form an analytical apparatus (Figure 3). High sensitivity and selectivity of biosensors enables toxicant determination at a level of trace and ultratrace [24].

Receptor-based biosensors acquire selectivity as a result of natural affinity of the properties of proteins or their fragments for specific substances referred to as complementary ligands. The receptor interacts selectively with a given ligand, forming a thermodynamically stable complex. This association is conditioned by the size and shape of the receptor pocket and complementary ligand, as well as the hydrogen bond, intercharge and Van der Waals interactions [28,29]. Catalytic biosensors make use of biocatalysts which recognize and bind chemical compounds, catalyzing their chemical change with simultaneous release of products which are then determined with an optical or electrochemical transducer. Microbiological sensors make use of the metabolic functions of living organisms. Bioanalytical material could be isolated antibodies or enzyme systems, which constitute a very specific, selective biological layer. However, due to their high costs and short life, they are usually replaced by bacterial, yeast and fungal colonies or fragments of living organisms. Their high tolerance for pH and temperature changes, and low costs make it possible to analyze complex mixtures, monitor the state of the natural environment or determine toxicity and detect mutagenes. The disadvantage of microbiological sensors is a long response time. Due to the application of the biological operation principle, biosensors enable accurate measurement of xenobiotic concentration in a given ecosystem component, and toxicity of anthropogenic pollutants [30].

Biomonitoring can be defined as a process in which the "analytical instruments" used, i.e. plant and animal organisms or their fragments, provide continuous, real-time analytical information [31,32]. Bioindication is a research activity allowing us to obtain a picture of the ecological situation on the basis of its important element (e.g. species, ecological form, population, association or community). Bioindicators are biological indicators of environmental quality, characterizing environmental conditions. Their tolerance is usually limited, so their presence or absence, and health state enable to determine some physical and chemical components of the environment without complicated measurements and laboratory analyses. Bioindicators may be divided into those responding to environmental changes in a visible way (morphological and physiological changes) and those whose reactions are invisible, but which cumulate different substances (pollutants) whose concentrations may be determined. According to another division, qualitative and quantitative bioindicators can be distinguished. The former indicate the fact that a given species occurs in a given ecosystem, the latter allow to determine the (optimum) number/concentration of representatives of a given species in a given ecosystem [27].

The indicatory properties of living organisms are used first of all in environmental quality analysis and environmental pollution assessment. They allow to determine the rate, level and range of present and future man-induced changes in the natural environment [24]. Bioindication is focused on searching for organisms that accumulate toxic substances, as their concentrations in such organisms provide the basis for estimating the level of environmental pollution with these substances. Toxic substances may

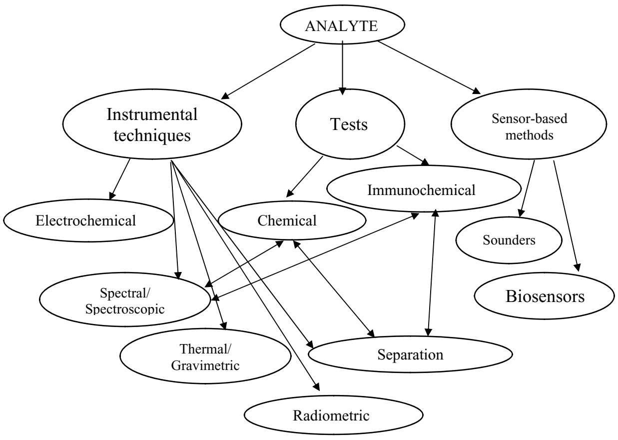

Fig. 2. Ecoanalytic techniques [26].

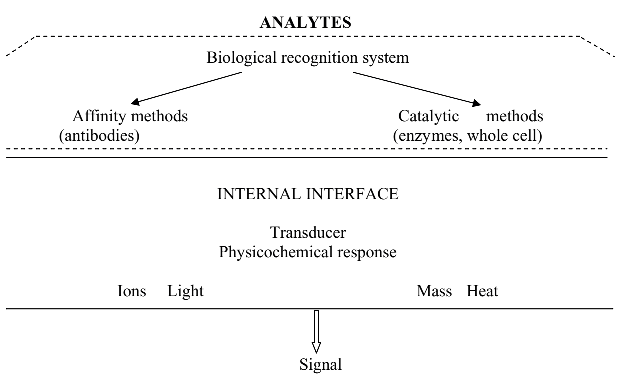

Fig. 3. Biosensor design and analyte recognition system [32].

accumulate in both plant and animal organisms. Biomonitoring is based on the correlation between toxic substance concentration in the environment and living organisms, expressed as the biological concentration factor (BCF) – equation 2 [33].

$$\text{BFC} = \frac{\text{concentration in the organism}}{\text{concentration in water}} \qquad (2)$$

Harmful substances can penetrate into the organism and be used for satisfying physiological needs (e.g. energy metabolism, growth, production) or accumulated in some tissues. Some of them, not used by the organisms, are excreted to the environment, often as metabolites. The concentrations of compounds that underwent bioaccumulation may be different, depending on numerous factors such as: pollutant concentration and physicochemical forms, properties of semipermeable membranes, physiological condition of the organism, physical characteristics of the environment, kind and amount of food, level of its contamination, kind of organisms and kind of pollutants [24, 25, 30].

Plants as Bioindicators

Plant organisms play an important role in their natural habitats - they supply oxygen, control organic substance circulation and biological balance of the soil and bottom deposits, provide food and shelter to other organisms [35]. Phytoindicators are more and more frequently used for ecosystem quality assessment due to their sensitivity to chemical changes in environmental composition and the fact they accumulate pollutants. The use of plants as bioindicators has many advantages, including low costs, the possibility of long-term sampling and high availability. Their disadvantage is the necessity to take into account the physical conditions, impact of environment properties (growth rate disturbed by large amounts of pollutants, soil type and fertility, humidity) and genotype diversity in a given population. Lower plant organisms (grasses, mosses, lichens, fungi and algae) are used most often in analyses of atmospheric depositions, soil quality and water purity. Responses of trees and shrubs to the presence of pollutants are also observed. The assimilatory organs of trees, especially coniferous ones (pine, fir, spruce), are characterized by the capacity to accumulate air pollutants, which makes them suitable for the determination of residues of pesticides, polychlorinated biphenyls (PCBs), pentachlorophenol (PCP), hexachlorobenzene (HCB), hexachlorocyxlohexane iosmers, dioxins and furans. Numerous and visible changes, like needle loss, crown thinning, changed bark color, increased needle fragility, enable us to estimate the level of environmental pollution [35-37]. A method of bioindicatory assessment of forest health was developed in the 1980s. The effects of pollution are determined only for trees from the principal crop, i.e. superior and co-dominating ones, aged at least 50 years. The evaluation criterion is assimilatory organ loss and discoloration. The levels of defoliation and discoloration provide the basis for determining five classes of tree stand damage.

Mosses and lichens are applied as indicators of environmental pollution due to their capacity to accumulate and store heavy metals and other toxins [24,38]. Typical examples of a biological indicator of air pollution are lichens. Their major advantage is response repeatability in various habitats. Regardless of the investigation site and differences in the species composition, destruction zones are easy to distinguish. Due to their specific anatomic, morphological and physiological characters, lichens are among the organisms that die first as a result of excessive air pollution. On the basis of the correlation between the level of industrialization and occurrence of sensitive lichen species, Kiszka and Bielczyk proposed an original scale in Poland [39, 40] enabling the determination of the air concentration of SO_2 (Figure 4) based on the Hawksworth and Rose scale [41].

Common application of pesticides, especially herbicides, and their adverse effects on the natural environment contributed to fast development of bioanalytical methods based on plant material. Algae (green, blue-green, and diatomes), duckweed, aquatic and terrestial macrophytes are frequently used in toxicity tests.

Green algae *Selenastrum capricornutum* [42], *Scenedesmus quadricauda* and *S. subspicatus* [43] are often applied because they are easily available. Due to their structure (single cells) and the fact that they contain a photosynthetic dye (*chlorophyll a*), microalgae can be successfully used in flow cytommetry. This method enables separation of particular species of these organisms by fluorescence measurement, which in turn allows the performance of biotests based on several species [42, 44-46]. Microalgae cultures immobilized on a special medium are also applied to wastewater treatment aimed at removal of heavy metals, nitrogen and phosphorus compounds [43].

Among various species of vascular plants used for biotesting, the most popular is duckweed (*Lemna minor* and *L. gibba*), characterized by breeding ease and fast proliferation. According to literature data none of the duckweed or algal species tested so far shows high sensitivity to chemical substances. This is probably the reason why the vast majority (about 90%) of acute toxicity tests and tests concerning bioaccumulation of toxins and their metabolites are carried out using animal organisms instead of plant material [34].

High concentrations of xenobiotics in plants allow us to employ simple measuring methods, and the popularity of the above plant species enables biomonitoring in different geographical regions, on a continental or even global scale.

Bioindicators of Aquatic Ecosystems

The organisms used as bioindicators must be characterized by much higher sensitivity than the best chemical indicators. Aquatic organisms accumulating pollutants allow us to detect them even when their water concentrations are too low to be detected. An example may be determination of radioisotope activity in plankton, which is several times higher than in water.

Sometimes the level of toxic substances in the abiotic part of a given area is low and does not suggest any threat to the environment, even in the case of further pollutant leakage. Analysis based on bioindicators may at the same time show that the concentration of toxic substances in living organisms is so high that its further increase may result in irreversible damage to particular populations or the whole organic world in the biotope examined.

To make global analyses uniform, international organizations have established a set of principles to be followed during toxicity determination, and compiled a list of indicatory organisms. Bioindicators should be selected according to the following criteria [25,47]:

- sedentary life,
- abundance, wide distribution,
- simple procedure of identification and sampling,
- high tolerance for the pollutants analyzed,
- population stability,
- high accumulating capacity.

The water purity state should be determined using organisms sensitive to pollution, characterized by a narrow range of tolerance. The following tests and bioindicators can be applied to analysis of water and sewage toxicity [2]:

- test based on *Chlorella vulgaris* – a unicellular green alga, widespread in fresh waters. Diluted sewage solutions are introduced into laboratory algal cultures, then absorbance is measured with a spectrophotometer in the visible range;
- test based on *Daphnia magna Straus* – a crustacean living in fresh waters. Young organisms are placed in crystallizers with sewage solutions of different concentrations. The count of bioindicators showing the test effect (organism immobilization) is determined after 24 and 48 hours. These data allow to determine sample toxicity;
- test Spirotox, based on the protozoan *Spirotostomum ambiguum*, present in clean rivers and lakes. Ciliates are placed in the sample and observed under slight magnification. The cells of these very sensitive organisms undergo dissolution (lysis) when affected by toxicants. Sample toxicity is determined by its dilution, causing lysis of 50% of the population;
- test Microtox, which consists in measurement of the natural luminescence of bacteria *Vibrio fischeri*, suspended in the solution of the sample analyzed. Toxic chemical compounds inhibit the activity of bacterial enzymes, which reduces the intensity of luminescence. The measurement is performed by the spectrophotometric method.

One of the criteria of water cleanliness is the fecal pollution index, referred to as the coli index, showing the degree of pollution with intestinal pathogenic bacteria. Also the so-called saprobiotic index is applied to evaluate running water purity. Water quality is determined on the basis of the count of indicatory organisms in a given site, and the catalogue value of the saprobiotic index [48-53].

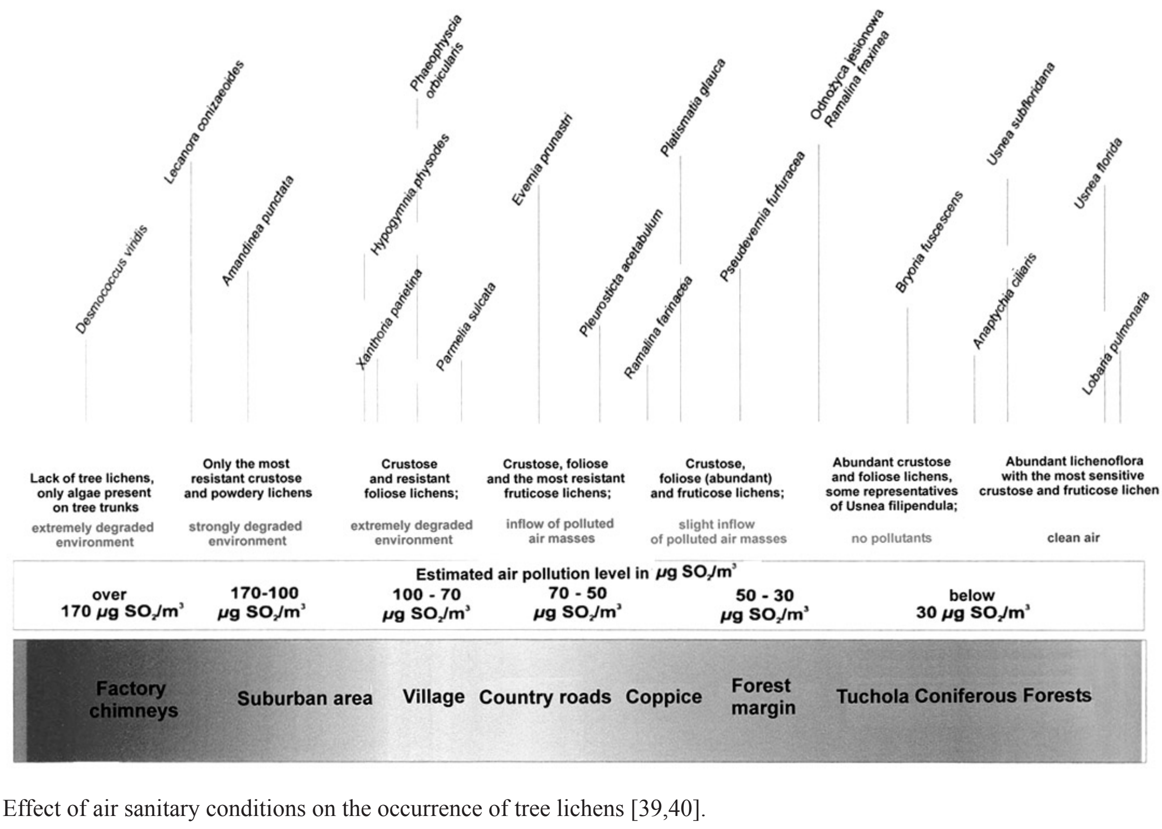

Fig. 4. Effect of air sanitary conditions on the occurrence of tree lichens [39,40].

Table 2. Occurrence of selected bioindicators depending on water purity class.

Oligosaprobiotic zone	β-mesosaprobiotic zone	α-mesosaprobiotic zone	Polysaprobiotic zone
Diatoms *Ceratoneis arcus* *Meridion cerculare* Chrysophyte *Hydrulus foetidus* Red algae *Betrachospermum vagum* Zoobenthos *Perla* sp. Caddis-flies *Molanna angustata*	Snails *Planorbis corneus* *Viparus viparus* *Lymne stagualis* Common mayflies *Ephemera vulgata* Diatoms *Melosira granurata* *Melosira variens* Blue-green algae *Microcistis aeruginoza* Bivalves *Pisidium amnicum*	Fungi *Leptomitus lapteus* Single diatoms *Navicula viridula* Zoobenthos *Asselus aquaticus* *Erpobdella octoculata* Bivalves *Spherium corneum*	Bacteria *Spherotilus nataus* *Zoogla ramigera* *Bacterium cyrusii* *Thiothrix nivea* *Beggioata* Zoobenthos Dipteran's larvae *Chironomus plumosus* *Eristalomya*

The suitability of particular animal species as bioindicators depends on their specific requirements towards the environment. Table 2 presents selected indicatory organisms typical of different water purity classes. The oligosaprobiotic zone is characterized by the presence of all systematic groups, corresponds to the first water purity class and is suitable for Salmonidae breeding. The most common bioindicators here are dipteran's larvae, hemipterans and caddis-flies. The β-mesosaprobiotic zone (second water purity class) is suitable for breeding fish other than the family Salmonidae. The most popular bioindicators here are snails and diatoms. In the α-mesosaprobiotic zone (third water purity class) bioindicators are first of all fungi, whereas in the polysaprobiotic zone (fourth water purity class) - bacteria [53].

According to the Regulation by the Minister of Environmental Protection, Natural Resources and Forestry of November 5, 1991 (Journal of Laws. No 116 item 503) surface waters in Poland may be divided into three classes:

Class I - consumption water, water for plants that need drinking water, water for Salmonidae breeding,

Class II - water for breeding fish other than the family Salmonidae, water for farm animals and recreation purposes,

Class III - water for plants that do not need drinking water, water for field irrigation, water for gardening (horticultural crops).

Heavily polluted waters, where pollutant concentration exceeds the admissible values for the above classes, are defined as classless waters.

Today the indices of water cleanliness are also determined on the basis of the species composition and count of different organisms (e.g. plankton, periphyton, benthos), as well as analysis of matter production and destruction processes. In clean waters a state of equilibrium is maintained between these processes. An increase in organic matter supply results in the domination of destruction over production and macroconsumption. The only consumers left in the ecosystem are destructors. The presence or absence of certain indicatory species (algae, insects, crustaceans, fish) may provide detailed information on the purity or pollution state of aquatic ecosystems [52, 53].

One of the criteria of water cleanliness is a qualitative and quantitative evaluation of benthos – plant and animal organisms living at the bottom of water bodies [54]. Phytobenthos and zoobenthos reflect the environmental quality state, are easy to collect (so-called bottom sampling), usually live for over a year and find it difficult to move. However, these organisms differ in their tolerance for the concentration and type of pollutants. Effective accumulators of pollutants are mollusks, often applied as bioindicators of water pollution with heavy metals [55,56]. The properties of some populations of these organisms, their capacity to accumulate toxicants, as well as the fact that they are widely spread, present in large quantities and easy to identify, make them valuable bioindicators of ecosystem pollution. Commonly used bioindicators are also bivalves, which – due to their physiology – act as water filters. They accumulate lipophilic substances (e.g. PAHs, PCBs) in their fatty tissue. Depending on the species, they live in fresh or salt water. Their feeding ground is the bottom of a water body. They purify water of suspended organic matter, i.e. small plant (phytoplankton) and animal (zooplankton) organisms. Bivalves (*Bivalvia*) are used as bioindicators in sensor units collecting information on water pollution. Under normal conditions bivalves filter water and their shells are open. At the moment of pollutant release to water they stop filtering and tightly close their shells. People usually believe that the presence of crayfish confirms water cleanliness. However, some species of these crustaceans, e.g. *Orconectus limsus* (*Cambarus affinis*) or *Pacifastacus leniusculus*, which come from North America, can very easily adapt to different environmental conditions. They appeared in European waters at the end of the 19th century and within the next hundred years colonized almost half of their area, threatening the existence of native crayfish species, like *Astacus astakus* or *Astacus leptodactylus*. *Orconectus* *limsus* is especially aggressive, due to its great reproductive potential, intensive migrations and high tolerance for changing environmental conditions – it can be found in irrigation canals or heavily polluted water bodies. Therefore, in order to assess water cleanliness using crayfish as bioindicators it is necessary to distinguish between their species, as only native crayfish – very sensitive to environmental changes – can be referred to as water purity testers [57].

Periphyton is also used in surface water biomonitoring. It includes benthic biota covering plants or objects. The species composition and biological condition of periphyton are analyzed. These primary producers, with a very high number of species, are characterized by high sensitivity and short-term responses to exposure to harmful substances. Some species are well-known for their sensitivity and tolerance for environmental changes.

Also macrophytes – big aquatic or waterside plants (floating, immersed, emersed) are used as bioindicators. They provide shelter and food to various organisms, and their lack may indicate population reduction and problems with water quality (e.g. too high turbidity or salinity, presence of herbicides).

Fish as Bioindicators of Water Cleanliness

Fish have been used as water pollution bioindicators for many years, taking into account their species diversity, numbers and health state. It is very important that water is the only biotope in which fish populations are present. In the case of an ecological disaster they have no possibility of escape. At the same time they usually constitute the last link of the food chain. It follows that they are directly affected by what is going on among producers (phytoplankton and higher plants) or lower consumers (zooplankton, protozoans, small crustaceans). The condition of the top of the trophic pyramid reflects the state of the whole ecosystem, closely correlated with the state of a given part of the natural environment. Lukas used some fish species, e.g. herring (*Clupea harengus*), sprat (*Sprattus sprattus*), cod (*Gadus morrhua*), flatfish (*Pleuronectes*), and mew's eggs (common gull - *Larus canus*, the family *Laridae*) as bioindicators in his studies on water pollution with xenobiotics, carried out on the Baltic Sea and North Sea from the beginning of the 1970s [58].The results obtained at the initial stages of his studies often indicated that the admissible DDT level had been exceeded is sea food with a high lipid content (cod's liver, herring). Also, the concentration of chloorganic compounds in fish was extremely high. When the use of DDT (dichlorophenyl-trichloroethane) was prohibited, its content of fish decreased considerably within a few years, but the concentrations of metabolites (DDD, DDE) increased [59,60]. In the next years the studies were also conducted on marine mammals and concerned other strongly toxic xenobiotics (dioxins, pesticides). Due to their lipophilic properties, such compounds accumulate in fatty tissues of fish, turtles, seals, sea-birds and other organisms inhabiting aquatic ecosystems.

Research is usually conducted on fish of the family *Salmonidae*. They are characterized by a narrow range of tolerance and high sensitivity, especially as concerns the water oxygen content and pollution connected with it. Numerous authors report frequent development defects in fish, caused by the presence of chemical compounds affecting their organisms during sex differentiation. Some chemical compounds are characterized by estrogenic properties and disturb the endocrine functions of fish. An example may be nonylphenol polyethoxylates (NPnEO) and products of their degradation [61]. The studies performed on rainbow trout (*Salmo gairdneri irideus*) describe the estrogenic activity of this group of compounds. Nonylphenol mimics the effects of natural estrogen - estradiol and binds to estrogen receptors, inducing vitellogenin synthesis in hematocytes (Figure 5). This disturbs natural steroid metabolism, has an adverse effect on spermiogenesis, and causes hermaphroditism in fish (the formation of intersex gonads) [62-64]. The presence of vitellogenin (specific protein contained in the egg yolk) in male fish indicates the presence of xenoestrogens in the environment.

Fish are valuable bioindicators as it is relatively easy to determine their numbers, biological diversity and behaviors. Changes in water oxygen content, increased turbidity or the presence of mineral compounds and toxic substances may result in their hyperexcitability, eyeball projection out of the eye socket, awkward swimming, laying upside-down, equilibrium disturbances, or even death. In such a case the state of the whole population deteriorates very quickly, but fish can also recover within a very short time. They are less sensitive than lower organisms to natural micro-environmental changes, which makes them suitable for evaluation of regional and macro-environmental changes.

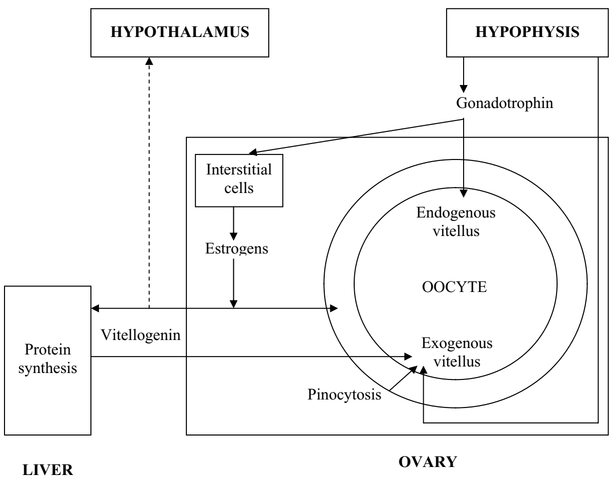

Fig. 5. Process of vitellogenesis (yolk formation) [65].

Conclusions

Due to a wide variety of chemical compounds, the problem of toxicity of polluting substances is difficult to define. However, appropriate interpretation of research results and environmental changes allow us to assess environmental pollution by xenobiotics and their degradation products.

The development of immunotoxicology and molecular biology makes it possible to use new, more effective monitoring techniques, determining the effects of xenobiotics on humans and animals. The studies on their influence on immune and adaptation mechanisms, conditioning survival in a given environment, seem to be especially important. To obtain a complete and reliable picture of the ecosystem, it is necessary to compare information provided by particular bioindicators. Environmental pollution constitutes a serious threat, so biomonitoring methods should be constantly improved, to enable prediction and control of potential environmental hazards. Nowadays it is a well-known fact that each ecosystem component can provide valuable information about degradation of the natural environment and dangers to human and animal health resulting from it. Beyond a doubt, acquiring knowledge about ecological tolerance and its application to practice can be of benefit to us all.

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