MINISTRY OF EDUCATION, CULTURE AND RESEARCH
INSTITUTE OF ZOOLOGY

GUIDANCE ON THE MONITORING OF WATER QUALITY AND ASSESSMENT OF THE ECOLOGICAL STATUS OF AQUATIC ECOSYSTEMS

Chisinau, 2020

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It is elaborated in the frame of the project no. 20.80009.7007.06 Determining the changes of aquatic environment, assessing the migration and impact of pollutants, establishing the patterns of the functioning of hydrobiocenoses and preventing the negative effects on ecosystems, AQUABIO (State Program 2020-2023). Some research findings within ongoing international projects BSB 27 MONITOX and BSB 165 HydroEcoNex (Joint Operational Program Black Sea Basin 2014-2020 of the European Union) were also used.

The Guidance on the monitoring of water quality and assessment of the ecological status of aquatic ecosystems is addressed to young researchers, including PhD and MS students, specialists in the field of environment and all those who desire to acquaint themselves with the theoretical and applied aspects of the research of aquatic ecosystems.

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SUMMARY

I MONITORING PROGRAMS

Sampling of water, suspensions, bottom sediments and hydrobionts is the first very important stage in carrying out the physico-chemical and biological analysis of water. The use of sophisticated analysis equipment and methods cannot diminish the impact of mistakes made in the process of sampling.

The methods used for sampling, field measurements, chemical and hydrobiological analysis of samples shall be validated and documented in accordance with the international standard EN ISO/CEI 17025, which is accepted at national level (SM SR EN ISO/CEI 17025:2006), and other standards related to sampling (SM SR EN ISO 5667-1:2011; SM SR ISO 5667-4:2007; SM SR EN ISO 5667-6:2011).

Sampling conditions and requirements differ in dependence of the type of water, program and aim of monitoring.

It is difficult to develop comprehensive guides or recommendations, but there are always basic requirements or principles for sampling in the case of physico-chemical and biological monitoring of aquatic ecosystems:

- collected materials shall be representative and shall correspond to the selected program of monitoring;
- methods of collection and transport of samples must influence as less as possible the physico-chemical and biological composition of the samples collected;
- volume of collected material must be sufficient in order to perform the analysis in accordance with the selected laboratory methods and techniques;
- recipients (vessel, containers) must be made of inert materials; in most cases, the sampling will be done in polyethylene or other plastic flasks, and in glass recipients – in the case of monitoring of some organic compounds;
- all sampling procedures must be strictly documented, in order to avoid any difficulty in identification of sample in the laboratory;
- samples shall be collected by qualified personnel and in accordance with safety standards (it is mandatory to train staff in order to ensure appropriate safety conditions, including wearing a life jacket).

The national monitoring system consists of two types of monitoring programs: surveillance and operational monitoring.

Surveillance monitoring of surface waters, including transboundary rivers, is carried out by the State Hydrometeorological Service based on the stationary network of hydrological stations. The network of surveillance monitoring of surface waters from the Republic of Moldova is specified in the Regulation on monitoring and systematic evidence of the state of surface and underground waters (2013). The network is developed according to the hydrological, ecological and hydrochemical status of the water body, based on preliminary investigations, which include the collection and analysis of information on water class and quality, types of use of the water body, its ecological status, sources of point and diffuse pollution, and the distance from the stations of hydrological observation. Moreover, the Indicative List of the Main Pollutants, proposed in Annex VIII of the Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy, shall be taken in account.

The monitoring network of the quality of surface waters, in dependence of the set up objectives, including research, also includes common transboundary monitoring sectors (or sections), established on the basis of collaboration agreements or treaties, with the stipulation in common monitoring programs of the number and the location of the monitoring points, the sampling schedule, the way the information is disseminated, as well as the joint assessment of the water quality of the transboundary ecosystems. The main sampling points are fixed by GPS coordinates.

The peridiocity of sampling depends on the type of monitoring, which includes the given point of observation.

The frequency of monitoring is established by taking into account the variability of parameters that can change both under the influence of natural conditions and as a result of anthropogenic impact.

The frequency of monitoring must make it possible to estimate the ecological status and quality of surface water in each water body by establishing and maintaining a sufficient number of monitoring points, so as to ensure a consistent and comprehensive description of the status of surface waters within each district of hydrographic basins, and to detect the tendencies of the evolution of anthropic factor.

For surface waters which are exposed to different quantitative and qualitative risks, the monitoring network and the frequency of monitoring must be sufficient to allow the assessment of human impact and prevention of ecosystem degradation. In exceptional cases (accidental pollution), the sampling is coordinated based on emergency conditions. Samplers should take into account the possible chemical, bacteriological, virotic or zoological hazards in some rivers or streams.

In the case of surveillance monitoring, sampling aims to assess the status of all waters within each hydrographic basin or sub-basin (according to the points and indicators set up in the Regulation on monitoring and systematic evidence of the state of surface and underground waters (2013)), necessary for: validation of the procedure of impact assessment, assessment of the trend of long-term variation of the quality and quantity of water resources, elaboration of criteria for keeping the evidence of water bodies at administrative-territorial level, as well as elaboration of the programs of optimization of national monitoring system.

Operational monitoring is performed for water bodies that may not meet the objectives of water protection.

In the case of investigative monitoring, the sampling aims to identify the causes of exceeding the limits of water quality requirements, to certify the factors due to which the water body cannot achieve environmental objectives, and to identify the size and impact of accidental pollution.

The material is collected from polluted areas (upstream and downstream of the source of pollution) according to an emergency plan or program, in order to obtain the necessary information for development of special measures, which aim remediation of effects of accidental pollution.

According to ISO 5667-1:2006, three main tasks of sampling are established:

- monitoring of water quality for the implementation of short-term actions;
- monitoring of water quality for the identification of long-term changes;
- monitoring of water quality for the identification of sources of pollution.

Different types of programs of sample collection are distinguished. Thus, a program of quality control includes verification of compliance with water quality standards and norms. Such programs are most commonly used by the services of state control and surveillance.

A program of assessment of water quality includes the determination of substance concentrations in a certain period. Programs can be episodic, designed for a specific study, short-term (for rare, but systematic observations) or long-term (for routine, systematic observations). Short-term and long-term programs are also based on scientific investigations aimed to assess the status of the studied waters.

The program of research of pollution consists in identifying the sources of pollution, determining the concentrations and processes of migration of pollutants in the water body. Such programs are based on knowledge of the nature of pollutants and the frequency of contamination. The last determines the periodicity of sampling. The research programs foresees not only the ascertainment of state and identification of determinant factors, but also the obtaining of materials for the elaboration of new methodologies, the establishment of new quality indicators, especially integral ones, based on bioindication, biotesting and ecotoxicological investigations. They consist of the simultaneous sampling of water, suspensions, sludge and aquatic organisms, aiming the complex qualitative and quantitative investigation of processes of chemical migration and flows of matter and energy, decipherment and evaluation of production-destruction processes, self-purification and secondary pollution processes.

All types of monitoring programs must contain the list of main parameters, the methods of their analysis and the sampling program, which includes the location of sampling points, sampling frequency, types of samples, sampling methods and techniques, methods of sample handling. Many procedures are already subject of international (ISO), the European Union or national standards (e.g. GOST).

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II MICRO- AND MACROELEMENT ANALYSIS OF ENVIRONMENTAL COMPONENTS

2.1 CHEMICAL AND PHYSICO-CHEMICAL METHODS FOR THE STUDY OF ENVIRONMENTAL COMPONENTS

The migration capacity of microelements, nutrients, pollutants and toxins, as well as their forms of migration are conditioned by their properties, as well as by the value of pH, the degree of oxidation, the presence of complexing agents, suspended substances, vital activity of hydrobionts, etc. Thus, one of the specialization facets of the Laboratory of Hydrobiology and Ecotoxicology of the Institute of Zoology is the ability to analyze environmental quality indicators (surface and groundwater, suspensions, silts, aquatic organisms, etc.), by using of the standardized and validated methods. Another facet is the analysis of different types of samples, others than liquid or semi-liquid, such as those of a vegetable (phytoplankton, phytobenthos, macrophytes) and animal nature (zooplankton, zoobenthos, fish). The third facet is the vastness of the analysed quality parameters.

In its practice, the Laboratory of Hydrobiology and Ecotoxicology uses a wide range of methods to perform quantitative determinations.

Titrimetry

Titrimetry is a common laboratory method in quantitative chemical analysis and is often used to determine the unknown concentrations of an analyte to be identified. A range of titrimetric methods are applied in the Laboratory of Hydrobiology and Ecotoxicology (Tab. 2.1).

Table 2.1. Titrimetric methods and indicators

Nr.	ND[1] or validated method	Parameter	Titrimetry
1	SM SR EN ISO 25663:2012	Total nitrogen (Kjeldahl)	Neutralization
2	SM SR ISO 5664:2007	Ammoniacal nitrogen	Neutralization
3	SM SR EN ISO 9963-1:2007	Total and permanent alkalinity	Neutralization
4	SM SR EN ISO 9963-2:2007	Carbonate alkalinity	Neutralization
5	SM SR ISO 6058:2012	Calcium	Complexometric
6	SM SR ISO 6059:2012	Calcium, magnesium (sum)	Complexometric
7	SM SR ISO 9297:2012	Chlorides	Precipitation
8	SM SR EN 25813:2011	Dissolved oxygen	Redox
9	SM SR EN ISO 8467:2006	Permanganate index	Redox
10	SM SR ISO 6060:2006	Chemical oxygen consumption	Redox
11	SM SR EN ISO 1899-2:2007	Biochemical oxygen consumption	Redox

Gravimetry

Gravimetric analysis is one of the most accurate methods of determination. Its basic principle consists in bringing a component to be determined, existing in solution, in the form of a practically insoluble (or precipitated) product, which must have a known and constant composition. The precipitate is filtered off from the rest of the components of solution and purified by washing. After an appropriate heat treatment, it is weighed. The quantity of the determined component is calculated from the resulting mass. The parameters determined by gravimetry are presented in Table 2.2.

[1] ND – normative document

Table 2.2. Gravimetric methods

Nr.	ND or validated method	Parameter
1	SM SR EN ISO 25663:2012	Substances (matter) in suspension
2	SM SR ISO 5664:2007	Ammonium

Spectrometry

The interaction of electromagnetic radiation with the substance takes place along the entire spectrum composed of ϒ, χ, ultraviolet, visible, infrared rays, microwave and radio waves. Depending on the energy of radiation, the interaction is manifested by absorption or emission spectra, diffusion of electromagnetic waves or changes in the properties of the substance. Spectroscopy is the experimental method, which measures and interprets this interaction.

UV-VIS spectrophotometry

UV-VIS spectroscopy is spectrometry in the range of visible wavelengths, as well as adjacent ranges, such as ultraviolet and infrared ($10^{-7} \div 10^{-6}$ m). The specificity of UV-VIS spectroscopy, compared to other types of spectroscopy, is that the vast majority of matter structures, which are larger than atoms, i.e. molecules, interact with the electromagnetic field of the UV-VIS domain by resonance. The identification of these interactions allows the discovering of the molecule identity and characters, in particular, of organic and coordinating compounds, with conjugated cyclic polyene systems, which are distinguished by high selectivity.

The quantification of the UV-VIS radiation intensity, transmitted in function of concentration and the thickness of the layer to be analyzed, at a certain wavelength, represents the fundamental law of radiation absorption:

$$-\log T = \log \frac{I_o}{I} = \varepsilon c l = A \qquad (1)$$

where: T – transmittance; A – absorbance; I_0/I – fraction of the intensity of the incident radiation that is transmitted by the sample; ε – extinction coefficient or absorption molar coefficient, expressed in $L \cdot mol^{-1} \cdot cm^{-1}$; c – analyte concentration expressed in mol/L; l – length, in cm, traveled by radiation through the analyte solution.

The Lambert-Beer equation (1) is the basis of quantitative spectrometric determinations. The parameters determined by spectrophotometric methods are presented in Table 2.3.

Table 2.3. Spectrophotometric methods

Nr.	ND or validated method	Indicator
1	SM SR ISO 6332:2006	Total iron
2	SM SR ISO 7890-3:2006	Nitrates
3	SM SR EN 26777:2006	Nitrites
4	SM SR ISO 7150-1:2005	Ammonium
5	SM SR EN ISO 6878:2011	Total phosphorus

Atomic absorption spectrometry

The phenomenon of atomic absorption occurs when the atom from the fundamental energy state absorbs energy at certain wavelengths and enters an excited state (Fig. 2.1). The absorbed energy leads to the cleavage of the valence electron orbitals, probed by the migration of electrons to energy levels other than the initial ones. The identification and

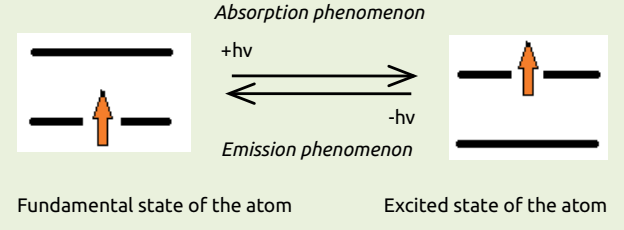

Fig. 2.1. Phenomenon of absorption and emission

quantification of this energy absorbed subsequently can be converted into units of concentration of the element to be analysed. Thus, if the atoms of an element to be analysed will be oriented in the path of a monochromatic light beam, then the amount of energy absorbed will increase proportionally to the number of atoms that pass into the excited energy state. The dependence between the amount of energy absorbed and the amount of element in the standard samples with known concentrations is used successfully in analytical determinations of samples with unknown concentrations, their atomic absorption being measured.

In order to transform the aerosol of samples into vapor of atoms, at which moment they absorb the energy, the atomic absorption spectrometry uses some methods of evaporation and atomization: 1) in the flame; 2) electrothermal atomization in graphite furnace; and 3) with the use of chemicals.

For the flame atomization, gas mixtures are used, which, on combustion, provide high temperatures, about 1900-2800 °C, such as those of air-acetylene or nitrogen(I) oxide-acetylene.

When using an electrothermal atomization system, all the atoms to be analysed in a sample are transformed into atomic vapors practically at the same time, in a closed system, such as that of a graphite furnace and remain in the atomization system until their absorption is measured. For this reason, the sensitivity of the determinations is higher and the volume consumption is reduced – about $5 \div 50 \cdot \mu L$ of sample.

Chemical atomization is applicable for the determination of ultra-microquantities of mercury and elements, which form volatile hydrides (SeH_2, AsH_3, SbH_3, BiH_3, SnH_4). The method involves the use of reagents that convert the element to be analysed into a volatile compound, usually a hydride, which is transported by an inert gas in a quartz reservoir heated to about 900 °C. The hydride, being very reactive, at this temperature decomposes with the formation of atoms of the corresponding elements.

The atomic absorption spectrometric methods used to determine the chemical elements are presented in Tab. 2.4.

Table 2.4. Atomic absorption and emission spectrometric methods

Nr.	ND or validated method	Parameter	Atomization technique
1	SM ISO 9964-1:2013	Sodium	Flame
2	SM ISO 9964-2:2013	Potassium	Flame
3	SM SR EN ISO 7980:2012	Calcium, Magnesium	Flame
4	SM SR ISO 8288:2006	Co, Ni, Cu, Zn, Cd, Pb	Flame
5	SM SR EN 1483:2012	Mercury	Chemical
6	SM SR EN ISO 12020:2012	Arsenic	Chemical
7	SM ISO 17378-2:2015	Ar, Sb	Chemical
8	ISO/TS 17379-2:2013	Se	Chemical
9	SM SR EN ISO 15586:2011	*3d*, Cd, Pb	Electrothermal
10	SM SR EN ISO 11885:2012	*Elements*	Inductively coupled plasma

Atomic emission spectrometry

Atomic emission spectrometry is based on the phenomenon of desorption (emission) of light energy (Fig. 2.1). The principle of the method consists in the vaporization and excitation of the atoms of the analysed sample, the separation of the emitted radiations in function of the wavelength, the recording, identification and quantification of the obtained signals (Tab. 2.5).

After separating the emitted radiation by a monochromator, they are focused on the radiation detector, which is usually a photoelectric detector. The qualitative analysis is made on the basis of the positions in the spectrum (of the wavelengths) of the emitted radiation, each element emitting a characteristic spectrum (Fig. 2.2). Quantitative analysis is done by measuring the intensity of the emitted radiation.

Table 2.5. Flame tests of the solutions of some elements

						
Lithium	Sodium	Potasium	Rubidium	Strontium	Boron	Copper

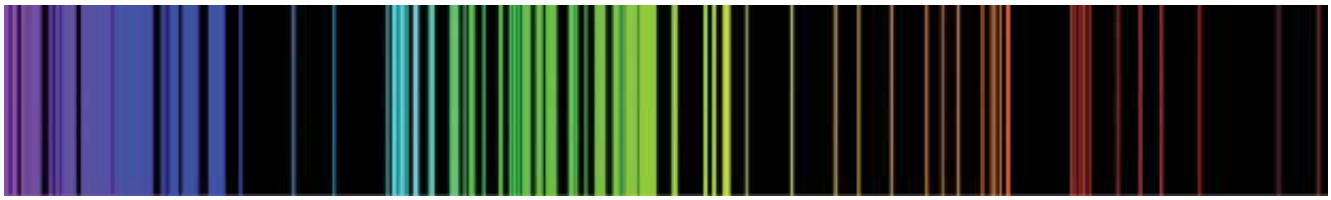

Fig. 2.2. The emission spectrum of iron

Due to its high temperature, plasma is the best way to vaporize and excite atoms in atomic emission spectrometry.

The torch with inductively coupled plasma consists of three concentric quartz tubes, the inner tubes being shorter (Fig. 2.3). Around the outer tube at the top are two induction coils (water cooled) of a radio frequency generator. The aerosol sample is inserted through the central tube. To put in function the torch, a stream of argon is introduced between the concentric tubes, then the ionization of argon is initiated with the help of electric sparks. The resulting ions and electrons interact with the oscillating magnetic field (H) produced by the induction coils. Argon plasma, which is a good electrical conductor by containing many free electrons, interacts with the magnetic field. This induces circulation of eddy currents in the formed plasma, which contributes to the increase in temperature. The plasma thus formed has the appearance of a flame in the upper part of the quartz tube, with a very high temperature, approximatively 9000 K.

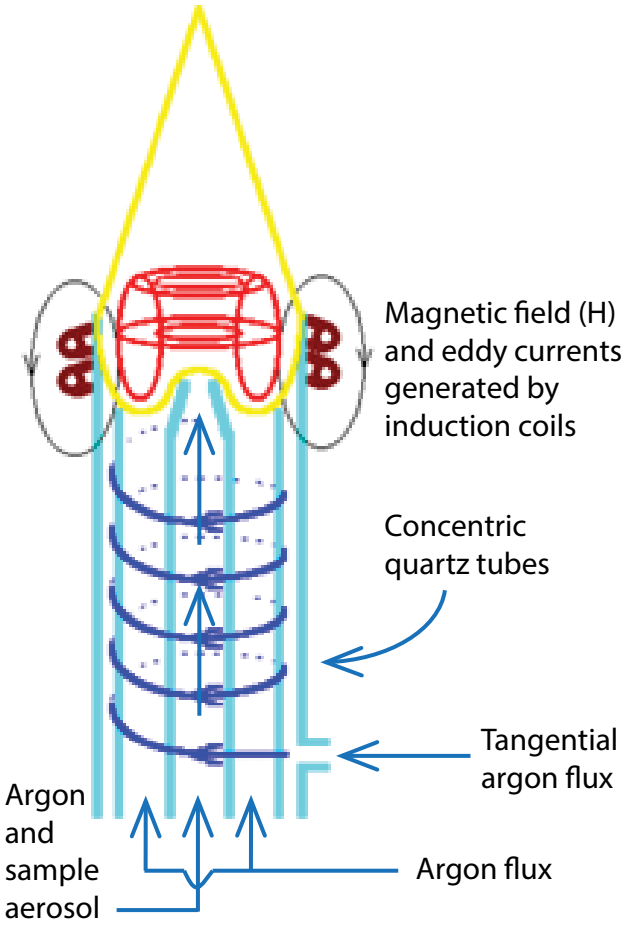

Fig. 2.3. Scheme of an inductively coupled plasma torch

Chromatography

Chromatography is the method of separating two or more components based on the difference in affinity between a stationary and a mobile phase. The stationary phase represents a chromatographic column loaded with specific adsorbent materials, and the mobile one – a carrier gas or a liquid. Detection and identification of separate components is performed using high-sensitivity related equipment, such as detectors.

The scheme of a chromatograph consists of: 1) mobile phase source; 2) device for regulating and measuring the mobile phase flow; 3) sample introduction device; 4) thermostat; 5) chromatographic column; 6) detector; 7) chromatogram recording and processing device (PC and computer software).

In particular, gas chromatography (GC) and high performance liquid chromatography (HPLC) are used for high sensitivity determinations in the monitoring and control of environment quality.

Gas chromatography

The specificity of gas chromatography is the determination of compounds that can be vaporized, without decomposition, within wide temperature limits, –80 ÷ +400 °C and concentrations of the order of parts per million (ppm). This is achievable by using: 1) the capillary column with an inner layer of stationary phase; 2) a gas as a mobile phase; 3) specific detectors FID, ECD or MS.

The gas, most often helium, will ensure rapid mass transfers between the mobile and stationary phases, high diffusion velocities of the components and, in this way, the distribution equilibria can be established very often in a short time. The low viscosity of the gases makes it possible to use long spiral columns (15-75 m), with a small diameter (0.1-1 mm,) which ensure the method short retention times, selectivity and high resolution.

The detection of separate components is done continuously, quickly and with great sensitivity. This is based on the change of a physical or chemical property of the carrier gas, when the components of the sample appear in it. This change is transformed into an electrical signal, which is recorded after amplification. The detector must have: high sensitivity, selectivity for the components to be analysed, fast response, wide range of proportionality between signal and component quantity, low background noise, stability to fluctuations of working parameters.

Qualitative analysis in GC consists in identifying the separate components (peaks) and is performed by the retention time, defined as the time elapsed from the introduction of the sample to be analysed in the chromatographic column until the maximum peak of that component appears (Fig. 2.4). The retention times of the components in the test sample are compared with those of the chromatographic standards.

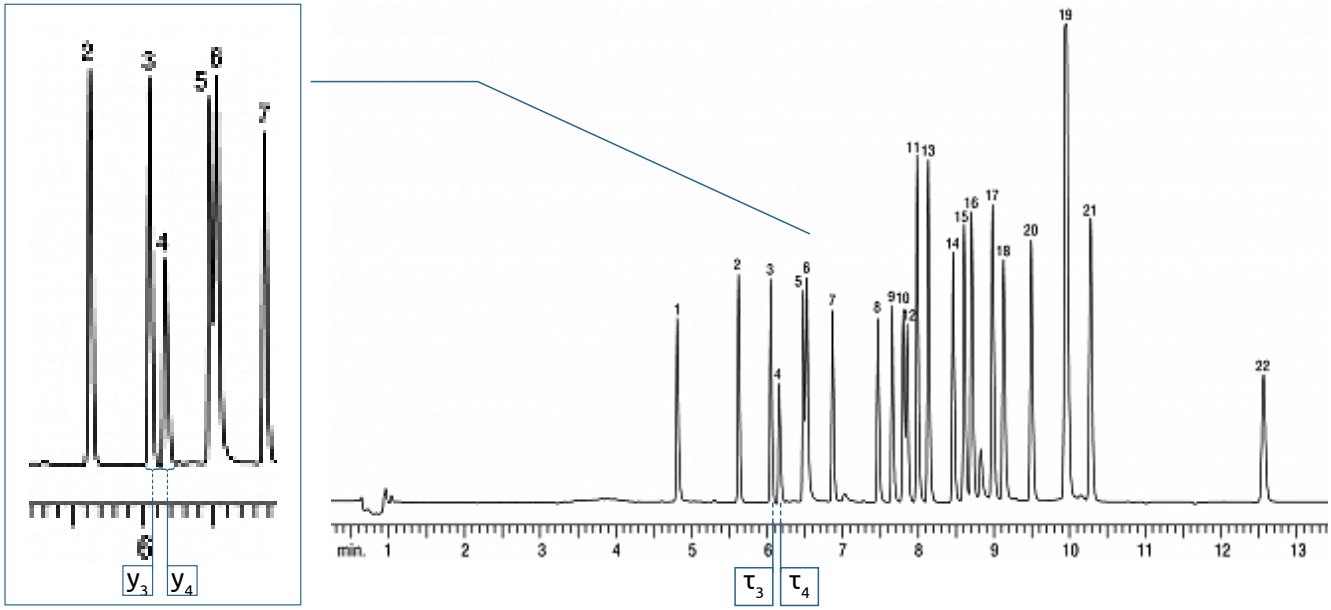

Fig. 2.4. Part of the chromatogram of a mixture of organochlorine pesticides on the CLP chromatographic column

The effectiveness of chromatographic separations is evaluated by resolution, which for two components (Fig. 2.4) is defined by the equation:

$$R = \frac{\Delta\tau}{y_3 + y_4} \quad (2),$$

where: R – resolution; y_3, y_4 – the width of the peaks, expressed in units of time, $\Delta\tau$ – the distance between peaks.

$$\Delta\tau = \tau_4 - \tau_3 \quad (3)$$

where: τ_3 and τ_4 – position of peaks, expressed in units of time.

Quantitative analysis is performed by measuring the surfaces of the chromatographic peaks and correlating them with the concentrations of these components. Thus, the detector highlights the separate and eluted components of the carrier gas flow, by emitting electrical signals proportional to their concentration.

Gas chromatography with mass spectrometer (GC-MS) is one of the most important ways to identify volatile and semivolatile ecotoxicants of anthropogenic nature. The advantage of using GC-MS, compared to routine GC-ECD and/or GC-FID methods, is explained by the fact that it combines gas chromatography and mass spectrometry. GC-MS is superior because it provides detailed structural information for most components of the gas phase. The identification of unknown organic pollutants is performed by comparing the MS spectrum obtained from the analysis with the known reference spectra placed in the NIST data libraries. Thus, GC-MS is applicable in areas that require both qualitative identification and quantitative measurement of components in complex mixtures. It is very suitable for applications of pollutants in aquatic ecosystems, because the sensitivity for some components is less than 1-100 ρg.

HPLC chromatography

HPLC, as a method of qualitative and quantitative analysis, is used for the separation, identification and quantification of organic and inorganic compounds, such as several mineral anions. HPLC is often used when some compounds cannot be analysed with GC, for example, if being vaporized in the capillary column, they decompose or change their composition and structure. Thus, the vast majority of analyses are performed at temperatures of 25-35 °C.

The analysis apparatus is the HPLC or UHPLC chromatograph. This involves the use of a column loaded with the stationary phase, a pump that ensures elution with the mobile phase (s) of the column and a detector, which shows the retention times and identifies the molecules.

The retention time depends on the interaction between the stationary phase, molecules to be analysed and solvents used. Those organic solvents, which form any miscible combination with water and/or other organic liquids, are used as the mobile phase, usually methanol and acetonitrile. Often, in the practice of HPLC, acidic, basic, salt solutions, buffer solutions, which help to separate the analysed components, are used as the mobile phase.

2.2 STEPS FOR PREPARING THE SAMPLES TO BE ANALYSED

The separation of the components to be analysed from liquid and semi-liquid samples is performed by filtration. The filtration can be made immediately after sampling (in the field), by using comfortable and easy in operation equipment (Fig. 2.5). This facilitates the subsequent preparation of samples for laboratory analysis and has some advantages, such as simplicity, speed, time saving and accessibility.

For conditioning of the samples to the state of liquid aggregation, required for further analyses with the application of high-performance automated or semi-automated equipment, the instrumental methods are applied (Tab. 2.6).

Fig. 2.5. Use of the mobile filtration system

Table 2.6. Laboratory equipment for the preparation of samples for analysis using the specified methods

Nr.	Equipment	Parameter	Applicable to method or ND
1	DK6 Heating Digester, VELP Scientifica	Nitrogen after Kjeldahl Macroelements (AAS)	SM SR EN ISO 25663:2012 SM ISO 9964-1, 2:2013 SM SR EN ISO 7980:2012
2	Steam distillation unit UDK 127, VELP Scientifica	Ammonium ions	SM SR ISO 5664:2007
3	HotBlock SC 154, Environmental Express	Fluorides Macroelements (AAS, ICP-OES), Microelements (AAS, ICP-OES), Toxic and heavy metals (AAS, ICP-OES)	SM SR ISO 10359-2:2011 SM SR EN ISO 11885:2012 SM SR EN ISO 17294-2:2012 SM SR EN ISO 15586:2011 SM SR EN ISO 15587-1, 2:2011
4	HotBlock SC 151, Environmental Express		
5	SpeedWave 4 microwave sample preparation system, Berghof		
6	Filtration system Sartoriu Stedim Biotech	-	-

1) Distillacid BSB-939-IR System (Berghof, Germany)

For its needs of water and high purity acids, applicable for ultramicroanalysis of microelements and toxic elements, the laboratory uses the Distillacid BSB-939-IR system (Berghof, Germany), which is a subboiling type purifier (Fig. 2.6).

Contactless heating of acids, by means of an IR lamp, creates a state of equilibrium between the absorbed radiation and the heat of evaporation of the liquid, which is reached approxatelly at 10 °C below the boiling temperature of the given acid (or water). This state of equilibrium ensures a gentle distillation and slow evaporation. The productivity of the distillate is slow, about 1-2 litres in 24 hours. As all the components of this system are made of extrapure PFA and PTFE, the obtained distillate has an extraordinary purity and the quality of the reagent corresponds to "ultrapure" or "for inorganic trace analysis" types.

Fig. 2.6. Distillacid BSB-939-IR unit

2) SpeedWave four (SW-4) microwave sample preparation system (Berghof, Germany)

The microwave oven is designed for digestion/extraction and/or chemical pyrolysis procedures at temperatures of up to 300 °C and pressures of up to 100 bar, for further use of samples for AAS and ICP-OES spectrometric analyzes. If a subsequent pretreatment is done, the samples can also be analysed by using the UV-VIS spectrophotometric method. The mixtures to be analysed are heated by microwave irradiation. Concentrated acids such as nitric, hydrochloric, hydrofluoric, phosphoric and sulphuric, as well as their mixtures, are used for acid digestion.

Fig. 2.7. Microwave digestor SpeedWave four (SW-4)

Exclusion of contamination and loss of analytes, short time for the digestion process are listed among the advantages of this equipment. All the components that come into contact with the digestive reagents are made of extrapure PFA and PTFE and the samples subjected to the digestion process are in hermetically sealed vessels (Fig.2.7). The digestion process, depending on the matrix of the analysed sample, takes from a few minutes to a few tens of minutes.

3) DK6 Heating Digester (VELP Scientifica, Italy)

It is a digester designed according to the traditional Kjeldahl principle, with an aluminum heating block, which offers excellent thermal homogeneity, with a maximum working temperature of 450 °C. It is applied in the determination of organic nitrogen by the Kjeldahl method (SM SR EN ISO 25663: 2012), which involves the mineralization with selenium (IV) oxide or mixtures of copper and potassium sulphate, and also in the case of sample preparation for the determination of macroelements by the flame AAS (in the table 2.6, see SM ISO 9964-1: 2013; SM ISO 9964-2: 2013; SM SR EN ISO 7980: 2012).

4) UDK Digester Steam Distillation System (VELP Scientifica, Italy)

It is a semi-automatic distillation equipment, designed to perform laboratory determinations of organic nitrogen by the Kjeldahl method (SM SR EN ISO 25663: 2012) and of ammonium ions (SM SR ISO 5664: 2007), on samples with difficult matrices (water, soil and silts). It is a programmable device, so it is possible to automatically control the distillation time, addition of reagents and the elimination of waste resulting from distillation. It has an alarm system for the level of reagents, as well as for the flow of water during cooling.

5) Filtration system Sartoriu Stedim Biotech

It is used to determine the content of suspensions in water samples (Fig. 2.8). It is a three-position stainless steel filtration system with a vacuum pump. Thus, from one to three water samples can be filtered simultaneously or gradually, being necessary only filter paper with blue band Ø = 45mm. Special porosity filters are used in research of bacterioplankton. It is a simple equipment in operation, convenient and fast, which not requires a special care.

6) HotBlock SC 151 and SC 154 (Environmental Express, USA)

The main element of this equipment is the heater mat, which is made of graphite and corrosion-resistant Kydex®[2]. It has been designed for the digestion with concentrated acids, especially of liquid samples, for metal determinations (Fig. 2.9). The temperature range varies from ambient temperature to 150 °C. The heat is provided by a low-power graphite heater mat, which provides uniform heating on all sides of the block. It is possible to create digestion programs, where the number of steps, temperature of each step, heating rate in °C/minute (or the temperature gradient) and the time of keeping the samples at the required temperature are programmed.

Fig. 2.8. Water filtration through Sartoriu Stedim Biotech

Fig. 2.9. Digestor with graphite mattress HotBlock SC 151

[2] https://en.wikipedia.org/wiki/Kydex

At the same time, it allows the operator to prepare 54 samples (of 50 mL) simultaneously. The strong point of this equipment is that it offers the possibility to use plastic digestion vessels, which permits the preparation of samples for micro- and ultra-microanalysis of the elements. The advantages of using this equipment are simplicity, speed, saving of energy and expensive reagents, and operational comfort.

2.3. CHEMICAL ANALYSIS EQUIPMENT

1) AAnalyst 400 atomic absorption spectrometer (Perkin Elmer, USA)

The AAnalyst 400 spectrometer consists of optical system, atomization system and accessories for sampling for manual and automated analysis AS-800 (Fig. 2.10).

The optical system consists of a carousel with 4 lamps, a monochromator, background corrector with deuterium lamp and a detector. It is equipped with 11 lamps and 3 atomization systems, acetylene-air flame, electrothermal graphite atomizer HGA 900, MHS-15 hydride generator, which allows the determination of 21 chemical elements, such as Na, K, Mg, Ca, Sr, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Pb, Hg, As and Se. WinLab32 software is used for data processing.

Fig. 2.10. AAnalyst 400 atomic absorption spectrometer: 1 – spectrometer; 2 – electrothermal atomization furnace with graphite reservoir; 3 – carousel with 4 lamps; 4 – autosampler AS-800.

The AAnalyst 400 atomic absorption spectrometer is applicable to samples with a simple or medium complexity matrix, such as water samples, and deuterium lamp background correction is sufficient. This equipment, which is characterized by superior analytical sensitivity, is applicable in the determination of trace elements, especially toxic ones, such as Ni, Cu, Zn, Cd, Pb, etc.

2) Thermo Scientific iCAP 6200 Duo ICP-OES spectrometer (Thermo Fisher Scientific, United Kingdom)

The spectrometer (Fig. 2.11) belongs to the category of ICP-OES spectrometers (OES – optical emission spectrometer, ICP – inductively coupled plasma). It is a tool designed for simultaneous multi-element quantification on liquid samples, assembled with "analysis-ready sample introduction parameters", so that users no longer need to optimize pump speed, RF plasma power and inert gas flow, which makes the operator's job much easier. Duo Plasma Viewer allows axial and radial reading of spectral information, which greatly improves the limits of sensitivity and detection for toxic elements. The EMT torch (Enhanced Matrix Tolerance) simplifies the handling of samples with difficult matrices.

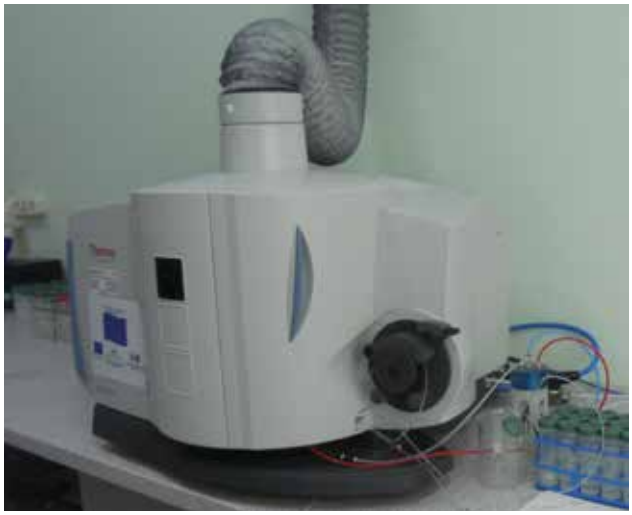

Fig. 2.11. Thermo Scientific iCAP 6200 Duo ICP-OES spectrometer

iTEVA software allows the user selecting template methods, saving time and, thus, avoiding the requirement to develop the methods.

The application of this equipment, according to the method SM SR EN ISO 11885: 2012, allows the simultaneous multielemental analysis of a water sample in about 3-4 minutes and the quantification of about 62 chemical elements.

3) *SPECORD® 210 PLUS UV-VIS spectrophotometer (Analytik Jena, Germany)*

Fig. 2.12. SPECORD® 210 PLUS UV-VIS spectrophotometer

The advantages of using this UV-VIS spectrophotometer (Fig. 2.12) are that it has a monochromator with a concave diffraction grating, which ensures a low level of diffused light. The analysis using two thermostatic detectors contributes to the stability of the results over time and an improved detection limit. In addition, the construction of the "double beam" optical system performs simultaneous measurements of the working and reference signals, which greatly improves the accuracy. Monochromator slot variable from 0.2; 0.5; 1; 2 and 4 nm allows measurements on different types of samples, with the possibility to select the best signal-to-noise ratio.

The spectrometer is also equipped with a cell holder, with 8 positions. With "WinASPECT" software, it is able to measure absorption, transmittance, reflectance and energy in the wavelength range between 190-1100 nm for liquid and solid samples. Operation in the 4 working modes – "Scan Mode", "Step Mode", "Time Scan" and "Wavelengths" – allows qualitative identifications, as well as quantitative determinations of concentrations and kinetic parameters.

4) *Clarus 500 GC chromatograph with autosampler and TurboMatrix HS 40 Trap (Perkin Elmer, USA)*

Fig. 2.13. Clarus 500 and TurboMatrix HS 40 Trap system

The gas chromatographic analyser (1) is equipped with 2 injectors, A and B (2), capillary columns and a thermostat (3), 2 detectors (4), FID (flame ionization detector) and ECD (electrone capture detector), autosampler (5) and TurboMatrix HS 40 Trap unit (6) (Fig. 2.13).

The injectors are the accessories responsible for purging and transporting the resulting gas flows to the columns, therefore, they are equipped with programmable heating systems. The role of the thermostat is to maintain the temperatures and/or the heating gradient and, effectively, to obtain the conditions, which ensure reproducible analyses and eliminate the sources of uncertainty, dependent on sudden temperature fluctuations.

Column separation efficiency is essential in GC analysis, therefore, the selection of a column with certain phases is of high importance, namely, of the polarity and hydrophobicity characteristics of the stationary phase compared to the analytes. Clarus 500 GC Chromatograph is equipped with 5 chromatographic columns namely: Elite 5 MS (30m × 0.32mm ID, 1.00 µm film); Elite CLP (30m × 0.32 mm ID, 0.50 µm film); Elite 1301 (30m × 0.32mm ID, 1.00 µm film); Elite 624 (75m × 0.53mm ID, 3.00 µm film) and Elite 225 (30m × 0.32mm ID, 0.25 µm film).

Being a non-polar column, Elite 5 MS is applicable for the separation of analytes such as C_5-C_{40} saturated hydrocarbons and their halogenated derivatives, waxes, aromatic hydrocarbons, petroleum products, pesticides, mercaptans and sulphur-containing compounds, solvents, flavorings, perfumes, etc.

The Elite CLP column is mainly used for the determination of chlorinated pesticides and herbicides according to EPA methods 504, 608, 619, 8081, 8151, etc.

The Elite 1301 column is a column with medium polarity, which is provided by the phase consisting of 6% cyanopropylphenyl and 94% dimethylpolysiloxane. It is applicable for analyses on compounds with variable polarity in the same sample, especially pesticides and insecticides

with multiple functional groups: hydroxy-, oxo-, carboxy-, haloformyl-, carboxamido-, alkoxy-, cyanate-, thiocyanate-, etc.

The phenylmethylpolysiloxane (phenyl cyanopropyl) phase gives the Elite 225 column an increased polarity, which offer the possibility to use it for the analysis of carbohydrates, sterols, fatty acids and their esters, etc.

The Elite 624 column is a special one and was developed for determinations according to the EPA 624 method. It is used for the analysis of volatile substances (VOA), such as BTEX (representatives of the BTEX group: benzene, toluene, ethylbenzene, 1,2,3-methylbenzene, 1, 2,4-methylbenzene, 1,3,5-methylbenzene, o-xylene, m-xylene, p-xylene), halogenated derivatives of methane (trihalomethanes), ethane, propane, ethene, butadiene, phenol, aniline, alcohols, dibenzofuran, vinyl chloride, etc.

The autosampler is an accessory for the automatic injection of analyte solutions, prepared preliminary by extraction with solvents, purification and concentration from analysed samples.

FID is a destructive detector, the detection principle of which is based on the exceptional sensitivity to ions formed during the combustion of substances in an ultrapure hydrogen flame. It is characterized by an extraordinary selectivity for organic compounds, which contain bonds and groups of C-C, C-N atoms and can measure the concentrations of substances from very low to very high, in a linear range of 10^7 concentration units.

ECD is a non-destructive detector and is used to detect compounds that contain atoms with high electronegativity. Although it has a limited range of detectable compounds, due to its high selectivity, being in general about 10-1000 times more sensitive than an FID and 10^6 times more sensitive than a TCD (thermal conductivity detector), the ECD detector finds its application in the analysis of halogenated compounds.

To lead the analysis process, the analyser display or a computer on which the TotalChrom software is installed (version 6.3.1 license) is used. The equipment permits to analyse automatically up to 82 samples. The "Duo" mode allows simultaneous separations and identifications on 2 columns with detection at FID and ECD detectors, respectively.

For the analysis of volatile substances, the TurboMatrix HS 40 Trap system is coupled to the Clarus 500 GC chromatograph. The TurboMatrix HS 40 Trap unit is designed for automatic vapor sampling from the airtight space of the thermostat bottle. Thermostation can be programmed at one or, in steps, at several desired temperatures. If it is also equipped with a hatch, then it is possible to capture in repetition the analyte vapors, thus ensuring a reproducible result and high sensitivity. Up to 40 bottles can be loaded into the carousel, of which up to 12 bottles are thermostated in the thermostat, which ensure a maximum productivity. The thermostatic algorithm is optimized and adjusted for maximum continuous flow.

This equipment opens new horizons in routine analysis. Basically, it frees the operator from the preparation of the samples to be analyzed. It is enough just to seal the samples in bottles. As next step, the equipment performs all actions in automatic mode. The endowment with these accessories is imperative and makes the Clarus 500 gas chromatograph unique as an analysis tool, indispensable for a high-performance laboratory in the field of ultra-microquantitative analytical determinations of anthropogenic pollutants.

5) Flexar FX 20 UHPLC chromatograph (Perkin Elmer, USA)

As in the case of HPLC or UHPLC (Ultra High Performance Liquid Chromatography) the mobile phase is a liquid or mixture of liquids, the one that sets this phase in motion is the binary pump. Depending on the pressure used by the pump, HPLC and UHPLC are distinguished.

The Flexar FX 20 system (Fig. 2.14) is a UHPLC chromatograph, composed of five

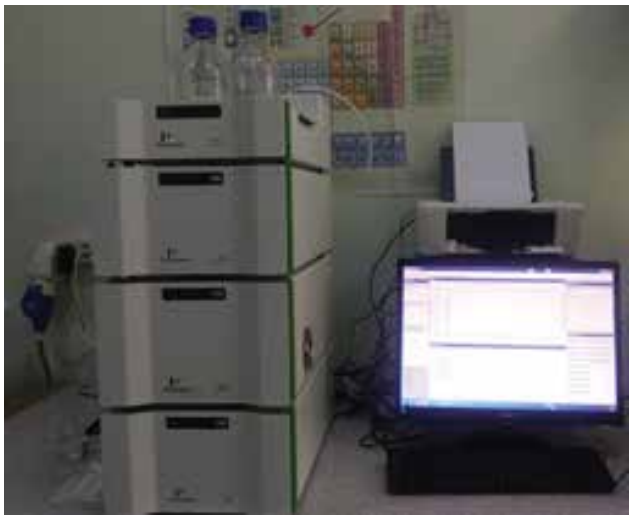

Fig. 2.14. Flexar FX 20 UHPLC chromatograph

modules: solvent degasser, binary pump, injector, column thermostat and UV-VIS detector. The chromatograph is run via the "Chromera" computer software. The degasser, which only requires connection to a sourse of electric power supply, and the injector are not programmable. The role of the degasser consists only in the degassing of mobile phase. The binary pump ensures the flow of the mobile phase through the system. It is a programmable component and in the analysis process it is necessary to program the flow of the mobile phase, the composition of the mobile phase and the maximum pressure.

The injector has a fixed volume in the loop, 20 µL, but, if necessary, it can be replaced with a loop with the desired volume – 5, 10 or 50 µL. Its role is limited only to the introduction of the sample into the liquid phase flow. It is loaded manually by using the syringe. It can be partially loaded by injecting volumes which differ from the loop volume.

For reproducible results, it is important that the separation of the analytes per column is performed at a stable temperature. Therefore, a thermostating of the chromatographic column in the furnace is performed by programming the temperature.

The UV-VIS detector ensures the detection of compounds eluting from the column. Several wavelengths λ (in nm) can be programmed to obtain in the analysis process a maximum sensitivity for all analytes.

Reduction of analysis time, low consumption of ultrapure solvents, at the same time, high sensitivity to analytes are some of the performances of Flexar FX 20 chromatograph. The analysis priorities of the Flexar FX 20 chromatograph cover a wider class of anthropogenic pollutants, which contain in their composition thermosensitive functional groups, such as oxo-, carboxy-, cyanate-, thiocyanate-, nitro- groups, but also nonsteroidal anti-inflammatory drugs, contraceptives, vitamins and other types of drugs.

III CHEMICAL COMPOSITION OF NATURAL WATERS

What can water be replaced with and what does “clean water”, “drinking water”, “polluted water”, “dirty water” and simply “natural water” mean? There are no simpler questions, but, at the same time, no more complicated ones.

If to consider that water is a chemical, then it can be stated that its formula is frighteningly simple: H_2O, meaning it contains two hydrogen atoms and one oxygen atom. The expression “Well, he doesn’t even know the formula of water” circulates among chemists, what expresses the absolute lack of knowledge in the field of chemistry. Despite its simplicity, water exists in three states – vapour, liquid and solid body, but at the same time, its chemical formula remains unchanged. The formula is the same, but their properties are difficult to compare. Thus, if we freeze the water and then defrost it, we will obtain water, which will differ from the first one not only by taste, but also by the action on human body. Only three atoms, however, depending on their mutual position, the spatial arrangement of water molecules, there is a radical change in the properties of water. Therefore, the structure of water still attracts the attention of researchers.

Water is a substance that has special properties for dissolving other substances, so there is no such compound as H_2O in nature, but there is a complex solution, which is constantly changing in time and space. Natural water can have no taste, but also it can be very sweet, sweet, brackish, bitter, bitter-salty, salty and in the form of brine, depending on its amount of dissolved salts and ratio of salts. In addition, it can be transparent, turbid, coloured, depending on the presence of suspended particles, colloidal substances and coloured ones. Water can have different odours – from the smell of freshness to the smell of putrefaction and spoiled eggs (in the presence of hydrogen sulphide in the water). The diversity of natural waters is immense, it being determined by its origin, physico-chemical and biological parameters.

By their chemical composition, the natural waters are extremely diverse. More or less similar waters by composition can be found, but never identical waters. Waters are distinguished not only by the chemical elements and the total concentration of the dissolved substances, but also by the quantitative ratio of the components and the form of their compounds. Thus, the chemical composition of natural waters is a complicated set of mineral and organic substances, which occur in various forms of ion-molecular and colloidal salts.

Conventionally, there can be highlighted five groups of substances in natural waters:

- dissolved gases (oxygen, carbon dioxide, nitrogen, methane, etc.);
- main ions (hydrogen carbonate and carbonate, chloride and sulphate anions, calcium, magnesium, sodium and potassium cations);
- nutrients (ammonium, nitrate, nitrite, phosphate ions, iron, silicon);
- organic substances of natural origin (albumins, lipids, carbohydrates, petroleum products) and xenobionts (detergents, pesticides, herbicides, biphenyl polychlorides (BPCs), polychlorodibenzodioxins (PCDDs), dibenzofurans (PCDFs), etc.);
- microelements (Cu, Zn, Mn, Co, Mo, V, Pb, Cd, As, Hg, Se, Sr, F, etc.)

Currently, investigations on the quality of surface waters aim to solve a set of problems related to the protection of natural waters against pollutants. Water is an important factor in the ecological balance, and its pollution has more or less serious consequences on the living environment. All mentioned above demonstrates the importance of systematic hydrochemical research in solving the problems of sustainable use of surface waters.

The traditional or classical methods, applied for practical purposes to assess the quality of drinking water, used in households and for irrigation, are briefly described in this chapter.

3.1. DISSOLVED GASES

Oxygen

Dissolved oxigen is found in water as O_2 molecules. Its amount in water depends on two groups of processes: the first contributes to the increase of its concentration and the second – to the decrease. The process of oxygen absorption from the atmosphere and its elimination by the aquatic vegetation, as result of photosynthesis, enriches the waters with oxygen. O_2 desorption in the atmosphere, oxidation processes (biological, biochemical and chemical) decrease the oxygen concentration.Process of photosynthesis can be represented schematically by the equation:

$$CO_2 + H_20 + 470\ KJ\ HV/\ CHLOROPHYLL = (CH_2O)+O_2.$$

The O_2 production by photosynthesis takes place only in the water layer, where sunlight enters. Animal respiration is a process opposite to photosynthesis, accompanied by the formation of new molecules of water and carbon dioxide, with oxygen consumption, which can be schematically described by the equation:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 2820\ KJ.$$

For many fish species the decrease of oxygen content up to 46-50% of saturation, that means reaching concentrations lower than 4 mg/L at a water temperature of 20˚C, is a critical situation, which leads to the decrease of their growth rate. Mass loss of fish and intensification of the activity of some species of bacteria, which are able to obtain the necessary oxygen from the reduction of sulphate ions from the water, occur at concentrations of dissolved oxygen lower than 1-2 mg/L.

The O_2 content ranges 0-14 mg/L and shows seasonal and diurnal oscillations. In summer, during the intense development of algae and higher aquatic plants, in windless days, when the water mixing is reduced, there may take place the oversaturation of water with oxygen in the surface water layers. Oversaturation can reach values of up to 200%. Such processes are also recorded in winter, when the increased development of diatom algae takes place. Oversaturation of water with O_2, as well as its deficiency has a negative influence on the development of hydrobionts.

Determination of dissolved oxygen

There are, currently, several tests and different types of oxygen meters for determination of the dissolved oxygen directly at the water source. However, the most common is Winkler's method, which is based on the oxidation of manganese (II) hydroxide to manganese (III) hydroxide, which in an acid medium removes iodine from potassium iodide in an amount equivalent to oxygen dissolved in water. Iodine is titrated with sodium thiosulphate.

The water is collected in separate bottles with a fixed volume. The bottles are completely filled, without aerating the water, closed with stoppers, and verified if no air bubbles are inside. Immediately 1 mL of manganese sulphate or manganese chloride solution and 1 mL of alkaline potassium iodide solution are introduced. The stopper is put back and the content of the bottle is shaken. In the presence of oxygen, a brown precipitate forms, and in its absence, the precipitate remains white. After complete deposition of the precipitate, 1-2 mL of HCl or H_2SO_4 (1:1) are added. The bottles are closed again and the content is shaken well until the precipitate is completely dissolved. The content is quantitatively poured in an Erlenmeyer flask and titrated with a 0.01N thiosulphate solution until yellow (straw colour) is obtained. 1 mL of starch is added and the content turns blue. Then the titration is continued until the blue colour disappears.

The content of dissolved oxygen in water (mg O_2/L) is calculated according to the equation:

$$O_2 = \frac{nN \cdot 8 \cdot 1000}{V - 2}$$

where: n – volume of thiosulphate used at titration, mL;
8 – equivalent mass of oxygen;
N – normality of thiosulphate solution;
1000 – recalculation for 1 litre of sample;
V – volume of sample, mL;
2 – volume of reagents added to fix the oxygen, mL.

Using Winkler's table, which includes the values of the equilibrium of saturation of water with dissolved oxygen at different temperatures, and based on the determination of the oxygen concentration, the saturation of analysed water is calculated.

Reagents:

- *50% $MnSO_4 \cdot 6H_2O$ or 40% $MnCl_2 \cdot 4H_2O$;*
- *alkaline potassium iodide solution (30 g NaOH and 15 g KI are dissolved in a small volume of distilled water; the volume of solution is brought to 100 mL with distilled water);*
- *0.5% starch solution (0.5 g of starch are taken for each 100 mL of distilled water and heated until boiling; it is used fresh or preserved (with salicylic acid));*
- *sulphuric acid diluted with distilled water (1:3);*
- *0.1 N sodium thiosulphate (it is prepared with distilled water free of CO_2 (boiled and cooled));*
- *0.01 N sodium thiosulphate (it is prepared from 0.01 N sodium thiosulphate by dilution).*

Determination of biochemical oxygen consumption (BOC_5) in water

Biochemical oxygen consumption (BOC_5) is the amount of oxygen consumed by microorganisms over a period of 5 days for the biochemical decomposition of organic substances, contained in water at 20 °C. The water to be analysed is collected in two bottles of known volume under the same conditions as for the determination of dissolved oxygen. Oxygen is fixed in one of the bottles and the second bottle is kept in the darkness at a temperature of approximately 20 °C for 5 days. The determination of dissolved oxygen in first bottle is carried out as described earlier in the text. After 5 days, the dissolved oxygen in the second bottle is determined, under the same conditions as for the first bottle.

Determination of permanganate chemical oxygen demand (COD_{Mn})

The essence of the method consists in the oxidation of organic substances from water with potassium permanganate in a sulphate medium by boiling:

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \rightarrow 2MnSO4 + K_2SO_4 + 10CO_2 + 8H_2O$$

Excess of permanganate after boiling is determined by the iodometric method. The amount of organic substances in the sample must ensure an excess of permanganate of at least 40% at the end of reaction. Otherwise, the sample is diluted.

2-3 glass capillaries are added to 100 mL of water sample, for boiling regulation, then 5 mL of sulphuric acid (1:3) are added and all solution is heated. Just at the beginning of boiling, 10 mL of 0.01 N $KMnO_4$ are pipetted into solution, the boiling round-bottom flask is closed with a stopper-condenser and the solution is boiled for exactly 10 minutes. If during boiling the colour of permanganate disappears or becomes brown, the analysis is repeated, diluting the sample previously. After boiling, the sample is cooled slightly. Approximately 0.5 g of dry potassium iodide is added and the sample is titrated with 0.01 N thiosulphate until pale yellow. Then, 1 mL of starch is added and the sample is titrated until the colour disappears. Similarly, the blank (control) determination – in 100 mL of distilled water – is performed. Calculation:

$$C = \frac{8N \times (a_1 - a_2) \times 1000}{V}, \text{ mgO/L,}$$

where: N – normality of thiosulphate solution;
a_1 – volume of thiosulphate used at the titration of control sample, mL;
a_2 – volume of thiosulphate used at the titration of sample, mL;
V – sample volume, mL.

Reagents:

- *0.1 N $KMnO_4$ solution (3.2 g are dissolved in 1 L of distilled water);*
- *0.01 N $KMnO_4$ solution (100 mL of 0.1 N solution are brought to 1 L with distilled water (it is ready for use after few days));*
- *0.01 N sodium thiosulphate (2.5 g are dissolved and the solution is brought to 1 L with distilled water, or the standard volumetric solutions are used);*
- *0.5% starch solution;*
- *sulphuric acid (1:3) (1 volume of acid is poured in 2 volumes of distilled water).*

Determination of dichromate chemical oxygen demand (COD_{Cr})

Chemical oxygen consumption with potassium dichromate is also called dichromate oxidability. The essence of the method consists in the oxidation of organic substances in water with potassium dichromate, which takes place in an acid environment in the presence of catalyst.

The water sample with a volume of 20 mL or less, brought with double-distilled water up to 20 mL, is poured into a round bottom flask (with ground glass joint) with 2-3 glass capillaries for boiling regulation. 10 mL of 0.025 N solution of potassium dichromate are added, and carefully – 30 mL of silver sulphate solution. The flask is connected to the reflux condenser and the solution is boiled evenly for 2 hours. After cooling, the condenser is removed, it being washed with double distillate (25 mL). The content of flask, by washing the flask walls with 100 mL of double distillate, is transferred to an Erlenmayer flask and the solution is cooled again. Then, indicator drops are added and the excess of potassium chromate is titrated with ammonium-iron (II) sulphate solution until the indicator colour changes from blue – reddish to green – bluish. The blank determination is performed similarly. Calculation:

$$C_{mg} = \frac{8N \times (a_1 - a_2) \times 1000}{V},$$

where: C_{mg} – concentration of dissolved oxygen, mg/L;
a_1 – volume of ammonium-iron (II) sulphate solution used at titration of blank sample, mL;
a_2 – volume of ammonium-iron (II) sulphate solution used at titration of sample, mL;
N – normality of ammonium-iron (II) sulphate solution;
V – sample volume, mL.

Reagents:

- *0.25 N potassium dichromate solution (12.58 g of $K_2Cr_2O_7$, dried previously for 2 hours at a temperature of 105°C, are dissolved and the volume is brought to 1L with distilled water);*
- *0.25 N ammonium-iron (II) sulphate solution (Mohr's salt) (98 g of ammonium-iron (II) sulphate are dissolved in double distilled water, 20 mL of concentrated sulphuric acid are added, the solution is cooled and, by distillation, is brought to 1 L);*
- *0.025 N ammonium-iron (II) sulphate solution (Mohr's salt) (it is obtained by dilution of 0.25 N solution by 10 times);*
- *solution of silver sulphate in concentrated sulphuric acid (13 g of silver sulphate are dissolved in 1 L of acid);*
- *indicator – N-phenylanthranilic acid solution (0.25 g are dissolved in 12 mL of 0.1 N NaOH and diluted with distilled water up to 250 mL).*

Carbon dioxide

Carbon dioxide is found in natural waters in the form of CO_2. The oxidation processes of organic substances are its source in surface waters, and volcanic gases – in groundwater. Its content varies from a few micrograms to 3-4 thousand milligrams per litre. In rivers and lakes the concentration, in most cases, not exceeds 20-30 mg/L. CO_2 has a vital role for plants and animals, being a main source of carbon. In addition, CO_2 increases the solubility capacity of water, being a source of HCO_3^- and CO_3^{2-} ions. Some of it (up to 1%) reacts with water:

$$CO_2 + H_2O = H_2CO_3.$$

The content of carbon dioxide in aquatic systems depends largely on the pH value. At values of 4.5 and lower, there is only free carbonic acid in natural waters, but at pH values higher than 8.3 the content of carbonic acid is negligible.

Water with high concentrations of carbon dioxide becomes aggressive to concrete. This property is characteristic for waters, where the concentration of carbon dioxide is higher than the concentration of equilibrium with HCO_3^- in the aquatic system. The exchange of carbon dioxide between the atmosphere and the Planetary Ocean is a factor that determines the earth climate and the thermal regime in the atmosphere.

Determination of carbon dioxide

The concentration of carbon dioxide, in most cases, is calculated based on the values of pH, water temperature, content of hydrogen carbonates and carbonates. For slightly mineralized waters, the method of titration with NaOH or Na_2CO_3, in the presence of the phenolphthalein indicator, is used directly at the sampling site.

Hydrogen sulphide

Hydrogen sulphide is a product of bacterial decomposition and biochemical oxidation of sulphur-containing albumins. Hydrogen sulphide oxidizes easily to form sulphur and sulphates. Its presence in the surface layers of natural waters is a proof of the occurrence of high amounts of protein substances. It is a very toxic substance for hydrobionts, especially for fish larvae and fry.

Hydrogen sulphide occurs in low concentrations in the water deep layers, and in winter and summer, when the water temperature is high, the oxygen content is low and the water is polluted with sulphide-containing organic substances. Hydrogen sulphide is found in water as non-dissociated H_2S molecules, hydrosulphide ions (HS^-), and less often – as sulphide ions (S^{-2}).

Determination of hydrogen sulphide

Several physico-chemical methods are used for determining this dissolved gas. One of the most simplified is the photometric method with dimethyl-para-phenylenediamine, which forms methylene blue with hydrogen sulphide in the presence of acids and iron salts. The water is preserved directly at the sampling site with a solution of dimethyl-para-phenylenediamine and iron chloride (2.5 mL of each substance per 100 mL of sample) and after 30 minutes or up to two days is analysed spectrophotometrically at λ667 nm (red filter).

Nitrogen, methane, ethane, propane

Molecular nitrogen has a low chemical activity, but it is necessary for plants and animals. Its content not exceeds 10-16 mg/L. It participates indirectly in the processes through ammonia, nitrite and nitrate ions, being an inert gas. Nitrogen dissolved in surface waters comes mainly from the atmosphere. This dissolved gas plays a role of buffer in the process of diluting or removing from the water layers of other gases, primarily carbon dioxide and dissolved oxygen.

Methane, ethane and propane exist in natural waters in a dispersed molecular state. These gases form in the deep layers of water in the process of decomposition of organic substances, when the content of dissolved oxygen is low, usually in winter or summer (periods with high water temperatures).

Hydrogen

Hydrogen ions are the most widespread in the nature, their concentration in natural waters is low. Due to the low values, the concentration of hydrogen is calculated by using the equation $pH = -\lg [H^+]$, or is expressed in mol/L.

The concentration of hydrogen ions determines the acid or basic character of water through the pH index, which is a qualitative property of water. The hydrogen concentration in natural waters ranges from 10^{-4} to 10^{-9} mol/L. The change in water pH is linked to the processes of formation and decomposition of organic substances, on which the decrease or increase of the concentration of carbonic acid depends. The pH values show both seasonal and daily oscillations. The pH values are in a negative correlation with the content of carbonic acid and directly depend on the oxygen content; pH serves as a good index of the reduction-oxidation processes that take place in natural waters.

In river waters, the pH varies from 6.5 to 8.5, in underground waters – from 5.5 to 7.5, in atmospheric precipitation – from 4.5 to 6.5, in oceans – from at 7.9 to 8.3, in mines and springs – from 1.0 to 4.5.

The pH value influences the development and vital activity of aquatic organisms, stability and migration of chemical elements, action of water on concrete and metals, which is of particular importance for various hydroconstructions.

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3.2. MAIN IONS, MINERALIZATION, HARDNESS

The main ions are among the most stable components of water and their concentrations are in a fairly clear dependence on physical and geographical factors and, in particular, on the composition of mountain rocks, soils in the hydrographic basin of rivers, lakes or other aquatic ecosystems. In the waters flowing in the north-south direction the values of the sum of ions increase along the river course; the highest values are characteristic for the period with the lowest water level and speed, and the lowest values – opposite, for those with high water speed and volume (high spring waters and summer-autumn floods).

The chemical compounds represented by the anions of hydrogen carbonates and carbonates, sulphates, chlorides and cations of calcium, magnesium, sodium and potassium are, according to several classifications of the quality of natural waters, called the basic elements, or the structural elements of the chemical composition and quality of water. Namely the sum of these anions and cations determines the mineralization or salinity of waters, and several classifications of natural waters are based on the correlation between these ions.

It is well known that the waters in which hydrogen carbonates and calcium predominate are the sweetest and refer to the fresh waters of hydrogen carbonate class, calcium group ($C^{\underline{Ca}}$). Those waters which contain large amounts of sulphates and magnesium are more or less bitter, slightly salty or salty and refer to the waters of sulphate class, magnesium group ($S^{\underline{Mg}}$). Waters in which chlorides and sodium predominate have a salty taste and refer to the chloride class, sodium group ($Cl^{\underline{Na}}$), but, if chlorides and magnesium predominate, the waters have a very bitter taste, being, in most cases, salt or very salt ($Cl^{\underline{Mg}}$).

If the river or lake fresh waters switch from the hydrogen carbonate class, calcium group to hydrogen sulphate, or sulphate, or chloride class, the water quality suffer a radical transformation.

Unfortunately, the regulations on the quality of natural waters and monitoring of surface waters, which were approved in 2013, not include the aspect of the correlation between the main ions in the assessment of water properties and quality. For example, the total mineralization can be quite small – less than 300 mg/L, but it is dominated by hydrogen carbonate and sodium ions. In this case, the water can harm human health. The correlation between the main ions is very important in the case of determining the type of water usage – irrigation, supply of drinking water.

Hydrogen carbonates, carbonates and alkalinity

Alkalinity of natural waters is the sum of anions of weak acids (carbonates, hydrogen carbonates, silicates, borates, sulphites, hydrogen sulphites, sulphides, hydrogen sulphides, anions of humic acids, phosphates) and is determined by the amount of strong acids needed to neutralize 1 dm^3 of water.

Over 80% of freshwater refer to hydrogen carbonate class, due to the predominance of HCO_3^- ions among the major anions; namely the hydrogen carbonates are those ions which determine the alkalinity. The sources of CO_3^{2-} and HCO_3^- in surface waters are carbonate rocks (limestones, marls, dolomites, etc.). In addition, the wastewaters of chemical and food enterprises, those of the production of soda (sodium hydrogen carbonate) are one of the sources of penetration of carbonate and hydrogen carbonate ions in surface waters. However, the primary factors that determine the dynamics of these ions in surface waters are the composition and characteristics of mountain rocks in the hydrographic network and the hydrological peculiarities of rivers.

In river waters the content of hydrogen carbonate and carbonate ions varies from 30 to 400 mg/L, being directly correlated with the total mineralization in slightly mineralized waters, as they are dominant in these waters.

Along with the pH values, those of carbonate alkalinity of the water serve as ground for calculating the balance between carbonates and calcium in surface waters, assessing water quality and, correspondingly, the possibilities for its use in irrigation and construction (e.g., calculation of the aggressiveness of water in relation to mortar).

Unfortunately, these parameters are not included in the national regulations on monitoring of surface water and groundwater and requirements for their quality. The ratio between the main ions is a major indicator in determining the quality of drinking water, irrigation water and the aggressiveness of water in relation to concrete, metals, etc.

Determination of alkalinity

Alkalinity is the sum of concentrations of the anions of weak acids, primarily of carbonic acid, which occur in the water. The method of determining alkalinity ($HCO_3^- + CO_3^{2-}$) by reverse titration is based on the reciprocal action of hydrogen carbonate and carbonate ions with a strong acid and the formation of H_2CO_3, which decomposes into CO_2 and H_2O.

100 mL of the sample are placed in an Erlenmeyer flask of 250 mL. There are added 10 drops of indicators and such quantity of 0.05N HCl so that the sample becomes pink. Then another 1-2 mL of HCl are added and carbon dioxide is eliminated by aerating (purging) the sample with air without CO_2 during 5-7 min.

The sample is titrated with the solution of 0.05N of borax until the appearance of the stable green colour. Calculation:

$$C(HCO_3^- + CO_3^{2-}) = \frac{(N_1 \times a_1 - N_2 \times a_2) \times 1000}{V}, \text{ mmol/L}$$

$$C_x(HCO_3^- + CO_3^{2-}) = C(HCO_3^- + CO_3^{2-}) \times 61.02, \text{ mg/L},$$

where: $C(HCO_3^- + CO_3^{2-})$ – concentration of hydrogen carbonate and carbonate ions, mmol/L;
$C_x(HCO_3^- + CO_3^{2-})$ – concentration of hydrogen carbonate and carbonate ions, mg/L;
N_1 – concentration of HCl, mol/L;
a_1 – volume of HCl, mL;
N_2 – concentration of borax ($Na_2B_4O_7$), mol/L;
a_2 – volume of borax used to reach the inflection point, mL;
V – sample volume, mL;
61.02 – molar weight of bicarbonate (HCO_3^-) ion.

Reagents:

- *0.05 N $Na_2B_4O_7$ solution;*
- *0.05 N HCl solution;*
- *Indicator solution (0.5 g of methyl orange in 100 mL of ethyl alcohol+ 4 mL of 1% water solution of methyl blue).*

Sulphates

Sulphates are present, in fact, in all surface waters and are part of the category of the most important anions. The main source of sulphates in surface waters is the chemical disintegration and dissolution of sulphur-containing minerals, especially gypsum, and the oxidation of sulphides and sulphur. Sulphates are found in large quantities in industrial discharges of enterprises using sulphuric acid, for example, of hydrolysis factories. Also, sulphates penetrate into surface waters through household wastewater and those of enterprises of agricultural production.

In the absence of oxygen, under the action of sulphate-reducing bacteria, sulphates are reduced to hydrogen sulphide and sulphides, which are again oxidized to sulphates with the appearance of oxygen in the natural waters. Plants and other autotrophic organisms use sulphates dissolved in water in the process of synthesizing protein substances. After the death of living cells, heterotrophic bacteria release the sulphur of proteins in the form of hydrogen sulphide, which easily oxidizes to sulphates in the presence of oxygen.

The concentration of sulphates in water of rivers and freshwater lakes varies between 5 and 100 mg/L. The concentration of sulphates in surface waters demonstrates visible seasonal oscillations and usually correlates with changes in total mineralization of water. Therefore, the most important factors, which determine the content of sulphates, are the variable ratio between surface and groundwater runoff, oxidation-reduction processes, the state of hydrobionts and human household activity.

Increased sulphate contents worsen the organoleptic properties of water and have a certain physiological action on the human body. As sulphates have diuretic properties, their maximum allowable concentration is strictly regulated through normative acts. The taste limit of magnesium sulphate is within 400-600 mg/dm^3, and of calcium sulphate – 250 mg/dm^3. The presence of sulphates in industrial and drinking water can be both useful and harmful.

Determination of sulphates

The gravimetric method is based on the determination by weighing of $BaSO_4$, which is formed by the reciprocal action of sulphates and salts of barium. Mode of determination:

100 mL of sample are placed in a heat-resistant beaker, 5 drops of methyl orange + 2 mL of HCl (1: 1) are added and the mixture is heated to boiling. Then, by stirring continuously, 5 mL of barium chloride are poured dropwise, and the mixture is left for 8-12 hours for complete deposition of the precipitate. The entire amount of precipitate is moved quantitatively on a paper filter (blue tape), which is previously moistened with ethyl alcohol or boiling distilled water, in order to ensure a higher density. Precipitate on the filter is washed with hot distilled water until the chlorides are removed from the filtrate. Filter is passed into a previously weighed crucible, which is placed in a muffle furnace and calcinated at 800°C until the precipitate turns white (about 1-2 hours). The crucible is cooled in the desiccator and weighed. The calcination is repeated until the weight of the precipitate becomes constant. Calculation:

$$C_x(SO_4^{2-}) = \frac{p \times 1000 \times 0.4115}{V}, \text{ mg/L},$$

where: $C_x(SO_4^{2-})$ – sulphate concentration, mg/L;
p – weight of barium sulphate precipitate, g;
V – sample volume, mL;
0.4115 – gravimetric factor.

For recalculation of sulphate concentration in mmol/L, it is necessary to multiply the obtained result by 0.0104.

Reagents:

- *5% barium chloride solution;*
- *0.5% methyl-orange water solution;*
- *95% ethyl alcohol;*
- *HCl 1:1.*

Chlorides

The primary sources of chlorides in natural waters are magmatic mountain rocks with chlorine-containing minerals, and the saliferous rock layers. Chlorides are the anions with the highest migration capacity, which is explained by their high solubility, low sorption by suspended substances and low degree of use by aquatic organisms. In addition, unlike SO_4^{2-} and CO_3^{2-}, chlorides not tend to form associated ion pairs. The concentration of chlorides in surface waters reveals obvious seasonal oscillations, which correlate with the change in total water mineralization.

In the waters of rivers and those of freshwater lakes, the chloride content varies from tenths of a milligram to tens, hundreds and even thousands of milligrams per liter. Significant amounts of chlorides enter the water because of the discharge of industrial and household wastewater into the hydrographic network.

The high chloride content diminishes the taste qualities of water, makes it less useful as drinking water and limits its use in many technical and household areas, including irrigation of agricultural land. Chlorine concentrations, their oscillations, including diurnal ones, can serve as a criterion for pollution of water bodies with household wastewater discharges.

Chloride ions represent the dominant anions in highly mineralized waters. In the presence of sodium ions in water, the concentration of chlorides above 250 mg/dm^3 gives the water a salty taste.

Determination of chlorides

The method is based on the poor solubility of silver chloride, which is precipitated when silver nitrate is added to water containing chloride ions.

25, 50 or 100 mL of the sample are placed in an Erlenmayer flask, if necessary, adding distilled water to bring the sample to 100 mL, 1 mL of potassium chromate solution is added and the titration with silver nitrate solution is done, by stirring constantly, till the yellow-brown colour is reached. Calculation:

$$C(Cl^-) = \frac{aN \times 1000}{V}, \text{ mmol/L}$$

$$C_x(Cl^-) = C(Cl^-) \times 35.45, \text{ mg/L},$$

where: $C(Cl^-)$ – concentration of chlorides, mmol/L;
$C_x(Cl^-)$ – concentration of chlorides, mg/L;
a – volume of silver nitrate solution, which was used at titration, mL;
N – concentration of silver nitrate ($AgNO_3$), mol/L;
V – sample volume, mL;
35.45 –molar weight of chloride ion.

If necessary, determination of chloride concentration is made in distilled water (control).

Reagents:

- *0.05 N silver nitrate solution (4.4937 g of salt are dissolved in a small volume of distilled water; the volume of solution is brought to 1 L with distilled water);*
- *potassium chromate solution (10 g of K_2CrO_4 are dissolved in a small volume of distilled water; the volume of solution is brought to 1 L with distilled water).*

Calcium

Limestones, dolomites, gypsum, silicates containing calcium and other metamorphic and sedimentary rocks are natural sources of calcium in surface waters. The content of calcium cations rarely exceeds 1 g/dm^3 in river waters, oscillating, in most cases, between 20 and 60 mg/L. There are well-observed seasonal oscillations of the concentration of calcium in surface waters. Calcium ions are decisive indicators in assessing water quality as a source of life and ion balance, the aggressiveness of water to concrete and metal. Wastewater from the silicate, metallurgical, chemical, and glass manufacturing and effluents from agricultural lands contains large amounts of calcium.

Determination of calcium

The method is based on the formation of a complex, with a low degree of dissociation, of calcium ions with the trilon B in an alkaline medium.

25 or 50 mL of sample are placed in an Erlenmayer flask. Distilled water is added up to the 100 mL level. 2 mL of 2N NaOH + the combined indicator are added. The titration is made with trilon B solution until the colour changes from dirty green to blue. Calculation:

$$C(Ca^{2+}) = \frac{aN \times 1000}{2 \times V}, \text{ mmol/L}$$

$$C_x(Ca^{2+}) = C(Ca^{2+}) \times 40.08, \text{ mg/L},$$

where: $C(Ca^{2+})$ – concentration of calcium, mmol/L:
$C_x(Ca^{2+})$ – concentration of calcium, mg/L;
a – volume of trilon B used to reach the inflection, mL;
N – concentration of trilon B, mol/L;
V – sample volume, mL;
40.08 – molar weight of calcium ion.

Reagents:

- *0.005 or 0.02 N trilon B – see Hardness;*
- *combined indicator (0.2 g of murexid + 0.5 g of naphthol green + 100 g sodium chloride (everything is ground into a mortar and stored in a dark flask));*
- *2N NaOH (80 g of NaOH are dissolved in 1 L of distilled water).*

Magnesium

Magnesium, like calcium, penetrates surface waters mainly as result of the chemical disintegration and dissolution of dolomites, marl and other minerals. The magnesium content in surface waters varies visibly, more often ranging 1-30 mg/L; as a rule, the highest concentrations are observed during the low water season, and the lowest – during the floods.

Considerable amounts of magnesium can enter water bodies together with the wastewater of metallurgical, textile, silicate and other enterprises.

Hardness

Hardness is a property of natural water that depends, especially, on the presence of dissolved salts of calcium and magnesium. Under natural conditions, calcium and magnesium ions and those of other alkaline earth metals, which determine the hardness, enter the water as result of the interaction of dissolved CO_2 with carbonate minerals, of the other processes of dissolution and chemical disintegration of mountain rocks. Sources of these ions include microbiological processes, which take place in the soils of the water catchment area, in bottom sediments and also in the wastewater of various enterprises.

Water hardness varies widely. The hardness of surface waters oscillates obviously in dependence of the season, the highest values being reached at the end of winter, and the lowest – during the spring floods.

According to classical methods, the hardness is calculated in mg-eq/dm^3, or mmol/ dm^3, and in German, French, English, or American degrees. The hardness of 1 mg-eq/dm^3 is equal to 2.804 German degrees, 3.511 English degrees, 5.005 French degrees, or 50.045 American degrees. According to the classification of Aleokin O.A., water with hardness below 4 mg-eq/dm^3 is considered soft, from 4 to 8 mg-eq/dm^3 – middle hard, from 8 to 12 mg-eq/dm^3 – hard, and over 12 mg-eq/dm^3 – very hard.

The total hardness ranges from units to tens and, sometimes, to hundreds of mg-eq/dm^3. At the same time, the temporary hardness (that caused by carbonates) makes up to 70-80% of the total hardness, but in some cases the hardness caused of magnesium ions can reach 50-60%.

The increased hardness worsens the organoleptic properties of water, giving it a bitter taste, and affects the digestive organs. For preservation of the human health, the total hardness of drinking water shall range 2-4 mg-eq/L, the maximum allowable concentration (MAC) being of 10.0 mg-eq/L.

Determination of water hardness

Hardness is conditioned by the presence in the water, first of all, of calcium and magnesium ions. The method for determining the hardness is based on titration of the sample with the solution of tetrasodium salt of ethylenediaminetetraacetic acid (trilon B) in an alkaline medium with chromogen black indicator.

25 or 50 mL of sample are placed in an Erlenmayer flask. Distilled water is added up to the 100 mL level. 5 mL of buffer solution and 5-7 drops of indicator (or 10-15 mg of dry mixture) are added. The titration is made until red colour changes to blue colour. Calculation:

$$C_x = \frac{aN \times 1000}{2 \times V}, \text{ mmol/L}$$

where: C_x – water hardness, mmol/L;
N – titrant (trilon B) concentration, mol/L;
a – volume of trilon B used to reach the inflection, mL;
V – sample volume, mL.

Reagents:

- *0.05 or 0.02 N trilon B solution (3.75 g of trilon B powder are dissolved in 1 L of distilled water, but preferable to use standard volumetric solutions);*
- *$NaCl+NH_4OH$ buffer solution (20 g of ammonium chloride are dissolved in 100 mL of concentrated ammonia and the solution is brought to 1 L by adding distilled water);*
- *indicator (solution: 0.5 g of chromogen black indicator are dissolved in 10 mL of buffer solution and brought up to 100 mL with ethyl alcohol; dry – 0.5 g of indicator are ground together with 50 g of sodium chloride).*

Method of calculation of magnesium concentration

Calculation is made according to the formula:

$$C(Mg^{2+}) = a - b, \text{ mmol/L},$$

$$C_x(Mg^{2+}) = C(Mg^{2+}) \times 24.34, \text{ mg/L},$$

where: $C(Mg^{2+})$ – concentration of magnesium, mmol/L,
$C_x(Mg^{2+})$ – concentration of magnesium, mg/L,
a – water hardness, mmol/L;
b – concentration of calcium ions, mmol/L;
24.31 – molar weight of magnesium ion.

Sodium and potassium

Sodium and potassium migrate in surface waters mainly in dissolved form. The concentration of sodium in river waters ranges between 0.6 and 300 mg/dm^3, that of potassium varies around 19 mg/L, depending on the physical and geographical conditions and geological features of the river hydrographical basin. The main sources of sodium and potassium penetration into surface waters are volcanic and sedimentation rocks, soluble native sodium salts (chlorides, sulphates, carbonates). In addition, sodium, potassium and their compounds reach the natural waters by household and industrial wastewater, the surface runoff both from the agricultural lands and urbanized territories.

Determination of sodium and potassium ions

Among the classical methods of determining these ions are those of flame atomic absorption or optical emission, newer – with inductively coupled plasma (ICP). In addition, the calculation method is used, based on the fact that the sum of the main anions is equal to the sum of cations. Thus, the sum of calcium and magnesium is subtracted from the sum of sulphates, hydrogen carbonates and chlorides (in equivalents).

Mineralization

The total content of all mineral substances, which are detected at the chemical analysis of water, is called total mineralization, or salinity, or sum of ions and is usually expressed in mg/dm^3.

Many production enterprises, farms, drinking water supply companies have certain requirements for water quality, in particular for its mineralization, because water containing a large amount of salts negatively affects plant and animal organisms, production technology and, finally, the quality of production. Highly mineralized water causes the formation of stone on the walls of boilers, the appearance of corrosion, salinization of soils. For drinking water and that used for irrigation of agricultural lands, a mineralization of 1000 mg/L is set as limit. For human health, the favourable mineralization is of 200-400 mg/L.

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3.3. NUTRIENTS

This group of chemical substances includes nitrogen compounds (ammonium, nitrite and nitrate ions), phosphorus, silicon, as well as the organic compounds of nitrogen and phosphorus.

Ammonium ions

Ammonium nitrogen is found in the nature in the form of ammonium ions and, partially, in the form of not dissociated molecules of NH_4OH; the ratio of these forms is an important indicator of water quality and depends of the water pH and temperature. In aquatic ecosystems the presence of ammonium ions is related to the metabolism of hydrobionts. The increased concentration of ammonium ions contributes to the deterioration of the sanitary condition of the water body, intensification of pollution of surface and underground waters, first of all, with household and agricultural discharges, those of food, chemical, coke industries. The transition from oligotrophic to meso- and eutrophic aquatic ecosystems increase both the absolute concentration of ammonium ions and their share in the total balance of mineral nitrogen.

Determination of ammonium ion content (N-NH_4^+) with Nessler reagent

The Nessler reagent method is the most widely used and widespread method of determining ammonium ions in surface waters. The limit of determination is 0.05-4 mgN/L. The principle of the method consists in the reaction of ammonium ions, in a basic medium, with potassium tetraiodomercurate ($K_2[HgI_4]$), with formation of an yellow-brown complex (oxymercur ammonium iodide). The colour intensity is proportional to the content of ammonium ions in the analysed sample.

50 mL of sample to be analysed are transferred into the volumetric flask (50 mL), 1 mL of Seignette salt is added and the flask is mixed well. Then, 1 mL of Nessler reagent is added. The obtained solution is mixed well and left for 7-10 minutes to develop the colour. After 10 min, the absorbance of solution (λ = 400) in cells of optical path length of 10 mm against sampled water as a reference is measured.

Reagents:

- *the Nessler reagent;*
- *sodium and potassium double tartrate solution (50%) (50g of $KNaC_4H_4O_6*4H_2O$ are dissolved in double distilled water, diluted up to 100 mL with double distilled water, and 0.2-0.5 mL of the Nessler reagent are added);*
- *basic ammonium chloride standard (0.2965 g of NH_4Cl are dissolved in double distilled water and the volume of solution is brought to 1 L; 1 mL of standard solution contains 0.1 mg NH_4^+);*
- *working solution of ammonium chloride (50 mL of standard solution of ammonium chloride are diluted with double-distilled water in a 1 L flask; 1 mL of working solution contains 0.005 mg NH_4^+).*

Nitrites

The presence of nitrites in natural waters is an indicator of fresh pollution. Nitrites form an intermediate stage in the process of bacterial oxidation of ammonium ions to nitrates (nitrification takes place only under aerobic conditions) and, opposite, in the reduction of nitrates to nitrogen and ammonia (denitrification occurs under conditions of oxygen deficiency). The increased content of nitrites in surface waters depends on the decomposition of organic matter under conditions of a slower oxidation of NO_2^- ions in NO_3^-, which is related to the pollution of aquatic ecosystem. The widespread use of nitrites as preservatives in the food industry is, also, a source of pollution of surface waters.

Determination of nitrites (NO_2^-) with Griess reagent

Nitrite ions form with Griess reagent (mixture of alpha-naphthylamine and sulphanylic acid) a diazonium compound, which colours the aqueous solution from pink to red (the intensity of colour increases with the increase of concentration). The method is used to determine the nitrite content in surface waters, if it ranges 0.007-0.35 mgN/L. The presence of nitrites in water denotes its contamination with decomposing organic matter. Nitrites indicate a certain time of water pollution, because the transformation of organic substances into nitrites takes time (days, weeks).

50 mL of water sample are poured into the volumetric flask (50 mL), 0.1 g of Griess reagent are added. The solution is mixed well and left for 40 minutes to develop the colour. After 40 minutes, the absorbance of solution (λ = 540 nm) in cells of optical path length of 10 mm against sampled water as a reference is measured.

Reagents:

- *dry Griess reagent;*
- *sulphanilic acid (0.5 g of sulphanilic acid [$(NH_2)-C_2H_4-SO_2H$] are dissolved in 150 mL of 12% acetic acid; the prepared solution can be stored for several months in a dark glass bottle with a polished stopper);*
- *12% acetic acid;*
- *$NaNO_2$ standard solution, 250 mgN/L (0.6157 g of pure salt for analysis are dissolved with distilled water in a 500 mL flask);*
- *$NaNO_2$ working solution, 5 mgN/L (5 mL of standard solution is diluted with distilled water in a 250 mL flask; the solution is used freshly prepared).*

Nitrates

The processes of nitrification and denitrification, the discharge of industrial and household wastewaters, runoff from agricultural lands and the metabolism of hydrobionts are the determining factors of the nitrate content in surface waters. The concentration of nitrates in surface waters reveals seasonal variations, which amplitude can serve as an indicator of the eutrophication of aquatic ecosystem. The concentration of NO_3^- ions in unpolluted surface waters not exceeds tens of micrograms per 1 dm^3. With the intensification of eutrophication, the concentration of nitrate nitrogen increases (up to $n \times 10^{-1}$ mg/dm^3) and, correspondingly, its share in the sum of mineral nitrogen.

Determination of nitrates (NO_3^-) with sulphosalicylic acid

Spectrophotometric method with salicylic acid is used, due to the formation of nitro derivatives between nitrates and salicylic acid in acid medium of yellow colour. The intensity of the colour is directly proportional to the content of nitrates.

10 mL of water sample are taken in a 50 mL beaker. 1 mL of sodium salicylate (freshly prepared) is added. The beaker is placed in the oven (or in the water bath) for evaporation and kept until geting dried. After cooling the beaker to room temperature, 1 mL of concentrated sulphuric acid (ρ=1.84 g/l) is added, by moistening well the walls. Beaker is left for 10-15 minutes. Meanwhile, by its slow rotating movements, the steaming down of the acid on the walls is reached, and as result, the total dissolution of crust occurs. After 15 minutes, the walls of beaker are washed well with distilled water and the content is transferred quantitatively into 50 mL volumetric flask. 7 mL of NaOH (10N) is added and the solution is brought to mark with distilled water. The flask is closed with the stopper and mixed well. After 10 minutes, the absorbance of solution (λ=400) in cells of optical path length of 10 mm against distilled water as a reference is measured.

In the case of water with high nitrate content, 1 mL of water sample will initially be taken.

Reagents:

- *standard solution (I) of sodium nitrate (0.7218 g of KNO_3 are dissolved in distilled water, 1 mL of chloroform is added and the volume of solution is brought with distilled water up to 1 L;*
- *working solution (II) of sodium nitrate (10 mL of standard solution (I) are poured in a 100 mL flask, the solution is diluted and brought to the mark with distilled water; 1mL of working solution contains 0.01 mg of nitrate nitrogen);*
- *sodium tartrate solution (30 g of salt are dissolved in 70 mL of distilled water);*
- *sodium salicylate solution (0.125 g of salt are taken in a 25 mL flask, diluted and the volume of solution is brought to the mark with distilled water);*
- *10N sodium hydroxide.*

All forms of mineral nitrogen, including gaseous ones, are in a permanent circuit and mutual transformations; therefore, the total concentration of mineral nitrogen is often used as indicator of the nitrogen saturation (or surplus) of phytoplankton development. This criterion is used, as a whole, to assess production-destruction processes and the level of trophicity in aquatic ecosystems.

Organic nitrogen

In hydrochemistry, the notion of "organic nitrogen" refers to the nitrogen in the composition of organic substances, such as proteins and proteids, polypeptides, amino acids, amines, amides, urea. The ratio between mineral nitrogen and organic nitrogen is an important indicator in assessing the water quality and pollution processes in aquatic ecosystems. The increase of the share of organic nitrogen in total nitrogen denotes pollution of the ecosystem with allochthonous organic substances.

Determination of organic nitrogen by the Kjeldahl method

The method of determining the content of organic nitrogen consists in the mineralization of the water sample with concentrated sulphuric acid, under certain temperature conditions and in the presence of the catalyst. Upon heating, the nitrogen is removed in the form of ammonia,

which in the presence of sulphuric acid changes to ammonium sulphate (mineralization of organic nitrogen). Then resulted ammonium sulphate decomposes in a strongly alkaline medium by forming ammonia and alkaline sulphate. Ammonia is separated by distillation and trapped in a known volume and excess of sulphuric acid (with a determined titre). The excess acid is titrated at the end of distillation with a solution of sodium hydroxide of known concentration. The difference between the initial amount of sulphuric acid and the excess determined by titration with sodium hydroxide is the amount of sulphuric acid, which fixed the ammonia as ammonium sulphate.

100 mL of water sample are poured into Kjeldahl digestion vessels, 2 mL of concentrated sulphuric acid and approximately 0.5-0.7 g of catalyst are added. The vessels are heated at a temperature of about 60 °C until approximately 30 mL of the total volume is left. Then the vessels are closed with condensers and the mineralization temperature is raised to 300-350°C. The end of the step is indicated by the green colouring of the solution in the vessel. After this, it is recommended to heat for another 40 minutes, in order to ensure the complete mineralization of compounds, which are more resistant to decomposition. The Kjeldahl vessels are cooled to room temperature. Each Kjeldahl vessel is then introduced into the distillation apparatus, to which 8 mL of NaOH (33%) are automatically added. The vapours, which pass through the distillation system, will be collected in the recipient, which contains 10 mL of H_2SO_4 (0.01N) and 8 drops of Groak indicator. The distillation process lasts 20-30 minutes. The content of recipient, in which the vapours have been trapped, will be titrated with sodium hydroxide solution (0.01N) until the pink colour turns green.

Reagents:

- *33% sodium hydroxide (preparation of 300 mL of solution: 1) 99 g of NaOH are dissolved in 200 mL of double-distilled water in a 1 L flask, 2) the lower meniscus of solution is noted (marked) on the walls of the flask, after that another 300 mL of double-distilled water are added, 3) the flask with solution is placed on the electrical grid and evaporates to the mark);*
- *0.1 N sulphuric acid solution;*
- *0.01N sulphuric acid solution;*
- *catalyst (10 g of $CuSO_4$ + 100 g of K_2SO_4 + 2 g of metallic Se; the mixture is homogenized in a mortar).*

Total nitrogen

Total nitrogen is defined as the sum of mineral and organic nitrogen in natural waters. Nitrogen containing compounds are found in surface water in dissolved, colloidal and suspended state and can pass from one state to another under the influence of physico-chemical and biochemical factors. The average concentration of total nitrogen in natural waters varies to a large extent and depends on the trophicity of aquatic ecosystem: in the oligotrophic ones it changes in the range of 0.3-0.7 mg/dm^3, mesotrophic – 0.7-1.3 mg/dm^3, and eutrophic ecosystems – 0.8-2.0 mg/dm^3. Thus, total nitrogen is an integral indicator of the trophicity of lakes and rivers.

Mineral phosphorus

Phosphorus is one of the most important indicators of trophic status in natural aquatic ecosystems. Phosphorus mineral compounds penetrate the natural waters as a result of the disintegration and dissolution of rocks containing orthophosphates and also with the runoff from the surface of the water catchment network in the form of ortho-, meta, pyro- and polyphosphate ions (from fertilizers, detergents, additives intended to prevent the formation of stone in boilers, etc.). Phosphorus mineral compounds are also formed in the process of biological decomposition of plant and animal remains. Excess of phosphates in water can be caused by the presence in the water body of a mixture of fertilizers, components of household wastewater, decomposing biomass. The main form of inorganic phosphorus at water pH values above 6,5 is HPO_4^{2-} ion (about 90%). In acid waters inorganic phosphorus is mainly present in the form of $H_2PO_4^-$.

Usually, the concentration of phosphates in natural waters is very low – n×10^{-2}, rarely n×10^{-1} milligram of phosphorus per 1 dm^3, but in polluted waters it can reach a few milligrams per 1 dm^3.

Content of phosphorus compounds changes in dependence of the season, because it depends on the correlation of the intensity of photosynthesis process and those of biochemical oxidation of organic substances. The minimum concentrations of phosphates in surface freshwaters are usually observed in spring and summer, the maximum ones – in autumn and winter; in sea waters – in spring and autumn and, respectively, in summer and winter.

Content of dissolved phosphates in water of 50 μg/L is recommended as a norm for the ecological well-being of aquatic ecosystems.

Determination of orthophosphates content with molybdate

This method is valid for the determination of orthophosphates and total phosphorus in drinking water, as well as in natural surface waters. Phosphate ion reacts with ammonium molybdate in an acid medium, with synthetisation of a blue coloured complex. Water sample is poured into a 50 mL flask, 1 mL of ammonium molybdate and 2-3 drops of tin chloride solution are added. Solution is left for 10 minutes to develop the colour. Absorbance of solution (λ = 670 nm) in cells of optical path length of 10 mm against sampled water as a reference is measured.

Reagents:

- *ammonium molybdate solution (250 g of $(NH_4)_6Mo_4O_{24}\times4\,H_2O$ are dissolved in approximately 600 ml of double distilled water, 337 mL of sulphuric acid (98%) are added; after cooling the solution is brought to 1 L with double distilled water);*
- *tin chloride (II) solution (0.1 g of Sn are taken in a 10 mL tube, 2 drops of $CuSO_4$ (5%) and 2 mL of concentrated HCl are added; the obtained mixture is placed on the water bath and heated until the tin dissolves completely; after cooling, double-distilled water is added up to the volume of 10 mL);*

Organic phosphorus

Natural phosphorus compounds (except synthetic organic phosphorus compounds) penetrate the natural waters as a result of hydrobiont metabolism, decomposition of perished organisms, exchange of substances with bottom sediments. Organic phosphorus compounds occur in surface waters in dissolved, colloidal form and suspensions.

Total phosphorus

The notion of “total phosphorus” defines the sum of mineral and organic phosphorus. As in the case of nitrogen, the exchange of phosphorus between its mineral and organic form on the one hand and between aquatic organisms, on the other hand, is the main factor that determines its concentration. The concentration of total phosphorus in dissolved state (mineral form plus organic) in unpolluted natural waters changes from 5 to 200 μg/dm^3.

Phosphorus is the biogenic element, which often limits the increase of the productivity of water bodies. Penetration of excess of phosphorus compounds from the water catchment area in the form of mineral fertilizers, with wastewater from animal farms, with insufficiently treated household and industrial water lead to the sudden, uncontrolled growth of plant biomass in aquatic ecosystems, especially, in those with a slow flow or stagnant waters. The trophic status of aquatic ecosystem changes, being accompanied by restructuring of the entire aquatic community, which leads to the prevalence of putrefaction processes. The latter, in turn, increase turbidity, salinity, the number of bacteria.

One of the possible aspects of the eutrophication process is the intense multiplication of blue algae, many of which increase the toxicity of water to other hydrobionts. Substances eliminated by these organisms belong to the group of organic compounds containing phosphorus and sulphur. In accordance with the requirements of the global environmental monitoring system, the determination of the total phosphorus content (dissolved and in suspensions, in the form of organic and mineral compounds) is also included in the natural water monitoring programs.

Silicon

Silicon compounds are permanently found in inland waters in ionic and colloidal form in concentrations from 1 to 20 mg/L, in marine waters – from 0.5 to 3.0 mg /L, in groundwater – from 20 to at 40 mg/L, and in some thermal waters – up to 2000-4000 mg/L. In the waters of the northern areas with low concentrations of the main ions, the concentration of silicon exceeds 50% of the mineral substances and becomes a component part of mineralization. In the periods of the most intense development of diatom algae, in spring and autumn, the concentration of silicon in the water decreases. Silicon participates in the formation of diatom frustules and the skeleton at siliceous and radiolar sponges. In thermal waters, silicon is one of the most important biogenic or nutrient sources for the development of microorganisms.

Iron

Iron is a very important oligoelement, being part of the haemoglobin of aquatic organisms. Iron is encountered in water in ferrous, ferric or colloidal form, mainly due to the presence of humic acids. The bivalent or trivalent state of iron depends on the pH of the water and the content of dissolved oxygen. In high quantities, iron becomes toxic for aquatic organisms. At concentrations of 1-2 mg/L, iron affects the taste and smell of water (likely of rust). In freshwaters, iron ions and their dissolved compounds are usually found in concentrations below 0.5 mg/L (in oxygenated waters), but in groundwaters and acid waters they often increase to 50 mg/L. In well-aerated waters, at concentrations above 0.1 mg/L, the dissolved forms pass into the colloidal ones. Then, they pass in suspended form, then precipitate, causing increased turbidity, rust, changing the taste and smell of the water. Iron concentrations above 0.2 mg/L make the water unsuitable for most industrial uses. For this reason, water deironing is frequently practiced.

3.4. MICROELEMENTS

The variety of migration forms and the large number of chemical elements, which are considered as microelements, have led to the fact that microelements, depending on their concentrations and the influence on one or another biological system, are also called trace elements, microcomponents, elements of life, biometals, toxicants, heavy metals, etc. The main sources of microelements in surface waters are mountain rocks, soils, atmospheric precipitation and, also, technogenic factors, which currently, according to the degree of influence on aquatic ecosystems, have become commensurable with natural ones. In surface waters, microelements have a very large role as biocatalysts, by preventing or stimulating life processes.

The migration capacity of microelements in surface waters and their forms of migration are conditioned by the properties of the elements themselves, the physico-chemical particularities of the environment (specifically, by the redox conditions, the value of pH, temperature, presence of complexing agents, of suspended substances), the vital activity of hydrobionts, etc.

Microelements have three main forms of migration in surface waters – real soluble form, in suspension and colloidal form. There are different methods for the separation of soluble forms of microelements from those in suspension, most of which are based on filtration, sedimentation, centrifugation. Free ions, complex compounds, ionic pairs, associations are the soluble forms of microelements. A whole complex of factors conditions the dynamics of the ratio between the soluble and suspended forms in the migration of microelements: the water pH and mineralization, quantity and composition of organic substances and suspended substances, hydrological regime of rivers and vital activity of hydrobionts.

Of particular importance are the sorption properties of suspended substances and bottom sediments. The sorption properties are determined, in turn, by the presence of clay particles, hydroxides of iron, manganese, aluminium, granulometric and mineralogical composition. The highest adsorption is characteristic for suspended substances and bottom sediments enriched with clay particles and organic substances. In connection with this fact, in the rivers of the southern latitudes most of the microelements migrate in the form of suspended substances, and in the northern ones – conversely, the dissolved forms predominate over the suspended ones.

In lakes, where the amount of suspended substances is considerably lower than in rivers, the role of suspended migration forms decreases sharply, but the importance of biological factor and bottom sediments increases. Suspensions are the main form of migration in the case of tin, bismuth, titanium, silver, aluminium, cobalt, lead. In most cases, the content of titanium, vanadium is higher in large fractions, and of manganese, nickel, copper, molybdenum, lead, zinc – in finely dispersed suspended particles.

The correlation between suspended and dissolved forms of migration of microelements in river waters is important in assessing their influence on living systems. It has a great significance in hydrogeochemical research, characterizes the denudation processes in the river hydrographic basins. The relative mobility of microelements, which is determined as the ratio of their suspended forms to their summary content (in solutions + in suspensions), indicates the state of microelements in water and characterizes the direction of exogenous processes on the water catchment area.

In order to study the migration of microelements in aquatic ecosystems, it is extremely important to establish the regularities of their migration in the system "water-suspensions-bottom sediments-hydrobionts". The role of bottom sediments is substantial in levelling the peak concentrations of microelements, and, under certain conditions, bottom sediments can become sources of secondary pollution of the water layer. The composition of silts is very helpful in establishing the history of the aquatic ecosystem, as they represent reliable indicators of the pollution of aquatic ecosystems.

The research of the accumulation of microelements in hydrobionts is accompanied, in turn, by difficulties, conditioned by the need to highlight many factors, such as: taxonomic and age differences of hydrobionts, their physiological state, physico-chemical parameters of the environment and the peculiarities of chemical elements. Without assessing these factors, the information about the accumulation of microelements in food chains is often fragmentary, and the researchers' conclusions – contradictory.

Biomonitoring of metals in aquatic ecosystems, in order to establish the tolerance limits and evaluate the resistance of aquatic plants and animals under the instability of the physico-chemical properties of aquatic environment, is a major problem for several reasons. First, these investigations contribute significantly to solving of a range of fundamental problems – establishing the evolution of species diversity of hydrofauna, deciphering the mechanisms of regulation of species density, processes of biological productivity, trophic structure of communities, circuit and flow of chemical elements in the food chains. The applied aspect consists in the protection of the gene pool of aquatic fauna and flora, elaboration of the recommendations regarding the restoration and sustainable capitalization of aquatic resources.

The biological monitoring of microelements is founded on the principle of permanent control, assessment and forecast of the status of aquatic ecosystems, based on research and identification of the regularities of their migration in the system "water – hydrobionts", as function of a range of factors (i), revelation of the regularities and level of accumulation of microelements in aquatic plants and animals and determination of their functional role in the biogenic migration of chemical elements (ii), assessment of the buffer capacity of aquatic ecosystems (iii), and the identification of organisms for biomonitoring and bioindication of the state of aquatic system (iv).

According to one of the main conceptions of biogeochemistry, organisms and biocenoses not only adapt to the chemical factors of the environment, but in turn change the composition of the environment in accordance with the needs of living beings in the process of development and reproduction. In this regard, one of the criteria for establishing the allowable level of metal contents in biological objects is the determination of dependencies between their concentration and the intensity of hydrobiont production in the ecosystem. If the multifactorial influence of natural conditions within the hydrographic basins and the physiological-chemical characteristics of hydrobionts are not taken into account, a non-objective image of the situation can be obtained, because the microelements simultaneously represent vital elements and toxic elements. Establishment of flows and revelation of the regularities of metal accumulation in aquatic plants and animals, along with those of production-destruction processes, grounded the concept of assessment of the buffer capacity of aquatic ecosystems from Moldova, in dependence

on the dynamics of the content of 14 metals. Optimal or favourable concentrations for ecosystem functioning (no influence on production-destruction processes), allowable concentrations (gradually decrease the primary production) and extreme or critical concentrations for aquatic ecosystems (primary production decreases sharply and reaches zero) have been established. Thus, aquatic ecosystems can be attributed to one of categories: unpolluted, polluted and heavily polluted or dirty.

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IV BACTERIOPLANKTON

The basic rules of work, regardless of the field of application of microbiological methods, are as follows:

- collection and transport of samples in conditions similar to natural (container with refrigerant);
- exclusion of the possibility of accidental contamination during sample processing (sample processing should be carried out in a special room – niche or box);
- additional protection of personnel, if there is a risk of contamination with pathogens (e.g. wastewater).

There are several categories of essential equipment, instruments and glassware in the laboratory of microbiology, such as:

- laboratory glassware: conical Erlenmeyer flasks of various capacities, Berzelius beakers, Foureau-type cylindrical-conical flasks, volumetric flasks, flasks with ground stopper, Bunsen flask for filtering solutions, tweezers, Petri dishes, pipettes of different volumes;
- water bath or temperature-controlled electric bath;
- thermostats;
- water distillation apparatus: for obtaining of distilled and double distiiled water;
- balances: pharmaceutical balance with a weighing limit of 5 g, technical balance, analytical balance.

The accuracy of laboratory analyses requires a thorough cleaning of the glassware used. Washing is mainly necessary in order to remove grease from the walls, due to which they are not wetted evenly. Washing methods: a) regular washing with warm water and liquid detergent; b) washing with hot trisodium phosphate solution; c) washing with organic solvents (alcohol, ethyl ether, acetone, dichloroethane). Sterilization of glassware is performed by several processes, the most common being the wet or dry heating.

The most common method of seeding microorganisms is by incorporation: 1 mL of water sample (or its decimal dilutions) is sterilely transferred, using a graduated pipette, into sterile Petri dishes. A culture medium, with a temperature of 45°C, is poured over. The content of the Petri dishes is gently mixed with a rotating motion, left to solidify and then incubated in a thermostat at the required temperature. The same method of growing on solid media can be used for determination of the physiological groups of microorganisms in a water sample by using decimal dilutions [1].

The following parameters are used to study the functional activity of bacterioplankton in hydrobiological practice: total number of bacteria (Ntot), biomass (B), bacterial production (P) and destruction (R).

To assess the ecological state of water bodies, the number of saprophytes (Nsapr) is determined, as well as the number of microorganisms from other functional groups of bacterioplankton, namely: ammonifiers, denitrifiers, organic-phosphorus-mineralizing bacteria, amylolytic and cellulolytic bacteria, hydrocarbon-degrading bacteria, phenol-degrading bacteria, etc. It should be noted that neither bacterioplankton nor zooplankton are included in the group of biological elements of the WFD classification system of water bodies. However, some countries, including the Republic of Moldova, at the level of their national regulations take into account the quantitative parameters of bacterioplankton for assessing the quality of surface waters (Table 4.1).

Table 4.1. Bacterioplankton parameters used in the assessment of the quality of surface waters in the Republic of Moldova [2]. Note: WQC – water quality class

Hydrobiological parameter	Unit	WQC I	WQC II	WQC III	WQC IV	WQC V
Total number of bacteria	million cells/mL	1.0	2.0	5.0	7.5	>7.5
Number of saprophytes, 22°C	thousand cells/mL	0.5	2.5	5.0	7.5	10

4.1. METHOD FOR DETERMINING THE TOTAL NUMBER OF BACTERIA

The total number of bacteria is determined by the method proposed in 1932 by Razumov I.A. This method consists in filtering a certain volume of the water sample through a membrane filter with a pore size of 0.23-0.40 μm, installed in the Zeits apparatus fixed in the Bunsen flask [3]. The filter is marked before placing.

Before use, membrane filters are boiled in distilled water, which is changed 2-3 times (or in fresh water filtered through a membrane filter with pore size of 1.5-2.5 μm, in order to remove zoo- and phytoplankton).

The amount of water, which shall be filtered, depends on the type of water body, the expected bacteria content in the water and the diameter of the filter used. After water filtration, the membrane filter is removed from the apparatus and placed on the filter paper soaked in formalin for fixation of microorganisms. After drying, the filters are stained in Petri dishes with a (3-5) % solution of erythrosine for 4-24 hours, and then washed, in order to remove the colorant, by transferring them on wet filter papers until a slightly-pink print is obtained. For counting of microorganisms, a part of the filter (1/2-1/4) is placed on a glass slide and microscoped under immersion. It is recommended to examine 20 fields of view.

The determination of the total number of bacteria (Ntot) is performed according to the following formula:

$$X = \frac{S \cdot N}{s \cdot V},$$

where: X – total number of bacteria, million cell/mL; S – filtering area of the membrane ffilter, m^2; s – total area of calculated fields of view, m^2; N – average number of bacterial cells in the examined field of view; V – volume of filtered water, mL.

4.2. METHOD FOR DETERMINING BACTERIAL PRODUCTION AND DESTRUCTION

Bacterial production (P) is calculated according to the method proposed in 1955 by Ivanov M.V., which was subsequently used by many scientists [3-7]. The water sample is preliminary filtered through a filter with a pore size of 1.5-2.5 μm, to remove zoo- and phytoplankton. The filtrate is poured into flasks, which are closed with ground stoppers and placed under water level (*in situ* – in the water body where sample was taken; in laboratory – in a container with water under conditions of temperature and lighting closest to natural at the time of sampling) for 12-24 hours. The total number of bacteria in the sample is determined at the beginning (N_0) and at the end (Nt) of incubation. In parallel, in the natural sample, in which zooplankton and phytoplankton are present, the total number of bacteria is determined, in order to take into account the nutrition of zooplankton with bacteria. Specific productivity (Cw) and bacterial biomass production (P) are calculated using the formulas proposed by Zaica I.E. [8]:

$$C_w = \frac{lnN_t/N_0}{t}, \qquad P = C_w \cdot B,$$

where: N_0 and Nt – total number of bacteria before and after incubation of the filtered water sample; t – incubation time; C_w – specific productivity; B – biomass of bacteria in water.

To determine bacterial destruction, a water sample is filtered through a membrane filter with a pore size of 1.3 μm, incubated in a water body for 24 hours in dark oxygen flasks with ground stoppers. An unfiltered sample is incubated in parallel. Previously, the concentration of dissolved oxygen is determined in the sample by the Winkler method, and the total number of bacteria by the Razumov method. After 24 hours, the total number of bacteria and dissolved oxygen are determined in both flasks. The difference between the oxygen content in a filtered and unfiltered sample shows the amount of oxygen consumed by microflora (R).

It is known that part of the food used by bacteria is spent within catabolic metabolism (*R*), part – within anabolic metabolism (*P*), the other part of the food remains unused (*K*). Thus, the consumption (ration) of bacteria (*C*) is formed from the sum of these three quantities:

$$C = P + R + K.$$

In applied microbiology, a coefficient that reflects the efficiency of assimilation of food by bacteria for the reproduction of biomass is widely used as the coefficient of growth efficiency (K_2), calculated by the following formula:

$$K_2 = \frac{P}{P + R}.$$

Knowing K_2 and one of the parameters of the physiological activity of bacteria (*P* or *R*), it is possible to calculate the necessary parameter, for example *P*, according to the formula:

$$P = \frac{K_2 \cdot R}{1 - K_2}.$$

This method is a theoretical one, being applied only in preliminar calculations, in order to estimate approximately *P* and *R*.

4.3. METHODS FOR DETERMINING PHYSIOLOGICAL GROUPS OF BACTERIOPLANKTON

Determination of only the total number of bacteria (Ntot) is not sufficient, if the knowledge of the mineralization processes of organic matter is targeted. The use of generally accepted traditional methods [3, 6] makes possible to assess the processes of transformation and regeneration of biogens (N, P, C, S, etc.) by determining the number of representatives of various physiological groups of microorganisms involved in these processes.

Methods for determining saprophytic microorganisms

The group of saprophytic microorganisms includes mesophilic aerobes and facultative anaerobes, capable of forming colonies on nutrient agar of the corresponding composition, visible when magnified by two times. When assessing biological pollution of surface water bodies, as well as establishing a water quality class, the following is determined:

- number of saprophytic microorganisms growing at 20-22°C within 72 hours; this indicator group of microorganisms is mainly allochthonous microflora introduced into the water body as result of anthropogenic pollution, including fecal pollution;
- number of saprophytic microorganisms growing at 37°C within 24 hours; this is an indicator group of microorganisms, which, in addition to the allochthonous one, determine the autochthonous microflora of a given water body.

The ratio of the number of these groups of microorganisms allows concluding about the dynamics and intensity of the self-purification process. Saprophytes growing at a temperature of 20°C are active participants in the self-purification process of water bodies. More saprophytic microorganisms grow at 20°C than at 37°C. This difference in numbers is more evident at the end of the self-purification process. In areas where contamination with household wastewater occur, the numerical values of both groups are close [9].

Nutrient medium for the cultivation of saprophytic microorganisms:

Nutritious meat peptone agar	Peptone, g	10.0
	Meat extract, g	10.0
	NaCl, g	5.0
	Agar, g	20.0
	Distilled water, mL	1000

From each sample, 1 mL is inoculated and 1 mL from one or two dilutions, choosing the volume of water for inoculation so that from 20 to 300 colonies grow on the Petri dishes. The selected volume is inoculated in two replicates. Two Petri dishes with inoculations of one replication are placed in a thermostat and incubated at a temperature of (37 ± 1)°C for (24 ± 2) hours. Two other Petri dishes with inoculations are incubated at a temperature of 20-22°C for (72 ± 2) hours.

After incubation, all colonies visible at 2-x magnification are counted. Counting should be done only on plates, which contain from 20 to 300 isolated colonies. When inoculated with 1 mL of undiluted water, counts on plates with any number of colonies less than 300 and at least two plates. The result is expressed as the number of CFU (colony forming units) per 1 mL (cm^3) of water sample.

Methods for determining the microorganisms involved in the nitrogen cycle

The nitrogen cycle includes four phases, namely: fixation of molecular nitrogen, ammonification, nitrification, and denitrification. With the exception of the stage of proteolysis, which precedes ammonification, in which micro-fungi also participate, bacteria realize all phases of the nitrogen cycle.

a) Nitrogen-fixing bacteria

Nitrogen-fixing bacteria can be cultured both on liquid media using the dilution method (in this case, the result is calculated according to the Mac Crady Table), and on solid nutrient media (in this case, the result is expressed in CFU per 1 mL of water sample). The optimal medium for the detection of free-living nitrogen-fixing bacteria of the genus *Azotobacter* is Fedorov's medium [3].

Nutrient medium for the cultivation of bacteria of the genus *Azotobacter*:

Fedorov's liquid medium	Mannitol, g	20.0
	K_2HPO_4, g	0.3
	$CaHPO_4$, g	0.2
	$MgSO_4 \cdot 7H_2O$, g	0.3
	K_2SO_4, g	0.2
	NaCl, g	0.5
	$FeCl_3 \cdot 6H_2O$, g	0.1
	$CaCO_3$, g	5.0
	Microelement solution, mL	1.0
	Distilled water, mL	1000
Microelement solution	H_3BO_3, g	5.0
	$(NH_4)_2MoO_4$, g	5.0
	KI, g	0.5
	NaBr, g	0.5
	$ZnSO_4 \cdot 7H_2O$, g	0.2
	$Al_2(SO_4)_3$, g	0.3
	Distilled water, mL	1000

The microelement solution is prepared separately, sterilized with flowing steam under pressure in an autoclave and used as needed, taking the necessary volume with a sterile pipette.

To determine the number of nitrogen-fixing anaerobic bacteria *Clostridium pasteurianum*, a water sample, or its decimal dilutions, is inoculated onto a solid or liquid nutrient medium. The classical medium for cultivation of *Clostridium pasteurianum* is Vinogradskiy's medium:

Vinogradskiy's medium for cultivation of *Clostridium pasteurianum*	Glucose, g	20.0
	K_2HPO_4, g	1.0
	$MgSO_4 \cdot 7H_2O$, g	0.5
	NaCl, g	0.01
	$MnSO_4$, g	0.001
	$FeSO_4 \cdot 7H_2O$, g	0.001
	$CaCO_3$, g	40.0
	Distilled water, mL	1000

Nutrient medium for the cultivation of ammonifiers:

Liquid medium for cultivation of ammonifiers	L-Asparagine, g	0.2
	KH_2PO_4, g	0.125
	K_2HPO_4, g	0.125
	$MgSO_4 \cdot 7H_2O$, g	0.125
	NaCl, g	0.125
	$FeSO_4$, g	0.001
	Distilled water, mL	1000
	pH – adjusted to 7.0-7.2	

The culture medium should be colourless. Sterilization of the medium is carried out in an autoclave with flowing steam under pressure of 1 atm within 20 min. For cultivation of bacteria, 1 mL of diluted sample is added to the medium. The diluted sample is incubated in 3, 4 or 5 replications. Incubation is made at 25-30°C for 6 days. At the end of incubation, a drop of Nessler's reagent is added. An orange colour indicates the presence of ammonia in the test tube. The number of ammonifying bacteria is calculated using the Mac Cready Table. This medium can be used in solid form by adding 20 g of agar. The diluted sample is poured into Petri dishes in 2-3 replications, incubated at 25°C for 5 days. The grown colonies are counted and expressed by the number of CFU/mL.

b) Determination of nitrate reduction processes. Denitrification

Denitrifying bacteria are a heterogeneous group of over 125 different species identified in over 50 genera, which represent 10-15 % of bacteria population in water, soil and bottom sediments. This group of bacteria, together with denitrifying fungi and archaea, is capable of performing denitrification as part of the nitrogen cycle.

Denitrifying bacteria are determined on a mineral medium with the addition of sodium citrate, using the incorporation method. Sterilization of the medium is carried out in an autoclave with flowing steam under pressure of 1 atm within 20 minutes.

Siltay's medium for cultivation of denitrifiers	Sodium citrate, g	2.0
	KNO_3, g	1.0
	K_2HPO_4, g	1.0
	KH_2PO_4, g	1.0
	$MgSO_4$, g	1.0
	$CaCl_2$, g	0.2
	Bromothymol blue indicator, mL	0.01
	Distilled water, mL	1000
	pH – adjusted to 7.0-7.2	

c) Determination of oxidation processes of nitrogen compounds. Nitrification

Nitrifying bacteria are chemolithotrophic microorganisms, which obtain their energy by the oxidation of inorganic nitrogen compounds. Nitrification in nature is a two-step oxidation

process of ammonium (NH_4^+) or ammonia (NH_3) to nitrate (NO_3^-) carried out by two ubiquitous bacterial groups. The first reaction is oxidation of ammonium to nitrite by ammonia oxidizing bacteria (AOB) represented by the genus *Nitrosomonas*. The second reaction is oxidation of nitrite (NO_2^-) to nitrate by nitrite-oxidizing bacteria (NOB), represented by the genus *Nitrobacter*. For the cultivation of nitrifying bacteria, two types of Vinogradsky's medium are used [3].

Vinogradskiy's medium for the first phase of nitrification	$(NH_4)_2SO_4$, g	2.0
	K_2HPO_4, g	1.0
	$MgSO_4 \cdot 7H_2O$, g	0.5
	$CaCO_3$, g	30.0
	NaCl, g	2.0
	$FeSO_4 \cdot 2H_2O$, g	0.4
	Distilled water, mL	1000

Sterilization of this medium is carried out in an autoclave with flowing steam under pressure of 0.5 atm for 10 minutes. All salts must be checked for NO_2^- or NO_3^-. The $CaCO_3$ reagent often contains large amounts of nitrates and nitrites. In this case, it is necessary to recrystallize (by boiling several times with distilled water).

Inoculated samples are incubated at 25°C. After 5 days, the presence of NO_2^- and NH_4^+ in cultures is determined using Griess reagent (for first phase bacteria) and Nessler reagent (for second phase bacteria). The total number of nitrifying bacteria is calculated from the Mac Crady Table. The cultures must be studied under a microscope.

Vinogradskiy's medium for the second phase of nitrification	$NaNO_2$, g	1.0
	Na_2CO_3, g	1.0
	NaCl, g	0.5
	K_2HPO_4, g	0.5
	$MgSO_4 \cdot 7H_2O$, g	0.3
	$FeSO_4 \cdot 2H_2O$, g	0.4
	Distilled water, mL	1000

Sterilization of this medium is carried out in an autoclave with flowing steam at 120°C within 15 minutes. The number of nitrifying bacteria of the second phase is calculated from the nitrate content in the sample after incubation (reaction with phenoldisulphonic acid).

Methods for determining the microorganisms involved in the phosphorus cycle

Two groups of microorganisms are involved in the phosphorus cycle: 1) bacteria that dissolve insoluble phosphates such as $Ca_3(PO_4)_2$ and Na_3PO_4; 2) bacteria that mineralize organic phosphorus. To determine bacteria of the first group, a mineral medium, enriched with corn extract, is used:

Mineral medium with corn extract	Glucose, g	10.0
	$(NH_4)_2SO_4$, g	1.0
	K_2SO_4, g	0.2
	$MgSO_4$, g	0.2
	$FeSO_4$, g	0.01
	0.02 % corn extract, mL	250
	Agar, g	20.0
	Distilled water, mL	750

After sterilization of the medium, 2.0 g of Na_3PO_4 and 3 g of $CaCl_2 \cdot 6\ H_2O$, previously sterilized with alcohol, are added to it. Colonies, around which lysis zones have formed, are counted.

For determination of bacteria, which mineralize organic phosphorus, a medium, enriched with potato extract, is used. The extract is prepared as follows: 500 g of potatoes are boiled in 1 litre of water over low heat for 2 hours until the volume of water is reduced to 500 mL.

Mineral medium with potato extract	Glucose, g	3.0
	$(NH_4)_2SO_4$, g	0.2
	KCl, g	0.1
	NaCl, g	0.1
	$MgSO_4 \cdot 7H_2O$, g	0.1
	$CaCO_3$, g	30.0
	1 % phenol solution, mL	4.0
	Potato extract, mL	100
	Agar, g	20.0
	Distilled water, mL	900

Methods for determining the microorganisms involved in the carbon cycle

The carbon cycle involves heterotrophic bacteria (aerobic and anaerobic) that decompose starch, pectin, cellulose, etc. Amylolytic bacteria can be determined by seeding the analyzed water sample on Bredemann's nutrient medium. Sterilization of the medium is carried out in an autoclave with flowing steam at 120°C within 30 minutes.

Bredemann's medium for cultivation of amylolytic bacteria	K_2HPO_4, g	0.5
	KCl, g	0.05
	NaCl, g	0.05
	$FeCl_3 \cdot 6H_2O$, g	0.05
	$MgSO_4 \cdot 7H_2O$, g	0.15
	L-Asparagine, g	5.0
	0.5 % starch solution, mL	200
	Bromothymol blue indicator, g	0.001
	Agar, g	12.5
	Distilled water, mL	500

Aerobic cellulolytic bacteria can be determined by seeding the analyzed water sample on Hutchinson's nutrient medium.

Hutchinson's medium for cultivation of cellulolytic bacteria	K_2HPO_4, g	1.0
	$CaCl_2 \cdot 6H_2O$, g	0.1
	NaCl, g	0.1
	$FeCl_3 \cdot 6H_2O$, g	0.01
	$MgSO_4 \cdot 7H_2O$, g	0.3
	$NaNO_3$, g	2.5
	Distilled water, mL	1000
	pH – adjusted to 7.0-7.2	

The medium is poured into test tubes (no more than 2 mL). Two strips of filter paper are inserted into each tube so that one edge is above the medium. The tubes are sterilized in an

autoclave. After inoculation, the samples are incubated at the temperature of 25°C. This medium can be used in solid form by adding agar to a concentration of 2 %.

Methods for determining the phenol- and hydrocarbon-degrading microorganisms

Various organisms, including actinomycetes (*Nocardia paraffinae*), fungi (moulds), yeasts (*Candida lipolytica, Candida tropicali*), anaerobic and aerobic bacteria (*Pseudomonas fluorescens, Corynebacterium petrophillum, Mycobacterium paraffinicum*, etc.), can degrade phenol and hydrocarbon compounds in aquatic and soil environment. The listed microorganisms are not strictly specific for degradation of these organic substances, thus, their detection on nutrient media containing petroleum hydrocarbon or phenol is not a direct evidence of contamination of the water body with oil products or phenolic compounds. However, the presence of phenol- and hydrocarbon-degrading microorganisms in the water sample indicates the presence (current or slightly earlier) of organic substances, which served as a substrate for the accumulation of these microorganisms in the given sampling station [10].

Number of phenol-degrading microorganisms can be determined by seeding the analyzed water sample on Yegorova's culture medium [11]. Sterilization of the medium is carried out in an autoclave with flowing steam at 120°C for 20 minutes. Before use, 15 mL of 1 % phenol are added to 1 L of this mineral medium. The samples are incubated at 25°C for 6 days. This medium can be used in solid form by adding agar to a concentration of 2 %.

Yegorova's mineral medium for cultivation of phenol-degrading microorganisms	K_2HPO_4, g	1.0
	$CaCl_2$, g	0.1
	NaCl, g	0.2
	$FeCl_3$, g	0.02
	$MgSO_4$, g	0.2
	$MnSO_4$, g	0.01
	$(NH_4)_2SO_4$, g	0.1
	$(NH_4)_2HPO_4$, g	0.5
	Distilled water, mL	1000

Hydrocarbon-degrading microorganisms can be determined by inoculating the analyzed water sample on a solid nutrient medium of the following composition:

Medium for cultivation of hydrocarbon-degrading microorganisms	K_2HPO_4, g	1.0
	NaCl, g	5.0
	$NH_4H_2PO_4$, g	1.0
	$MgSO_4 \cdot 7H_2O$, g	0.2
	Petroleum hydrocarbon, g	5.0
	Agar, g	15.0
	Distilled water, mL	1000
	pH – adjusted to 7.0-7.2	

The preparation of a solid nutrient medium for the cultivation of hydrocarbon-degrading bacteria is carried out as follows: the mineral base is prepared and pH is adjusted to 7.0-7.2. This mineral base with agar are sterilized by autoclaving with flowing steam under pressure of 1.5 atm and temperature (121±1)°C for 15 minutes. The hydrocarbon component is sterilized by filtration through a microporous filter (pore size 0.2 μm) and added to the mineral base immediately after autoclaving. The hot mixture is dispersed (with ultrasound or with a magnetic stirrer) until a homogeneous suspension is obtained. After cooling to 45°C, the nutrient medium with petroleum hydrocarbon is ready for use [12].

4.4. MONITORING OF WATER QUALITY BASED ON MICROBIOLOGICAL INDICATORS

A trophicity and saprobity classifier of surface water bodies is proposed, by using a range of bacterioplankton indicators, such as the total number of bacteria (Ntot), number of saprophytic bacteria (Nsapr), number of phenol-degrading (Nph) and hydrocarbon-degrading (Nhc) microorganisms (Table 4.2), based on own long term research experience and literature data [13-14].

Table 4.2. Assessment of the trophicity status and saprobity of surface waters of the Republic of Moldova according to bacterioplankton indicators

Water quality class	Trophicity status	Saprobity category	Water quality	Ntot, million cells/mL	Nsapr, thousand cells/mL	Nsapr/ Ntot ratio, %	Nph, cells/mL	Nhc, cells/mL
I	Oligotrophic	Oligosaprobic	Very good	≤ 0.5	≤0.5	≤ 0.05	<10	<1
II	Mesotrophic	Oligome-sosaprobic	Good	0.6 – 2.0	0.6 – 2.0	≤ 0.10	11 – 100	1- 10
III	Eutrophic	β-mesosap-robic	Moderately polluted	2.1- 5.0	2.1- 10.0	≤ 0.30	101- 1000	11 – 100
IV	Polytrophic	α-mesosap-robic	Polluted	5.1- 10.0	10.1- 25.0	≤ 0.50	1001-10000	101- 1000
V	Hypertrophic	Polysaprobic	Highly polluted	> 10	>25	> 1.0	>10000	>1000

Water quality class I (very good) corresponds to surface waters in which the total number of bacteria not exceeds 0.5 million cells/mL, of saprophytic bacteria – not exceeds 0.5 thousand cells/mL, of phenol-degrading bacteri – not exceeds 10 cells/mL, and of hydrocarbon-degrading bacteria – not exceeds 1 cells/mL. The water is suitable for all types of water use. The vital activity of aquatic organisms is not disturbed. The ecosystem is characterised as oligotrophic, and the aquatic environment – as oligosaprobic.

Water quality class II (good) corresponds to surface waters in which the total number of bacteria not exceeds 2.0 million cells/mL, of saprophytic bacteria – not exceeds 2.0 thousand cells/mL, of phenol-degrading bacteria – not exceeds 100 cells/mL and of hydrocarbon-degrading one – not exceeds 10 cells/mL. Water is good for drinking purposes after a simple treatment. The ecosystem is characterised as mesotrophic, and the aquatic environment – as oligomesosaprobic.

Water quality class III (moderately polluted) corresponds to surface waters in which the total number of bacteria not exceeds 5.0 million cells/mL, of saprophytic bacteria – not exceed 10.0 thousand cells/mL, of phenol-degrading bacteria – not exceeds 1000 cells/mL, and that of hydrocarbon-degrading bacteria – not exceeds 100 cells/mL. Moderate attributes of degradation are observed in the functioning of the aquatic ecosystem, which entails some disruption of the normal life of aquatic organisms. A simple treatment of water is not enough for using it with drinking purpose. The trophicity status of ecosystem corresponds to eutrophic one, the category of saprobity of aquatic environment – to β-mesosaprobic one.

Water quality class IV (polluted) corresponds to surface waters in which the total number of bacteria not exceeds 10.0 million cells/mL, of saprophytic bacteria – not exceeds 25.0 thousand cells/mL, of phenol-degrading bacteria – not exceeds 10000 cells/mL, and the number of hydrocarbon-degrading bacteria are no more than 1000 cells/mL. Water can be used for drinking purposes only after an intens complex treatment. The functioning of aquatic ecosystems shows attributes of major alterations of hydrobiological and hydrochemical parameters. The ecosystem is classified as polytrophic, and the aquatic environment – as α-mesosaprobic.

Water quality class V (highly polluted) corresponds to surface waters in which the total number of bacteria is higher than 10.0 million cells/mL, of saprophytic bacteria – higher

than 25.0 thousand cells/mL, of phenol-degrading bacteria – higher than 10000 cells/mL, and of hydrocarbon-degrading bacteria – higher than 1000 cells/mL. The functioning of aquatic ecosystems shows serious attributes of degradation. The vital activity of hydrobionts and, especially, fish farming is severely affected. The trophicity of the ecosystem corresponds to the hypertrophic status, the saprobity of aquatic environment – to polysaprobic category. Water cannot be used for drinking purposes.

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V PHYTOPLANKTON. PRIMARY PRODUCTION OF PHYTOPLANKTON AND DESTRUCTION OF ORGANIC MATTER

5.1. TAXONOMIC IDENTIFICATION OF ALGAE, ESTIMATION OF DENSITY AND BIOMASS OF PHYTOPLANKTON

Identification of algae species from phytoplankton composition is performed at optical microscopes, using the identification guides in force: Gollerbah, Poleanskii (1951), Matvienko (1954), Zabelina-Kiseleov, Proshkina-Lavrenko (1951), Dedusenko-Shcegoleva, Gollerbah (1962), Kiseleov (1954), Popova (1955), Dedusenko-Shcegoleva, Matvienko (1959), Moshkova, Gollerbah (1986), Palamari-Mordvintseva (1982), Vinogradova, Gollerbah, Zauer, Sdobnikova (1980), Kondratieva (1968), Moshkova (1979), Palamari-Mordvintseva (1984, 1986), Matvienko-Dogadina (1978), Tsarenko (1990), etc. (cited after [5]).

Phytoplankton density is estimated by counting the algae cells in the Najota (0.01 cm^3), Ucinski (0.02 cm^3), or Goryaev (0.9 cm^3) chambers [1, 5, 7].

As not the whole, but only a part of sample is analysed at the microscope, it is very important to shake the sample well, in order to have a homogeneous distribution of algae and reduce the error. Then, using a pipette, a drop of the sample is taken and placed in the counting chamber. The chamber is covered with a lamella and, after the algae sedimentation, the species are identified and the cells are counted. Simultaneously, the parameters necessary to assess the cell volume are measured. For each sample it is necessary to identify taxonomically and count all the cells of the species in at least 3 chambers. The arithmetic mean of obtained results is calculated. Depending on the number of organisms in the sample under study, the chamber must be counted in whole or in part. The cell is considered as the unit of counting.

Phytoplankton density is calculated according to the formula:

$$N = \frac{nv_1}{v_2\omega},$$

where: N – number of cells in 1 cm^3 of water, n – number of cells in the chamber with an approximate volume of 1 mm^3, v_1 – volume of sample concentrate, v_2 –volume of counting chamber, ω – initial volume of sample.

If the initial volume of sample and the volume of sample concentrate are constant (ω = 250 cm^3, v_1= 5 cm^3), then the following formula is used:

$$N = n \times 20$$

Density is calculated separately for each species, and then the densities are added together, in order to obtain the phytoplankton density in the analysed sample [7].

Phytoplankton biomass is calculated as sum of the species biomasses, for which it is necessary to calculate the average mass of algae cells in the given sample. Various methods for determining the volume of algae cells are known. The most accurate is the stereometric method, when the body of algae is likened to a figure or a combination of geometric figures. The cell volume is calculated according to the formulas known in geometry, based on the linear dimensions of given organisms (DIN EN 16695). The relative density of freshwater algae is considered equal to 1.0-1.55. Sometimes, mean volumes can be used for different species of algae. The calculated volume of each cell is multiplied by their number and the value of biomass is obtained, expressed in mg/L or g/m^3, with an accuracy of 0.01. The biomass is calculated for each species, then added, to obtain the total biomass of phytoplankton in the investigated sample [5].

5.2. PRIMARY PRODUCTION OF PHYTOPLANKTON AND DESTRUCTION OF ORGANIC MATTER

Primary production and destruction of organic matter are important features of the state of the aquatic ecosystem. Intense production of organic matter during the abundant development of phytoplankton causes eutrophication of aquatic ecosystems. Currently, the self-cleaning index is increasingly used to determine the water quality – the ratio of gross primary production to the summary destruction of plankton.

In order to determine the primary production of phytoplankton, the method of exposed bottles in oxygen modification is used [7]. Oxygen modification allows determining both the primary production (in light bottles) and the destruction (in dark bottles), thus, it is possible to calculate the gross and net production. The dissolved oxygen is measured by using the Winkler titration method (ISO 5813:1983).

Production bottles

For the method of exposed bottles in the oxygen modification, light (transparent) glass bottles with polished stoppers are used. Preference is given to quartz bottles, which not influence the penetration of solar radiation. Bottles with a volume of 100-500 mL are used, depending on the productivity of the investigated ecosystem. Usually, the production bottles with the following volumes are chosen: 100 mL – for eutrophic water bodies, 100-250 mL – mesotrophic, 250-500 mL – oligotrophic water bodies.

Prior to determination of production, all bottles are calibrated by volume. Dark (opaque) bottles are used to determine destruction. For this, the bottles are painted black and wrapped in tinfoil. Also, there are used bags made of thick black fabric, which not allows penetrating the sunlight.

Technics of bottle exposure

In order to determine the primary production, at different water horizons, as a rule, 2-3 light bottles and one dark are exposed, but it is also allowed an equal number of bottles. For this, metal or wood frames are used, to which the production bottles are fixed in a vertical position. The frames with the production bottles are fixed to a chain and immersed in water at the corresponding horizons.

Duration of bottle exposure has a high importance in determining the primary production. The phytoplankton exists inside bottles in isolated conditions and a prolonged exposure leads to a sudden change of environmental conditions (increase of pH, over saturation with oxygen, consumption of biogenic elements, etc.), which will differ considerable from those of the water body. In this case, large errors can occur in determining production and destruction. The bottles should preferably be exposed for 24 hours. Some authors proposed an exposure period of 2-6 hours [11].

In order to determine the production in a water column (under 1 m^2), it is necessary to collect samples and expose them in production bottles at several levels of the photic layer. The lower limit of the photic layer, where the production is equal to the destruction (compensation point), corresponds to the depth at which only 1% of the surface solar radiation penetrates.

The selected water horizons for determination of the primary production must correspond to the depths at which 100, 75, 50, 25, 10 and 1% of the surface solar radiation penetrate. White Sekky disk (diameter – 20 cm) or the pyranometer are used for identification of the lower limit of the photic layer; this limit corresponds to the triple depth of transparency (S) according to the white disk. The exposure horizons must be as follows: 0S; 0.25S; 0.5S; 1S, 2S, 3S.

Production bottles and reagents for oxygen fixation ($MnCl_2$ and KI+NaOH) are stored and transported in special boxes. The bottles are arranged in the order of collecting samples from the horizons, for each horizon four production bottles are needed: two for determining the initial oxygen concentration, one light for determining production and one dark for determining destruction.

Collection, exposure and fixation of samples

Transparency is measured with the white Sekky disk and the depth of the photic layer is determined at the given sampling point. Samples from corresponding horizons are taken with the collector. Bottles are filled with water from the collector in such a way that to avoid the water aeration during handling and appearance of air bubbles inside. Bottles are filled completely, closed with the stoppers and exposed at the corresponding horizons. Exposure starting time is noted. Samples selected for the determination of the initial oxygen concentration, at all investigated horizons, are fixed. Oxygen is fixed by pipetting 1 mL of $MnCl_2$, then 1 mL of alkaline KI solution (KI + NaOH). When adding the reagents, the pipette must be held directly above the surface of the sample. 2 mL of sample, which are lost at the addition of reagents, are taken into account later. After fixation of oxygen, the bottle is closed with a stopper and shaken until the content is homogenised. In the presence of oxygen a reddish – brown precipitate forms, and in its absence the precipitate remains white. The fixed samples are kept in the darkness for 3 to 24 hours.

Titration and assessment of the content of dissolved oxygen in samples

Sediment formed after fixation is dissolved by adding 5 mL of concentrated H_2SO_4 or concentrated HCl (the tip of pipette is kept immediately below the surface of the solution). The bottle is closed and turned upside- down several times until the complete dissolution of sediment. Whole sample is transferred into an Erlenmeyer flask by rinsing the bottle few times with distilled water. Titration is made with 0.01 N thiosulphate solution until a yellow colour (straw colour) is obtained. 1-2 mL of starch are added (a blue colour appears), and the sample is titrated until the blue colour disappears completely. Respectively, the volume of thiosulphate used in the titration of each sample is recorded.

Calculation of primary production

The content of dissolved oxygen in water (mg O_2/L) is calculated according to the formula:

$$O_2 = \frac{nNK \cdot 8 \cdot 1000}{V - 2},$$

where: n – volume of thiosulfate used to titrate the sample (mL), 8 – equivalent mass of oxygen, N – normality of thiosulphate solution, K – coefficient of normality of thiosulphate, 1000 – recalculation for 1 liter of sample, V – volume of bottle, in which the sample was fixed, 2 – volume of sample lost at fixation [7].

If: $V_{init.}$ – the content of oxygen in the bottles until exposure; V_{light} – the content of oxygen in light bottle after exposure; V_{dark} – the content of oxygen in dark bottle after exposure; t – time (hours), then primary production A [mgO_2/(L · hour)] and destruction of organic matter R [mgO_2/(L · hour)] is calculated according to formulas:

gross production: $A_{gross} = (V_{light} - V_{dark})/t,$

net production: $A_{net} = (V_{light} - V_{init.})/t,$

destruction: $R = (V_{init.} - V_{dark})/t.$

Estimation of daily primary production and destruction of organic substances under 1 m² of water surface

Value of primary production is obtained by constructing the curve of the vertical distribution of primary production. After this, the area covered by the curve and, respectively, the production in 1m^3 of the water column are determined. The ratio between the total area and the area of 1 m^3 is found. By multiplying the production in 1 m^3 of water at the received figure, the value of the production in a water column, specifically, under 1 m^2 of water surface is obtained.For the determination of the primary production (gO_2/m^{-2}), the following formula is applied:

$$A = K_1A_1 + K_2A_2 + \ldots + K_nA_n,$$

where: $A_1, A_2 \ldots, A_n$ – production at the horizons 1, 2, etc., mgO_2/m^3;
$K_1, K_2, \ldots, K_n$ – coefficients, which depend only on the choice of the horizon.

The coefficients K are calculated by dividing the area of the broken line into several trapezoids. The area of trapezoid is calculated:

$$A = \frac{a + b}{2} h,$$

where: a, b – lengths of trapezoid bases, h – trapezoid height.

The height of each trapezoid is the distance (in meters) between the neighbouring horizons, and the bases are the values of production at these horizons. The sum of the trapezoidal areas represents the value of the production under 1 m^2 of water surface.

Knowing the values of primary production in $mgO_2/L \cdot hour$, or under 1 m^2 of water surface, knowing the length of the day for the researched area, it is possible to calculate the value of daily primary production:

$$A \text{ mg } O_2\text{/day} = A \text{ mg } O_2\text{/L/hour } (T - 2)$$

where: $T - 2$ – length of the day minus 2 hours (within one hour after sunrise and one hour until sunset, the angle of fall of the sun rays is very small, the amount of solar radiation is reduced, therefore the process of photosynthesis not takes place).

The primary production under 1 m^2 of water surface serves as a characteristic of the productivity of the water column in the researched area of the water body.

The destruction of organic substances in 1 m^3 of the water column ($mgO_2/L \cdot hour$) is obtained by calculating the arithmetic mean of the values obtained at different horizons. The value of the destruction of organic substances for 24 hours can be calculated as follows:

$$R \text{ (mg } O_2\text{/L)/24 hours} = A \text{ mg } O_2\text{/L/hour x 24 hours.}$$

Value of the destruction in the water column, under 1 m^2 of water surface, is calculated as the product of the destruction of organic substances in 1 m^3 of water in 24 hours and the depth of water body, expressed in meters, at the sampling point.

5.3. ASSESSMENT OF WATER QUALITY BASED ON PHYTOPLANKTON

With the purpose to estimate the degree of water pollution according to hydrobiological indices, different methods, which are based on the systems of indicator organisms, have been proposed [6, 9]. There are recommended quantitative methods of R. Pantle, H. Buck (1955), M. Zelinka, P. Marvan (1961), which represent the improved modifications of the system proposed by R. Kolkwitz, M. Marsson (1908, 1909) (cited after [8]).

Saprobic analysis is based on the assessment criteria delivered by the classic saprobic system (1977). According to the method of saprobic value of M. Zelinka and P. Marvan (1961) and based on the list of indicator species [9], the Rotshine saprobic index is calculated by using a formula modified by I. Toderas [8]:

$$S_R = \sum_{i=1}^{i=n} N_i G_i S_i / \sum_{i=1}^{i=n} N_i G_i,$$

where: N_i – density of i species; G_i – indicative weight[3] of i species; S_i – saprobic value[4] of i species.

Pantle and Buck saprobic index is calculated with an accuracy of 0.01 according to the formula:

$$S_{P\text{-}B} = \Sigma (sh)/\Sigma h,$$

where: h – frequency of each species found; s – saprobic indicator value of each species.

[3] Note of editors: indicative weight is also called weighting factor.
[4] Note of editors: saprobic value is also called saprobic valence.

The value of h is found in the six-step grid of frequency values, which are determined by the values of the relative abundance of the species [7]. (Tab.5.1).

Assessment of the quality class of surface waters according to phytoplankton is made based on the limit values, which are specified in Annex 1 Environmental quality requirements for the surface waters of the Regulation on the environmental quality requirements for the surface waters (2013) (Tab. 5.2).

Table 5.1. Correlation between the relative abundance and frequency of species

Number of individuals of each species, % of total number	Frequency, h
< 1	1
2-3	2
4-10	3
10-20	5
20-40	7
40-100	9

Table 5.2. Environmental quality requirements for the surface waters (phytoplankton)

Parameter (*group*)	Acronym	Unit	Quality class I	Quality class II	Quality class III	Quality class IV	Quality class V	Regulated parameter
Pantle and Buck Saprobic Index, phytoplankton			1.5	2.0	3.0	3.5	4.0	*
Phytoplankton, biomass	F	[mg/L]	<0.5	1.5	2.5	5.0	10	*
Production/destruction ratio	A/R	Self-cleaning Index Pollution Index	1	0.8 1.2	0.7 2.5	0.5 5.0	<0.2 >5.0	

* In regions where no enterovirus studies are performed, it may be used as an indirect indicator of possible viral contamination.

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VI MACROPHYTES

Macrophytes are one of the biological quality elements for the classification of ecological status of surface waters, alongside the phytoplankton, benthic invertebrate fauna, and fish fauna, which are recommended by the Directive establishing a framework for Community action in the field of water policy (Directive 2000/60/EC, WDF) [1]. Also, the requirements for the use of macrophytes in monitoring are foreseen in such European Directives as the Directive concerning urban waste-water treatment (91/271/EEC) and the Directive concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC).

6.1. QUALITATIVE AND QUANTITATIVE RESEARCH OF MACROPHYTES

In the first decade of twenty one century, an European standard (EN 15460:2007) for the survey of macrophytes in lakes was developed. This guidance standard received the status of Romanian standard (SR EN 15460:2008), which, later, was approved by the Republic of Moldova as a Moldovan Standard (SM SR EN 15460:2012).

The data, which can be obtained by the application of method proposed by the standard SM SR EN 15460:2012, includes the composition and abundance of the aquatic macrophyte flora. It is important to mention that the term "aquatic macrophytes" is defined in the document as large plants of fresh water, which are easily seen with the naked eye, including all vascular plants, bryophytes, stoneworts (Characeae) and macro-algal colonies.

The proposed method uses belt transects of 2-5 m width, which shall protrude into the lake at right angles with respect to the shore line [4]. Transects can be virtually or physically delimited. Transects can be marked with a submersible measuring tape with a cement weight. Another way of transect marking is shown in Fig. 6.1. All transects must be of the same width. The number of belt transects to be surveyed around a stagnant water body depends on the spatial arrangement of the aquatic plant stands, and on the adjacent land use types (e.g. settlements, recreation areas, agricultural lands, shrub etc.). Anyway, at least four transects shall be surveyed in the first shore reach for each land use type present around a lake, even if species number saturation (SNS = the number of species not increases in an additional transect) is reached with a smaller number of transects. In other shore reaches with the same type of land use only one transect needs to be surveyed, if at least one new species occurs or one species is missing (±1). Otherwise, the original approach (4 transects minimum) must be followed.

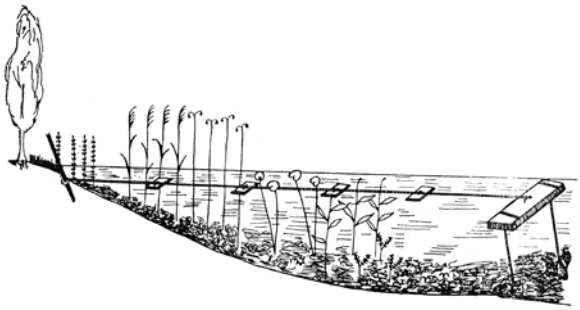


Fig. 6.1. Transect delimitation by using a rope [9]

For the full length of the belt transect, regarding each depth zone (usually 0-1 m, 1-2 m, 2-4 m, 4-8 m, and deeper than 8 m) or any other subdivision of the transect, Plant Mass Estimates (which is not synonymous to "biomass") are made for each species occurring there. The Plant Mass Estimates are not based on the area (% cover), but on the three-dimensional development of the species. Five levels are distinguished: 1 = rare; 2 = occasional; 3 = frequent; 4 = abundant; 5 = very abundant [9].

Another approach consists in the application of a scale for macrophyte abundance (DAFOR) (Tab.6.1).

Table 6.1. DAFOR scale

Level	Abundance description	Coverage, %
D	dominant (very abundant)	> 75
A	abundant	51- 75
F	frequent	26-50
O	occasional	11-25
R	rare	1-10

Surveys are made by walking along the belt transect, if the water body maximum depth is approximately 1.0 m, fulfilling all safety requirements. Otherwise, the survey is performed by diving, and by using, for example, a boat. The surveyor shall use a hydroscope (Fig. 6.2) and sunglasses with polarizing coating, which reduce the reflections on the water surface and, thus, considerably improve the level of visibility.

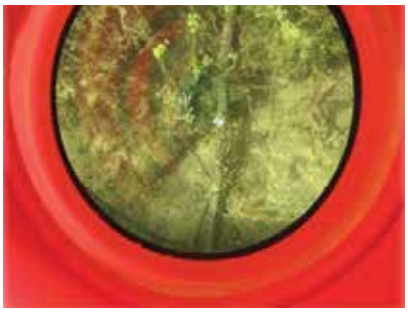
1  2  3 

Fig. 6.2. Hydroscope: 1 – aspect[7], 2 – mode of usage[8], 3 – obtained image[9]

If the water is very clear, the use of a rake for getting the plant material up to the surface in order to assess the Plant Mass Estimates is suggested. Rakes with extended shaft can be operated with high efficiency in waters with depths less than 3.5 m. The "raking" is not the best solution, because it can result in: 1) missing smaller species and those intermingled in deeper layers of plant stands and invisible from the surface and 2) wrong estimates of the plant mass. In water depths beyond 4 m depth, and in turbid conditions, a greater rate of misinterpretation and failing species recognition must be expected. Therefore, for the assessment of the Plant Mass Estimates the diving equipment is strongly recommended. In water bodies not deeper than 2 m wetsuit, mask and snorkel are sufficient. In deeper lakes SCUBA (SCUBA – abbreviated form of self-contained underwater breathing apparatus) equipment (Fig. 6.3) is necessary. In this case, only trained and certified personnel shall be involved.

1  2 

Fig. 6.3. Equipment for underwater research: 1 – wetsuit, mask and snorkel[10]; 2 – SCUBA[11]

Experienced personnel shall be able to identify in field conditions the most of macrophytes up to species level. Otherwise, the plants are collected in plastic bags or containers, which are labelled in advance, and transported to the laboratory. A special attention shall be given to the plant parts, which play an important role in the species identification. Moreover, as the raking method is a destructive one, the sampling personnel must recognize the endangered and vulnerable species from the investigated area.

It is recommended to create collections of aquatic plants, mostly in the classical forms of herbarium. Some plant organs (e.g. fragile leaves, reproduction organs) can be preserved in ethylic alcohol. In conclusion, the above described methods are, mainly, semi-quantitative, based on a descriptive scale with visual assessment of species structure and abundance. They

5 https://www.bootszubehoer.de/sonstige/aquascope-unterwassersichtgeraet/ a-7441/
6 http://www.carpealsace.com/t11714-sceau-translucide-pour-boat
7 http://www.pecheur.com/achat-aquascope-plastimo-10157.html
8 http://www.decathlon.co.uk/C-10883-diving
9 http://www.openwateradventures.com/

are considered to be time-efficient and meeting the preconditions for assessing the ecological status of rivers and lakes.

Various types of grabs and grapnels – with two or more arms, in the form of a rake – are used in qualitative study of macrophytes (Fig. 6.4).

The frequency of monitoring primary depends on objectives of monitoring programs. According to Georg A. Janauer (2002) [4], each lake littoral should be assessed in total at least once every ten years or whenever marked changes in environmental conditions (e.g. changes in macrophyte composition noticed during the monitoring) have occurred. WFD proposed as guideline the period of three years for monitoring of macrophytes in all types of inland waters, but underlying that additional monitoring during different seasons of the same year shall be carried out, when necessary.

Fig. 6.4: 1 – a type of rake-grapnel[12]; 2 – grapnel with few arms[13]; 3 – sampling of macrophytes with a grapnel[14]; 4 – usage of a rake-grapnel[15]

According to the Regulation on monitoring and systematic evidence of the state of surface and underground waters of the Republic of Moldova (2013), macrophytes are listed among the hydrobiological parameters, which shall be monitor in the mandatory monitoring points of aquatic ecosystems. The recommended frequency of macrophyte sampling is two times during the vegetation season (April-September) both in rivers and lakes. Diversity, density and biomass (the presence of sensible taxa and invasive taxa) are the indicative parameters of macrophytes.

Within the quantitative research of macrophytes (determination of the number of stems, biomass and occupied area) different frames are widely used, which vary in size (1.0, 0.5, 0.25 m^2, etc.), shape (square, rectangular, round) and construction material (wood, metal, for example, aluminium pipes, or synthetic compounds). Partially or completely demountable or foldable frames are easier to transport.

The type of frame fixing depends on the type of plant community. Thus, in the case of studying small plant communities, located at shallow depths (up to 0.2-0.3 m), the frame is placed above the community and the plants are collected manually. In submerged, floating plant communities and those that rise slightly above the water level (up to 1 m) the frame is also placed on the surface of the water, but is fixed to the bottom with two pillars, located at opposite corners of the frame. Obviously, if the plants rise far above the water level, the frame cannot be installed above the plants, so it is practically built in water and in this case, it is often not necessary to fix the frame with pillars. All works with frames are possible to be carried out only at depths of up to 2 m. Counting or cutting of plants in open areas of the water body can be done only if the weather is quiet – wind and waves make it difficult to use the frames, and sometimes – even impossible.

The collection of aquatic plants for the determination of biomass is performed by several methods. As rule, the determination of phytomass is carried out during the period of the most abundant plant development, which is considered to be the period of their mass flowering. The notion "phytomass" means only the biomass of the part above the substrate, which includes the part in the water and, possibly, in the air and excludes the roots of the plants.

[10] http://efe-uk.com/products?f% 5B0%5D =field_product_ref %253A field_ main_ heading%3A698
[11] http://valleynaturalist.blogspot. md/201108_01_archive.html
[12] http://plantecol.co.uk/surveys.htm
[13] http://main-stream-resources.com/nestle'_waters.htm

The determination of phytomass is made on square or rectangular lots, usually of 0.25-1 m^2, during the mass flowering of the edifying species. Lots are delimited by described above frames or ropes (Fig. 6.5a). The number of lots depends on the surveyed area (if the squares are small, then their number is larger), water mirror occupied by plant communities, complexity of plant communities, for example, the number of layers, number of species, density of plants.

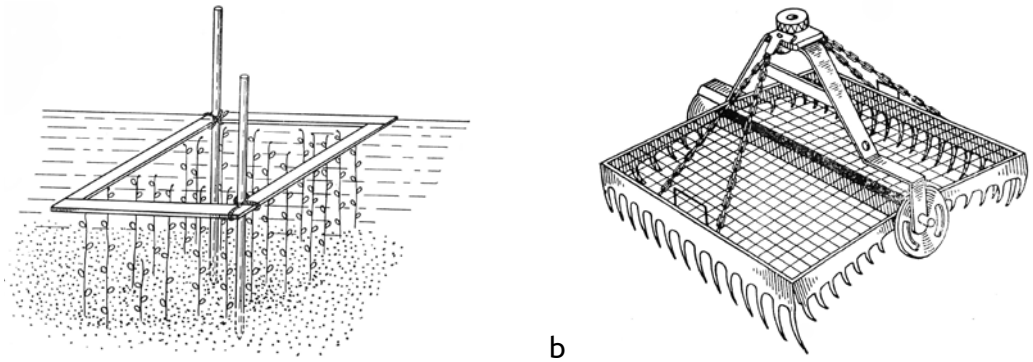
All plants located within the plots are cut at the level of substrate and weighed. Depending on the research needs, the sample wet mass, dry mass or absolutely dry mass is determined.

Cutting (mowing) of plants, in fact, is the most difficult part of biomass determination. In shallow places (depths of up to 0.6 m) the plants fixed to the substrate are cut with a knife, scissors, secator or sickle. Those that are not fixed to the substrate are harvested manually or with a net. Disturbance of soft sediments should be avoided, as this will reduce the visibility.

At depths of more than 1-1.5 m, the plants are cut with a scythe from the boat. In this case, two people are involved in the sampling process – one person harvests, and the second person gathers the plants that come to the surface of the water. It should be mentioned that in such cases the scythe must be endowed with a long snath – it must rise above the water by 1-1.5 m.

Mowing is done with sudden movements of small amplitude. All harvested plants are arranged in one direction (the lower parts – on one side and the tips – on the other side), along the boat, on polyethylene films or canvas, or other strong, heavy cloth. In the case of collecting submerged plants, on the surface of which a lot of water is retained, the film shall be arranged so that to avoid the pouring of accumulated water into the boat.

Also, various quantitative grab samplers are used in research of aquatic plants, some of them being suitable for the collection of macrofauna. For example, for the collection of phytomass in communities composed mostly of submerged and floating leaf plants, the Bernatovici grab is most frequently used (Fig. 6.5b). To collect a sample of phytomass from 1 m^2, the grab is lowered and raised six times. Another tool, used for cutting plants on a strictly determined area (0.25 m^2) at shallow depths, is a combination of fork and frame.



a b

Fig. 6.5: a – fixing of the frame before the plant cutting; b – grab designed by S.Bernatovichi for sampling of aquatic plants [9]

If possible, the plants are washed, cleaned of concretions and classified into groups at the stage of sampling. The sample is labelled with indication of the number of sample, name of the water body, type of phytocenosis, place, date and depth of sampling, type of bottom (visual identification), type and area of sampling. The plants are wrapped in film or wet cloth, tied and transported to the laboratory. Such samples can be kept fresh for 1-2 days, with the condition of being stored in a cool place. It is not recommended to place and transport submerged plants in vessels with water, this practice requiring much more effort and creating more inconvenience to those working in the boat [9].

The laboratory initial handling consists of cleaning and washing the plants. With this purpose, the metal nets are used, but sometimes, for example, in the case of submerged and floating plants, which are more fragile – pieces of cloth (plants are wrapped in cloth and submerged in water). If necessary, this operation must be repeated several times. To remove water from the surface of the plants, they are shaken off and placed on nets or paper. Later, the plants are classified in dependence of species and group (submerged, emerged, floating, etc.).

If the quantity of submerged and floating plants is big, then they are weighed in bags. Larger emergent plants are tied in small bundles before weighing, but they shall not be broken in any case. After weighing the wet mass, in dependence of research interest, biometric measurements are carried out (e.g., stem height and diameter in the lower part of plants), the plants are counted (preferably, separately the plants at vegetative stage and those at generative stage), the leaf area and their number are determined, etc.

Air-drying of plants can be done both indoors and outdoors, but, in the second case, the plants must be protected from rain. The plants can be dried indoor in the extended form, and outdoor – in bags. Before being put in bags, the long stems of the plants are bent or cut. Likewise, thicker stems can be cut longitudinally for a faster drying. During the drying, the bags shall be turned over and the plants inside of them mixed. The complete drying of the plants can be established visually (the leaves break when bent) or by repeated weighing during drying (until a constant mass is obtained).

In order to determine the absolutely dry mass, a certain quantity of sample (a few plants or a certain quantity of the whole sample, which was grinded in advance) is dried in the drying oven at 105ºC. Phytomass (or plant biomass) is expressed in the mass of plants (wet, dry or absolutely dry) per unit area of the water body – g/m^2, kg/m^2, t/km^2, etc. [9]. The annual production of aquatic plants is calculated on the basis of data obtained for phytomass, using certain coefficients, and is expressed in units of mass per units of area or volume of the water body (mg/L, g/m^3) or in energy units per unit of surface area (kJ/m^2) or volume of water (J/L, kJ/m^3). Investigation of such parameters of aquatic vegetation as dynamics of both phytomass and plant density, the growing rate of plants, dynamics of occupied areas, and, related to the last, the mapping of aquatic vegetation, require seasonal, annual and multiannual complex researches.

6.2. MACROPHYTES IN THE BIOINDICATION/BIOMONITORING OF WATER QUALITY AND ECOLOGICAL STATUS OF WATER BODIES

The usage of macrophytes in bioindication of water quality has both advantages and disadvantages. As advantage is considered the fact that macrophytes are usually fixed to the bottom and because of this they are visible and can be easily identified, also they are good indicators of suspended solids and nutrient enrichment. As disadvantages are not well documented responses to pollution (i), often tolerance of intermittent pollution (ii), mostly seasonal occurrence (iii) [3].

Taking into account that plants intensely assimilate biogenic elements, the species composition and abundance (depending on density, biomass, cover area, etc.) of macrophytes are used as indicators of organic matter pollution and eutrophication of water bodies. Kolkwitz R. and Marsson M. (1902, 1908, 1009, cited after [3]), the authors of the Saprobic system, initially included aquatic plants in this system, as their ecological approach relied on biological communities and not purely on indicator species. Later, with the development of taxonomy, in the Saprobic system the emphasis was put on indicator species.

A list of aquatic plants, which can be used as indicators of saprobity, can be found in [10]. The plants are classified in five zones of saprobity. According to this list, the higher aquatic plants grow mainly in oligosaprobic and the β-mesosaprobic zones (for example, *Elodea canadensis, Lemna gibba, Potamogeton perfoliatus, Ceratophyllum demersum, Myriophyllum spicatum,* etc.). Such species as *Fontinalis antipyretica*, *Hydrocharis morsus-ranae*, *Sagittaria sagittifolia,* etc., are oligo–β-mesosaprobic.

Some authors consider such species of macrophytes as *Ceratophyllum demersum, Ceratophyllum submersum, Isoetes lacustris, Potamogeton praelongus, Fontinalis antipyretica, etc.,* as good indicators of acidification of water bodies, as *Hydrocharis morsus-ranae, Lemna gibba, Lemna minor, Myriophyllum spicatum, Potamogeton crispus, Spirodela polyrhiza,* etc. – of their eutrofication [8]. A range of species, as *Ceratophyllum demersum, Ceratophyllum submersum, Elodea canadensis, Hydrocharis morsus-ranae, Potamogeton lucens, Potamogeton nodosus, Potamogeton perfoliatus, Spirodela polyrhiza,* charophycean algae (*Charophyceae),* are recommended for the monitoring of pollution with heavy metals.

Macrophytes have been excluded during the revision of the Saprobic system in Germany (Friedrich, 1990, cited after [3]), being considered that autotrophic plants cannot survive in conditions of a very severe organic pollution. Moreover, in the case of photoautotrophic organisms, it was wanted to avoid the interaction between the indication of saprobity and the indication of the trophic state (Friedrich, 1990, cited after [3]).

Probably, due to the deficiencies of the use of macrophytes in bioindication and, in particular, in the saprobic system, they were not included as indicators of water quality in the Regulation on the environmental quality requirements for the surface waters of the Republic of Moldova (2013) [5], unlike other two groups of hydrobionts – phytoplankton and benthic invertebrates.

The difficulty of identifying the indicator species of aquatic plants is determined by their high ecological plasticity. Thus, many species have a large distribution area and, in different physical and geographical conditions, they can be found in aquatic bodies, which trophicity differs considerably. In addition, for most aquatic plant species there is insufficient information on their ecology (environmental preferences, e.g. physical, chemical and hydrological factors) and physiology [9].

Even the higher aquatic plants are a more conservative indicator of water quality, compared to planktonic (phyto- and zooplankton) and benthic communities, lasting changes in species composition, phytomass and areas covered by plant stands indicate the transformation of ecosystems and change in water quality.

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VII ZOOPLAKTON

Freshwater zooplankton communities consist of three main groups of invertebrates: rotatorians, copepods and cladocerans. When collecting samples of zooplankton, it is necessary to take into account that each of these groups has species adapted to various habitat types: pelagic, coastal (with vegetation) and benthic.

The basic principles of selection of sampling stations, the main techniques and tools used in the sampling of zooplankton in rivers and stagnant waters (natural and artificial lakes, ponds), as well as the standard requirements for processing and preservation of zooplankton samples are described in detail in [2]. Each zooplankton sample, if it is not processed in alive state, must be fixed. As a rule, zooplankton samples are fixed with 40% formalin in such a way that the final concentration of formalin in the sample is 4% (1 part formalin to 9 parts of water). Formalin, which is used in fixing zooplankton samples, must have a neutral pH and should be sediment-free. Each sample should be labeled and recorded in a special register.

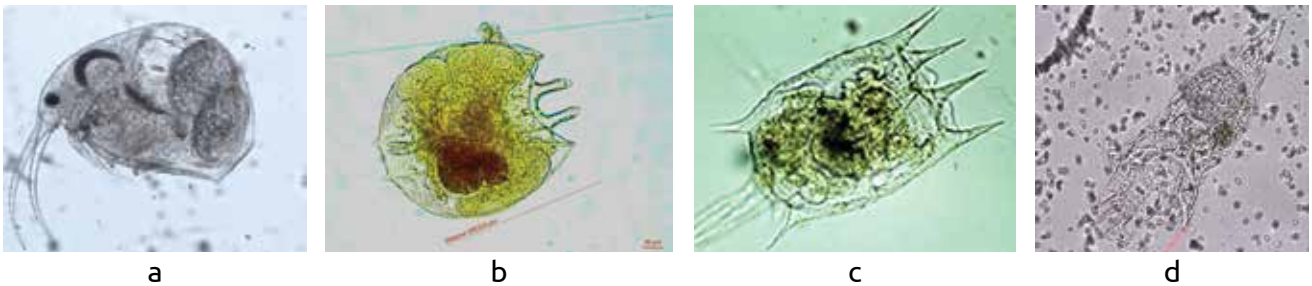
The analysis of zooplankton samples under laboratory conditions aims to: 1) determine the structure of this community; 2) estimate the density and biomass of the main taxonomic groups of zooplankton; 3) evaluate zooplankton production; 4) assess the water quality based on zooplankton indicator species.

7.1. TAXONOMIC IDENTIFICATION OF ZOOPLANKTON

For the determination of the taxonomic structure of zooplankton, both qualitative and quantitative evidence are used. The purpose of processing qualitative samples of zooplankton is to determine the organisms to the lowest systematic category. Immature forms of copepods are identified up to the level of the suborder (Cyclopoida, Calanoida or Harpacticoida), or, if possible, up to the genus level. Adult cladocerans (Fig.7.1a) and copepods are identified at the species level.

Rotatorians (also called rotifers) are identified up to the genus level and, if possible, up to the species level (Fig. 7.1 b-d). Some rotatorians may be difficult to identify due to their contracted state. In this case the identification shall be made by examination of their chitinous mouthparts after use of the 5% sodium hypochloride solution as a bleaching agent [12].

It is recommended to collect samples – duplicates, which are not fixed and processed immediately after collection. In the living state, mostly small forms of rotifers without shells (Synchaeta, Floscularia, etc.) are determined [22].



a b c d

Fig. 7.1 Cladoceran *Bosmina longirostris* (a) and rotifers *Platyias quadricornis* (b), *Brachionus calyciflorus* (c), *Rhinoglena frontalis* (d)

Prior to processing, the sample is concentrated by centrifugation or removal of water by means of a siphon, the lower part of which is covered with a mesh with a mesh size of 40 µm. Using a pipette, the plankton is transferred from the sediment of concentrated sample on a slide and visualized under binoculars (eyepiece size – from 10x to 80x), and then under a microscope (100x – 400x) (Fig.7.2). A slide cover glas is used for the microscopic examination, in

order to perform a microscopic preparation. During the microscopic analysis, all zooplankton organisms are identified, with the help of specialized identification keys: Rotatoria [12, 17, 18, 20], Cladocera [3, 5], Crustacea [4, 19, 21], Copepoda [1] and others.

7.2. MEASUREMENTS OF QUANTITATIVE PARAMETERS OF ZOOPLANKTON COMMUNITIES

Quantitative samples are used to examine the taxonomic structure and evaluate the functional parameters of zooplankton – density, biomass and production.

The determination of the density is based on the counting of the adult individuals of each species and at their stages of development. Depending on the density of organisms, the sample may be concentrated or, conversely, diluted with water.

The quantitative sample of zooplankton is poured into a graduated glass and brought to the required volume (25, 50, 100 cm^3) with distilled or filtered water. The sample, brought to the respective volume, is shaken well, then, with the help of a pipette, 1-5 mL of sample are transfered into a Bogorov chamber. The individuals of each species are examined under the binoculars or microscope. This operation using the Bogorov chamber is performed at least two times. The rest of the sample is transferred, for example, to a Petri dish and examined under binoculars to identify less common and large species.

Fig. 7.2. Microscop *Axio Imager A.2 (Zeiss)*

For each analyzed sample a form is completed. The number of organisms found in the sample portion is divided to the total sample volume, this indicator being recorded as the density. The calculations are performed as following: the average number of individuals registered is calculated, for example after vizualization of the sample three times (e.g. 21 + 18 + 15) / 3 = 18). Knowing the volume of the sample (e.g., 100 mL), a recalculation coefficient is obtained, in accordance to the vizualized volume (e.g., 5 mL subjected to visualization, then 100/5 = 20). By multiplying the coefficient with the average value of the density of the individuals of a species, the number of individuals of this species in the sample is obtained (for example: 18 * 20 = 360). After determining the number of individuals in the sample, their density (number of individuals/1 m^3) is calculated using the following formula [22]:

$$X = \frac{n \times 1000}{v},$$

where: X – the number of individuals in 1 m^3 of water, ind./m^3, n – number of individuals in the sample, v – volume of water filtered via the conical mesh Apshtein type, in litres.

This operation is repeated separately for each species or other systematic group, e.g., order.

The next step in processing of a zooplankton sample is calculation of biomass and production. Biomass expresses the weight of zooplankton and is calculated by multiplying the density by the average individual mass of the species. Data on the individual mass of zooplankton species are available in the specialized literature.

Estimation of the secondary production and productivity involves the application of various methods, including direct measurements of the physiological parameters of hydrobionts, as well as indirect methods based on the calculation of structural-functional parameters of zooplankton

populations (such as density, biomass, age structure and sex, duration of development of different stages, etc.). The values of these parameters can be determined experimentally or can be calculated according to the existing graphs and tables in the specialized literature [9, 16].

A widely used method is to estimate population productivity as a biomass growth rate per day. In this case, the daily productivity of zooplankton can be calculated by the formula:

$$P = \Sigma_{i=1}^{n} P_i, \quad \text{where} \quad P_i = C_w \cdot B_i,$$

where: P – daily production of zooplankton, mg/m^3/day, P_i – daily production of the species or each age group, mg/m^3/day, C_w – specific rate of biomass growth per day at a given water temperature, day^{-1}, B_i – total biomass of the species, mg/m^3, n – number of species.

Cw values can be calculated using growth curves, which are constructed for each species or group of species (in the case of copepods – for each age group), according to established methods.

The P/B ratio (day^{-1}) is the rate of biomass renewal in a unit of time. This coefficient has a constant value only in populations with a stable structure of age groups and in stable environmental conditions. In real ecosystems, the P/B coefficient indicates an average value over a period of time, which is largely influenced by water temperature. For example, if the production of zooplankton in a monitored area of the aquatic ecosystem is 10 mg/m^3/day, but the biomass is 40 mg/m^3, then it turns out that P/B = 0.25 day^{-1}, thus each day 25% of biomass is renewed [6].

Depending on the research objectives, the trophic structure of zooplankton is also established, which is determined not only by the size and productivity of hydrobionts, but also by the structural and functional characteristics of their food collection organs [13]. The species in the composition of zooplankton form two trophic levels: primary consumers (C1 level) and secondary consumers (C2 level), each of which includes representatives of different taxonomic groups. The assessment of the structural-functional parameters of the zooplankton communities, differentiated by the trophic groups, allows the analysis of the C1/C2 ratio for the monitored ecosystems in temporal and spatial aspect.

7.3. CALCULATION OF BIOCENOTIC INDICES

The relative abundance of zooplankton is expressed in percentage (%) and is calculated by dividing the number of individuals or biomass of a species to the total number (N) of individuals in the sample or total biomass of the sample:% N = (n_i / N)×100; % B = (b_i / B)×100, where n_i – the number of individuals of species *i*; b_i – biomass of species *i*; N – total number of individuals in the sample; B – total biomass of the sample.

Based on abundance, the index called dominance (D) is calculated. For example, based on the abundance, it was established that the structural complex of zooplankton included in the summer of 2012 in the Prut river: eudominant species – 13, dominant species – 15, subdominant species – 10 and receding species – 8. The figures indicate the lack of significant predominance of certain species in the structure of zooplankton and, consequently, the specific aquatic ecosystem is characterized by a rather high degree of ecological stability. Based on the gravimetric abundance (biomass), the dominance index is calculated according to the model proposed by Dziuban [14]:

$$Id = \frac{B_i}{\Sigma B_i} F,$$

where: B_i – biomass of the species *i*, F – frequency ($F = \frac{a \times 1000}{A}$, where a – the total number of samples containing *i* species, A – total number of samples).

The classification of species according to their occurrence frequency can be determined as follows:

C1 – accidental species – presence in 1-25% of samples;
C2 – accessory species – presence in 25.1-50% of samples;
C3 – constant species – presence in 50.1-75% of samples;
C4 – euconstant species – presence in 75.1-100% of samples.

Ecosystem biodiversity can be expressed by species richness, or by the index of diversity, equitability, similarity, etc.

The degree of affinity of zooplankton communities is calculated using the Sorensen index [11]:

$$Iaf = \frac{2c}{(a + b)} \times 100\%,$$

where: a and b – the total number of species recorded in two ecosystems under investigation, c – the number of species which are common to both ecosystems.

The biological diversity is calculated according to the Shannon equation in Wilhm's modification:

$$Ish = -p_i log_2 p_i,$$

where: p_i – the contribution of species i to the formation of community biomass ($p_i = b_i/B$, where b_i – biomass of species i; B – total community biomass).

The Shannon-Wiener Diversity Index is recommended by the Water Framework Directive for assessing the ecological status of all types of surface water. The index is calculated according to the following formula:

$$H = -\Sigma_{i=1}^{S} P_i lnP_i,$$

where: S – the number of species; P_i – the ratio between the number of individuals of the species i (N_i) and the total number of individuals in the sample (N): $P_i = N_i/N$.

Species diversity indices are highly informative for the assessment of the state of an aquatic ecosystem as a whole, which characterize not only the taxonomic structure of a community, but also its seasonal state, the boundaries of various complexes. Indices of species diversity reflect well the changes in the structure of the community, which occur during seasonal succession. At the initial stages of succession in the spring, the species diversity grows rapidly, in the summer season, as a rule, it stabilizes, and in autumn and especially in winter it sharply decreases. Correlation between the values of the species diversity index and the trophic status of a lake, according to the indicators of the development of the zooplankton community, is as follows: 2.6-4.0 – oligotrophic status, 2.1-2.5 – mesotrophic, 1.0-2.0 – eutrophic, less than 1.0 – an indicator of extreme environmental conditions [14].

7.4. EVALUATION OF WATER QUALITY BASED ON ZOOPLANKTON INDICES

The estimation of water quality according to hydrobiological indices is performed either by: 1) comparing the communities of hydrobionts in the researched station with those in the reference aquatic ecosystems, or 2) studying the indicator species.

Zooplankton communities characterize the state of the aquatic ecosystem, and some zooplankton species are used to indicate the water quality.

For the purpose of estimating the quality of water in aquatic ecosystems according to zooplankton communities, the most widespread and convenient method to use is the saprobic approach, namely the application of the Saprobic Index of Pantle and Buck, in the modification of Marvan and Dziuban [15], which is calculated according to the formula:

$$S = \frac{\Sigma S_i \times G_i \times N_i}{\Sigma G_i \times N_i},$$

where: S – Saprobic Index; s_i – saprobic value of i species; G_i – indicative weight of i species; N_i – density of i species.

Below an example of calculating the saprobic index is given (Tab. 7.1).

Table 7.1 Example of determining the Saprobic Index according to the method of Pantle and Buck (in the modification of Marvan and Dziuban).

Species	Saprobic zone	*N*	*s*	*G*	*G×N*	*s×G×N*
Keratella cochlearis	β – o	240	1.55	2	480	744
Keratella quadrata	*o – β*	200	1.55	2	400	620
Lecane luna	*o – β*	600	1.55	2	1200	1860
Brachionus calyciflorus	β – α	350	2.50	3	1050	2625
Trichocerca sp.	-	100	-	-	-	-
Asplanchna priodonta	*o – β*	1500	1.55	1	1500	2325
Daphnia longispina	β	500	2.05	1	500	1025
Chidorus sphaericus	β	400	1.75	1	400	700
Acantocyclops vernalis	β	120	1.85	3	360	666
Cyclops strenus	β – α	370	2.25	2	740	1665
Total					**6630**	**12230**

Note: the saprobic value (s) and the indicative weight (G) of the species are indicated according to the bibliographic sources [10, 23]

By applying the formula indicated above, we obtain S = 12230/6630 = 1.84. Thus, according to the saprobic index, calculated based on zooplankton, the studied aquatic ecosystem corresponds to the oligosaprobic area, and the water can be characterized as of good quality (quality class II), in accordance with Tab. 7.2.

Table 7.2 Classification of water quality of aquatic ecosystems by hydrobiological parameters (zooplankton) [7]

Water quality class	Water quality	Saprobic zone	Pantle and Buck Saprobic index
I	Very good	ksenosaprobic	≤ 1.5
II	Good	oligosaprobic	>1.51- ≤ 2.0
III	Moderately polluted	β –mesosaprobic	>2.0 – ≤ 2.5
IV	Polluted	α – mesosaprobic	>3.0 – ≤ 3.5
V	Highly polluted	polysaprobic	>3.5 – ≤ 4.0

A zooplankton sample to be representative, the presence of at least 7-10 indicator species is required.

In conclusion, the standard procedure for estimating the ecological status of the aquatic ecosystem according to zooplankton includes the following steps:

- determination of the total number of taxa and the number of taxa in the main taxonomic groups;
- determination of the total number of individuals and the number of individuals in each group;
- determination of the total biomass and the biomass of the main taxonomic groups;
- establishing the constant and dominant species, as well as the species-indicators of saprobity (name, share in the total number, saprobity);
- completion of the Taxonomic List for each sampling station;
- calculation of the saprobic index for the sampling station or for the monitoring sector;
- analysis of the results of water quality.

Based on all the parameters and indices mentioned, and taking in account their importance, the multimetric index of zooplankton can be calculated (at river basin level), for example:

- total number of taxa (N) 30%;
- Shannon-Wiener diversity index (H) 30%;
- saprobic index (SI) 30%;
- the ratio between the abundance of crustaceans and that of rotifers (NCr / NRot) 10%.

The National Surface Water Monitoring System of the Republic of Moldova includes zooplankton as a hydrobiological parameter (diversity, density, biomass, saprobic index) to be systematically monitored [8], but the limit values for assessing water quality for zooplankton communities were not yet developed in the Regulation on quality requirements for surface waters [7]. In order to assess the ecological status and classify the water quality according to the saprobic index calculated based on indicator zooplankton organisms, the values of saprobic index calculated based on phytoplankton, which are given in mentioned above regulation, are used.

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VIII MACROZOOBENTHOS

The European Union's Water Framework Directive, adopted in 2000, regulates new approaches to water management. It raises the attention on the role of biological indicators and harmonization of methods for assessing water quality using aquatic organisms. In most countries, macrozoobenthos is used as a basic group in bioindication, with special attention paid to pollution-sensitive taxa, with the use of the indicator-species system and biotic indices.

8.1. SAMPLING OF BENTHIC INVERTEBRATES AND PRELIMINARY PROCESSING OF SAMPLES

The choice of substrate is the starting point in the sampling of macrozoobenthos. For the purpose of hydrobiological monitoring, the most populated substrates are selected, with the most diverse fauna of benthic invertebrates, these being the most informative in assessing water quality.

The substrate must be located on the best oxygenated sectors of the bottom of the water body; in aquatic ecosystems with a slow exchange of water such conditions are created in the littoral area, and in rivers – in the area of banks and wading areas. In order to obtain comparable information on benthic fauna at different collection stations, it is desirable to collect samples in similar habitats.

Benthic invertebrates are animals living on the bottom of water bodies, in the benthic water layers and on different types of substrates, being, basically, represented by the following taxonomic groups: oligochaets, chironomids, mollusks, crustaceans, trichopteras, ephemeropteras, plecopteras, etc.

Sampling of benthic invertebrates is carried out in accordance with international and national standards [1-9]. Likewise, the methods unanimously accepted in hydrobiology are used [14-21, 23]. Depending on the type of substrate (stony, sandy, muddy, macrophyte, periphyton), the type of water body (lentic, lotic, deep or shallow) and the group of invertebrates researched, the following unanimously accepted collection tools and methods are used: grabs (e.g., Petersen and Ekman-Birge), trawls, dredges, frames, nets (nylon nets № 38), scoops (nylon nets №23), artificial substrates, bores and manual sampling. Based on the quantitative samples, the density (individuals/m^2), biomass (g / m^2) and the taxonomic diversity are determined and in the qualitative samples – the taxonomic diversity of the macrozoobenthos is identified.

Methods and tools for collecting macrobenthos are presented in detail in a number of relevant textbooks [10, 22, 23]. After collection, the benthic samples are transferred to a vessel with water for washing, using a sieve or a net. The sample is further transferred to a plastic vessel with a volume of 200-1000 mL and fixed. For fixation (preservation) 96% ethyl alcohol or 37% formalin is used, which is added to the sample until a final concentration of 70% and 3.7%, respectively, is achieved. If the sample not needs to be preserved, then it should be kept at a temperature of 1-5 °C and processed in the first 24 hours after sampling [3]. Each sample must be labeled, indicating the number of the sample, the water body, the point and date of collection, the depth, the type of substrate and the number of repetitions of the sampling.

The first steps in the processing of macrozoobenthos samples consist of washing and sorting them. For this purpose, under laboratory conditions, the samples are washed under running water, using a set of sieves of different sizes. Larger samples by volume are sorted and processed by selecting a sub-sample. In the case of a poorer sample by number of species or individuals, a complex sample of several samples is collected. Complex samples and sub-samples can contain up to 500 ± 20% of macrobenthos individuals.

8.2. DETERMINATION OF DENSITY, BIOMASS AND SPECIES COMPOSITION

The washed sample or the selected part of the sample is transferred to the Bogorov chamber, where with the help of a binocular the benthic organisms are selected, determined by groups (or species) and their density is calculated by direct counting of the identified organisms.

After this, the hydrobionts are weighed. The biomass is determined after the hydrobyonts are preliminary dried until the disappearance of wet spots on the filter paper. The weighing is performed by an analytical balance, for example ABS 80-4 Kern with a level of accuracy of 0.0001 g. The density and biomass of the organisms are recalculated in ind./ m^2 and g/m^2, respectively. When collecting quantitative samples of benthos using the Ekman-Birge and Petersen graber samplers, with a catchment area of 0.025 m^2, for the recalculation of the density and biomass at 1 m^2, the result obtained is multiplied by 40.

For the determination, the small organisms or their parts are placed on the glass slide, adding a drop of water and covering it with a cover glass. Thus, a temporary preparation is obtained, which is analyzed under a microscope. Specialized methodological guidelines describe in details how to prepare permanent or temporary preparations with the addition of glycerin or alkaline solutions (for lighting). The identification is carried out till the lowest possible taxonomic level, by using specialized key identification guides [14-20].

The number of taxa are determined for each sample.

Indicator species have to fulfil a number of criteria: to be sufficiently spread over a large area, to play an important functional role in the ecosystem, to show a sufficiently rapid reaction to changes in environmental conditions, the ecology of the species must be sufficiently known.

In case of unfavorable conditions, the diversity of species in a biocenoses decreases, while the density of resistant species increases. With an increasing pollution level, the species sensitive to pollution (e.g. rheophilic, oxyphilic species, adapted to oligo- and oligo- β-mesosaprobe conditions), are decreasing in their numbers. These include, first of all, the larvae of insects of the orders Ephemeroptera, Trichoptera, Plecoptera. In moderately and heavily polluted waters, the representatives of Ephemeroptera, Trichoptera, Plecoptera are missing, except for the representatives of the Baetidae, Caenidae families. In the reference conditions, the number of EPT taxa (Ephemeroptera, Trichoptera, Plecoptera) shall not be below of 13-15 species. Some species-indicators of the oligosaprobic and oligo-β-mesosaprobic conditions are presented in Figs. 8.2-8.12, and of the polysaprobic conditions (species with a reduced sensitivity to water pollution, which increase their number in case of pollution) – in Fig. 8.13-8.14.

To mention that the below photos (Fig. 8.2-8.14) have been made in the Laboratory of Hydrobiology and Ecotoxicology of the Institute of Zoology with the use of SteREO Discovery V8 (Zeiss) and Axio Imager A.2 (Zeiss) microscope (Fig. 8.1) by Munjiu O.

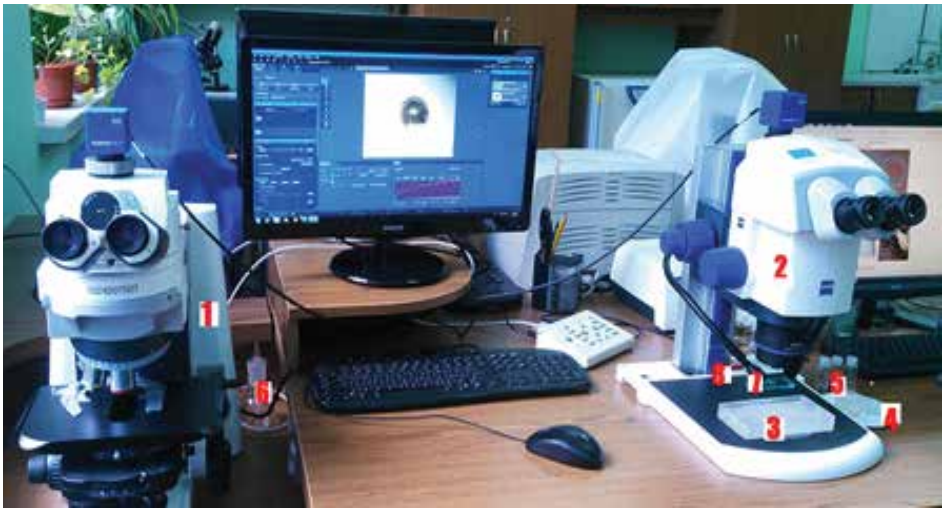

Fig. 8.1: 1. microscope; 2. binocular; 3. Bogorov chamber; 4. Petri dishes; 5. glass bottles, 10 mL; 6. vessel with alcohol; 7. microscope glass slide; 8. cover glass (Photo: O. Munjiu)

Fig. 8.2 Ephemeroptera *Ephemera vulgata* (*Linnaeus*, 1758) – oligo-, β-mesosaprobic, S=1.4

Fig. 8.3 Plecoptera *Brachyptera* sp.div oligosaprobic, S=0.90

Fig. 8.4 Ephemeroptera *Palingenia longicauda* (Oliver, 1791) – oligo-, β-mesosaprobic, S=1.3

Fig. 8.5 Trichoptera *Neureclipsis bimaculata* (Linnaeus, 1758), oligo-, β-mesosaprobic, S=1.4

Fig. 8.6 *Trichoptera Molanna* sp.div., oligosaprobic, S=1.00

Fig. 8.7 Trichoptera *Phryganea* sp.div none – oligo-, β-mesosaprobic, S=1.50

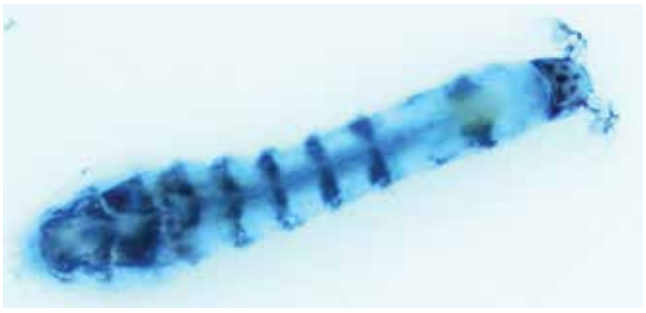

Fig. 8.8 *Simuliidae* – rheophilic, oligosaprobic, S=1.15

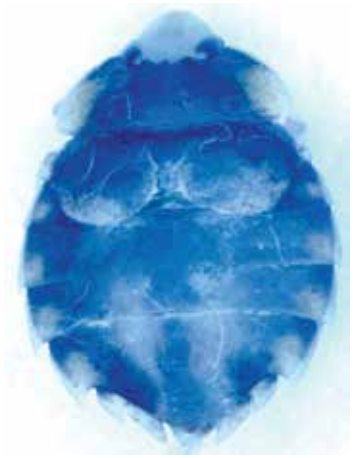

Fig. 8.9 *Aphelocheirus aestivalis* (Fabricius, 1803) – oligo-, β-mesosaprobic, S=1.5

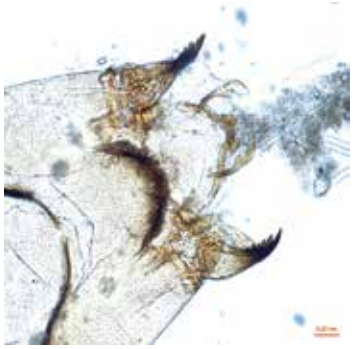

Fig. 8.10 *Diamesa* sp.div none –oligo, β-mesosaprobic, S=1.50

Fig. 8.11 *Chaetogammarus ischnus behningi* (Martynov, 1919) – oxiphylous

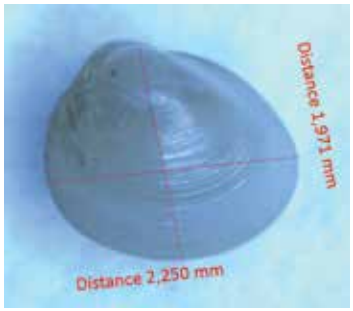

Fig. 8.12 *Pisidium casertanum* (Poli,1791) – oligosaprobic, S=1.15

Fig. 8.13 *Tubifex tubifex* (O.F.Muller) – polysaprobic, S = 3.80

Fig. 8.14 *Chironomus plumosus* (Linne,1758) – polysaprobic, S = 3.80

8.3. ASSESSMENT OF WATER QUALITY BASED ON MACROZOOBENTHOS COMMUNITIES

The evaluation of water quality according to macrozoobenthos indices is performed according to the methods approved for this purpose:

- TBI Index (abreviated from the Trent Biotic Index);
- EPT Index (Ephemeroptera, Plecoptera, Trichoptera);
- BMWP Index (Biological Monitoring Working Party);
- ASPT Index (Average Score per Taxon);
- number of species of macrozoobenthos (biodiversity);
- assessing the saprobity according to Pantle and Buck and its modification proposed by Zelinka and Marvan.

The simplest and most commonly used index is TBI, which was proposed by Woodiwiss in 1964 [cited in 21]. After determining the macrobenthos groups in the analyzed sample, at the intersection of the respective rows and columns, the value of the TBI Index is calculated (Tab. 8.1), then the water quality is evaluated (Tab. 8.2).

Table 8.1 Determination of TBI index [21]

Presence of the indicator group	The number of indicator species	Total number of groups of benthic organisms present in the samples				
		0-1	2-5	6-10	11-15	16+
Plecoptera larvae (Plecoptera)	More than one species	-	7	8	9	10
	One species	-	6	7	8	9
Ephemeroptera larvae (Ephemeroptera)*	More than one species	-	6	7	8	9
	One species	-	5	6	7	8
Trichoptera larvae ** (Trichoptera)	More than species	-	5	6	7	8
	One species	4	4	5	6	7
Gammarus	All above species are missing	3	4	5	6	7
Asellus	All above species are missing	2	3	4	5	6
Oligochaeta or Chironomidae larvae		1	2	3	4	5
All above species are missing		0	1	2	-	-

* excepting *Baetis rhodani*
** including *Baetis rhodani*

The list of groups of benthic macronevertebrates, developed by Woodiwiss, includes: Planariidae (with each species separately), Oligochaeta, Hirudinea, Mollusca, upper crustaceans, Plecoptera, Ephemeroptera, Trichoptera (to be calculated separately each family), Megaloptera, Chironomidae, Diptera larvae, aquatic species of Coleoptera, Heteroptera, Acariformes. However,

Woodiwiss considered as separate groups: oligochaete *Nais*, ephemeroptera *Baetis rhodani* and chironomid *Chironomus thummi*.

Table 8.1 is based on the research results, according to which at the occurrence of pollution and its intensification certain groups of macrozoobenthos disappear in a certain order. First, the most sensitive species – Plecoptera, Ephemeroptera and Trichoptera – disappear.

Table 8.2 The value of the TBI Index and the evaluation of water quality [22]

The value of TBI index	Saprobity zone
0-2	polysaprobic
3-5	α – mesosaprobic
6-7	β – mesosaprobic
8-10	oligosaprobic

Below we present an example of calculating this index. For example, the following macronevertebrates were identified in the sample: 1) Ephemeroptera: *Palingenia longicauda*, *Heptagenia sp.;* 2) *Gammarus sp*.: *Chaetogammarus sp.;* 3) Oligochaeta; 4) Chironomidae; 5) Mollusca.

In the given sample, no Plecoptera were detected, instead two species of Ephemeroptera were recorded, therefore only the third row of Table 8.1 is applied in this case (Ephemeroptera: more than one species). In addition to ephemeropteras, also molluscs, *Gammarus sp*., oligochaetes and chironomids, in total 5 groups, were identified in the sample. In conclusion, the value of the TBI Index calculated for this sample is 6 points. According to Table 8.2, this value of TBI Index indicates the investigated aquatic environment can be classified as a β – mesosaprobic zone, with moderately polluted water.

The EFA Index is calculated on the basis of species from Ephemeroptera, Plecoptera and Trichoptera families, which are very sensitive to pollution. The number of species is determined for each sample taken and then summed. For reference stations, the EPT Index is 13-15 species [22].

The BMWP system takes into consideration the level of sensitivity of invertebrates to pollution. The identified zoobenthic families are assigned a score between 1 and 10. The BMWP Index is calculated as the the sum of the values for all families present in the sample. Values higher than 100 are associated with clean streams, while the scores of heavily polluted streams are lower than 10 (Tab. 8.3). Based on the values of the BMWP Index, the water quality is evaluated (Tab. 8.4).

Table 8.3 The biological score attributed to different groups of zoobenthos by the Biological Monitoring Working Party [22]

	Taxa	Score
Ephemeroptera	Siphlonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae	10
Plecoptera	Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Chloroperlidae	
Hemiptera	Aphelocheiridae	
Trichoptera	Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae	
Decapoda	Astacidae	8
Odonata	Lestidae, Agriidae, Gomphidae, Cordulgasteridae, Aeshnidae, Corduliidae, Libellulidae	
Trichoptera	Psychomyiidae, Philopotamiidae	
Ephemeroptera	Caenidae	7
Plecoptera	Nemouridae	
Trichoptera	Rhyacophilidae, Polycentropodidae, Limnephilidae	

	Taxa	Score
Mollusca	Neritidae, Viviparidae, Ancylidae	6
Trichoptera	Hydroptilidae	
Bivalvia	Unionidae	
Amphipoda	Corophiidae, Gammaridae	
Odonata	Platycnemididae, Coenagriidae	
Heteroptera	Mesoveliidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae	5
Coleoptera	Haliplidae, Hygrobiidae, Dytiscidae, Gyrinidae, Hydrophilidae, Clambidae, Helodidae, Dryopidae, Elmidae, Chrysomelidae, Curculionidae	
Trichoptera	Hydropsychidae	
Diptera	Tipulidae	
Diptera	Simuliidae	
	Planariidae, Dendrocoelidae	
Ephemeroptera	Baetidae	4
Megaloptera	Sialidae	
Hirudinea	Piscicolidae	
Mollusca	Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae	3
Mollusca	Sphaeriidae	
Hirudinea	Glossiphoniidae, Hirudidae, Erpobdellidae	
Isopoda	Asellidae	
	Chironomidae	2
	Oligochaeta (whole class)	1

Table 8.4 The value of BMWP in evaluating of the water quality [22]

Value of the index	Water quality
>150	Very good
101-150	Good
51-100	Moderate
26-50	Poor
<25	Very poor

The ASPT Index is based on the same score allocation table as the BMWP, but the total score obtained is divided by the number of taxonomic groups identified in the sample: ASPT = BMWP / number of taxonomic groups. Depending on the value of the ASPT Index, the water quality is assessed: > 5 – excellently good; 4.5-4.9 – very good; 4.1-4.4 – good; 3.6-4.0 – not very good; 3.1-3.5 – moderated; 2.1-3.0 – poor; 0-2.0- very poor [22].

To assess the ecological status of water basins, the EQI Index is calculated as a correlation between the value of any parameter in the given sample collection station and the value of the same parameter in the reference station (Tab. 8.5).

To assess the diversity of macrobenthic species, the Shannon Index (1963) is applied, which is considered to be the most informative and convenient to use. Index values greater than 3 correspond to clean water, from 1 to 3 – moderately polluted water, equal to 1 – polluted water (dirty).

Table 8.5 The value of EQI indices based on ASPT and BMWP scores [22]

Water quality	EQI for ASPT	EQI for BMWP
Very good	1.00 and above	Less than 0.85 or above
Good	0.90-0.99	0.70-0.84
Relatively good	0.77-0.89	0.55-0.69
Moderate	0.65-0.76	0.45-0.54
Poor	0.50-0.64	0.30-0.44
Very poor	Less than 0.50	Les than 0.3

The Shannon Index provides a quantitative estimate of community structure and is calculated as follows:

$$H = -\sum n_o \cdot log_2 n_o,$$

where: n_o – the relative abundance of the species in the sample [22].

The method of assessing Pantle-Buck saprobity and its modification by Zelinka-Marvan requires the collection and processing of quantitative samples and the identification of species in the sample up to the species level or the lowest possible taxon, thus applying this method requires a lot of time and high professional skills [22]. The statistical confidence of the obtained results is ensured by the presence of at least 10-12 groups of indicator species.

The main lists of indicator species and their saprobic values, taking into account the regional peculiarities of indicator species, are available in the literature [13, 23]. For example, in the Laboratory of Hydrobiology and Ecoxicology of the Institute of Zoology a general table of indicator species is used, which includes over 800 names (Tab. 8.6).

Table 8.6 The general table of indicator species (fragment)

Indicator taxa	s_i	G_i	Bibliography
Chaetogaster spec. none	2.30	2.00	[25]
Chaetonotus maximus none	1.40	3.00	[23]
Chaetopteryx villosa Fabricius	1.30	2.00	[13]
*Chaoborus sp.*div none	2.25	1.00	[23]
Cheumatopsyche lepida Pictet	1.90	3.00	[13]
Chironomus anthracinus Zetterstedt	2.20	3.00	[13]
Chironomus plumosus (L.)	3.80	4.00	[13]
Chironomus semireductus none	2.30	4.00	[24]
*Chironomus sp.*div none	3.30	2.00	[13]
Chloroperla apicalis Newman	1.70	3.00	[13]
Chloroperla tripunctata (Scopoli)	0.70	2.00	[13]
Choroterpes picteti (Eaton)	1.80	4.00	[13]
Cincinna piscinalis (Mull.)	2.40	3.00	[13]
Cladotanytarsus gr.mancus Walker	1.50	3.00	[24]
Cleistosimulium argenteostriatum (Strobl)	0.30	4.00	[13]
Clitelio arenarius (O.F.Muller)	3.20	2.00	[25]
Cloeon dipterum (L.)	2.05	2.00	[23]

The Saprobic Index is calculated on the basis of data on the saprobic value, the indicative weight and the abundance of indicator species:

$$1)\quad S_i = \frac{\sum_i S_i \times A_i}{\sum_i A_i}, \qquad 2)\quad S_i = \frac{\sum_i S_i \times A_i \times G_i}{\sum_i A_i}$$

where: i = number of the i species; s_i = saprobic value of the i species; A_i = abundance of the i species; G_i = indicative weight of the i species; 1) Pantle–Buck; 2) Zelinka-Marvan modification.

The values of the Saprobic Index, calculated on the basis of macrozoobenthic organisms, depend not only on the water quality, but also on the types of substrate existing in the area of sample collection. This factor will be taken into account when comparing different samples. The values of the Saprobic Index are used to assess water quality (Tab. 8.7).

Table 8.7 The value of Saprobic Index and evaluation of water quality [11]

Water quality class	Water quality	Value of the Saprobic Index
I	Very good	≤ 1.8
II	Good	≤2.3
III	Moderately polluted	≤2.7
IV	Polluted	≤3.2
V	Highly polluted	> 3.2

The values of the Saprobic Index can vary from 0 to 4 and are interpreted on the Kolkwitz-Marsson scale as follows: 0-0.50 – xenosaprobic conditions, 0.51-1.50 – oligosaprobic, 1.51-2.50 – β -mesosaprobic, 2.51-3.50 – α-mesosaprobic, 3.51-4.00 – polysaprobic [21].

Equipment and materials required for the collection and processing of macrozoobenthos:

1. Grabs Ekman, Petersen
2. Dredge
3. Scraper
4. Fishnet
5. Gaz Mill №10,23,38
6. Frames
7. Artificial substrates
8. Thermometer
9. Analytic balance
10. Binocular
11. Microscope
12. White cuvettes
13. Jars of plastics or glass with thread cap (200-1000 ml)
14. Tweezers
15. Dissection needle
16. Pipette
17. Petri dishes
18. Bogorov chamber
19. 10 ml and 50 ml glass bottles (flasks)
20. Alcohol
21. Formalin
22. Microscope glass slides and covers
23. Cup
24. Rope
25. Field diary
26. Pencil
27. Key identification guides
28. Box for transporting of the samples

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IX FISH FAUNA

9.1. GENERAL PRINCIPLES OF THE MONITORING OF FISH FAUNA

Assessment of water quality only on the basis of physical and chemical parameters not always provides full information on the effects that pollution or damage of biotopes have on aquatic organisms or on the health of the ecosystem, and "pollution waves" may pass unnoticed between two samplings. In order to obtain a more complete picture of water quality, the assessment should be extended to biological components, which can store information at the structural and functional level, in time and space, etc.

It is almost impossible to carry out an integrated monitoring of abiotic and biotic parameters even in the simplest structured ecosystem, therefore, one of the most important objectives is to find alternatives, as efficiently as possible, to the complicated, meticulous, too expensive and often with delayed effect research.

Various groups of living organisms (from bacteria to mammals) are used to monitor the health of the environment, and investigations are performed at different levels of integration and organization of life (from intracellular to those higher than population).

The concept promoted by the Water Framework Directive (WFD) [5] on the surface water status is based on a new, integrative approach (ecosystem approach), which differs fundamentally from previous approaches to water quality – they relied mainly on physico-chemical parameters, the biotopic and biocenotic components being less taken into account. According to FWD, fish fauna becomes a mandatory element in assessing water quality. In this way, a new approach to good ecological status is launched in terms of ecosystem health and low levels of chemical pollution.

According to WFD, the assessment of the ecological status of aquatic systems is based on biological, physico-chemical and hydromorphological elements. The ecological status is specific to each type of ecosystem (lotic, lentic, large, medium or small) and, based on comparison with the reference conditions (undisturbed, real or hypothetical), it can be characterised as high, good, moderate, poor or bad.

The main approaches in monitoring of quality biological elements are the following:

- saprobic approach – a widespread method based on the Saprobic system developed by Kolkwitz and Marson (1908, 1909);
- diversity approach, which has three components: species richness (number of species), distribution (spatial characteristics) and abundance (quantitative characteristics);
- biotic approach – a complex method of assessing the state of ecosystems based on the state of different ecological groups (ecological guilds) in relation to the environment; with this purpose, all the species, which are characteristic for the given type of habitat and exploit the natural resources in a similar way, are taken into account and classified according to the mode of nutrition, reproduction, tolerance to the alternation of environmental gradients, etc. (this information will serve as input data in the bioindication process);
- functional approach, which is focused on biochemical, physiological processes, etc.

All these approaches are important and useful in the process of ecological monitoring, but obtaining of a more complete picture requires their complex use, each method having as advantages, as some eloquent difficulties. For example:

1. in the saprobic approach the emphasis is put mainly on organic environmental pollution, while currently anthropogenic pollution has a much more complicated composition; in addition, if an ecosystem is affected by several factors of chemical stress, the use of saprobic approach becomes difficult, because indicator species respond differently to different sets of stressors;
2. in the diversity approach the main postulate focuses on the interaction and strong interdependence between biotopic and specific diversity, while ecosystems will a low diversity of habitats, but not affected from anthropogenic point of view may exist;

3. in the case of the biotic approach, fish, as an object of study, are characterized by a fairly expressed mobility, therefore they can significantly distort the results, if a local or short-term pollution in a medium or large aquatic ecosystem occurs;
4. the functional approach is based, in particular, on studies at the level of organism and even at lower organisation levels of life, what proved to be less representative in the case of insignificant pollution, fish, for example, showing a wide response rate, with a high potential for rapid recovery.

It is not sufficient to know if there are fish in water or not. It is necessary to know what kind of fish are there, how numerous and healthy they are. Some of the most common problems that fish have to face are: oxygen deficiency and organic pollution, thermal pollution, phonic pollution, various obstacles to migration, pollution with persistent synthetic compounds, radioactive pollution, etc. Fish can also be used indirectly as bioindicators, namely by the presence of certain parasites, their degree of invasion, functional state, etc. [24, 26].

In most cases, sublethal concentrations of pollutants are registered in the natural aquatic ecosystems of the Republic of Moldova, excepting the uncontrolled cases, when the large amount of pollutant and the short discharge time cause catastrophic ecological situations. Namely, the negative changes which provoke at first sight "invisible" reactions occur at various levels of life organization. The specialist must identify them operatively and interpret them correctly.

A special attention in the monitoring process is paid to the action of toxicants on the fish fauna. Pollutants have a direct negative influence at various levels of integration and organization of life:

- molecules – structural degradation, additional disintegrations of ATP;
- cells – inhibition of synthesis and self-regulation processes, cell autolysis, disorders in the process of cell division, etc.;
- tissues and organs – worsening of food assimilation, detachment and deformation of muscle fibres, pathologies of excretion, reproduction systems, disorders of the immune system, etc.;
- organisms – delayed growth, various pathologies, decreased reproductive capacity, death;
- populations – decrease of the number of individuals, reduction of the age structure, increase of the share of females in the population, etc.;
- ichthyocenosis – simplification of species structure and disappearance of sensitive species, dysfunction of trophic relationships, etc. [27].

The toxicants accumulate in organisms in certain places, depending on their physico-chemical peculiarities and the degree of affinity with the biological substrates. The knowledge of these regularities is very important in the diagnostic process. Usually, the liposoluble chlororganic pesticides accumulate in high fat organs, phosphororganic compounds (phosphororganic pesticides) – in parenchymal organs, detergents – in the gills and walls of the digestive tract, heavy metals – in epithelial tissue, liver, gills, etc. [18 -20].

As result of the ecotoxicological monitoring of the main aquatic ecosystems of the Republic of Moldova, which consisted of highlighting the sources, concentration, migration and impact of heavy metals in the system "water – solid suspensions – silts – hydrobionts", a very important conclusion was made, which is often neglected in standards, systems and methods for assessing environmental quality: set up values for assessing the well-being of aquatic ecosystems, based on CMA (maximum allowable concentration), not always work. In the case of heavy metals, their necessary and vital important concentration and that with toxic effect, and even lethal one for hydrobionts, can be placed in a very narrow range of values. Moreover, the same concentration can be optimal for a group of hydrobionts and, opposite, lethal for another. The same concentration of a heavy metal can have various effects in different aquatic ecosystems, even in the same ecosystem, if environmental parameters change (water hardness, pH, dissolved oxygen, antagonistic/synergistic interactions between chemical elements, temperature, etc.) [20].

Based on the assessment of the influence of 14 heavy metals on production-destruction processes and decipher of the regularities of metal accumulation in hydrobionts, a new concept on assessing the well-being of aquatic ecosystems was elaborated. According to this concept, the concentrations of heavy metals which not influence the production-destruction processes in

the ecosystem are considered „optimal", those that condition the insignificant decrease in the intensity of these processes are considered "admissible", and concentrations which suddenly decrease not only primary production of phytoplankton, but also the destruction of organic matter are considered „critical". Correspondingly, the status of aquatic ecosystems refers to the categories "good", "moderately polluted", "heavily polluted" or "degraded" [cited after 2].

Within this methodology, there were also taken in account the concentrations that were "favourable", those that influenced "moderately" or even became "toxic" for different species of hydrobionts, including fish, at different ontogenetic stages.

At the level of organs and organ systems, under the influence of pollutants, the most eloquent morpho-functional changes are found in the liver, kidneys, spleen, reproductive, nervous and endocrine systems. The liver plays an important role in the process of detoxification of pollutants. As rule, under harmful conditions, the relative weight of this organ increases. The highest values are found in geographical areas with chronic pollution, where the weight of the liver can increase by 5-7 times compared to normal one (Fig. 9.1).

Fig. 9.1 Hepatic hypertrophy at *Carassius gibelio* (Bloch, 1782)

As consequence of particularly high doses of toxicants, degenerative processes predominate over those of defence, which, however, not stop; necrosis processes take place actively, as does the degeneration of lipids into hepatocytes and their replacement with connective tissue. The kidneys also participate in the detoxification of the body and in polluted environments they react by hypertrophy and sensitization of biochemical defence mechanisms [22, 23]. Mobilization of lipid metabolism through the process of active accumulation of fat in the fish body plays an important role in toxic environments. Through this mechanism, the body prepares for possible unfavourable conditions by slowing growth and active accumulation of energy reserves. Increase of the fattening coefficient demonstrates the "high energy payment" of fish body for detoxification and survival [22].

If the adaptive potential is unable to withstand the impact of factors, numerous teratogenic changes occur, expressed by harmful mutations (morphological, physiological or biochemical), with often lethal effects: deformation of the fins and vertebral column, with deregulation of swimming capacity), dysfunction of vision, the "pug" (dog breed) head shape, underdevelopment of mouthparts, and, especially, of the lower jaw, gills, etc. [26] (Fig. 9.2).

At the level of the reproductive system, the polluting factor can cause multiple significant dysfunctions. As example, the total resorption of sexual products in the phases of trophoplasmic growth may indicate a sudden worsening of nutrition conditions, overpopulation, the influence of toxicants, inaccessibility to spawning grounds, etc. [30, 31].

Fig. 9.2 Diversity of morphological pathologies and their frequency in ichthyocenosis becomes an important indicator of the intensity of anthropogenic impact in the ecosystem

In all cases, the response of the reproductive system to the action of environmental factors is very diverse and depends, in large part, on their intensity and the bio-ecological characteristics of the taxon. The most common disorders of reproductive system in fish collected in different aquatic

ecosystems of the Republic of Moldova are: asymmetric development of gonads, their abnormal shape, early sexual maturation, modification of the duration of oogenesis and spermatogenesis, shifting timing of reproduction, cases of mass resorption of sexual products in their final stages of growth and development, decrease of fertilization capacity, decrease of the share of individuals capable to reproduce, abortion of eggs with lysis of follicular membranes, etc. [15, 28, 29].

Also, in unstable ecological conditions, there is an increase in the share of interspecific hybrids in the ichthyocenoses of the aquatic ecosystems of the Republic of Moldova. Unfavourable conditions during the reproduction period can cause disturbances in the process of gametogenesis and, respectively, the modification of the spawning terms. As consequence, when favourable conditions return, there may be overlaps in the reproduction of several species of fish in the same spawning area, which leads to interspecific hybridization (a phenomenon with increasing frequency in the Dniester River after the construction of the Novodnestrovsk dam).

At the individual and population level, the structural-functional state of fish is evaluated using various ecological, ichthyological classical and modern methods, unanimously recognized [1, 3, 4, 8, 11, 12, 14, 16, 17, 21, 25].

Examination of fish is performed usually by their counting, measuring and weighing; in order to determine their age, the sampling of scales or other hard tissues (operculum, otolithes, pharyngeal teeth) is made; the identification of sex and stage of development of gonads are performed; the degree of infestation with parasites and the frequency of individuals with malformations are established.

In order to put in evidence the fish growth rate, a series of biometric measurements are done. The following characteristics are determined via biometric studies:

- metric characteristics: length, thickness;
- gravimetric characteristics: total weight, weight without viscera, weight of ingested food;
- meristic characteristics: number of scales in the linea lateralis, number of fin rays, number of gill rakers, etc.

The specialized literature (identification guides) is used to determine the taxonomic affiliation [1, 8, 16, 21]. Individuals with unclear characters (e.g. hybrids, juveniles) are preserved in 4% formalin solution and identified later in the laboratory conditions.

9.2. CALCULATION OF SOME INDICES AND COEFFICIENTS BASED ON FISH EXAMINATION

Based on biometric measurements, the following biometric indices and coefficients are calculated:

- profile index – ratio between the total body length (L) and the body height (H);
- thickness index – ratio between the maximum body thickness (G) and the total body length (L) multiplied to 100; the higher this index is, the more valuable from economic point of view the fish is;
- circumference index (Kiselev Index) – ratio between the body standard length (l) and the body circumference (C);
- fattening coefficient (Fulton Coefficient) – ratio between the body weight (W) and the cube of its body standard length (l), multiplied to 100; this coefficient is also called „condition coefficient" – the better the fish feeds, the higher the value of this coefficient is.

For determining the fish age the anatomic method is used, which is based on the analysis of scales, bones and other rough organs.

Determination of fish sex ratio has both theoretical and practical importance in revealing the reproductive capacity of fish populations in the studied ecosystems.

As a close correlation exists between gonad weight, egg number and body weight, different coefficients, e.g. fertility coefficient and gonadosomatic ratio, are calculated with the purpose of determining the female prolificacy.

Fertility coefficient (Fc) or Behning Index is calculated for female broodfishes after their sacrification and counting of number of eggs in ovaries. Calculation is made by the following formula:

$$Fc = \frac{l \times W}{N},$$

where: l – body standard length, W – body weight, N – total number of produced eggs.

Gonadosomatic ratio or Gonadosomatic Index (GSI) is based on the correlation between the ovarian weight and female body weight and the following formula is applied:

$$GSI = \frac{OW \times 100}{W},$$

where: OW – ovaries weight, W – body weight.

The Bertanlanffy growth equation can be used to estimate the growth rate of different fish species. The calculation of the growth parameters k and t_0 can be performed by prior fixation of the value of l_∞ as input value [11]. This method has been used by authors in some previously published scientific papers, especially for species with short life cycle, for which the maximum empirical gravimetric values correspond to reality, their populations having a complete and well-balanced structure. Value of l_∞ can be also found in unanimously recognized scientific sources (e.g., www.fishbase.org).

Also, there is applied the Ford-Walford equation, which allows estimating the maximum theoretical physiological increments in real and concrete conditions (time and space), based on empirical data [33]. The Ford-Walford equation requires the calculation of the value of l_∞, used for the description of the Bertanlanffy growth equation.

Thus, the length of fish at age t will be calculated according to the equation:

$$l_t = l_\infty (1 - e^{-k(t-t_0)}),$$

and, correspondingly, the weight of fish body at age t will be calculated according to the equation:

$$w_t = w_\infty (1 - e^{-k(t-t_0)})^3,$$

where: l_t – standard length of fish at age t;
w_t – weight of fish at age t;
l_∞ – maximal theoretical length of fish;
w_∞ – maximal theoretical weight of fish, g;
k – constant of growth;
t_0 – theoretical age when the length of fish is equal to ”0”;
e – base of natural logarithm.

Following the application of a series of mathematical transformations, the given equations can be brought to the following linear equations:

$$l_{t+1} = a + bl_t,$$
$$w_{t+1}^{\frac{1}{3}} = a + bw_t^{\frac{1}{3}},$$

For the calculation of coefficients a and b, the method of least squares was applied:

$$a = \bar{y} - b\bar{x},$$

$$\mathrm{b} = \frac{n\sum xy - \sum x \sum y}{n\sum x^2 - (\sum x)^2},$$

From analytical point of view, the correlation between the length and weight of fish is descried by the equation:

$$w = a \cdot l^b,$$

where: w – body weight, g;
l – standard length of fish, cm;
a – constant equal to w when $l = 1$;
b – exponential coefficient.

Correlation coefficient r_{xy} was calculated according to the equation:

$$r_{xy} = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}},$$

The exponential coefficient b = 3, if the geometric similarities (harmonic balance) of the body shape are maintained during the fish growth. If b> 3, then positive allometry is found, and b <3 indicates a negative allometry (favouring the increase in length).

For calculation of the parameters *a* and *b* of equation, the method of least squares (its linear logarithmic form) is applied:

$$lgw = a + blgl.$$

As result of calculations, a series of data are obtained regarding the fish growth in a given ecosystem. These data allow making a comparison between populations of the same species in different ecosystems/water bodies (different ecological conditions), or between populations of different species from the same water body (similar ecological conditions).

When assessing the age structure of populations, it is sufficient to undertake measurements on a sample of at least 30 individuals/species (especially in the case of rare species), but the accuracy of data representation increases with the increase of the number of measurements [3].

At individual and population level, under the influence of anthropogenic pressing, there are various structural and functional dysfunctions, this being an important aspect in the biomonitoring process.

In conditions of a high toxicity, but with sublethal effect, the abundance of all age groups decreases, being selected only tolerant individuals with a slow growth rate (for example, in heavily polluted areas of small rivers). Under stressful conditions, such as anthropogenic pollution, small sized species with high population densities (*r*-strategic) predominate, while in unaffected environments the share of large sized K-strategic species, which demonstrate relatively low population densities, increases [2, 34].

Data on well-studied fish species (e.g., *Abramis brama, Rutilus rutilus, Aspius aspis, Perca fluviatilis, Esox lucius,* etc.) in different environmental circumstances can be very valuable in the process of biomonitoring and bioindication. In this respect, the statistical analysis of morphometric and meristic parameters (with the determination of dispersion, standard deviation, standard error, coefficient of variation, Student's t-test in comparative analysis, etc.) can reveal an important ecological picture of environmental welfare.

In the case of large fluctuations of environmental gradients and high fishing pressure, the use of the K-type strategy is not biologically justified – late maturation, spending of large amounts of energy on somatic growth and long life cycle increase the chances of being caught before reproduction, which means before contributing to the perpetuation of the species.

Appearance of dwarf forms is a compensatory reaction mechanism, in order to maintain the population reproductive potential of the species despite the negative changes of environmental factors [32].

As result, early-maturing coastal ecofenas pass easily through the meshes of fishing nets and get advantage in access to spawning grounds, compared to the fast-growing deep forms, which are often caught until they reach the spawning grounds, in this way the humans acting as an indirect factor of artificial selection [2].

In the current ecological conditions of intensification of the anthropic pressing and modification of the abiotic conditions, substantial changes are also found at the level of the ichthyocenosis structure.

Studies on polluted ecosystems have shown that chemical stress tends to be accompanied by a reduction in biomass, abundance and species richness, compared to undisturbed ecosystems. Many researchers have tried to use these structural indices as tools for monitoring and assessing the state of ecosystems, because they express some quantitative relationships and some relationships between groups of species inside a biocenosis and, thus, allow a more complete and correct characterization of the structure and role of different species in the biocenosis functioning, as well as the comparison between biocenoses (in our case, of ichthyocenoses).

9.3. SYNECOLOGICAL ANALYSIS

In deciphering of the relationships established between different species within the ecosystem and hierarchies within the ichthyocenoses, the application of a set of mathematical methods, which are known as synecological analysis, is of huge help. This type of analysis allows us to accurately identify the species which have the largest share in the ecosystem, in terms of energy exchanges with the environment, the characteristic species of a biotope, or species that have accidentally penetrated into the researched area; also, the interrelationships between the species that make up the biocenosis can be established with enough precision [3, 4, 7]. The finding of the disappearance or appearance of some species in biocenoses, especially of the indicator ones, can serve to highlight the direction of ecological successions, which means the species richness can become an important tool in the bioindication process. However, for a more detailed analysis of the cenotic structure, it is necessary to undertake quantitative investigations, which allow detecting the most insignificant changes.

Currently, more than 20 indicators are proposed, which characterize the properties of diversity: 1) the higher number of species leads to higher biodiversity; 2) the value of biodiversity is directly proportional to the equitability of representation of the abundance of species in the biocenosis.

In this situation, the researcher has to make the choice on the most appropriate indices to be applied in the study.

Depending on how they are calculated, the ecological indices are divided in two distinct categories:

1. analytical indices, based on the raw field data;
2. synthetic indices, based on analytical indices; synthetic indices are used to highlight the interrelationships between species, communities or cenoses.

Numerical abundance (A) reflects the absolute number of individuals of a species, which are present in a control capture. The assessment of numerical abundance is important for putting in evidence the role and share of the species in the given ecosystem. For this purpose, five classes are used: 0-absent; I- rare; II-relatively rare; III-abundant; IV-very abundant.

Relative abundance (Ar) expresses in percentages the participation of each species to the studied ichthyocenosis and is calculated according to the equation:

$$Ar = \frac{n}{N} 100,$$

where: n – number of individuals of species A in control capture, N – total number of individuals (of all species) in control capture.

The same five classes, as in the case of numerical abundance, are used: 0 – absent (Ar ranges 0-10%); I – rare (11-30%); II – relatively rare (31-50%); III – abundant (51-70%); IV – very abundant (71-100%).

Frequiency (F) reveals the number of samples, in which a species was registered, in relation to the total number of samples. According to this index, the species are classified as: very rare (below 10%), rare (10-29%), relatively rare (30-49%), relatively common (50-69%), common (F exceed 70%).

Constancy (C) indicates the continuity of a species appearance in a given biotope. It is a structural indicator, which shows the contribution of a species to the building up of the structure of biocenosis. The values of constancy are expressed in percentages. It is considered that the higher the value of constancy is, the better the species is adapted to the conditions offered by hydrobiotope. The following equation is used:

$$C = \frac{p}{P} 100,$$

where: p – the number of samples (control fisheries) in which the species A is found, P – total number of carried out control fisheries.

In dependence on the values of constancy, there are four species classes: C1 – accidental species (C< 25%), C2 – accessory species (C= 25.1 – 50%), C3 – constant species (C= 50.1 – 75%), C4 – euconstant species (C> 75%).

Dominance (D) is used at the percentage representation of individuals of one species reported to the number of individuals of all species, thus expressing the relative abundance. It is an indicator of production, which demonstrates the share of each species in the formation of biomass. It is calculated using the equation:

$$D = \frac{Ni}{Nt} 100,$$

where: *Ni* – number of individuals of species *A*, *Nt* – total number of individuals of all species.

In dependence with the obtained values of dominance, the species are classified in the following classes: D1 – subrecedents (D<1.1%), D2 – recedents (D= 1.1- 2%), D3 – subdominants (D= 2.1 – 5%), D4 – dominants (D= 5.1-10%), D5 – eudominants (D > 10%).

Ecological Significance Index (W) represents the correlation between the structural indicator (constancy, C) and production indicator (dominance, D) and reflects eloquently the position of species *A* in ichthyocenosis. It is calculated according to the equation:

$$W = \frac{C_A \cdot D_A \cdot 100}{10000},$$

Based on this index, the species are divided into five classes: W1 (W< 0.1 %), W2 (W=0.1 – 1%), W3 (W= 1.1 – 5 %), W4 (W= 5.1 – 10 %), W5 (W>10%). Class W1 corresponds to accidental species, classes W2 and W3 – to accessory species, and classes W4 and W5 – to species characteristic for given biocenosis.

Simpson Index (Is) estimates the probability that two randomly drawn up individuals from an ichthyocenosis belong to the same species. It is calculated according to equation:

$$l_s = \sum_{i=1}^{s} p_i^2$$

where: p_i – share of individuals by which the species *i* is present in biocenosis.

Diversity Index (H(S)) or *Shannon – Wiener Index* represents the ratio between the total number of species in an ichthyocenosis and total number of individuals. It reflects the mode of cenotic organization and the degree of structural stability of ichthyocenoses. True diversity is calculated according to the equation:

$$H(S) = \frac{K}{N(Nlog_{10}N - \Sigma_{r=1}^{S} Nrlog_{10}Nr)},$$

where: *K* – conversion factor for changing the base of logarithm from 10 to 2, which is equal to 3.321928, *N* – total number of individuals, *Nr* – number of individuals of species *r*, *S* – total number of species.

Equitability (e) is calculated by equation:

$$e = \frac{S'}{S},$$

where: S' – theoretical number of species, expressed by H(S), *S* – observed number of species.

The use of diversity indices in the bioindication process must be done with great discernment. For example, it is found that in the case of an increasing dynamic gradient (e.g., intensifying pollution), the specific diversity expressed only by the Shannon index may not decrease continuously. Opposite, often an increase in diversity can occur. This is the result of increased equitability, due to the diminution of population abundance of dominant species, which have proven to be the most sensitive to pollution.

Aquatic ecosystems are heavily affected by chemical stress, due to the tendency of pollutants to distribute homogeneously and rapidly in the active mixing zone. Under these conditions, the modification of the chemical particularities of the environment will eliminate some sensitive species and benefit others more resistant.

Chemical stress can be expressed by replacing "more competitive, but more sensitive" species with stress-tolerant species. In some cases, there may occur a real "blooming" of opportunistic species, which are normally excluded or marginalized by competition or predation.

In the current ecological conditions, when the anthropogenic pressure on the natural aquatic ecosystems of the Republic of Moldova is maintained, demonstrating an already chronic character, the changes at the level of ichthyocenosis structure are the most noticeable. In addition to the reduction of species diversity, with the "irreversible loss" of stenobiont fish species, the numerical supremacy of small sized eurytope species, highly prolific and competitive, with a high expansive potential (e.g., *Alburnus alburnus, Pseudorasbora parva, Carassius gibelio, Rutilus rutilus, Blicca bjoerkna*, etc.) is registered.

Assessment of the environment quality can be done not only based on species, which are hypersensitive to changes of environmental factors, but, also, based on the presence in the hydrobiotope of the species resistant to pollution, their abundance being an indicator of unfavourable ecological status. In this way, the phenomenon of bioinvasion can, also, be used to assess the quality of aquatic ecosystems. The most representative species of fish and their associations in the ichthyocenoses of small rivers from the Republic of Moldova, which can serve as models in the assessment of the ecological conditions, are presented in Tab. 9.1.

Table 9.1 Species and associations of bioindicators in the ichthyocenoses of small rivers from the Republic of Moldova

Ichthyocenosis not affected by anthropic factor		**Ichthyocenosis with moderately anthropic modifications**		**Ichthyocenosis intensively affected by anthropic factor**	
Dominant species	Associations of dominant species	Dominant species	Associations of dominant species	Dominant species	Associations of dominant species
Gobio sp., Romanogobio sp., Barbatula barbatula, Phoxinus phoxinus, Squalius cephalus, Cottus sp, Alburnoides bipunctatus, Salmo trutta, Thymallus thymallus	*Gobio sp., Romanogobio sp., Barbatula barbatula*	*Alburnus alburnus, Perca fluviatilis, Gymnocephalus sp., Rhodeus amarus, Neogobius fluviatilis, Squalius cephalus, Leuciscus leuciscus, Cobitis sp.*	*Perca fluviatilis , Gobio sp., Romanogobio sp., Gymnocephalus sp.*	*Carassius gibelio, Pseudorasbora parva, Cobitis sp., Perccottus glenii, Rutilus rutilus, Pungitius platygaster*	*Carassius gibelio, Pseudorasbora parva, Cobitis sp.*
	Squalius cephalus, Alburnus alburnus, Gobio sp., Romanogobio sp.		*Gymnocephalus sp. Neogobius fluviatilis, Perca fluviatilis*		*Carassius gibelio, Pseudorasbora parva, Pungitius platygaster*
	Alburnoides bipunctatus, Alburnus alburnus, Gobio sp., Romanogobio sp.		*Alburnus alburnus, Perca fluviatilis, Rutilus rutilus*		*Lepomis gibbosus, Pseudorasbora parva, Carassius gibelio*
	Phoxinus phoxinus, Cottus sp., Salmo trutta , Thymallus thymallus		*Rhodeus amarus, Perca fluviatilis*		*Carassius gibelio, Rutilus rutilus*
			Rhodeus amarus, Gobio sp., Romanogobio sp., Cobitis sp.		*Perccottus glenii, Cobitis sp.*
					Perccottus glenii, Rutilus rutilus, Carassius gibelio

This classification is based more on the principles of the Saprobity system developed by Kolkwitz and Marson, who used aquatic invertebrates as study objects. In the same time, this classification, in addition to the advantage of a quick and simple assessment, has disadvantages, one of them being the fact that the assessment criterion relies on organic pollution, while currently the character of anthropogenic impact is much more complex (thermal, radioactive, toxic, hydromorphological, etc.).

Some species such as *Alburnus alburnus, Neogobius fluviatilis, Carassius gibelio, Perca fluviatilis, Rhodeus amarus, Pseudorasbora parva*, etc., because of their exceptional hydrobiotic potential, can demonstrate high quantitative values in different types of ecosystems, including those with a different anthropic pressure.

This classification of the quality of small river ecosystems according to fish bioindicator species is not a rigid one and is characterized by fairly wide variability limits. The cause, as was

mentioned above, consists not only in the sudden changes in the gradients of environmental factors in the last period, but also in the different response of fish species, depending on their reaction norm, namely, their adaptive potential.

By highlighting the changes provoked in the structure of ichthyocenoses of large and middle rivers (within the boundaries of the Republic of Moldova), as result of anthropogenic interference of hydrobiotope (fragmentation, clogging, eutrophication) and excessive selective fishing, six ecological groups of fish were identified. By their significant contribution to the ichthyocenotic structure, these fish ecological groups reflect the three qualitative levels of ecosystem condition/status (I – good, II – fair, III – poor) (Tab. 9.2).

Table 9.2 Characteristic ecological groups of fish for the different levels of ecological condition of lotic, medium and large ecosystems from the Republic of Moldova

Good condition	I. Lithophilic and cryophilic rheophilic potamodromous fish species with large spawning grounds in upstream sectors of the Dniester and Prut Rivers (within the boundaries of the Republic of Moldova) – *Chondrostoma nasus, Leuciscus leuciscus, Barbus petenyi*, *Barbus barbus, Acipenser ruthenus, Squalius cephalus, Lota lota, Salmo trutta fario, Oncorhynchus mykiss, Thymallus thymallus, Alburnoides bipunctatus, Phoxinus phoxinus*. The presence of these species in sufficient quantities indicates the appearance of a hydrobiotope little affected anthropically by the processes of clogging, eutrophication and fragmentation. II. Migratory and semi-migratory large and medium sized fish species, which breed in the riverbeds – *Huso huso, Acipenser stellatus, Acipenser gueldenstaedtii, Acipenser nudiventris, Alosa immaculata*; among semi-migratory fish *Pelecus cultratus* shall be firstly mentioned. Nowadays, the populations of these species have been substantially reduced in the Dniester and Prut Rivers, due to the worsening of the reproduction conditions, with the clogging of the sandy-stony substrate (lithophilic and psammophilous species) or of the modification of the flow regime in dammed sectors (pelagophilous species). Pelagophilous species record a dramatic decrease in population prolificacy, due to decrease of the number of laid down egg portions (e.g., *Alosa immaculata*) and increased mortality of floating eggs, as result of decreased flow rate (e.g., *Pelecus cultratus, Alosa immaculata*).
Fair condition	III. Semi-migratory species, which have adapted reproductively to the modification of abiotic conditions – *Rutilus heckelii, Abramis brama, Vimba vimba, Sander lucioperca*. Some representatives formed opportunistic local populations (*Rutilus heckelii* in Dubasari reservoir, *Vimba vimba* in Costeşti-Stanca reservoir), and others joined the group of potamodromous or sedentary species, making short reproductive migrations only within the riparian ecosystems (*Sander lucioperca, Abramis brama, Cyprinus carpio, Vimba vimba*). IV. Indigenous limno-rheophilic, potamodromous or sedentary species of medium and large size – *Cyprinus carpio, Silurus glanis, Aspius aspius, Esox lucius, Abramis brama, Sander lucioperca, Leuciscus idus*. In the past, these species actively used floodplains, meadow lakes and communication channels for reproductive or nutritional purposes, often transiting between these two types of aquatic ecosystems (lentic and lotic). Nowadays, however, due to the drastic reduction of wetland areas and overfishing during migration, the representatives of this group have become numerically vulnerable, most of them switching to a sedentary reproductive lifestyle.
Poor conticion	V. Opportunistic freshwater species, allogenic or indigenous, usually, sedentary from reproduction point of view, polyphilous or phytophilic, small and rarely medium sized species. Such species as *Pseudorasbora parva, Percottus glenii, Lepomis gibbosus* and *Carassius gibelio* shall be firstly mentioned as representatives of the group of allogenic and intervenient fish species. The group of opportunistic indigenous species includes *Alburnus alburnus, Blicca bjoerkna, Perca fluviatilis, Rutilus rutilus, Gymnocephalus cernua, Rhodeus amarus, Cobitis* sp. The presence of fish species from this ecological group in exaggerated quantities reveals, first of all, changing environmental conditions, with large alternations of values of environmental gradients, active processes of limnification of riparian ecosystems and an exaggerated selective fishing pressure. VI. Intervenient fish species, with opportunistic reproduction types (e.g., presence of brood pouch, construction of nests, etc.), nutrition types (can switch easily to predation, malacophagy), small sized, with a large reaction norm in relation to abiotic gradients – *Gasterosteus aculeatus, Pungitius platygaster, Syngnathus abaster*, *Gobiidae*, *Atherina boyeri, Clupeonella cultriventris*, etc. Due to changes of abiotic conditions in these rivers, as result of multiple anthropogenic hydrobiotope fragmentation (having many negative secondary consequences, such as decreased flow rates, increased mineralization, clogging of substrates, spreading of aquatic vegetation, etc.), marine and liman species have advanced upstream of these rivers. The „rule of supremacy of fish species with a predatory type of nutrition in marine ecosystems" offered them considerable advantages in the freshwaters of the Republic of Moldova, which are rich in prey and devoid of native ichthyophagous species (as result of intense selective fishing), having dramatic consequences on the diversity of hydrobionts and the bioproductive potential.

It can be stated that the acute lethal pollution leads to the total extinction of species, regardless of the stage of ecological succession of ecosystem, with the subsequent establishment of "toxicoresistant pioneer" species, which often have an invasive effect. Instead, in the case

of a chronic chemical exposure, the selective removal of sensitive species takes place, with the substitution of dominant species by opportunistic ones. Changes occur quickly if the dominant species are very sensitive to disturbing factors. If the exposure time is long enough, the regression tendencies can become irreversible even after the stress factor is removed.

The degree of invasion of various parasites in the ichthyocenosis can significantly contribute to the process of monitoring of the state of aquatic ecosystems. In some ecosystems significant anthropogenic pressure causes the accumulation of pollutants in hydrobionts, which, in turn, reduces their degree of resistance in the host-parasite relationship, often causing epizootic states [2].

The possibility of using parasites in fish as bioindicators is justified by their double influence: from the external environment and from the host organism.

Thus, one of the essential factors, which influences and determines the degree of parasitic invasion in fishing communities, is the physiological state of the host organism. As example, if the fish nutrition conditions have worsened in an aquatic ecosystem, then individuals will enter the winter period weakened, which will make them especially vulnerable in spring, when the parasitoses start to activate (Fig. 9.3).

Fig. 9.3 Weakened after wintering fish are easily attacked by *Saprolegnia sp.*

Among the most significant factors, which have currently stimulated the spread of ichthyozooanthropocenoses in the conditions of the Republic of Moldova, can be listed [24]:

1. decrease of the flow speed of water streams, as result of the multiple anthropic fragmentation of the riverbed;
2. excessive development of aquatic vegetation, which, in turn, determines the modification of the hydrological and thermal regime of rivers, organic and mineral pollution, intense clogging of biotopes;
3. active eutrophication of aquatic ecosystems leads to an increase in the abundance of final, intermediate and complementary hosts (planktonic crustaceans, molluscs, oligochaetes, fish, etc.), ensuring the success of the finality of the life cycles of fish parasites;
4. reduction of flood areas, which leads to the concentration of birds (hosts) on limited areas and to the active contact of contaminated individuals with healthy ones;
5. illicit fishing and the decrease in ichthyophagous species in competition with large fluctuations in environmental gradients cause the excessive numerical development of small sized fish, subsequently serving as important vectors for the transmission of parasitoses;
6. catastrophic sanitary and ecological state of reservoirs and of their adjacent areas;
7. active self-expansion and anthropochoria of allogenic species;
8. poor infrastructure of the epidemiological system (monitoring, prophylaxis and counteracting of parasitoses), etc.

9.4. EVALUATION OF THE CONDITION OF NATURAL AQUATIC ECOSYSTEMS BASED ON THE STRUCTURAL-FUNCTIONAL STATE OF THE FISH FAUNA

According to the Water Framework Directive, there were elaborated principles of assessment of the condition of natural aquatic ecosystems based on the structural and functional state of fish fauna (with some additions of the authors), by using of 5 quality categories [2, 3, 5].

1. High condition- the composition and abundance of species correspond entirely or almost entirely to the anthropically unchanged conditions. Migratory and semi-migratory species are present in optimal quantities, as well as the local limnophilous and rheophilous stenobionts,

which are sensitive to anthropic disturbances. The age structure of populations of large sized species is well balanced, with an optimal share of higher age groups. The continuity of age groups reveals favourable and stable conditions of reproduction in time and space. The diversity of fish fauna is represented by several key native species, with maximum contribution in maintaining the ichthyocenotic functionality at different trophic levels (e.g., *Chondrostoma nasus, Leuciscus leuciscus, Squalius cephalus, Barbus barbus, Salmo trutta fario, Sander lucioperca, Aspius aspius, Silurus glanis, Tinca tinca, Abramis brama, Phoxinus phoxinus*, etc.) and a large number of accessory species, which coexist and remain stable and constant in the characteristic habitats, occupying well-defined niches.

2. Good condition – an insignificant reduction of the composition and abundance of the accessory and stenobiont fish is registered (Fig. 9.4).

Fig. 9.4 In a "healthy" lotic ecosystem, the stenotope native species have satisfactory abundances (*Zingel zingel* and *Sabanejewia balcanica*).

The abundance of opportunistic euritope species increases slightly and signs of a decrease in the share of characteristic stenotope species appear: limnophilous in lentic ecosystems (*Tinca tinca, Carassius carassius, Misgurnus fossilis, Umbra krameri*, etc.) and rheophilous ones in lotic ecosystems (*Alburnoides bipunctatus, Salmo trutta fario, Thymallus thymallus, Phoxinus phoxinus, Cottus sp., Barbatula barbatula, Sabanejewia balcanica, Zingel streber, Zingel zingel*, etc.). The diversity and abundance of migratory and semi-migratory fish species is not significantly affected, but there are signs of disturbance in the age structure, with the dominance of young age groups and "gaps" in structure continuity. The spatial distribution of indigenous fish species demonstrates some small signs of fragmentation caused by anthropogenic activities. Trophic levels within the ichthyocenosis maintain a "healthy" pyramidal structure. The share of allogenic and intervenient species is insignificant, being effectively controlled by the pressure of native species (but it can be in a slight continuous increase).

3. Moderate condition – a continuous reduction of the diversity of autochthonous ichthyofauna is registered – up to 30% compared to the initial, reference diversity (based on the disappearance of stenotope species). Share of euritope, opportunistic and generalist polyphagous, phytophilic or polyphilous species increases substantially. Diversity and share of migratory and semi-migratory species undergo substantial negative changes. Share and continuity of lithophilic and psammophilic species decrease in lotic ecosystems, but they are still sufficiently represented (*Squalius cephalus, Chondrostoma nasus, Barbus barbus, Gobio sp., Romanogobio sp*. *Gymnocephalus sp*., etc.) (Fig. 9.5).

Fig. 9.5 In a river ecosystem, the presence in the catches of *Gymnocephalus cernua*, *Romanogobio vladycovi*, *Sander lucioperca*, *Perca fluviatilis*, which are species sensitive to pollution, denotes a moderate ecological status

The age structure of large sized species shows a reduction in the higher age groups and a decrease in the abundance of other groups. The abundance of species with a short life cycle increases, and the trophic level of predators is more represented by facultative ichthyophagous species (e.g., *Perca fluviatilis*, *Gymnocephalus sp.*, *Squalius cephalus*) or by the young age groups

of obligatory eurytope ichthyophagous species (*Sander lucioperca, Aspius aspius, Silurus glanis*). Also, the share of opportunistic polyphagous or monophagous species (phytophagous, malacophagous, ichthyophagous) increases in the trophic structure of ichthyocenoses. The fish biomass of eutrophic ecosystems is limited as result of active extractions by fishing and the proliferation of small sized species (with high trophic coefficients), or, opposite, demonstrates high values as result of the prosperity of opportunistic medium-sized species (*Rutilus rutilus, Abramis brama, Perca fluviatilis, Blicca bjoerkna*) in environments, which are trophicaly rich (due to the intake of allochthonous nutrients). Native ichthyocenosis gradually cedes in front of allogenic and intervenient taxa, but not everywhere.

4. Poor condition – a reduction of autochthonous ichthyofaunistic diversity with more than 30% in comparison with initial one is recorded. Migratory and semi-migratory species practically disappear, and the ichthyocenotic structure is dominated by several allogenic, indigenous or intervenient species, with short and medium life cycle and wide ecological value (e.g., *Perccottus glenii*, *Carassius gibelio*, *Rutilus rutilus*, *Alburnus alburnus*, *Pseudorasbora parva*, *Lepomis gibbosus*, *Blicca bjoerkna*, *Perca fluviatilis*, *Cobitis* sp., *Pungitius platygaster*, *Syngnathus abaster*, *Gobiidae*, etc.); stenotope species register an abundance decline or are extinct (Fig. 9.6).

Fig. 9.6 *Perccottus glenii*, *Cobitis sp.* and *Carassius gibelio* – species resistant to critic concentrations of dissolved oxygen in water

The higher age groups of large sized species are practically absent, and the other ones are represented intermittently and by few individuals. Spatial distribution is diminished and strongly fragmented. Substantial changes occur at the intrapopulational level through the proliferation of slow-growing ecofenas. Ichthyocenotic structure is dominated by the polyphilous species with portioned reproduction and long reproductive period. In the trophic structure, opportunistic polyphagous species have a great significance, with a pronounced trophic flexibility and easy adaptation to the available resources. Due to the disturbances of the production-destruction processes in the ecosystem, the fish productivity also decreases substantially.

5. Bad condition – ichthyocenosis is formed by few toxicoresistant species (*Rutilus rutilus*, *Carassius gibelio*, few species of *Cobitis*, *Perccottus glenii*, etc.), with low abundances, discontinuous spatial distribution and a high degree of morpho-functional pathologies. The ecosystem production-destruction processes are strongly affected. In exceptional cases, the loss of all hydrobionts in the ecosystem and the degression of ecosystem are recorded.

The significance of the small-scale temporal and spatial fluctuations of the abundances of indicator species is difficult to interpret, if there are no data from long-term studies, which could indicate their limits of natural variation. Ichthyocenotic successive changes, especially in the structure of the representative fish species for a certain type of ecosystem ("core species"), which do not necessarily have to occupy a eudominant or dominant position, allow reconstructing the history of the environmental conditions, highlighting of the speed, intensity, character and cause of climate or anthropogenic changes over time [2].

9.5. APPLICATION OF THE INDEX OF BIOTIC INTEGRITY (IBI)

Ecological conditions in a water body can be evaluated based on the Index of Biotic Integrity. For this purpose, the multiannual data on the structure and functional state of ichthyocenoses of different natural aquatic ecosystems shall be available [6, 9, 14]. Biotic integrity represents the ability of an environment to sustain and maintain integrated and adapted communities of organisms, which have a specific composition, diversity and a functional organization comparable to that of less affected habitats (cited after [13, 14].

In order to integrate biotic components in the systems of assessment of ecological conditions, the following variables must be taken into account: composition, distribution and abundance of biotic components, the ratio between sensitive taxa and resistant to disturbance taxa, diversity within the given compartment, etc.

IBI is the solely recognized index in some countries, which use aquatic vertebrates (fish) in biomonitoring and bioindication. It was applied for the first time in USA by Karr et al. (1981) and later underwent multiple changes both in the country of origin and in Europe (cited after [14]), [6, 9]. One of its original forms is presented below (Tab. 9.3).

Table 9.3 Metrics used to determine the Index of Biotic Integrity (IBI), adapted after Karr et al. (1986), Miller et al. (1989) (cited after [14])

Category of metrics	Metrics	Rating of metric (score)		
		5	3	1
Species composition and richness	Total number of species (from existing ones)	>90%	50 – 90%	<50%
	Total number of cyprinids (group of leading species)	>45%	20 – 45%	<20%
	Total number of salmonids	>5%	1 – 5%	<1%
	Total number of all other species	>20%	5 – 20%	<5%
	Total number of autochthonous (native) species	>68%	35 – 67%	<34%
	Number of introduced species (acclimatized)	<1%	10%	>10%
	Total extinct species	0 sp.	2 sp	>2 sp
Trophic composition	Percentage of zoobentophagous species	>45%	20- 45%	<20%
	Percentage of carnivorous species	>5%	1 – 5%	<1%
	Percentage of carnivorous and zooplanktonophagous species	<20%	20 – 45%	>45%
	Percentage of herbivorous and detritophagous species	<25%	25 – 50%	>50%
Fish abundance and population condition	Total biomass (g/100 m^2) (in function of the dimensions of water body, of other factors)	>1500	500 – 1500	<500
	Total number of individuals (ind./100 m^2), of which:	>100 ind.	2 – 10 ind.	<2 ind.
	Percentage of hybrids	0%	0 – 1%	1%
	Percentage of individuals with anomalies, tumors, diseases	0%	0 – 1%	>1%

The first attempts of the monitoring of fish fauna were made in Europe after the 1990s in such countries as France, Belgium, Spain and Poland, where it became an officially recognized procedure, and then – in Romania, the Baltic countries, etc., where testing has been still conducted. Currently, the system of assessment and classification of aquatic bodies based on fish fauna is applied in Europe in a modified form, under the name of EFI + (European Fish Index). This relatively young European experience was systematized and developed through the FAME program [9].

The metrics selected and used for the calculation of EFI + are related to two broad categories: salmonid waters and cyprinid waters. In some particular situations it is difficult to delimit the two types of water bodies. In these cases, the role of specialist's opinion and competence increases, in particular, of his level of knowledge of ecological characteristics of the habitat and

of the structure of the given cenosis.

An important advantage of applying IBI is based on the possibility of analyzing the fishing community by using the parameters, which integrate the three structural levels of organization of living things:

- individual level – by quantifying the health of fish; in the case of aquatic ecosystems affected by high anthropogenic pressure, essential disturbances at the morphological and physiological level appear;
- population level – by quantifying the age structure of representative populations (normally, the structure of a population must be well balanced and complete);
- fishing community (ichthyocenosis) level – by quantifying of the species richness, abundance and/or relative biomass, of the ecological groups (trophic, reproductive, topic guilds).

In general, IBI includes 10-12 metrics, which are adapted according to the structural-functional state of the ichthyocenoses in the studied region, in order to preserve their ecological significance given by Karr et al. (1986) and Miller et al. (1989) (cited after [6, 13-14]). Their choice is the result of a compromise between stability and sensitivity. A score of 5, 3 or 1 is assigned to each metric according to whether its value aproximates, deviates somewhat from, or deviates strongly from the value expected at a comparable site, which is relatively undisturbed and considered as reference. The scores of metrics are then summed to determine the value of IBI.

It is also worth to mention that within a lotic ecosystem the value of IBI is much higher in the downstream piscicolous areas, compared to the stations located in the upper sectors of the river [6].

Depending on the sum of the scores of the chosen metrics, the obtained results are assigned to one class of ecological bonitation, which reflects the health condition of this ecosystem, based on the principle of interaction of organisms with the environment (ecosystem principle) (Tab. 9.4).

Table 9.4 Total Index of Biological Integrity (IBI) scores, integrity classes, and the qualification of those classes after Karr et al. (1986), Miller D. et al. (1989) (cited after [14])

Total IBI score in small rivers	Total IBI score in large rivers and lakes	Bonitation class (integrity class of site)	Qualification
37-40	57-60	I	Excellent
-	53-56	II	Excellent-Good
30-33	48-52	III	Good
-	45-47	IV	Fair-Good
23-27	39-44	V	Fair
-	36-38	VI	Poor-Fair
12-20	28-35	VII	Poor
-	24-27	VIII	Poor – Very poor
<13	<23	IX	Very poor

However, it has been currently found that the European Fish Index (EFI) is sensitive to pressures on water quality and not an enough good indicator to highlight the hydromorphological pressures [10], which are so evident in the conditions of the Republic of Moldova. At the same time, the convenience of IBI is mentioned by many authors, as the sampling and identification of fish species, especially in shallow rivers, are simple, fast and inexpensive procedures [6].

As mentioned earlier, IBI has undergone numerous changes over time and in different countries, being adapted to concrete regional conditions, but all these changes „not have the right" to influence the possibility of intercalibration and comparison of data obtained by similar studies. As example, the changes of the metrics for the aquatic ecosystems of the Republic of Moldova and their scientific argumentation are given in the work of Bulat et al. (2014).

The IBI method, as mentioned above, is not without shortcomings. The most significant shortcoming is that this assessment system not covers all quality components and all investigation

compartments, and not has a frequency to ensure high levels of confidence and accuracy in assessing water bodies. There are also shortcomings in identifying the boundaries of separation of integrity classes. The main conclusion of the Romanian research teams is that this index is reliable in detecting global alterations of aquatic ecosystems, but its sensitivity is mediocre, no doubt due to the quality and quantity of used data. There is still lots work on establishing both the most significant metrics, which make up the IBI, the optimal reference values, and the impact factor with the lowest values of "starting" the negative structural-functional changes [6].

Among the advantages of this index can be mentioned the simple calculation system based on scores and the complex multimetric analysis (with the neglect of some insignificant inaccuracies). It should be mentioned that the expert shall possess extensive knowledge in the field and, peculiarly, undertaken long-term studies in the ecosystem under investigation – knowledge of the "history" and the peculiarities of the changes produced under the influence of anthropogenic factors. Moreover, when using this index, some subjective influences persist, with tendencies to overestimate the ecological gravity [2].

In conclusion, it can be stated that IBI permits to perform a complex analysis in the frame of the biomonitoring of aquatic bodies and to compare the results of different studies, but can not be used as the only indicator in assessing the welfare of aquatic ecosystems [2].

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