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METODE DE EVALUARE SI MONITORIZARE
A CALITATII APELOR IN ROMANIA
WORKSHOP
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All authors are responsible for submitting manuscripts in comprehensible US or UK English and ensuring scientific accuracy.

Picture on front cover: Logo of the Workshop

Methods for Assessing and Monitoring Water Quality Based on Biotic Communities in Romania, 17th – 19th October 2008, Cluj-Napoca, Romania

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FOREWORD

TO THE PROCEEDINGS OF THE WORKSHOP:

METHODS FOR ASSESSING AND MONITORING WATER QUALITY BASED ON BIOTIC COMMUNITIES IN ROMANIA
17TH – 19TH OCTOBER 2008
"Babeş-Bolyai" University, Faculty of Biology and Geology, Department of Taxonomy and Ecology, Cluj-Napoca, Romania

The present issue of STUDIA UNIVERSITATIS BABEŞ–BOLYAI, Series BIOLOGIA, includes the papers presented during the workshop **Methods for assessing and monitoring water quality based on biotic communities in Romania [Metode de evaluare şi monitorizare a calităţii apei pe baza comunităţilor biotice în România],** held in October 2008 at Babeş–Bolyai University of Cluj-Napoca, Romania.

The main objective of the workshop was to bring together specialists in the assessment and monitoring of water quality, in order to discuss the adequate methods that should be used at a national level.

The 2000/60/EC Water Framework Directive represents the European law in the field of water policy and establishes a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater. Regarding the quality elements for the classification of ecological status of surface waters, the researchers should focus on the following biological elements: composition and abundance of aquatic flora (macrophytes and algae), composition and abundance of benthic invertebrate fauna and composition, abundance and age structure of fish fauna.

Romania, as a Member State, must meet the European Union requirements for both inland and marine water bodies. However, the lack of national methods of assessing and monitoring water quality based on biotic communities led to the acute need to bring together the specialists within the framework of the workshop.

The discussions were, indeed, extremely fruitful and targeted to specific subjects, namely planktonic and benthic algae, macrophytes, macroinvertebrates and ichthyofauna (from inland and marine habitats). The valuable conclusions of the workshop were included in a memo that was sent to The Ministry of Environment and Sustainable Development and to The Romanian Waters National Administration. The recommendations regarding the target biotic communities are detailed below.

For monitoring the algal communities from running waters, the representative community is the benthic one. According to the European regulations, the composition and abundance of benthic algae must be considered, and identifications must be made to the species level. Different indices, like the saprobity, diversity or biotic indices must be calculated (e.g. The Diatom Biotic Index, elaborated in France). For planktonic algal communities, representative for standing waters, the monitoring method should be based on the qualitative structure, relative abundance, trophicity, saprobity, diversity and biotic indices, together with biomass estimations (the Utermöhl method) and chlorophyll *a* determinations. **For aquatic macrophytes** from inland lotic ecosystems, the specialists recommend the use of the Kohler index, adapted by Janauer, which also includes diversity, abundance and dominance estimations. For macrophytes from inland lentic ecosystems, different methods are recommended, e.g. the Schaumburg trophic index.

For monitoring **the aquatic macroinvertebrates**, a multi-parameter approach is recommended, including diversity indices, functional groups and biotic indices. It is very important to develop a Romanian biotic index for benthic macroinvertebrates, or to adapt European indices to the particular habitat conditions of our country. Data accuracy depends on the sampling technique. That is why a specific recommendation of the specialists is the standardization of the sampling methods on a national scale, taking into consideration European standards (e.g. the AQEM method).

For monitoring **the ichthyofauna** from running waters, the specialists recommended the implementation of the European Fish Index (EFI+), which represents the result of an ongoing European project. On the other hand, fish fauna from large rivers or lakes is difficult to monitor, so the methods are not yet fully developed and standardized.

For **marine ecosystems**, the following indices are recommended in monitoring the aquatic macroinvertebrates: AMBI and BENTIX. They should be tested in order to establish the tolerance limits for Romanian species. Moreover, not only the benthic communities should be considered in marine monitoring programs, but also the planktonic and nektonic ones.

As a general recommendation, the workshop participants identified the need to differentiate the basic monitoring studies (done by the national authority in the field – the Romanian Waters National Administration) and the biodiversity researches that should be done by renowned specialists in different taxonomical groups, who could provide accurate and updated species lists, habitat preferences, functional aspects etc.

The papers presented at the workshop included four general reviews concerning the algal communities (L. Momeu, L. Ş. Péterfi, Assessment of the Ecological State of Rivers Based on Benthic Algae, Especially Diatoms), the macrophytes (E. Schneider, Aquatic Macrophytes in the Danube Delta – Indicators for Water Quality and Habitat Parameters), the aquatic macroinvertebrates (C. Ciubuc, The Water Framework Directive and the Research Methodology for Macroinvertebrate Communities) and the ichthyofauna (K. W. Battes, Monitoring the Ecological Status of Running Waters Based on Fish Communities). The other papers deal with aquatic biodiversity on algal communities (L. Momeu, R. Cnab, Preliminary Study on Autumn Diatom Communities from the Crişul Alb River, Ineu Section) or on aquatic invertebrate communities (A. Avram, K. P. Battes, M. Cîmpean, R. Kasza, A Preliminary Data on Zooplankton and Aquatic Invertebrates from the Fînațele Clujului Nature Reserve (Transylvania, Romania)). Water quality assessment studies were also included in the workshop program, for inland running waters (A. Avram, M. Cîmpean, A. Jurcă, N. Timuş, Water Quality Assessment using Biotic Indices Based on Benthic Macroinvertebrates in the Someşul Mic Catchment Area) and for marine habitats (V. Surugiu, An Overview of the Methods Used in the Assessment of the marine Environmental Quality, Based on the Analysis of the Zoobenthos).

The workshop was organized by the research team at the Laboratory of Aquatic Ecology, Faculty of Biology and Geology, Babeş – Bolyai University (Reader PhD Laura Momeu, Teaching Assistant PhD student Karina Paula Battes, PhD student Mirela Cîmpean and PhD student Anca Avram).

Karina P. Battes

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ASSESSMENT OF THE ECOLOGICAL STATE OF RIVERS BASED ON BENTHIC ALGAE, ESPECIALLY DIATOMS

LAURA MOMEU[1] AND LEONTIN ŞTEFAN PÉTERFI[1]

SUMMARY. The present paper presents the main aspects concerning monitoring programs of running waters for assessing their ecological status. It is very important to know the characteristics of lotic ecosystems, the role of algae as primary producers, as well as the legal framework ruling monitoring programs. Since the representative algal community for running waters in the upper, middle and sometimes lower reaches is the benthic one (periphyton), the paper depicts the importance of choosing the proper type of substratum and algal sampling, together with the correct manner of sample handling. In concordance with the literature but also with personal data, the present paper shows the importance of using biotic indices in assessing the ecological status of running waters.

Keywords: running waters, benthic diatoms, biotic indices, modern concepts, truncated normal curve

Introduction

The premises which stand on the base of this approach concern three categories of problems. On one hand, there are those referring to the characters of lotic ecosystems, those of the algae as group with major importance in the running water biocoenoses, and the existing legal framework. There is no intention here to analyze exhaustively these, but only to point out some of the essential elements which are absolutely necessary to understand the complexity of issues involved in the water quality evaluation mechanism in streams based on aquatic organisms, especially on algae.

The characteristics of lotic ecosystems

With reference to the problems of the first category it should be emphasized that after 1970 numberless investigations in river ecology (in a broad sense) have been finalized in defining and introducing of new theories like the "river continuum" concept (Vannote *et al.*, 1980) (Fig. 1), the "nutrient spiraling" (Newbold *et. al.*, 1982) (Fig. 2), and also the concept of "drift" (Tanaka, 1960), that of the "flash flood" and the "tributary hierarchy" or "stream order" (Allan, 1995), as well as some new aspects of accidental physical factors (the hazard) or of those decisive biotic ones (competition, predation, parasitism) in stream community structuring (Wetzel, 2001).

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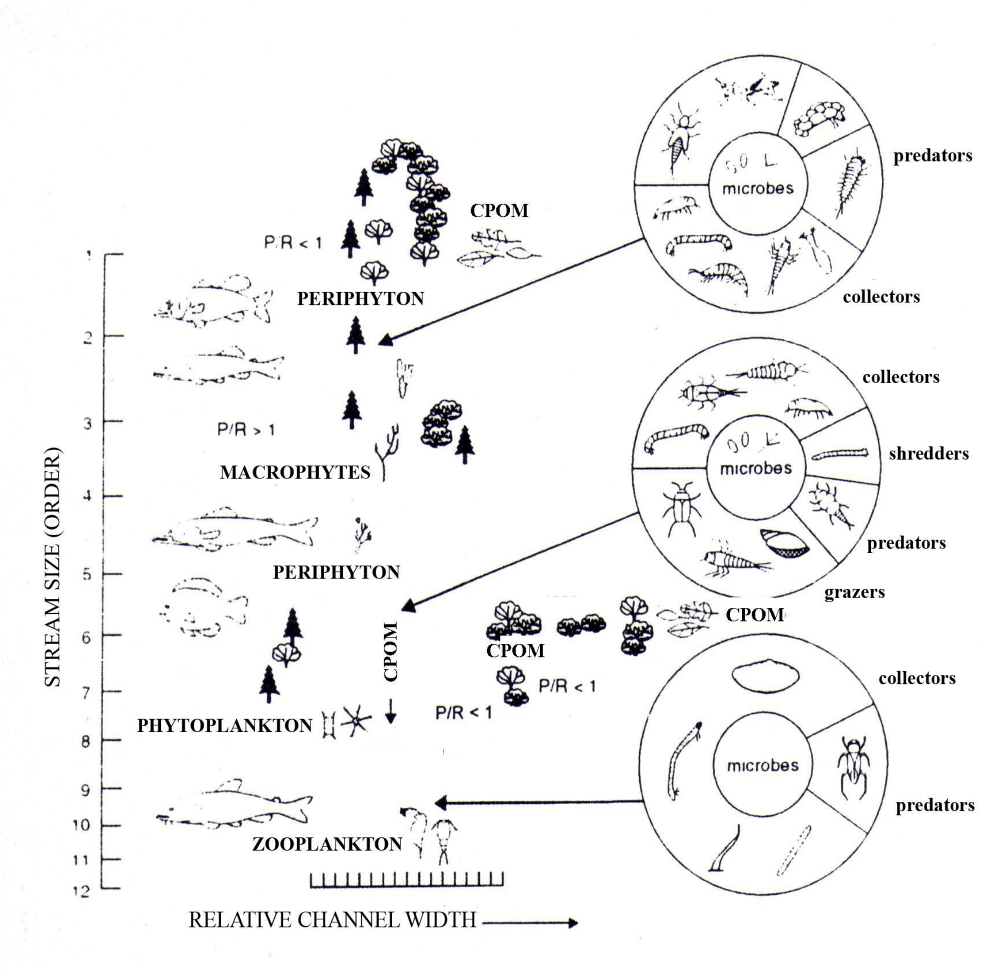

Fig. 1. The "river continuum" concept (redrawn from Vannote *et al*., 1980)

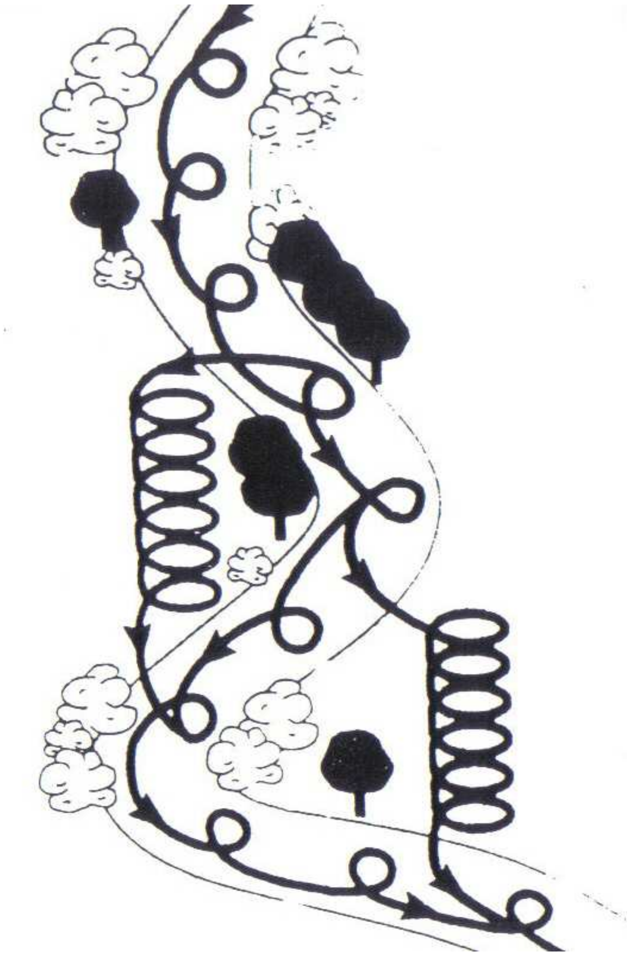

Fig. 2. Nutrient spiralling in two – compartment stream
(redrawn from Newbold *et al*., 1982; Ramade, 2002)

In all these cases there have been emphasized the importance of physical processes in the streams, the most important being the hydrological and geomorphologic ones and their interactions with biotic factors, without neglecting the chemical factors, essential when autotrophic organisms like algae are involved.

It is important to point out the diversity of running waters, starting with torrents, springs, rivulets, rivers and finally ending with large streams, some of them with catchment areas extending on entire subcontinents. There should also be mentioned the existence of great many microhabitats, the highly mosaic pattern of these and the outstanding dynamics of processes which take place in the river-bed. This diversity concerns the physical-chemical and biotic factors in both, the proper river-bed and the catchment area as well, factors inducing and influencing the structural and functional patterns of the stream as ecosystem. The input into the river of matter and energy, the exchange of matter and energy with the riparian zones, influences the biodiversity in the interaction processes between abiotic and biotic factors. These processes are universal, due to the fact that they take place in all running water types, but they exhibit different patterns according to each particular system, becoming often unique for one river or for another.

Special attention should be paid to the unwanted effect of human activities on the ecological health of running waters, being evident many of the ecological disasters due to the lack of medium- or long-range forecasts in this field.

Characteristics of algae

The second problem refers to the algae, as autotrophic aquatic organisms, belong to the so called Thallophytes, exhibiting outstanding heterogeneity not only as the organization of thalli, cell structure and physiology, but concerning their ecology too. To emphasize their structural heterogeneity is worth mentioning that they belong to two different kingdoms (Regnum) according to the five kingdom concept of Margulis and Schwartz (2000): Cyanobacteria (Cyanoprokaryota) – with prokaryotic organization of cells, classified into the kingdom Monera (Bacteria) and eukaryotic algae, the majority, belonging to the kingdom of Protista. The pattern of heterogeneity is equally valid for every particular algal group of any rank (phylum – class – order – genus). The acceptance of such taxonomic approaches presumes, first of all, to give up some usual concepts and terms and to adopt new and adequate ones (Cyanobacteria or Cyanoprokaryota instead of Cyanophyta; planktonic algae instead of phytoplankton; better to use benthic algae than phytobenthos).

The algae, generally, are known as photoautotrophic, photochemotrophic, able of oxygenic photosynthesis, this capability being the common feature of both prokaryotic cyanobacteria and eukaryotic algae. But, many eukaryotic forms are able of facultative or obligate heterotrophy. Sometimes, there is present either phagotrophy or auxotrophy versus autotrophy, myxotrophy being often mentioned in some algal groups, which developed the adaptative nutritional strategy to switch over to heterotrophic nutrition in some environmental conditions. In the field of ecology, the various algal groups exhibit equally high heterogeneity. Most of them are primarily aquatic organisms, but sometimes, due to their high ecologic plasticity, could become adapted for terrestrial ecosystems too. In aquatic environment the algae grow in the most diverse ecosystem types, marine or continental, freshwater, brackish or saline, with running or standing water, occurring sometimes even in the most extreme conditions (on the surface of snow, glaciers or in thermal springs).

The importance of algae inhabiting streams and other aquatic ecosystems consists in their role of primary producers, being organisms capable to use inorganic energy sources in their photosynthetic processes producing organic matter (Fig. 3). Besides algae, the so called "higher plants" or macrophytes including ferns and mosses (Fig. 3), as well as some photoautotrophic and chemosynthetic bacteria, are equally primary producers. On the other hand, the algae are important as food source for herbivores. Especially in the context of our discussion, should be highlighted that some of the algal species are particularly important as indicators of water quality for the ecosystems they inhabit.

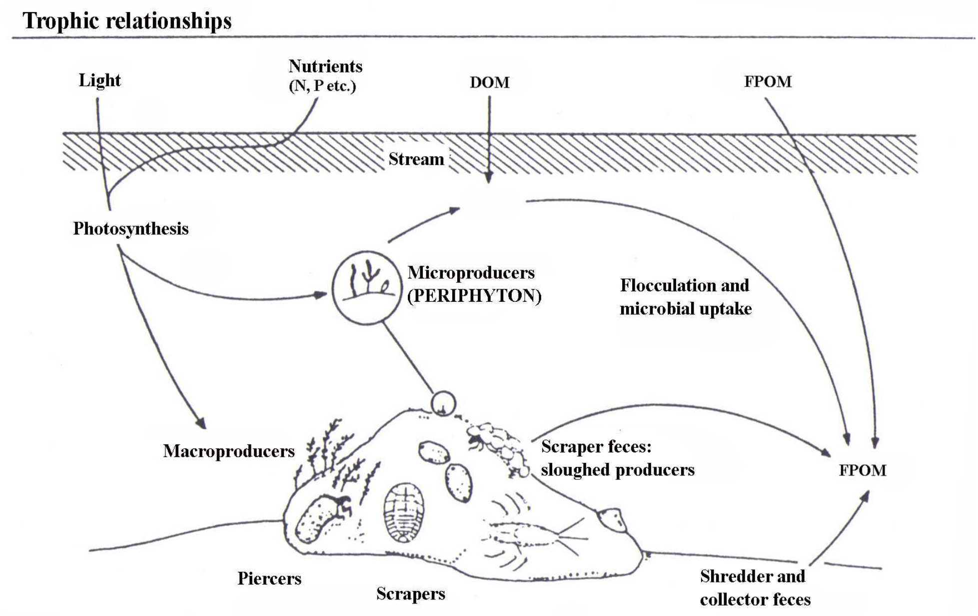

Fig. 3. The role of algae as primary producers (redrawn from Allan, 1995)

The algae occurring in rivers develop two main community types: periphyton (benthic community) and plankton (pelagic, drifting community). The periphyton is defined as complex structured community, inhabiting the bottom substratum – rocks, boulders, gravel, sand, silt, but also any submersed objects, including cables, walls, pillars, submersed aquatic plants etc. Planktonic algae inhabit the water column.

The periphyton of the running waters consists primarily of diatoms (Bacillariophyta), the dominant algal group in many rivers of the temperate zone, especially in their upper and middle course (Fig. 4). The same is true for the Romanian running waters, as concerns particularly the Transylvanian ones (Momeu and Péterfi, 2004; 2007; Florean *et al.*, 2006, Voicinco *et al.*, 2005). Besides diatoms the other algal groups occurring in the river periphyton are cyanobacteria (Cyanoprokaryota), green algae (Chlorophyta), golden algae (Crysophyta), euglenoid flagellates (Euglenophyta) and red algae (Rhodophyta). Except rhodophytes some members of the other groups are also present in the plankton community of the rivers. According to Vannote *et al.* (1980) the periphyton is the community which develops as a rule on the upper rivercourses, but often inhabits their middle or even on lower courses too. By the contrary, plankton community develops in big rivers only, especially in their lower courses. Otherwise, on all surfaces of stream substrata illuminated in a proper degree, irrespective that they are in the smallest rivulets or in big rivers, sustain several types of benthic algal assemblages (sub-communities) according to the nature of substratum, namely epilithon, epipsammon, epipelon, epiphyton, each with a diversity of organisms developing special adaptation mechanisms, necessary to attach and maintain on the level of substratum. Therefore, assessing the ecological status of running waters should be based on periphytic communities, dominated by diatoms (Lowe and Pan, 1996).

The main environmental factors that affect the development of river periphyton are: light, temperature, nutrient availability, current velocity, and nature of substratum, flash flood – among the abiotic factors, competition, pressure of herbivores (grazing) and parasitism – among biotic ones. The action of these factors is simultaneous, their changes take place in the same time, the importance of a particular factor limiting the growth of periphyton is not easy to establish. The adaptation degree of algae to environmental changes, of natural origin or caused by human influences, is very different. Some algae exhibit wide range of ecological plasticity, being of eurybiotic nature, (eurythermic, euryhalinic, euryionic etc.). The others are more specialized, with narrow limits of adaptation, belonging to the stenobiotic group of algae. The stenobiotic forms are valuable and fine indicators in estimating water quality of aquatic ecosystems.

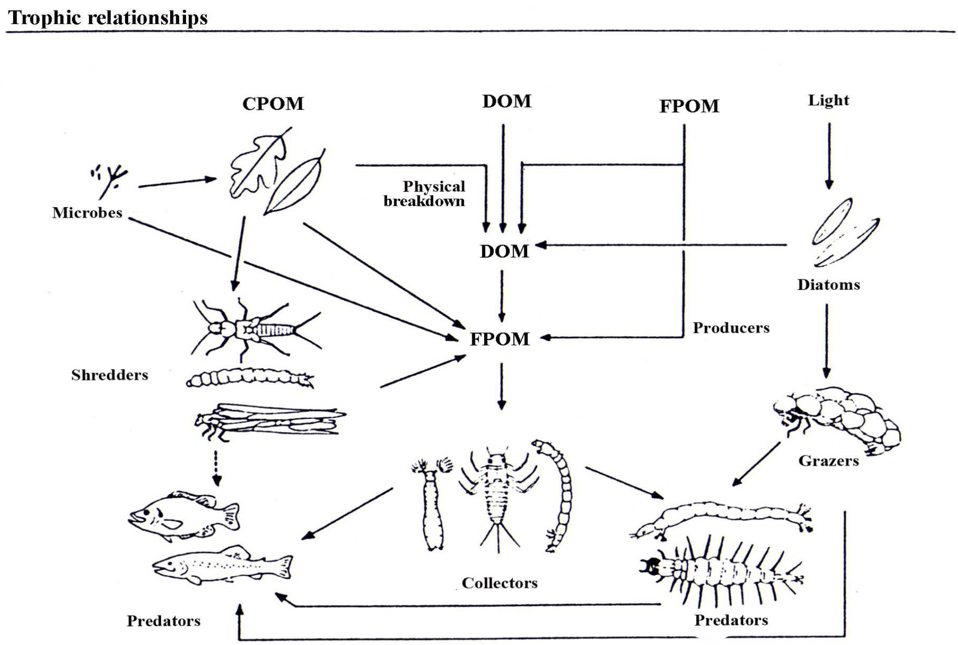

Fig. 4. Position of diatoms in the food web of the river ecosystem (redrawn from Allan, 1995)

The main characteristics of the algae which make them to be used as indicators for the estimation of the ecological state of aquatic ecosystems are:

- Short life cycles, rapid multiplication in favorable conditions, prompt answer to environmental changes, being “sentinel organisms” which integrate the effects produced;
- Many and varied resting stages which survive harsh environmental conditions;
- Several and varied distribution (dispersion) strategies: by water, air, animals, human;
- Occurrence in all aquatic ecosystem types, lotic and lentic, freshwater, saline or brackish, even in extreme environmental conditions.

As far back as in the beginning of the last century stenobiotic algae had been included in the first system of estimating water quality based on bioindicators, namely in the “saprobian system” (Kolkwitz and Marsson, 1908) besides other groups: bacteria, micromycetes, insect larvae etc. The system has subsequently been improved (Sladecek, 1973). Instead of estimating simply the affiliation of the species to one of the saprobic categories (xeno-, oligo-, beta-, alpha-), the investigators proposed the calculation of various saprobic indices Pantle and Buck, 1955; Zelinka and Marvan, 1961). Such approach presumed not only qualitative observations, but quantitative investigations too.

Monitoring procedures, based on living organisms quantify the ecological health state of a certain aquatic basin, integrating in the case of algae, that are primary producers, the effects of the environment and reflecting the typical conditions of the environment comparatively with the actual point values which can be measured exactly by physical and chemical parameters. Even continuous monitoring of physical and chemical factors might miss some aspects with great impact upon key organisms in the aquatic communities due to their synergic interferences, life being the basic monitoring mean of environmental quality.

The legal framework of running water monitoring

In accordance with the recent normative rules (Water Framework Directive 2000/60/EC, Law No. 107/1996, Law No. 310/2004) referring at aquatic environment, the quality of evaluation on the ecological state of rivers should be improved, primary being the biological elements, without forgetting, of course, the hydro-morphological and physical and chemical factors, as well as the human impact factors which might affect both physical-chemical and biotical indicators.

In streams, the quality elements to be fulfilled in estimating their ecological state, according to the present laws, are the “composition and abundance of the aquatic flora”, in the case of algae – to establish both qualitative and quantitative structures (composition) of the communities, essential to compute various indices.

Recommended types of substratum and algal sampling in monitoring programs

According to the investigations carried out in Transylvanian rivers and taking into consideration the statements of Vannote *et al.* (1980), Kelly *et al.* (1995) and Wetzel (2001), the present authors consider that the really representative biocoenoses in running waters (Fig. 5 and 6) are the periphyton ones. Therefore, the methods of sampling, preserving and laboratory treatments will be considered briefly next, together with the most important indices, commonly used for the estimation of river water quality. Finally, it should be stated that the ecological quality of rivers is the result of corroboration of all quality data obtained, biological, physical-chemical and hydro-morphological.

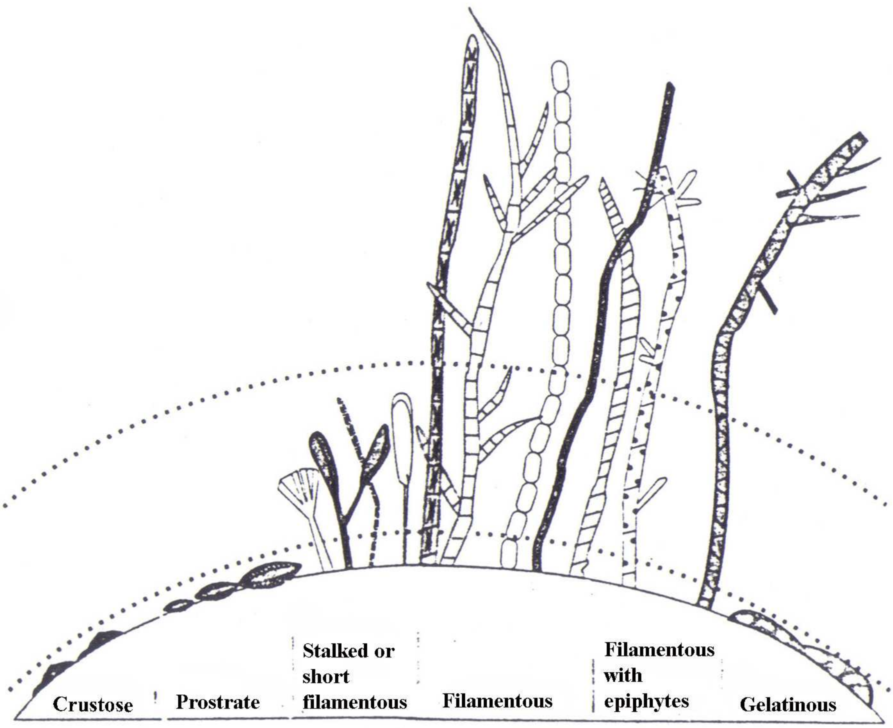

Fig. 5. Features of periphyton growing on hard substrate (redrawn after Allan, 1995).

The establishing of locations, of the stands to be sampled for their algal communities, the number and type of samples, the time and periodicity of sampling should be carried out according the aim of the investigations in the monitoring processes of various types (integrated, supervision, operational or investigation monitoring).

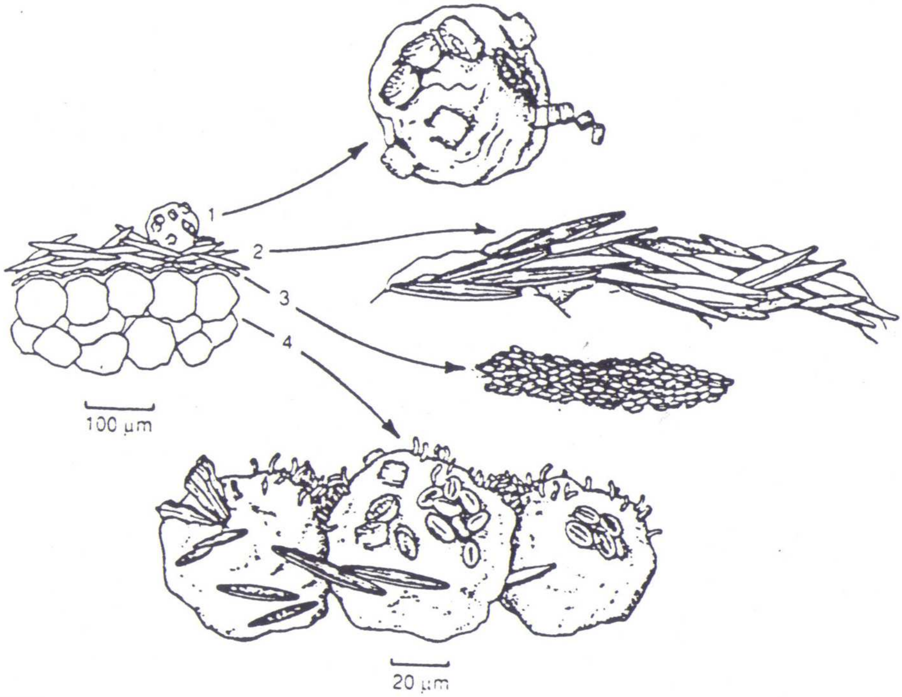

Fig. 6. Features of epipsammic periphyton (redrawn after Allan, 1995).

Due to the fact that in streams the nature of substrate is highly heterogenic it is particularly important to establish the right type to be sampled for the algae. Some of the investigators (Kelly *et al.*, 1995) recommended sampling the algal communities growing on the same, standard substrate in all stands, or on a substrate that best reflects the typology of the river. Diatoms being the dominant algal group, Kelly *et al.* (1995) suggest to collect the samples from hard surfaces (epilithon – rocks, boulders, cobbles) when they are available and from the most characteristic one in each stand. Winter and Duthie (2000) recommend especially for biomonitoring the sampling of a single substrate type, the most frequent one, and the selection of a second one, when the first one is not present in all stands. The second substrate should preferably be submersed plants, but diatoms might be sampled from the surface of silt (epipelon) or sand (epipsammon) when the first ones are absent. The sampling techniques are the usual ones (Patrick, 1977) by scraping, and/or brushing the surface of hard substrates, or by sucking up from the mobile ones, by washing and/or brushing the stems of submerse plants etc. Sometimes the periphyton might be removed from previously measured, standard areas of the substrate surface.

Another problem of major importance in obtaining correct and reliable results, is the identification of diatoms, observed using oil immersion lenses, exclusively in preserved, permanent mounts (preps) employing up to date standard methods (Barber and Haworth, 1981). Among the criteria used in the determination of diatoms should be mentioned the size, shape and structure of frustules; their ecological preferences (except eurybiotic species), are very valuable criteria in estimating the ecological state of river and in water quality assessments. These concerns the trophic and saprobic level of the water, but also some physical and chemical features (parameters), like water temperature, pH, conductivity, salinity level, the presence of some heavy metals in the water or excess of calcium etc. (Patrick, 1977)

The quantitative data are useful to compute diversity indices (Shannon-Wiener, Simpson etc.) or equitability (Washington, 1984), as well as the saprobity ones (Zelinka and Marvan, 1961). According to recent stage of development new ways of approach are necessary, either by reconsidering the use neither of some indices, nor by introducing new ones, in case of rivers, indices based on diatoms. The reliability and use of the saprobic indices tends to be restricted, the major shortcoming is that they reflect only momentary situations, therefore are useful only together with other above mentioned indices or recently introduced and better ones. In several European Union countries is frequently used the Biological Diatom Index (Prygiel and Coste (eds.), 2000), considered one of the essential indices for the assessment of the ecological state of rivers. In the elaboration of the computing formula there have been involved a great many investigators; over 1330 samples were collected from over 940 stands, in France, more then 600 diatom species being involved in calculating the BDI index. Parallel there have been measured 14 physical and chemical parameters of the waters, and finally based on the BDI values 5 water quality classes were established: excellent, good, moderate, poor and bad. Recent investigations carried out in the Arieş River drainage area, financially supported by the CNCSIS project, type A 1329, emphasize that the BDI values (Prygiel and Coste (eds.), 2000), and the saprobity indices (Zelinka and Marvan, 1961) calculated for the Arieş River are in concordance with the other biotic indices employed: macroinvertebrate and fish biotic indices (Momeu and Péterfi, 2007; Momeu, 2008). BDI and SI have been applied in other Transylvanian rivers too: Someşul Rece, Someşul Cald, Someşul Mic and Someşul Mare (Battes *et al.,* 2004; Rasiga *et al.,* 1996; Florean *et al.,* 2006; Voicinco and Momeu, 2005) and they proved to be equally useful in assessment of ecological state in the investigated rivers.

Less commonly used in Europe, as well as in Romania, the model of truncated normal curve proposed by Patrick *et al*. (1954). The results obtained by employing the model for the study of epilithic diatom communities of the Arieş, Cheile Turului, Cheile Turzii and Ampoi watersheds well illustrated changes in the qualitative and quantitative structures of the diatom communities due to pollution stress (Péterfi and Momeu**,** 1984, 1985; Kozma *et al*., 2001; Momeu and Péterfi, 2004; Biró-Halmágyi *et al*., 2004) (Fig. 7). The same method applied in mineral springs distributed in the Harghita and Covasna counties seemed to be equally useful; there has been demonstrated that the structural changes in community structure depend on the diversity and amount of dissolved minerals in the spring waters (Péterfi *et al.*, 1983; Péterfi *et al.*, 1988).

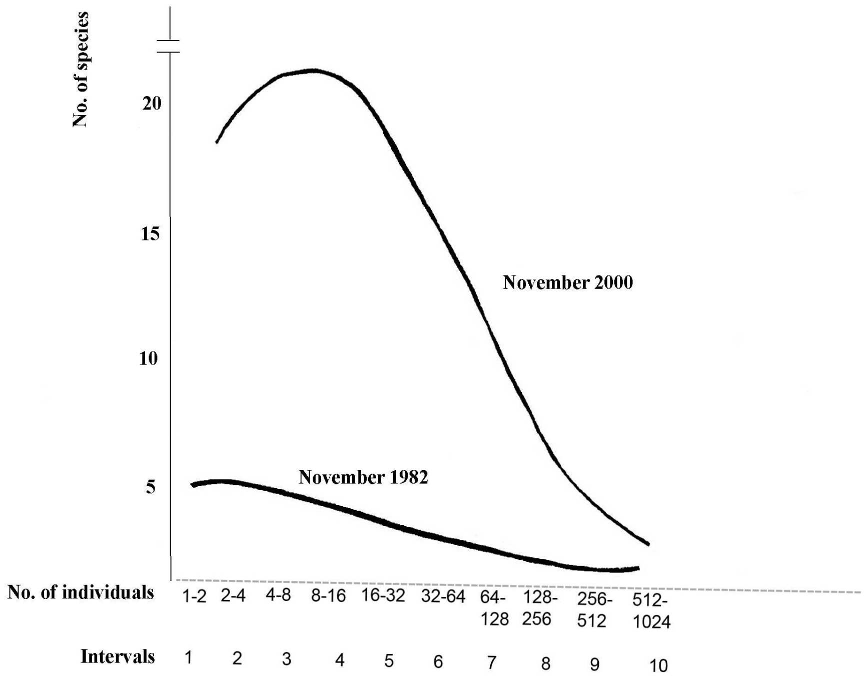

Fig. 7. The truncated normal curve model for algal communities sampled downstream Zlatna waste water treatment plant in two different years (Momeu and Péterfi, 2004)

The results obtained based on the above mentioned indices and methods, should be supplemented by biomass estimations, measuring the bio-volumes or by analyzing the chlorophyll *a* content. In all cases the results should be correlated with the hydro-morphological and physical and chemical parameters of the watercourses.

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AQUATIC MACROPHYTES IN THE DANUBE DELTA – INDICATORS FOR WATER QUALITY AND HABITAT PARAMETERS

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SUMMARY. The diversified types of water bodies in the Danube Delta, their hydrological regime and trophic condition offer the basis for a large range of aquatic macro- and microhabitats. These are settled by various macrophytes and their communities and are distributed along ecological gradients. The aim of our research has been to make out the differentiation between various types of water bodies (standing and running waters) on the basis of the indicator value of water macrophytes. This concerns not only water quality i.e. the trophic condition of the water bodies, but also physical parameters.

Studies showed that only a few species are bound to running waters, other species occur in both water types, i.e. in slowly running waters abundant in suspended solids and in standing waters showing different degrees of turbidity or with clear water in more isolated lakes. A large range of macrophytes is only characteristic of lakes occupying different ecological niches in these water bodies.

After having studied the water macrophytes for several years in the Danube Delta/Romania and after having been able to monitor some sampling points in chosen areas (Babina, Cernovca, Popina, Fortuna, Isac-Uzlina), the species' indicator values became much more precise in their different evolution stages. They demonstrated the dynamics of the sites in the studied areas and at the same time showed the possibility to use water macrophytes as perfect indicators for water quality and as indicators for water dynamics, including the regime of suspended solids, the filtering capacity of vegetation and sedimentation processes.

Keywords: types of water bodies, macro- and microhabitats, water macrophytes, ecological gradients, water quality indicators, habitat quality indicators, macrophyte communities

Introduction

The Danube Delta is characterized by a diversified network of water bodies. On the one hand there are different types of running waters starting from the main branches of the Danube (category I), large secondary natural branches (category II), large channels with the flow into secondary branches (category III), small and narrow channels connecting the lakes, smaller streams ("gârle" and "japşe") (category IV), temporary water courses, flood channels, channels that are characterized by the intervention of man (dragging) and artificial canals. On the other hand the Delta shows different types of standing waters of various sizes, so e.g. isolated lakes surrounded by reeds, others connected to smaller and larger water courses influenced by them, temporary small standing waters and artificial standing waters such as basins for fish culture. Numerous small waters, such as the flood channels, change their aspect with

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fluctuating water levels. In times of high water levels they temporarily become running waters and vice versa, at low water levels they temporarily become standing waters, the water body being disconnected from the running water dynamics.

Hydrological regime, hydromorphological processes and trophic condition of the water bodies constitute the basis for a large diversity of aquatic macro- and microhabitats. These are colonized by various macrophytes and their communities, distributed along ecological gradients. The habitats are differentiated by their spatial structure and the species composition of the macrophyte communities. Ecological gradients exist between the various types of water bodies depending on their size, water depth, hydraulic residence time, their substrate (organic or mineral). They do also exist within individual water bodies, from their border lines up to their centre. This differentiation within a one and only water body depends on its width, depth, flow velocity, turbidity / content of suspended solids. When considering these factors, the repartition of the water macrophyte communities reflects the habitat conditions.

From the abiotic habitat parameters the followings are very important as evaluation elements: shore structure, sediment type (silt, sand, organic or mineral substrate), the connectivity type which is responsible for nutrient input, flow velocity class (low 0.3 m/s; medium 0.35-0.65 m/s; high >0.7 m/s), transparency respectively lighting conditions as well as the type of land use in the water's surroundings (Janauer *et al.,* 2006). Of course, the aquatic vegetation is also immediately affected by interventions such as rectifications, the construction of new channels, dykes and further hydraulic measures. The latter occur e.g. in the form of bank protections that may dramatically influence the development of plant communities in shore-adjacent, dynamic areas.

The classification of the ecological condition of the waters depends on a number of components as is noted in the WFD Water Framework Directive of EU. These are a biological component, a hydromorphological component providing the basis for the biological one, a chemical and physical-chemical component which is to be considered as complementary to the biological one all the same. It applies to water temperature, oxygen content, salinity, pH value and nutrient content. Toxic substances have to be considered as well given that they may considerably affect the habitats and their biodiversity.

The water's ecological condition affects the abundance of the aquatic vegetation and the respective composition of their communities. The species composition of aquatic plant communities and the abundance-dominance of species in general is subject to natural fluctuations that are determined by the dynamics of the water levels and the hydrological regime as a whole as well as by the dynamics of sediments. Further influences on the composition of species are due to human intervention and have to be considered when classifying the macrophyte communities (Janauer *et al.,* 2006).

Given that the aquatic vegetation is very sensitive to changes and fluctuations of environmental factors, it may act as indicator for both water quality and changes in physical parameters, habitat changes going along with the latter. This is not just a matter of species, but rather a matter of the combination of species and how they do occur in specific communities.

The aquatic vegetation of the Danube Delta has repeatedly been subject to various studies. It is however only in recent times that macrophytes have been increasingly used for the evaluation of the trophic condition. Within the frame of a comprehensive study (Oosterberg and Staras (eds.), 2000) mainly focussed on the area of the Isac-Uzlina lake complex but also other area, an evaluation scale for the Danube Delta lakes has been elaborated on the basis of the aquatic vegetation.

The objective of our study was to find out whether there is a difference between macrophyte settlements in the Delta's lakes as compared to its water courses and which could be the results with regard to the evaluation of the ecological condition.

Material and Methods

To come up with this task we chose the Isac-Uzlina lake area with varying lakes and running water types of different categories. It has been studied during the 2003 vegetation period, a follow-up of the studies took place a year later. Studies carried out in other areas of the Delta have been considered as a reference as well, even though these consisted in the long-term monitoring of the restoration areas Babina, Cernovca and Popina situated in the north-east of the Delta as well as polder Fortuna situated in the central Delta area (Marin and Schneider, 1997; Lagendijk and Schneider, 2000; Schneider *et al*., 2008). Further individual observations and inventories from other parts of the Delta have been included all the same. In doing so, physical-chemical parameters have been measured and the sampling sites were determined in a way to include all types of water bodies, in particular running waters, small and large channels, canals, temporary flood channel etc. For a better recording of the aquatic vegetation, transects of 10 to 10 m have been drawn in long straight waters, natural channels and canals. Inventories were taken inside these transects to record their alignment along ecological gradients The data collection of water macrophyte communities has been conducted by means of the Braun-Blanquet 7-point scale method for the evaluation of abundance-dominance.

In some waters the Kohler method (Kohler, 1978; Schneider and Melzer, 2005; Schranz, 2005) has been applied as well respectively it has been tested for a number of studied waters. In these cases the waters have been structured in sections and the species occurring in a specific section were estimated according to a 5-point scale: 1- very rare, 2 – rare, 3 – sporadic, 4 – frequent, 5 very frequent.

However, given that the majority of the waters have been recorded according to the above-mentioned method of Braun-Blanquet (1964), we also preferred to choose this method to allow a better comparability of the waters as for their mean abundance-dominance values. For the graphic presentation of the plant communities the mean abundance-dominance values of individual species have been calculated for samples of one site respectively one water body and have been compared to the values of communities from another site. The studies realized beyond the Isac-Uzlina lake complex provided comparison possibilities allowing to control the observations respectively the results. Thanks to multiannual samplings taken at the same sampling spots it became possible to prove, within the frame of the monitoring program and in individual areas, abundance-dominance value fluctuations of individual species on various sites over several years (Schneider *et al*., 2008). Surveys made in various parts of the Danube Delta and the comparison of their sites allowed to establish site requirements and ecological amplitude of the individual species for various aquatic plants and their communities.

Results and discussion

The results obtained in the Isac-Uzlina complex allow at least partly to document the differences and similarities of the macrophyte vegetation in lakes and running waters. 10 aquatic plant vegetation types have been recorded (Coops and Hanganu, 2000) that may be considered as being representative of the Delta's standing waters (Tab. 1, column 1). These types have also been confirmed by our surveys.

Table 1.

Water macrophyte community types in lakes and slowly running water courses of different sizes in the Danube Delta, in particular in the area of Isac-Uzlina

Standing waters – lakes following Coops and Hanganu (2000)	Slowly running waters Schneider (mscr.)
Potamogeton crispus	-
Potamogeton pectinatus	*Potamogeton pectinatus*
	Potamogeton pectinatus, P. crispus & P. perfoliatus
-	*Potamogeton pectinatus, P. perfoliatus & Vallisneria spiralis*
-	*Potamogeton nodosus*
Myriophyllum spicatum	-
Stratiotes aloides	*Stratiotes aloides* (old Gârlas)
Potamogeton lucens	-
Trapa natans	*Trapa natans*
Potamogeton trichoides	-
Nuphar luteum	*Nuphar luteum*
Ceratophyllum demersum	*Ceratophyllum demersum*
Nitelopsis obtusa	-
-	*Nymphoides peltata*

As a result of our studies and evaluations, the macrophyte communities studied in the water courses of the Isac-Uzlina area may be attributed to 9 different types (Tab. 1, column 2), various species compositions becoming apparent. Besides a pure community consisting of *Potamogeton pectinatus* which occurs in the lakes as well (sometimes together with *Potamogeton trichoides* and *Potamogeton berchtoldii*), the channels show two more communities where *Potamogeton pectinatus* occurs together with *Potamogeton crispus* and *Potamogeton perfoliatus* and a further one combining *Potamogeton pectinatus* with *Vallisneria spiralis*. Small settlements of the latter together with *Potamogeton perfoliatus* have been observed in the Litcov-Canal (category II), it does however form broader stands in the Sontea-Canal (north of polder Fortuna). Moreover, *Potamogeton pectinatus* overlaps with the *Potamogeton nodosus* community, the latter being the only one that is really bound to running waters (Fig. 1).

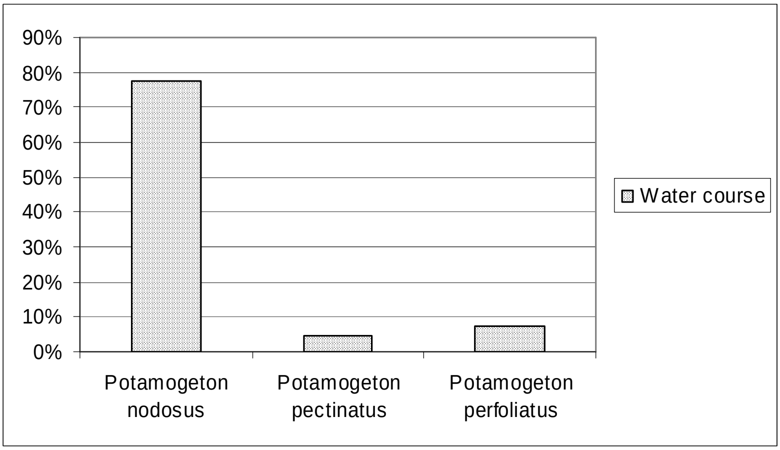

Fig. 1. *Potamogeton nodosus* type vegetation characteristic for water courses in the Delta

Beyond the studied area and in this group of real stand-forming species of running waters one may find *Potamogeton nodosus* as well as *Potamogeton gramineus* and *Ranunculus trichophyllus*. Further communities observed in stretched water courses respectively channels of category three and four such as community of *Stratiotes aloides*, *Trapa natans*, *Nuphar luteum*, *Ceratophyllum demersum*, *Nymphoides peltata* have been found in the two studied water groups. They occur on those sections of the waters where the water is hardly flowing but is temporarily characterized by undulations and water level fluctuations. It has to be noted however that e.g. *Nymphoides peltata* even occurs in the shore area of sections with slowly flowing water of the Chilia branch (downstream Periprava). *Stratiotes aloides*, together with *Hydrocharis morsus-ranae*, are mainly characteristic of the former water courses called „Gârla", as has been established in small lateral waters of the Perivolovca-Canal such as Taranova canal, or gârla leeding to lake Gorgostel and others (category III). The old Gârla in the areas of Babina, Cernovca and Popina are mainly characterized by blanket-like coverages of *Stratiotes* stands (Lagendijk and Schneider, 2001; Schneider *et al.*, 2008).

All types of plant communities recorded and species analysed in the Isac-Uzlina area may be incorporated, as for their nutrients, in the category of what is considered on a European level as nutrient-tolerant types of communities respectively species (Penning *et al*., 2008). They range from species indicating moderately eutrophic waters to species of hypertrophic conditions (Tab. 2).

Table 2.
The studied macrophytes indicators for water quality and habitat parameters.

Type of water	**st**	**vsl**	**slr**								
Trophic degree				**m**	**meu**	**eu**	**hy**				
Transparency								**cl**	**med**	**low**	**vl**
Species											
Potamogeton pectinatus	+	+	+		+	+	+	+	+	+	+
Potamogeton crispus	+	+	+		+	+	+	+	+	+	+
Ceratophyllum demersum	+	+	+		+	+	+	+	+	+	+
Potamogeton perfoliatus	+	+	+		+	+		+	+	+	
Potamogeton nodosus		+	+		+	+		+	+	+	
Ranunculus trichophyllus	(+)	+	+		+	+		+	+		
Nymphoides peltata	+	+	+		+	+	+	+	+	+	
Vallisneria spiralis	+	+			+	+		+	+		
Trapa natans	+	+	+		+	+		+	+	+	
Nuphar luteum	+	+	+		+	+		+	+	+	
Nymphaea alba	+	(+)			+	+		+			
Stratiotes aloides	+	+			+	+		+	+		
Hydrocharis morsusranae	+			+	+	+		+			
Utricularia vulgaris	+			+	+			+			
Myriophyllum spicatum	+	+		+	+			+	+		
Potamogeton trichoides	+			+	+	+		+			
Potamogeton berchtoldii	+			+	+			+			
Potamogeton lucens	+	(+)			+	+		+			
Elodea nutalii	+	(+)			+	+		+			
Lemna trisulca	+			+	+			+			

st = standing water, **vsl** = very slowly running water, **slr** = slowly running water, **m** = mesotrophic, **meu** = moderate eutrophic, **eu** = eutrophic, **hy** = hypertrophic, **cl** = clear, **med** = medium transparency, **low** = low transparency, **vl** = very low to no transparency

The mean values for the Danube Delta's eutrophic lakes range between 0.05-0.19 mg/l as for their total phosphorous content and between 0.9-4.1 mg/l for their total nitrate content (Oosterberg and Staras (eds.), 2000, see also Moss *et al.*, 2003; Moss, 2008; Solimini *et al.,* 2006). During the hot summer of 2003 the values were very high as a result of high temperatures and low water levels. In August of that year a total phosphorous content of 0.556 mg/l has thus been measured in lake Cuibul cu Lebede, in September Uzlina lake showed a total phosphorous value of up to 0.412 mg/l and lake Isac a value of 0.536 mg/l). This was an indication of increased eutrophication. In summer 2007 the total phosphorous values did not exceed the characteristic values of eutrophic waters (Moss, 2008).The monthly values of total nitrate exceeded the mean values for eutrophic waters during the vegetation period as from May to October and reached their maximum values in July in lake Cuibul cu Lebede with 12.261 mg/l. This value dropped in the following but was still very high in October with 7.314 mg/l (measurements and analysis realised by the staff of Danube Delta National Institute). In general, high total phosporous values are recorded rather in spring and are connected to the floods (Oosterberg and Staras (eds.), 2000).

When comparing the specific plant communities occurring both in lakes and in water courses one may observe that there are no significant differences as for their species composition and abundance-dominance values (Fig. 2, 3, 4). Besides the waters' nutrient content other parameters such as flow velocity, transparency (measured by means of the Secci disc), shore structure and substrate type play an important role as for the composition and species abundance of the communities. Depending on flow velocity and connectivity towards the Danube's main branches implying a higher sediment load and thus turbidity, the number of species decreases given that a lower number of species is in a position to cope well with these conditions and to adapt to them (Tab. 2).

Our studies revealed that among all stands-forming species *Potamogeton pectinatus* is the species with the broadest ecological amplitude. It reaches from moderately eutrophic to distinctly eutrophic waters and occurs both in lakes and in running waters (Fig. 2). The species does also cope well with wave dynamics and water level fluctuations. Fennel-leaved pondweed (*Potamogeton pectinatus*) occurs in clear waters, in waters with a moderate suspended sediment load as well as in waters with a high suspended sediment load and distinct turbidity. In combination with *Potamogeton crispus* and *Ceratophyllum demersum* it is an indicator of eutrophic waters, the high abundance-dominance values of all three species pointing out conditions of strongly eutrophic waters.

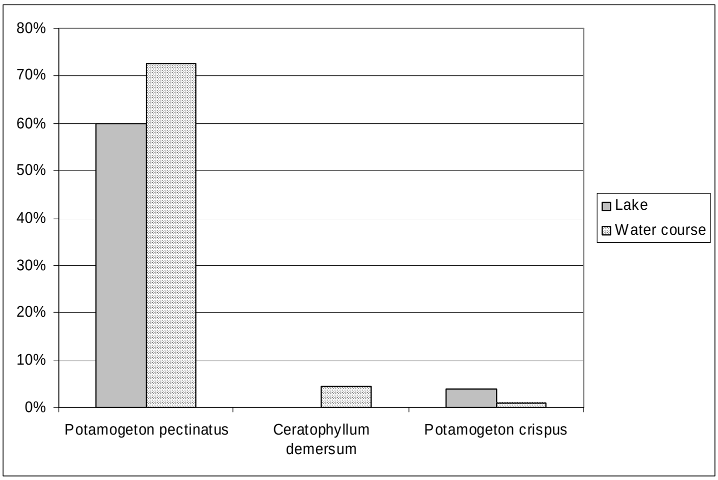

Fig. 2. Comparison of abundance-dominance of *Potamogeton pectinatus* type vegetation in lakes and running waters

Nymphoides peltata may be considered as an indicator species for strongly eutrophic to hypertrophic conditions in the studied slowly flowing waters (0.3 m/s) with low transparency and high abundance values. In a species combination with *Hippuris vulgaris* (polder Popina) it does however indicate moderately eutrophic site conditions.

Trapa natans occurs both in moderately and in strongly eutrophic waters. Even though it does mainly occur in lakes, these are usually connected with running waters of the categories I, II or III and are well adapted to heavy water level fluctuations and stronger currents (Fig. 3). This is why in the running waters and lakes studied it may be found in those places that are temporarily characterized by a stronger current. Comparable conditions occurred as well immediately adjacent to the openings of the restored polder Babina. High abundance-dominance values and an opulent vegetation indicate strong eutrophication conditions. In some cases the dense rosettes with their stipes and aquatic roots operate as traps for sediments and filter for extremely suspended sediment-abundant waters.

Stratiotes aloides which occurs in smaller elongated water courses, smaller waters type Gârla (categories III and IV) in combination with *Utricularia vulgaris* and also with *Hydrocharis morsus-ranae* as well as *Lemna trisulca* usually is an indicator for moderately eutrophic waters with a low carbonate content. In some spots, so as e.g. in Popina (Lagendijk and Schneider, 2000), the combination is complemented by *Nymphaea alba*.

Nuphar luteum usually indicates moderately eutrophic to strongly eutrophic conditions. In combination with *Nymphaea alba*, as for instance in Gârla Popina, it is a characteristic indicator for moderately eutrophic waters. The species occurs in lakes and also on the borders of channels, with very low water current (Fig. 4).

The plant communities and abundance-dominance values of specific species are subject to dramatic yearly fluctuations that may be documented in particular by means of long-term monitoring on chosen sampling spots (Fig. 5).

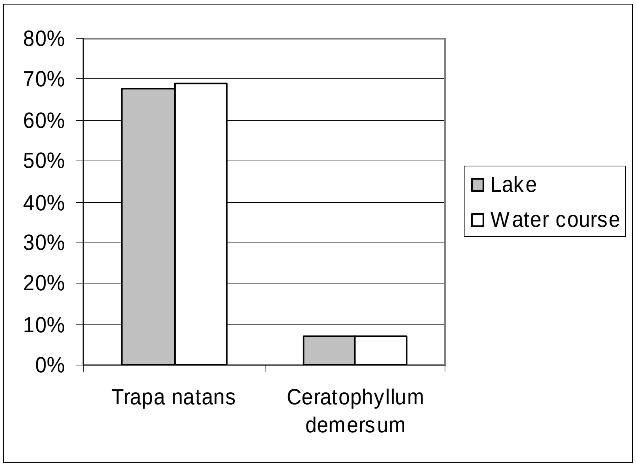

Fig. 3. Comparison of abundance-dominance of *Trapa natans* vegetation type in lakes and running waters

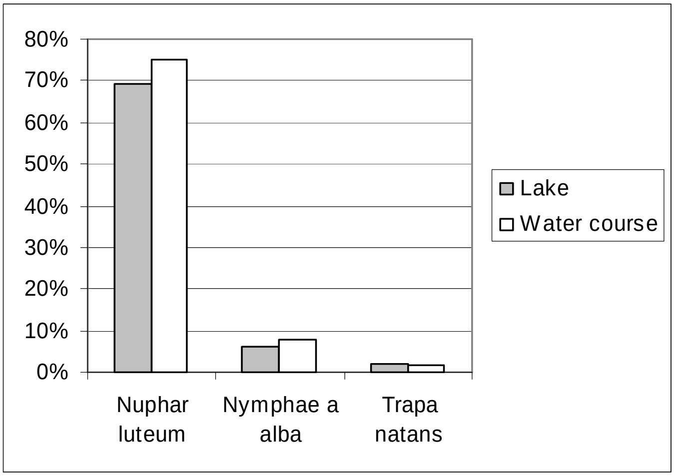

Fig. 4. Comparison of abundance-dominance of *Nuphar luteum* type vegetation in lakes and water course (Perivolovca canal)

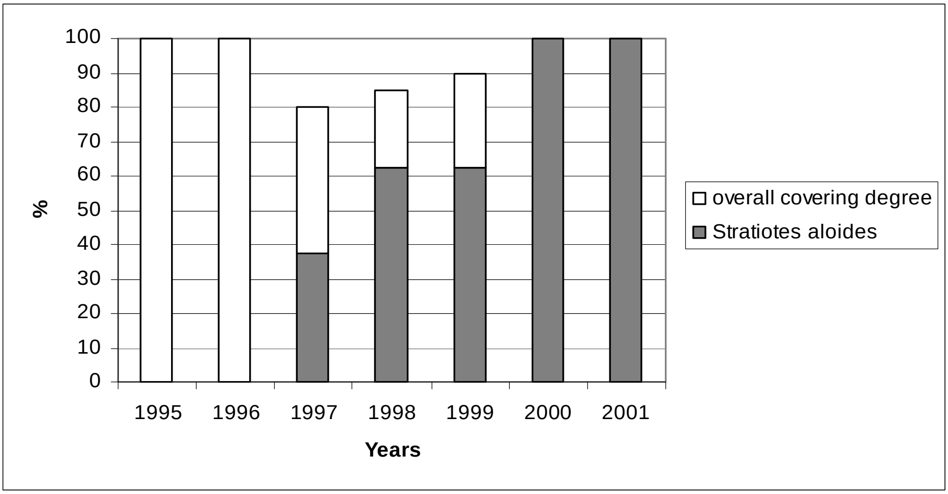

Fig. 5. Changes of the covering degree represented on the base of abundance-dominance mean values following the monitoring programme realised in the restored area Babina, North-East of the Danube Delta

In the case of restored areas they do for instance back up as well the resettlement processes that may be evaluated accurately only in the course of the years. The specific species and their characteristic combinations prove, by means of their indicator value, to which extent their characteristic biogeochemical and ecological functions do redevelop subsequent to the restoration measures effected.

In that case they serve the control of success and allow a forecast as for the future development in the restored areas. *Utricularia vulgaris* for instance is an indicator for a re-established filter function, as it occurs along the borders of the channels in those places where the clear waters that are filtered by the reeds enter the water bodies. On those surfaces of the restored areas that are subject to periodical shallow indundations (e.g. Babina and Cernovca), water macrophytes such as *Azolla filiculoides, Ranunculus circinatus* or *Zannichellia palustris ssp. pedicellata* still indicate a slight salinity. These areas were subject to soil salinization at the time of the polders, the process of washing-out has already taken place subsequent to their reconnection to the flood regime of the Danube River (Schneider *et al.*, 2008).

Conclusions

Water table dynamics, import of nutrients in connected lake systems, depth of water bodies, transparency, silt or sandy deposits on the bottom of the lakes and channels etc. are of crucial importance for the repartition of macrophytes and their communities.

In dependence of the hydrological regime from one year to the other, clearly visible differences arise between the abundance-dominance of species and their distribution within the communities.

It becomes apparent from the comparison of plant communities in standing and slowly running waters of the Isac-Uzlina area and even beyond i.e. of further areas studied in the Danube Delta, that besides a few exceptions and regarding the vegetation types determined, no major and significant differences have been observed. It has however to be stated that the stands of running waters generally show lower species numbers, especially with increasing flow velocity and high sediment load, given that only few species cope with these conditions.

As resulted from the studies only few species, such as for instance *Potamogeton nodosus*, *Potamogeton gramineus*, *Ranunculus trichophyllus* (the latter only partly), are bound to running waters.

Species such as *Potamogeton pectinatus, Potamogeton perfoliatus*, *Potamogeton crispus*, *Ceratophyllum demersum* occur in both types of waters: in slowly running waters and in lakes, more or less rich in suspended solids and with a higher turbidity in running waters and connected lakes, less turbidity or clear waters in more isolated lakes. A number of species find theirselves in a transition situation as they sometimes occur in very slowly running waters, but also in lakes where they support water table changes and dynamics caused by waves (*Nymphoides peltata, Nuphar luteum, Stratiotes aloides, Trapa natans*).

Species showing a broad ecological amplitude (such as for example *Potamogeton pectinatus*) may only serve as water quality indicators in specific species combination. This is the case when they occur together with species showing a tighter ecological amplitude.

In restoration areas the species may serve as indicators for redeveloped, characteristic ecological and biogeochemical functions and thus be used in an evaluation for the control of success.

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THE WATER FRAMEWORK DIRECTIVE AND THE RESEARCH METHODOLOGY FOR MACROINVERTEBRATE COMMUNITIES

CONSTANTIN CIUBUC[1]

SUMMARY. The Water Framework Directive represents the legal basis for the assessment of all types of water bodies in Europe. The present paper represents a general overview concerning the implementation of the Water Framework Directive in Romania, with special attention on its requirements, targets and special problems encountered. Macroinvertebrate community represents the focus of the second part of the paper. The methods used in macroinvertebrate monitoring studies are reviewed.

Keywords: macroinvertebrates, Management Plans, pressures, The Water Framework Directive.

Introduction

The Water Framework Directive ensures the framework required for water resources management according to the sustainable development principles, represented by the quantitative and qualitative control of aquatic basins. The main target is to achieve “good status” of water bodies until 2015.

The Management Plan of the catchment area(s) represents the implementation tool of the Water Framework Directive. It establishes the target-objectives for a six year period and it proposes measures for achieving the “good status” of water bodies.

According to the 2000/60 Water Framework Directive provisos, the Danube states, Romania included, must contribute to the elaboration of the Danube River catchment area Management Plan.

The signatory states of the International Commission for the Protection of the Danube River (I.C.P.D.R.) decided that the Management Plan of the Danube catchment area would have two parts.

Part A – the general plan, includes the important problems at the catchment scale, with transboundary effects, and refers to: the main river courses with an area that exceeds 4000 sq. km; the lakes with a surface larger than 100 sq. km; transboundary aquifers larger than 4000 sq. km and the Danube, the Danube Delta and coastline water bodies.

Part B refers to the national Management Plans of the Danube countries. According to the Romanian legislation 310/2004, the National Management Plan of Romanian Waters consists of eleven Basin Management Plans. Moreover, River

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Basin Committees were established for every catchment area, according to the law no. 107/1996 and the Government Decision (HG) no. 1212 from the 29th of November 2000. These River Basin Committees establish the Management Schemes for every catchment area in Romania.

General characterization of the Romanian catchment areas

Surface waters – categories:

There are six categories of water bodies found in Romania, as follows: permanent rivers - 55535 km, representing 70% from the total Romanian water courses; not permanent rivers – 23370 km, representing 30% from the total Romanian water courses; 117 natural lakes having a surface larger than 0.5sq. km (half of which in the Danube Delta); 255 dam reservoirs with a surface exceeding 0.5 sq. km; transboundary waters – 174 km and coastline waters – 116 km (Fig. 1).

Ecoregions, types and reference conditions

Four ecoregions were identified in Romania: 10 – the Carpathian Mountains; 11 – the Pannonian Plain; 12 – the Pontic area; 16 – the Russian Plain. Other two ecoregions were proposed: the Transylvanian Plateau (sub ecoregion 10s) and the Black Sea ecoregion.

The types of the Romanian water courses and lakes were defined based on unique national methods (Şerban and Jula, 2002), taking into consideration the abiotic parameters of the systems A and B recommended by the Water Framework Directive. The analysis made by using these methods in the Romanian catchment areas led to the definition of 32 water course types.

Identification of pressures

Hydromorphological pressures (Fig. 2) affect most of the Romanian water courses from the analyzed catchment areas. However, the most important hydromorphological pressures are caused by:

- 255 dam reservoirs (the most important ones are: the Iron Gates I and II, Stânca Costeşti, Izvorul Muntelui, Vidra and Vidraru)
- 7100 km of dykes and 6600 km of river regularization works (the most important are on the following rivers: the Râul Negru River, representing 83.3% from its length; the Bega – 79%; the Olt – 74.5%, the Jiu – 69%, the Crasna – 63.5%; the Dâmbovița – 61%, the Berheci - 60%; the Buzău – 45%; the Barcău 44%). The Danube River is dammed up to 80% of its length in the Romanian sector.
- 175 water derivations, including canals (e.g. Timiş-Bega; Argeş-Dâmbovița; Ialomița-Dâmbovița; Ialomița-Mostiştea etc., and the canals: The Danube – the Black Sea; Poarta Albă – Midia Năvodari; Bega; Siret – Sitna; Siret – Bărăgan).
- 138 water abstraction sources, collecting important water quantities; and 147 important water restitutions.

The percent of significant pollution sources from the total point sources analyzed is 80%.

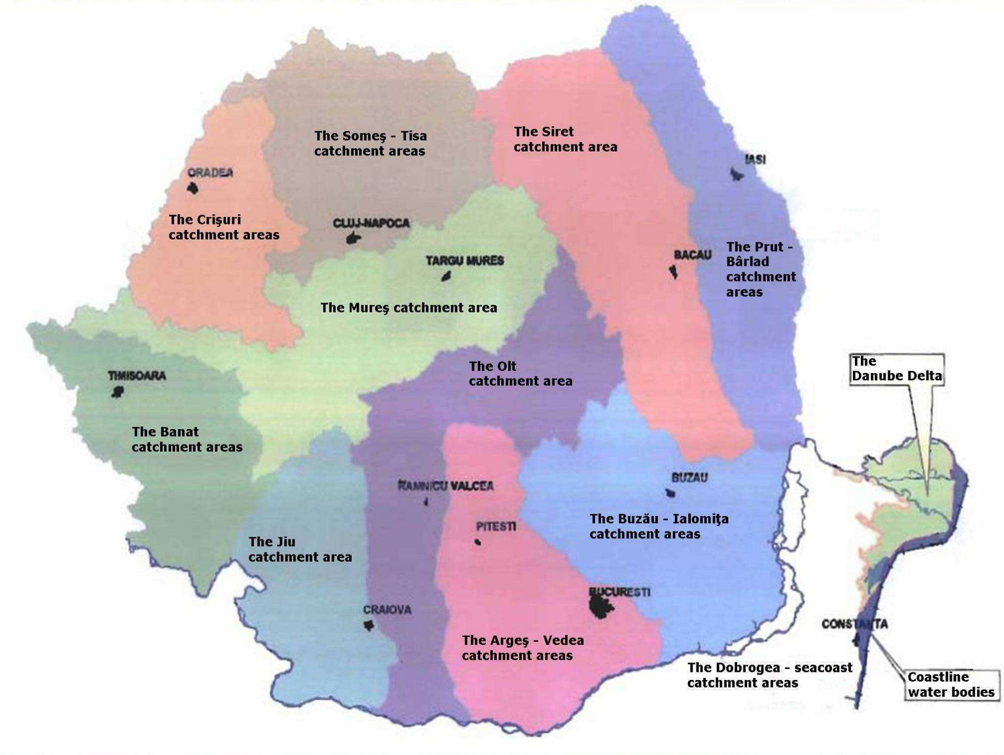

Fig. 1. The Romanian catchment areas and coastline water bodies (National Management Plans, 2004).

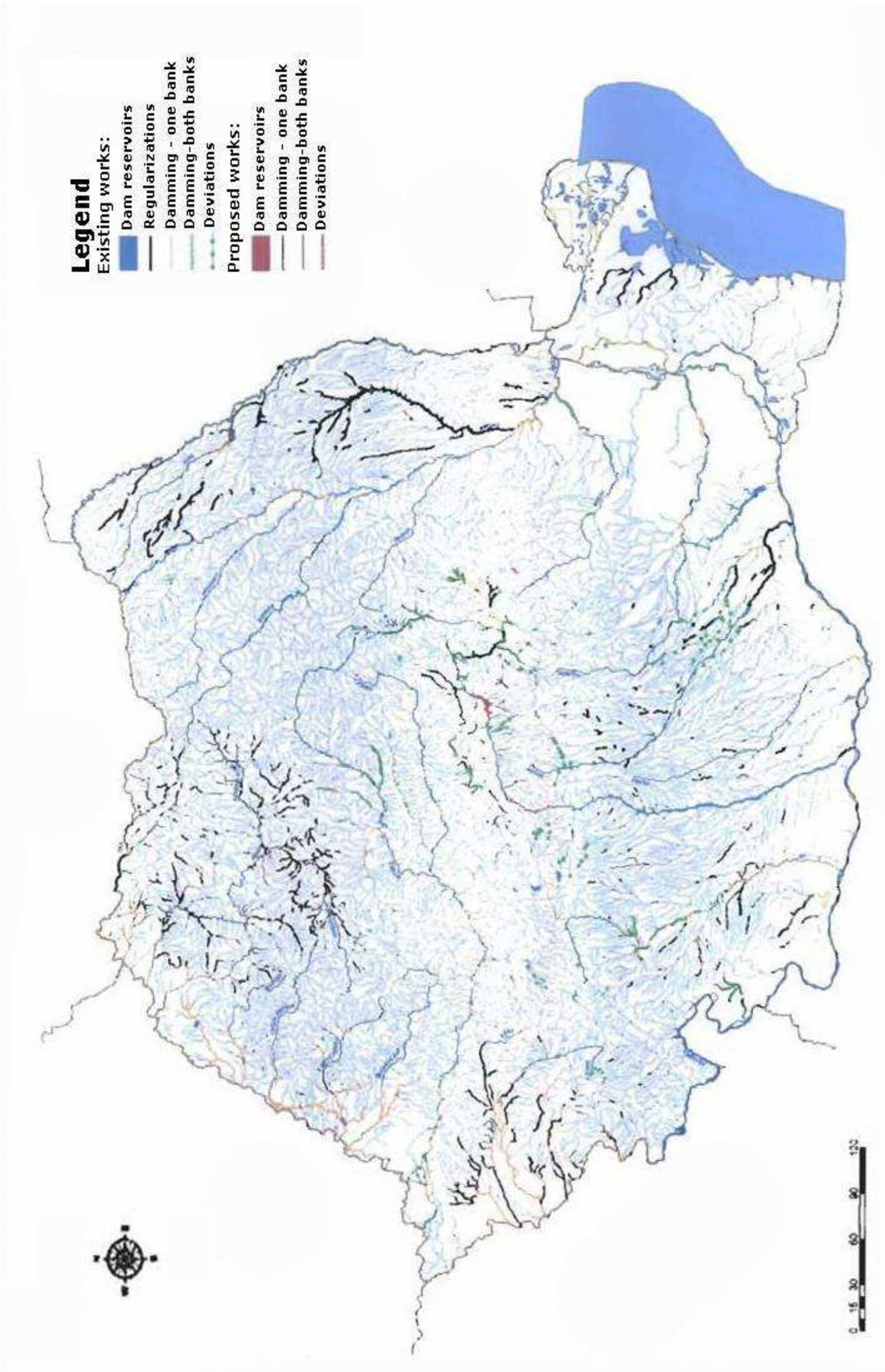

Fig. 2 Hydromorphological pressures in Romanian water bodies (National Management Plans, 2004).

Diffuse pollution sources include mostly: chemical fertilizers used in agriculture, pesticides, livestock, human settlements etc. (Fig. 3). Chemical fertilizers varied in the analyzed catchment areas between 0.39 and 8.7 kg P/ha and between 6.91 and 23.6 kg N/ha, respectively, usually lower than the average values recorded in the Danube catchment area (5.9 kg P/ha and 31.4 kg N/ha).

The pesticide quantities used for killing pests ranged between 0.08 kg/ha and 1.17 kg/ha in the analyzed catchments, thus recording lower values compared to the Danube catchment (1.39kg/ha representing the average value in seven countries). Livestock located in the analyzed catchments had a density that varied between 0.16 and 0.65 cows/ha, compared to the average value in the Danube basin (0.45 and 0.55 animals/ha, depending on the calculation method).

In rural areas, the most important diffuse pollution sources are located in the localities from the vulnerable regions. Human settlements, both rural and urban, represent important pollution sources by means of the poor connection to the sewerage network (4.1% in rural areas and 47% in urban ones).

However, there are several problems in identifying significant pressures. First, there are not enough monitoring data, especially the content of priority substances, priority/dangerous ones and heavy metals in the wastes coming from the point pollution sources. Secondly, there are no data for the calculation of the pollutant loads reaching surface waters from diffuse sources. On the other hand, there are not enough data concerning the species biodiversity for most of aquatic ecosystem types.

The Water Framework Directive (Directive 2000/60/EC) represents the legal basis for the assessment of all types of water bodies from Europe.

The implementation of the Water Framework Directive offers an assessment system of ecological quality for rivers from the European Union based on benthic macroinvertebrates, together with the aquatic flora and ichthyofauna.

The ecological status of a water body must be defined comparing the composition of the biological community of the river or lake with the natural reference conditions.

The assessment guidelines of water quality led to a “new” evaluation of the systems that should meet all criteria.

Methods and criteria used for the macroinvertebrate communities

Benthic invertebrates represent “organisms that live on the river substratum (hard mineral sediments, logs, macrophytes, algal filaments etc., in freshwater habitats (AQEM, 2002). The organisms are considered to be large enough to be caught with a net having the mesh size between 100 and 500µm.

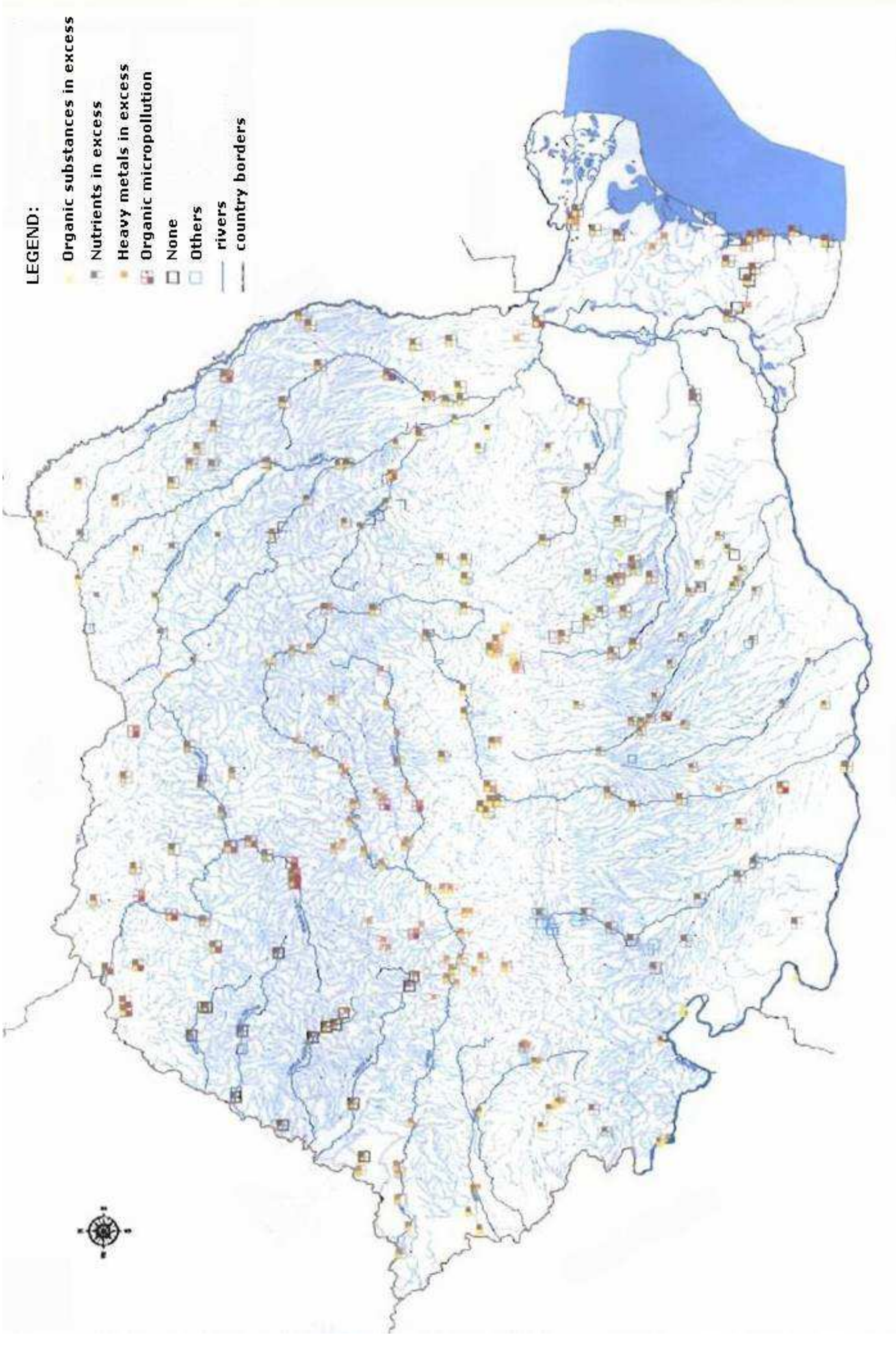

Fig. 3 The main pollution sources in Romanian water bodies (National Management Plans, 2004).

Macroinvertebrates represent good indicators of water quality. They can reflect different impacts on the river, thus a holistic assessment of the aquatic ecosystem is obtained. Benthic macroinvertebrates can identify acidity, habitat degradation and some changes of morphometrical parameters of the river.

The following steps must be considered in macroinvertebrate community analyses (AQEM, 2002):

1. First, it's necessary to distinguish between the "**representative sample**" and the "**sampling area**". The first represents the sample that must be representative from a biological point of view for the study river. "The sampling area" can cover a section of several hundreds meters along the river. The sampling area will be considered for monitoring.

2. The length of a representative sample depends on the river width and on the variability of its habitats. As a general rule, it must not be shorter than 20 meters and it must cover all the river width. It also must be representative for a minimum analysis of 500m length or 100 multiplied by the average of the river width.

3. For the analysis of a lotic ecosystem the following characteristics must be considered:

- the river morphology
- the habitat composition
- the sample must reflect the habitat composition from the study area. For example, if the sampling area includes wooden dams, sampling must be avoided in the regions with dead wood, because they are not representative.
- the bank vegetation (the sample must include the characteristic structure and density of the bank vegetation)
- the sample must reflect the presence of lentic and lotic zones throughout the study area. If both lentic and lotic zones are representative, then both will be considered.
- human impacts – it's better to avoid the regions near bridges (upstream or downstream human-made bridges) or wooden dams, unless they are typical for the study region.
- pollution sources – if the point source of pollution affects only a small section from the study area, the sample must not be collected from the region close to the pollution source, but from the area where the water is well mixed.
- the perturbation – if the macroinvertebrate communities from the study area are included in other monitoring programs, the region will not be selected

4. The procedure of field analyses

The AQEM method is based on the monitoring of major habitats reported to their percent in a sample unit. One sampling includes 20 samples collected from all types of microhabitats representing more than 5% from the area. The 20 locations must be distributed according to microhabitat distribution. For example, if the habitat from the sampling region is represented by 50% sand, then 10 samples must be collected from the sandy habitat. The structure and microhabitat categories must be established in sampling protocols. The total collection surface represents 1.25 sq. m from the riverbed.

5. The coverage of all microhabitats is considered (even of those less than 5%) based on the list of microhabitats identified in the field. Microhabitats with mineral and organic substrata are considered to be a whole that should be 100%. Based on the estimation of habitat coverage, the number of samples is established in the sampling protocol. For example, if a sample consists of 50% gravel, 25% sand and 25% coarse particulate organic matter (CPOM), than 10 samples will be collected from gravel habitats, 5 from sand and 5 from CPOM.

6. Sampling takes place from downstream on going upstream.

7. The net goes vertically into the water, tilted towards the upstream region of the water current. The substratum (together with the benthic macroinvertebrate fauna) must be dislocated at a depth of 10-15 cm, from a square surface (0.25 x 0.25m) upstream the net. Rocks are washed and the substratum is swept up for the collection of sessile organisms. Pieces of wood or smaller rocks should be put in plastic bags for later collection of attached organisms.

Different sampling tools can be used, depending on the microhabitat types. For example, the Surber can be used in waters with strong currents. For slow – flowing waters, the Surber tool can be used but the sediments must be dislocated by stirring up the water.

8. The sample taken from the river must be washed into the net, so that all the material collected should be preserved.

9. If there are different regions in the sampling area, both lotic and lentic, than the macroinvertebrate fauna should show the differences between the two types of habitats. If the study requires further information, the collected samples could be treated separately for lotic and lentic habitats. Usually the number of samples differs from lotic (e.g.13) and lentic regions (e.g.7) – depending on their relative importance. If there are problems differentiating between lotic and lentic, then 10 samples will be taken from each habitat.

10. The collected material must be sorted. The wood pieces and the small rocks must be removed after being analyzed and cleaned of sessile organisms. Small debris can be analyzed in the field, to be more time-efficient. However, large and fragile organisms (e.g. Ephemeroptera – the Mayflies) or species that cannot be preserved (belonging to e.g. Tricladia or Oligochaeta groups) must be sorted in the laboratory. These organisms must be collected in separate bottles, without sediments. For the South European rivers, the samples can be totally sorted in the field, if there are enough human resources and time.

11. The large organisms that can be easily identified on the spot must be released into the river after analyses.

12. The sample must be washed. The whole sample will be passed through a dense mesh (1000 μm for sand and 2000 μm for rocky substratum). This procedure can be made in the field or in the laboratory.

13. The storage of the sample must be made in a simple bottle. The sample is preserved with 4% formaldehyde or 95% alcohol. Sample preservation prevent carnivorous organisms (like Adephaga, Rhyacophilidae, Sialidae, some Gammaridae

etc.) to eat other macroinvertebrates before the taxonomical and ecological analyses. The final concentration of the alcohol must be 70%, and the water from the sample must be partially removed before the alcohol is added.

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MONITORING THE ECOLOGICAL STATUS OF RUNNING WATERS BASED ON FISH COMMUNITIES

KLAUS W. BATTES[1]

SUMMARY. The present paper represents a review of the most common methods used in assessing water quality of running waters based on fish communities. Monitoring the ichthyofauna offers valuable information on the ecological status of aquatic ecosystems, because most of the times changes occurring in fishes reflect the changes from lower trophic levels, caused by human impacts but also by natural ecological factors. The newest trends in fish-based methods for assessing European running waters are presented, including the Index of Biological Integrity and the European Fish Index.

Keywords: ichthyofauna, ecological status, running waters, monitoring

Introduction

As a result of multiple human impacts (water pollution, river regularization works, introduction of alien species, the presence of invasive species etc.) the structure and functions of native aquatic ecosystems changed, sometimes up to a complete transformation. Thus, assessing the present ecological status of aquatic ecosystems allows us to understand the true impact of human activities.

The EU 60/2000 Water Framework Directive stipulates the introduction of the „good ecological status" concept regarding water bodies. It also stipulates that the focus of water quality monitoring should change from physical and chemical parameters to the biological and ecological ones.

The aim of the Directive is to establish a framework for the protection of surface, inland, transitional, coastal and ground waters, together with the prevention of future alteration considering the protection and improvement of the ecological status of aquatic ecosystems.

The following biotic communities represent the main focus of the Water Framework Directive for determining the present ecological status of surface waters (especially running waters) using ecological indices: structure and abundance of aquatic flora (macrophytes and algae), structure and abundance of benthic macroinvertebrate community and the structure and abundance of fish fauna.

For fish fauna, the quality elements stipulated by the Water Framework Directive include at least three quality classes: **very good and good status** (the structure and abundance of fish associations are similar to the ones existing before

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major human impacts in a proportion of 80%) – quality classes I and II – codes blue and green; **moderate ecological status** (minor changes in the structure and abundance of fish communities occurred) – class III, code yellow; and **poor and bad ecological status** (ichthyofauna structure and functions are strongly affected) - classes IV and V, codes orange and red.

Aquatic ecosystem monitoring based on fish communities

Several parameters and indices characterizing fish associations are used in determining the ecological status of ichthyofauna.

The accuracy of the results depend firstly on using standardized sampling methods (European Standard CEN/TC/230) and on making correct taxonomical identifications (according to the updated fish species list after Kottelat and Freyhof (2008), accepted all over Europe).

Another important step is the calculation of several quantitative parameters in order to assess correctly the fish communities. These parameters include the numerical abundance, the total biomass and the fish stock given in individuals per 100 sqm. or grams per 100 sqm.

In order to establish the ecological rank of every fish species collected, the following ecological indices must be estimated: the abundance **- A**, the dominance **- D**, the constancy **- C** and the index of ecological significance – **W.** According to the values of these indices, fish species can be dominant, leading, characteristic, complementary, associated or accidental.

The red list of fish species is a major task in assessing the ecological status of fish communities. It must be made according to precise methods of determining the level of endangerment. Extinct species – **EX**; endangered species – **EN**; vulnerable species **- VU**; rare species – **R**; not evaluated – **NE** and data deficient – **DD** represent some of the categories used in establishing the level of endangerment of fish species. It can be established very accurate by collecting a minimum number of 1000-2000 individuals / catchment area and by relating the number of very rare individuals (lower than 10) to the total capture (Tab. 1).

Table 1.

The score used in assessing the level of endangerment of fish communities

The level of endangerment	**Number of individuals / A***		**Stock****	**Ecological significance (W)**
	Number	**Percent**	**(ind./100 sqm)**	
Critical status	2	0.20	0.01 (0.02)	W < 0.05
Endangered	< 20	< 2.00	0.05 (0.1)	W < 0.1
Vulnerable	< 100	<10.00 (15.00)	0.50 (1.0)	W < 0.5 (1.0)

* number of individuals / minimum 1000 individuals captured

** stock calculated at a minimum of 15 - 20 samplings

The Index of Biological Integrity (IBI)

The biological integrity represents the ability of an ecosystem to maintain its balance as a whole, thus including all its components (genes, species, higher systems) and processes (mutations, demography, biological interactions, nutrient dynamics, energy flow and population parameters) specific to a natural habitat. Biological integrity can also be defined as the ability of an ecosystem to sustain integrated and adapted communities, with a structure, diversity and organization characteristic to slightly affected habitats (Fausch *et al.*, 1984; Fausch *et al.*, 1990). The Index of Biological Integrity was introduced in the late 20th century in order to assess the ecological status of running waters in the United States of America. It was adopted and used extensively all over the world (Hughes *et al.*, 1998). A first variant of the index elaborated by Karr (1981) and Karr and Dudley (1981) was adapted for some water courses in Romania (Battes, 1999). This index uses 15 metrics, included in 3 groups.

The first one is the **species richness**, including the following parameters: the total number of species existing before the impact, the total number of cyprinid species; the total number of salmonid species; the total number of other species; the total number of native species; the total number of introduced species and the total number of extinct species.

The second group refers to the **trophic structure of fish populations** – estimating the percent of benthivorous (insectivore) species, carnivore species, together with euryphagous, herbivore and detritivore species.

The third group includes **the population abundance and its ecological status** – meaning the number of individuals / 100 sqm., the biomass (g/100 sqm.), the number of hybrids and the number of sick individuals, with tumors or anomalies (tumors, lesions, malformations etc.). Every parameter is scored with 1 point (low), 3 points (average) and 5 points (good).

The integrity classes used for assessing the ecological status of fish communities were nine at first, but they were reduced to 5, in order to correspond to the quality classes used for other biotic indices. Thus, former classes 1 and 2 (excellent and excellent/good) became **very good**; former classes 3 and 4 (good and moderate/ good) became **good**; former classes 5 and 6 (moderate and low/moderate) became **moderate**; former classes 7 and 8 (low and low/very low) became **poor,** while the 9th class (very low) became **bad**. Tables 2 and 3 present the calculation method for the score and the integrity classes for fish communities.

Table 2.

Categories of metrics used for the calculation of IBI, with the score (5 – maximum score; 3 – average score; 1 – minimum score)

Metrics		Score 5	Score 3	Score 1
Fish community structure and species richness	The total number of species existing before the impact	>90% (abundant)	50 - 90% (constant)	<50% (rare)
	Total number of cyprinid species	>45%	20 - 45%	<20%
	Total number of salmonid species	>5% (300 ind./km)	1 - 5% (100 - 300 ind./km)	<1% (100 ind./km)
	Total number of other species	>20% (6 sp.)	5 - 20% (3 - 5 sp.)	<5% (2 sp.)
	Total number of native species	>68%	35 - 67%	<34%
	Total number of introduced species	<1%	10%	>10%
	Total number of extinct species	0 sp.	2 sp	>2 sp
Trophic structure of fish populations	Percent of benthivorous (insectivore) species	>45%	20 45%	<20%
	Percent of carnivore species	>5%	1 - 5%	<1%
	Percent of euryphagous species	<20%	20 - 45%	>45%
	Percent of herbivore and detritivore species	<25%	25 - 50%	>50%
Population abundance and its ecological status	Total biomass (g/100 sqm)	>1000	100 - 1000	<100
	Total number of individuals (ind./100 sqm)	>100 ex	10 - 100 ex	<10ex
	Number of hybrids	0%	0 - 1%	1%
	Number of sick individuals, with tumors or anomalies	0%	0 - 1%	>1%

Table 3.

Integrity classes according to the score granted for small and average rivers

No	Quality class	Small rivers (w < 5 m)	Average rivers (5 m < w > 20 m)	Integrity class
1	Very good (code blue)	> 37	> 53	**I**
2	Good (code green)	30 / 37	45 / 52	**II**
3	Moderate (code yellow)	23 / 29	36 / 44	**III**
4	Poor (code orange)	12/ 22	24 / 35	**IV**
5	Bad (code red)	< 12	< 24	**V**

w – river width

Fish indices used in Europe

Table 4 presents different calculation methods for diverse fish indices at national scale from several European countries for assessing the ecological status of aquatic ecosystems characteristic to running waters (Jepsen and Pont (eds.), 2007).

Table 4.
Different indices based on fish communities from several European countries

Country	National method	Type	Notes
Austria	AT – FIA	multi-parameter index; 9 metrics; score 1 – 5; fish zones	official method
Germany	DE – FIBS	metrics: 6-9; score 1-5; integrity classes: 1-5	official method
Spain-Catalonia	SP – IBICAT	metrics: 1-3; river types: 5	developing
Sweden	SE – VIX	metrics: 6; score: 0-1; integrity classes: 5	developing
The Netherlands	NL – FI	metrics: 8; score: 0-1	official method
France	FR – FBI	metrics: 7; score: 1-100	derived from the American IBI
Lithuania	LT – LFI	metrics: 12; score: 1-7	based on ecological guilds and indicator species
Belgium – Valona Luxemburg	BWLUX IBIP	metrics: 12; score: 6-30	based on standard IBI
Belgium – Flanders	BF – IBI	metrics: 8-9; integrity classes: 5	official method, adapted after Karr's classic method
Finland	FIN – FIFI	metrics: 5; specific river types, at national level	unofficial method, based on standard IBI
Czech Republic	CZ – FI	metrics: 3; score: 0-1; reference sites	developing
Italy	IT – FITNESS	decision-support system	developing
Portugal	PT – PoFI	metrics: 5-6; 3 types of rivers	official method

The European Fish Index

The FAME project - Fish Based Assessment Method for Ecological Status of European Rivers took place between 2002 and 2004 and included 25 institutions from 12 countries. The main output of the project was the European Fish Index – EFI that assesses the ecological status of Western and Northern European rivers.

This index uses two types of variables. The first group refers to fish communities and includes 10 metrics belonging to the following ecological functional groups: trophic structure (density of insectivorous species; density of omnivorous species), reproduction guilds (density of phytophilic species; relative abundance of lithophilic species), physical habitat (number of benthic species; number of rheophilic species), migratory behavior (number of species migrating over long distances;

number of potamodromous species) and capacity to tolerate disturbance in general (relative number of intolerant species; relative number of tolerant species) (FAME Consortium 2004).

The second group of variables characterizes the habitat conditions and the human impacts (coordinates, altitude, slope, flowing regime, distance to source, riverbed geology etc.) together with details concerning the sampling technique, the sampled surface etc.

The ecological status of the rivers is expressed as an index ranging from 1 (high ecological status) to 0 (bad ecological status). There are five ecological status classes: high, good, moderate, poor and bad.

The index has been developed for sites located in Western and Northern Europe and can not be applied or should be applied with caution in other areas, characterized by different fish fauna (Schmutz *et al.*, 2007) That is why the ongoing project **"Improvement and spatial extension of the European Fish Index – EFI+"** attempts to address these limitations of the EFI, including in the project consortium countries from Central and Eastern European region as well as from Mediterranean areas. A new fish species list was made based on Kottelat and Freyhof (2008), including endemic species from different ecoregions from Europe.

One main objective of the EFI+ project is to evaluate the applicability of the existing European Fish Index and make the necessary improvements to the existing EFI in Central and Eastern Europe ecoregions, by identifying metrics taking into account the higher species richness in the Danube basin and integrating ecological traits of endemic species of Central and Eastern European catchments. However, there were only few Central and Eastern European countries which had sufficient and adequate data for a participation in the EFI+ project. The improvement and adaptation of the existing European Fish Index is therefore within the framework of the EFI+ project mainly concentrated on Polish, Hungarian and Romanian rivers.

279 sampling sites were considered for data collection in Romania, located in nine catchment areas in the Siret basin the Suceava; the Moldova; the Bistrița, the Trotuş; the Şuşita; the Putna; the Rîmnicu Sărat; the Buzău; the Bîrlad) (Fig. 1).

For data delivery, environmental data were considered for the prediction of reference conditions, including: the altitude, the presence of lakes upstream, the distance from source, the natural flow regime, the wetted width, the geology, the water source type, the actual river slope, the valley slope, the size of catchment, the catchment name, the presence of a former floodplain, the valley form, the geomorphological river type, the naturally dominant sediment. Variables describing the location, name of the sampling site and the fishing date were also important (site code, date, latitude, longitude, river name, site name), together with variables describing the sampling methods (sampling strategy, method, fished area).

Pressure categories and types were detailed for every sampling site, considering the connectivity (the existence of migration barriers at the catchment and river segment scale), hydrological alteration (impoundment, hydropeaking, water abstraction, water use, collinear connected reservoir, hydrograph modification, temperature, flow velocity increase, reservoir flushing, sedimentation), morphological alteration (channelization, floodprotection), water quality (toxic priority substances, acidification, national water quality indices, the existence of artificial eutrophication, organic pollution, organic siltation) and the navigation intensity.

Fig. 1. The location of the sampling sites considered for data delivery used to develop the new European Fish Index

Fish data collected for index modelling included variables describing the location of the sampling site, variables describing the sampling methods, catch data and variables describing length data.

The new European Fish Index will be developed this year and it will become a standardized method for assessing ecological status of running waters in Europe, according to the Water Framework Directive.

Conclusions

The standardization and unification of fish-based monitoring methods for running waters in Europe represents, by means of the elaboration of the unique index EFI a progress in assessing the ecological status of these aquatic ecosystems. This index will help simplify the problems in monitoring, protection and reconstruction of aquatic ecosystems from the European rivers. Moreover, it will contribute to the development of monitoring programs for different catchment areas, national or transboundary.

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PRELIMINARY STUDY ON AUTUMN DIATOM COMMUNITIES FROM THE CRIŞUL ALB RIVER, INEU SECTION

LAURA MOMEU[1] AND RAMONA CNAB[1]

SUMMARY. The present paper represents the first study concerning benthic diatom communities from the Crişul Alb River, Ineu section. Qualitative samples were collected in autumn 2007 from five sampling sites located near Ineu locality, on the main river course. Most of the species identified in the study area were cosmopolitan, but many were eutrophic or hypertrophic, due to natural conditions but also to human impacts present in the region.

Keywords: diatom community, the Crişul Alb river, ecological valences, substratum nature, saprobic values

Introduction

The Crişul Alb River has its source from the Pietrele Albe calcareous massif, on the Western side of the Bihorului Mountains, at 980 m a.s.l. (Oancea, 2002). The catchment area covers all relief units: mountains, hills and plains. The Bihorului and Codru Moma Mountains borders the catchment area to the North, while the Zarand and Metaliferi Mountains to the South. The piedmont region looks like an elongated golf, bordered by the following hills: Codru Moma, Hălmagiul, Bradul, Tăuț, Cuied and Almaş (Coteț, 1957). The plains are characterized by intense Quaternary alluvial processes, with gravel deposits alternating with marl, sands and clays that become thicker and thicker on going Westwards (Pop, 2005).

The geological substratum consists of calcareous rocks and cristaline dolomites in the mountains, cristaline schists, marls, clay deposits and gravel in depressions, sands, gravel, boulders and clays in piedmont areas and clays, sands, loess, pit deposits formed on cristaline schists and marls in the plains (Mutihac, 1990).

The climate is temperate-continental to subcontinental-moderate with oceanic influences. It records several topoclimatic differences due to Western and South-Western air masses with high humidity, but also due to tropical warm air masses and cold, polar ones (Mustățea, 2005). This fact has influences on the temperatures and rainfall regime. The last one records an annual average value of 450-650mm in the plains, 700-900mm in piedmont regions and 1000-1200mm in the mountains (Mustățea, 2005).

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The geological substratum nature, rock alteration processes, temperature, humidity and vegetation are the most important factors that influence the types of soil in the Crişul Alb catchment area. They form a true mosaic, including not only zonal but also azonal ones (Anastasiu, 1988; Florea *et al*., 1968).

The mosaic structure of the vegetation is caused mainly by the pedological conditions, the climatic regime and the fact that the Crişul Alb catchment area is oriented to the West (Pop, 1968; Ardelean, 1999)

The great variety of relief conditions, climate, geological substratum, soils and vegetation from the Crişul Alb catchment area have important influences on the algal community structure.

The present research was motivated by the lack of previous studies concerning diatoms from the Crişul Alb River. The present paper is included in the framework of actual world-wide biological research trends, of the conservation, protection and capitalization of biodiversity according to the sustainable development principles. Moreover, the EU Water Framework Directive stipulates the assessment of river ecological status based mainly on aquatic organisms. Algae, as primary producers, are frequently used in river monitoring programs, short-term or not.

The main target of the present paper is the study of the qualitative structure of the diatom community (Bacillariophyta), because diatoms are the dominant algal group living in rivers, especially in the upper and middle reaches, but sometimes on the lower ones as well, depending on the substratum nature (Foerster *et al.*, 2004). Human impacts affecting the qualitative structure of the diatom community from the Crişul Alb, Ineu section, will be also considered.

Material and Methods

The samples were collected in October 2007 from the middle Crişul Alb River, from five sites (Fig. 1). The first sampling site (I) was located 5 km upstream Ineu locality, while the last one (V) was situated near the city limits of the city, on going downstream. According to the literature (Winter and Duthie, 2000), epilithic samples were collected because hard substratum was characteristic to all sampling sites, even if in different proportions.

Diatom samples were collected using classical methods like scraping, brushing or washing the rocks and boulders. The samples were preserved in the field with 4% formaldehyde.

The biologic material was analyzed in the laboratory according to standardized methods (Barber and Haworth, 1981). Diatom taxonomical identifications were made from permanent slides with a Nikon Eclipse E400 microscope (equipped with a 100X immersion objective), according to usual diatom keys.

Considering the qualitative structure of diatom community, the similarity between the five sampling sites was calculated, based on Jaccard similarity index

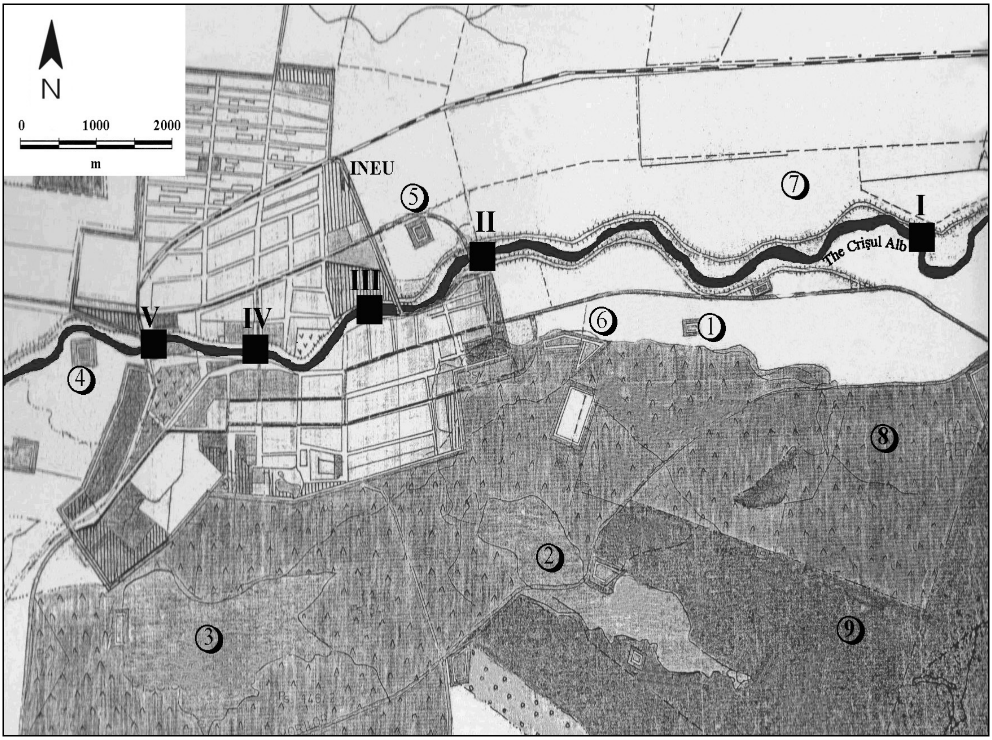

Fig. 1. The location of the five sampling sites on the Crişul Alb River, Ineu section (I-V – the sampling sites; 1- the waste dump of Ineu locality; 2, 3 – fish ponds; 4 - waste water treatment plant; 5 – water abstraction plant; 6 – sheep stables; 7 – croplands; 8 – pastures; 9 - forests (source: Office of Civil Defense and Emergencies, Ineu Townhall)

Results and discussion

A number of 121 diatom taxa was identified at the five sampling sites located on the Crişul Alb River, 118 species and 3 varieties, belonging to 27 genera (Tab. 1). Genus *Navicula* had the highest number of species (24), followed by *Nitzschia* (19), *Cymbella* and *Surirella* (9), *Gomphonema* (8) and *Fragilaria* (7), while the other genera had fewer species (1 to 4) (Tab. 1).

The high number of diatoms identified in the Crişul Alb River, Ineu section, showed an increased eutrophication of the water. This process was caused not only by natural environmental factors (Mutihac, 1990, Anastasiu, 1988), but also by human impacts. Many cropland areas, pastures and fish ponds were located within the river catchment area, in Ineu section, while the city waste dump was located on the bank of the river. Moreover, the lack of a sewage system in Ineu led to discharges of domestic and industrial waste waters into the Crişul Alb River.

Table 1.

List of diatom species identified in the Crişul Alb River, Ineu section

No.	Taxa / Sampling sites	I	II	III	IV	V
1.	*Achnanthes delicatula*	+	-	-	-	-
2.	*Achnanthes hungarica*	-	+	+	+	-
3.	*Achnanthes lanceolata*	-	+	+	+	+
4.	*Achnanthes minutissima*	+	+	+	+	+
5.	*Amphipleura pellucida*	-	-	-	+	-
6.	*Amphora libyca*	-	+	+	+	+
7.	*Amphora ovalis*	+	+	+	+	+
8.	*Amphora pediculus*	+	+	+	+	+
9.	*Amphora veneta*	+	+	+	+	+
10.	*Anomoeoneis sphaerophora*	+	-	-	-	-
11.	*Bacillaria paradoxa*	+	+	+	+	+
12.	*Caloneis silicula*	+	+	+	+	+
13.	*Cocconeis pediculus*	+	+	+	+	+
14.	*Cocconeis placentula*	+	+	+	+	+
15.	*Cyclotella meneghiniana*	+	+	+	+	+
16.	*Cyclotella pseudostelligera*	-	+	+	-	-
17.	*Cyclotella stelligera*	+	-	+	-	-
18.	*Cymatopleura elliptica*	+	+	+	+	+
19.	*Cymatopleura solea*	+	+	+	+	+
20.	*Cymbella caespitosa*	-	+	+	+	-
21.	*Cymbella cistula*	-	+	+	+	+
22.	*Cymbella lanceolata*	+	+	+	+	+
23.	*Cymbella minuta*	+	+	+	+	+
24.	*Cymbella prostrata*	+	+	+	+	+
25.	*Cymbella silesiaca*	+	+	+	+	+
26.	*Cymbella sinuata*	+	+	+	+	+
27.	*Cymbella tumida*	+	+	+	+	+
28.	*Cymbella uniseriata*	+	-	-	-	-
29.	*Diatoma ehrenbergii*	-	-	+	+	-
30.	*Diatoma moniliformis*	+	+	-	-	+
31.	*Diatoma vulgaris*	+	+	+	+	+
32.	*Epithemia adnata*	-	+	-	-	-
33.	*Epithemia sorex*	-	+	-	-	-
34.	*Fragilaria arcus*	-	-	-	-	+
35.	*Fragilaria capucina* et var. *vaucheriae*	+	+	+	+	+
36.	*Fragilaria dilatata*	+	-	-	-	-
37.	*Fragilaria parasitica*	+	+	+	+	+
38.	*Fragilaria pulchella*	+	-	-	-	-
39.	*Fragilaria ulna*	+	+	+	+	+
40.	*Frustulia saxonica*	+	-	-	-	-
41.	*Frustulia vulgaris*	+	+	+	+	+
42.	*Gomphonema acuminatum*	-	-	-	+	-
43.	*Gomphonema affine*	+	+	+	+	-
44.	*Gomphonema augur*	-	+	+	+	+
45.	*Gomphonema clavatum*	+	+	-	-	-
46.	*Gomphonema olivaceum*	+	+	+	-	+
47.	*Gomphonema parvulum*	+	+	+	+	+

Table 1. (continued)

No.	Taxa / Sampling sites	I	II	III	IV	V
48.	*Gomphonema pseudoaugur*	-	-	-	-	+
49.	*Gomphonema truncatum*	+	+	+	+	+
50.	*Gyrosigma acuminatum*	+	+	+	+	+
51.	*Gyrosigma attenuatum*	+	+	+	+	+
52.	*Gyrosigma nodiferum*	+	-	-	-	-
53.	*Gyrosigma scalproides*	+	+	+	+	+
54.	*Hantzschia amphioxys*	+	+	+	-	+
55.	*Melosira distans*	-	+	-	-	-
56.	*Melosira granulata*	+	+	+	+	+
57.	*Melosira varians*	+	+	+	+	+
58.	*Navicula ambigua*	-	+	-	-	-
59.	*Navicula bacillum*	+	-	-	-	+
60.	*Navicula capitata*	+	-	+	+	+
61.	*Navicula capitatoradiata*	+	+	+	+	+
62.	*Navicula cincta*	+	+	+	+	+
63.	*Navicula contenta*	-	+	-	-	-
64.	*Navicula cryptocephala*	+	+	+	+	+
65.	*Navicula cryptotenella*	-	+	+	+	+
66.	*Navicula cuspidata*	-	+	+	+	+
67.	*Navicula decussis*	+	+	-	-	-
68.	*Navicula goeppertiana*	+	-	-	-	-
69.	*Navicula lanceolata*	-	+	+	-	-
70.	*Navicula minima*	+	-	-	-	-
71.	*Navicula minuscula*	+	-	+	-	-
72.	*Navicula mutica*	+	-	-	-	-
73.	*Navicula pupula*	+	+	+	+	+
74.	*Navicula pygmaea*	-	+	-	-	-
75.	*Navicula radioasa*	-	+	-	-	-
76.	*Navicula rhyncocephala*	+	+	+	+	+
77.	*Navicula tripuncata*	+	+	+	+	+
78.	*Navicula trivialis*	-	-	-	-	+
79.	*Navicula veneta*	+	-	-	-	-
80.	*Navicula ventricosa*	+	-	-	-	-
81.	*Navicula viridula*	+	+	+	+	+
82.	*Neidium ampliatum*	+	-	-	-	-
83.	*Neidium bisulcatum*	-	+	-	-	-
84.	*Neidium dubium*	+	+	+	-	-
85.	*Nitzschia acicularis*	+	+	+	+	+
86.	*Nitzschia amphibia*	-	+	+	+	-
87.	*Nitzschia clausii*	+	-	-	-	-
88.	*Nitzschia constricta*	+	+	+	+	+
89.	*Nitzschia dissipata*	+	+	+	+	+
90.	*Nitzschia dubia*	+	-	-	-	-
91.	*Nitzschia flexa*	-	-	-	-	+
92.	*Nitzschia fruticosa*	-	+	+	+	-
93.	*Nitzschia heufleriana*	+	-	-	-	-
94.	*Nitzschia hungarica*	+	+	+	+	-
95.	*Nitzschia linearis*	+	+	+	+	+
96.	*Nitzschia palea*	+	+	+	+	+

Table 1. (continued)

No.	Taxa / Sampling sites	I	II	III	IV	V
97.	*Nitzschia paleacea*	-	-	+	+	+
98.	*Nitzschia sigma*	-	+	+	+	+
99.	*Nitzschia sigmoidea*	-	+	-	-	-
100.	*Nitzschia sinuata* var. *tabellaria*	+	+	+	-	+
101.	*Nitzschia subacicularis*	-	+	-	-	-
102.	*Nitzschia sublinearis*	-	-	+	+	-
103.	*Nitzschia umbonata*	+	-	-	-	-
104.	*Pinnularia borealis*	+	-	+	+	-
105.	*Pinnularia rupestris*	+	+	-	-	-
106.	*Rhoicosphenia abbreviata*	+	+	+	+	+
107.	*Rhopalodia gibba*	-	+	-	+	+
108.	*Rhopalodia gibberula*	-	-	-	+	-
109.	*Stauroneis anceps*	+	-	-	-	-
110.	*Surirella angusta*	-	+	+	+	+
111.	*Surirella brebissonii*	+	+	+	+	+
112.	*Surirella crumena*	-	+	-	-	-
113.	*Surirella linearis*	+	+	+	-	+
114.	*Surirella minuta*	-	-	+	+	+
115.	*Surirella ovalis*	-	+	-	-	-
116.	*Surirella roba*	-	-	+	+	-
117.	*Surirella robusta* et var. *splendida*	-	-	-	-	+
118.	*Thalassiosira baltica*	+	-	-	-	-

From a quantitative point of view, the dominant species were represented by cosmopolitan elements (*Nitzschia* sp., *Fragilaria* sp., *Achnanthes minutissima*, *Diatoma vulgaris*). Species indicating critical saprobic levels were identified downstream the sampling site II (Nit*zschia paleacea, N. sigma, N. constricta, N. clausii*). The number of diatom species ranged between 86 at the first two sampling sites and 66 at site V (Fig. 2).

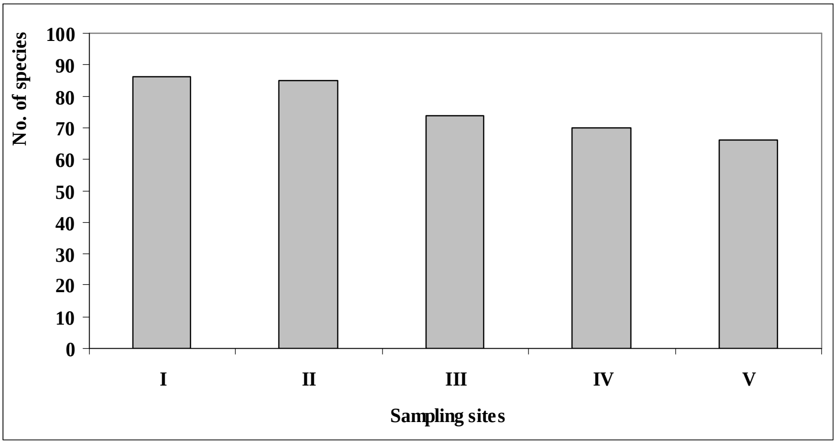

Fig. 2. Number of diatom species identified in the five sampling sites from the Crişul Alb River, Ineu section.

This decrease of species number on going downstream was caused by changes in the substratum (larger mud and sand areas in spite of rock and boulders) but also by human impacts. Nine indicator species of critical saprobic level (*Gomphonema augur, Gomphonema pseudoaugur, Navicula cuspidata, N. ambigua, N. trivialis, Nitzschia paleacea, N. sigma, Surirella angusta* and *S. minuta*) were identified only downstream from site II, located near the waste dump of Ineu locality (Fig. 1). These nine species represented almost one third of all indicator species of critical saprobian levels identified in the study area (β-α mesosaprobic, α mesosaprobic, α-polisaprobic, polisaprobic).

Among the dominant species which were represented in most cases by cosmopolitan elements, several species indicating critical saprobic levels were identified downstream site II (e.g. species belonging to *Nitzschia* genus).

From the point of view of the type of substratum characteristic for the study area, epilithic elements were well represented (e.g. species belonging to the following genera: *Achnanthes, Cymbella, Gomphonema, Navicula*). However, epipelic species were identified too (*Gyrosigma nodifera, Navicula ambigua, N. cuspidata, N. trivialis, Nitzschia sigma*) due to the presence of some areas covered in mud in the riverbed. Epiphytic elements were also found: *Cocconeis placentula, C. pediculus, Epithemia adnata*.

A high number of diatoms were cosmopolitan elements (*Cymbella minuta, Cymbella sinuata, Hantzschia amphioxys, Fragilaria ulna, Nitzschia palea, Navicula tripunctata, Gomphonema parvulum, Sururella brebissonii* etc.). A large number of diatoms known to be alkaliphilous (Patrick, 1977) were also found (*Amphora ovalis, A. pediculus, A. veneta, Cymatopleura solea, Gomphonema acuminatum, Navicula lanceolata, Nitzschia acicularis, N. dissipata, Rhoicosphaenia abbreviata* etc.). Elements known to be tolerant to heavy metals (*Achanthes minutissima, Fragilaria capucina* var. *vaucheriae*) were also identified, even among the dominant species, probably due to the pollution coming from some tannery shops that existed in the area before the 90's.

According to the river continuum concept (Vannote *et al*., 1980), because the Crişul Alb River entered its lower reaches downstream Ineu, but also due to numerous human impacts, many eutrophic and hypertrophic elements were found, like: *Gyrosigma acuminaturm, Navicula cincta, N. cryptocephala, N. veneta, N. viridula, Nitzschia fruticosa, N. umbonata, Surirella robusta*. Some halophilous species were present too: *Cyclotella meneghineana, Navicula cuspidata, N. ambigua, N. pygmaea, Nitzschia constricta*. However, allochthonous species coming from the river catchment area were also identified, like the following oligotrophic species: *Neidium dubium, N. ampliatum, Stauroneis anceps,* and the following mesotrophic species: *Cymbella lanceolata, Nitzschia heufleriana*. Other allochthonous elements were aerophilous species like *Navicula minuscula* and *Pinnularia borealis* or euplanktonic elements like *Cyclotella pseudostelligera* and *Melosira granulata*.

The saprobic level of the water assessed based on indicator species usually refers to isolated situations. However, there are some interesting aspects regarding saprobic values reflected by diatom species from the Crişul Alb River. Thus, over 30% species indicated a critical saprobic level, out of the 89 species with indicator value (Rott, 1997). On the other hand, more than 20% species belonging to xeno, oligo and oligo-β mesosaprobic group were present only in one or two sampling sites, most of them allochthonous elements, coming from the river catchment area (*Navicula contenta, Neidium ampliatum, N. dubium, N. bisulcatum, Pinnularia borealis, Stauroneis anceps*) (Fig. 3). Thus, the presence of species indicating critical saprobic levels should be a clear alarm sign, showing high quantities of organic matter in excess. These results are in concordance with the papers published by other authors on other rivers (Voicinco *et al*., 2006; Momeu *et al*., 2007).

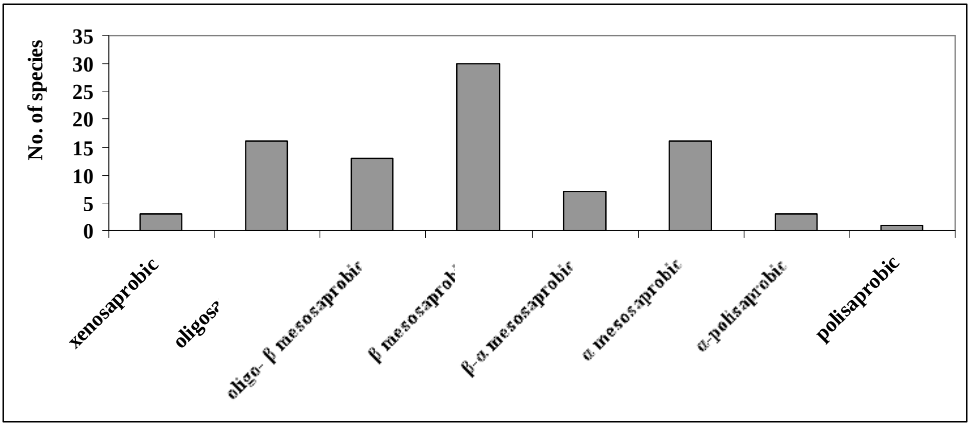

Fig. 3. The number of species with different saprobic indicator values in the Crişul Alb River, Ineu section

Fig. 4 shows the similarity between the five sampling station, the five diatom communities respectively, based on the Jaccard index. It recorded high values, exceeding 0.5 in all cases, because the stations were situated close to each other. The highest value, exceeding 0.8, was characteristic to diatom communities coming from sites II and III, located inside Ineu locality. They differed not only from the two algal communities located downstream (IV and V), but also from the one upstream (I).

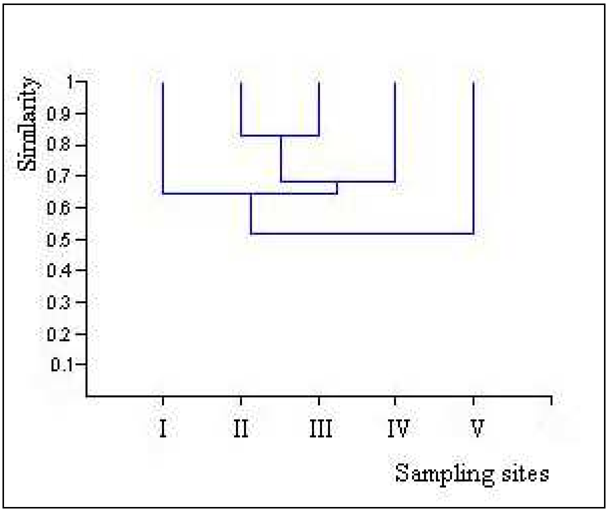

Fig. 4. The similarity between the diatom communities characteristic to the five sampling sites in the Crişul Alb River, Ineu section

Conclusions

1. 121 taxa were identified from the biological material from the Crişul Alb River, Ineu section (118 species and 3 varieties) showing a high species biodiversity.
2. Because of the hard nature of the substratum in the study area, epilithic species were well represented, together with epipelic ones (found at the surface of mud areas)
3. Considering the ecological valences of the species, most of the taxa identified in the Crişul Alb River were cosmopolitan, many eutrophic and hypertrophic, due to natural environmental conditions but also to human impacts (the waste dump, cropland areas, pastures, waste waters etc.)
4. Numerous alkaliphilous species, together with halophilous and halobiotic species were identified at the five sampling sites.
5. Domestic and agriculture pollution led to the accumulation of organic matter, having as a direct consequence the development of algal species indicating critical saprobic level, which should be a clear alarm sign for the institutions that monitor water quality from the Crişul Alb River, Ineu section.

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WATER QUALITY ASSESSMENT USING BIOTIC INDICES BASED ON BENTHIC MACROINVERTEBRATES IN THE SOMEŞUL MIC CATCHMENT AREA

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SUMMARY. The present paper presents the results of using biotic indices in assessing water quality based on benthic macroinvertebrates from 16 sampling sites located in the upper and middle Someşul Mic catchment area. Four biotic indices were considered: B.M.W.P. (Biological Monitoring Working Party), A.S.P.T. (Average Score Per Taxon), S.G.B.I. (Standardized Global Biotic Index) and E.B.I. (Extended Biotic Index), used in different European countries. Qualitative macroinvertebrate samples were collected from 16 the sampling sites. Laboratory analyses included taxonomical identifications to the levels required by the four biotic indices. The results showed good and very good water quality at the sampling sites located in the upper Someşul Mic catchment area (on the Someşul Rece and Someşul Cald Rivers). The water quality changed on going downstream, reaching the worst status downstream Cluj Napoca.

Keywords: water quality, biotic indices, macroinvertebrates, the Someşul Mic River

Introduction

In concordance with the Water Framework Directive, each European country must develop its own national system of monitoring and water quality assessment based on biotic communities. Numerous indices are used in water quality assessment studies, based on different aquatic communities: algae, macroinvertebrates, macrophytes and ichthyofauna.

The present paper represents a water quality assessment study of the Someşul Mic River on its upper and middle catchment areas. 16 stations were selected for benthic macroinvertebrate sampling and four biotic indices were calculated in order to show different assessment methods for water quality: B.M.W.P. - Biological Monitoring Working Party, A.S.P.T. -Average Score Per Taxon, S.G.B.I. - Standardized Global Biotic Index and E.B.I. - Extended Biotic Index.

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Water quality assessment studies considering benthic macroinvertebrates from the Someşul Mic catchment area were made by Găldean *et al.* (1999) and Cîmpean (2004). Details on geographical and hydrological characteristics of the Someşul Mic catchment area are included in Battes *et al.* (2000-2001) and Avram *et al.* (2006).

Material and Methods

The 16 sampling sites, representative for the study area (considering the altitude, substratum, shadowing degree, human impacts etc.) were located not only on the main river course, but also on its main tributaries (Fig. 1, Tab. 1). Seven stations were located on the Someşul Rece, seven on the Someşul Cald and two on the Someşul Mic River.

Macroinvertebrate qualitative samples were collected by means of a benthic 250 μ mesh size net. The samples were preserved in the field with 4% formaldehyde. In the laboratory they were sorted and identified to different taxonomical levels, depending on the specific requirements of the four indices considered for the present paper.

The first index used for water quality assessment is B.M.W.P. (Biological Monitoring Working Party), developed in United Kingdom in the 90's (Walley and Hawkes, 1996, 1997) and subsequent adapted for Spain and Poland. It requires identifications up to family level for all taxonomical groups. The second index, A.S.P.T. (The Average Score Per Taxon) represents the value obtained by dividing the B.M.W.P. score to the total number of families from the considered sample. The third index calculated for the present paper, S.G.B.I. - Standardized Global Biotic Index (AFNOR, 2000), used in France, also requires identification up to family level for all taxonomical groups. The last index, E.B.I., Extended Biotic Index (Ghetti, 1997) is integrated in the Italian environmental legislation. It requires identifications to the genus level for Plecoptera, Ephemeroptera, Turbelaria and Hirudinea; and to the family level for the rest of the macroinvertebrate groups.

These biotic indices have different calculation methods, but their values indicate five water quality classes, from I, which represents very good water quality, to V, the worst one.

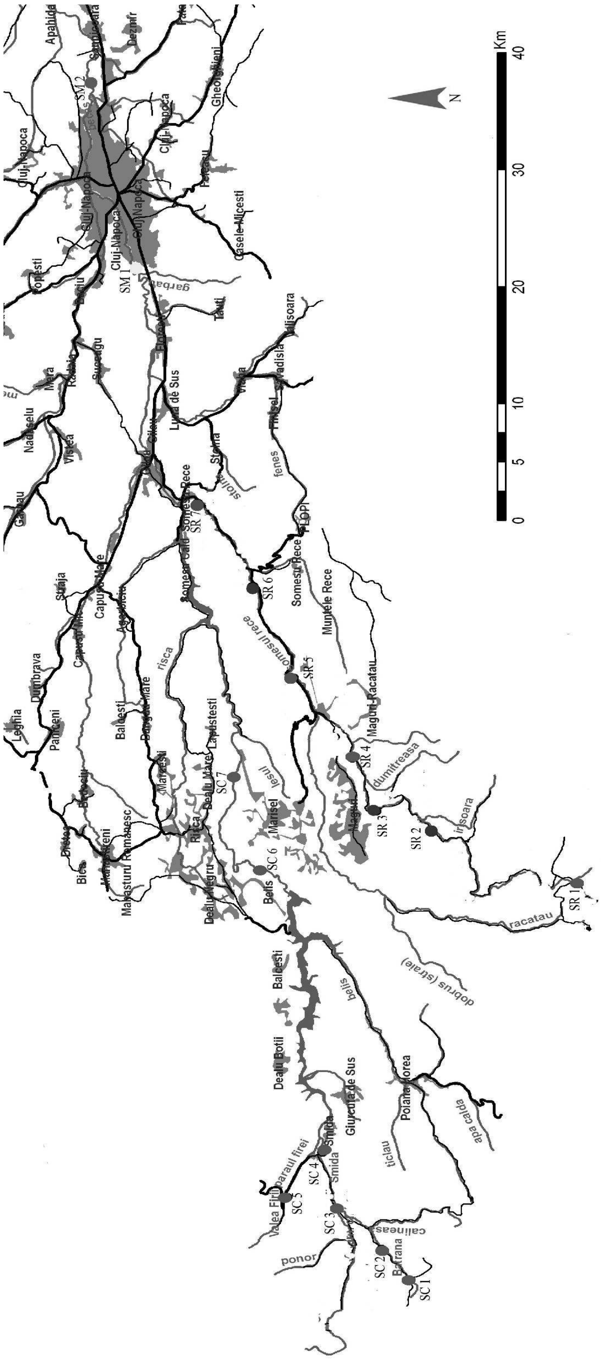

Fig. 1 The location of the 16 sampling sites within the Someşul Mic catchment area

Table 1.

The 16 sampling sites located in the Someşul Mic catchment area

Sampling site code and name		Altitude (m a.s.l.)	Coordinates	Maximum water depth (m)	Riverbed width (m)
SR 1	**Junction – river sources**	1467	N 46^{0}29’29’’ E 23^{0}04’06’’	0.20	2
SR 2	**Upstream the derivation from the Arieş River basin**	1139	N 46^{0}34’49’’ E 23^{0}06’31’’	0.30	20
SR 3	**Derivation from the Arieş River basin**	1035	N 46^{0}36’53.7’’ E 23^{0}07’25.8’’	0.70	20
SR 4	**Upstream Măguri Răcătău**	803	N 46^{0}37’45’’ E 23^{0}09’52’’	0.20	6
SR 5	**Downstream Măguri Răcătău**	662	N 46^{0}39’56.6’’ E 23^{0}13’34’’	0.40	8
SR 6	**Upstream Gura Rîştii**	528	N 46^{0}41’25’’ E 23^{0}17’48’’	0.20	7
SR 7	**Hydrological point**	445	N 46^{0}43’27.2’’ E 23^{0}21’38.7’’	0.25	15
SC 1	**Bătrîna**	1213	N 46^{0}35’38.1’’ E 22^{0}45’48’’	0.40	4
SC 2	**Bătrîna – natural deforestation area**	1094	N 46^{0}36’40’’ E 22^{0}47’14’’	0.30	4
SC 3	**The Someşul Cald upstream junction with the Bărîna**	1035	N 46^{0}38’18.4’’ E 22^{0}49’03.4’’	0.60	10
SC 4	**Smida**	994	N 46^{0}38’51.4’’ E 22^{0}51’42.9’’	0.70	30
SC 5	**Valea Firii**	1065	N 46^{0}40’10.8’’ E 22^{0}49’37.4’’	0.30	8
SC 6	**Ruseşti**	636	N 46^{0}42’06.6” E 23^{0} 08’53”	0.40	15
SC 7	**Upstream Tarnița**	550	N 46^{0}42’10.8” E 23^{0}12’15.1”	0.50	8
SM 1	**Grigorescu**	354	N 46^{0}45’51.3” E 23^{0}32’ 28.8”	0.60	35
SM 2	**Waste water treatment plant**	310	N 46^{0}47’29.1” E 23^{0}41’7.9”	0.70	25

Results and discussion

Several physical and chemical parameters were measured in the field at the 16 sampling sites (Tab. 2).

Table 2.

Physical and chemical parameters measured in the 16 sampling sites from the Someşul Mic catchment area

Site code	Water temperature (°C)	Conductivity (µS/cm)	Salinity (mg/l)	Dissolved oxygen (mg/l)	Dissolved oxygen (%)	pH
SR 1	6.8	12.62	6.67	8.68	71.1	6.76
SR 2	5.1	17.94	9.63	9.61	75.3	7.47
SR 3	4.7	22.6	12	10.8	84	7.47
SR 4	6.2	65.3	34.7	11.61	93.8	7.96
SR 5	6.7	61.6	32.7	9.83	80.4	8.04
SR 6	7.7	93.3	49.6	11.01	92.2	7.89
SR 7	7.6	92.5	49.2	9.17	76.6	8.05
SC 1	5.9	145.9	77.5	7.19	57.4	8.65
SC 2	5.7	99.2	52.7	3.62	28.7	8.36
SC 3	7.2	86.9	46.1	7.8	64.5	8.62
SC 4	7.8	84.3	44.8	8.51	71.1	8.16
SC 5	6.5	131.7	70	8.96	72.8	8.42
SC 6	6.3	46.3	24.5	11.31	91.6	7.5
SC 7	6.7	59.9	31.9	11.07	90.6	7.59
SM 1	-	-	-	-	-	-
SM 2	-	-	-	-	-	-

The decrease in temperature on the Someşul Rece River (SR3 sampling site) was probably caused by the water derivation from the Valea Dumitreasa. Conductivity and salinity values were low at the sampling sites located upstream the Someşul Rece dam (SR1, SR2, SR3), but they increased downstream of the dam, where the river was recomposed entirely from its tributaries. At all the stations located on the Someşul Cald River, conductivity and salinity values were higher compared to the ones recorded on the Someşul Rece, probably due to differences in the geological substratum – the Someşul Cald collected waters coming from calcareous regions, while the Someşul Rece from cristaline schists. As concerns the pH values, they were neuter to alkaline, except for the SR1 site, where they were slightly acid, due to the influence of a near-by peat bog. Dissolved oxygen values measured in the Someşul Rece catchment area indicated well oxygenated mountain streams. At the stations located on the Someşul Cald River, dissolved oxygen values recorded a sudden drop at SC2, probably due to the natural deforestation occurred in 2007 as a result of a strong storm. Large areas of woodland were destroyed, causing high quantities of organic matter to be carried into the river.

53 taxa were identified at the 16 sampling sites from the Someşul Mic catchment area (Tab. 3). Order Diptera was represented by 9 families, out of which Chironomidae and Limoniidae were present at all stations. Order Ephemeroptera included 10 genera, out of which *Baetis* was found at all sites, except for SM2. 12 genera were identified belonging to Order Plecoptera; *Leucra* and *Nemoura* were present at all stations from the Someşul Cald and Someşul Rece catchment areas, but not downstream the junction of the two rivers. *Arcynopteryx* was identified only at the SR1 sampling site, at the Someşul Rece River source. 8 families belonging to Order Trichoptera were found, the most frequent being Hydropsychidae and Limnephilidae.

The highest number of taxa present at one sampling site was 38 (recorded at SR2), and the lowest 6 (SM2). At SC2 the relatively low number of taxa was probably caused by low dissolved oxygen values due to large deposits of organic matter coming from the mountains as a direct result of deforestation induced by storm.

From all the biotic indices considered for the present study, the Extended Biotic Index (E.B.I.) was the least sensitive to hydromorphological impacts and to natural environmental factors. For example, E.B.I. indicated very good quality at SR7, located near a bottom sill (and thus having very low water velocities) and at SC6, situated between several hydroelectric reservoirs (the Fîntînele - Beliş - Tarniţa "chain" of dam reservoirs). Moreover, sites characterized by restrictive natural conditions like SR1 (located in a peat bog region) and SC5 (located on a tributary that collected waters with low temperatures coming from a karstic region) were characterized also by very good water quality according to E.B.I.

The other indices used (B.M.W.P. - Biological Monitoring Working Party, A.S.P.T. - Average Score Per Taxon, S.G.B.I. - Standardized Global Biotic Index) showed these natural pressures or human impacts for all the sampling sites (Tab. 4).

On the Someşul Mic River, at SM1, situated upstream Cluj Napoca, the values of all biotic indices showed a sudden decrease in water quality with at least one quality class compared to all the sampling sites located upstream (Tab. 4). This might be due to the influences induced by the localities situated upstream from the site, but also to hydromorphological impacts (the Fîntînele - Beliş - Tarniţa – Someşul Cald – Gilău "chain" of dam reservoirs and the presence of flood-protection works built along the banks, leading to a complete extinction of riparian vegetation). At SM2, located near the waste water treatment plant of the city of Cluj Napoca, all indices indicated the worst quality class, probably due to the heavy impact caused by the city and its waste water treatment plant.

Table 3.
List with benthic macroinvertebrate taxa identified in the Someşul Mic catchment area at the 16 sampling sites

TAXA / SAMPLING SITES	SR1	SR2	SR3	SR4	SR5	SR6	SR7	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SM1	SM2
Phy. Nematoda	+	+	+	+	-	+	+	-	-	-	-	+	+	-	+	+
Sphy. Turbellaria																
Dugesia	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-
Cls. Hirudinea																
Erpobdella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Cls. Oligochaeta	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cls. Bivalvia	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-
Cls. Gastropoda																
Ancylus	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+	-
Hydrachnidia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Sphy. Crustacea																
Cls. Ostracoda	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Ord. Amphipoda																
Fam. Gammaridae	-	-	-	-	+	-	+	-	+	-	-	+	-	+	-	-
Scls. Copepoda	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
Ord. Coleoptera																
Fam. Dytiscidae	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Fam. Gyrinidae	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-
Fam. Elmidae	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-
Ord. Diptera																
Fam. Athericidae	-	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-
Fam. Empididae	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Fam. Tabanidae	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Fam. Ceratopogonidae	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Fam. Chironomidae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fam. Simuliidae	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Fam. Psychodidae	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	-
Fam. Limoniidae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fam. Tipulidae	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Ord. Ephemeroptera																
Baetis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Caenis	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-
Ephemerella	+	+	+	-	+	+	-	-	-	-	-	-	-	-	+	-
Torleya	-	-	-	+	+	+	-	-	-	+	+	+	-	+	-	-
Ephemera	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-

Table 3 (continued)

TAXA / SAMPLING SITES	**SR1**	**SR2**	**SR3**	**SR4**	**SR5**	**SR6**	**SR7**	**SC1**	**SC2**	**SC3**	**SC4**	**SC5**	**SC6**	**SC7**	**SM1**	**SM2**
Ecdyonurus	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
Epeorus	-	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-
Rhithrogena	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Habroleptoides	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-
Habrophlebia	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
Ord. Megaloptera																
Sialis	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Ord. Plecoptera																
Capnia	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-
Leuctra	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Amphinemura	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-
Protonemura	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	-
Nemoura	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Rhabdiopteryx	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Chloroperla	-	+	+	-	+	-	-	-	+	+	+	-	+	+	-	-
Dinocras	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Perla	-	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-
Isoperla	+	+	+	+	+	+	-	+	-	-	+	+	-	+	-	-
Arcynopteryx	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perlodes	+	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-
Ord. Trichoptera																
Fam. Hydropsychidae	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
Fam.Polycentropodidae	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-
Fam. Philopotamidae	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Fam. Brachycentridae	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-
Fam. Goeridae	+	+	+	-	-	-	-	+	+	-	-	-	-	+	-	-
Fam. Limnephilidae	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Fam. Sericostomatidae	-	+	+	+	+	+	-	+	-	+	-	-	+	+	-	-
Fam. Rhyacophilidae	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	-
TOTAL TAXA	**28**	**38**	**35**	**32**	**34**	**36**	**24**	**28**	**19**	**23**	**26**	**22**	**28**	**25**	**14**	**6**

Table 4.

The quality classes calculated according to the four biotic indices, for the sampling sites considered in the Someşul Mic catchment area (BMWP - Biological Monitoring Working Party, ASPT - Average Score Per Taxon, SGBI - Standardized Global Biotic Index and EBI - Extended Biotic Index)

SAMPLING SITES		BMWP Quality classes	ASPT Quality classes	SGBI Quality classes	EBI Quality classes
SR 1	**Junction – river sources**	II	III	II	I
SR 2	**Upstream the derivation from the Arieş River basin**	I	II	I	I
SR 3	**Derivation from the Arieş River basin**	I	II	I	I
SR 4	**Upstream Măguri Răcătău**	I	II	II	I
SR 5	**Downstream Măguri Răcătău**	I	II	II	I
SR 6	**Upstream Gura Rîştii**	I	II	I	I
SR 7	**Hydrological point**	III	III	II	I
SC 1	**Bătrîna**	I	II	II	I
SC 2	**Bătrîna – natural deforestation area**	II	II	II	I
SC 3	**The Someşul Cald upstream junction with the Bărîna**	II	II	II	I
SC 4	**Smida**	II	II	II	I
SC 5	**Valea Firii**	III	II	II	I
SC 6	**Ruseşti**	II	III	II	I
SC 7	**Upstream Tarniţa**	II	II	II	I
SM 1	**Grigorescu**	IV	IV	III	II
SM 2	**Waste water treatment plant**	V	V	V	V

Conclusions

53 benthic macroinvertebrate taxa were identified in 16 sampling sites. They represented the basis for water quality assessment from the Someşul Mic catchment area. In the upper reaches of the river, water quality was very good and good according to most of the indices, except for two sites where the values were lower due to restrictive natural environmental factors (SR1 and SC5); and two stations where water quality decreased probably due to hydroelectric works (SR7 and SC6).

In the middle reaches of the river, water quality was very low, probably due on the one hand to large hydroelectric works and on the other hand to influences coming from several important localities.

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PRELIMINARY DATA ON ZOOPLANKTON AND AQUATIC INVERTEBRATES FROM THE FÎNAȚELE CLUJULUI NATURE RESERVE (TRANSYLVANIA, ROMANIA)

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SUMMARY. The present study represents the first inventory of zooplankton and aquatic invertebrate taxa from three pools included in the Fînațele Clujului Nature Reserve, which is a botanical protected area since 1932. The samples were collected in June 2008 and only qualitative analyses were carried out. Zooplankton cladocerans and cyclopoid copepods, together with benthic water mites and Mayflies were identified up to genus or species level. One water mite species is recorded for the first time in the Romanian fauna.

Keywords: aquatic habitats, zooplankton community, aquatic invertebrates, Fînațele Clujului Nature Reserve, biodiversity

Introduction

Fînațele Clujului Nature Reserve lies only 4 km North-East from the city of Cluj Napoca and has been a protected area for more than 75 years. It is well-known due to its special relief, dominated by the presence of characteristic ridges (glimee, copîrşaie) (Morariu and Călinescu, 1965) and the steppe vegetation common on the Southern slopes of these geomorphological units. The nature reserve shelters a high variety of habitats, a specific fauna but most of all a very rich vegetation, mostly characteristic to dry areas – xerophilous, but also mesophilous and woody. Near the water bodies present in the nature reserve there is a characteristic hygrophilous vegetation.

In time, many botanists and zoologists studied the terrestrial flora and fauna, while the water bodies included within the protected area were ignored. Thus, the present paper contributes to the knowledge of the biodiversity of these particular areas.

Material and Methods

The samples were collected on June the 13th 2008 from three pools located within the Fînațele Clujului Nature Reserve (Tab. 1). Qualitative zooplankton samples were taken using a 55 µm mesh zooplankton net. The samples were preserved with sucrose solution according to Haney and Hall method (1973) and in 4% formaldehyde. Aquatic invertebrates were collected with a 250 µm qualitative hand-net and they were also preserved in 4% formaldehyde.

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Zooplankton and major aquatic invertebrate taxa were identified from pool no. 1, while water mites and mayflies individuals coming from the three pools were considered for taxonomical identifications.

Zooplankton crustaceans and cyclopoid copepods, together with water mites and Mayflies were identified to the species or genus level using taxonomical keys for each group.

Results and discussion

Physical and chemical parameters of the three water pools were measured in the field in Fînațele Clujului Nature Reserve. Table 1 depicts the location and the altitude of the sampling sites, together with the values of physical and chemical parameters considered for this study.

Table 1.

The coordinates and the physical and chemical parameters measured in the three pools from the Fînațele Clujului Nature Reserve

Sampling sites:	Pool 1	Pool 2	Pool 3
Coordinates	N 46° 50’ 02,9’’ E 23° 38’15,5’’	N 46° 50’ 02,8’’ E 23° 38’10,6’’	N 46° 50’ 04,2’’ E 23° 37’44,1’’
Altitude (m)	492	495	508
pH	7.97	9.72	9.28
Conductivity (μS/cm)	16.41	16.85	16.59
Salinity (mg/l)	8.6	8.98	9.06
Dissolved oxygen (mg/l)	7.37	6.93	10.6
Water temperature (^{0}C)	21.1	21.8	21.6

pH values were neuter to alkaline in the three aquatic habitats, while conductivity and salinity recorded normal values for freshwater habitats. The dissolved oxygen values were measured near the surface, thus no hypoxia or anoxia phenomena were recorded.

The most frequent cladoceran species was *Daphnia curvirostris* (Tab. 2). It is known to live in shallow standing waters, with alkaline pH and high organic matter load (Fig. 1). Another well represented species was *Chydorus sphaericus* (Fig. 2),a cosmopolitan species identified in all types of water bodies (Negrea, 1983). *Simocephalus exspinosus* was also found in Fînațele Clujului Nature Reserve, together with species belonging to *Ceriodaphnia*, *Alona* and *Scapholeberis* genera (Fig. 1 and 2). Ovigerous and non-ovigerous parthenogenetic females were identified for all cladoceran taxa, except for *Alona* sp., where only non-ovigerous parthenogenetic females were collected. *Eucyclops proximus* represented the most common cyclopoid copepod species idenfied in pool no. 1 from Fînațele Clujului Nature Reserve (Fig. 3). It is adapted to different environmental conditions, living in waters with diverse characteristics (Damian-Georgescu, 1963). *Megacyclops viridis* was the other copepod species represented by adults, both males and females. Copepodites and nauplii, larvar stages in copepod development, were also present (Tab. 2).

Most cladoceran and copepod species present in pool no. 1 from the Fînațele Clujului Nature Reserve recorded the β-mesosaprobic indicator value (Negrea, 2002; Pleşa and Müller, 2002).

Table 2.

List of zooplankton taxa from pool no. 1 and their frequency of occurence

TAXA	The frequency of occurrence
Subphylum Crustacea, Class Branchiopoda, Suborder Cladocera	
Daphnia curvirostris Eylmann, 1887	✱✱✱✱
Chydorus sphaericus O.F. Müller, 1776	✱✱✱
Simocephalus exspinosus Koch, 1841	✱
Ceriodaphnia sp.	✱
Alona sp.	✱
Scapholeberis sp.	✱
Subphylum Crustacea, Subclass Copepoda, Order Cyclopoida, Family Cyclopidae	
Eucyclops proximus Fischer, 1851	✱✱
Megacyclops viridis Jurine, 1820	✱
Copepodites	✱✱
Nauplii	✱✱
Others	
Calanoid copepods	✱✱
Ostracods	✱
True fly larvae (Diptera: Chironomidae)	✱
True fly larvae (Diptera: *Chaoborus* sp.)	✱
True bugs (Heteroptera)	✱
✱ - rare; ✱✱ - common; ✱✱✱ - frequent; ✱✱✱✱ - very frequent	

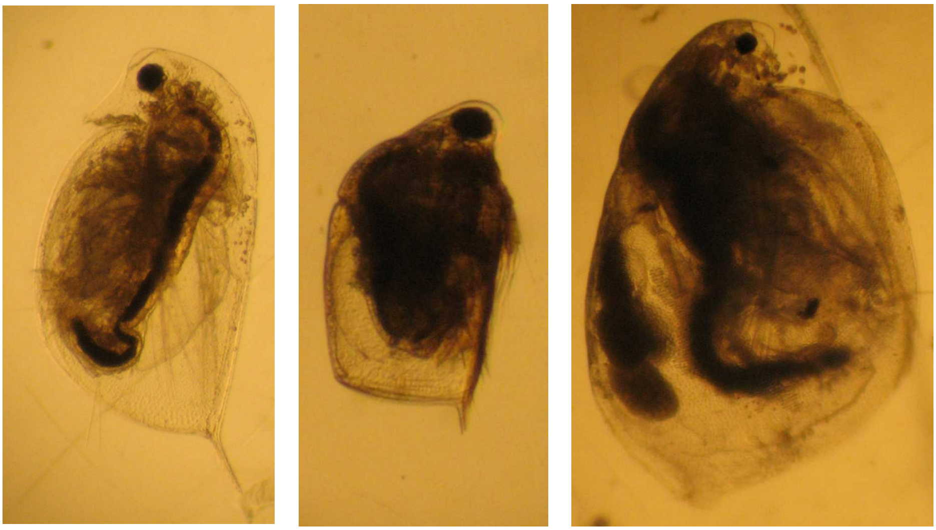

Fig. 1. Three cladoceran species identified in Fînațele Clujului Nature Reserve: *Daphnia curvirostris* (left); *Scapholeberis* sp. (middle); *Simocephalus exspinosus* (right)

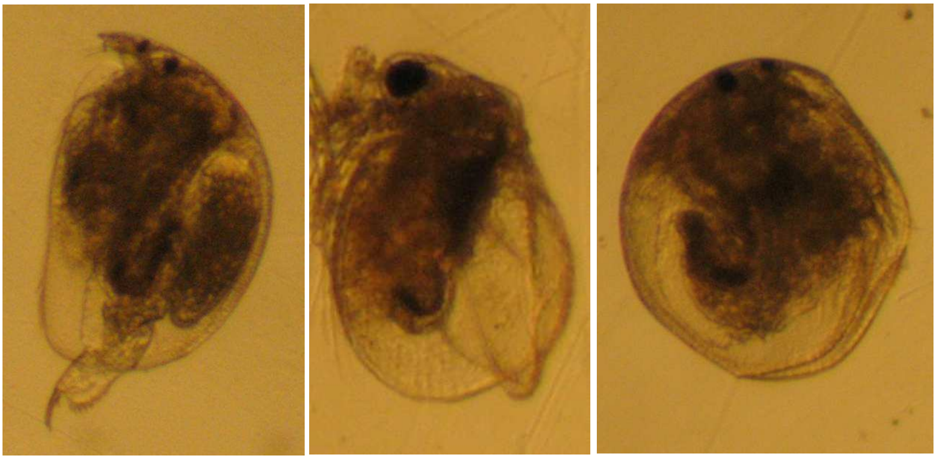

Fig. 2. Ovigerous or non-ovigerous cladoceran females identified in Fînațele Clujului Nature Reserve: *Alona* sp. (left); *Ceriodaphnia* sp. (middle); *Chydorus sphaericus* (right)

Fig. 3. Two copepod species from the pools of Fînațele Clujului Nature Reserve: *Eucyclops proximus* – ovigerous female (left) and *Megacyclops viridis* - non ovigerous female (right)

In case of macroinvertebrate community, 10 taxonomical groups were identified (Tab. 3). Ostracoda and Gastropoda recorded the highest abundance, exceeding together 77% from the total number of macroinvertebrate organisms. Insects (Heteroptera, Coleoptera, Odonata, Chironomidae, Strationidae and Ephemeroptera) recorded more than 22%.

Table 3.

List of macroinvertebrate groups collected from pool no. 1 in Fînațele Clujului Nature Reserve

Taxa	Percentage numerical abundance (%)
Cls.Hirudinea	0.1
Cls. Gastropoda	23.1
Hydrachnidia	0.3
Cls. Ostracoda	54.4
Ord. Heteroptera	11.1
Ord. Coleoptera	1.3
Ord. Odonata	0.4
Fam. Chironomidae	4
Fam. Stratiomidae	0.7
Ord. Ephemeroptera	4.6

Water mites were also identified to species or genus level. *Hydrodroma pilosa*, found in pool no. 3, is common to both standing and running waters from 0 to 560 m a.s.l. (Gerecke, 1991). This species is recorded for the first time in Romanian fauna (Fig. 4).

Piona genus (Fig. 5) was collected from pool no. 1. It is also typical to standing waters, including 30 species in Europe, out of which 22 in Central Europe (Gerecke, 1994). *Arrenurus* genus was found in all three pools, but only as deutonimph, thus identifications to the species level were impossible (Fig. 6). This genus is characteristic to standing waters, including 150 species all over Europe, out of which 85 in Central Europe (Gerecke, 1994).

Cloeon dipterum (Ephemeroptera) was identified in pools 1 and 2. It is known to be living in both standing and running waters, in bank vegetation, being a poor swimmer (Silina, 1994).


Fig. 4. *Hydrodroma pilosa* **-** idiosoma – ventral view (left), gnathosoma (right)


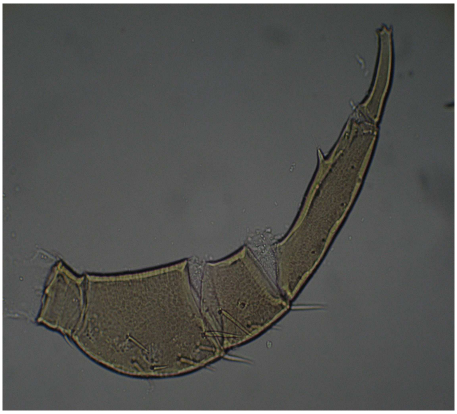

Fig. 5. *Piona* sp. – idiosoma – ventral view (left), palp (right)

Fig. 6. *Arrenurus* sp. (deutonimph idiosoma – ventral view)

Conclusions

Even if the present paper represents a first attempt to characterize the aquatic habitats included in Fînațele Clujului Nature Reserve, considering only qualitative samples on zooplankton and macroinvertebrate communities, it represents the first research on this topic, thus a valuable step in characterizing biotic diversity from this special area of protection.

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AN OVERVIEW OF THE METHODS USED IN THE ASSESSMENT OF THE MARINE ENVIRONMENTAL QUALITY, BASED ON THE ANALYSIS OF THE ZOOBENTHOS

VICTOR SURUGIU[1]

SUMMARY. The methods and techniques most often used in the assessment of the marine environmental quality, based on the zoobenthos, are reviewed and re-evaluated. These methods, grouped into four main categories (univariate methods, graphical methods, multivariate methods, and biotic indices) are examined for their reliability and applicability for the marine pollution monitoring.

Keywords: ecological quality; benthic communities; marine environment; diversity indices; biotic indices

Introduction

The implementation of the Water Framework Directive (2000/60/EC), the Habitats Directive (92/43/EEC), the Integrated Coastal Zone Management proposal (ICZN) and the Bathing Water Directive (76/160/EEC) by the Member States of the European Community has emphasized the elaboration of methods for the assessment of the *Ecological Quality Status* (EcoQ) of the marine and coastal environments. The evaluation of the marine environmental quality is based on the analyses of the structure and composition of different biotic components of the ecosystem (*e.g.* phytoplankton, zoobenthos, macroalgae and nekton etc.) and by their comparison with a reference site/state.

For the evaluation of the health of the marine and coastal environments most often are used estimates of the *benthic macrofauna* condition (Pearson and Rosenberg, 1978; Billyard, 1987), because benthic organisms present a series of advantages:

- reflect environmental conditions not only for the moment of the sampling but also for a longer period prior to sampling;
- are more or less sedentary (*i.e*. are unable to avoid the deterioration of the water and sediment quality) and are thus useful for the study of the local effects of pollutants;
- have relatively long life spans, indicating good integration to the water and sediment quality conditions;
- belong to various systematic categories (*i.e.* exhibit different levels of tolerance to different levels of environmental stress) and can be classified accordingly to their response to the disturbing factor;

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- are commercially important or represent an important food source for economically valuable species;

- play an important role in the cycling of the organic matter, nutrients and other chemicals between the sediment and the water column.

Pearson and Rosenberg (1978) describe an empirical model of the effects of organic enrichment upon macrozoobenthos. This model states that the first detectable change consists in the increase in the number of species, followed by an increase in biomass and then an increase in numerical abundance. Abundance increases drastically at relatively high levels of organic load and is given by the exponential development of the so-called *opportunistic species*. As the organic load increases further, the biomass, the number of species and the number of individuals decrease rapidly as anaerobiosis installs (Fig. 1).

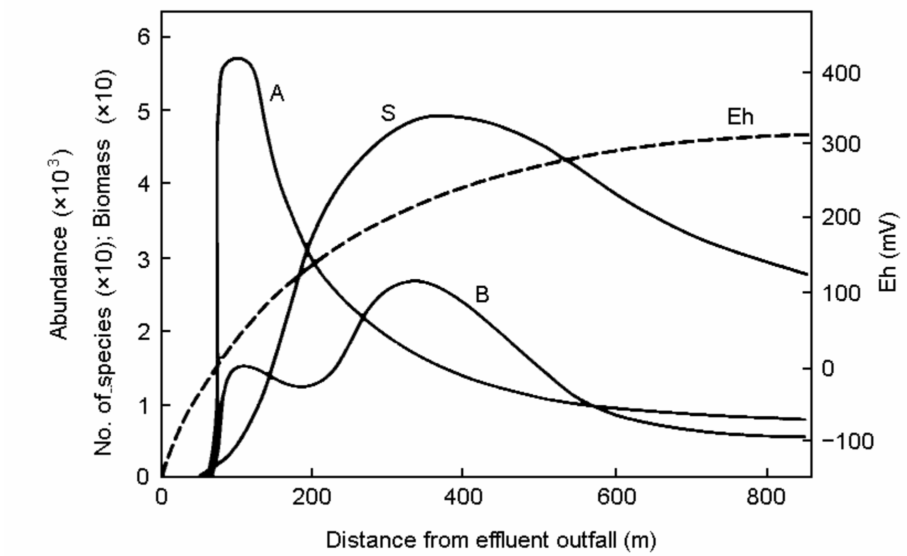

Fig. 1. Diagrammatic representation of changes in benthic macrofauna in terms of species diversity (*S*), numerical abundance (*A*), and the total biomass (*B*) (modified from Pearson and Rosenberg, 1978)

Based on this model a bewildering variety of biological methods for the evaluation of the ecological status of marine and coastal waters has been proposed. Basically, all these methods can be grouped into the following four main categories (Washington, 1984; Elliott, 1994; Clarke and Warwick, 1994; Simboura and Zenetos, 2002; Diaz *et al.*, 2004; Borja and Dauer, 2008): univariate methods, graphical representations, multivariate tools and biotic indices.

Univariate methods

The univariate techniques quantify the pollution stress or other disturbance through the information on the community structure patterns, based on the paradigm that the more diverse a community is, the less it is considered to be affected by environmental stress. However, the Intermediate-Disturbance Hypothesis (Connell, 1978) suggests that the highest diversity occur not in stable systems, but in communities having intermediate levels of disturbance. In essence, univariate methods reduce all the information on the community structure to a single statistic in the form of a *diversity index* (Clarke and Warwick, 1994).

In the simplest case the diversity is expressed as the total number of species present in a sample or in a given area, usually termed as *species richness* (*S*). According to the Pearson-Rosenberg paradigm, generally, the number of species decreases with the increase of pollution levels (Fig. 2). Species richness is a common measure used by marine biologists, providing a satisfactory estimate of the diversity and having good discriminant ability.

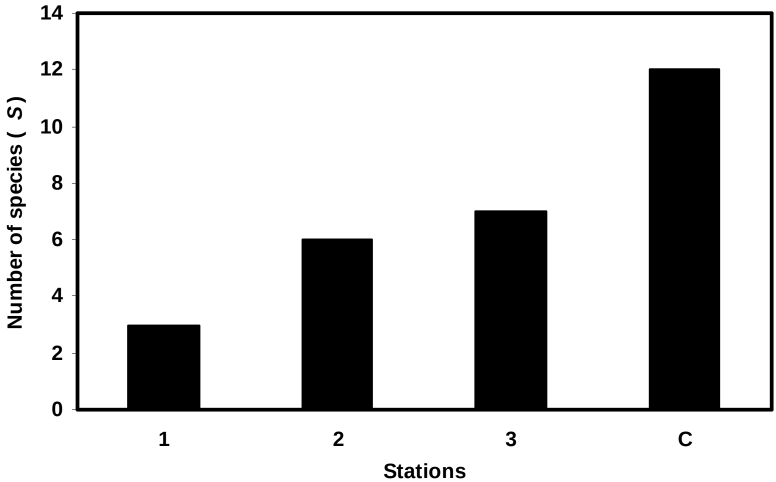

Fig. 2. Variation of the total number of polychaete species (*S*) with the distance from a waste-water effluent: 1 = 0 m, 2 = 50 m, 3 = 100 m, C = control (from Surugiu, 2008)

The main disadvantage of using the species richness in this simplified form is that it does not take into account the species abundance. Also, the species richness is strongly dependent on the sample size: the greater is the number of individuals sampled, the more species there are likely to be present. In order to reduce this drawback, in the assessment of the disturbances induced by organic pollution upon benthic communities is employed the ratio between the total number of species (*S*) and the total number of individuals (*N*) in a sample or in a given area

(Sanders, 1968; Pérès and Bellan, 1973). However, this ratio is usually very small (many individuals for by far few species). This is why in the marine ecology more favoured is the *Margalef's index* (Margalef, 1958):

$$d = \frac{S - 1}{\log N}$$

The main disadvantage of all species richness indices, including Margalef's index, is that the distribution of individuals among species is not taken into account. Also, species richness is dependent on the habitat type (Simboura and Zenetos, 2002).

Diversity indices overcome the problem by including both the number of species and the relative abundance (dominance) of each species. There are a plethora of diversity indices, which can be divided into two broad categories: dominance indices and information-statistic indices (Washington, 1984).

Dominance indices include Berger-Parker diversity index (Berger and Parker, 1970) and Simpson's diversity index (Simpson, 1949). Both indices are easy to calculate and to interpret and have low sensitivity to sample size. The major disadvantage of all dominance indices, however, is that they are weighted toward the most abundant or dominant species. Simpson's index uses information from a broader array of species in community than the Berger-Parker index do and therefore may be regarded as more accurate.

Indices based on the information theory, such as Shannon-Wiener (Shannon and Weaver, 1949) or Brillouin (Brillouin, 1951), reflect both the species richness and the evenness and are biased toward rare species. Brillouin index is considered as having better discriminant ability, but is more difficult to compute in practice (but not in the computational era!).

By far the most commonly used diversity index in the evaluation of the pollution in marine benthic communities is the *Shannon's* or *Shannon-Wiener diversity index*:

$$H' = -\sum_{i=1}^{S} p_i \log p_i$$

where p_i is the relative abundance (or the relative biomass) of the species i.

Over the time the Shannon-Wiener formula become some kind of "$E = mc^2$" in ecology. Sometimes this index is erroneously called the Shannon-Weaver index because Shannon published his paper in a book together with Weaver (Shannon and Weaver, 1949). Wiener and Shannon independently developed the formula, so it must bear both names (Washington, 1984).

The particular strength of the Shannon-Wiener formula is that it incorporates both the species richness and equitability components. In the calculation of the Shannon's diversity the logarithm to the base 2 is usually preferred because it provides the results in "bits per individual" or "bits per biomass" units (Clarke and

Warwick, 1994; Borja *et al*., 2000). Occasionally $\log_e$ or $\log_{10}$ is used. This is why the comparison between different values of *H'* is possible only when the same logarithm base has been used.

Equitability, also termed *evenness*, expresses how evenly the total number of individuals in a sample is distributed between each species. The equitability is the reciprocal of the dominance: the higher is the equitability, the lower is the dominance. The equitability is most frequently expressed as *Pielou's evenness index* (Pielou, 1966):

$$J' = \frac{H'}{H_{\max}}$$

where *H'* is the calculated (actual) value of the Shannon-Wiener diversity index and $H_{\max}$ is the maximum possible diversity.

The maximum diversity is achieved when all species are equally abundant. So the formula also can be rewritten as:

$$J' = \frac{H'}{\log S}$$

The levels of pollution stress, reflected by the variation in any of univariate metrics (species richness, diversity or evenness), can be assessed only by comparisons of spatial or temporal data sets (Fig. 2 and 3). Hence, the use of the univariate methods in assessing Ecological Quality Status requires the definition of ranges of variation of diversity indices for each ecological quality class and for each habitat type (Simboura and Zenetos, 2002).

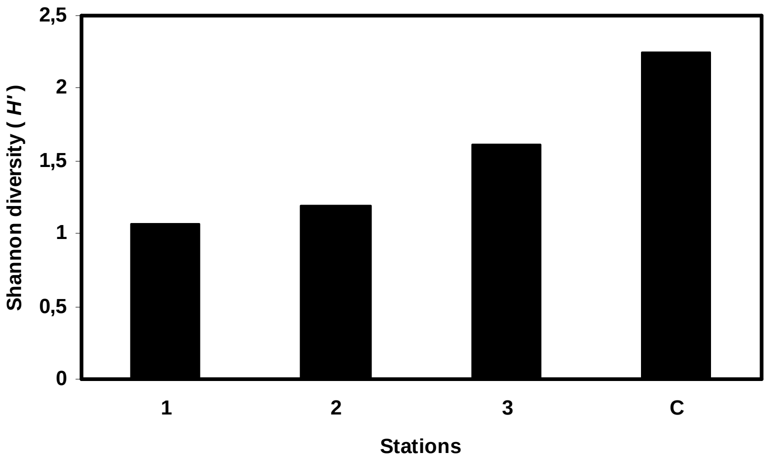

Fig. 3. Variation of the Shannon diversity index (*H'*) with the distance from an wastewater effluent: 1 = 0 m, 2 = 50 m, 3 = 100 m, C = control (from Surugiu, 2008)

For instance, for Mediterranean waters, Simboura and Zenetos (2002) have established that the values of *H‘* range (log base 2), depending on the habitat type, between 0.57 for polluted areas and 6.68 for pristine areas. They also provide, for muddy-sandy habitats, the values of the Shannon diversity index corresponding to each of the five WFD ecological quality classes (Table 1). Zettler *et al*. (2007) indicates for the southern Baltic Sea lower values, ranging from 0.06 to 4.81 (Table 1).

Table 1.

Classification scheme of the Ecological Quality status (EcoQ) based on the values of the Shannon-Wiener diversity index

EcoQ	Quality class	*H‘* (after Simboura and Zenetos, 2002)	*H‘* (after Zettler *et al*., 2007)
I (high)	Undisturbed/reference community	$H‘>5$	$H‘>4$
II (good)	Slightly polluted	$4<H‘\leq5$	$3<H‘\leq4$
III (moderate)	Moderately polluted	$3<H‘\leq4$	$2<H‘\leq3$
IV (poor)	Highly polluted	$1,5<H‘\leq3$	$1<H‘\leq2$
V (bad)	Azoic to very highly polluted	$H‘\leq1,5$	$H‘\leq1$

The weakness of all diversity indices is that they are influenced by sample size, by the sampling methodology and by the level of taxonomic expertise. Also, these indices are dependent on the habitat type being analysed (Simboura and Zenetos, 2002).

Graphical methods

Graphical techniques, also termed *distributional* or *curvilinear representations*, are a class of methods which are also widely used in the assessment of the pollution effects on the macrobenthic communities. Distributional methods summarise the patterns of the relative abundance of species in a form of a curve or histogram (Gray, 1981; Clarke and Warwick, 1994). Graphical representations are a more accurate measure of diversity and incorporate more information on community structure than do simple diversity indices. This category of methods is considered as being intermediate between univariate techniques and the full multivariate analyses (Clark and Warwick, 1994).

Rarefaction curves (Sanders, 1968) are graphs of the number of individuals (the *x*-axis) against the number of species (the *y*-axis). In more diverse the community the rarefaction curve is steeper and more elevated (the ratio *S/N* is greater).

×2 geometric abundance classes (Gray and Pearson, 1982) are histograms of the number of species categorised into geometrically-scaled abundance classes (*e.g*. class 1 is represented by a single individual in a sample, class 2 by 2-3 individuals, class 3 by 4-7 individuals, class 4 by 8-15 individuals etc.) falling in each abundance range. In unpolluted situations there are many rare species and the curve is very steep, extending across few abundance classes. In polluted situations

there is a reduction in number of the rare species (the first one or two geometric classes) and an increase in abundance of the opportunistic species (the higher geometric classes) so that the species abundance distribution is more flatter, extending over many more abundance classes (Fig. 3). Gray and Pearson (1982) indicate that the species of the intermediate abundance classes 3 to 5 are the most sensitive to pollution-induced changes, thus suggesting an objective way to select indicator species.

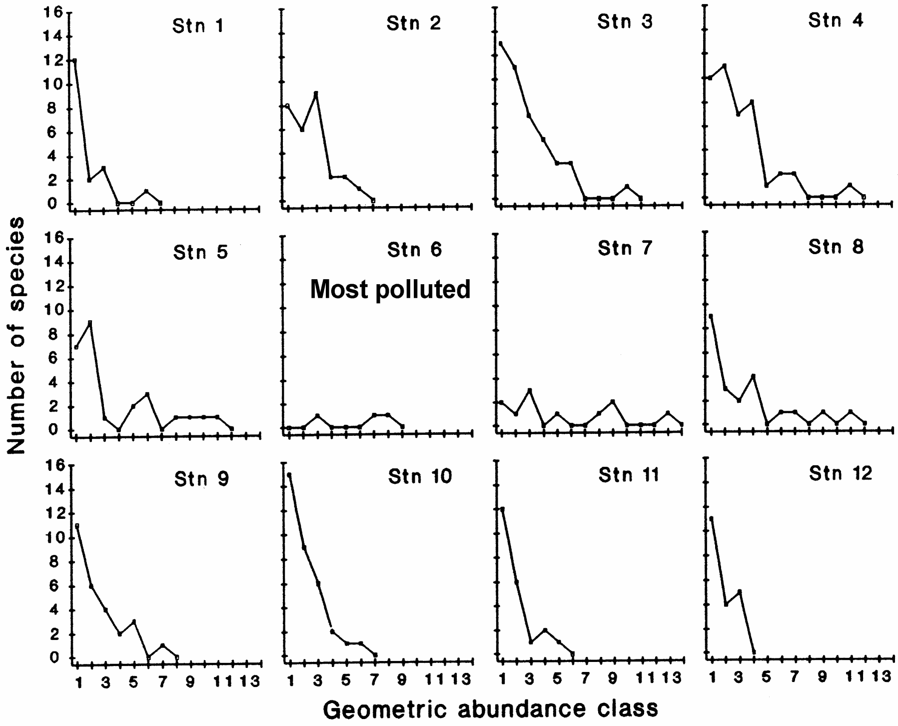

Fig. 3. Plots of ×2 geometric abundance classes (from Clarke and Warwick, 1994)

Rank abundance (dominance) diagrams (Gray, 1981) are plots of proportional abundance or biomass of each species against its rank in terms of abundance or biomass. Basically, there are three typical shapes of the rank abundance curves: the geometric series, lognormal, and broken-stick (Gray, 1981). The distribution of individuals among species is most equitable in the broken-stick and less equitable in the geometric series. In more polluted stations the curve is more "J-shaped", showing high dominance of abundant species, whereas in less polluted stations the curve is much flatter, indicating high evenness (Fig. 4).

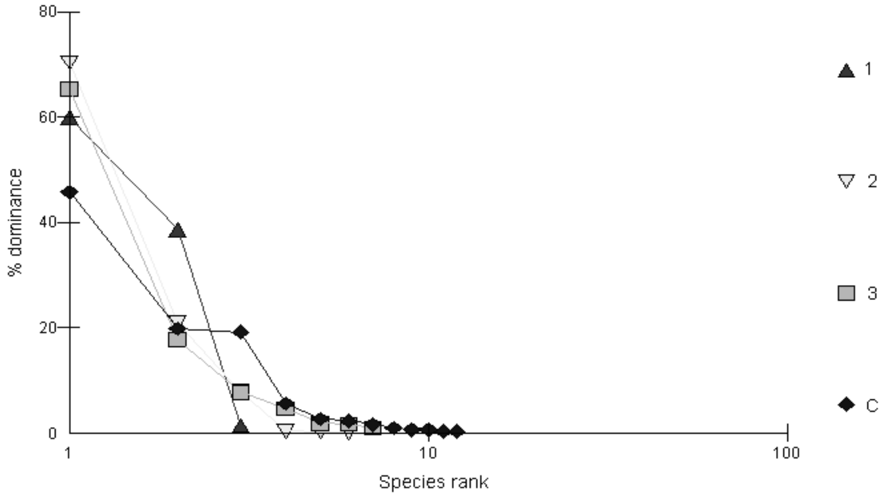

Fig. 4. Rank dominance plots (the rank of each species is plotted on a decimal logarithm scale) for 4 stations situated at different distances from a sewage outlet: 1 = 0 m, 2 = 50 m, 3 = 100 m, C = control (from Surugiu and Feunteun, 2008)

k-dominance curves (Lambshead *et al*., 1983) are cumulative ranked abundances plotted against the species rank (or log species rank). The communities with higher diversity will have a less elevated the curve (Fig. 5). The advantage of *k*-curves against rank abundance curves lies in the fact that the differences between polluted and unpolluted situations are better visualised.

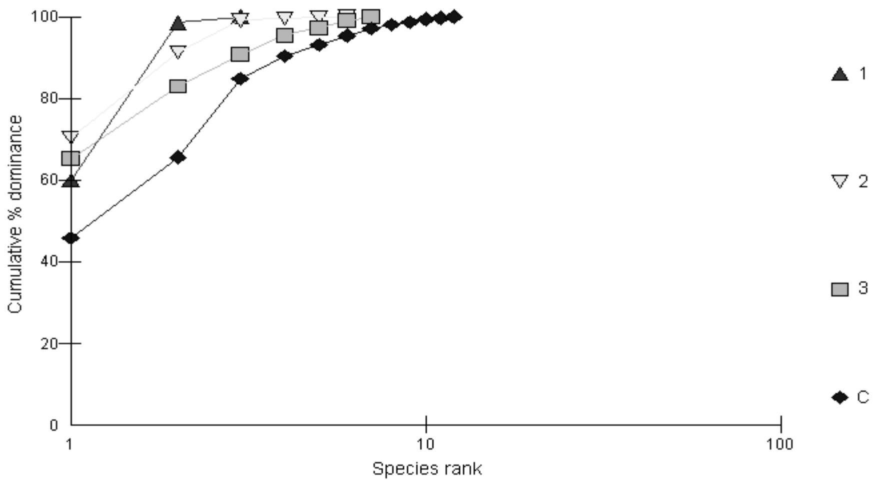

Fig. 5. *k*-dominance curves (x-axis logged) for 4 stations situated at different distances from the sewage outlet: 1 = 0 m, 2 = 50 m, 3 = 100 m, C = control (from Surugiu and Feunteun, 2008)

ABC (Abundance-Biomass Comparison) curves (Warwick, 1986) are based on the superimposition of the *k*-dominance curves of the species abundance on the analogous *k*-dominance curves of the species biomass on the same plot (Fig. 6). In undisturbed sites the biomass curve will lie above the abundance curve for its entire length because the biomass is dominated by one or a few large-bodied conservative species, each represented by few individuals (the so-called *k*-strategists). In strongly polluted sites the biomass curve will drop below the abundance curve throughout its length. This is explained by the loss of large bodied conservative species and by the rise of dominance of small-bodied opportunists (the so-called *r*-strategists).

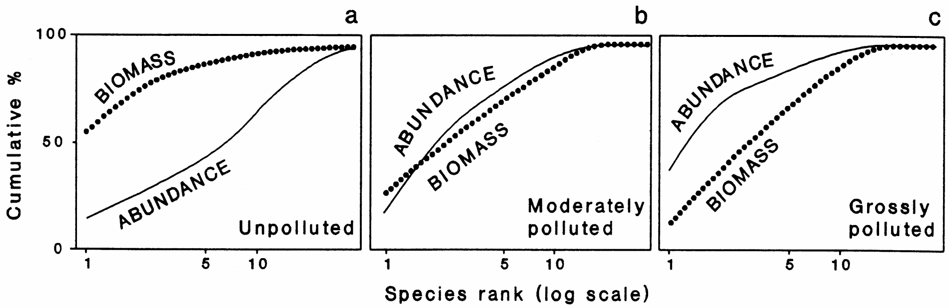

Fig. 6. Hypothetical ABC curves for unpolluted, moderately polluted and grossly polluted conditions (from Clarke and Warwick, 1994)

The ABC plots have proved a reliable tool in detecting disturbance effects because changes in diversity can be assessed without reference to a temporal or spatial series of data (Warwick, 1986).

Multivariate methods

Multivariate techniques take into account changes in taxa and "base their comparisons on the extent to which different data sets share particular species, at comparable levels of abundance" (Clarke and Warwick, 1994). All multivariate techniques are based on the *similarity coefficients*, which calculate the proportion of species common to the communities being compared. Many similarity coefficients have been proposed, the more commonly advocated coefficients are the "simple matching" coefficient, the Jaccard coefficient, the Sorensen coefficient, and the Bray-Curtis coefficient.

The *"simple matching" coefficient* represents the probability that a single species picked at random will be present in both sites or will be absent in both sites:

$$S_{jk} = \frac{a+d}{a+b+c+d} \cdot 100$$

where a is the number of species common to both sites, b is the number of species present in site j but absent in site k; c is the number of species present in site k, but absent in site j and d is the number of species absent in both sites.

The weakness of the "simple matching" similarity coefficient is that it is affected by the number of species that are absent from both of the sites being compared (and which is usually difficult to know in practice).

The *Jaccard coefficient* (Jaccard, 1908) is more sensible because it depends only on the number of species present in one of the sites and represents the probability of a single species picked at random to be present in both sites:

$$S_{jk} = \frac{a}{(a+b+c)} \cdot 100$$

The *Sorensen coefficient* (Sorensen, 1948) is similar to Jaccard coefficient, but is considered better than that because it weights matches in species composition between two samples more heavily than mismatches:

$$S_{jk} = \frac{2a}{(2a+b+c)} \cdot 100$$

All the similarity coefficients presented above have the main drawback in reducing the biological information to a simple presence or absence of each species. Thus, to a rare species is given the same weight as to a very common one.

The *Bray-Curtis coefficient* (Bray and Curtis, 1957) is generally regarded as the most suitable for ecological data which tend to have many zero values and where abundances tend to be over-dispersed among replicates (Washington, 1984; Clarke and Warwick, 1994). It includes abundance (or biomass) of individuals in each species. Also, the Bray-Curtis measure is not dependent on the species which are jointly absent in both areas. This coefficient allow, by choosing the severity of the transformation of the original data (no transformation → $\sqrt{y}$ → $\sqrt{\sqrt{y}}$ / log(1+y)→ +/−) to focus all the attention either on the dominant species or on the rare ones. The Bray-Curtis similarity coefficient is calculated using the following equation:

$$S_{jk} = \left[1 - \frac{\sum_{i=1}^{p} |y_{ij} - y_{ik}|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})}\right] \cdot 100$$

where p is the number of species, y is the abundance or biomass value of the species i and j and k represent any of two sites.

When two sites are identical, the similarity measure is 100% (correspondingly dissimilarity is 0%) and when they have no species in common, similarity is 0% (and dissimilarity is 100%).

The calculation of coefficients permits either the *clustering* (or *classification*) of the sites into groups which are mutually similar, or the *ordination* of the sites in a two- or three-dimensional space.

The starting point of the *cluster analysis* is a table or matrix giving the similarity values between every pair of sites, as measured by one of the similarity coefficients. The method of *hierarchical agglomerative clustering* fuses the two most similar sites in the matrix into a single cluster. The analysis proceeds by successively fusing similar sites or clusters into larger groups until all are combined. These groupings are displayed in a single figure called *tree diagram* or *dendrogram* (Fig. 6). There are several techniques for joining sites into clusters (*e.g.* single-linkage, complete linkage), but the *group-average linking* appear to be most favoured for marine pollution studies.

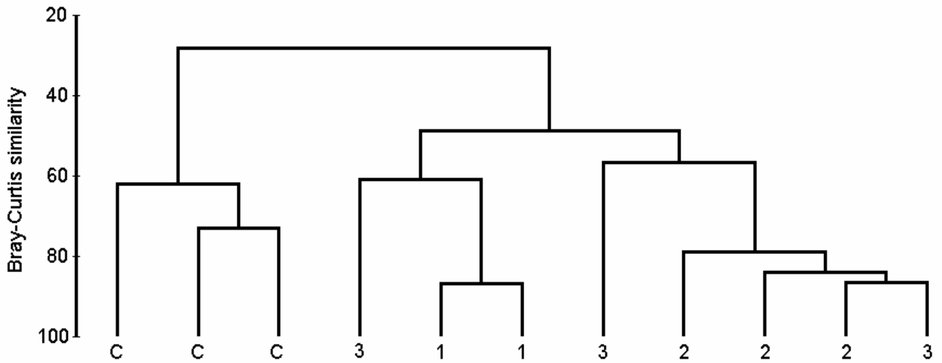

Fig. 6. The dendrogram for hierarchical clustering of the samples taken at different distances from a sewage effluent (1 = 0 m, 2 = 50 m, 3 = 100 m, C = control), based on Bray-Curtis similarities (from Surugiu, 2008)

The dendrogram is a particularly appropriate representation of the community structure in the case where the sites are expected to form distinct groups.

The *ordination* is a two- or three-dimensional plot which attempt to “map” the sites in such a way that the distances between sites reflect their corresponding dissimilarities in community structure. The representation by an ordination is usually more appropriate where there is a more continuous gradation of the species composition pattern across the sites in response to environmental gradients. There is a bewildering array of ordination methods in common use: Principal Co-ordinates Analysis (PCoA), Correspondence Analysis (CA), Detrended Correspondence Analysis (DECORANA) etc. However, two ordination techniques have become particularly common in ecological studies: the Principal Component Analysis and the Multi-Dimensional Scaling.

The *Principal Component Analysis* (PCA) is one of the earliest ordination techniques and is still commonly employed. The PCA is an ordination plot, usually in two or three dimensions, which transposes the positions of sites on conventional Cartesian coordinates. PCA is especially useful in assessing the physico-chemical status of the environment. It also can provide an interpretation of the main axes of the plot.

The *Multi-Dimensional Scaling* (MDS) is considered as one of best ordination techniques (Warwick and Clarke, 1994). This ordination plot attempts to place all sites, usually in two- or three-dimensional space, in such a way that the rank order of the distances between sites on the map exactly agrees with the rank order of the matching dissimilarities, taken from the triangular similarity matrix. Sites that plot closely together contain similar assemblages and sites that plot further apart contain more dissimilar assemblages (Fig. 8).

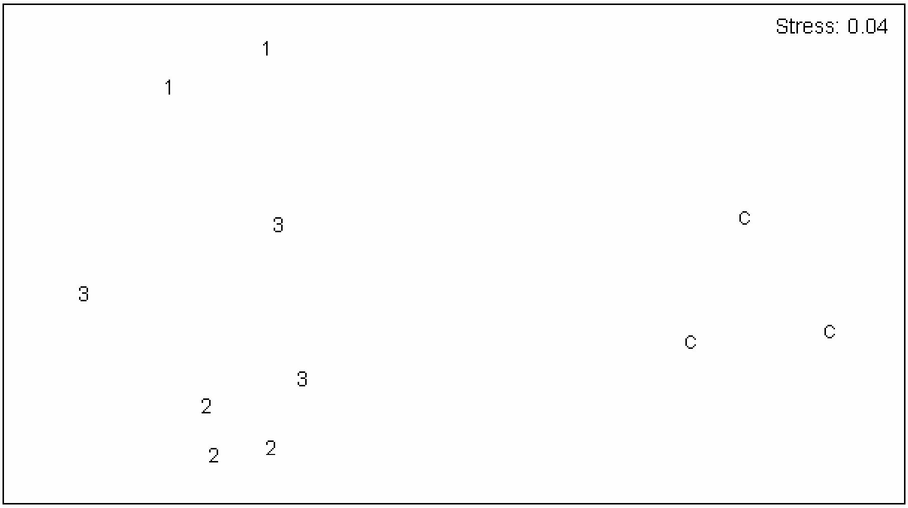

Fig. 8. The MDS ordination plot illustrating similarity of 11 samples taken at different distances from a sewage effluent (except the station 1 where one sample contained no fauna), based on Bray-Curtis similarities from root-transformed densities: 1 = 0 m, 2 = 50 m, 3 = 100 m, C = control. Note that points closer together on the plot are more similar and vice versa (from Surugiu, 2008)

Multivariate analyses have been used also at higher taxonomic levels, such as genus, family or even phylum (Warwick, 1988).

Biotic indices

The recently proposed tools in detecting the effects of pollution stress include various *biotic indices*. The methods grouped in this category are based on the proportions of the so-called *indicator species* (sensitive/opportunistic) in respect to the total fauna. However, these methods require the definition of reference levels for each habitat type and the definition of the ranges of variation for each quality class to use for classification purposes (Simboura and Zenetos, 2002). Also, biotic indices are limited in their application to the geographical areas in which the tolerance lists were compiled or to a particular type of pollution, usually organic (Washington, 1984). Like multivariate methods biotic indices take into account changes in taxa.

For the evaluation of the organic pollution were employed the ratios between the total number of the representatives of groups of organisms with different tolerances to pollution such as, for instance, Nematode/Copepod (Raffaelli and Mason, 1981) or Capitellidae/Spionidae from polychaetes (Losovskaya, 1983). However, the use of these ratios was much questioned because at higher taxonomic levels there is greater likelihood that they contain taxa with different tolerances to stress (Surugiu, 2005).

For the assessment of the marine environmental quality, Bellan (1980) proposes an "*Annelid Pollution Index*", which is based on the ratio of the summed dominances of "polluted water sentinel species" (*Platynereis dumerilii*, *Theostoma oerstedi*, *Dorvillea rudolphii*, *Cirratulus* cf. *cirratulus*, etc.) to that of the "pure water sentinel species" (the species of the genus *Syllis* or *Amphiglena mediterranea*). In polluted environments this ratio provides always values greater than 1, while in pristine or slightly polluted waters this ratio is inferior to 1 (Bellan *et al*., 1988).

The *AMBI biotic index* (Borja *et al*., 2000) is based upon the relative distribution of sensitive/tolerant species groups. It produces a continuous range of values between 0 (reference site) and 6 (strongly polluted), the value of 7 being characteristic for azoic sediments.

This biotic index derives from the *biotic coefficient* which is calculated on the basis of the percent abundances of the each of the five *ecological groups* distributed according to their sensitivity or tolerance to a disturbance (*GI* – species very sensitive to organic enrichment; *GII* – species indifferent to pollution; *GIII* – tolerant species; *GIV* – second-order opportunistic species; *GV* – first-order opportunistic species), and which are weighted proportionately according to the following formula:

$$BC = \frac{\left[(0 \times \%GI) + (1{,}5 \times \%GII) + (3 \times \%GIII) + (4{,}5 \times \%GIV)(6 \times \%GV)\right]}{100}$$

The *BENTIX biotic index* (Simboura and Zenetos, 2002) resembles the previous, but the number of ecological groups is restricted to only three (*GI* – sensitive species; *GII* – tolerant species and second-order opportunistic species; *GIII* – first order opportunistic species):

$$BENTIX = \frac{\left[6 \times \%GI + 2 \times (\%GII + \%GIII)\right]}{100}$$

Thus, the BENTIX biotic index is simpler and easier to use. Also, in the BENTIX the classification scale is reversed to produce a continuous series of increasing values from 0 (azoic sediments) to 6 (pristine community), which are distributed into five ecological quality classes as defined by WFD (Table 2).

Table 2.
Classification scheme of the Ecological Quality status (EcoQ) based on various biotic indices

EcoQ	**AMBI (Borja *et al*., 2000)**	**BENTIX (Simboura and Zenetos, 2002)**	**BQI (Rosenberg *et al*., 2004)**
I (High)	AMBI ≤ 1.2	4.5 ≤ BENTIX < 6.0	BQI ≥ 16
II (Good)	1.2 < AMBI ≤ 3.3	3.5 ≤ BENTIX < 4.5	12 ≤ BQI < 16
III (Moderate)	3.3 < AMBI ≤ 4.3	2.5 ≤ BENTIX < 3.5	8 ≤ BQI ≤ 12
IV (Poor)	4.3 < AMBI ≤ 5.5	2.0 ≤ BENTIX < 2.5	4 ≤ BQI < 8
V (Bad)	5.5 < AMBI ≤ 7.0	BENTIX = 0	BQI < 4

The advantage of the BENTIX index is that it is largely independent from the sample size and the habitat type and is moderately affected by the degree of taxonomic effort required to obtain the index, which denote the “robustness” of the index. Also, it is characterised by good “effectiveness”, reflected in a high discriminating power between ecological classes (Simboura and Zenetos, 2002).

Especially for the assessment of the environmental quality status in accordance with the European Water Framework Directive, Rosenberg *et al*. (2004) propose the *Benthic Quality Index* (BQI). This index is calculated by combining into a single formula the tolerances of indicator organisms to environmental disturbances, the relative abundance or each species and the species richness:

$$BQI = \left[\sum_{i=1}^{n} \left(p_i \cdot ES50_{0.05i} \right) \right] \cdot \log_{10} \left(S + 1 \right)$$

where p_i is the mean relative abundance of the species i, $ES50_{0.05i}$ is the tolerance value of this species (calculated objectively according to Hurlbert’s formula) and S is the mean number of species.

According to Zettler *et al*. (2007) the BQI values in the southern Baltic Sea varied between 0 and 25. The ecological quality is determined by taking the highest value as reference value and by dividing the continuous scale between 0 (“bad” EcoQ status) and this reference value (“high” EcoQ status) into the five WFD classes of equal size (Tab. 2). The particular strength of the BQI is that it has better discriminant ability in distinguishing impacted from un-impacted sites relative to AMBI or BENTIX. However, in order to improve the accuracy of this index, are required analyses of extensive data sets.

Conclusions

The univariate methods (the Margalef's species richness index, the Shannon-Wiener diversity index and the Pielou's evenness index) applied to the polychaete populations influenced by a municipal sewage discharge provided good results in the evaluation of the marine environmental health (Surugiu and Feunteun, 2008; Surugiu, 2008). Except the Pielou's evenness index, there was a gradual reduction of the values of the various diversity indices with increasing levels of organic load. The high equitability at intermediate levels of disturbance is explained by the fact that there were few opportunistic species represented by approximately equal number of species. The graphical methods (ranked species abundance curves and *k*-dominance curves) had a better visual appeal as a means of detecting the effects of organic enrichment. These indicated an increased dominance in areas influenced by domestic wastewaters and an increased equitability in unpolluted sites. Best results in the assessment of the organic pollution stress were achieved by the multivariate techniques (clustering, MDS and PCA). The two latter were suitable especially in the detecting pollution along a more or less continuous organic enrichment gradient. The biotic indices were not yet applied in the determining the ecological status of the Romanian Black Sea coast, because the species encountered must be first assigned to the ecological groups outlined by each method. However, we must expect an extensive use of the various benthic indices in the future because of their simplicity and reliability.

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