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Manual for biological monitoring of rivers and lakes/reservoirs in B&H

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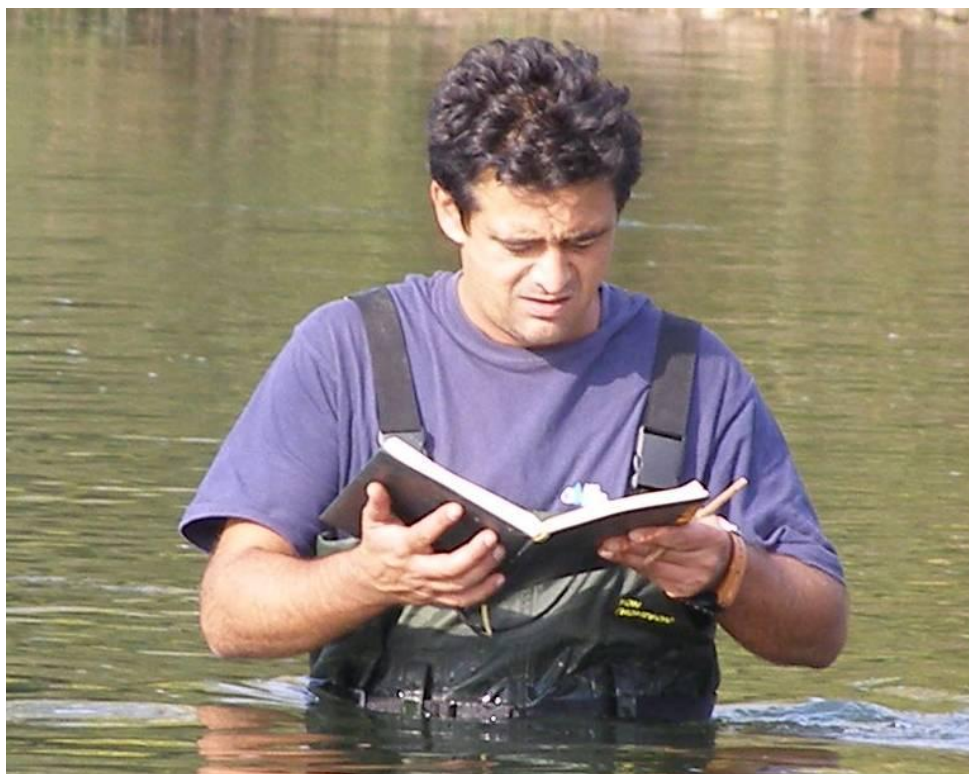
Abbreviations

| | |
|---------|--|
| AQEM | Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates |
| AWB | Artificial Water Bodies |
| B&H | Bosnia and Herzegovina |
| BQEs | Biological Quality Elements |
| EQO | Environmental Quality Objectives |
| EQS | Environmental Quality Standards |
| EU | European Union |
| EU WFD | EU Water Framework Directive (2000/60/EC; WFD) |
| GEF | Global Environment Facility |
| GIS | Geographical Information System |
| GIGs | Geographic Intercalibration Groups |
| HMWB | Heavily Modified Water Bodies |
| HyQE | Hydro-morphological Quality Elements |
| ICPDR | International Commission for the Protection of the Danube River |
| IPS | Specific Pollution Sensitivity Index |
| JDS | Joint Danube Survey |
| MHS | Multihabitat Sampling |
| NORMAN | Network of Reference Laboratories and Related Organisations for Monitoring and Bio-monitoring of Emerging Environmental Pollutants |
| OSS | Open Source Software |
| PHS | Priority Hazardous Substances |
| PME | Plant Mass Estimate |
| PS | Priority Substances |
| RC | Reference Conditions |
| RBM EG | River Basin Management Expert Group |
| RBMP | River Basin Management Plan |
| SWB | Surface Water Bodies |
| TNMN | Trans-National Monitoring Network |
| TOC | Total Organic Carbon |
| UNDP | United Nations Development Programme |
| WB | Water Bodies |
| WFD CIS | WFD Common Implementation Strategy |
| WISE | Water Information System for Europe |

1 Introduction

The main objective of the project was to establish a basis for setting up the biological monitoring system in Bosnia and Herzegovina (B&H) that meets the requirements outlined in the WFD. The existing knowledge combined with new findings obtained during the two project's field surveys resulted in the proposal of methodologies, which are outlined in the presented **Manual for biological monitoring of rivers and lakes/reservoirs in B&H** (further referred to as "Bio-monitoring manual").

Detailed findings and results supporting the statements and conclusions in the Bio-monitoring manual are summarised in the project's Mid-term and Final report. As an overall important outcome of the project it has been recognized that available data are not sufficient to make the final conclusions on the methodologies to be used for routine monitoring. Therefore it was suggested that national experts should further develop and adjust the proposed biological and hydro-morphological methods based on future monitoring results.



2 GENERAL FRAMEWORK

The main goal of the WFD is to achieve good status of surface water bodies, i.e. both their good ecological and good chemical status. The ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters. Basic unit for the ecological status as well as for the chemical status of surface water is water body. The ecological status assessment has to be type-specific which implies that establishment of national typology and delineation of water bodies are essential prerequisites for the development of the WFD-compliant monitoring schemes.

In the Annex V of the WFD the biological quality elements (BQEs), comprising of aquatic flora and fauna, are defined for classification of the ecological status of rivers and lakes. For defining the ecological status it is necessary to set reference conditions and class boundaries for all relevant BQEs and supporting physico-chemical, chemical and hydro-morphological quality elements. Detailed information on BQEs and supporting quality elements are required to assess the ecological status, providing evidence of the natural conditions, undisturbed or nearly undisturbed conditions, and various degree of disturbance, indicating the significance of the anthropogenic impact. The above mentioned information can only be obtained by detailed and well designed monitoring structure.

For establishment of an effective monitoring system, type-specific reference values for selected metrics for relevant BQEs and supporting quality elements have to be determined. The type-specific evaluation of the ecological status is based on the reference situation concerning the natural, unimpacted community structures. The most important requirement for an exact definition of such conditions is to have large data sets referring to those sites in each water body type that are either having the smallest or lacking anthropogenic impact.

In addition, optimum sampling frequency, sampling methodology, as well as procedures of analysis and evaluation have to be identified. BQEs have to be defined by the selected biological traits reflecting the composition and structure of the ecosystem and they have to also reveal the relevant pressures/stressors. All these characteristics are expressed in the form of metrics/indices. Based on these metrics, multi-metric or single-metric approach is used for the ecological status assessment. The assessment should be type-specific, and, stressor-specific, at the same time.

2.1 Typology

According to the WFD for each surface water category, the relevant surface water bodies within the river basin district have to be differentiated according to type.

River typology can be done using either system A or system B. If system A is used, the surface water bodies within the river basin district shall first be differentiated by the relevant ecoregions (in accordance with the geographical areas shown on the relevant map at Annex XI of the WFD), catchment size, altitude and geology. System B is based on combination of obligatory factors (descriptors) and optional factors.

Lakes typology also uses system A or system B. System A is based on ecoregion, mean depth, size surface area and geology. System B is similarly like in case of rivers based on combination of obligatory factors (descriptors) and optional factors.

Typology is a baseline for classification systems for assessment of the ecological status, while for the different types of rivers or lakes different classification scheme can be developed and used.

2.2 WB delineation

The identification of water bodies is, first and foremost, based on geographical and hydrological determinands. However, the identification and subsequent classification of water bodies must provide for a sufficiently accurate description of this defined geographic area to enable an unambiguous comparison to the objectives of the WFD. This is because the environmental objectives of the WFD, and the measures needed to achieve them, apply to “water bodies”.

The general method for water body delineation is given in the CIS Guidance Document No. 2: Identification of Water Bodies. The basic criteria are:

- Surface water category;
- Physical characteristics;
- Water type;
- Water status;
- Heavily modified or artificial character;
- Pressures and impacts;
- Protected areas.

The identification of water bodies is important for the selection of the representative sampling site (sampling point, sampling stretch). Representativeness of sampling is a crucial point for determination of the ecological status with high confidence and accuracy.

2.3 Monitoring structure

The monitoring network has to be designed so as to provide a coherent and comprehensive overview of ecological and chemical status within each river basin and shall permit classification of water bodies into five classes consistent with the normative definitions of the WFD.

On the basis of the characterisation and impact assessment of the rivers and lakes water bodies for each period to which a river basin management plan applies, it should be established a surveillance monitoring programme and an operational monitoring programme. Countries may also need in some cases to establish programmes of investigative monitoring.

Parameters which are indicative of the status of each relevant quality element should be monitored. In selecting parameters for BQEs countries have to identify the appropriate taxonomic level required to achieve adequate confidence and precision in the classification of the quality elements.

Objectives of surveillance monitoring are to provide information for:

- Supplementing and validating the impact assessment procedure;
- The efficient and effective design of future monitoring programmes;
- The assessment of long-term changes in natural conditions;
- The assessment of long-term changes resulting from widespread anthropogenic activity.

Surveillance monitoring has to be carried out of sufficient surface water bodies to provide an assessment of the overall surface water status within each catchment or subcatchments within the river basin district. Therefore sampling points or sampling stretches should be representative for water body. Representativeness of sampling point is a crucial point for assessment of the results.

Surveillance monitoring has to be carried out for each monitoring site for a period of one year during the period covered by a river basin management plan for:

- Parameters indicative of all biological quality elements;
- Parameters indicative of all hydromorphological quality elements;
- Parameters indicative of all general physico-chemical quality elements;
- Priority list pollutants which are discharged into the river basin or sub-basin, and
- Other pollutants discharged in significant quantities in the river basin or sub-basin.

The WFD gives minimum required frequencies of sampling for the above parameters. However, to receive sufficient database of results which can be used for development of the reliable assessment system (classification schemes for individual parameters) higher frequencies have to be selected.

2.4 Reference conditions and classification schemes

For each surface water body type, type-specific hydromorphological and physico-chemical conditions have to be established representing the values of the hydromorphological and physico-chemical quality elements specified as surface water body type at high ecological status. Type-specific biological reference conditions have to be established, representing the values of the biological quality elements specified for that surface water body type at high ecological status.

Reference condition is a state in the present or in the past corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensification of agriculture, and with only very minor modification of physico-chemistry, hydromorphology and biology.

There are a few approaches to the selection of reference conditions:

- Reference sites;
- Historical data;
- Modelling;
- Expert judgement;
- Reference sites from other countries.

Usually a combination of some of the above approaches is used when developing reference values for classification schemes. In the Annex 5 of the WFD there is a description of the normative definitions of ecological status classifications which have to be fulfilled.

Normative definitions:

- a) Quality elements (biological, hydro-morphological and physico-chemical);
- b) Quality of the structure and functioning of aquatic ecosystems associated with surface waters:
 - Species diversity;
 - Abundancy/biomass/density;
 - Sensitive taxa;
- c) Sensitivity to the stressors/pressures (hydromorphological, eutrophication/nutrients, organic pollution, acidification).

All normative definitions should be included into classification schemes for individual relevant quality element (parameter) via selected metrics (indices). Selection of the metrics has to be proved by testing. Reliable metric should show differences on the scale of the antropogenic impact.

Beside reference value determination an important issue is setting of boundaries between classes (high-good, good-moderate, moderate-poor, poor-bad). There are a few approaches for boundary setting protocol in the Guidance document No. 10: River and lakes – Typology, reference conditions and classification systems, which can be used.

For individual water body types, harmonisation of BQEs among each other within classification scheme should be done as well as the harmonisation of BQEs and supporting quality elements.

3 BIOLOGICAL QUALITY ELEMENTS FOR RUNNING WATERS

3.1 Phytoplankton

Based on the existing data supported with the experience and results from the project's surveys of selected water body types in B&H in October 2008 (Mid-term Report, 2009) and May 2009 (Final Report, 2009) it is evident that phytoplankton is not relevant BQE for the surveyed types of running waters.

3.2 Phytobenthos

Algae are important primary producers in many surface waters of temperate regions. This makes this organism group interesting for use as a bio-indicator to monitor long-term changes in aquatic ecosystems, especially as it is related to eutrophication. Preferentially in running rivers, phytobenthos is considered to be a suitable parameter to determine the impact of nutrient pollution, because the organisms are generally sessile and therefore represent the nutrient status at the sampled location.

The WFD requires to monitor phytobenthos and macrophytes as one quality element. There are a few approaches to this issue in Europe:

1. Both elements are investigated separately, phytobenthos covers non-diatom phytobenthos and benthic diatoms;
2. Macrophytes are investigated together with non-diatom phytobenthos, benthic diatoms are analysed separately;
3. Macrophytes and benthic diatoms are analysed separately (non-diatom phytobenthos is not included).

Based on the knowledge reflecting the situation in B&H and experience from other countries it is proposed to use the second option.

3.2.1 Sampling procedure

Sampling of benthic diatoms is based on EN 13946: Water quality. Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers.

At each sampling station must be used basic principles of sampling site selection:

- Select segment of river that has substrate suitable for sampling;
- Length should be approximately 10 m or more;
- Prefer site with prevailing fast flowing lentic areas (greater diversity of hard substrates), avoid lotic slow flowing areas with sediment;
- Selected substrate must be completely submerged;
- Select euphotic areas, avoid the shadow, not well exposed areas and river banks;
- Hydrological situation should be stable during four previous weeks.



Figure 3.2-1 Basic principles of the selection of the sampling site for phytobenthos.

Equipment and accessories for sampling:

- Plastic tray (white, size e.g. 30 x 20 cm);
- Plastic bottle with distilled water to wash stones;
- Toothbrush, knife, tweezers;
- Sampling containers;
- Rubber boots;
- Gloves;
- Sampling protocol;
- Preservative solution (Formaldehyde, Lugol solution, Ethanol).

Principle steps of sampling:

- Take preferably hard substrates;
- Stones – epilithon - are recommended (size of 64 – 256 mm);
- Minimum of 5 stones is taken from suitable zones in sampling site (pebbles or cobbles);
- Fast wash out of substrate in the flow of the river (if necessary – in order to remove sediments or other pollution);
- Move selected substrate (at least 5 stones) to the plastic tray with small amount of water;
- Scrab the substrate surface;
- Size of sampled surface PER SAMPLED SURFACE-STONE in total must be larger than 10 cm²;
- Toothbrush must be well washed and should not be used at different sites without lab washing (or use one-off toothbrush);
- After scrabbing – sample should be moved to the sampling bottle;

- Preserve sample:
 - Short time preservation: Lugol solution – 1-5 drops to 100 ml;
 - Long time preservation: Formaldehyde (HCHO) – to the final concentration of 4 % in the sample or 70% Ethanol;
- Label sample container;
- All field information record in the standardised field protocol (Annex 1).



Figure 3.2-2 Examples of the sampled stones.

Minimum requirement for monitoring of phytobenthos and macrophytes (water flora other than phytoplankton) according to the WFD is once within three years. This requirement is accounting for available monitoring structure and assessment methods. However, for the development of a national method for assessment of ecological status based on phytobenthos and macrophytes a database with sufficient good quality data is a must. Based on this, a frequency of twice a year for investigation of benthic diatoms is recommended in B&H for the first two years, preferably in spring (March – May) and autumn (September – November). A care should be taken that the hydrological situation on selected sampling sites is stable during four weeks before sampling. The sampling can be combined together with the collection and follow up determination of benthic invertebrates.

3.2.2 Pretreatment of diatoms

In the laboratory benthic diatoms have to be treated before preparation of permanent slides for determination and enumeration. As a first step fast observation of physiological status of the diatoms in the preserved sample should be done.

Pretreatment consist of following steps:

- Elimination of large inorganic particles;
- Concentration of diatoms using sedimentation (1 cm = 1 hour) or centrifugation;
- Treatment of diatoms:
 - Elimination of organic content of frustules using strong solution for oxidation (40% hydrogen peroxide - H₂O₂; recommended);
 - Use of concentrated hydrochloric acid (HCl) for removing carbonates.

- Mounting of diatom solution on slides:
 - Prepare suitable solution (10-15 valves in the field);
 - Move the small amount of homogenous sample on the cover slide;
 - Let it dry on air (do not warm on heat!);
 - Mount with suitable medium (Naphrax, Pleurax, Hyrax);
 - Label base slide.
- The density of diatom valves on slide is very important for identification and enumeration of benthic diatoms. Therefore it should be checked by microscopy during preparation of diatom solution.



Figure 3.2-3 Treatment of benthic diatoms.

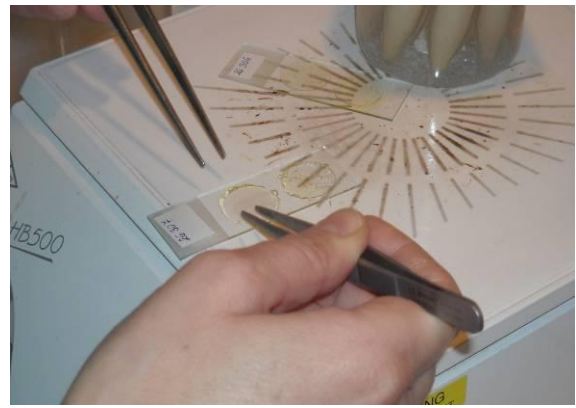
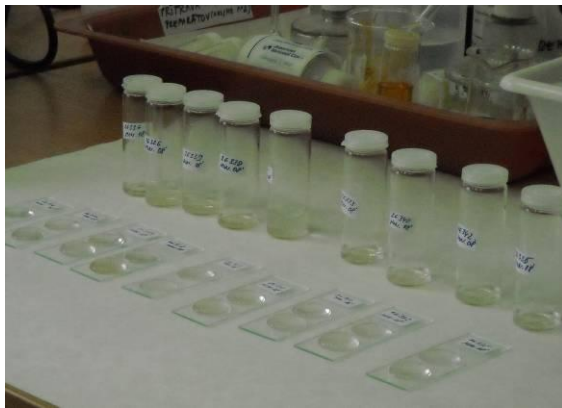


Figure 3.2-4 Mounting of diatom solution on slides.

3.2.3 Identification and quantification of benthic diatoms

For identification and quantification of benthic diatoms follow the instructions of the EN 14407: Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. Method for identification and enumeration of diatoms in permanent slides based on relative abundance.

Relative abundance (%) is a proportion of species within certain amount of counted specimens. The principle is based on counting and identification of diatom valves in the microscope up to selected number of valves (until certain number of valves is reached). Counting up to 300 – 500 valves is recommended in random transects using a light microscope with Differential Interference Contrast at 1000 x magnification.

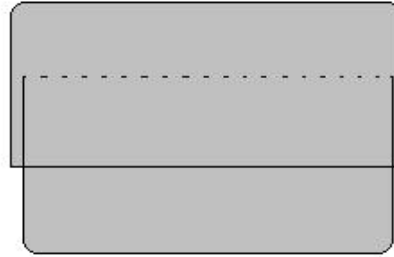


Figure 3.2-5 Diatom frustule, consisting of two diatom valves.

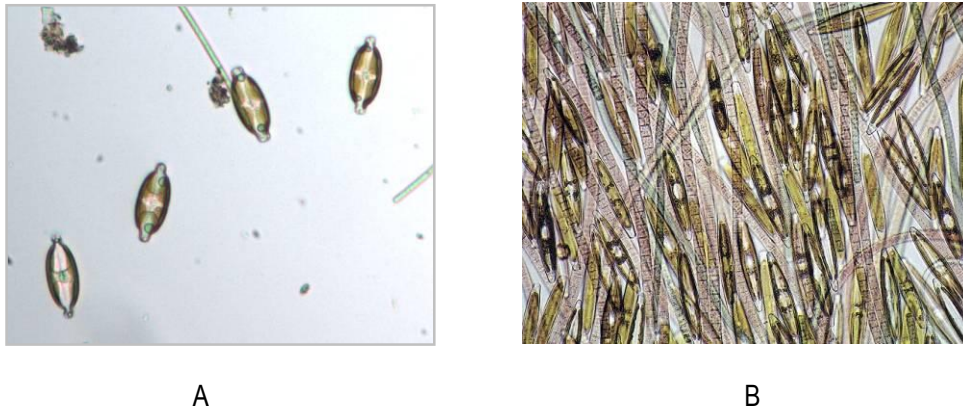


Figure 3.2-6 Density of diatoms on slide: A - recommended, B – inconvenient.

The EN 14407 does not recommend nomenclature for determination of benthic diatoms. Systematic level of determined taxa depends on the assessment method used. In order to avoid higher uncertainty in the assessment it is recommended to determine all taxa with the relative abundance of 5% at least to the species level. List of recommended determination keys and other literature sources for identification of benthic diatoms is given in Chapter 7.1.

3.2.4 Benthic diatom data processing

Using method as described in previous chapters the diatom inventory (taxa list and relative abundances of each taxa in %) will be available for each sampling site within certain type of rivers. For assessment of ecological status relevant metrics should be calculated. It is recommended to use OMNIDIA software to calculate diatom indices.

OMNIDIA can serve for a first initial period also as a basic national database. Later on the raw data as well as processed data can be transferred to any other database. Among the advantages is also its compatibility with other databases in Europe and the fact that this tool is also used in the process of intercalibration.

OMNIDIA calculates 17 various indices which are available for benthic diatoms. Some of the calculated indices are presented for selected rivers in the Mid-term Report (Mid-term Report, 2009).

The most used index is the IPS (Coste in Cemagref, 1982) „Specific Pollution Sensitivity Index“, which was developed in France as a national assessment index (Prygiel et al., 2002) for detection of total water pollution. Indicator taxa are divided into 5 classes according to their sensitivity to pollution and into 3 classes according to their indicative weight. IPS was tested and selected as an appropriate tool for water quality evaluation in the United Kingdom (Kelly et al., 1995), France (Prygiel & Coste, 1993), Finland (Vilbaste, 2004), Hungary (Acs et al., 2004, Van Dam et al., 2005), Poland (Kwandrans et al., 1998), Luxembourg and Slovakia (Hlubikova et al. 2007) and in Spain (Gomá et al., 2004).

The applicability of the index in many different European regions is of a great advantage in light of the WFD requirements, because the same metric used in more countries facilitates the intercalibration of national assessment methods. For this purpose, IPS was successfully used as the intercalibration metric of diatoms within the intercalibration exercise of the Central Baltic Geographical Intercalibration Group. On the other hand, IPS was developed from a large database, involves large number of diatom taxa in the calculation and includes most of the species of the OMNIDIA database. This feature can explain why IPS successfully works in many countries rather than other indices.

3.3 Macrophytes

Macrophytes are an important component of aquatic ecosystems and can be used to facilitate the monitoring of ecological status. Surveying of aquatic macrophytes based on requirements of the WFD and related methods are mentioned in the standard EN 14 184 (2003). Methods for surveying aquatic macrophytes in running and standing waters specify a procedure for the purpose of assessing ecological status, using aquatic macrophytes as the BQE. These methods include the species composition and abundance of the aquatic macrophyte flora.

Aquatic macrophytes are represented by larger plants of freshwater which are easily seen with the naked eye, including all aquatic vascular plants, bryophytes, stoneworts (Characeae) and macro-algal growths. Macrophytes of aquatic ecosystems can occur in three growth forms:

- Helophytes (usually rooted under water with floating or submerged leaves, or totally free floating at the water surface);
- Hydrophytes (plants usually rooted under water with emergent leaves, typically growing in marginal or marshy areas);
- Amphiphytes (plants growing in the same survey unit on the bank, similarly as a helophyte, and at the same time in the water body as hydrophyte).



Figure 3.3-1 Helophytes (*Carex* sp., *Caltha pallustris*).

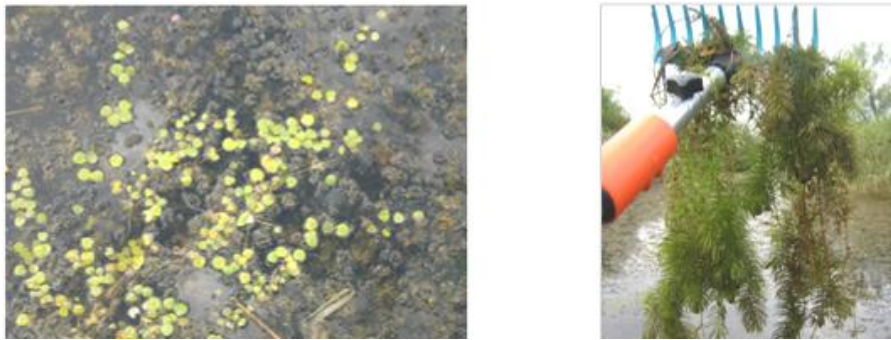


Figure 3.3-2 Hydrophytes (*Spirodela polyrhiza*, *Ceratophyllum demersum*).



Figure 3.3-3 Amphiphytes (*Veronica beccabunga*, *Butomus umbellatus*).

3.3.1 Macrophyte survey

Macrophyte survey in running waters can be undertaken at different levels of sophistication. For routine assessment of ecological status, the survey in short length of river (50 – 500m) representing certain water body is sufficient.

Survey technique is based on recording and quantification of determined macrophyte species on the survey stretch. In shallow sites, upstream wading in a zig-zag manner across the river is recommended, so that disturbed sediments do not interfere with macrophyte observation and identification. Where it is not safe to wade (due to fast current or the nature of the substratum) observations should be made from the bank instead. In large rivers it is recommended to use the boat and travel upstream across survey stretch in a zig-zag manner or along left and right bank (1 river kilometer).

The presence of aquatic macrophytes in the river of defined river lengths can be recorded by relative abundance or macrophyte biovolume or biomass. The values of plant mass estimate (PME) in the scale from 1 to 5 (1 = rare; 2 = occasional; 3 = frequent; 4 = abundant; 5 = very abundant taxa) it is recommended to use. This is not identical with biomass (= kg / unit area), but it is equivalent to “amount of species”, to “3-D-amount”, or biovolume, of a plant stand or species patch, in a general sense (KÖHLER & JANAUER, 1997).

Equipment and accessories for sampling:

- Binoculars;
- Maps;
- Clipboard, recording sheets, water resistant pens/pencils, waterproof labels/pens;
- Plastic bags, small specimen tubes;
- Tape measure calibrated in meters;
- Hand lens with appropriate magnification;
- Identification keys and field guides;
- Wading suit;
- Polarising sunglasses;
- Global Positioning System (GPS)-instrument;
- Rake and/or grapnel;
- Underwater viewing aid/aqua-scope.

For deeper waters:

- Boat and necessary equipment such as e.g. engine;
- Grapnel on the rope.

Timing of initial and subsequent surveys is very important. Comparative surveys in subsequent years should be undertaken at the same time of the year as in previous years (uncertainty of resulting from differential seasonal growth are minimised). The development of macrophyte growth vary between years as it is highly dependent on water depth, physical disturbance, flow regime, solar radiation and water temperature. For these reasons pilot visits to specify optimal survey occasion for concrete river survey stretch are recommended.

Selection of representative sites

The ecological status of the representative site is measured in terms of the deviation of the selected metrics relevant for particular BQEs at the representative site compared with the reference value of the metrics. The number and location of sites should allow a representative flora to be recorded, reflecting the human impact within a river reach and including exposed and shaded stretches. Natural conditions of substrate, water depth, bank site shading, flow type, etc. should normally be similar to the conditions at the reference site, so that differences in the flora due to anthropogenic impact can be distinguished more easily from changes due to hydromorphological factors.

The actual river length surveyed at the representative site should be of sufficient length to reflect adequately the diversity of plant species characteristic for this ecological type of river reach. The lengths of individual survey stretches need not be the same since much of the data can be compared directly by mathematical scaling. Surveyors should be aware that the number of species recorded will increase with increasing the length of river surveyed. Therefore, it is recommended that each survey length is the same as for the corresponding reference site and ideally the same survey length should be used for sites on the same river/water body types. When using belt transects, belts of the same width should be used.

It is important to be able to locate accurately the survey site during subsequent surveys. It may be appropriate to use markers or permanent landmarks such as field boundaries, trees, etc. Following the state-of-the-art procedures marking of survey stretches and reference sites should be geo-referenced (preferably by GPS), which

also facilitates easy processing in Geographic Information Systems (GIS). Data transfer and central data acquisition as well as the production of quality status maps will then be easier, especially with respect to long-term trend monitoring.

Habitat parameters, which are needed to be recorded in the field protocol (Annex 2):

- Survey stretch name;
- Identification in drawing;
- Latitude N, longitude E, altitude, river km, estimated length;
- Width of stretch, evaluated part of stretch (whole/RS/LS), depth bank;
- Structure sediment, type flow, class connectivity;
- Type of land use;
- Type of Secchi depth transparency;
- Shading (%).

Determination and quantification of aquatic macrophytes, which should be recorded in the field protocol:

- Macrophyte name;
- Herbarium or sample number;
- Growth form (HY, HE, AM);
- Quantification (from 1 to 5).

Recording and quantification of determined macrophytes on survey stretch:

In shallow sites wade in a zig-zag manner across the channel:

- Wade upstream in a manner that disturbed sediments do not interfere with macrophyte observation and identification;
- Where it is not safe to wade (due to fast current or the nature of the substratum) observations should be made from the bank instead;

In large river use the boat and travel upstream across survey stretch in a zig-zag manner or along the left and right bank (1 river kilometer).

Recording and quantification of macrophytes determined on survey stretch - example of macrophyte abundance scales (plant mass estimate (PME) is 3-dimensional development volume amount in the whole water column):

| SCALE | DESCRIPTOR | CLASS |
|-------|---------------|------------|
| 1 | Rare | < 0,1 % |
| 2 | Occasional | 0,1 to 1 % |
| 3 | Frequent | 1 to 5 % |
| 4 | Abundant | 5 to 10 % |
| 5 | Very abundant | >10 % |

3.3.2 Macrophyte identification

Determination is a key step in the method:

- Personal should be able to identify most macrophytes and macroalgae to species level (or macroalgae and cyanobacteria to the genus level) in the surveying field, using the appropriate keys and guides;
- The same samples can identified (confirmed) in the laboratory (macroalgae, bryophytes or samples of macrophytes, which are not certainly determined in the field);
- Macroalgae and cyanophyta should be identified by light microscopy in the laboratory up to 24 hours after sampling;
- It is recommended to prepare database of photographs, resp. micro-photographs for later confirmation;
- List of related determination keys and other relevant literature is given in Chapter 7.1.

Qualitative and quantitative data from aquatic macrophytes surveys serve as the basis for establishment of the database, which is then used for further development of the evaluation system and calculation of indices. Some of macrophyte biological indices are listed below:

- TIM - trophic index of macrophytes (Germany);
- IBMR - macrophyte biological index (France);
- MTR - mean trophic rank (United Kingdom);
- RI - reference index (Germany).

An obvious problem is that different taxa are dominant in different regions and different water body types, but countries may develop region specific indices based on other data (chemical data, data on other BQE's, HYMO data, etc.).

3.4 Benthic invertebrates

Benthic invertebrates are commonly applied for the integrated quality assessment of rivers based on the requirements of the WFD. They may offer numerous advantages in biomonitoring, which explains their reputation as the most commonly used group in assessing water quality. Among the reasons are:

- 1) It is generally well known group, which enables reliable identification and predicting of response to stress;
- 2) Organisms belonging to this group are generally characterized by low mobility. Thus, use of macroinvertebrates in monitoring enable effective spatial analyses of the effect of stressors;
- 3) There is an array of widely distributed species among the group, which allow extensive use of same indicators;
- 4) It is diverse component of the aquatic environment, which offers a spectrum of responses to the stress;
- 5) Sampling can be done easily, using simple inexpensive equipment (Rosenberg and Resh 1993).

Benthic invertebrates are widely used in bio-monitoring

- ✎ Advantages in use:
 - ✎ Generally well known group
 - ✎ Group comprise organisms that are static in regard to habitat
 - ✎ A lot of widely distributed species
 - ✎ Diverse component of aquatic ecosystem
 - ✎ Easy sampling, well defined for shallow waters
- ✎ Disadvantages in use:
 - ✎ Identification of some taxonomic groups is time consuming (Chironomidae, Oligochaeta, Amphipoda)
 - ✎ Development of sampling procedures for large rivers is still an on-going process

3.4.1 Selection of the relevant sampling points

The selection of sampling sites is dependent on the goal of the investigation. For the WFD-compliant monitoring, water body is the unit of interest to deal with. Therefore the sampling site should be representative for the water body to be assessed by means of BQEs, i.e., the site should reflect the conditions of the whole water body. On the other hand, if the basic purpose is to gain the most complete species list (e.g., for nature conservation) the criteria for site selection are different as they should provide selection of sites with maximum habitat diversity and, consequently, maximum species richness.

Sampling site represents the stretch of the stream, or part of the lake/reservoir, which is a subject of assessment/investigation. In general, the length of the stretch should be 50 m for small rivers and between 50 and 100 m for medium sized and large rivers. In very large rivers, the longer sector could be the unit selected for evaluation of the status of water body.

Sampling near specific technical structures (bridges, weirs, fortified banks, dams) should be avoided. These structures cause a deviation in current velocity, substratum, macroinvertebrate community so that biological characters are not representative for that particular river reach.

Selection of sampling points

- ✎ Selection of sampling points depends of the purpose of the investigation:
 - ✎ For monitoring programme, sampling point representative for the stretch should be selected
 - ✎ For research programmes, the sectors with the most diverse habitat structure should be selected
- ✎ Avoid sampling near technical structures

3.4.2 Sampling time and frequency

The frequency of monitoring should not be less than suggested by the WFD. Minimum sampling frequency recommended by the WFD for benthic invertebrates is twice per three years. Sampling should be performed preferably during periods of spring-early summer and late summer-early autumn, depending on water body and monitoring type.

Monitoring frequency for benthic invertebrates could be increased if necessary. For routine monitoring, the frequency should be 2-4 times per year. For the basin wide comparison, one sampling per year is acceptable.

In the initial phase of monitoring system reconstruction according to the WFD objectives, it is better to reduce monitoring frequency (not less than minimum recommended according to the WFD), but at the same time to cover more sampling sites in the short time period (to minimize seasonal influence) and during the same period of the year in order to provide comparable data. The monitoring could be continued in the following years, within the same period of the year (for a particular water body type). This approach provides collection of relevant spatial data, necessary for evaluation of typology and development of the assessment system.

The performing of the monitoring in a defined period allows for data comparison. If one sampling has been planned per year (survey), it is recommendable that sampling in large rivers should be performed in periods of low water conditions to make it more easy and safe. Several international surveys on large Central European rivers were organized in the low water discharge period (August-September) such as JDS1 in 2001, AquaTerra Danube Survey in 2004, JDS2) and early October (International Tisza Research 2001). A selection of the appropriate sampling period in very large rivers is not influenced very much by the seasonal dynamics of the most sensitive insect groups because in this running water type the role of EPT taxa is rather limited. In case of smaller rivers, it is recommended to focus on sampling in the period of April to June/July, depending on stream type and altitude classes. The species richness is expected to be much higher in this period than in late summer and autumn.

Sampling frequency

- ✱ Minimum frequency of monitoring is twice per 3 years

Sampling period

- ✱ For small to medium sized rivers, the most preferable sampling period is from beginning of April to end of June
- ✱ For large and very large rivers, it is the best to collect the samples during low water conditions (for temperate zone of northern hemisphere period August-September)

3.4.3 Sampling

Standardised reliable sampling procedure is the basis of efficient monitoring programme. The aim of collecting abundance data of the macroinvertebrate community on species level is, similarly to the other BQEs – to enable the assessment of ecological status of water bodies during WFD-compliant monitoring, which provides the frame for an effective river basin management.

Sampling methods for aquatic macroinvertebrates in running and standing waters are different. Methods for surveying aquatic macroinvertebrates in running and standing waters are specified by the international standard EN 27828: 1998.

Large EU framework projects were dealing with the „Multihabitat sampling” (MHS) in small and medium sized, wadeable rivers. Sampling in large and very large rivers (characterized by deep, non-wadeable zones) is not harmonised yet at the EU scale. This methodological problem is currently discussed in the ECOSTAT and Intercalibration Expert Groups from the two point of views: (i) sampling methods and (ii) assessment tools for the determination of ecological status that implicitly contains the problems and difficulties of establishing the referential conditions within large, lowland river catchments.

However, until now the multi-habitat sampling method was recommended for the wadeable streams (AQEM 2002). Similar principle (i.e. the MHS) is suggested for large and very large rivers but widely accepted methodology concerning the estimation of abundance is still missing. Stagnant waters and lakes just became the study objects of a recent large EU 7th FP programme (WISER) that was formulated among different Geographic

Intercalibration Groups (GIGs) organized by the Lake Intercalibration Group of the Joint Research Center (Ispra, Italy).

On the other hand, the sampling in water bodies in which considerable areas are covered with dense aquatic macrophytic vegetation (according to AQEM procedure, considerable area is the area that exceeds 5% of the area that is the target of the investigation) is not properly defined and a lot of effort should be focused on the issue. In the case of dense macrophyte cover, the sampling procedure should comprise the evaluation according to volume (individuals per volume of vegetation). Obvious example of such methodological problem is research performed in May 2009 within the current project on the sampling site upstream Knežina, the Bioštica River (site 16). The sampling stretch is characterised by dense cover of aquatic vegetation. With regard to cross section, more than 80% of the stream is covered by macrophytes. In such conditions, there is no sense to evaluate the number of individuals per square meter, since the whole space is populated with macroinvertebrates.

During the field work in B&H small and medium sized wadeable streams were sampled where several AQEM and Kick and Sweep (K&S) samples were collected simultaneously, in order to compare effectiveness of different sampling techniques in different sampling locations and river types. In case if the river contained non-wadeable parts and sections, the multi-habitat K&S sampling was used in the wadeable littoral zone of the river.

Having in mind above mentioned methodological problems regarding macroinvertebrate sampling, the intention was to suggest the most appropriate procedure to be discussed and further elaborated for use in routine monitoring in B&H.

Wadeable rivers:

The **AQEM sampling method** is focused on a multi-habitat scheme designed for sampling of major habitats proportionally according to their presence within a sampling reach (AQEM 2002, www.aqem.de, www.eu-star.at/frameset.htm). Replicate samples should be taken according to the proportion of the main habitat types described in the AQEM protocol. Sample consists of the 20 “replicates” taken from all microhabitat types at the investigated sampling reach (e.g., 100 m long) sharing of at least 5% coverage. A “replicate” is a stationary (0,25 x 0,25 m) sampling accomplished by positioning the net and disturbing the substrate for a distance that equals the square of the frame width upstream of the standard net with the coarse mesh of 500 µm. The 20 replicates should be distributed according to the share of microhabitats. The sampling is made in transversal profile.

The first step is the detailed classification of mineralogene and biogene microhabitats as given in Annex 3.

The second step is the estimation of the percentage cover of each microhabitat type. Ten (10) different segments are taken for that in separate field protocols (Annex 4); finally the average value of each microhabitat ratio is determined. Microhabitats having less than 5% are signed only in the protocol, but not sampled.

The third step is setting of the number of sampling units according to percentage ratio of relevant micro-habitats. One sub-sample has to be taken per each of 5% cover values, altogether 20 sub-samples have to be distributed along the investigated river stretch.

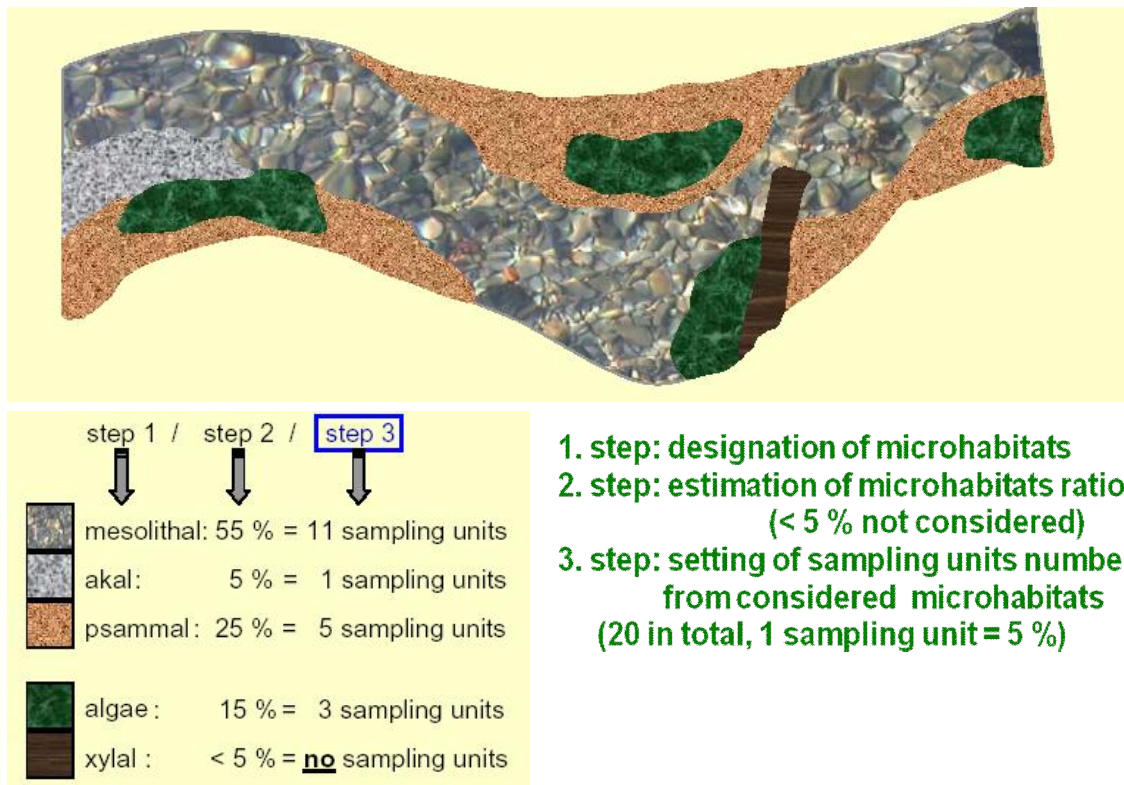


Figure 3.4-1 Example of microhabitat designaton in a theoretical investigation site according to the "multi-habitat sampling" method (Elexova, 2007). Note that replicants should be distributed according to the ratio of microhabitats – evaluated in 5% step ratios!

Sampling starts at the downstream end of the reach and proceeds upstream. When kick sampling, hold net vertically with the frame at right angles to the current, downstream from feet, and disturb the stream bed vigorously by kicking or rotating the heel of your boot to dislodge the substratum and the fauna within a depth of at least 10-15 cm. Cobbles and large stones have to be moved by hands, swept or brushed the surfaces to dislodge clingers and sessile organisms. To dislodge the animals from the interstices of the sediments, the substrate should be disturbed with a screwdriver or similar device. The surface of soft sediments and fine or organic materials should be sampled by pushing the hand-net gently through the uppermost 2-5 cm of the substratum. For sampling sediment in the slow-flowing areas it can be disturbed using the hands, in the normal fashion, and then a current created by pushing water through the net with the hands to trap the animals. Large wood and stones can be removed after being rinsed and inspected for clinging or sessile organisms.

The maximum depth of the sampled bottom is 15 cm (in case of large stones) and few cm in case of fine bottom material. The content of the sampling hand net should be emptied regularly to the container to avoid the accumulation of large volume in it. Generally, the following instructions should be kept in mind during sampling biotic microhabitats:

- The bed surface (25x25 cm) with the water column above, together with vegetation within this area, has to be covered by sampling, in case of sampling microhabitats with aquatic macrovegetation;
- Organisms are washed down from sampled vegetation (by means of hand-net);
- Mineral substrate from corresponding bottom is included, as well;
- Floating vegetation is sampled together with bottom material;
- The sampling of dominant vegetation should be followed.

Summarizing the AQEM sampling procedure it should be emphasized that:

1. The method is applicable only in case of very shallow water bodies. Generally, the term wadeable corresponds to waters that are deep less than 1 m (it is rather dangerous to make sampling action especially in rapid rivers, not only because of the strong current, but the slimy algal layer on the stones and unstable bottom as well).
2. Large transparency is required for the visual microhabitat estimation, turbid waters are problematic for the AQEM sampling.
3. Sampling in deeper and/or turbid waters require considerable amount of field experience in order to apply proper strategy!

Medium sized, large and very large rivers

In large and very large rivers the application of MHS methodology requires another approach, due to different topological, dimensional and statistical considerations. In this case, the estimation of individual numbers per unit of surface area (i/m^2) is not relevant, because the large area of the river bed is either not wadeable or not transparent for visual observation in all available habitat types, and, consequently: NOT SAMPLED.

In this case the sampling could be taken:

- Using the Kick and Sweep (K&S) method;
- In the shallow littoral zone;
- During low water discharge conditions;
- The procedure has to be standardised for time that is proportional to the ratio of the given habitat type (EN 27828: 1998).

In such situation the results are comparable to each other providing semi-quantitative data that is referring to the actual abundance conditions. A hand net with 500 μm mesh size (according to the standard) should be used for sampling up to a water depth of 1.5 m. The majority of habitat types at the bank zone should include in the sampling action. Beside of the difficulties several EU Member States apply AQEM sampling method only in the shallow littoral zone during low water level. The natural substrate of the wadeable littoral zone in the large sized rivers can be recommended for sampling. Preferably quantitative and microhabitat proportional sampling should be done. Kick and sweep method can be used similarly as described above, or, other quantitative samplers can also be used as, e.g., those described in ISO 9391 (Sampling in deep waters for macro-invertebrates - guidance on the use of colonization, qualitative and quantitative sampling). The selection of the method depends on the depth of the river, on the type of substrate and several other factors. If different area is sampled, this should be taken into account during the final quantification.

Summarizing the sampling procedures for large and very large rivers it should be emphasized that:

1. The standardized AQEM can be applied but only restricted to the shallow littoral zone;
2. WFD-compliant methods are still not available for large rivers, due to the problems of sampling and the lacking definition of reference conditions in large lowland rivers.

Due to the fact that bank region of medium sized, large and very large rivers, in general, is the most populated and the most diversified area considering macroinvertebrate community, it is recommended that focusing on this part of aquatic ecosystem is satisfactory and economically efficient approach for routine status/potential assessment. However, for investigative monitoring and scientific research, it is necessary to investigate the deeper part of the river as well, in order to obtain also the information about the diversity and relative abundance

of taxa within macroinvertebrate community in this area. Also, it is necessary to regularly investigate the deeper part of the river, however less frequently, to get necessary data about dynamics of this, often considerable part of the river.

Typical examples of the water bodies belonging to large rivers, which were investigated within the project, are sites Žitomisljić, upstream and downstream Glavatičevo, the Neretva River (site 1, 11 and 12), as well as sites on the Bosna River, Dobož and Modriča (sites 9 and 10).

On those sites, part of the river is wadeable, while another part is deep, or the fast current velocity disable applying hand net sampling procedure. Under such conditions, AQEM procedure could be applied within the wadable zone. The rest of the river could be sampled by benthological dredga, since the fast flow does not allow the use of benthological grab (Van Veen or Eckman). However, during our investigation, the most diverse and dense macroinvertebrate community was recorded within shallow zone, which support the approach that status evaluation could be done according to the data from this region. Sampling of **water bodies with dense vegetation cover** is, as it was emphasized above, the special case.



Figure 3.4-2 Sampling site Žitomisljić, the Neretva River – an example of large, non-wadeable river.

The quantification procedure requires a combination of evaluation of number of individuals per volume (within the area covered with macrophytes) and number of individuals per square meters (within the area not covered, or not densely covered). This procedure is not developed. According to our experience, in such conditions, it is the most efficient to use semi-quantitative hand net sampling. This approach, although does not provide information about number of individuals per defined area or volume, provides comparable datasets. The majority of the metrics used for status/potential assessment based on benthic macroinvertebrates could use semi-quantitative data (number of individuals per sample, number of individuals per effort-unit, number of individuals per time sampling unit, even the abundance expressed via logarithmic scale – 1-5 – Canyi 2002 or 1-9 – Pantle & Buck 1955). Taking into the consideration the above advantages, as well as the assumption that routine monitoring sampling should be time efficient and consequently economically reasonable, the semi-quantitative hand net sampling procedure could be recommended for further discussions on its potential use in routine monitoring in water bodies with dense macrophyte cover. Such water bodies are frequent on mountain plateaus, often within peat bog areas, as well as within smaller lowland rivers.



Figure 3.4-3 Typical example of the water body with dense macrophyte cover – the Bioštica River, upstream settlement Knežine.

It should be mentioned that the preliminary comparison of suggested techniques has been done. According to the results obtained within this project, as well as based on previous research within different types of aquatic ecosystems, results obtained by AQEM procedure and semi-quantitative hand net sampling procedure, are comparable. Although this hypothesis should be tested on more data to ratify the reliability of comparison, it is approved that the data obtained by both approaches on general taxa richness within the community, as well as the ratio of the principal groups and taxa is similar.

Sampling

- ✎ For small rivers, AQEM procedure is recommended
- ✎ For non-wadeable watercourses, the sampling within shore region by using hand net is suggested to be used in routine monitoring
- ✎ For more detailed investigation, it is advisable to collect the samples from deeper region, as well, by using benthological dredging (in fast water) or grab (Van Veen or Eckman, in the case of slow, soft bottom rivers)

3.4.4 Sample treatment

After collecting the material, large parts should be removed from the sample (stones, parts of the wood, etc.). The sample must be sieved through a coarse mesh of 500 µm to remove soft sediment. This procedure reduces the volume of the sample.

The biological material is transferred to sample containers. The size of sample container depends of the volume of the sample. In general, sample container of 1000 ml, or larger should be used.

If large amount of samples is expected to be collected, durable plastic bags could be used for sample storage, in order to reduce the space needed for storage (Note: if the samples are sorted in plastic bags, only formaldehyde could be used as preservative, because ethyl alcohol could decompose the plastic). Samples should be preserved either with formaldehyde to the final concentration of the 4% or with ethanol having the final concentration of 70%.

It is advisable to store samples in boxes with closure (e.g. hand refrigerators) during the transport to protect samples and to prevent evaporation.

The necessary minimum number of sorted individuals should be 500 per sample. If there are too many individuals in the sample, subsampling should be done – it has to be homogenized and then sorted by means of sorting equipment (sub-sampler) in the laboratory (Figure 3.4.3.4). In this way the proportionality of present organisms should be provided. Sub-sampler is divided to 30 squares. Sub-samples being in five randomly selected quadrates will be selected.

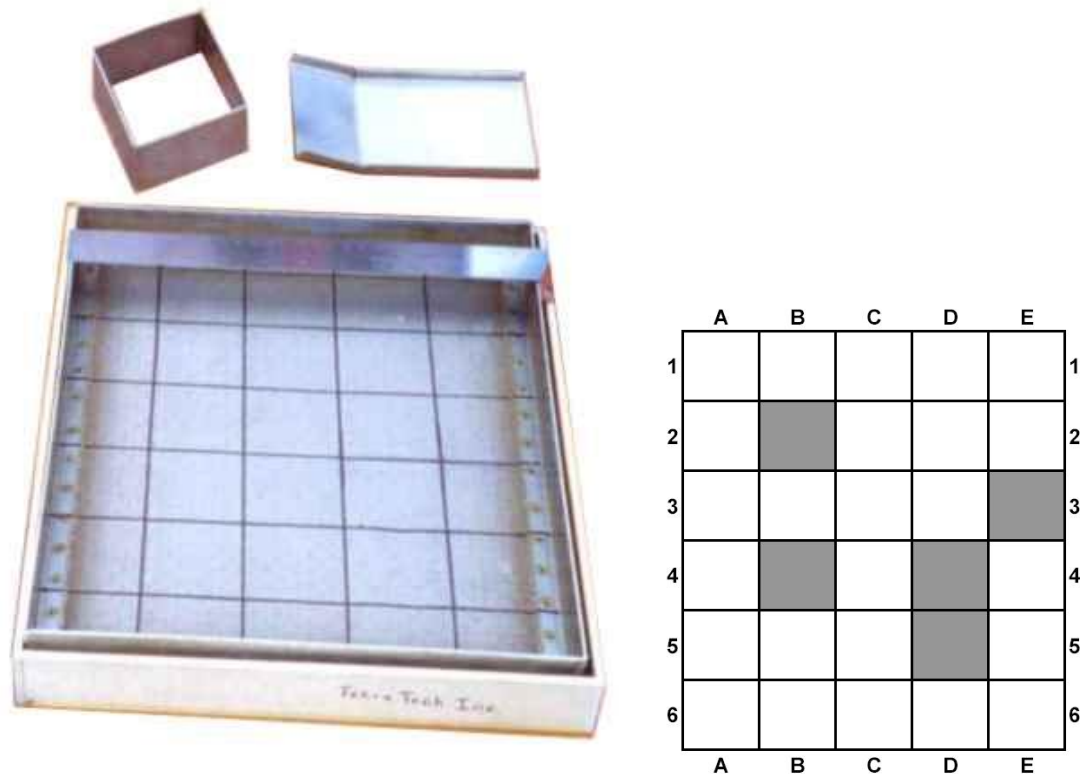


Figure 3.4-4 Sub-sampler made of 30 x 36 cm, stainless steel frame with net (500 µm) and split up into 5 x 6 square fields (30 in total). Random selection of 5 square fields are taken, min. 500 individuals of organisms have to be selected (Elexova 2007).

Sample treatment

- ✂ Reducing the sample volume
 - ✂ Removing large parts
 - ✂ Sieving
- ✂ Preservation
- ✂ Packing
- ✂ Preparation for transport

3.4.5 Labeling

Appropriate labeling is an essential part of sampling. It is recommended to add labels to the inside and outside of sampling containers or bags. Labeling can be done directly on the vessel by permanent marker, or on the labeling paper. If the label is on the labeling paper, it is recommended to cover the label with transparent tape. Inside labels should be written with a soft-lead pencil on non-recycled, high-quality paper.

Labels should include basic information on the sample such as:

- Station number;
- River name;
- Site name;
- Date;
- Sample number.

The data on samples taken, together with the label information, should be entered in the field data book

Labeling

- ✎ Proper labeling is essential part of sampling
- ✎ Durable pens and labels should be used
- ✎ Labels should include:
 - ✎ Station number
 - ✎ River name
 - ✎ Site name
 - ✎ Date
 - ✎ Sample number

3.4.6 Identification of the organisms

As detailed as possible identification of the benthic invertebrates is recommended. Species level taxa list, whenever possible, can be used for calculation of the metrics/indices.

Suggested level of taxa identification per taxa group:

| Taxa group | Recommended level of identification | Taxa group | Recommended level of identification |
|------------------------|-------------------------------------|--------------------|-------------------------------------|
| <i>Turbellaria</i> | Genus, species | <i>Trichoptera</i> | Genus |
| <i>Oligochaeta</i> | Family, genus | <i>Odonata</i> | Species, genus |
| <i>Hirudinea</i> | Genus, species | <i>Megaloptera</i> | Species, genus |
| <i>Mollusca</i> | Genus, species | <i>Heteroptera</i> | Genus, species |
| <i>Crustacea</i> | Species, genus, family | <i>Coleoptera</i> | Genus, species |
| <i>Plecoptera</i> | Genus | <i>Diptera</i> | Family, genus |
| <i>Ephemeroptera</i> : | Genus, species | <i>Hydracarina</i> | Presence |

For the routine monitoring, it is necessary to develop “operational taxa list” – the list of taxa usually used in biological monitoring. The taxa list could be developed based on the suggested level of taxonomic identification.

Identification

- ✎ The identification to the species level is preferred
- ✎ In some cases the identification to higher taxonomical level is acceptable
- ✎ There is a need for development of operational taxa list

3.4.6 Quantification of the organisms

Individual squares of sub-sampler are counted and the determination of the organisms is completed. The necessary number of counted organisms is 500 individuals. Then the number of organisms is converted to 30 squares of sub-sampler. However, also lower numbers may occur in some samples. The final total number of organisms for individual taxonomical groups refer to the total sampled area of 1,25 m². So, individual numbers per m² unit of surface area can be calculated.

If the semi-quantitative approach is used (i.e. in case of large rivers where AQEM is not applicable) the *relative abundance* is expressed as number of individuals per sample, based on percentage participation and by using 1-5 values of abundance classes (Csanyi 2002). Other scales for expression of relative abundance scale could be used, as for example 1-9 scale (Pantle & Buck 1955).

Quantification

- ✎ The quantitative relations within community could be expressed as:
- ✎ Abundance per square meter
- ✎ Percentage participation of taxa
- ✎ According to selected relative abundance scale

3.4.7 Data handling and storage

As a minimum, following data have to be stored:

- a) General data (river, sampling point, coordinates, date of sampling, estimation of substrate...);
- b) List of macro-invertebrate species of the individual sample with abundance (abundance per sq. meter, relative abundance according to selected abundance scale or as percentage participation) Note: for further calculation, it is advisable to express abundance in
- c) Calculated metrics for relevant sample.

However, the specific database should be developed for storage of biological data. Database should provide the easy interface for data upload, the effective filter that prevents inaccuracy in entering species names, possibility for easy calculation of relevant metrics and automatic evaluation of ecological status (type specific). Thus, the database should contain specific background data on sampling site (type) and species (operational taxalist with taxa codes, taxonomic information, data on taxa biology – autecological information).

Data handling and storage

- ✖ The data needed for effective status evaluation should be comprised
- ✖ Development of specific database should be the priority
- ✖ The database should provide the effective interface for status evaluation

3.4.8 Selection of relevant metrics and ecological status assessment method

Based on the analyses of response to stress, the appropriate biological metrics should be selected. The selection should be based on type-specific approach. Thus, the metrics that reflect the type characteristics and the level of stress within particular water type should be chosen.

Also, some general criteria should be taken into the consideration during the selection of metrics:

- The experience in previous use;
- Accuracy and effectiveness of the method;
- Possibility of use in intercalibration (is it widely used method, or not);
- The possibility of method adaptation for use in local/regional conditions;
- The simple, universal biological traits are preferred.

Based on presented criteria, as well as on the investigation performed within the project (Mid-term report 2009), the following metrics could be suggested for further elaboration for assessment of the ecological status:

- Abundance (relative and/or abundance per m²);
- Saprobic indices (several indices could be used – Pantle & Buck 1955, Zelinka & Marvan 1961, German system, Austrian system, Check System – AQEM 2002);
- Trophic index (RETI/PETI);
- Original BMWP/ASPT score (Armitage et al. 1983), or modifications (AQEM 2002), system tested within this project (Hungarian modification – see Mid-term report 2009);
- Total number of taxa;
- The number of taxa within selected taxa group;
- The relative abundance of selected taxa groups;
- Percentage participation of EPT taxa (AQEM 2002);
- The ratio of selected functional feeding groups (AQEM 2002);
- Balkan Biotic Index (Simic & Simic 1999).

For the calculation of the relevant metrics the background database of the benthic-invertebrate species and their relevant characteristics are needed. The ASTERICS software can be used for calculation of the metrics (it contains more than 150 metrics such as saprobic indices, tolerance measures, zonation metrics, current preference, microhabitat preference, diversity measures, feeding types, locomotion types, composition measures, richness measures, abundance).

Selection of the metrics for evaluation, as well as the method of assessment of ecological status highly depends on testing/analysis and should be done in advance and then agreed upon by all involved experts.

As a first step, the metrics, which was used within the framework of the inter-calibration exercise of EC GIG can be applied.

It should be underlined that metrics comprised within ASTERICS software are developed for different geographical regions. Some of important and widely used metrics are of limited use for streams in B&H, due to the character of the region and specific macroinvertebrae fauna.

Thus, ASTERICS software offers use of different saprobic indices developed for Germany, Austria, Czech Republic or The Netherlands. As the basis for the calculation of these, basically similar indices, the different input lists of indicator organisms with the data on saprobic valence and indicator weight are used. These lists were developed for particular area and should be modified for use in particular conditions in B&H.

The similar conclusion could be drawn for original version of BMWP and ASPT indices, as well as for the modification of these indices for different European regions - indicator list should be modified for the investigated region.

However, it is recommended to modify indicator lists for saprobic system and BMWP/ASPT for wider area, as for example for Balkan, or Western Balkan area.

The concept of "sensitive organisms" is of particular interests, since, if the basic lists of taxa that are sensitive to particular stress, or group of stress factors are defined, it could give the stressor specific information. As in the case of saprobic system, the area specific indicator list should be developed.

From the other hand, some general metrics comprised by ASTERICS software could be used for definition of type specific evaluation system in B&H, since the background database of the tool comprehends extensive database with information on taxonomy end general biological (ecological) characteristics (autecological data) of extensive range of taxa (more than 12.800 taxa). Thus, ASTERICS software could be used for fast and easy evaluation of total number of taxa per sampling unit, the number of taxa of particular taxonomic group in the sample, relative abundance of particular taxonomic group, percentage participation of each macroinvertebrate group, EPT index and FFG relation. The use of ASTERICS software enables easy calculation/production of the array of selected metrics that could be the part of the assessment system.

Metrics

- ✎ Appropriate selection of metrics should be done
- ✎ Effective, easy to use, reliable metrics should be chosen
- ✎ There is a need for metrics modification to be effectively used in B&H

3.5 Fish

Although fish were not included in the project, it is one of the BQEs that can reflect to the hydromorphological pressures. Method of sampling, identification and quantification is generally based on the European standard EN 14011 Water quality. Sampling of fish with electricity.

In principle, the same type of sampling on the various habitats or substrates applies to fish. The number of random tests for a species in any water body depends on its rarity and subsequently the chance of hitting on that species.

Fish is relevant BQE for all streams. Selection of the sampling sites has to be representative for the habitats in the river. In the rivers with high environmental stress, where lower species diversity occurs, the increase of the

number of sampling points is needed to obtain good results on abundance and age structure of the present species. It should be measured in the appropriate flow and temperature period from the point of view of fish growth. Water temperature must not be lower than 5°C. Repetition sampling should be done in the same period and in the same flow conditions as the first one.

Sampling procedure should be based on the EN 14011. Fish monitoring is carried out in large rivers/water bodies from sailing ships, which means that the monitoring point cannot, in fact, be entirely accurate. The same area is located with the aid of GPS. Changing conditions (the absence or presence of large sections of water plants, for example) can, however, influence the choice, and a certain amount of leeway must be allowed. In smaller waters, fishing is usually carried out from the river bank and it is easier to work from fixed points. Minimum length, where to fish sample are given in following table.

| Size of river | Minimum length |
|---|--|
| Small stream, breadth < 5 m | 20 m, sampling of the whole stream breadth |
| Small river, breadth from 5 m to 15 m | 50 m, sampling of the whole river breadth |
| Large river and channel, breadth > 15 m | > 50 m bank of the river from one side or on the both bank sides |
| Large shallow river, depth < 70 cm | 200 m ² |

Approximately 200 individuals of fish is sufficient for evaluation but the total area of sampling have to be more than 100 m².

Based on the morphological features each fish species have to be identified. In case of uncertainty preserved sample should be transferred to the lab for additional determination.

Measurement of the fish length (preferable tail length) have to be recorded in mm (accuracy of measurement is 10 mm). In case of the length over coverage of the age classes, other identification structures should be taken (otoliths, scales). When the number of some species is over 30 in the sample, identification of the age is sufficient.

Data storage:

- a) General iotic data (river, sampling point, coordinates, date of sampling, others.....);
- b) List of fish of the individual sample with abundance and length;
- c) Calculated metrics for relevant sample.

The detailed steps of the method should be harmonized with regards to the method of assessment. It is recommended to use the metrics used within the process of intercalibration.

4 BIOLOGICAL QUALITY ELEMENTS FOR LAKES

4.1 Phytoplankton

Phytoplankton is an important BQE in the stagnant waters (lakes, reservoirs). Normative definitions of the WFD requires to assess ecological quality of phytoplankton from the point of view of abundance and composition. Frequency of the sampling is during vegetation season within the year (preferably April – October).

Equipment, chemicals and preservatives

- Microscope with adequate facilities (required lenses with magnification 10x, 20x, 40x, 100x with oil immersion and objective micrometer or other appropriate measuring devices);
- Inverted microscope for quantification;
- Ground and cover slides, Pasteur pipettes, preparation needles;
- Counting chamber;
- Planktonic net for phytoplankton species diversity determination with a mesh size of 10 µm;
- Tubular samplers for example the type Friedinger;
- Denatured alcohol for cleaning the ground and cover slides;
- Preservative solution (Lugol solution, formaldehyde 36% solution);
- The determination keys.

4.1.1 Sampling, preservation, transportation and storing of samples

Sampling and preservation of samples is performed in accordance with EN ISO 5667-1, EN ISO 5667-3, STN and STN ISO 5667-4 ISO 5667-6. There is a new standard under development regarding to the quantitative and qualitative sampling of phytoplankton in inland waters (guideline).

Samples are taken into clean sampling containers and filled to 4/5 of its volume. For sampling through zoning a tubular sampler is used (e.g. type of Friedinger, Ruttner). For the quantitative and qualitative determination of phytoplankton free water is taken from selected depth. To determine the Cyanophyta/Cyanobacteria in stagnant waters, at the sampling site a sample is taken at the depth in the range from 0 cm to 30 cm.

If it is not possible to process a sample within 24 hours after sampling or it is required by the purpose of the analysis, the sample is preserved by Lugol solution to slightly yellow in color or formaldehyde to the resulting concentration of 4%. Not preserved sample is transferred to the laboratory in cooling boxes (maximum temperature of 8 °C) and in the processing period it is kept in the dark at a temperature of 2 °C to 5 °C.



Figure 4.1-1 Integral sampler by UWITEC.

4.1.2 Procedure for the determination of phytoplankton

Phytoplankton determination is based on:

- Species diversity of algae and Cyanophyta/Cyanobacteria;
- Identification of individual phytoplankton taxa at the lowest possible taxonomic level;
- Quantification in number of individual/cells per taxa per unit volume;
- Phytoplankton is determined only in qualitative way → the result obtained by counting a very small cells (smaller than 2 mm) would be probably loaded with significant statistical error → as far as the organisms constitute a significant proportion in the sample it is appropriate to express their quantification by indicating the content of the chlorophyll-a;
- The results of analysis of the phytoplankton - a list of taxa of present algae and Cyanophyta/Cyanobacteria and the number of individuals/cells per volume.

Quantification is counting of cells according to the European standard EN 15 204 (2004): Water quality – Guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique).

In order to promote a random distribution of plankton in the sedimentation chamber, the sample and all equipment used should be of a similar temperature. Usually, an acclimatization period to room temperature of some 12 h is adequate but this depends upon actual ambient temperatures and the sample volume.

The first critical step in preparing a sample for microscopic analysis is homogenisation of the sample.

After homogenisation, a known volume of sample should be used to fill the counting chamber either using a pipette or pouring from the sample bottle into a graduated measuring cylinder or directly into the chamber.

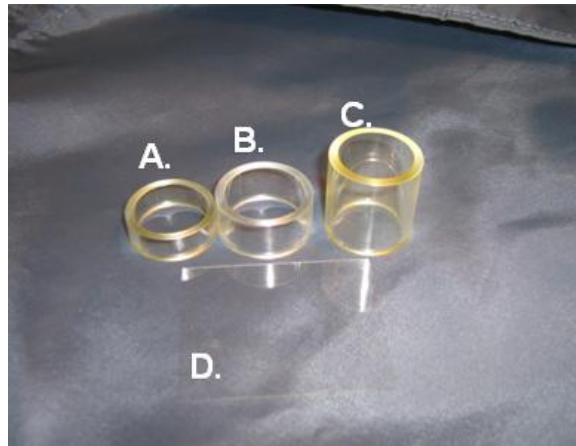


Figure 4.1-2 Sedimentation Utermöhl counting chamber (A. for chamber with a height of 1 cm and diameter of 2,4 cm: 4,5 ml; B. for chamber with a height of 2 cm and diameter of 2,6 cm: 10,6 ml; C. for chamber with a height of 3 cm and diameter of 2,4 cm: 13,6 ml; D. cover glass thickness of 0,17 mm).

Random distribution allows for simple and uniform counting strategies and statistical procedures to assess measurement uncertainty. The chamber should be filled directly from the sample bottle. The exact volume depends on the phytoplankton density, the volume of the counting chamber and its surface to volume ratio. Larger sub-sample volumes (up to 100 ml) will be required from oligotrophic waters. At high phytoplankton biomass a dilution step may be necessary to ensure that the concentration of particles is sufficiently low to prevent clogging of particles by adhesion and to optimise the counting process. Dilution should be performed with relatively large volumes (e.g. using a graduated measuring cylinder). When using small volume pipettes (1 ml to 5 ml) with removable tips, the end of the tip should be cut off to widen the opening to a diameter between 3 mm and 4 mm, to ensure that large taxa such as *Ceratium* are not excluded. Since cutting the tip will affect the accuracy of the pipette, calibrate each individually.

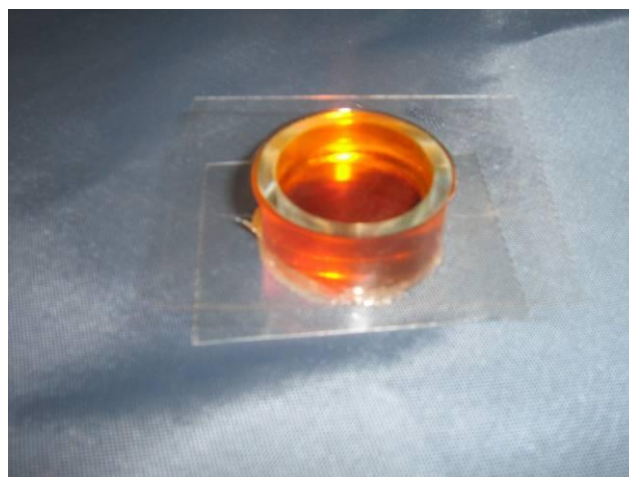


Figure 4.1-3 Utermöhl chamber after filling by sub-sample.

When all particles are settled, the overall distribution pattern should be judged using a stereo-zoom microscope to confirm a random distribution. At a low magnification, large-scale patterns can be observed easily, though the smallest particles might be overlooked.

A taxon list should be compiled before the quantitative analysis of the sample starts. The main objective of this qualitative analysis is to provide an overview of phytoplankton composition prior to the detailed quantitative analysis. This may determine the counting strategy and also helps subsequent identification of the preserved sample. Additional information may also be apparent that is of relevance to the condition of the sample (e.g. it may be useful to make a note of colony size in a fresh sample, if the colonies disintegrate upon preservation). Several approaches are suitable for the compilation of this list. If possible, a preliminary study of living samples should be made as preservation often obscures diagnostic identification characteristics.

A preliminary taxon list should be available for consultation during the final analysis of the preserved sample. Any observed differences should be investigated. Any taxa that are uncommon in the waterbody/region should be highlighted and expert confirmation sought. Once differences have been resolved, the numbers of algae present in the quantitative sample should be counted. Taxa found in the preliminary analysis but having been absent in the quantitative sample should be recorded as such.

Proper identification should be controlled by setting up one or more taxon lists depicting all taxa identified by the analysts in a laboratory. This should specify the taxonomic level to which identification is required. Reference should be made to keys, guides and Floras relating to the relevant geographical area and preferably in the working language of the analyst and more general and well-accepted Floras (Chapter 7.1) with detailed keys. Care should be taken to check descriptions of species and not just to match illustrations.

The list of all taxa identified by analysts within the laboratory should include identification characteristics and relevant references in the literature for each taxon. Drawings or digital photographs of taxa observed should be made and retained as a reference collection. Taxa should never be identified beyond the level at which the analyst is confident.

The chamber area to be counted will also depend on the objective of the study as well as on the composition and density of the phytoplankton sample itself. There are three alternative counting strategies when using sedimentation chambers:

- a) Counting a number of randomly-selected fields;
- b) Counting transects, or
- c) Counting the whole chamber.

The latter may be appropriate for low densities of phytoplankton (or detecting rare species), or for counting large species whose distribution in the chamber may not be random.

For randomly selected fields, use a square or grid eyepiece graticule to delimit the counting field and a tally is kept of the number of fields counted.

The number of fields or algal objects to be counted can be decided according to the level of precision or detection required, as the precision/detection limit depends on the number of algal objects/fields. The precision of a count may be expressed either as the standard error or as a proportion of the mean, or as the 95% confidence limits as a proportion of the mean. In most routine counts, the precision should usually be pre-set.

Counts of whole colonies and filaments can be made by applying a similar rule. Generally this requires a lower magnification (200 ×) than when counting cells. When intact colonies/ filaments are surveyed and are large with respect to the field of view, an objective basis should be agreed for determining whether it should be counted in a particular grid or not. For instance, colonies are counted when the extreme tip at the right or left side lies inside the field and filaments when the centre cell lies inside the field.

If the total number of cells is required for colonial/filamentous algae, there are three alternative approaches:

- Direct counts of cells;
- Making separate counts of the number of colonies/filaments and the mean number of cells per colony/filament;
- Desintegration of colonies into their constituent cells.

4.1.3 Phytoplankton biomass

A summary parameter for phytoplankton biomass widely used is concentration of chlorophyll-a. Method is based on ISO 10260: Water quality. Spectrophotometric determination of concentration of chlorophyll-a. Basic principle of this chemical method is separation of algae from water sample and extraction of algal pigments using hot ethanol. Concentration of chlorophyll-a is measured by spectrophotometry and evaluation is done based on difference of measured absorbancy at 665 nm before and after adding acid into extract. Concentration of chlorophyll-a is expressed as $\mu\text{g/l}$ or mg/m^3 .

Result of the method of quantification (according to the EN 15 204) can be transformed into volume biomass using special mathematical formulas for individual taxa of phytoplankton and abundance of the taxa.

4.1.4 Evaluation of the results

The method for determination of phytoplankton results to the taxa list and abundance of individual taxa. Based on this data of species diversity and abundancy some indices can be calculated (Padisak et al., 2006; Reynolds et al., 2002). Additionally concentration of chlorophyll-a and phytoplankton abundance (number of cells per volume) are used as separate metrics within classification schemes used in various European countries.

Especially the concentration of chlorophyll-a was used for intercalibration of lakes within Central Baltic Geographical Intercalibration Group.

4.2 Phytobenthos

Based on the experience and results from survey of selected lake water types in B&H in October 2008 (Mid-term Report, 2009) and May 2009 (Final Report, 2009) it is evident that phytobenthos is not relevant biological quality element for the surveyed types of lakes, resp. reservoirs (cf. also Chapter 2).

Other types of lakes should be surveyed prior to setting the relevancy of phytobenthos (benthic diatoms) as a relevant BQE. The litoral sampling of benthic diatoms is generally used and other steps of the method (sample pretreatment, identification and quantification) are the same as in case of running waters.

4.3 Macrophytes

Macrophytes are one of the four obligatory BQEs identified in the WFD, and should be used in the ecological classification of lakes. In addition to their important ecological role, the use of macrophytes as indicators of ecological status in standing waters is based on the fact that certain species and species groups are indicators for specific standing water types and are adversely affected by anthropogenic impact. Method for surveying macrophytes in lakes is based on European standard EN 15460.

In certain situations the lack of macrophytes is also a natural characteristic of certain types of aquatic habitat. As in case of phytobenthos, results from survey of selected lake water types (reservoirs) in B&H in October 2008

(Mid-term Report, 2009) and May 2009 (Final Report, 2009) it is evident that macrophytes are not relevant biological quality element for the surveyed types of reservoirs.

Equipment and accessories for sampling:

- Binoculars;
- Maps;
- Clipboard, recording sheets, water resistant pens/pencils, waterproof labels/pens;
- Plastic bags, small specimen tubes;
- Tape measure calibrated in meters;
- Hand lens with appropriate magnification;
- White plastic trays;
- Identification keys and field guides;
- Wading suit;
- Polarising sunglasses;
- Global Positioning System (GPS)-instrument;
- Rake and/or grapnel;
- Underwater viewing aid/aqua-scope.
- For deeper waters:
 - Boat and necessary equipment such as e.g. engine;
 - Grapnel on the rope;
 - Underwater 'drop' camera, waterproof camera mounted to a cable or pole;
 - Equipment to measure water depth (a hand-held echo-sounder);
- Equipment for collecting additional data
 - Conductivity meter;
 - Depth meter;
 - pH meter;
 - Secchi disc.

4.3.1 Macrophyte surveying

Lake macrophyte surveys can be undertaken at different levels of sophistication and intensity. This can involve the surveying of:

- Several discrete transects or short lengths of shoreline in lakes with shallow littoral zones;
- Multipoint transects or longer lengths of shoreline as a mean of defining the best positioning for survey locations under approach;
- Continuous transects or stretches of shoreline;
- Complete survey of the lake, in special cases.

The most widely used method for surveying macrophytes in lakes is based on belt transects. This approach allows mapping of the distribution of individual species and abundance of aquatic macrophytes, it provides robust data sets that can be used to generate indices and metrics, and it is a cost effective means of data collection. The number and positioning of survey locations at which belt transects are surveyed should allow a representative flora and the extent of colonisation to be recorded, reflecting the extent of human impact on the lake. These decisions should be made, as far as is possible, before the initiation of fieldwork.

It is strongly recommended that the important and/or representative sections of the shoreline are walked prior to survey. Preliminary walks will also provide information on seasonal variation of the aquatic vegetation. Where this is not possible due to the extent of the helophyte reed growths this may be undertaken from a boat or using aerial photographs. Sufficient transects will need to be surveyed to generate representative data, particularly with respect to identifying the maximum number and abundance of species, but also to ensure that changes in the macrophyte assemblage due to anthropogenic factors such as land-use can be distinguished from changes due to natural factors, such as differences in geology, altitude, or exposure.

Transects can be of varying widths and lengths (usually 1 m to 5 m in width) but this should be standardised as far as possible, particularly when comparing lakes that are defined as having the same or very similar hydromorphological characteristics. Each transect should be surveyed to the depth at which no further plant growth occurs, where possible, confirming this by the use of divers or drop cameras. In very shallow lakes (< 3 m mean depth) there may not be a defined end to the aquatic macrophyte colonisation area. Alternative strategies that may be considered include setting a maximum length to the belt transect, surveying across the entire width of the lake (suitable for smaller lakes) or applying the principle of stopping observations when no new taxa are identified.

Timing of surveys

As far as possible, macrophyte surveys of lakes should be undertaken between late spring and early autumn (usually May to late September, but this is dependent on local climatic conditions), when macrophyte growths will be optimal.

Transect surveying

A transect survey comprises:

- Survey along the transect conducted by either diving or by boat (both requiring suitably trained personnel);
- Survey of the shallow littoral and wash zone by wading along the shoreline to either side of the transect.

Belt transects between 2 m and 5 m can be effectively surveyed by a single diver in one run taking into account national safety practices. When plant stands are non-uniform or mosaic-like, several such belts should be surveyed to ensure a record of all species and abundance levels present. Starting from the shore the diver moves along the transect line identifying and estimating the abundance of individual species. When wider belts are used (e.g. 20 m wide) the diver should zig-zag over the whole width of the transect.

Where a boat is used, the boat handler should be familiar with operating a dive boat (i.e. the recovery of divers from the water) and there should be an air-horn on the boat to warn other users of the presence of divers in the water.

Macrophyte material is obtained by either dropping the double-headed rake grapnel or multi-point grapnel with an attached rope or using a rake with an extendable handle in water depths up to 3,5 m. The boat is stopped at a

known distance along the transect. The double headed rake or grapnel is dragged across the lake bottom for a few metres to collect samples. The rake should be deployed until no new taxa are obtained. The material is removed from the rake and identified. If necessary, specimens are retained for subsequent identification in individually labelled plastic bags. Where local water conditions allow, the extent of macrophyte colonisation should be observed directly using a viewing tube. In deeper waters a drop camera can be used with a surface recorder to locate and identify submerged hydrophytes and the extent of their colonisation.

In order to identify the maximum number of aquatic macrophyte species, a search along the shoreline and shallow littoral areas should be undertaken for each transect. Typically some bryophytes are restricted to these areas as they are adapted to periodic inundation. This surveying may be particularly important where the slope of the lake is very gradual and where level fluctuations may affect large areas of lakes enhancing colonisation by amphibious species (amphiphytes – species distributed almost equally in the water and on the shore).

Recording and quantification scales for macrophytes

Record the date of the survey, the name of the surveyor and any other relevant information that will assist the execution of future surveys. Record any observations relevant to the survey transect, such as depth, water turbidity, sediment type, adjacent land-use, water uses, shading and evidence of recent changes in the water level.

Record all macrophyte species present in each transect on the survey data sheet. Collect samples of plants for later verification for Bryophyta, algae, *Ranunculus* species, *Callitriche* species, narrow leaved *Potamogeton* species and Charales and any other uncertain taxa. Samples should be kept in voucher collections. Where necessary, specimens should be retained in national collections following verification of species which are rare or difficult to identify.

For each transect, estimate and record the area/cover or three-dimensional development/volume/amount of each species, for example as Plant Mass Estimates (PME) or using an abundance scale such as in the following table.

| Scale | Abundance | |
|-------|--------------------------|------------------|
| | Descriptor | Percentage Cover |
| 1 | Rare | < 1 % |
| 2 | Occasional | 1 to 10 |
| 3 | Frequent | 10 to 25 |
| 4 | Abundant | 25 to 75 |
| 5 | Dominant (very abundant) | > 75 % |

Where species are identified that are not present on the checklist, record these in an appropriate section of the recording sheet.

4.3.2 Macrophyte identification

Personnel should be able to identify most macrophytes and macroalgae to species level (or macroalgae and cyanobacteria to the genus level) in the field, using the appropriate keys and guides.

The same samples or samples of macrophytes, which are not certainly determined in the field can be identified in laboratory (macroalgae, bryophytes).

Macroalgae and cyanophyta should be identified by light microscopy in the laboratory up to 24 hours; it is recommended to prepare database of photographs, resp. micro-photographs for later confirmation.

List of related determination keys and other relevant literature is given in Chapter 7.1. If necessary, confirmation by an independent national/regional expert should be sought, particularly for difficult groups.

Qualitative and quantitative data from surveying of aquatic macrophytes in lakes create basis for calculation of the indices. The classification should be type-specific.

4.4 Benthic invertebrates

According to the requirements of the WFD, benthic invertebrates are not applied commonly for the routine monitoring of lakes and especially not for reservoirs. The practical use of this particular BQE for the lake water quality assessment is under discussion. An EU-wide Framework Project (acronym WISER) has been launched in 2009 in order to clarify various issues of the lake water research with particular interest in aquatic macroinvertebrates.

In fact, majority of the methodological problems concerning benthic invertebrates for integrated lake quality assessment is still under discussion within the international expert community. An obvious use of the macroinvertebrate data can be understood in case of the surveillance monitoring, when the long-time scale ecological changes are of concern.

4.4.1 Selection of the relevant sampling location

Similarly to the site selection for running waters, the representative site should be chosen for lakes/reservoirs. However, there is no widely accepted opinion about particular areas that should be part of regular sampling procedures for lakes and reservoirs. Two kinds of habitats and localities could be of interest for such purposes:

- The shallow, wadeable littoral zone characterized by euphotic zone and obvious occurrence of aquatic macrophytes (usually stock of submerge taxa);
- The open water bottom region characterized by considerable (non-wadeable) depth.

These two different types of localities require different sampling procedure and strategy. The general criteria of site election, on the other hand, are that the sample should be representative to the water body, either to the littoral or to the deep zone.

In general, the difference within the condition within the zones of the body of standing water should be taken into the consideration. Special attention should be given to lakes with pronounced thermal stratification.

In general, three different approaches could be distinguished – (i) monitoring based on evaluation of deeper part of the lake, (ii) monitoring based on the evaluation of littoral zone and (iii) combination of these two approaches. Selection of sampling sites depends on the selected approach.

In the initial phase of monitoring establishment, the sampling should cover different depth zones of the lake. After the initial phase, the selection of the most representative sampling sites could be done.

The representative sampling sites should reflect the situation that is present in the significant part of the lake (not only locally, within few narrow localities) and which influence bottom fauna in considerable extent.

Selection of sampling sites

- ✿ In general, three different sampling approaches are possible for lakes/reservoirs:
 - ✿ Sampling littoral zone,
 - ✿ Sampling deeper part of the lake
 - ✿ Combination of these two approaches
- ✿ Selection of sampling site depends on the selected approach
- ✿ Sampling sites should cover different depth zones in the initial phase of monitoring
- ✿ Based on results of initial phase of monitoring, the most appropriate sites should be selected

4.4.2 Sampling time and frequency

Similarly to the river sampling, the frequency of monitoring should not be less than suggested by the WFD. Minimum sampling frequency recommended by the WFD for benthic invertebrates is twice per three years. Sampling should be performed preferably during periods of spring-early summer and late summer-early autumn, depending on lake- and monitoring type.

Sampling frequency

- ✿ Minimum frequency of monitoring is twice per three years

4.4.3 Sampling

Similarly to the river sampling, the aim of collecting abundance data of the macroinvertebrate community on species level is to facilitate the estimation of ecological status of lake water bodies during the WFD-compliant surveillance monitoring.

The sampling method in shallow, littoral zone covered by vegetation of lakes is generally hand net sampling that is specified by the international standard (EN 27828: 1998).

Deep stagnant water bodies require the use of grab sampler like the Ekman-Birge, ponar or Van Veen grab. All of these equipments have defined surface area that makes the sample to provide quantitative data on the bottom fauna.

Sampling

- ✿ Sampling with hand nets in littoral zone
- ✿ Deep zone sampling by using different grabs – Van Veen, Ekman, Ponar grabs

4.4.4 Sample treatment

Sample treatment is practically the same as in case of samples collected in running waters.

1. The complete sample must be sieved through a coarse mesh of 500 µm.
2. The biological material is transferred to sample containers (1000 ml or larger).
3. Preserved either with formaldehyde to final concentration of the 4% or with ethanol having the final concentration of 70%.
4. There is no prefixed necessary minimum number of sorted individuals per sample. If there are too many individuals in the sample, it has to be homogenized and then sorted by means of sorting equipment (sub-sampler) in the laboratory. In this way the proportionality of present organisms should be provided. Sub-sampler can be the same as it is specified in the AQEM protocol. Sub-samples being in five randomly selected quadrates will be selected.

Sample treatment

- ✎ Reducing the sample volume
 - ✎ Removing large parts
 - ✎ Sieving
- ✎ Preservation
- ✎ Packing
- ✎ Preparation for transport

4.4.5 Labeling

Similarly to the river sampling, the appropriate labeling is an essential part of sampling. It is recommended to add labels to the inside and outside of sampling containers or bags. Labeling can be done directly on the vessel by permanent marker, or on the labeling paper. If the label is on the labeling paper, it is recommended to cover the label with transparent tape. Inside labels should be written with a soft-lead pencil on non-recycled, high-quality paper. Labels should include basic information on the sample such as: the station number, site name, the lake or stream name, and the date. The data on samples taken, together with the label information, should be entered in the field data book.

Labeling

- ✎ Proper labeling is essential part of sampling
- ✎ Durable pens and labels should be used
- ✎ Labels should include:
 - ✎ Station number
 - ✎ River name
 - ✎ Site name
 - ✎ Date
 - ✎ Sample number

4.4.6 Identification of the organisms

Similarly to the river monitoring, as detailed as possible identification of the benthic invertebrates is recommended. Species level taxon list, whenever possible, can be used for calculation of the metrics/indices.

| Taxa group | Recommended level of identification | Taxa group | Recommended level of identification |
|-----------------------|-------------------------------------|--------------------|-------------------------------------|
| <i>Turbellaria</i> | Genus, species | <i>Trichoptera</i> | Genus |
| <i>Oligochaeta</i> | Family, genus | <i>Odonata</i> | Species, genus |
| <i>Hirudinea</i> | Genus, species | <i>Megaloptera</i> | Species, genus |
| <i>Mollusca</i> | Genus, species | <i>Heteroptera</i> | Genus, species |
| <i>Crustacea</i> | Species, genus, family | <i>Coleoptera</i> | Genus, species |
| <i>Plecoptera</i> | Genus | <i>Diptera</i> | Family, genus |
| <i>Ephemeroptera:</i> | Genus, species | <i>Hydracarina</i> | Presence |

Identification

- ✦ The identification to the species is preferred
- ✦ In some cases the identification to higher taxonomical level is acceptable
- ✦ There is a need for development of operational taxa list

4.4.7 Quantification of the organisms

Based on the sample Individual numbers per m² unit of surface area can be calculated.

If the semi-quantitative approach is used (i.e. in case of littoral samples where quantitative (i.e. grab) sample is not taken easily, the relative abundance is expressed, based on percentage participation using 1-5 abundance class values (Csanyi 2002).

Quantification

- ✦ The quantitative relations within community could be expressed as:
 - ✦ Abundance per square meter
 - ✦ Percentage participation of taxa
 - ✦ According to selected relative abundance scale

4.4.8 Data storage

As a minimum, following data have to be stored:

- a) General data (name of lake, sampling point, coordinates, date of sampling, estimation of substrate);
- b) List of macro-invertebrate species of the individual sample with relative abundance (relative number of individuals per m²);
- c) Calculated metrics for relevant sample.

The same procedure of data storage is needed as for running waters, which require the preparation of specialized database.

Data storage

- ✘ The data needed for effective status evaluation should be comprised
- ✘ Development of specific database should be the priority
- ✘ The database should provide the effective interface for status evaluation

4.4.9 Selection and calculation of relevant metrics and ecological status assessment method

The following data, metrics could be used for the determination of the ecological status:

- Relative abundance of taxa (per m²);
- Biotic index (and/or Saprobic index) (several indexes will be calculated);
- Trophic index (RETI/PETI);
- Total number of taxa;
- Relevant groups of taxa for further determination at the species level should be identified.

For the calculation of the relevant metrics the background database of the benthic-invertebrate species and their relevant characteristics could be used with the help of the ASTERICS software (it contains more than 150 metrics such as saprobic indices, tolerance measures, zonation metrics, current preference, microhabitat preference, diversity measures, feeding types, locomotion types, composition measures, richness measures, abundance – for details, see AQEM 2002).

In case of lakes the selection of appropriate metrics for the optimal interpretation of the ecological status of stagnant water bodies is not widely accepted yet. Until now there were no significant steps taken forward in the framework of inter-calibration exercise of different GIGs, concerning lakes.

Metrics

- ✘ Appropriate selection of metrics should be done
- ✘ Effective, easy to use, reliable metrics should be chosen
- ✘ There is a need for metrics modification to be effectively used in B&H

5 QUALITY ELEMENTS SUPPORTING BIOLOGICAL QUALITY ELEMENTS

5.1 Physico-chemical quality elements

WFD surveillance monitoring has to be carried out for each monitoring site for a period of one year during the period covered by a river basin management plan for:

- Parameters indicative of all biological quality elements,
- Parameters indicative of all hydromorphological quality elements,
- Parameters indicative of all general physico-chemical quality elements,
- Priority list pollutants which are discharged into the river basin or sub-basin,
- Other pollutants discharged in significant quantities in the river basin or sub-basin.

The “physico-chemical quality elements” identified in the Annex V of the WFD, mean the “chemical and physico-chemical elements supporting the biological elements” listed in Section 1.1 of Annex V for each surface water category, except those for which an Ecological Quality Standard (EQS) has been set at EU level.

For the general physico-chemical quality elements, the the Annex V of the WFD specifies that in order to achieve good ecological status/potential the values for these general quality elements must not reach levels outside the range or exceed the levels established so as to ensure:

- (a) The functioning of the (type specific) ecosystem;
- (b) The achievement of the values specified for the BQEs.

The ranges and levels established for the general physico-chemical quality elements must support the achievement of the values required for the BQEs at good status or good potential, as relevant. Since the values for the BQEs at good status will be type-specific, it is reasonable to assume that the **ranges and levels established for the general physico-chemical quality elements should also be type-specific**. Several types may share the same ranges or levels for some or all of the general physico-chemical quality elements.

| General physico-chemical quality elements | | |
|---|--------------------------|-------------------|
| Oxygenation conditions | | |
| 1 | Dissolved oxygen | O ₂ |
| 2 | Biological oxygen demand | BOD ₅ |
| 3 | Chemical oxygen demand | COD _{Cr} |
| Thermal conditions, salinity and acidification status | | |
| 4 | Water temperature | T |
| 5 | pH | pH |
| 6 | Conductivity | EC |
| 7 | Alkalinity | - |
| 8 | Acidity | - |
| Nutrient conditions | | |
| 9 | Ammonium | N-NH ₄ |

| General physico-chemical quality elements | | |
|---|------------------|-------|
| 10 | Nitrates | N-NO3 |
| 11 | Total nitrogen | Ntot |
| 12 | Ortophosphates | P-PO4 |
| 13 | Total phosphorus | Ptot |

Sampling for these parameters is based on the standards EN ISO 5667 (1-19). Analyses should be provided according to the well known European and ISO standards together with biological quality elements.

5.2 Other pollutants discharged in significant quantities in the river basin or sub-basin

The WFD also requires that *other pollutants* also need to be monitored if they are discharged in significant quantities in the river basin or sub-basin. No definition of 'significance' is given but quantities that could compromise the achievement of one of the Directive's objectives are clearly significant, and as examples, one might assume that a discharge that impacted a "Protected Area", or caused exceedance of any national standard set under Annex V 1.2.6 of the Directive or caused a biological or ecotoxicological effect in a water body would be expected to be significant.

In other words, potential effects of all pollutants, which are not regulated at the EU level (all except 41 priority substances from the EQS Directive (2008/105/EC), must be taken into account at the assessment of the ecological status. In order not to monitor and study a whole universe of the chemical substances a river basin specific substances should be identified. Once identified, environmental quality standards (EQS) for these substances should be established at the national or river basin level and the pollutants must be included into the regular monitoring schemes and monitored at least four times in one year during the period covered by a river basin management plan

A number of prioritisation procedures are currently under discussion at the EU level, using hazard (ecotoxicity) and exposure (use and occurrence) risk assessment strategies. An approach of the United Kingdom (UK Environment Agency, 2007) might be recommended for use in B&H before the discussion at the EU level is concluded. First steps towards the identification of Bosna and Neretva river basin specific substances have already been done during the project (Mid-term Report, 2009).

One of the implications of the above is that ecotoxicological screening of water and/or sediment samples and screening of "unknown" substances using mass spectrometric techniques should be a part of the surveillance monitoring as a minimum during the starting period of the river basin management cycle. The list of river basin specific substances should then be revised at least once in five years. The samples (water and sediment) for such screening should be taken from the same sites and at the same time as samples of other BQEs relevant for the particular water body.

Large part of B&H belongs to the Danube river basin. In order to fulfil minimum national obligations towards the International Commission for the Protection of the Danube River (ICPDR) a participation of the B&H laboratories in the monitoring of the so-called "Surveillance monitoring 2" scheme with parameters as indicated below is recommended. More details on the background of selection of the parameters can be found in the report of the UNDP-GEF Danube Regional Project, 2007.

| Parameter | Surveillance Monitoring 2 | |
|--|---------------------------|-------------------------|
| | Water | Water |
| | concentrations | load assessment |
| Flow | anually / 12 x per year | daily |
| Temperature | anually / 12 x per year | |
| Transparency (1) | anually / 12 x per year | |
| Suspended Solids (5) | anually / 12 x per year | anually / 26 x per year |
| Dissolved Oxygen | anually / 12 x per year | |
| pH (5) | anually / 12 x per year | |
| Conductivity @ 20 °C (5) | anually / 12 x per year | |
| Alkalinity (5) | anually / 12 x per year | |
| Ammonium (NH ₄ ⁺ -N) (5) | anually / 12 x per year | anually / 26 x per year |
| Nitrite (NO ₂ ⁻ -N) | anually / 12 x per year | anually / 26 x per year |
| Nitrate (NO ₃ ⁻ -N) | anually / 12 x per year | anually / 26 x per year |
| Organic Nitrogen | anually / 12 x per year | anually / 26 x per year |
| Total Nitrogen | anually / 12 x per year | anually / 26 x per year |
| Ortho-Phosphate (PO ₄ ³⁻ -P) (2) | anually / 12 x per year | anually / 26 x per year |
| Total Phosphorus | anually / 12 x per year | anually / 26 x per year |
| Calcium (Ca ²⁺) (3, 4, 5) | anually / 12 x per year | |
| Magnesium (Mg ²⁺) (4, 5) | anually / 12 x per year | |
| Chloride (Cl) | anually / 12 x per year | |
| Atrazine | anually / 12 x per year | |
| Cadmium (6) | anually / 12 x per year | |
| Lindane (7) | anually / 12 x per year | |
| Lead (6) | anually / 12 x per year | |
| Mercury (6) | anually / 12 x per year | |
| Nickel (6) | anually / 12 x per year | |
| Arsenic (6) | anually / 12 x per year | |
| Copper (6) | anually / 12 x per year | |
| Chromium (6) | anually / 12 x per year | |
| Zinc (6) | anually / 12 x per year | |
| p,p'-DDT and its derivatives (7) | see below | |
| CODCr (5) | anually / 12 x per year | |
| CODMn (5) | anually / 12 x per year | |
| Dissolved Silica | | anually / 26 x per year |
| BOD5 | anually / 12 x per year | |

- (1) Only in coastal waters
(2) Soluble reactive phosphorus SRP
(3) Mentioned in the tables of the CIS Guidance document but not in the related mind map
(4) Supporting parameter for hardness-dependent eqs of PS metals
(5) Not for coastal waters
(6) Measured in a dissolved form. Measurement of total concentration is optional
(7) ; In areas with no risk of failure to meet the environmental objectives for DDT and lindane the monitoring frequency is 12 x per a RBMP period; in case of risk the frequency is 12 x year

Similar to biology results, collection of the data on *other pollutants* and ecotoxicology measurements together with relevant metadata in well designed databases is of utmost importance for their future correct interpretation. In order to ensure compatibility with the future upgrades of the Water Information System for Europe (WISE) database system, the formats of the ICPDR (www.icpdr.org) and pan-European NORMAN (www.norman-network.net) databases are proposed as examples to be followed.

5.3 Hydro-morphological quality elements

The expression **Hydro-morphology** brings together two subjects: Hydrological regime and Morphological conditions. **Hydrological regime** is dealing with quantity and dynamics of water flow while **Morphological conditions** refer to the shape of particular water body, i.e. river/channel width, depth and slope variations, structure and substrate of bed, structure of riparian zone.

Recent characterisation of water bodies according to requirements of the WFD at the EU member states level has identified that a high proportion of surface water bodies are *at risk* of failing to meet the “good status” due to hydro-morphological pressures and a high percentage of surface water bodies (SWB) are provisionally identified as Heavily Modified Water Bodies (HMWB). The terms Heavily Modified Water Body (HMWB) and Artificial Water Body (AWB) understand stretches with significant physical alterations by human activities on the hydro-morphology, which prevent the achievement of good ecological status. It may be river, lake, transitional or coastal water bodies. Amongst the main driving forces are hydropower production, navigation and flood protection. The types of hydro-morphological pressures in SWB which should be considered and combined are dams, weirs, barrages, dredging, dredged material placement, land claim, shoreline reinforcement, aggregate extraction, fish and shellfish harvesting and commercial fishing as habitat modification.

The main objective to study hydro-morphology is to extend knowledge of the hydro-morphological pressures and impacts resulting from human activities; to identify potential areas of conflict between the policies and to be able to recommend reconciliation using existing or new instruments and measures. Focus should be put on navigation, hydropower and flood protection.

The WFD requires hydro-morphological assessment activities to be carried out in order to enable better understanding of biological and chemical data. More precisely, the classification of the ecological status of a surface water body is based on biological elements, while hydro-morphological, chemical and physico-chemical elements are supporting elements.

The definitions for high, good and moderate ecological status in rivers considering hydro-morphological quality elements are given in the following table.

| HYDRO-MORPHOLOGICAL QUALITY ELEMENTS | STATUS | | |
|--------------------------------------|--|---|---|
| | HIGH | GOOD | MODERATE |
| Hydrological regime | The quantity and dynamics of flow, and the resultant connection to ground waters, reflect totally, or nearly totally, undisturbed conditions. | Conditions consistent with the achievement of the values specified above for the biological quality elements. | Conditions consistent with the achievement of the values specified above for the biological quality elements. |
| River continuity | The continuity of the river is not disturbed by anthropogenic activities and allows undisturbed migration of aquatic organisms and sediment transport. | Conditions consistent with the achievement of the values specified above for the biological quality elements. | Conditions consistent with the achievement of the values specified above for the biological quality elements. |

| HYDRO-MORPHOLOGICAL QUALITY ELEMENTS | STATUS | | |
|--------------------------------------|--|---|---|
| | HIGH | GOOD | MODERATE |
| <i>Morphological conditions</i> | Channel patterns, width and depth variations, flow velocities, substrate conditions and both the structure and condition of the riparian zones correspond totally or nearly totally to undisturbed conditions. | Conditions consistent with the achievement of the values specified above for the biological quality elements. | Conditions consistent with the achievement of the values specified above for the biological quality elements. |

The hydro-morphological quality elements (HyQE) must be taken into account when assigning water bodies to the high ecological class and the maximum ecological potential class. For other status/potential classes, the hydro-morphological elements are required to have “conditions consistent with the achievement of the values specified for the biological elements”. Thus the assignment of water bodies to the good, moderate, poor or bad status/ecological potential classes may be made on the basis of the monitoring results for the biological quality elements. This is because, if the biological quality elements relevant to good, moderate, poor or bad status/potential are achieved then, by definition, the condition of the hydro-morphological quality elements must be consistent with that achievement and would not affect the classification of the ecological status/potential.

To achieve those goals a better understanding of relationship between hydrology and morphology, between hydro-morphology and ecology and hydrodynamic (dynamic nature of water bodies) and tolerance and adaptability of flora and fauna is a prerequisite. Physical and chemical features should be used to derive **river types** in the legislative context of the WFD. Describing and identifying river ‘types’ enables the results of hydromorphological surveys from similar types to be compared. Together with definitions of ‘high status’, type specific, ‘reference conditions’, it gives possibility to compare quality of rivers in an equitable and ecologically meaningful way.

Monitoring frequencies shall be selected taking into account the variability in parameters resulting from both natural and anthropogenic conditions. The monitoring frequency defined by the WFD is given in the following table.

| HYDRO-MORPHOLOGICAL QUALITY ELEMENT | RIVERS | LAKES | TRANSITIONAL | COASTAL |
|-------------------------------------|------------|---------|--------------|---------|
| Continuity | 6 years | x | x | x |
| Hydrology | continuous | 1 month | x | x |
| Morphology | 6 years | 6 years | 6 years | 6 years |

The hydro-morphological quality elements (HyQE) and hydrological parameters have to be recorded when studying and characterising river reaches, which is important for data processing, interpretation, and presentation of the results. Currently, standards mainly focus on morphological parameters of water bodies and river continuity, not considering sufficiently hydrological aspects. The collection of hydro-morphological data should started before the field survey, gathering information from different sources as topographic, geological and hydrological as well as historical maps, aerial photographs and GIS databases layers and other background documents regarding different water management issues such as water use, flood protection and water quality protection. In general a wide range of parameters useful for characterisation of a river in terms of its habitat features should be collected and stored. Three areas should be considered during surveys: (i) river channel, (ii) banks and riparian zone and (iii) floodplain, while following categories of data should be in focus (see Annex 6):

I Basic data:

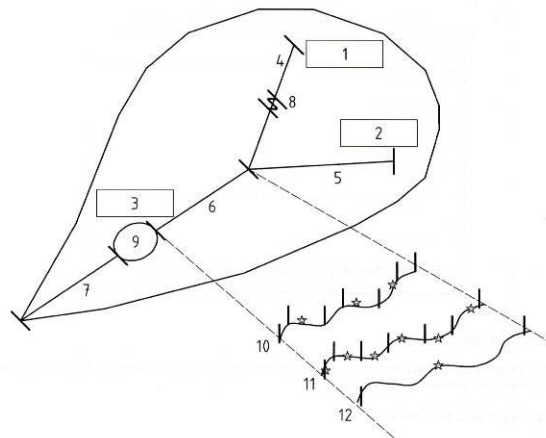
- a) Site related information**, i.e. site description as maps, registration number and scale; stream name and system; catchment area (km²) and distance to the mouth of the river (river km); longitude/latitude/altitude of sampling site (m above sea level); stream order (Strahler system) and stream type; slope of the valley floor (%); eco-region(s), sub-region(s); size class based on catchment area, according to the WFD; geology (dominant type); photographs, short description, etc.
- b) Sample related information - stream morphology and hydrology at sampling site:** valley form/cross section; riffles, pools; debris dams; logs at sampling site; bank and bed fixation; other transverse structures; pulse releases; water abstraction; stagnation; torrent modification; channelling for navigation; straightening; cut-off meanders; scouring [m below surface]; culverting; fire incident; waste and other human impacts on the stream morphology and hydrology.

II Additional data - sample related Information

- a) Hydrology at the sampling site** as hydrologic stream type; cross section/river geometry, velocity (m/s); characteristic discharges (m³/s), slopes, substrate pattern and size, etc.
- b) Shoreline and floodplain morphology** as; impoundments and woody riparian vegetation/average width at sampling site; shading at zenith and natural vegetation/removal/lack at left and right shoreline, floodplain vegetation; land use¹, non-native woody riparian vegetation and number of standing water bodies in the floodplain.

Additionally to the river attribution, the **river reach** and **survey units**' selection is fundamental for survey strategy and assessment. Every individual catchment needs first to be divided into river type(s) and then to the component reaches and survey units as shown on Figure 4.4.9-1.

¹ 1 km length



- | | | | |
|---|----------|----|------------------------------------|
| 1 | Type A | 7 | Reach C2 |
| 2 | Type B | 8 | Waterfall |
| 3 | Type C | 9 | Lake |
| 4 | Reach A1 | 10 | Sample survey units within a reach |
| 5 | Reach B1 | 11 | Continuous survey units |
| 6 | Reach C1 | 12 | Single survey units |

Figure 5.3-1 A hypothetical catchment showing the main types of approach to hydromorphological survey, set within the context of river scale ('type', 'reach', 'survey unit') (* survey unit)

Factors used to define river reach boundaries are significant change in geology, valley form, slope, discharge (inflow of significant tributary/change in stream order), land use and sediment transport (lake, reservoir, dam, major weirs).

Different survey strategies can be used for the survey concerning the assessment of river reaches, by defining and studying possibly smaller contiguous survey units, larger single units (e.g. covering the whole reach of the river) or locating randomly the survey units within the reach. When the aim of the assessment is to provide an overall evaluation of the river stretch (e.g. for WFD purposes) data from distinct surveys units should be combined considering their relative length. The collected data should support the assessment of the two banks separately, and the field survey should be preceded or followed by the use and interpretation of all the existing information available for the study site.

The length of a survey unit is dependent on the purpose of the assessment and the size of the river¹. If continuous survey is used, survey units should be 100 m, 500 m, 1 km, or variable lengths according to the degree of morphological uniformity. If the sampling option is chosen, it is recommended that the length of survey sites should be 100 m, 500 m, or 1 km. Where the main purpose of survey is to assist in operational river management, more detailed collection of data on river features may be required.

¹ CEN 14614 "A Guidance Standard for Assessing the Hydromorphological Features of Rivers"

AQEM site protocol recommends to record human impacts on stream morphology separately for the sampling site itself, upstream and downstream section. These stretches are defined as follows:

“sampling site”

- small streams ($A [km^2] < 100$): 250 m upstream and downstream of sampling point;
- medium sized streams ($100 < A [km^2] < 1000$): 500 m upstream and downstream of sampling point;
- medium sized streams ($100 < A [km^2] < 1000$): 1000 m upstream and downstream of sampling point.

“upstream”

- small streams ($A [km^2] < 100$): 5 km upstream of sampling point;
- medium sized streams ($100 < A [km^2] < 1000$): 10 km upstream of sampling point;
- medium sized streams ($100 < A [km^2] < 1000$): 50 km upstream of sampling point.

“downstream”

- small streams ($A [km^2] < 100$): 5 km downstream of sampling point;
- medium sized streams ($100 < A [km^2] < 1000$): 10 km downstream of sampling point;
- medium sized streams ($100 < A [km^2] < 1000$): 50 km downstream sampling point.

Lateral survey boundaries need to encompass all floodplain features that may be present. For large, active, rivers in their lower reaches these features could extend several kilometers from the channel. Where the river valley is less than 100 m wide, it is possible for surveys to include the river and its floodplain. A standard distance of 50 m on either side is recommended for all other watercourses. A category of ‘special features’ should be used to ensure that any features of ecological or conservation importance but beyond the 50 m boundary are included as well. Where embankments are present, hydromorphological field survey should not extend beyond them. However, notes should be made to allow a description of the *potential* floodplain area and features that could be present if embankments were absent.

Regarding **season of hydro-morphological assessment** it should be conducted during the low flow periods, avoiding extremely dry seasons. The times at which monitoring is undertaken shall be selected so as to minimise the impact of seasonal variation on the results, and thus ensure that the results reflect changes in the water body as a result of changes due to anthropogenic pressure. Moreover, the investigation period must be selected to allow a good description of the vegetation features of the site. Additional monitoring during different seasons of the same year shall be carried out, where necessary, to achieve this objective. The hydro-morphological survey, when feasible, should be carried out in the same period of the biological survey.

Concerning the **selection of reference sites** and/or the definition of acceptable reference conditions, four main criteria are proposed dealing with:

- (i) Bed and bank characteristics;
- (ii) The freedom of lateral movement of the river;
- (iii) The free movement of biota and sediments along the river continuum and
- (iv) The condition of the riparian vegetation.

Reference conditions (RC) should be identified within each river type reflecting totally, or nearly totally, undisturbed conditions, using the following criteria:

(i) Bed and bank character

RC: Lacking any artificial instream and bank structures that disrupt natural hydromorphological processes, and/or unaffected by any such structures outside the site; bed and banks composed of natural materials.

(ii) Planform and river profile

RC: Planform and river profiles unmodified by human activities.

(iii) Lateral connectivity and freedom of lateral movement

RC: Lacking any structural modifications that hinder the flow of water between the channel and the floodplain, or prevent the migration of a river channel across the floodplain.

(iv) Free flow of water and sediment in the channel

RC: Lacking any instream structural modifications that affect the natural movement of sediment, water and biota.

(v) Vegetation in the riparian zone

RC: Having adjacent natural vegetation appropriate to the type and geographical location of the river.

If reference conditions for any particular type cannot be found, they may be sought in other countries or regions, by modeling, or by using expert judgment. When reporting, channel, banks and floodplain information should be expressed in different outputs.

Hydro-morphological assessment should be done for all HyQE groups and to cover the three broad zones of river environments: (i) river channel; (ii) river banks/riparian zone; (iii) floodplain. To identify sites or reaches that should be classified as “*high status*” under the WFD, attention should focus on river geometry, substrate, flow regime, bank structures and modifications and vegetation types. River vegetation, organic debris and land use of aspects should be considered too. For operational monitoring under the WFD, the selected HyQE should be the most sensitive ones to the existing pressures on hydromorphology. Remote sensing methods such as aerial photography, video recording, or satellite imagery are recommended where appropriate as they can yield valuable data on large-scale features (e.g. extent of riparian zones, location of embankments, river planform, artificial structures).

A cost-effective analysis has to be taken into account in order to optimize the ratio between resources invested in data collected (staff, time, equipment, money, etc) and the added value the data to be collected could provide.

Field survey should be preceded and/or followed by exhaustive use and interpretation of all available data and carried out by walking along the river bank. Using a boat can help in seeing channel and bank features in places not easily accessible from the banks. In general, entering the channel to check features is recommended wherever possible. Field recorders need a good understanding of the survey method, and familiarity with the features recorded. Completed survey forms (Annex 7) should be accompanied by photographs of the site with details of the location carefully recorded. This is important for reporting purposes as well as providing a record for future comparisons. Safety issues are paramount when surveying rivers. Surveyors should conform to EU and national Health and Safety legislation, and any additional guidelines appropriate for working in or near rivers.

Member States have carried out previously a number of different river morphology projects and/or national programs with the intention of developing methods for hydro-morphological assessment, being suitable for application under the WFD. To mention for instance the Austrian nation-wide method, the French “SEQ Physique”, the German “LAWA-vor-Ort”, the United Kingdom “River Habitat Survey” (RHS) or the Italian “River Functionality Index (I.I.F.). The first four have been used as a foundation for producing CEN standards to fulfil the WFD requirements. The extent of difference from reference condition is used to place a site or reach in one of five classes according to its degree of modification as shown in the following table.

| | RIVER CHANNEL (including flow conditions) | BANKS / RIPARIAN ZONE (integration of left and right banks) | FLOODPLAIN (integration of left and right floodplain) |
|-----------|---|---|---|
| Class I | nearly natural | nearly natural | with very high ecological value |
| Class II | slightly modified | reinforcements in small sections | with high ecological value |
| Class III | moderately modified | reinforcements in large sections | with moderate ecological value |
| Class IV | severely modified | continuous bank reinforcements | with low ecological value |
| Class V | totally modified | totally modified banks | totally modified |

This should be achieved by assessing data from field survey and other sources (e.g. maps, remote sensing). Whilst an overall assessment of quality should be determined, it is essential that individual quality assessments for the channel, banks and floodplain are kept separate and can be used as three distinct outputs. An example of assessment method is shown in Annex 6.

Whilst the WFD does not require hydro-morphology to be reported in five classes, CEN 14614 standard ¹ recommends the use of an equivalent 5-band classification system in which reference conditions (high status) are defined as Class 1, and the remaining classes as 2-5. For the purposes of this standard, use of the WFD terms such as 'good status' and 'moderate status' should be avoided as they are linked entirely to WFD assessments of biological conditions. Where maps of hydro-morphological quality are produced, it is recommended that the following colours are used: Blue - Class 1 (reference conditions); Green - Class 2; Yellow - Class 3; Orange - Class 4 and Red - Class 5.

Collected data and information from field sheets should be transferred to databases. It is important that no errors occur when transferring data from field sheets to databases. Suitable quality assurance methods should be used, such as double entry of data onto databases by two different operators, followed by tests to ensure the results are identical. Random testing should also be carried out on hydromorphological quality assessments and other applications to ensure that consistent results are obtained from the same data

The procedures for assessing hydromorphological survey data should take into account purpose of assessment. For the purpose of identifying sites or reaches in reference condition under the WFD, applied methodology should consider the present level of assessment methods used by national hydromorphological service, national needs and habitats specificities. It should provide guidance to enable a basic assessment of the extent of difference from reference conditions. Further development of national methods and inter-comparison of the results that they produce should lead to harmonised assessments based on type-specific predictions of the occurrence of physical features within a river.

¹ EN 14614: 2004 "Water Quality - Guidance standard for assessing the hydromorphological features of rivers"

6 General conclusions and recommendations

The presented Bio-monitoring manual was developed using the general guidelines of the WFD combined with a specific knowledge of the project team obtained in B&H. Within the project, the most important elements of on-site sampling activity carried out according to the requirements of the WFD-compliant monitoring were introduced to the national experts together with the theoretical basis. The sampling, sample processing and taxonomic determinations were performed for illustrating the WFD compliance. However, it should be stressed that one of the primary sources of information – two field surveys conducted within the project - provided only a snapshot of the actual situation. With regard to the limited availability of the historical datasets and the fact that the aquatic flora and fauna is region specific, it must have been concluded that further improvement of the *WFD-compliant ecological status assessment* methodologies is necessary.

Several data collection and evaluation methodologies were tested within the project, however, the interpretation of results summarised in the project's Mid-term and Final report should serve only as an example or a guideline for the implementation of a WFD-compliant monitoring under the specific B&H conditions. Methodologies for the fully WFD-compliant ecological status assessment could only be proposed if more detailed monitoring of all quality elements were performed with WFD-prescribed frequencies for at least one year. The two basic purposes of such a detailed and intensive data collection would be (i) to collect relevant and consistent dataset that covers the whole gradient of pollution range in B&H, and, (ii) to identify genuine reference sites that are lacking significant anthropogenic impact for all types of rivers and lakes in B&H. Only with such data available a proposal of good (working) classification scheme (including selection of metrics) would be possible.

A recommended solution for the development of the WFD-compliant assessment system would be to continue in the systematic data collection within a framework of a well-designed monitoring programme. Here, several issues, which might still need an attention are indicated below:

1. A need to establish national method for assessment of ecological status based on aquatic flora and fauna has been recognised. Following B&H specific issues should be taken into account:

- Water bodies delineation;
- Establishment of a national typology;
- Monitoring of water bodies according to the requirements of the WFD (BQE together with supporting QE fitting to the normative definitions);
- Derivation of reference sites/reference values;
- Selection of relevant metrics (according to the normative definitions);
- Testing of relevancy of the metrics from the point of view of pressures;
- Boundary setting protocol (derivation of boundaries between classes/status);
- Establishment of type specific classification schemes;
- Harmonisation of individual BQE classification schemes between each other;
- Harmonisation of BQE schemes and supporting QE schemes.

2. Risk analysis should be worked out, in order to find out which water bodies are *at risk*, *possibly at risk* and *not at risk*. Information about pressures (e.g. hydromorphology, organic pollution, nutrient pollution/eutrophication, acidification, pollution by hazardous substances), available results on chemical and biological water quality, land use and possible point and diffuse sources of pollution should be taken into account.

3. There is a need to establish relevant structure of monitoring (based on previous information), which should propose/optimize:

- Network of sites (especially representative sites for surveillance monitoring and relevant stressor sites for operational monitoring);
- Selection of relevant determinands;
- Sufficient frequencies;
- Responsible organisations for sampling, analysis and evaluation of results;
- Assessment of water bodies;
- Training for responsible persons (sampling methods, analytical/chemical methods, biological methods);
- Technical infrastructure (laboratories, equipment etc.);
- Availability of determination literature and software for calculation of metrics.

4. A national web-based database should be established for biological, chemical and hydromorphological data in order to ensure (i) intercomparability of the monitoring data from different agencies responsible for monitoring, (ii) proper interpretation of the monitoring results and (iii) compatibility of the national/river basin data formats with WISE and other European database systems (e.g. ICPDR).

The most important steps towards fulfillment of the WFD requirements are the establishment of (i) the national water typology and (ii) type specific referential conditions for all BQEs. Next to the sites already included in the national monitoring scheme it is important to extend the sampling network to those locations or sections of the investigated rivers which are impacted in less extent to anthropogenic effects in order to collect more data for the basic comparison/reference.

It should be emphasised that several taxonomic uncertainties have to be clarified as well. There are quite a few taxa that are characteristic for this part of the Balcan region. Further research is necessary to reveal the real taxonomic information of those species.

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7.2 Determination literature of the main taxonomic groups of aquatic macroinvertebrates

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Annexes:

- Annex 1** Field protocol for phytobenthos sampling in streams/rivers
- Annex 2** Water macrophyte survey protocol
- Annex 3** Details of the AQEM sampling protocol
- Annex 4** Form “Site related information: Site description”
- Annex 5** Sampling protocol for surface water
- Annex 6** Hydromorphology assessment scheme example
- Annex 7** Site Protocol

C. Sampling

1. Type of sampled community

benthic diatoms

all present groups

2. Type of sampled substrate

Description:

boulders (above 40 cm)pieces
 stones (above 20 - 40 cm)pieces
 pebbles (above 6 - 20 cm)pieces
 gravel (above 0,2- 6 cm)pieces
 sandpieces
 fine sediment (mud, silt)pieces
 macrophyte vegetationpieces
 dead vegetationpieces
 artificial substratepieces
 otherspieces

2. Type of sampled substrate

Description:

.....pieces
pieces
pieces
pieces
pieces
pieces
pieces
pieces
pieces
pieces

3. Sampled habitat

stream
 litoral
 other

3. Sampled habitat

stream
 litoral
 other

4. Size of scrapped area (cm²):

4. Size of scrapped area (cm²):

5. Sample

no. of samples:
 no. of sub-samples:
 note:

4. Sample

no. of samples:
 no. of sub-samples:
 note:

6. Sample processing

not preserved
 preserved formaldehyde
 other:

5. Sample processing

not preserved
 preserved formaldehyde
 other:

D. Other determinands

1. Special abiotic conditions

ice floas extreme low water level
 extreme high water level other:

2. Other observations (e.g. possible substrate stability disturbance)

.....

3. Physico-chemical determinands

oxygen [mg/l]:
 pH:
 conductivity [μ S/cm]:
 alkalinity [mol/l]:
 acidity [mol/l]:

Sampling has been provided according to the SOP No.

Stamp:

Sampled by:
 Name Signature

Approved by:
 Name Signature

Annex 2 Water macrophyte survey protocol

Water Macrophyte Survey Protocol

| | |
|----------------------------|--|
| Date of survey (from – to) | ID No.: |
| Survey was provided by: | Documentation: <input type="checkbox"/> photo / <input type="checkbox"/> herbarium |
| River..... | <input type="checkbox"/> Lake..... |
| Survey sampling site: | |

Abiotic site parameters

| Surveyed stretch | 1 | 2 | 3 | 4 | 5 |
|------------------------------|---|---|---|---|---|
| Mark of drawing | | | | | |
| Latitude | | | | | |
| Longitude | | | | | |
| River km | | | | | |
| Estimated length (m) | | | | | |
| River width (m) | | | | | |
| Surveyed part of the stretch | | | | | |
| Depth (m) | | | | | |
| Bank structure | | | | | |
| Type of sediments | | | | | |
| Flow class | | | | | |
| Type of connectivity | | | | | |
| Type of land use | | | | | |
| Transparency (Secchi) | | | | | |
| Shading (%) | | | | | |

Notes:

ANNEX TO THE MACROPHYTES SURVEY

- Approach to the sampling site,
- Drawing,
- Photo No.....)
- Others.....

The plant mass

- 1 rare <0,1%
- 2 occasional 0,1 - 1%
- 3 frequent 1-5%
- 4 abundant 5-10%
- 5 very abundant >10%

Bank structure (CORINE)

refers to the upper littoral, extending over water level during mean discharges

- 1 large blocks of the stones (used for bank stabilisation and river regulation, often on each side of the main channel)
- 2 gravel
- 3 sand
- 41 fine inorganic material, gentle/flat slope
- 42 fine inorganic material, steep/vertical slope
- 5 concrete or other artificial embankment material
- 6 floating mats

Sediment type (CORINE)

this part of the substrate in the littoral of the river or other type of water body where the aquatic vegetation is growing

- 1 solid rocks
- 2 gravel
- 3 sand
- 4 fine inorganic material
- 5 artificial material (concrete, asphalt, ...)
- 6 detritus or other organic material

Flow class (CORINE)

- 1 no flow
- 2 low flow, just visible to cca 30 cm/s
- 3 medium flow (35 to 65 cm/s)
- 4 high flow (>70 cm/s)

Connectivity type (CORINE)

- 99 main channel (with international commercial navigation)
- 98 big secondary channel (directly connected to the main channel)

- 1 small side arm (of considerably smaller width than the main channel)
- 21 oxbow (with open inflow from the lower end)
- 22 oxbow (with open inflow from the upper end)
- 3 oxbow (semi-separated, no permanent vegetation in the connecting stretch, flooded minimum every 2 years)
- 4 oxbow (separated, growth of higher vegetation in connecting stretch, flooded in intervals longer than 2 years)
- 5 no type at present
- 61 water body outside the flood protection dam, running water
- 62 water body outside the flood protection dam, still water
- 7 no type at present
- 81 lake with surface water connection
- 82 lake without surface water connection

Land use type (CORINE)

- 1 artificial surfaces
- 11 urban fabric (urban centres, discontinuous built up areas) and in the context of this study use 11 for cities and towns and 11908 for villages)
- 12 industrial, commercial and transport units (road and rail networks and associated land, port areas, shipyards)
- 13 mine, dump and construction sites (mineral extraction site gravel-, sand-, clay-pits, dump sites)
- 14 artificial, non agricultural vegetated area (parks, cemeteries, sport facilities such as golf grounds, open-air theatres...)
- 2 agricultural areas
- 21 arable land (including also fallow lands up to 3 years abandoned, greenhouses)
- 22 permanent crops (vineyards, orchards, berry fruit plantations, hop plantations, oil-bearing rose plantations, willow plantations)
- 23 pastures
- 24 heterogenous agriculture areas
- 3 forest and semi-natural areas
- 31 forest and semi-natural areas
- 311 broad-leaved forest
- 312 coniferous forest
- 313 mixed forest
- 32 scrub
- 33 open space with little or no vegetation (beaches, dunes, sand, river banks, bare rock)
- 4 wetlands
- 41 inland wetlands
- 411 inland marshes
- 412 peat bogs

Annex 3 Details of the AQEM sampling protocol

Mineral microhabitats can be sorted as the following natural ones:

- **megalithal** (boulders >40 cm);
- **macrolithal** (boulders 20-40 cm);
- **mesolithal** (cobble 6-20 cm);
- **microlithal** (pebbles 20-60 mm);
- **akal** (fine gravel 0.2-2 cm);
- **psammal** (sand 0,06-2,0 mm);
- **argylal** (silt and clay >6 μ m).

Artificial substrates are called **technolithal** microhabitats such as the characteristic rip-rap bank enforcement, or any kind of concrete river bed structures, both are handled separately during sampling.

The same distinct biotic substrates or organic microhabitats are as follows:

- filamentous macroalgae;
- submerged macrophytes;
- emerging macrophytes;
- floating parts of riparian vegetation;
- xylal (wood trunks and branches);
- CPOM (coarse particulate organic matter);
- FPOM (fine particulate organic matter)
- bacterial stuffs;
- organic debris in the splash zone.

According to the AQEM protocol **the first step** is the visual microhabitat estimation for the designation procedure.

Annex 4 Form "Site related information: Site description"

| | | | | | | | |
|--|--|------|--|---|--|--------------|--|
| site name | | date | | sample no. | | investigator | |
| Site related information: Site description | | | | | | | |
| 1 map (No., scale) | | | | 2 stream name | | | |
| | | | | 3 stream system (river flowing into the sea) | | | |
| | | | | 4 country | | | |
| | | | | 5 federal state | | | |
| | | | | 6 map no. | | | |
| | | | | 7 longitude (degree, min, sec) | | | |
| | | | | 8 latitude (degree, min, sec) | | | |
| | | | | 9 distance to source [km] | | | |
| | | | | 10 stream order (Strahler system) | | | |
| 12 subregion (if applicable) | | | | 13 ecoregion and ecoregion no. | | | |
| 14 altitude of sampling site [m a. s. l.] | | | | 15 altitude class | | | |
| 16 catchment area [km ²] at sampling site | | | | 17 size class based on catchment area | | | |
| 18 Geology (dominant type) | | | | 19 geology class | | | |
| 20 stream type (mark system and fill in name) <input type="checkbox"/> System A <input type="checkbox"/> System B | | | | | | | |
| 21 photographs (a. downstream) | | | | (b. upstream) | | | |
| <div style="border: 1px solid black; width: 100%; height: 100%;"></div> | | | | <div style="border: 1px solid black; width: 100%; height: 100%;"></div> | | | |
| 22 short description | | | | | | | |
| | | | | | | | |

| site name | date | sample no. | investigator |
|--|----------------------|--|--|
| Sample related information, to be recorded at each sampling date (copy if necessary) | | | |
| 23 MINERAL SUBSTRATES (5% steps, mark substrates <5% with 'X') | | % of coverage (5% classes), sum of mineral and biotic microb. = 100% | no. of replicates for sample |
| | | | x = artificial substrate technolithal |
| hygropetric sites water layer on solid substrates | <input type="text"/> | <input type="text"/> | <input type="checkbox"/> |
| megalithal >40 cm large cobbles, boulders and blocks, bedrock | <input type="text"/> | <input type="text"/> | <input type="checkbox"/> |
| macrolithal >20 cm to 40 cm coarse blocks, hand-sized cobbles, with a variable percentages of cobble, gravel and sand | <input type="text"/> | <input type="text"/> | <input type="checkbox"/> |
| mesolithal >6 cm to 20 cm fit to hand-sized cobbles with a variable percentage of gravel and sand | <input type="text"/> | <input type="text"/> | <input type="checkbox"/> |
| microlithal >2 cm to 6 cm coarse gravel, (size of a pigeon egg to child's fist) with variable percentages of medium to fine gravel | <input type="text"/> | <input type="text"/> | <input type="checkbox"/> |
| akal >0.2 cm to 2 cm fine to medium-sized gravel | <input type="text"/> | <input type="text"/> | |
| sand and mud | <input type="text"/> | <input type="text"/> | |
| silt, loam, clay (inorganic) | <input type="text"/> | <input type="text"/> | |
| phytal floating stands or mats of macrophytes, lawns of bacteria or fungi, and tufts, often with aggregations of detritus, moss or algal mats | <input type="text"/> | <input type="text"/> | |
| algae filamentous algae, algal tufts | <input type="text"/> | <input type="text"/> | |
| submerged macrophytes macrophytes, including moss and Characeae | <input type="text"/> | <input type="text"/> | |
| emergent macrophytes e.g. Typha, Carex, Phragmites | <input type="text"/> | <input type="text"/> | |
| living parts of terrestrial plants fine roots, floating riparian vegetation | <input type="text"/> | <input type="text"/> | |
| xylal (wood) tree trunks, dead wood, branches, roots | <input type="text"/> | <input type="text"/> | |
| CPOM deposits of coarse particulate organic matter, e.g. fallen leaves | <input type="text"/> | <input type="text"/> | |
| FPOM deposits of fine particulate organic matter | <input type="text"/> | <input type="text"/> | |
| organic mud mud and sludge (organic) = peil | <input type="text"/> | <input type="text"/> | |
| debris organic and inorganic matter deposited within the splash zone area by wave motion and changing water levels, e.g. mussel shells, snail shells | <input type="text"/> | <input type="text"/> | |
| sewage bacteria, -fungi and sapropel sewage bacteria and -fungi, (Sphaerotilus, Leptomitus), sulphur bacteria (e.g. Beggiatoa, Thiothrix), sludge | <input type="text"/> | <input type="text"/> | |
| → sum = 100% | | sum = 20 | |

Annex 5 Sampling protocol for surface water

stream sample stagnant water (lake) sample other.....

| | | | |
|---|--------------------|--------------------------|-------------------------|
| Sample No: | | | |
| Reason: | | | |
| Name of stream/lake: | | | |
| Sampling site: | | | |
| Latitude: | | | |
| Longitude: | | | |
| Date of sampling: | | | |
| Time of sampling: | | | |
| Visual status of water body: | | | |
| Visual status of sample: | | | |
| Flow estimation <input type="radio"/> small <input type="radio"/> smiddle <input type="radio"/> high <input type="radio"/> other..... | | | |
| Estimation of stream width m | | | |
| Used sampling equipment : <input type="radio"/> SOP..... | | | |
| <input type="radio"/> sampling rod <input type="radio"/> Friedienger | | | |
| <input type="radio"/> sampling container <input type="radio"/> other..... | | | |
| Sample preservation: <input type="radio"/> according to SOP <input type="radio"/> other..... | | | |
| Sample filtration: <input type="radio"/> yes <input type="radio"/> no | | | |
| Sample transportation: <input type="radio"/> according to SOP <input type="radio"/> other..... | | | |
| Range of required determinands:: <input type="radio"/> sampling programme <input type="radio"/> other..... | | | |
| Notes: | | | |
| Field measurements | | | |
| Water temperature | °C | Alkalinity | mmol/l |
| Air temperature | °C | Eh | V |
| pH | | Transparency | cm |
| Dissolved oxygen | mg/l | Radon – time of sampling | hour min. |
| Conductivity | mS/m | CO ₂ | mg/l |
| Acidity | mmol/l | | |
| Intensity of colour | | 1 - no | 2- light |
| | | 3 - light | 4 - dark |
| Colour | 1 - no | 2 – yellow-brown | 3 - green |
| | 4 - yellow | 5 – light brown | 6 – yellow-green |
| | 7 - green | 8 - brown | 9 - other |
| Odour | 1 - no | 2 – no marked | 3 - marked |
| Odour type | 1 – phenols like | 2 – chlorine like | 3 – mould like |
| | 4 – mould like | 5 – soil like | 6 – pharmaceutical like |
| | 7 – chemicals like | 8 – fish like | 9 – no identify |

Sampling is provided according to the SOP

Sampling provided by:

Name:

Signature

Stamp

Agreed by:

Name:

Signature

Annex 6 Hydromorphology assessment scheme example

| | RIVER CHANNEL (including flow conditions) | BANKS / RIPARIAN ZONE (integration of left and right banks) | FLOODPLAIN (integration of left and right floodplain) |
|---------------------------------|---|---|---|
| Class I (1,0 . 1,4) | nearly natural | nearly natural | with very high ecological value (EPF = 320 - 640) |
| Class II (1,5 . 2,4) | slightly modified | reinforcements in small sections | with high ecological value (EPF = 150 - 319) |
| Class III (2,5 . 3,4) | moderately modified | reinforcements in large sections | with moderate ecological value (EPF = 70 - 149) |
| Class IV (3,5 . 4,4) | severely modified | continuous bank reinforcements | with low ecological value (EPF = 0 - 69) |
| Class V (4,5 . 5,0) | totally modified | totally modified banks | totally modified |

Evaluation of the Ecological Potential¹

$$FPT \cdot FPW \cdot (AFC \cdot PAT) = EPF$$

FPT: Floodplain type
FPW: Floodplain width
AFC: Assessment factor (CORINE . Data)
PAT: Percentage of area type (%AFC)
EPF: Ecological potential of floodplain

ASSESSMENT RANGE:
 low EPF EPF = 0 - 69
 moderate EPF EPF = 70 - 149
 high EPF EPF = 150 - 319
 very high EPF EPF = 320 - 640

| | | |
|---------------------------------|-----------------------------------|---|
| Floodplain type (FPT) | recent floodplain | 4 |
| | polder/diversion stretch | 2 |
| | former floodplain | 1 |
| Floodplain width in km (FPW) | 0 . 1 | 1 |
| | 1 -2.5 | 2 |
| | 2.5 . 5.0 | 3 |
| | > 5.0 | 4 |
| Landcover (CORONA . Data) (AFC) | forests (PAT %) | 4 |
| | swamp/waters (PAT %) | 4 |
| | meadows (PAT %) | 3 |
| | heterogeneous agriculture (PAT %) | 2 |
| | farmland (PAT %) | 1 |
| | settlements (PAT %) | 0 |

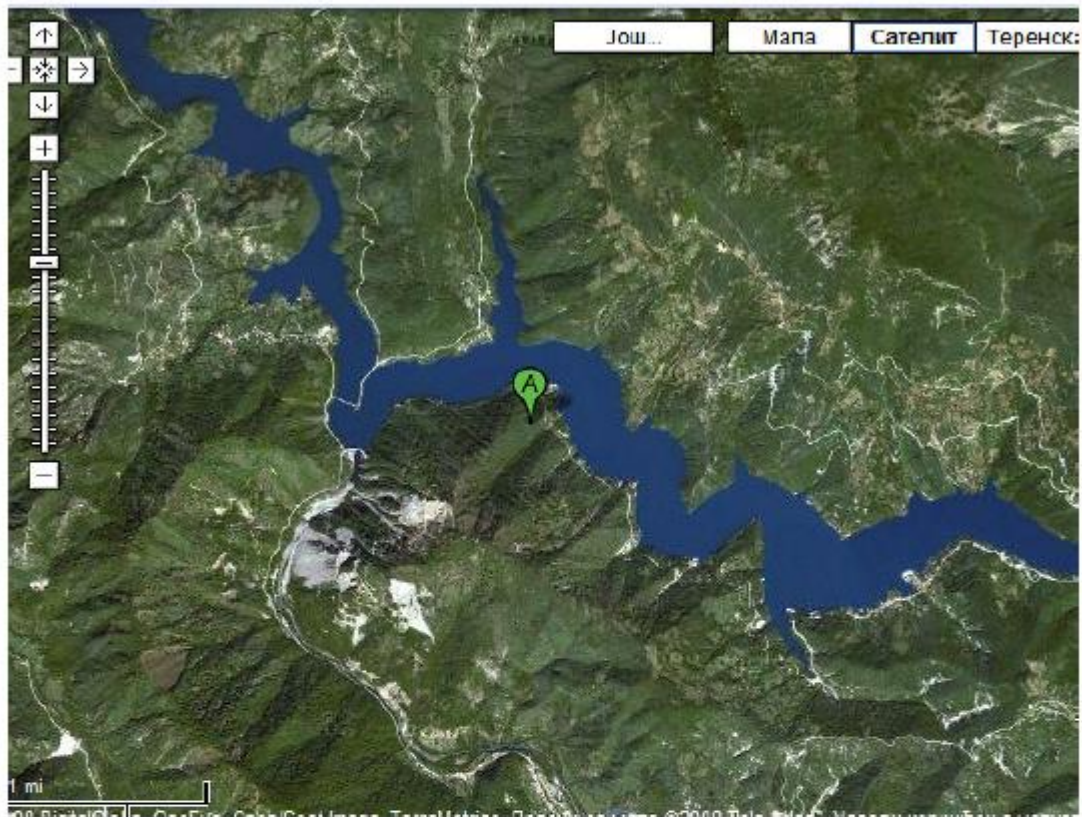
¹ Danube Pollution Reduction Programme "Evaluation of wetlands and floodplain areas in the Danube river basin", Final report, May 1999

Annex 7 Site Protocol

| | | | |
|-----------------------------|--------------------------|--|-------------|
| Location | Date | Time | Prepared by |
| I - SITE DESCRIPTION | | | |
| 1 Sketch | | | |
| | | | |
| 1.2. River/Stream name | 1.3. Watershed/sea | 1.4. Catchment area (km ²) | |
| 1.5. River kilometer (r km) | 1.6. Stream type | 1.7. Stream order | |
| 1.8. Latitude (°, ', ") | 1.9. Longitude (°, ', ") | 1.10. Altitude (m asl) | |
| 1.11. Source of the data | | | |
| 1.12. Comment | | | |

II - SITE DESCRIPTION

2.1. Sketch stream section and sampling points









| | | |
|--|--------------------------|---|
| 2.2. River/Stream name | 2.3. Watershead/sea | 2.4. Catchment area (km ²) A = |
| 2.5. River kilometer (r km) L = | 2.6. Stream type | 2.7. Stream order |
| 2.8. Latitude (°, ', ") | 2.9. Longitude (°, ', ") | 2.10. Altitude (m asl) |
| 2.11. Eco-region and Eco-region number | | 2.12. Eco-subregion (if applicable) |
| 2.13. Comment | | |

| III - SAMPLE RELATED INFORMATION TO BE RECORDED AT EACH SAMTLING DATE | | |
|---|--|---|
| 3.1. Sketch cross section and longitudinal profile | | |
| | | |
| 3.2. Average stream width (m) $B_{avr} =$ | 3.3. Average stream depth (m) $h_{max} =$ | 3.4. Average stream velocity (m/s) $V_{max} =$ |
| 3.5. Maximum stream width (m) $B_{max} =$ | 3.6. Maximum stream depth (m) $h_{max} =$ | 3.7. Maximim stream velocity (m/s) $V_{max} =$ |
| 3.8. Water level (m) | | 3.9. Discharge (m ³ /s) |
| 3.9. Comment | | |

| III - SAMPLE RELATED INFORMATION (continuation) | | |
|--|--|---|
| 4.1. Stream Density [km/km^2] at least 20 km^2 within catchment area or outside if geology is comparable. | | |
| 4.2. Catchment geology at (10% steps) | | |
| <input type="checkbox"/> acid silicate rocks | <input type="checkbox"/> flysch& molasse | <input type="checkbox"/> marine deposits |
| <input type="checkbox"/> mafic silicate rocks | <input type="checkbox"/> lacustrine deposits | <input type="checkbox"/> organic formations |
| <input type="checkbox"/> carbonate rocks | moraines | <input type="checkbox"/> loess |
| <input type="checkbox"/> alluvial | <input type="checkbox"/> sander | 100% |
| 4.2. land use in catchment area (at 10% steps) | | |
| <input type="checkbox"/> deciduous native fore | <input type="checkbox"/> naturally unvegetated | <input type="checkbox"/> pasture |
| <input type="checkbox"/> coniferous native fore | <input type="checkbox"/> alpine heath | <input type="checkbox"/> clear-cutting |
| <input type="checkbox"/> mixed native forest | <input type="checkbox"/> standing waters | <input type="checkbox"/> urban sites (resid.) |
| <input type="checkbox"/> wetland (mire) | <input type="checkbox"/> non-native forest | <input type="checkbox"/> urban sites (industrial) |
| <input type="checkbox"/> open grass-/bushland | <input type="checkbox"/> macchie | <input type="checkbox"/> others: |
| <input type="checkbox"/> reeds | <input type="checkbox"/> crop land | 100% |
| 4.3. presense of lakes in the stream continuum upstream of | 4.4. width of the floodplain [m] | 4.5. means slope of the valley floor [%] |
| 4.6 mean slope of the thalweg [%] | 4.7 valley form | |
| | <input type="checkbox"/> canyon | <input type="checkbox"/> meander valley |
| | <input type="checkbox"/> V-shaped valley | <input type="checkbox"/> U-shaped valley |
| | <input type="checkbox"/> trough | <input type="checkbox"/> plain floodplain |
| 4.8 land use in the floodplain 1 km (at 10% steps) | | |
| <input type="checkbox"/> deciduous native fore | <input type="checkbox"/> naturally unvegetated | <input type="checkbox"/> pasture |
| <input type="checkbox"/> coniferous native fore | <input type="checkbox"/> alpine heath | <input type="checkbox"/> clear-cutting |
| <input type="checkbox"/> mixed native forest | <input type="checkbox"/> standing waters | <input type="checkbox"/> urban sites (resid.) |
| <input type="checkbox"/> wetland (mire) | <input type="checkbox"/> non-native forest | <input type="checkbox"/> urban sites (industrial) |
| <input type="checkbox"/> open grass-/bushland | <input type="checkbox"/> macchie | <input type="checkbox"/> others: |
| <input type="checkbox"/> reeds | <input type="checkbox"/> crop land | 100% |
| 4.9. Comment | | |

| III - SAMPLE RELATED INFORMATION (continuation) | | | |
|---|---|---|--|
| 5. Human impacts on stream morphology upstream and downstream (see comments) | | | |
| upstream | | | |
| 5.1 Number of other transverse structures | 5.2 torrent modification <input type="checkbox"/> yes <input type="checkbox"/> no | 5.3 channeling for navigation <input type="checkbox"/> yes <input type="checkbox"/> no | 5.4 straightening <input type="checkbox"/> yes <input type="checkbox"/> no |
| 5.5 removal of CWD <input type="checkbox"/> yes <input type="checkbox"/> no | 5.6 cutoff meanders <input type="checkbox"/> yes <input type="checkbox"/> no | 5.7 scouring [m below surface] <input type="checkbox"/> yes <input type="checkbox"/> no | 5.8 culverting <input type="checkbox"/> yes <input type="checkbox"/> no |
| downstream | | | |
| 5.9 Number of other transverse structures | 5.10 torrent modification <input type="checkbox"/> yes <input type="checkbox"/> no | 5.11 channeling for navigation <input type="checkbox"/> yes <input type="checkbox"/> no | 5.12 straightening <input type="checkbox"/> yes <input type="checkbox"/> no |
| 5.13 removal of CWD <input type="checkbox"/> yes <input type="checkbox"/> no | 5.14 cutoff meanders <input type="checkbox"/> yes <input type="checkbox"/> no | 5.15 scouring [m b.s] <input type="checkbox"/> yes <input type="checkbox"/> no | 5.16 culverting <input type="checkbox"/> yes <input type="checkbox"/> no |
| upstream | | | |
| 5.17 Number of dams retaining sediment | | 5.18 cumulative height of dams ret. sediment [m] | |
| downstream | | | |
| 5.19 Number of dams obstructing migration | | 5.29 cumulative height of dams obstructing migration [m] | |
| 6. Human impacts on hydrology upstream and downstream | | | |
| upstream: | | | |
| 7.1 length of stretches with residual flow [m] | 7.2 purpose of water abstraction <input type="checkbox"/> irrigation <input type="checkbox"/> hydropower <input type="checkbox"/> others | | 7.3 pulse releases <input type="checkbox"/> yes <input type="checkbox"/> no |
| downstream: | | | |
| 7.4 length of stretches with residual flow [m] | 7.5 purpose of water abstraction <input type="checkbox"/> irrigation <input type="checkbox"/> hydropower <input type="checkbox"/> others | | |
| 7. Human impacts on floodplain upstream and downstream | | | |
| upstream: | | | |
| 7.1 impoundments (% of length) <input type="checkbox"/> 0% <input type="checkbox"/> 10% <input type="checkbox"/> 20% <input type="checkbox"/> 30% <input type="checkbox"/> 40% <input type="checkbox"/> 50% <input type="checkbox"/> 60% <input type="checkbox"/> 70% <input type="checkbox"/> 80% <input type="checkbox"/> 90% <input type="checkbox"/> 100% | | | |
| downstream: | | | |
| 7.2 lack of natural woody vegetation <input type="checkbox"/> yes <input type="checkbox"/> no | | 7.3 non-native woody vegetation <input type="checkbox"/> yes <input type="checkbox"/> no | |
| downstream: | | | |
| 7.4 impoundments or dams (% of length) <input type="checkbox"/> 0% <input type="checkbox"/> 10% <input type="checkbox"/> 20% <input type="checkbox"/> 30% <input type="checkbox"/> 40% <input type="checkbox"/> 50% <input type="checkbox"/> 60% <input type="checkbox"/> 70% <input type="checkbox"/> 80% <input type="checkbox"/> 90% <input type="checkbox"/> 100% | | | |
| 8. Pollution upstream of sampling site | | | |
| 8.1 source pollution <input type="checkbox"/> yes <input type="checkbox"/> no | | 8.2 non-source pollution <input type="checkbox"/> yes <input type="checkbox"/> no | |
| 8.3 sewage overflows <input type="checkbox"/> yes <input type="checkbox"/> no | | 8.4 eutrophication <input type="checkbox"/> yes <input type="checkbox"/> no | |
| 8.5 acidification <input type="checkbox"/> yes <input type="checkbox"/> no | | 8.6 liming <input type="checkbox"/> yes <input type="checkbox"/> no | |
| 8.7 mining <input type="checkbox"/> yes <input type="checkbox"/> no | | 8.8 toxic substances <input type="checkbox"/> yes <input type="checkbox"/> no | |

| IV - SAMPLE RELATED INFORMATION TO BE RECORDED JUST ONCE | |
|--|---|
| 9. Stream morphology and hydrology at sampling site | |
| 9.1 mean depth at bankfull discharge [cm] | |
| 1 _____ 2 _____ 3 _____ 4 _____ 5 _____ | |
| 6 _____ 7 _____ 8 _____ 9 _____ 10 _____ | mean value _____ |
| 9.2 shading at zenith (foliage cover) | |
| <input type="checkbox"/> 0% <input type="checkbox"/> 20% <input type="checkbox"/> 40% <input type="checkbox"/> 60% <input type="checkbox"/> 80% <input type="checkbox"/> 100% | |
| 9.3 average width of natural woody vegetation | |
| | right _____ left _____ shoreline |
| 9.4 channel form | |
| <input type="checkbox"/> meandering |  |
| <input type="checkbox"/> braided |  |
| <input type="checkbox"/> anabranching |  |
| <input type="checkbox"/> sinuate |  |
| <input type="checkbox"/> constrained (natural) |  |
| <input type="checkbox"/> constrained (artificial) |  |
| 9.5 presence of standing water bodies in the floodplain (number at sampling site) | |
| side arms connected to the river/stream temporary side arms recently disconnected from the river/stream permanent side arms recently disconnected from the river/stream | side arms abandoned years ago in the process of silting up standing water bodies located in the floodplain and fed by tributaries other types (specify) _____ no standing water bodies present _____ |
| 9.6 number of debris dams (POM accumulation > 0.3 m) | 9.7 number of logs (>10cm) at sampling site |
| + | + |
| 9.8 left shoreline covered with woody riparian vegetation at sampling site | |
| <input type="checkbox"/> 0% <input type="checkbox"/> 10% <input type="checkbox"/> 20% <input type="checkbox"/> 30% <input type="checkbox"/> 40% <input type="checkbox"/> 50% <input type="checkbox"/> 60% <input type="checkbox"/> 70% <input type="checkbox"/> 80% <input type="checkbox"/> 90% <input type="checkbox"/> 100% | |
| 9.9 right shoreline covered with woody riparian vegetation at sampling site | |
| <input type="checkbox"/> 0% <input type="checkbox"/> 10% <input type="checkbox"/> 20% <input type="checkbox"/> 30% <input type="checkbox"/> 40% <input type="checkbox"/> 50% <input type="checkbox"/> 60% <input type="checkbox"/> 70% <input type="checkbox"/> 80% <input type="checkbox"/> 90% <input type="checkbox"/> 100% | |

| 10. Human impacts on stream morphology at sampling site | | | |
|---|---|--|--|
| 10.1 dams number _____ and cumulative height _____ | | 10.2 other transverse structures number _____ and cumulative height _____ | |
| 10.3 bank fixation (at 10% intervals) left shoreline _____ concrete without seams _____ concrete with seams _____ stones _____ wood _____ trees _____ stone plastering with interstices _____ stone plastering without interstices _____ other materials _____ no bank fixation | | 10.4 bank fixation (at 10% intervals) right shoreline _____ concrete without seams _____ concrete with seams _____ stones _____ wood _____ trees _____ stone plastering with interstices _____ stone plastering without interstices _____ other materials _____ no bank fixation | |
| 10.5 bed fixation (at 10% intervals) _____ concrete without seams _____ concrete with seams _____ stones _____ wood | | _____ trees _____ stone plastering with interstices _____ stone plastering without interstices _____ other materials _____ no bank fixation | |
| 10.6 stagnation <input type="checkbox"/> yes <input type="checkbox"/> no | 10.7 torrent modification <input type="checkbox"/> yes <input type="checkbox"/> no | 10.8 channel. for navig. <input type="checkbox"/> yes <input type="checkbox"/> no | 10.9 straightening <input type="checkbox"/> yes <input type="checkbox"/> no |
| 10.10 removal of CWD <input type="checkbox"/> yes <input type="checkbox"/> no | 10.11 cut-off meanders <input type="checkbox"/> yes <input type="checkbox"/> no | 10.12 scouring [m b.s.] <input type="checkbox"/> yes <input type="checkbox"/> no | 10.13 culverting <input type="checkbox"/> yes <input type="checkbox"/> no |

| 11. Human impacts on hydrology at sampling site | | | |
|--|--|---|---|
| 11.1 pulse releases <input type="checkbox"/> yes <input type="checkbox"/> no | 11.2 % residual flow in comparison to unimpaired sites (worst condition in course of the year) | 11.3 purpose of water extraction irrigation <input type="checkbox"/> <input type="checkbox"/> hydropower others _____ | |
| 12. Human impacts on floodplain at sampling site | | | |
| 12.1 impoundments at sampling site <input type="checkbox"/> yes <input type="checkbox"/> no | 12.2 removal/lack of floodplain vegetation <input type="checkbox"/> yes <input type="checkbox"/> no | 12.3 non-native woody riparian vegetation <input type="checkbox"/> yes <input type="checkbox"/> no | |
| 13. Pollution at sampling site | | | |
| 13.1 source pollution <input type="checkbox"/> yes <input type="checkbox"/> no | 13.2 non-source pollution <input type="checkbox"/> yes <input type="checkbox"/> no | 13.3 sewage overflows <input type="checkbox"/> yes <input type="checkbox"/> no | 13.4 eutrophication <input type="checkbox"/> yes <input type="checkbox"/> no |
| 13.5 acidification <input type="checkbox"/> yes <input type="checkbox"/> no | 13.6 liming <input type="checkbox"/> yes <input type="checkbox"/> no | 13.7 mining <input type="checkbox"/> yes <input type="checkbox"/> no | 13.8 toxic substances <input type="checkbox"/> yes <input type="checkbox"/> no |