



Project no. **INCO-CT-2005-003659**

Project acronym: **ASSESS-HKH**

Project title: **Development of an Assessment System to Evaluate the Ecological Status of Rivers in the Hindu Kush-Himalayan Region**

Instrument: **Specific targeted research or innovation project**

Thematic Priority: **Specific measures in support of international co-operation;  
A.2.1 Managing humid and semi-humid ecosystems**

**Deliverable No. 8 – Part 2**  
**Manual for sorting of benthic invertebrates from rivers in the HKH region –  
standard operation procedure within ASSESS-HKH**

Due date of deliverable: **Month 23**

Actual submission date: **Month 23**

Start date of project: **April 15<sup>th</sup> 2005**

Duration: **36 months**

Organisation name of lead contractor for this deliverable:  
**Kathmandu University, Dhulikhel, Nepal KU**

Revision [Final]

<b>Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)</b>		
<b>Dissemination Level</b>		
<b>PU</b>	Public	✓
<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
<b>RE</b>	Restricted to a group specified by the consortium (including the Commission Services)	
<b>CO</b>	Confidential, only for members of the consortium (including the Commission Services)	

Authors:

Anne Hartmann (BOKU – University of Natural Resources and Applied Life Sciences Vienna)

Patrick Leitner (BOKU – University of Natural Resources and Applied Life Sciences Vienna)

Otto Moog (BOKU – University of Natural Resources and Applied Life Sciences Vienna, project co-ordinator)

Subodh Sharma (Kathmandu University, Nepal, Leader WP 4)

Ilse Stubauer (BOKU – University of Natural Resources and Applied Life Sciences Vienna)

E-mail for correspondence: [anne.hartmann@boku.ac.at](mailto:anne.hartmann@boku.ac.at)

In co-operation with:

Alternate Hydro Energy Centre (AHEC), Roorkee, India

Bangladesh University of Engineering & Technology (BUET), Dhaka, Bangladesh

National Environment Commission Secretariat (NECS), Thimphu, Bhutan

Pakistan Council of Research in Water Resources (PCRWR), Islamabad, Pakistan

University of Duisburg-Essen (UDE), Germany

*This research work is funded by the European Commission under the 6<sup>th</sup> Framework Programme contributing to priority "Specific measures in support of international co-operation (INCO); A.2.1. Managing humid and semi-humid ecosystems".*

*Contract number: INCO-CT-2005-003659*

*Co-ordinator: Prof. Dr. Otto Moog, BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria; E-mail: [otto.moog@boku.ac.at](mailto:otto.moog@boku.ac.at).*



## Contents

<b>1. Foreword</b> .....	<b>4</b>
<b>2. Normative References</b> .....	<b>4</b>
<b>3. Objectives of the Deliverable</b> .....	<b>4</b>
<b>4. Precaution</b> .....	<b>5</b>
<b>5. Sieving, sorting, identification, preservation and labeling</b> .....	<b>5</b>
5.1. Sieving.....	5
5.2. Sorting .....	7
5.3. Identification .....	7
5.4. Preservation.....	8
5.5. Storage of the sorted samples .....	8
5.6. Labelling .....	9
<b>6. Quality assurance and quality control in the lab</b> .....	<b>9</b>
<b>7. Further Remarks</b> .....	<b>10</b>
<b>8. References</b> .....	<b>11</b>

## **1. Foreword**

This working document has been prepared and tested by the ASSESS-HKH consortium. Due to the agreement made at the 2<sup>nd</sup> ASSESS-HKH project meeting in Rajendrapur, Bangladesh (13<sup>th</sup> to 17<sup>th</sup> December 2005) basically the whole sample has to be sorted and *no subsampling* should be applied. If few species occur in masses or samples consist of a huge amount of sandy or muddy sediments the RIVPACS technique can be used to save time. For details on subsampling consult the manual on RIVPACS sorting and recording (Furse & Gunn 2002).

## **2. Normative References**

This method incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed in the references

## **3. Objectives of the Deliverable**

The main objective of this deliverable is to provide complete and standard procedure on sorting of macroinvertebrate in the rivers of the HKH region for use in river quality assessment.

The general objectives of this include:

- Describe the sorting procedures in use in European partner countries,
- Incorporate experiences gathered by both European and Asian partners into the procedure,
- Suggest procedures for HKH river monitoring which are achievable and sustainable.

This deliverable describes the procedure used to remove (pick) the benthic invertebrates from a benthic sample; to sort the animals according to taxonomic groups, to enumerate and to transfer them to dishes and/or vials. When the invertebrates removed with this method are subsequently identified, a description of the benthic invertebrate community present at the site

sampled is generated and can be used for further evaluation of the ecological status assessment of running waters. The data is an integral part of biological assessments of rivers and streams in the HKH-region.

#### 4. Precaution

Macroinvertebrate samples preserved with Formalin are processed best in the laboratory under controlled conditions. Aspects of laboratory processing include sieving, sub sampling (if required), sorting and identification of organisms. All steps of sieving and sorting must be done in a fume cupboard or under a fume extractor (if procurable). If no fume extractor is available the decantation of the preservative should be done outdoor or in nature. At least a good aerated room should be available for sorting.



Figure 1: Fume extractor, for washing and sieving the samples

#### 5. Sieving, sorting, identification, preservation and labeling

##### 5.1. Sieving

Samples preserved with Formalin must at least be stored for two weeks before being treated further. The preservative liquid must then be decanted from the samples thoroughly with tap water before the sample is treated.

Concerning the disposal of formalin (formaldehyde) and ethanol the rules and instructions of the individual countries are to be applied.

Before sorting, the complete sample must be passed through a set of sieves in order to gently rinse the fine material from the sample under running water. By sieving the sample is split up into different portions: from coarse to fine fractions. For samples from soft-bottom streams (sand) use sieves from 1000  $\mu\text{m}$  to 500  $\mu\text{m}$  mesh size. For samples from gravel and hard-bottom streams use from 2000  $\mu\text{m}$  to 500  $\mu\text{m}$  mesh size. In addition, a coarse sieve may be used to retain stones and CPOM. If no sieves are available handnets of adequate mesh sizes can be used.



Figure 2: Set of sieves to be used for separating the coarse and the fine fraction



Figure 3: Rinsing the separated coarse and fine fractions through the sieves

If the sample was stored in more than one container, the contents of all containers for a given sample should be combined at this time. The sample should be mixed gently by hand while rinsing to achieve a homogeneous distribution of the content.

After rinsing the animals attached to the remaining large organic material (whole leaves, twigs etc.) should be sorted. The remaining large organic material can be discarded. The sorted (filamentous) algae and macrophytes should be kept as a part of the sorted rest of the sample (e.g. for a subsequent determination of algae's biomass).

The material retained is spread thinly over the bottom of a large pan (white plastic tray; (e. g., 30 x 50 cm) and covered with a thin layer of water for picking out the animals.

## 5.2. Sorting

**The sample must be sorted completely in the lab (all specimens should be removed).** The animals sorted in the lab should be separated into systematic units. The systematic units correspond to highest potential taxonomic level that can be identified by the sorting personnel. For this purpose each sorting lab shall use a sorting protocol containing the following information:

- Information on the sampling site (see chapter labelling)
- List of sorted systematic units plus number of specimens per unit.

Use one Petri dish per each systematic unit that can be addressed as a discrete taxonomic category. This process is termed as pre-determination. The taxonomic resolution of pre-determination can be enhanced in a following procedure after having finished the sorting. After pre-determination the content of each Petri dish has to be stored in a waterproof plastic vial inclusive preservation and labelling.

**While separating the animals into systematic units, the number of sorted specimens must be counted and recorded** (enumerated = number of specimen per systematic unit).



Figure 4: Animals have to be separated into systematic units while sorting

## 5.3. Identification

All aquatic macroinvertebrates in the sample, including caddis and Dipteran pupae, have to be identified to highest potential taxonomic level. Terrestrial taxa are not part of the samples. Adults (aerial stages of aquatic animals),

empty mollusc shells, empty puparia, empty caddis cases are kept separately but are not part of the sample. Fragments of damaged specimens can cause errors particularly in the calculation of abundances. In case of fragments use only head and thorax, single heads, not single abdomens, legs or other smaller parts.

All samples should be dated and recorded in a sample data form upon reception by laboratory personnel. All information from the sample container label should be included on the sample log sheet. If more than one container was used, the number of containers should be indicated as well. All samples should be sorted in a single laboratory to enhance quality control.

#### 5.4. Preservation

After the minimum time of storage in Formalin (two weeks), samples should be transferred from fixative (e.g. formalin) to preservative (ethanol) if they are kept for more than a few weeks before sorting. Rare or fragile organisms that have been sorted in the field and stored separately have to be preserved in 70% ethanol after replacing the alcohol a number of times to ensure that there is an adequate concentration in the sample.

Similarly, the animals sorted in the lab are stored in 70% ethanol. Ideal is storing the organisms in waterproof plastic vials.

Preserved samples must be stored at cool temperatures, away from any heat source and preferably in the dark to minimise the loss of colour.

If specimens are sent to outside taxonomists, posting has to be done properly to prevent any damage and all sample information has to be recorded in a log book for dispatched samples.

#### 5.5. Storage of the sorted samples

Make sure that enough containers are available to keep separated the remaining from sorted samples. In some cases it might be necessary to determine some features later on that haven't been regarded so far (e.g. if one wants to correlate the fauna with the amount of filamentous algae or FPOM or CPOM).

If you may run short on containers there is also the possibility to store the remainings in plastic bags (that are used to deep freeze food).

## 5.6. Labelling

After sorting, vials containing identified and counted specimens should be labelled in pencil or laser printer on a slip of waterproof paper placed inside the vial. The following information should be included:

Label for vials, example Bhutan:

Project: ASSESS-HKH	Sampling site: Gedu Chhu
sampling code: H02CG013	Sampling date: 03. 12. 05
investigator code: G.K.Chh*	Sorting date: 15. 01. 06
sorter code: PaDe*	Taxonomic group: Baetidae

## 6. Quality assurance and quality control in the lab

(partly based on: ENVIRONMENT AGENCY 1999a)

The aims of quality assurance and control in the lab are to minimise errors in the treatment of biological samples and thus secure the validity of the biological assessment results. One must distinguish between the general improvement of the treatment of the samples in the laboratory (as a part of “quality assurance”) and the quality control by an auditor. This chapter does not cover any aspects of auditing.

### Important elements of quality assurance in the lab are:

Treatment of the samples during the process of sieving and sorting (compare chapter 5)

In order to minimise damage to specimens in the process of sieving, e.g. loss of gills, legs and tails:

- rinse very gently and never use a high-pressure spray when you separate specimens from substratum e.g. by means of a hose attached to the tap
- never swirl the sample violently in a bucket or sieve

---

\* G. Karma Chhopel

\* Pasang Dema

- decant water very carefully
- when the sorting is adjourned, the tray with the sample must be stored at a cool place (ideally in a fridge) to avoid evaporation of the water.

When picking out the specimens from the sieved samples a soft pair of forceps should be used in order to minimise damage to the animals. Use suitable and regularly serviced dissection microscopes (binoculars with a magnification between 5x and 50x) and microscopes for identification (minimum magnification 100x). The working environment should be well illuminated. It is recommended to cover the specimens with enough liquid to avoid reflections of light. Sometimes it may be necessary to break mollusc shells and poke caddis cases to check for occupants.



Figure 5: Well illuminated work environment

## 7. Further Remarks

### Fitness of the lab personal staff:

Sorting usually is a hard and very time-consuming work. Samples, which are easy to sort may be finished in few hours, laborious samples may consume more time. The work area should always be well lit and health implications should be considered during the whole process of sample treatment in the lab. A good physical condition during the process of treating the samples contributes to a good result in the process of biological evaluation. Short, regular breaks from the sorting every hour are highly recommended. If the tray is left for longer breaks, the sample should be covered completely in order to reduce evaporation.

## 8. References

- Barbour, M.T., J. Gerritsen, B.D. Snyder & J.B. Stribling 1999. Rapid bioassessment protocols for use in wadable streams and rivers: periphyton, benthic macroinvertebrates and fish. 2nd Edition. EPA 841-B-99-002. USEPA, Office of Water, Washington, D.C.
- Caton, L.W. 1991. Improved sub-sampling methods for the EPA "Rapid Bioassessment" benthic protocols. Bulletin of the North American Benthological Society 8(3), 317-319.
- Environment Agency 1999a. Procedures for collecting and analysing macroinvertebrate samples. Environment Agency Document no BT001, 176 pp.
- Environment Agency 1999b. Procedure for quality assurance for RIVPACS compatible macro-invertebrate samples analysed to the taxonomic level needed for the BMWP-Score. Environment Agency Document No. BT 003, 72 pp.
- Illies, J. 1978. Limnofauna Europaea. Gustav Fischer Verlag, Stuttgart.
- ISO 7828. Water quality – methods of biological sampling – guidance on handnet sampling of aquatic benthic macro-invertebrates. Ref. No. ISO 7828-1985 (E).
- Karr, J. R. & E. W. Chu 1999. Restoring life in running waters: better biological monitoring. Island Press, Washington DC.
- Moog, O., A. Chovanec, J. Hinteregger & A. Römer 1999. Richtlinie zur Bestimmung der saprobiologischen Gewässergüte von Fließgewässern. Bundesministerium für Land- und Forstwirtschaft, Wien.
- Rosenberg, D.M. & V.H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: Rosenberg, D.M. & V.H. Resh. Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.