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Water Resources and Environmental Data (ENI/2021/425-550)

TRAINING REPORT

MZB & PHB TRAINING ARMENIA

Date:	Training Lead:	Output n°:
Nov 6-9, 2023	Wolfram Stockinger Daša Hlúbiková **Main partner:** NEA HMC	**1.4**

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1. Scope of the training

The target of this training was to teach the Armenian experts on all essential aspects of laboratory processing and analysis of benthic macro-invertebrates and diatoms. The main objective of the training was that participants understand the essential and correct analytical routine of the two biological elements focusing on both methodological aspects and correct identification and get familiar with their morphology, terminology, and identification literature. In addition, the macroinvertebrate training aimed to profound the current knowledge of the Armenian experts and verify their level of expertise by proficiency testing exercise. Besides identification and routine methodology, the training also aimed at exchange of expertise in ecological monitoring for the Biological Quality Elements benthic invertebrates and phytobenthos.

In particular, the training focused on the following subjects:

Benthic diatoms:

- correct laboratory diatom routine techniques of e.g. laboratory treatment of diatom samples, slides' preparation and microscopic analysis assuring compliance with WFD standards through data analysis;
- diatom morphology and taxonomy focusing on the key features important for taxa identification – presentation of genus-specific and species-specific features; differential criteria on genus and species level; importance of micrometry, DIC contrast and good microscopic techniques to visualize the key features);
- use of correct diatom identification guides, literature and online sources; guide to correct routine using diatom literature making sure that participants use the correct identification guides and reliable online sources to decrease the risk of taxa misidentification;
- develop awareness of individuals of all the possible sources of uncertainty in routine diatom analysis to minimise risks of incorrect taxa identification and consequent assessment and to develop correct analytical routine.

Benthic macro-invertebrates:

- repetition of methods of macroinvertebrates' samples processing, quantification and analysis including species identification;
- preparation for proficiency testing aiming at verification of the quality of macro-invertebrate taxa determination and the taxonomic skills of Armenian experts;
- improve and profound the identification skills and determination level (correctness, taxonomic level of individuals) of Armenian experts;
- improve the knowledge of participants on ecological preferences of the different taxa and indicator groups;
- training on correct assessment and evaluation of human impacts based on macro-invertebrates.

Institutions involved

DWS Hydro-Ökologie GmbH acted as trainers.

HMC and NAS RA supplied equipment and sent participating experts.

2. Main results / outputs of the training

Armenian experts showed great interest in acquiring knowledge about the two biological elements and the related topics. In total 10 experts from two different institutions (6 HMC and 5 NAS RA) participated to the trainings, plus one additional student from NAS RA joined the diatom training in the afternoons. Two international experts on macro-invertebrates and diatoms (from DWS Hydro-Ökologie) lead the training activities parallelly each focusing on one biological element. HMC supplied equipment and laboratory material for the training.

Armenian experts split into two training groups based on their level of expertise and interest. In general, each group was trained one day on diatoms and one day on macro-invertebrates. However, participants in each group could switch between groups upon request to get more profound training in the desired area. *Such smaller training groups* allowed close interaction-both verbal and collaborative among the participants and the trainers. All Armenian experts had thus opportunity to gain the same knowledge in both fields or develop the knowledge only in the desired area.

Two training groups were created:

Group 1: 4 (5) NAS RA experts + 1 HMC expert (Day 1: Diatom training, Day 2: half a day macroinvertebrate training, half a day of diatom training)

Group 2: 6 HMC experts (Day 1: Macroinvertebrate training, Day 2: diatom training)

2.1. Diatom training

2.1.1. Diatom methodology, laboratory equipment

The training started with overview of the available routine laboratory methodology and microscopic equipment.

- The laboratory has sufficient laboratory equipment at disposal to prepare the diatom samples and diatom slides, although the correct diatom mounting medium is missing.
- The Armenian experts treated benthic diatom samples and prepared diatom slides before the training following instructions of the online diatom training from May 2023 and the diatom video.
- The Armenian experts showed to be able to treat the benthic diatom samples correctly using the hot hydrogen-peroxide method. The samples were sufficiently cleaned, all organic material was removed, slides were correctly mounted, however, the subsamples on the slides were too diluted and they used incorrect mounting medium called Eukitt®. This is a quick-hardening mounting medium, which has refractive index between 1.490-1.5, which is

not sufficient for diatom identification. The refractive index for diatom mounting medium must be at least 1.65 to visualise many weakly silicified or tiny morphological details of diatom valves. The possible mounting media to be used are either Naphrax®, which can be purchased from several suppliers or Pleurax, which can be prepared in the laboratory. Both diatom mountants including contact details on suppliers and preparation procedure of Pleurax were presented during the Diatom training in May 2023.

- Based on these findings we concluded that the experts would benefit from a laboratory manual for diatom samples and slides preparation including preparation of the diatom mounting medium, which could be part of the routine laboratory documentation. We therefore prepared a 'Manual for diatom samples' treatment and slides' preparation'.
- Since the Armenian experts do not have any suitable diatom mountant and couldn't make good quality slides, the trainer took 10 diatom subsamples to mount the diatom slides of sufficient quality and will deliver the slides back to HMC experts for microscopic analysis.
- The microscope available for diatom identification is Optika B-600T equipped with 100x achromatic objective for oil immersion. Despite of it, immersion oil was not available in the laboratory, but the expert team was able to provide it upon request. The level of microscope is basic. The achromatic 100x objective with oil immersion has sufficient magnification for species identification, but the resolution is sufficient only for large or middle-sized taxa with strongly silicified valves. Small taxa, such as *Achnanthidium*, *Sellaphora* etc., were at the resolution limit for correct species identification, because morphological details of species bellow 10 were only hardly visible. The achromatic objective does not provide sufficient contrast to resolve the very fine details of small taxa or less silicified species. We therefore recommend trying to upgrade the 100x achromatic objectives to plan- or plan-apochromatic objectives, if possible. Microscopic micrometer was not available.

2.1.2. Species identification, microscopic equipment

The main part of the training focused on diatom species identification. The course of the training, topics and outcomes are summarised below.

- Participants were trained during practical microscopic session using the available microscope.
- The trainer provided a microscope digital camera (microQ SP-51) attached to the microscope ocular so that all participants could see the microscopic field on a monitor. This allowed them to see all the taxa discussed at the same time and to focus on the correct morphological features.
- The diatoms slides used for microscopy during the training were mainly provided by the trainer and came from different river types covering broad spectrum of human impacts from Austria and Slovenia, mainly coming from mountain streams similar to Armenian river types. Slides prepared by Armenian experts were used as well, but due to the unsuitable diatom mounting medium, not all morphological features of diatoms were visible, therefore we focused mainly on diatom slides provided by the trainer.
- During the training, participants became familiar with the key diatom terminology and key morphological features of diatom valves needed for identification on genus and species level, such as symmetry, dimensions, shape, raphe, apices, striation, valve view, pleural view, stigma, rimoportulae etc. They became familiar with the most common

diatom genera in river benthos under different levels of human impacts and became familiar with ecological preferences of the taxa. Identification of all the taxa always reached species level despite of the beginners' level of the participants. This helped them to understand all steps of correct species identification. Because microscopic micrometer was not available, practical micrometry could be explained only theoretically.

- The participants became familiar with the most important diatom identification literature and were trained how to use it. The HMC experts also obtained a diatom book on identification of the most common diatom species in freshwater river benthos in Europe (*Lange-Bertalot, H., Hofmann, G., Werum, M. and Cantonati, M. (2017). Freshwater Benthic Diatoms of Central Europe: Over 800 Common Species Used in Ecological Assessment. English edition with updated taxonomy and added species. Koeltz Botanical Books, Schmitten-Oberreifenberg, 942 pp.*). Other identification sources were presented as well, such as identification guides from the different French regions, which are freely available and other diatom webs such as https://diatoms.org and http://symbiont.ansp.org/dntf/.
- The knowledge of most participants was on beginners' level, although NAS RA experts had experiences with diatom identification and could thus advance faster.
- Except for the diatom morphology and taxa identification, also diatom ecology, and bioindication was discussed. Participants were interested in the available diatom-based methods for ecological status assessment and the possibility to calculate the different diatom indices using the diatom software Omnidia.

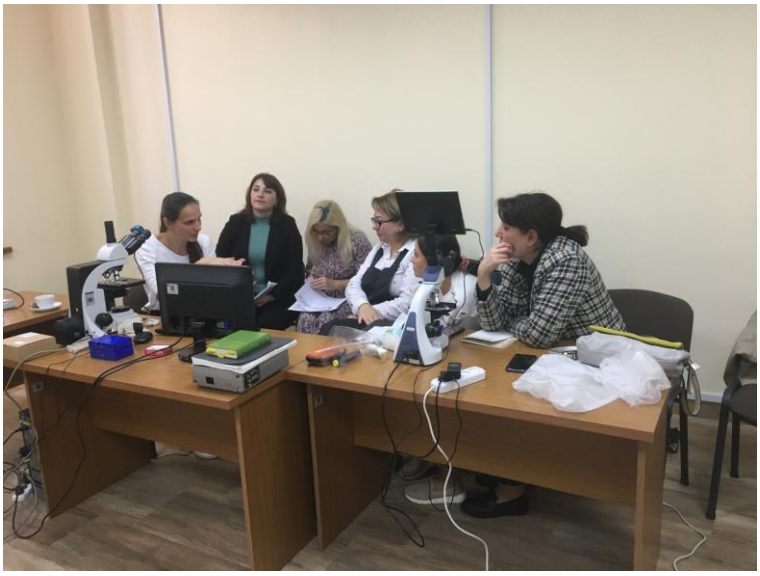

Figure 1: Diatom training of NAS RA experts and one HMC expert, November 7, 2023.

2.2. Benthic invertebrates training

The training programme differed depending on the participants' level.

The first day of training day started with HMC experts who have experiences with taxa identification to groups or genus level and are familiar with laboratory techniques.

Training during the second day had similar structure, but as the NAS RA participants had beginners' level of knowledge, thus the topics were adapted. The NAS RA participants were trained during a half a day during the second day.

Last hours of the second day of training were dedicated to more detailed identification of macro-invertebrates upon request of one HMC expert.

- Training started with planning of the proficiency test. Samples with individual animals from Austria were transferred to HMC experts to be determined with deadline at the end of the year 2023.
- The Armenian colleagues from HMC gave some invertebrates to the trainer requiring verification of their determination.

Main part of the training was dedicated to determination of samples, confirmation of taxa determination on different taxonomic levels and to ecological notes to the taxa and their value for assessment. In particular, the training with HMC experts focused on:

- Discussion about time of larval development (weeks to up to three years, depending on size, environmental influence, and general aspects of groups)
- Comparison between development of algae, invertebrates and fish and hints for impacts during this time.
- Morphology and adoption to environmental necessities (concerning velocity, bottom surface, feeding habits)
- Special Introduction to identification of Plecoptera to families and partly genus with ecological notes.
- Possibilities for identification of Diptera
- Identification of various groups including Ephemeroptera, Trichoptera, Hirudinea and Mollusca.
- Discussion on literature of central Europe and the value for Armenia. Russian literature would be a further help.

In addition to practical microscopic sessions and discussion, a quiz was prepared by a trainer and the participants were asked to take part in a quiz competition. The purpose was to test their knowledge and launch discussion.

- The quiz proved to be very interesting for the participants and helped them to get involved in the discussion and think about many subjects especially related to ecological needs of macroinvertebrates, anthropogenic impacts, and their relevance in assessment.

Figure 2: Benthic invertebrate training of HMC experts, November 7, 2023.

Figure 3: All participants of the training at HMC.

2.3. Recommendations

2.3.1. Diatom training

- The overall impression of the training was very good. The expert teams, both from HMC and NAS RA were highly motivated to learn. They seemed to understand all the methodological issues, the necessity of suitable equipment and the importance of correct identification. We greatly appreciate their effort in sample preparation, which was a brand-new field of work, but they exhibited creativity and sufficient laboratory skills to perform well. They exhibited comprehensiveness of the subject during microscopic sessions and despite of their beginner-level were able to individually identify some of the diatom taxa on genus level and even on species level correctly after one-day training. Our recommendation to participants is to keep in touch with diatom analysis and dedicate as much time as possible to species identification and morphology to keep the obtained knowledge. Their current skills and abilities are still on beginners' level, but they have sufficiently good background to advance and improve their knowledge in the future. This however requires their personal effort and motivation to develop the gained skills and knowledge.
- To reach the level needed for analytical routine, the background knowledge of all participants obtained during this training needs to be further developed and upgraded. This is achievable only if participants keep studying the diatom identification literature to become familiar with the diatom morphology and devote as much time as possible to microscopic analysis of benthic diatoms. Only such continuous and intensive contact with the subject allows them to further advance and gain the experience and sufficiently broad knowledge, which is essential for correct analytical routine.
- We strongly suggest upgrading the microscopic equipment, by purchasing:
  - a microscopic micrometer

  (the current light microscope is not equipped with micrometer, which is essential for diatom micrometry. Species dimensions, fibulae/striae density can be only measured with a micrometer and without it a correct species identification without experiences is impossible)

  - plan-achromatic or plan-apochromatic 100x oil objective

  (the currently available 100x objective is achromatic and analysts would benefit from having a better optics for diatom identification. Some diatoms' specific morphological features are at the limit of visibility with the light microscope and high-quality optics is essentials)

  - differential interference contrast (DIC, or Nomarski contrast)

  (specific contrasts available for light microscopy that help to visualise the features at the limit of light microscopy. The best to be used for diatoms is the Interference differential contrast (DIC), but a phase contrast also helps in identification. An upgrade of the microscope with the DIC or phase contrast equipment (together with the objectives) would be very helpful)

- We recommend the Armenian experts to purchase or prepare suitable diatom mounting medium with sufficient refractive index (higher than 1.65). The currently available Eukitt® medium is not sufficient for diatom analysis. All information on diatom mediums is available in the 'Manual for treatment of benthic diatom samples and preparation of permanent diatom slides' prepared within this project.
- We are convinced that Armenian experts would benefit from a reference sample analysis (diatom slides with a list of diatom taxa present on each slide), which helps beginners to get used to the microscopic features of the diatom taxa and helps to verify and correct their knowledge.

2.3.2. Macro-invertebrates

- The overall impression of the training was very satisfying. The Armenian experts were willing to profound their knowledge.
- The microscope used for invertebrates is sufficient for getting to families or genus. But it is quite useless without a proper light. We strongly recommended them to be more active and creative in adapting the basic equipment to their needs and in this special case try to make an effort to get sufficient light for microscopy. A common table office lamp would be sufficient and at low cost.
- There is already a lot of literature for most groups in the laboratory available, but most of it refers to fauna from Central Europe. In this case it is important that experts know that identification to species level may be incorrect due to specific regional characteristics. We therefore recommend identifying taxa to genus level with the available literature but be very careful with identification to species if no specific literature from their region is available.
- We strongly recommend that HMC experts start developing and building their own animal reference collection, so that beginners can be trained more efficiently.
- In conclusion, the knowledge of the participants is on a good basic level, but should certainly be further improved, which was difficult in local conditions. Participants would benefit from training in well-equipped laboratory with all the available literature.

3. Other important issues for Programme implementation

The team of Armenian experts showed to be very motivated to learn, which is a promising start for a successful advance in this field. The Armenian team showed effort to develop their knowledge, but due to some technical issues (specific equipment missing for microscopy and sample preparation for both macro-invertebrates and diatoms and insufficient literature for macro-invertebrates) slowed down the training process. Moreover, correct species identification requires both sufficient knowledge and experience. We are convinced that the Armenian experts would benefit from additional training in well-equipped laboratory with good microscopic equipment and identification literature. We would also

recommend that experts make some effort to improve their level of English, which is not only necessary for communication with experts in the field but also for a full comprehension of scientific literature.

4. Time Table

Time	*Activity*	*Comments*
	Monday 06/11/2023	
06:25	Start Vienna – Yerevan	Flight number: W42891
13:00	Arrival Yerevan airport	Transfer to Hotel
Afternoon	Ibis Yerevan Center Hotel	
	Tuesday 07/11/2023	
tbd	Travel to Laboratory	
9:00-12:00	Training	Training: Diatoms (NAS RA+1 HMC) / Macroinvertebrates (HMC)*
12:30-13:00	Lunch break	Lunch
13:30-17:00	Training	Training: Diatoms (NAS RA+1 HMC) / Macroinvertebrates (HMC)*
	Wednesday 08/11/2023	
tbd	Travel to Laboratory	Vahagn will pick up experts at Hotel
9:00-12:00	Training	Training: Diatoms (HMC) / Macroinvertebrates (1 HMC+NAS RA) *
12:00-13:30	Lunch break	Lunch will be provided
13:30-17:00	Training	Diatoms (HMC+NAS RA) / Macroinvertebrates (1 expert HMC) *
	Thursday 09/11/2023	
5:20	Start Yerevan - Vienna	Flight via Athens. Flight numbers: A3899 and A3860
10:00	Arrival Vienna airport	

* Two coffee breaks of 30 minutes each day were included.

5. Next steps / to do's

STEP/TASK	RESPONSIBILITY	DEADLINE	FOLLOW UP
Proficiency testing – Analysis of macro-invertebrates delivered to HMC laboratory to be done Armenian experts.	HMC experts	30.12.2023	

6. Annexes

List of Participants:

International Experts

Institution	*Name*	*Function / Position*
DWS Hydro-Ökologie	Wolfram Stockinger	Surface Water Expert - Biology
DWS Hydro-Ökologie	Daša Hlúbiková	Surface Water Expert - Biology
EU4Environment	Vahagn Tonoyan +374-99-55-04-87	Program representative (AM)

HMC (Armhydromet)

Institution	*Name*	*Function / Position*
HMC	Vardan Karyan	SW Expert - Biology
HMC	Hayk Minasyan	SW Expert - Biology
HMC	Rima Avetisyan	SW Expert - Biology
HMC	Zanazan Nersisyan	SW Expert - Biology
HMC	Ani Stepanyan	SW Expert - Biology
HMC	Varduhi Petrosyan	SW Expert - Biology

Institute of Zoology and Hydroecology NAS RA

Institution	*Name*	*Function / Position*
NAS RA	Lusine Hambaryan	SW Expert - Biology
NAS RA	Armine Mamyan	SW Expert - Biology
NAS RA	Anahit Hovsepyan	SW Expert - Biology
NAS RA	Tehmine Khachikyan	SW Expert – Biology
NAS RA	Lilit Stepanyan	student