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EU4Environment in Eastern Partner Countries:

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

# TRAINING REPORT

# MZB & PHB TRAINING GEORGIA

| Date: | Training Lead: | Output n°: |
|---|---|---|
| **Nov 20-23, 2023** | Wolfram Stockinger<br>Daša Hlúbiková<br>**Main partners:**<br>NEA Laboratory<br>Tbilisi, Georgia<br>Azelab, LLC of the Ministry of Ecology and Natural Resources of Azerbaijan | **1.4** |

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*Date*
Dec 14, 2023

Implementing partners

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# 1. Scope of the training

The target of this training was to teach the two teams of experts on all essential aspects of laboratory processing and analysis of benthic macro-invertebrates and diatoms and to profound their current knowledge. 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 at verification of the level of expertise of the two teams by proficiency testing exercise. Besides identification and routine methodology, the training also allowed exchange of expertise in ecological monitoring for the Biological Quality Elements benthic invertebrates and phytobenthos between the teams.

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, importance of DIC contrast and good microscopic equipment;
- use of correct diatom identification guides, literature and online sources;
- 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 the experts;
- improve and profound the identification skills and determination level (correctness, taxonomic level of individuals) of the 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.

NEA Laboratory of Tbilisi (Georgia) supplied equipment and sent participating experts, Azelab, LLC of the Ministry of Ecology and Natural Resources of Azerbaijan sent participating experts.

## 2. Main results / outputs of the training

In total 10 experts from two different countries participated in trainings, 3 from Azerbaijan and 5 from Georgia. NEA supplied equipment and laboratory material for the training.

The experts split into two training groups based on their interest and field of work, one group was trained on diatoms and another on macro-invertebrates. Two international experts on macro-invertebrates and diatoms (from DWS Hydro-Ökologie) lead the training activities parallelly, each focusing on one biological element. Participants in each group could switch between training teams upon request to get more profound training in the desired area.

Within Georgian team, 4 experts participated in diatom training and 1 expert on macroinvertebrate training during both days.

The two Azerbaijani experts participated in diatom training during the first day and on the second day Illaha Gurbanova took part in macro-invertebrate training during half-a day and spend the second half a day again with diatomists' team. The experts had thus opportunity to gain the same knowledge in both fields or profound the knowledge in the desired area.

The Azerbaijani team showed to be very motivated in acquiring the knowledge in all aspects of the training of both biological elements. The Georgian team was highly motivated to learn the laboratory routine of benthic diatoms and interested mainly in technical aspects (laboratory and microscopic equipment).

**Day 1 (Nov 21)**: Diatom training: Elene Kobauri (NEA), Ani Gabrichidze (NEA), Maia Papiashvili (NEA), Nino Korchilava (NEA), Illaha Gurbanova (Azelab), Ramina Abdullayeva (Azelab), Jeyhum Muradov (Azelab)

Macroinvertebrates' training: Irakli Kordzaia (NEA)

**Day 2 (Nov 22)**: Diatom training: Elene Kobauri (NEA), Ani Gabrichidze (NEA), Maia Papiashvili (NEA), Nino Korchilava (NEA), Illaha Gurbanova (Azelab), Ramina Abdullayeva (Azelab), Jeyhum Muradov (Azelab)

Macroinvertebrates' training: Illaha Gurbanova (Azelab), Irakli Kordzaia (NEA)

### 2.1.1. Diatom methodology (samples' treatment and slides' preparation), laboratory equipment, microscopic equipment

The training started with revision of the available equipment and of the procedure of diatom samples' preparation for microscopy. Both Georgian and Azerbaijani participants were asked to prepare their diatom samples for microscopic analysis following instructions of diatom training in April-May 2023 prior to training. Both teams treated the diatom samples (using hot hydrogen peroxide method) and tried to prepare diatom permanent slides.

- Labotatory equipment for samples' preparation in NEA laboratory was sufficient and allowed correct application of the methodology, except, the diatom mounting medium was missing.
- Georgian participants treated their diatom samples using the hot hydrogent method, dried the diatom suspension on slides, but did not mount the diatoms as they the diatom mountant was not at disposal and they didn't try to prepare the mountant on their own. However, the unmounted slides were too dense for observation anyway. So the first part of the training focused on samples' preparation.
- The whole process of samples' and slides' preparation was demonstrated using the diatom samples collected in Georgia. Slides were mounted using Naphrax® and all participants obtained a small sample of the Naphrax® medium for one more preparation, if needed.
- Participants from Azerbaijan prepared and mounted the diatom slides very well despite of no previous experience and were even able to prepare the diatom mountant (Pleurax) correctly. They were advised how to fine-tune the method to obtain the best results.
- 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', which was distributed after the training.
- Due to the lack of time, the slides' preparation procedure had to be accelerated during the training, which usually reduces slides' quality. Therefore, 3 diatom subsamples from Azerbaijan and 4 diatom subsamples from Georgia were taken to the DWS laboratory to be well processed and mounted in sufficient quality and will be delivered back to the national teams for microscopic analysis.
- The microscope for diatom analysis available in NEA laboratory, which was OPTIKA B-600T S/N 215035, did not have any 100x objective, only objectives with lower magnification were available. Despite of the effort, the 100x was not found in the laboratory and the Georgian participants were not sure whether it was available at all. It had to be concluded that the microscope was insufficient for diatom identification. Diatoms can be correctly identified only using a 100x objective with oil immersion and ideally equipped with DIC contrast. Fortunately, we found an operational microscope Kyowa in the microbiological section that was equipped with 100x oil immersion objective and showed to be sufficient. This was further used during the training for taxa identification. Microscopic micrometer was not available.

### 2.1.2. Species identification

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 Kyowa 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.

**Figure 1. Diatom training of NEA and Azelab participants, November 21, 2023.**

- 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, covering a broad spectrum of conditions and impacts. Slides prepared during the training and by Azerbaijani experts were used as well.
- 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. Both teams 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/.

- 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. Therefore the last part of the training focused on demonstration of the diatom software Omnidia.

**Figure 2. Diatom training. NEA's participants, November 22, 2023.**

## 2.2. Benthic invertebrates' training

The training programme differed depending on the participants' level.

The first day of training day started with one Georgian expert only, who had experiences with taxa identification to groups or genus level and was familiar with laboratory techniques.

- 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.
- Taxonomy of samples from the river Debet was done together and then the ecological values and preferences of the taxa were discussed and determined.

Training during the first half of the second day had similar structure, but only one Azerbaijani biologist, Ilaha Gurbanova took part in it. The Georgian biologist Irakli Kordzaia left und returned

in the afternoon. Last hours of the second day of training were dedicated to more detailed identification of macro-invertebrates upon request of the returned Georgian biologist.

Main part of the training was dedicated to determination of invertebrates, 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 the experts focused on:

- 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 selected families and partly genus with ecological notes.
- Discussion about time of larval development (weeks to up to three years, depending on size, environmental influence, and general aspects of groups)
- Identification of various groups including Ephemeroptera, Trichoptera, Hirudinea and Mollusca.
- Discussion on literature of central Europe and the value for the Kaukasus region. Russian literature would be a further help.

In addition to practical microscopic sessions and discussion, a quiz was prepared by a trainer on first day. The purpose was to test their knowledge and launch discussion.

- The quiz proved to be very interesting for the participant 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.

## 2.3. Recommendations

### *2.3.1. Diatom training*

- The overall impression of the training was satisfying. The motivation of the participants to advance differed, most likely based on experience and their field of work. Whilst most of the NEA participants were mainly interested in laboratory techniques and samples' preparation, where they were very eager to learn, the biologists of Azelab were highly motivated to advance in all fields of diatom research and bioindication.

- All participants have acquired the necessary skills to perform the laboratory procedure of samples' preparation, they understood all the methodological issues and the importance of suitable equipment. We greatly appreciate their effort in samples' preparation, which was a brand-new field of work, but they exhibited creativity and sufficient laboratory skills to perform well.

- The participants exhibited comprehensiveness of the subject during microscopic sessions and despite of their beginners' level and the language barrier, they were able to individually identify some of the diatom taxa on genus level and even on species level correctly after the two-day training. 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 immersion objective

  (the current microscope OPTIKA B-600T S/N 215035 does not have any available 100x objective. The microscope used for diatom training was obtained from in the microbiological laboratory.)

  - 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 that the Georgian biologists purchase or prepare a suitable diatom mounting medium with sufficient refractive index (higher than 1.65). 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 both teams 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 satisfying, the Azerbaijani expert was eager to profound her knowledge. The same was with the Georgian expert on the first day. On the second day he joined only for a few hours.
- The microscope (binocular) used for invertebrates is sufficient for getting to families or genus. For further taxonomy the preparation of slides and a microscope with an magnification of up to 100x would be necessary.

- 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 the national 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

Both teams exhibited the ability and potential to develop sufficient skills and knowledge needed for correct routine praxis. However, correct routine involves not only sufficient knowledge, but also sufficient laboratory equipment, experience, and personal motivation. Biologists must understand that upgrading their knowledge in this field is a long-term process and they must be sufficiently motivated to constantly educate. We are convinced that both teams would benefit from additional training. 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 20/11/2023** | |
| 06:50 | Start Vienna – Tbilisi | Flight via Istanbul<br>Flight numbers: TK 1890, TK 382 |
| 16:45 | Arrival Tbilisi airport | Transfer to Hotel |
| Afternoon | Ibis Budget Hotel | |
| | **Tuesday 07/11/2023** | |
| tbd | Travel to Laboratory | |
| 10:00-12:00 | Training | Training: Diatoms (NEA+Azelab) / Macroinvertebrates (NEA)* |
| 12:30-13:00 | Lunch break | Lunch |
| 13:30-18:00 | Training | Training: Diatoms (NEA+Azelab) / Macroinvertebrates (NEA)* |
| | **Wednesday 08/11/2023** | |
| tbd | Travel to Laboratory | |

| | | |
|---|---|---|
| 10:00-12:00 | Training | Training: Diatoms (NEA+Azelab) / Macroinvertebrates (Azelab)* |
| 12:00-13:30 | Lunch break | Lunch |
| 13:30-18:00 | Training | Training: Diatoms (NEA+Azelab) / Macroinvertebrates (NEA)* |
| | **Thursday 09/11/2023** | |
| 13:00 | Start Tbilisi- Vienna | Flight numbers: A9 681 |
| 13:45 | 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. | NEA and AZELAB biologists | 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 | Zurab Jincharadze | Program representative (Georgia) |

NEA laboratory, Georgia

| *Institution* | *Name* | *Function / Position* |
|---|---|---|
| NEA | Elene Kobauri | SW Expert - Biology |
| NEA | Ani Gabrichidze | SW Expert - Biology |
| NEA | Irakli Kordzaia | SW Expert - Biology |
| NEA | Maia Papiashvili | SW Expert - Biology |
| NEA | Nino Korchilava | SW Expert - Biology |

Azelab, LLC of the Ministry of Ecology and Natural Resources of Azerbaijan

| *Institution* | *Name* | *Function / Position* |
|---|---|---|
| Azelab | Illaha Gurbanova | SW Expert - Biology |
| Azelab | Ramina Abdullayeva | SW Expert - Biology |
| Azelab | Jeyhum Muradov | SW Expert - Field sampling |