EU4Environment in Eastern Partner Countries:
Water Resources and Environmental Data (ENI/2021/425-550)

REGIONAL REPORT ON GENOME SEQUENCING

OUTPUT 1.4.2

Funded by
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ABOUT THIS REPORT

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ABOUT EU4ENVIRONMENT – WATER RESOURCES AND ENVIRONMENTAL DATA

This Programme aims at improving people's wellbeing in EU's Eastern Partner Countries and enabling their green transformation in line with the European Green Deal and the Sustainable Development Goals (SDGs). The programme's activities are clustered around two specific objectives: 1) support a more sustainable use of water resources and 2) improve the use of sound environmental data and their availability for policy-makers and citizens. It ensures continuity of the Shared Environmental Information System Phase II and the EU Water Initiative Plus for Eastern Partnership programmes.

The programme is implemented by five Partner organisations: Environment Agency Austria (UBA), Austrian Development Agency (ADA), International Office for Water (OiEau) (France), Organisation for Economic Co-operation and Development (OECD), United Nations Economic Commission for Europe (UNECE). The programme is principally funded by the European Union and co-funded by the Austrian Development Cooperation and the French Artois-Picardie Water Agency based on a budget of EUR 12,75 million (EUR 12 million EU contribution). The implementation period is 2021-2024.

https://eu4waterdata.eu

CONTENTS

List of abbreviations

ADA....................... Austrian Development Agency
BQE....................... Biological Quality Elements
DoA....................... Description of Action
DG NEAR.............. Directorate-General for Neighbourhood and Enlargement Negotiations of the European Commission
EaP....................... Eastern Partners
EC......................... European Commission
EECCA.................. Eastern Europe, the Caucasus and Central Asia
EMBLAS................ Environmental Monitoring in the Black Sea
EPIRB.................... Environmental Protection of International River Basins
ESCS..................... Ecological Status Classification Systems
EU......................... European Union
EUWI+................... European Union Water Initiative Plus
GEF....................... Global Environmental Fund
ICPDR.................... International Commission for the Protection of the Danube River
INBO..................... International Network of Basin Organisations
IOW/OIEau........... International Office for Water, France
IWRM.................... Integrated Water Resources Management
NESB..................... National Executive Steering Board
NFP....................... National Focal Point
NGOs..................... Non-Governmental Organisations
NPD....................... National Policy Dialogue
OECD..................... Organisation for Economic Cooperation and Development
RBD....................... River Basin District
RBMP.................... River Basin Management Plan
Reps...................... Representatives (the local project staff in each country)
ROM...................... Result Oriented Monitoring
ToR........................ Terms of References
UBA....................... Umweltbundesamt GmbH, Environment Agency Austria
UNDP.................... United Nations Development Programme
UNECE.................. United Nations Economic Commission for Europe
WFD...................... Water Framework Directive

Country Specific Abbreviations Armenia

EMIC .... Environmental Monitoring and Information Centre (until January 2020)
HMC .... Hydrogeological Monitoring Centre (since February 2020)
MNP .... Ministry of Nature Protection
SCWS .... State Committee on Water Systems
SWCIS .... State Water Cadastre Information System of Armenia
WRMA .... Water Resources Management Agency

Country Specific Abbreviations Azerbaijan

Azersu JSC .... JSC Water Supply and Sanitation of Azerbaijan
MENR .... Ministry of Ecology and Natural Resources
WRSA .... Water Resources State Agency of Ministry of Emergency Situations

Country Specific Abbreviations Georgia

MENRP .... Ministry of Environment and Natural Resources Protection
NEA .... National Environment Agency
NWP .... National Water Partnership

Country Specific Abbreviations Moldova

AAM .... Agency "Apele Moldovei"
AGMR .... Agency for Geology and Mineral Resources
AMAC .... Association of Apacanals
EAM .... Environment Agency Moldova
MoAgri .... Ministry of Agriculture (of the Republic of Moldova)
MoENV .... Ministry of Environment (of the Republic of Moldova)
Moldova .... Republic of Moldova
SHS .... State Hydrometeorological Service

Country Specific Abbreviations Ukraine

MENR .... Ministry of Ecology and Natural Resources
NAAU .... National Accreditation Agency of Ukraine
SAWR .... State Agency of Water Resources
SEMS .... State Environment Monitoring System
UkrHMC .... Ukrainian Hydrometeorological Center

Executive Summary

This report summarizes the main activities carried out in Armenia, Azerbaijan, Georgia, Moldova and Ukraine in the framework of the Eu4Environment Water Resources and Environmental Data Program for activitiy 1.4.2. ”Novel Approaches to Water Monitoring are further promoted”. The implemented activities comprised preparatory meetings, questionnaire elaboration/circulation/evaluation, theoretical and practical trainings and preparation of workshops and trainings. In particular, the trainings in Armenia and Moldova are described in detail.

1. Introduction

Activity 1.4.2. was divided into two operational phases: (I) the inception phase, followed by (II) the training phase. The inception phase lasted until August 2023 and beyond, clearly demonstrating that whole genome sequencing is a specialised and complex topic and poses a challenge for non-specialists, including programme representatives from the respective countries. It was difficult to quickly raise awareness of the topic at the technical and regulatory level. Accordingly, searching for, contacting and obtaining information from relevant experts in the respective countries proved difficult and delayed activities. As a result of the slower developments, the end of the project was postponed twice at the request of the UBA, from April 2024 to August 2024 and then from August 2024 to the end of 2024. Apart from our activities, other non-European interest groups/organisations (ICAP, Gynco Bioworks Collaboration) had launched activities in the field of whole genome sequencing from wastewater samples in Ukraine and Georgia. As a result, the two countries were largely ‘outsourced’. At the request of the UBA, activities in Azerbaijan were reduced during the inception phase starting in autumn 2023 and subsequently came to a standstill. As a result, the resources made available to us were used in the other countries, but minor resources were kept in reserve for participation in online meetings and for assistance with questions. Communication with Ukraine remained sporadic, with sporadic participation in meetings organised by Ukraine. A multi-day on-site training course was organised and conducted in Armenia (early 2024) and Moldova (late 2024). Collaboration with Georgia took place exclusively online and was significantly more intensive than, for example, communication with Ukrainian colleagues. However, this was sufficient to provide appropriate support. In all three countries, SARS-CoV-2 was sequenced from wastewater with the help of activity 1.4.2. All three countries have the technical equipment and know-how to carry out the relevant analyses. Due to external support in Ukraine and the breakdown in communication with Armenia, the situation there is unclear. The respective laboratories in Georgia, Armenia and Moldova lack the financial support for sustainable monitoring.

2. PURPOSE

Promotion of a joint concept as a draft for a necessary wastewater-based epidemiological sentinel surveillance system for practical implementation in each country with a focus on variants.

• Support for increased cooperation between the relevant health and environmental/water authorities;

• Raising awareness among stakeholders through virtual and in-person events;

• Sharing the experience gained through the EU wastewater sentinel system and national wastewater-based epidemiological sentinel surveillance systems on the potential use of variant testing by the public health sector;

• Assessing the necessary capacities, capabilities, equipment, sampling logistics and analysis methods for sequencing;

• Providing training on sample preparation and specific PCR analysis methods for whole genome sequencing for COVID-19 wastewater monitoring, including initial data production;

3. Activities and results

3.1. Inception-Phase

At the start of the project, an internal kick-off meeting was held in mid-April 2023 to get to know the Austrian project partners (Appendix A01). This was followed in mid-May 2023 by a workshop with the programme representatives from the respective countries (AM, AZ, GE, MD, UA), including an introduction to whole genome sequencing for the programme representatives in order to raise awareness of the topic (Appendix A02). In accordance with the work contract and the jointly discussed strategy, the necessary information for carrying out the activity from the respective countries was to be obtained as far as possible during the initial phase by means of a questionnaire. A corresponding questionnaire for collecting essential points in the initial phase was drawn up and forwarded to the programme representatives for completion. The questionnaire contained a comprehensible summary of the topic of whole genome sequencing as well as suggestions for contact persons to further familiarise the programme representatives with the topic and facilitate their work. The questionnaire was designed to obtain information on the following points: (I) Suitable institutions and their infrastructure, (II) Assessment of capacities, capabilities, equipment and analysis methods for sequencing, and (III) Contact persons at the technical and organisational level. (Appendix A03). Feedback on the questionnaire from the individual countries was modest. By the end of August, no fully completed questionnaires had been submitted to Carinthia University of Applied Sciences. After consulting with the programme representatives, we were informed (feedback from Armenia and Moldova) that the complex topic was causing difficulties for the programme representatives, who are primarily water experts and therefore unfamiliar with the topic. The search for suitable contact persons in the respective countries was time-consuming, even though contact persons were suggested by the Carinthia University of Applied Sciences in the questionnaire. As part of the activity, the Carinthia University of Applied Sciences participated online in the 'Technical Working Group for coordination of the Wastewater Surveillance Implementation in Ukraine' at the beginning of May 2023. Independently of our activity, the US institutions ICAP, PATH and Gynco Bioworks Collaboration were working on setting up a monitoring programme in Ukraine. Data presented showed considerable progress in determining the concentration of SARS-CoV-2 in wastewater and in whole genome sequencing. Wastewater monitoring in Ukraine was well advanced. As a result of this development, after consultation with the UBA, the resources made available to us were focused on the remaining countries. The situation had developed similarly in Georgia, where from February 2023 to probably early 2024, the US CDC had unexpectedly launched a WBE programme with the Georgian NCDC (Lugar Centre). In mid-June 2023, a training week on sampling, sample processing, concentration determination from wastewater samples and whole genome sequencing took place in Georgia, independent of Activity 1.4.2 and the Carinthia University of Applied Sciences. According to the agenda for the training week provided to us (Appendix A05), the Georgian NCDC and ICAP were in charge of the event. The Carinthia University of Applied Sciences did not participate in the training. As in Ukraine, the company Gynco Bioworks Collaboration was active in the field of whole genome sequencing. As a result of this development, the resources made available to us were initially focused on the remaining countries, Armenia and Moldova. In order to increase the visibility of the activity at a higher (ministerial) level in the respective countries, the UBA organised a high-level workshop at the beginning of July 2023 (Appendix A06). In addition to representatives from the individual countries, representatives from the European Commission also participated. The Carinthia University of Applied Sciences participated in the meeting to answer any technical questions that might arise, but did not give a presentation. After consultation with the UBA, the Carinthia University of Applied Sciences took the initiative to identify contact persons for whole genome sequencing in Armenia, Moldova and Azerbaijan. Potential experts in the respective countries were identified based on database entries in relevant sequence databases (GISAID). Initial contact with the potential collaboration partner in Armenia, Arsen Arakelyan, Institute of Molecular Biology NAS RA, was made by the UBA and expanded upon by Carinthia University of Applied Sciences after positive feedback. In mid-August 2023, a meeting took place between Mr Arakelyan, the Armenian programme representative and the Carinthia University of Applied Sciences. As a result of the meeting, mutual cooperation in the field of whole genome sequencing was agreed. Subsequently, on-site training was carried out at the end of January 2024. Maia Alkhazashvili from the Lugar Centre Georgia contacted UBA in February 2024 because she was having problems implementing the analysis protocol for whole genome sequencing from the previous partner (Gynco Bioworks Collaboration). A corresponding revision of the protocol within the framework of Activity 1.4.2 solved the problems. Contact with the collaboration partners from Moldova, Olga Burduniuc and Mariana Apostol from the Agenția Națională pentru Sănătate Publică (ANSP), was established at the end of 2023 and was facilitated by the UBA. An initial meeting on the topic of whole genome sequencing of SARS-CoV-2 from wastewater took place at the end of November 2023 (Appendix A07). It took another year before training could also take place at the ANSP laboratory in Chişinău in December 2024.

3.2. Trainings-Phase

3.2.1. Armenia

After the programme representative in Armenia got in touch with their mates at the Institute of Molecular Biology NAS RA in Yerevan, they set up an initial online meeting to talk about the topic in August 2023 (Appendix A08). This was followed by an online workshop in October 2023, during which the content and technical details of an on-site training course were agreed (Appendix A09). The requirements for whole genome sequencing of SARS-CoV-2 from wastewater in Armenia differed from those in Austria, for example. The sample volume would be significantly lower. The sequencing method and platform commonly used in this field (Illumina SBS, Miseq Illumina) would have led to very high analysis costs per sample in the Armenian setting. For potential wastewater monitoring in Armenia, a special protocol with low sample throughput was therefore developed for the more cost-effective MinION sequencing platform (Oxford Nanopore, Oxford UK) (Appendix P01). This was to ensure that even with the small number of samples available in Armenia, analysis could be carried out cost-effectively and promptly. In accordance with the protocol, reagents and consumables were brought to Armenia for the analysis of 12 samples. On-site training began with an in-depth theoretical introduction for a broad audience (laboratory technicians, students, institute staff) (Appendix I01 and A10). The technical infrastructure at the institute was modern and on a par with or better than the laboratories at the Carinthia University of Applied Sciences. On a technical level, the two sides were also on an equal footing (Annexes I02 and I03), as the COVID-19 pandemic had ensured that the staff had gained extensive experience in the detection and sequencing of SARS-CoV-2 from human samples. The analysis protocols developed for Armenia in Activity 1.4.2 (Appendix P01) could be implemented without any problems at the Institute of Molecular Biology NAS RA in Yerevan. However, wastewater samples from Yerevan, extracted at the Armenian National Centre for Disease Control and Prevention, could not be successfully sequenced. The samples were not adequately purified and showed an unusual brownish colour and precipitates (Appendix I04). It seems likely that colleagues at the Armenian National Centre for Disease Control and Prevention had problems with sample preparation. To circumvent the problem, a purified wastewater sample brought from Austria was processed during the training and successfully sequenced (Appendix A15). For the sequencing specially adapted for Armenia, a correspondingly adapted bioinformatic data analysis was prepared (Appendices P02a and P02b) and carried out with the bioinformatician at the institute. It was shown that the Institute of Molecular Biology NAS RA in Yerevan has the appropriate infrastructure and competent personnel for whole-genome sequencing of SARS-CoV-2 from wastewater in Armenia. With the help of Activity 1.4.2, an analysis protocol tailored to Armenian needs was developed and implemented on site. For wastewater monitoring, quality assurance in the preparation of wastewater samples should be reviewed again. However, the Institute of Molecular Biology NAS RA lacks the financial support for sustainable wastewater monitoring. A corresponding mission report can be found in the appendix (Appendix M01).

3.2.2. Georgia

After the UBA contacted the Lugar Centre in Tbilisi in February 2024, an online meeting was held to discuss the problems and needs surrounding whole genome sequencing of SARS-CoV-2 from wastewater in Georgia (Appendix A11). In further in-depth meetings in March 2024 (Appendices A12 and A13), problems with the implementation of the analysis protocol at the technical level were addressed. After reviewing and revising the analysis protocols used locally and consulting with colleagues at the University of Nebraska Medical Centre, who use a similar protocol to their colleagues in Georgia, suggestions for modifying the procedure were made (Appendix P03). According to the colleagues (Appendices A14a and A14b), the modifications significantly improved the sequencing results. This specifically addressed the request of the colleagues in Tbilisi, enabling successful sequencing of the wastewater samples without on-site training. To verify this, prepared wastewater samples were to be sequenced and compared in Georgia and Austria. A protocol for sample transport was drawn up specifically for this purpose in order to transport the samples to Austria in a stable condition and enable them to be sequenced (Appendix P04). Five samples were sent to Austria in accordance with the protocol (P04) and sequenced by the project partner (Meduni Vienna) at the Centre for Molecular Medicine as part of activity 1.4.2. Unfortunately, the analysis could not be carried out successfully. It is likely that the samples degraded during transport despite chemical stabilisation. In summary, the Lugar Centre in Tbilisi probably has adequate infrastructure and competent personnel to carry out molecular biological analysis and corresponding bioinformatic evaluation as part of wastewater monitoring. However, sustainable financing for continuous monitoring must also be found here.

3.2.3. Moldova

After a lengthy inception phase involving activities in Moldova, on-site training was planned for early December 2024. Colleagues at the ANSP in Chişinău had the greatest need for support in the provision of reagents and equipment. Despite alternative sequencing options, our colleagues opted for the Illumina platform (Miseq, Illumina), which can be very expensive per sample when sample volumes are low. However, the relevant analysis protocols from the manufacturers of the reagents and equipment required for whole genome sequencing could be used without further modification. A corresponding agenda was drawn up for the training (Appendix A16) and completed on site at the ANSP virology laboratory at the beginning of December (Appendix M02). Except for a missing device for size analysis of nucleic acid fragments for quality assurance during sample preparation, the infrastructure in the ANSP virology laboratory was state-of-the-art. As part of activity 1.4.2, it was therefore decided to purchase a low-cost device for size analysis of nucleic acid fragments for quality assurance as a temporary measure. Unfortunately, the device was not ready for use in time for the on-site training. Many devices were purchased in the wake of the COVID-19 pandemic or AIDS surveillance (WHO). The staff was well trained and was able to work through the protocols without any problems. However, as in Armenia, the extraction of genomic material from wastewater was not performed correctly, see Mission Report (Appendix M02). During the training, 23 wastewater samples from Chişinău and one human sample from Moldova were analysed. For cost reasons, the wastewater samples were purified using a Russian extraction kit (manufacturer unknown). The extracts were cloudy and showed a distinct pellet. The human sample was purified using a corresponding extraction kit from Qiagen. Regardless of this, the analysis protocol was completed without incident. However, during the bioinformatic evaluation, it became clear that the wastewater samples contained hardly any usable sequence data, whereas the human sample did, see Appendix A17. It is assumed that this is due to the ‘inexpensive’ extraction. Appropriate personnel were trained for the bioinformatic evaluation. In addition, an analysis pipeline with automated report generation was made available (Appendix P05). As in Armenia and Georgia, Moldova also has the infrastructure and competent personnel for whole-genome sequencing of SARS-CoV-2 from wastewater. However, here too there is a lack of sustainable funding for long-term wastewater monitoring.

4. Outlook

To continue capacity building, regular meetings should be held to promote cooperation between international and local institutions. Inter-laboratory tests can ensure the quality of sample preparation and sequencing. Investments in modern laboratory equipment and technologies are necessary in part to ensure the efficiency and accuracy of sequencing. It is important to secure sustainable financial support from governmental and non-governmental organisations. Finally, the public and decision-makers should be made more aware of the importance of whole genome sequencing and wastewater monitoring.

5. Appendix

- A01_EU4EnvWD_AGENDA_Internes Kick-off Meeting Externe ExpertInnen.pdf
- A02_2023 05 12 EU4ENV_Covid.pdf
- A03_Questionnaire_2023_FA_RM.pdf
- A04_05-2023_Agenda_Technical Working Group Ukraine Agenda.pdf
- A05_12.06.23 Georgia Wastewater Surveillance Protocol Implementation training program_525v2.pdf
- A06_WBE Workshop practical information.pdf
- A07 _231121_Meeting_Moldova.pdf
- A08_Meeting_Armenia.pdf
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- A10_On-site-Training.pdf
- I01_IMG_2253.JPG
- I02_IMG_2267.JPG
- I03_IMG_2274.JPG
- I04_bad samples.jpg
- P01_P01_Protocol_Library-Prep_Armenia.pdf
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- P02b_waste_water_analysis_pipline_armenia.sh
- M01_EU4EnvWD_AM_mission-plan report_2024 02_07_RM.pdf
- P03_Modifikation to xGen Protocol.pdf
- P04_Stabilizing of TNA.pdf
- A14a_WG_ WW Project,Georgia.pdf
- A14b_Results Georgia.zip
- A15_A15_HAC_freyja.zip
- A16_Schedule_Sars-CoV-2 Sequencing Workshop.pdf
- M02_EU4EnvWD_MD_mission-plan_report_2024_12_09_FA.pdf
- A17_HandOver_Moldowa.zip

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