SUMMIT

SOFIA UNIVERSITY
MARKING MOMENTUM
FOR INNOVATION AND
TECHNOLOGICAL TRANSFER

Funded by
the European Union
NextGenerationEU

National Recovery and Resilience Plan

OF THE REPUBLIC OF BULGARIA

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National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Sofia University - Marking Momentum for Innovation and Technological Transfer

Contract BG-RRP-2.004-0008 for the financing of project "Sofia University - Marking Momentum for Innovation and Technological Transfer“ under pillar 2 "Establishing a network of research higher education institutions in Bulgaria", component "Innovative Bulgaria" from National Recovery and Resilience Plan part of the program to accelerate economic recovery and transformation through science and innovation

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REPORT

of the group "Microbiological risks in the environment", project SUMMIT 3.2.4.,

for the period 04.2023 – 31.03.2024

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The main goal of the project is to create a new laboratory for working with microorganisms pathogenic to humans and animals, isolated from the environment, including wastewater. The project develops Wastewater-based epidemiology (WBE) as a new scientific activity in Bulgaria.

Main activities for the first year:

1. Infrastructure and logistics. Building a BSL2+ laboratory and purchasing the necessary equipment and supplies.
2. Selection of wastewater treatment plants and preparation of a program for monitoring pathogens.
3. Development of new and/or adaptation of existing procedures and protocols of isolation, concentration, purification and detection, molecular and classical, of pathogens in environmental samples.

4. Launch of secondary research task and pilot research. Isolation and cultivation of viruses and bacteriophages from wastewater.

5. Administrative activities and personnel management.

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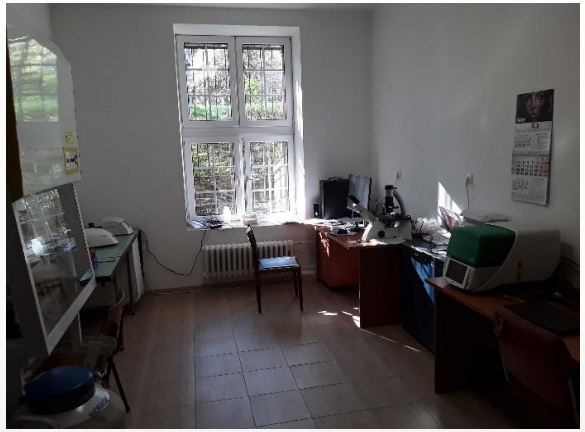
✓ *Infrastructure and logistics. Building a BSL2+ laboratory*



PCR testing room

BSL-2+ wastewater processing room

Timeline of activities during the year

2023

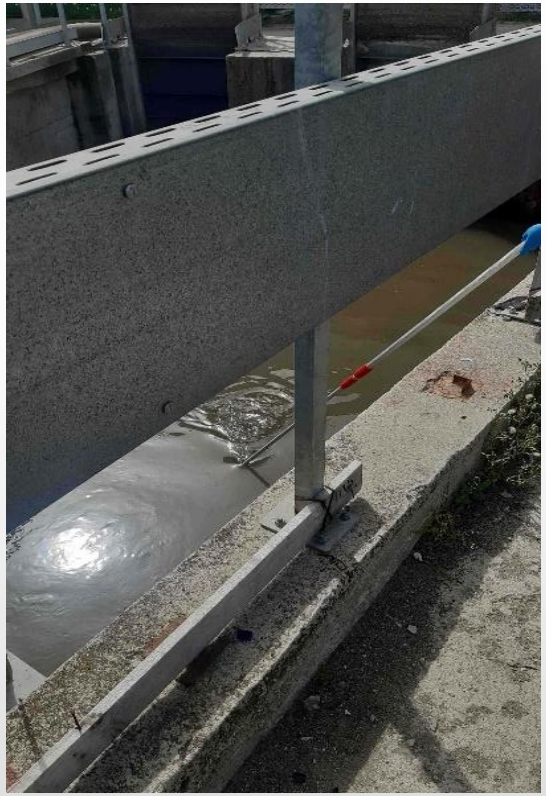
- April, formation of the scientific team.
- May, the documentation for a public procurement tender for the equipment.
- August, the first activity of the new scientific group is a field study on seawater quality at 11 beaches.
- September, completed renovation of the laboratory.
- November - December, the main part of the ordered scientific equipment was delivered.

2024

- 14.01.24. First test sampling from the wastewater of the Kubratovo Water Treatment Plant. **Actual start of experimental and research work in the new laboratory!**
- January, testing wastewater processing procedures.
- February, development of *in house/lab* qPCR protocol for pathogen identifications. Virus cultivation.
- February, first results of the wastewater analysis – adenoviruses and coronaviruses were found.
- March, isolation and cultivation from wastewater of bacteriophages.

✓ *Selection of wastewater treatment plants*

Sampling point in WWTP Sofia



Sampling in WWTP Plovdiv

WWTP for permanent (all seasons) monitoring

- WWTP Kubratovo, Sofia. the capital of Bulgaria.
- WWTP Plovdiv, the second largest city of the country.
- WWTP Varna, the largest city on the Black Sea coast.
- WWTP Burgas, the fourth most populous city.

A selection of additional WWTPs in tourist locations is forthcoming.

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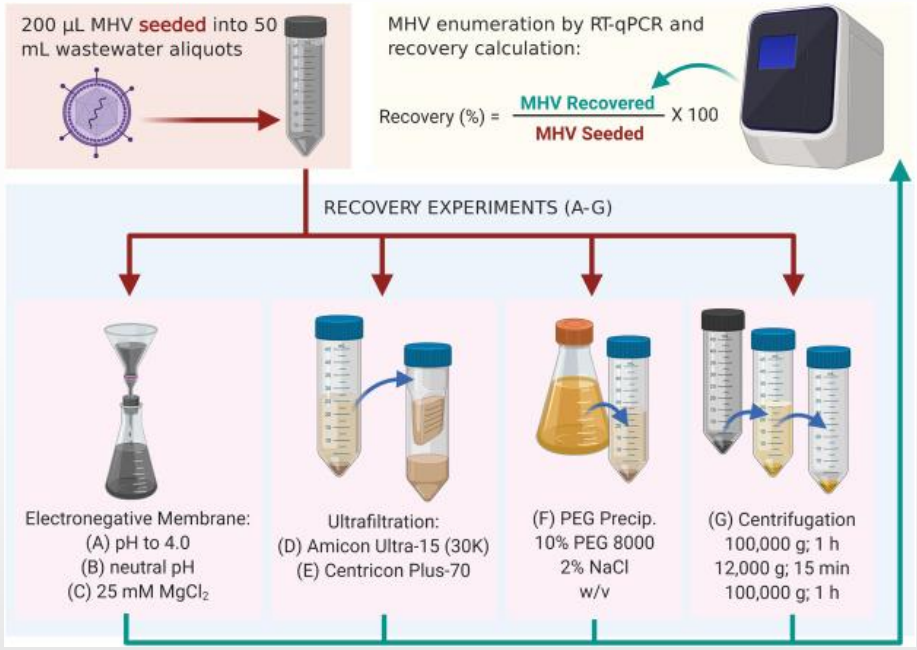
✓ *Main activity - virus testing in wastewater*



Presentation of different methods of concentration of viruses from water.
https://doi.org/10.1016/j.scitotenv.2020.139960

1. Selection of appropriate wastewater processing procedures.

2. Testing protocols for virus concentration, DNA and RNA extraction, inhibitor removal, etc.

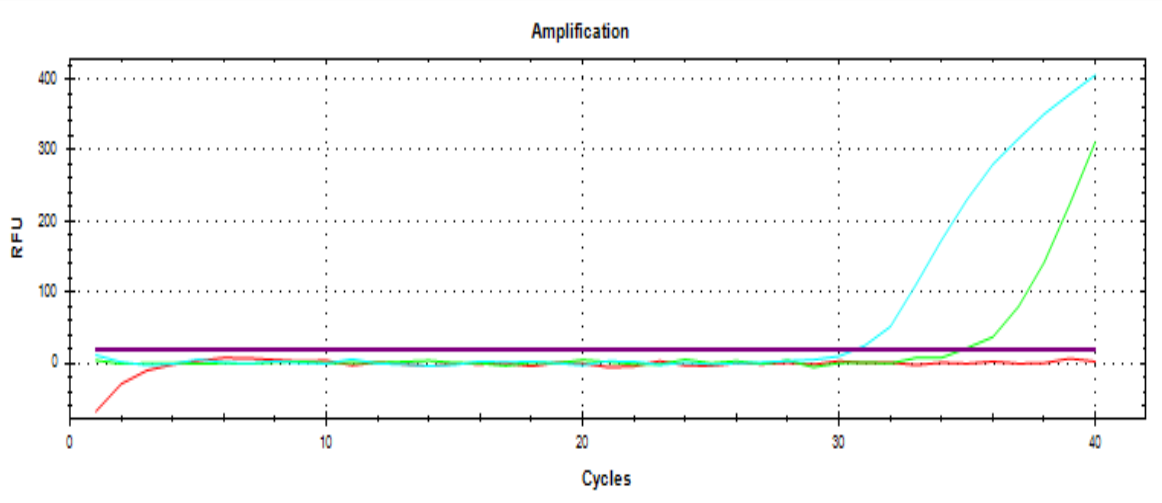
Our selection includes two main procedures:

- concentration of the viruses by PEG 8000 and high-speed centrifugation.
- salt/detergent lysis of viruses and bacteria and subsequent alcohol precipitation and/or concentration of nucleic acids by filtration.

Output result of wastewater processing:

- purified and concentrated DNA/RNA from viruses and bacteria.
- concentrated viral fraction of intact virions stored in liquid nitrogen.

✓ *Main activity - virus testing in wastewater*



Detection of traces of SarsCoV-2 in real wastewater sample. Amplification of sequences from the N2 (CDC) gene. Positive control (7500 copies/µl) in blue, negative control in red, positive sample (Sofia-27.03.2024) in green.

Viruses studied in wastewater are divided into separate packages. The first includes the following viruses, mainly respiratory: influenza A, influenza B, Sars-CoV-2, enteroviruses (coxsackievirus B3), respiratory syncytial viruses A and B and human adenoviruses.

We chose to use quantitative polymerase chain reaction (qPCR) with TaqMan chemistry, for the detection of viruses in wastewater. For each virus, the corresponding synthetic positive control oligonucleotides were selected.

An *in house* qPCR protocol was developed for each individual viral target. A duplex variant of TaqMan chemistry is implemented.

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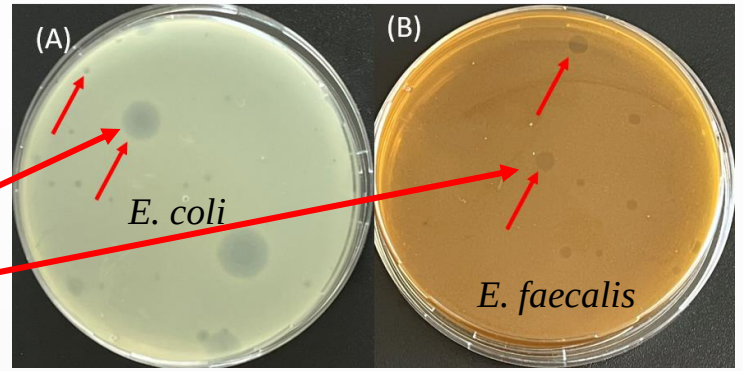
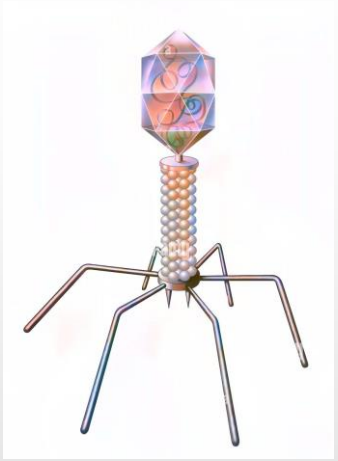
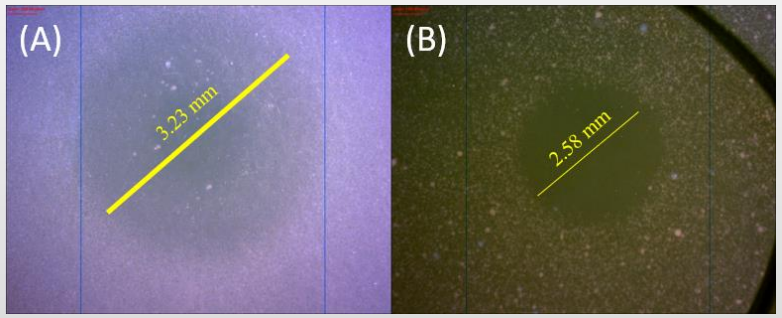
- ✓ *A secondary tasks - isolation of therapeutic phages from wastewater*

- Bacteria used as target species:
  - ✓ *Escherichia coli* (9 strains);
  - ✓ *Enterococcus faecalis* (10 strains);
  - ✓ *Salmonella* spp. (2 strains);



- The samples were collected at the entrance of the wastewater facility in Kubratovo;
- The initial phage isolates were chosen for isolation on the bases of their macromorphological characteristics:





Different plaques from phage lysis

- In total, 75 clear plaques from bacterial lysis were observed on petri dishes;

- A total of 15 phages, destroying three of the most harmful bacteria: *E. coli*, *E. faecalis and Salmonella* were isolated and partly characterized;

- These phages are the first members of the first collection of potentially therapeutic phages, effective against harmful bacteria, in Bulgaria.

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✓ *A secondary tasks – virus cultivation*

Why do we grow live viruses in the lab?

1. We add our cultured viruses to the wastewater to ensure that we can detect and isolate them alive from there. Ours, and any other virus.

Human mastadenovirus C type 1 (a double-stranded DNA virus, non enveloped), Human coronavirus 229E (a single-stranded (+) RNA virus, enveloped), and bacteriophage BsXeu269p/3 are cultivated and used as positive controls.

2. With qPCR, we detect viral nucleic acids. We have no way of knowing which virus is infectious and poses a danger. By infecting cells, we detect "live" viruses.

3. Through cultivation we can discover new, unknown and unexpected viruses.

Cytopathic effect in HeLa cells infected with Adenovirus used as control in wastewater processing.

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✓ *Administrative activities and personnel*

Personnel:

- The scientific team includes 6 part-time researchers (2 hours per day).
- One full-time sampling technician.
- One part-time administrative secretary.
- Starting next month, we will be joined by three part-time technicians, including two students.

Administrative activities:

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