MINT PROJECT

Research protocol

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City: Bialystok, Poland

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Sampling locations

WATER:

1. River Biala in Bialystok - a river flowing through a large agglomeration and located at frequently visited sites before reaching the Supraśl River
2. River Biala in Fasty - River Biala after receiving water from a sewage treatment plant
3. River Supraśl after its confluence with the Biała River - material taken in Fasty after the confluence of the two rivers
4. River Supraśl before entering Bialystok - river flowing through small towns
5. River Narew before Łapy - a river flowing for over 100 km without meeting any big towns

SOIL:

1. Forest soil - taken from the forest on the outskirts of the town
2. Bottom sediment - from the Biebrza river in the Biebrza National Park
3. Arable field - approximately 60 kilometres from Bialystok
4. Bottom sediment - from the bottom of the river where the Biała River flows into the Supraśl River
5. Forest soil - taken from the Zwierzyniecki forest in the city centre
6. Main street in Bialystok - Tunnel of Fieldorf Nil

Why did we choose these places?

The sites chosen will allow us to compare the condition of rivers flowing through large agglomerations with those flowing only through small towns and villages. We will also be able to observe the changes in the bacterial flora of the river before and after the contact with the sewage treatment plant. We will investigate the amount of e.coli bacteria in the water from the city as well as from the national park, and the amount of mould and fungi in the soil near these rivers.

What will the results be used for?

The results will serve us to assess the non-invasiveness of the sewage treatment plant on the bacterial flora and the amount of fungi in the water and soil.

Our expectations:

We expect that water from the Biała river flowing through the city will contain more microorganisms than that from the Narew river running for several tens of kilometres without contact with larger towns. We expect the greatest number of bacteria, yeasts and moulds from the Biała river in Fasty - after receiving water from the sewage treatment plant.

We expect the highest number of microorganisms from 3 soil samples - soil from near the river in Fasty, behind the city sewage treatment plant. Soil sample from Antoniukowskie orchards should show single colonies of bacteria and fungi. The lowest number of soil biological contaminants is expected from samples from gardens in Łapy and in front of the WWTP.

1. Equipment and reagents

- petri dishes with a prepared microbiological medium for the determination of the total number of microorganisms, total yeast and moulds and coliforms

- test tubes with aluminium caps

- 100 ml conical flasks

- automatic pipettes

- Sterile pipette tips

- autoclave

- Magnetic stirrer

- hothouse

- 0.8% NaCl solution (physiological saline)

2. Methodology

To carry out the intended research, we will use extraction of soil samples with physiological saline (0.8% NaCl). In the extract obtained, we will estimate the total number of bacteria and the population of yeasts and moulds. In water, in addition to the total number of bacteria, we will determine the content of coliforms. For this purpose we will use petri dishes with a dry microbiological medium. Coliforms are one of the indicators of water pollution, commonly known as faecal bacteria. And one strain - Escherichia coli - is responsible for the most common water contamination.

3. Making the determination

3.1. Water

Petri dishes were filled with 1 ml of water and incubated in a greenhouse:

- 48 h at 30oC for the total number of microorganisms

- 5 days at 30oC for total number of yeasts and moulds

- 24 h at 37oC for coliforms

In 2 samples, the colonies were so numerous that it was impossible to count them. It was necessary to perform cultures again from diluted samples. For this purpose, tubes containing 9 ml of 0.8% NaCl solution were prepared. The tubes with their contents were sterilised in an autoclave. Into the prepared tubes 1 ml of the tested water was introduced and by successive dilutions the concentration equal to 10-3 was obtained. The samples prepared in this way were sieved on petri dishes and incubated in the conditions described above.

3.2. Soil

Into 100ml conical flasks, 45ml of 0.8% NaCl solution was introduced, covered with aluminium foil and sterilised in an autoclave. To the flasks thus prepared, 5g of each of the soils tested was added and extracted on a magnetic stirrer for 20 minutes. In the case of total mould, fungi and coliform counts, cultures were taken directly from the extract obtained by injecting 1ml directly into the plates. For total microbial counts it was necessary to dilute to 10-3 in the same way as for water samples. Incubated under conditions as described above.

4. **Characteristics of the microorganisms to be determined**

- **Total microorganism count -** this is an indicator of the total number of culturable bacteria. These are psychrophilic organisms, they die below 0°C and above 30°C, and thrive best at 15°C. It is considered that there is no danger of disease if the number of these bacteria **does not exceed 100 in 1 ml of water.**
- **Yeasts and moulds -** Yeasts are microscopic fungi that are commonly found in nature and can cause microbiological contamination in all food industries. They can be introduced with the raw material as well as secondary contamination from personnel or air. Yeasts are also the largest group commercially used by humans. Moulds are filamentous fungi that cause as much as 8% of diseases in plants. Moulds are a harmful microflora due to the formation of secondary metabolites of mycotoxins.
- **Coliforms -** In Poland they are accepted as an indicator of water pollution in sanitary terms.Coliforms themselves are mostly not harmful to humans. Their presence, however, indicates the presence of other pathogenic bacteria.

  If coliforms are found in water, the water must not be drunk without boiling. The danger lies in the fact that the appearance of this bacteria means that there may also be pathogenic microorganisms in the water, dangerous for humans. However, it should be emphasised that coliforms can be pathogenic under certain conditions. It causes infections of the urinary system and inflammation of the meninges in newborns. According to the regulation, coliforms **must not be present in 100 ml of tested water.**

5. Research results:

WATER:

Comparing the content of fungi and yeast in the waters from the rivers, the water from the Supraśl River was the most prominent. The water from the river Narew also contained one larger colony, in the water from the river Biała connected to the river Supraśl and the river Biała behind the treatment plant single yeasts and fungi were noticeable. In the water from the river Biała in the city we observed one individual. The above data are from tests on an undiluted sample. At higher dilutions, no yeasts or fungi showed up.

Coliform bacteria were present in only two samples.The water from the Biała river in the city centre contained one bacterium, but the water from the Supraśl river in front of the city contained a very large colony of about 960 individuals.

The highest total number of microorganisms was detected in the sample from the Narew River. A trace content was also found in the sample from the Supraśl River. No microorganisms were detected in the other samples.

SOIL:

Comparing the content of fungi and yeasts in the soil, the forest soil showed the highest amount. Soil from the Gen. August Fieldorf "Nil" tunnel also showed a high content. The other samples showed almost no yeasts and fungi. The above data are from tests on an undiluted sample. At higher dilutions no yeasts or fungi showed up.

Coliform bacteria were not detected in any of the soil samples.

The highest total number of microorganisms at a dilution of 10-3 was detected in a sample from the Fieldorf Nil tunnel. Soil samples collected in the arable field and in the zwierzyniecki forest also showed high contents. About 30 colonies were detected in a sample taken at the junction of the White and Supraśl rivers. The other samples oscillated around 10 colonies.

6. Conclusions

The waters from the Biała River in the city centre and the Biała River that flows behind the treatment plant contained the least microorganisms. These waters pass sanitary inspections and are not polluted with any hazardous substances. The Biała river connected to the Supraśl river and the Narew river contained more pollutants because these are rivers flowing through swamps and small villages. The highest number of microorganisms was observed in the Supraśl river flowing through densely populated villages near the city. Bacteria, yeasts and fungi thrive best where rivers are accessible to people and are not subjected to detailed examination and, if abnormalities are detected, to treatment.