

# BACTERIOLOGIC AND ENZYMATIC ACTIVITIES IN SEDIMENTS SAMPLES 

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## Determining the main physicalchemical water parameters

- temperature,
- pH,
- concentration O2,
- conductivity.



## Collected samples

- Sampling to determine indicators of water sanitary and ecophisiological main groups of microorganisms in the river bed was about 1 meter from the shore in glass containers.
- They used glass bottles with ground stoppers, 500 ml and 1000 ml , previously sterilized in an oven at $180^{\circ} \mathrm{C}$ for 60 minutes.
- Sampling of sediments for determining the quantitative and qualitative enzymatic activity of the riverbed was about 50 cm from the shore using the Draga Ekman.
- They used glass containers with lockable lids, sterilized in an oven.



## Enzymatic study

- The following enzymatic activities were quantitatively established for the sediment samples:
- catalase (CA),
- urease (UA),
- reduction of trivalent iron (RFE),
- actual dehydrogenase (ADA),
- potential dehydrogenase (PDA),
- phosphatase (PA).
- The reaction mixture for CA consisted of 3 g sediment + 10 mL phosphate buffer +2 mL solution H2O2 3\%.
- The incubation was carried out at room temperature for 1 h .
- Then the reaction was stopped by adding 10 mL de $\mathrm{H} 2 \mathrm{SO} 4 \mathrm{4N}$ and 78 mL distilled water and the solution obtained was filtered through filter paper.
- From the obtained filtrate is taken 25 mL and is introduced in an Erlenmeyer glass.
- Then is added 2.5 mL H2SO4 4 N and the solution is titrated with a solution of KMnO 40.05 N until appears a weak pink coloration (Drăgan-Bularda, 2000).
- CA were expressed as $\mathrm{mg} \mathrm{H} 2 \mathrm{O} 2 / \mathrm{g} . d . \mathrm{s}$.

- Actual dehydrogenase activity (ADA) and potential (PDA) were determined using described methods (Casida et. al., 1964).
- The reaction mixture consisted of 3 g sediment +0.5 mL TTC $3 \%$ solution +1 mL distilled water and for PDA is added 1 mL glucose solution $3 \%$.
- The incubation was carried out at 37 oC for 48 h .
- ADA şi ADP were represented as mg formazan/g.d.s. measuring absorbance at 485 nm .
- Dehydrogenase is an enzyme produced by microorganism in the soil which acts on organic compounds in soil and releases hydrogen which is transferred to compounds that accept $\mathrm{H}+$.
- Urease activity (UA) is expressing the decomposition intensity of urea in $\mathrm{NH} 3+\mathrm{CO} 2$.
- The reaction mixture consisted of 5 g sediment + 2 mL toluene +5 mL phosphate buffer +5 mL urea solution.
- The mixture was incubated at 37 oC for 24 h .
- The formed ammonia is extracted with a KCl solution and is determined by nesslerization, and is measured the absorbance at 445 nm (Drăgan-Bularda, 2000).
- Trivalent iron reduction activity (RFE) can be used as ecotoxicological test to evaluate the pollutants' effect over the microbiota of the soil or sediments.
- To determine this activity the following reaction mixture was used: 3 g sediment +5 mL glucose solution $4 \%+0.5 \mathrm{~mL}$ ferric chloride solution ( FeCl 3 ) and 5 mL distilled water.
- The incubation was carried out at $28^{\circ} \mathrm{C}$ for 48 h .
- After incubation in each tube was added 5 mL solution KCl 2 M , after which the content is filtered through filter paper.
- From filtrate is taken 7 mL and added 1 mL of $\alpha, \alpha^{-}$ dipiridil solution. For the obtained solution is measured the absorbance at 240 nm (Filimon, 2007).
- Determination of phosphatase activity (PA) involving the use of reaction mixtures consisting of 3 g sediment, 2 ml toluene, $10 \mathrm{ml} 0.5 \%$ phenilphosphat disodium.
- Incubation of samples is done at $37^{\circ} \mathrm{C}$ for 48 hours.
- The phosphatase activity was expressed in mg phenol as a result from the hydrolysis of the phenilphosphat disodium $/ 3 \mathrm{mg}$ dry sediment (Krámer and Erdei, 1959).


## Enzymatic indicator of the sediment quality (EISQ)

- EISQ = 1/n $\Sigma \mathrm{Vr}(\mathrm{s}) / \mathrm{Vmax}$ (i) where:
- EISQ - enzymatic indicator of the sediment quality,
- n - number of activities,
- Vr (i) - individual, real value,
- Vmax (i) - theoretical, maximum, individual value.


## Bacteriological study I

- Key-samples of 10-1 and 10-6 have been used, starting with one gram of soil.
- Elective and nutrient environment inoculation has been performed with an ml of every soil dilution. Incubation has been made at 28 C for 7-21 days, depending on the established ecophysiological group.

- In order to quantitatively determine the nitrogenfixing bacteria (NFB) any kind of culture environment without nitrogen is used, such as the Ashby environment, which has the following chemical composition: K2HPO4 $0.5 \mathrm{~g}, \mathrm{NaCl} 0.5 \mathrm{~g}$, MgSO4 0.2 g , K2SO4 $0.1 \mathrm{~g}, \mathrm{CaCO} 5 \mathrm{~g}$, sucrose 5 g.
- Samples have been incubated for a week at $27^{\circ} \mathrm{C}$.
- For the aerobic nitrogen fixing bacteria a veil has been established above the environment or a kind of ring on the tube walls.
- The veil could be fluorescent, greenish yellow ( $A$. vinellandi) or brown (A. chroococum). For anaerobic fixing bacteria (Clostridium sp.), positive tubes feature the appearance of gas bubbles (Zarnea, 1994).


Fixatori de azot aerobi
(A. chroococum, A. vinelandii)


Fixatori de azot anaerobi
(Clostridium sp.)

- For the cultivation of amonifying bacteria (AMB) the following chemical composition is used: NaCl 0.5 g , peptone 2 g , distilled water 1000 ml . The culture environment's pH should be 7.9.
- Samples are incubated at 22 C for 14 days in anaerobic conditions.
- Highlighting the ammonia, as a result of the amonifying bacteria activity, is accomplished with the help of a specific color reaction, adding one or two drops of Nessler reagent.
- An intense yellow is obtained, with or without precipitation (Cusa, 1996).
- The culture environment for nitrifying bacteria (NB) has the following chemical composition: standard saline traces 50 ml , (NH3)SO4 $0.5 \mathrm{~g}, \mathrm{CaCO} 31 \mathrm{~g}, 950 \mathrm{ml}$ distilled water.
- Samples have been incubated for 20 days, at a temperature of 28 C .
- The nitrifying bacteria diphenylamine-sulfuric acid has also been studied.
- The presence of nitrifying bacteria is translated into the appearance of an intense bluish color at high concentrations, sometimes surpassing the concentration limit (Dunca et al., 2007).

- Culture environment for denitrifying bacteria (DNB) has the following chemical composition: standard saline solution 50 ml , KNO2 20 g , glucose 10 g , KCO3 5 g , oligoelements solution 1 ml , distilled water 1000 ml .
- Studied samples are incubated at 28 C for 7-15 days.
- Denitrifying bacteria is highlighted by adding diphenylamine-sulfuric acid.
- Tubes in which the nitrate disappeared are colorless (Dunca et al., 2007).

- In order to establish the probable number of sulphate-reducing bacteria (SRB) such as Desulfovibrio, the multiple tubes method has been used Cusa (1996).
- The Starkey environment has been also used Domagala et al. (1992), with the following chemical composition: Na3PO4 $5 \mathrm{~g}+\mathrm{NH} 4 \mathrm{Cl} 1,0 \mathrm{~g}+\mathrm{K} 2 \mathrm{HPO} 4$ $0,5 \mathrm{~g}+\mathrm{MgSO} 4 \times 7 \mathrm{H} 2 \mathrm{O} 2,0 \mathrm{~g}+\mathrm{Na} 2 \mathrm{SO} 40,5 \mathrm{~g}+$ $\mathrm{CaCl} 2 \times 2 \mathrm{H} 2 \mathrm{O} 0,1 \mathrm{~g}+(\mathrm{NH} 4) \mathrm{Fe}(\mathrm{SO} 4) 2 \times 6 \mathrm{H} 2 \mathrm{O}$ 0,01 g.
- Positive tubes are considered to have a black precipitate at the bottom (iron sulfide) DraganBulandra (2000).
- In order to determine the iron-reducing bacteria (IRB) the Ottow culture has been used Ottow et al. (1968).
- This Ottow culture has been chemically modified by the following composition: Glucose $20 \mathrm{~g}+$ Peptone $5 \mathrm{~g}+$ yeast extract $0,5 \mathrm{~g}+\mathrm{MgSO} 4 \times 7 \mathrm{H} 2 \mathrm{O} 0,2 \mathrm{~g}+\mathrm{K} 2 \mathrm{HPO} 43$ $\mathrm{g}+\mathrm{KH} 2 \mathrm{PO} 40,8 \mathrm{~g}+\mathrm{KCl} 0,2 \mathrm{~g}+\mathrm{Fe} 2 \mathrm{O} 3 \times 3 \mathrm{H} 2 \mathrm{O} 1 \mathrm{~g}$.
- Fe2+ ions, produced by iron-reducing bacteria, as a reduction of $\mathrm{Fe} 3++\mathrm{Fe} 2 \mathrm{O} 3$, can be highlighted by adding to 0.7 ml of culture 0.1 ml of reactive $\alpha$, $\alpha$-dipiridil.
- The red or pink color indicates the presence of $\mathrm{Fe} 2+$ ions Dragan-Bulandra (2000).


## BISQ - bacterial indicator of the sediment quality

- $\operatorname{BISQ}=1 / n \times \Sigma \log 10$ N, where:
- BISQ - bacterial indicator of sediment quality,
- n - number of ecophysiological groups,
- N - number of bacteria appertaining which belongs to each ecophysiological group.


## Bacteriological study II

- Determination of the total number of bacteria that grow at $37^{\circ} \mathrm{C}$ (mesophilic) is made by seeding or decimal dilutions of the sample by the method incorporated into a solid nutritive medium incubated at $37^{\circ} \mathrm{C}$ for 48 hours followed by counting developed colonies.
- The result is expressed as the number of colony forming units (CFU) in 1 ml . The calculation is done using the following formula:
- Total mesophilic bacteria (CFU / ml) $=\Sigma(\mathrm{nxd}) / \mathrm{N} \times \mathrm{V}$, where: n - number of colonies were grown in a Petri dish; d - the inverse of the sample dilution sown; N - number of Petri dishes and counted; V-volume of sample taken into work in ml (Drăgan-Bularda, 2000, STAS 3001/91).

- The presence of coliform bacteria (total coliforms) is emphasized by presumptive test (presumptive test) însământând water and/or decimal dilutions in a number of vials and test tubes with liquid enrichment medium (medium-lauryl sulfate broth), the reaction was positive evidenced by a confirmatory test on solid medium (agar-lactose medium-eosin-methylene blue (GEAM)) at $37^{\circ} \mathrm{C}$ after 24 hours.
- Considering the number of positive tubes confirmed calculate the probable number of coliform bacteria (total coliforms).
- The number of coliform bacteria (total coliforms) is calculated by: Coliform bacteria $=(\mathrm{n} 1+\mathrm{n} 2++\mathrm{V} 2+$ V3 n3/V1) • $100 \mathrm{ml}=$ totali/ 100 coliforms, where: n1, n2, n3 = number of colonies grown on each membrane filter characteristic; $\mathrm{V} 1, \mathrm{~V} 2, \mathrm{~V} 3=$ volume filtered in the same sample in ml (STAS 3001/1991).

- Termotolerante coliform bacteria (fecal coliform) is put out the bottles and tubes based on presumptive positive test for coliform bacteria (total coliforms) by confirmation in the selective liquid (broth-bile-lactose-bromocresol purpura (MacConkey medium).
- Medium-bile-lactose broth, brilliant green (medium BBLV)) at a temperature of $44^{\circ} \mathrm{C}$ in 24 hours.
- Taking into account the number of positive tubes at a temperature of $44^{\circ} \mathrm{C}$ is calculated the probable number of coliform bacteria termotolerante (fecal coliforms).


BULION BILA LACTOZA VERDE BRILIANT (BBLV)

- The presence of fecal streptococci is emphasized by presumptive test sample and/or decimal dilutions in a number of vials and test tubes with liquid enrichment medium (broth medium Na -azide) at $37^{\circ}$ $C$, as evidenced by the positive reaction a confirmatory test in a liquid selective medium (medium-broth-bromocresol PurPur Na azide) at a temperature of $44^{\circ} \mathrm{C}$ for 48 hours.
- Considering the number of positive tubes confirmed calculate the probable number of faecal streptococci (Drăgan-Bularda, 2000, STAS 3001/1991).


AGAR AZIDÅ-BILA-ESCULINA


BULION AZIDĂ DE SODIU BROMCRESOL-PURPUR

