Samara State Medical University
Common, Bioinorganic and Bioorganic Chemistry Department

Chemotherapeutic effect of humic substances low mineralized silt sulphide muds on pathogenic and opportunistic microorganisms

assistant of the Common, Bioinorganic and Bioorganic Chemistry Department
Yury V. Zhernov

Topicality:

- The problem of poly-resistance of pathogens to chemotherapeutic drugs.
- The use of antimicrobial drugs leads to adverse effects in humans: the cytotoxic reaction, dysbacterioses, allergenic effects and the immunosuppressive state.

The unique materials include

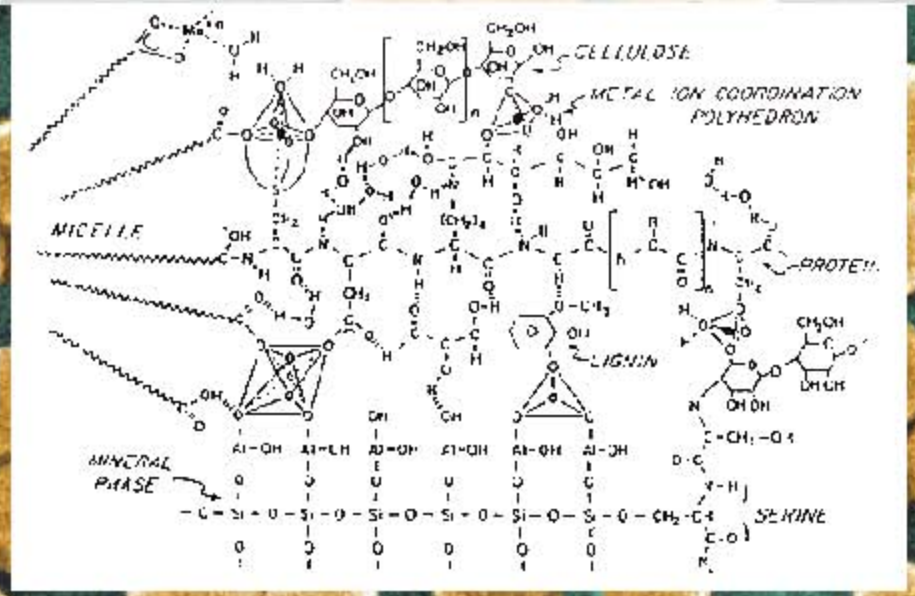
humic substances!

Franz Carl Achard
Франц Карл Ахард
(28.04.1753 - 20.04.1821)

Franz Karl Achard was the first who isolated humic substances from peat in its pure form, and described it.

Ernst Felix Immanuel Hoppe-Seyler
Эрнст Феликс Иммануэль Гоппе-Зейлер
(26.12.1825 - 10.08.1895)

Ernst Hoppe-Seyler was the first who isolated and described the last fraction of humic substances in a sequence of hymatomelanic acid.



Paul Ehrlich
Пауль Эрлих
(14.03.1854 - 20.08.1915)

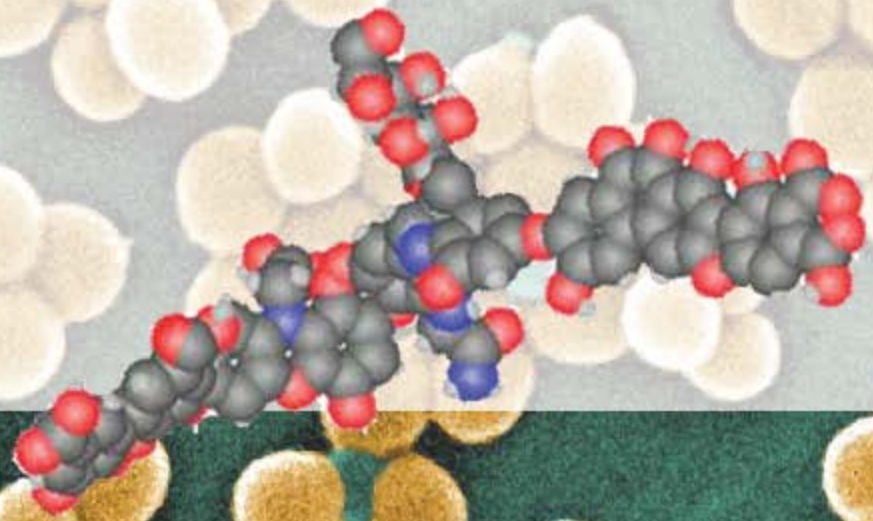
The founder of chemotherapy. Paul Ehrlich in 1891, developed the concept of "Magic bullets" – der Zauberkugel.

Ohne Hast, ohne Rast

In his searches for drugs for trypanosomiasis, he analyzed more than five hundred of dyes and pigments (including humic acid).

Objective:

Study of the influence of the drugs peloid humic series and their derivatives on pathogenic and opportunistic microorganisms, as a promising basis for the development of chemotherapeutic drugs.



Fractions of humic substances of low mineralized silt sulphide muds (peloids) of lake Molochka of the sanatorium «Sergievsk mineral water» (0,25%; 0,1%; 0,01%):

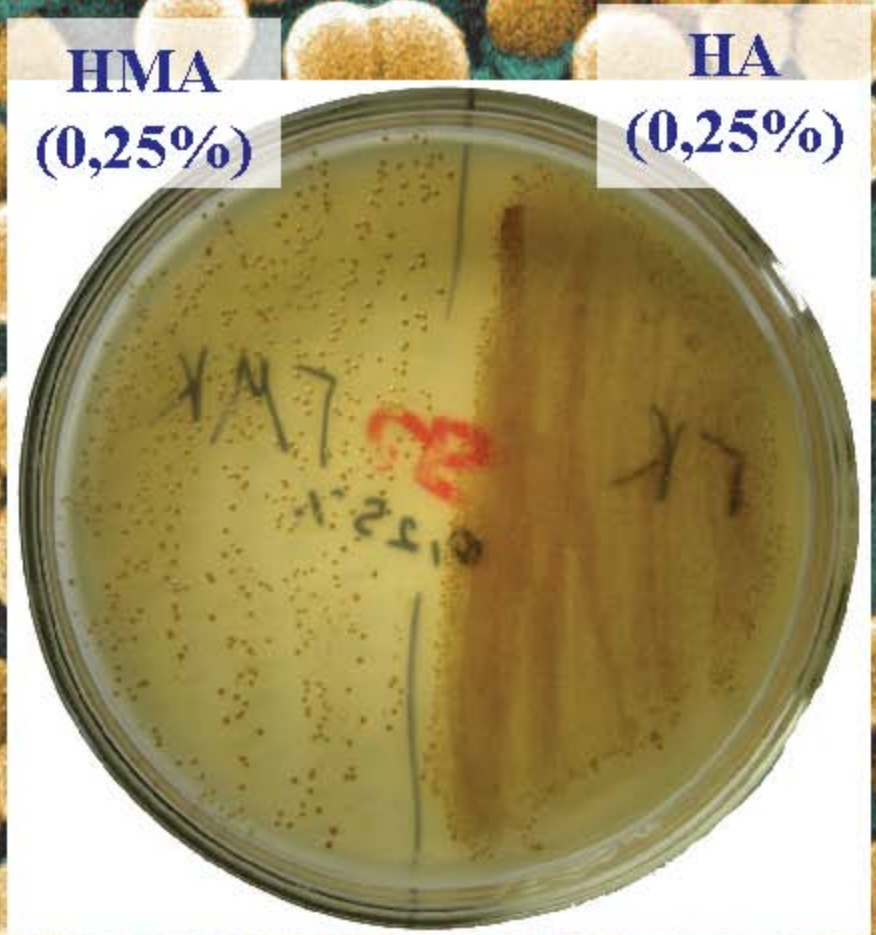
- Humic acid (HA);
- Hymatomelanic acid (HMA);
- Fulvic acid (FA);
- Humus acid (HsA).

The stages of our research:

1. The study of bacterial contamination of fractions of humic substances and their derivatives – humates, fulvates and hymatomelanates and their purification.
2. The study of environmental toxicity of drugs in biological objects.
3. The study of the antibacterial activity of drugs.
4. The study of antimycotic activity of drugs.
5. The study of antimikobakteritic (anti-TB) activity of drugs.
6. An IR spectroscopic study of humates of different nature, for a more detailed understanding of the mechanism of action on the cells of microorganisms.

Stage 1

The study of bacterial contamination of fractions of humic substances and their derivatives.



Saburo agar

blood agar

Results:

- The samples of humic substances peloids have been contaminated by bacteria of the family *Enterobacteriaceae*, the so-called sanitary-indicative microorganisms.
- The researched fractions of humic substances peloids are a good substrate for microbial growth. Sterilization of the samples for further study humic substances was performed by subtly spilling substances with the help of ultraviolet irradiation within sixty minutes.
- The researched fraction of humates, fulvates, hymatomelanates were not contaminated by microorganisms, which makes it possible to believe in their high antimicrobial activity.

Stage 2

The study of environmental toxicity of drugs in biological objects.

The study of environmental toxicity of humic substances was carried out using protococcoid unicellular green algae ***Scenedesmus quadricauda***.

Results:

All peloids accelerate the growth of *Sc. quadricauda*. Effect was observed in direct proportion to the concentration in comparison with control samples.

Stage 3
The study of the antibacterial activity of drugs.

The test-cultures of microorganisms were used to determine the antibacterial activity of substances:

1) Gram-negative bacteria –
Escherichia coli (ATCC 25922),
Pseudomonas aeruginosa (ATCC 27853).

1) Gram-positive bacteria –
Staphylococcus aureus (ATCC 25293),
Bacillus subtilis (ATCC 6633).

Mueller-Hinton agar

Ingredients	grams / liter
Meat infusion	300,00
Casein hydrolyzate	17,50
Starch	1,50
Agar-agar	17,00
Final pH (at 25 ° C)	7,3 ± 0,2

As a nutrient medium for determining microorganism sensitivity to antimicrobial drugs, according to standard National Committee for Clinical Laboratory Standards (Clinical and Laboratory Standards Institute, NCCLS / CLSI) to study the chemotherapeutic activity, we have chosen Mueller-Hinton agar.

The active component of the derivatives of humic substances is a **metal cation**.

The humic components options:

1. Increased penentrability through biological membranes;
2. Masking bactericidal component;
3. Tropism drug to microorganisms;
4. Reducing the toxic effect of metals on macro-organisms.

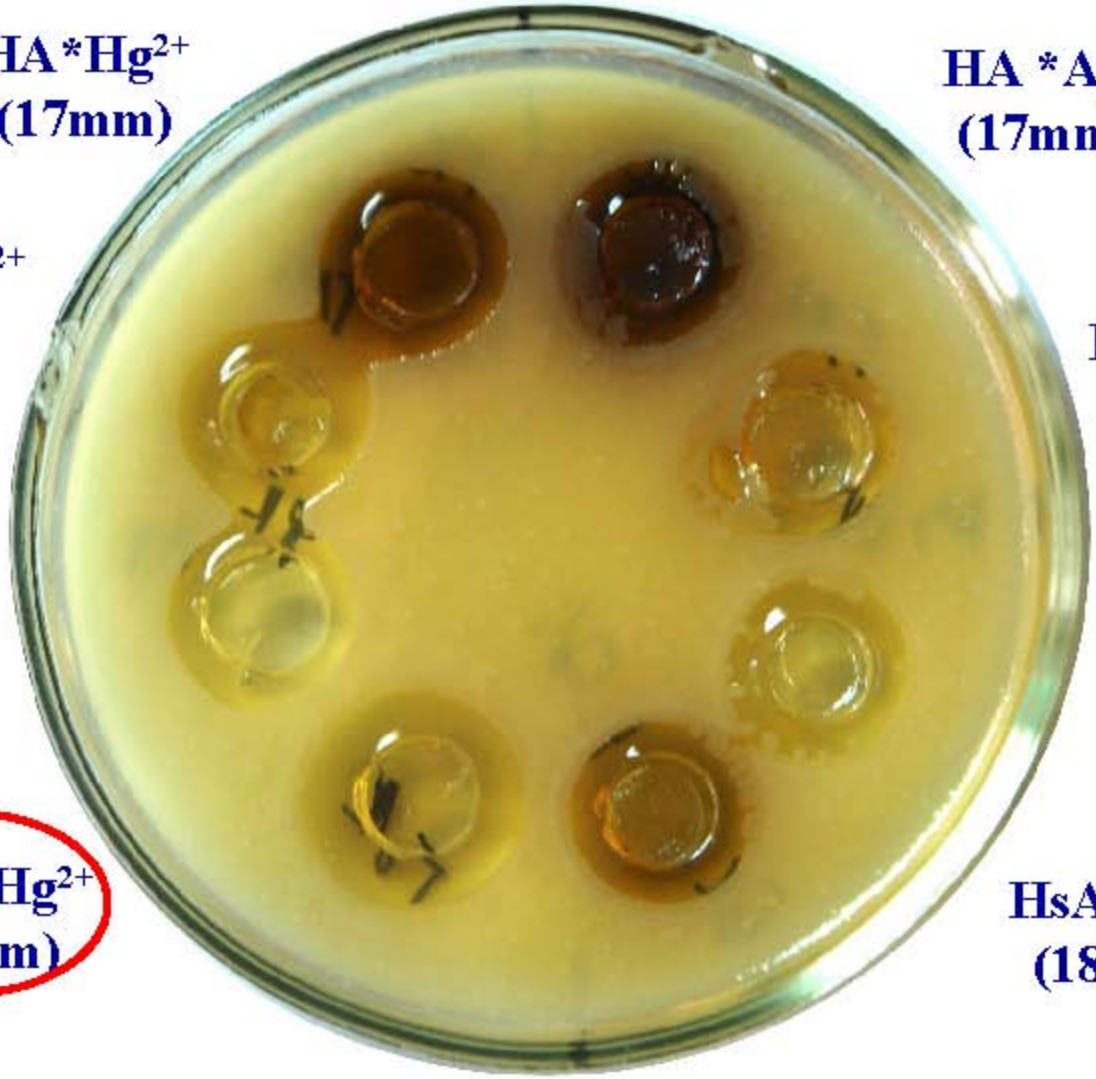
Results:

- Hymatomelanic, fulvic, humic, humus acid peloids have no cytolytic activity against the studied microorganisms.
- But often a static activity to an increase in *E. coli, Ps. aeruginosa* – all studied Gram-negative microorganisms.
- Stimulate the growth of the investigated Gram-positive microorganisms – *St. aureus, B. subtilis* with respect to the control.

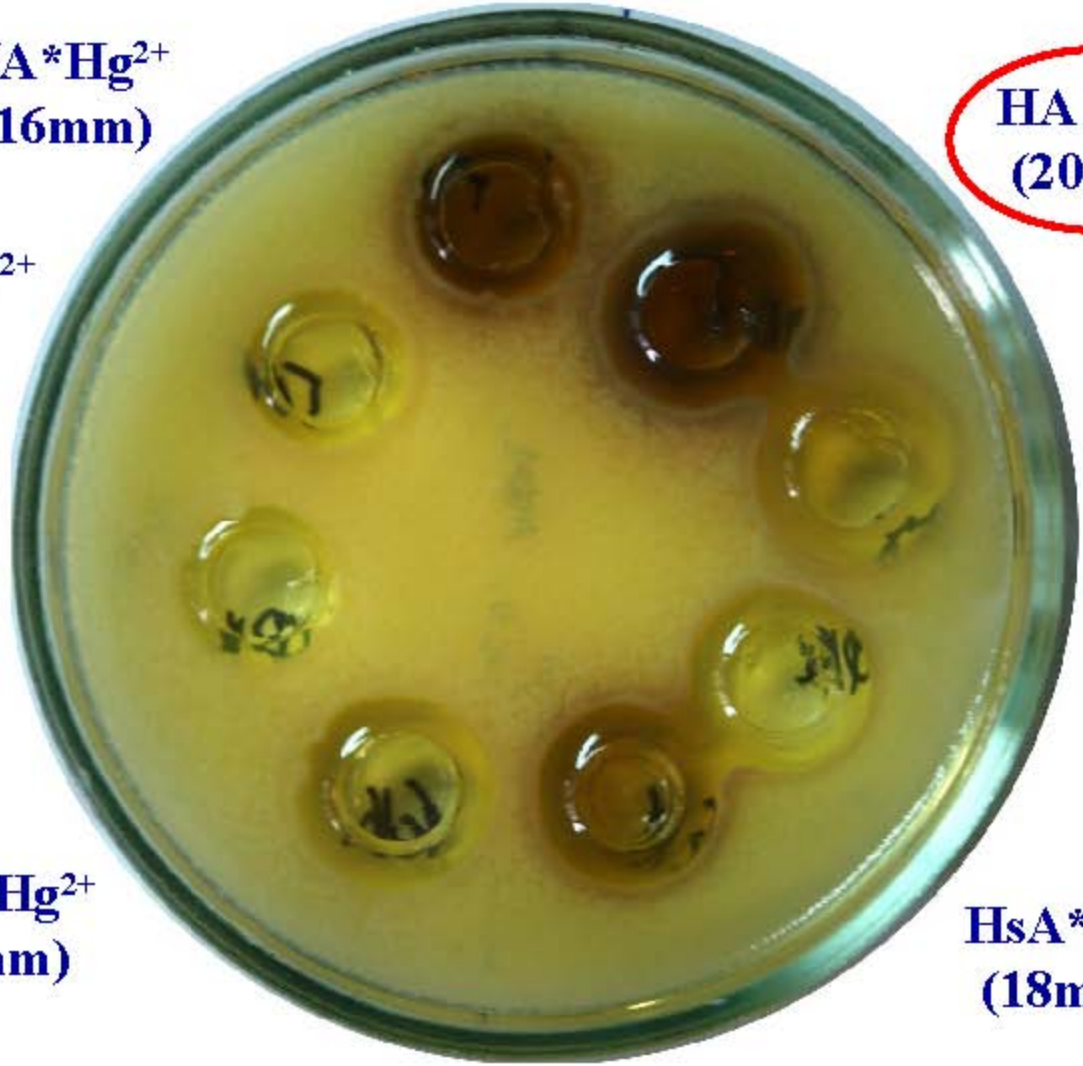
Heterogeneity of the experimental results, and a clear link of rendered effects of humic substances peloids with tinctorial properties of micro-organisms suggests that there is a "fulcrum" of peloids.

Results:

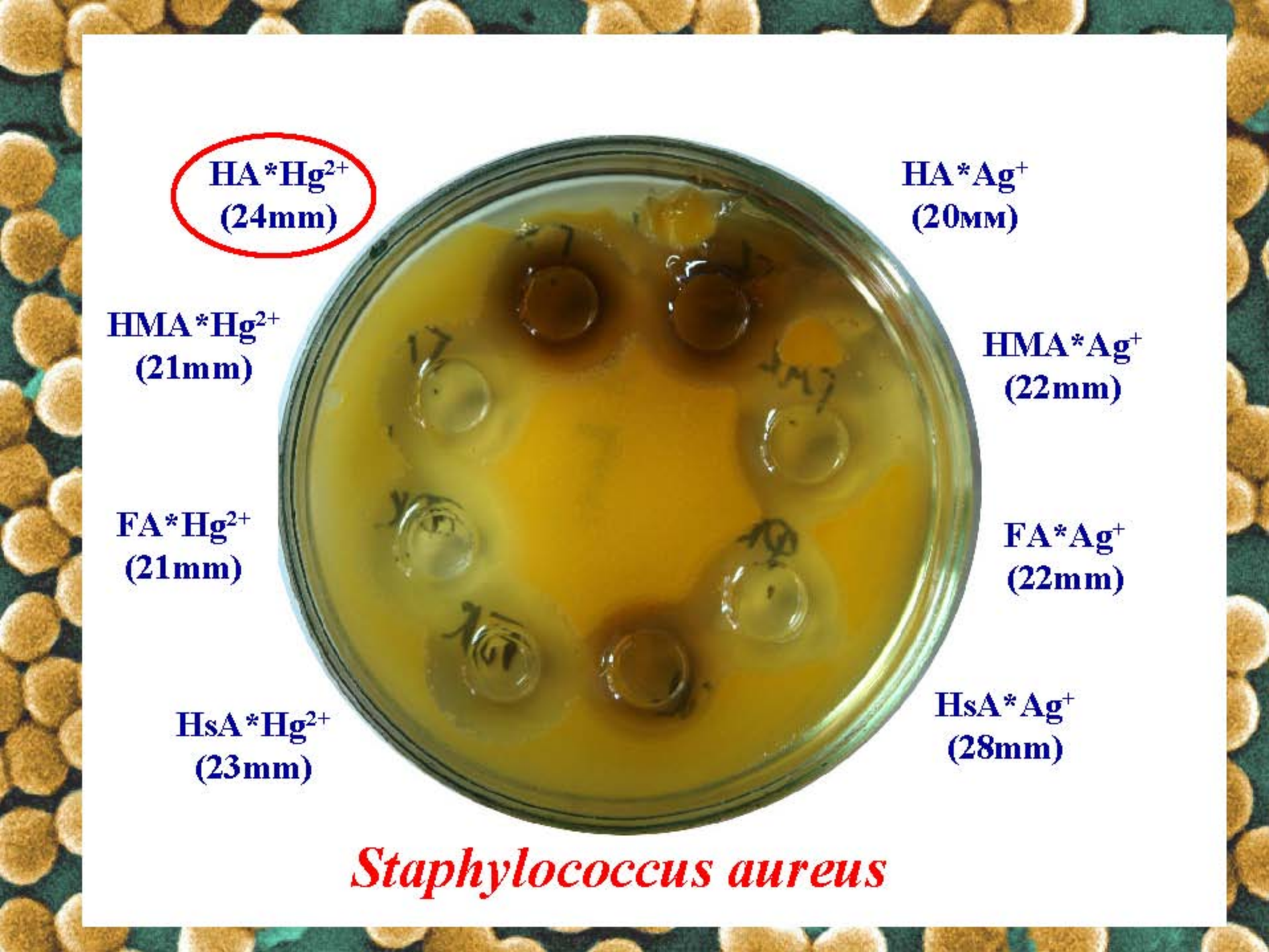
- The studied chelates of humic substances with cations of mercury (II), silver, iron (II) in all studied concentrations exhibit cytolytic activity to the test cultures of microorganisms.
- The humates of zinc and magnesium do not have cytolytic activity.
- The greatest antimicrobial effect of chelate complexes have 0,25% of humic substances: *E. coli* (zone growth suppression 20±0,1mm) – HsA*Hg2+; *Ps. aeruginosa* (20±0,1mm) – HA*Ag+; *St. aureus* (24±0,1mm) – HA*Hg2+; *B. subtilis* (16±0,1mm) – chelate complexes of humic substances with Ag+.

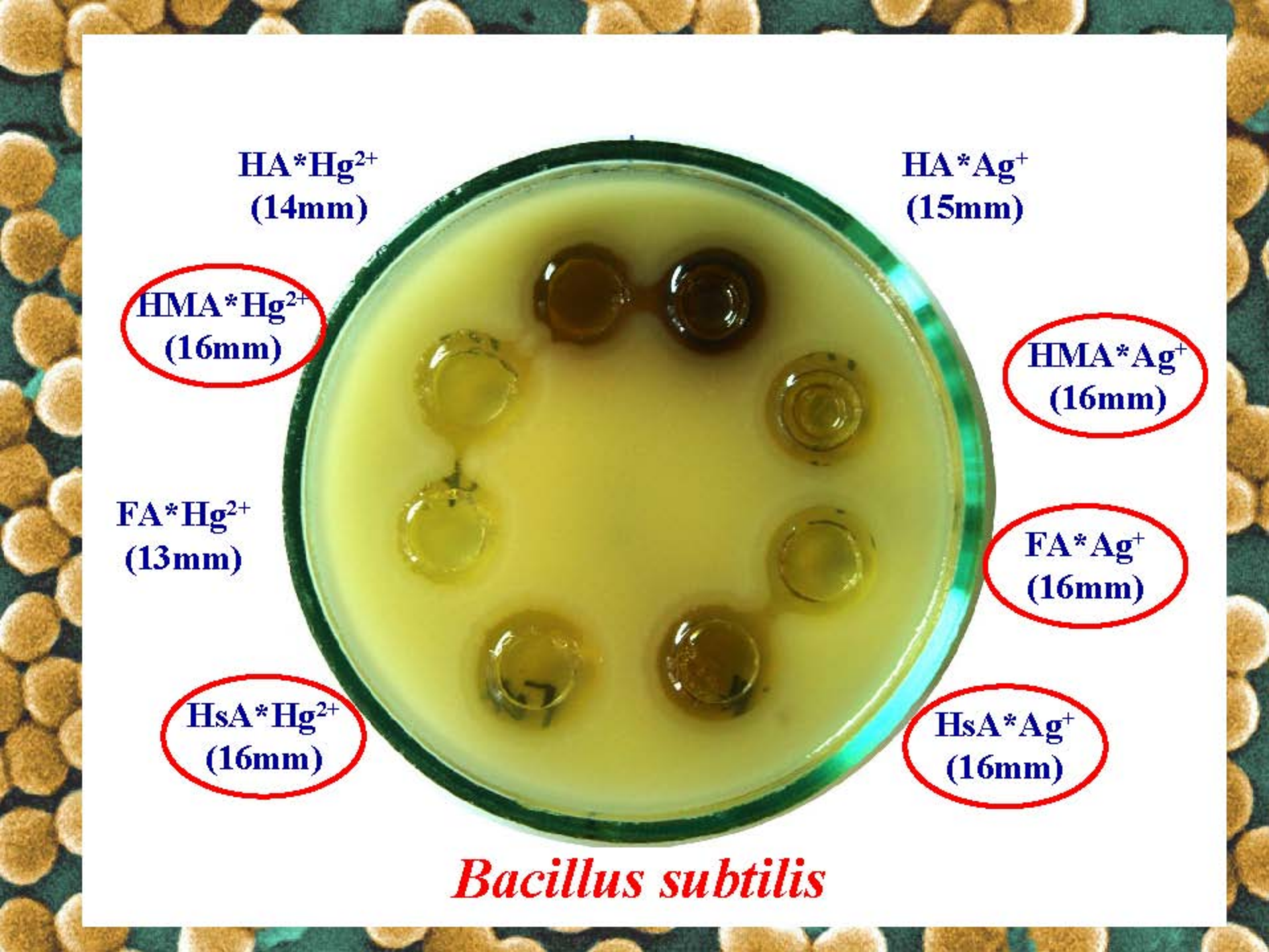


Escherichia coli



Pseudomonas aeruginosa


HA*Hg2+
(24mm)
HA*Ag+
(20мм)
HMA*Hg2+
(21mm)
HMA*Ag+
(22mm)
FA*Hg2+
(21mm)
FA*Ag+
(22mm)
HsA*Hg2+
(23mm)
HsA*Ag+
(28mm)
Staphylococcus aureus

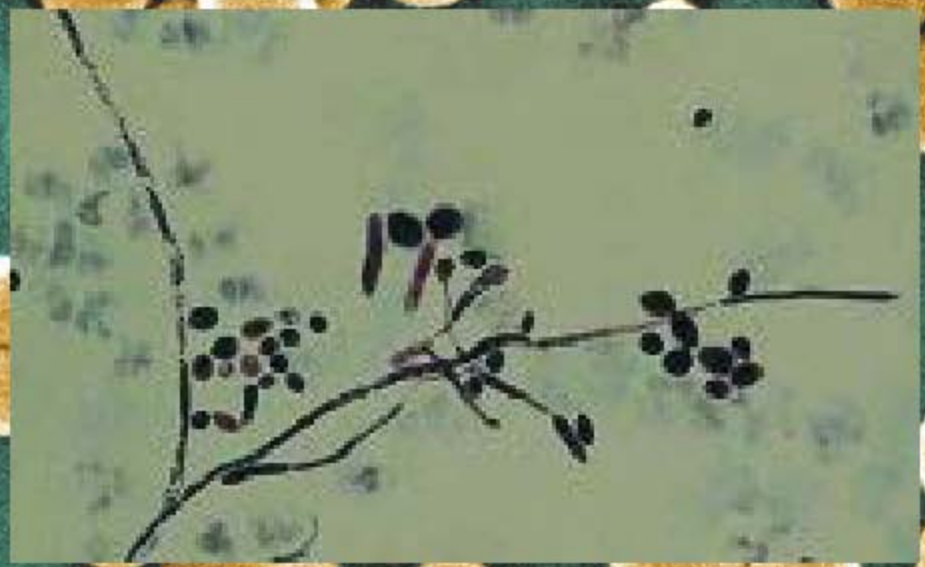

HA*Hg2+
(14mm)
HA*Ag+
(15mm)
HMA*Hg2+
(16mm)
HMA*Ag+
(16mm)
FA*Hg2+
(13mm)
FA*Ag+
(16mm)
HsA*Hg2+
(16mm)
HsA*Ag+
(16mm)
Bacillus subtilis

Stage 4

The study of antimycotic activity of drugs.

The test-cultures of microorganisms were used to determine the antimycotic activity of substances:

Yeast-like fungi – *Candida utilis* (LIA-01), *Candida albicans* (museum culture).



Results:

- The test samples of humic substances peloids exhibit cytolytic activity towards the growth of *Candida*. With the growth of fungi the area around the colonies is highlighted in yellow pigment – a product of metabolism of fungi humic acids.
- Humates, fulvates and hymatomelanates metals exhibit antimycotic activity towards *C. utilis* at all concentrations.
- Solutions of chelate complexes of humic acids with silver show the greatest fungicidal effect on *C. utilis* (25 ± 0,1 mm)

HMA (0,25%)

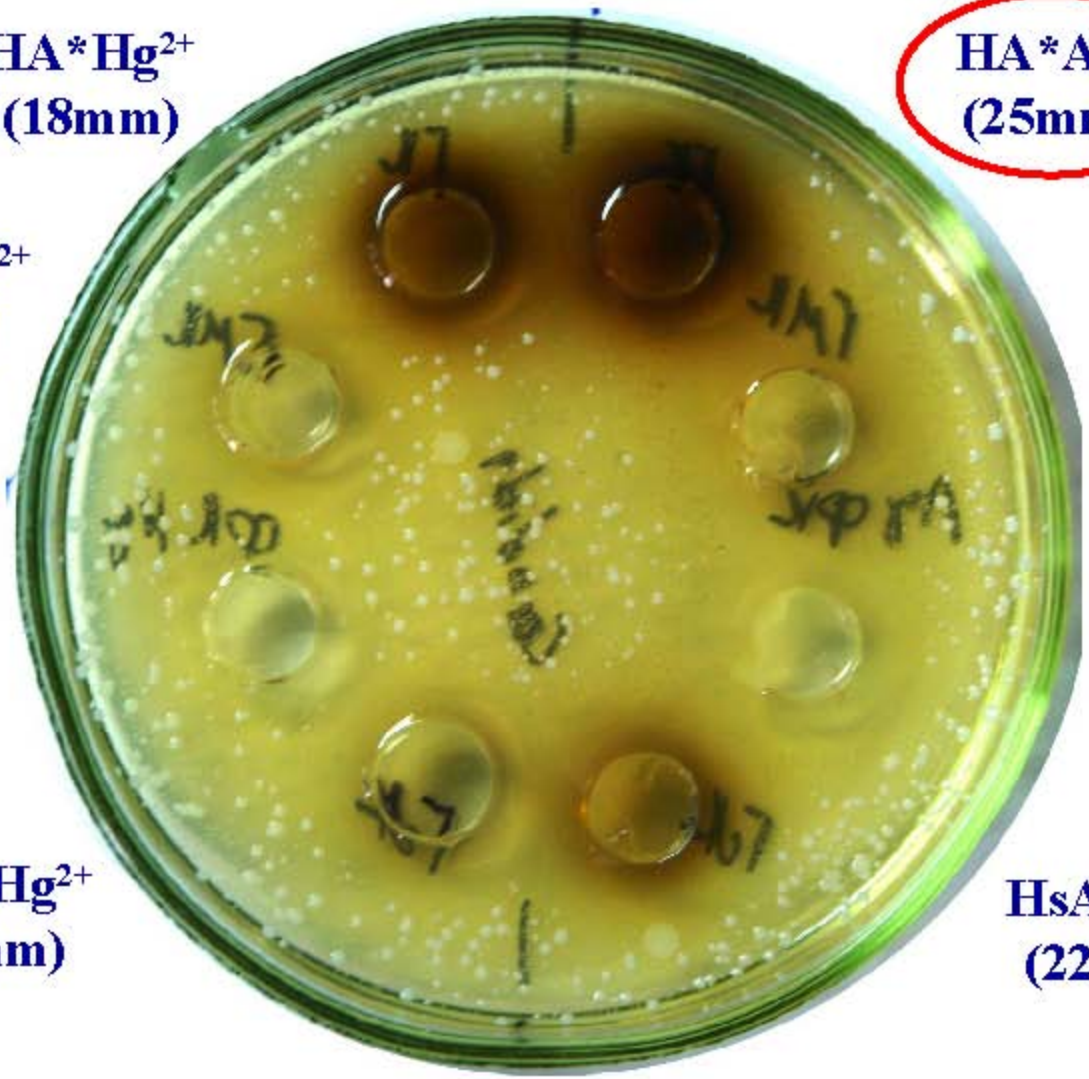
HA (0,25%)

HMA (0,1%)

HA (0,1%)

Yellow pigment - a product of the metabolism of humic acids

Candida utilis



Candida utilis

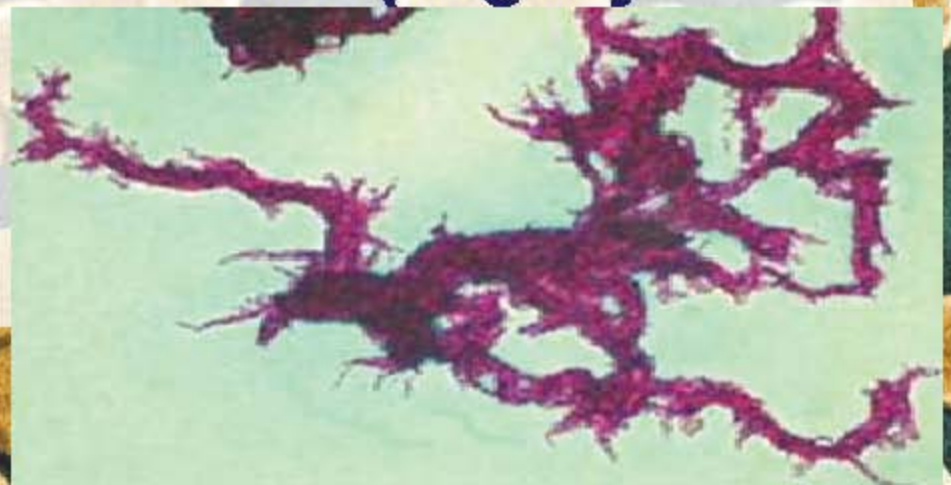
Stage 5

The study of antimikobakteritic (anti-TB) activity of drugs.

The test-cultures of microorganisms were used to determine the anti-TB activity of substances:

1) ***Mycobacterium tuberculosis*** **(H37Rv),**

2) ***Mycobacterium tuberculosis*** **(highly antibiotic-resistant strain (HRS) isolated from patients).**



Lowenstein-Jensen medium

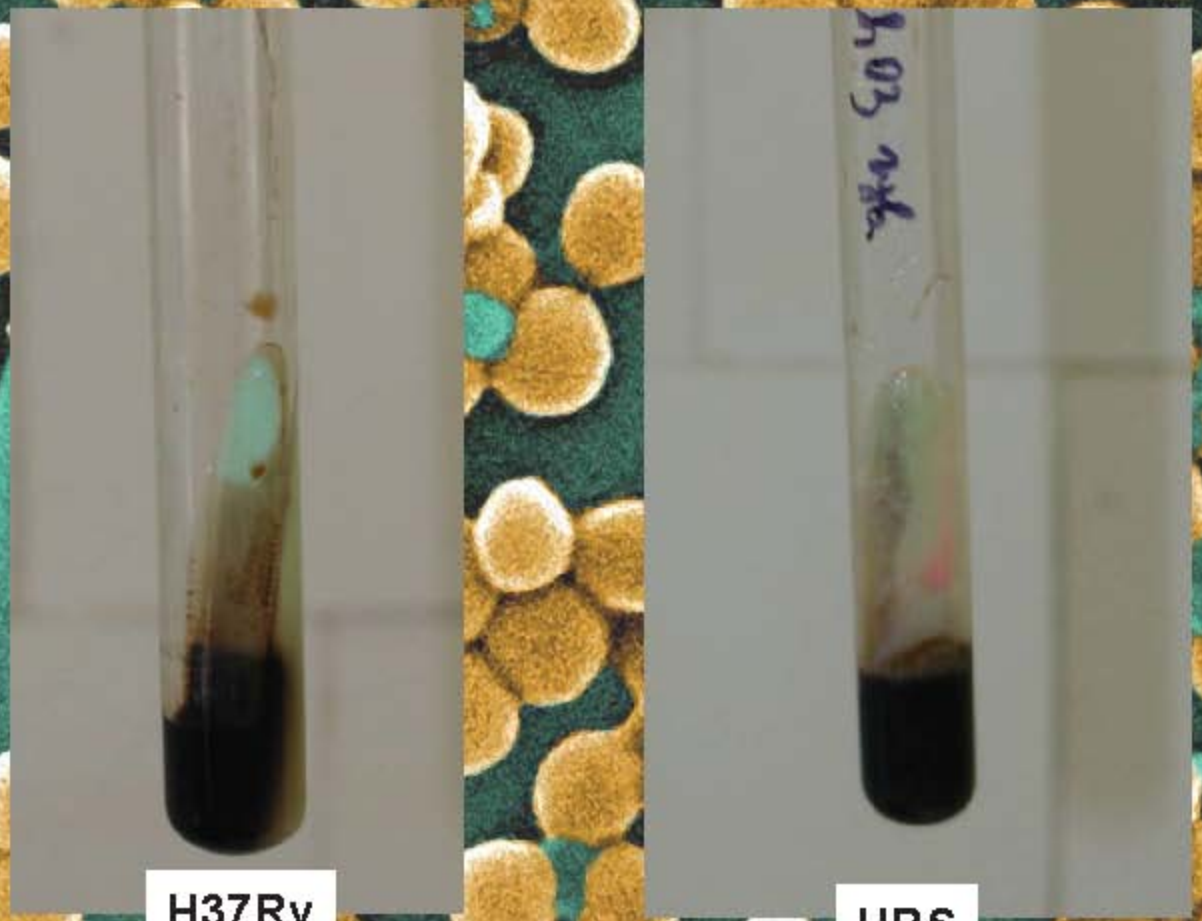
Composition, g per 600 ml H_2O

L-asparagine	3,6
Potassium dihydrogen	2,6
Magnesium sulfate	0,26
Magnesium citrate	0,6
Starch	30,0
Malachite green	0,4

Make 1000 ml of sterile egg masses

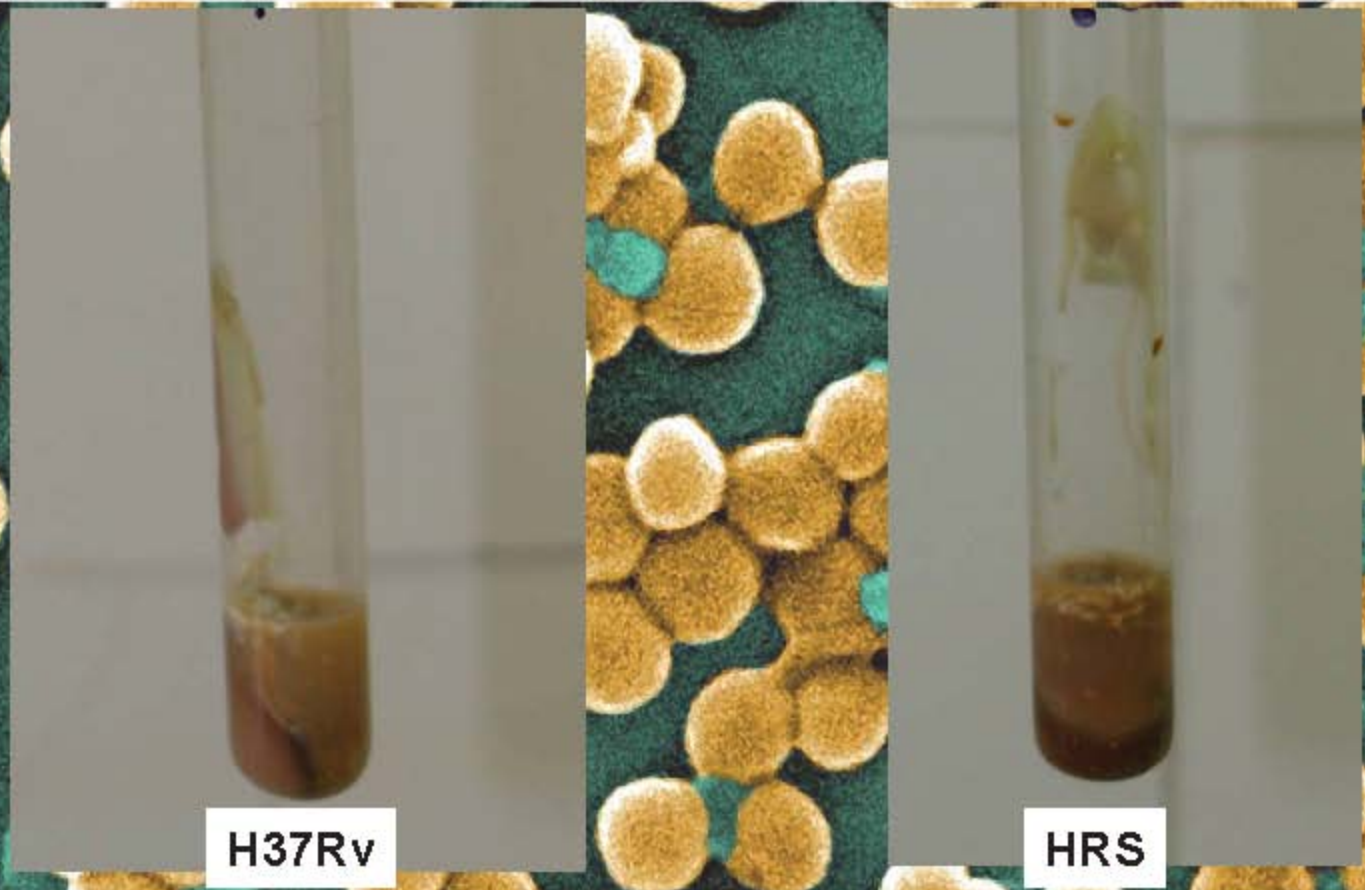
- During the study of antimikobakteritic (anti-TB) activity in control tubes without addition of humic substances peloids, the growth of *M. tuberculosis* H37Rv and the highly pathogenic strain were observed. (2+/5)

- In test tubes containing 1 ml of 1.6% humic acid an increase in *Mycobacterium tuberculosis* H37Rv and the highly pathogenic strain (4+/5) was observed. Interaction with the environment organoleptically was not observed.

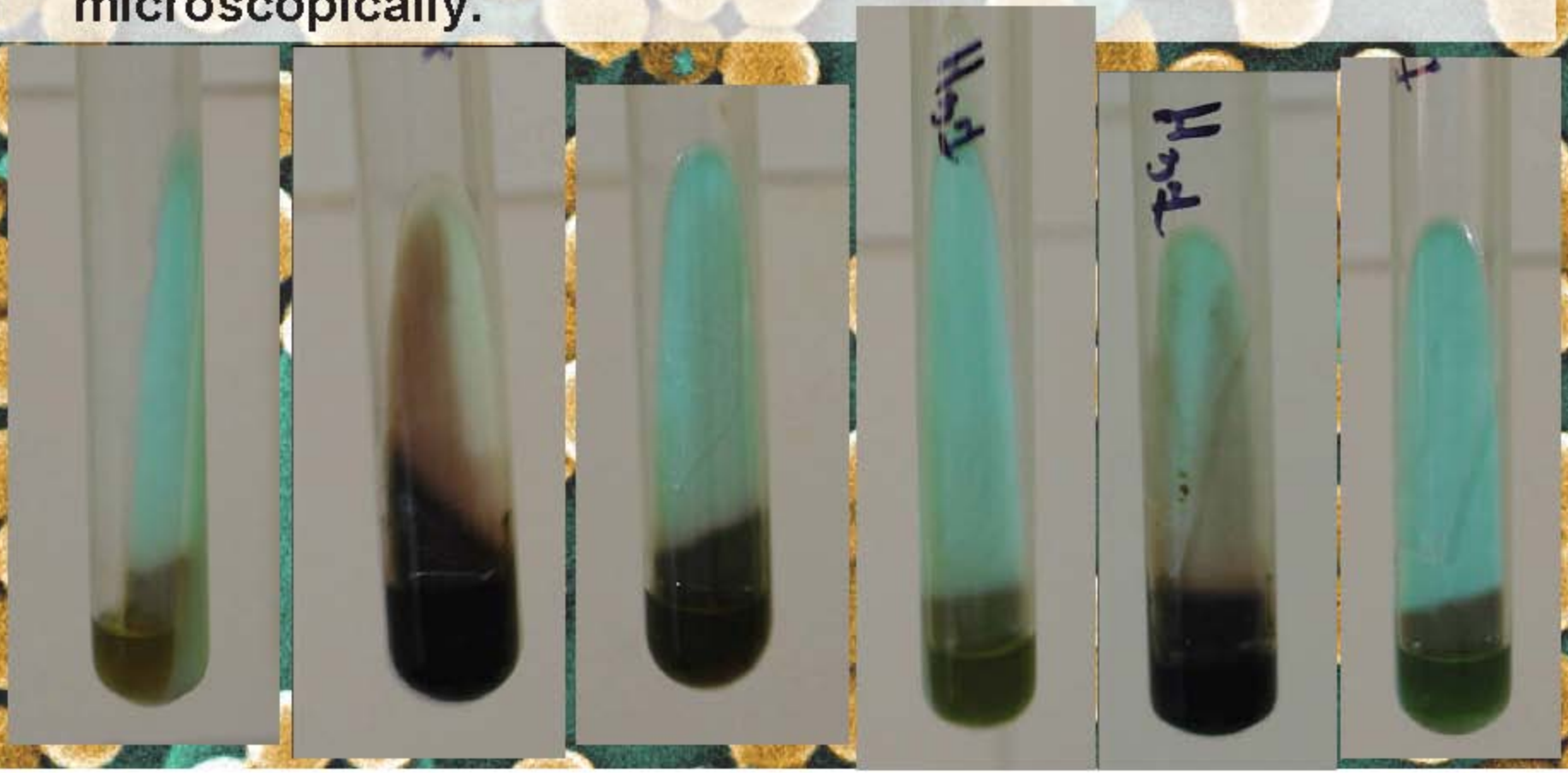


- In test tubes containing 1 ml of 1% of fulvic acids an increase in Mycobacterium tuberculosis H37Rv and the highly pathogenic strain (5+/5) was observed. Interaction with the environment was the discoloration of the medium.

- In test tubes containing 1 ml of 1% hymatomelanic acids an increase in Mycobacterium tuberculosis H37Rv and the highly pathogenic strain were observed. But due to loss of structural integrity of the environment (interaction with the environment) it is impossible to assess the criterion of growth.

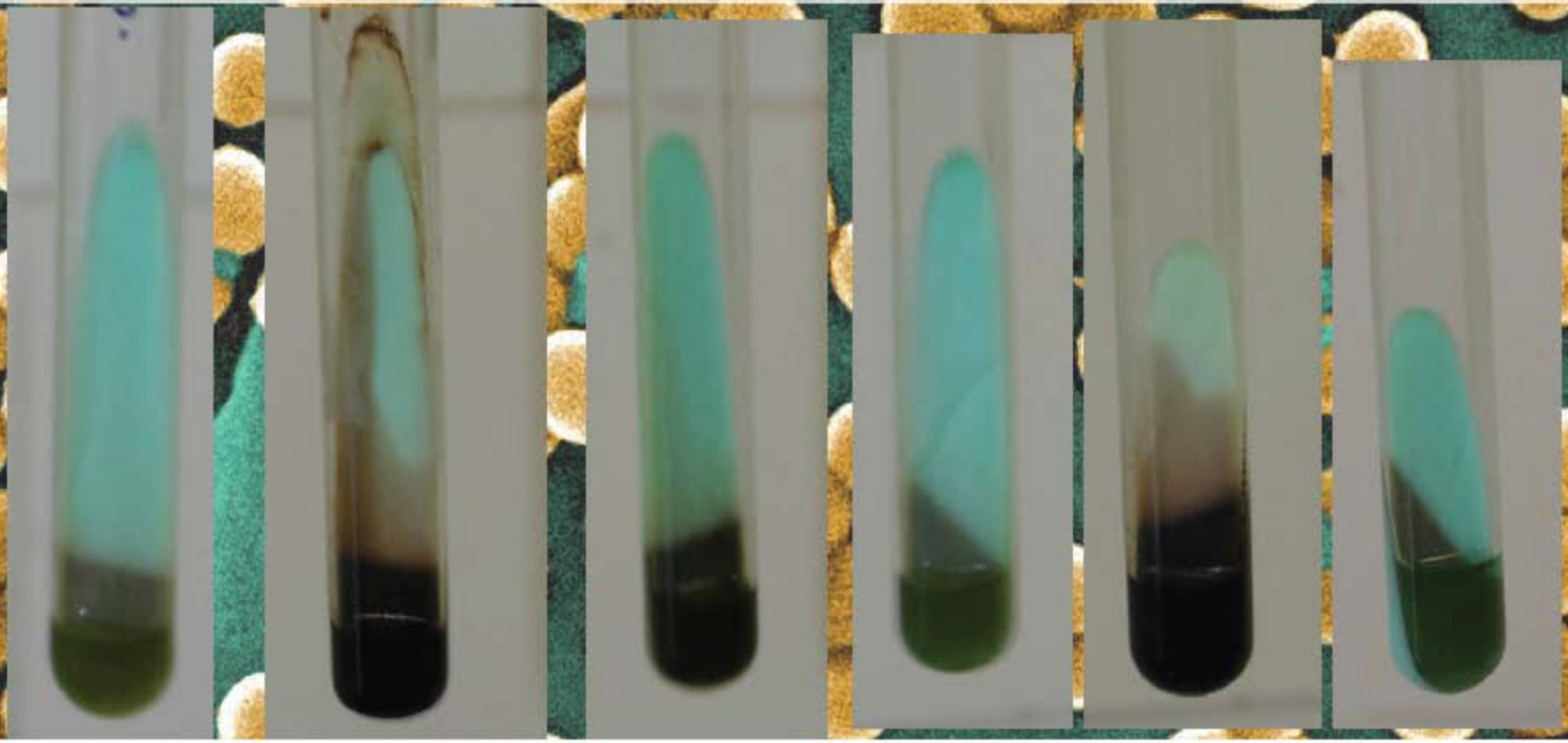


- In test tubes containing 1 ml chelates humic solid matter around with silver ions, and mercury (II) after 3 months of incubation, M. tuberculosis H37Rv growth was observed, the antimikobakteritic effect was confirmed microscopically.



FA*Ag+ HA*Ag+ HMA*Ag+ FA*Hg2+ HA*Hg2+ HMA*Hg2+

- In test tubes containing 1 ml chelates humic solid matter around with silver ions, and mercury (II) after 3 months of incubation, M. tuberculosis (highly antibiotic-resistant strain isolated from patients) growth was observed, the antimikobakteritic effect was confirmed microscopically.



FA*Ag+ HA*Ag+ HMA*Ag+ FA*Hg2+ HA*Hg2+ HMA*Hg2+

Conclusions:

- Humic substances low mineralized silt sulphide muds are the optimal components for further elaboration on their basis of the selective and differential-diagnostic nutrient medium for the mycobacteria and other pathogenic and opportunistic microorganisms.
- Chemotherapeutic study of chelate complexes of humic peloids substances characterize them as a promising substance for further development based on these broad-spectrum antimicrobial action, including anti-TB drugs of natural origin.

- Further work will be done in the direction of studying the spectrum of humic peloids on several other microorganisms – pathogenic streptococcus, HIV, HAV, HBV, HCV, etc., as well as the study of the immunogenicity of humic substances.
- We plan to develop applique drugs on the basis of chelate complexes of humic peloids sequence for the treatment of infections, including long nonhealing wounds, not responding to treatment by traditional medicines during several months. The prevalence and availability of raw materials contributes to their low cost, which has socio-economic importance.

Thank you for your attention!