AGRI-KNOWS

Knowledge Transfer in Agriculture as Added Value in Environment Protection

Javni razpis/ AGRI - KNOWS/ Prenos znanja v kmetijstvu kot dodana vrednost pri zaščiti okolja

Javni razpis/ AGRI - KNOWS/Trasferimento delle conoscenze in agricoltura come valore aggiunto per la tutela dell'ambiente

Public procurement/ AGRI - KNOWS/ Knowledge transfer in agriculture as added value to environment protection

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Translated by Klara Levstek

Šempeter pri Gorici, 28.2.2014

2007-2013

cooperazione territoriale europea
programma per la cooperazione transfrontaliera
Italia-Slovenia

evropsko teritorialno sodelovanje
program čezmejnega sodelovanja
Slovenija-Italija

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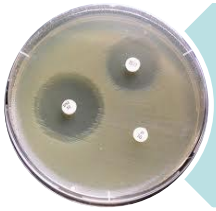
Projekt sofinancira Evropski sklad za regionalni razvoj

THE PURPOSE OF THE RESEARCH

The purpose of the research was to see how susceptible the microorganisms are to penicillin. For this purpose we used antibiogram.

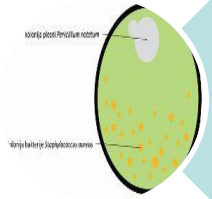
PROCEDURE



ANTIBIOGRAM

PREPARING SOIL AND WATER SAMPLES

INOCULATION OF WATER AND SOIL MICROORGANISMS ON SOLID MEDIA



COUNTING COLONIES ON INDIVIDUAL GROWTH MEDIA

I. ANTIBIOGRAM

- *Antibiogram is a method which is used for defining bacterial susceptibility to antibiotics or their resistance to them.*

- *The substances used in that method are chemotherapeutics, which destroy microorganisms. Antibiotics are one type of them.*

We distinguish 3 antibiograms:

ANTIBIOGRAM BASED ON DISC DIFUSION METHOD
(Antibiotic discs or straps are applied on a solid culture medium.)

ANTIBIOGRAM BASED ON DILUTION METHOD *(Bacterial growth is observed in a series of liquid media with different antibiotic concentrations.)*

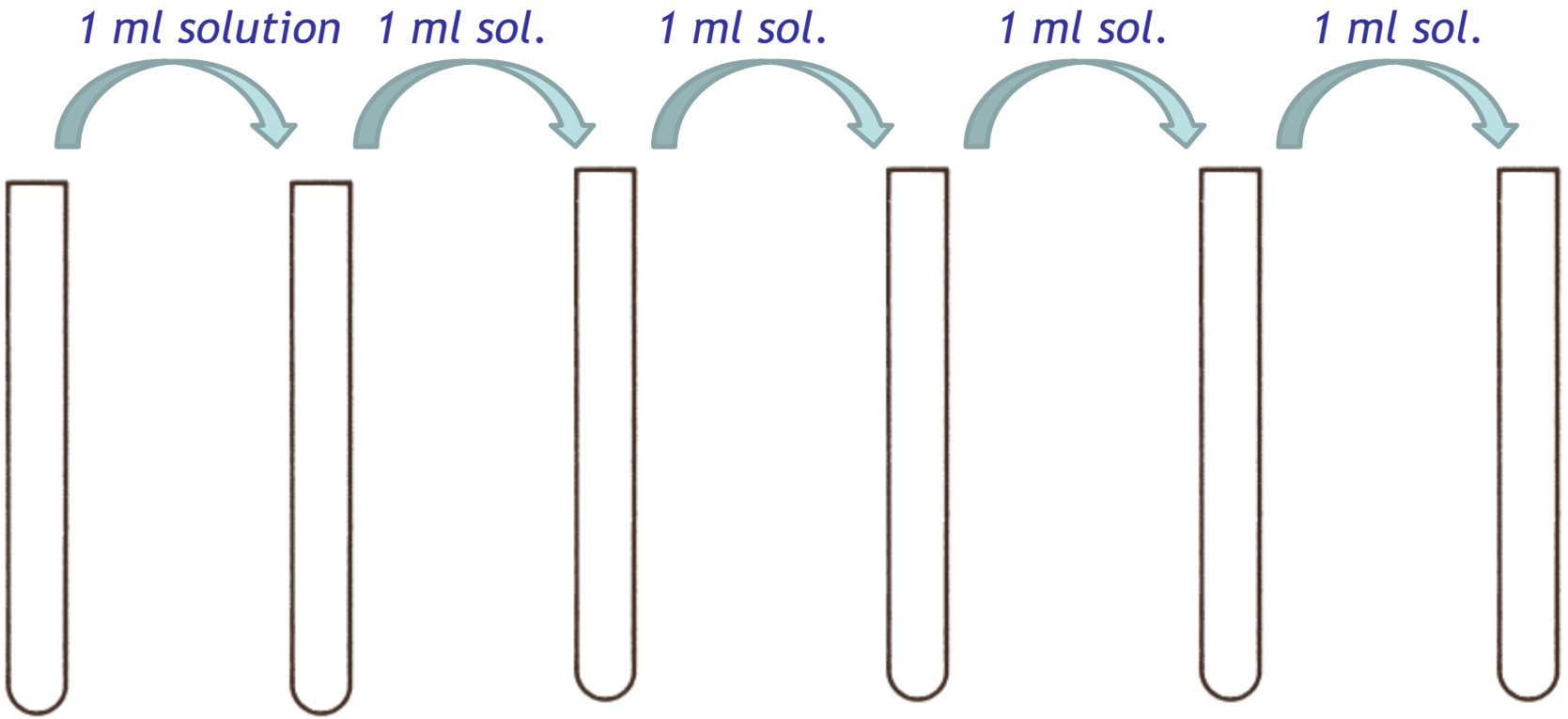
CUMULATIVE ANTIBIOGRAM *(Different bacteria are inoculated on a bacterial growth medium, which contains an antibiotic.)*

Procedure:

-In a sterile Erlenmeyer flask we prepared a soil suspension (10 g soil + 90 ml sterile physiological solution).

-We shook the suspension on a chemical shaker for 1 hour.

-In test tubes, we prepared 6 different penicillin solutions: from 10^{-1} *to* 10^{-6} *.*

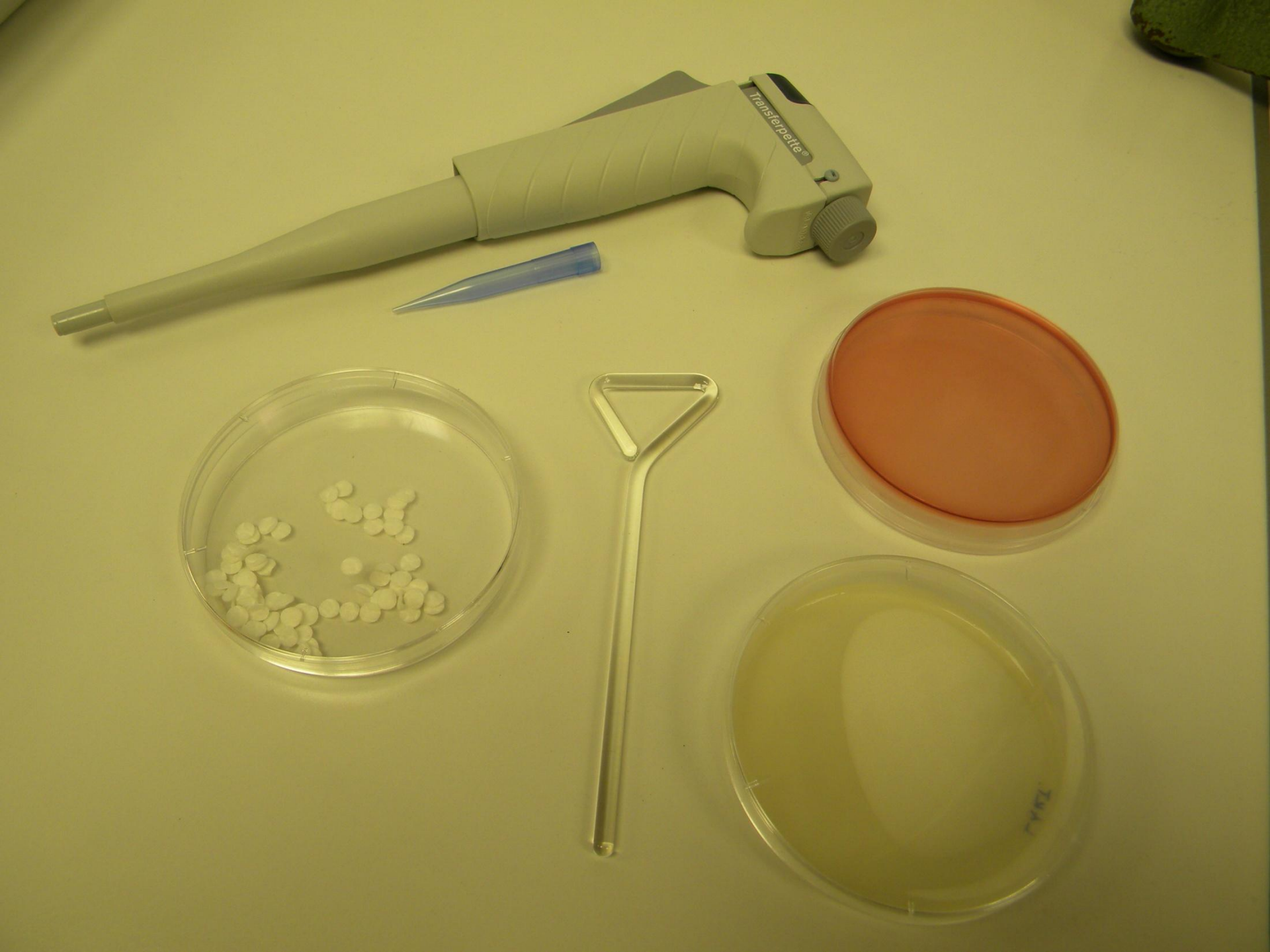


1 ml penicillin

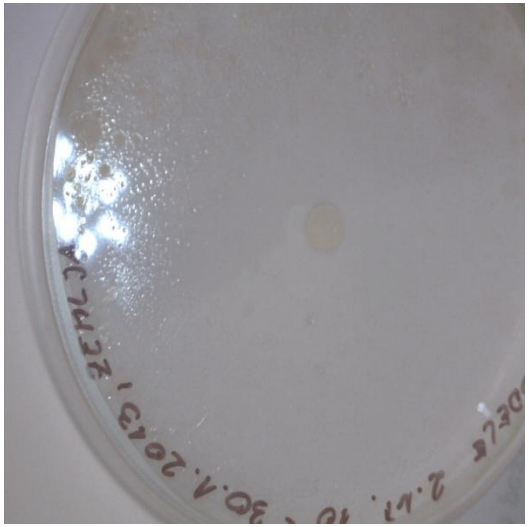
+9 ml sterile physiological solution

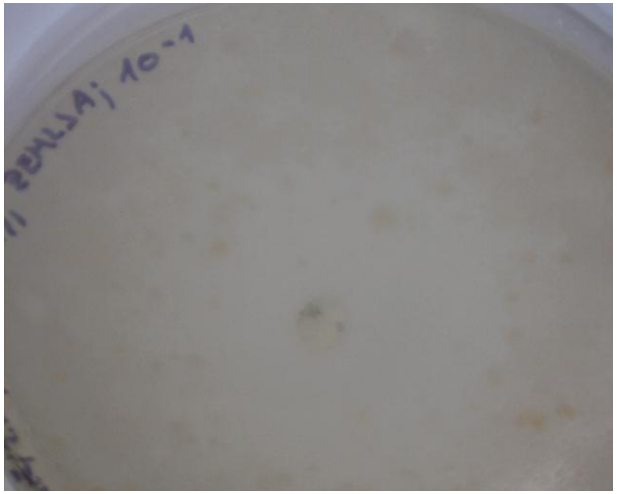
10^{-1}

- *With a pipette, we applied 0.1 ml soil suspension on each of the 6 media.*
- *Using a spatula, we spread the soil sample evenly over the whole surface of a petri dish. Afterwards we dried it by the fire for 5 minutes.*
- *Using tweezers, we put into each petri dish a disc, which had been soaked in one of the different penicillin solutions before.*


Transferpette®

Results:





CHOOSING A GROWTH MEDIUM

GROWTH MEDIA are substrates for growing microorganisms in lab conditions.

We distinguish different culture media:

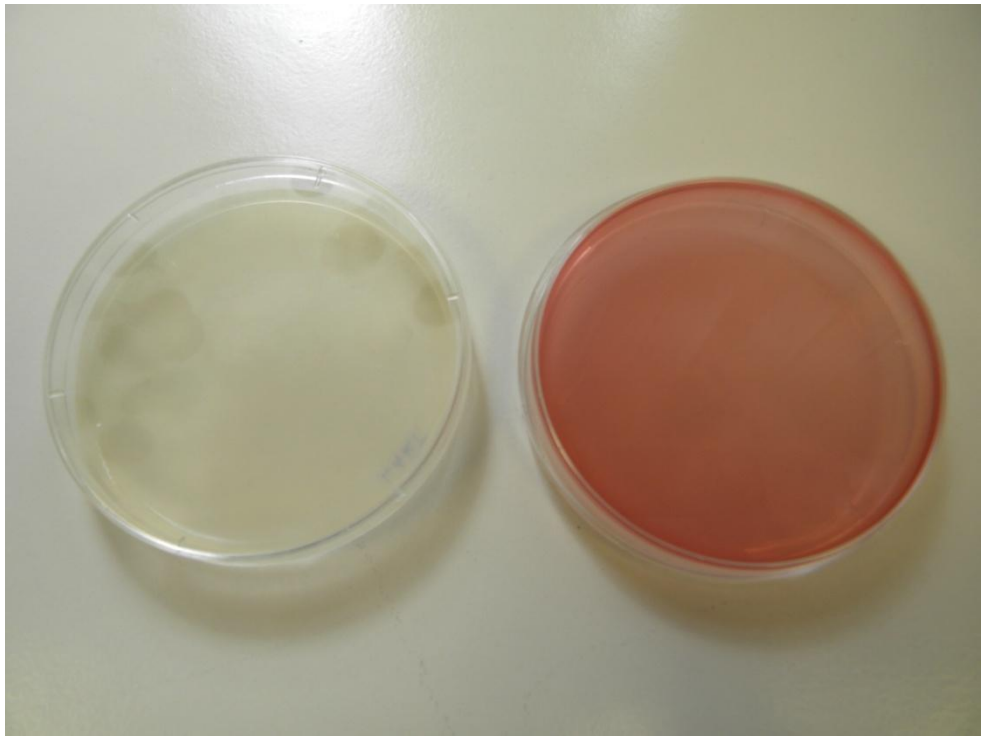
- *COMPLEX MEDIA: They are rich complex media where most heterotrophic bacteria can grow.*

- *SELECTIVE MEDIA: They contain ingredients that encourage the growth of specific bacteria and inhibit the growth of others.*

- *In our research we used two types of growth media:*

a) nutrient broth (PKE)

b) MacConkey Agar
(a selective growth medium for coliform bacteria)




1.05465.
Microbiology
MacCONKEY-Agar
MacCONKEY agar
Agar MacCONKEY
Agar MacCONKEY
MERCK
HRANILNI
AGAR
Biolife
NUTRIENT AGAR

Materials:

- a soil or water sample
- A sterile medium
- An inoculation loop
- A lab burner

How are microorganisms inoculated on a medium?

All microbiological tests must be done aseptically.

This means that we deal with all contaminants and bacterial cultures in such a way that there is no invasion of undesired microorganisms. At the same time we take care not to spread any microorganisms.

Glassware, objects and utensils as well as growth media have to be sterile.

We use the flame from a gas burner.

We flame sterilize the inoculation loop and glassware.

II. INOCULATION OF MIKROORGANISMS ON SOLID MEDIA (WATER)

This experiment was done out to see how much penicillin is leached into water.

1.

- Making columns from plastic tubes, filling them with soil

2.

- Polluting the columns with penicillin and pouring rainwater over

3.

- Inoculating microorganisms from rainwater on a solid medium, counting colonies, analyzing all samples

4.

- Inoculating microorganisms from soil on a solid medium, counting colonies, analyzing all samples

1. Making the columns

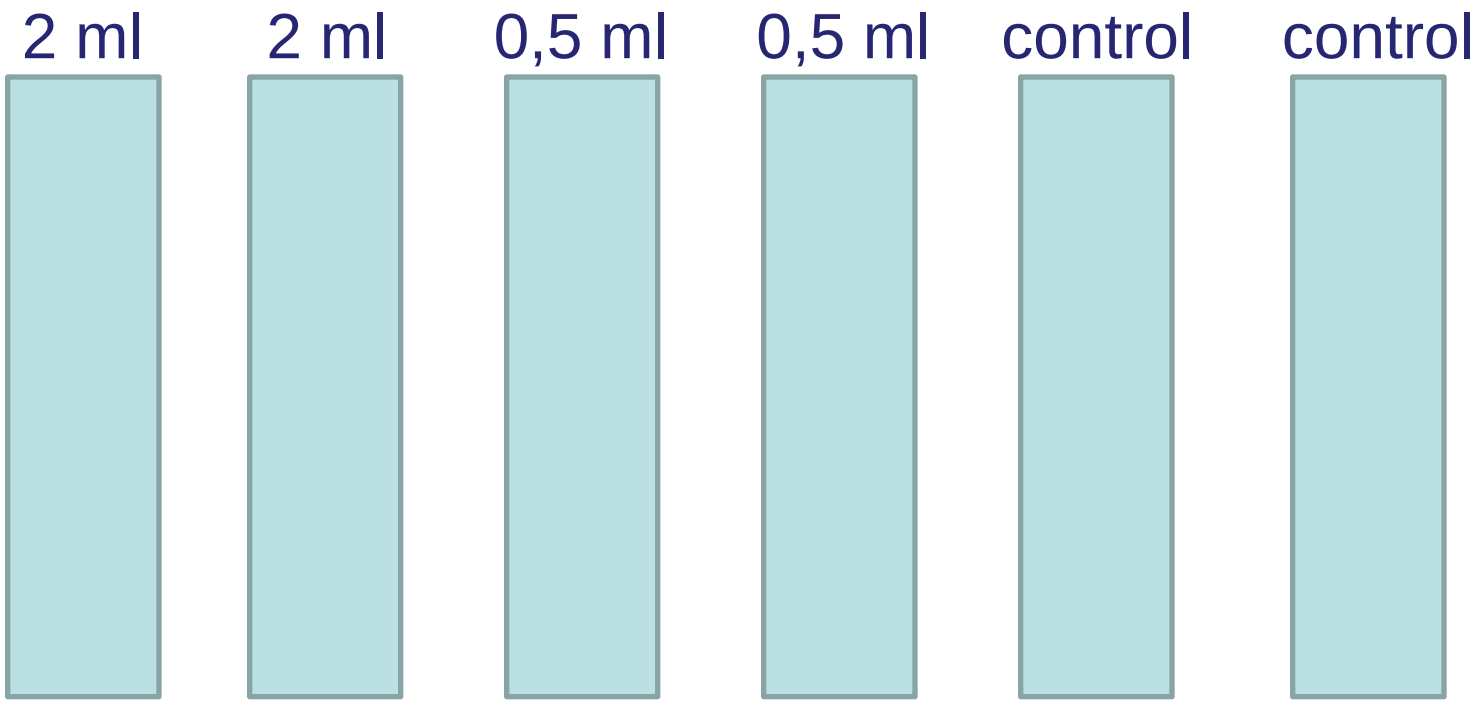
PROCEDURE

- We took six columns (plastic tubes): 10 cm in diameter, 3 mm thick and 50 cm high. All the columns had a cone shape on the bottom.

- At the bottom of the column, we place a piece of plastic net and pour Over 40 ml course sand. Then we add another piece of plastic net and cover it with 50 ml fine sand.

- Afterwards we gradually filled in the columns with 4 kg soil, which we had taken from our school green house. After each kilo we stopped and compressed the soil. Then we continued.
- Then we attached a plastic hose to the conical bottom of the columns. The hose was about 150 cm long.
- Finally, we poured water over the columns. We had to make sure that the water height in the column and in the hose was 60 cm. In that way we reached the same pressure. After two days we released the water out of the hose.

2. Polluting the columns with penicillin



3. Preparing water samples

- Into each column, we poured 400 ml rainwater. This rainwater went through the soil and slowly flowed out through the hose.

-We collected that water in a measuring cup. Then we analyzed it.

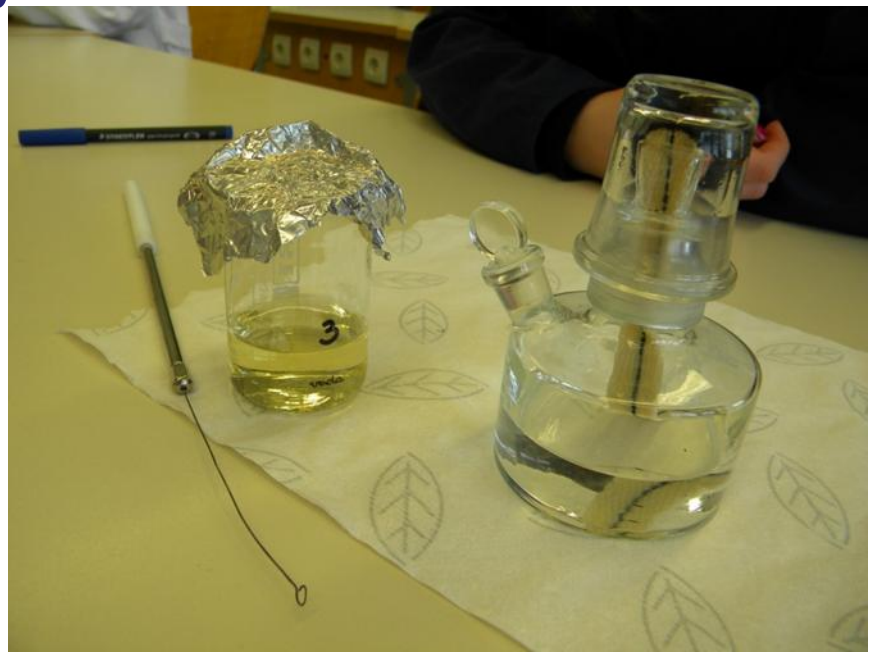
- We repeated the procedure 4 times.

4. Inoculating microorganisms on the medium

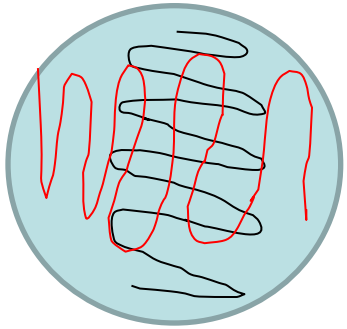
- We used the flame from a gas burner. We flame sterilized the inoculation loop and glassware.

- We regularly cleaned the worktop with disinfectants.

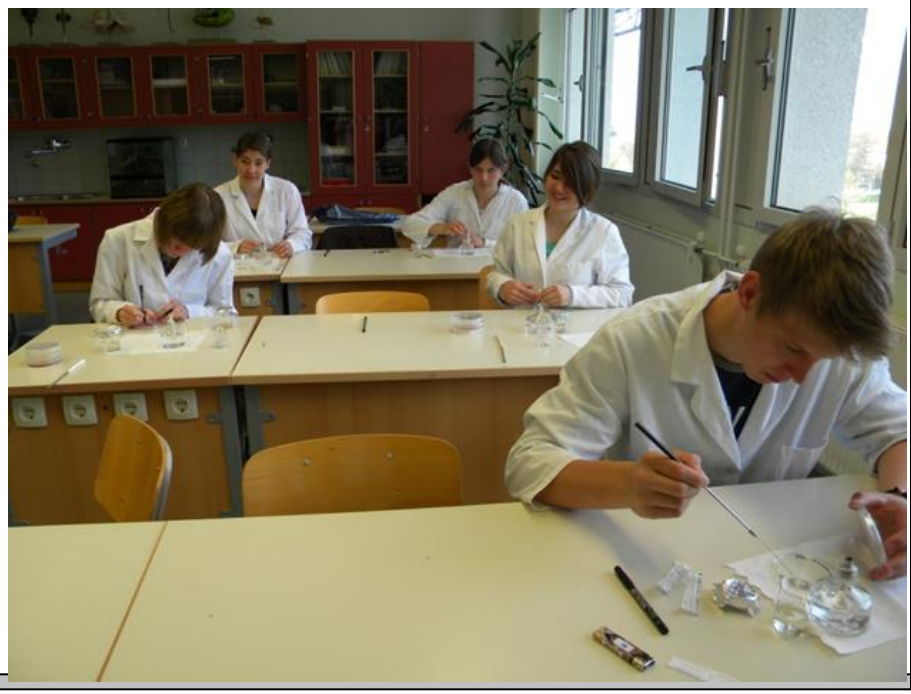
- We inoculated microorganisms from rainwater on both growth media, using a flame sanitized inoculation loop.



- We took one drop of water aseptically on the growth medium.



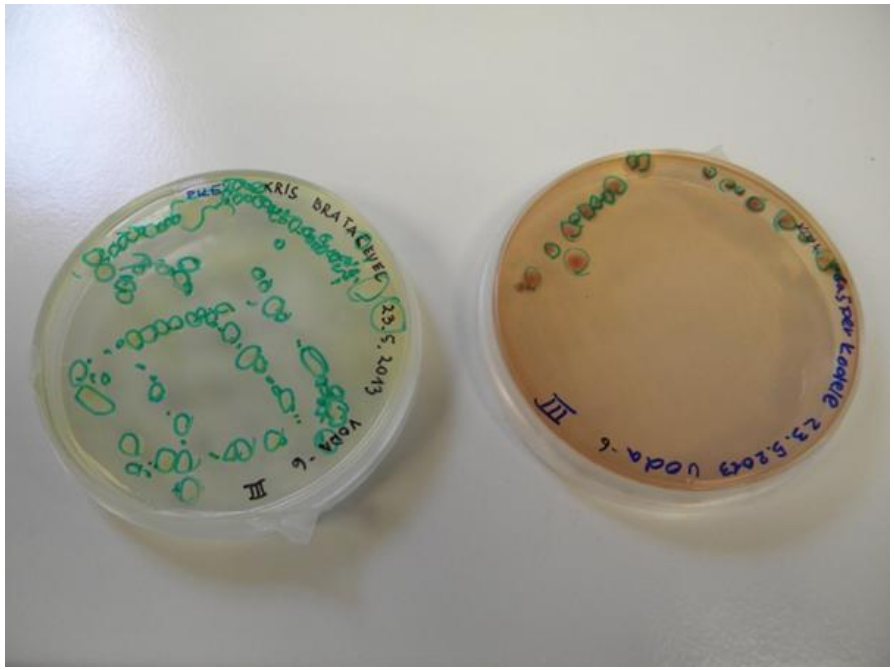
- We spread the water drop over the petri dish with wavy movements.
- Afterwards we flame sterilized the inoculation loop until it started glowing.
- Additionally, we applied another water drop on the growth medium and made a wavy line, which was perpendicular to the first one.
We got a twisted pattern.



5. The growth of microorganisms:

- We incubated the growth medium at 28 °C.

6. Results:



III. INOCULATION OF MICROORGANISMS ON SOLID GROWTH MEDIA (SOIL)

This experiment was carried out in order to see how many antibiotics stayed in soil.

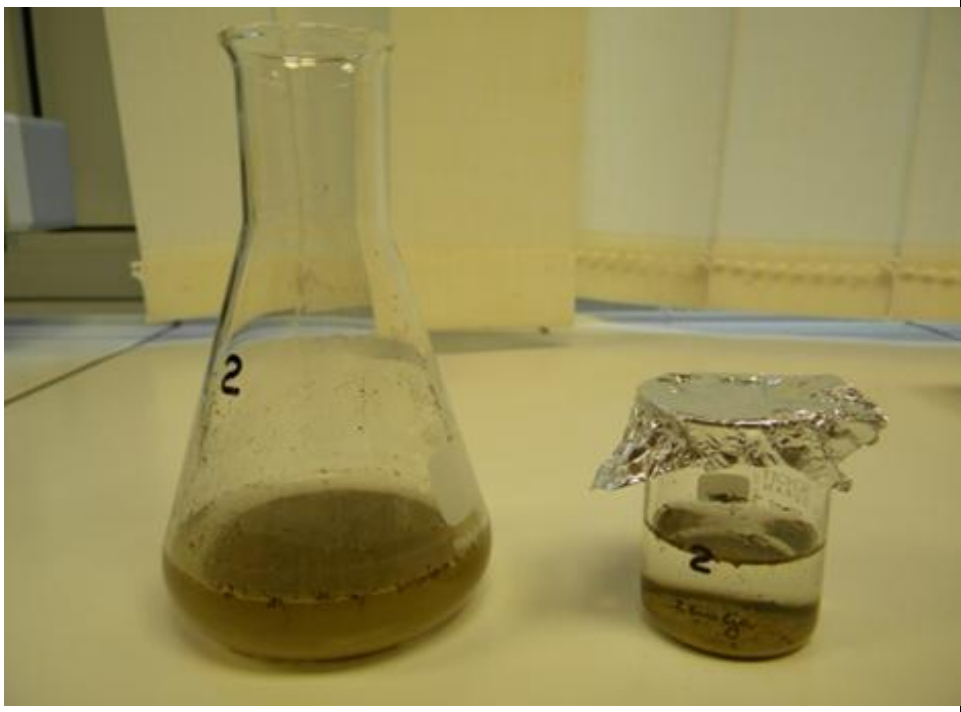
1. Preparing a soil sample

- In an Erlenmeyer flask we weighed 10 g soil and poured over 90 ml sterile physiological solution.

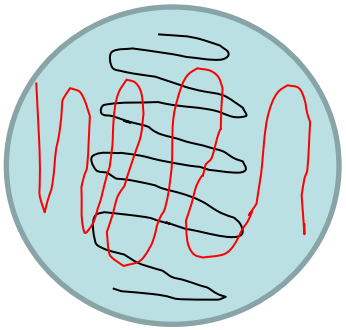
- We put the Erlenmeyer flask on a chemical shaker at speed 2 for one hour.

2. Inoculating microorganisms on the medium

-From that suspension we inoculated microorganisms on both growth media, using a flame sanitized inoculation loop.



- We spread the drop over the petri dish with wavy movements.
- Afterwards we flame sterilized the inoculation loop until it started glowing.
-Additionally, we applied another water drop on the growth medium and make a wavy line, which was perpendicular to the first one.

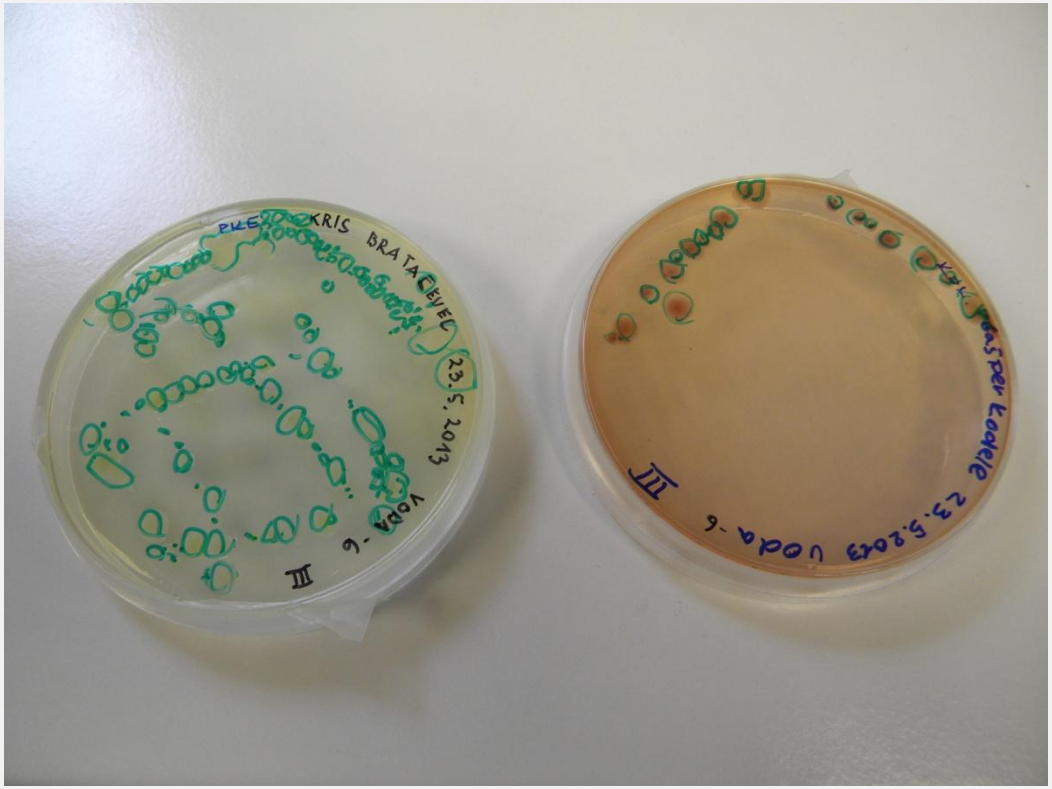


3. The growth of microorganisms:

- We incubated the growth medium at 28 °C.

4. Counting colonies

- The number of colonies which grew on each medium



IV. RESULTS

NUMBER	DATE OF WATER ANALYSIS	DATE OF SOIL ANALYSIS
I.	18/4/2013	18/4/2013
II.	26/4/2013	
III.	23/5/2013	
IV.	28/5/2013	28/5/2013

	1ST COLUMN		2ND COULMN		3RD COULMN		4TH COULMN		5TH COULMN		6TH COULMN	
	PKE	KOLI	PKE	KOLI	PKE	KOLI	PKE	KOLI	PKE	KOLI	PKE	KOLI
I.	243	46	195	80	1O1	14	108	10	167	9	131	9
II.	200	uncountable	153	141	135	26	141	19	157	8	uncountable	8
III.	71	87	81	102	155	60	176	45	160	12	131	12
IV.	uncountable	174	uncountable	uncountable	uncountable	176	uncountable	uncountable	uncountable	22	uncountable	22

AGRI-KNOWS

Prenos znanja v kmetijstvu kot dodana vrednost v varovanju okolja

Šempeter pri Gorici, 28.2.2014

Hvala za vašo pozornost!

Grazie per l'attenzione!

Thank you for your attention.

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Projekt [AKRONIM/NASLOV PROJEKTA] je sofinanciran v okviru Programa čezmejnega sodelovanja Slovenija-Italija 2007-2013 iz sredstev Evropskega sklada za regionalni razvoj in nacionalnih sredstev

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