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

PROJECT:

PENICILLIN INFLUENCE ON THE GROWTH OF MICROORGANISMS IN WATER AND SOIL

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1. ANTIBIOGRAM

1.2. INTRODUCTION

An antibiogram is a method which is used for determining 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.

Types of antobiograms:

- **ANTIBIOGRAM BY THE DISC DIFFUSION METHOD** (applying antibiotic discs or straps)
- **ANTIBIOGRAM BASED ON DILUTION** (bacterial growth in a series of liquid bacterial growth media with different antibiotic concentrations)
- **CUMULATIVE ANTIBIOGRAM** (inoculating different bacteria on a bacterial growth medium, which contains an antibiotic)

1.2. CHEMICAL TEST PURPOSE

The purpose of the research was to realize the antibiogram use and to determine the susceptibility of microorganisms to penicillin.
The antibiogram used in the research was based on dilution, whereby different penicillin concentrations were applied.

1.3. PREPARING DIFFERENT PENICILLIN CONCENTRATIONS

1.3.1. MATERIALS

- test tubes,
- test tubes stands,
- an Erlenmeyer flask,
- a chemical shaker,
- a soil sample,
- penicillin,
- a micropipette (1 ml, 0.1 ml),
- a spatula,
- a bacterial growth medium.

1.3.2. PROCEDURE

- In a sterile Erlenmeyer flask, we prepare a soil suspension (10 g soil + 90 ml sterile physiological solution).
- We have the soil suspension stirred on a chemical shaker for 1 hour.
- In test tubes, we prepare 6 different penicillin solutions: from 10^{-1} to 10^{-6}.
- With a pipette, we apply 0.1 ml soil suspension on each of the 6 bacterial growth media.
- We spread the samples evenly over the whole surface of a petri dish and let it dry near the fire for 5 minutes.
- Using tweezers, we put into each petri dish a disc, which has preliminarily been soaked in one of the different penicillin solutions.

2. INOCULATION OF MICROORGANISMS FROM SOIL ON SOLID BACTERIAL GROWTH MEDIA

2.1. PREPARING TWO BACTERIAL GROWTH MEDIA

Bacterial growth media are substrates for growing microorganisms in lab conditions. There are different types of bacterial growth media:

a. Complex bacterial growth media (rich and complex culture media where most heterotrophic bacteria can grow)
b. Selective bacterial growth media (They contain ingredients that encourage the growth of specific bacteria and inhibit the growth of others.)

In the research, we used two different culture media: nutrient broth (PKE) and MacConkey Agar (a selective bacterial growth medium for coliform bacteria).

All microbiological tests must be done aseptically. This means that all contaminants and bacterial cultures are dealt with in such a way that there is no invasion of undesired microorganisms and, at the same time, no spreading of microorganisms. Glassware, objects and utensils as well as bacterial growth media have to be sterile.

We use flame from a gas burner to flame sterilize the inoculating loop and the glassware.

2.2. PREPARING A SOIL SUSPENSION

2.2.1. MATERIALS

- a soil sample,
- sterile bacterial growth media (PKE and MacConkey Agar),
- an inoculating loop,
- a gas burner,
- an Erlenmeyer flask,
- a chemical shaker,
- scales,
- a spatula,
- physiological solution.

2.2.2. PROCEDURE

- In an Erlenmeyer flask we weigh out 10 g soil and pour it over with 90 ml sterile physiological solution.
- We put the Erlenmeyer flask on a chemical shaker at speed 2 for 1 hour.
- We regularly clean the worktop with disinfectants.
- We inoculate microorganisms from the soil suspension on both bacterial growth media, using a flame sterilized inoculating loop.

2.2.3. RESULT

We count the colonies which have grown on each culture medium.

3. INOCULATION OF MICROORGANISMS FROM WATER ON SOLID BACTERIAL GROWTH MEDIA

The experiment was carried out in order to see how many antibiotics stay in soil and how many are leached into water.

The procedure consists of four steps:

1) MAKING COLUMNS FROM PLASTIC TUBES FILLED WITH SOIL
2) POLLUTING THE COLUMNS WITH PENICILLIN and POURING RAINWATER OVER THE COLUMNS
3) INOCULATING MICROORGANISMS FROM RAINWATER ON A SOLID BACTERIAL GROWTH MEDIUM, COUNTING COLONIES, ANALYZING ALL SAMPLES
4) INOCULATING MICROORGANISMS FROM SOIL ON A SOLID BACTERIAL GROWTH MEDIUM, COUNTING COLONIES, ANALYZING ALL SAMPLES

3.1. MAKING COLUMNS FROM PLASTIC TUBES FILLED WITH SOIL

3.1.1. MATERIALS

- a measuring cup,
- scales,
- plastic tubes for columns (6),
- plastic net,
- long plastic hoses (6),
- different sand fractions (coarse and fine),
- soil. (In our case it was taken from our school nursery.)

3.1.2. PROCEDURE

- We take 6 plastic tubes: 10 cm in diameter, 3 mm thick and 50 cm high. The tubes, called also columns, have a conical 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 add 4 kg soil. After adding a kilo, we stop and compress it. Then we continue.
- To the conical bottom of the columns, we attach a plastic hose, which is about 150 cm long.
- In the end, we pour water over the columns. We must make sure that the water height in the column as well as in the hose is 60 cm in order to reach the same pressure (the principal of communicating vessels). After two days we release the water out of the hose.

3.2. POLLUTING THE COLUMNS WITH PENICILLIN

3.2.1. MATERIALS

- penicillin,
- rainwater,
- glass measuring cups (6).

3.2.2. PROCEDURE

- In the first and second column, we add 2 ml liquid penicillin. In the third and fourth column we add 0.5 ml penicillin. In the fifth and sixth column we do not add any penicillin because they are used as controls.
- Into each column we poured 400 ml rainwater. The rainwater will permeate through the soil and flow out through the hose. We collect this water in 6 glass measuring cups in order to analyze it - the same way as the soil from the columns.
- We repeat the procedure four times.

3.3. INOCULATION OF MICROORGANISMS FROM WATER ON SOLID BACTERIAL GROWTH MEDIA

3.3.1. MATERIALS

- water samples drained from the columns,
- two sterile bacterial growth media (PKE and MacConkey Agar),
- an inoculating loop,
- a gas burner.

3.3.2. PROCEDURE

- We use flame from a gas burner to sterilize the inoculating loop and glassware.
- We regularly clean the worktop with disinfectants.
- We inoculate microorganisms from rainwater on both bacterial growth media, using a flame sanitized inoculating loop. We take one drop of water aseptically on the bacterial growth media.
- We spread water over the petri dish with circular movements in the shape of a thread.
- Afterwards we flame sterilize the inoculating loop until it starts glowing.
- Additionally, we carry another drop of water on the two culture media and make a wavy line, which is perpendicular to the first spread.

3.3.3. RESULT

We count the colonies which have grown on each culture medium.

3.4. INOCULATION OF MICROORGANISMS FROM SOIL ON SOLID BACTERIAL GROWTH MEDIA

3.4.1. MATERIALS

- soil samples from the columns,
- two sterile bacterial growth media (PKE and MacConkey Agar),
- an inoculating loop,
- a gas burner,
- an Erlenmeyer flask,
- a shaker,
- scales,
- a spatula,
- physiological solution.

3.4.2. PROCEDURE

- In an Erlenmeyer flask we weigh out 10 g soil and pour it over with 90 ml sterile physiological solution.
- We put the Erlenmeyer flask on a chemical shaker at speed 2 for 1 hour.
- We regularly clean the worktop with disinfectants.
- We inoculate microorganisms from the soil suspension on both bacterial growth media, using a flame sanitized inoculating loop. We take one drop of the suspension aseptically on the bacterial growth media.
- We spread the drop over the petri dish with circular movements in the shape of a thread.
- Afterwards we flame sterilize the inoculating loop until it starts glowing.
- Additionally, we carry another drop of the soil suspension on the two culture media and make a wavy line, which is perpendicular to the first spread.

3.4.3. RESULT

We count the colonies which have grown on each culture medium.