Surveying microorganisms in the saline fields of Setthaw Township in the province of Kalasin

Miss. Padtarawadee Chantasan Mr. Phasit Ritloetwiriya Miss. Pornpansa Seema
Mr. Nopparat Ongkakas

Mr. Chumpol Charisarn Miss. Thrantip Chantharanima
Kalasinpittayasan School, Mueang Kalasin District, Kalasin Province

Abstract

In Kalasin Province There is a lot of agricultural land. There are some areas where plants can be grown. Some areas cannot grow crops. and plants that produce low yields Therefore, soil properties (EC and pH values) were surveyed in the area of Kalasin Province. The results from the inspection found that when measuring the EC (dS/m) and pH values of saline soil in the Ban Ton area, Lup Subdistrict, Mueang Kalasin District, Kalasin Province, it was found that the soil surface area had a salinity (EC) of 5.98 dS/. m has a pH value of 4.00, at a depth of 10 cm has a salinity value (EC) of 6.96 dS/m, has a pH value of 5.00, at a depth of 15 cm has a salinity value (EC) of 7.74 dS/m, has a pH value of 5.25, found microorganisms in 3 types of fungi. Results of measurement of EC (dS/m) and pH values of saline soil in the Ban Phon Sim area, Hua Na Kham Subdistrict, Yang Talat District, Kalasin Province. It was found that the soil surface area had a salinity (EC) of 6.03 dS. /m has a pH value of 4.25, at a depth of 10 cm has a salinity value (EC) of 8.81 dS/m, has a pH value of 4.50, at a depth of 15 cm has a salinity value (EC) of 8.99 dS/m, has a pH value of 6.00 and when Microorganisms were examined and separated and found to be Gram-positive bacteria. Gram-negative bacteria and 1 type of fungus, respectively.

Introduction

In Kalasin Province There are many agricultural areas, some of which can be grown. Some areas cannot grow crops. and plants that are grown give low yields. One of the reasons why plants cannot be grown or have low yields is from the problem of soil salinity. Affects water uptake by plants, causing dehydration and an imbalance of nutrients in the soil. Because it has sodium content and a lot of chloride This makes it toxic to plants. In addition, farmers have used agricultural chemicals to improve saline soil continuously for a long time, causing the soil surface to deteriorate. Especially in the area of Ban Phon Sim, Hua Na Kham Subdistrict, Yang Talat District, which has more than 2,000 rai of saline soil that cannot grow crops according to the season, resulting in lower yields. In the dry season, water in the saline soil will seep into the soil surface and leave salt stains. which is an obstacle to planting crops in the next round (Kalasin Provincial Agricultural Office 2023). Therefore, the study is interested in exploring the saline soil area. In order to gain in-depth information about the salinity of the soil to look at the types of microorganisms and bacteria that are present in saline soil in order to gain insight into the functions of microorganisms, such as their ability to tolerate salinity from salt. Promote the availability of nutrients and helps the overall ecosystem to be resilient. It can lead to the development of sustainable farming practices. Including selecting salt-tolerant plants and designing experiments using microorganisms to improve soil quality in saline environments. In addition, it can support Understanding the ecological adaptation of microorganisms in extreme environments Studying the growth of organisms in saline soils can reveal microbial survival strategies and potential biotechnological applications of great importance in terms of improving agricultural practices in that environment. It's salty soil. enhancing soil health and the discovery of the potential of microorganisms The team therefore conducted a survey of soil salinity data in the area of Ban Ton, Lub Subdistrict, Mueang Kalasin District. and Ban Phon Sim, Hua Na Kham Subdistrict, Yang Talat District, it was found that there were several rai of rock salt fields, with each rai having a large amount of salt deposits, causing the villagers who owned the farms to be unable to plant crops and produce the expected yield.

objective

To survey microorganisms in the saline soil area of Ban Phon Sim, Hua Na Kham Subdistrict, Yang Talat District, Kalasin Province.

Experimental procedure

1. Fielding the coordinates of the location

1, Ban Ton, Thung Si Mueang Road, Lub Subdistrict, Mueang Kalasin District, Kalasin Province 46000, geographic coordinates 16.419001, 103.535909,

2, Ban Phon Sim, Hua Na Kham Subdistrict, Yang Talat District. Kalasin Province 46120 Geographic coordinates 16.409815,103.259814

2. Collection of microorganisms

The process of collecting bacteria by collecting samples of microorganisms in saline soil is carried out as follows: Materials and equipment

1. Collect samples of saline soil.

2.Equipment for separating microorganisms

2.1 Sample collection bag

2.2 Digging equipment (shovel)

3. pH measurement tool

4. Soil EC measurement tool

3. Check the pH value of saline soil.

1. Dig a small hole in the soil. Use a spade or shovel to dig a hole with a depth of 2- 4 inches. Crush the soil inside the hole and remove any branches or foreign objects.

2. Fill the hole with water. Use distilled water (not tap water), you can find it at the store. Rainwater is slightly acidic. And bottled or tap water is slightly alkaline. Fill the hole with water until you have a muddy puddle at the bottom.

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4. Hold it for 60 seconds and take a reading. pH is usually measured on a scale of 1-14, although equipment may not have this wide range.

- pH of 7 indicates that the soil is neutral.

- pH value above 7 indicates that the soil is alkaline .

-pH value of less than 7 indicates that the soil is acidic.

5. Take measurements at several different points in the garden. Reading a single point at a time may give an incorrect reading. The best way is to find the average of the pH of the entire plot if they all have the same value. You can adjust the soil condition as desired. If any one point is different from the rest, You may need to "treat" the soil at that point.

4. Measuring the salinity of soil (EC soil).

1. Plow the soil around the point you want to check.

2. Use the tail of the soil salinity meter to stick it in.

3. Wait for the value to stabilize and then record the result.

5.How to make microbial culture food Culture media.

Unknown chemical components, both type and quantity (Artificial media or Non-synthetic medium). This culture medium will contain many organic substances obtained from extracts from plant or animal tissues, such as peptone (Peptone), yeast extract (Yeast extract), beef extract (Beef extract) or Malt extract, etc. This type of culture media therefore helps in the growth of many types of microorganisms. Culture media that fall into this group include liquid food (Nutrient broth or NB), solid food (Nutrient agar or NA), Potato dextrose agar (PDA) or Trypticase soy broth, etc. Components of NB liquid food include

Beef Extrax. 3 grams

Peptone 5 grams

Distilled water 1 liter

Adjust pH to be between 6.8 - 7.2

Solid food ingredient NA. It is similar to NB, but agar is added to harden the food with another 15 grams per liter.

The food commonly used to grow fungi in the laboratory is PDA food (PDA or Potato dextrose agar), which has the following ingredients

Potatoes. 200 grams

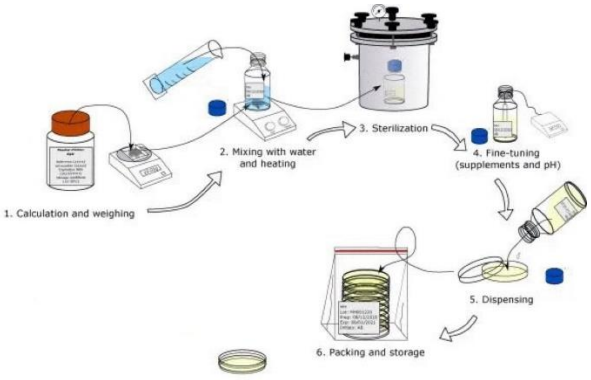
Dextrose 20 grams

Agar 15 grams

Distilled water 1 liter

Adjust pH between 5.0 - 5.5

Steps for preparing culture media. The medium with agar added is normally 15 grams per liter of medium. In growing microorganisms, solid media is placed in a glass dish (petri dish) or in a test tube with an inclined surface called a slant or slope. Food in a test tube with an unangled surface is called a deep tube to be used to grow microorganisms that require oxygen. Little growth -Semisolid media is microbial culture media with a lower amount of agar added than solid media, approximately 0.5% or less -Liquid media (Broth)



Picture 1: Process for making culture media.

6. Separation of microorganisms from saline soil

Is carried out as follows. Materials and equipment

1. Petri dish

2. Alcohol burner (Alcohol Burner)

3. Loop

4. Triangular glass rod (Spreader)

5. Micropipette size 1000 microliters and 2000 microliter

6. Pipette tip (pipette tip) size 200 ul and 1000 ul

7. Alcohol

8. Incubator

9. Sterile cabinet (Lamina air flow)

10. Distilled Water

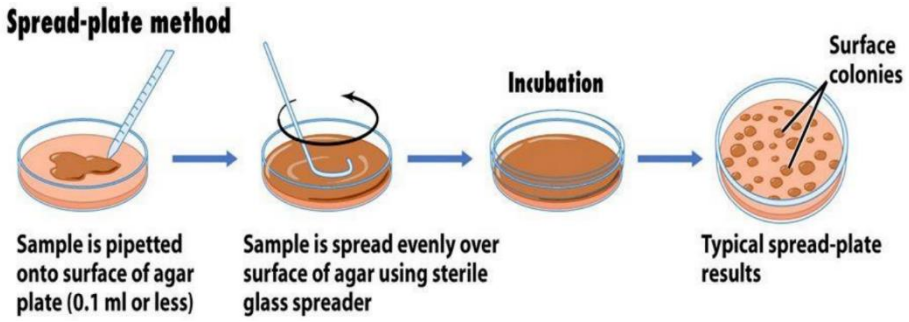
11. Plastic microcentrifuge tube (Micro Centrifuge Tube)

7. Separation of microorganisms in saline soil by dilution.

Microorganisms were separated by dilution at three levels: 10 times, 100 times, 1000 times. The experiment was done in a sterile cabinet using alcohol to wash hands before testing. Use a micropipette to measure the amount of distilled water mixed with the soil sample by shaking it to mix the water and soil in a plastic microcentrifuge tube. Then wait for the soil to dissolve and place it in another plastic microcentrifuge tube that must be diluted 100 times and 1,000 times. Then use a micropipette to measure the amount of bacteria in the diluted tube. 1,000 times and put it in a culture dish to see what types of microorganisms there are in the salty soil sample. Steps for separating and purifying germs

After the organizers had grown the cultures in Petri dishes.

We then separated each culture from one Petri dish in order to know what the microorganisms looked like and what kind of growth they had. In this step, you will get fungus and also bacteria. After selecting duplicate cultures, the cultures will be placed in an incubator for 1-2 days. In order for the germs to grow well.



Picture 2. Culture method.

8.Criticizing germs in a petri dish.

Use a loop to burn the fuel, then wait for it to cool. Then touch the fuel to scratch on solid media. In every line of drawing, the Loop used should be changed or the fire should be burned every time to kill it. If there is too much germs, the germs will be very dense in the first scratch and then gradually become less dense in the last scratches. Each cell can be separated from each other. After being incubated at temperature and the appropriate time for each type of microorganism, each cell will be separated into individual colonies.

9. Microbial staining Materials and equipment.

1. Alcohol burner (Alcohol Burner)

2. Ring Loop

3. Crystal violet

4. Iodine solution

5. 95% Alcohol

6. Distilled water

7. Glass slide

8. Safranin O

Algorithm for staining microorganisms.

When the organizers have the desired microorganisms, they will be stained by taking a glass slide and dropping one drop of distilled water onto the glass slide. Then the

microorganisms were swirled over the distilled water point and waited for it to dry. Then put it through fire 2-3 times, drop crystal violet dye (Crystal violet) down to cover the smear mark, leave it for 30 seconds, wash off the paint with gently running distilled water, drip iodine solution to cover the smear mark and discard. For 30 seconds, wash off the dye with gently running distilled water, wash off the dye with 95% Alcohol for about 10 to 20 seconds, then wash off with distilled water and dye with Safranin O: Leave for 30 seconds, rinse with distilled water, and leave the slide to dry completely.

10. Using a microscope to know the characteristics of microorganisms.

After the organizing team dyed the microorganisms it is then taken under a microscope to distinguish the nature of the microorganisms whether they are bacteria or fungi. at 1000 times magnification

Experimental results

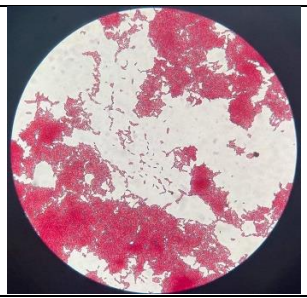
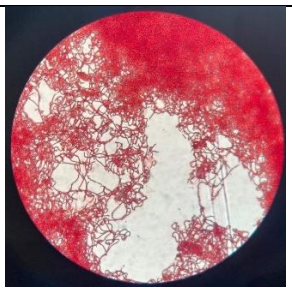
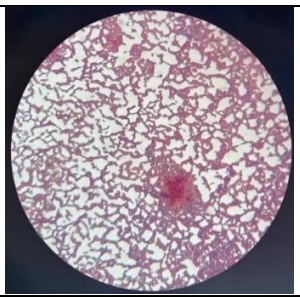
From the microbial survey in the saline rice fields of Sinthao District, Kalasin Province, the experimental results are as follows

1.survey results

The survey of EC (Electrical Conductivity) and pH values in saline soil.

Table 1: Microbial Survey in the Ban Totan Area, Tambon Lhub, Muang Kalsin District, Kalsin Province.

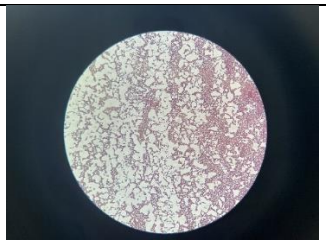
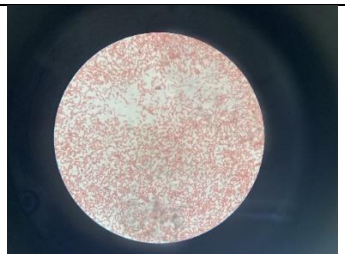
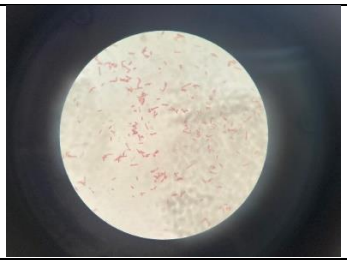
Point	EC (dS/m)	pH	Microbial characteristics
1. Surface soil at 5cm depth	5.98	4.00	
2.Depth at 10 cm	6.96	5.00	
3.Depth at 15 cm	7.74	5.25	

		
Fungi on the soil surface at 5 cm depth	**Fungi at a depth of 10 cm**	**Fungi at a depth of 15 cm**

From Table 1, the results of measuring EC (dS/m) and soil pH in the saline soil of Ban Totan, Tambon Lhub, Muang Kalsin District, Kalsin Province, reveal that the surface soil has an EC value of 5.98 dS/m and a pH of 4.00. At a depth of 10 cm, the EC value is 6.96 dS/m with a pH of 5.00. At a depth of 15 cm, the EC value is 7.74 dS/m with a pH of 5.25. Additionally, three different types of fungi with distinct hyphae were found in the area

Table 2: Microbial Survey in the Phonsim Village Area, Huanakham Sub-district, Yang Talat District, Kalasin Province.

Point	EC (dS/m)	pH	Microbial characteristics
1. Surfacesoil at 5cm depth	6.03	4.25	
2.Dept at 10 cm	8.81	4.50	
3.Dept at 15 cm	8.99	6.00	

		
Surfacesoil at 5cm depth **Positive gram bacteria**	**Depth at 10 cm** **Negative gram bacteria**	**Depth at 15 cm** **fungus**

From Table 2, the results of measuring EC (dS/m) and pH values of saline soil in Ban Tohn, Tambon Luhp, Mueang Kalasin District, Kalasin Province, are as follows:Surface Soil:EC: 6.03 dS/mpH: 4.25Depth 10 cm:EC: 8.81 dS/mpH: 4.50Microorganisms: Positive gram stain bacteria, negative gram stain bacteria, and one type of fungi.Depth 15 cm:EC: 8.99 dS/mpH: 6.00Microorganisms: Positive gram stain bacteria, negative gram stain bacteria, and one type of fungi.

Discussion of experimental results

The study on microorganisms in the saline fields of Setthaw Township in Kalasin Province involved surveys in two areas: Ban Tohn, Tambon Luhp, Mueang Kalasin District, and Ban Phon Sim, Tambon Hua Na Kham, Yung Talat District. The surface and subsurface soil EC and pH values were measured at different depths. In Ban Tohn, at 5 cm depth, the EC was 5.98 dS/m with a pH of 4.00. At 10 cm, the EC was 6.96 dS/m with a pH of 5.00, and at 15 cm, the EC was 7.74 dS/m with a pH of 5.25. In Ban Phon Sim, at 5 cm, the EC was 6.03 dS/m with a pH of 4.25. At 10 cm, the EC was 8.81 dS/m with a pH of 4.50, and at 15 cm, the EC was 8.99 dS/m with a pH of 6.00 Additionally, the researchers identified microorganisms in the salty soil by diluting and staining samples. In Ban Tohn, fungi were found at all three depths, while in Ban Phon Sim, positive gram stain bacteria were found at 5 cm, negative gram stain bacteria at 10 cm, and fungi at 15 cm.

Summarize the experimental results

From the experiment, it can be summarized that in the saline fields of Setthaw Township in Kalasin Province, there are six types of microorganisms discovered. At a soil depth of 5 cm with EC value of 5.98 dS/m and pH of 4.00, three types of fungi were found. At a soil depth of 10 cm with EC value of 6.96 dS/m and pH of 5.00, fungi were also identified. Furthermore, at a soil depth of 15 cm with EC value of 7.74 dS/m and pH of 5.25, another type of fungi was observed. In the area around Ban Phon Sim, at a soil depth of 5 cm with EC value of 6.03 dS/m and pH of 4.25, bacteria of positive gram stain type were discovered. At 10 cm depth with EC value of 8.81 dS/m and pH of 4.50, bacteria of negative gram stain type were found. Finally, at 15 cm depth with EC value of 8.99 dS/m and pH of 6.00, another type of fungi was identified.

Reference documents

Soil Salt Management Research and Development Group Land Management Research and Development Division Department of Land Development (Department of Land Development).

Managing the soil salinity problem. Retrieved on 8 June 2023,

from : https://www.ldd.go.th/Web_Soil/salty.htm

Neonics Company Limited (M.P.P.). EC values in soil. Retrieved 9 June 2023,

from : https://www.neonics.biz/agricultural/ec-in-soil/

Hanna Instruments (Thailand) Co., Ltd. (2022/02/03). pH in soil (acid and alkaline). Retrieved on

9 June 2023, from: https://www.hannathailand.com/2022/02/03 Nianwan Meecharoen (2017/08/11). Culture media, microbial culture, culture media, culture media used in microbiology. Retrieved on 7 July 2023, Tonan Asia Autotech (2019/08/04). Article Gram Staining Gram staining. Searched when

10 July 2023, from: https://www.tonanasia.com/gram-staining/

Teerawat Suksai's blog (2019/02/20). Techniques for bacterial gram staining. Retrieved on 10 July 2023,

from : https://agro-industry.rmutsv.ac.th/agro/?q=th/Blog/533-687-create-blog-entry