MEDICINES FROM MICROBES

A science investigation pack for teachers of 9-11 year olds

CENTRE for INDUSTRY EDUCATION COLLABORATION

Supported by the Gatsby Charitable Foundation

Contents

Introduction

AGE RANGE

The activities in this book provide an opportunity for children to think about micro-organisms and come to understand that they can be both helpful and harmful, depending on the context. The activities are written for use with children in Years 5 and 6, although the work can be modified for use by other age groups.

CONTEXT

The activities are put in a real life context, that of biotechnology and the development of new medicines, with the need to find the best conditions for mould growth. This approach makes the work more relevant and enjoyable for the children.

An area of rapid development in the biotechnology industry at present centres on the use of plants, moulds and micro-organisms as a natural resource for medicinal materials. Many of the folk remedies of yesteryear do have some medicinal properties, and the 'newspaper article' used with the children in this unit mentions some of them. For example, willow bark contains salicylic acid, the active ingredient of aspirin, and Fleming's discovery of penicillin was due to him noticing that micro-organisms would not grow in close proximity to the mould on an agar plate.

Many of the antibiotics produced for the treatment of human and both pet and farm animal diseases are isolated from moulds that are fermented to maximise their growth. Since they are living things, they need a source of food, and the correct conditions of warmth, moisture and air. Interestingly, however, the mechanisms that produce the antibiotic during the fermentation process are not fully understood. At least one production company points out that many of today's processes result from years of experience of maximising the yield of antibiotic, rather than a complete understanding of why they work. There is also an interesting link with another teaching unit, *Runny Liquids*. Some companies' antibiotics are isolated from a mould that produces a very thick, viscous broth. The difficulties of stirring such viscous liquids provides the context for the unit *Runny Liquids*.

Thus, any new antibiotic produced by fermentation of moulds must ensure that the growing conditions for the organism are optimum. The context of the activities is that of finding the best types of conditions to grow moulds on bread or yogurt, to assist a bio-technology firm in developing a new antibiotic.

ACTIVITIES

The activities take 2 x 2 hours, separated by a fortnight of daily observations and recording of 10 minutes, since the moulds take some time to grow and develop. Detailed teacher support and information are provided in the Activity notes, as well as a framework for learning activities.

The activities will be most effective if developed after some initial introduction to micro-organisms through the notion of 'germs'. Time should be spent beforehand in giving some explanation of the work of Edward Jenner, Louis Pasteur and others who developed the 'germ' theory, so that children understand that there are both helpful and harmful micro-organisms. Information and useful websites are provided in Appendix 1. Appendix 2 suggests some cross-curricular links. Appendix 3 provides information on links with ICT, whilst Appendices 4-5 provide lesson plans and an assessment grid for one investigation.

The Activity sheets should help the children record various aspects of their investigation. At Key Stage 2 children are expected to '*...talk about their work and its significance, and communicate ideas using a wide range of scientific language, conventional diagrams, charts and graphs*'. It is hoped that the Activity sheets will increase the children's enjoyment of science by appreciating the variety of ways in which they can record their work. The clue cards are intended to support differentiated teaching in the primary classroom.

ACTIVITY SUMMARY

Title	Description	Timing
1 Growing mould	The children are introduced to the activity through a fictitious newspaper article which promotes discussion of the type of conditions which might encourage mould growth. They then investigate these conditions.	2 hours
2 Recording growth	The work continues with regular observation and recording of the progress of the mould growth.	10x10 mins per day
3 Reporting to the industry	The children draw conclusions from their results, using line graphs to inform their decisions as to the best conditions for mould growth. They present their findings for discussion. They write an e-mail/letter to the industry suggesting ways the industry could most effectively grow mould.	2 hours

1. Growing mould

2 hours activity

The children are introduced to the activity through a fictitious newspaper article which promotes discussion of the type of conditions which might encourage mould growth. They then investigate these conditions.

OBJECTIVES

- Describe how living things are classified into broad groups according to common observable characteristics and based on similiarities and differences, including micro-organisms
- To appreciate that micro-organisms are living things that are too small to be seen
- To appreciate that micro-organisms may be either beneficial or harmful
- To plan and set up an investigation into mould growth

RESOURCES

(Per group of 4)

- Activity sheets 1- 5
- Clue cards
- 6 transparent seal-able freezer bags
- 6-8 slices of fresh bread
- Refrigerator or freezer compartment
- Scissors
- Sticky tape
- 2 water droppers
- 1 beaker of water
- Adhesive labels

OR

- 4 plastic Petri dishes for yogurt
- 2 pots of natural yogurt

INTRODUCING THE ACTIVITY (30 minutes)

Use the 'newspaper article' on Activity Sheet 1 to introduce the problem of finding the best conditions in which to grow moulds[1]. In this article, the firm wants details, backed up by evidence and measurement of the children's investigations. The illustration in the article shows bread going mouldy, but are these the only things to go mouldy? Questions to encourage discussion are suggested below.

- *Which other foods have you seen growing mould?*
- *Based on things you know can you suggest any conditions that might cause moulds to grow?*
- *Think about where the food is stored. Do you think it is warm or cold? Dry or moist? Light or dark?*
  Use words like 'micro-organism' and 'microbes', as well as the common 'germs' to familiarise the children with these terms.
- *Can you suggest/brainstorm how we could test which conditions produce the most mould?*
- *What 'medium' or material/foodstuff should we use to grow the moulds on?*

The teacher should also explain the safety implications of growing microbes. They may be beneficial, but equally they may not, so they will be contained in sealed freezer bags. Discuss how the tests can be kept 'fair', e.g. same sized pieces of bread, same loaf.

Safety note

Mouldy foods should be kept in sealed plastic bags.

MAIN ACTIVITY (70 minutes)

In groups of four, the children should decide the conditions they will test, and plan how to record their results. The clue cards (provided on Activity sheet 2) can be used to support the children's planning and setting up of their investigations. The teacher can provide the cards if groups request them, to give suggestions for setting up the method, and for recording results. Knowing which clue cards the group has requested can give the teacher an idea of how confident the children are in aspects of planning their investigatory work, and may even help provide an assessment tool for the teacher.

Key questions for the children to decide upon are:

- how the test is to be set up
- what will actually be done
- how the results are to be recorded.

Activity sheet 4 provides a more structured alternative or addition to the clue cards.

1 Moulds are visible, but other micro-organisms which cause food to decompose (e.g. bacteria) are not. Many micro-organisms release large amounts of spores into the air to which some people are allergic.

The experiments can be done using either fresh sliced bread or natural yogurt. Each slice of bread can be cut vertically so that the same slice of bread is used for each pair of conditions. They are then put into freezer bags and sealed, with details of the date and conditions marked on them.

If using yogurt it should be spread across the bottom of plastic Petri dishes so that the whole bottom surface is covered with a thin layer. The Petri dishes should be sealed with sticky tape. Teachers will need to be aware that mould will take a few days to grow, and quicker results are not likely with either bread or yogurt. Typically, it takes about six days for mould to grow.

Once the various test conditions have been decided, the groups set up the tests, clearly labelling their bags with the conditions, and date of commencement of the tests. However, even if 2-3 groups test each set of conditions, there will be scope for comparisons. Living things do not all do exactly the same thing, and these differences can be used to stress the variation in living things and their response to conditions.

PLENARY (20 minutes)

Encourage the children to explain what they expect to see happen in their investigation. In this case, ask the children:

- What do you think will happen to the pieces of bread in your test?
- Which one will grow the most mould?
- Are your predictions based anything else you have seen before?

Using Activity sheet 5 they can record their results and ideas in an organised format.

News Post

Wednesday *48p*

Mouldy food produces new medicine!

Funding worries put breakthrough at risk

New discovery

A small local company at the cutting edge of new bio-technology has run into difficulties with the development of its latest discovery. The company, NewBioTech (NBT), has been working for some time with different plants and foods to try to extract ingredients which might make new medicines.

The Director of NBT, Dr. Smail, explained "Everyone knows that many common plants contain ingredients which can help us. If you are stung by a nettle, you can rub it with a dock leaf to take the itch away. Once upon a time, willow bark was boiled in water to make a drink which cured headaches. Even bread was used in poultices in some cases!"

In the latest discovery, NBT found that a mould growing on food seemed to stop other micro-organisms growing around the mould. "We wondered if this mould could be used as a medicine," said Dr. Smail. "If it stopped other micro-organisms growing, we wondered if it would stop bacteria, which are micro-organisms too. This could be a break-through in treating things like simple cuts, which so often get infected by bacteria in dirt. We think this mould might produce a new antibiotic."

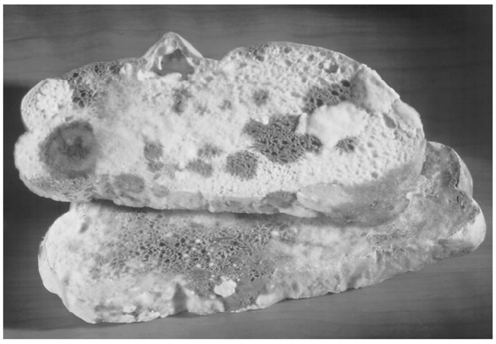
Funding

However, NBT have run into difficulties. They only have a small research fund for developing new ideas, and have to rely on grants from other groups which are interested in their work. "If another firm thinks that our discovery might help their work, then they will give us some money to help develop the ideas," explained Dr. Smail. "Sometimes the government will help, too."

Unfortunately, this time no-one has come forward with offers to help with the development costs.

Appeal

The firm is looking for assistance from other groups. "We have been able to grow the mould, but we need to find the best conditions for growing it. We will need to produce large amounts to make antibiotics," said Dr. Smail.



One suggestion is that school research groups could help. Unlike small firms like NBT, who can spare just one or two people to experiment, school groups can gather lots of data very quickly. Dr. Smail was enthusiastic. "We would love to hear from a school, if they can help us find the best conditions for growing moulds. It does not matter what food is used either. Every piece of information is helpful! If the information gives us an idea of the actual amount of mould produced for each condition, that would be really useful."

Clues for the method

Clue 1.

Decide on the conditions you want to try growing the mould in. For example, you could . . .

Compare warm against cold conditions.

or Compare moist against dry conditions.

or Light conditions against dark conditions.

or Warm and moist against cold and moist.

or ...what do you think?

Clue 2.

Make sure you have only changed **ONE** thing!

For example, you could use the same sized slice of bread, in the same size of bag, (or yogurt in a Petri dish) but ONLY CHANGE THE CONDITIONS from

warm to cold

or moist to dry

or light to dark

or . . . what you decide!

Clue 3.

Cut out 2 pieces of bread the same size.

Put them into 2 different freezer bags.

Put one bag in one of the conditions you have chosen, (e.g. warm) and the second into the other conditions (e.g. cold).

Clues for recording results

Clue 1.

Can you think of a way to record how mouldy the bread or yogurt becomes? Remember that **words** like 'a little bit mouldy', or 'quite mouldy' will not mean very much to the scientists at NewBioTech! They will need **evidence** of some kind.

Clue 2.

You could draw the pieces of bread, colouring the areas of mould that appear. You could use a different colour for each observation.

Clue 3.

One way of describing how mouldy the bread is would be to describe it in words with a scale.

E.g.

☐	= 0	No mould
▒	= 5	Quite mouldy
▓	= 10	Completely covered in mould

OR can you think of a better way?

Clue 4.

Use a sheet of clear OHP plastic marked with a grid of 1- centimetre squares. Lay it over the bread. Count the number of squares that have some mould inside them, or colour in those squares on the OHP, using a different colour each day.

Activity 4: Our predictions

Circle the conditions that **you think** will encourage the most mould to grow:

cold   wet   light   warmth

moist   dark   dry   hot

Explain why you think this: ____________________

What do you think will be the best way to record the amount of mould that has grown every day (or every other day)?

Why do you think this might be the best way?

Activity 5: Recording the growth of the mould

Type of food used: ____

1. Fill in the date column on each day you make a recording.
2. Write in each numbered Column the conditions you are using. There are 5 columns, but you don't have to use them all.
3. Each day, record the number of squares containing mould.

Day	Date	1. ________	2. ________	3. ________	4. ________	5. ________
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						

2. Recording growth

10x10 mins per day

The work continues with regular observation and recording of the progress of the mould growth.

OBJECTIVES

- To know that the effects of micro-organisms can be observed over a period of time
- To appreciate the time required for growth
- To observe and record results at consistent, regular intervals

APPROXIMATE DURATION:

10 minutes per daily recording session.

RESOURCES

(Per group of 4)

- Recording sheets/grids as chosen by each group
- Activity sheet 6

INTRODUCING THE ACTIVITY (5 minutes)

Remind the children of the overall task, to find the best conditions for mould growth to assist the industry. Check that they have remembered their chosen method for recording their results. Remind them not to open the bags or Petri dishes to check the contents, for the safety reason given below.

DAILY OBSERVATIONAL RECORDING

The children record the mould growth on their slices of bread or on the surface of the yogurt. These sessions should be carried out daily, or possibly every other day, and preferably at roughly the same time each day.

The clue cards provided on Activity sheet 3 suggest three methods for recording results (i) pictorial recording, (ii) numerical scale (representing *no mould*, to *completely covered in mould*) or (iii) a grid (to be printed on acetate) to ascertain the area of mould coverage (by counting the number of squares containing some mould). More able children could calculate the coverage as a fraction or percentage of the piece of bread. Use of a numerical scale will allow the results to be plotted on a graph. Least able children could draw the pieces of bread each day resulting in a visual representation of the mould growth.

Apparent changes will not be seen for about a week, which will reinforce the notion that living things take time to grow. However, the teacher can use these observations to show that some living things, like humans, grow very slowly over several years, but others like moulds can grow relatively quickly: in days.

The observations will probably spread over some 14-21 days, so some ideas will have to be developed as to recording changes over the weekend, when the children will not be able to observe their bread. For example, the most able children can leave a space in the recording sheets for Saturday and Sunday, the results plotted graphically, and the missing results *interpolated* into the graph (that is, the missing results of coverage can be estimated and placed on the graph perhaps in a different colour to show that they are not measured, but merely estimated).

Safety note

Mouldy foods should be kept in sealed plastic bags and the lids of Petri dishes should be taped down.

3. Reporting to the industry

2 hours activity

The work continues with regular observation and recording of the progress of the mould growth.

OBJECTIVES

- The children draw conclusions from their results, using line graphs to inform their decisions as to the best conditions for mould growth.
- They present their findings for discussion.
- They write an e-mail/letter to the industry suggesting ways the industry could most effectively grow mould

RESOURCES

- Activity sheet 1
- Computer
- Spreadsheet/graphing program, e.g. RM Windowbox *Starting Graphs* or Microsoft *Excel*

OR

- Graph paper
- Rulers and pencils

INTRODUCING THE ACTIVITY (15 minutes)

Using the newspaper article as a reminder, discuss what has been done to try and help the company. Explain that the information must now be presented to the company so that they can easily understand the investigation, and what the results show. The information about the mould growth was collected using drawings and/or numbers (fractions, percentages, amount of cover). The best way of presenting the numerical information is by making bar and line graphs. Discuss with the children what the graph will look like if the mould grew quickly, compared to the graph showing slow growth. *(You would expect to see the line or bars rising steeply for rapid growth, but much less steeply for slow growth.)*

MAIN ACTIVITY (90 minutes)

Children producing numerical data begin by graphing their results. (Those producing drawings could perhaps join other groups, if appropriate.) Examples of spreadsheet programs available include the RM Windowbox *Starting Graphs* or Microsoft *Excel*. This is more appropriate for more able children. For those producing a table of results, these will need to be copied into a spreadsheet, as the example overleaf, using *Excel*, shows.

Each group then presents their findings. Where appropriate data can be collected on the board, so that the class can discuss and compare them to conclude which set of conditions will lead to best mould growth.

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Finally, the children can write an e-mail or letter to the company, explaining which conditions they should use to grow their antibiotic mould and giving their reasons. The e-mail/letter should include attachments depicting their test and recorded data.

Bread	Number of Squares with mould			
Day	Warm	Cold	Dry	Moist
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	3	0	1	1
8	4	0	1	3
9	11	0	1	4
10	17	0	3	6
11	45	0	3	6
12		0		
13	55	0	10	13
14		0		
15		0		
16		0		
17	90	0	49	23

Note: blank squares on days 12, 14-16 indicate that no measurements were made. The Chart Wizard will take these into account when it generates the graph or bar chart. Some sample graphs are shown in *Appendix 3*.

PLENARY (15 minutes)

To draw together all their findings, ask the children:

- Did all the tests give similar results, or did some tests, supposedly identical, give different results?
- Why do you think this was?

It can be stressed that this does not mean that their tests were 'wrong', just that living things do not always do what is expected. When scientists set up tests, they have constructed some idea of what they think causes the events to take place, and run the tests to confirm their ideas. It can be very interesting when things do not go according to plan, and something unexpected happens! The scientist then has to go back and check the work for obvious errors, before reconstructing the ideas in the light of the new facts. The new ideas are then tested to see if they work.

Appendix 1: Background information for teachers

EDWARD JENNER (1749 - 1823) SMALLPOX VACCINATION AND INOCULATION

Until recently smallpox was a major scourge, and it took over from bubonic plague as the major killer disease during the 18th century, particularly of infants and young children. It had a high mortality rate, and caused serious disfigurement or blindness to the few survivors. However, by the early 20th century it was rare in Britain and Europe, due to the pioneering work of the Gloucestershire doctor, Edward Jenner, in the 18th century. Jenner noticed that those people who worked with cows, particularly milkmaids, often suffered from cowpox, a related disease to smallpox. They usually caught it if they had open cuts or sores on their hands, and continued to milk, or work with, cows. However, it was not a serious disease, and sufferers quickly recovered with no ill effects. However, Jenner also noticed that these people very rarely caught smallpox, or if they did, recovered quite quickly. He thought that they must gain some form of immunity by first catching cowpox.

He originally tested his theory on his young son, aged one and a half, in 1789, when he inoculated him with swine-pox, similar to cowpox, followed by a smallpox inoculation. It had no ill-effects, and seemed to work. He then further tested and confirmed his theory in 1796 on a young boy in his village, James Phipps, by collecting some of the pus from a cowpox sore from the dairy maid Sarah Nelmes, then scratching the boy's arm and rubbing the pus into the open scratch. The boy caught a very mild form of cowpox, but although exposed to smallpox, did not develop the disease. So confident was Jenner in his vaccination that he made several attempts to deliberately infect the boy with smallpox, but with no success! As a result of his work, vaccination became free for all infants in 1840, and became compulsory in Britain in 1853. In 1980 the World Health Organisation declared that smallpox had been eradicated throughout the world.

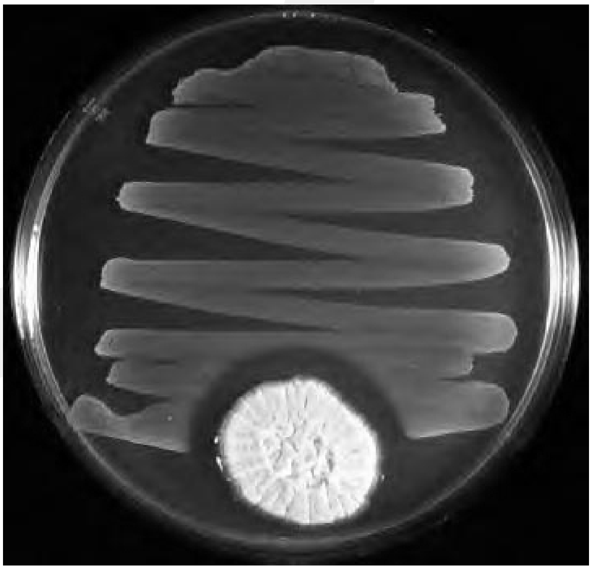
LOUIS PASTEUR (1822 - 1895)

Even following the work of Jenner at the end of the 18th century, in the mid-19th century many people still believed that disease was caused by polluted air, or miasma. However, Louis Pasteur, a French chemist, showed by his researches that it was the presence of germs in the air which were the link to disease. Asked by a wine company to discover why their wine was turning sour during the fermentation process, he showed that it was due to living germs of various kinds, which were visible under the microscope. He developed a process for killing the germs by boiling the wine and then cooling it, which he called pasteurisation. He then set about proving that the germs came from the air with his famous experiments using flasks with curved necks. Taking liquids like wine, and destroying the living germs by boiling, he showed that only the flasks of liquid which allowed free access by germs were affected. Even though air may get into the flask, fermentation or putrefaction does not take place if a piece of cotton wool, or a mere bending of the neck of the flask, keeps germs from entering. This is all that is required after sterilization to keep organic solutions quite sterile.

Following this work, Pasteur went on to show why Jenner's vaccinations worked, and developed vaccinations for chicken pox, cholera, diphtheria, anthrax and rabies. Interestingly, he also recommended that surgical instruments should be boiled before an operation to kill any germs on them, but unfortunately most surgeons ignored this advice! We had to wait 60 years or more, until the beginning of the 20th century for true aseptic surgery to be developed.

SIR ALEXANDER FLEMING (1881 – 1955)

Following the First World War, during which Fleming had been a captain in the Army Medical Corps, he returned to his bacteriological research in an attempt to find something that would attack infections in wounds. (He had noted how many wounded soldiers died from simple infections.) In 1928, while putting a pile of Petri dishes into cleaning mixture, he noticed one on which a mould had begun growing. However, what caught his attention was that it had contaminated a staphylococcus culture, a common bacteria, and stopped its growth, killing it.



An example of penicillin inhibiting staphylococcus growth (Taken from Access Excellence@ the National Health Museum. http://www.accessexcellence.org/AE/AEC/CC/chance.html)

On investigation he found that the mould was from the penicillium family, which he then named penicillin. (The blue mould on bread is another form of penicillium.) Further work showed that it was non-toxic to humans and could be used in treating many bacterial infections harmful to humans. However, little interest was taken in his report made in 1929, and it was not until the Second World War that Howard Florey and Ernst Chain developed Fleming's work. All three shared the 1945 Nobel Prize for physiology/ medicine, however.

FLORENCE NIGHTINGALE (1820 - 1910)

Florence Nightingale was the daughter of wealthy parents and as such was not expected to make her own living. After initial opposition from her parents she trained as a nurse in Egypt, Germany, France and lastly in London.

In 1854 Florence, with a group of 38 other nurses, arrived at the military hospital in Scutari to nurse the wounded from the Crimean War. She was appalled by the complete lack of sanitation, hygiene and fresh food. Many soldiers succumbed to typhus, cholera and dysentery. 1 in 6 deaths in the Crimea were ascribed to wounds received on the battlefields; the other 5 were attributed to the unsanitary hospital conditions.

By implementing basic hygiene techniques such as keeping the men clean, changing bed linen and giving them fresh food, the mortality rate fell from 60% to 2.2% by the spring of 1855.

Florence Nightingale is remembered as the gentle 'lady with the lamp' but this belies the truth. In fact most of the changes she implemented in the Crimea, and the many more at home, were largely brought about by her collection of data and statistical analysis. She was the first woman to be elected to the Statistical Society for her contribution to army statistics and comparative hospital statistics.

Her many projects included, introducing sanitary nursing to the British Army, transforming nursing from an occupation largely for women of ill repute to a respectable profession, establishing a nursing school at St Thomas's Hospital, summarising the principles of nursing in a book in 1860, and revolutionising the public health system in India.

FURTHER INFORMATION

Some useful websites for this information include:

- http://www.accessexcellence.org
- http://www.bbc.co.uk/schools
- http://www.sc.edu/library

The first website is of more relevance to the teacher, for it includes an article on the element of chance on various scientific discoveries. As Pasteur's famous aphorism puts it, 'Chance favours the prepared mind!' In Fleming's case, he was already thinking deeply about the antibiotic properties of moulds, and the 'chance' observation of the mould inhibiting and killing the staphylococcus growth on the agar plate was all that was needed to spark his further investigations. There are many other examples, and several are mentioned in the article. Using search engines on the other two websites, children can investigate aspects of medicine, and the lives of Jenner, Pasteur, Fleming and Nightingale.

Of real relevance to the activities in this unit, however, is the fact that with the need to produce sufficient quantities of medicinal penicillin during the war, the British pharmaceutical industry was unable to cope with the increased demand. Ernst Chain sailed to the USA to find the technology for mass-production of the drug. He in fact turned to a beer brewing technology to produce the huge amounts of mouldy liquor needed for penicillin production, which then underwent a slow purification process to produce the usable penicillin for military use.

Children may be puzzled as to where the mould comes from, since it has changed from something invisible into a coloured patch. They will need reminding that just because they cannot see something does not mean that it does not exist, just that it is too small to be seen.

Similarly, if they ask why perhaps some of the moulds are different colours, it can be pointed out that just as flowers come in different colours and shapes, so do moulds.

Appendix 2

DISCUSSION WORK WHICH LINKS TO OTHER CURRICULUM AREAS

Jenner's work can provide a useful discussion point on the testing of new drugs and medicines, in the light of recent controversies on the use of animals in drug testing. Would the children like to have been James Phipps? What do they think James' parents might have thought about Jenner's work on their son? What sort of questions might they have asked?

- *"Is it safe?"*
- *"Will our boy die?"*
- *"Can you be certain it will work?"*

What answers could Jenner have given them?

- *"Trust me, I'm a scientist."*
- *"Look at the evidence I have got."*
- *"No, I am not certain, but I'm pretty sure it will work."*

Similarly, his work can be used to illustrate that he had **planned his investigation** when he inoculated his own son. For instance, the children can be asked why he decided to use his own young son for the first test. Why did he not try it out on himself? *(He recognised that smallpox was a major threat to young children, rather than adults.)*

Fleming's work following the First World War shows that people were still dying from simple infections following surgery just 60 or 70 years ago. Have things improved since then? Recent newspaper reports in 2003 suggest that hospitals are dangerous places, where it is very easy to become infected with bacteria unrelated to the cause of the hospitalisation in the first place. Why should this be, after all the work done by Fleming and his followers? *(The answer seems to be that the bacteria are becoming resistant to antibiotics, because we use them too much! This is the reason for so much research being carried out into new forms of antibiotic.)*

There is scope for some work on the advances made in nursing and the treatment of diseases during the Victorian era, examining the work of Florence Nightingale and her methods of reducing mortality in the military hospital at Scutari during the Crimean War, or the work of Joseph Lister on the development of antiseptics.

Appendix 3

CHART 1

The bar chart was generated by highlighting the column of figures for warm conditions, and going to the Chart Wizard in Microsoft Excel to generate this chart.

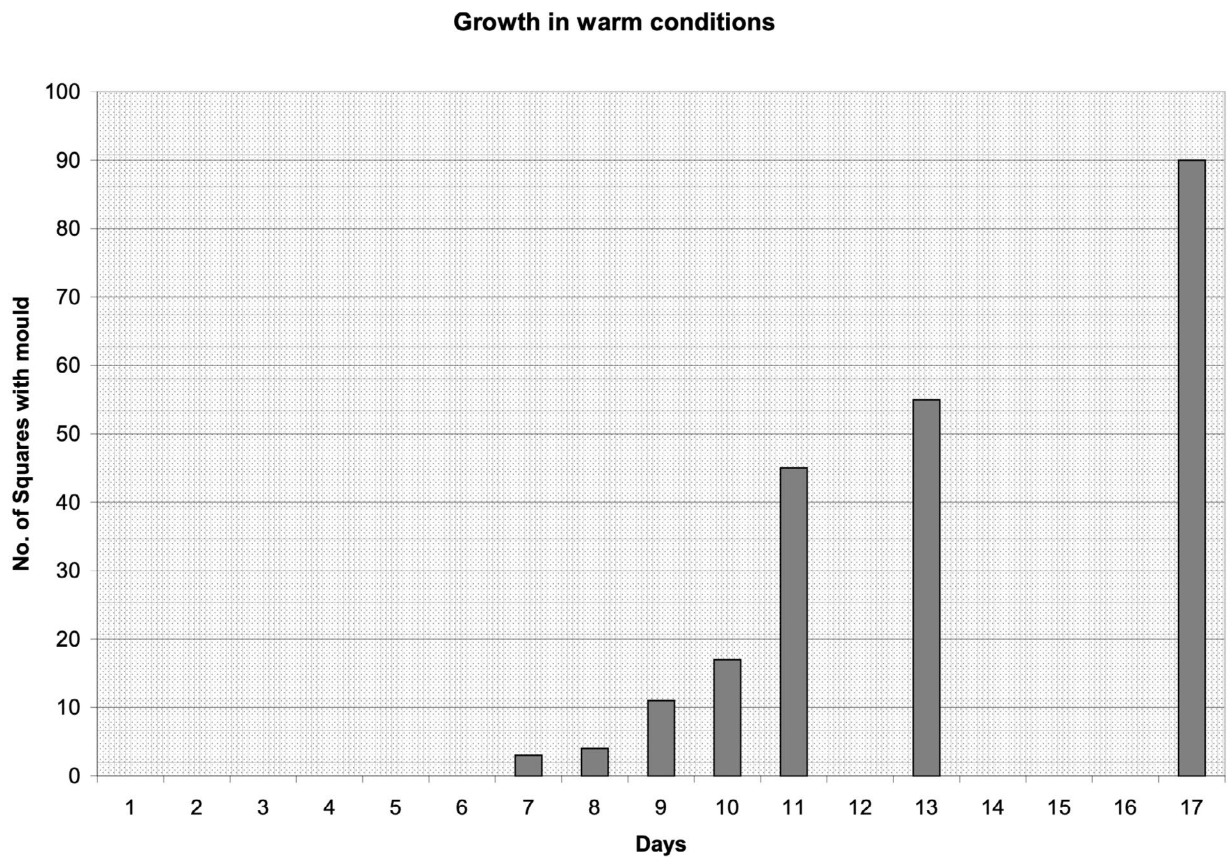


CHART 2

To produce a line graph two sets of data are being used, 'Days' and 'Number of squares containing mould,' which are highlighted, before clicking on the Chart Wizard. From the chart type options presented, choose 'Scatter' and press and hold the Preview button to see the graph. Follow the instructions, leaving the second step, the Data Range as it stands, and putting the titles and axis names in the spaces provided in the third step. Always ensure that the button for a 'New Sheet' is highlighted before clicking Finish. This ensures that the graph or chart will be displayed separately from the Table of Results. The graph of the above is shown below.

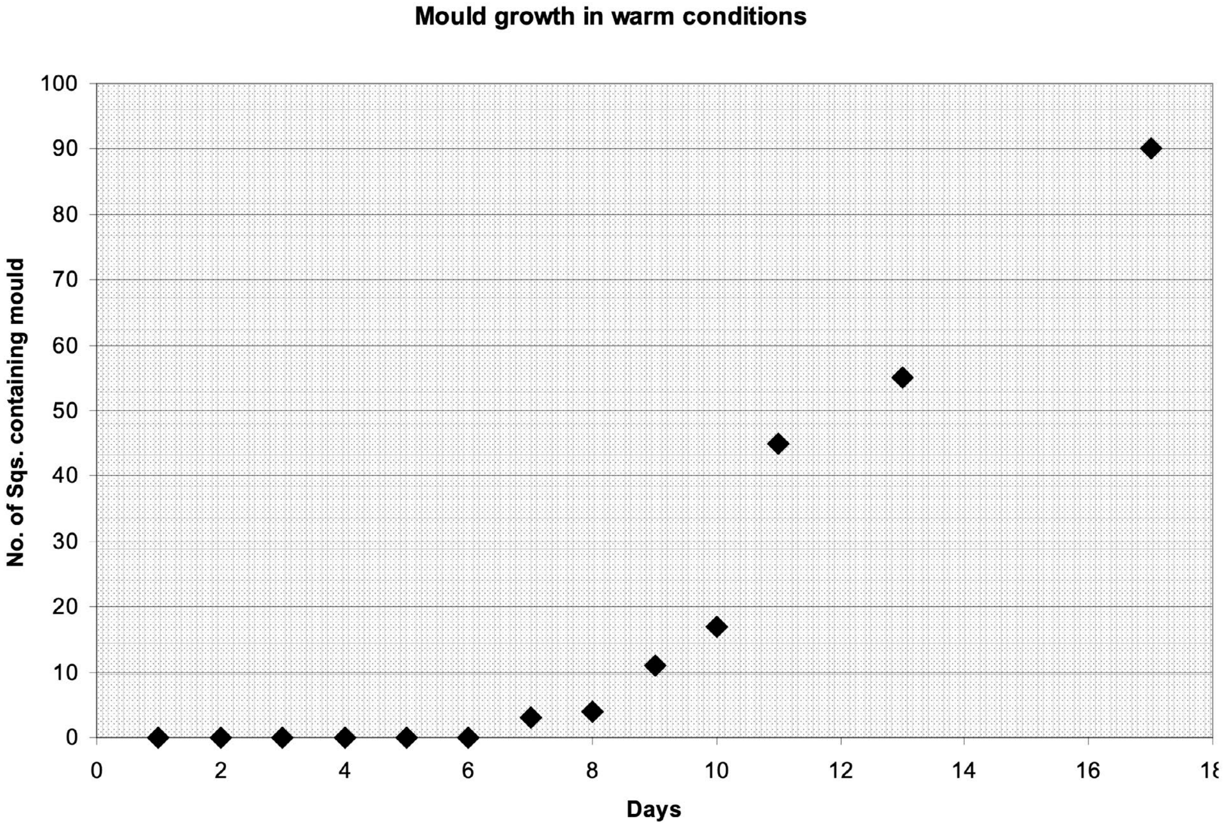
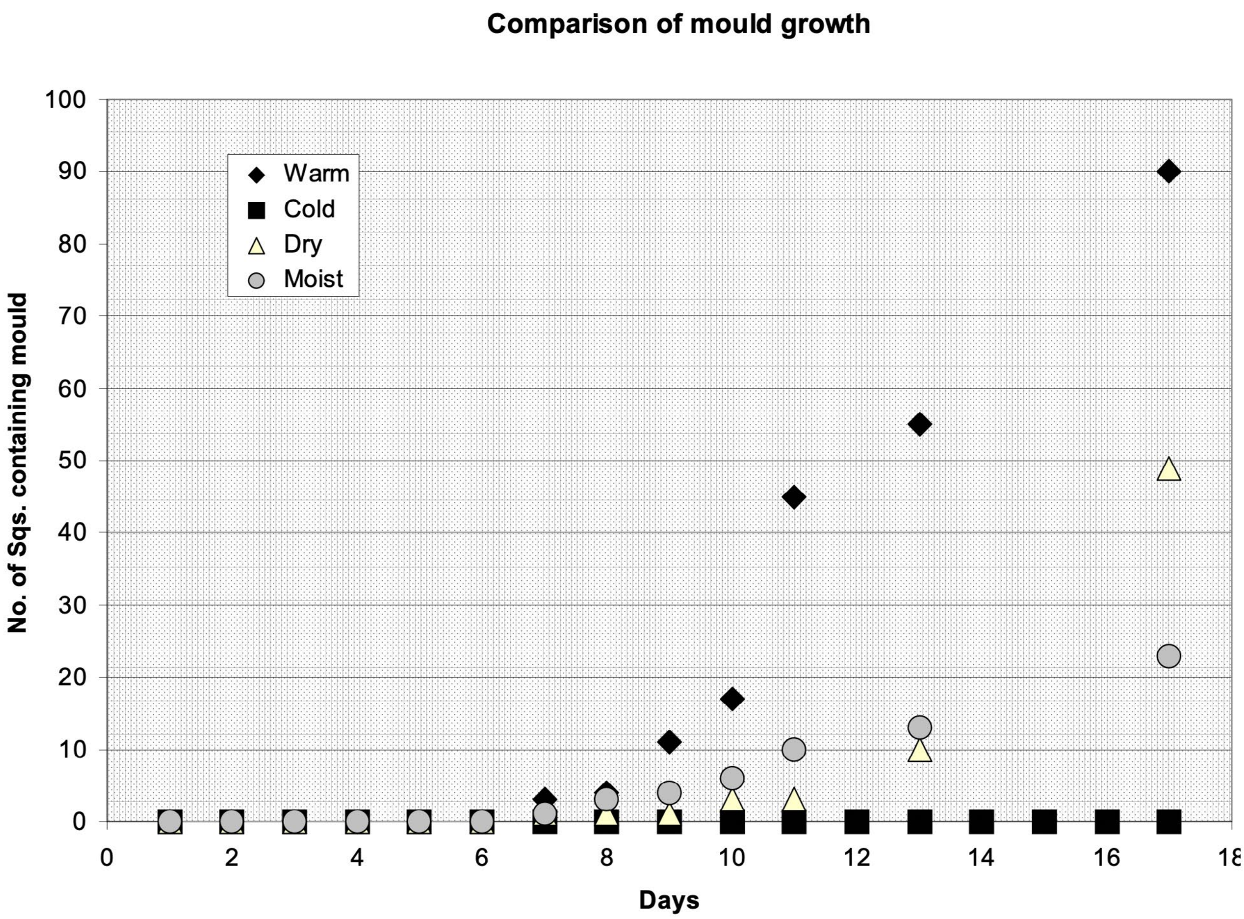


CHART 3

The conditions of growth, and their effect rate of mould growth can be compared by highlighting the whole table, choosing 'Scatter' as before, without any joining lines, and producing another separate chart. Again, the example below illustrates the type of graph generated.



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