looking at decomposers -Micro biology experiment

Posted by Anonymous on Fri, 2016-06-03 08:35

looking at decomposers -Micro biology experiment: This micro biology experiment looks at fungi and bacteria on grass. With the Biological controls ACT and the concerns of late about cultivation unknowns bacteria on agar plates from swabbing surface in a PC1 LAB in a school environment I wondering if this decomposers experiment should be conducted?

I would be collecting samples from the school grounds and the possible fungi spore that would be in the grass samples are Stinkhorns, Cyanthus Striatus, Leratiomyces ceres and chlorophyllum molybdites these are very common fungi in the school grounds. But for the bacteria this is harder to say. Other than the common grass bacillus we have been know to have Melioidosis in the soil in Cairns BUT in saying this not to our knowledge in the school soil. Also we have cane fields close to the school i was wondering if I should be concerned about the possible of rat urine on the grass and therefore possibility of Lymphocytic choriomeningitis. it is common here from handling sugar cane during harvest time which it is now. The rats leave the field so the oval area could be affected

The experiment requires to wipe non sterilized grass cutting of agar plates and sterilized grass cutting over agar plates and incubate for 24hrs at 37C

So give this I am wondering if my concerns are justified. Could you please look into this and give me some safety guidelines please?

Voting:

No votes yet

Year Level:

7
8
9
10
Senior Secondary

Laboratory Technicians:

Showing 1-1 of 1 Responses

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Submitted by sat on 14 June 2016

In brief:

School policies: Currently there are differences between the state/territory educational jurisdictional policies on whether certain microbiological activities can be carried out. Schools are advised to check what activities are permitted in their jurisdiction/school sector before proceeding to work with any microorganisms.

It is important to be aware of the possible hazards and risks, so that appropriate controls can be put into place before working with microorganisms.

Through further correspondence we have obtained further details of this activity. There are several concerns with this activity.

Collection of grass specimens: Precautions would be required for the collection of grass samples to reduce the risk of exposure to unknown microorganisms.

Unknown microorganisms: The activity you describe will produce 'wild cultures' which will not be identified and hence the possibility of isolating pathogens. The Petri dishes should be sealed and never opened after incubation and whilst conducting observations. Due to your tropical location you have indicated a number of potential microorganisms of concern.

Sterilising forceps: Sterilising forceps in a Bunsen flame for 1 minute will result in the forceps becoming extremely hot and the risk of severe burns. Forceps are safely sterilised by wrapping in foil and placing in an autoclave or pressure cooker at 15psi, 121^{o}C for 15minutes. Several forceps can be sterilised for each group at a time. An alternative is to soak the forceps in 70% alcohol for 10min, air dry and wrap in aluminium foil avoiding touching the ends.

Paper bags in a hot oven for 15 minutes: The method does not specify the temperature of the oven for sterilising. A hot air oven needs to be run at 160^{o}C for 2-3 hours to sterilise[1]. The 15 minutes suggested will not be sufficient to sterilise and regular paper bags will not survive the high dry heat temperatures[2]. Special sterilisation paper bags are available for use in steam sterilisers.

Incubation temperature: The method specifies an incubation temperature at around 37^{o}C. The recommended incubation temperature for schools is at temperatures of 30^{o}C or below to avoid the growth of human pathogens.

Decontamination procedure: The method does not explain that the plates should be sterilised in an autoclave or pressure cooker prior to disposal.

Science ASSIST recommends that before schools embark on working with microorganisms they should ask the following questions and perform a site specific biological risk assessment:

- Do the school facilities comply with the requirements of PC1 laboratories? Generally, Australian school science laboratories are classified as Physical Containment level 1 (PC1) and this is only **if** they conform to the requirements specified in Section 5 of AS/NZS 2243.3:2010 Safety in Laboratories – Microbiological safety and containment. At this level they are only suitable for work with microorganisms where the hazard levels are low, and where laboratory personnel can be adequately protected by standard laboratory practice [3]. Microorganisms that are classified as Risk Group 1 are the only group that should be handled in PC1 laboratories.
- Does the school have the necessary equipment for sterilisation and decontamination procedures?
- Do the staff have training in microbiological skills?
- What microorganism is being used?
- What manipulations are being performed with the microorganism? Are methods being used to eliminate or minimize exposure to potentially infectious material via aerosols, splashes, ingestion, absorption and accidental inoculation?
- Are any staff or students wishing to participate in microbiological activities immunocompromised or immunosuppressed (Include those who are pregnant or may become pregnant, or are living with or caring for an immunocompromised individual)? These individuals are more prone to infections. If so, it has been suggested that they should consult a doctor to determine whether their participation is appropriate [4].

Biological risk assessment: According to *Biosafety in microbiological and biomedical laboratories* (BMBL)[5], the following five steps should be considered:

1. Identify agent hazards and perform an initial assessment of risk.
2. Identify laboratory procedure hazards
3. Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment.
4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.
5. Review the risk assessment with a biosafety professional,

If after conducting a detailed risk assessment you have determined that your school can manage the risks, the following procedures are recommended to prevent the growth of any pathogenic microorganisms.

- When culturing from the environment, samples should **not** be taken from areas likely to contain human pathogens.

- The type of media used should not select for pathogens.
    - **Nutrient agar** is a simple media which supports the growth of a wide variety of bacteria and moulds and is recommended for use in school laboratories.
    - Selective media designed to select for more fastidious microorganisms and pathogens such as Blood and MacConkey Agar **should not** be used.

- The lid and base of the Petri dish should be taped with 4 pieces of sticky tape [6] to allow for aerobic conditions and to prevent accidental opening of the plate during incubation. Plates can be sealed with sticky tape or preferably Parafilm completely around their circumference prior to allowing students to examine them. This will prevent any exposure to moisture or drips that may seep out of the Petri dish which are potential sources of infection, as well as keeping the lid securely attached to the base. All observations of any 'wild cultures' must occur with the Petri dish taped. Wild cultures should never be subcultured in a school laboratory.
- When handling micro-organisms it is important to use aseptic techniques at all times. A significant risk associated with microbiology is the generation of microbial aerosols, where fine droplets of water containing cells and/or spores are released into the air.
- Aseptic technique is a fundamental skill in microbiology
    - to avoid the contamination of culture media with unwanted microbes,
    - to prevent contamination of personnel and work surfaces and
    - to prevent microbes from being accidently released into the environment.
- Cultures should be incubated at **temperatures of 30^0C or below** to avoid the growth of potential human pathogens that are adapted to human body temperature.
- The agar plates should be sterilised before disposal. When observations are complete the plates should be decontaminated by sterilising in an autoclave or pressure cooker before disposal into the waste bin. Agar plates must be placed into an autoclavable bag, such as an oven bag, for sterilisation at 110kPa/15psi, 121^0 C for 15-20 minutes in an autoclave or pressure cooker before disposal.

Science ASSIST has previously answered several questions relating to microbiology, see:

Inoculating agar plates and sealing them

Bacteria

Additional information:

Microbiology of soils: Soils contain a diverse range of microorganisms which include bacteria, fungi, algae and protozoa which are involved in the decomposition of plant materials as well as being involved in maintaining soil fertility and recycling nutrients. The rhizosphere (the area closely associated with the roots) of plant material is where much of the microbiological activity takes place[7]. The soil microbial community is influenced by many factors such as temperature, moisture, acidity or alkalinity, oxygen levels, organic matter and soil porosity.

Bacteria and fungi are the most important microorganisms involved in the decomposition process of plant materials.

Bacteria are the most predominant microorganisms present and play an important role in the

early stages of decomposition of organic material, some are nitrogen fixers, some are sulfur oxidisers and others help develop humus in soils and contribute to the smell associated with high organic matter[8]. Some bacteria are very sensitive to changes in the soil environment, while others have features such as resistant spores that allow them to remain in the soil for long periods[8]. Some can cause disease in plants, animals and humans. Bacillus species, Pseudomonas species[8] and Clostridium species are some examples of bacteria found in the soil environment. There is also the emergence of the soil bacterium Burkholderia pseudomallei which is implicated in the infectious disease of humans and animals in the tropics[9]. This organism is able to be cultivated on Nutrient agar[10].

Fungi have roles in plant disease, organic matter decomposition and specialized functions in the rhizosphere. They dominate in the later stages of decomposition. Examples of fungi commonly isolated from soils include Penicillium, Aspergillus, Fusarium and Mucor species [11].

Other: There is also the possibility of microorganisms being introduced via animal excretions in the area. Lymphocytic Choriomeningitis (LCM) if present may be a consideration in your situation. LCMV infections can occur after exposure to fresh urine, droppings, saliva, or nesting materials from infected rodents[12].

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