TWO DAY ONLINE WORKSHOP

ON

LAB SAFTEY AND WASTE MANAGEMENT

04th – 05th JUNE, 2021

Organized by

Sri Venkateswara College

(under the aegis of IQAC)

University of Delhi (South Campus),
Benito Juarez Road, Dhaula Kuan, New Delhi-21

Foreword

An essential component of all science departments are the laboratory technicians whose committed involvement is essential for the progressive growth of the department. The technicians are responsible for the day to day running of the laboratory based tasks, which include preparation of solutions, sample preparation and storage, testing, measuring and recording of preliminary preparatory work, purchase and maintenance of instruments and consumables. They also provide all the required technical support to enable the laboratory to function effectively while adhering to correct procedure and safety guidelines.

Laboratory safety is of great concern these days as the nature of experimental work is no longer confined to conventional old experiments. The ongoing advances in automation and information technology require technicians to operate more sophisticated laboratory equipment and ensure safe work conduct and practices. In addition to performing above mentioned tasks, laboratory technicians should be trained well to avoid health risks and accidents, be in a position to act appropriately in case of emergencies and to minimize the environmental burden and risks caused. They also need to be trained in handling the different types of waste generated in the laboratory such that laboratories adhere to environmental norms. The avoidance of safety risks for the personnel working in a laboratory requires them to be equipped with the knowledge of possible hazards they are likely to encounter(chemicals, biological agents or radioactivity). Workshops and conferences afford valuable training opportunity as well as motivational tool for staff members.

The goal therefore of this two-day workshop is to provide the participants a theoretical as well as a practical overview of the general laboratory procedures, safety measures and the waste disposal methods to be adopted in a Science laboratory. It will acquaint the participants with the scientific knowhow, and make all users aware of safety and emergency protocols (do's and don'ts) that must be followed. The aim is to give them general safety guide lines. While it does cover a wide variety of hazards -- chemical, electrical, biological, etc. – the document does not cover all possible hazards. A reference manual has been provided to the participants to make it simple and informative.

The college also acknowledges TTD management for their constant guidance, encouragement and for providing infrastructural facility.

Workshop for Laboratory Staff on
'Lab Safety and Waste Management'

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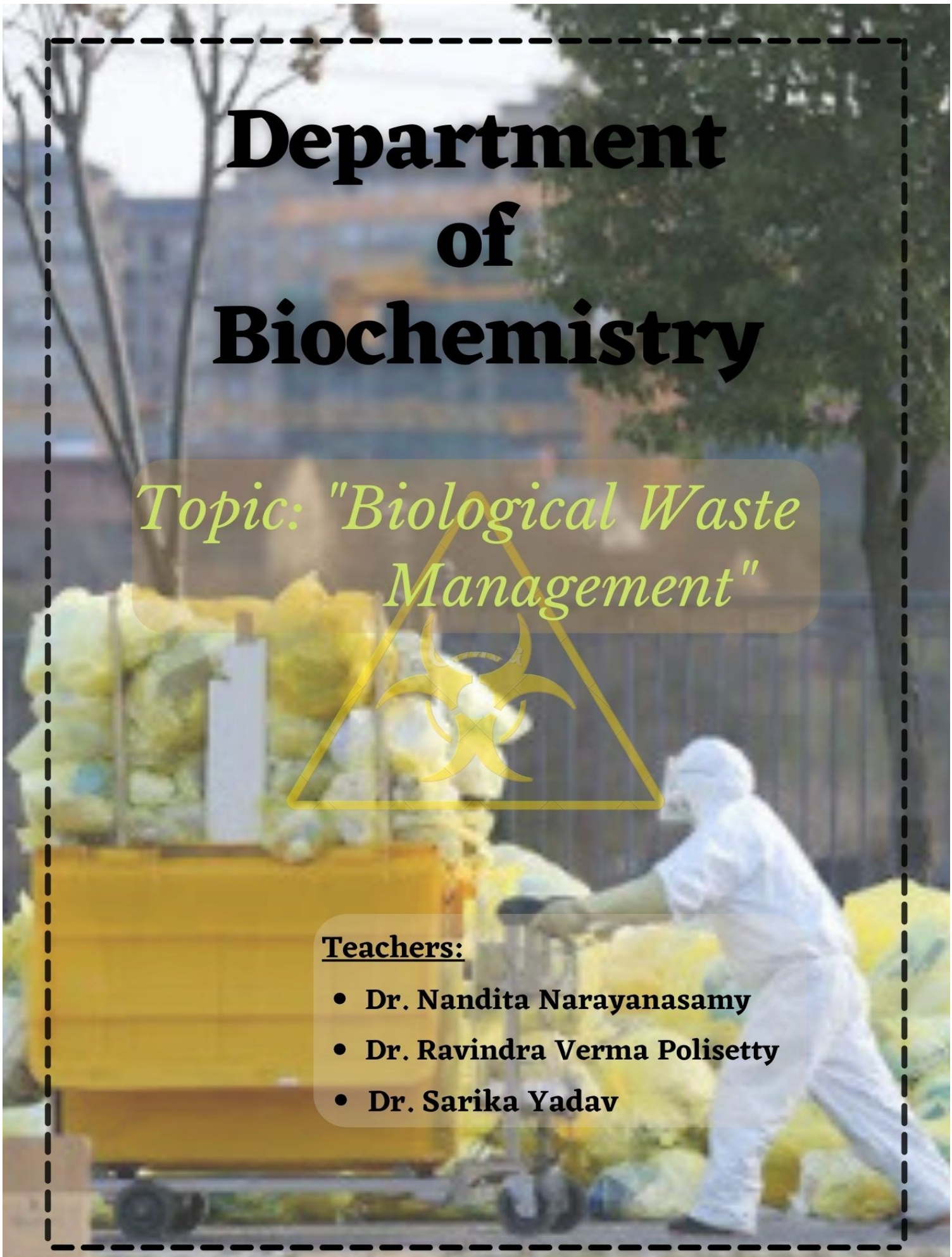


Department of Biochemistry

Topic: "Biological Waste Management"

Teachers:

- **Dr. Nandita Narayanasamy**
- **Dr. Ravindra Verma Polisetty**
- **Dr. Sarika Yadav**

Department: BIOCHEMISTRY

INTRODUCTION: Biological Waste Management

1. OBJECTIVES:

- ✓ Provide detailed information to laboratory staff about laboratory waste categories, segregation, treatment and disposal methods.
- ✓ Provide technical and managerial considerations for the biological waste management process.

2. METHODOLOGY:

- ✓ Discuss about different types of Biological waste such as Clinical waste, culture waste, sharps, gels and plastic wastes.
- ✓ Methodologies regarding the storage and treatment of different biological waste
- ✓ Methodologies regarding the disposal of the treated waste

3. OUTCOMES:

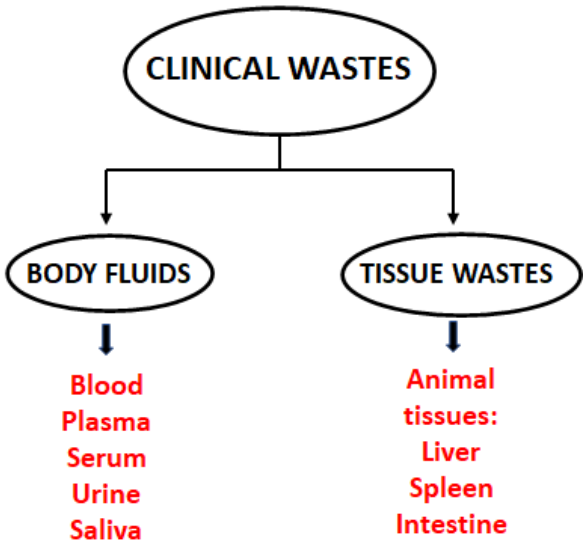
- ✓ Laboratory staff will gain insight about the importance of biological waste segregation.
- ✓ They will learn about the methods of management and disposal of biological waste generated in the laboratory.
- ✓ Laboratory staff will be more aware about the hazards that biological wastes pose to the people working in the laboratory and also to environment and society at large.

Figure 1: International Biohazard symbol

Section 1: CLINICAL WASTE MANAGEMENT

- What are clinical wastes in an undergraduate laboratory?

Any waste arising from medical, nursing, dental, veterinary, pharmaceutical or similar practice, investigation, treatment, care, teaching or research, or the collection of blood for transfusion is considered as a clinical waste. This waste may cause infection to any person coming in contact with it. It may also include wholly or partly of human or animal tissue, or other body fluids, excretions, or other pharmaceutical products, swabs or dressings, syringes, needles which may pose harm to others through injury or infection.



- What are the hazardous consequences of untreated clinical waste?

Clinical waste may:

✓ Contain infectious agents;
✓ Be genotoxic;
✓ Contain toxic or hazardous chemicals or pharmaceuticals;
✓ Contain sharps.

Immediate hazardous effects:

Biological waste may contain pathogenic microorganisms. Pathogens in it, may enter the human body by a number of routes viz through a puncture/abrasion/ cut in the skin; through the mucous membranes; by inhalation or by ingestion.

Long term hazardous effects:

Biological waste is highly hazardous, when it is mixed with municipal solid waste and is dumped in landfills. This can also lead to environmental pollution, apart from posing serious public health risks. Animals are more susceptible to bio-medical waste ingestion and contact.

- Different Treatment methods for clinical waste and disposal:

Blood /Saliva/Urine Sample

- Sample (Blood/ saliva/urine) is treated with 1% Sodium Hypochlorite or 1% Perchloric acid and then discarded in an autoclavable bag for decontamination and further placed in YELLOW color-coded bin.

- Spillage: Cover spillage with absorbent paper towels sufficient to prevent the spread of the spillage and to absorb it. Take 1% Sodium Hypochlorite or 1% Perchloric acid in paper towels and place over the spillage. Leave for 2 minutes for the released chlorine to act. Dispose of absorbent towels and gloves appropriately as biological waste in a Yellow colored bin.

- Containers/tubes containing Blood /Saliva/Urine/any Biological Sample must be treated with 1% Sodium Hypochlorite or 1% Perchloric acid before washing or discarding.

Animal tissues:

- Frequently used animal tissues in undergraduate lab are Liver, Kidney, Spleen, intestine.

- Place all animal waste in plastic bags labeled 'ANIMAL WASTE FROM BIOSCIENCES FOR DISCARD

- Do not allow discarded animal specimens to accumulate in teaching laboratories. NEITHER THROW IN THE DUSTBIN DIRECTLY.

- Animal tissues are disposed off either by INCENERATION (Figure 2) or by BURIAL in an institutional designated space.

Figure 2: Laboratory incinerator

Section 2: Culture Waste Management

Microbiology is a science that investigates the biology of microscopic organisms by growing them in populations called **cultures**. Microbial culture media may be in the form of a liquid called a "broth" or solid or semi-solid forms, either in tubes or in culture dishes (Petri plates) (Figure 3). To ensure that we culture only the specific bacteria we want, and nothing else from the environment, a set of strict **aseptic techniques should be implemented**, which protects us from the bacteria in the cultures, and also protects our cultures from contaminants in the environment. In addition, specific laboratory rules must be followed for containment of microbial cultures in the laboratory, for the safety of all.

Culture in Liquid media

Culture on Solid media

Figure 3: Types of media used for microbial culture

- **Microbial lab Safety Practices and Procedures**

- All bacteria are potential pathogens that may cause harm under unexpected or unusual circumstances. A person with compromised immune system or a recent extended illness should be away from the lab until they have completely recovered.

- Wear gloves when working with cultures, and when your work is completed, dispose of the gloves in the biohazard garbage bin. Safety glasses or goggles are also recommended.

- In the event of an accidental spill involving a bacterial culture, completely saturate the spill area with disinfectant, then cover with paper towels and allow the spill to sit for 10 minutes. Then carefully remove the saturated paper towels, dispose of them in the biohazard waste and clean the area again with disinfectant.

- Long hair should be pulled back to keep it away from bacterial cultures and open flame.

- Make sure that lab benches are completely cleared (everything either thrown away or returned to storage area) before you leave the lab.

- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas.

- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Careful management of needles and other sharps are of primary importance.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Know where specific safety equipment is located in the laboratory, such as the fire extinguisher and the eyewash station.
- Recognize the international symbol for biohazards (Figure 1), and know where and how to dispose of all waste materials, particularly biohazard waste.
- All of the equipment and supplies used in experiments involving bacterial cultures should be sterilized. This includes the media you use and also the tools used for transferring media or bacteria, such as the inoculating instruments (loops and needles) and pipettes for liquid transfer.
- Disinfect your work area both BEFORE and AFTER working with bacterial cultures. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

- **Disposal of Microbiological Waste:**

Microbial waste includes microbial cultures, media, culture plastics, sharps and glassware. All the biohazard waste must be sterilized before it can be included in the waste stream.

- SOLID: Place in a properly labeled, leak proof container; disinfect by thermal autoclaving (Figure 4) or chemical treatment with Sodium Hypochloride and place in a dumpster for disposal in the Landfill.
- LIQUID waste should be disinfected by thermal autoclaving or chemical treatment with bleach and then discharged into the Sewer System.
- Excess proteinaceous material can clump and cause drain clogging. Grinding of treated waste may be necessary.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using the above methods

Figure 4: Autoclave.

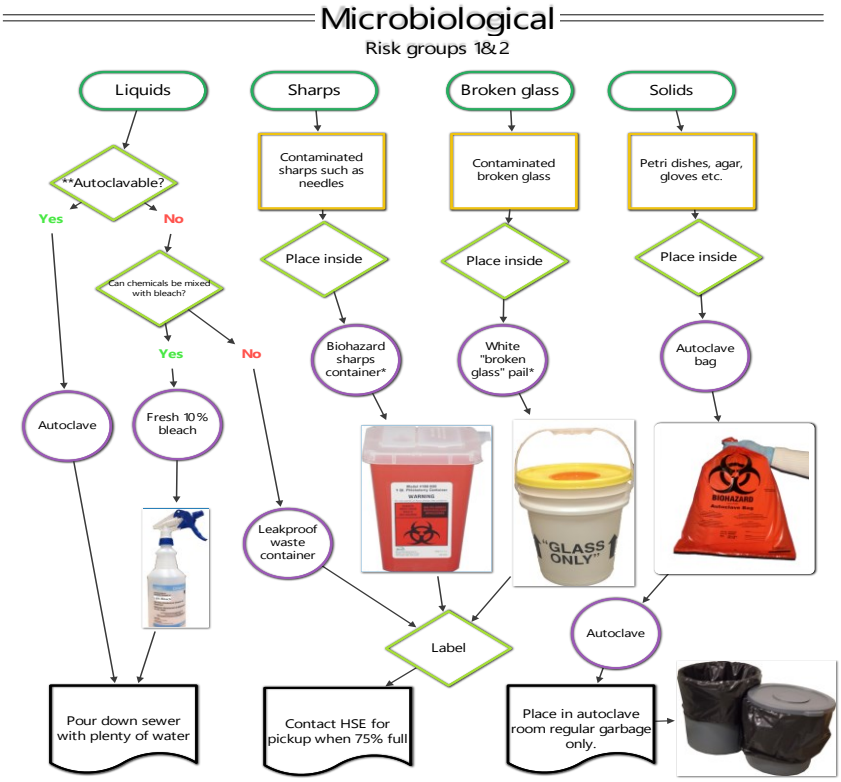


Figure 5: Methods for microbial waste management

Section 3: Sharps

❖ What are Sharps?

Sharps are any object with corners, edges, or projections that when not handled properly or disposed into regular trash are capable of cutting or piercing skin of the garbage handler or tearing the trash bag.

❖ Examples of sharps:

- Hypodermic needles
- Syringes, including tubing, with or without needles
- Blades (scalpels, razors)
- Sharp wires and appliances
- Glass capillary tubes
- Microscope slides and covers contaminated with an infectious agent
- Pasteur pipettes contaminated with an infectious agent
- Serological pipettes contaminated with an infectious agent
- Laboratory glassware contaminated with an infectious agent
- Pipette tips contaminated with an infectious agent
- 'Plasticware' made from plastic polymers which shatter on breakage (culture flasks, petri dishes)
- Glass wool.

❖ How do you dispose sharps?

Sharps can be separated into 3 categories

- Contaminated with Biological wastes like tissue pieces, body fluids or microbial agents
- Contaminated with Chemical waste
- Non-Contaminated

✓ Non- contaminated sharps can be disposed directly into a **'Sharps Container'**.

✓ Contaminated sharps need to be processed before disposal.

❖ Procedure for processing Biological waste contaminated sharps

Biohazardous waste containing sharps that must be sterilized or otherwise rendered non-infectious prior to disposal must be done so using the following methods:

✓ Autoclave using (as a minimum) standard operating procedures established for the sterilizers being used.

✓ Chemical sterilization through the use of appropriate disinfectant e.g.10% Sodium Hypochlorite (bleach), quaternary ammonia compounds and glutaraldehyde

<u>Once processed the sharps may be disposed into a Sharps container</u>

❖ What is a Sharps container?

Sharps containers are boxes of varied dimensions having required specifications. All sharp containers must meet the following standards; they should be:

✓ rigid

✓ non-breakable and puncture resistant

- ✓ impervious to moisture and leak proof
- ✓ have a self-closing lid
- ✓ red in color with a universal biohazard label / chemical waste label. (Figure 6)

❖ Sharp containers MUST:

- Be stored near where the waste is generated and segregated from other waste
- Have their lids in place while in use

- Sharps containers MUST NOT:
- be filled greater than the 2/3 fill line
- be discarded in the regular trash
- contain free liquids, such as full culture tubes or filled syringes (Fig 6)

Figure 6: Sharp cutters and disposal bags

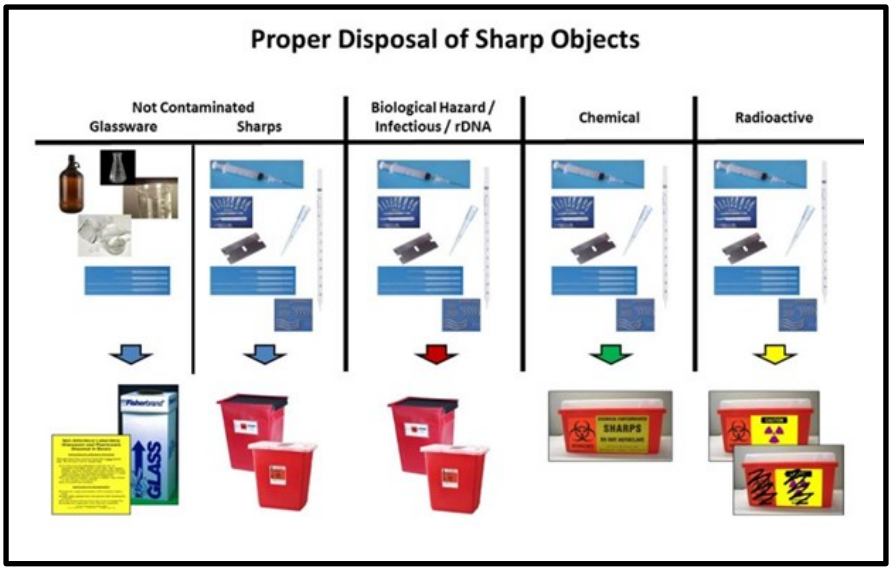


Figure 7: Methods of disposal of different sharp Objects

Section 4: Storage, use and disposal of hazardous material used in a laboratory

- **What are the hazards?**

Certain gels and stains routinely used in a modern Biology laboratory are classified as hazardous chemicals. The risk is encountered during preparation of reagents, conduct of experiment as well as disposal of the reagents after use.

Acrylamide, ethidium bromide, and other stains used in gels are hazardous materials that can cause long term health effects. Exposure can occur through inhalation, ingestion, and skin absorption. Ethidium bromide, binds to DNA and is a potent mutagen capable of causing genetic damage. Acute exposure can cause irritation to the eyes, mouth, skin, and upper respiratory tract. The monomer acrylamide used in the preparation of polyacrylamide gels is a carcinogen and may also cause adverse reproductive and nervous system health effects. While toxicity and potential for exposure substantially decrease after polymerization, exposure remains a concern as complete polymerization cannot be assured.

Casting gels can present hazards from chemical exposure and burns when heating. Physical hazards are presented during the heating done during the preparation of a gel. Spilling and splashing may occur during the heating, mixing, and pouring of the hot liquefied gel, which poses a burning hazard to the body including the face, hands, and upper body.
Running gels also presents hazards of electrical shock. Electrocution is also a potential hazard as typical voltages of 100 V can be applied across gels resulting in 25 mA of current. And lastly gels and stains must be disposed of properly and not in the normal trash

How Can Exposures to these Hazardous Agents Be Minimized?

As with any other hazardous material, in laboratories also we need to conduct a thorough risk assessment and employ the hierarchy of controls (Figure 8) to minimize risk when working with any hazardous material in an Undergraduate laboratory. Some specific applications of the hierarchy of controls to these hazards are listed below.

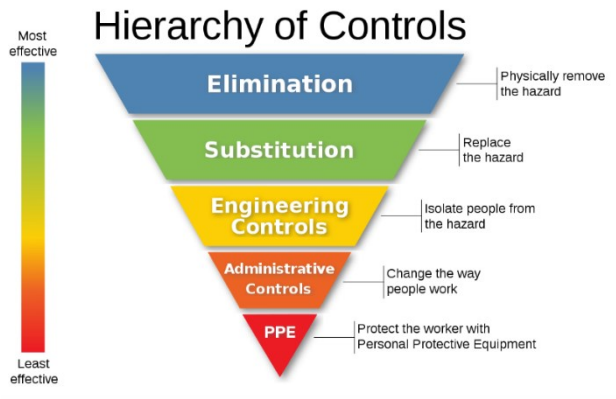


Figure 8: hierarchy of controls to minimize risk associated with hazardous material

1. **Elimination/Substitution**

- Reassess experimental protocols such that high concentration products can be eliminated or substituted with low concentrations or less hazardous substitutes, wherever possible.
- Purchase premade solutions of stains, ethidium bromide and acrylamide at desired concentrations to avoid working with the solid forms which pose a greater inhalation hazard.

2. **Engineering Controls**

- Decrease inhalation exposure by using engineering controls by designating a contained specific area with local exhaust ventilation for dispensing, mixing, and pouring (e.g. chemical fume hood). Local enclosures and fume hoods are good options. All work with solvents for staining and destaining should be conducted in a fume hood.
- Ensure routine maintenance of such local exhaust ventilation systems and check that chemical fume hoods are operating at the proper face velocity before each use.

3. **Administrative Controls**

- Locate nearest eyewash and wash area and confirm that they are accessible and within 50 ft. of the work area. This is particularly important when working with corrosive stains and chemicals
- Ensure all gel apparatus is in good working order. Do not use damaged connectors or cables that are frayed. Use power supplies that have load protections.
- Designate and label areas in the lab where hazardous material is stored and to be used.
- Cover areas where gels are to be poured with a bench cover and change out when it becomes contaminated or is spilled on.
- Clean areas where hazardous materials are weighed, dispensed, or used on a regular basis to reduce accidental exposure.
- When heating liquids in the microwave, use boiling stones (Teflon) to ensure that a nucleation site is always present in heated solutions or ensure constant stirring or shaking.

4. **Personal Protective Equipment**

Always wear appropriate PPE when making gels that includes

- Safety glasses or chemical splash goggles and face shield (when heating liquids in a microwave)
- Lab coat and closed toed shoes,
- Nitrile gloves and
- Thermal gloves when handling hot materials

5. **Waste Handling**

- Contaminated debris like bench covers, pipette tips, gloves should be bagged, labelled and disposed in a hazard container.
- Contaminated glass should be placed in a puncture resistant container before placing in the solid waste hazardous container.
- Stock solutions of ethidium bromide and acrylamide should can be disposed of as hazardous waste in their original container.
- Used stains also should be contained in air tight containers and placed in hazard waste container. The destaining solutions can be recycled by passing through activated carbon.
- Buffers containing any amount of acrylamide or ethidium bromide should be placed in leakproof containers and disposed in hazard waste container.

- Used gels can be photobleached by exposure to sun for 24hrs and then bagged and placed in a labelled solid hazardous waste container.

References/ Interesting Weblinks:

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3. National Institute of Environmental Health Science. https://www.niehs.nih.gov/health/topics/agents/acrylamide/index.cfm
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5. Millipore Sigma Ethidium Bromide Safety Data Sheet.
6. Agency for Toxic Substances & Disease Registry. Toxic Substances Portal – Acrylamide. https://www.atsdr.cdc.gov/phs/phs.asp?id=1113&tid=236
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Figure 9: Biological waste segregation

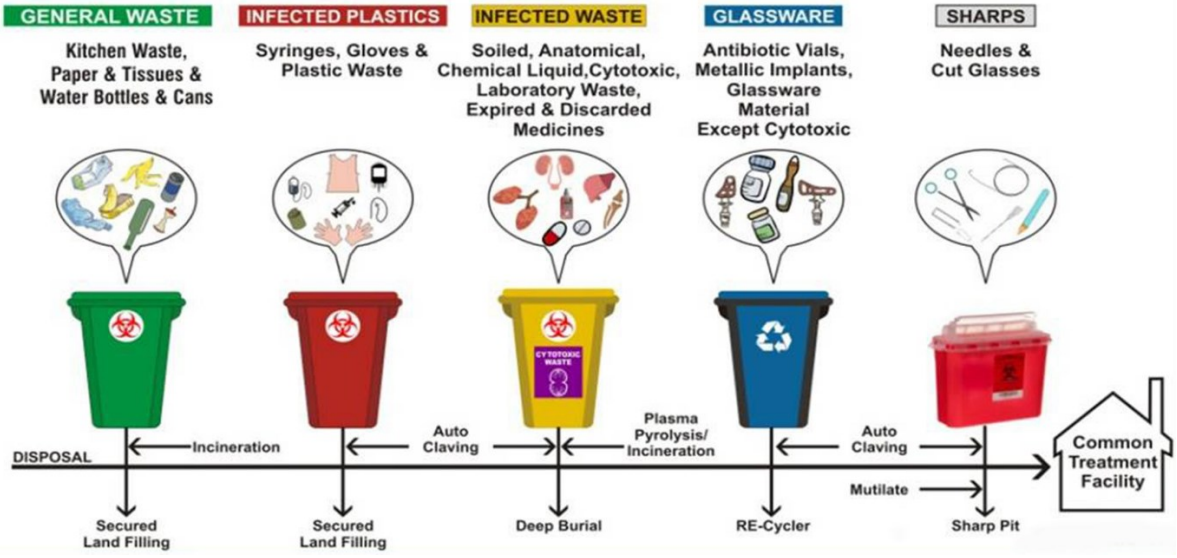

Figure 10: Color coding for the Biological waste segregation

Department of Botany

Topic: "Collection & Management of Plant Specimens"

Teachers:

- **Dr. Sunila Khurana**
- **Dr. Amit Vashishtha**
- **Dr. Pamil Tayal**
- **Dr. Tabassum Afshan**

Department: BOTANY

INTRODUCTION

OBJECTIVES:

The Department of Botany caters students of B.Sc. (Hons.) Botany, B.Sc. Life Sciences, B.Sc. (Hons.) Biological Sciences and all other students of various disciplines opting for General elective (GE) offered by our department. Botany laboratories for routine practical's deals with the variety of plant material be it healthy or diseased for different types of taxonomical, ecological, physiological, anatomical, biochemical and pathological related studies. To meet the requirements, a training to lab staff is required to wisely use of fresh and diseased plant material (without disturbing the ecological flora and biodiversity), and maintenance of collected field specimens.

METHODOLOGY:

The workshop will be organized in online mode to train lab staff for preparing and maintaining herbaria records and maintenance of botanical museum where herbarium sheets are stored. Herbaria document the world's flora and provide a constant and permanent record of botanical diversity. This role is increasingly important as the rate of habitat destruction increases and climate change precipitates rapid changes in species' ranges and all aspects of their ecology. Therefore, the methodology of the present workshop to be organized by our department will focus on:

1. Collection and preservation of disease- free plant material for accurate preparation of herbarium sheets.
2. Proper arrangement, and maintenance of preserved herbarium specimens for long to understand about the changes imposed in present day species by ecological fluctuations.
3. Regular monitoring and mandatory measures to follow of repository to avoid pathogen attack. Pre-planting preparatory steps to follow to avoid pathogen infestation.
4. Understanding the symptoms and disease types in plants so as to avoid the dissemination of pathogenic spores in Botanical Garden and Botanical Museum to prevent the spread of the disease.

OUTCOMES:

At the end of the session, we expect that the learners will understand the importance of plants specimens. During Botanical excursions, lab staff will be able to collect good flora from their native habitat and will bring them to laboratories for preservation. They will be able to evaluate the importance of herbaria and botanical garden. The workshop will help technical staff to develop skills in laboratory and field, develop an understanding of microbes and their adaptive strategies.

Section 1: Collection and Preparation of Herbarium sheets

A herbarium (plural herbaria) is a critical resource for ecological, biodiversity, taxonomy, evolutionary, plant anatomy, palynology and ethnobotany and plant pathological research/ teaching and are generally associated with botanical gardens , research or educational organizations. It is a primary data source of dried, mounted on appropriate sheets, and labelled plant specimens that are arranged to enable easy retrieval access and archival storage. Herbaria consist of specimens that have been collected over broad geographic ranges and over many years and kept in pigeonholes of steel or wooden cup-boards.

The word "Herbarium" was derived from Herbar means plant specimens and arium means an artificial place. Tournefort (1700) used the term Herbarium as an equivalent to Hortus siccus and Linnaeus also used this term. Luca Ghini (1556) is the sole initiator of the art of herbarium making, who started collecting, drying and pasting plant specimens on paper. However, in 18th Century, Linneaus started a new method in which he mounted his specimens on single sheets and started storing them horizontally, which is a method now followed by almost all the museums and herbaria in the world.

The herbarium helps as a support in teaching botany students. Many specimens, which the teachers would like to show to their students, may not be available fresh at the time of giving the course. In such situations, available specimens/herbarium sheets in the herbarium serve the purpose of teaching undergraduate students.

Objectives of herbarium

1. To enable preparation of new monographs and floras.
2. To provide facilities for identification of any material including new taxa.
3. To assemble data for education and research.
4. To preserve specimens of any material including historic importance.
5. To provide material for specific research/education as in ecological, biodiversity, taxonomy, evolutionary, plant pathological, plant anatomy, palynology and ethnobotany and also for molecular research.
6. To bring together in a relatively permanent form specimens for comparative morphological or phylogenetic studies
7. Conservation of plant specimens.

List of Herbarium in Delhi:

1. **Herbarium**

   Botany Department
   University of Delhi
   Delhi-110007

2. **Herbarium**
   Division of Mycology & Plant Pathology
   Indian Agricultural Research Institute
   New Delhi-110012

3. **National Herbarium of Cultivated Plants (NHCP)**
   National Bureau of Plant Genetic Resources (NBPGR)
   New Delhi-110012

4. **Raw Material Herbarium & Museum**
   National Institute of Science Communication
   Dr. K.S. Krishnan Marg
   New Delhi-110012

Requirements for collection of specimens

- Field notebook to record habitat and location information.
- Soft lead pencils for writing
- Global Positioning System (GPS) instrument
- Small altimeter for measuring elevations.
- Gardening gloves to prevent injury when handling irritating or thorny specimens.
- Strong bags for storing branches or carrying individually bagged collections.
- Waterproof tags and permanent felt markers.
- A digger (Khurpi) tool for digging underground stems, bulbs, corms, and roots.
- Pruning cutters to cut plant parts (e.g., fruit, cones, flowers, buds, leaves, bark) or for trimming large, woody plants to appropriate size.
- Plant press (Figure 11 and 12)

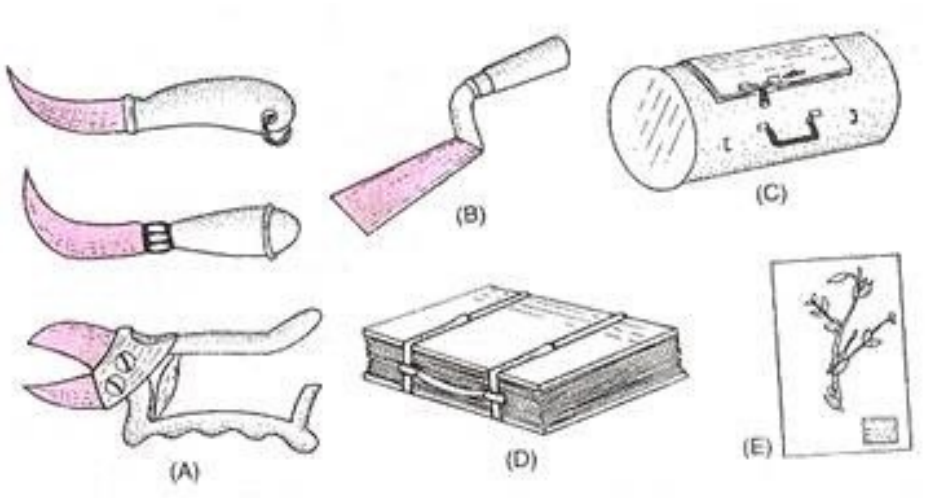

Figure 11: Tools for plant collection A. Cutters, B. Digger (Khurpi), C. Vasculum, D, plant press E. Herbarium sheet.

Procedure for collection of specimens:

- Select specimens in good condition, free of insect damage, rust, or disease (if you are not collecting specimen for lant pathology)
- Select plants with mature parts (well-developed leaves, stems, roots, flowers, and/or fruits or other reproductive structures).
- Select specimens that represent the range of variation in the population, not just atypical specimens.
- Collect entire plants when possible, even if they are large (the plant can be divided for pressing). Collect enough plant material from each species to fill two standard herbarium sheets (42 x 28 cm).
- Collect at least stems, leaves, and flowers or fruit of herbaceous plants, and twigs, leaves, and flowers or catkins of trees and shrubs.
- Collect extra flowers and fruit for later dissection.
- Retain as much of the root system as possible. Remove excess soil as it may cause disfiguration and deterioration of some plants.
- Place all specimens of a single species from one locality into one collection bag.
- As each specimen is collected, assign a unique collection number (see Recording the Data).
- A plant collection without accompanying data is of no use to the scientific community. Keep a careful record of collection data and field observations in a field notebook using a consistent, clear, and legible style. You can use the information later for the herbarium label or for preparing a collection report. File the completed field notebook as a permanent record.

Poisoning, pressing and drying the specimen:

- Poisoning kills the plants and prevents the formation of abscission layer and thereby the leaves, flowers and fruits will be intact with the specimen (twig) will not be getting detached from the plant.
- The poisoning is generally done by dipping the whole plant in a saturated solution of mercuric chloride in ethyl alcohol (usually 20 gm in a litre of alcohol). The plant is again put between the blotters in the presser till it gets completely dried.
- Mercuric chloride is corrosive for metals, and hence enamel trays and disposable gloves are used.
- Lauryl Pentachloro-phenate (LPCP) is also used (3.75% in white spirit) for poisoning the specimens. It is safer than mercuric chloride and leaves the plant features more intact.
- The solution can also be applied to mounted specimens by spraying. Then, the specimens are spread out for pressing and drying. Pressing equipment is the main piece of equipment is a plant press.

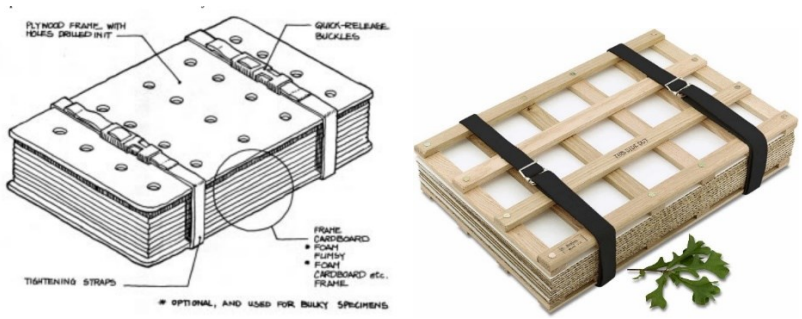

Figure 12: Plant press

Basic techniques of a specimen press

- Separate the frame parts. Make a separate stack for each part if you have plenty of room and it is not windy. Otherwise arrange the materials in the order they will be used: back panel, cardboard, newsprint, blotter (for damp plants, put on both sides of paper), foam (for large branches put on both sides of paper), cardboard, paper, etc.
- Fold a large number of the newsprint sheets in half to form 45 × 30 cm folders (flimsies).
- Lay the straps at one end of the table (both in the same direction). Place one back panel on top of the straps, and place two cardboard separator sheets on the panel.
- Place a folded newsprint sheet on another cardboard sheet (alternate the sides of the newsprint openings within the press to prevent a large bulge on one side). Write the field number on the bottom right corner of the newsprint sheet, inside and outside.
- Write the family name, if known, on the outside as well.
- Place plants to be pressed on the right half of the newsprint folder

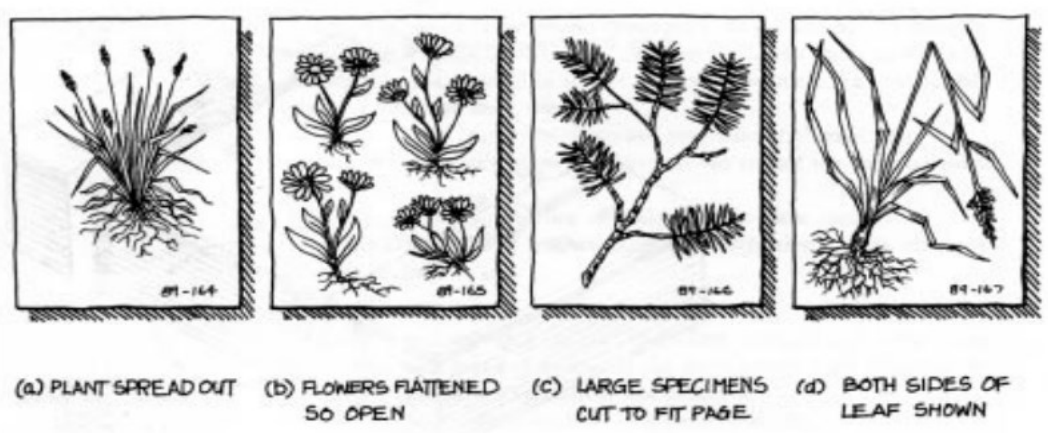

Figure. 13 Arrange the plants carefully with a minimum of overlap.

- Arrange the plants carefully with a minimum of overlap as mentioned in Figure 13.
- **Drying** is a crucial step in preserving collected plant material. To ensure that a specimen retains its colour and does not become brittle or scorched, the moisture must be removed rapidly, while using only a moderate heat. Good air circulation will speed up the process. Make sure the corrugated cardboard still has air spaces in between and is not crushed flat with use.
- During warm, dry weather, tie the press onto the roof-rack if you are travelling by car. The air will flow through the lattice panels and the corrugated separators when the vehicle is moving.
- Place the press inside a well-ventilated vehicle parked in a sunny spot.
- Plants will dry reasonably well in a heated room in a week if you change the papers regularly

Mounting, Stitching and labelling:

- The dried plant specimens are now ready for mounting on herbarium sheets. Fixing the processed plant specimen on herbarium sheet is called mounting.

- A standard herbarium sheet is 28 cm (breadth) x 42 cm (length) and usually made up of heavy long-lasting white handmade paper or thick sheet. The sheet is usually stiff and flexible so as to prevent damage during the handling of mounted specimens.
- The common technique is pasting specimens to sheet with natural glue (usually Gum Arabic). Small quantity of copper sulphate or thymol crystals or may be added to the glue as insect repellent.
- It is advised to have a paper bag/pouch attach to the herbarium sheet to keep any seed/fragments detached from the specimens. Now the herbarium specimen sheet pasted with a label usually at the right-side bottom corner.
- Sometimes large stems, large fruit that are still connected to a branch, or large fruit that burst open may not, with handling, stay taped to the mounting board. In these cases, heavier-grade mounting board can be used and these difficult parts are sewn to the board for added security.

Figure 14: Mounting of dried plant specimens on herbarium sheets

- Use heavy duty thread and a large needle, puncture the board either side of the stem, take the thread through the underside of the sheet and back up to the top of the specimen. Tie off using a reef knot (being careful not to overtighten and thus tear the sheet), move the knot a little to the side of the specimen and trim off any excess. In the case of roundish, large fruit use a criss-cross pattern of sewing by making two holes on either side and tying the threads together in the middle. (Figure 15)
- Extremely large, wide leaves can be sewn on either side of the mid-rib, if the mid-rib is firm enough, instead of using tape at the tip of the leaf.

Recently the Nihon Vogue-sha, Japanese company is offering a new approach/technique to botanical plant preservation for herbarium specimens. The "Plant Specimen Preparation Kit" utilizes a simple and reliable vacuum pump and a unique air press to ensure that plant specimens are beautifully preserved with their natural colors retained (Anonymous, 2016).

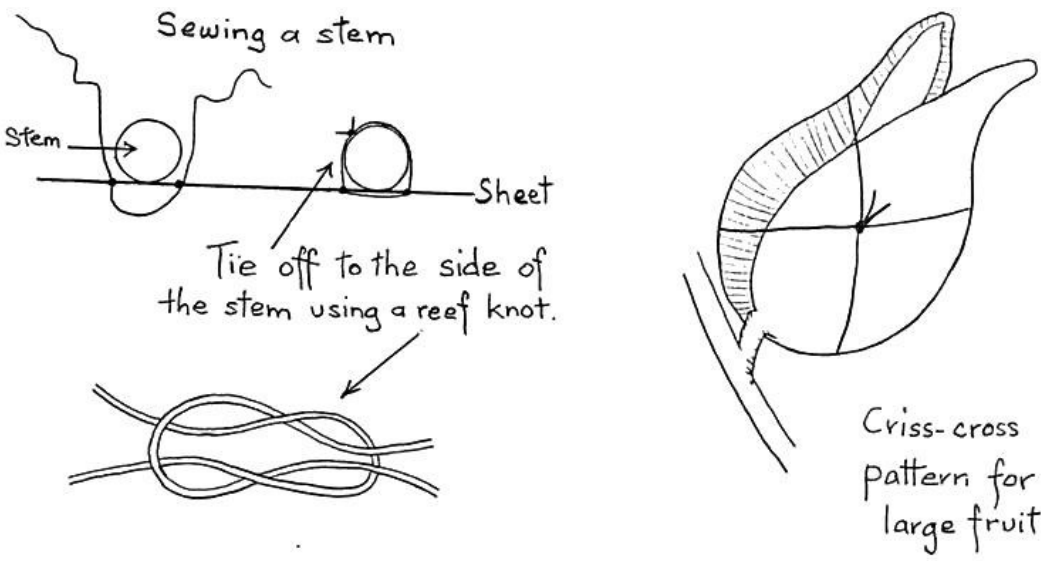

Figure 15: Sewing of specimen

Figure 16: Label of specimen

Deposition of Specimen:

Mounted, labelled and treated specimens are finally incorporated in a herbarium, where they are properly stored and looked after. Small herbaria arrange specimens alphabetically according to family, genus and species. Larger herbaria, however, follow a particular system of classification. Most herbaria usually follow Bentham and Hooker or Engler and Prantl system of classification.

Significance of Herbarium:

1. It provides valuable data and material for teaching and research.
2. Helped in all kinds of taxonomic researches and teaching classes.
3. It aids in assessment of conservation status of a taxon.
4. It serves as a fundamental resource of identification of almost all plants of country and even world.
5. The specimen tag carries all the information about habitat, habit, local name, flower color, etc., of plant specimen for further botanical, ethnobotanical phytogeographical studies etc.
6. As source of estimation of global biodiversity.
7. It is a base line for data on distribution and abundance of keystone species.
8. It helps in distributional range and population biological studies.
9. It may provide seeds of extinct plant species for further studies.
10. It may be useful as source of material for anatomy, palynology cytotaxonomy biochemistry, pharmacognosy etc. studies.
11. Herbarium serves as an aid in teaching plant taxonomy.

General Precautions while collection of plant specimens:

1. Before conducting field experiments, the site should be thoroughly surveyed for relevance of study as well as safety.
2. Permission from authorities should be taken, especially in case samples have to be collected.
3. First aid kits should be readily available
4. Light and comfortable full sleeves clothes, and boots should be worn to avoid mosquito and insect bites

Section 2: Management of preserved specimens - Botanical Museum

Herbarium sheets are often attacked by museum pests, fungi, etc. The practice of preventive conservation is the management of fluctuations in temperature, light, relative humidity, pests, and pollutants that can deteriorate museum collections.

Understanding the practice of preventive conservation as well as the 'Agents of Deterioration' (Physical forces, light, temperature fluctuations, and pests) is most important when trying to protect, display, and store collections. For instance, specimens with sugary or large quantities of nectar / water exudates are also particularly attractive to fungi and need special care during drying to ensure that they dry fast enough to prevent mold growth.

- Dried and pressed plant specimens can be stored best in archival grade boxes, or tied in bundles in cardboard folders for long-term storage.
- A reoccurring threat to the longevity of herbarium specimens is insects such as silverfish, book lice (psocids), cigarette or tobacco beetles (*Lasioderma*), dermestids, drugstore beetles (*Stegobium paniceum*), a number of which find dried plants palatable. Proper storage in secure and stable shelving will allow for less stress and movement to affect plant collections.

- If fungal growth occurs on specimens, it can be brushed with 95% ethanol or methylated spirits (denatured alcohol). However, this may alter the specimen for chemical and other investigative research and only kills the fungus present on the specimen, not preventing further problems of fungal growth.
- **Deep Freezing:** the bundles of specimens are placed in clear plastic bags. The bags are then sealed and excess air is pushed out. The mouth of the bags are heat sealed with the specimen inside or the specimen can be placed inside polythene bags and sealed with parcel tape. Then the sealed bags are placed into a deep freezer for at least 14 days at a temperature of -18 °C, or for 72 hours if freezing at -30 °C.
- **Anoxic treatments:** it involves the usage of bags impermeable to oxygen, containing the specimens inside and oxygen scavenger chemicals. Small anoxic environments starve the pests of oxygen and the specimens become free from pest attack.
- Small sticky tapes placed in hidden areas of the herbarium and herbarium cases to trap the insects. Such traps are monitored regularly after every 15-20 days and replaced with new traps.
- Pheromone traps (Figure 17) involves the use of natural scents which insects use to communicate with each other. Certain insects are attracted to these traps from the surrounding area and are very effective. Specific traps are available for drug store beetles, Indian meal moth, cigarette beetles and ware-house beetles.

Figure 17: Pheromone traps in the botanical garden to capture insects

- Insect electro-cutters are useful for detecting and controlling flying insects. These emit ultra-violet light that attracts flying insects particularly flies and moths.
- Mounted specimen may also be treated with mercuric dichloride or copper sulphate. To prevent them from attack, powdered naphthalene balls or gamaxene powder be also spread from time to time. This ensures durability and long life of the herbarium sheet.

Figure 18: Dry Specimen Treated and Displayed in transparent containers

- The preservation of wet specimen requires periodical replenishment of the specimen. Therefore, the focus should be on maintenance of correct shape and configuration of the fertile parts. For this purpose, a saturated solution of copper acetate in 50% Glacial acetic acid is made as Mother Stock. This mother stock is then diluted in water at a ratio of 1:4, filled in wide-mouthed air tight glass jars (Figure 19). The preservation medium in the specimen jar needs to be changed preferably every 3-4 weeks.

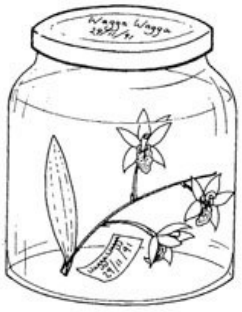

Figure 19: A Preservation Jar having Wide Mouth

Note: The museum manual therefore, must make documented provisions for calendar for these periodical changes in the medium. So also, there should be a provision in the manual for mobilizing a new sample at regular intervals depending upon the source of specimen.

- **Use of repellents -** Chemicals with an offensive odour are kept in herbarium cases to keep pests away from specimens. Naphthalene and p-dichlorobenzene are commonly used repellents. They are available in powdered form and are put in small muslin bags kept in pigeonholes. P-dichlorobenzene is more toxic and prolonged exposure of the chemicals to workers should be avoided. For people working 8 hours a day in a 5 day per week schedule, the upper exposure level for naphthalene is 75ppm and p-dichlorobenzene is 10ppm.

Management of field plants – In the Botanical Garden

Controlling plant diseases with cultural practices and use of chemicals involves a combination of preventing the conducive environment for pathogen growth and improving the growing conditions for maximum plant health. In most cases, gardeners should employ a multitude of cultural practices to produce healthy, disease-free plants that grow vigorously. A few examples are noted below.

Cultural methods - In the Botanical garden, rotating crop to a different crop-bed/ spot each year is a popular cultural control used to disrupt year-to-year pest cycles, as is removing and destroying old plants at the end of the growing season.

- Warming the soil under sun before planting can avoid seed rots (soil-solarization).
- Planting a mix of plant species may reduce or slow the spread of disease if resistant plants are planted among more susceptible plants.

- Watering lawns and gardens early in the morning so the plants can dry quickly and remain dry through the night is an effective cultural practice for some fungal or bacterial diseases.

- Watering ornamentals at the base of the plant, as opposed to using overhead irrigation, will minimize leaf wetness duration and prevent foliar diseases.
- Fertilize plants at the correct time of year. Tall fescue and Kentucky bluegrass fertilized with nitrogen too late in the spring or during the summer are more susceptible to brown patch or Pythium blight.
- Pruning and training plants in ways that promote air circulation around leaves and that allow more light penetration creates a healthy environment that discourages infection.
- Planting a tree or shrub correctly and in the right type of location gives the plant a better chance of resisting diseases and other problems. Diagnostic clinics often see plants that died simply as a result of improper planting.

Chemical methods - Finally, chemicals such as fungicides, bactericides or nematicides are sometimes necessary to control problematic plant diseases. Chemical controls can be an effective and necessary part of an IPM program, but it is important to properly identify the problem and determine if a pesticide is warranted and, if so, to use it correctly.

- When using chemicals, always read and follow the label carefully. Apply compounds properly, and respect environmental health and safety information indicated on labels. This information can include special directions for mixing and application, limits to application on windy days or before predicted rain events, or buffer areas needed between application areas and wells or watercourses.

Physical Methods - Physical methods include the use of fumigant. Fumigation is a method of pest control that completely fills an area with gaseous pesticides or fumigants to poison the microbes within. Fumigant at a required temperature and pressure exist as a vapor form, when released it penetrates objects or enclosed areas in concentrations that kill by interfering with the respiratory function pest organisms.

- **Soil fumigation** - Most fumigants require a soil temperature between 10-30 ^{o}C at a soil depth of 6 inches to activate. Since you'll need to apply the fumigant prior to planting, spring is an ideal time to apply the chemical. A sheet of plastic over the fumigated soil prevents the fumigant from escaping into the air. Sealing the area for up to 2 weeks is best done immediately after applying the fumigant. Rocks or soil help hold down the edges of the plastic, ensuring the fumigant is kept inside. The gas passes through the soil and controls pests that live there, including nematodes, fungi, bacteria, insects and weeds. (Figure 20)

Figure 20: Soil fumigation in Botanical Garden

- Chamber Fumigation - Chamber fumigation is effective at above 20°C and relative humidity of 55-60%. Formaldehyde vapor (commercially available as formalin) is an extremely effective biocidal agent. Before room fumigation, hydrochloric acid and chlorine-containing disinfectants must be removed from the area.

1. Seal off the room (Laboratory/ Laminar Hood/ Botanical Museum). Never allow personnel in the environment when the area is being decontaminated, as highly toxic agents are used. Place boundary tapes or other obvious notices to alert anyone that they are not allowed to approach or attempt to operate the hood until decontamination is complete. (Figure 21)
2. Notify the institution's safety officers of the date and time that the decontamination is to be done, as well as the type of fumigation agent being used (formaldehyde or hydrogen peroxide).
3. Prepare the fumigation solution - in a petridish, mix 30 ml of formalin and 3g of $KMnO_4$, keep the pertidish in every corner of the room and evacuate immediately. Seal the arear properly for atleast 18-20 hour.
4. After the fumigation is complete, switch ON the exhaust fan and ceiling fans for 2-3 hours to evacuate the fumes form the room.

Figure 21: Fumigation inside cabinets and laminar air flow

Safety Precautions

Before performing a fumigation, the applicator needs to understand clearly the hazards and problems associated with the use of fumigants. Most fumigants are highly toxic to all forms of life, including humans, animals, plants, and even microbes. Fumigation is a highly specialized operation that requires equipment, techniques, and skills not generally used for applying other types of pesticides. Applying a fumigant may be time consuming and expensive, usually requiring more labor than other pest control methods.

Understanding the pathology of plants:

Phytopathology taken from the Greek word where, Pathos means suffering and logos means study of therefore it is the study of suffering plants. And plant disease is the study of microorganisms and environmental factors that cause diseases in plants. Organisms that cause infectious disease include fungi, oomycetes, bacteria, viruses, viroid etc. Plant pathology also involves the study of pathogen identification, disease etiology, disease cycles, economic impact, plant disease epidemiology, plant disease resistance, how plant diseases affect humans and animals, pathosystem genetics, and management of plant diseases.

GENERAL SYMPTOMS OF PLANT DISEASES

Plant Pathogens induce different reactions in the hosts. This results in creation of abnormalities which appear on the plants. The evidence of the abnormalities in the appearance of the disease plant brought about by the pathogens after host pathogen interaction is called symptoms.

- As a result of infection, a number of physiological changes occur in the plants such as change in respiration, photosynthesis nitrogen fixation, transpiration etc.
- Basically, host respiration increases therefore photosynthesis rate is reduced and there is an increase in transpiration rate.
- These lead to anatomical and morphological changes in the entire plant (vegetative and floral).
- These changes are expressed in visible changes- Myxomycetes and phycomycetes: it forms galls, scalps, warts, rot, blight, white rust, damping off, downy mildew.

BACTERIAL PLANT DISEASES

a) CITRUS CANKER: Causal organism: *Xanthomonas citri*

- It is gram negative bacteria.
- It is a rod-shaped bacteria with a size of 1.5*2*0.5*0.75 µm
- Aerobic bacteria
- Possess single flagellum
- It attacks the leaves, twigs and fruits.
- Favorable conditions for the spread of this disease are moisture and strong winds.
- It survives in parasitic form, canker leaves, bark for long periods.

Symptoms:

- In young leaves it appears as lesions as white small specs which later on develop into brown necrotic spots (1-2 mm in diameter)
- They become raised to form white spongy eruption.
- As the lesions enlarge the white eruption begin to collapse and brown depression appear which grows as crater.
- Margin of lesion has greasy appearance. As the disease progresses, the crater portion becomes greyish white and is surrounded by yellow halo on the leaves crater is 1-9 mm in diameter while in twigs and fruits is 1cm in diameter.
- These lesions are due to hypertrophy or hyperplasia.

b) Bacterial Blight of Rice: Causal organism: *Xanthomonas oryzae*

- It causes wilting of seedlings, yellowing and drying of leaves.
- The disease is most likely to develop in areas that have weeds and stubbles of infected plants. In general, the disease favors temperatures at 25−34°C, with relative humidity above 70%.
- It is commonly observed when strong winds and continuous heavy rains occur, allowing the disease-causing bacteria to easily spread through ooze droplets on lesions of infected plants.
- Bacterial blight can be severe in susceptible rice varieties under high nitrogen fertilization. (Figure 22)

Symptoms:

- Small, green water-soaked spots develop at the tips and margins of fully developed leaves.
- Then they expand along the veins, merge and become chlorotic then necrotic forming opaque, white to grey colored lesions that extend from leaf tip down along the leaf veins and margins.
- Both bacterial blight and bacterial leaf streak can occur simultaneously and are difficult to distinguish.

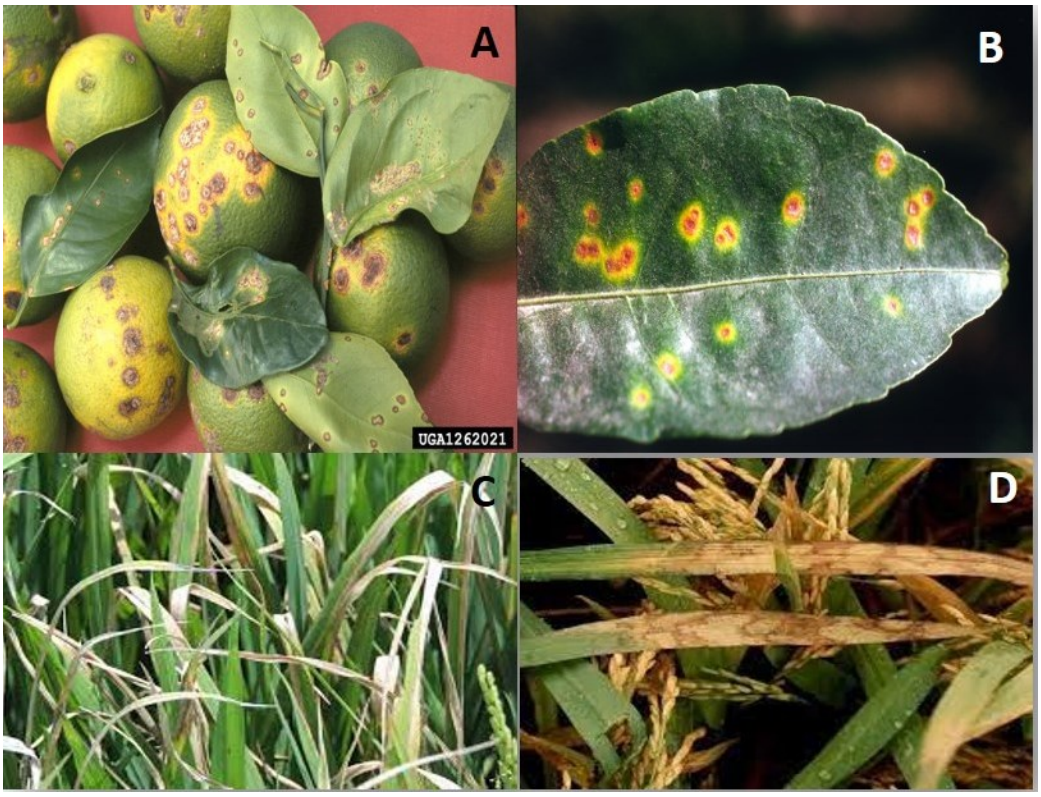

Figure 22: Bacterial disease symptoms caused by (A and B) – *Xanthomonas citril*; (C and D) - *Xanthomonas oryzae*

FUNGAL PLANT DISEASES

a) Early Blight of Potato: Causal Organism: *Alternaria solani*

- It is a fungal pathogen that produces a disease in tomato and potato plants called early blight.
- The fungus is readily cultured on artificial media such as V8 juice where it produces a deeply pigmented gray/black hairy colony.
- The mycelium is haploid and septate, becoming darkly pigmented with age.
- Sporulation in culture can be stimulated by exposure to fluorescent light. The asexual conidia are borne singly or in a chain of two on distinct conidiophores. The beaked conidia normally possess 9–11 transverse septae.

Symptoms:

- Symptoms of early blight occur on fruit, stem and foliage of tomatoes and stem, foliage and tubers of potatoes.
- Initial symptoms on leaves appear as small 1-2 mm black or brown lesions and under conducive environmental conditions the lesions will enlarge and are often surrounded by a yellow halo.
- Lesions greater than 10 mm in diameter often have dark pigmented concentric rings. This so-called "bullseye" type lesion is highly characteristic of early blight.
- As lesions expand and new lesions develop entire leaves may turn chlorotic and dehisce, leading to significant defoliation. Lesions occurring on stems are often sunken and lens-shaped with a light center, and have the typical concentric rings.

b) **White Rust of Crucifers: Causal Organism:** *Albugo candida*

- Mycelium intercellular with knot like structure called haustoria.
- It is an obligatory parasite.
- It bears sporangia in chains.
- Each sporangia germinates to produce 4-8 zoospores, which reinfect the host plant.
- Moist favors this disease.

Symptoms:

- All the parts of plant are attacked except the roots.
- In local infection, pustules develop on leaves and stem.
- The pustules appear shiny white or yellow in color.
- Several pustules coalesce to form a large patch.
- Host epidermis ruptures and white powdery mass of spores appear on the leaf surface. (Figure 23)
- When young stem and inflorescence are attacked, fungus grows inside the host plant tissue and form deformities.
- Leaf is rarely distorted and sori develop on lower surface of the leaf.

Figure 23: Fungal disease symptoms caused by (A and B) –*Alternaria solani*; (C) –*Albugo candida*

VIRAL PLANT DISEASES

(a) Tobacco Mosaic Virus (TMV) Disease: Causal Organism: TMV (Tobacco Mosaic Virus)

- It is a rod-shaped virus having 15nm diameter and is 300nm long. (Figure 24)
- It is a single stranded RNA which is thermostable.
- It survives in leaves and stocks in soil and seeds.
- Initially this virus infects the seedling then it infects throughout the season.

- It enters through wounds and then enters the parenchymatous tissue, moves from one cell to another and multiplies until it reaches phloem from there it infects whole plant.

Symptoms:

- Mottling(patches) on leaves
- Curling of leaves
- Chlorosis
- Dwarfism
- Distortion of leaves
- Stunting of younger plant by curling/distortion of leaves
- Dark green areas are thicker and appear elevated while light green areas are thin which are chlorotic.

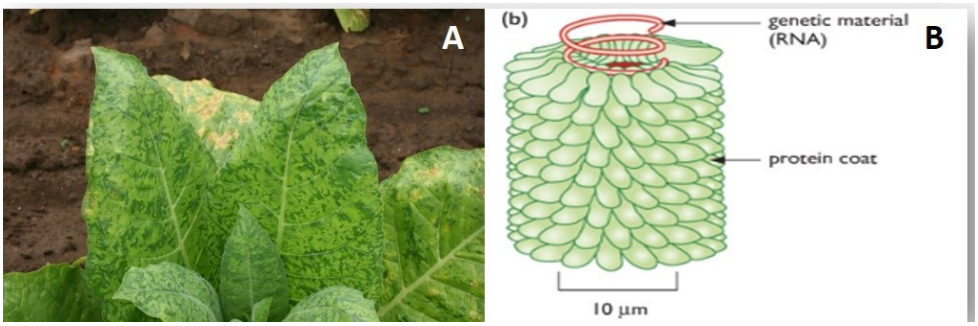

Figure 24: Viral disease symptoms on the surface of leaves and its causal agent i.e. Tobacco Mosiac Virus

(b) Yellow Vein Mosaic Virus: Causal Organism: Yellow Vein Mosaic Virus or Hibiscus Virus I (Figure 25)

- Transmitted by white flies (*Bemesiatabaci*)
- Bhindi leaf hopper (*Empoascadevastans*)
- Several weeds like *Ageratum, Malvastrum*are susceptible to this virus.

Symptoms:

- Vein clearing followed by vein chlorosis.
- Veins become thick and prominent.
- In severe cases chlorosis extends to interveinal area and cause complete yellowing of leaf.
- Fruits become dwarf and malformed.

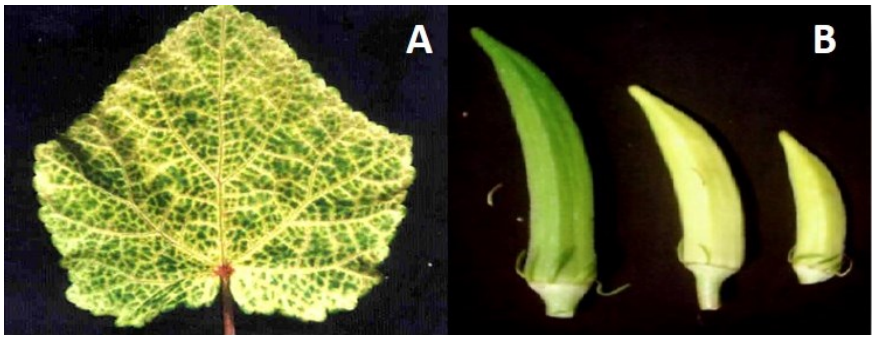

Figure 25: Viral disease symptoms caused by Hibiscus virus

PRECAUTIONS WHILE HANDLING DISEASED PLANT MATERIAL

1. Diseased plants should be burned after harvest.
2. Use of disease resistant varieties.
3. Sanitation of the field should be done on a regular basis.
4. Workers in the field should wash their hand with 3% thisodium phosphate and soap.
5. Equipment used during harvesting should be sterilized.
6. Use of pesticides, fungicides can be done in order to eradicate the pathogens.

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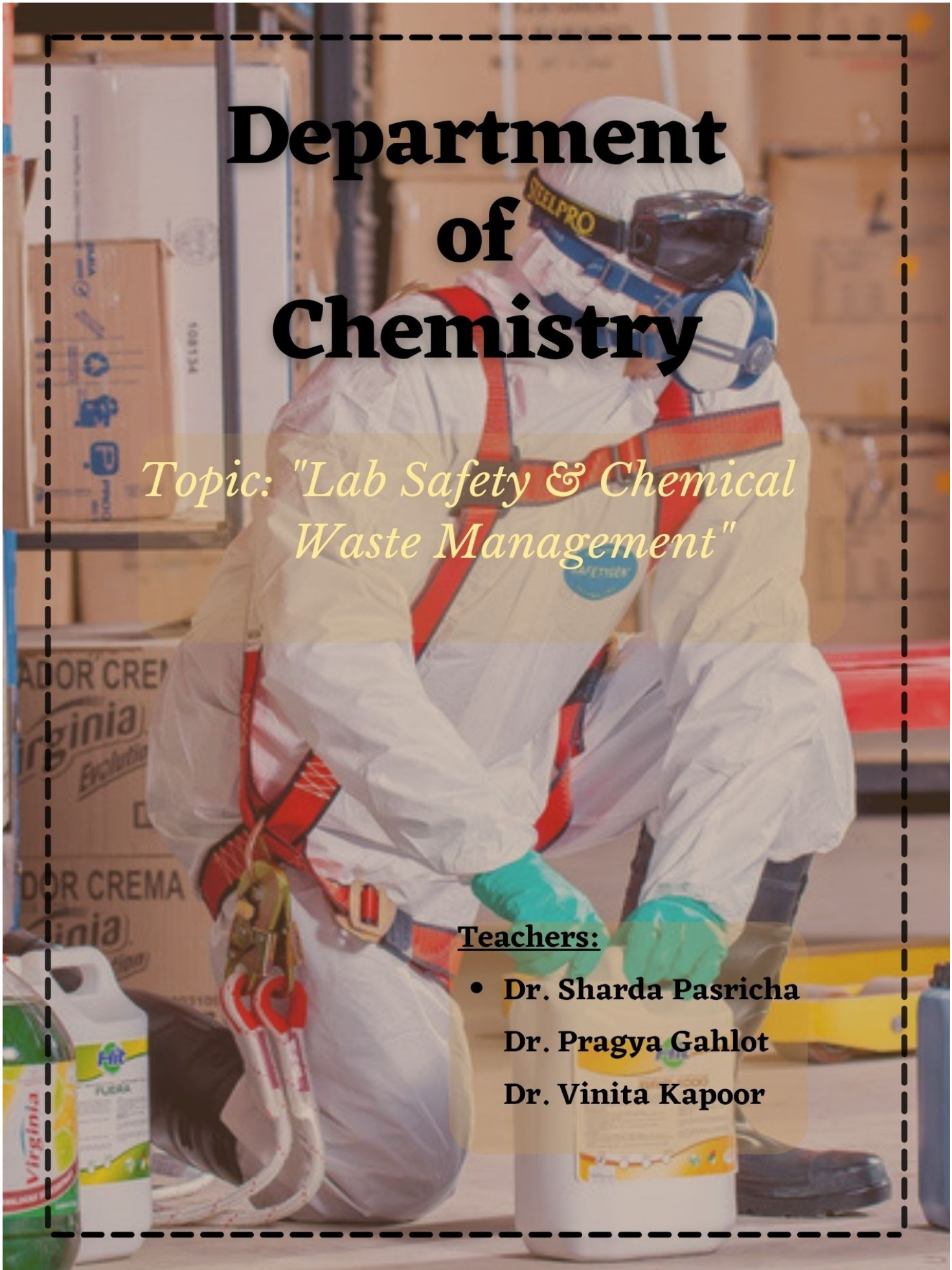

Department of Chemistry

Topic: "Lab Safety & Chemical Waste Management"

Teachers:

- **Dr. Sharda Pasricha**
  Dr. Pragya Gahlot
  Dr. Vinita Kapoor

Department: CHEMISTRY

OUTCOMES:

Through this two-day workshop we wish to provide the participants with an overview of the general laboratory procedures and safety measures to be followed. This reference manual has been drafted for the participants in a simplified and informative manner. The participants would:

- ***Learn*** *how to work safely in the lab*
- ***Know about*** *the first-aid and anti-dotes in case of chemical exposure and accidents*
- ***Ensure*** *reduction in environmental pollution by following the 3R principle*
- ***Reduce*** *the amount of untreated and corrosive waste being discarded in the sink.*

SECTION 1: LAB SAFETY

1.1. GENERAL LAB SAFETY INSTRUCTIONS

It is highly recommended that the following precautions and safety measures must be followed while handling of chemicals, solutions and preparing the reagents:

1. Always wear a lab-coat, mask and safety goggles while working in the lab.
2. Wear N95 gloves while dealing with highly poisonous/irritant chemicals.
3. Lab should always be well ventilated.
4. Follow the specific procedure with protocol and amounts/volumes of chemicals and solvents to be used for the reagent to be prepared. This not only prevents accidents and wastage of chemicals but is also an eco-friendly practice.
5. Use clean and dry glass apparatus while preparing the reagents.
6. Store the reagents after proper labelling (*name of reagent, date of preparation, hazard warning as per the instructions*) in clean apparatus.
7. Dispose-off any waste paper or any kind of solid waste properly.
8. Maintain a safe distance while using the chemicals. Hold containers away from the body when transferring a chemical or solution from one container to another.
9. Make sure all flammable solventsand volatile chemicals are used in the fuming cupboard only.
10. Add concentrated acid to water slowly. **Never add water to a concentrated acid**!
11. Never leave containers of chemicals/reagents open.
12. Wash hands properly after handling chemicals every time.

Figure 26: General lab safety instructions

1.2. PREVENTION, SAFETY AND FIRST-AID IN LAB ACCIDENTS

A lab worker should be well versed in laboratory safety procedures. Alertness and caution are the things of requisition for anyone concerned with laboratory. One is surrounded by many dangers from corrosive substances, poisonous, explosive, inflammable chemicals etc. Almost all the chemicals except a few used in a laboratory are lethal poisons. Appropriate methods of safe handling, handling the situations, rendering the first aid in case of accidental happenings are to be well known to technicians.Accidental happenings in a laboratory can be caused by-

1) Acids and alkalis
2) Toxic, harmful and irritating chemicals
3) Heat
4) Fire
5) Explosion
6) Electrical shock.

1.2.1 ACIDS AND ALKALIS:

Acids, alkalis and phenols which are corrosive in nature are used extensively in a laboratory. Corrosives cause damage to the living tissues when they come into contact. They can cause bodily damage due to a) Spilling or splashing on skin, eyes. b) Accidental swallowing during mouth pipetting c) Contact with lip and tongue.

1.2.1.1 First aid in case of Swallowing of acids:

1) Make the victim drink 5% soap solution or 8% Mg $(OH)_2$ or white portion of two eggs combined with 500 mL of water or at least plenty of ordinary water.
2) Make the victim gargle with soap solution.

1.2.1.2 First Aid in case of contact of acid with lip and tongue:

1) Immediately rinse in tap water.
2) Bathe the affected area with 2% aqueous solution of sodium bicarbonate.

1.2.1.3 First aid in case of accidental swallowing of alkalis:

1) Rinse the mouth with tap water and make the patient drink 5% acetic acid solution or lemon juice.
2) Make the victim drink 3-4 glasses of water.
3) Consult a physician.

1.2.2 TOXIC, HARMFUL AND IRRITATING CHEMICALS:

Toxic and harmful chemicals are those which can cause death or serious illness. Irritating chemicals are those which irritate the skin, mucous membranes or the respiratory tract. **Potassium cyanide is well known and the most lethal poison. Xylene, formaldehyde and ammonia vapours are skin and mucous membrane irritants. Phenyl hydrazine, Aniline, nitro compounds, Aromatic hydrocarbons etc are harmful to respiratory ducts, skin and whole body. Iodine, chloroform, sodium nitroprusside, mercuric nitrate, sodium azide, barium chloride and methanol etc. also fall into the category of toxic, harmful and irritating chemicals.**

1.2.2.1 First aid in case oftoxic, harmful and irritating chemicals:

1) If some substance falls on the hands, wash thoroughly with a brush.
2) When liquid bromine spills on skin or clothes or working benches, pour 20-40% Hypo solution and rinse with plenty of water. Apply Vaseline to skin.
3) If a noxious gas like Cl_2 or Br_2 vapour is inhaled, breathe in a dilute solution of NH_3.
4) A lethal poison like Potassium cyanide causes immediate death. However, when not so lethal poisons are accidentally swallowed:
    a) Spit immediately
    b) Rinse with lot of tap water.
    c) Induce vomiting by drinking warm salt water.

5)In case of injury by broken glass ware containing infectious specimens:

a) Wash the wound immediately.
b) Squeeze to bleed for several minutes.
c) Bathe the wounded area with antiseptic lotion.
d) Wash thoroughly with soap water.
e) Bathe second time with antiseptic lotion and consult a physician.

1.2.3 HEAT BURNS:

1.2.3.1. First Aid in case of minor burns:

1) Pour cold water or ice-cold water over the affected part.
2) Apply mercurochrome or acriflavine ointment to the affected part. Glycerol or cotton wool soaked in alcohol can also be used.
3) Apply a dry gauze dressing loosely.
4) Do not tear the blisters formed over the burns.
5) Consult a physician

1.2.4 FIRE:

Sources of fire in a laboratory are:

1) Inflammable liquids-Inflammable liquids are those which have low flash points. Eg. Ether, acetone, CCl_4, alcohol, benzene, toluene etc.
2) Gas-Gas supply can be source of fire accidents due to
   a) Leakage in the gas pipe line
   b) Gas cylinders left on
   c) Gas delivery points turned off.
3) Electricity- Electricity is the source of fire due to short circuit in the pipeline.

1.2.4.1. Extinguishing of fire:

- Water should not be used to extinguish fire caused by inflammable liquids. Sand can be used. Do not try to blow off such a fire. Use fire extinguishers in case of a big blaze.
- If inflammable liquid in a vessel takes flame, immediately disconnect supply of fuel to flame. Make non - available, the oxidant gas i.e. air to the fire in the vessel by closing with porcelain, metal, wooden, glass plate or towel.
- If burning liquid spills on the floor, extinguish the fire with sand.
- If fire happens in a laboratory, immediately turn off gas supply, electrical supply and use fire extinguishers. If fire has not caught organic liquids, water can be used.
- If fire is due to electrical short circuiting, immediately put of the main switch and use sand, carbon dioxide fire extinguisher to extinguish fire. Do not use water to extinguish electrical fire.
- Extinguish the fire on a man by means of a woollen blanket. Wrap the victim and hold for 1-2 minutes.

1.2.5 Explosion:

- Explosion causes fire and devastating damage. Explosion is caused in the laboratory by explosive chemicals and heat.
- Explosive chemicals are those which can explode on heating or exposure to flames or friction. For example, Picric acidshould be stored under water. If it dries, it can cause explosion.
- Heat produced enormously during chemical reactions can cause explosion. e.g.,heat produced when sodium reacts with water.

1.2.6 Electric Shock:

Electric shock is caused by faulty equipment, particularly when they are handled by wet hands. Since low-voltage alternating current (220 V) is used in a laboratory, electric shocks are rare. These can however be easily prevented by-

1) Timely checking of the instruments.
2) Earthing of the instruments.

When electrical shock occurs, someone has to put off the main switch immediately.

1.3. SAFETY SYMBOLS AND THEIR MEANINGS

Observe the following symbols and remember the meaning each one conveys.

SYMBOL	MEANING
	**EXPLOSIVE** Chemicals that can cause an explosion as they contain great amount of stored energy and Should therefore be handled carefully.
	**FLAMMABLE** Chemicals that catch fire easily under a given set of conditions and must therefore be used with utmost care
	**OXIDISING** Chemicals that are oxidizing in nature and may lead to highly exothermic reactions when they are in contact with other chemicals particularly Flammable ones. They should be stored in a cool place away from flammable chemicals / objects
	**GASES UNDER PRESSURE** Used for compressed gases, liquefied gases, refrigerated liquefied gases and dissolved gases.
	**CORROSIVE** Highly reactive chemicals that damage human tissues. Wear gloves while handling them.

	**DANGEROUS TO ENVIRONMENT** Chemicals that pose danger to life. They should not be thrown in the laboratory sinks and bins without the permission of the laboratory personnel
	**TOXIC** Chemicals that are poisonous and can damage an exposed part of the body
	**CARCINOGENIC** Cancer causing chemicals that can readily enter human tissues and seriously disturb the body's Complex processes.
	**IRRITANT** An immediate skin, eye or respiratory tract irritant

SOME OTHER SYMBOLS –

SYMBOL	MEANING
	**HIGH VOLTAGE** Always stay clear of areas marked with this sign as coming in contact with the electricity will mean serious injury or death.
	**RADIOACTIVE** Highly reactive chemicals that can be fatal to life even if used in minute quantities depending on their half-life.
	**BIOHAZARD** Biohazards are micro-organisms which can potentially harm or even kill living organisms. It is recommended that you always wear a face mask and anti-bacterial gloves when dealing with such substances.
	**LASER BEAM HAZARD** Letting your skin to come in direct contact with a laser beam can be very dangerous and can have somedisastrous consequences. Eye is the most susceptibleone which can cause partial or complete blindness.

1.4. FIRST AID MEASURES AND ANTI-DOTES WHILE HANDLING SOME COMMON CHEMICALS

S.No.	Chemical name	Health hazard and symptoms	First aid	Antidote	Safety symbol
1.	Aniline	**Inhalation:** coughing, sneezing, headache, fatigue, dizziness, methemoglobinemia, anoxia **Ingestion:** injury to the kidney, liver **Skin contact:** skin sensitization, Causes skin irritation. Harmful if absorbed	**Inhalation:** Remove victim to fresh air and seek medical help. **Ingestion:** Rinse mouth thoroughly. Ask the victim to drink sips of water. **Skin contact:** immediately flush the skin with plenty	Individual should be administered supplemental oxygen and methylene blue.	 Toxic  Irritant

		Eye contact: may cause severe eye irritation, blurred vision and photophobia	of water for 30 min and then wash with soap. **Eye contact:** rinse with plenty of water and seek medical help immediately.		 Corrosive  Health hazard
2.	Benzoyl chloride	**Inhalation:** lung cancer, reproductive damage, Cough. Shortness of breath. Sore throat. **Ingestion:** harmful if swallowed, severe burns in mouth, Abdominal pain. Shock or collapse **Skin contact:** skin cancer, skin burn, pain, blisters **Eye contact:** Redness. Pain. Severe deep burns	**Inhalation:** move to fresh air, get medical aid immediately **Ingestion:** Loosen tight clothing such as a collar, tie, belt or waistband. **Skin contact:** immediately flush skin with plenty of water, Cover the irritated skin with an emollient, Get medical attention immediately. Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream **Eye contact:** immediately flush eyes with plenty of water	Immediately flush contaminated eyes with gently flowing water. Move victim to fresh air and seek medical aid immediately.	 Toxic  Corrosive
3.	Bromine	**Inhalation:** severe choking, fatal **Ingestion:** nervous, circulatory and renal disturbances, gastrointestinal tract burns **Skin contact:** severe burns, ulceration and measle like eruptions **Eye contact:** causeseye burns, lachrymatory	**Inhalation:** move the victim to fresh air **Ingestion:** give the victim a cupful of water and seek medical aid **Skin contact:** wash with plenty of water and get medical aid.	Fresh air, and seek medical aid immediately.	 Danger  corrosive

			Eye contact: flush eyes with plenty of water.		
4.	Copper sulphate	**Inhalation:** May cause ulceration, respiratory tract irritation with possible burns. **Ingestion:** severe gastrointestinal tract irritation with nausea, vomiting and possible burns, bloody stools and vomit, low blood pressure, jaundice and coma. **Skin contact:** skin irritation and possible burns. **Eye contact:** may cause conjunctivitis, ulceration, and corneal abnormalities. Causes eye irritation and possible burns.	**Inhalation:** move to fresh air **Ingestion:** give 2-4 cupful of milk or water. **Skin contact:** Get medical aid. Flush skin with plenty of water **Eye contact:** flush eyes with plenty of water	Chelation therapy and methylene blue	 irritant
5.	EDTA	**Inhalation:** irritation of the mucous membrane and upper respiratory tract. **Ingestion:** gastrointestinal irritation with nausea, vomiting and diarrhoea, kidney injury, muscle cramps **Skin contact:** skin irritation, redness and pain **Eye contact:** eye irritation, redness and pain	**Inhalation:** move to fresh air. **Ingestion:** rinse mouth and drink 2-4 cupful of milk or water **Skin contact:** seek medical aid **Eye contact:** flush eyes with plenty of water	Chelation therapy	 irritant
6.	Ethyl acetate	**Inhalation:** respiratory tract irritation, narcotic effects. **Ingestion:** irritation of the digestive tract, central nervous depression. May cause headache, nausea, fatigue, and dizziness.	**Inhalation:** move the victim to fresh air **Ingestion:** Get medical aid **Skin contact:** flush skin with plenty of water.	Move the victim to fresh air and wash affected areas with plenty of water.	 flammable

		Skin contact: skin irritation **Eye contact:** eye irritation	**Eye contact:** flush eyes with plenty of water, Get medical aid		
7.	Formalin	**Inhalation:** irritation of respiratory tract, sore throat, coughing and shortness of breath. **Ingestion:** severe abdominal pain, vomiting, headache and diarrhoea **Skin contact:** irritation, redness and pain **Eye contact:** irritation, redness and pain	**Inhalation:** move victim to fresh air and seek medical aid **Ingestion:** rinse mouth with water and give warm water to drink **Skin contact:** wash with plenty of water and get medical aid. **Eye contact:** flush eyes with plenty of water.	No specific antidote available	 Danger
8.	Hydrochloric acid	**Inhalation:** May cause severe irritation of the respiratory tract with sore throat, coughing, shortness of breath and delayed lung edema. **Ingestion:** May cause corrosion and permanent tissue destruction of the oesophagus and digestive tract. **Skin contact:** Contact with liquid is corrosive and cause severe burns and ulceration. **Eye contact:** May cause irreversible eye injury. Vapour or mist may cause irritation and severe burns.	**Inhalation:** Remove victim to fresh air. **Ingestion:** Get medical aid immediately, give a cupful of water. **Skin Contact:** immediately flush skin with plenty of water while removing contaminated clothing and shoes **Eye Contact:** immediately flush eyes with plenty of water. Get medical aid immediately.	Drink plenty of water, followed by milk of magnesia or an antacid. Eat banana.	 Corrosive hazard
9.	Liquor ammonia	**Inhalation:** Burning sensation. Cough. Laboured breathing. Shortness of breath. Sore throat. Nausea, vomiting (emesis), abdominal pain, and burns of the mouth,	**Inhalation:** Fresh air, rest. Half-upright position. **Ingestion:** Do not induce vomiting **Skin contact:** rinse with plenty of water	Fluorescein examination,rinse with plenty of water.	 corrosive

		throat, oesophagus, and stomach. **Ingestion:** Nausea, vomiting (emesis), abdominal pain, burns of mouth, throat, oesophagus, and stomach, swelling of lips, mouth, and voice box (larynx), severe corrosive damage or burns of mouth, throat and stomach. **Skin contact:** redness. Pain. Blisters. Skin burns **Eye contact:** Rapid eye irritation and burning sensation.	**Eye contact:** rinse with plenty of water		 Acute toxic
10.	Mercuric sulphate	**Inhalation:** fatal, may cause respiratory tract irritation, acute poisoning, tightness in chest, coughing, pain **Ingestion:** severe toxicity, necrosis, pain, vomiting **Skin contact:** skin irritation, redness, pain, blisters **Eye contact:** irritation, pain	**Inhalation:** move the victim to fresh air **Ingestion:** get medical aid **Skin contact:** Wash off immediately with plenty of water **Eye contact:** Rinse immediately with plenty of water	Fresh air, drink water and seek medical aid.	 Danger
11.	Methanol	**Inhalation:** nausea, headache, vomiting, dizziness and incoordination blurred, double and/or snowy vision, and blindness **Ingestion:** May be fatal or cause blindness if swallowed, may cause gastrointestinal irritation with nausea, vomiting and diarrhoea, systemic toxicity with acidosis, central nervous system depression, characterized by	**Inhalation:** move victim to fresh air **Ingestion:** get medical aid **Skin contact:** wash skin with plenty of water **Eye contact:** flush eyes with plenty of water	Immediately seek medical aid.	 Flammable  Health hazard  Acute toxic

		excitement, followed by headache, dizziness, drowsiness, and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death **Skin contact:** skin irritation, may cause defatting of the skin and dermatitis **Eye contact:** painful sensitization to light, eye irritant			
12.	Nitric acid	**Inhalation:** Cough. Sore throat. Burning sensation. Shortness of breath. Laboured breathing. **Ingestion:** Burns in mouth and throat. Burning sensation behind the breastbone. Abdominal pain. Vomiting. Shock or collapse. **Skin contact:** Pain. Yellow staining of the skin. Serious skin burns. **Eye contact:** Redness, Pain. Severe burns	**Inhalation:** move victim to fresh air **Ingestion:** seek medical aid **Skin contact:** wash with plenty of water **Eye contact:** flush eyes with plenty of water.	Keep patient quiet and maintain normal body temperature, fresh air, drink water	 Oxidiser  corrosive
13.	Phenol	**Inhalation:** Sore throat. Burning sensation. Cough. Dizziness. Headache. Shortness of breath. Laboured breathing. Unconsciousness. **Ingestion:** Sore throat. Burns in mouth and throat. Convulsions. Abdominal pain. Diarrhoea. Shock or collapse.	**Inhalation:** Fresh air, rest. Half-upright position **Ingestion:** rinse mouth, give 2-3 cups of water to drink **Skin contact:** Remove contaminated clothes. Rinse skin with plenty of water	Charcoal can be used.	 Health hazard  Acute toxic

		Skin contact: skin burns. Numbness. Convulsions. Collapse. Unconsciousness. **Eye contact:** Pain. Redness. Loss of vision. Severe burns.	**Eye contact:** Rinse with plenty of water		 corrosive
14.	Phosphorous pentachloride	**Inhalation:** Sore throat. Cough. Burning sensation. Shortness of breath. Laboured breathing **Ingestion:** burning sensation. Shock or collapse. Abdominal pain. **Skin contact:** Pain. Redness. Blisters. Skin burn **Eye contact:** Pain. Redness. Severe deep burns. Loss of vision	**Inhalation:** Fresh air, rest. Half-upright position **Ingestion:** Rinse mouth. Give one or two glasses of water to drink. Refer for medical attention **Skin contact:** remove contaminated clothes. Rinse skin with plenty of water or shower. **Eye contact:** rinse with plenty of water	2-3% solution of copper sulphate or silver nitrate	 corrosive  Acute toxic
15.	Potassium dichromate	**Inhalation:** irritation of nose, throat, and bronchial tubes can occur, with cough and/or wheezing. **Ingestion:**Nausea. Vomiting. Abdominal pain. Burning sensation. Diarrhoea. Shock or collapse. **Skin contact:** skin irritation, burns, redness, pain **Eye contact:** redness, pain, blurred vision	**Inhalation:** Fresh air, rest. Half-upright position **Ingestion:** rinse mouth and give plenty of water to drink **Skin contact:** rinse with water and then wash with soap **Eye contact:** flush eyes with plenty of water	flush contaminated eyes with gently flowing water. Do not induce vomiting. If vomiting occurs, lean patient forward or place on left side. Keep patient quiet and maintain normal body temperature.	 Health hazard  Acute toxic  corrosive

16.	Potassium permanganate	**Inhalation:** Burning sensation. Cough. Sore throat. Shortness of breath. Laboured breathing. **Ingestion:** Burning sensation. Abdominal pain. Diarrhoea. Nausea. Vomiting. Shock or collapse. **Skin contact:** Redness. Skin burns. Pain **Eye contact:** Redness	**Inhalation:** Fresh air. Half-upright position. **Ingestion:** drink water **Skin contact:** rinse with water **Eye contact:** rinse with water	induce vomiting and follow with thorough gastric lavage, demulcents, glucose	 Irritant  Oxidiser  Health hazard
17.	Sodium hydroxide	**Inhalation:** may lead to chemical pneumonitis and pulmonary edema, severe irritation of upper respiratory tract with coughing, burns, breathing difficulty, and possible coma. burns to the respiratory tract. **Ingestion:** severe and permanent damage to the digestive tract, gastrointestinal tract burns, severe pain, nausea, vomiting, diarrhoea, and shock. **Skin contact:** skin burns and ulcers **Eye contact:** eye burns. May cause blindness. May cause chemical conjunctivitis and corneal damage.	**Inhalation:** remove to fresh air **Ingestion:** give a cupful of water **Skin contact:** wash skin with plenty of water, seek medical aid **Eye contact:** flush eyes with plenty of water	No specific antidote but burns can be treated	 corrosive
18.	Sodium thiosulphate	**Inhalation:** respiratory tract irritation	**Inhalation:** fresh air, rest **Ingestion:** rinse mouth	Fresh air, ventilation, drink water.	

		Ingestion: gastrointestinal irritation. **Skin contact:** skin irritation **Eye contact:** redness, mild eye irritation	**Skin contact:** rinse and then wash with soap **Eye contact:** rinse with water		irritant
19.	Sulphuric acid	**Inhalation:** irritation of the respiratory tract with burning pain in the nose and throat, coughing, wheezing, shortness of breath and pulmonary edema. **Ingestion:** severe and permanent damage to the digestive tract. Causes gastrointestinal tract burns. **Skin contact:** skin burns **Eye contact:** severe eye burns. May cause irreversible eye injury. May cause blindness. May cause permanent corneal opacification.	**Inhalation:** Remove victim to fresh air **Ingestion:** give a cupful of water **Skin contact:** flush skin with plenty of water **Eye contact:** flush eyes with plenty of water	Give one-fourth litre of water or milk or milk of magnesia or lime water.	 danger

1.5. SAFETY MEASURES TO BE TAKEN WHILE HANDLING SOME COMMON REAGENTS

While preparing reagents, one should be aware of correct handling, procedure, storage and safety hazards. One of the most common accidents which happen is while opening the bromine capsule to prepare bromine solution. However, with adequate knowledge and care, accidents can be avoided in the chemistry lab. Listed below are some reagents in *alphabetical order* and the precautions to be taken while storing and handling them.

S.No.	Chemical name	Safe storage and Handling
1.	Acetic Acid	• Keep container tightly closed. • Keep container in a cool, well-ventilated area. • Unused acetic acid should be discarded at the end of the day.
2.	Ammonium hydroxide solution	• Do not allow it to come in contact with eyes, skin or clothing. • Keep container tightly closed. • Do not store in direct sunlight. • Isolate from oxidizing materials and acids. • Store in a cool, dry, well-ventilated area away from incompatible substances.
3.	Bromine water	• Keep in a dark coloured bottle.

		• Keep container in a cool, well-ventilated area. • Elemental bromine is toxic and causes burns. Do not ingest or breathe gas/fumes/ vapor/spray. Avoid contact with eyes. • Always wear suitable respiratory equipment/ N95 mask while handling.
4.	Dilute Hydrochloric Acid	• Avoid adding water. Slowly add acid to water to prevent spattering or boiling. • Wash thoroughly with soap and water after handling. • Keep container tightly closed. Store in a cool, dry, well-ventilated area. • Concentrated hydrochloric acid (fuming hydrochloric acid) forms acidic mist. Both the mist and the solution have corrosive effects on human tissue, with the potential to damage respiratory organs, eyes, skin, and intestines.
5.	2,4 –Dinitrophenyl-hydrazine solution (DNP or Brady's reagent)	• Do not breathe dust. • Do not allow solution or solid to come into contact with the skin. • Store in Oxidizer Storage Area with other oxidizers and away from any combustible materials.
6.	Eriochrome black-T reagent	• Emits toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxides and sulphur oxides under fire conditions. • In case of contact with the eyes, may cause irritation, rinse immediately with plenty of water and seek medical advice.
7.	Iodine solution	• Keep away from incompatibles such as oxidizing agents, reducing agents, metals. • Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 25°C. • Avoid direct sunlight.
8.	Methyl orange	• Protect from freezing and direct sunlight. • Avoid contact with eyes, skin, and clothes. • Wash thoroughly after handling.
9.	Molisch’s reagent	• Store with alcohols, glycols, amines and amides. • Contains ethyl alcohol, severe fire risk. Store in a dedicated flammables cabinet. If a flammables cabinet is not available, store in flame resistant can. • Use and dispense in a hood.
10.	Nessler’s reagent	• Do not discharge onto the ground or into water course. • In case of accident, seek medical advice immediately. • Keep containers tightly closed. • Protect from light, including direct sunrays
11.	Phenolphthalein	• Harmful if swallowed. • May cause irritation. Avoid breathing vapours, or dusts. • Use with adequate ventilation.
12.	Picric acid	• It is advisable to store picric acid wet with at least 30% water and in rubber stoppered flasks.
13.	Potassium dichromate	• Use with adequate ventilation.

		• Minimize dust generation and accumulation. Avoid breathing dust. • Avoid contact with eyes, skin, and clothing. • Do not store in direct sunlight. Store in a cool, dry area away from incompatible substances.
14.	Potassium permanganate	• Solid $KMnO_4$ is a strong oxidizer and thus should be kept separated from oxidizable substances. • As an oxidizer that generates the dark brown product MnO_2, potassium permanganate rapidly stains virtually any organic material such as skin, paper, and clothing. Lemon juice is enough to quickly remove colour. • Crystalline solid $KMnO_4$ can cause serious eye injury also it is a skin and inhalation irritant, and can be fatal if swallowed.
15.	Silver nitrate solution	• Silver Nitrate is to be stored in dark brown glass bottle. Keep bottle in cabinet and away from sunlight. • Silver Nitrate should not be stored in Polyethylene bags or bottles as it reacts with plastic and it turns "gummy". • Avoid release to the environment.
16.	Sodium hydroxide	• Caustic soda solution is highly corrosive and can be hazardous to personnel. • Product should be stored between 29° to 38°C. • It is a deliquescent salt and has a strong affinity for moisture. It should be stored in air-tight plastic container. • Do not allow solid or solution to come into contact with your skin. When preparing solutions swirl the liquid constantly to prevent "hot spots" developing.
17.	Tollen's reagent	• Wear safety glasses. • Do not breathe dust. • Do not allow solution or solid to come into contact with the skin. • The reagent should be freshly prepared and stored refrigerated in a dark glass container. It has an approximate shelf-life of 24 hours when stored in this way. After the test has been performed, the resulting mixture should be acidified with dilute acid before disposal. These precautions are to prevent the formation of the highly explosive silver nitride.

Section 2: CHEMICAL WASTE MANAGEMENT

Waste disposal is an integral part of any science laboratory. As teachers or students perform demonstrations or laboratory experiments, chemical waste is generated. These wastes should be collected in appropriate containers and disposed offaccording to regulations. The proper **handling, reduction, treatment and disposal of chemical waste** should be our prime focus as chemists due to major environmental concerns that these chemicals may cause if discarded into the land or water ecosystems.

In order to minimize the amount of waste generated and to handle it safely, following steps may be considered.

(i) Spend time planning and preparing for the activity. Review the properties of the chemicals required and the products generated using resources such as the Material Safety Data Sheets (MSDS). If the reactants or products require special disposal or create unique hazards, then modify the experiment to use safer materials.

(ii) Review the MSDS for safety hazards of a chemical before ordering it.

(iii) Use small-scale or microscale procedures. These **reduce waste, produce less fumes, save on resources, and reduce preparation time**.

(iv) Make waste reduction/disposal a routine in every activity, students will develop a culture of concern for the environment and accept it as part of their responsibility.

(v) Sometimes laboratory explosions occur from inappropriate mixing of wastes, such as mixing nitric acid waste with organic wastes, so **be sure that waste materials are compatible**.

(vi) Collect all compatible waste solutions with similar properties in a well-labelled container.

(vii) Dispose off waste immediately, following the appropriate regulations. Disposal of small amounts of waste is easier and quicker than disposal of larger, stockpiled amounts.

2.1. The PRINCIPLE of 3Rs

Figure 27: The 3 R's

2.1.1. REDUCE

(i) To lessen the usage of chemicals and thereby, decrease the chemical waste being generated, the students can be given a small quantity of starting compound (0.25 g or 0.5 g) to start with for any preparation. This practice is not only environment friendly but is also economical and drastically reduces the expenses for procuring chemicals.

(ii) Semi-micro kits can be used in qualitative organic and inorganic analysis and students should be encouraged to use 2 to 5 mL test tubes. The usage of 10 to 20 mL test tubes should be completely stopped. This will ensure lesser volumes of chemicals as well as reagents being used in the lab and dumped in the sink.

(iii) Groove tiles and Spot-testsmay be used for functional group analysis etc. wherever possible, in order to reduce the amount of chemicals and reagents required.

(iv) Work with dilute solutions as far as possible.

2.1.2. REUSE

(i) The product of one experiment can be used as a starting material for another experiment. After the students leave the lab, the synthesized compound may be collected and stored in well labelled containers mentioning the name of the compound along with the date. These may be handed over to the respective teachers for use in other experiments such as compound analysis, recrystallization, determination of melting point, preparations etc.

(ii) The derivative prepared in organic analysis can be re-used by giving it to students of some other batch/ class for the extra element detection experiments. Therefore,the compounds synthesized by one batch of students or class of students can also be re-used by another batch/ class of students.

(iii) Instead of making sample notebooks and pasting the samples for examination record purpose, pictures of the compound can be stored as a proof by the students and teachers. The compound prepared by them may be collected by lab staff and stored for later use (as mentioned in the point (i) above). Thiswill ensure that the sample notebooks and files, once discarded, do not pose any health hazard to the ecosystem or animals. Wastage of paper is reduced this way.

(iv) Some products of precipitation like silver chloride and barium sulphate can be used for cation-anion analysis experiments instead of giving fresh sample of chemical from new bottle.

(v) The left-over solutions from titration experiment should not be discarded in the sink. Itcan be rather stored in labelled glass jars (*with name of solution, concentration of solution and date*) and maybe later used by some other class or batch for a similar experiment. Similarly, the standard solutions prepared by all the students should not be discarded into the sink. It can be collected in a labelled glass jar and given as an unknown sample solution to another batch for determining the strength during titration.

2.1.3. RECYCLE

(i) All the organic solvents can be distilled and used again and should not be discarded in the sink. The solvents may be collected after each class and placed in labelled glass jars and later handed over to the teacher for distillation.

(ii) Bottles containing chemicals leftover in them should never be discarded as such. They can rather be categorized depending upon the compound, into different cartons. Later with the help of the teacher, the compound can be recrystallized or distilled (as per the physical state) and may be used again as new.

(iii) Broken glass pieces, discarded glassware should not be disposed in the common dustbins. The plastic and glass waste should be segregated and given for recycling whenever possible.

2.2. SAFE DISPOSAL OF CHEMICAL WASTE

In case of chemicals and waste where the disposal is absolutely necessary, there are some key points that need to be kept in mind. Adequate pre-treatment, processing and waste segregation during disposal play an important role.

2.2.1. DISPOSAL OF CHEMICALS WITH LARGE QUANTITY OF WATER

There are some chemicals including drying agents like calcium chloride, magnesium sulphate, sodium sulphate, phosphorous pentoxide etc. that can be safely drained with large quantities of water. Small amounts of alcoholic reagents, fine TLC grade silica and alumina can also be safely drained with large quantities of water. Disposal strategies in lab are laid down and approved for only a handful of chemicals so far, however this can go a long way in cutting down the volume of chemicals dumped in water treatment plants.

2.2.2. MATERIALS ON THE "RED LIST"

No material on the "red list" should ever be washed down a drain. These are highly toxic chemicals. These include

- Compounds of the following elements- antimony, arsenic, barium, beryllium, boron, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, silver, tellurium, thallium, tin, titanium, uranium, vanadium, and zinc.
- Organohalogen, organophosphorous or organonitrogen pesticides, triazine herbicides, any other biocides.
- Mineral oil and hydrocarbon
- Cyanides,Fluorides and nitrites
- Poisonous organosilicon compounds, metal phosphides and phosphorous element

2.2.3. SAFE DISPOSAL OF LIQUIDS / SOLUTIONS

(i) Store used organic solvents separately.
(ii) Store halogenated organic liquids separately.

(iii) A mixture of chloroform and acetone can explode so these should never be stored together.
(iv) Store the following inorganic solvents separately: –

a) Waste acid solutions

b) Waste base/ alkaline solutions

c) Waste inorganic solutions

Once these waste acid solutions and waste base solutions are stored safely, they may be disposed judiciously. Precipitation technique can be used to separate out all the heavy metals. Then the supernatant liquid can be drained out and its pH can be adjusted to the range of 7 to 8 using the waste acid/base solutions. This liquid may then be drained out and disposed safely.

2.2.4. SAFE DISPOSAL OFORGANIC SOLVENTS / COMPOUNDS

Use incineration technique for the safe disposal of: -

(i) Soluble organic waste including most organic solids

(ii) Paraffin and mineral oil (from oil baths and pumps)

(iii) Halogenated organic solvents

2.2.5. SAFEDISPOSAL OF SOLID WASTE

Separation of solid waste into separate bins is very important. Glassware/ broken glass pieces, plastic, chemical waste and paper waste should be segregated so that different treatments of waste may be done. Filter papers, Whatman papers and waste paper can be collected and put in a one feet deep bio-degradation pit.(Figure 28)

Figure 28: Separation of solid waste into separate bins

References/ Interesting Weblinks:

1. https://www.youtube.com/watch?v=WQyObsamnMw
2. https://www.youtube.com/watch?v=qXHrXE4cJME
3. https://www.redcross.org.uk/first-aid/learn-first-aid/choking
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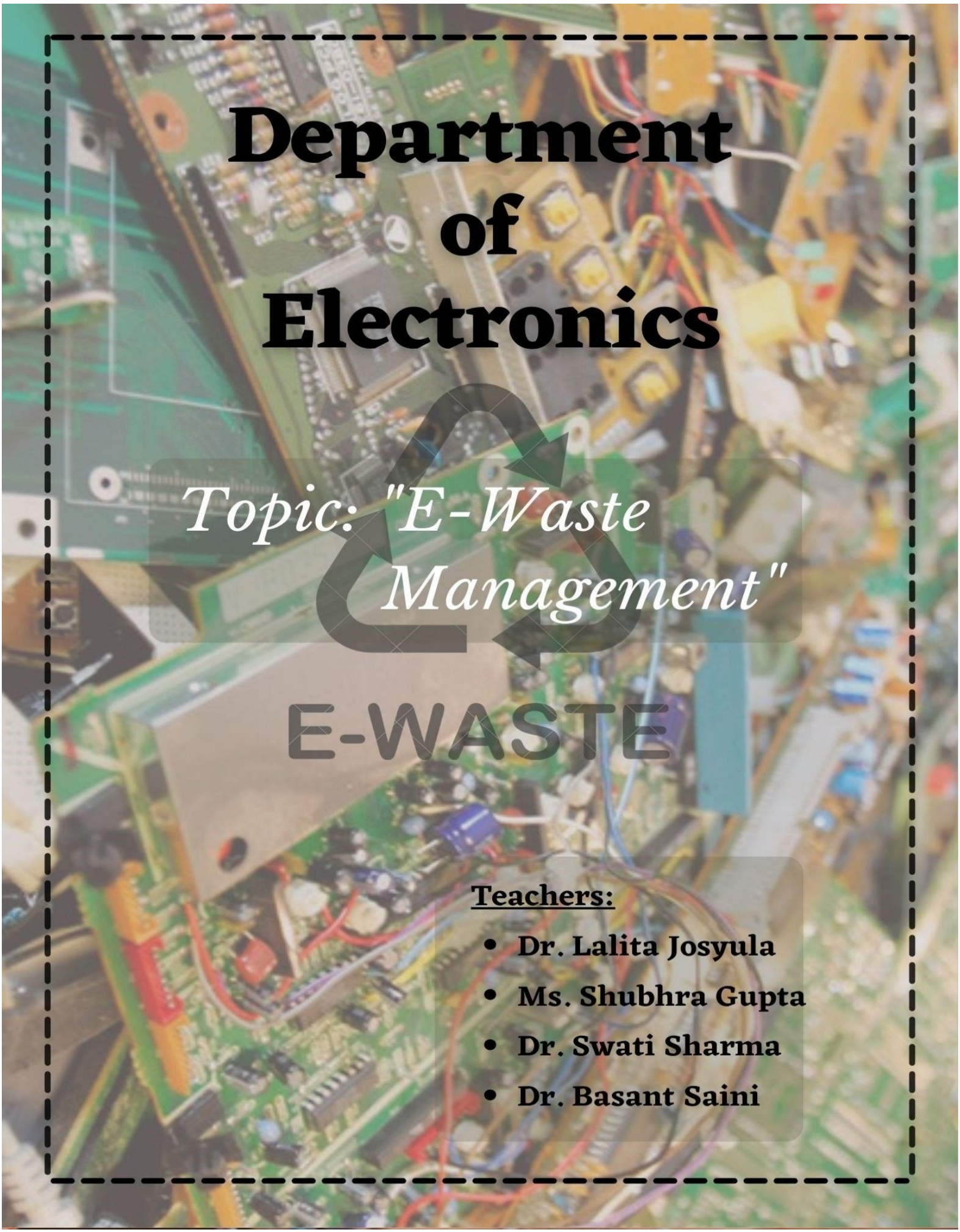

Department of Electronics

Topic: "E-Waste Management"

Teachers:

- **Dr. Lalita Josyula**
- **Ms. Shubhra Gupta**
- **Dr. Swati Sharma**
- **Dr. Basant Saini**

Department: Electronics

INTRODUCTION:

OBJECTIVES:

With the rapid expansion of technology e-waste or electronic waste has become a growing concern for human health and environment. Consequently, volume of electrical and electronic products continue to grow every year. E-waste refers to the electronic product which can be discarded after the end of its useful life. This includes but not limited to laboratory equipment and components, household appliances, medical equipment, information technology and telecommunication equipment. Most of the people are unaware of the hazardous effect of disposing e-waste carelessly. When incinerated or placed in landfill sites, the toxic components that are considered harmful are released. This can pose a serious threat to human health and environment. Proper segregation and dismantling can reduce the problem associated with e-waste by reuse or recycling. This would also lower the extraction of raw material for new electronic products which may otherwise lead to resource depletion. The objective of this technical session is to provide an insight into e-waste handling, effective management practices and challenges along with safety concerns in using electrical and electronic equipment.

METHODOLOGY:

The technical session would be strictly online with interactive session and active participation of target audience. The course would include lecture on e-waste management practices and safety measures on their handling. Illustrations and diagrams along with textual content for smooth learning in online mode would be included in the presentation. In order to develop interest amongst the participants and to make them comfortable, verbal discussion or discussion on chat window will be made. Learning through short videos and clips will also be included in the session as video based learning will encourage the participants to learn and understand effectively the issues arising due to improper e-waste disposal so that proper measures can be taken before it's too late. Also, case study on some of the equipment would also be presented to identify the key issue and related harmful effects. Existing government policies and action taken up by industries for recycling of e-waste will be presented. E-learning approach on the topic will be beneficial to bring the learners with different skills and knowledge at the same level. Assessment exercise for self-assessment of the participants at the end of the session will be provided.

OUTCOMES:

Department of Electronics will organize the e-waste training for the lab staff to make them understand how e-waste is generated and now becoming the fastest growing waste stream in the world. They will be able to recognize and realize consequences of improper disposal of e-waste and the grave danger it poses to our environment. They will gain the cognizance of the process involved in recycling of E-waste, its benefits and the organizations where it is carried out. They will be able to think that how the lack of implementation of policies regarding e-waste collection and recycling have health and ecological risk. They will be able to know the current e-waste management initiatives and there is an urgent need to implement proper strategies as large number of workers from slum area are involved in segregation of metals and components without knowing the risk it is posing to their own health. The illustrations, diagrams, textual content, short video clips will enable the attendees to learn and accept the fact that hazardous nature of e-waste is another concern for the environmental problems worldwide. In addition to this attendees will be able to learn regarding

safe handling of electronic equipment in day to day life and in laboratories. The participants will be kept actively involved in the training program.

Section 1: E - Waste Management

- **What is E- waste?**

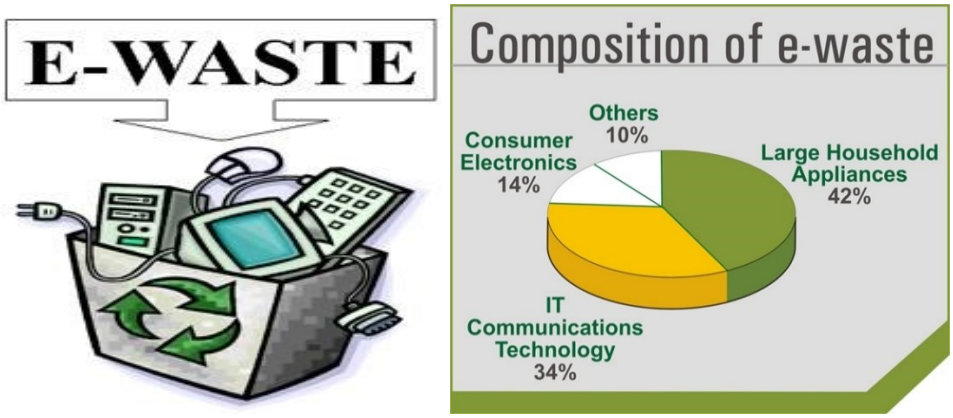

Figure 29: composition of e-waste

Electronic waste or e-waste is generated when electronic and electrical equipment become unfit for their originally intended use or has become obsolete. These can include computers, servers, mainframes, monitors, compact discs (CDs), printers, scanners, copiers, calculators, battery cells, cellular phones, transceivers, TVs, iPods, IC's, chips, etc. With rapid advancement of technology, older models get fast replaced with newer models which has led to an exponential increase in e-waste generation. E-waste typically consists of metals, plastics, cathode ray tubes (CRTs), printed circuit boards, cables, and so on. The presence of toxic substances such as lithium, mercury, nickel, polychlorinated biphenyls (PCBs), lead etc. makes scientific recycling of e-waste a high priority task. Processing these in a crude manner with rudimentary techniques such as incineration can be very harmful for human, animals as well as the environment. Valuable metals such as copper, silver, gold, and platinum could also be recovered from e-wastes, if they are scientifically processed.

Figure 30 : E-Waste management

- **Process of E-waste Recycling**

Due to widespread environmental degradation, recycling of e-waste has become a growing trend. E-scraps are typically sophisticated and manufactured from diverse elements such as metals, plastics, glass, circuit boards, etc. which can be recycled and reused for new products manufacturing. The first step of recycling includes collecting and transporting the e-waste from user to the recycling facility. Next the waste is shred and sorted either manually or mechanically. Then the dust is extracted and metals are magnetically separated from shredded waste. The plastic and glass materials are separated using water separation followed by purification of the water stream. The final stage involves preparing the recycled materials for sale as raw materials to produce new electronics. The step by step process is explained below:

1. **Collecting and Transporting**
   This is the first step of recycling e-waste. In majority of cases, the consumers can contact the e waste vendor, who then collects the material directly from their homes. Sometime, recyclers also place take-back booths or collection bins in specific places. When these bins get filled, the recyclers then transport the e-wastes to recycling facilities and plants.
2. **Shredding and Sorting**
   After collecting and transporting, the next step is to shred and sort the e-waste. Shredding involves breaking e-waste into smaller pieces for proper sorting. With the use of hands, these tiny prices get sorted and then manually dismantled. This is typically labour-intensive as waste items are, at this stage, separated to retrieve different parts. After this, the materials get categorized into core materials and components. Then, these items get sorted into various categories. Typically, these category includes items that you can re-use as they are and those that require further recycling processes.
3. **Dust Extraction**
   The tiny waste particles get smoothly spread via a shaking process on the conveyor belt. The smoothly spread e-waste pieces then get broken down even further. At this point, the dust gets extracted and discarded in an environmentally compliant manner. This way, there is no environmental degradation.

4. **Magnetic Separation**

   After the dust is separated, a strong overhead magnet helps to separate steel and iron from other wastes. This successfully recycles the steel from the waste stream. Sometimes, mechanical processes may also be required to separate circuit board, copper, and aluminium from other wastes particles.

5. **Water Separation**

   Water separation technique is then employed to separate the glass from the plastic. The leads that contain glass are sent to smelters for use in the production of batteries, x-ray tubes, and new CRTs.

6. **Purification of Waste Stream**

   The next thing is locating and extracting leftover metals from plastics to purify the waste stream further.

7. **Preparing Recycled Materials for Sale**

   The final stage is preparing recycled materials for sale. Here, the materials separated during SSS get prepared for sale as raw materials to produce new electronics.

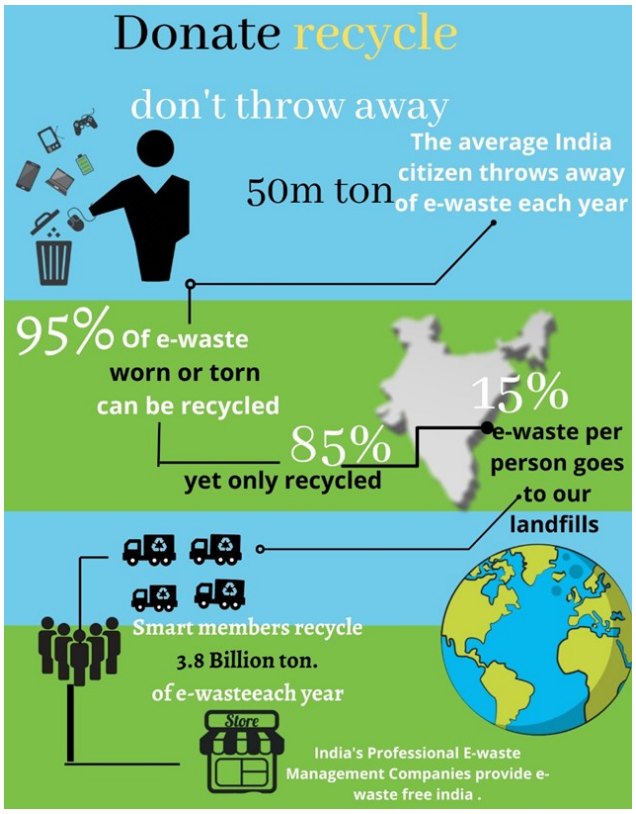

Figure 31: various modes of preparing recycled material for sale

- **Benefits of E – waste Management**

E-waste Recycling Helps to Conserve Available Natural Resource as it helps recover valuable materials from electronic products. So the manufacturers can now obtain raw materials from recycled waste which in turn saves and conserves natural resources. Recycling prioritizes Environmental Protection as it involves properly handling and processing of hazardous and toxic substances such as lead, mercury, cadmium. It also helps in improving economic condition of the society by creating jobs such as professional recyclers. Usually, uncollected e-wastes get dumped at incinerators and landfills which pollute the water and soil. By recycling e-waste, the amount of e-wastes pilling up at these places is considerably reduced saving the environment from its harmful effects.

E-waste Recycling organisations in Delhi –NCR

1. Resource E Waste Solution Pvt Ltd
   518 FIE PATPARGANJ INDUSTRIAL AREA Near Haridarshan Building
   011 4940 2735

2. Greeniva Recycler Pvt. Ltd.
   Industrial Area, Kanti Nagar New Delhi, Delhi
   011 6515 0035

3. E Waste Recyclers India
   New Delhi, Delhi
   099901 33388

4. Greenzon E-waste Recycler Delhi
   New Delhi, Delhi
   098112 06076

5. Adatte E-Waste Management Private Limite
   New Delhi, Delhi
   097899 78420

Section 2: Safety Measures

Science lab safety rules and symbols are needed for the well-being of staff and students working in science laboratories. In order to ensure the workers safety some signs and symbols are globally developed and adopted as illustrated in figures 32 and 33.

Figure 32 : GHS Pictograms

Figure 33: NFPA rating

"Safety First" should be the prime priority when handling electricity and electronic equipment in the lab. Whenever a workstation or an equipment is installed it must comply with the standard installation regulations. The following points should be followed in order to avoid any electrical hazard in laboratory:

- We must always disconnect a plug by pulling on the connector body not the cable.
- While servicing any device it must be removed from the circuit first.
- Fuses or circuit breakers shall never be bypassed. Keep electrical service and breaker panels accessible at all times.
- Keep all cords and wires out of foot traffic areas and do not roll chairs over electrical cords or wires.
- Electrical equipment and connections should not be handled with wet hands, nor should they be used after liquid has been spilled on it.
- All lighting stands must be properly secured.
- Avoid using extension cords. Extension cords are intended for temporary use only.
- Never overload a circuit. High wattage appliances like freezers, refrigerators, copy machines and laboratory equipment must be plugged directly into a wall holder. It must not be supplied power via extension cords or power strips.
- Do not use any machine that smokes, sparks, or appears defective in any way. Immediately remove damaged or defective office machines from service.
- Avoid stretching or pinching cords. Check cable, cords, and connectors periodically and immediately replace any items that show signs of cracking, chipping or other deterioration.
- Attention for proper earthing of electrical equipment.
- A grounded tool has a three-conductor cord with a three-pronged plug that must be plugged into a grounded outlet.
- Never clip off ground pins on three-wire appliances or use two-wire adapters to wed incompatible equipment. All electrical repairs must be done by qualified individuals

For equipment like Oscilloscope, Function Generator, power supply etc. and potentiometer special care must be taken while using them. We should adhere to the following points to avoid electrical hazards:

- Use only the power cord specified for each product and certified for the country of use.
- When operating with AC power, this device is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making connections to the input or output terminals of the product, ensure that the device is properly grounded.
- Do not operate the devices with covers or panels removed.
- Do not slide sensitive components over any surface. Do not touch exposed connector pins. Handle sensitive components as little as possible.

- Power supplies are equipped with three wire line cords which ground the chassis to power line ground. Do not cut off or disable the ground plug.
- For function generators before applying power, ensure that the line selector is in the proper position for the power source being used.
- To prevent equipment overheating, provide proper ventilation.
- In potentiometer zero deflection cannot be obtained is the emf of the cell connecting in primary circuit must be more than or equal to the emf of the cell of secondary circuit.
- Cross section area of wire should be uniform else potential gradient will not be constant.
- Current through the potentiometer wire should not be passed for a long duration otherwise heating up of wire results in resistance change and hence potential gradient will also change.

Figure 34 : Electronics laboratory equipment

Sensitive electronic circuits and electronic components have to be handled with great care. The inappropriate handling of electronic component can damage or destroy the devices. The devices can be destroyed by driving to high currents through the device, by overheating the device, by mixing up the polarity, or by electrostatic discharge (ESD). Therefore, always handle the electronic devices as indicated by the handout, the data sheet or other documentation. An ESD event is a rapid transfer of charge from one object to another in an attempt to become electrically neutral. Electrostatic charge is most commonly created by the contact and separation of two electrically nonconductive materials. The amount and type of charge (positive or negative) depends on the materials involved. The following common materials, often found in business and laboratory environments, are all sources of static electricity:

- Common plastic bags
- Common packing tape
- Paperwork
- Common untreated plastic materials
- Styrofoam parts

Correct handling and care of electronic instruments in chemistry, biochemistry, zoology, botany and physics laboratory such as pH meter, conductivity meter, spectrophotometer, and calorimeter (figure 35) has been enlightened as follows.

- A pH electrode is fragile and one should not be tempted to use it as a stirring glass rod when adjusting pH.

- A pH meter needs to be calibrated daily with the help of standard buffer solutions.
- Avoid temperature fluctuations and never keep the pH meter exposed to direct sunlight as pH readings are temperature sensitive.
- Glass is rapidly attacked by hydrofluoric acid so never keep the electrode in HF solution as it can lead to irreparable damage of the electrode.
- After every soaking the electrode should be rinsed with distilled water. Droplets adhering to electrode should be gently dried with tissue paper and not rubbed or wiped.
- The conductive electrode of conductivity meter should be cleaned thoroughly by stirring it with a mild detergent or isopropyl alcohol.
- The electrodes of conductivity meter are sensitive so should not be stroked on a hard surface. They should be cleaned with tap water, wiped with a soft tissue paper and recalibrated before every use.
- Simples precautions while using spectrophotometer includes: allowing lamp to warm up, correct wavelength of light should be used specific to particular application, fingerprints and spilt sample should be wiped off the outside of the cuvette before measuring. Before carrying the setup procedure, calibration has to be performed to ensure measurement accuracy.
- Since calorimeter is used to determine the heat flow in the chemical reaction, the solution should not be stirred vigorously as it may add extra heat to the chemical reaction and fluctuates the readings.

Figure 35: Commonly used instruments in science laboratories

Prevention is the area where you can make the biggest difference. A number of common sense rules can be applied. These rules do not require additional materials but are extremely effective in preventing static damage.

- Always keep your workbench clean and clear of unnecessary material, particularly common plastics.
- Return ESD-sensitive items to their ESD-protective containers when not actively working with the items
- Do not hold ESD-sensitive items like semiconductor device (diodes, transistors, integrated circuits) against your clothing.
- Don't touch sensitive items (e.g. metal oxide semiconductor field effect transistors (MOSFETs), Operational Amplifiers, Logic gate).

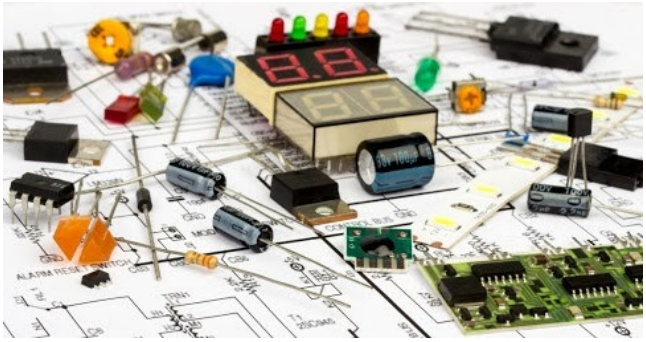

Figure 36: Basic electronics laboratory components

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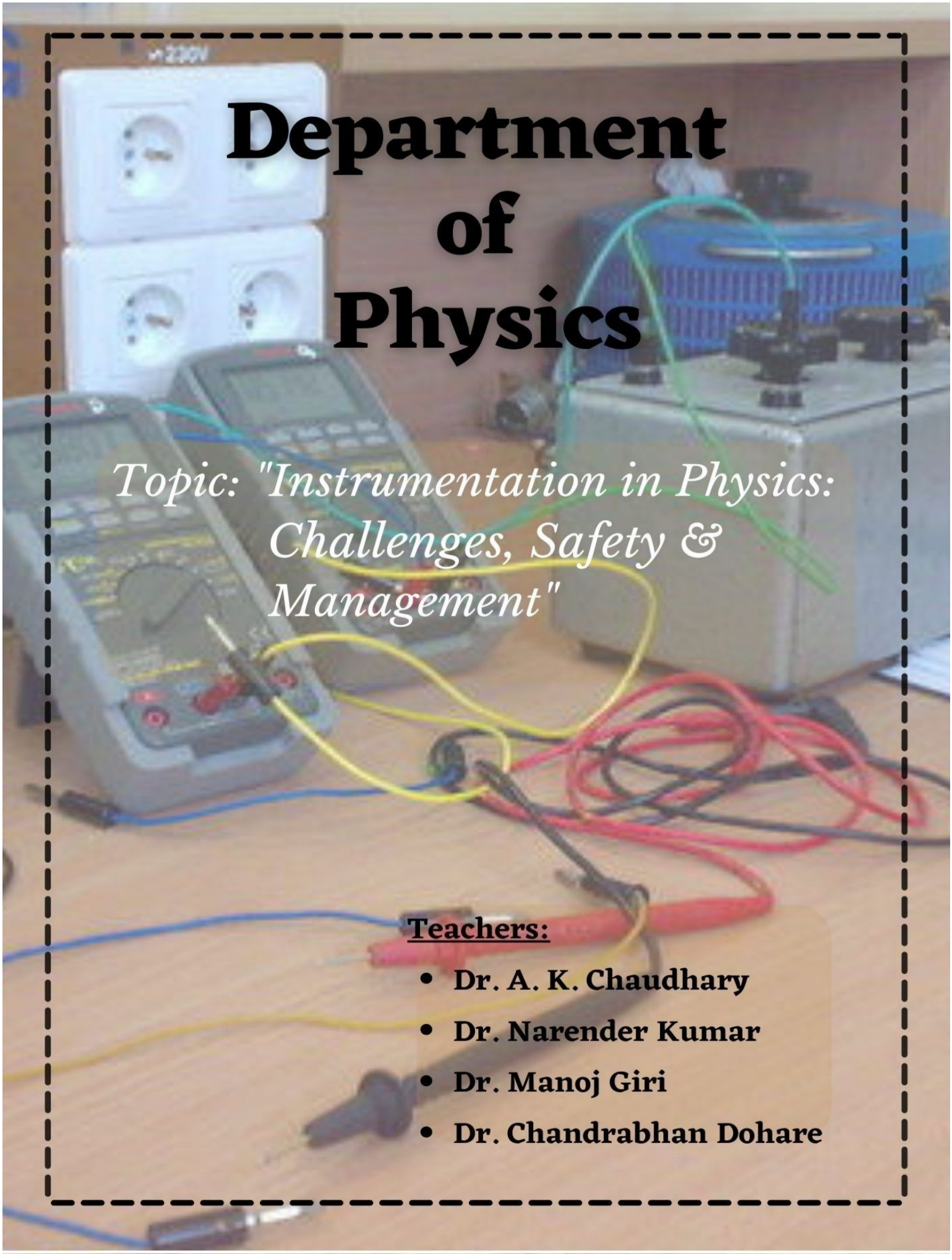


Department of Physics

Topic: "Instrumentation in Physics: Challenges, Safety & Management"

Teachers:

- **Dr. A. K. Chaudhary**
- **Dr. Narender Kumar**
- **Dr. Manoj Giri**
- **Dr. Chandrabhan Dohare**

Department: PHYSICS

INTRODUCTION

The study of instrumentation and, associated safety and its management in physics provides many touchstones to everyday lab applications for i.e., LCR Meter, DC. Voltage source, Rheostat, Inductor, Capacitor, Ammeter, Battery, Voltmeter and Multi-meter, electric motor, fly wheel, pendulum, and pumps etc. However, laboratory activities in this area are not without danger. Although in the field of radiation and ionization, and the use of ionizing radiation sources in graduation, it is necessary to have planned safety protocols in place. In the radiation technology, non-ionizing radiation consists of electromagnetic radiation that lacks sufficient energy to ionize matter. These may include the use of lasers, microwaves and infrared radiation in the physics laboratory. The most common non-ionizing radiation equipment used in physics laboratories is the laser. Safety specifications vary depending on the class of laser instrument being used. Working with vacuums system, has the potential of an implosion and the possible hazards of flying glass, splattering chemicals and fire. Potential risks must be carefully considered.

OBJECTIVES:

We will cover the following segments:

(i) Safety measures for electrical setup (s) and the management of grounding problem
(ii) Instrumentation and safety measures related to Radiation and Ionization
(iii) Instrumentation and safety concern to Vacuum technology
(iv) Safety measures and management for Laser & optical instruments
(v) Instrumentation for advanced research instrumentsi.e. XRD, SEM

METHODOLOGY:

The technical session would be strictly online with interactive session and active participation of target audience. The course would include lecture on instrumentation and, its safety and management practices on their handling. Illustrations and diagrams along with textual content for smooth learning in online mode would be included in the presentation. In order to develop interest amongst the participants and to make them comfortable, verbal discussion or discussion on chat window will be made.

OUTCOMES:

Through this two-day workshop, we wish to provide the participants with an overview of instrumentation and general physics laboratory procedures and safety measures. After having good understanding of safety measures of above-mentioned instruments and technology, students are able to handle the Laser and optical instruments, vacuum pump, Electrical and Mechanical setup.

SECTION 1: INSTRUMENTATION, SAFETY MEASURES AND MANAGEMENT

The most basic physics laboratory equipment includes fume hoods, desks, tables, benches and gas, water and vacuum lines. Safety equipment may include gloves, goggles and eyewash stations.

1.1 MECHANICAL:

The study of mechanics in physics provides many touchstones to everyday applications. However, laboratory activities in this area are not without danger. Raw or manufactured samples in the physics laboratory merit various tools for processing. Physicists sometimes use a mortar and pestle to grind samples. Other processing implements include **polishers, sonicators, ultracentrifuges,** and **other materials testing** apparatus. A hydraulic press and stainless steel die set may be used in making pellet samples for property measurements. Always use caution when dealing with projectiles, falling objects, moving equipment, exposed belts, heavy iron piece, powerful permanent magnets, sharps such as knives and razor blades, and springs. (Figure 37)

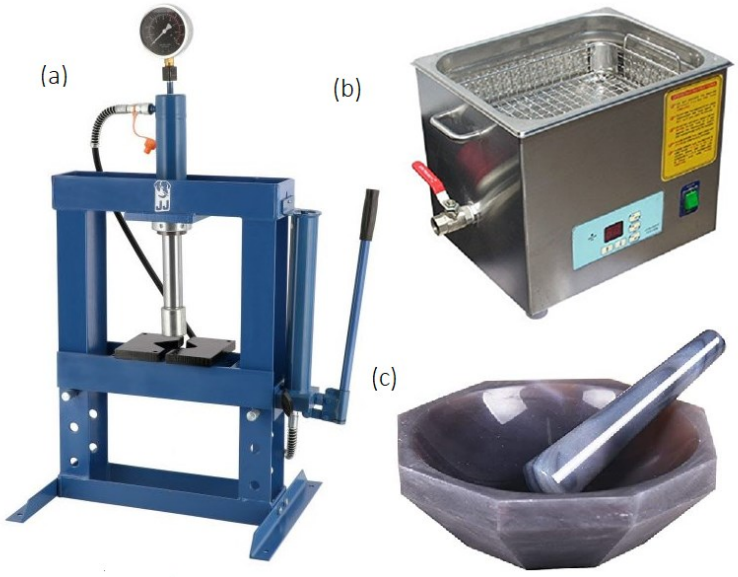


Figure 37: (a) Hydraulic Press (b) Sonicator (c) Mortar and Pestle

1.2 IONIZING AND RADIATION EXPERIMENTS:

Although the use of ionizing radiation sources in graduation, it is necessary to have planned safety protocols in place. i.e **Ionization chamber, Evaporation technique experiments (e-beam, Sputtering technique, thermal evaporation), Beta decay experiment etc. (Figure 38)**

The following safety procedures should be reviewed and adopted prior to dealing with radioactive materials:

- Select only low-level alpha and beta emitters.
- To prevent accidental entry of radioactive materials into the body, high standards of cleanliness and good housekeeping must be maintained in all laboratories where radioactive materials are present and/or used.
- Visitors are not allowed without approval of chemical hygiene officer

- Always use gloves when handling more than a few hundred counts per minute. Wear protective clothing (lab coats, masks, shoe covers) as needed.
- When work is completed each person will clean up his own work area and arrange for disposal or proper storage of all radioactive materials and equipment.
- Laboratories shall provide special radioactive waste containers. These shall bear the words “Caution, Radioactive Waste” and a warning to janitors against handling.

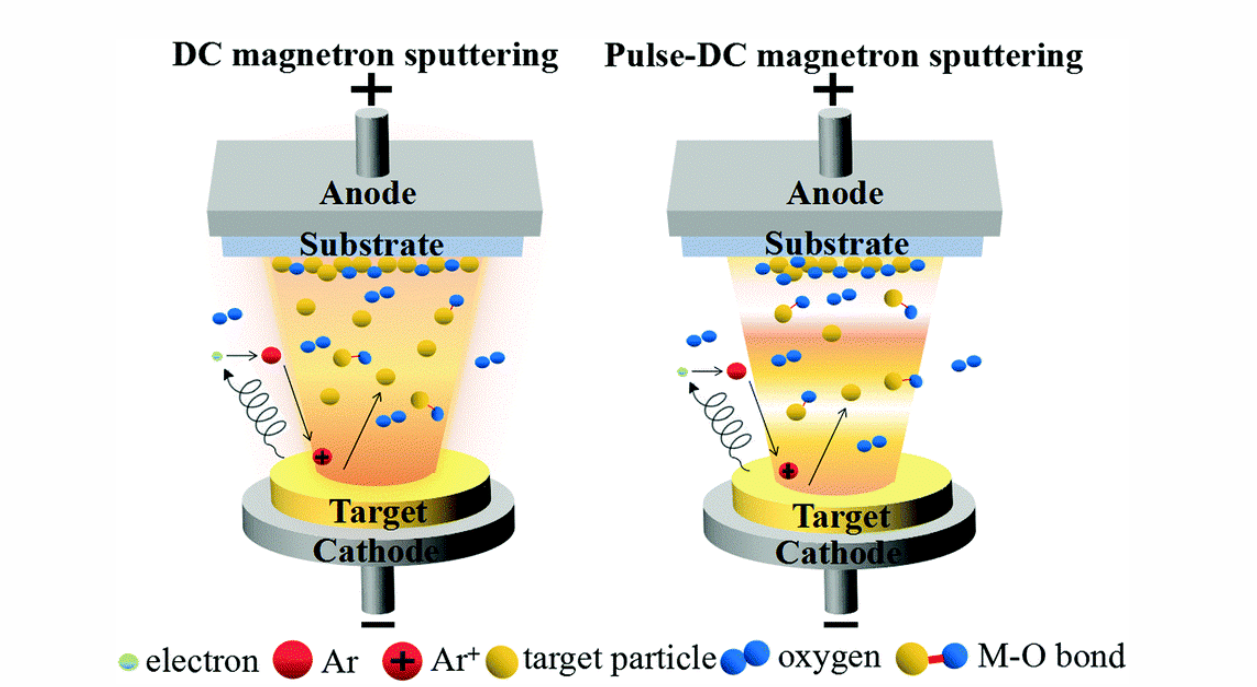


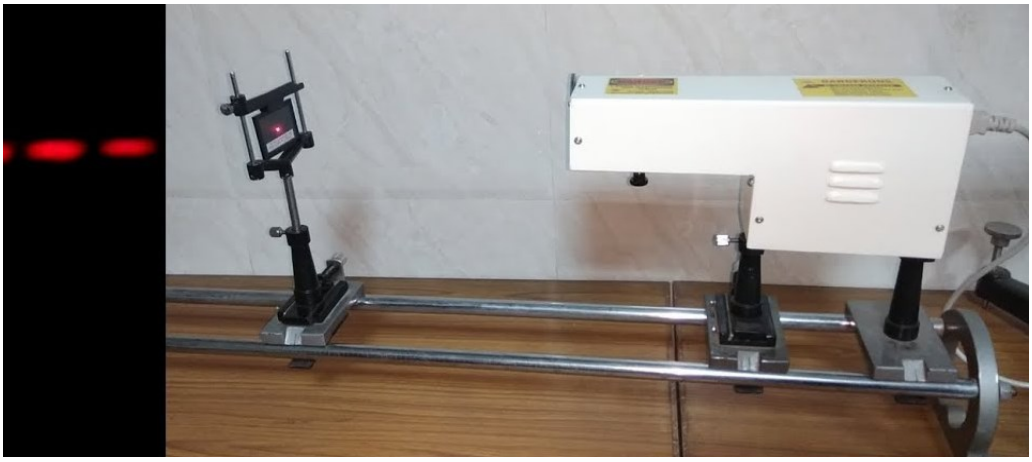
Figure 38: Sputtering Set up

1.3 OPTICS AND LASER EXPERIMENTS:

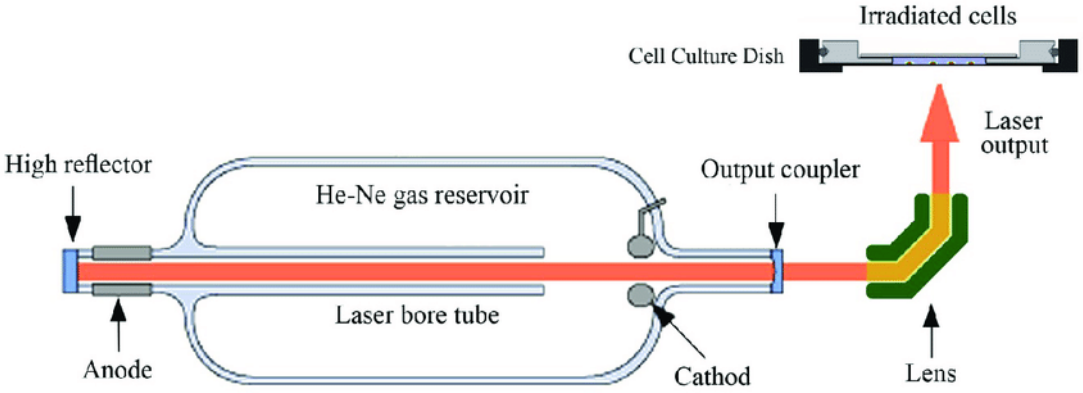
Non-ionizing radiation consists of electromagnetic radiation that lacks sufficient energy to ionize matter. These may include the use of lasers, microwaves and infrared radiation in the physics laboratory. The most common non-ionizing radiation equipment used in physics laboratories is the laser. Safety specifications vary depending on the class of laser instrument being used. **i.e. He-Ne laser (Figure 39 a and b), CO_2 laser, Pulse Laser Deposition (PLD), Photo-Photoluminescence etc.**

- Remove all watches and rings before changing or altering the experimental setup. Shiny jewellery can cause hazardous reflections.
- Before a laser operation, prepare a detailed operating procedure outlining operation.
- Cover all exposed wiring and glass on the laser with a shield to prevent shock and contain any explosions of the laser materials. Be sure all no energized parts of the equipment are grounded.
- Set up the laser so that the beam path is not at normal eye level, i.e., below 3 feet (9 meters) or above 5 feet (2 meters).
- Whenever a laser is operated outside the visible range (such as a CO_2 laser), a warning device must be installed to indicate its operation.
- Use shields to prevent strong reflections and the direct beam from going beyond the area needed for the demonstration or experiments.
- A key switch to lock the high voltage supply should be installed.

- Do not aim the laser with the eye. Direct reflection can cause eye damage.
- Do not use sunglasses to protect the eyes. If laser safety goggles are used, be certain they are designed for use with the laser being used.



(a)



(b)

Figure 39 : (a) He-Ne Laser (b) Pictorial Laser diagram

1.4 VACUUM SYSTEMS:

Use only pressurized or evacuated items that are designed for such an activity. The instrument related to vacuums technology has the potential of an implosion and the possible hazards of flying glass, splattering chemicals and fire. Potential risks are always there and must be operated carefully. Equipment at reduced pressure can be prone to rapid pressure changes forcing liquids through an apparatus i.e. **vacuum Pump, CVD set up, Evaporation technique experiments (e-beam, Sputtering technique, thermal evaporation) etc. (Figure 40)**

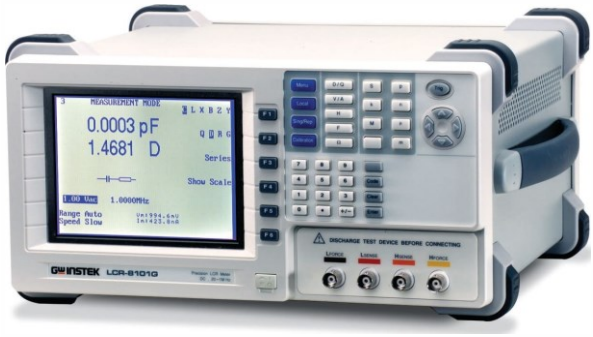
- Always use safety glasses or goggles with ANSI Z81 ratings.
- Place vacuum apparatus out of harm's way so an accidental hit is minimized. Placement of transparent plastic around the apparatus helps prevent injury from flying glass in case of an explosion.
- Protect vacuum pumps with cold traps and vent the exhaust into an exhaust hood.
- Do not allow water, solvents and corrosive gases to be drawn into vacuum systems.

Figure 40: Left side: Vacuum unit, Right side: Rotary pump

1.5 ELECTRICITY EXPERIMENTS:

The following equipment's are used i.e **LCR Meter, DC.Voltage source, Rheostat, Inductor, Capacitor, Ammeter, Battery, Voltmeter and Multi-meter etc. (Figure 41 a-c)** Consider the following safety specifications in working with electricity:

- Know where the master switch is for electricity in the laboratory in case of an emergency.
- When unplugging cords, always pull cords from the plug at the electrical receptacle and never pull the cords from the wire.
- Use only ground fault interrupt circuits (GFI) protected circuits (proper grounding)
- Be water phobic when working around electricity. Never use water or have wet hands when dealing with cords, plugs or electrical equipment. Never run a cord near or over a sink.
- Never plug damaged electrical equipment into a wall receptacle. This includes frayed wires, missing ground pin and bent plugs.
- Never overload circuits as they will overheat and cause power outages or fires.



(a)

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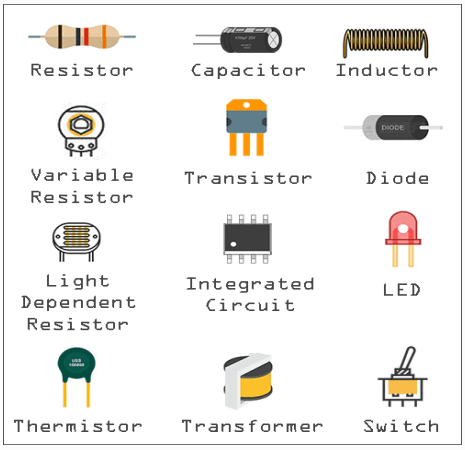

(C)

Figure 41: (a) LCR Meter (b) Voltmeter (c) Electronic components

1.6 ANALYZER INSTRUMENTS:

Numerous instruments perform analysis on samples in physics laboratories. Some examples include impedance analyzers, particle analyzers, optical multichannel analyzers, semiconductor parameter analyzers, spectrum analyzers, capacitance-voltage (CV) analyzers, and X-ray diffractometer for characterizing crystalline materials and identifying phases. **(Figure 42 a-c)**

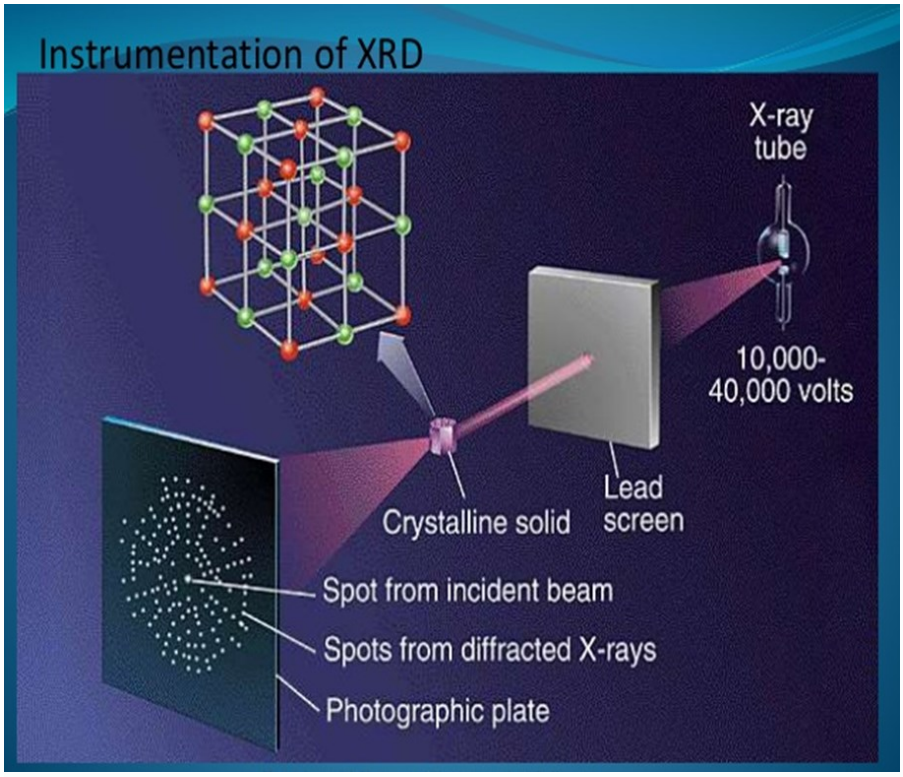

Figure 42 (a): Instrumentation of XRD

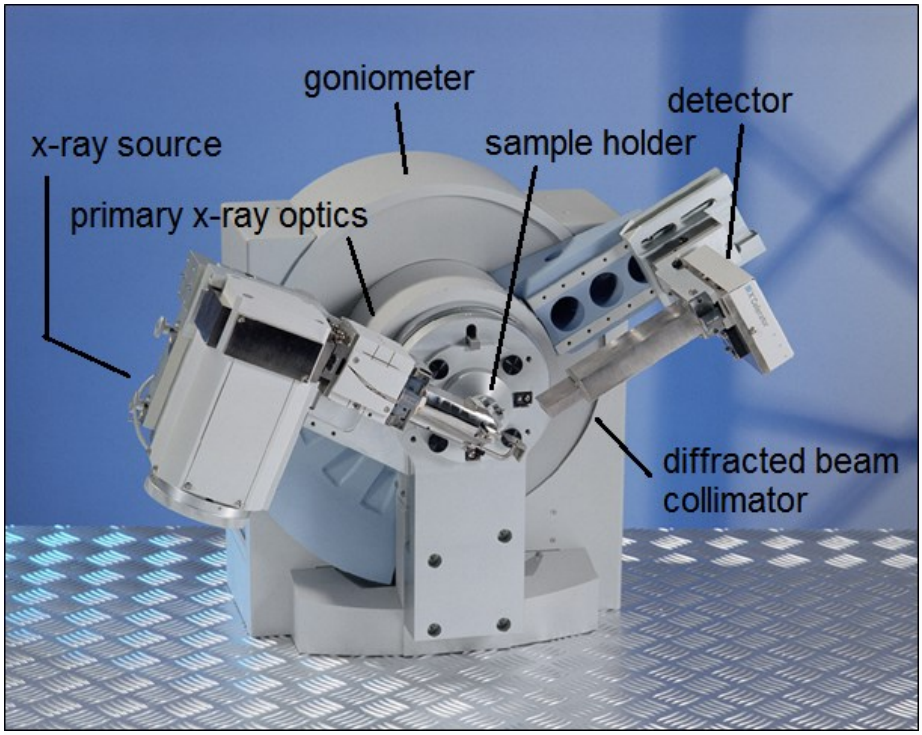

Figure 42 (b): XRD diagram

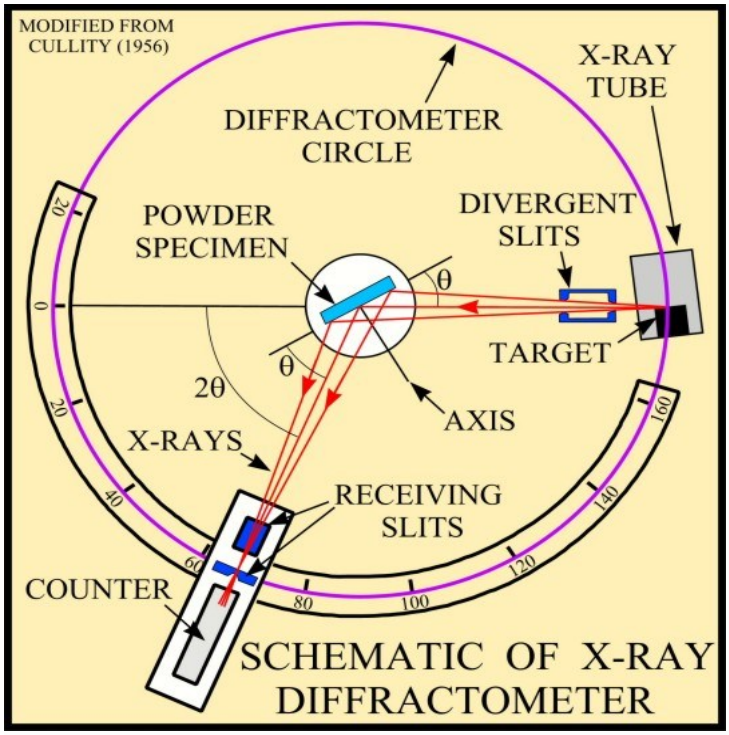

Figure 42 (c): XRD (geometrical diagram)

1.7 VERY ADVANCED MICROSCOPIC INSTRUMENTATION:

The optical microscopic dealt with the following instruments (Figure 43)

(i) Scanning Electron Microscopy (SEM)

(ii) Scanning Transmission Microscopy (TEM)

(iii) Atomic Force Microscopy (AFM)

(iv) Optical Microscope

(v) RAMAN spectroscope

These above mentioned instruments are very sensitive sophisticated in term of electromagnetic lens, inbuilt Laser. These instruments should be used very carefully in the presence of expert and having good prior knowledge of Optics and, Electricity and Magnetism.

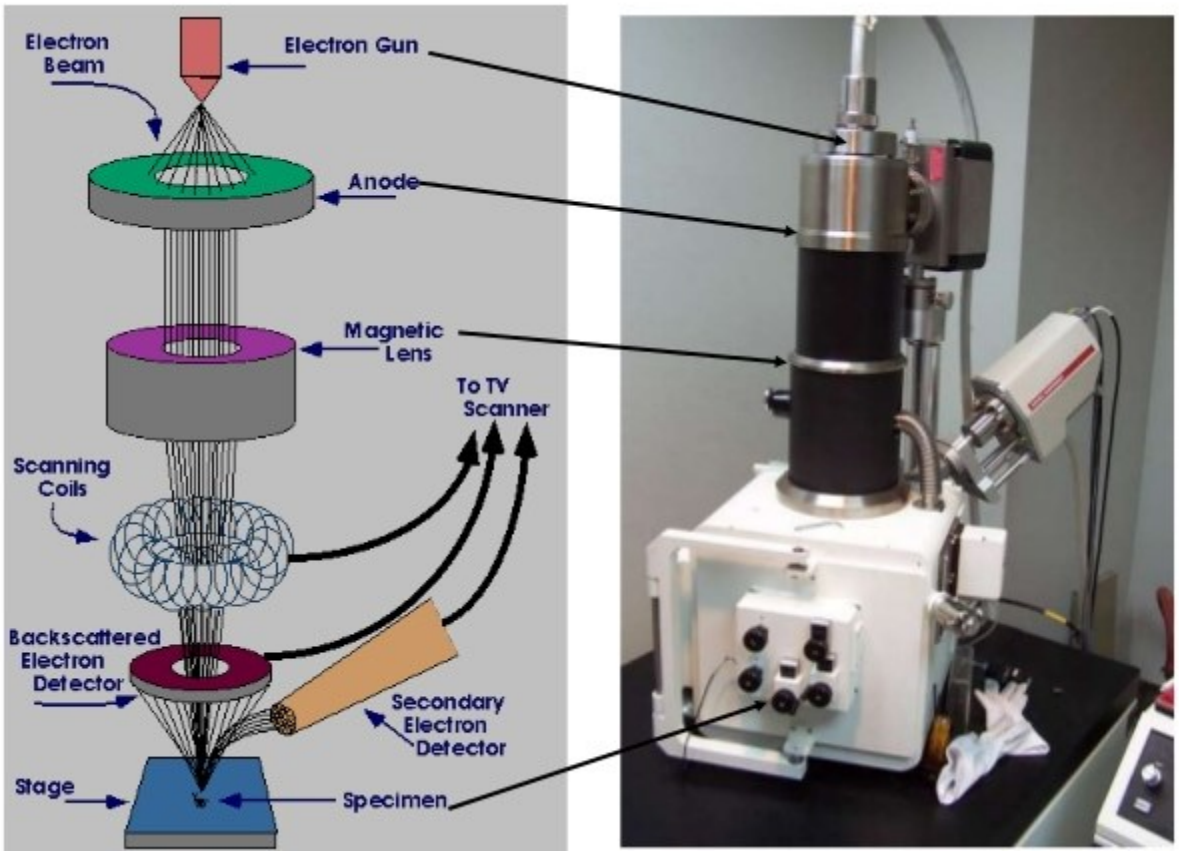

Figure 43: Scanning Electron Microscopy (SEM)

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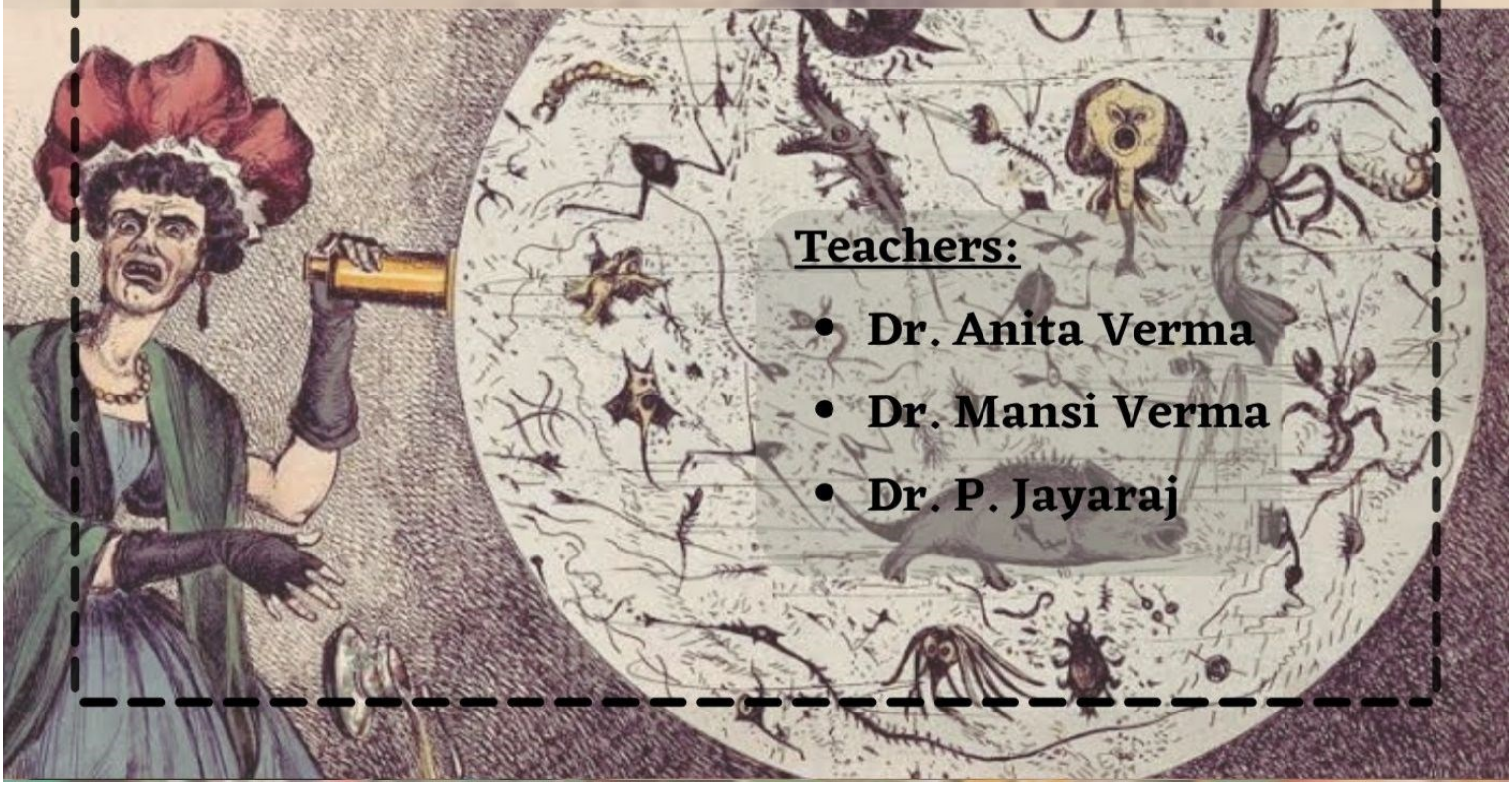
Department of Zoology

Topic: "Safety measures on microscopes & Zoological museum upkeep"



Teachers:

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- **Dr. P. Jayaraj**

Department: Zoology

INTRODUCTION

OBJECTIVES:

The Department of Zoology caters students of B.Sc. (Hons.) Zoology, B.Sc. Life Sciences, B.Sc. (Hons.) Biological Sciences and all other students of various disciplines opting for General elective (GE) offered by our department. To meet the requirements of practical's, we have skilled lab staff. But with the change in curriculum and methods, a routine training to lab staff is required for proper maintenance of equipment used in zoology laboratories. Microscopes are one of the indispensable instruments for our regular practical needs, be it for blood cell counting, observing microscopic animals, sections of various organisms, for viewing life cycle of various pests, studying mitosis-meiosis, and observing various staining methods. Hence, it is necessary to train lab staff for proper maintenance of microscopes.

Apart from the laboratories, Zoology department also has a museum for keeping preserved animals, bones for teaching osteology and slides for showing histology of various organisms. As the dissections are banned, these museum specimens are our heritage to at least make our students familiar with chordates and invertebrates. Therefore, the objectives of the present workshop to be organized by our department are:

1. Use of different kinds of microscopes.
2. Maintenance of microscopes.
3. Maintenance of museum specimens.
4. Biosafety in museum.

METHODOLOGY:

The workshop will be organized in online mode to train lab staff for practices and safety measures on instruments and museum specimen handling. For this, we will be following the following methodology:

- Providing lab staff updated guideline regarding care and maintenance
- Departmental Instrument and purchase committee to inspect the laboratory instrument
- Departmental Museum upkeep and maintenance committee coordinates with museum curator for museum related issues.
- Providing updated manual to Lab technicians for ready reference
- Use of PowerPoint presentations to make it more interactive.

OUTCOMES:

Handling microscopes and museum specimen is always a challenge for lab staffs due to their regular use in zoology. Many of the microscopes are not even suitable for observing slides at 40X due to the dust/stain /xylene on lenses and we spend a lot of money on AMC of these microscopes. Also, as there is blanket ban on dissections, core zoology studies are limited to museum specimen observation. But as the specimens are preserved in formalin, it is necessary to know how to tackle spillage of formalin and how regularly formalin should be replaced in specimen jars. At the end of this session, we expect the laboratory staff to learn healthy practices to maintain and operate microscopes. Also, after this workshop, they will be confident to deal with formalin spillage to avoid any severe accident caused this this harmful chemica

Section 1: Microscopes

Background:

Our naked eyes can only visualize objects that:

1. fall under visible range of light
2. are smaller than 1mm (1 mm is also difficult to see)

When we talk about visible range, it is necessary to know what the electromagnetic spectrum is. Visible light falls under the wavelength of 400-750 nm (Figure 44). Anything above or below this range is not visible to us unless we use specific instruments. For example, we can view flowers, butterflies etc. but we cannot see DNA/RNA/proteins unless we use UV light.

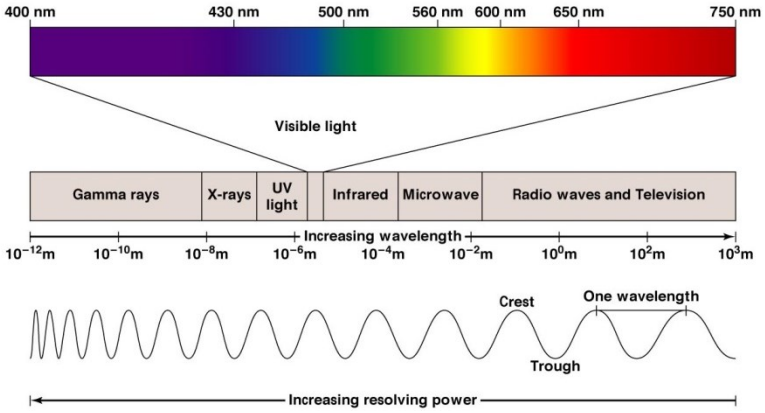


Figure 44: Components of electromagnetic spectrum

Likewise, we can see a building or a dot marked by a pencil, but we cannot see bacteria in curd! This is where the use of microscope comes.

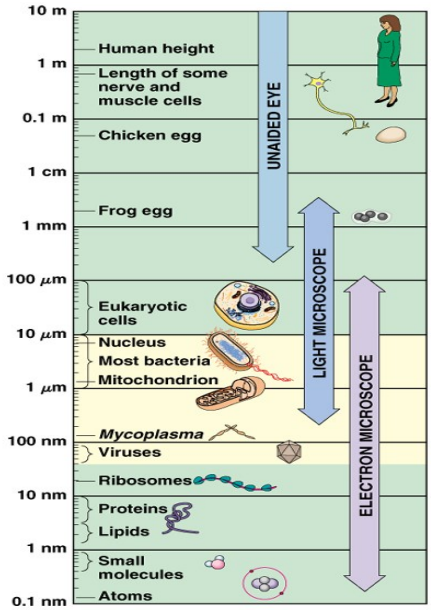


Figure 45 : Resolution of various types of microscopes

So, **Microscope** is an instrument used to see objects that are too small to be seen by the naked eye; and the science of studying such small objects using microscope is called as **microscopy**.

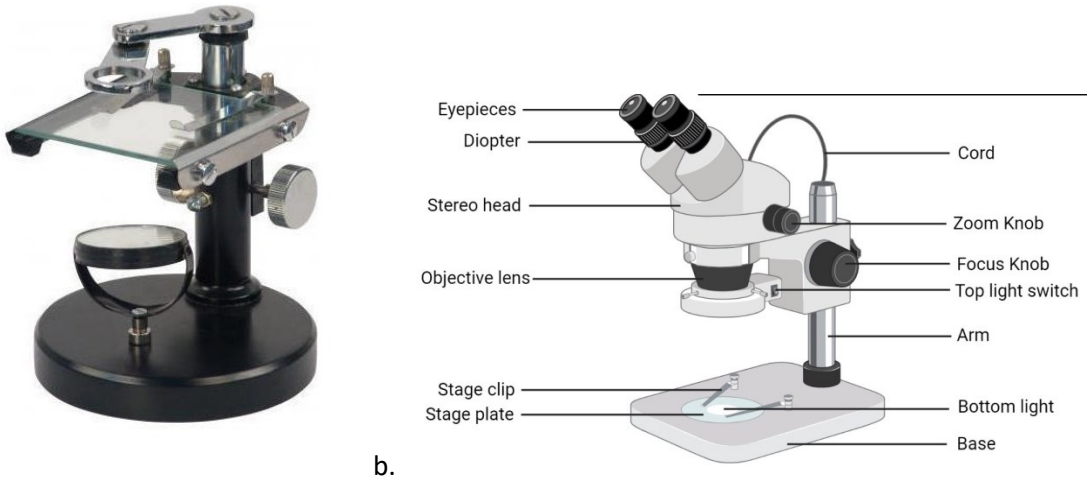
Depending upon the use, microscopes can be categorized as:

1. Dissecting Microscope
2. Compound Microscope /Light Microscope (Monocular and Binocular)
4. Phase contrast Microscope
5. Fluorescence Microscope
6. Electron Microscope (TEM, SEM)

We commonly use only the above three kinds of microscopes, i.e. dissecting microscopes, compound microscopes, binocular microscopes for routine experiments. In this exercise we will elaborate the instrumentation part, working principle, cleaning and precautions while using microscopes.

Dissecting Microscope

A dissecting microscope is a low power stereoscopic microscope; designed for low magnification observation (5x-250x) of a sample. It is used in many applications especially during taxonomic studies, embryo separation, entomology etc. It uses light source reflected from the surface of object. There are two major types of magnification systems in stereo microscopes, 1) fixed magnification achieved by objective lenses; 2) zoom or pancratic magnification, capable variable degree of magnification. This microscope has comparatively simple operating functions. (Figure 46 a and b)



a. b.

Figure 46 :Image source: https://microbenotes.com/dissecting-microscope/

Compound Microscope

These are modern light microscopes. They are named compound as they have two lens systems, i.e., the oculars and the objectives. Compound microscopes can further be classified as monoculars (one eye piece) and binoculars (two eye piece), but the basic structure remains same for both (head, arm, base). (figure 47 and 48)

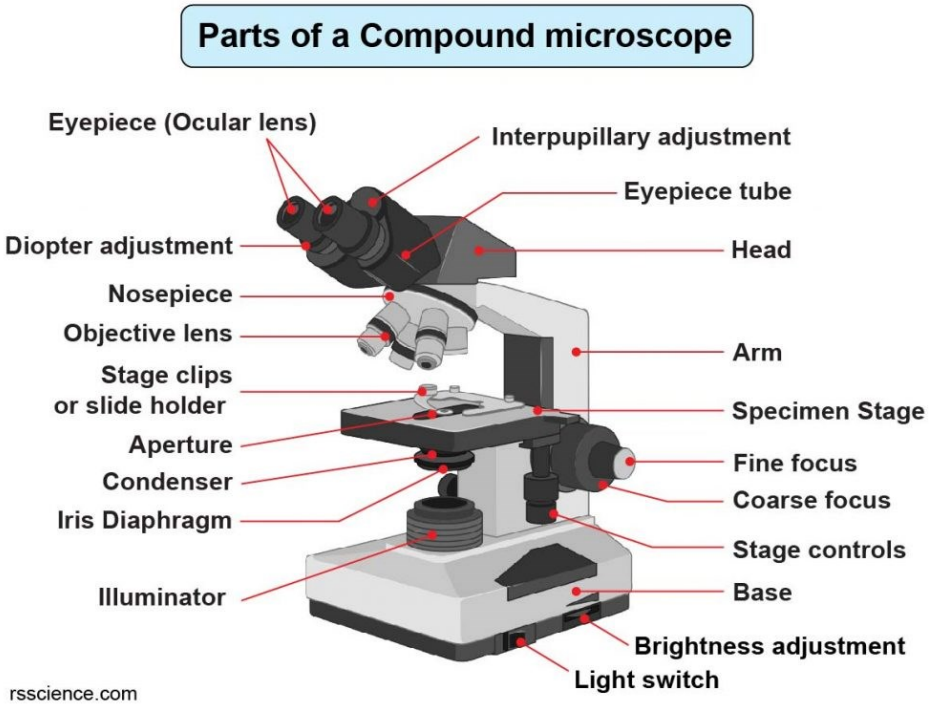
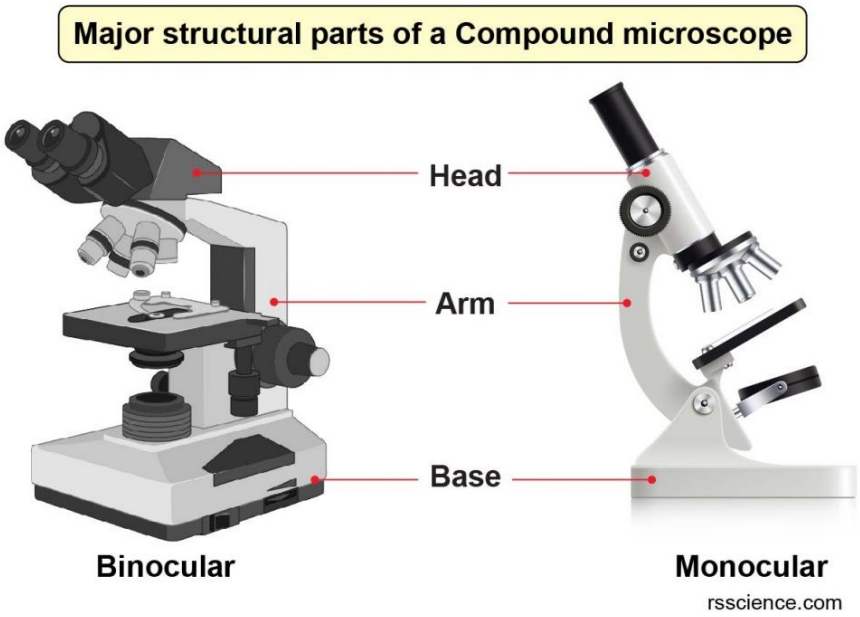


Figure 47: Parts of Compound microscope



https://rsscience.com/compound-microscope-parts-labeled/

Figure 48 : structural parts of compound microscope

Objective Lenses	• There are more than one objective lenses. • primary lenses; can have magnification of 4x, 5x, l0x, 20x, 40x, 50x and l00x.
Stage	• platform below the objective lens on which the object to be viewed is placed. • light beam passes and illuminates the specimen
Stage Clips	• two stage clips one on each side of the stage. • used to hold the slide in place.
Diaphragm	• located on the lower surface of the stage. • used to control the amount of light that reaches the specimen
The Adjustments	• two adjustment knobs: fine and coarse adjustment knobs • coarse adjustment knob helps in improving the focus of the low powers • fine adjustment knob helps in adjusting the focus with higher magnification.
Condenser (Light Microscope)	• illuminates the specimen and controls the amount of light and contrast.

Figure 49: **Information present on lens**

General Measures:

1. Always carry microscopes by the arm and set them flat on your desk.
2. Keep your slide to be viewed with the cover slip (Do not put coverslip while observing specimen under immersion oil.
3. Always start and end with the low power Objective with the lowest magnification.
4. Adjust the Knobs to obtain a field of visibility.
5. Then focus step wise in the higher magnification.
6. Do not remove slides with the high power objective into place - this will scratch the lens.
7. Clean the eye piece and objective.
8. Microscopes should be stored with the low power Objective clicked into place.
9. Always wrap electric cords and cover microscopes before returning them to the cabinet.

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Cleaning Microscope Lenses:

1. Keep the lenses covered when not in use.
2. If lens is dirty, then locate the dirt. If dirt is seen in all powers, then it is on the outside of the eyepiece lens.
    a. Try to blow off the residue. Once blown clean, lightly wipe the lens with Kimwipes or Kodak Lens Tissue
3. Cleaning with solvents: use distilled water first. Add 2-3 drops of distilled water on paper and clean the lens. If it does not work, clean lens with >90% Isopropyl alcohol. For further cleaning, a stronger solvent like Xylene can be used.
   (Note: Never use acetone on plastic parts).

4. For cleaning 100x objective with immersion oil, wipe excess oil with Kimwipe. Else wipe it with Xylene, or turpentine using Kimwipe.
   (Note: Never use water, alcohol or acetone for cleaning immersion oil)
5. For cleaning fungus from lens, 2% hydrogen peroxide can be used (2%, hydrogen peroxide, 4% ammonia in distilled water). Soak the lens for one hour in this mixture and then clean the lens with Kimwipe. Finally, keep the lens elements in front of a UV light for 20 minutes in a dust-free environment.

Section 2: Zoological museum upkeep, maintenace and biosafety

Introduction

Zoological museum:

- Zoological museum is a scientific, cultural, and educational institutions in which animal collections are assembled
- The collection includes microscopic elements to fluid preserved specimens, models and skeletal material. The scientific collection is always systematically displayed. Collections are mounted in glass cases (large specimens are often displayed in open). Microscopic specimens are displayed through permanent slides, micrographs, sketches or enlarged models.
- Labels and explanatory texts contain information on taxonomic status of the animal, its geographic distribution, distinctive biological features and economic value.

Specimen Preservation

- Specimen preservation means “long-term” preservation of organisms either plant or animal in the best possible condition. So that it can be accessed in future as reference collection for scientific purposes”
- Many chemical methods are used to preserve both vertebrate and invertebrate specimens for maintenance purposes.

Labelling

- Once the specimen is preserved it is very important that it should be taxonomically labelled. This information may either be printed on a label which is attached to the specimen or may be recorded in a notebook. If a notebook is used the data should be identified by a number; a tag bearing the same number should be attached to the specimen.
- The taxonomic information on the specimen should include: Phylum-
- Sub Phylum-
- Class-
- Sub class-
- Order-
- Genus-
- Species- if available

Maintenance of the museum

- All collections should be stored in secure, environmentally controlled conditions.
- To minimize deterioration, keep specimens away from light sources.
- The museum should be kept at a stable relative humidity level of between 45% and 55%. This may require a humidifier or dehumidifier.

- Temperature levels should be as stable as possible, between 10°C and 22°C (thus requirement of an air conditioner).
- Museums are never completely immune to pests. Most pests will lay eggs inside specimens and the young stages (larvae) will cause the most damage.
- Pests can be reduced by regular vacuuming and cleaning of the museum and banning food and drink from the area.
- Pests can be monitored with insect traps (such as the sticky trap). Quarterly checks of the traps will show the types of pests entering museum. Inspect your collections at least twice a year to monitor for any pest activity.

Fixation

Fixation is the process whereby the organism is prepared so that the post-mortem changes are arrested. This generally involves chemicals that penetrate the tissues of the dead creature and solidify the body by inducing covalent bonds that cross-link constituent molecules. The fixatives used for whole or parts of animals always have some side effects including, in some, shrinkage and in others expansion. There is generally a loss of colour, but the important effect is the hardening of the components of the animal so that it may then be stored for indefinite periods.

Preservatives and their usage

1. Formalin(Fixative mostly)

Usage:

- It is used for **vertebrates only.**
- It is avoided for **long-term storage** since it is acidic and difficult to handle.
- Mostly formalin is used where color is important since alcohol dissolves most colors almost immediately.
- It penetrates more rapidly and internal organs remain in better condition

Procedure to prepare formalin solution for specimen preservation and storage

10% neutral buffered formalin (100 ml) 37% formaldehyde solution - Potassium phosphate monobasic - 0.4 g Potassium phosphate dibasic - 0.65 g Distilled water - 90 ml

(note :10% **formalin** is a 1:**10** dilution of 100% **formalin** in water, i.e. 1 part saturated formalde- hyde in water diluted with 9 parts plain water. Since 100% **formalin** contains 40% **formaldehyde**, a 1:**10** dilution would contain 4% **formaldehyde**.)

(please see Links for detailed preparation of buffered formalin)

Precaution:

1. Inhalation of formalin fumes is harmful &causes extreme discomfort to nose and eyes.
2. Contact with fluid causes severe irritation to the Skin
3. Contact with sore or raw spots results in extreme pain.
4. It is **carcinogen.**
5. Hand should be rinsed after usage.

Storage:

1. It should be kept in safe, water-tight, spill-proof bottles,
   e.g. pep-bottles, It should always be *clearly labelled.*

Table 1 : Common types of preservation strategies for different specimens

Animal phyla

Phylum/phyla	Common types of preservation					Biological notes
	Dried	Skeleton/ shell	Fluid	Cast	Taxidermy	
Porifera (Sponges)	√	√	√			Different species of sponges produce calcareous, siliceous or elastic skeletons on which their cell layers are arranged. Dried specimens do not contain any of the cells so fluid preservation (of the smaller species) is required to preserve the living components. Some sponges are too large for fluid preservation
Cnidaria, Ctenophora, endoprocts, and ectoprocts	√		√			Corals (parts) may be dried (losing their organic components) but jellyfish, sea anemones, etc. for the soft tissue medusa and polyps must be preserved in fluid
Platyhelminthes			√			Many are huge tapeworms, both long and fragile. These are often wound round a clear plate
Annelids, nemerteans, nematodes, priapulids, and other worms			√	√		A huge variety of size and complexity with some annelids having tentacles, eyes, jaws and long chaetae
Mollusks	√	√	√	√		A huge variety of body forms from slugs and snails to mussels to giant squid mean that fluid preparation may need a few drops to many liters of preserving fluid
Onychophorans			√			These velvet worms are relatively small and most often fluid-preserved
Echinoderms	√	√	√			Most echinoderms can be fixed and then dried, but their complex internal architecture is then lost. Some are fluid-preserved so that windows can be cut into their outer shell to exhibit their internal organs in situ
Arthropods	√		√			Insects, in particular, are dried and set with pins in adherence to entomological customs. Large crustaceans may be cleaned, and the exoskeletons set as a dried specimen
Chordates		√	√	√	√	Many techniques exist, particularly for vertebrates as they are kept for hunting trophies as well as for scientific research and museum collections

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Arrangement of specimens

- This is the most fundamental issue in the storage of a zoological collection. Lack of proper arrangement of the specimens makes the entire collection good-for-nothing
- Numerical arrangement requires a species index that lists the species represented in the collection and catalogue number of all the specimens. Without such an index, it would be necessary to search through the entire collection or the numerical catalogue in order to assemble all representatives of a particular taxon. Moreover, in numerical arrangement, if a container is misplaced, it is practically lost.
- In collections arranged according to zoological classification, misplacement is immediately detected from the name on the container. It is also more economical in space, as a number of specimens of the same taxon can be stored in one container, with labels on each.
- **Specimen cards:** Specimen card for each specimen with all the details, viz., sex. locality, date of collection, collector or donor, condition of the specimen, registration or catalogue number, external and cranial measurements, keeping place, etc., should be prepared and arranged in a card cabinet according to the zoological classification. Consultation of these cards will often serve the purpose of the scientists and reduce the incidences of handling and removal of the specimens from storage.

Safe Handling

- Wearing disposable nitrile gloves will eliminate any transfer of skin oils and perspiration to specimens.
- Always handle specimens with gloves, and if cleaning them, always use dust extraction/fume cupboard and wear a dust mask. Pinned entomology specimens are normally handled using specialized curved and gripping forceps.
- Old specimens can be very dry and brittle and pins may be corroded; remove specimens from their drawer very slowly and carefully.
- **Replace the preservatives regularly** to ensure that the specimens are completely immersed in the preservative. The jars should be closed tightly to prevent evaporation of the preservative **(Figure 50 a and b)**
- **Check the fluid levels in jars annually and that fluids are not discolored or contaminated. Advice should be sought about topping up. (Figure 50 c)**
- **Fragile or loose labels can be archived in polyester envelopes and the data recorded onto new labels, using pigment ink, to be stored inside the jar. If you do not have the equipment to work on conservation problems for spirit collections, contact a conservator or curator for advice.**

a. 

b. 

The lid of the jar has cracked and the alcohol has fully evaporated

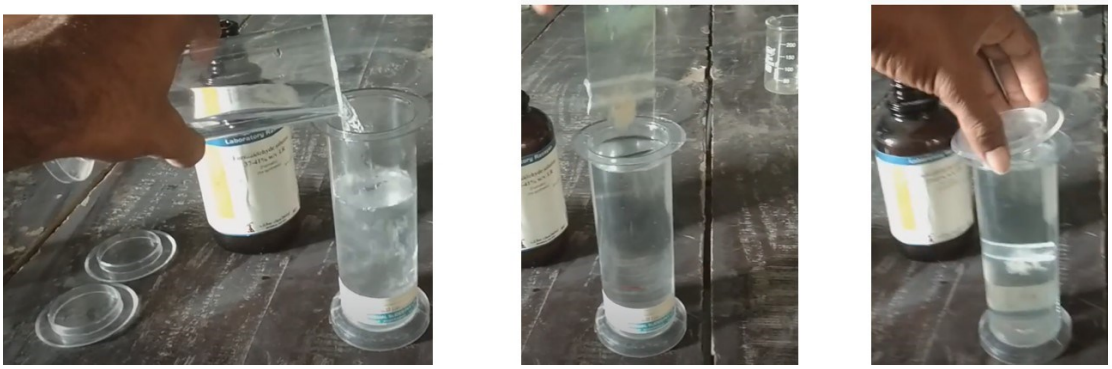
c. 

Figure 50: a) and b) are representative specimens which needs topping up with preservative solution. c) Representative image showing refilling and securing of a biological specimen.

Reasons to Replace Preservative Solutions

Reasons for transferring specimens to a new storage fluid includes:

• When removing the specimen from a fixative to a preservative

• When the preservative has become acidified (usually due to instable products in the preservative such as formaldehyde)

• Due to health and safety mandates

• For research purposes or other use for the specimen

• Because the preservative method has been deemed inappropriate

• If extracted lipids cause sufficient acidification to damage bone or other tissues

• If extracted lipids (particularly with oily marine species) may discolour the specimen or make it difficult to handle or use for research purposes

Changes in colour, turbidity, or opacity of a liquid may indicate that a fluid should be changed (opacity is generally a better indicator than colour, e.g., petroleum ether may cause naphtha to form in the fluid). A simple turbidity test will

give a relative estimate of lipids in the preservative--pipette a small quantity of the preservative into a petri dish of clean water on as dark background and observe any formation of whitish turbidity.

(Note: please see the provide links for instructions on topping up of specimens with preservatives)

	Box Number	Slide Contents	Description on slide	Numbers on slide	Number of slides	Notes
Information	Box reference number on front of box	Name of specimen on slide	Any aditional descriptions e.g. location and date	Any numbers associated with slide, possibly relating to catalogue (e.g.291/3) or box (e.g. B8)	The amount of that particular slide within collection	Any additional information e.g. green dot
Example Slide	B8	Hydroida,Gymnoblastea, Corynidae, Syncoryne, eximia	Calf Island 7/07	C.a.9	1	Blue dot

Figure 51: Format for cataloguing slides

- Slides should be checked annually and, if serious deterioration is noticed, a microscope slide conservator should be contacted. Slides can be cleaned of surface dirt using cotton wool swabs or buds dampened with deionized water. Slides should be labelled and arranged as per format in order to catalogue or order new slide **(Figure 51).**

BIOSAFETY

formalin safety (protocol for liquid spills)

- Skin contact with the spill can result in allergic reactions, blistering, and hives among other symptoms. If skin does come in contact, immediately rinse with water and a mild detergent for a minimum of 15 minutes. If irritation persists, seek medical attention right away. It is critical for every facility to have a clearly communicated process for hazardous material spills.
- Depending on the severity of the spill, it may be acceptable for laboratory personnel to clean up the spillage. Appropriate training should be provided prior to beginning work in the facility.
- It is important to note that protective gear should be worn at all times including eyewear and gloves.
- water spray may be used to help reduce the vapors released. If the spill is fairly small, paper towels may be used for absorption and **then placed in a marked sealed bag.**
- A **spill kit** should be easily accessible for larger, yet still manageable spills, which contains items such as a **neutralizer and a marked hazardous waste bag.** The **neutralizer** can simply be spread onto the formalin to quickly soak up the moisture. Ensure that disposal of any material is compliant with the facility's procedures, as well as state and federal

measures. Before returning to work after a spill, any contaminated equipment should be thoroughly cleaned**. (Figure 52)**

Figure 52: The spill kit should contain items such as absorbent pads, booms, pillows, gloves, disposal bags and formalin neutralizing agent

Categories of spills

Minor Spills	Major Spills
A single leaking specimen container containing 10% formalin or paraformaldehyde	Multiple broken specimen containers that contained 10% formalin (perhaps from a collapse of a shelf).
A broken specimen container that contained up to 100 ml of 10% formalin or paraformaldhyde	A partial or full container containing more than 100 ml of 35% formaldehyde onto a bench or the floor
A partial of full container (greater than 100 ml) of 35% formaldehyde in a chemical fume hood	A stock bottle (500 ml or greater) of 10% formalin onto a bench or the floor
A splash of concentrated formaldehyde or paraformaldehyde onto a surface	A single lab staff member who becomes injured and drops 1-2 specimen containers

- **Personal Protective Equipment (PPE)**
  Gloves: Nitrile or neoprene are recommended
- Eye/face protection: Safety goggles
- Maximum protection = safety goggles with face shield and a Lab coat that is completely snapped or buttoned
- An impervious apron can be used over a lab coat

(Note please see the provided links to know more about biosafety)

Knowledge required by museum curator and lab staff:

- **Required Skills**
- knowledge of museum archival storage methods and techniques, including detailed knowledge of museum cabinets and proper storage and protective materials in order to research, design, and order custom-made museum storage supplies and equipment.
- Knowledge of proper storage methods for the specific requirements of the wide variety of biological materials in the collections, to prevent aging and deterioration of specimens.
- Knowledge of the range of environmental hazards that affect museum collections, and the recording and prevention of such threats.

- Knowledge of museum pest control materials and techniques including prevention of pest problems, pest control chemicals and their proper application, laws governing pesticides and their applications, identification of pest species, freezing as a pest control method, and safety procedures.
- Knowledge of current strengths of the museum, and needs in the teaching collections, based on the mission and goals of the museum.
- Knowledge of preparation techniques for vertebrate specimens, including preparation of bird and mammal taxidermy skins and mounts, preparation of skeletal specimens using live dermestid beetles and other methods, and preparation and storage of specimens in alcohol.
- Knowledge of museum's written collections management policies and procedures manual, supervision of all access to and loans of museum materials, and facility with database software used in building and maintaining museum digital catalogs.
- Ability to supervise and enforce all museum policies.
- Ability to manage assignment of keys.
- Excellent communication skills, both oral and written.
- Knowledge of all state, federal, and local laws and regulations regarding transactions involving animal specimens or parts thereof.
- Knowledge of and ability to accurately identify animal specimens or parts thereof, in order to avoid personal liability for museum activities.
- Knowledge of and ability to take preventative actions to prevent injury, infection, or other damage to workers in the museum.
- Knowledge of supervisory personnel skills, including hiring, training, and disciplinary actions.
- Excellent interpersonal skills, both oral and written, and a desire to communicate with labs staff and faculty
- Demonstrated ability to operate personal computers, related word processing, spreadsheet and database software with the ability to learn other software.

References/ Interesting Weblinks:

1.https://www.microscopeworld.com/t-cleanlens.aspx

2. **Buffered formalin preparation https://www.youtube.com/watch?v=b-Hk21dmMmQ**

3.**This video demonstrates the Identification of specimens which require toping up with preservatives (formalin/Alcohol)**https://www.youtube.com/watch?v=15teKU7j6Hw

4.**This is a formaldehyde safety training video will teach your employees how to safely use this chemical**.
https://www.youtube.com/watch?v=oACJSfENFQg