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Biodeterioration of Library Resources and Possible Approaches for their Control

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Abstract

Biodeterioration is “any undesirable change in the properties of a material caused by the vital activities of the organisms” A study was undertaken to find out the microflora associated with the library environment, decaying books and papers. Microflora were isolated on Nutrient Agar and Potato Dextrose Agar by using Air Trapping Method and Biodeterioration paper part technique. Total 11 bacteria and 8 fungi were isolated, out of which *Bacillus subtilis* and *Staphylococcus aureus* were the predominant bacteria where as *Aspergillus* spp. Were the mostly found associated fungi. Both bacteria and fungi shows positive activity of Cellulase degradation. Approaches were done to control microbial growth by modification of ink i.e. by making combination of pen and printer ink with different dyes Lactophenol Cotton Blue and Gentian violet in different ratio. The best result was obtained by using pen ink with Lactophenol Cotton Blue and printer ink with Lactophenol Cotton Blue to both fungi and bacteria.

Keywords: Antibacterial, Antifungal, Library, Lactophenol Cotton Blue, Cellulase

1. Introduction

A library is a repository of wisdom of great thinkers of the past and the present. It is a social institution charged with the responsibility of disseminating knowledge to the people without any discrimination. The holdings of the libraries are the priceless heritage of mankind as they preserve facts, ideas, thoughts, accomplishments and evidences of human development in multifarious areas, ages and directions.

Paper is an organic substances composed of cellulose from green plants which produce fibers suitable for paper making. Cellulose is the most abundant polymer found on the Earth. Cellulases are a group of fibrolytic enzymes which cooperatively hydrolyze plant cell wall fibers into glucose, cellobiose or oligosaccharides (Murad and Azzaz, 2010; Chinedu *et al.*, 2010) [16, 25]. Deterioration of library material due to the fungal and bacterial growth is a worldwide problem and a cause of extensive damage to precious books and manuscripts (Sunandan Baruah *et al.*, 2008) [6].

The common microorganisms which are responsible for deterioration of library material or paper are Fungi and Bacteria but the most common is fungi. Microfungi which produce too many spores and can be easily transported, form the significant part of the bioaerosols in the external and internal atmosphere of the surrounding and they can contaminate all kinds of surface. There are 20 thousand to 2 million fungus spores in 1 m³ of air and this is one of the significant causes of skin and respiratory diseases (Ceter and Pinar, 2009). Fungus are a large heterogeneous group of plant organisms. The fungal spores are present in the earth, water and air and remain in a dormat state for long periods. These spores sprout and grow when they have the required moisture and heat. Generally fungi grow at temperature range of 15-35 °C. In libraries fungal growth is known as mould or mildew and they appear as brown/black vegetative growth on paper. Besides fungus, bacteria also decompose cellulose in paper. Damage can occur because of mechanical stress or enzymatic action, because moulds can produce a wide range of enzymes (proteinases, gelatinases, cellulases) which are able to destroy the component materials of library and archival collections. The most common fungi that grow on paper as the *Aspergillus* and *Penicillium* spp.

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Besides fungus, bacteria also decompose cellulose in paper. The common bacteria are *Staphylococcus aureus* and *Bacillus subtilis*.

Book in library especially bound ones offer good substrate for microbial growth as the binding glue, cloth covering and paper support (Tilak and Vishwe, 1976, Atluri and Padmini, 2002) [20].

Human exposure to airborne microorganisms may result in a variety of adverse health effects including infectious diseases (Brachman, 1974) [9], allergic and irritant responses (Agarwal and Shivpuri, 1974) [3], respiratory problems (A I Doory 1984, Tilak, 1989) [4] and hypersensitivity reaction (Sooley and Hyatt, 1980).

Paper and books stores carry all kind of knowledge through the barriers of time and pass them on to the youngsters in the future. The microbial deterioration of paper material such as books, journals, manuscripts and decorative wall paper etc., is a serious problems throughout the World in museums, libraries and archives. Most modern book papers have a relatively short life span, which can be further reduced by improper storage environments. They deserve to be maintained and conserved in their original condition in some special places such as libraries and museums, in order to prevent them from deterioration by environmental conditions. That's why all of these the need of this work is essential.

An attempt has been made in the present study to isolate, identify and optimize cellulases producing microorganisms from decaying paper and to check out possible remedy with LCB with different combination of ink and other dyes to enhance the growth of paper deteriorating microorganisms i.e. Fungi and Bacteria.

2. Materials and Methods

Present investigation has been undertaken to study the microorganisms (fungi and bacteria) which are responsible for paper deterioration and to find out the possible remedy of ink to enhance the growth of paper deteriorating microorganisms with the help of Lactophenol Cotton Blue. The experiment was carried out by adopting the following materials and methods.

2.1 Methods Adapted

1) Collection of samples

The experiment was carried out by surveying various sections of Shivaji college library, Departmental library of microbiology, Akola and from old books kept in home, Fungal and bacterial colonies on books were recorded. During course of study the samples were collected from both infected objects coming from different places to be conserved in our laboratory and the internal air. Paper sample were collected in plastic bags. Then paper sample carried to laboratory for further investigation.

2.2 Isolation of bacteria from paper sample

Two methods for the isolation of paper deteriorating bacteria from library places and paper samples were carried out.

- **Air trapping method**

Isolation of bacteria in the air inside libraries was studied by using petriplate exposure method. Media (NA) containing petriplates were placed in different sections of library on the

top of book shelves, in the middle and on the floor. Petri dishes were exposed to air for 15 minutes. These plates were incubated at 30-37 °C for 24 hours. After incubation the individual bacterium were inoculated on petriplate containing Nutrient Agar by streak plate method and they were maintained on agar slant by sub culturing once in a week. (Upadhyay and Jain, 2012) [12].

- **Agar plate method**

Paper from books cut into small cubes (1 cm) then transferred directly with the help of sterile forceps into Petri dish contain sterilized Nutrient agar and incubated at 30-37 °C for 24 hours. Then the bacterial colonies were identified according to morphological and microscopic characteristics (Pitt *et al.*, 1992).

2.3 Isolation of fungus from paper sample

Various bio-deteriorated paper samples were collected from various sections in library. Sample mycoflora were isolated by using Agar plate method (APM), air trapping method, Biodeterioration paper part technique.

- **Agar Plate Method (APM)**

Paper from books cut into small cubes (1 cm) then transferred directly with sterile forceps into Petri dish contain sterilized Potato Dextrose Agar (Mix 1/100 ml antibiotic i.e. erythromycin at the time of preparing PDA to inhibit the growth of bacteria), do not touch the sample paper. Then the plates were incubated at 25 °C for 4-6 days. Fungi colonies were identified by morphological and microscopic characteristics (Pitt *et al.*, 1992).

- **Air trapping method**

Isolation of fungi in the air inside libraries was studied by using the petriplate exposure method. Media (mix 1 antibiotic per 100 ml) containing petriplates were placed in different sections of library, on the top of book shelves in the middle and on the floor. Petri dishes were exposed to the air for 15 minutes. These plates were incubated at 25-28 °C for 3-4 days. After incubation period the individual mycoflora were inoculated petriplate containing Potato Dextrose Agar (PDA) by cotton swab technique.

- **Cotton swab technique**

The individual mycoflora were transferred using sterilized inoculating needle into test tube containing 1 ml of Sterilized distilled water (SDW). After that this suspension were inoculated on petriplate containing PDA using sterilized cotton buds swabbed over the surface of petriplate. The dishes were incubated at 25 °C for 4-6 days (Abdel-kareem, 1997).

- **Bio deteriorated paper part technique**

In bio deteriorated paper part technique very small paper separated from the original ancient paper objects, paper cuts into small cubes and transferred using sterilized forceps into sterilized distilled water. Then this water transferred to the Petri dish containing PDA (mix 1 antibiotic per 100 ml) and plates were incubated at 25 °C for 4-6 days. Fungi colonies were identified according to morphological and microscopic characteristics (Abdel- Kareem, 1997).

2.4 Identification

• Colonial morphology of isolates

Presumptive identification of the bacterial colonies was done by observing their individual appearance on the selective and differential media used for isolation. For example, yellow colour of *S.aureus* on Mannitol Salt Agar. Whereas fungi colonies observing on Potato Dextrose Agar. For example Black color colony of *A. niger* and cottony white colony of *Fusarium* on Potato Dextrose Agar. Purified colonies of bacteria were further characterized using Gram staining while the fungal colonies were characterized by Lactophenol Cotton Blue staining (Basu, 1980) [7].

• Biochemical test

The confirmation of the organisms was done on the basis of conventional cultural and biochemical characteristics (Bergy's, 1986) [23].

Sugar fermentation, Indole production, Methyl red, Voges prausker, Citrate utilization, for bacterial isolates was carried out.

2.5 Cellulase activity of Bacteria and Fungi

After the pure culture formation spot inoculation of individual bacterium and mycoflora was inoculated on Nutrient agar containing 0.5 g/100 ml cellulose powder. The plates were incubated at 37 °C for 48 hours. At the end of incubation, if the test organism were positive the zone of inhibition were observed, it is recorded in millimeters (mm) (Sakthivel *et al.*, 2010).

2.6 Antibacterial and Antifungal testing quality of mixed paper dyes with Lacto phenol Cotton Blue and Gentian Violet

(i) Inoculum preparation

• Bacteria

The bacterial inoculum was prepared by inoculating a loopful culture of test organism in a 5 ml of Nutrient broth and incubated at 37 °C for overnight till a moderate turbidity was developed.

• Fungus

The inoculum for fungus i.e. spore suspension of selected fungus was prepared by inoculating a loopful spores or fungus culture in test tube containing 1-2 ml of Sterilized Distilled Water. Then the suspension directly used for testing.

ii) Preparation of ink mixture

For testing of mixed paper dyes the two paper ink were used, the printer ink and pen ink. (Kamel *et al.*, 2014) [11].

- The solution were prepared from printer ink + lactophenol cotton blue in a ratio (1:1), (1:2), (2:1), (2:2) and the alone lactophenol cotton blue.
- The solution of pen ink + lactophenol cotton blue were prepared in ratio (1:1), (1:2), (2:1), (2:2) and the alone lactophenol cotton blue. and
- The solution of printer ink + Gentian violet were prepared in ratio (1:1), (1:2), (2:1), (2:2) and alone Gentian violet.

iii) Testing the quality of mixed dyes with LCB and Gentian violet

For antibacterial and antifungal testing the filter paper discs prepared by using ordinary office two hole puncture, paper discs with approximate diameter of 6 mm were punched out one by one from a sheet of filter paper (whatman No.14), the discs placed in vials, sterilized by oven and allowed to cool. Then the blank discs were soaked in known concentration of solution. (Disc without any addition used as control). Then used for sensitivity test for both bacteria and fungus. After incubation period bacteria at 37 °C for 24-28 hrs and fungi at 25 °C for 4-6 days, zone of inhibition were obtained, it is recorded in millimeters (mm). Measurements were made from both sides of the slope and their average accepted (Al. Refai, 2006) [5].

Photo Plate



Photo 1: Collection of Paper Samples from Library Environment

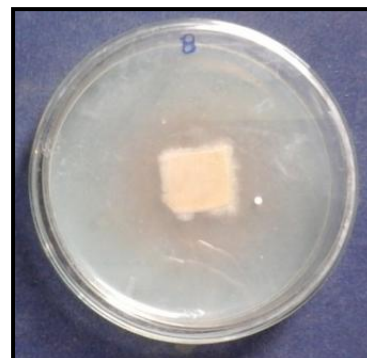


Photo 2: Isolation of Bacteria on Nutrient Agar by Agar Plate Method

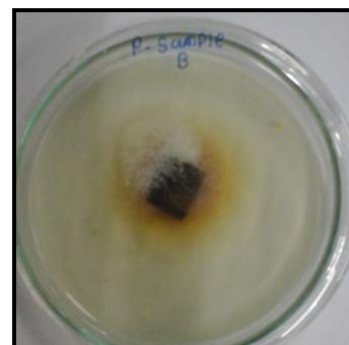


Photo 3: Isolation of Fungi on Potato Dextrose Agar by Agar Plate Method



Photo 4: Isolation of Bacteria by Air Trapping Method

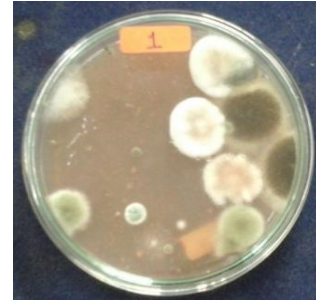
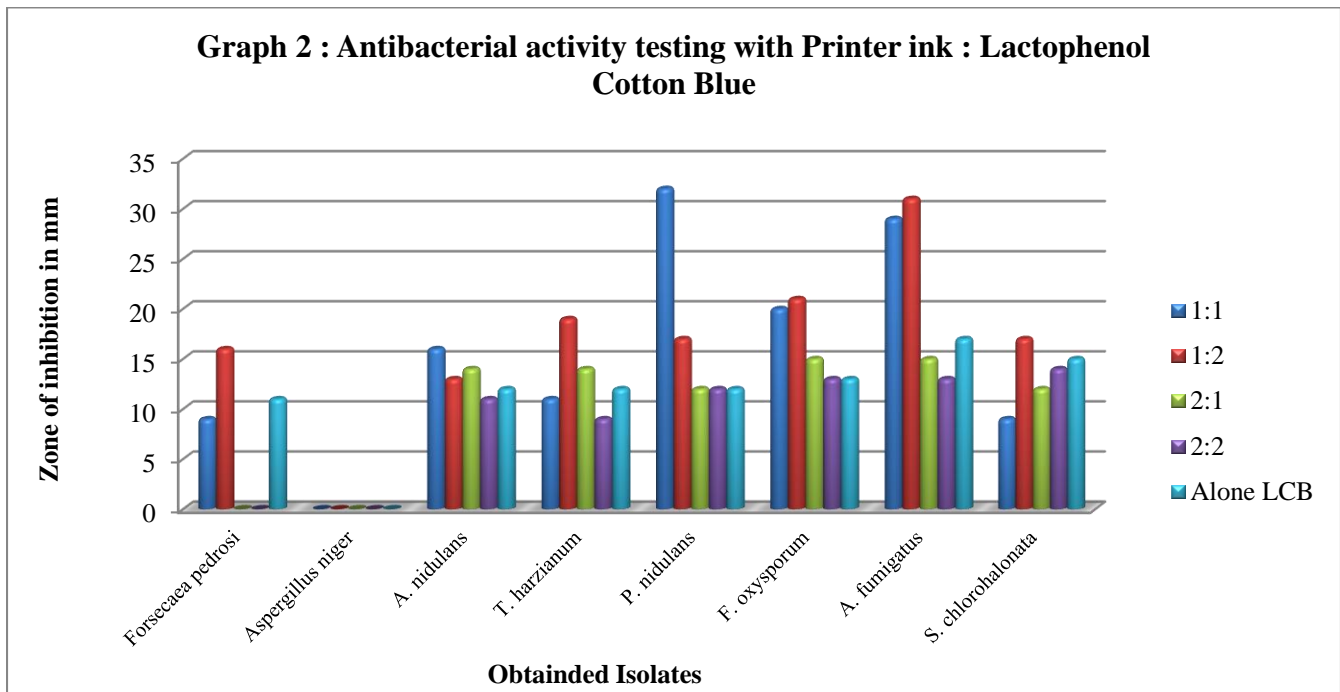
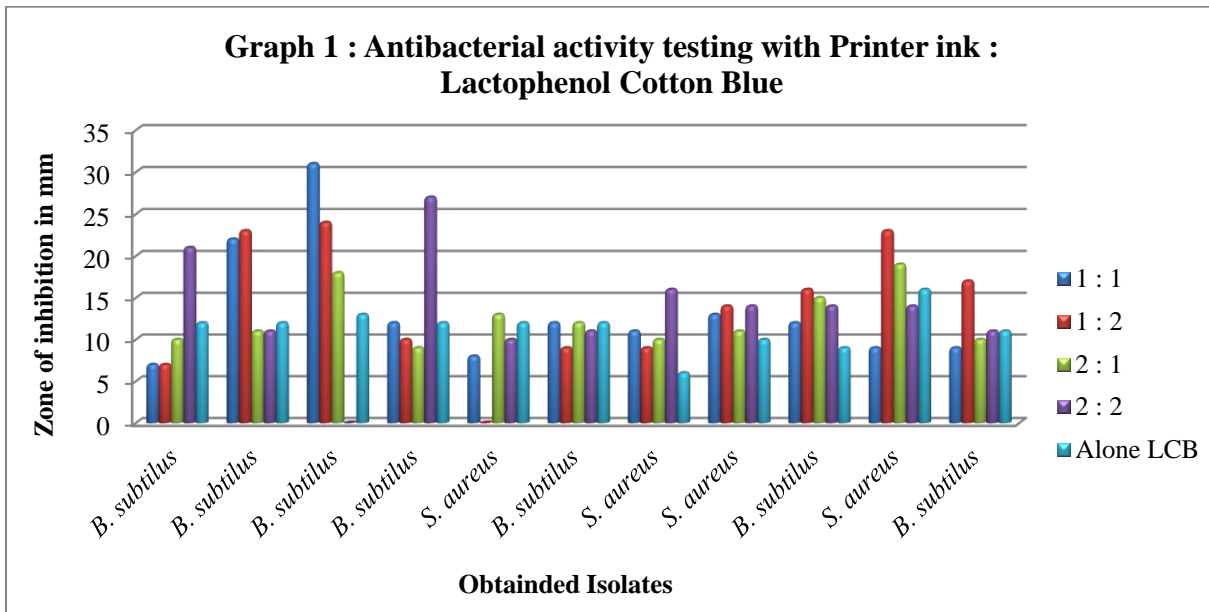
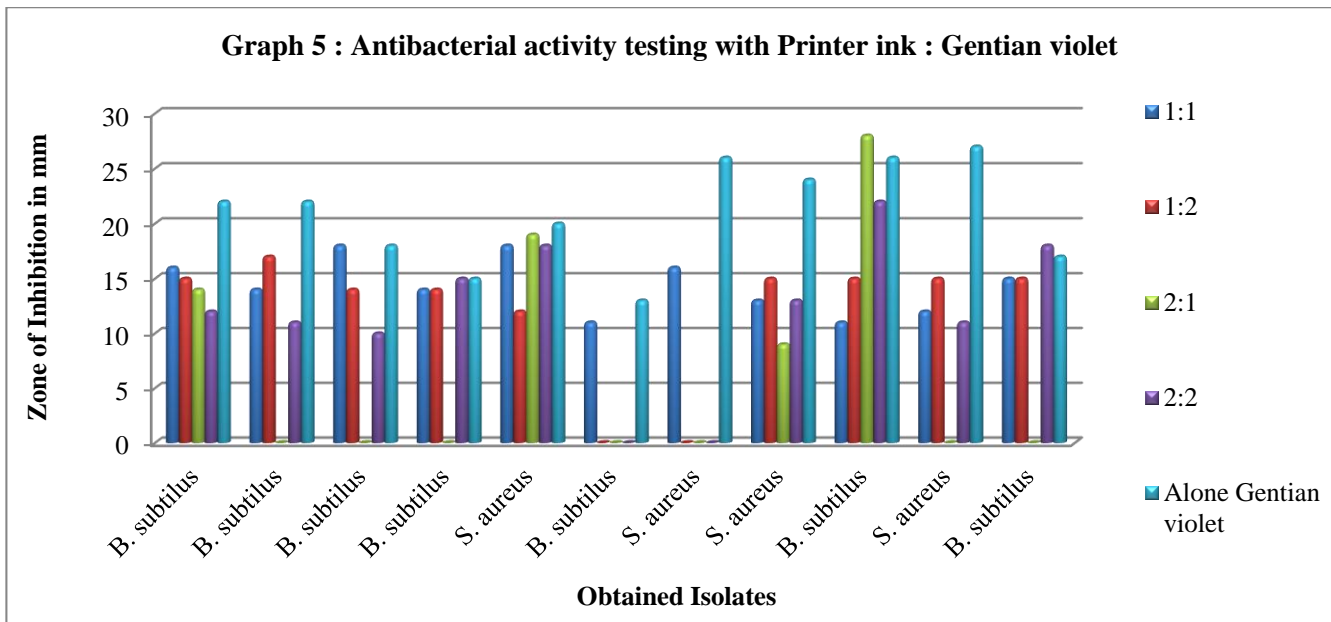
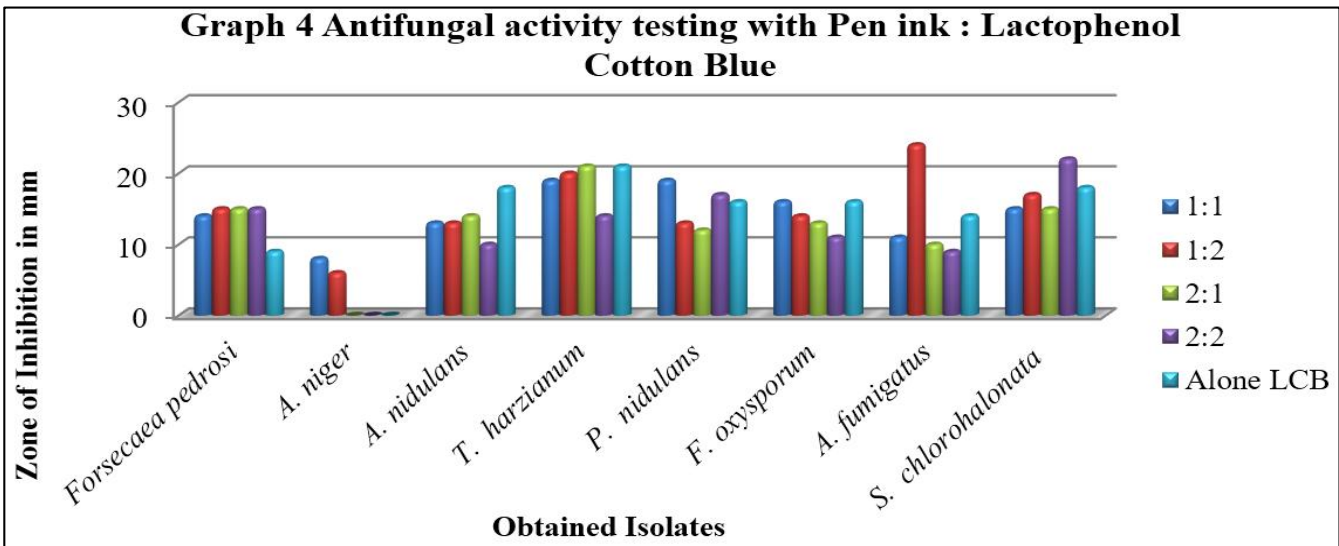
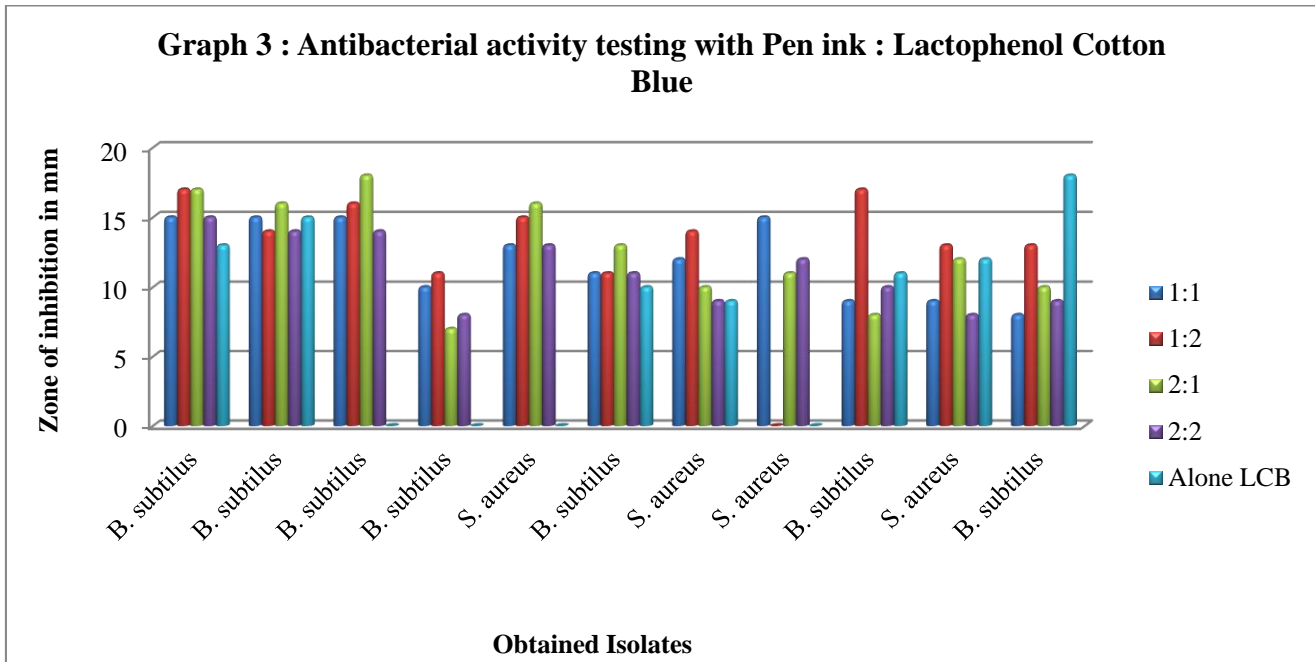


Photo 5: Isolation of Fungi by Air Trapping Method

3. Results and Discussion

A library is a repository of wisdom of great thinkers of the past and the present. So the importance of preserving and maintaining printed materials is crucial for librarians and specialists in libraries and information centers. Fungi are decomposing organisms that play the main role in destroying and degrading carbon and residue of nitrogen such as wood and paper.





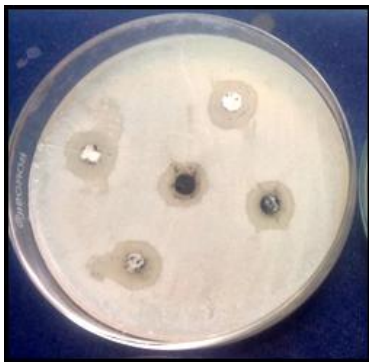
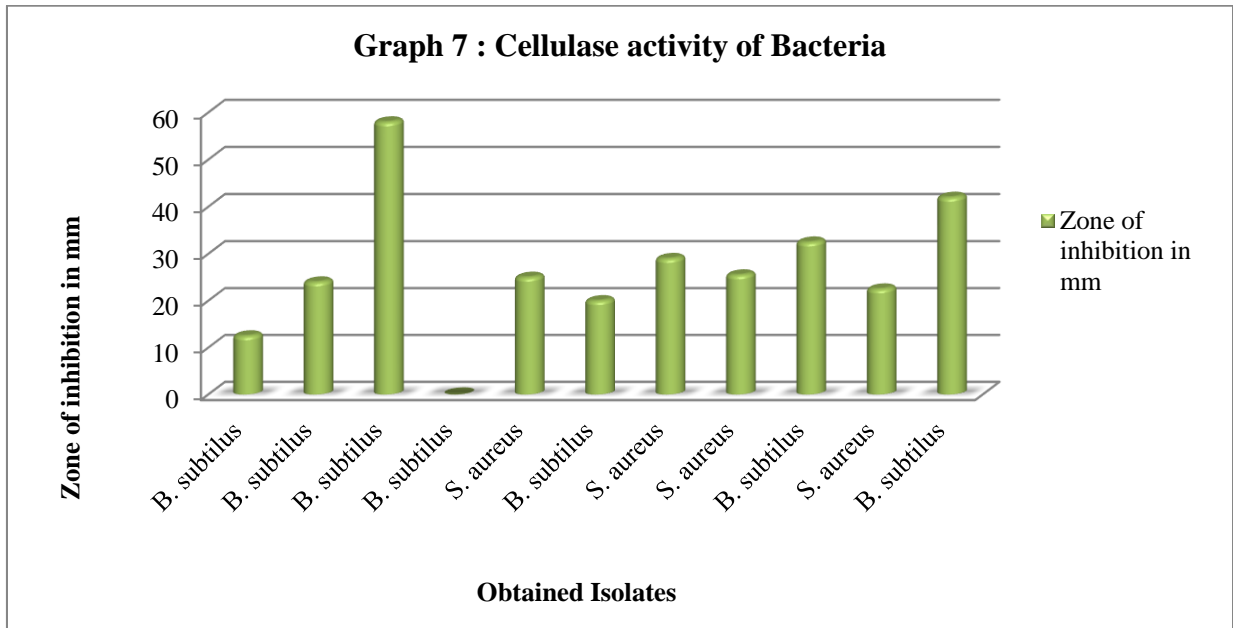
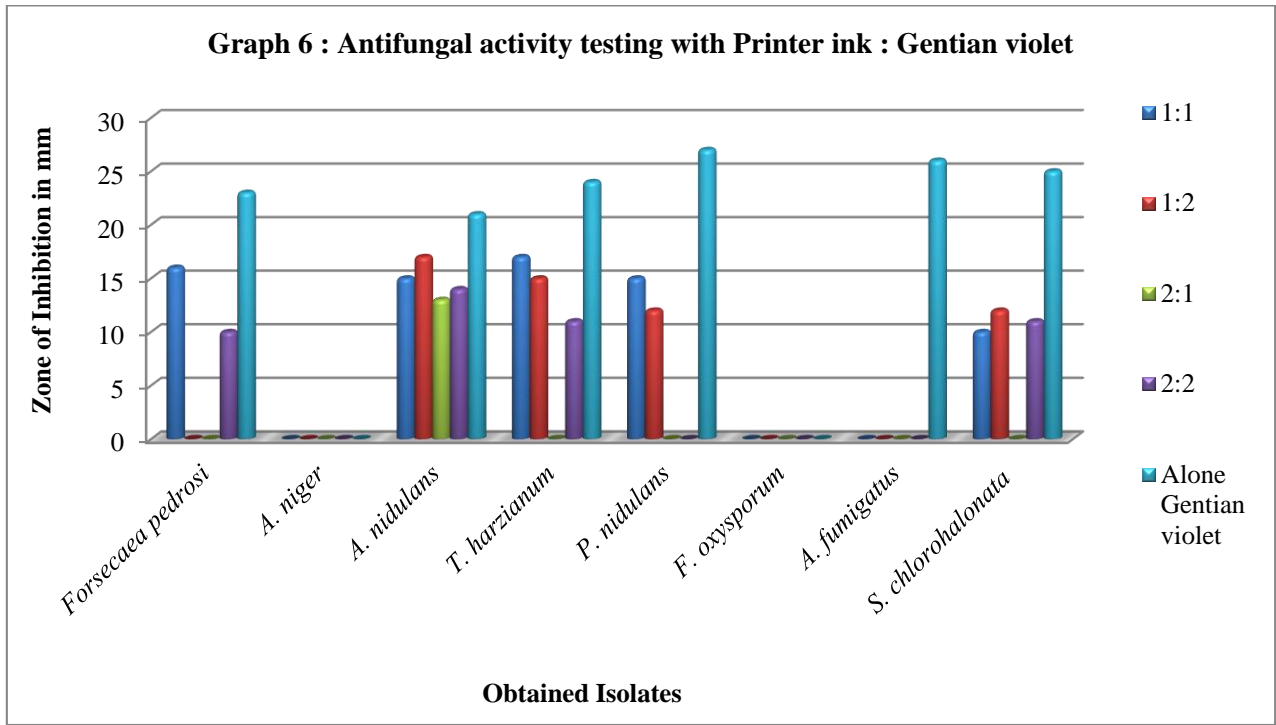


Photo 6: Antibacterial effect of Printer ink: LCB against *Bacillus subtilis*

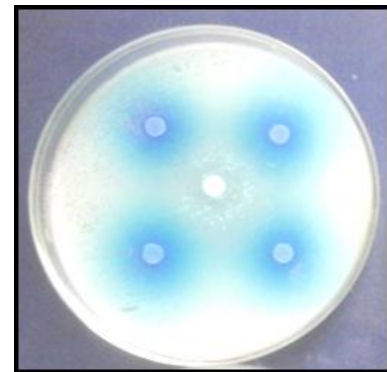


Photo 7: Antibacterial effect of Pen ink: LCB against *Bacillus subtilis*

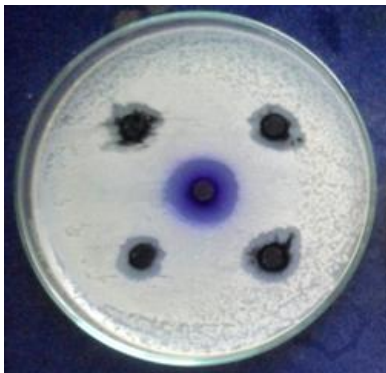


Photo 8: Antibacterial effect of Printer ink: Gentian violet against *S. aureus*

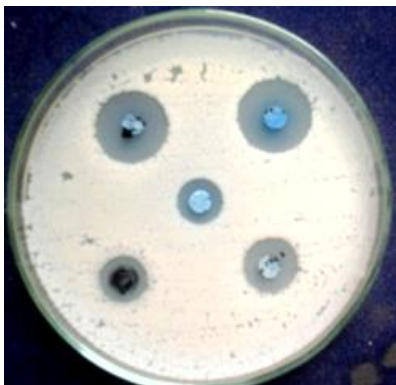


Photo 9: Antifungal effect of Printer ink: LCB against *Fusarium oxysporum*

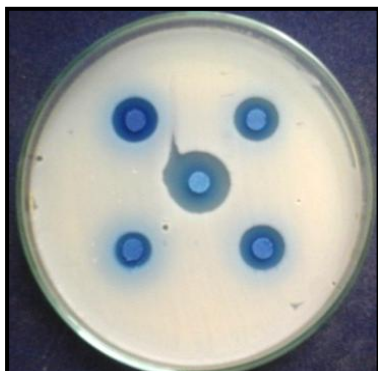


Photo 10: Antifungal effect of Pen ink: LCB against *Aspergillus nidulans*

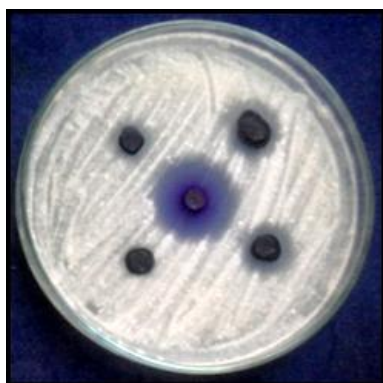


Photo 11: Antifungal effect of Printer ink: Gentian violet against *Trichoderma harzianum*

In this survey, samples of the air from library area different surfaces of books and also surfaces of shelves in libraries were examined for the presence of fungi and bacteria. In this study, the open plate method was used for assessment of fungal spores and bacteria in the air of libraries; the plates containing Potato Dextrose Agar and Nutrient Agar were put in altitude of 1.5 meter from the floor in the middle point of library exposing to the air for 15 minutes. Also sampling from surfaces of shelves and books were performed using moist sterile swabs after which the swabs were cultured on PDA medium at sterile conditions. Then, the plates were closed, labeled, and transferred to laboratory. All plates were put in an incubator at 25-30 °C and examined for fungal growth for 20 days. Mould fungi grew on culture media were identified by macroscopic and microscopic standard procedures.

A total of 11 bacteria and 8 fungi were isolate from library places and decaying books and papers. The isolates were confirmed on the basis of conventional, biochemical characteristics. The most frequently encountered bacteria were *Bacillus subtilis* and *Staphylococcus aureus* (Table No. 1 and 2) The fungi were identified on the basis of growth on media and on the basis of microscopic examination and the most frequently encountered fungi were *Aspergillus*, *Fusarium etc* (Table No.3). Reports from Shansian *et al.*, (2006) ^[19] and Guiamet *et al.*, (2011) ^[24] showed the fungal contamination to library material and to historical manuscripts by most of the fungal genera. They showed that most fungal genera were *Aspergillus*, *Penicillium*, and *Mucor* sp. could damage the paper.

Generally, the paper ink is a complex medium, composed of solvents, pigments, dyes, resins, lubricants, solubilizers, surfactants, particulate matter, fluoresces, and other materials. The pH of paper ink and printing ink was (7) that is mean it is act as favorable medium for microorganism contamination which cause of paper degradation by their extracellular cellulase enzyme. While the prepared mixture (ink with lacto phenol) in ratio 1:1, 1:2, 2:1, 2:2 and alone LCB were decreased the pH in to (5, 3.7, 4.2) respectively. That's mean producing of unfavorable pH for growth of isolated bacteria and fungi. While the prepared mixture (ink and lactophenol) treated the pH effect by inhibition of bacterial and fungal growth because it acts as antimicrobial.

According to Ponnambalam *et al.*, (2011) ^[18], cellulases enzymes produced mainly by microbial sources, starting from prokaryotic organisms like bacteria, and protozoan to eukaryotic organisms that catalyze the cellulolysis. Cellulases are inducible enzymes that are synthesized by microorganisms during their growth on cellulosic materials e.g. Paper, As a result of analysis of carboxyl cellulose compose of the paper, that it enhances enzyme activity of cellulase which produced by bacteria and fungi, finally it causes degradation of paper. In addition to phenol composition of lactophenol which acts as antimicrobial for bacterial and fungal growth.

Graph No. 1 shows the inhibitory effect of Printer ink and LCB on bacterial growth. The present study was carried out with filter paper disc soaked in different concentrations of printer ink: Lactophenol Cotton Blue in ratio (1:1), (1:2), (2:1), (2; 2) and alone LCB. These concentrations of mixed dyes use for testing their inhibitory effect and the result in the form of zone of inhibition was in between 10 mm – 27 mm for all 11 bacteria, are *Bacillus subtilis* and *Staphylococcus*

aureus, except isolate 5 i.e. *S. aureus* show negative result at concentration 1:2. Similar observations was made by Fouad H. Kamel *et al.*, (2014) ^[11], about the use of mixed dyes at different concentration which shows different inhibitory effect on bacterial growth. *B. subtilis* is very sensitive to Printer ink: Lactophenol Cotton blue at ratio 2: 2 while the *S. aureus* is resistance to 1: 2 ratio.

Graph No. 2 shows the inhibition of fungal growth in filter paper soaked in alone LCB and printer ink: LCB in ratio (1:1), (1:2), (2:1), and (2; 2). Zones was obtained between 9.0 mm – 32 mm showing positive result for 7 fungi are *Aspergillus niger*, *A. nidulans*, *Trichoderma harzianum*, *Penicillium nidulans*, *Fusarium oxysporum*, *A. fumigatus* and *Stachybotrys chlorohalonata* while the isolate no 1 i.e. *Forsecaea pedrosi* show negative result at all concentrations and also at alone LCB. *Penicillium nidulans* is very sensitive to Printer ink: Lactophenol Cotton Blue at ratio 1: 1 whereas, the *Forsecaea perbroisi* is resistance to all ratios.

Graph No. 3 shows the inhibitory effect of bacterial growth, the filter paper disc soaked in pen ink: LCB in ratio (1:1), (1:2), (2:1), (2:2) and alone LCB. The filter paper used in the present study soaked in the different concentrations of mixed dyes for testing their inhibitory effect and positive result was obtained for all 11 bacterial isolates i.e. *B. subtilis* and *S. aureus*, zone obtained between 8.0 mm – 18 mm, while the isolate no.3, 4 and 5 i.e. *B. subtilis*, *B. subtilis* and *S. aureus* show the negative result at alone LCB and isolate no. 8 i.e. *S.aureus* show no zone at ratio 1:2 and alone LCB.

Graph No. 4 shows the antifungal activity testing with pen ink: LCB. Concentrations of mixed dyes for testing their inhibitory effect the filter paper disc soaked in pen ink: LCB in ratio (1:1), (1:2), (2:1), (2:2) and alone LCB. The result was positive for all fungi and zone obtained is in between 6.0 mm – 24 mm but the isolate no.2 i.e. *A. niger* show the negative result at ratio 2:1, 2:2 and alone LCB. According to Fouad H. Kamel *et al.*, (2014) ^[11] different combination of dyes and inks in different concentrations is responsible for producing unfavourable pH for growth of bacteria and fungi and acts as antimicrobial, similarly phenol composition of Lactophenol acts as antimicrobial for bacterial and fungal growth.

Gentian violet also inhibited the remaining fungi and bacteria. The gentian violet mixed with pen ink and printer ink and alone Gentian violet shows positive result.

As the Graph No. 5 shows the Antibacterial inhibitory effect of bacteria with printer ink: Gentian violet. For testing the inhibitory effect the filter paper disc soaked in mixed dyes of printer ink: Gentian violet in ratio (1:1), (1:2), (2:1), (2:2) and alone Gentian violet, the positive result obtained and zone obtained between 10 mm – 28 mm, while the isolate no. 2, 3 and 4 i.e. *B. subtilis* show negative at ratio 2:1. Isolate no. 6 i.e. *B. subtilis* show negative at ratio 1:2, 2:1 and 2:2 whereas isolate no.7 i.e. *S.aureus* show the negative result at ratio 2:1 and 2:2 and isolate no. 10 and 11 i.e. *S. aureus* and *B. subtilis* show negative at ratio 2:1.

Graph No. 6 show the antifungal activity with pen ink and Gentian violet. The activity of pen ink with gentian violet for inhibition of fungal isolate not obtained satisfactory result. Half and more negative result were obtained at different concentrations whereas half less result were obtained positive at different concentrations.

Isolate no. 1 *Forsecaea pedrosi* show the zone 16 mm at 1:1 ratio 10 mm at 2:2 ratio and 23 mm at alone gentian violet whereas negative at conc. 1:2 and 2:2. Isolate no.2 *A. niger* show the negative at all concentrations and also for alone gentian violet. Isolate no. 3 *A. nidulans* show the positive at all concentrations and zone between 13 mm – 21 mm. Isolate no. 4 *Trichoderma harzianum* shows the positive result at all conc. And zone obtained between 11 mm – 24 mm except at conc. 2:1 show negative result.

Isolate no. 5 *Penicillium nidulans* show zone 15 mm at ratio 1:1, 12 mm at ratio 1:2 and 27 mm at alone gentian violet whereas negative result obtained at ratio 2:1 and 2:2. Isolate no. 6 *Fusarium oxysporum* show negative at all conc. and alone gentian violet. Isolate no. 7 *A. fumigatus* show negative at all concentrations, show positive at alone gentian violet. Isolate no. 8 *Stachybotrys chlorohalonata* show the zone 10 mm at 1:1, 12 mm at 1:2, 11 mm at 2:2 and 25 mm at alone gentian violet, whereas show negative at ratio 2:1. The result regarding the use of different dyes was also studied by Bragulat *et al.*, (1991) ^[10], during their course of study, 13 different dyes such as auramine, gentian violet, phenol red, methylene blue and many more were tested to study the inhibitory effect of these dyes on growth of different fungi.

Printer dye and pen ink allowed adequate colony development of the bacteria and fungi. That means the dyes used in printing and writing on paper have no any inhibitory effect, strains tested while controlling rapidly. While in the case of plates contains filter paper soaked in the dyes: lactophenol cotton blue alone, printer dye mixed with lactophenol cotton blue and ink dye mixed with lactophenol cotton blue and there was no any fungal and bacterial growth that mean these dyes have been reported as antibacterial effect and mold-spreading inhibitors at different situation. Printer dye mixed with Gentian violet there was no fungal and bacterial growth that mean these dye also reported as antifungal and antibacterial effect.

In present study, fungal and bacterial isolates were tested for their cellulose activity (Graph No. 7 and 8). Out of 11 bacterial isolates the 7 bacteria were *Bacillus subtilis* and 4 were *Staphylococcus aureus*. The all 4 *S.aureus* and 6 *B. subtilis* demonstrated positive results and observed zones between 12.5mm – 58 mm after 48 hours of incubation at 37 °C on Nutrient Agar containing 5% cellulose powder, whereas isolate no. 4 i.e. *Bacillus subtilis* show the negative result. Out of 8 fungi 7 fungi i.e. *Forsecaea pedrosi*, *Aspergillus niger*, *A. nidulans*, *Trichoderma harzianum*, *Penicillium nidulans*, *Fusarium oxysporum* and *Stachybotrys chlorohalonata* demonstrated positive results and observed zones between 12.2 mm - 35.5 mm, while isolate no 7 i.e. *A. fumigatus* show negative result after 48 hours of incubation at 28 °C on Nutrient Agar containing 5% cellulose powder, as that indicated by the change and disappearance or zone of inhibition on Petri plates which indicates the production of extracellular enzymes by the applied bacteria and fungi. Our result are in accordance with the Bennett and Faison (1997) ^[8]. According to them fungi can hydrolyse a wide variety of polymers including cellulose, as a result of their efficient degradative enzyme. Similar results were obtained from Adamo *et al.*, (2003) ^[2] about the cellulytic activity.

Results obtained in this study could make the development of new antifungal stain possibly happen. Lactophenol can be

mixed with paper printing and writing ink for preventing paper and book deterioration in the libraries and archive.

4. Conclusion

Biodeterioration of library material is a worldwide problem and it cause great damage especially to unique manuscripts and books stored in libraries.

Following conclusions are drawn from the complete study.

- The sources of deterioration and degradation of library resources were the bacteria and fungi.
- The possible combinations of paper and printer ink with different dyes are the best method to prevent fungal and bacterial growth on library material in order to prevent library resources.

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