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Indoor Fungal Populations Inhabiting Cement Structures - Remedial Measures

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Abstract: *Fungal populations are ubiquitous in nature and vary in composition depending on the substrata. Impact of fungal populations on human health conditions depends on type of species-invasive conditions with respect to tissue and association leading to detrimental conditions. A variety of indoor fungi can be noticed in air, on cement walls in case of seepage, submergence during flooding or water logging. Fungal species viz. Penicillium, Mucor, Aspergillus ,Cladosporium spps may inhabit such wet and effected surfaces, which can affect the cosmetic appearance and also result in allergic reactions. The current study of indoor walls of the residential/ commercial constructions exposed to seepage indicated the presence of Aspergillus niger, Ascotricha charatarum, Fusarium solani, Aspergillus spp 2, Penicillium spp., Cladosporium spp., Phoma spp., Stachybotrys spp. Ascospora spp., Curvularia spp, Alternaria spp. Some of the fungal inhabitants proved to possess allergen capabilities. Combinations of commonly available house-hold materials were used to control fungal growth on cement walls. The investigations revealed that remedial measure 1 found to be effective in controlling growth in the effected regions, spreading further and also from re-infestation for longer period than the other.*

Key words**:** *cement walls, control, fungi, indoor, remedy*.

I. Introduction

Mold growth in homes is the hot topic in the house construction industry today. Mold is a member of the fungi family with thousands of varieties and exists in every indoor and outdoor environment. Molds have mycelia and reproduce by releasing tiny spores into the air. Fungi are common, widely distributed and grow vigorously on paper, wood, plaster, paint, leather and fabric that contain 12% to 15% water, adjust to temperature changes more readily than higher life forms. Drought-resistant fungi (*Aspergillus* and *Penicillium*) grow best at 21.1 to 32.2°C, slow at 10°C, stop growing at 1 to 4.4°C, easily survive freezing/zero, but do not die. A wide array of biologically active substances such as mycotoxins is produced by the fungi. These substances are anti-predatory and pro-territorial-protective and ensure perpetual existence of fungi in quite hostile circumstances. Mycotoxins are anti-viral, anti-bacterial, anti-protozoan, anti-insect, anti-animal & anti-human. Fungi and their biological metabolites (mycotoxins) are silent and relentless attackers of human health. The health related problems are due to the spores and mycelia of fungi and molds which can cause allergy, Immediate Type -1 hypersensitivity, Extrinsic allergic alveolitis, Allergic broncho-pulmonary aspergillosis, Allergic fungal sinusitis, immune suppression diseases, asthma, skin allergies etc. Several strains which appear as black, white or multi-colored stains or fuzz can lead to health problems, if allowed to flourish. Children, senior citizens and people with weakened immune systems may be more vulnerable to mold exposure.

Mumbai with opportunities at galore and hectic building activity of sky scrapers is endowed with rich yearly monsoons. Monsoons also cover a spectacular view with a nice cover of mold growth on the floor and walls of residential apartments of buildings particularly above 15-20yrs of age during the season. Moisture caused by plumbing leaks and seepage is the primary cause of mold growth in buildings; followed by water from floods and rains. The cement walls of the constructions with internal seepage either due to rains or leakages or air-conditioners also support the indoor fungal growth. The increasing growth of indoor fungi and molds is a matter of concern for health hazards it may pose to the occupants of the buildings for eg."Sick Building Syndrome" caused by moisture and mold growth.

Human beings have 3 requirements in common with molds as favorable conditions to grow and survive such as (1)Temperature- Between 40 and 100° F; (2). Food Source : Organic material (wood, dust, paper, etc.) and, (3). Moisture : Water, or water vapor (high humidity, plumbing or roof leaks etc.) and are typically available in any household.

Indoor mould composition and constitution has been well studied as airborne spores [1,2]. The normal household carpets, air conditioner filters, wet tiles of bathroom, wooden surfaces or leather surfaces are ideal substrates for the growth [2]**.** Newly constructed energy efficient buildings and residences are susceptible to mould infestation due to poor construction practices, substandard material used, and construction techniques which can allow the entrapment of moisture in the exterior of walls, creating an environment conducive to mould growth. Wood and drywall products are sources of mould growth while, clay brick, concrete and

concrete block products do not support fungal mold growth [3]. A similar infested conditions were reported in US , Northern Europe, Sweden where flood submerged conditions occurred and in UK where poor construction and condensation were the reasons [4].

Building related illness are well defined diseases associated with fungal contamination of buildings. The standard for the air microflora has been mentioned as 50 CFU/m^3 (single sps) or 150 CFU/m^3 (mixture of sps) to consider the indoor conditions as normal. However the values may vary with seasons. A study conducted on indoor fungal and bacterial contamination on house hold environment in Riyadh, Saudi Arabia considered the walls, carpets and Air conditioners and indicated the presence of a variety of bacteria and fungi [2]. *Alternaria* spp, *Aspergillus niger, A.flavus*, *Rhizopus* spp. *Penicillium* spp on the bed room walls covering 7% of the total population, and around 6% were bacteria of the total populations were observed during the study [2]. The other studies include mould growth inside the building and Indoor aerobiology and related health aspects [5,6]. Reviews and reports by Environmental health Directorate, Canada [7] and EPA reports about the indoor fungi and limits also constitute important references to study fungal species on hard substrata.

Research on fungal spores were of considerable importance while studying isolation and identification of the fungi of indoor environments [8,9], but the aspects of control or remedial measures were given less importance. However, different antifungal compounds such as IPBC, OIT, DCOIT , Zinc pyrithone, Carbendazium were studied with respect to plants and the fungal diseases[10]. Gaseous fungicides such as ClO_2 is a strong oxidant, capable of circumvent the problem of overlooking of growth sites ,penetrate into crevices, wall cavities and other hard to access areas and treats the inaccessible mold colonies. It is also capable of sterilizing the contents inside a contaminated structure [11]. The fungicides have negative impact on ecosystem and food chain, hence ecofriendly measures are of importance

II. Materials and Methods

2.1**. Sample collection**: Samples were collected from infested cement walls of normal households, offices/educational institutions of old buildings with seepage or internal leakages where the walls and wooden cupboards were affected. Sterile bottles were used to collect the samples. Surface sampling is used to confirm the nature of the suspected microbial growth on environmental surfaces to measure the relative degree of contamination and identify the types of present fungi [12]. In this approach, samples are collected by adhesive tape onto a surface and scrapings.

a. Adhesive tape sampling: transparent cellophane tape was struck on the walls with intact fungal blisters. The cellophane tape was collected in sterile bottles once the blisters break [13,14]

b. Scrapings of contaminated materials (Environmental Health Directorate, Canada, 1995). Contaminated structures were scraped with sterilized scalpel and collected into sterile plastic bottles[7].

2.2. **Isolation and Identification:** Samples were dissolved in sterile saline and serial dilutions up to 10^{-4} were prepared. 0.1 ml sample was used for spread plate technique and incubated at room temperature. Sabouraud , Cellulose, Carrot Potato agar and Potato dextrose agar media were prepared and used for culturing purposes[15]. Different fungal/mold species were isolated on sabouraud agar and sent for conformation and identification to ARI, Pune. The samples of the cultures were archieved at ARI, Pune.

2.3**. Extraction of allergens**: The fungal extractions were prepared from *Aspergillus niger*, *Penicillium*spp., *Ascotricha charatarum, Fusarium solani, Cladosporium spp. Stachobotrys spp. Alternaria spp* using coca's buffer from spores and phosphate buffer from mycelium . Allergens were extracted from fungal samples of mycelium (1/10 w/v). and spores with CoCa's solution and phosphate buffer (1/4 w/v), homogenized and supernatant was stored at lyophilized conditions [16]. The allergen capabilities of isolated fungal species were assessed within 24 hours using different techniques viz., Counter immunoelectrophoresis [17], Crossed Immune Electrophoresis and Double diffusion [18]. Standardized IgE antiserum sample (359KU/L) were used to check the antigenic capacity of the fungi. The protein (antigens) extracted from the spores and buffer extractions using molecular biology techniques such as PAGE and the molecular weight of the separated proteins was assessed based on standard Protein molecular markers from Merck , Bangalore.

2.4.**Anti-fungal Control / remedial compositions** : MIC was checked with two combinations of antifungal chemical combinations

2.4.1. Anti-fungal spray 1 (BHVW) A mixture of household materials like boric acid, hydrogen peroxide, Venigar, and water was considered to check the fungal control (BHVW).10ml of vinegar,10ml of hydrogen peroxide,100gm of Boric acid dissolved in 1ltr of water was prepared.

2.4.2. Anti-fungal Spray 2 (CCCW): Mixture of Cupric sulphate and Calcium carbonate mixture (carborundum) was also considered for the control (CCCW). 1gm of Calcium Carbonate and 1 gm of Cupric sulphate were dissolved in 200ml of distilled water.
Antifungal Mixtures were sprayed (one time) from a distance of 10 cm equaled to quantity of 0.32g ± 0.05 (empty plate). 1.25g ± 0.05 (approx 2.5 ml) spray was required for agar plates.
Both the mixtures were sprayed on petriplates containing the fully grown fungal cultures and the results were observed at periodic intervals.

2.4.3 **Fungal growth control checks**: fungal growth after treatment were checked by two methods by reinnoculation.
a. Reinnoculation of treated fungal samples on agar plates: The samples of the treated fungi loopfuls were re-innolculated on Sabouraud agar plates after specific time gaps (1wk,2wks.3wks 4wks) , incubated at room temperature and results observed.
b. Reinnoculation of treated fungal samples in agar broths: A loopful fungal culture from the treated plates were inoculated in the Sabouraud's broth and the growth was checked periodically for 3 months.

2.**5 Fungal Inhibition concentration tests** : MIC tests were performed by adding 4ml, 2ml and 1ml of Antifungal mixtures of BHVW and CCCW were added to 16ml, 18ml and 19ml of Sabouraud agar and poured into petri plates [19]. MIC tests were performed by inoculating the plates with different isolates of fungal cultures and incubated at RT for 48 hrs to 72 hrs.

The house hold fungal control mixture was checked in vitro and in vivo conditions. 10mg/ml, 5mg/ml and 2.5mg/ml of boric acid mixture was used for assessing the susceptibility of the fungal species during the study and the efficacy was checked. Triplets were made for accuracy at every level. The chemicals used for the preparation of media, for culture purposes, Electrophoretic techniques such as PAGE, Immuno electrophoresis, immunodiffusion, extraction of antigens were of analytical grade.

III. Results

Altogether 15 different types of fungi and bacteria were encountered from the samples of cement walls which were identified and confirmed by ARI, Pune. The fungal species belonged to genera – *Aspergillus niger, Ascotricha charatarum*, *Fusarium solani*, *Aspergillus* spp 2, *Penicillium* spp., *Cladosporium* sp., *Phoma* spp., *Stachybotrys* spp., *Ascospora* spp., *Curvularia* spp, *Alternaria* spp, *Rhodotorula* spp., and actinomycete species such as *Nocardia* spp., *Pseudonocardia* spp.

The fungal extractions were prepared from *Aspergillus niger*, *Penicillium* spp., *Ascotricha charatarum, Fusarium solani, Cladosporium sp. Stachobotrys sp. Alternaria sp* using coca's buffer from spores and mycelium. The protein (antigens) extracted from the spores and buffer extractions were isolated using SDS-PAGE. The banding patterns were seemed to be similar indicating similar molecular weight of the antigen proteins in both the spore and mycelial extractions.

The counter immunoelectrophoresis, crossed immunoelectrophoresis and immunodiffusion techniques were used to assess the antigen capabilities of fungal mycelia and spore extractions against the IgE antiserum. The identified species of fungi showed precipitin band with the serum sample indicating their capabilities as antigens. (**Fig**1.)

Antifungal mixtures were sprayed on Sabouraud , Potato Carrot Agar, Cellulose and Potato Dextrose agar plates containing fungal populations on agars indicated the death of fungal populations. No regrowth pattern was observed even after 1 month for the BHVW mixture in contrast to the CCCW mixture. The fungal growth was not observed in any type of the media irrespective of the time with respect to the BHVW mixture. Neither Sabouraud Agar plates nor broth showed fungal growth at any interval for the BHVW mixture. In contrast, the second mixture (CCCW) could not control the growth (**TABLE-1)**

A comparison of the fresh mixture and 6months old preparation of BHVW showed the similar level of efficacy irrespective of their preparation, as long as they are kept in dark, closed conditions.
Fungal Inhibition concentration tests revealed that the minimum concentration of inhibition was 1ml of BHVW mix with 19 ml of Sabouraud agar where no fungal growth was observed at RT for 1week. In the case of BHVW,.fungal growth was not observed even after 3months, indicating the strong antifungal contro.l The CCCW mixture found to be ineffective as the regrowth of fungus was noticed.

The BHVW mixture was also sprayed on infested wall from 10 cm distance in vivo conditions. The repeated infestation by fungal populations was not observed even after 2yrs. The conditions of before and after the spray on the infested walls was very evident (Fig.2) and after painting also there was no fungal growth

The BHVW spray is a mixture of vinegar (diluted acetic acid), hydrogen peroxide (strong antioxidant) and Boric acid with acidic characteristics. It should be kept away from children as it may effect skin

temporarily. It is safe, smells for a period of 5 min. and ecofriendly, has impact on the fungal community for a period (longevity) of aprox 3-4 years.

The mixture was proven to be equally effective on the wooden surfaces containing fungal infestations without any changes on the structure or change on the painting pattern. The spray does not show any marking on the unaffected walls or discoloration even after a prolonged period.

IV. Conclusion

Previous Studies on fungal species and water damaged building materials [20,21] revealed a similarity with some of the species observed during the current study. *Penicillium chrysogenum* and *Aspergillus versicolor* are the most common fungal species in water-damaged buildings Specific associations were noticed between mycobiotas and the different building materials, viz., (i) *Acremonium* spp., *Penicillium chrysogenum*, *Stachybotrys* spp., *Ulocladium* spp., association with gypsum and wallpaper, (ii) *Arthrinium phaeospermum*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Trichoderma* spp., yeasts, with different types of wood and plywood, and (iii) *Aspergillus fumigatus*, *Aspergillus melleus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Chaetomium* spp., *Mucor racemosus*, *Mucor spinosus*, with concrete and other floor-related materials [20]. Inner wall materials used in buildings, such as prefabricated gypsum favors the growth of *Stachybotrys chartarum; Aspergillus* and *Penicillium* grow superficially on painted surfaces; *Aureobasidium pullulans* can deteriorate the paints and, Acrylic painted surfaces are attacked by *Alternaria*, *Cladosporium*, and *Aspergillus* [21]

In normal house hold conditions, the normal remedial measures such as removal of the reservoirs of fungal growth, prevention of saturating the air and building materials with moisture, checking the musty odors (a sign of mold growth in a building) and visible signs of mold or moisture and elimination; maintaining the relative humidity levels (< 40% - heating season, 60% - cooling season) and proper ventilation; sealing of the outlets and sill plates, insulation of pipes and ductwork in humid spaces can minimize condensation in these areas and regular inspection and cleaning of AC drains will help to maintain healthy conditions indoors. The other treatments which include gas treatment with ClO_2, antifungal agents including uv radiation are successful in inactivating mold and bacterial colonies. But, the other side effects such as corrosive properties (chlorine dioxide gas) require care to avoid degradation of the contents as well as the components of the structure [11] and respiratory impacts

Aged buildings during rainy season, buildings or houses affected with seepage or water leakages often create favorable conditions for the fungal infestations on the wooden cupboards, furniture and cement walls. Indoor fungal infestations can be controlled by spraying cheap and household materials available. Vinegar (Used in the kitchen for preservation and anti-dandruff treatment), Hydrogen peroxide (antioxidant, used to clean wounds), Boric Powder (used for carom board and insect repellant) could be used for controlling indoor fungal populations. The current study revealed that when sprayed with said composition the treated surface has long lasting resistance to fungal infestation by way of controlling the fungal mycelia growth and spores. It causes no harm on the painting/ color of the wall and can be repainted easily. The spray has no side effects with respect to respiratory problems as it the strong smell is for a short duration of 15 min. It should be kept away from the children as it has pH value in region of 4.5 and, away from light conditions.

Architectural design has the potential to influence the microbiology of the built environment, and can cause implications for human health and well-being [22]. The knowledge of indoor micobiomes' ecological preferences and negative impacts will help us to design buildings with better conditions in future which can provide well being for human populations.

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Table-1 : Efficacy of treatment methods used on fungal growth on seepage affected walls

	Treatment and Condition		MIC (1ml of medium in 20ml of medium)		Re innoculation on normal sabouraud's Agar plates					Spray On infected walls		
					1wk	2wk	3wk	4wk	12 wks	In vivo		Overall efficacy
TYPE OF SPRAY	Before	After	Sabouraud Agar plates	Sabouraud broth						before	After 12 wks	
BHVW	+	-	-	-	-	-	--	-	-	+	-	good
CCCW	+	-	-	+	-	-	+	+	+	+	+	-

Note : + growth ; - no growth

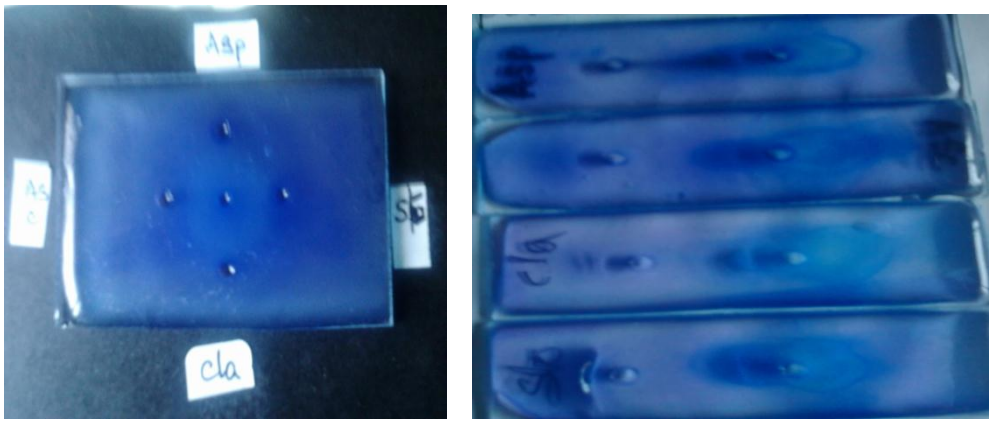

Fig.1 Radio immunodiffusion and crossed immuno electrophoresis results of the fugal extracts (Note: cla-*Cladosporium sps.,Asp-Aspergillus sps, Sto-Stochobotrys sps., Asc-Ascotrichium spps.)*

INFESTED WALLS CEMENT WALL BEFORE TREATMENT

INFESTED WALLS CEMENT WALL AFTER TREATMENT

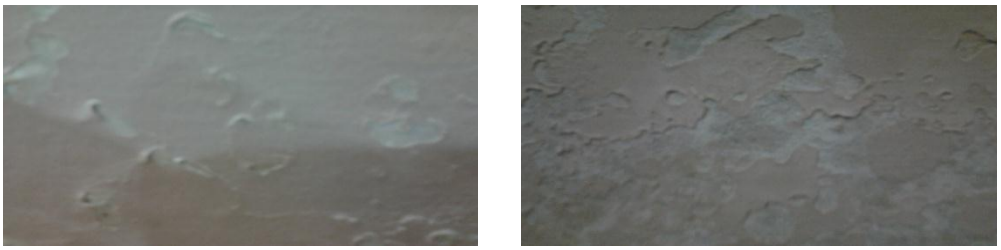

Fig.2 : In vivo study to assess the effectiveness of the treatment.