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OLIGODYNAMIC ACTION OF METALS ON FUNGAL DETERIOGENS OF HISTORICAL PALACE

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ABSTRACT

Metals have always been a part of our day to day life. Infrastructures of most of the monuments are basically made with metals and metal ions are also present in soil which significantly affects the metabolism of microbes. Present study was designed to assess the effect of metal ions on deteriorating fungal speciesof Kaliadah Palace, Ujjain (M.P.) India namely *Talaromycespurpurogenus, Curvulariasp, Aspergillusniger, Fusariumsp, Aspergillusflavus, Alternariasp* & *Eurotiumamstelodami*. Fungi showing resistance to metal ions might be helpful in the mycoremediation strategies by renovating the monuments of historical importance.

Key words: *Oligodynamic, deteriogens, monuments, fungi, mycoremediation.*

I. INTRODUCTION

The word Oligodynamic has been taken from Greek word Oligo means ‘few’ and Dynamic means ‘force’. It has been discovered by Nageli, (1893) as toxic effect of metal ions on living organisms like bacteria, fungi, algae, spores etc. even in relatively low concentration. The oligodynamic action of metals on bacteria serves as a basis of many patented processes for the sterilization of milk, water, etc., but the application of heat is usually cheaper and more efficacious. A strange effect that is sometimes observed is that an antiseptic in low concentration may not only fail to check growth, but may even exert a marked stimulating action on growth of micro-organisms. This effect would appear to be more marked with mould fungi than with bacteria (Galloway and Burgess, 1946).

Metals have always been a part of our day to day life. Brass door knobs have been used from a long time in homes and even in monuments as it is apparent that it makes itself free from any germs within certain period of time. Keeping drinking water overnight in brass vessel is thought to be good for health purposes. It is also suggested to cook green vegetables in iron pan. There has been a tradition of using silver utensils at homes of rich people and palaces of Kings. Most of the antiseptics having mercuric chloride and formalin inhibit enzyme action as well as the growth of micro-organisms. Copper, in traces of 0-5 part per million or less, suppresses algae, protozoa, and many bacteria. Zinc salts are cheap and fairly efficient as antiseptics, and nickel salts also exert an antiseptic action. Silver and gold in the form of colloidal solutions are effective bactericides for specific cases.

Infrastructures of most of the monuments are basically made with metals. Metals are also present in soil. Metals ions significantly affect the metabolism of microbes. Though stone deteriorating fungi have direct contact with soil, they select it as the main source of metals for saprophytic species, they need to balance the metal input from the atmosphere. The effects of certain metal (e.g. copper, brass) are inhibitory at moderate concentrations.Soil microbial biomass is mainly composed of fungi and bacteria. Phospholipid fatty acid analysis has also been used to differentiate between the fungal and bacterial components of the soil microbial biomass (Rajapaksha*et al.*, 2004 ; Kelly *et al.,* 1999).The view that fungi are found more tolerant of heavy metals as a group than bacteria is often stated by scientists (Hiroki, 1992). In addition, different biomass determinations or plate counting techniques have also indicated that heavy metals affect bacteria and fungi in soil differently. This was initially inferred by comparison of metal tolerance of pure culture isolated of soil microorganisms (Rajapaksha*et al.,* 2004; Babich and Stotzky, 1978).Iron is essential in trace amount for growth of some microorganisms while some metals like mercury and silver have been proved to be toxic even in small concentration (Bhatnagar, 2008). Cuero *et al.,* (2003) supported the existence of mixed state of metal ions in nature and its effect on cellular and molecular levels in fungi.

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Metal ions may also alter the genetics of the fungi and these gene products are supposed to involve in mechanism of accumulation and/or resistance to heavy metals in them (Cuero *et al.*, 2001, 2000, 1998; Gadd and White, 1989; Failla, 1977).The exact mode of action of these metal ions is still not clear but they are thought to denature protein of the target cells by binding to reactive groups resulting in their precipitation and inactivation (http://www.tested.com/science/life/453961-oligodynamic-effect-how-some-metals-kill-bacteria.html). Hambidge, 2001 also suggest that positively charged copper ion distorts the cell wall by bonding to negatively charged groups and allowing the silver ion into the cell. In this context, present study is designed to determine the oligodynamic action of metal ions in order to know about effect of metal on growth of palace fungi.

II. MATERIALS AND METHODS

In order to investigate the effect of metals on seven test fungi namely i.e. *Talaromycespurpurogenus, Curvulariasp, Aspergillusniger, Fusariumsp, Aspergillusflavus, Alternariasp*and *Eurotiumamstelodami*, the inoculum preparation of conidial or sporangiospores suspensions was adjusted using a spectrophotometer with a test inoculum in the range 0.4 × 104 to 5 × 104 CFU/ml. The optical density (OD) at 530nm required is i.e. for *Aspergillus*the OD=0.09-0.11; for *Fusariumsp* OD=0.15 - 0.17; for *Alternaria*the OD=0.2-0.05% (Pandey*et al.*, 2011; Bhatnagar, 2008). Tween 80 was added as wetting agent to facilitate the preparation of innoculums (*http://www.mycology.adelaide.edu.au/Laboratory_Methods/Antifungal_Susceptibility_Testing/overview.html).*

Prepared suspensions of test fungi were gently spread over freshly prepared plates of Potato Dextrose Agar using sterile cotton swabs and metal coin was placed in the centre of plate and plates were then incubated at 28±2°C for 48-72 hours (Benson, 2010). At the end of incubation period, the plate was observed for zone of inhibition, which was measured with ruler and expressed as average of duplicates and standard error (Table 1.2). Details of the coins used in the study are shown in Table 1.1.

Table 1.1: Details of coins used in the study

S. No.	**Coin**	**Year of Issue**	**Shape**	**Composition**	**Weight**	**Diameter**
1		1997	Circular	Cupro-nickel	9.00 gms	23mm
2		1862	Circular	Silver	11.66 gms	30.3-30.55mm
3		1957 – 1961	Circular	Bronze (97% Copper + 2.5% Zinc + 0.5% Tin)	1.5gms	16 mm
4		1962 – 1963	Circular	Nickel-Brass Alloy (79% Copper + 20% Zinc + 1% Nickel)	Same as above	Same as above
5		1964	Circular	Bronze	1.85 gms.	16mm

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6		1988-98	Circular	Ferritic Stainless Steel (82% Iron, 18 % Chromium)	1.85 gms	12mm
7		1972 – 1990	Circular	Copper–Nickel alloy (75% Copper + 25% Nickel)	2.8gms	12mm
8		1990	Circular	Stainless steel	2.00gms	16mm
9		1920	Circular	Brass	2.00 gms	10mm
10		1938	Circular	Brass	2.00 gms	10mm
11		1959	Circular	Brass	2.00 gms	10mm

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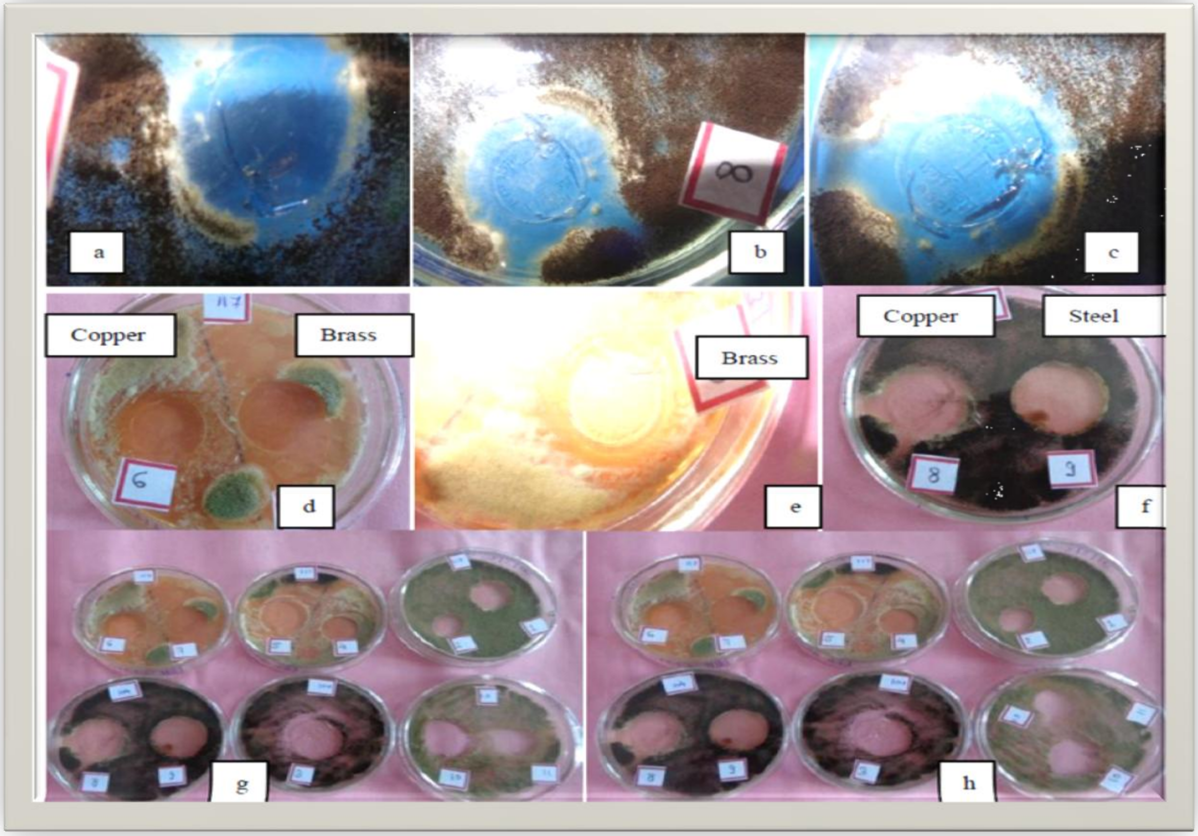

Plate 1: Oligodynamic action of metals on growth of test fungi (a-c A. niger, copper coin; d- A. flavus, copper and brass coin; e- A. flavus, brass coin; f-A. niger, copper, steel coin;d-h Effect of different metal coins over test fungi)

Table 1.2: Oligodynamic action of metals on test fungi

Metals used	Zone of Inhibition (in mm*)						
	Talaro-mycespurpurogenus	*Curvulariasp*	*Aspergillusniger*	*Fusarium sp*	*Alternaria Sp*	*Eurotiu-mamstelodami*	*Aspergillusflavus*
Silver	-	-	-	-	-	-	-
Gold	-	-	-	-	-	-	-
Aluminium	-	-	-	-	-	-	-
Copper	-	-	12.8±0.1	-	-	-	10.4±0.2
Brass	-	-	11.2±0.3	-	-	-	7.0±0.4
Steel	-	-	-	-	-	-	-

III. RESULT AND DISCUSSION

Effect of metals coins of silver, gold, aluminium, copper, brass and steel were assayed against *Talaromycespurpurogenus, Curvulariasp, Aspergillusniger, Fusariumsp, Aspergillusflavus, Alternariasp*and *Eurotiumamstelodami*. Out of which, *Talaromycespurpurogenus, Curvulariasp, Fusariumsp, Alternariasp*and *Eurotiumamstelodami*were found resistant to every metal whereas, copper and brass exhibited inhibitory activity against *Aspergillusniger*and *Aspergillusflavus*. Oligodynamic action of copper and brass were found to be significant against monument fungi.

Some fungi are capable to tolerate the deleterious effect of metal ions and skillfully manage to grow and survive whereas some differ in their physiological response to such condition and hence get inhibited. Present study shows that the copper and brass were the only two metals able to inhibit the growth of very common airborne fungi of *Aspergillus*species i.e. *A. niger*and *A. flavus*.

Our results are in corroboration with previous findings. Antimicrobial effects of different metals have been dealt with precision from a long time. According to Galloway and Burgess, 1946; Copper, in traces of 0-5 part per million or less, suppresses algae, protozoa, and many bacteria. Konieczny and Rdzawski, (2012) also explained the antibacterial potential of copper and copper compounds. Copper sulfate, which is frequently used as a biocide, is much more effective for algae and fungi than bacteria (Cepero*et al.*, 1992; Richardson, 1988; Dawson, 1982; Ware, 1978; Salle, 1943). Copper was reported to inhibit *Actinomucorelegans, Aspergillusniger, Bacterium linens, Bacillus megaterium, Bacillus subtilis, Brevibacteriumerythrogenes, Candida utilis, Penicilliumchrysogenum, Rhizopusniveus, Saccharomyces mandshuricus, and Saccharomyces cerevisiae*in concentrations above 10 g/l (Chang and Tien, 1969). Aakyan and Rabotnova (1966) reported complete inhibition of *Candida utilis*(formerly, *Torulopsisutilis*) at 0.04 g/l copper concentrations. Recent study by Rasool and Iram, (2014) reported that copper at high concentration is toxic to *A. terreus*and *A. alternata.*

There are also researches showing antimicrobial effects of other metals like silver, stainless steel etc. Hambidge, (2001) explained the mode of action of silver that positively charged copper ion distorts the cell wall by bonding to negatively charged groups and allowing the silver ion into the cell Azevedo and Cassio, (2010). Michels, (2006) reported that toxic *E. coli* O157:H7 remain viable for weeks on stainless steel S30400. Epi-fluorescence photographs have demonstrated that it is almost completely killed on copper alloy C10200 after just 90 minutes at 20 °C. In 2008, research specifically governed by "United States Environmental Protection Agency" was granted by EPA, stating copper alloys kill more than 99.9% of Methicillin-resistant *Staphylococcus aureus*(MRSA) within two hours. Michels*et al.*, (2009) reported similar results from research conducted at the University of Southampton (UK) to compare the antimicrobial abilities of copper and several non-copper proprietary coating products to kill MRSA. We got similar kind of results with copper but stainless steel was not able to inhibit any of the test fungi. Reason behind it may be the source from where the fungi were isolated and its physiology. Test conditions are also an important parameter for finding the exact oligodynamic action of metals towards fungi. Fungi have been widely used in bioremediation of industrially polluted soils and waters, specifically in the removal of hydrocarbons and heavy metals (Potin*et al.*, 2004; Khan, 2001; Akhtar and Mohan, 1995). Finding the exact action of fungi showing resistance to metal ions might be helpful in the mycoremediationstrategies.

IV. CONCLUSION

*Aspergillus*is the causative organism of Aspergillosis and due to its rich sporulation, they able to manifest easily and even cause respiratory problems to the visitors and animals, birds wondering at the palace. Use of copper and brass in renovating /restoring the monument can help in avoiding the threat of these fungi. Although, coins used in the study differed in diameter and weight, also containing small concentration of other metals also, so there is a need to find out exactly the mode of action of metal alone and its synergistic effect with exact concentration of metal causing toxicity or resistance to growth of a particular species.

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