Protection of decorative limestone against microbial and chemical causes of organic stain

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Abstract

Decorative limestone of Northern Galala is frequently used as cladding material for both interior and exterior purposes. This decorative limestone should be achieved a series of requirements supporting its usage for different construction applications. Some of these requirements are physico-mechanical properties while other called Aesthetic properties which deal with homogeneity in appearance and color of stone surface. The recent study concerned with microbial and chemical causes which have been changed the original color of Northern Galala decorative limestone by organic stain agents such as tea and coffee. On the other hand, it have been focused on the preliminarily treatment of such Aesthetic stone defects by using a chemical mixture as protective material that recommended to be added to stone surface before its usage particularly in interior purposes such as kitchen upon innovative, sustainability and green building basics. The achievement for the aim of the recent study has been required using microbiological (antibacterial and antifungal) and also mineralogical investigation tools such as stereomicroscope and XRD (X-Ray diffraction).

Introduction

Materials such as natural stone, wood and water considered as the oldest sustainable materials whereas it improves energy efficiency, reduce waste and pollution conserves natural resources and have long life-span. Generally, decorative natural stone and particularly, decorative limestone represented one of the main important and valuable subject for engineers and others such as architectures.

Decorative limestone as a type of decorative stone can be defined sedimentary originated ornamental stones usually called (compact limestone) (hardened lime) or

as stated in **[1]** called (recrystallized limestone) which means limestone subjected to advanced stages of digenesis or recrystallization without metamorphism. It becomes widely used allover Egypt as ornamental and decoration building stones for both exterior and interior purposes.

Two main factors have been controlled such decorative natural stones **[2]** involving:

1. Aesthetic (Visual) characteristic,
2. physico-mechanical requirements.

In the last decade, the term of durability or sustainability should be added as the third required marketing criteria that should be present in decorative natural stone as it should be used keeping its original characterization for long period of time.

Hence, the modern studies carried out on such natural decorative stone should be not stopped on the evaluation and characterization of decorative natural stone but it should be extent to involve natural stone defects, factors affecting on their original criteria and also their interaction with surrounding environmental parameters.

On the other hand, some researchers have been concerned with physical, chemical and biological factors affecting deterioration of marble and decorative limestone for instance**[3-5]** particularly on microbial action or bio deterioration have been appeared in such many actions as surface deposits, discoloration, pitting or accelerated weathering.

The recent study have been exhibited the effect of discoloration or stain as one of the defect have been faced decorative limestone. Firstly, the definition of stone stain should be mentioned as change or alteration and discoloration of the original color of stone by organic or /and inorganic agents. Organic stain agents involves: Foods, drinks, plants, tobacco, cosmetics, urine and dyes while, Inorganic includes: minerals oxidization, efflorescence, moisture, accumulation of hard water.

The hazardous of stone stain started simply with decreased the decorative stone color stability and its accumulation led to shortening its life cycle of used decorative stone then finally, may be reached to additive cost by necessary

changing the old damaged stained stone by new one. All of the previous negative effects of stain on natural decorative stone mean decrease in its durability and consequently, sustainability which conflict with green building principles. Moreover, the recent study has been selected tea and coffee as two dominant organic stain agents and followed their effects on decorative limestone. It should be noticed that study of the organic stain includes both of its chemical and microbial causes.

Last but not least the aim of the current research is preliminarily treatment or coating decorative limestone by chemical mixture before (pre stain stage) or after (post-stain stage) its usage in different construction purposes to overcome the hazardous of the stain generally and particularly organic stain and consequently, decrease cost of the building construction especially in building components made of natural stone.

Materials and Sampling

Generally, the recent research includes the following studied materials (decorative limestone, organic stain agents and treatment material). Each one of the previous used materials will be described briefly in the following:

Firstly, decorative limestone has been represented by Egyptian Northern Galala limestone .Geographically it have been extracted from Northern Galala quarries which located in Suez governate. Geologically, Northern Galala limestone belongs to Northern Galala plateau of Mokattam Group (Middle Eocene) as (Fig.1). The selection of Northern Galala as decorative limestone based on its relatively low cost than other decorative limestone types and consequently, it's widely distribution in Egyptian building particularly, for interior purposes such as kitchen [6].

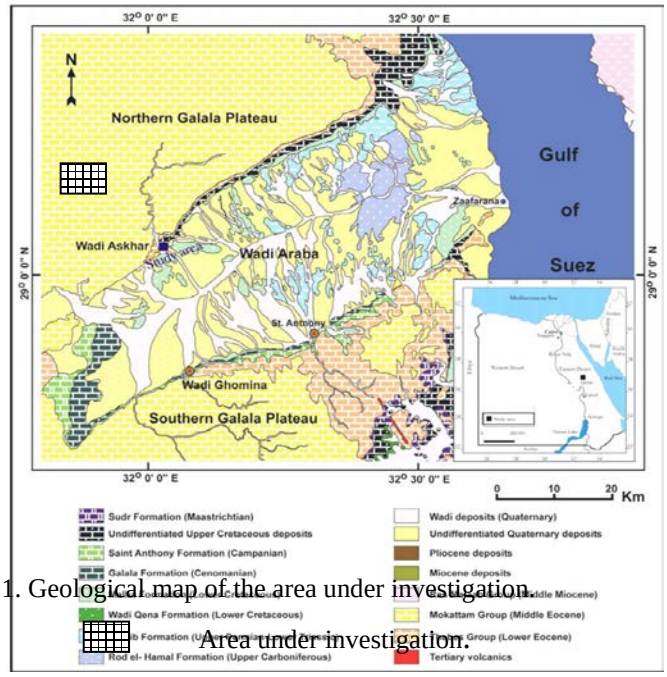

Fig.1. Geological map of the area under investigation.

Secondly, the choice of tea and coffee drops as two organic stain agents. It based on its characterization as it represents the most popular Egyptian drinks that in contact with used interior decorative limestone (Northern Galala) particularly in kitchens.

The third main used material in the recent research is treatment material which commercially called (base Pirhana) and chemically consists of ammonium hydroxide and hydrogen peroxide in a certain ratio. As stated treatment material used in both cases before staining as coated material and after staining as removing staining material.

Sampling

The studied samples can be divided into two main types:

The first were unpolished bulk samples collected from one of the recently used Northern Galala plateau limestone quarry and were prepared according to each standard test method for different techniques particularly for investigation of decorative limestone characterization such as mineralogical and chemical composition.

On the other hand, The second one were polished prepared samples for study the interaction between organic stain agents (tea and coffee drops) with decorative

limestone were cuboid measured (2*2*0.5cm) and should be have been cut from polished decorative limestone ready for its final usage**.**

Methodology

The current research includes different laboratory techniques that have been agreed with achieve its main aim. It can be classified into:

1. Stone characterization, it can be involved the textural, mineralogical and chemical composition of the studied Northern Galala decorative limestone. As it can be studied texturally by petrographical examination using transmitted polarizing microscope and based on [7]. Mineralogically, it has been investigated by XRD (X-Ray diffraction model X Pert ProPhillips MPD PW 3050/60 X-ray differctometer). Chemically, the major and trace oxides of studied decorative limestone have been analysis using X-Ray Fluorescence (XRF) model (Phillips PW 1400 Spectrometer, Holland). The percentage of $CaCO3_{\%}$ were measured according to [8] while acid insoluble residue A.I.R% followed [9].

2. Organic stain Ageing, Representative cuboids of the studied samples subjected to 15cycles of tea and coffee organic stain ageing cycles. Each one of these cycles consisting of:

- 10 ml of tea or coffee have been added on cuboids surface for a period of time 2 hrs.
- The stained cuboids dried at 105 ^{0}c for 16 to 18 hours using dryer.
- The previous two steps have been repeated for 15 cycles.

3. Microbiological examination

All microbiological examinations in the recent study have been carried out for polished decorative limestone cuboids measured (2*2*0.5cm). The studied samples subjected to different microbiological examination divided into: blank samples and stained samples by tea and by coffee.

Microbiological examinations include antifungal and antibacterial activity using different microorganisms strains. Firstly, fungal spps includes (*Asperagillus niger, Fusarium oxysporum ,Pencillium notatum and Candida albians).* Secondly, bacterial

strains involve (*E coli*, *Staphylcoccus auries*, *Enterococcus Feacalis and Bacillus subtillus).*

3.1. Antifungal Activity

Fungal Spps.

The tested fungal strains were isolated from drinking water and identified according to [10].

Preparation of Media

Sabronud dextrose agar medium was prepared and adjust pH to 7 and sterilized by autoclaving at 15 ibs pressure 121 ^{0}C for 15 min according to **[11]** .

Antifungal Assay

Petri plates containing 20 ml of autoclaved and solidified Sabroud dextrose agar medium were inoculated by 100 µl of prepared spore's suspension by spreading on agar surface. Prepared cuboids of limestone impregnated, gently pressed down to ensure contact petridishes with tested samples incubated at 28 °C for 28days then Check each plate after incubation time and record results as the rating which recommended by **[12-15]** for each fungal Spps.

3.2. Antibacterial Activity

Four bacterial strains were used for the antibacterial activity, two of them were gram positive strains namely *Enterococcus faecalis* NCTC 775/ATCC 19433 and *Bacillus subtillis* NCTC 10400/ATCC 6633 others were gram negative strains namely *Escherichia coli* NCTC 12241/ATCC 25422 and *Staphylcoccus aureus* NCTC 10788/ATCC 6538. These test organisms were maintained and stored at -20ºC.

Preparation of Media

Nutrient agar medium was prepared and adjust pH to 7 and sterilized by autoclaving at 121ºC for 15 min.

Antibacterial Assay

Petri dishes containing 20 ml of autoclaved of each strain and solidified nutrient agar medium were inoclumed by 100 µl of prepared inoculum were plated onto agar surface followed by impregnated prepared cuboids of limestone of each tested sample, gently pressed down to ensure contact and incubation at 37°C for 24 hrs. Check each plate after incubation time and record results as the rating which recommended by [13], [14], for each bacterial strain. All of the obtained microbiological examination results were compared to the following rating table. The presence of some fungal and bacterial Spps on tested samples was examied and identificated using stereo-microscope (Olympus GX71, Japan).

Table (1) Rating of microbial growth according to [12],

Observed growth on specimens	Rating
None	0
Traces of growth (less than 10 %)	1
Light growth (10 – 30 %)	2
Medium growth (30 – 60 %)	3
Heavy growth (60 – 100 %)	4

Results and Discussion

1. Decorative limestone characterization

Textural and mineralogical composition

Petrographically, studied decorative limestone as microfacies showed that the major constituents are bioclasts (skeletal grains). More than 80% of these skeletal grains are Nummilites with sparitic size (> 10 µm). Moreover, some lithoclasts (fragmented rock particles) and minute quartz grains are embedded into this micritic matrix (Fig.2). Spary calcite cement replacing micrite in the core of Nummilites were also been observed as in (Fig.2). It can be classified petrographically as Packstone [16].

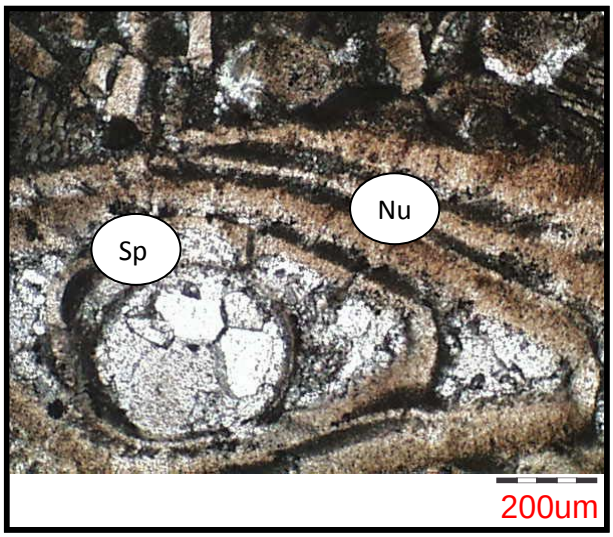

Fig.2. **Photomicrograph shows sparry calcite filling Nummilites core.**

SP: Sparry calcite Nu: Nummilites

Mineralogically, the obtained results from XRD revealed that the studied decorative limestone essentially consisted of calcite as it has been detected by its main peak at 2θ theta= 29.45 (Fig.3).It can be also observed the presence of quartz mineral as minor component detected by its main peak at 2θ theta= 26.65 (Fig.). Dependence on the mineralogical composition of the studied samples it can be classified as high-calcium limestone **[17].**

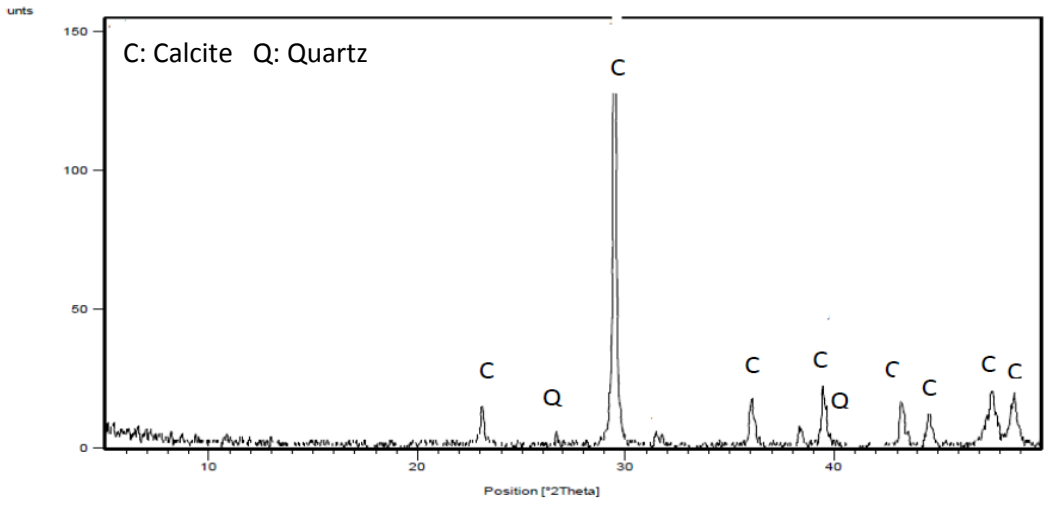

Fig.3. Representative X-ray diffractogram of the studied samples

Chemical Composition

The entire chemical composition of the representative studied decorative limestone samples including oxides, chlorides, sulphates, acid insoluble residue (AIR), $CaCO_3$ and loss of ignition (LOI) is listed in Table(2). The obtained results exhibited that CaO is the main oxide with an average value 56.3 by wt %. On the other hand, all others are recognized as minor constituents. Consequently, the average of (L.O.I) 42.3% and also calcium carbonate percentage were measured 99.28%. Based on the average chemical composition particularly MgO % by wt% the studied decorative limestone have been classified as high-calcium limestone according to [18].

Table (2): The average complete chemical composition of the studied decorative limestone samples.

Chemical components %	Average
SiO_2	0.32
Al_2O_3	0.12
Fe_2O_3	0.02
CaO	56.3
MgO	0.3
Na_2O	0.04
K_2O	0.02
P_2O_5	0.01
SO_3	0.04
Cl	0.01
LOI	42.38
Total	99.48
A.I.R	0.46
$CaCO_3$	99. 28

Decorative limestone staining:

The interaction between the studied decorative limestone and organic stain agents (tea and coffee) involves two main effects:

First one called chemical effect while the other one is microbial effect as illustrated in (Fig.4.). The chemical effect exhibited by change the original color of decorative limestone as reaction product. As decorative limestone adsorbed organic stain agents (tea & coffee) Ca (II) ions leached from its calcite crystals and mediated to form such reaction product **[18].** For example: Theaflavins and Thearubigin pigments forming brown spots on decorative limestone surface by black tea as reaction products [18] as detected by stereomicroscope and illustrated in (Fig.5).

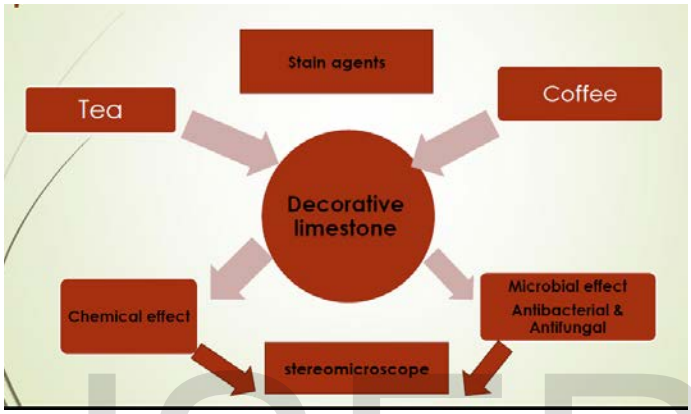

Fig.4. Schematic diagram shows different effects of organic stain agents on decorative limestone.

Fig.5.Macro and micro variation in original color of decorative limestone surface as observed by stereo microscope.

The second effect that called microbiological effect involving antibacterial and antifungal activity. It has been recognized for three types of samples a) Blank decorative limestone (b) tea stained decorative limestone c) coffee stained decorative limestone .All of each

type examined before and after treatment with chemical mixture (base Pirhana).The accumulation of microorganisms on the studied decorative limestone surface has been observed clearly by stereo- microscope. It ca be illustrated in Fig (6) for bacteria and fungi.

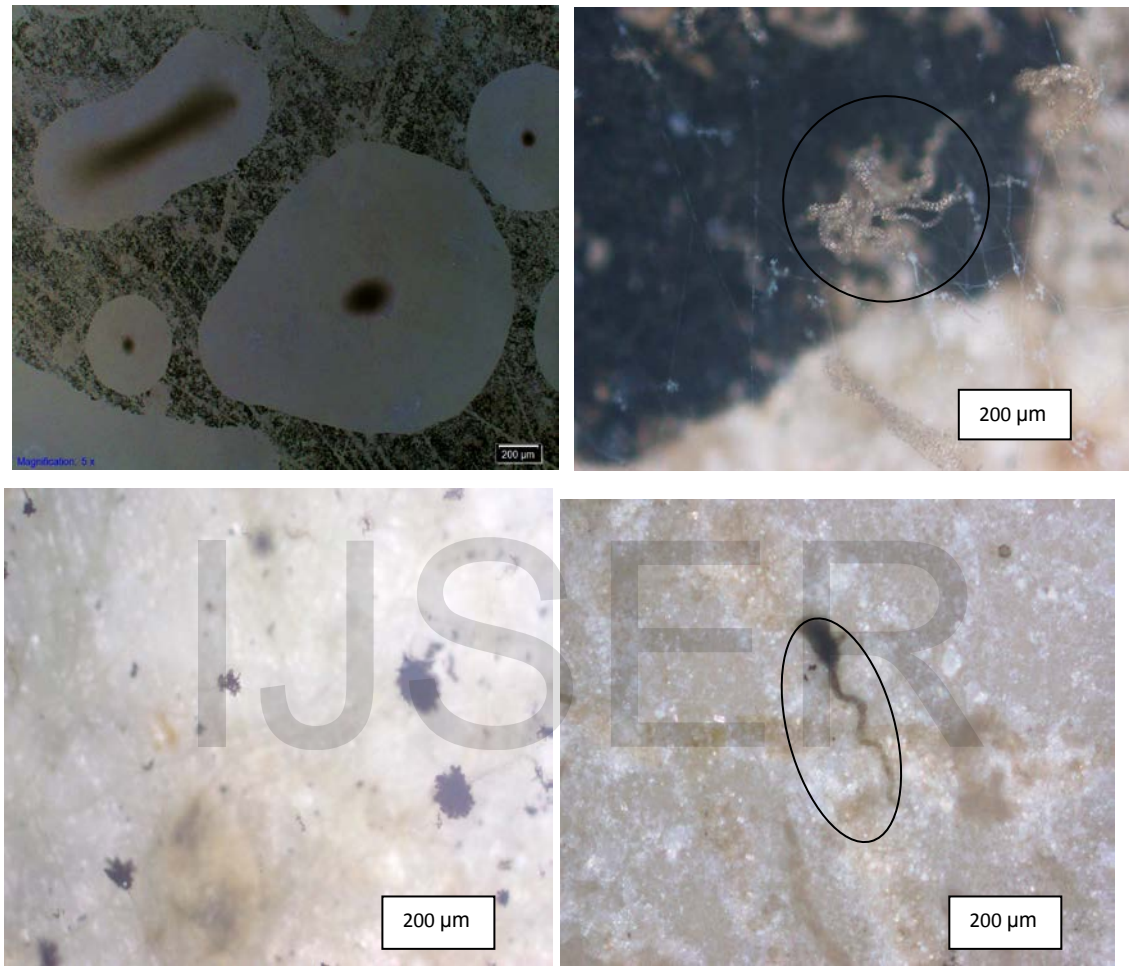

Fig.6. stereophotomicrographs illustrated the presence of different microorganisms on studied decorative limestone surface.

The descriptive investigation of microbiological accumulation by stereo-microscope followed by quantitative analysis of existed microorganisms formed by organic staining on the studied limestone. This quantitative analysis called microbiological activity.

The obtained results of microbiological activity can be summarized in tables (3 and 4) where, presented graphically in (Figs.7 and8). According to the mentioned results it can be concluded that:

In case of *E.coli* (a)Blank sample heavily growth with rating 4(60-100%),whereas after treatment it show antibacterial activity with light growth (10-30%) and rating 2, (2)sample stained with tea has no antibacterial activity with rating4(60-100%) whereas it showed antibacterial activity with rating (0) none growth, finally, samples stained with coffee there was small difference in rating before(60-100%) and after(30-60%) treatment where they had no antibacterial activity.

The best removal was achieved in treatment of blank examined with *Staphylcoccus auries* with rating 0% and none growth followed by samples stained with tea with rating 1 and <10% growth. Whereas, samples stained with coffee there were slight difference before and after treatment with removal 40%.

Samples stained with tea showed antibacterial activity against *Enterococcus fecalis* before and after treatment with rating (0) and none growth.

Treated blank and samples stained with tea showed high antibacterial activity before and after treatment (rating: 0 & none growth) against *Bacillus subtillis.*

Table (3) showed antibacterial activity of the 3 examined decorative limestone samples with tested bacterial strains.

	Blank		Tea		Coffee	
bacterial satrains	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
E.coli	4	2	4	0	4	3
Staphylcoccus auries	2	0	3	1	4	3
Enterococcus fecalis	2	0	0	0	4	4
Bacillus subtillis	2	0	0	0	4	1

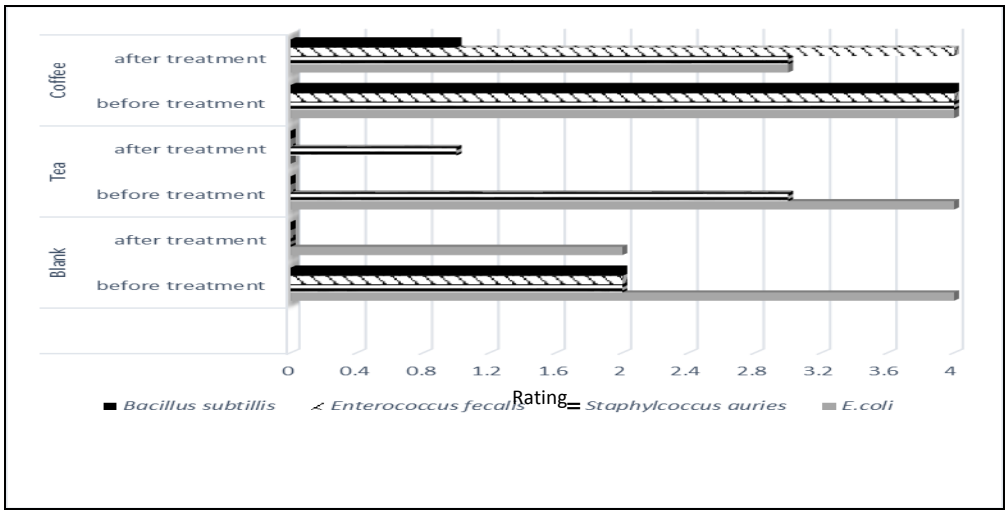

Fig. 7.Antibacterial activity of the three examined limestone samples.

The obtained microbiological results revealed also that the best% removal of for fungal spp from examined samples appear in decolourization step was more efficient in case of limestone stained with coffee with low growth 10% with *Asperagillus Niger*. Blank treated sample showed antifungal activity against *Fusarium Oxysporum* with light growth and rating 2 (10-30%). There was no difference between before and after treatment in antifungal activity against *Pencillium notatum*. The studied samples stained with tea received the best removal %(70) with rating 2 and light growth for *Candida albians.*

Table (4) showed antifungal activity of the 3 examined decorative limestone samples with tested fungal strains.

Fungal satrains	Blank		Tea		Coffee	
	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
Asperagillus niger	4	3	3	2	4	1
Fusarium oxysporum	3	2	3	3	4	4
Pencillium notatum	4	4	4	4	4	4
Candida albians	4	4	4	2	4	4

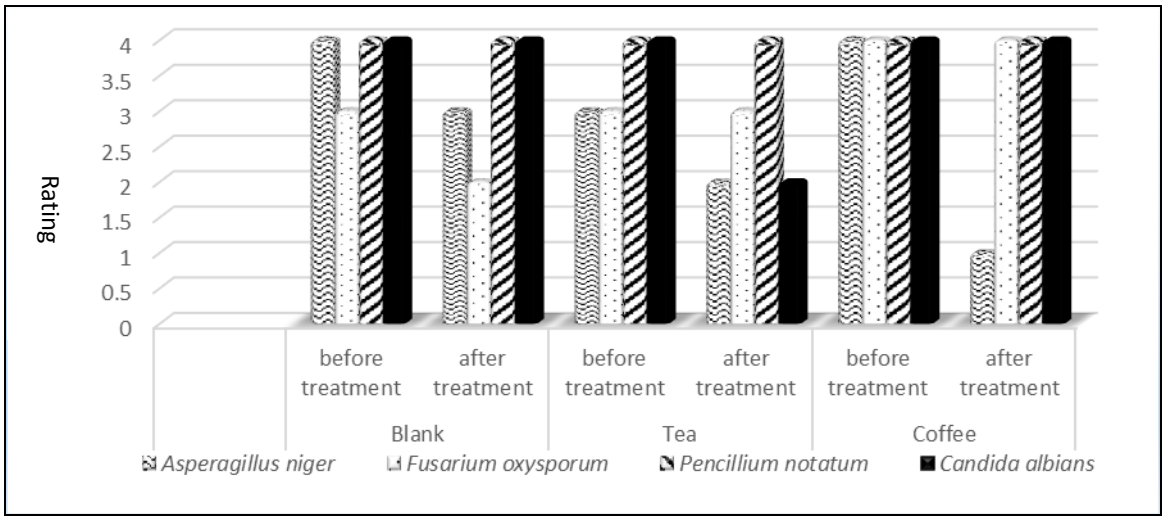

Fig. 8.Antifungal activity of the three examined limestone samples.

Preliminarily Treatment and its significance:

To overcome all of the previous negative effects of organic stain on the studied decorative limestone a mixture such as base pirahna has been used in preliminarily treatment. The usage of base pirahna has been occurred in two mechanisms: Firstly as cleaning agent in post staining stage as illustrated in Fig. (9). the tea stained decorative limestone with brown tarnish has been changed to its original blank color. Moreover, the base pirhana as treated material ca be also used as coated protective material in a pre-staining stage as showed in Fig (10). As the treated samples with base pirhana have been kept its original blank color even though post subject to tea and coffee as organic stain agents. The mechanism using base pirhana as cleaning and or / as coating material based on its chemical characterization. As it is an oxidizing agent which can be removing most of organic matter from decorative limestone surface moreover, add more (OH) to hydroxylase most surface of decorative limestone then it becomes more hydrophilic.

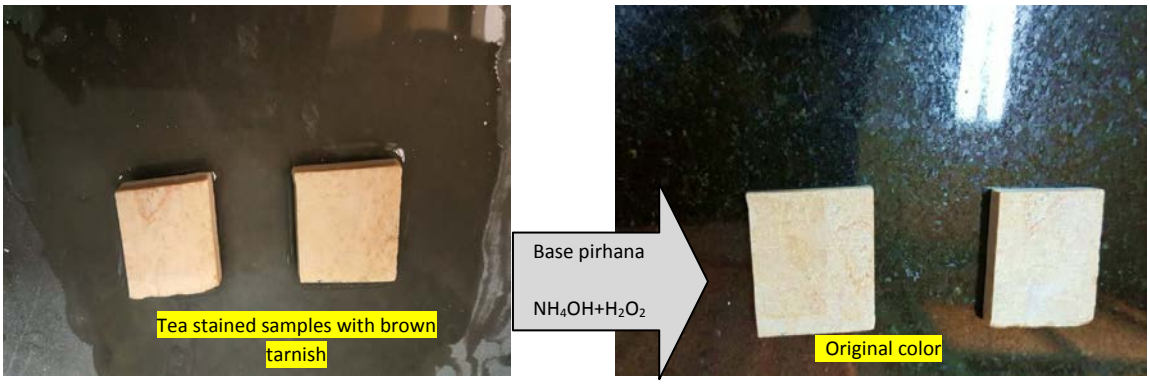

Fig. Returning the original blank color of the studied decorative limestone by using base pirhana.

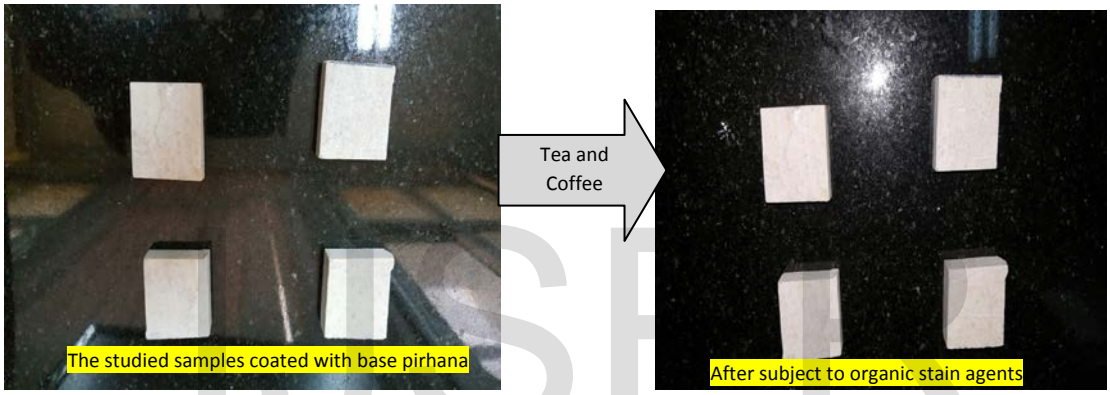

Fig. 9.Returning the original blank color of the studied decorative limestone by using base pirhana.

Discussion

Firstly, as decorative limestone successively adsorbed organic stain agents (tea & coffee) Ca (II) ions leached from its calcite crystals and mediated to form such reaction product which led to change its bulk original color.

Secondly, base pirhana considered as an oxidizing agent containing NH_4OH and H_2O_2 which can be removing most of organic matter from decorative limestone surface by adding more (OH) to hydroxylase and consequently, surface of decorative limestone more hydrophilic.

Conclusion and Recommendations:

1. The studied decorative limestone belongs geologically to Mokattam Group (Middle Eocene).

2. Mineralogically and chemically the studied decorative limestone have been classified as high calcium limestone.
3. The studied decorative limestone subjected to successive cycles of organic stain by tea and coffee.
4. The organic stain led to change the original color of the studied decorative limestone and decrease its sustainability.
5. The base pirhana used as treated material for removing organic stain from the surface of studied decorative limestone.
6. It can recommended to use base pirhana not only as cleaning agent for removing organic stain in post-staining stage but also as coated material to protect in pre staining stage.

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