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Interactions between microorganisms and composite material with marble & granite filler

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Interactions between microorganisms and composite material with marble & granite filler

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Abstract. It is promising to develop new composite materials which have antimicrobial activity for many applications; this is our goal in this study. All experiments were done to achieve this an important objective by studying the interaction between the new composites and some pathogenic reference microorganisms. The produced composite was manufactured from industrial wastes. Recently, many research groups in all fields have focusing work on removing the wastes and recycling it as useful materials. In this study, the preparing new composite from marble and granite wastes with thermoplastic matrix PMMA was investigated. This composite is considered as new type of micro- nano composite because it has fillers in the nano and micro sizes. Pathogenic microorganisms, used in this study, obtained from the American type culture collection (ATCC; Rockville, MD, USA), yeast and one pathogenic fungus were applied. The evaluation of analysis was done by spectrophotometer SEM and EDX techniques. The suitable applications of new composite material were mentioned based upon the interaction recorded in the study. The study ends with recommendation for future modifications in the new composite materials to improve its resistances to microorganism.

1. Introduction

Exposure to microorganisms, such as fungi, bacteria, viruses and their biological by-products, may cause a lot of defects in materials. The type of defects takes different forms; it may be lead to change in materials micro-structure, change in chemical composition, change in dimension which leads to harmful and cracks in the structure [1,2]. Contact of materials and machine parts with pathogenic microorganisms and their by-products inside or outside parts usually occurs by interaction between moisture, lubricant and different substances which contain microorganisms during manufacturing and operation [3,4]. In order to achieve the acceptable level of interaction between materials and microbes in different application like buildings, machinery and daily life applications, the contacting technology with fungi and other pathogens should be minimized or eliminated [5]. The pathogens distribute and develop daily to resist used antibiotics and antimicrobial materials [6]. For that, researchers work hard to find solution for this problem. Some constructions made to face the problem of pathogens distribution in different sectors like production of new biomaterials [7], development of antimicrobial nanomaterials [8] and converting the wastes to useful substances [9]. Prevention and protection of the material must be related to the factors causing interaction between materials and microorganisms which can be summarized in three main actions;

1. There must be a reservoir (i.e., location where an unusually high concentration of microorganisms is present).
2. The microorganisms must be released into the air.

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3. The microorganisms must be allowed to reproduce favorable conditions are needed for reproduction to occur [10]. For example, fungal growth is usually optimized when moisture levels are high. Figure 1 shows the micro-organisms types and effects [11].

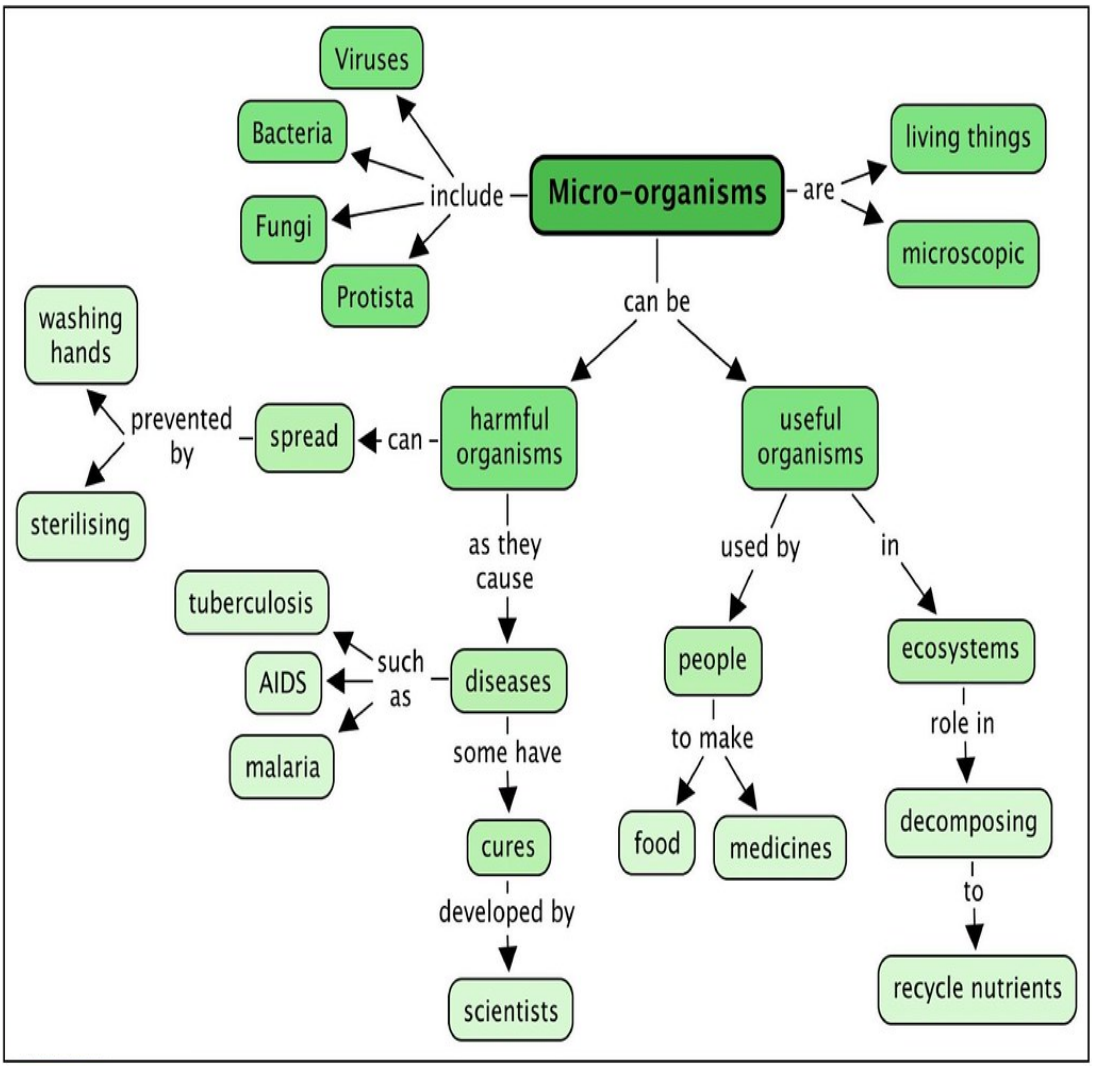


Figure 1. The micro-organisms types and effects [11].

Fault diagnosis, qualitative and quantitative analysis are the promising techniques to evaluate the interaction between microorganisms and materials from the material point of view, re-maintenance and preventive maintenance program should be implemented that provides regular inspections [12].
Inspection techniques must be followed by treatment methods before cracks initiation and propagation to the critical size. Management requirements for the materials exposed to contamination leads to failure by microorganisms must include commissioning, operation, maintenance, repair, replacement, and expansion of new and existing material [13]. Different techniques such as coating, surface alloying, painting, lubrication and material replacement by new materials may be used [14]. The selection of best technique to eliminate and/or reduce the interaction results between material and microorganism depend on different factors; some of them related to materials and their operation environment conditions and others related to the type and amount of microorganism, the factors may be correlated and interrupt together [15]. In other words, we can say that as the interaction between microorganism and material are complicated process the protection techniques are also need so many precautions to be considered [16]. In this trend, the present study focused on converting the granite

and marble wastes to useful materials having antimicrobial activity to face the problems causing by interaction between materials and pathogenic microbes. The main focus of the paper is to investigate the interaction between microorganisms and the developed composite material.

2. Experimental Work

2.1 Materials

The matrix used in this study is poly methyl methacrylate (PMMA). It is a thermoplastic agent prepared from monomer (methyl methacrylate) by additional polymerization process; the form of polymer granule was in less than 5mm diameter.

2.2 Filler

Marble & granite refuses consists of wet refuse (Sahla). It is a type of the wet refuse where its percentage of water reaches about 70%. It is sticky and it is produced from the processes of leveling and polishing. Commercially it is known as Al Sahla. The sticky refuse is heavy in weight and big in size. Lime 28-32%, Silica 3-30%, Magnesium oxide 20 to 25%, Iron oxides 1-3%, and losses 20-45%. After drying, the particles size ranges from 50 to 300 μm for marble wastes, while granite wastes particle size ranged from 150 to 500 μm.

2.3 Preparation of wet refuse

The mixture is automatically pushed to the drying room. The heaters were adjusted at 120°C for period from three to four h. For guaranteeing the drying process, wet refuse (Sahla) is got out of the dryer in the form of fragile masses which are transferred by a conveyor to the automatic turning over room and made in the form of powder suitable for mixing [17-18].

2.4 Manufacturing of the composites

Plastic powders and treated marble and granite wastes were mixed together in the solid state with 30% volume fraction. The mixture was heated at the required temperature about 300 -400° C, according to its components. The heating rate depends on the type of the joining material; compression 160-200 Kg/cm^2 and cooling were done for heated mixture. Nano and micro -composite material was fabricated and the new composite material was distinguished with the light weight and the ability to endure scratch and damping capacity comparing with the natural marble and /or granite. It was also labled for the easy formation in different colors and shapes [19].

2.5 Preparation of pathogenic microbe's cultures and making interaction with produced composites

Pathogenic microorganisms, used in this study, were obtained from the American type culture collection (ATCC; Rockville, MD, USA). Two gram-positive bacteria, two gram-negative bacteria, yeast and pathogenic fungus were applied [20]. The details of pathogenic microorganisms subjected for this study were represented in table 1.

Table 1. Pathogenic microorganisms used to study their interaction with produced compounds.

Type	Microorganisms
Gram +ve Bacteria	Bacillus cereus ATCC- 12228
	Staphylococcus aureus ATCC-47077
Gram -ve Bacteria	E. Coli ATCC- 25922
	Salmonella typhi ATCC 15566
Fungi	Candida albicans ATCC- 10231
	Aspergillus niger ATCC- 16888

A loopful of bacterial growth from culture slope was inoculated into Minerals salt medium (MSM) broth medium and incubated at 30°C under shaking conditions (100 rpm) for 48 h. After incubation period, 100 µl of growing cultures were inoculated into flasks (250 ml) containing 100 ml of MSM broth medium and the treatments (surface mechanical attrition treated sheets at times and frequencies, also etched as treated surfaces) were added to flasks [21]. The inoculated flasks were incubated at 37°C for 7 days under shaking conditions with speed (120 rpm). In case of fungal strain, 100 µl of spore suspensions were inoculated into flasks (250 ml) containing 100 ml of MSM broth medium (pH 5.5) and the sheets were added. The flasks were incubated under shaking conditions for 7 days at 30°C. The microorganism growth was recorded by two methods: -

1. Weighting the cell biomass daily by digital electrical balance of fungal strain and measuring the changing in cultures optical density using spectro-photometer (Jenway UV/Visible- 2605 spectro-photometer, England) at wavelength 550 nm [22].
2. Measurement of the changing in pH using digital Orion pH meter (model 420A).

Composite material exposed to microorganism in disc shape with 10mm length and 16mm diameter.

2.6 Scanning Electron Microscope (SEM)
The specimens were examined by scanning electron microscope (SEM) operating at a nominal accelerating voltage of 30kv. Specimen preparation is very simply accomplished by cutting a thin slice of the specimen containing the surface of interest, the samples were inserting into the specimen chamber for direct examination of the effects microorganisms on the structure [23].

2.7 Energy dispersive X-ray "EDX"
The quantitative method of elemental analysis of the samples has been examined by SEM JSM-T200 at 25KV acceleration voltage, 20mm working distance, and magnification 200x, (1peak omitted 0.02 KeV). Each value is at least an average of 2 readings.

3. Results and discussion
Pathogenic microorganisms had distribution in various environments; agriculture, industrial, medical and municipal waste water. These pathogens cause many problems toward people, animals and plants [47, 48]. The global researchers have continuously research about new technology for pathogens disinfection such as using plant extracts nano-materials and new formulations. In this study, we used the new formulation (micro-nano composite) from marble and granite wastes to obtain composite materials with behavior of antimicrobial activity.

Six microbial pathogens (St. aureus**,** B. cereus**,** E. Coli**,** S. typhi.**,** C. Albicans, A. niger**)** were developed in specialized and liquid environments together for a week under optimal growth conditions and microbial growth was monitored during the growth period. Samples were extracted and washed. The microorganisms in the study range were divided into three main categories G^+ bacteria include (St. aureus, B. cereus), G^- bacteria include (E. Coli, S. typhi) and fungi which include (A. niger, C. Albicans). the interaction between each category and the new thermoplastic based composite with marble and granite filler were studied [24]. The influence of pH on the relative importance of the decomposer in thermoplastic base micro and nano composite. The behaviour of composite was investigated along a continuous pH gradient. This experimental location provides a uniform pH gradient, ranging from pH 7 or 5.5, incubated at 30 °C under shaking conditions (100 rpm) for 48 h, it has been continuously grown for more than 7 days as shown in figures (2), (3) and (4), respectively, which represent the rate of change in basicity for bacteria G+, bacteria G^- and fungi, respectively. The changing in microbial growth with time give indication about the interaction between microorganisms and composite material figures (5), (6) and (7) show these variations of weight for all microorganisms in the study range. The bacteria G+ specially, the composite material control sample basicity remains constant at normal basicity PH 7 for 4th days and in 5th day, it dropped down to PH 6.8, the decrease in basicity is continue with time and reach PH 6.5. The rate of basicity decrease with B. cereus, it is decreased and just reach B cereus PH 6.8 after 7 days while St. aureus have the same behaviour of the control sample as shown in figure (2). The bacteria G^- such as E. Coli basicity is dropped down faster after only three days, the rate of decrease continue at the same rate and reach PH 6.7 at the end of the 7 days, S. typhi has the behaviour but the rate of decrease is less, it reaches 6.9 PH at the end of the 7 days while rate of decrease began after 4 days figure (3). The fungi basicity as shown in figure (4) yeast (C. Albicans) have the same behaviour of the control sample while the A. niger is dropped down after only 2 days and reach to PH 2.2 at the end of the 7^{th} day. The fungal growth was recorded by weighting the cell biomass daily. The weight changes with microbial growth almost have the same behavior for all microorganism under study. The results in figures (5), (6) and (7) showed that when water was absorbed by capillary action to the materials, fungal and bacterial growth started fast and was abundant in the composite materials with time above 20 %. Such a limit value could not be defined for fungal contamination in the composite material because of complex behavior of moisture covering. The condensation under the varying RH and temperature conditions caused only restrained fungal growth in the materials [25-27].

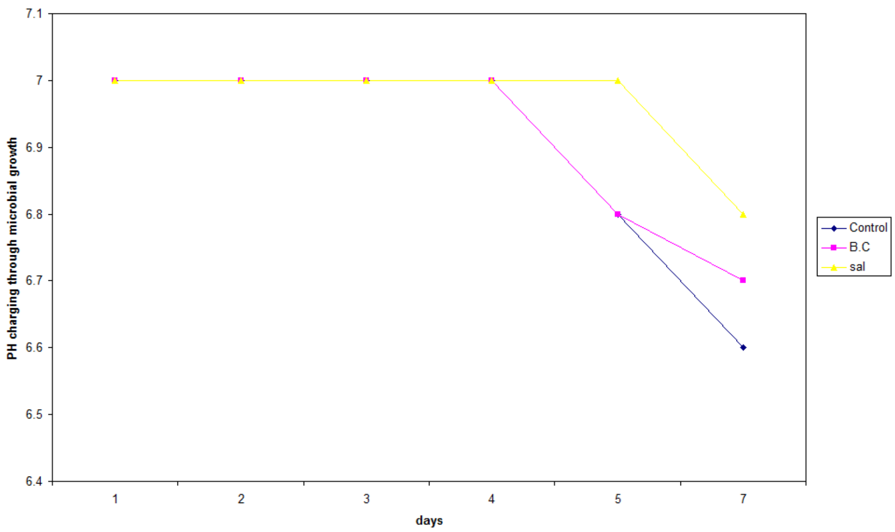


Figure 2. PH (basicity) change during microbial growth for G^+ bacteria.

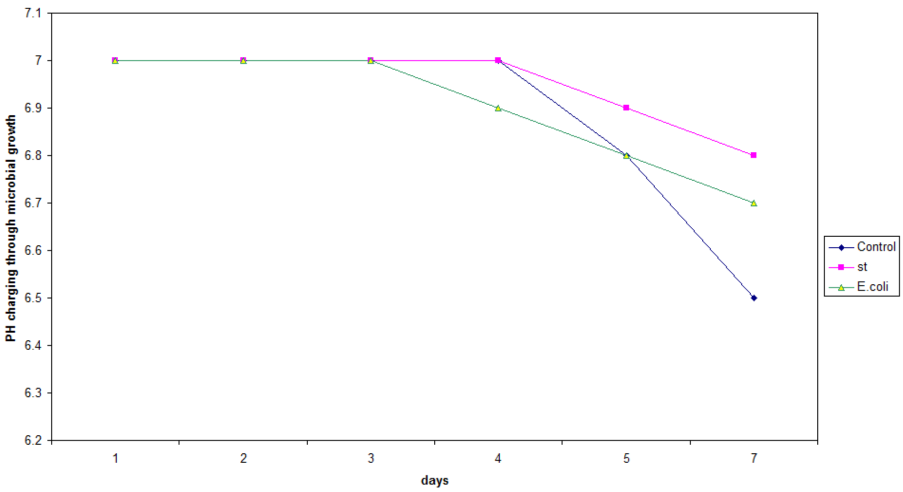


Figure 3. PH (basicity) change during microbial growth for G^- bacteria.

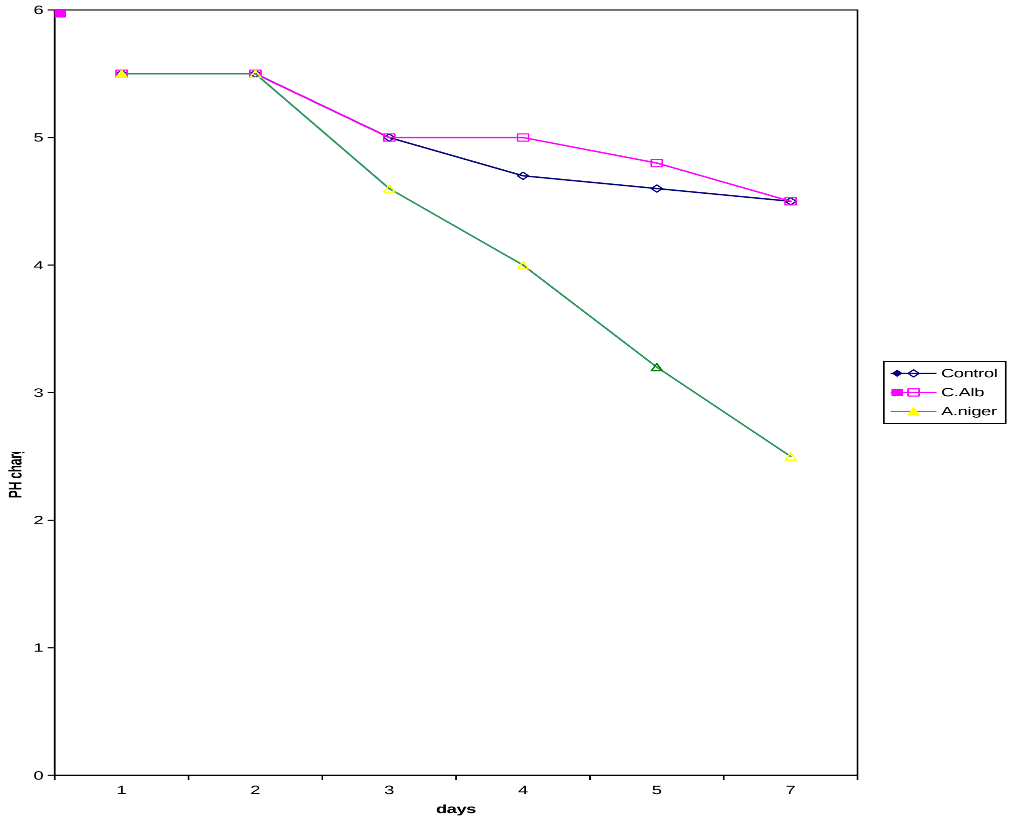


Figure 4. PH (basicity) change during microbial growth for fungai.

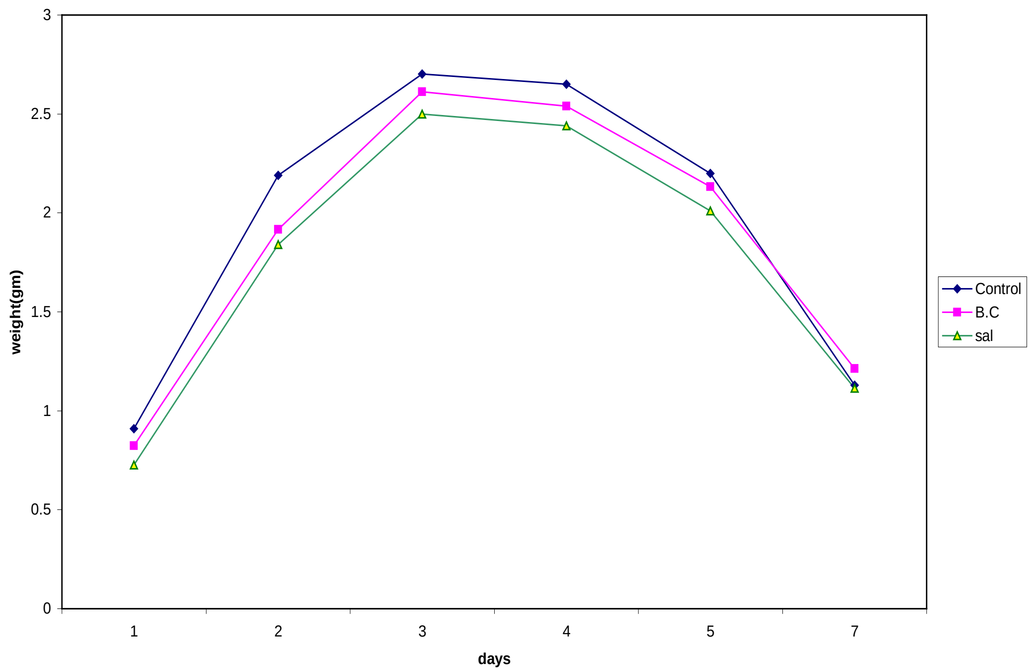


Figure 5. Weight change during microbial growth for G^+ bacteria.

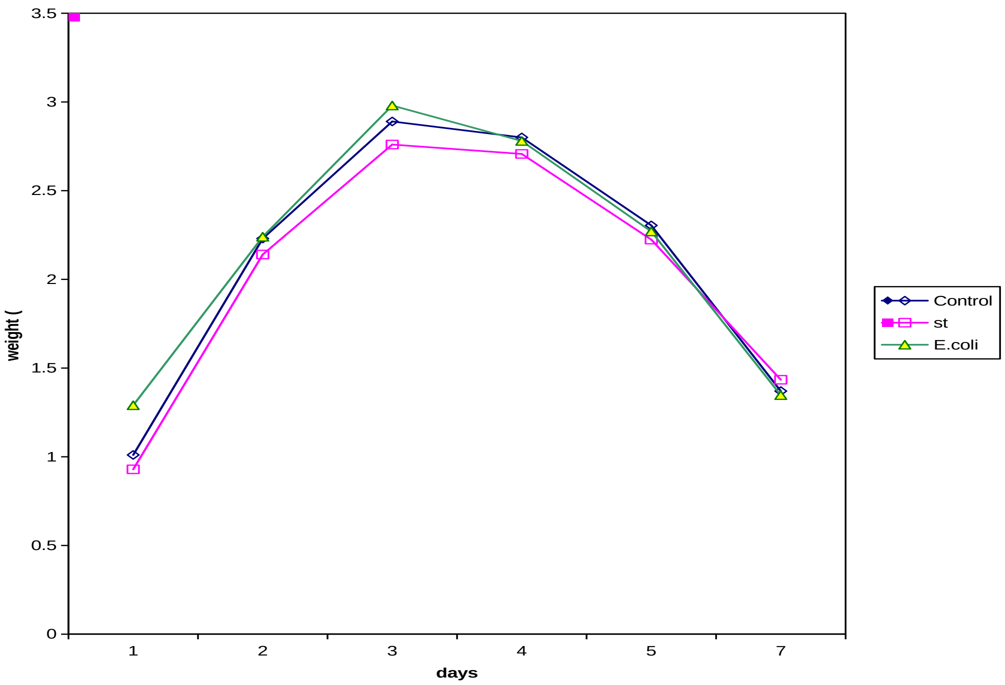


Figure 6. Weight change during microbial growth for G$^-$ bacteria.

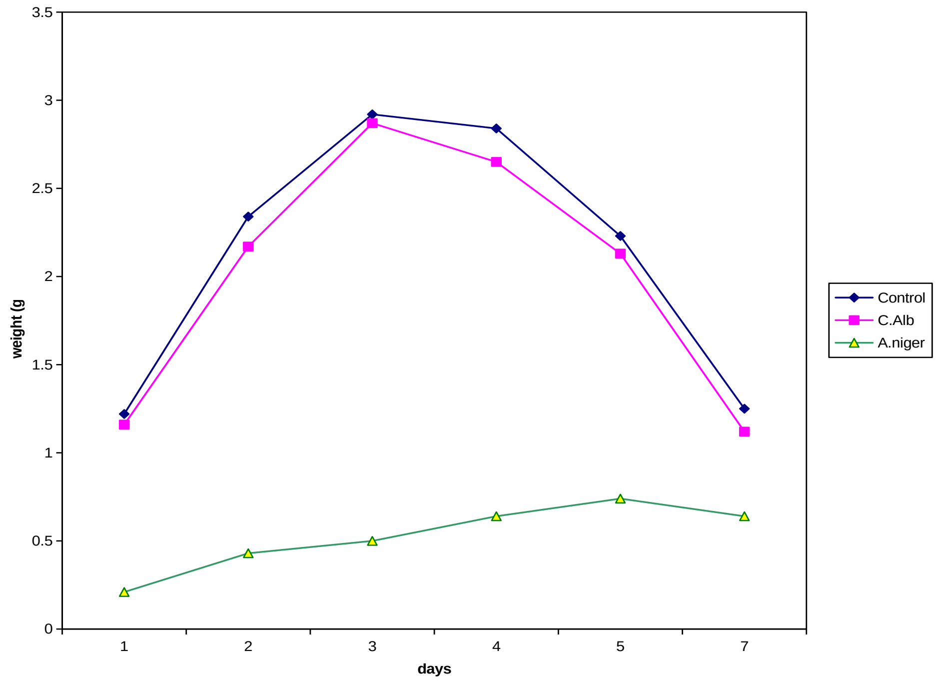
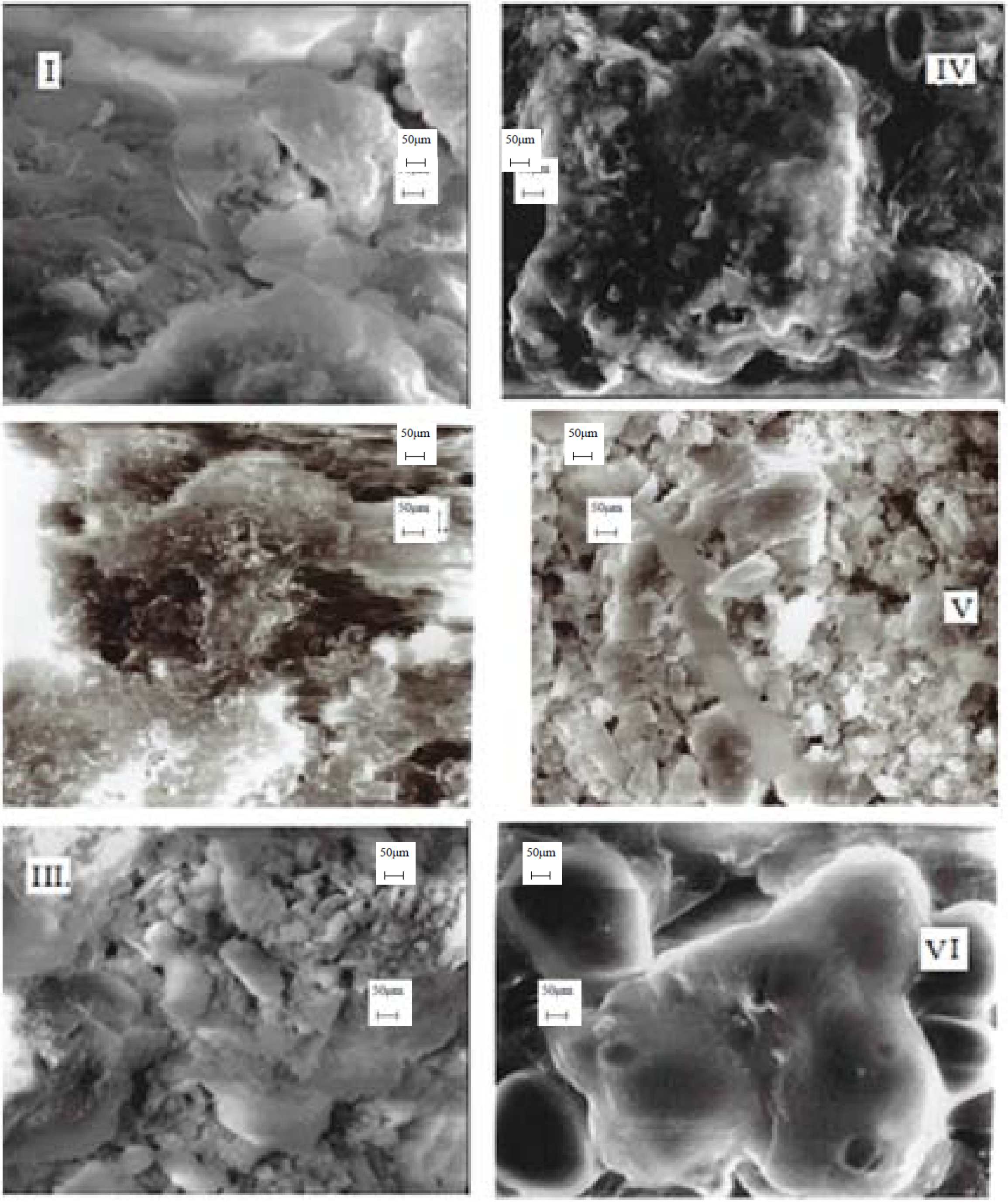


Figure 7. Weight change during microbial growth for fungai.

The shapes of the growth curves were different depending on the nutrient medium used and the species tested. Microbes can interact in the environment with materials/surfaces in so many ways, the interactions lead to change in micro structure and chemical composition of the composite material. The discs of the produced composite were incubated with the tested pathogenic microbes for 7 days and then examined for their surface changing using scanning electron microscopy (SEM). The pictures produced from SEM were illustrated in figure 8. No clearly changing was detected in the internal structure of composites, just few changing resulting from microbial growth or metabolities adhesion onto discs surface were detected. The elemental analysis by EDX as represented in figure (9), changing in elemental profile of composite surface was noted.

Certain species of fungi is the engines of the process of decomposition. Fungi is the major organisms decomposing of organic substances. Decomposition can be a problem on a large and small scale. Organic describes any material made up of molecules containing carbon and hydrogen atoms. The matrix of the composite material under consideration is PMMA is considered organic. Decomposition is a complex process. Organic matter is broken down into carbon dioxide and the mineral forms of nutrients like nitrogen. It is also converted into fungi and bacteria through these organisms feeding on the organic material and reproducing. Scientists call the organisms that decompose organic matter decomposers, saprobes or saprotrophs. Decomposition is made up of a number of sub -processes. Consider the decomposition of matrix which is the binding element in the structure, it breaks the matter into smaller pieces in a process called fragmentation. This is an important step because smaller fragments have more surface area to support the growth of fungi. Fungi growth is especially affected by fragment size, since fungi can penetrate substances more easily than bacteria or other microorganisms.

The fast drying (RH 30%) seemed to decrease the viability of fungi but along with the experiment fungal flora was modified to tolerate fluctuating conditions and the drying at RH 50% had only a slight effect on the viability of fungi. In the case of yeast there was a decrease in the amount of organic matter, which means that Yeast interact with the matrix and swelling of matrix was done by yeast. Strong precautions must be considered in using Composite material in applications have Yeast (Candida albicans ATCC- 10231), which has bad reaction with the organic matrix. Staphylococcus aureus ATCC-47077 is spread in hospitals and it is recommended to use composite material with marble filler samples under study in hospital floors and walls. The adverse effect appears clearly for Staphylococcus aureus ATCC-47077 with the composite material with granite filler, the matrix erosion appears clear in SEM photos and decrease in oxygen and hydrogen percentages appear in EDX analysis, so using of these materials in hospitals is prevented. Salmonella typhi ATCC 15566 did not have any obvious effect on the composite materials with marble and /or granite filler used in poultry raising wards and poultry processing places. Environmental factors influence rate of bacterial growth such as acidity (pH), temperature, water activity, macro and micro nutrients, oxygen levels, and toxins. Conditions tend to be relatively consistent between bacteria with the exception of extremophiles



Marble **Granite**

I. Control marble II. Staureus Marble III. Albicans Marble
IV. Control Granite V. Staureus Granite VI. Albicans Granite

Figure 8. Scanning electron microscopy image for interaction between microorganisms and composites.

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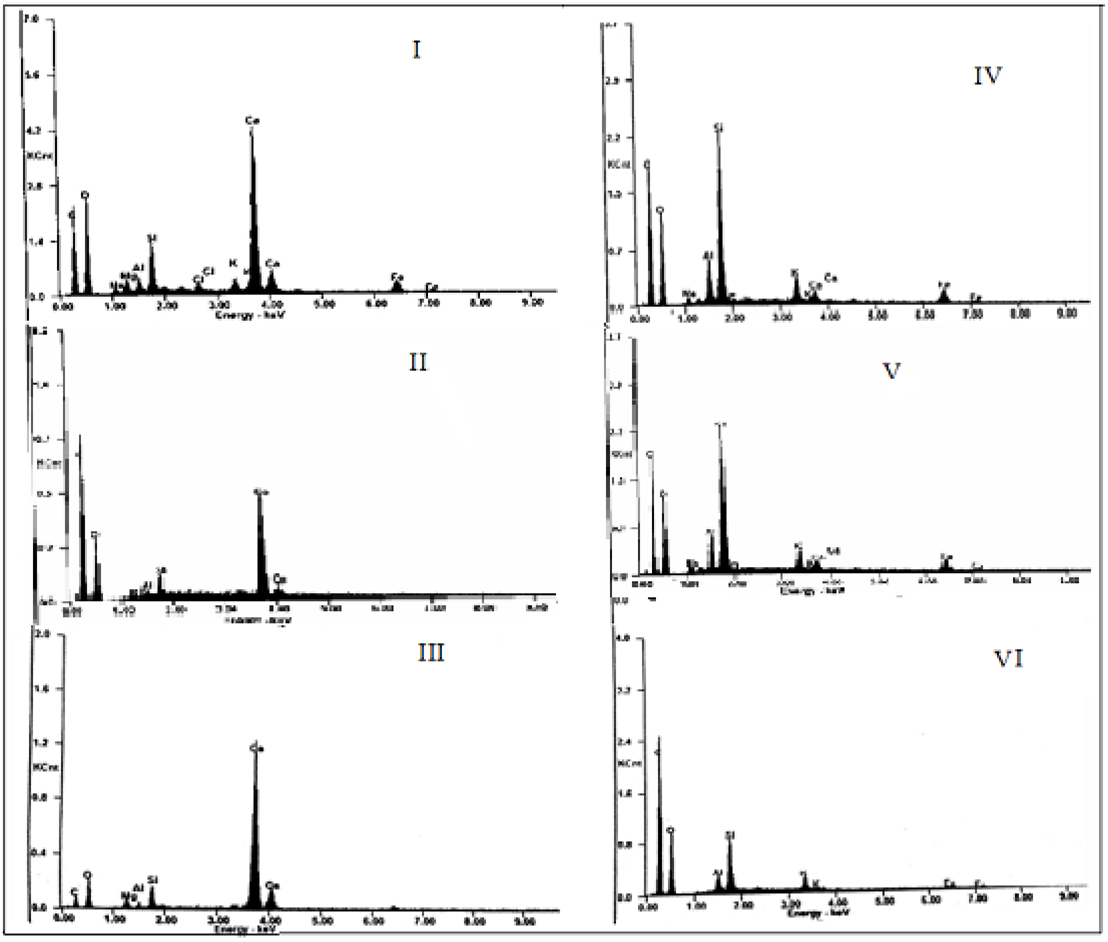

Marble **Granite**

I. Control Marble II. Staureus Marble III. Albicans Marble
IV. Control Granite V. Staureus Granite VI. Albicans Granite

Figure 9. EDX images for interaction between microorganisms and composites**.**

4. Conclusions

- The interaction between microbes and materials is function of time and type of microorganism, weight loss and change in basicity PH can be recorded significantly during the interaction of microorganism with composite material, the behavior depends mainly on the composition of composite materials and type of microorganism.
- Biofilm formation is considered as the primary step to start a degradation process, microorganism become in close contact with the surface and creating a micro environment that can be totally different from the bulk with distinct properties
- Six pathogenic microbes were developed in specialized and liquid environments
- For yeast there was a decrease in the amount of organic matter, which means that Yeast interact with the matrix and swelling of matrix was done by yeast. Strong precautions must be considered in using Composite material in applications have Yeast (Candida albicans ATCC-10231), which has bad reaction with the organic matrix.
- Staphylococcus aureus ATCC-47077 is spread in hospitals and it is recommended to use composite material with marble filler samples under study in hospital floors and walls.

- The adverse effect appears clearly for Staphylococcus aureus ATCC-47077 with the composite material with granite filler, the matrix erosion appear clear in SEM photos and decrease in oxygen and hydrogen percentages appear in EDX analysis, so using of these materials in hospitals is prevented.
- Salmonella typhi ATCC 15566 did not have any obvious effect on the composite materials with marble and /or granite filler used in poultry raising wards and poultry processing places. Environmental factors influence rate of bacterial growth such as acidity (pH), temperature, water activity, macro and micro nutrients, oxygen levels, and toxins. Conditions tend to be relatively consistent between bacteria.

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