DEVELOPING ANTIMICROBIAL SURFACES USING ION-EXCHANGED ZEOLITES

by

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"*this thesis is dedicated to*
my family and my friends…"

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ABSTRACT

DEVELOPING ANTIMICROBIAL SURFACES USING ION-EXCHANGED ZEOLITES

Microorganisms are normally found in nature such as in the air, water and soil, ubiquitously. They are significant for human health and ecosystem, and used particularly in the fields of food, environment, agriculture and bioengineering. Beside these beneficial effects of microorganisms, they can also be pathogenic for living systems. These infectious microorganisms affect millions of people every year around the World, and cause considerable amount of disease and death. Some of these microorganisms may be opportunistic pathogens. They are not normally pathogenic for humans; unless the immune system is suppressed or the amount in human microbial flora is increased. Antibiotics are well known drugs developed and used to control clinically important microorganisms. Unfortunately multiple drug resistance has been developed due to the extensive and misuse of antibiotics in long period of time. Therefore, new antibiotics and synthetic drugs are developed. Also to maintain the material hygiene antiseptics and disinfectants are developed; but they don't satisfy the demand for material hygiene. To cope with contamination of materials, scientists are interested in developing new materials that inhibit the microbial growth on the surface. So far some materials with antibacterial effects have been developed and introduced into the market; however, there has been no attempt to develop antimicrobial material.

The aim of this study was to develop novel antimicrobial agents which can be combined with different materials used for manufacturing antimicrobial surfaces. Thus, zeolites which were synthesized with different chemical compositions were ion-exchanged with heavy metals of Ag^{+}, Zn^{2+} and Cu^{2+}, and tested for antimicrobial activity against various microorganisms. Antimicrobial (antifungal, antibacterial and anticandidal) zeolites with Ag^{+}, Zn^{2+} and Cu^{2+} were selected based on *in-vitro* tests and then combined with different materials, including acrylic paint, dust paint, liquid paint, polypropylene and plaster of paris at different rates to develop antimicrobial surfaces.

The results in the present study showed that zeolites that have been ion exchanged with silver, copper and zinc solutions and have synthesis composition of $4.64Na_2O:Al_2O_3:3.2SiO_2:400\ H_2O$ and $2Na_2O:Al_2O_3:1.6SiO_2:200H_2O$ have antimicrobial effect against a great range of microorganisms including bacteria, fungi and yeast tested. The data also demonstrated that antimicrobial surfaces with different materials can be developed by combination of 7-15 weight per cent mixture with ion exchanged zeolites.

ÖZET

İYON DEĞİŞİMİ YAPILMIŞ ZEOLİTLER KULLANARAK ANTİMİKROBİYAL YÜZEYLER GELİŞTİRME

Mikroorganizmalar doğada havada, suda ve toprakta her zaman ve her yerde bulunurlar. Bunlar insan sağlığı ve ekosistem için önem taşımaktadırlar. Mikroorganizmalar insan sağlığı ve ekosistem için önemlidir ve özellikle gıda, çevre, tarım ve biyoteknolojide kullanılır. Bu faydalarının haricinde mikroorganizmalar canlı sistemleri için patojenik olabilirler. Bu bulaşıcı mikroorganizmalar dünyada her yıl milyonlarca insanı etkilemekte ve ciddi miktarda hastalıklara ve ölümlere sebep olmaktadır. Mikroorganizmaların insan hayatını etkileyen başka bir kısmı ise fırsatçı patojenlerdir. Bu mikroorganizmaların normal koşullarda insanlar üzerinde patojenik etkisi bulunmasa da; bağışıklık sisteminin baskılanmış olduğu durumlarda ya da insan mikrobiyal florasında sayıları arttığı durumlarda patojenik etki göstermektedirler. Antibiyotikler klinik olarak önemli olan mikroorganizmaları kontrol etmek için geliştirilmiş ilaçlardır. Ancak yanlış ve uzun süreli kullanım yüzünden mikroorganizmalar bu antibiyotiklere karşı direnç geliştirmiştir. Bu yüzden yeni antibiyotikler ve sentetik ilaçlar geliştirilmiştir. Ayrıca malzeme hijyenini sağlamak için antiseptikler ve dezenfektanlar geliştirilmiştir; ancak geliştirilen ürünler malzeme hijyenini sağlamaktaki talebi karşılamamaktadır. Malzemelerin kontaminasyonunu engellemek için bilim insanları, üzerinde mikroorganizma gelişmesini engelleyen yüzeyler geliştirmeye çalışmaktadırlar. Şimdiye kadar antibakteriyel etkisi olan bazı materyaller geliştirilip markete sunulmuştur; ancak, antimikrobiyal malzeme geliştirmeye yönelik bir girişimde bulunulmamıştır.

Bu çalışmanın amacı; antimikrobiyal yüzey oluşturmak için farklı malzemelerle kombine edilebilen yeni bir antimikrobiyal ajan geliştirmektir. Bunun için farklı kimyasal kompozisyonlarla sentezlenen zeolitler Ag^{+}, Zn^{2+} ve Cu^{2+} ağır metalleri ile iyon değişimi yapılarak farklı mikroorganizmalara karşı etkinlikleri test edilmiştir. İn-vitro sonuçlara göre Ag^{+}, Zn^{2+} ve Cu^{2+} içeren antimikrobiyal (antifungal, antibakteriyal ve antikandidal)

zeolitler akrilik boya, toz boya, sıvı boya, polipropilen levhalar ve alçı ile kombine edilerek antimikrobiyal yüzeyler oluşturulmuştur.

Bu çalışmanın sonucunda, gümüş, çinko ve bakır çözeltileri ile iyon değişimi yapılmış ve sentez kompozisyonları $4.64Na_2O:Al_2O_3:3.2SiO_2:400H_2O$ ve $2Na_2O:Al_2O_3:1.6SiO_2:200H_2O$ olan zeolitler, bakteri, mantar ve mayayı da içeren çok sayıda mikroorganizmaya karşı antimikrobiyal etki göstermektedir. Sonuçlar göstermektedir ki; ağırlıkça % 7-15 arasında iyon değişimi yapılmış zeolit karıştırılarak antimikrobiyal yüzeyle oluşturulabilinir.

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LIST OF SYMBOLS/ABBREVIATIONS

BEA	Zeolite Beta
BHA	Brain Heart Agar
CTAB	Cetrimonium bromide
D4R	Double Four Membered Ring
D6R	Double Six Membered Ring
FAU	Faujasite
GIS	Gismonite
HEU	Heulandite
LTA	Linde Type A
MFI	Mordenite Framework Inverted
MOR	Mordenite
NA	Nutrient Agar
NB	Nutrient Broth
PDB	Potato Dextrose Broth
PDA	Potato Dextrose Agar
Pp	Polypropylene
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SEM	Scanning Electron Microscopy
SMS	Sodium metasilicate pentahydrate
SOD	Sodalite Cages
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
XRD	X-ray Diffraction
ZMS-5	Zeolite Socony Mobil-5
Å	Angstrom
α-cage	Alpha cage
β-cage	Beta cage

1. INTRODUCTION

Microorganisms can live almost everywhere in the world. Some of them are beneficial for the environment and human being, while others are pathogenic to humans, animals or plants. Those pathogenic microorganisms cause serious diseases, prolong the hospital stay and medical care, they even cause death. After the discovery of penicillin, deaths caused by microorganisms were decreased. New antimicrobial organic based agents are developed but after a while microorganism developed resistance against those drugs. In order to control microorganisms in their flora, antimicrobial agents are modified with chemicals. However, these chemically modified drugs can cause adverse drug reactions and toxicity. Additionally, disinfectants are improved to provide surface hygiene, but their short term effect on surfaces is the major limitation for long term hygienic conditions. In other words, they may reduce or eliminate the target microorganism in a certain period of time, but not forever due to the development of resistance in long term.

Antimicrobial effects of metals are known for centuries. Especially silver is used as antimicrobial agent since ancient times. However, they show broad spectrum activity against microorganisms in the ion form. Thus, scientists have tried to develop carriers for heavy metals in order to prevent microbial contamination. In today's technology, developed materials are only effective against bacteria that is responsible for a small part of microbial originated infections.

Zeolites are aluminasilicates with balancing cations. As the balancing cations are loosely bound to the framework, they are easily exchangeable with heavy metal ions. In this perspective, when zeolites are ion exchanged with metal ions such as; silver, zinc and copper, they gain antimicrobial property.

The aim of this project is combining ion exchanged zeolites with various substances to develop new antimicrobial materials and surfaces.

2. THEORETICAL BACKGROUND

2.1. ZEOLITES

Zeolites were discovered in 1756 by Swedish mineralogist Axel Fredrik Cronstedt. Cronstedt discovered that a natural mineral that, was named later as stilbite, visibly lost water when heated. This new family of minerals, hydrated aluminasilicates was named as zeolithos from old Greek words “zeo” for “boiling” and “lithos” for “stone” [1, 2].

Zeolites are inorganic materials with large surface areas. Zeolites are defined by Smith in 1963 as “an aluminosilicate with a framework structure enclosing cavities occupied by large ions and water molecules, both of which have considerable freedom of movement, permitting ion-exchange and reversible dehydration” [3]. Zeolites have well-defined internal structures of uniform channels, channel intersections and/or cages. Water molecules and cations are located in those channel intersections and/or cages, with dimensions from approximately 0.2 to 1 nm [4]. Three-dimensional framework of interconnected SiO_4 and AlO_4 tetrahedra with linked channel systems and well-defined micro- and mesopores constitute the elementary building unit of zeolite. The aluminum ion is small enough to occupy the position in center of the tetrahedron of four oxygen atoms, and it is evident from this building principle that isomorphous replacement of and SiO_2 raises a negative charge in lattice. The net negative charge is compensated with exchangeable cation (sodium, calcium and potassium) [5, 6]. The backbone structure of zeolite was given in Figure 2.1 and the chemical composition of zeolite can be written as equation 2.1.

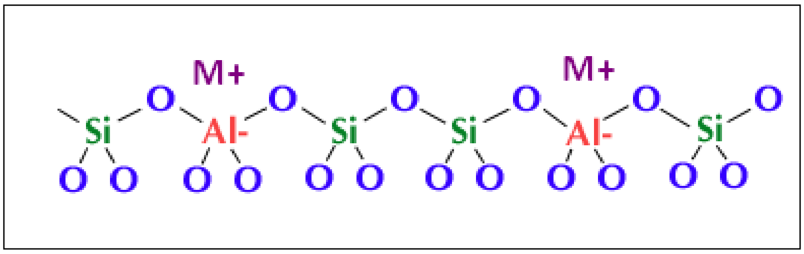


Figure 2.1. Structure of Zeolite

$$\_ \qquad (2.1)$$

Where M is cation with charge *m*, represents the framework composition. The sum of (x+y) gives the total number of tetrahedral in the unit cell. A negative charge is introduced into the framework with each tetrahedrally coordinated aluminum ion Al^{3+}. This charge need to be compensated by *x/m* M^{m+} cations. M^{m+} cations are electrostatically bounded to the host framework and mobile along the channels and w is the number of water molecules per unit cell [7].

The ratio x/y must be smaller than or equal to 1, since tetrahedral can only join to SiO_2 tetrahedra according to the Lowenstein rule. The amount of Al within the framework can change over a wide range, with Si/Al = 1 to ∞, the completely siliceous form being polymorphs of SiO_2. Lowenstein proposed that the lower limit of Si/Al = 1 of a zeolite framework arises because placement of adjacent AlO_4^- tetrahedra is not favored due to electrostatic repulsions between the negative charges. Therefore, Al-O-Al linkages in zeolitic frameworks are forbidden. The framework composition depends on the synthesis conditions. As the Si/Al ratio of the framework increases, the hydrothermal stability as well as the hydrophobicity increases [8].

The ratio of silicon/aluminum ratio in the lattice (n_{Si}/n_{Al}) can be represented as y/x, which is an important parameter for zeolites. The ratio of y/x indicates the content of mobile cations as well as the amount of acidic centers per unit cell [9]. Zeolites with low Silica/Alumina ratio are highly polar. Those types of zeolites are also potential ion conductors and excellent ion exchangers. Also, negatively charged the aluminum ions act as highly acidic sites by which zeolite can catalyze a number of chemical reactions [4].

Development of zeolite structure is shown in Figure 2.2. The primary building $(SiO_4)^{4-}$ tetrahedron and $(AlO_4)^{5-}$ tetrahedron are connected through their corners of sharing oxygen atoms and they form secondary building units. Then these secondary building units are connected and form wide polyhedras. Finally these polyhedras are connected directly or via secondary building units and form final zeolite structure.

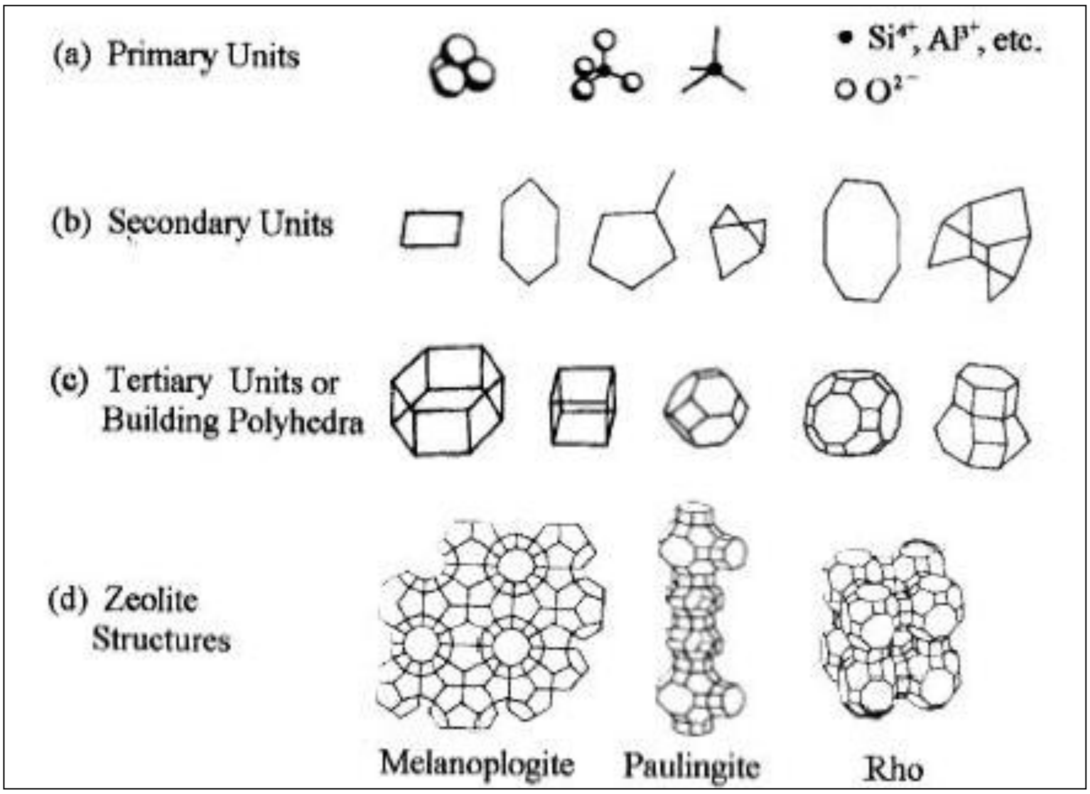
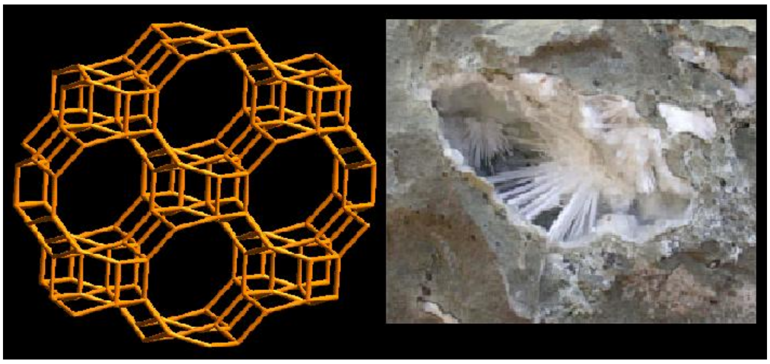


Figure 2.2. Development of zeolite structures [10]

While secondary building units are connected with each other; channels and interconnected voids are formed. According to synthesize temperature, the structural properties and the existing cation type in the structure, different pore or window diameters are formed. Pore openings are proportional with the number of atoms in the rings. For industrial applications, zeolites which have 8, 10, 12 rings in their framework structures are used. Their pore openings are 5, 6, 7 Å respectively. Meanwhile, zeolites having 4-6 rings in their structure have pore openings of 2.5 Å, those small pore openings allow passage of only very small molecules such as water. According to these pore openings molecular sieving ability of zeolites can be designated [11, 12].

The channel and cage systems form the main crystallographic characteristics of zeolites. Zeolite channels are classified according to the number of oxygen or central atoms of framework tetrahedra forming the window together with the geometrical dimensions of the two dimensional channel opening. Water content and/or cation species present in the channel system plays deterministic role on the sizes of the channel openings. Because of the unit cell dimensions are dependent on water content; the zeolite structures are quite flexible. But in some cases dehydration can lead to a collapse of the channel system. For example if laumontite is exposed to dehydration channel system of the zeolite collapses [13].



2.3. Laumontite [14]

For the zeolite properties zeolite cages are important as much as the channel systems. Zeolite channels can accept as a cage of infinite length and cages are voids with higher radius than the channel system. The only access to the cages can be achieved through the channel systems. Molecules bigger than channel system (formed within the cages or remaining from the synthesis process) are stuck in these cages. In some zeolites at the crossing of the channel systems supercages are emerged, such as FAU-type zeolites. In FAU-type zeolites they are formed by the tetrahedral crossing of channel systems [15].

2.2. TYPES OF ZEOLITES

Today zeolites include great number of natural and synthetic minerals with common structural characteristics. There are approximately 200 types of zeolites; about 40 of which occur naturally in the environment, and more than 150 zeolite types have been synthesized. Many of them occur naturally as minerals, and are extensively mined in many parts of the world including Turkey, USA... Others are synthetic, and are made commercially for specific uses, or produced for researches. Their primary differences are in their crystal structure, chemical composition and purity. Also, particle density, cation selectivity, molecular pore size and strength are some of the other differences between zeolites.

2.2.1. Natural Zeolites

Natural zeolites' unique properties, like cation exchange capacity and its multichannel structure, were recognized many years ago. Natural zeolites are formed with the chemical reaction of salt water and volcanic ash. Either by volcanic explosions that distributed enormous amounts of ash aluminosilicates created from alkali and alkali earths to the meteoric water in vicinity of volcano, or by the alteration of wind-blown volcanic dust accumulated in saline lakes. In both cases Figure 2.4 shows the formation of natural zeolites.

Generally for synthesis of high Si/Al ratio zeolites structure directing agents are used. Because of the absence of organic structure directing agents in nature; most natural zeolites occur at lower Si/Al ratios. Even it is difficult to make large crystals in the laboratory, sometimes natural zeolites are found as large single crystals in the nature [8].

Zeolites are sorted out according to difference in their chemical compositions, the size and arrangement of their crystal structures. The most common natural occurring zeolites are mordenite, cliniptilolite, chabaxite, erionite and phillipsite [4].

Clinoptilolite (HEU) and mordenite (MOR) are two natural zeolites that find extensive use for ion-exchange (radioactive), agricultural uses and as sorbents. Inspite of natural zeolites are cheap and abundant they contain impurities and they have low surface area. These undesired properties limit the usage of natural zeolites for catalytic activity. Erionite is another natural occurring zeolite which is toxic then the most potent forms of asbestos. It cause to formation of lung mesothelioma [8].

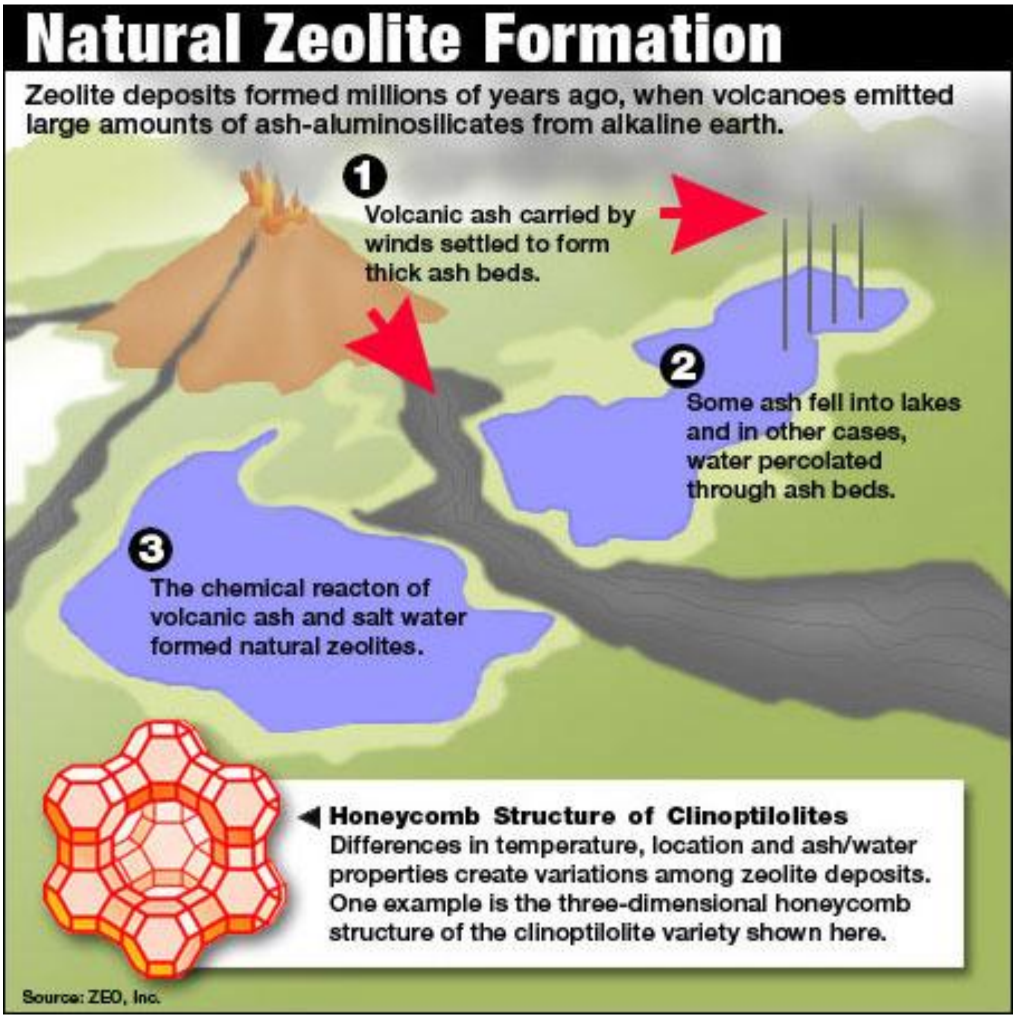


Figure 2.4. Natural zeolite formation [16]

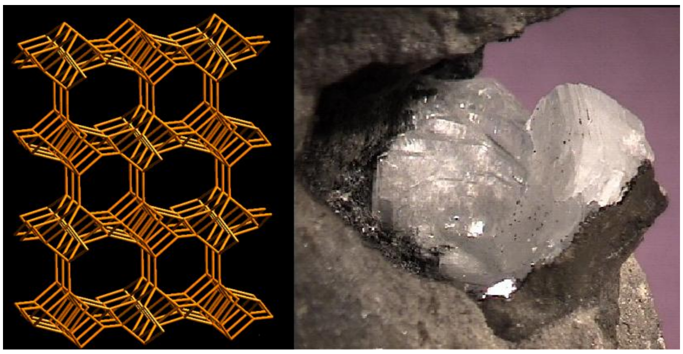
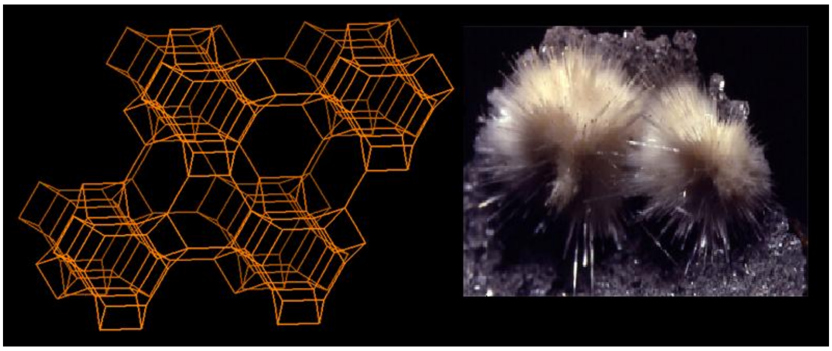


Figure 2.5. Clinoptilolite [14]

2.6. Mordenite [14]



2.7. Erionite [14]

2.2.2. Synthetic Zeolites

However, some zeolites has occurred naturally, a significant amount of them are synthesized for commercial use or created by scientists that study their chemistry. Although natural zeolites occur in large amount with no effort, they represent only limited atomic structures and properties. Synthetic zeolites have a wider range of properties and larger cavities than natural zeolites. Like natural zeolites, synthetic zeolites are also microporous crystalline aluminosilicate materials, with alkali or alkaline earth metals as counterions. As pictorially represented in Figure 2.8, they consist of a framework of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra, linked to each other at the corners by sharing their oxygens.

In Figure 2.8 the alkali metal cation is represented with M^+. The uniform channels and cages or cavities are formed by joining those tetrahedra and three-dimensional network structures are created. In zeolites that containing aluminum, negative charges generated from the difference in charge between the $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedral is balanced by cations M^+. The cations and water molecules present are located in the cages (cavities) and channels. These cations can be exchanged with metal ions via ion exchange [17].

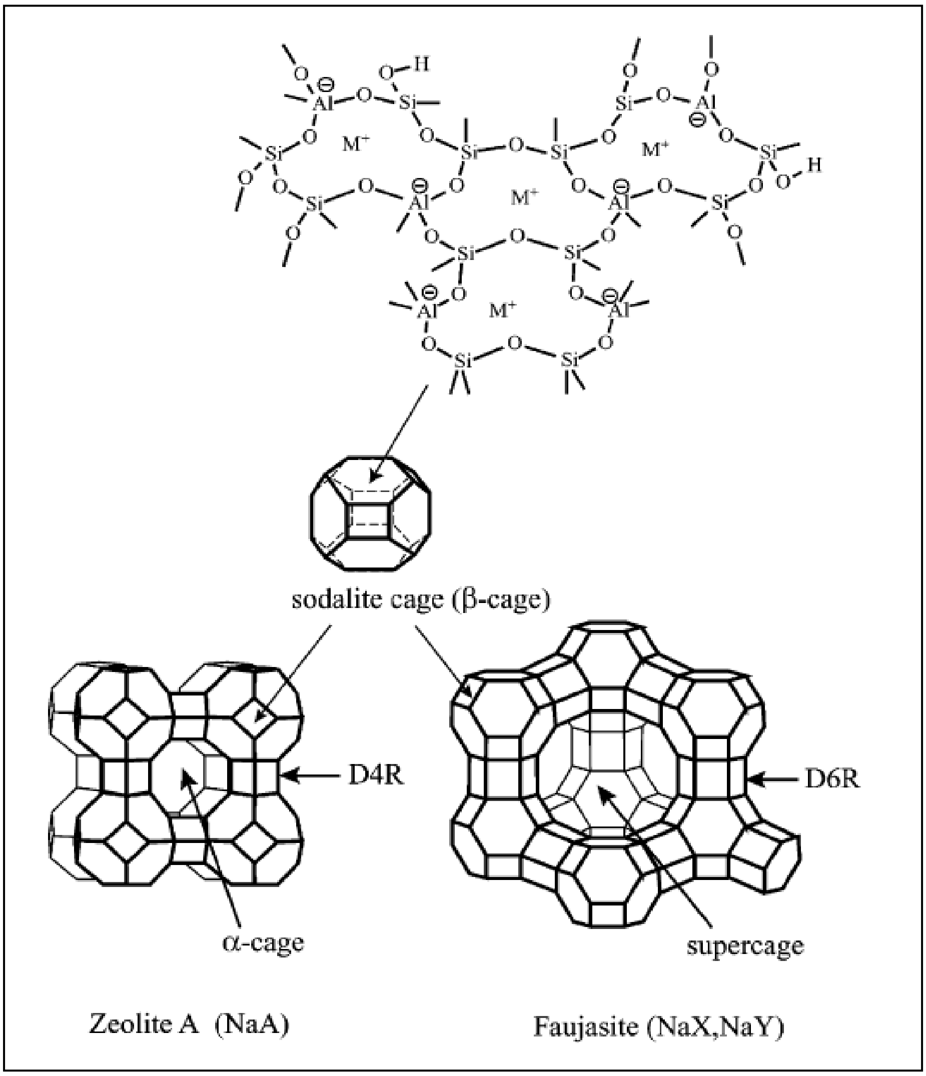


Figure 2.8. Framework structure of sodalite-based zeolites [17]

In 1930s the first zeolites were synthesized, but mass productions and the synthetic zeolite market did not develop until the 1960s. The large scale synthesis was performed for catalytic cracking in petroleum refining. More than 150 zeolites were synthesized by researchers in order to develop more efficient catalysts, thus zeolite catalysts had a huge contribution to the petroleum and old petrochemical industry. After zeolites began to displace phosphates in detergents in 1970s, demand for zeolites was dramatically increased [18].

In comparison with natural zeolites synthetic zeolites; are produced by consuming energy and chemicals while natural zeolites are formed spontaneously from natural rocks that contain minerals in it. Natural zeolites which have higher ratio of silica alumina which keeps the structure together, have more acid resistance than synthetic zeolites. However synthetic zeolites are more advantageous because; they have high purity, uniform pore size, and better ion-exchange abilities while natural zeolites always contain impurities and their chemical compositions varies from one sample to another. The new structures and compositions, and hence, the new physicochemical properties, enlarge the range of applications of zeolites [19, 20]

2.3. ZEOLITE SYNTHESIS

Zeolites can only be synthesized hydrothermally. Zeolites contain silica, alumina, and/or phosphorus species, metal cations, organic molecules, and water, which are converted into microporous crystalline zeolites via an alkaline supersaturated solution. This chemical process is named as zeolitization. In order to achieve a high yield of crystallization in an acceptable period of time zeolitization process is usually carried out at elevated temperatures [21].

Zeolites are generally synthesized by mixing aluminate and silicate solutions, usually with the formation of a gel, and by maintaining the mixture about temperatures of 100°C or more [22]. The synthesis process of zeolites consists of mixing of reactants, which is followed by nucleation and crystal growth steps. Figure 2.9 represents the formation steps of zeolite crystals.

Previously, the synthesis method for zeolite was based on mimicking the zeolite formation in nature which was following up the hydrothermal conditions to crystallize zeolite from aqueous gels composed of silica and aluminum sources in alkaline solutions [24]. This approach continued until the crystallization mechanisms and controlling factors are understood [25]. Then approximately 150 types of zeolites are improved. Zeolites can be classified in three groups according to their silica aluminum ratio. Those are high silica, intermediate silica and low silica zeolites.

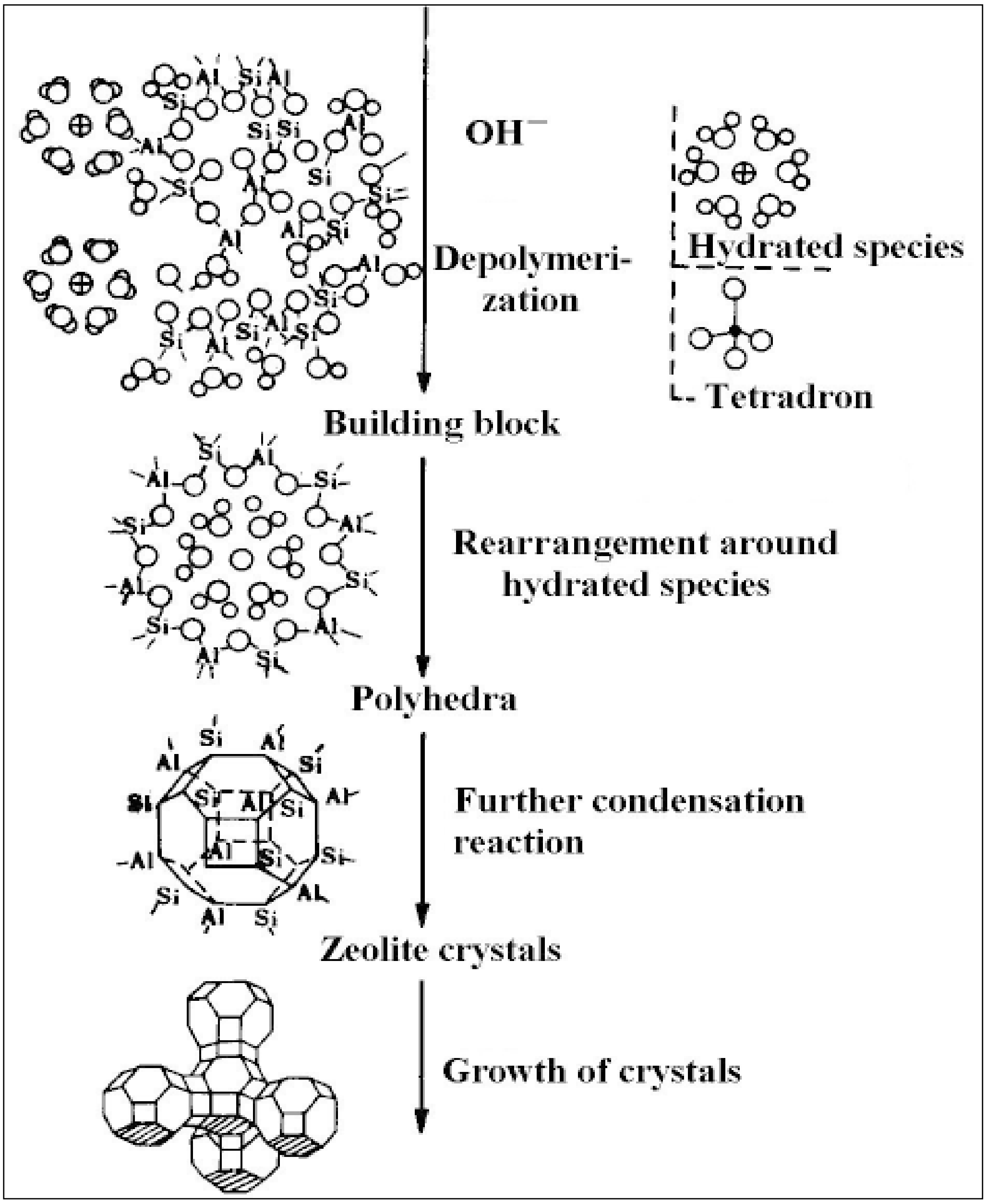


Figure 2.9. Schematic illustration of the solution-mediated transport [23]

The framework composition of the zeolite is controlled by the silica to alumina ratio. The Si/Al ratio of the zeolite affects the hydrophobic or hydrophilic nature of the zeolite. Al is a hydrophilic element where Si is hydrophobic. This ratio also affects the acid resistance of the zeolite. High aluminum content gives acidic sites, that acidic site makes zeolite useful for many applications. For catalytic cracking and isomerization applications high silica zeolites are useful. Acid resistance and thermal stability of zeolite are inversely proportional aluminum content. These effects can be summarized as follows: [26]

Increasing the silica / alumina ratio affects following physical properties of the zeolite [23]:

- Increases acid resistance.
- Increases thermal stability.
- Increases hydrophobicity.
- Decreases affinity for polar adsorbents.
- Decreases cation content.

Decreasing the silica / alumina ratio affects following physical properties of the zeolite:

- Increases hydrophilicity.
- Increases cation exchange properties [26].

Synthetic zeolites were firstly contain low silica or alumina rich zeolites The earliest synthetic zeolites were essentially Al-rich or had a low Si to Al ratio and their Al concentrations were equivalent to Si, however, Al-O-Al linkages have not been observed in zeolites [21]. Loewenstein was the first researcher who rationalized the absence of Al-O-Al linkages in tectoaluminosilicates on the basis of clusters of negative charges [27]. Thus, if Al-atoms do not have Al-atoms in their second coordination sphere, then $Si/Al \geq 1$. Low-silica zeolites are defined as having $1 \leq Si/Al < 2$, while intermediate Si/Al zeolites contain $2 < Si/Al \leq 5$. In the late 1960s, and early 1970s high-silica zeolites with $Si/Al > 5$ were synthesized. As the Si/Al ratio increases, the properties of zeolites are significantly altered. At higher Si/Al ratios, the crystals are hydrophobic. In the early 1980s, silica-free molecular sieves, which were based on the aluminophosphate compositions, were developed [28, 29].

Table 2.1. Some Zeolite Types according to Si/Al Ratio [8]

Low Si/Al ratio (1 to 1.5)	A,X
Intermediate Si/Al ratio (2 to 5) A) Natural Zeolites B) Synthetic Zeolites	 Erionite, Chabazite, Clinoptilolite, Mordenite Y, L, Large pore mordenite, omega
High Si/Al Zeolites (10 to 100) A) By thermochemical framework modification B) By direct synthesis	 Highly siliceous variants of Y, Mordenite, Erionite ZSM-5
Silica Molecular Sieves (Si/Al ratio = ∞)	Silicalite

2.3.1. Low Silica Zeolites

Low silica zeolites have silica alumina ratio of approximately 1. Zeolites A and X are examples for low silica zeolites. In the framework composition with a molar ratio of Si/Al ≈ 1 both zeolites are nearly saturated with aluminum. It is the maximum possible aluminum content for zeolite frameworks. With this highest aluminum tetrahedral they contain maximum number of balancing exchangeable cation capacity. Due to the cationic charges in the aluminosilicate framework which results in high field gradients, the most highly heterogeneous surface among porous materials is zeolites. Because of low silica zeolites surfaces are highly selective for water, polar and polarizable molecules; they can be used especially in drying and purification [30].

2.3.1.1. Zeolite A

Zeolite A has the framework structure of LTA (Linde Type A). Silica to alumina ratio of zeolite A is 1. It is made of sodalite (SOD) cages linked via double four membered rings (D4R). While four SOD cages and four D4R are connected eight member oxygen ring forms a pore which has a small diameter of 4.2Å [31, 32]. Its three dimensional pore structure runs perpendicular to each other in the x, y, and z directions. Channel systems which are connected to each other allow motion of the Na^+ ions and water molecules. The SOD units connected through double 4-membered ring (D4R) of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ to produce an α-cage with a diameter of 11.4 Å (the large cavity in the center of the structure). The cavity is surrounded by eight sodalite cages (truncated octahedra) connected by their square faces in a cubic structure. The unit cell is cubic; length of the unit cell is 24.61Å. Zeolite A has a void volume fraction of 0.47 and thermally decomposes at 700ºC [33]. Its chemical formula is $|Na^+_{12}\ (H_2O)_{27}|_8\ [Al_{12}Si_{12}\ O_{48}]_8$. Figure 2.10 shows the framework structure of zeolite A.

Just like other zeolites, Zeolite A is also synthesized in hydrothermal process. In basic aqueous solution alumina (usually sodium aluminate) and silica (usually sodium silicate) solutions are mixed to form a gel. NaOH can be used as the alkali agent or a solution quaternary ammonium salts, amines or other polar organics. Crystallization occurs between 70-300^{o}C therefore the gel is then heated to 70-300^{o}C. The zeolite is normally synthesized in the Na^+ form [34].

Zeolite A was the first zeolite to be synthesized on a large scale because of its industrial importance [9]. Since zeolite A has a very good ion-exchange capability, it is one of the most widely used zeolites [35]. Zeolite A is widely used in daily life due to their crystal structure and chemical resistance. It has a lot of application area such as sorbents (gases, liquids), cation exchangers (detergents), molecular sieves (membranes for small molecules) and catalysts in many industrial processes (e.g. oil hydrocracking). Zeolite A has an inner cavity is small enough to let specific structures can get into the cavity for reaction but also large enough to take place structure changing reactions. One of these type of reactions that zeolite A is used is paraffin cracking. Linear paraffins can enter from the small entry pores and within the supercage (alpha-cage) cracking can occur to produce smaller chain alkanes [36].

The loosely bounded extra-frameworks cations in zeolite A such as Na^+ are willing to exchange with other types of metal ions in aqueous solutions. Ca^{2+} and Mg^{2+} ions in the city water deactivate soap powder or detergent and causes inefficient cleaning. Thus detergent producers were using phosphate in order to soften water. After it was understood that, phosphate compounds are harmful for aquatic life; zeolites were started to use in detergent industry. Zeolites with exchangeable cations of sodium or potassium prefer to exchange out of the zeolite and are replaced by the "hard" calcium and magnesium ions in the water. In powder detergents and soaps mainly synthetic alumosilicate zeolites Linde Type A (Na -LTA) and Na-gismondine have been applied for these purposes. The zeolite materials are removed from the laundry by the washing process itself and are harmless to environment. As a result of these properties the largest use of zeolite A consumption is in the European detergent market of around 650,000 tons annually [37]. Typical concentrations of zeolite A in laundry detergents is in range from weight per cent of 20 to 34. LTA is also applied in water treatment for households and in water treatment plants for the removal of heavy metal ions from solution by ion exchange [15].

Zeolite A is a nontoxic safe compound. *In-vivo* tests on rats and mice did not indicate toxicity. Synthetic zeolite A is not absorbed in the diet by oral application and is not toxic to metazoan [36].

2.3.1.2. Zeolite X

Zeolite X has the same framework structure with naturally occurring mineral faujasite but silica alumina ratio was changed and zeolite X synthesized as aluminum rich. The unit cell is cubic and it is made of sodalite cages or β-cage which is the 14-hedron with 24 vertices. The sodalite cages are connected with oxygen atoms from the hexahedron faces of cage and form double six-rings (D6Rs, hexagonal prisms). As a consequence of these connections, supercage (large cavity) of faujasite zeolite which is accessible in three dimensions through 12-ring (24-membered) windows is formed. The supercage has a diameter of 11.8 Å and 12-membered rings has an open diameter of 7.4 Å [37]. Corners of these polyhedra are occupied by the Si and Al atoms. Each silicon and aluminum atoms are connected with oxygen atoms, and they are in the midpoint of these atoms. But to give near-tetrahedral angles oxygen atoms are displaced those midpoints through silicon and aluminum. Sodalite cage and supercage share single six-rings (S6Rs), and those S6Rs are entrance to the sodalite unit. Each unit cell has eight sodalite units, one supercage, 16 D6Rs, 16 12-rings, and 32 S6Rs. Figure 2.11 shows the framework structure of zeolite X. The negative charge of the aluminosilicate framework is balanced with exchangeable cations and these cations are found within the zeolite cavities. Zeolites X framework has a

Si/Al ratio between 1.0 < Si/Al < 1.5. No faujasite with Si/Al ratio of less than 1.0 has been prepared because of the unstable framework structure (Lowenstein's rule [27]). The void fraction for zeolite NaX is 0.50 and for NaY is 0.48. Zeolite X thermally decomposes at 793 ^{o}C [37]. Zeolite X has the chemical formula of $|Na_{88}(H_2O)_{220}|$ **[**$Si_{104}Al_{88}O_{384}$**]** [38,39] .

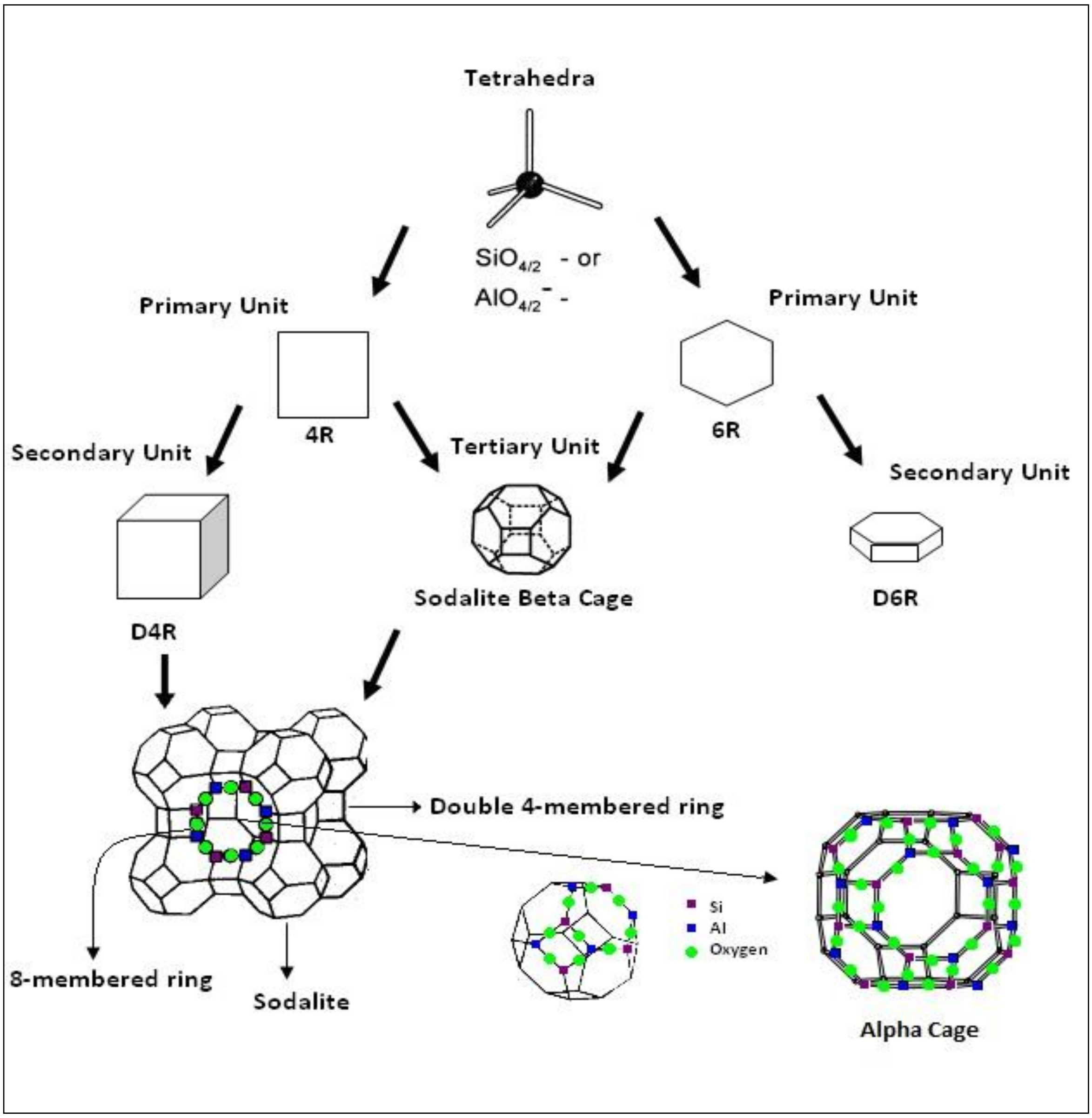


Figure 2.10. Framework structure of zeolite A [14]

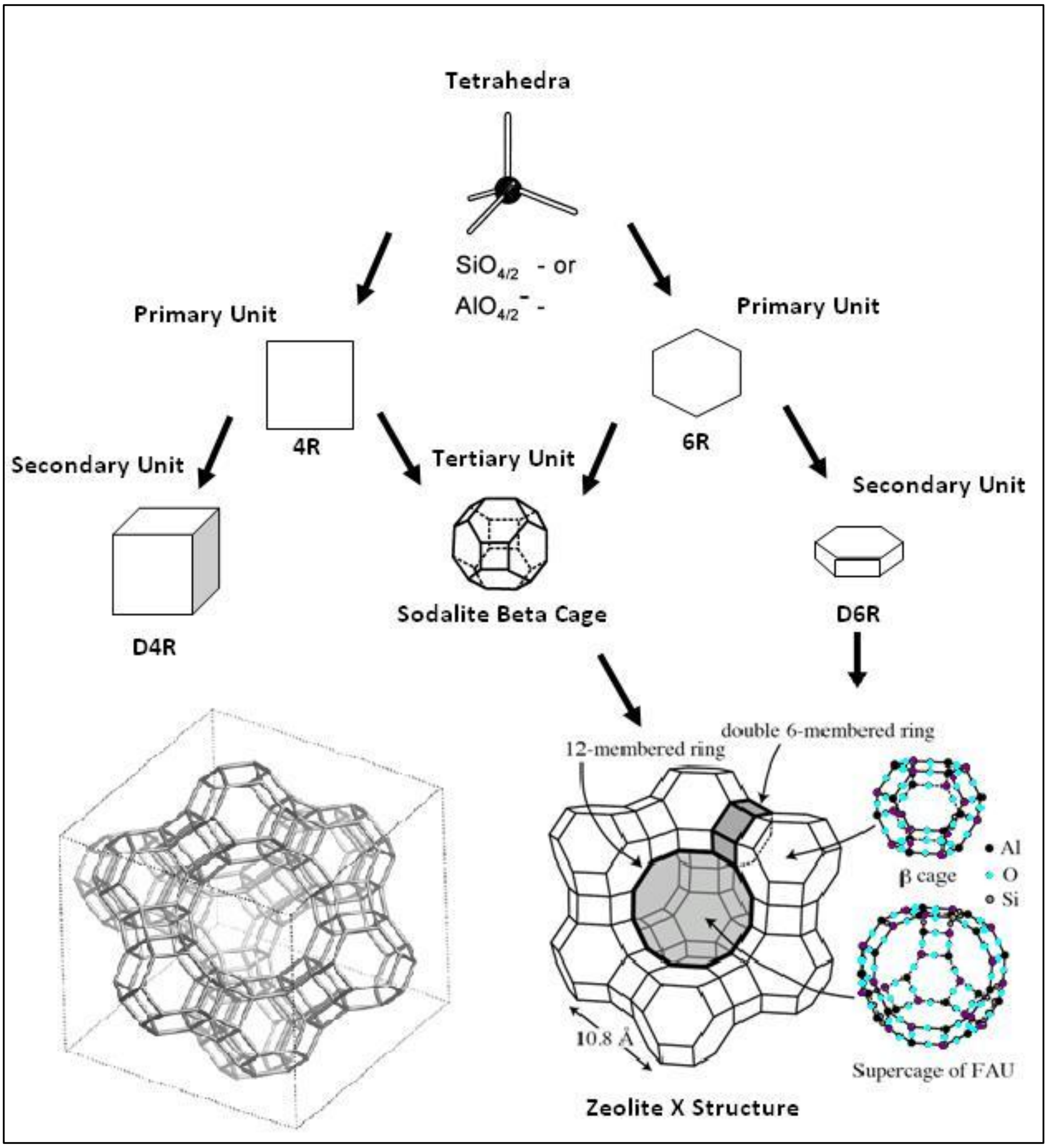


Figure 2.11. Framework structure of zeolite X [14]

2.3.2. Intermediate Silica Zeolites

This type of zeolites have silica alumina ratio between 2 and 5. Thus low silica zeolites accommodate high amount of tetrahedral aluminum in the zeolite frameworks, they provide a site of instability for attack by acid and water vapor of steam which make synthetic low silica zeolites less stable than their natural equipollents, which have superior stability characteristics reflecting higher Si/Al molar ratio of 3-5. Therefore, in order to upgrade stability characteristics against heat and acids, zeolites with higher content of silicon were synthesized in 1950s [37].

Zeolite Y which is one of the intermediate silica zeolites was started to use commercially in 1959. Zeolite Y has a Si/Al ratio from 1.5 to 3.0 and is used as molecular sieves. The framework topology of zeolite Y is just like a natural faujasite zeolite and low silica zeolite X [37]. Synthesizing zeolite Y with more aluminum according to zeolite X has improved the stability. This alteration in composition made zeolite Y based catalyst valuable for hydrocarbon conversion [1].

In the early 1960's an analogue of natural mineral mordenite with ratio Si/Al ≈ 5 was synthesized with larger pore size. This difference between the pore sizes of natural and synthetic mordenite brings new properties to the synthetic one. Synthetic mordenite with larger pore size can accept cations or molecules larger than 4.5Å, while natural mordenite can accept only molecules with diameter less than 4.2 Å. Moreover synthetic ones with NH_4^+- and H-exchanged forms can crack significant amounts of hexane at 450 °C while small pore natural mordenite cannot. In the early 1950's Type L zeolites with a Si/Al = 3.0 were discovered. They were adapted as commercial catalysts in selective hydrocarbon conversion reactions with their unique framework topology [40].

2.3.3. High Silica Zeolites

High silica zeolites have a silica alumina ratio between 5 and 100. Zeolite beta and ZMS-5 are the best known zeolites that have high silica alumina ratio. Zeolite Beta has a structure type of BEA and its formula is and ZMS-5 has a structure type of MFI with the chemical formula is , where n < 27. Figure 2.12 shows the framework structures of Zeolite Beta and ZMS-5. Left hand side of the figure shows BEA structure (for zeolite beta) and right hand side shows the MFI structure (for ZMS-5) [41]. Porous crystals represent heterogeneous hydrophilic surfaces; in contrast to low and intermediate Si/Al zeolites, high silica zeolites are more homogeneous with an organophilic-hydrophobic selectivity. They strongly adsorb organic molecules with low polarity while interacting weakly with water and other polar molecules. In addition to this extraordinary surface selectivity, small amount of aluminum in the framework provide cation exchange sites to the high silica zeolites. With this cation exchange ability, the introduction of acidic OH^- groups via the well-known zeolite ion exchange reactions which is essential to the development of acid hydrocarbon catalysis properties is obtained [1].

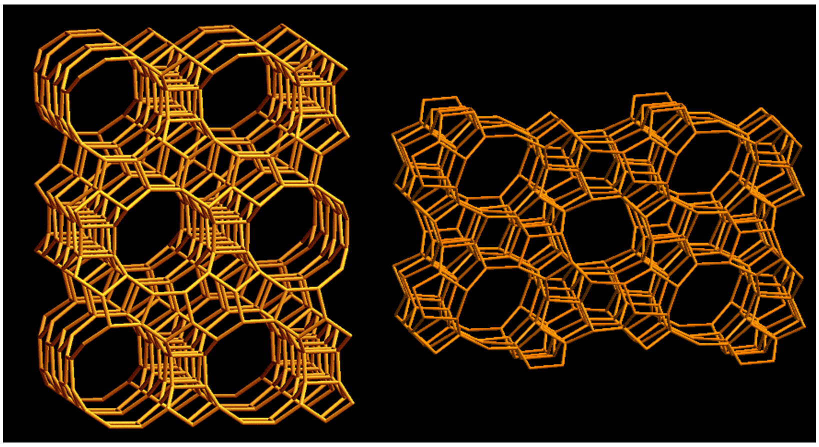


Figure 2.12. Framework structures of Zeolite Beta and ZMS-5 [14]

2.4. ZEOLITE CHARACTERIZATION

2.4.1. X-Ray Powder Diffraction

One of the most common methods to identify the zeolite structure and purity is X-ray powder diffraction [42]. X-ray tube, a sample holder and X-ray detector are the three basic components of the X-ray diffractometers. Tungsten filament in the X-ray tube is heated to speed up the electrons. Electrons accelerate in the electric field toward anode metal target which is a water cooled block of commonly Cu, but it can be also Ag, Mo, Mg Fe, Cr, containing target metal. Due to the electrons high energy, when they hit to the target anode an inner shell electron or target anode can scatter. Therefore an outer shell electron fills the gap produced by scattering and emits its extra energy as X-ray photon, by this way characteristic X-rays are produced. Figure 2.13 shows the cross section of sealed-off filament X-ray tube and the emergence of X-rays. The wavelength of these X-rays is characteristic of that target (Ag, Mo, Mg Fe, Cr) [43-45].

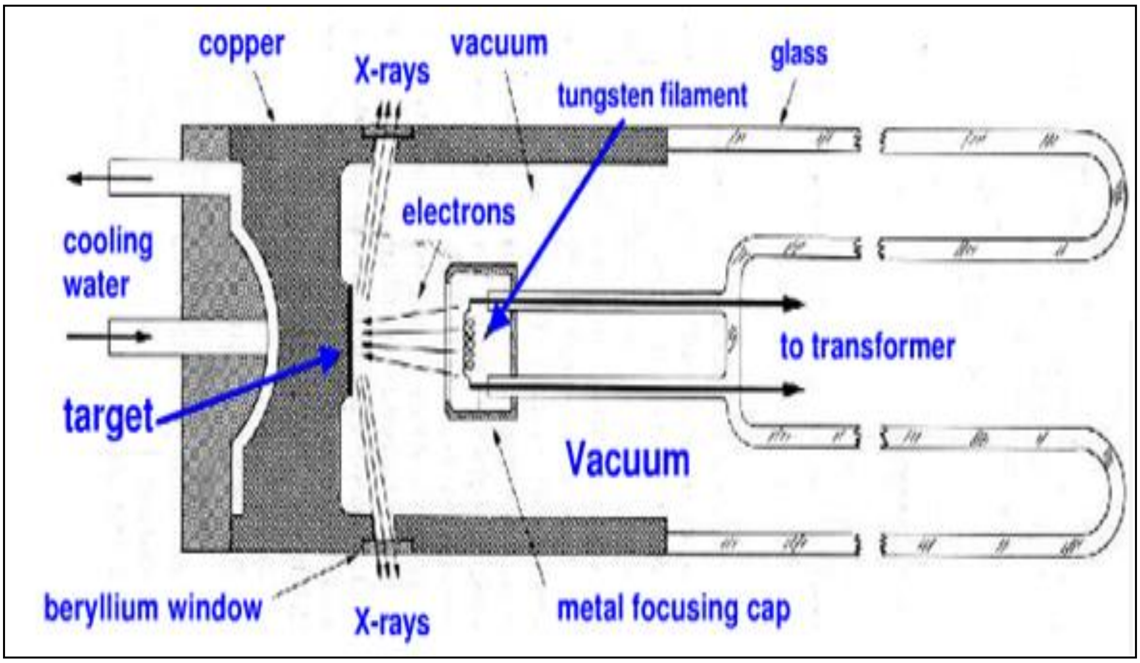


Figure 2.13. Cross section of the X-ray tube and the emergence of X-ray

Then these X-rays are collimated and directed onto a sample. The sample must be ground to a fine powder. The size of the particles must be between 0.1µm and 40 µm. If the particles are less than 0.1µm then peaks would broad, and if particles are bigger than 40 µm then less diffraction will occur. When X-ray photons collide with sample (electrons), some photons from the incident beam changes their directions where they originally travel. During the analysis sample and detector are rotated and the intensity of the reflected X-rays is recorded. Scattered X-ray signals are, then processed either by a printer or computer, converted into a count rate. Figure2.14 shows the process and Figure 2.15 is an X-ray diffraction instrument [43-45].

XRD is a nondestructive technique. It is used to identify crystalline phases and orientation of the materials. Also it determines structural properties such as; lattice parameters, strain, grain size, preferred orientation etc. X-ray diffractometer also measures thickness of thin films and multi-layers. Moreover it determines atomic arrangement of materials [44].

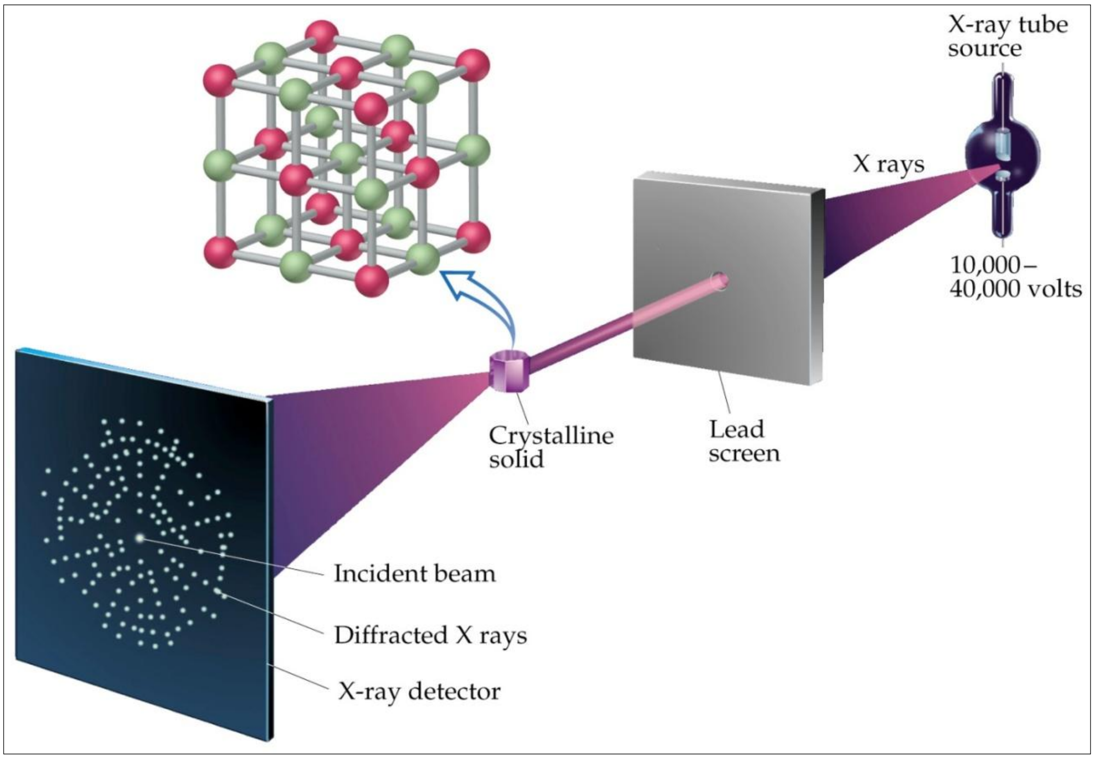


Figure 2.14. X-ray Diffraction Process

Figure 2.15. Bruker's X-ray Diffraction instrument [43]

2.4.2. Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) is the method of choice for determining the size and morphology of zeolite crystallites. Rather than light SEM uses electrons to form the image. There are three main components of SEM which are; optical column, sample chamber and computer screens for imaging. Electron gun at the top of the optical column produces a beam of electron. Electron beam is transferred vertically through the column which is vacuumed. When SEM is using, the electron-optical column and sample chamber must always be at a vacuum. There are two reasons for this situation. First if the column is in a gas filled environment, electrons will be scattered by gas molecules which would lead to reduction of the beam intensity and stability. Secondly other gas molecules, which could come from the sample or the microscope itself, could form compounds and condense on the sample. This would lower the contrast and obscure detail in the image. The electron beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. When the electron beam hits to the sample electrons and X-rays are scattered from the sample [46, 47]. Figure 2.16 shows the emission and Figure 2.17 shows the column of SEM.

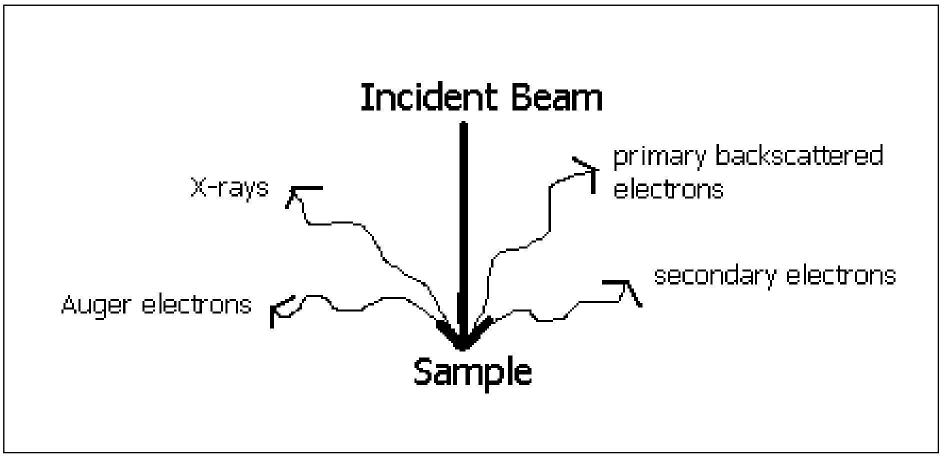


Figure 2. 16. Emmition of Electrons and X-rays when an electron beam hits to the sample [46]

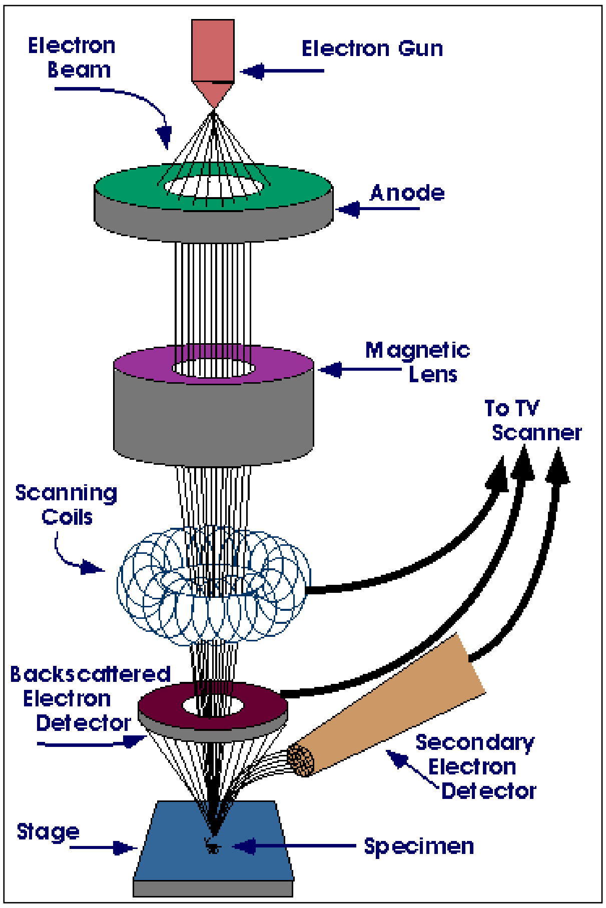


Figure 2. 17. Optical Column of the SEM [46]

Detectors in the column collect these X-rays, backscattered electrons, and secondary electrons emitted from the sample and convert them into a signal. Those signals are sent to a computer screen and final image is produced [46, 47].

In comparison to optical microscope the scanning electron microscope has many advantages. Optical microscopes use light where SEMs use electron which produce largely magnified images. SEM has a large depth of field that allows more of a specimen to be in focus at one time. The closely spaced features can be examined at a high magnification due to SEM produces images of higher resolution. Because the SEM uses electromagnets rather than lenses, the researcher has much more control in the degree of magnification [46, 47].

Figure 2.18. SEM Instrument

2.5. PROPERTIES AND APPLICATIONS OF ZEOLITES

2.5.1. Properties of Zeolites

According to silica amount in the structure thermal stability of zeolites varies over a large temperature range. Low-silica zeolites have the decomposition temperature about 700^{o}C, whereas such as silicalite, completely siliceous zeolites are stable up to 1300^{o}C. In the acidic medium low-silica zeolites are unstable, while high-silica zeolites are stable in boiling mineral acids but unstable in basic solution. Low-silica zeolites tend to have structures with 4, 6, and 8 member rings; meanwhile high siliceous zeolites contain 5 member rings. Low-silica zeolites have affinity to water (hydrophilic), whereas high-silica zeolites are tending to repel water (hydrophobic) and the transition occurs around Si/Al ratios of ~10 [8].

Silica alumina ratio in the structure of zeolites significantly varies cation concentration, siting, and exchange selectivity and these variations play an important role in adsorption, catalysis, and ion-exchange applications. Increment in Si/Al ratio decreases acid site concentration, whereas the acid strength and proton activity coefficients increase with decreasing aluminum content. Another very important property for zeolite characterization is internal surface area. These highly accessible internal surface areas of zeolites can compose more than 98 per cent of the total surface area. Internal surface areas vary between 300 to 700 m^2 per grams of zeolite [8].

2.5.2. Applications of Zeolites

The pore structures and the chemistry of the framework are both deterministic for the properties of porous materials. Increasing demand for materials with highly chemical and physical properties as such in zeolites has inspired scientists to make new porous materials with unique structures [48-50].

Zeolites have very wide range of application area in almost all fields of human life where chemical; biochemical and physicochemical processes are taking place. They can be used, for storing of molecules, for sieving and filtering, for ion exchange purposes and also for catalysis under nonoxidizing and oxidizing environment [51].

Zeolites unique crystal structure, which allows acting as molecular sieves, selectively sorts molecules based on particle sizes; therefore they are used to purify gaseous and liquid mixtures and solutions by sorption. Zeolites have a lot of application areas such as; they are used in the United States in pet litter, animal feed, horticulture (fertilizers and soil additives), laundry detergent and water purification [18].

In 1970's, as it was mentioned before in Section 2.3.3.1, in laundry detergent powders zeolites took the place of the phosphate compounds because of the environmental issues. About 80 per cent of the total zeolite production is consumed in laundry detergents and the current world production of synthetic zeolites is estimated to be about 1.4 million metric tons per year. In recent times, synthetic zeolites were begun to use as a hemostatic agent by the U.S. military to promote clotting of traumatic wounds. Furthermore, synthetic zeolites that are ion exchanged with copper and silver are being used as antimicrobial agents in dust masks, plastic films and paints. Also paints include heavy metal ion exchanged zeolites (antifouling paints) are used on undersea surfaces of boats to prevent or counteract the buildup of barnacles on these surfaces [18].

Although zeolites have various application areas such as; water treatment [6, 52-54], dentistry [55], space craft technology [56], food packing [57], hydrogen storage [58], gas sensors [59], cosmetics [60] etc. they are extensively used primarily in three applications as: adsorbents, catalysts and ion exchangers [8].

2.5.2.1. Zeolite in Adsorption

Zeolites adsorption property is based on the molecular sieving ability. Molecular sieving ability is the ability of preferentially adsorbing certain molecules, while excluding others (Figure 2.19). Molecules with smaller diameter then the pore size of the zeolites passes through the pores while molecules with higher diameter than the zeolite pore size are not able to pass, by this was molecules with larger diameter size are effectively sieved. This "sieve" effect can be utilized to produce sharp separations of molecules by size and shape [60].

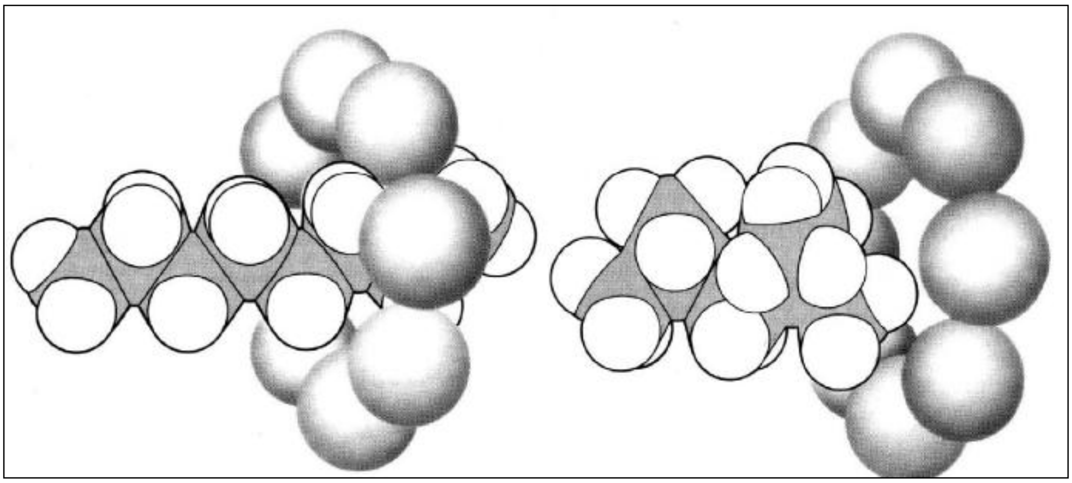


Figure 2.19. Zeolite molecular sieving property

As mentioned above in molecular adsorption uses the basic principle is the shape-selective property of zeolites but in other cases different types of molecules enter the zeolite, but some diffuse through the channels more quickly while leaving others stuck behind. Purification of para-xylene by the zeolites X or Y is an example for this case of adsorption [1, 51].

Due to the high affinity for water, cation containing zeolites are commonly used as desiccants. Also as a result of the ability to differentiate molecules on the basis of electrostatic interactions with metal ions they are used in gas separation. Moreover, silica zeolites which are hydrophobic adsorb organic solvents. Therefore, for the separation of molecules based on the difference of size, shape and polarity zeolites are widely used [61].

The particular affinity a species has for an internal zeolite cavity depends on electronic considerations. The strong electrostatic field within a zeolite cavity results in very strong interaction with polar molecules such as water. Non-polar molecules are also strongly adsorbed due to the polarizing power of these electric fields. Thus, excellent separations can be achieved by zeolites even when no steric hindrance occurs [62].

Adsorption based on molecular sieving, electrostatic fields, and polarizability are always reversible in theory and usually reversible in practice. This allows the zeolite to be reused many times, cycling between adsorption and desorption. This accounts for the considerable economic value of zeolite in adsorptive applications [62].

Resulting from the hydrophilic properties, cation-containing zeolites are extensively used as desiccants. On the bases of electrostatic interactions of cations with metal ions molecules can be differentiated in gas separation. On the other hand organic solvents can be adsorbed by using hydrophobic silica zeolites [51].

2.5.2.2. Zeolite in Catalyst Applications

Zeolites can act as a catalyst in chemical reactions. The chemical reaction takes place in the internal cavities of zeolites, which allows a greater degree of product control [63]. They can be used as acid catalysts, oxidation catalyst and be supports for active metals or reagents. Especially three properties of zeolites make them usable as catalyst, which are;

- Their exchangeable cations allow other cations (especially metals), with various catalytic properties, to introduce into their intracrystalline pore system.
- When exchangeable cations exchanged with H^+, zeolites can have very high number of very strong acidic sites.

- Zeolites have less than 1nm of pore diameters and they are with one or more discrete size [64, 65]. Zeolites are crystalline porous materials with pore dimensions in the same order as the dimensions of simple molecules; hence they possess molecular sieving properties when the shape and size of a particular pore system exert a steric influence on the reaction, controlling the access of reactants and products.

In other words third property can explain as; the shape and size of the pore system determines which reactions can take place in a particular zeolite. Zeolites are therefore said to act as 'shape-selective' catalysts. Figure 2.20.a shows reactant shape selectivity. Branched-chained hydrocarbons are the wrong shape to enter the zeolite pores. Only straight-chained hydrocarbons can enter and react to form smaller hydrocarbon fragments. These smaller fragments can easily leave the zeolite. Figure 2.20.b shows product shape selectivity. Reactants enter the zeolite and react to produce a mixture of products. Only one of these, 1,4-methylbenzene, can diffuse out of the zeolite channels. The other isomers are the wrong shape to leave the pores and so are trapped inside until they react to form the molecule, which can then leave.

Due to ease of recoverability and recyclability, low cost, leading to less waste and fewer byproducts and fewer impurities, often higher activity, higher efficiency, affording higher selectivity, ability to combine several catalytic steps, and reduce environment pollution by substitution of homogeneous catalysts used in the traditional chemical industry, make zeolites advantageous as catalyst [1, 66].

Environmentally unfriendly catalysts are replaced with zeolites in production of petrochemicals. Zeolites are non-hazardous and regenerable converse to more hazardous acid catalysts that have been used in the past such as solid phosphoric acid or hydrofluoric acid [65].

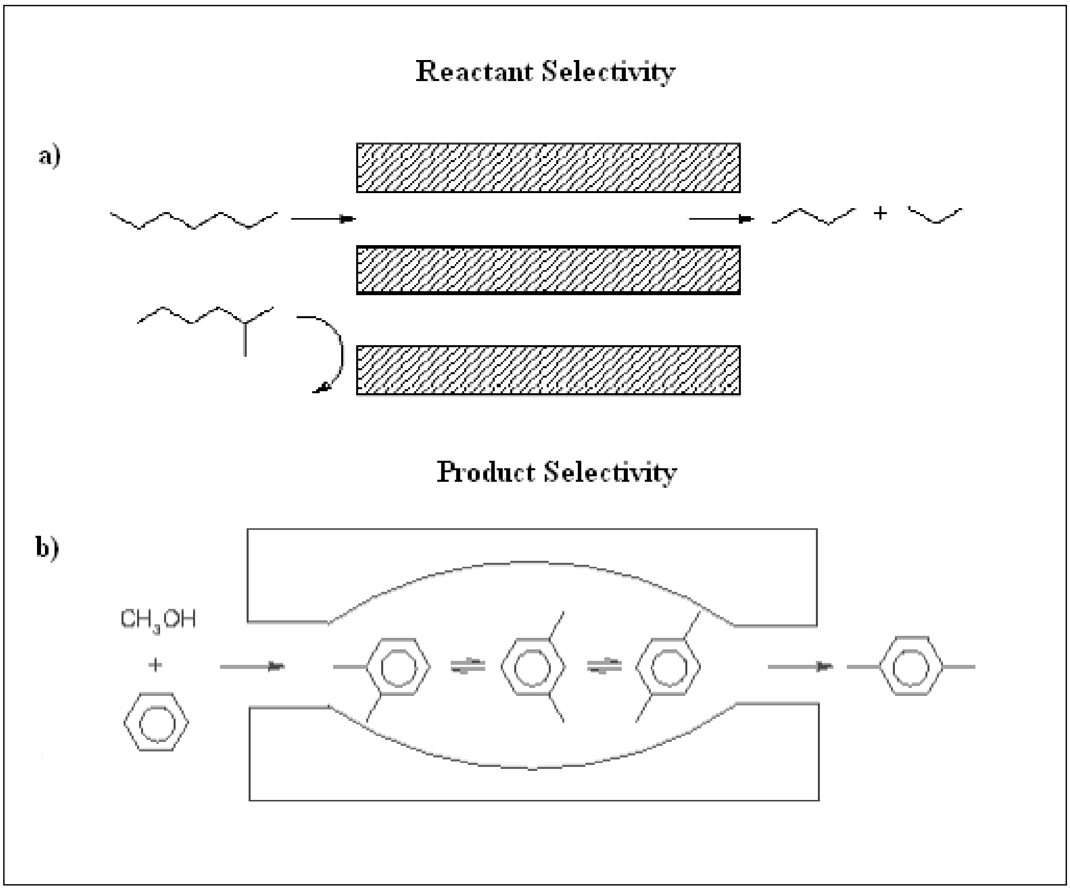


Figure 2.20. Reactant and Product Selectivity

Zeolites can take place in variety of reactions such as acid-base and metal induced reactions. The main industrial application areas are: petroleum refining, synfuels production and petrochemical production [67].

2.5.2.3. Zeolite in Ion Exchange

The ion-exchange process is a chemical reaction between ions in solid phase and ions in liquid phase. Certain ions in the solution are preferentially sorbed by the ion-exchanger solid and, since electroneutrality is maintained, the exchanger solid releases replacement ions back into the solution [12].

The cation exchange property of zeolite minerals was first observed 130 years ago. Three-dimensional framework of zeolites is composed of SiO_4 and AlO_4 tetrahedras. In the lattice a negative charge is raised because of the isomorphous replacement of Al^{3+} for Si^{4+}. This negative charge is balanced by the exchangeable cation (sodium, potassium and calcium).

These cations are loosely bound to the framework and they are exchangeable with certain cations in solutions [6, 62, 68]. Therefore zeolites are typical ion exchangers with their negatively charged porous framework and the small and mobile cations sitting in these pores. As it was mentioned before zeolites are widely used as builders in laundry detergents. Ion exchange property of zeolites makes them useful in laundry detergents. They give their loosely bounded cations such as sodium and take up calcium and magnesium ions. Thereby washing water which is free from magnesium and calcium ions would be softened. Cation exchange capacity varies with the aluminum content of the framework. As aluminum tetrahedral in the framework structure increases ion exchange capacity of the zeolite also increases. Obviously, highest possible aluminum content in the framework ($n_{Si} / n_{Al} = 1$) are desired for ion exchange applications. According to the silica alumina rations zeolite A is mostly preferred for ion exchange applications. Therefore zeolite A is preferred to use in detergents which have a huge market worldwide [19].

The main features of zeolites that make them advantageous for ion exchange are:

1. Cations can diffuse in order to undergo exchange between the solution and zeolites extra framework charges. Hence their well-defined framework structure, identical pores and channels lead to uniformity. The nature of these intracrystalline penetrations is important in ion sieving and diffusion control, and depends mainly upon the framework topology.
2. Because of their three-dimensional framework structure, most zeolites do not undergo any noticeable dimensional change with ion-exchange [2].
3. The aluminum atom content per unit cell of framework, defines the maximum number of negative charges available for cations. The ion-exchange capacity is directly related with this aluminum quantity present in the zeolite framework. Which means; low silica zeolites are favorable for ion exchange processes. The ion exchange property of zeolites can be altered by changing aluminum content in the framework.
4. The sieving and partial sieving effects of zeolites toward various cations are one of the most important features of them and have potential applicability in many areas [12].

The ion exchange property of zeolites is used in various areas. The most common applications are water softening, demineralization, desalting, and ammonia removal [69]. Ion-exchange ability of zeolites is also used for environmental and ecological interest, such as removal of several toxic substances including heavy metals, from industrial and municipal wastewater and treatment of some radioactive wastes [12].

2.6. MICROORGANISMS

Microorganisms were first time observed under the microscope by Anton van Leeuwenhoek microorganisms 1675 [70]. A microorganism or microbe is a microscopic organism that comprises either a single cell (unicellular) or, cell clusters, or no cell at all (acellular). Microorganisms include bacteria, fungi, archaea, and protists. Microscopic plants such as green algea, and animals such as plankton and planarian. However all microbiologists do not have same opinion about viruses. Some of them classify them as microorganism while others consider them as non-living. Although most of microorganisms are unicellular (single-celled), there are also multicellular organisms exist in nature and they are also microscopic. Nevertheless some unicellular protists and bacteria are macroscopic and visible to naked eye. Microorganisms (bacteria, fungi, archaea, protists, microscopic plants and some animals) can live in all parts of the biosphere where there is liquid water, including soil, hot springs, ocean floor, high in the atmosphere and deep inside rocks, in the deeper parts of the ocean within the Earth's crust, ubiquitously [71]. Microorganisms act as decomposers at nutrient recycling in ecosystem. Fixing nitrogen is a vital part of nitrogen cycle and this fixing process is carried out by microorganisms. Furthermore, some researchers reported that microorganisms may play a role in precipitation and weather [72]. Microorganisms are exploited in various areas such as; in biotechnology, both modern and traditional food and beverage preparation and have a lot of application in genetic engineering. Therefore they are essential for ecosystem [38, 72, 73]. Nevertheless pathogenic microorganisms can induce serious diseases which can even cause death to humans, animal or plants [72].

In the early 1900's infectious diseases were the most common reason of death all around the world [74]. Over the last century the number of deaths originated from microorganisms was dramatically decreased with the development of antimicrobial agents. However after some time the microorganisms started to develop resistance against antibiotics. Moreover because of the increased dosage of antibiotic usage reached the degree of resistance to a critical level that the currently used antibiotics in the clinics are majorly invalidated [75, 76]. The bacterial resistance problem tried to be solved by chemically modifying existing drugs and by developing new antimicrobial drugs. But unfortunately the handicap about this situation is; no one can quarantine to develop new antimicrobial agents contemporaneously with the microorganisms develop resistance to currently used antimicrobial drugs. Both Gram-positive and Gram- negative bacterial pathogens developing drug resistance in hospitals and in the community, and the evolution of the drug resistance threats human health by compromising our ability to treat serious infections [77, 78]. This challenging and dynamic pattern of infectious diseases and the emergence of bacterial strains resistant to many currently used antibiotics demand for longer-term solutions to this ever-growing and foreseeable problem [79, 80].

The demand and development of new, safe and cost-effective materials with antimicrobial properties were increased with public awareness and consciousness [81, 82]. Those developed materials which have ability to inhibit microbial growth on itself started to use in academic researches and technologic applications, such as water purification systems, medicinal devices, food packaging and storage, construction and textiles [81, 83]. Antimicrobial activity of materials brings some advantages like being chemically stable, thermally resistive, stands for longer time and user safety [84-86].

Most of the infections caught from hospitals (nosocominal infections) are medical device associated [87, 88]. Nosocomial infections prolog patients' length of stay in hospital and prolong period of drug therapy [89]. Moreover morbidity, mortality and hospital care costs; that are financed by government, private health insurance or personally; are dramatically increased because of these types of infections [90]. Bacterial infections can be treated by antibiotics. But those pathogenic microorganisms can mutate and gain resistant against antibiotics. Methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* developed resistance against methicillin antibiotics and they require new and efficient antibacterial agents [91].

2.7. ANTIMICROBIAL EFFECT OF ION EXCHANGED ZEOLITES

Zeolites are crystalline aluminosilicates which host water and cation within their pores. Their three dimensional structure is composed of $[AlO_4]^{5-}$ and $[SiO_4]^{4-}$ connected mutually via oxygen atoms. The negatively charged aluminosilicate lattice is balanced with metal cations of Na^+, K^+ or Ca^{2+} in order to be electroneutral [92] and these balancing cations are exchangeable with other metal ions such as silver, zinc, copper, nickel, cadmium, lead etc. present in the solution [93].

Some metal ions are essential for microorganisms in nano to micro-molar concentrations such as copper, zinc and nickel. However when the concentration of these metal ions increased to mili-molar concentrations, they are toxic to almost all kind of bacterial strains, only very few of them can tolerate to those high concentrations[94, 95]. Even thought solutions including Cu^{2+}, Zn^{2+} and Ni^{2+} ions are also antibacterial, zeolites carrying cations in the lattice exhibit different toxicity then free ions [93].

2.7.1. Silver and Silver Ion-Exchanged Zeolite as an Antibacterial Agent

As it was mentioned early in section 2.6 that some microorganisms are essential in our ecosystem while rests of them are pathogenic for people, animals or plants [71]. In order to get rid of those pathogenic microorganisms, inorganic antimicrobial agents stared to use which are more chemically stable, have higher thermal resistance and longer lasting action period with respect to organic antimicrobial agents which are alcohol, quaternary ammonium salt, thiabendazole, and ortho-phenylphenol [96]. Heavy metal ions have antibacterial activity. Among the all heavy metals, silver and silver containing materials have been used for antimicrobial agent very commonly since ancient times [97-99]. The reason is that silver has antiseptic effect against a wide spectrum of microorganisms, it has strong activity and it has low toxicity for humans [97, 100]. According to metallic silver, silver ions are more effective against microorganisms [97]. Metallic silver can show its antimicrobial property even in the aqueous mediums, in consequence of its ability to oxidize to the ionic silver species [73, 101, 102].

About the working principle of silver ion there are two possible claims. One of these is; bacterial cells which contact with silver ions take in silver and those silver ions inhibit the most vital enzymes, interfere with electron transport, bind to DNA and prevent DNA replication [103]. The other one is the generation of reactive oxygen species, which are produced possibly through the inhibition of respiratory enzymes by silver ion and attack the cell itself [104].

In order to metabolite and distribute silver to bone and soft tissues, silver ion binds to intracellular proteins especially albumins, and macroglobulins. Cysteine-rich proteins—metallothioneins (MTs) I and II in metabolically active cells of the wound margin are bound and induced by the adsorbed silver ions. MTs are a family of cysteine-rich proteins that bond to heavy metals and carry them; also they protect cells by binding toxic metal ions and reduce the risk of cell damage [105,106].

There are some disagreement between the scientists about the predominant route of silver in the human body, its transitory fashion or long-term accumulation in kidney, liver, and bone, and its excretion patterns in bile, urine, hair, and nail [107,108]. "For the excretion of silver from the body; biliary route dominates the urinary route; but, urinary silver measurement may provide a convenient index of silver absorption by all routes and serve as a guide to the total silver content of the body at blood levels of $<100 \mu g.L^{-1}$. Patterns of urinary excretion show irregularity at higher concentrations. While workers exposed to long-term environmental silver residues were biologically monitored it has been shown that; in hair, blood, urine, and faeces silver concentrations were raised. However, faecal silver represents that excreted in bile plus the 90% or more ingested with food and not absorbed into the circulation" [105].

One of the most advantageous properties of silver is that; it can easily put into a carrier in different forms, such as polymers [102, 109], metal oxides [101], silica [110], clays [111], synthetic [101, 109-113] and natural zeolites [109]. Therefore the most common antimicrobial additive being incorporated into solid materials is silver [114].

Silver is used as antimicrobial agent in various applications. For example silver sulfadiazine is used as antimicrobial agent for burn patient's treatment [114]. Another example is in the ancient Greek, silver coin was placed into the bottles in order to prevent microbial spoilage and prolong the liquids (milk, wine...) freshness [115, 116].

Silver is incorporated with materials in certain amount. The reason for using silver as additive within materials is; it is more cost effective than using silver as silver solution or plate [117]. Antibacterial effect of different silver compounds and silver ions in different carriers were studied by Lalueza et al [101]. As a result of the study antibacterial activity of silver including compounds was found that $AgNO_3$ > Ag-ZSM-5 > Ag_2O > commercial silver-exchanged zeolite (granular) > commercial silver-exchanged zeolite (pellets) > Ag nanoparticles. The amount of silver which is available for microorganisms, oxidation state of the silver species and the particle size of the material indicates the effect level of silver introduced compounds. Between these materials zeolite is the most promising carrier because it can adjust the release rate of silver ion [85, 100, 117-120].

“Zeolites can be used as antibacterial agents because the native cations in the zeolite structure can be exchanged with heavy metal ions that have antibacterial activity” [96]. The ion exchange reaction of zeolite with silver is [121];

$$ZNa + AgNO_3 \qquad ZAg + NaNO_3$$

Figure 2.21 is a model for ion exchanged zeolite A. In figure purple spheres indicates silver ions and they are located in the voids of the zeolite structure.

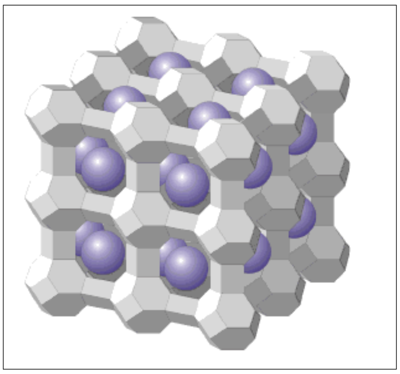


Figure 2.21. Crystal structure of zeolite A, the purple spheres represent silver ions [122]

2.7.2. Copper and Copper Ion-Exchanged Zeolite as an Antibacterial Agent

Copper is an essential metal for living organisms at low concentrations. It is used as cofactors for metalloproteins and enzymes. On the other hand, copper inhibits bacterial growth and is toxic to most microorganisms at high concentrations [123, 124]. There are two claims about the working principle of copper as biocidal. First one is, the copper ions replace with essential ions, block proteins functional groups and inactivate enzymes. The other one is hyperoxide free radicals which are produced by the membrane binds to copper, therefore, membrane integrity is altered [125].

E. coli O157:H7 is a specie of *Escherichia coli* and it is highly infectious, foodborne and waterborne pathogen. It creates serious public health challenge for the food processing industry. A study by Bill Keevil in 2001 showed that "that the highly toxic *E. coli* O157:H7 strain of bacteria survive for much shorter periods of time on copper and brass surfaces than on stainless steel" [126]. Another study performed by Kielemoes and Verstraete in 2001 showed that copper inhibits the adhesion of bacteria on biofilm development [127]. Casey and colloquies investigated copper containing toilet seat, set of tap handles and a warden trance door push plate of a hospital according to their microbial load with equivalent non-copper containing materials. As a result of the research copper containing surfaces host 90% or 100% lower *Clostridium difficile*, Methicillin-susceptible *Staphylococcus aureus*, Vancomycin resistant *Enterococcus faeceum* and *Escherichia coli* according to non-copper containing surfaces [128]. Also Hrenovic et. al. reported that Cu- and Zn-loaded zeolites are promising antibacterial materials for disinfection of secondary effluent water. They tested Cu- and Zn-loaded zeolites against *E. coli* and *S. aureus* found them effective against those bacterial strains [93].

2.7.3. Zinc and Zinc Ion-Exchanged Zeolite as an Antibacterial Agent

Zinc ion is necessary for all living systems in micro-molar concentrations. Zinc is present in metallo enzymes and provides membrane stabilization. Also, zinc binds to, histidine or other moieties on membranes, enzymes, or other proteins and alters their activity [129]. For human body, zinc is an essential antioxidant and anti-inflammatory agent. Zinc deficiency is resulted with delayed wound healing, immune dysfunction, growth retardation and neurosensory disorders. In human body, zinc ions are necessary for more than 300 enzymatic processes including bone growth and replication of DNA [130, 131].

Just like copper ion, zinc ion is essential for various metabolic activities in small quantities but higher concentrations are toxic for most of the living organisms [132]. There are several claims for the bactericidal action of zinc ion. The first one is zinc binds to the membranes of microorganisms and "prolongs the lag phase of the growth cycle and increasing the generation time of the organisms so that it take each organism more time to complete cell division" [133]. Another claim is; zinc ions bind to proteins and deactivate them. Then, they interact with microbial membrane and change the structure and permeability. Finally, lipids and proteins of the bacterial cell membrane are destructed by Zn^{2+} ions and this leads to a leakage of intracellular contents and after all the death of bacterial cells [134, 135].

Wound healing and bactericidal properties of zinc ion is used almost as much as silver ion in formulations, composites and coatings although the antimicrobial effect of Ag^{+} is much extensive than Zn^{2+} [116, 135, 137]. Furthermore, zinc zeolites have been incorporated with lots of materials for use in healthcare due to the bactericidal efficiency against various pathogens [118].

3. MATERIALS

3.1. CHEMICALS

For zeolite synthesis; sodium aluminate (Al_2O_3: 1.4 Na_2O), sodium hydroxide (NaOH: 0.07 H_2O), sodium metasilicate pentahydrate (Na_2O: SiO_2: 5 H_2O) and distillate water were used.

For ion exchange process of zeolites; silver nitrate ($AgNO_3$), zinc chloride ($ZnCl_2$), copper sulfate pentahydrate ($CuSO_4$. 5 H_2O) and anhydrous copper sulfate ($CuSO_4$) were used.

For microbial isolation; PBS (Phosphate Buffered Saline) solution which is consists of sodium chloride (NaCl), potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4), monopotassium phosphate (KH_2PO_4) and distilled water was prepared. Also hydrochloric acid (HCl) and sodium hydroxide (NaOH) used for pH alteration. For the microorganism innoculation brain heart agar (BHA), nutrient agar (NA), potato dextrose agar (PDA), sabouraud dextrose agar (SDA), tryptic soy agar (TSA), nutrient broth (NB), potato dextrose broth (PDB), sabouraud dextrose broth (SDB) and tryptic soy broth (TSB) were used.

For microbial identification; tryptic soy agar (TSA), sodium hydroxide (NaOH), hydrochloric acid (HCl), HPLC grade methanol (CH_3OH), HPLC grade methyl tert butyl ether (MTBE)-($(CH_3)_3COCH_3$), HPLC grade hexane (C_6H_{14}), pottasium hydroxide (KOH), czapek dox agar (CDA) and potato dextrose agars (PDA), sterile sea sand, 10x TE buffer (100 mM Tris/HCl pH8, 10 mM EDTA), 20% (v/v) SDS, proteinase K (10 µg/ml), sodium chloride (NaCl), Cetrimonium bromide (CTAB)-($(C_{16}H_{33})N(CH_3)_3Br$), chloroform/iso-amylalcohol ($CHCl_3/(CH_3)_2CHCH_2CH_2OH$), ammonium acetate ($CH_3COONH_4$), iso-propanol ($C_3H_8O$), ethanol (C_2H_6O), TE buffer (10x), agarose gel bands, primers (10 µg/ml), TBE buffer (0.5x), , magnesium sulfate ($MgSO_4$ 20 µg/ml), dNTPs (10 µg/ml), reaction buffer (10x), taq polymerase (10 µg/ml) were used.

For antimicrobial material tests, liquid paint, dust paint, acrylic paint, polypropylene plates and plaster were used.

3.2. LABORATORY EQUIPMENTS

For zeolite synthesis; laboratory scale, vacuum filtration system, and oven were used.

For ion exchange process of zeolites; laboratory scale, shaker, vacuum filtration system, and oven were used.

For microbial isolation; washing machine parts, shaker, heater, mixer, autoclave and incubator were used.

For microbial identification; Biolog^{TM} Microstation System, Microbial Identification System, Rotator , Water bath, Thermal cycler and agarose gel instruments, heater, mixer, autoclave, incubator, turbidimeter, vortex, 50 ml falcon centrifuge , microcentrifuge, UV spectrophotometer, Promega Wizard SV Gel and PCR Clean-UP System, laminar flow cabinet, nano-photometer

For zeolite characterization; SEM and X-ray Diffraction (XRD) instruments were used. Also for SEM analysis BAL-TEC SCD 005 Sputter Coater was used.

High density poly ethylene (HDPE) bottles, weighting dishes, spatulas, filter papers, watch glasses, elisa plates, pipettes, micropipettes, pipette tips, swaps, petri plates, plastic sterile drigalski spatulas, plastic sterile loops, erlenmeyer flasks, beakers, eppendorfs, Bunsen burner, falcon tubes, glass bottles, mortar and pestles and parafilm were used during the experiments.

4. METHODS

4.1. MICROBIAL ISOLATION

Microorganisms were isolated from the washing machine parts which were simulated for ten-years usage. Washing machines were provided by the project partner Vestel Inc. There were two different washing machines, first one was operated with heating system and the second one was operated with no heating system. By this way, the temperature factor on microorganisms could be considered. Microorganisms were isolated from different parts of the washing machine in order to consider the difference in humidity, temperature, detergent etc.. Parts of the washing machine are listed in Table 4.1.

Table 4.1. Parts of the washing machine used for microbial isolation

Backside Cauldron
Backside Lid of the Drum
Detergent Drawer
Drain Hose
Forefront Cauldron
Forefront Lid of the Drum
Inlet Hose
Middle Sheet of the Drum
Outlet hose with bellows
Plastic Agitator
Pulsator
Pump Chamber

Washing machine parts were placed in sterile erlenmeyer flasks containing 200 mL PBS (Phosphate Buffered Saline) solution. PBS solution was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 in 800 mL distilled water. Then pH of the solution was adjusted to 7.4 by adding HCl of NaOH. The prepared PBS solution was autoclaved in order to prevent any microbial contamination. The 500 mL Erlenmeyer flasks including 200 mL PBS solution and washing machine parts were placed in to shaker and shaken for 2 hours at 200 rpm. After 2 hours 100 µl PBS solution collected from each erlenmeyer flasks and 10^{-1} dilutions were inoculated with spreading technique by using plastic sterile drigalski spatulas onto five different agars. These were brain heart agar (BHA), nutrient agar (NA), potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and tryptic soy agar (TSA). These agars were kept at different temperature for 24-72 hours for cultivation. BHA and TSA were kept at 37 °C, NA was kept at 27°C, PDA and SDA were kept at room temperature (25 °C). After cultivation grown microbial colonies were purified and reinoculated onto fresh mediums with streaking technique by using plastic sterile loops. After cultivation each microorganism was stored with its proper method at -80°C and at room temperature.

4.2. MICROBIAL IDENTIFICATION

4.2.1. Bacterial Identification

4.2.1.1. Microbial Identification System (MIDI)

Whole-cell fatty acid methyl ester (FAMEs) analysis was conducted for the identification of microorganisms by gas chromatography [138]. Bacterial strains were transferred from long term stocks on to tryptic soy agar mediums (TSA) and incubated at 37°C for 48 hours. A loopful of cell was harvested from TSA plates and added to 1.2 M NaOH in 50% aqueous in a screw cap tube, then incubated at 100 °C for 30 minutes in a water bath. After saponified samples were cooled at room temperature for 25 minutes, they were acidified and methylated by adding 2ml of 54% 6 N HCl in 46% aqueous methanol and incubated at 80 °C for 10 minutes in water bath. After rapid cooling, methylated fatty acids were extracted with 2.5 ml of 50% methyl tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 minutes and the bottom phase was removed with sterile glass Pasteur pipette. The top phase was mixed with 3 ml 0.3 M NaOH and rotated for 5 minutes. After mixing for 5 minutes, the top phases were removed for analysis. Solutions required for the fatty acid extraction are listed in Table 4.2.

Table 4.2. Reagents and their ingredients used in fatty acid extraction

Reagent 1 (Saponification)	150 ml methanol (HPLC grade) was combined with 150 ml distilled water. 45 g NaOH (certified ASC) was added to the solution and stirred till the pellets dissolved
Reagent 2 (Methylation)	325 ml 6.00 N HCl was added to 275 ml methanol (HPLC grade) while stirring
Reagent 3 (Extraction)	200 ml methyl-tert-butyl ether (HPLC grade) was added to 200 ml hexane (HPLC grade) and stirred
Reagent 4 (Washing)	10.8 g NaOH (certified ASC) was added 900 ml distilled water and stirred till the pellets dissolved

4.2.1.2. Biolog System

The Biolog system consists of a microplate system including 95 different carbon sources and one control well, a turbidimeter and a plate reader connected to a computer system. Cultures of bacteria identified by MIDI were confirmed by Biolog Microplate System. Suspensions prepared for each strain to be tested in Biolog System were made from second subculture. Gram reaction and oxidase activity of bacterial strains were determined by 3% KOH method [139] and oxidase test [140] described before. Bacterial strains were transferred from long term stocks on to tryptic soy agar mediums (TSA) and incubated at 37°C for 48 hours. Cells were harvested with a sterile cotton swaps and suspended in a sterile 0.85% saline solution. By means of using biolog's turbidimeter the density of solutions inside the tubes were adjusted according to the different Biolog standards (about $4x10^8 – 6x10^8$ organisms per ml) provided by manufacturer for different bacteria types. The Biolog GN and GP microplates (Biolog, Hayvard, USA) preconditioned at 27°C for 2 hours and then inoculated by adding 100µl of each bacterial suspension into the reaction wells of the microplate using multichannel micropipetter. The microplates were capped, paraffined and incubated for 18-24 hours at 27°C. The color change reaction of each carbon sources was recorded by plate reader (Biolog, Hayvard, CA USA) and the results were compared with the Biolog GN and GP database with the Microlog Software (Biolog, Hayvard, CA USA).

4.2.2. Fungal Identification

4.2.2.1. Microscopic Identification

Fungal species were inoculated onto Czapek Dox and Potato Dextrose agars and sent to Prof. Dr. İsmet Hasenekoğlu, a lecturer in Erzurum Atatürk University (Erzurum, Turkey), an expert in fungal identification based on morphological properties under microscope observations. The ingredients for Czapek Dox agar were listed in Table 4.3.

Table 4.3. The ingredients in Czapek Dox Agar

Ingredients	Amount
Sucrose	30.0 mg
Sodium Nitrate	2.0 mg
Magnesium Sulfate	0.5 mg
Potassium Chloride	0.5 mg
Ferrous Sulfate	0.01 mg
Dipotassium Phosphate	1.0 mg
Agar	15.0 mg

4.2.2.2. DNA Based Identification

Further identifications of fungal species were performed by rDNA sequencing. Fungal species were inoculated onto fresh PDA plates and incubated at 25°C for 5 days. 100mg of agar including fungus mycelium was taken and put into centrifuge tubes containing 100mg sterile sea sand, 0.5ml of 10x TE buffer (100 mM Tris/HCl pH8, 10 mM EDTA), 50µl 20%(v/v) SDS and 3µl of 20mg/ml proteinase K were added into the tubes. Homogenization was carried out till a muddy structure is obtained. Afterwards, the solution inside the tubes was kept at 60°C for 30 minutes. The tubes were centrifuged for 5 minutes at 7000 rpm resulting in two phases. 500µl supernatant was transferred into a new centrifuge tube containing 194µl 5M NaCl and 70µl of 10% CTAB to have 1.4M salt concentration and incubated at 65°C for 10 minutes. 700µl chloroform/iso-amylalcohol (24:1v/v) was added to the mixture and 30 minutes incubation at 0°C was processed. The upper phase was transferred to a new tube and 325µl 5M ammonium acetate was added and the centrifuge tubes were kept on ice for 60 minutes. After thawing, the mixtures were centrifuged at maximum rpm for 10 minutes. Supernatant liquid was replaced into a new tube and incubated with 2μl 10mg/ml RNAse at 37°C. 0.55 volume of iso-propanol was added and centrifuged at 13,000 rpm for precipitation of DNA. The supernatant was discarded and the pellet was washed with 70% ethanol (v/v). The pellet was dried at room temperature for 20 minutes. The precipitated DNA was dissolved in 30μl TE/dH_2O and stored at -20°C for following steps. The purified DNA was run in 1% agarose gel to make sure about the purity of fungal genomic DNA of fungi isolates studied.

A primer set of ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for Polymerase Chain Reaction (PCR) to amplify a targeted reaction (600 to 650 bp fragment) on 18S and 5.8S rDNAs; of fungal isolates to be identified. PCR conditions were arranged as it is described in the literature previously [141]. PCR conditions and reagents used for the reaction are listed in Table 4.4. and Table 4.5. Obtained PCR products were run on 1% agarose gel for the confirmation of amplification.

Table 4.4. Amounts of used PCR reagents

	Amount
Reaction Buffer	2.5μl
MgSO4	2.5μl
dNTP	0.5μl
Forward Primer	0.5μl
Reverse Primer	0.5μl
Taq Polymerase	0.2μl
DNA	2.0μl
dH2O	16.3μl
Total	25μl

Table 4.5. PCR conditions

	Temperature	**Time**	**Cycle**
Initial Denaturation	96°C	2 minutes	1
Denaturation	96°C	1 minute	32
Annealing	58°C	1 minute	
Elongation	72°C	1 minute	
Final elongation	72°C	10 minutes	1

Amplified fungal DNA fragments were isolated from %2 agarose gel by Promega Wizard SV Gel and PCR Clean-UP System. The DNA bands on agarose gel were cut out with scalpels. DNA fragments were purified according to the manufacturer's instructions [142]. An additional agarose gel running step was applied with a small amount of fungal DNA to make sure about purification. The concentration and purity of DNA fragments were determined with nano-drop UV spectrometry (San Francisco, USA). Purified DNA fragments were sent to RefGen Co. (Ankara, Turkey) for sequencing. The results were read with the program Finch TV. The results were blasted using the official blast web site.

4.3. ANTIMICROBIAL ZEOLITE SNTHESIS

4.3.1. Zeolite Synthesis

During the project eleven types of zeolites, which have different Si/Al ratio, were synthesized. Formulas of the zeolites are listed at Table 4.6. Sodium metasilicate pentahydrate (SMS, $Na_2O.SiO_2.5H_2O$) and ludox (SiO_2. $5H_2O$) was used as silica source, sodium aluminate was used as aluminum source and sodium hydroxide was used as balancing cation.

Table 4.6. Zeolite Gel Formulations

Sample number	Synthesis gel formula	Silica Source
Sample 1	17 Na_2O : Al_2O_3 : 8SiO_2 : 666 H_2O	SMS
Sample 2	17 Na_2O : Al_2O_3 : 8SiO_2 : 666 H_2O	Ludox
Sample 3	10 Na_2O : Al_2O_3 : 6 SiO_2 : 650 H_2O	Ludox
Sample 4	10 Na_2O : Al_2O_3: 8 SiO_2: 650 H_2O	Ludox
Sample 5	4.64 Na_2O : Al_2O_3: 3.2 SiO_2:400 H_2O	SMS
Sample 6	6 Na_2O : Al_2O_3: 4 SiO_2: 300 H_2O	Ludox
Sample 7	6 Na_2O : Al_2O_3: 7 SiO_2: 300 H_2O	Ludox
Sample 8	2 Na_2O : Al_2O_3: 1.6 SiO_2: 200 H_2O	Ludox
Sample 9	3.39 Na_2O : Al_2O_3: 1.77 SiO_2: 116.9 H_2O	Ludox
Sample 10	2.5 Na_2O : Al_2O_3: 0.84 SiO_2: 194 H_2O	SMS
Sample 11	5 Na_2O : Al_2O_3: 1.35 SiO_2: 220 H_2O	SMS

4.3.2. Sample Calculation for Zeolite Synthesis

17 Na_2O: Al_2O_3 :8SiO_2 :666 H_2O

For this formulation 8 moles of silica (supplied from Na_2O: SiO_2: 5 H_2O (SMS)), 1 mole of aluminum oxide (supplied from Al_2O_3: 1.4 Na_2O), 17 moles of sodium oxide (supplied from NaOH: 0.07 H_2O), and 666 moles of water is required.

Silica, aluminum oxide and sodium oxide were not in the pure form therefore some calculations had to be performed.

Sodium metasilicate pentahydrate formula is $Na_2SiO_3.5H_2O$ which is same with Na_2O: SiO_2: 5 H_2O and molecular weight is 212.14g/mol.

Sodium aluminate formula is Al_2O_3: 1.4 Na_2O and molecular weight is 188.8g/mol.

Sodium hydroxide formula is NaOH: 0.07 H_2O and molecular weight is 41.25g/mol.

Whole silica could be obtained from SMS therefore for 8 moles of Silica 8 moles of SMS should be used.

Also whole aluminum could be obtained from sodium aluminate; therefore for 1 mole of aluminum oxide 1 mole of sodium aluminate should be used.

When SMS and sodium aluminate was used automatically some amount of sodium was gathered from those molecules.

For the zeolite formulation specified above 17 moles of sodium oxide is required. Sodium obtained from SMS is;

-

Sodium obtained from Sodium Aluminate is;

-

Sodium should be obtained from Sodium hydroxide is;

-

Also for the zeolite formulation specified above 666 moles of water is required.
Water obtained from SMS is;

-

Water obtained from Sodium hydroxide;

-

Water should be obtained from distilled water is;

-

Weights of the required amount were calculated by multiplying molecular weight and mole numbers of chemicals;

SMS weight is;

-

Sodium aluminate weight is;

-

Water weight is;

-

Total amount is;

-

For 1000 g of batch;

-
-
-
-

Required amount of chemicals were weighed out and put into polyethylene erlenmeyer flasks. While SMS or Ludox was put into one Erlenmeyer flask, sodium aluminate and sodium hydroxide were put into another Erlenmeyer flask. Required amount of water was divided into two parts and poured into those erlenmeyer flasks and those flasks were shaken till all chemicals were dissolved completely. Then those solutions were united in one erlenmeyer flasks and it was shaken until the homogeneous solution was obtained. While solutions were at different erlenmeyer flasks they were transparent and not viscous but when they were mixed solution got white and very viscous. Then those erlenmeyer flasks were put into the oven at 90^{o}C and kept for three days.

Table 4.7. Amount of chemicals employed for zeolite synthesis

TYPE	$Na_2O:SiO_2:5H_2O$ SMS	$SiO_2:5H_2O$ LUDOX	$Al_2O_3:1.4Na_2O$ Sodium aluminate	$NaOH:0.07H_2O$ Sodium hydroxide	H_2O Water	TOTAL
Sample 1	123.32	-	13.72	45.56	817.40	1000
Sample 2	-	86.19	13.56	92.44	807.81	1000
Sample 3	-	69.50	15.48	54.80	860.22	1000
Sample 4	-	91.90	14.46	54.34	893.3	1000
Sample 5	87.22	-	24.26	0.42	888.09	1000
Sample 6	-	77.09	24.26	48.76	646.09	1000
Sample 7	-	164.65	29.61	59.51	746.23	1000
Sample 8	-	60.79	51.62	12.54	875.05	1000
Sample 9	148.82	-	74.83	7.19	769.15	1000
Sample 10	46.84	-	49.63	5.64	897.89	1000
Sample 11	63.73	-	42.01	41.31	852.94	1000

After three days zeolites were synthesized and separated from the water by using vacuum filtration. Zeolites were obtained like white residue, because there was still water in it; therefore they put into oven for one night at 90^{o}C and water was evaporated. Next day zeolite was ground and received in powder form. Grinding was performed with mortal and paste or household coffee grinder.

4.3.3. Ion-Exchange Process

After zeolites are synthesized, solutions with different concentrations were prepared. Solutions were prepared with silver nitrate ($AgNO_3$), zinc chloride ($ZnCl_2$), copper sulfate pentahydrate ($CuSO_4$. H_2O) and anhydrous copper sulfate ($CuSO_4$). First 0.05 M $AgNO_3$, 1M $CuSO_4$. H_2O, 1M $CuSO_4$ and 2M $ZnCl_2$ solutions were prepared. Toward optimization $AgNO_3$ concentration was increased to 0.1M, 0.6M and finally 1M. Then 80g of zeolites were poured into 1L of those solutions and shaken for three days at dark medium and room temperature. At the end of three days ion-exchange process was over and zeolites were filtered at vacuum filtration and put into oven at 90^{o}C for 24 hours. Dry zeolites were grinded with mortar and pestle or ordinary household coffee grinder.

4.4. ZEOLITE CHARACTERIZATION

After zeolites are synthesized their chemical composition and structures must be identified. Therefore X-ray diffractometer and SEM was used.

4.4.1. X-Ray Diffraction

After the zeolite synthesis X-ray powder diffraction was used in order to identify the zeolite structure and purity. The initial step for X-Ray diffraction is preparing the sample for x-ray diffraction. There are some important points must be taken into consideration during the sample preparation process. First one is grinding the sample. When the sample is grinding the most important point is making it monosize. If sample is not ground as monosize x-ray peaks would be asymmetric. The other point that must be taken into consideration is the surface of the sample; sample surface must be flat and there must not be any cavity on the surface of the sample. If sample was not prepared correctly peaks would shift right hand side or left hand side of the graph. But also preferred orientation must be regarded. In a polycrystalline aggregate crystallographic orientation of each grain is different from its neighbors which mean all the grains are randomly distributed. This situation must be considered during the preparation step. Also the homogeneity of the sample is important. The sample must be homogeneous in order not to obtain different result from different point of the sample.

The next step is obtaining data from the x-ray diffractometer. The X-ray powder diffraction patterns were obtained using a Bruker D8 Advance Powder X-ray diffractometer with Cu K-radiation, operated at 20mA and 40 kV. 2θ values were between 5^o and 38^o and the scan speed was 3 seconds and degree step was 0.01.

After taking the graph the name of the sample was determined by using EVA program. In EVA there are vertical lines indicating the materials and these lines have to match with sample’s peaks.

4.4.2. SEM

SEM was used to obtain the size and morphology of zeolite crystallites. In order to prevent evaporation during the imaging zeolites were oven dried at 90^{o}C for one day. And they are covered with gold with BAL-TEC SCD 005 Sputter Coater in order to provide conductivity to zeolites. SEM images were obtained by using Zeiss EVO 40. Energy dispersive spectroscopy was at beam voltage of 10.00 kV.

4.5. ANTIMICROBIAL TESTS

4.5.1. Modified Disc Diffusion Assay

Antimicrobial activity of pure zeolites and ion exchanged zeolites were tested against each microorganism including bacteria, fungi and yeast species listed in Table 4.8. Antimicrobial activity of pure, Ag^{+}, Zn^{2+} and Cu^{2+} ion-exchanged zeolites were performed according to modified disc diffusion assay against 48 microorganisms belonging to 35 bacteria, 6 fungi and 6 yeast species, respectively. Of these microorganisms 32 bacteria and 3 fungi species tested in this study were isolated from different parts of washing machines provided by VESTEL Inc., and the remaining 3 bacteria, 4 fungus, and 6 yeast species were obtained from the microbial culture collection unit in Department of Genetics and Bioengineering at Yeditepe University. For disk diffusion method approved by National Committee for Clinical Laboratory Standards (NCCLS), 100 µl of suspensions containing 10^{8} cfu/ml bacteria, 10^{6} cfu/ml yeast, and 10^{4} spore/ml fungi were prepared from freshly grown cultures and spread on NA, SDA, and PDA respectively. 17 µl of autoclaved sterile water was added on blank disks, and those disks were dipped into ion-exchanged zeolite and pure zeolite by the way disks were covered with both metal loaded zeolite and pure zeolite. Those zeolite covered disks were placed on inoculated plates. Also as positive control ofloxacin disks (10 µg/disc) were placed on NA for bacteria and nystatin disks (30 µg/disc) were placed on SDA and PDA for yeast and fungi and as a negative control blank disks which were wetted with sterile water were placed on inoculated placed. Inoculated plates were incubated at 37^{o}C for 24 hours for bacterial strains, for 48 hours for yeast strains and 25^{o}C 72 hours for fungi isolates. Antimicrobial activity of zeolites was evaluated by measuring the inhibition zone against microorganisms.

4.6. PREPARING ANTIMICROBIAL MATERIALS

Antimicrobial test were performed on four different surfaces which were metal painted with dust paint, metal painted with liquid paint, polymeric surface painted with acrylic paint and polypropylene surfaces. Different types and concentrations of zeolites were used according to color and type of material. For white dust paint 2M type 5 and 8 zinc zeolites were used; zeolite was mixed to paint with 7%, 10%, 12% (w/w) ratio. For grey metal paint 1M type 5 and 8 silver zeolites were used; zeolite was mixed to metal paint with 10% (w/w) ratio. For acrylic paint 1M type 5 and 8 silver zeolites were used; zeolite was mixed to acrylic paint with 10%, 12% and 15% (w/w) ratio. For polypropylene surfaces both 1M type 5 and 8 silver zeolites and 2M type 5 and 8 zinc zeolites were used; zeolite was mixed to polypropylene with 7% and 10% (w/w) ratio. Polypropylene and polymer which were painted with acrylic paint were prepared by VESTEL Inc., dust metal paint was provided by VESTEL Inc., liquid metal paint was bought from market (Nitropol metal paint).

4.6.1. Antimicrobial Activity Tests of Prepared Antimicrobial Surfaces

Prepared antimicrobial surfaces placed into petri dishes and protective caps of petri dishes were marked with type of the zeolite, weight percent of zeolite in the paint or in the surface itself, microorganism name that will be contaminated to the surface. Then 1-2 ml of Sabouraud Dextrose Broth (SDB) was poured onto the prepared surfaces and surfaces were contaminated with various microorganisms by using cotton swabs. Surfaces were kept at room temperature and SDB was added every day as feed for microorganisms. As a negative control materials which have no ion exchanged zeolite were prepared. Difference between zeolite mixed and commercially used surfaces were observed.

Table 4.8. Microorganisms tested against ion-exchanged zeolite activity

Bacteria	**Yeast**	**Fungus**
Pseudoxanthomonas spp.	*Kluyveromyces marxianus*	*Aspergillus niger*
Gordonia rubropentinctuc	*Pichia membranifaciens*	*Alternaria alternata*
Nocardia globerula	*Candida albicans*	*Paecilomyces lilacinus*
Acinetobacter baumannii	*Candida glabrata*	*Fusarium oxysporum*
Stenotrophomonas maltophlia	*Candida parapsilosis*	*Botrytis cinerea*
Pseudomanas chlororaphis	*Candida krusei*	*Penicillium expansum*
Duganella zoogloeoides		*Penicillium vinaceum*
Staphylococcus hominis hominis		
Burkolderia glumea		
Corynebacterium		
Nocardia brasiliensis GC sub B		
Acinetobacter calcoaceticus		
Providencian heimbachae		
Pseudomonas putida - biotype A		
Acinetobacter genomospecies 3		
Pseudomonas flourescens biotype G		
Xanthomonas spp.		
Actinomadura cremea		
Pseudomonas maculicola		
Hydrogenophaga pseudoflava		
Bacillus coagulans		
Pseudomonas stewartii ss stewartii		
Chryseobacterium balustinus		
Chryseobacterium meningosepticum		
Nocardia transvalenssis		
Pediococcus acidilactici/parvulus		
Sphingomonas terrae		
Rhodococcus rhodnii		
Brevundimonas vesicularis		
Gordonia sputa		
Cellulosimicrobium cellulans		
Sphingomonas sanguinis		
Escherichia coli		
Staphylacoccus aureus		
Pseudomonas aeroginosa		

4.6.2. Recovery of Microorganisms from the Contaminated Surfaces

Antimicrobial surfaces of different materials including paints, polypropylene, and plasters by mixing optimum concentration of ion exchanged zeolites were flated with liquid medio of SDB, PDB and NB, and maintained on the bench and incubator for fungal, candidal and bacterial contamination for 2.5 years. 100µL of samples were taken once a month from the each surface and plated on SDA, PDA and NA after 4 fold dilution for growth of contaminated microorganisms (fungi, yeast and bacteria) on the surfaces by comparing with control plates with no ion exchanged zeolites.

5. RESULTS

5.1. MICROBIAL IDENTIFICATION

In this study, a total 48 microorganisms belong to 32 bacteria and 3 fungi species were isolated and identified from different parts of washing machines provided by VESTEL Inc. were listed at Table 5.2.

No yeast species was isolated from the cold and hot washing machine systems. Different bacterial and fungal species were isolated from both washing systems, except two bacterial species, *Pseudoxanthomonas* spp. and *Nocardia brasiliensis* GC sub B, which were present in both systems.

In addition to newly isolated microorganism from washing machines, 13 additional microorganisms including 3 bacteria, 4 fungi and 6 yeast species obtained from the culture collection unit of Genetics and Bioengineering Department at Yeditepe University were included on studies in this work which were listed in Table 5.1.

Table 5.1. The list of bacteria, yeast and fungus species obtained from the culture collection of GBE depatment

Bacteria	**Yeast**	**Fungus**
Escherichia coli	*Kluyveromyces marxianus*	*Aspergillus niger*
Staphylacoccus aureus	*Pichia membranifaciens*	*Alternaria alternata*
Pseudomonas aeroginosa	*Candida albicans*	*Fusarium oxysporum*
	Candida glabrata	*Botrytis cinerea*
	Candida parapsilosis	
	Candida krusei	

Table 5.2. The list of bacterial strains and fungal species isolated from two different washing machines

Washing machine with heating system	**Washing machine with no heating system**
Bacteria	**Bacteria**
Pseudoxanthomonas spp.	*Pseudoxanthomonas* spp
Nocardia brasiliensis GC sub B	*Nocardia brasiliensis* GC sub B
Pseudomonas maculicola	*Nocardia globerula*
Bacillus coagulans	*Acinetobacter baumannii*
Pantoea stewartii ss stewartii	*Stenotrophomonas maltophlia*
Chryseobacterium balustinus	*Pseudomanas chlororaphis*
Chryseobacterium meningosepticum	*Duganella zoogloeoides*
Nocardia transvalensis	*Staphylococcus hominis hominis*
Pediococcus acidilactici/parvulus	*Burkolderia glumea*
Sphingomonas terrae	*Corynebacterium*
Rhodococcus rhodnii	*Gordonia rubropentinctuc*
Brevundimonas vesicularis	*Acinetobacter calcoaceticus*
Gordonia sputa	*Providencian heimbachae*
Cellulosimicrobium cellulans	*Pseudomonas putida - biotype A*
Fungi	*Acinetobacter genomospecies 3*
Paecilomyces lilacinus	*Pseudomonas flourescens biotype G*
Penicillium vinaceum	*Xanthomonas* spp.
	Actinomadura cremea
	Sphingomonas sanguinis
	Hydrogenophaga pseudoflava
	Fungi
	Penicillium expansum

5.2. ZEOLITE SEM AND X-RAY IMAGES

At the beginning of the study 11 types of zeolites were synthesized than according to their stiffness and ease of grinding they are reduced to 7. Those were sample 1, sample 3, sample 5, sample 7, sample 8, sample 10 and sample 11.

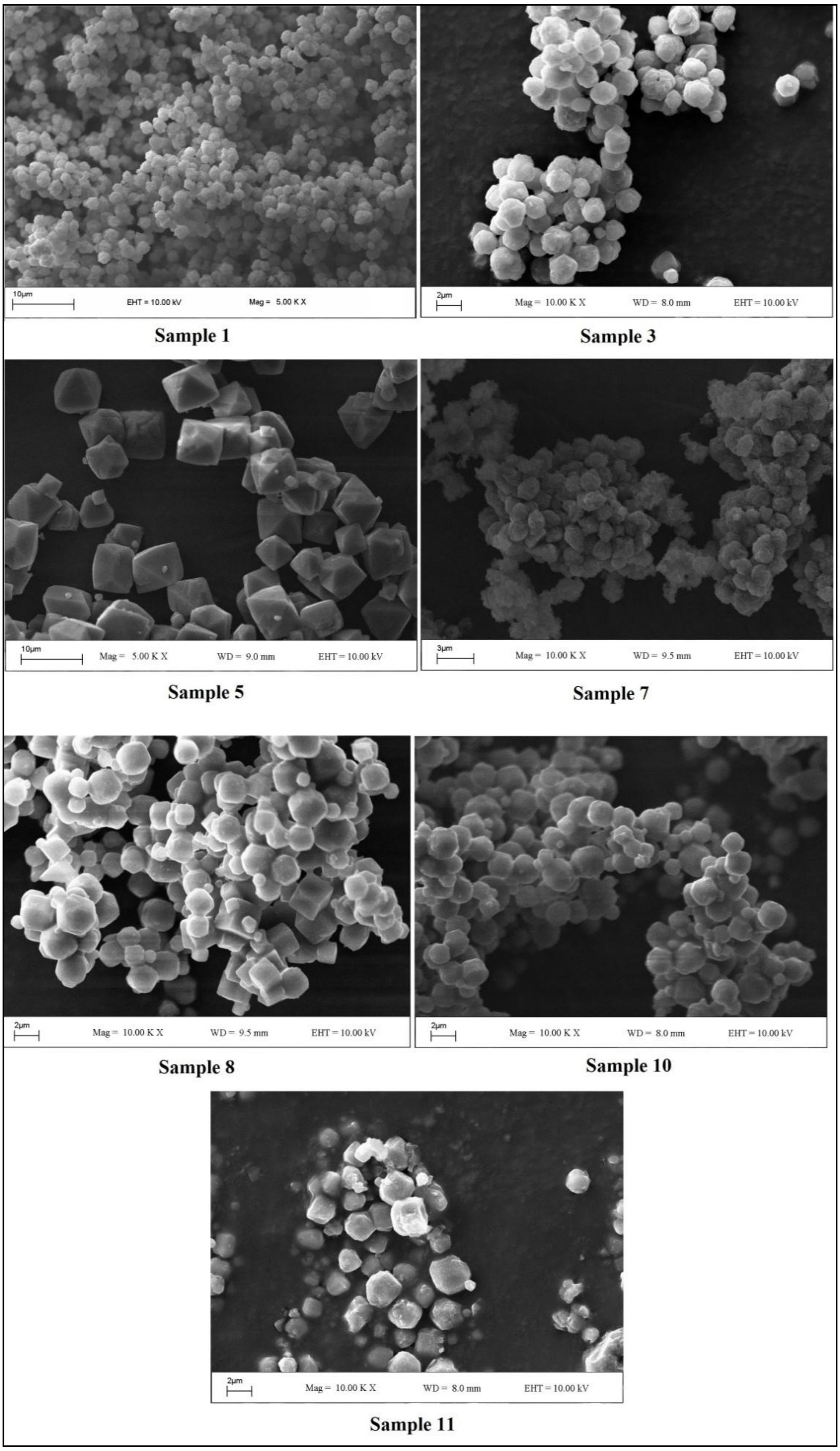


Figure 5.1. SEM Images of 7 Different Types of Zeolites

During the study zeolites which have different Si/Al ratio were synthesized and this Si/Al ratio determines if the zeolite type is zeolite A or zeolite X. It is known that zeolite A and zeolite X have cubic structure therefore SEM (Scanning Electron Microscope) images were visualized and compared with literature. SEM images are shown in the Figure 5.1. Supplementary details are given in Appendix A.

As it can be seen from the figure, except sample 5 and 7, all zeolites have cubic structure; however most clear cubic structure can be seen for sample 8. Zeolite 1 (FAU-NaX), 3 (FAU-NaX), 10 (LTA-Na) and 11 (LTA-Na) also have cubic structure but they have beveled edges which is very normal due to the lack of shaking during synthesis. All crystals grew in a batch and they overlapped each other therefore they were not have enough space for perfect growth. Sample 7 which is FAU-NaX was agglomerated during the synthesis. It could be prevented by mixing during the synthesis. Moreover, it also could be because of lack of time for synthesis, therefore shape of the structure cannot be determined easily. Sample 5 which is FAU-NaX has an octahedral shape. The ideal crystal system of the FAU-X is cubic but crystal habit could be octahedral. This octahedral structure of zeolite X is in agreement with the Warzywoda, Bac and Sacco's studies. They grew zeolite X crystals in both low earth orbit and ground and they found the structure of crystal as octahedral [143-145].

Another identification method used for zeolite characterization was X-Ray diffraction. X-Ray patterns were obtained and compared with ICDD-PDF chart (International Centre for Diffraction Data) and matched patterns were assumed as synthesized product.

As it can be seen from the figures all the peaks are symmetrical; that means all particles are monosize. Asymmetry on the peaks is a sign of variety in particle size, which is an undesired situation. Peaks are defined in narrow 2θ range which indicates good crystallinity.

Figure 5.2 shows the X-ray pattern for sample 1. As it can be seen from the figure there are two phases and both phases have cubic structure. Even if the chemical formula of sodium faujasite zeolite is described as $|(Na_{58}\ (H_2O)_{240}|\ [Al_{58}Si_{134}O_{384}]$, X-ray diffraction pattern which is indicated with red lines that have chemical formula of $Na_2Al_2Si_{2.5}O_{9.6}.2H_2O$ is also Zeolite NaX [146, 147]. Besides, if the coefficients of atoms in the formula of Na-FAU are reduced both formulas are very similar to each other. Blue lines which has a chemical formula of $Na_8(AlSiO_4)_6(OH)_2$-$2H_2O$ is the sodalite cages [148]. SOD were formed but could not combine via D6Rs to form zeolite X. the reason for this situation could be the absence of time.

Figure 5.3 shows the X-ray pattern for sample 3. As it can be seen from the figure there are four matches. Pink and green lines both represent sodalite cages with chemical formula of $Na_8(AlSiO_4)_6(OH)_2$-$2H_2O$. For the main peaks even if the blue lines peak intensities match better, red lines match every peak. Therefore red lines are chosen and the chemical formula of the zeolite is determined as Faujasite-Na $Na_2Al_2Si_{3.3}O_{10.6}$-7H2O. Moreover zeolite NaX has a cubic structure as expected.

Figure 5.4 shows the X-ray pattern for sample 5. According to the peaks all three compositions are possible but according to the intensities most possible one is the red one which is sodium aluminum silicate hydrate with composition of $Na_2Al_2Si_{2.5}O_{9.6}.2H_2O$. That formulation is also belongs to Zeolite NaX just as Sample 1.

Figure 5.5 shows the X-ray pattern for sample 7. There are three phases in the batch. First phase is represented by blue lines. It is sodium aluminum silicate hydrate with chemical formula of $Na_2Al_2Si_{2.5}O_{9.6}.2H_2O$ which is Zeolite NaX. The second one is represented by red lines and it is anhydrate sodalite cage with chemical formula of $Na_4Al_3Si_3O_{12}(OH)$. The third phase is represented with pink lines. It is sodium aluminum silicate hydrate with chemical formula of $Na_{3.6}Al_{3.6}Si_{12}O_{32}.14H_2O$ which is sodium gismonite (Na-GIS) [149]. Sodalite cage and zeolite NaX have cubic structure while Na-GIS has tetragonal structure. But in literature it has to be in the monoclinic structure [14].

Figure 5.6 and 5.7 show the X-ray patterns for sample 8 and 10 respectively. In both figures red lines are better choices due to the higher intensities. Both sodium aluminum silicate hydrates have $Na_{96}Al_{96}Si_{96}O_{384}.216H_2O$ which is exactly match with the chemical formula of LTA zeolite. The crystal structures are cubic for both zeolite 8 and 10.

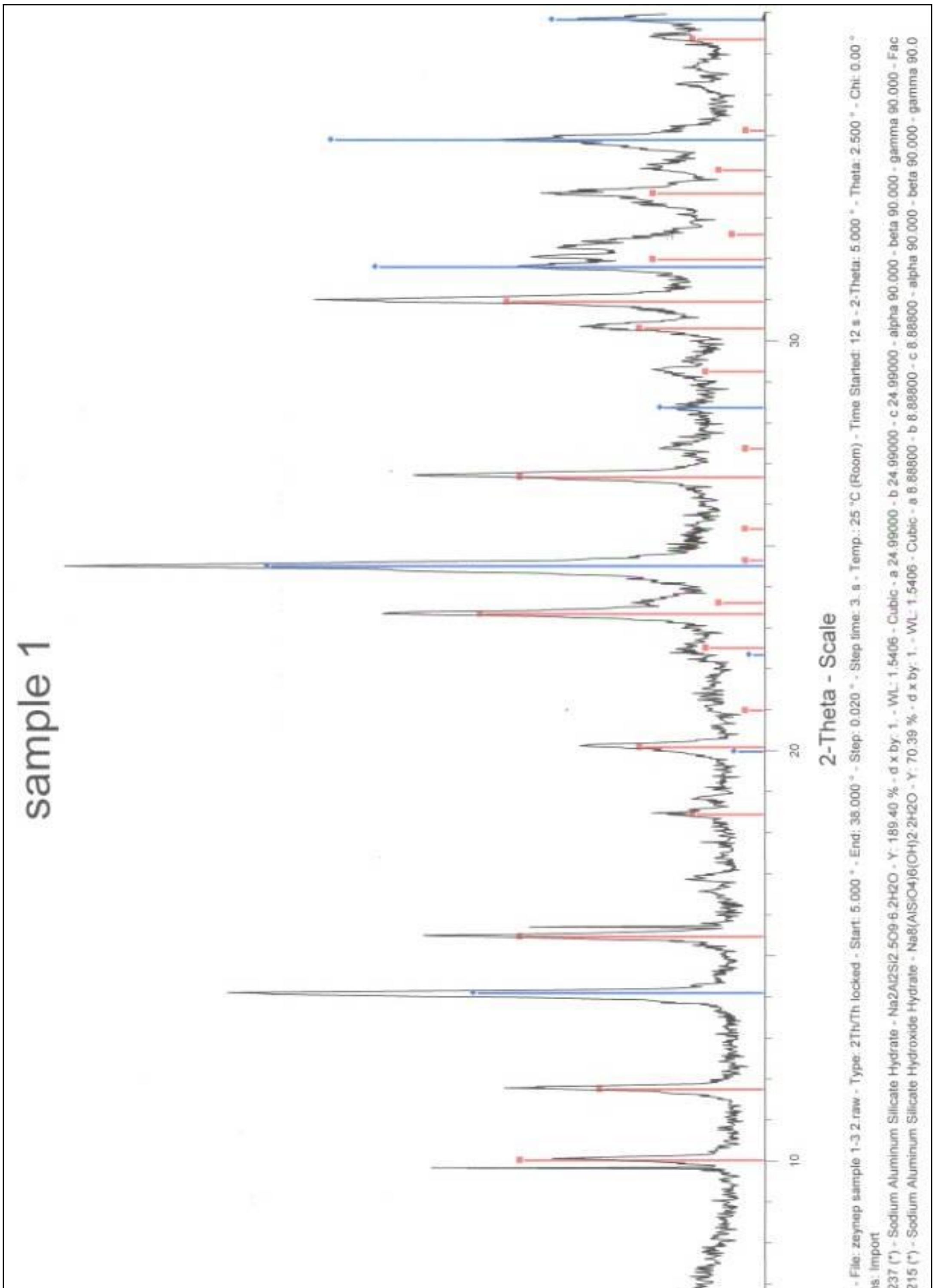


Figure 5.2. X-Ray Pattern and Matched Peaks for Sample 1

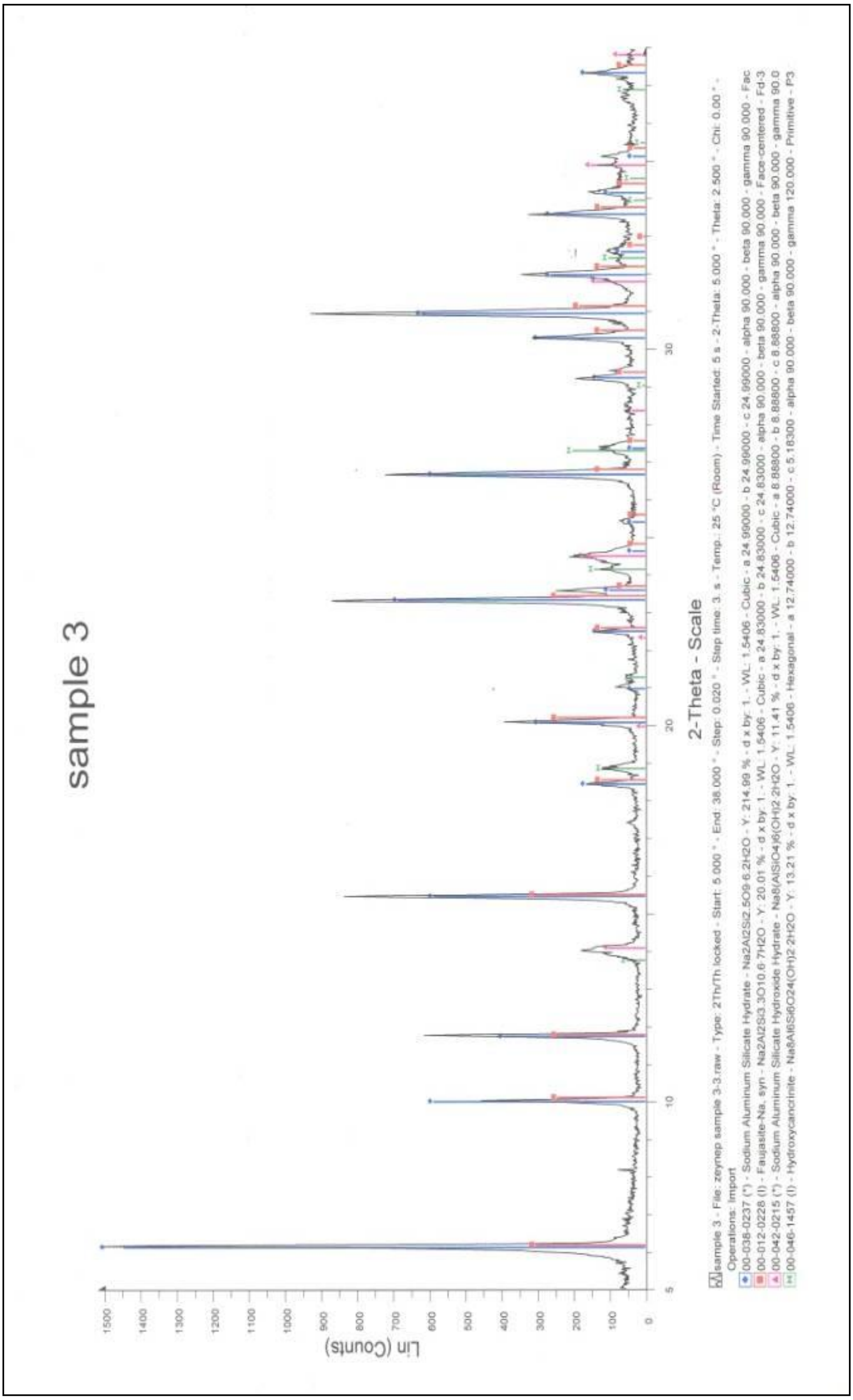


Figure 5.3. X-Ray Pattern and Matched Peaks for Sample 3

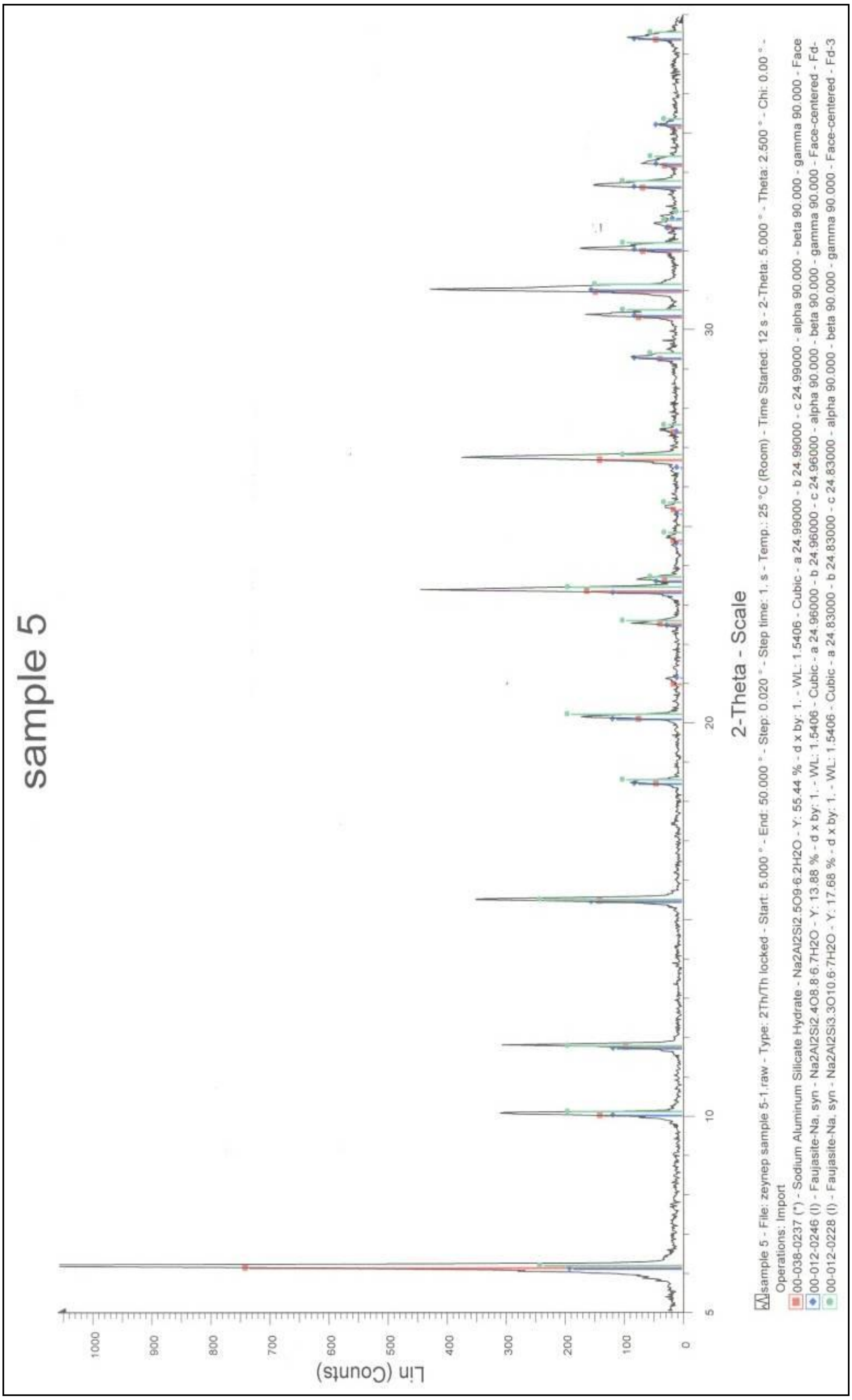


Figure 5.4. X-Ray Pattern and Matched Peaks for Sample 5

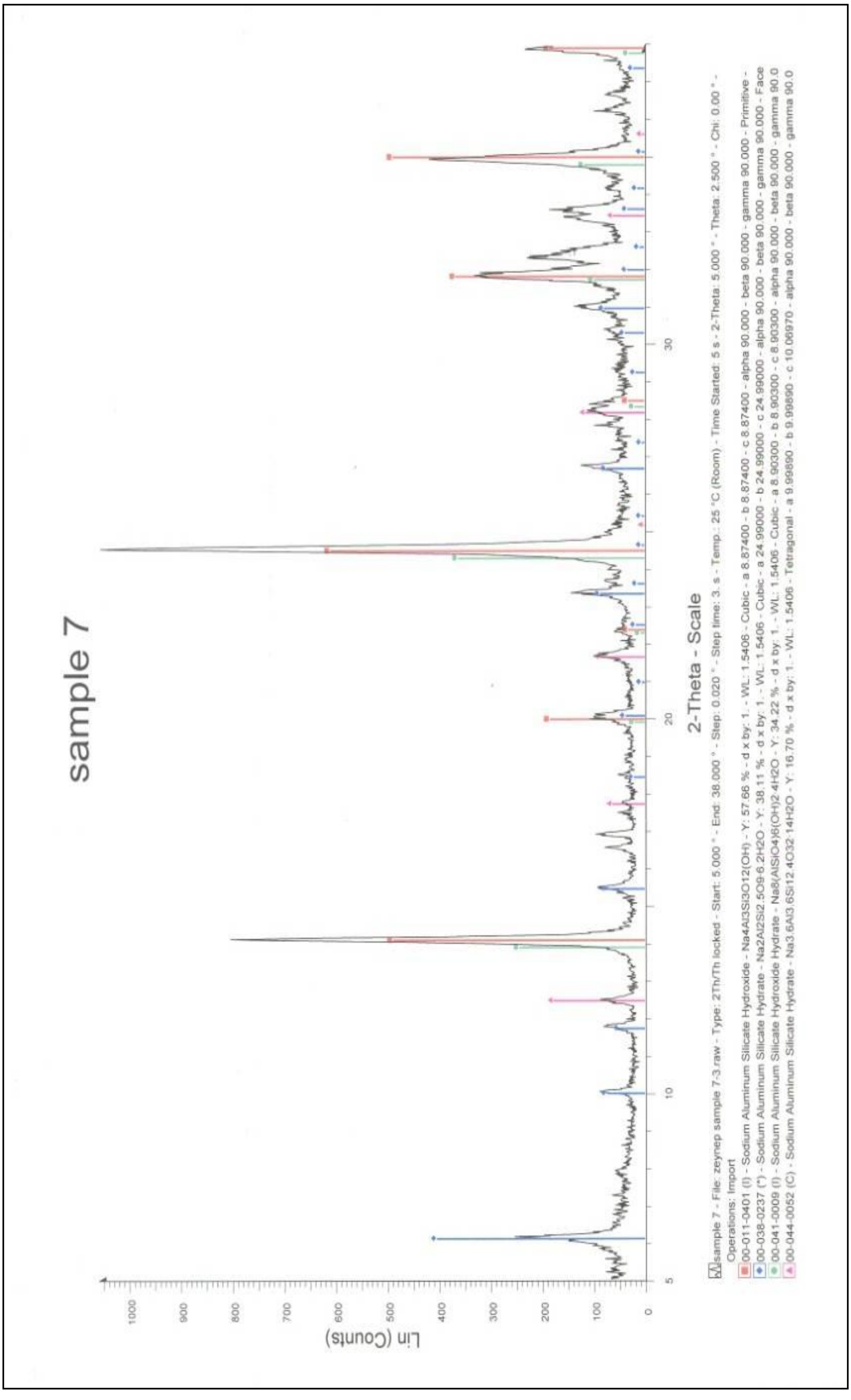


Figure 5.5. X-Ray Pattern and Matched Peaks for Sample 7

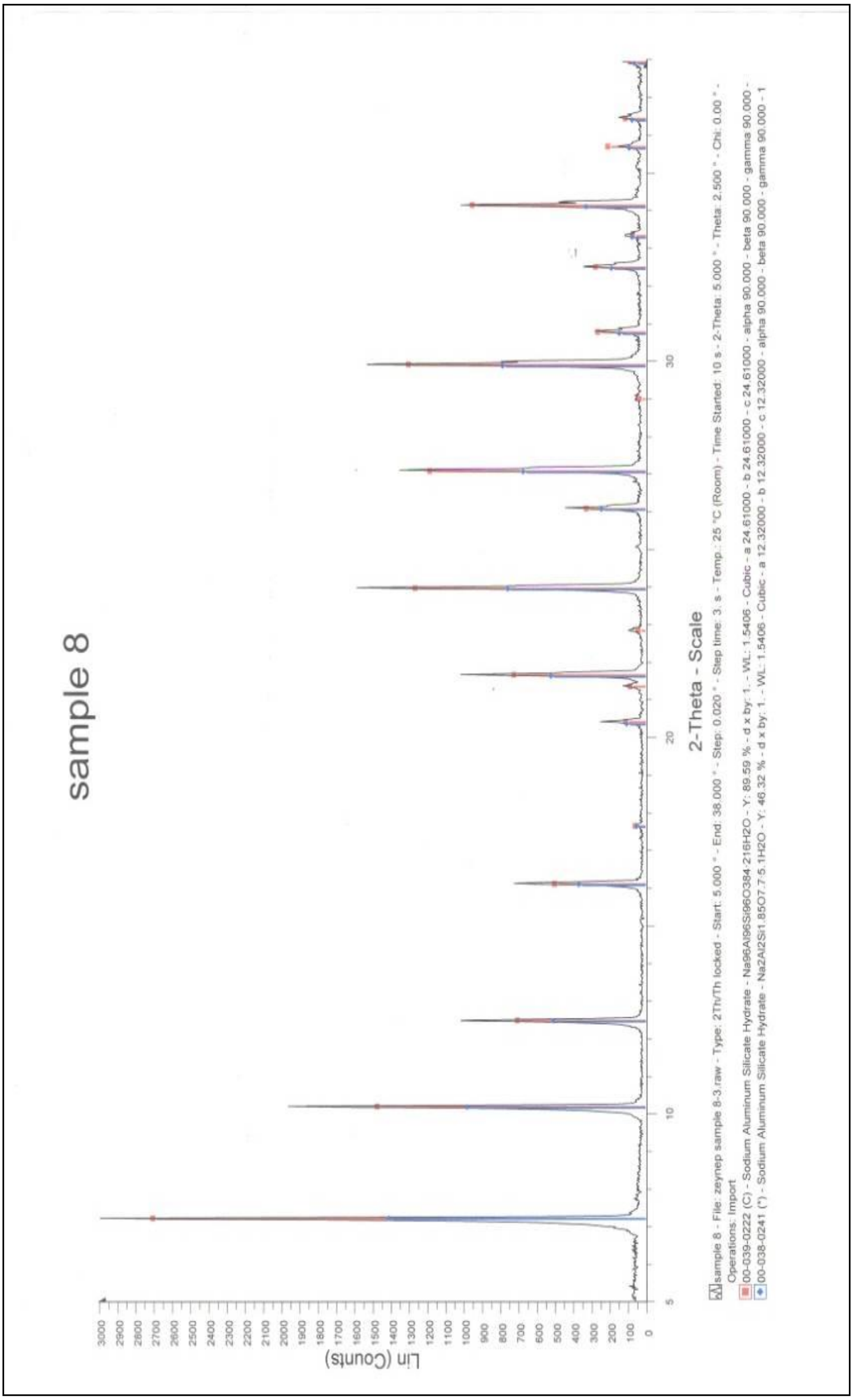


Figure 5.6. X-Ray Pattern and Matched Peaks for Sample 8

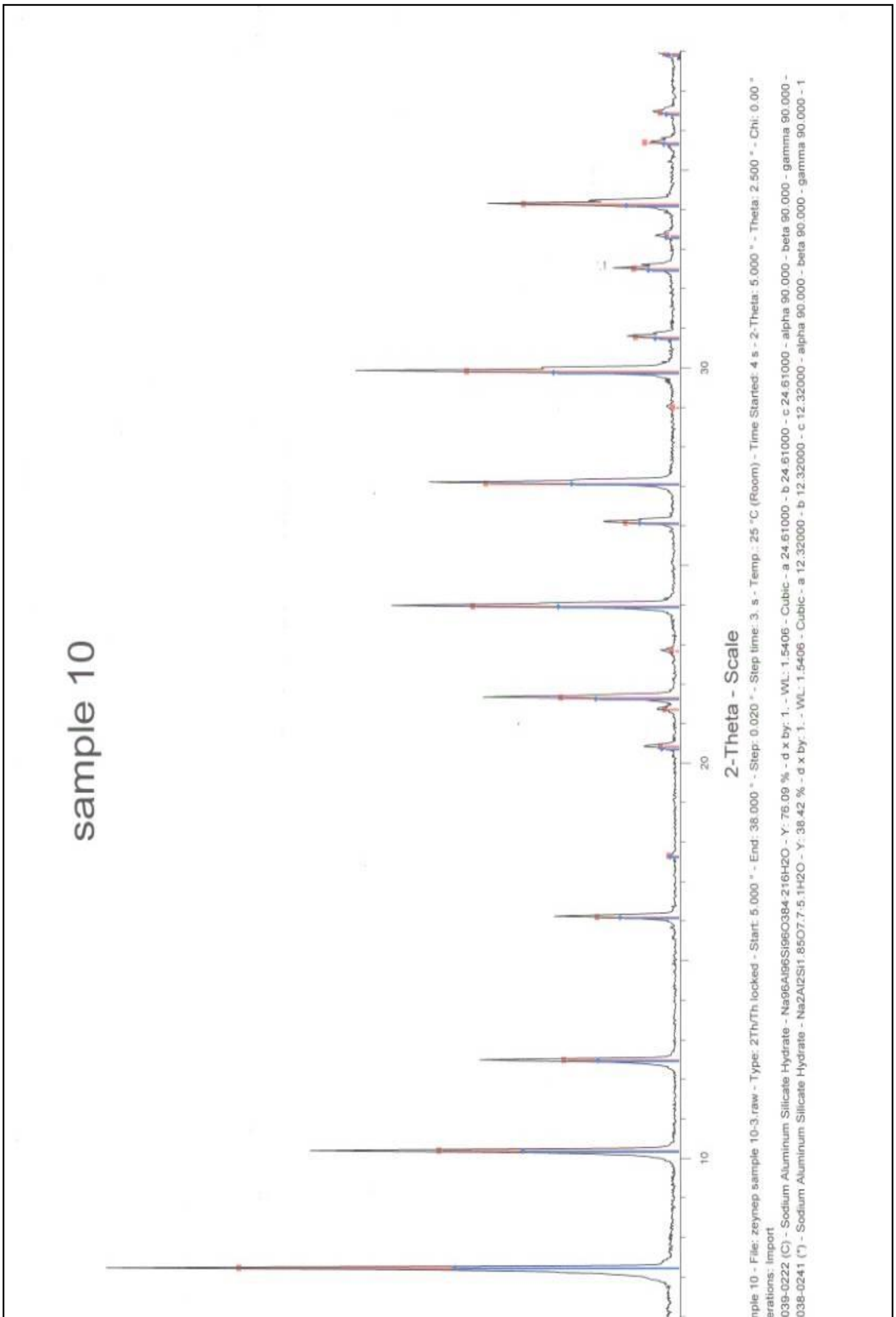


Figure 5.7. X-Ray Pattern and Matched Peaks for Sample 10

5.3. MODIFIED DISC DIFFUSION ASSAY RESULTS

5.3.1. Modified Disc Diffusion Tests of Ion Exchanged Zeolites

Ion exchanged zeolites were investigated for antimicrobial properties with modified disc diffusion assay. Modified disc diffusion assay was performed as mentioned at section 4.4. All type of zeolites were ion exchanged with 0.6 M $AgNO_3$, 2M $ZnCl_2$ and 1M $CuSO_4.H_2O$. Those 3 ion exchanged zeolite and mixture of those 3 were tested against 42 different microorganisms and test results are given in the Table 5.3, 5.4 and 5.5.

36 bacterial strains, 6 yeast species and 6 fungal species were tested during modified disc diffusion method. In Table 5.3, 5.4, 5.5 and 5.6 bold numbers shows maximum inhibition zone values. For FAU-X type of zeolite sample 5 and for LTA type zeolite sample 8 show maximum inhibition zones in average.

Clear zone around the ion exchanged zeolites and positive control indicates that there is no microbial growth around the effect area of ion exchanged zeolite and antibiotics of microorganisms. Behind the effect area microbial growth can be observed.

As it can be seen from the Figure 5.8 maximum inhibition zone developed around zinc ion exchanged zeolite 3 and 5 samples and inhibition zone radii are about 2.6 cm that is greater than positive control which is 1.8 cm. Among copper ion exchanged zeolites; zeolite 7 has maximum inhibition zone that has 1.7 cm radius. Copper zeolite 7 has inhibition zone almost equal to positive control. Among silver ion exchanged zeolites; zeolite 5 has maximum inhibition zone that has 1.5 cm radius. In figure N.C. indicates negative control which means zeolites that are not ion exchanged has no effect on microbial growth. P.C indicates positive control which is oflaxacin (antibiotic for bacterial stains). The most effective ion on *Escherichia coli* growth control is the zinc ion.

In some researches different complexes, including zinc at different concentrations showed that zinc ion has antimicrobial effect on bacterial species, mold and yeast species such as *Escherichia coli* (bacteria), *Staphylococcus aureus* (bacteria), *Bacillus subtilis* (bacteria), *Pseudomonas aeruginosa* (bacteria), *Aspergillus* spp. (mold), *Candida* spp. (yeast), and

Saccharomyces cerevisiae (yeast) [150-152]. In addition to those studies, He et al. (2010) reported that antifungal effect of ZnO on *Botrytis cinerea* and *Penicillium expansum* [153]. They demonstrated *Penicillium expansum* was more sensitive to ZnO than *Botrytis cinerea.* In our study results overlap with He's one. Zinc zeolite inhibits the growth of *P. expansum* more than *B. cinerea.*

In Figure 5.9 maximum inhibition zones against *Staphylacoccus aerous* were observed at zinc ion exchanged zeolites samples 3 and 5 as 2.8 cm where positive control nistatin created zone of 2.2 cm. this means zinc on exchanged zeolite showed greater inhibition zone then positive control. Copper ion exchanged zeolite 8 created an inhibition zone of 1.9 cm while silver ion exchanged zeolite 10 creating an inhibition zone of 1.6 cm. Consequently for *Staphylacoccus aerous* maximum inhibition zone was observed with zinc ion exchanged zeolite.

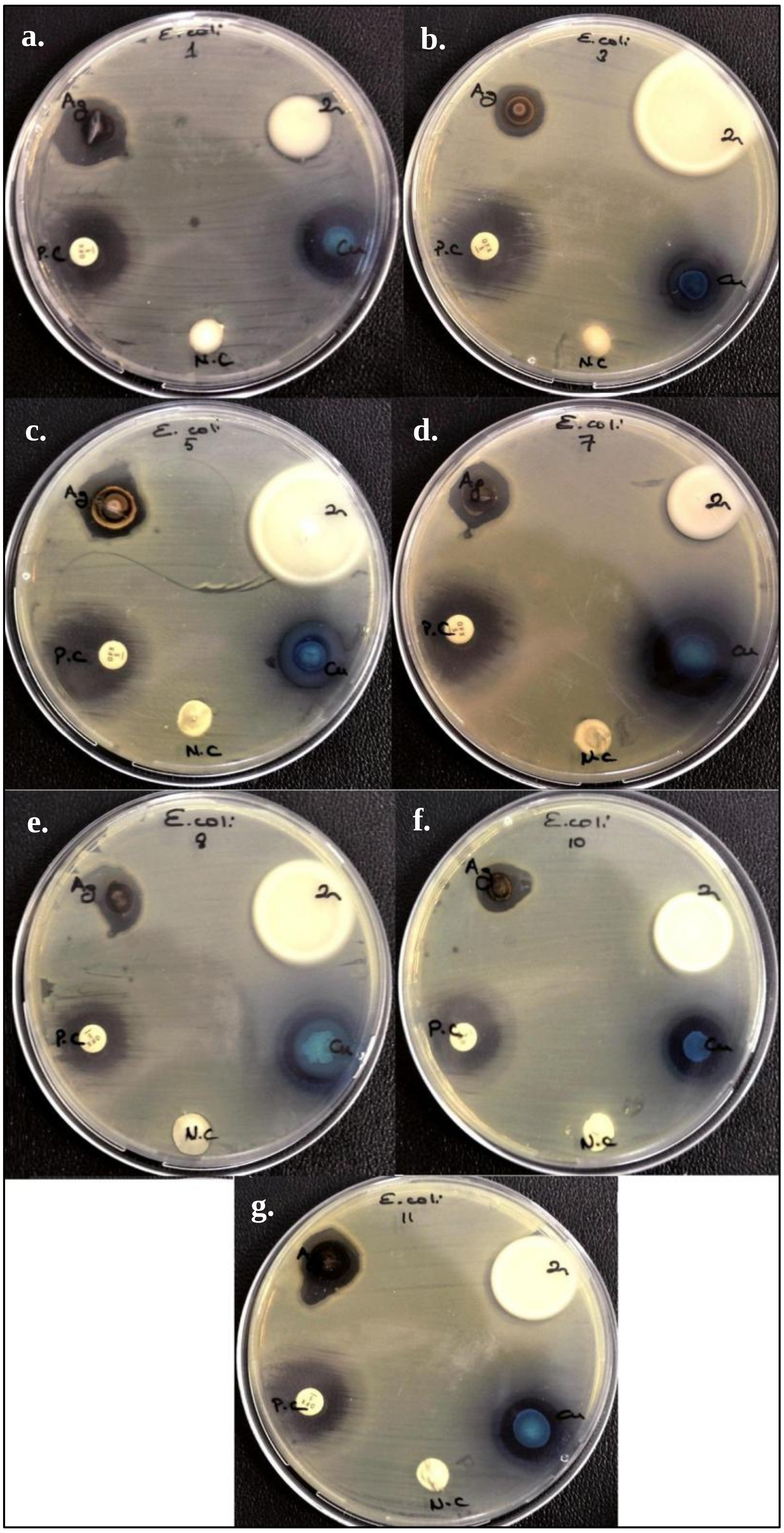


Figure 5.8. Inhibition zones (cm) around Ag^{+}, Zn^{2+} and Cu^{2+} Ion-exchanged Zeolites, Negative Control and Positive Control against *Escherichia coli* in Modified Disc Diffusion Assay; a. Sample 1, b. Sample 3, c. Sample 5, d. Sample 7, e. Sample 8, f. Sample 10, g. Sample 11

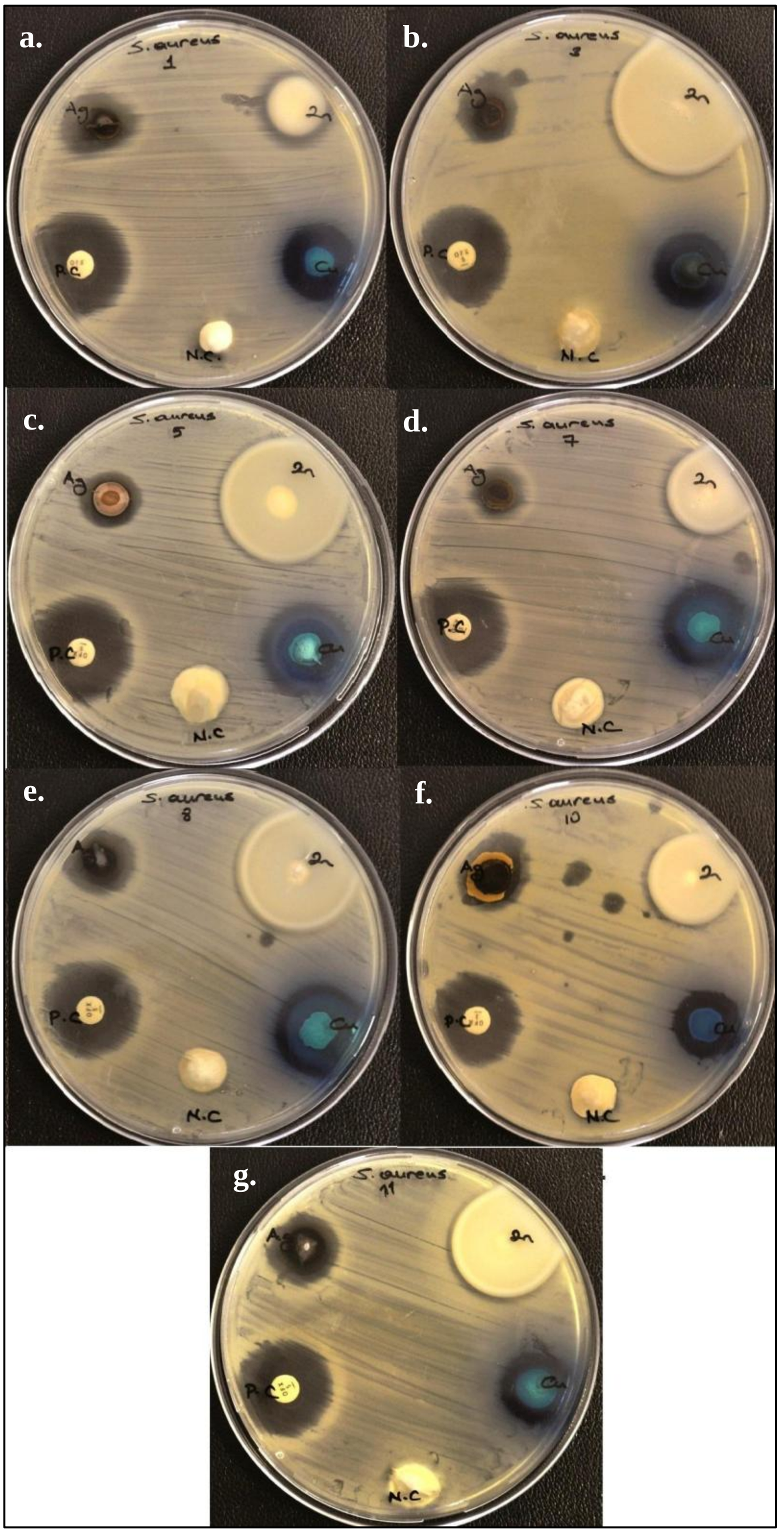


Figure 5.9. Inhibition zones (cm) around Ag^{+}, Zn^{2+} and Cu^{2+} Ion-exchanged Zeolites, Negative Control and Positive Control against *Staphylacoccus aerous* in Modified Disc Diffusion Assay; a. Sample 1, b. Sample 3, c. Sample 5, d. Sample 7, e. Sample 8, f. Sample 10, g. Sample 11

In Figure 5.10 microbial disc diffusion test was performed against yeast specie which is *Candida albicans.* As it can be clearly seen from the figure silver ion exchanged zeolites has an enormous effect on *Candida albicans.* Silver ion exchanged zeolite 5 has an inhibition zone of 4.3 cm radius which is 2.5 times of positive control that has an inhibition zone of 1.7 cm. Also copper and zinc zeolites have very satisfactory zones against *Candida albican;* copper ion exchanged zeolites 8 and 11 have an inhibition zone of 2.2 cm radius while zinc zeolite 8 has 2.4 cm zone of inhibition. Obviously silver has by far the best inhibition effect against *Candida albicans.*

Panacek et al. (2009) reported that even at low concentration silver shows a great anticandidal effect on *Candida* spp.[154]. Our results also are in agreement with the Panacek's study. Especially against *Candida albicans* and *Candida glabrata* silver zeolite have enormous effect.

Huang was reported that copper ions show antibacterial effect on *Acinetobacter baumannii* even at very low concentrations [155]. Belaid et al. showed that copper (II) complexes have no or little effect on mold and yeast species [156]. In our study copper shows antimicrobial effect on most microorganisms, except sample 11. Sample 11 almost has no effect on molds.

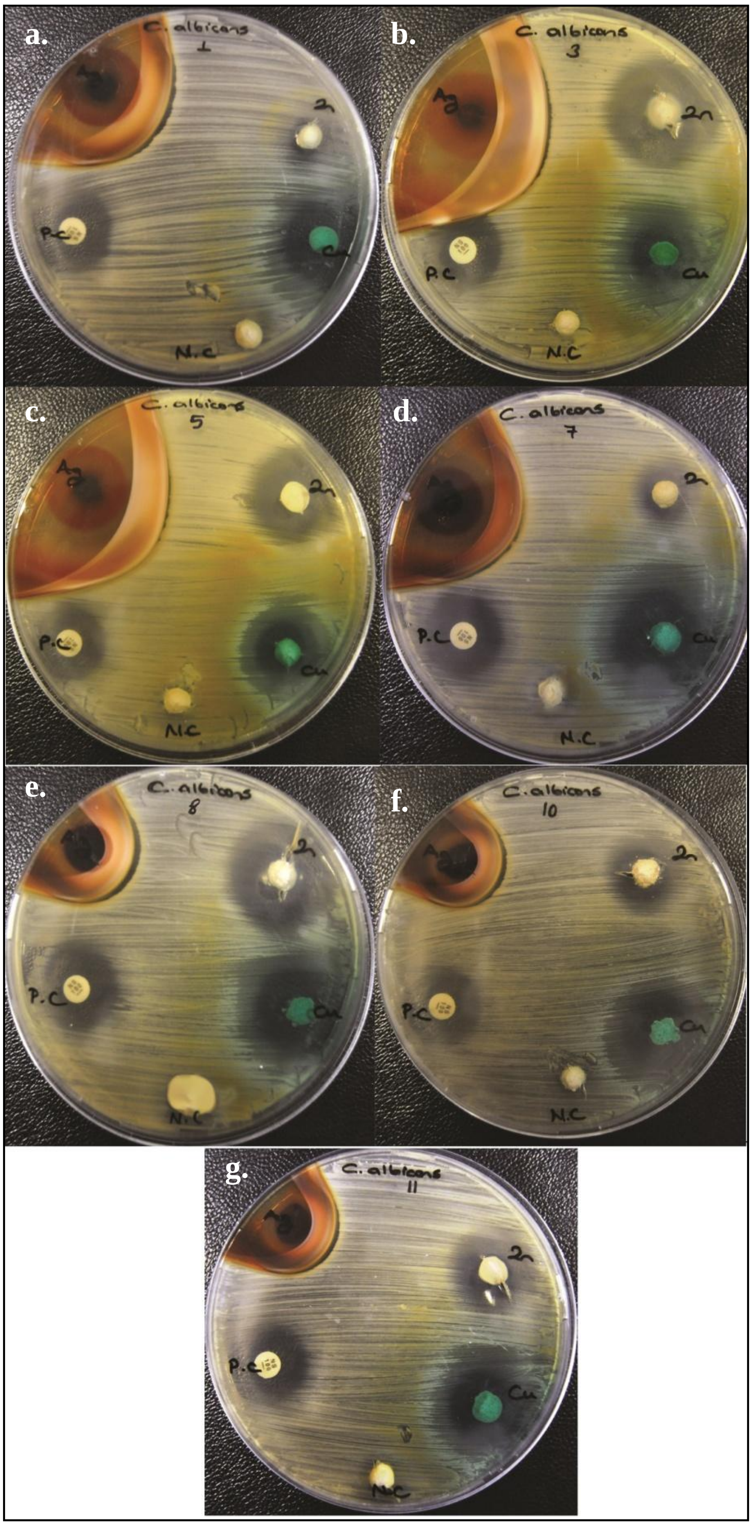


Figure 5.10. Inhibition zones (cm) around Ag^{+}, Zn^{2+} and Cu^{2+} Ion-exchanged Zeolites, Negative Control and Positive Control against *Candida albicans* in Modified Disc Diffusion Assay; a. Sample 1, b. Sample 3, c. Sample 5, d. Sample 7, e. Sample 8, f. Sample 10, g. Sample 11

Table 5.3. Inhibition zones around (cm) Ag^+ loaded zeolite discs on microorganisms tested in this study

	SILVER (Ag^+)							
Microorganism/Zeolite Type	**Sample 1**	**Sample 3**	**Sample 5**	**Sample 7**	**Sample 8**	**Sample 10**	**Sample 11**	**PC**
BACTERIA								
Pseudoxanthomonas spp.	2.2	2.1	**2.4**	1.9	2.2	2	1.9	3
Gordonia rubropentinctuc	1.5	1.5	**1.8**	**1.8**	1.4	1.3	1.3	2.3
Nocardia globerula	1.9	1.8	1.9	1.7	2.2	2.1	**2.3**	3
Acinetobacter baumannii	1.1	1	**1.3**	1.1	1.2	0.8	0.8	1.5
Stenotrophomonas maltophlia	1.6	1.6	**2.1**	**2.1**	1.5	1.5	1.6	2.7
Pseudomanas chlororaphis	**2.5**	2.2	**2.5**	2	2.4	1.8	2	3.3
Duganella zoogloeoides	1.2	1.6	1.4	1.4	**1.9**	1.3	1.3	1.2
Staphylococcus hominis hominis	1.2	1.2	1.2	1.1	**1.3**	0.6	1	1.9
Burkolderia glumea	1	1	1.3	1	**1.5**	1.1	1.3	2
Corynebacterium	1.2	1	**1.3**	**1.3**	1	0.9	1	1.8
Nocardia brasiliensis GC sub B	1	1.1	**1.2**	1	1.1	1	1.1	1.5
Acinetobacter calcoaceticus	1.1	**1.2**	1.1	1.1	**1.2**	1	1.1	1.1
Providencian heimbachae	1.2	1.2	**1.7**	1.6	1.4	1.2	1.3	2
Pseudomonas putida - biotype A	1.4	1.4	**1.7**	1.5	1.5	1.5	1.4	2.4
Acinetobacter genomospecies 3	1.2	1.1	1.4	1.3	1.1	1.1	**1.6**	1
Pseudomonas flourescens biotype G	**1.3**	**1.3**	1.1	**1.3**	**1.3**	0.8	1	1.4
Xanthomonas spp.	1.2	1.3	1.5	**2**	1.3	1.3	1.4	2
Actinomadura cremea	1.3	1.3	1.3	**1.5**	**1.5**	1.2	1.3	2
Pseudomonas maculicola	1.2	1.2	**1.8**	1.5	0.8	0.8	1.5	2.4
Hydrogenophaga pseudoflava	1.2	1.3	1.2	1.4	**1.6**	1.4	1.2	1.4
Bacillus coagulans	1	0.7	**1.2**	1.1	1.1	0.9	1	2.1
Pseudomonas stewartii ss stewartii	2.1	2.1	**2.4**	2.3	2.2	2.1	2	3.2
Chryseobacterium balustinus	2.1	2	**2.5**	2.4	2.3	2.1	2	3
Chryseobacterium meningosepticum	1.2	1.1	**1.4**	**1.4**	1.2	1.1	1.2	1.8
Nocardia transvalenssis	1.6	1.5	**1.8**	**1.8**	1.4	1	1.2	2.2
Pediococcus acidilactici/parvulus	1.8	1.8	**2**	**2**	1.9	1.6	1.8	2.4
Sphingomonas terrae	1.6	**1.8**	**1.8**	**1.8**	**1.8**	1.6	1.6	2.3
Rhodococcus rhodnii	1.8	1.6	2.2	1.9	**2**	1.6	1.7	3
Brevundimonas vesicularis	1.2	1.1	**1.5**	1.3	1.3	1	1.1	1.9
Gordonia sputi	1.2	1.1	**1.5**	1.4	**1.5**	1.2	1.4	1.7
Cellulosimicrobium cellulans	1.3	1.1	1.4	**1.5**	1.3	1.2	1.3	1.8
Sphingomonas sanguinis	1.5	1.6	1.7	**2.1**	1.5	1.3	1.6	2.5
Escherichia coli	1.4	1.2	**1.6**	**1.3**	1.3	1.1	**1.3**	1.8
Staphylococcus aureus	1.2	1.2	1.3	1.2	1.4	**1.6**	1.3	2.2
Pseudomonas aeroginosa	1.3	1.2	**1.7**	1.4	1.4	1.2	1.3	1.7
YEAST								
Kluyveromyces marxianus	1.4	1.3	**1.6**	**1.6**	1.4	**1.6**	1.4	2
Pichia membranifaciens	1.3	1.4	**1.7**	1.3	1.6	1	1.2	2.4
Candida albicans	3.5	4.1	**4.3**	2.1	2.3	2.1	3.2	1.7
Candida glabrata	4.1	**4.4**	**4.4**	3.9	3.3	3	3.1	2
Candida parapsilosis	0.8	0.7	**0.9**	0.8	0.7	0.7	**0.9**	2.2
Candida krusei	1.6	1.6	1.9	1.6	**2**	1.2	1.5	2.8
FUNGI								
Aspergillus niger	1.7	1.6	1.9	**2**	1.7	1.6	1.5	1.4
Alternaria alternata	1.9	2	**2.2**	2	**2.2**	1.9	1.8	2.5
Paecilomyces lilacinus	1.1	1.2	1.6	**1.8**	**1.8**	1.7	**1.8**	1.4
Fusarium oxysporum	**1.6**	1.1	1.4	1.4	**1.6**	1.1	1.4	1.3
Botrytis cinerea	1.8	1.6	**2.1**	1.8	1.5	1.5	1.3	2
Penicillium expansum	1.6	1.7	1.8	2	1.7	**1.9**	**1.9**	2.1
Ofloxacin and nystatin were used for bacteria and molds and fungus.								

Table 5.4. Inhibition zones around (cm) Cu^{2+} loaded zeolite discs on microorganisms tested in this study

	COPPER (Cu^{2+})							
Microorganism /Zeolite Type	**Sample 1**	**Sample 3**	**Sample 5**	**Sample 7**	**Sample 8**	**Sample 10**	**Sample 11**	**PC**
BACTERÍA								
Pseudoxanthomonas spp.	1.5	1.3	**1.6**	1.4	1.5	1.3	1.2	3
Gordonia rubropentinctuc	1.1	1.1	1.3	**1.4**	1.2	1.2	1.2	2.3
Nocardia globerula	**1.6**	1.4	1.2	1.2	1.4	1.3	**1.6**	3
Acinetobacter baumannii	1	0.6	0.6	**1.8**	0	0	0	1.5
Stenotrophomonas maltophlia	1.5	1.4	**1.8**	1.3	1.4	1.4	1.5	2.7
Pseudomanas chlororaphis	1.3	1.3	**1.5**	**1.5**	**1.5**	1.4	1.4	3.3
Duganella zoogloeoides	0	0	0	0	0	0	0	1.2
Staphylococcus hominis hominis	**1**	**1**	0	**1**	0.6	**1**	**1**	1.9
Burkolderia glumea	1	0.8	1	**1.1**	0.9	0.6	1	2
Corynebacterium	1.2	1.3	1	**1.4**	0	0	0.6	1.8
Nocardia brasiliensis GC sub B	**2**	1.5	1.7	1.8	1.6	1.6	1.3	1.5
Acinetobacter calcoaceticus	0	0	0	0	0	0	0	1.1
Providencian heimbachae	0	**1.5**	0.7	1.4	1.2	1.2	0.6	2
Pseudomonas putida - biotype A	1.6	**1.9**	0.6	1	1.1	1.1	1.3	2.4
Acinetobacter genomospecies 3	0	0	0	0	0	0	0	1
Pseudomonas flourescens biotype G	**1.3**	1.2	0	1.1	1	0	0	1.4
Xanthomonas spp.	1.3	1.3	1.2	1.2	**1.4**	1.2	1.1	2
Actinomadura cremea	1.2	**1.4**	1.3	1.2	1.1	0	0.6	2
Pseudomonas maculicola	1.1	1.1	1	**1.5**	1.1	0.8	0.9	2.4
Hydrogenophaga pseudoflava	1	1.2	**1.5**	1	0	0	0	1.4
Bacillus coagulans	**1.5**	**1.5**	1.2	1.3	1.3	1.2	1.2	2.1
Pseudomonas stewartii ss stewartii	**1.6**	**1.6**	1.5	**1.6**	1.5	1.5	1.4	3.2
Chryseobacterium balustinus	1.6	1.7	**1.8**	**1.8**	1.7	1.7	1.7	3
Chryseobacterium meningosepticum	1.3	**1.4**	1	**1.4**	1	1.1	1	1.8
Nocardia transvalenssis	1.1	**1.6**	1.5	1.5	1.2	1.1	1.3	2.2
Pediococcus acidilactici/parvulus	1.6	1.5	**1.7**	1.5	1.5	1.4	1.1	2.4
Sphingomonas terrae	1.5	**1.6**	1.2	1.1	1.5	0.8	1.2	2.3
Rhodococcus rhodnii	1.3	1.3	**1.6**	0	1.1	0	0	3
Brevundimonas vesicularis	1.4	**1.5**	1.4	1.3	1.2	1.1	1	1.9
Gordonia sputi	2	1.8	1.8	**2**	**2**	1.8	1.5	1.7
Cellulosimicrobium cellulans	**1.2**	**1.2**	1	1.1	0.6	0.6	**1.2**	1.8
Sphingomonas sanguinis	1	1.2	1.2	1.1	**1.4**	0.8	0.9	2.5
Escherichia coli	1.6	1.2	1.4	**1.7**	**1.4**	1.3	1.5	1.8
Staphylococcus aureus	1.6	1.3	1.4	1.7	**1.9**	1.6	1.5	2.2
Pseudomonas aeroginosa	0.8	0.9	0.7	**1.2**	1	1.1	1.1	1.7
YEAST								
Kluyveromyces marxianus	1.1	**1.9**	1.5	1.5	1.4	1.1	1.2	2
Pichia membranifaciens	1.2	1.2	1.2	**1.4**	**1.4**	0.8	0.8	2.4
Candida albicans	1.9	1.1	1.4	1.7	**2.2**	1.7	**2.2**	1.7
Candida glabrata	**2.1**	2.3	1.8	2.8	2.2	2.2	2	2
Candida parapsilosis	1.2	0	1	1.2	**1.3**	1	0.8	2.2
Candida krusei	**1.2**	**1.2**	**1.2**	**1.2**	1.1	1.1	1.1	2.8
FUNGUS								
Aspergillus niger	**1.3**	**1.3**	1.2	0	0.6	0	0	1.4
Alternaria alternata	1.2	**1.3**	**1.3**	1.1	1.1	1.2	1	2.5
Paecilomyces lilacinus	**1.4**	1.2	0	1.1	1.1	0	0	1.4
Fusarium oxysporum	**1.4**	1.3	0	1.2	0	0	0	1.3
Botrytis cinerea	1.2	1.4	**1.5**	1.3	1.2	1.1	0	2
Penicillium expansum	1	1.2	**1.3**	**1.3**	1.1	**1.3**	**1.3**	2.1
Ofloxacin and nystatin were used for bacteria and molds and fungus.								

Table 5.5. Inhibition zones around (cm) Zn^{2+} loaded zeolite discs on microorganisms tested in this study

	ZINC (Zn^{2+})							
Microorganism /Zeolite Type	**Sample 1**	**Sample 3**	**Sample 5**	**Sample 7**	**Sample 8**	**Sample 10**	**Sample 11**	**PC**
BACTERİA								
Pseudoxanthomonas spp.	1.3	1.4	**1.5**	**1.5**	1.2	1.1	1.1	3
Gordonia rubropentinctuc	0	1.2	**1.3**	1.1	0.8	1	1	2.3
Nocardia globerula	1.3	**1.5**	1.1	1.1	1.1	1.2	1.4	3
Acinetobacter baumannii	1.1	1.1	**1.2**	1.1	**1.2**	1	1	1.5
Stenotrophomonas maltophlia	1.3	1.6	**1.7**	1.5	1.4	1.6	1.6	2.7
Pseudomanas chlororaphis	1	1.2	1.6	**1.8**	**1.8**	**1.8**	1.6	3.3
Duganella zoogloeoides	0.8	1	**1.1**	0.6	1	1	0.9	1.2
Staphylococcus hominis hominis	**0.6**	1	0	0	0	0	0	1.9
Burkolderia glumea	0.6	1	**1.3**	1	0.7	0.8	**1.3**	2
Corynebacterium	0	0	0	0	0	0	0	1.8
Nocardia brasiliensis GC sub B	**1.5**	1	1.4	**1.5**	**1.5**	1	1.2	1.5
Acinetobacter calcoaceticus	0	0	0	0	0	0	0	1.1
Providencian heimbachae	1	**1.5**	1.1	1.1	1.4	1	1	2
Pseudomonas putida - biotype A	1.2	1.2	**1.3**	0.8	1.1	1.1	**1.3**	2.4
Acinetobacter genomospecies 3	**1.3**	1.2	**1.3**	**1.3**	1.2	**1.3**	1	1
Pseudomonas flourescens biotype G	0	0	0	0	0	0	0	1.4
Xanthomonas spp.	1	1.3	1.4	1.5	**1.6**	0.9	1.4	2
Actinomadura cremea	1	**1.2**	1.1	0	1.1	**1.2**	1.1	2
Pseudomonas maculicola	1.1	1.2	**1.6**	1.5	1.5	1.3	1.4	2.4
Hydrogenophaga pseudoflava	0	0	0	0	0	0	0	1.4
Bacillus coagulans	1.2	1.1	1.2	1	**1.3**	1.1	1	2.1
Pseudomonas stewartii ss stewartii	**2**	1.9	1.5	1.8	1.7	1.7	1.7	3.2
Chryseobacterium balustinus	1.4	1.4	**2**	1.5	1.8	1.8	1.6	3
Chryseobacterium meningosepticum	0	0	0	0	0	0	0	1.8
Nocardia transvalenssis	1.2	1.4	**1.6**	1.3	**1.6**	1.1	1.3	2.2
Pediococcus acidilactici/parvulus	1.2	1.2	1.3	1.3	**1.8**	1.3	1	2.4
Sphingomonas terrae	1.3	1.4	**1.8**	**1.8**	1.2	1.1	1.2	2.3
Rhodococcus rhodnii	1.2	1.2	1.2	1.2	1.1	**1.3**	**1.3**	3
Brevundimonas vesicularis	1	1.2	**1.6**	1.1	1.1	1.1	1.3	1.9
Gordonia sputi	1.4	**1.5**	1.4	1.2	1.2	1.4	1	1.7
Cellulosimicrobium cellulans	1	1	**1.3**	1.2	1.1	**1.3**	1.1	1.8
Sphingomonas sanguinis	1.1	1.2	**1.4**	1	1.3	1.2	1.3	2.5
Escherichia coli	1.4	**2.6**	**2.6**	1.6	1.9	1.4	1.8	1.8
Staphylococcus aureus	1.4	**2.8**	**2.8**	1.9	2.5	2.1	2.4	2.2
Pseudomonas aeroginosa	0	0	0	0	0	0	0	1.7
YEAST								
Kluyveromyces marxianus	1	1.1	**1.2**	**1.2**	**1.2**	1	**1.2**	2
Pichia membranifaciens	1.4	**1.7**	**1.7**	1.4	1.6	1.5	1.3	2.4
Candida albicans	1	2.3	2	1.6	**2.4**	1.6	1	1.7
Candida glabrata	1.3	**3.4**	**3**	1.9	3.2	2.3	2.1	2
Candida parapsilosis	1.4	1.5	**2.1**	**2.1**	1.8	1.4	1	2.2
Candida krusei	1	1.2	1.2	1.2	1.2	1.1	**1.4**	2.8
FUNGUS								
Aspergillus niger	**0.9**	0.8	0.8	0.8	0.7	0.8	0.8	1.4
Alternaria alternata	**2.1**	2	2	2	2	1.7	1.7	2.5
Paecilomyces lilacinus	1.1	1.1	1.1	**1.3**	**1.3**	**1.3**	**1.3**	1.4
Fusarium oxysporum	1	**1.3**	1.1	**1.3**	1.2	1.2	1.1	1.3
Botrytis cinerea	0.7	0.6	0.8	0.8	**1**	0.9	0.9	2
Penicillium expansum	1.1	1.3	1.2	**1.4**	1.3	1	1.2	2.1
Ofloxacin and nystatin were used for bacteria and molds and fungus.								

Table 5.6. Inhibition zones around (cm) the mixture of Ag^{+}.Zn^{2+} and Cu^{2+} loaded zeolite discs on microorganisms tested in this study

	MIXTURE							
Microorganism/Zeolite Type	**Sample 1**	**Sample 3**	**Sample 5**	**Sample 7**	**Sample 8**	**Sample 10**	**Sample 11**	**PC**
BACTERİA								
Pseudoxanthomonas spp.	1	1	**1.2**	**1.2**	1.1	**1.2**	1	3
Gordonia rubropentinctuc	1.1	1	**1.7**	1.1	1	1.2	1.4	2.3
Nocardia globerula	**1.5**	1.4	1.1	**1.5**	1.1	1.1	1.1	3
Acinetobacter baumannii	1	**1.1**	1	1	0	0	0	1.5
Stenotrophomonas maltophlia	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	1.4	0	2.7
Pseudomanas chlororaphis	1.3	1.3	1.4	**1.6**	1.5	1.5	1.2	3.3
Duganella zoogloeoides	1.4	1.4	1.4	**1.7**	1.3	1.6	1.5	1.2
Staphylococcus hominis hominis	0	**1.1**	1	1	1	1	0	1.9
Burkolderia glumea	1	0	1	**1.1**	0.9	0	0.8	2
Corynebacterium	1	1	1	1.2	1.2	**1.4**	1.2	1.8
Nocardia brasiliensis GC sub B	**1.5**	1.3	1.1	1.1	1.1	1.1	0	1.5
Acinetobacter calcoaceticus	1.1	1.2	**1.5**	1.2	1.4	1.4	1.3	1.1
Providencian heimbachae	**1.5**	**1.5**	0.9	1.3	1.2	1.1	0	2
Pseudomonas putida - biotype A	1.3	**1.7**	1	1	1.1	1.1	1.1	2.4
Acinetobacter genomospecies 3	1.3	1.2	1.3	**1.5**	1.4	**1.5**	0.9	1
Pseudomonas flourescens biotype G	0	0	0	0	0	0	0	1.4
Xanthomonas spp.	1.3	**1.5**	1.1	1.2	1.3	1.4	1.2	2
Actinomadura cremea	1.2	**1.4**	0	1.1	1	1	0	2
Pseudomonas maculicola	1.3	1.1	1.1	**1.4**	1.1	1	1	2.4
Hydrogenophaga pseudoflava	1.1	1.1	1.1	0.6	**1.2**	1.1	**1.2**	1.4
Bacillus coagulans	1.1	1.1	**1.2**	1	1	0.9	1	2.1
Pseudomonas stewartii ss stewartii	**1.5**	1.2	1.1	1	1	1.2	0.7	3.2
Chryseobacterium balustinus	1.4	1.5	1.5	1.4	**1.6**	1.4	1.1	3
Chryseobacterium meningosepticum	**1.6**	1.4	1.3	1.3	1.4	**1.6**	1.1	1.8
Nocardia transvalenssis	1	**1.5**	1	1	1	1.2	1	2.2
Pediococcus acidilactici/parvulus	1.6	**1.7**	1.6	1.3	2	1.2	1	2.4
Sphingomonas terrae	**1.5**	1.4	1.4	1.2	1.4	**1.5**	1.2	2.3
Rhodococcus rhodnii	1.4	**1.5**	1	0	1	0.9	1	3
Brevundimonas vesicularis	1.3	1.2	**1.5**	1.4	1.2	1.3	1.2	1.9
Gordonia sputi	1.1	1.3	1.4	**1.5**	**1.5**	1.4	1.1	1.7
Cellulosimicrobium cellulans	**1.4**	**1.4**	1.3	1.3	1.3	1	1.3	1.8
Sphingomonas sanguinis	1.3	1.4	**1.6**	1.5	1.4	1.4	1.3	2.5
Escherichia coli	0.7	**0.9**	**0.9**	0.7	0.7	0.7	0.7	2.4
Staphylococcus aureus	0.9	1.1	1.1	1.1	**1.2**	**1.2**	**1.2**	2.7
Pseudomonas aeroginosa	**1**	0.9	0.9	0.9	**1**	**1**	**1**	1.7
YEAST								
Kluyveromyces marxianus	1.4	1.6	1.5	1.3	1.3	**1.7**	1.5	2
Pichia membranifaciens	1.5	1	0.6	**1.6**	1.1	1.1	1.1	2.4
Candida albicans	1.8	2	**2.1**	1.2	1.8	1	1.7	1.7
Candida glabrata	2	**2.3**	2.1	2.2	2	1.8	1.3	2
Candida parapsilosis	0	0	0.9	1.1	**1.2**	0	0	2.2
Candida krusei	**1.2**	0.9	0.9	0.9	1.1	0.9	0	2.8
FUNGUS								
Aspergillus niger	0	0	0	0	0	0	0	1.4
Alternaria alternata	0	0	0	0	0	0	0	2.5
Paecilomyces lilacinus	0	0	0	0	0	0	0	1.4
Fusarium oxysporum	0	0.7	0	0	0	0	0	1.3
Botrytis cinerea	0	0	0	0	0	0	0	2
Penicillium expansum	1.1	1.2	1	**1.3**	0.8	0.8	0.8	2.1

Ofloxacin and nystatin were used for bacteria and molds and fungus.

Various concentrations of different metal containing solutions ($AgNO_3$, $ZnCl_2$ and $CuSO_4$) were prepared and ion exchanged with zeolites. In Table 5.3, 0.6 M $AgNO_3$ was prepared, ion exchanged with 7 different types of zeolites and antimicrobial effect on 48 different microorganisms were investigated. Silver-zeolites inhibited the microbial growth for all kinds of microorganisms. Moreover, effect zone of silver-zeolites were measured and it was observed that for some microorganisms silver-zeolite is more effective than the positive control. Sample 5 showed the maximum inhibition zone over 22 bacterial strain and 4 yeast species. Sample 7 and 8 showed maximum inhibition zone over 3 fungus species.

In Table 5.4, 1 M $CuSO_4$ was prepared, ion exchanged with 7 different types of zeolites and antimicrobial effect on 48 different microorganisms were investigated. However, copper-zeolite didn't inhibit the microbial growth for all kinds of microorganisms. 3 bacterial strains did not effected from any copper-zeolite which were *Duganella zoogloeoides*, *Acinetobacter calcoaceticus* and *Acinetobacter genomospecies* 3. *Acinetobacter baumannii*, *Hydrogenophaga pseudoflava* and *Fusarium oxysporum* didn't effected from any A type copper-zeolite. Sample 7 showed the maximum inhibition zone over 13 bacterial strains; sample 8 showed maximum inhibition zone over 3 yeast species and sample 5 showed maximum inhibition zone over 3 fungus species. According to these results it can be said that copper loaded zeolite X is more effective on microorganisms than copper loaded zeolite A.

In Table 5.5, 2 M $ZnCl_2$ was prepared, ion exchanged with 7 different types of zeolites and antimicrobial effect on 48 different microorganisms were investigated. Also just like copper loaded zeolite, zinc-zeolite was not effective on all kind of microorganisms. Zinc loaded zeolite could not inhibit *Corynebacterium*, *Acinetobacter calcoaceticus*, *Pseudomona flourescens biotype G*, *Hydrogenophaga pseudoflava*, *Chryseobacterium meningosepticum* and *Pseudomonas aeroginosa* growth. These microorganisms are all bacteria, zinc exchanged zeolites were effective on yeast and fungus species. Sample 5 showed the maximum inhibition zone over 16 bacterial strains and 4 yeast species. Sample 7 showed maximum inhibition zone over 3 fungus species.

Table 5.6 shows the inhibition zone of microorganisms for Ag-Zn-Cu ion exchanged zeolite mixture. One third of the mixture is composed of silver-zeolite, one third of the mixture is composed of zinc-zeolite and the last one third of the mixture is composed of cupper-zeolite. As it can be seen from the table mixture of those three metal loaded zeolite has no effect on any kind of fungus species but *Penicillium expansum*. Also zeolite mixture has no effect on the bacterial species *Pseudomonas flourescens biotype G.* Sample 3 showed the maximum inhibition zone over 11 bacterial strains and sample 8 showed maximum inhibition zone over 2 yeast species.

As an average for zeolite X, silver ion exchanged sample 5 showed maximum inhibition zone in 14 bacterial strains, 2 yeast species and 2 fungus species; for zeolite A silver ion exchanged sample 8 showed maximum inhibition zone in 7 bacterial strains, 1 yeast specie and 2 fungus species. Maximum inhibition zones were observed with 0.6 M silver ion exchanged zeolites. Therefore at first sample 5 and 8 silver loaded zeolites were mixed with dust and liquid paint and tested against microorganisms with modified disc diffusion method. 0.6 M silver ion exchanged zeolites were mixed with dust and liquid paints at 7%, 10% and 12% (wt/wt) but results were not satisfactory, hence molarity of the $AgNO_3$ was increased to 1M and tests were performed for on some microorganisms again. Figure 5.11, 12 and 13 shows some modified disc diffusion tests against some microorganisms and Table 5.7 shows the experimental results for some specific microorganisms.

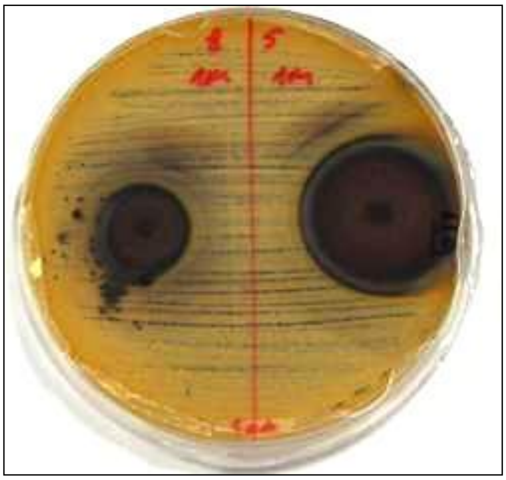


Figure 5.11. Inhibition zones (cm) around Ag^+ Ion-exchanged Zeolites 8 and 5 against *Pichia membranaefciens* in Modified Disc Diffusion Assay

Figure 5.12. Inhibition zones (cm) around Ag^+ Ion-exchanged Zeolites 8 and 5 against *Candida albicans* in Modified Disc Diffusion Assay

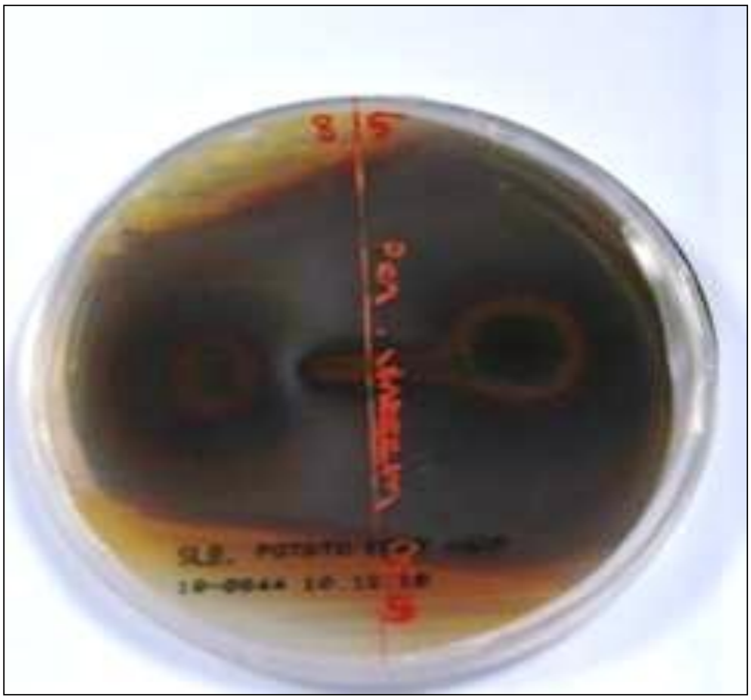


Figure 5.13. Inhibition zones (cm) around Ag^+ Ion-exchanged Zeolites 8 and 5 agaist *Penicillium vinaceum* in Modified Disc Diffusion Assay

Figure 5.10, 5.11 and 5.12 shows modified disc diffusion tests for 1M silver ion exchanged zeolites 5 and zeolite 8. Zeolite 8 was placed to the left hand side while zeolite 5 was placed to the right hand side of the petri dish on which the microorganisms were spread on the agar at the beginning of the test.

As it can be seen from the Figure 5.11, 5.12 and 5.13 silver ion exchanged zeolite 5 has more inhibitive effect on microorganisms than ion exchanged zeolite 8. When Table 5.7 is scanned we can see that 1M silver ion exchanged zeolite 5 has more inhibitive effect on almost for all microorganisms.

Table 5.7. Inhibition zone of 1M silver loaded zeolite samples on microorganisms

	1M Silver Ion Exchanged	
Microorganism/Zeolite Type	**Sample 5**	**Sample 8**
Bacteria		
Escherichia coli	1.2	1.1
Pseudomonas aeruginosa	1.3	1.5
Bacillus megaterium	1.2	1.6
Staphylococcus aureus	1.8	1.2
Yeast		
Candida albicans	4.5	2.4
Kluyveromyces marxianus	2.6	2.5
Pichia membranaefaciens	2.2	1.6
Fungus		
Botrytis cinerea	7.0	3.6
Fusarium oxysporum	7.0	4.4
Penicillium vinaceum	7.0	5.0
Penicillium expansum	6.0	4.0
Aspergillus niger	6.5	4.5
Alternaria alternata	7.5	4.5

5.3.2. Modified Disc Diffusion Tests of Silver Ion Exchanged Zeolites Mixed with Liquid Paint

After all microbial tests were performed and concentrations were determined 1M silver-zeolite 5 and 8 were mixed with liquid paint. Silver loaded zeolites were mixed with liquid paint at 10% (wt/wt) ratio and tested against microorganisms. Tests were performed against bacteria, yeast and fungus species with modified disc diffusion method. Results were positive therefore this formulation was applied on metal plates and microbial tests were performed as explained in Section 4.5. Figure 5.14, 5.15 and 5.16 show the modified disc diffusion tests against some microorganisms.

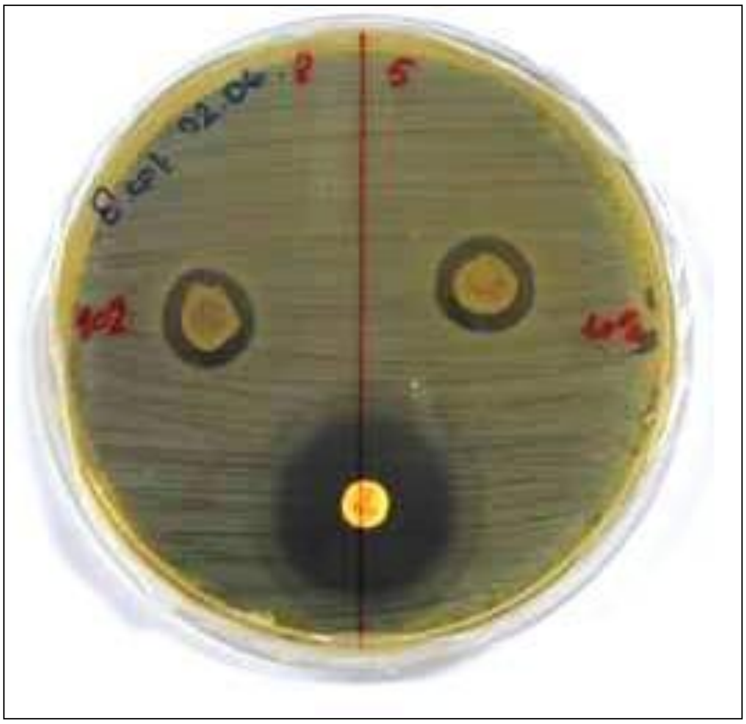


Figure 5.14. Modified Disc Diffusion Test of liquid paint containing 10% (wt/wt) Ag zeolite against *Escherichia coli*

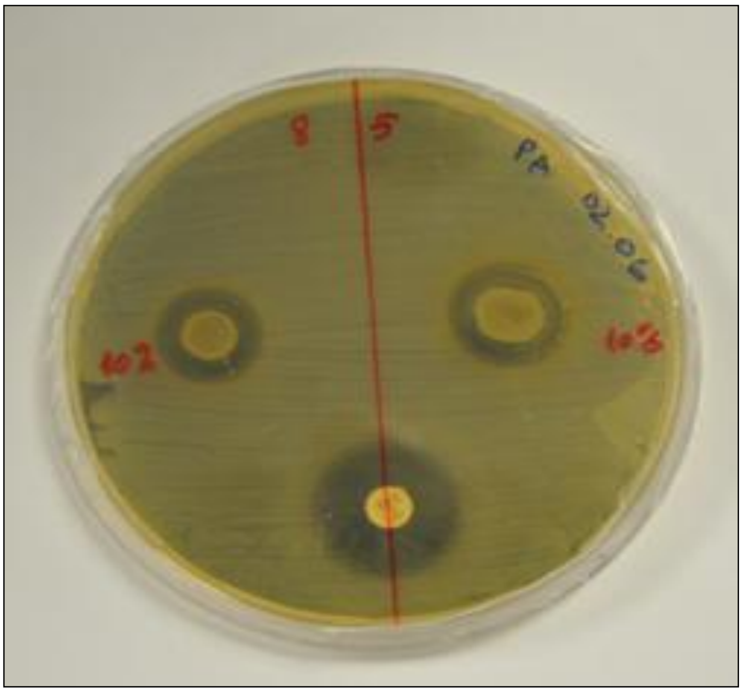


Figure 5.15. Modified Disc Diffusion Test of liquid paint containing 10% (wt/wt) Ag zeolite against *Pseudomonas aeruginosa*

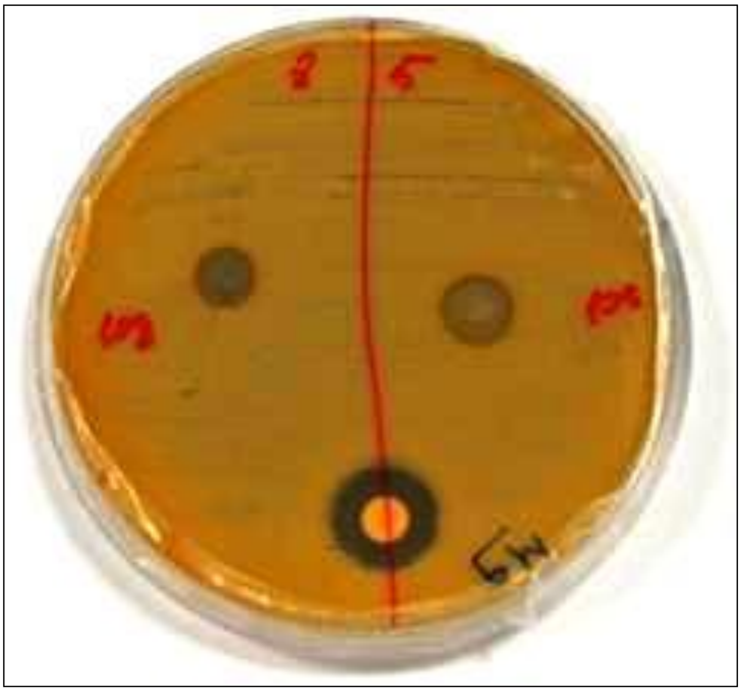


Figure 5.16. Modified Disc Diffusion Test of liquid paint containing 10% (wt/wt) silver zeolite against *Pichia membranaefaciens*

Figure 5.14 and 5.15 show antibacterial effect and Figure 5.16 shows the anticandidal effect of liquid paint mixed with silver ion exchanged zeolite 5 and 8. Zeolite was mixed into liquid paint at 10% (wt/wt) ratio. Zeolite 8 was placed to the left hand side of the petri dish while zeolite 5 was placed to the right hand side. In all Figures 5.14, 5.15 and 5.16 effect zone of zeolite is emerged around the liquid paint mixed with both zeolite 5 and 8. Thus; it can be said that liquid paint mixed with silver ion exchanged zeolite 5 and 8 are effective against microorganisms.

5.3.3 Modified Disc Diffusion Tests of Silver Ion Exchanged Zeolites Mixed with Dust Paint

Vestel Inc. paints outer surface of their washing machines with dust paint. Negatively charged dust paint is sprayed onto positively charged washing machine outer shell, so the paint holds on to surface. Metal outer part of the washing machine passes through a hot line and then dust paint melts on outer shell and sticks onto material. Therefore that kind of dust paint was provided from Vestel Inc. and mixed with silver zeolites in three different ratios (7%, 10% and 12% (wt/wt)). Later these mixtures were tested against microorganisms with modified disc diffusion method.

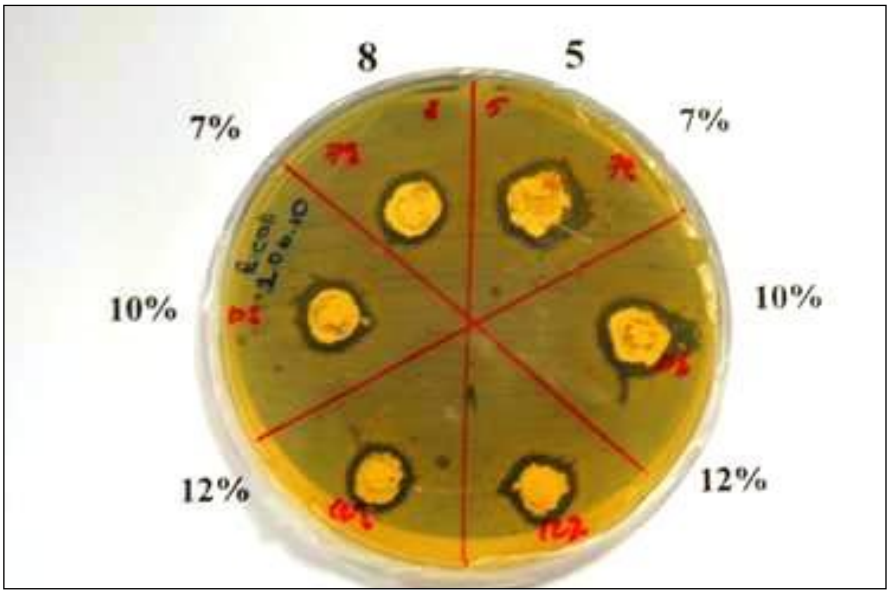


Figure 5.17. Antibacterial test for dust paint mixed with silver zeolite at various concentrations against *Escherichia coli*

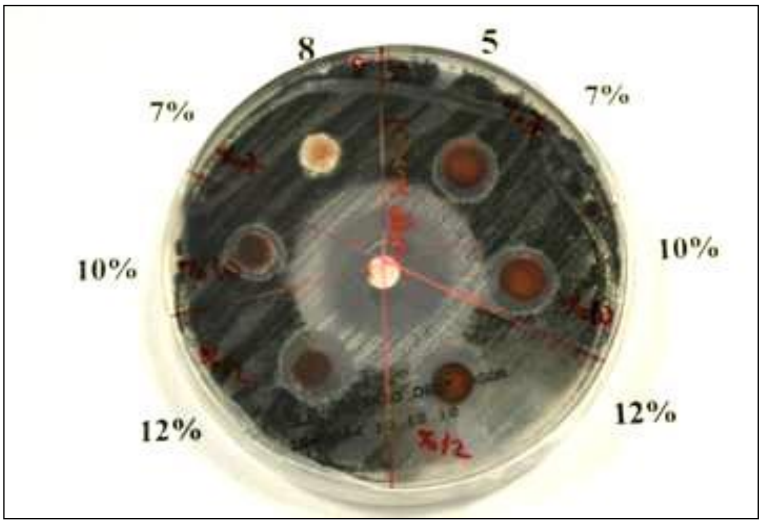


Figure 5.18. Antifungal test for dust paint mixed with silver zeolite at various concentrations against *Alternaria alternata*

Figure 5.17 and 5.18 show antibacterial and antifungal effect of dust paint mixed with silver ion exchanged zeolite 5 and 8 at various (7%, 10% and 12%) weight per centages. Zeolite 5 combinations were placed to the right hand side of the petri dish while zeolite 8 combinations were placing to the left hand side. Positive control discs were placed into the middle of the petri dishes. In Figure 5.18 7% zeolite 8 was not effective against a fungus specie which was *Alternaria alternata.* Therefore for sample 5 zeolite 7% was enought but for sample 8 zeolite weight per cent was increased to 10%.

5.4. DEVELOPING ANTIMICROBIAL SURFACES AND ANTIMICROBIAL TESTS

5.4.1. Metal Surfaces Painted with Liquid Paint and Zeolite Mixture

Subsequent to concentration and zeolite type optimizations, metal plates were cut off about 4cm x4cm size and painted with 10% (wt/wt) silver ion exchanged zeolite (1M) and liquid paint mixture. Then these metal plates were placed into plastic petri dishes and 2 mL SDB (Sabroud Dextrose Broth) was dropped onto painted metal surfaces. Later SDB was contaminated with *Aspergillus niger, Penicillium expansum* and *Botrytis cinerea*. 1 mL

SDB was added onto surfaces to provide nutrient for microbial growth and keep humidity stable.

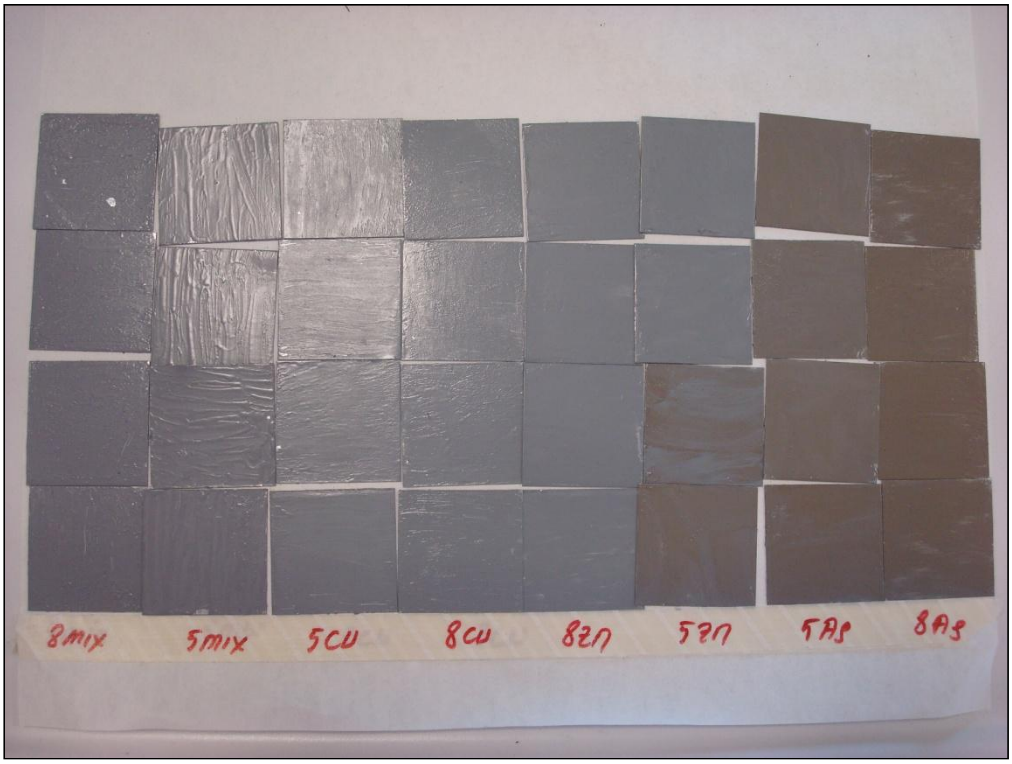


Figure 5.19. Metal Plates Painted with Liquid Paint and Different Types of Zeolite

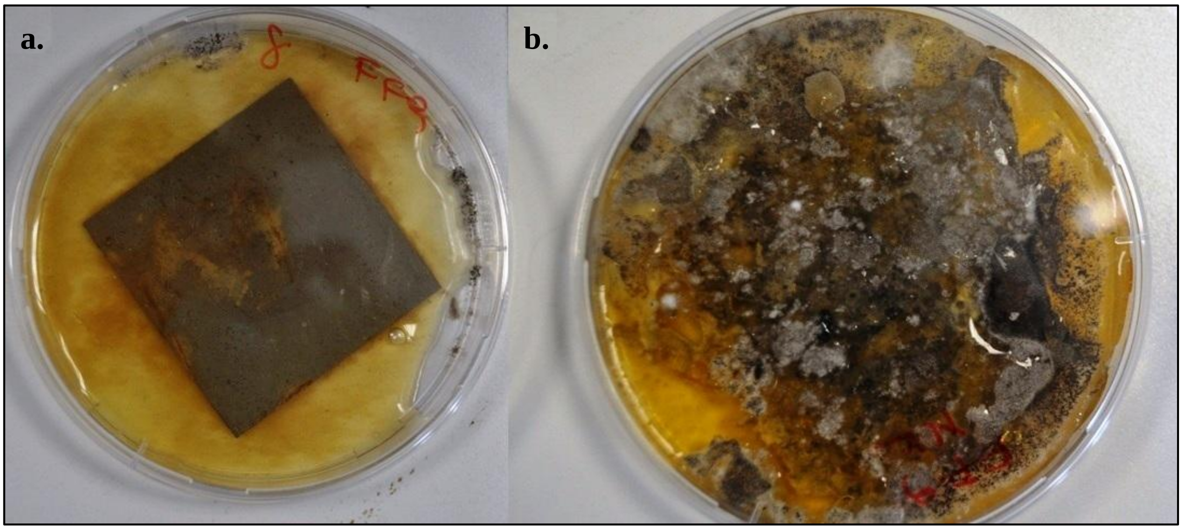


Figure 5.20. Growth of *Aspergillus niger* on metal plates painted with a. 10% silver zeolite liquid paint mixture b. commercial liquid paint (NC)

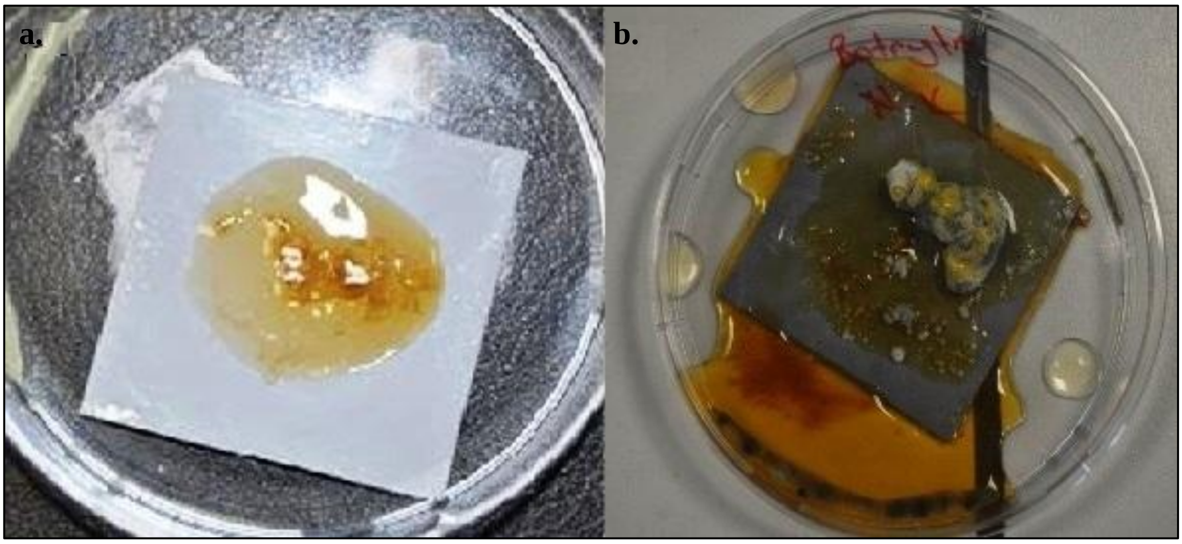


Figure 5.21. Growth of *Botrytis cinerea* on metal plates painted with a. 10% zinc zeolite liquid paint mixture b. commercial liquid paint (NC)

Figure 5.20 and Figure 5.21 represent 45th day after microbial contamination. In Figure 5.20.a and Figure 5.21.a metal plates were painted with 10% silver zeolite and 10% zinc zeolite respectively. The color difference arise from the color of the liquid paints, grey paint was used for silver zeolite while white paint was using for zinc zeolite. As it can be clearly seen from the figures ion exchanged zeolites inhibited the microbial growth while commercial paints supporting the phenomena. In Figure 5.20.a *Aspergillus niger* spores can be seen near the wall of petri dish but there is no spore on the metal plate, however metal plate painted with commercial paint in Figure 5.20.b *Aspergillus niger* spores cover all over the metal plate. In Figure 5.21.a *Botrytis cinerea* spores cannot be seen on the metal plate painted with 10% zinc zeolite mixed with liquid paint but for the case of metal plate painted with commercial paint in Figure 5.21.b *Botrytis cinerea* spores grow over the metal plate.

5.4.2. Metal Surfaces Painted with Dust Paint and Zeolite Mixture

Just like tests on metal plates painted with liquid paint zeolite mixture; after concentration and zeolite type optimizations, metal plates were cut off into squares o about 4cm*4cm size and painted with 10% and 12% (wt/wt) zinc and silver ion exchanged zeolite (2M and 1M respectively) and dust paint mixture. Then these metal plates were placed into plastic petri dishes and 2 mL SDB (Sabroud Dextrose Broth) was dropped onto painted metal surfaces. Later SDB was contaminated with *Penicillium expansum*, *Aspergillus niger* and *Botrytis cinerea*. 1 mL SDB was added onto surfaces to provide nutrient for microbial growth and keep humidity stable.

Figure 5.22, Figure 5.23, Figure 5.24, Figure 5.25, Figure 5.26 and Figure 5.27 represent 60th day after microbial contamination. The color difference between 10% silver zeolite and 10% zinc zeolite painted metal surfaces arise from the color of the liquid paints, grey paint was used for silver zeolite while white paint was using for zinc zeolite.

In Figure 5.22 zeolite 5 was mixed with dust paint and contaminated with *Penicillium expansum.* Figure 5.22.a represents the negative control; metal plate was painted with commercial dust paint and after 6 days *Penicillium expansum* spores were developed on painted metal surfaces. Figure 5.22.b, c, d, e represents metal plates painted with %10 zinc ion exchanged zeolite-dust paint mixture, %12 zinc ion exchanged zeolite-dust paint mixture, 10% silver ion exchanged zeolite-dust paint mixture and 12% silver ion exchanged zeolite-dust paint mixture. After 60 days there was still no microbial contamination over the plates. This test was performed till the observation of microbial growth on metal sheets. As result silver and zinc zeolite 5 mixed dust paint was inhibited the microbial growth for 4.5 months then fungus spores started to show off on the plates. These fastened tests are equal to about 9 years in normal life cycle. Every 15 days for fastened tests are equal to about 1 year.

Figure 5.23 plates were contaminated with *Penicillium expansum* but this time metal plates were painted with the zeolite 8 mixture, rest of the set up was the same with Figure 5.22. Results were also satisfactory for this setup; Figure 22 and 5.23 represent the 60th day of the experiment but experiment was kept on and after 140 days microbial contamination over the plates started to emerge. Silver and zinc zeolite 8 mixed dust paint inhibited the microbial growth for 140 days. Same negative control was used for zeolite 5 and 8 setups.

Figure 5.24, 5.25 and 5.26, 5.27 were contaminated with *Aspergillus niger* and *Botrytis cinerea* respectively. Figure 5.24 and 5.26 show metal plates painted with zeolite 5 mixtures while Figure 5.25 and 5.27 show metal plates painted with zeolite 8 mixtures.

Figure 5.24.a, 5.25.a, 5.26.a and 5.27.a shows the commercial paint contaminated with microorganisms and as it can clearly be seen from the figures microorganism spores cover the metal plate while others remain clear. To sum up; metal plates painted with dust paint and ion exchanged zeolite mixture inhibit the microbial growth for at least 4.5 months while commercial paint supports the microbial growth.

5.4.3. Polymer Surfaces Painted with Acrylic Paint and Zeolite Mixture

In order to make polymer parts that are painted with acrylic paint of the washing machines hygienic; ion exchanged zeolite 5 and 8 were sent to Vestel Inc.. Acrylic paint was mixed with silver ion exchanged (1Molar) zeolite at 10, 12 and 15 weight per centages (wt/wt) and plastic parts were painted with that combinations. Plastic parts were cut approximately same size, put into petri dishes and labeled. Same procedure for metal surfaces painted with liquid paint mixed with antimicrobial zeolites and metal surfaces painted with dust paint mixed with antimicrobial zeolites were repeated. Painted surfaces contaminated with *Aspergillus niger*, *Botrytis cinerea* and *Penicillium expansum*. Figures 5.28, 5.29 and 5.30 show the 7.5th month of the experiment.

Figure 5.28 shows the polypropylene (pp.) surfaces painted with acrylic paint mixed antimicrobial zeolite at different weight per centages contaminated with *Aspergillus niger*. Pp. plates in Figure 5.28.a and 5.28.b painted with 10% silver zeolite 8 and 12% silver zeolite 8 acrylic paint mixture respectively. As it can be seen from the figure *Aspergillus niger* spores covered the surface of the plastic. Also in Figure 5.28.d which represents the pp. plate painted with 10% silver zeolite 5 acrylic paint mixture, *Aspergillus niger* spores can be seen on the pp. surface. This means zeolite 8 10%, 12% and zeolite 5 10% is not efficient for controlling the *Aspergillus niger* growth on pp. surface. But in Figure 5.28.c, 5.28.e and 5.28.f there is no microbial growth on pp. plate painted with 15% silver zeolite 8, 12% silver zeolite 5 and 15% silver zeolite 5 acrylic paint mixtures control the microbial growth on pp. surfaces for 7.5 months which is equal to approximately 15 years in daily life.

In Figure 5.29 polypropylene surfaces contaminated with *Botrytis cinerea*. After 7.5 months as it can be seen from Figure 5.29.d and 5.29.e which are 10% silver zeolite 5 and

12% silver zeolite 5 acrylic paint mixtures lost their effect while Figure 5.29.a, 5.29.b, 5.29.c and 5.29.f which are 10% silver zeolite 8, 12% silver zeolite 8, 15% silver zeolite 8 and 15% silver zeolite 5 acrylic paint mixture kept their effect against *Botrytis cinerea*.

In Figure 5.30 polypropylene surfaces contaminated with *Penicillium expansum*. After 7.5 months as it can be seen from Figure 5.30.a, 5.30.b and 5.30.d which are 10% silver zeolite 8, 12% silver zeolite 8 and 10% silver zeolite 5 acrylic paint mixtures lost their effect while Figure 5.30.c, 5.30.e and 5.30.f which are 15% silver zeolite 8, 12% silver zeolite 5, 15% silver zeolite 5 acrylic paint mixture kept their effect against *Penicillium expansum*.

As a conclusion Silver ion exchanged zeolite 5 and 8 mixed with acrylic paint at 15% weight fraction are effective against these three kinds of fungus species for 7.5 months. If a pp. surface painted with these mixtures they will keep surface hygienic for 15 years.

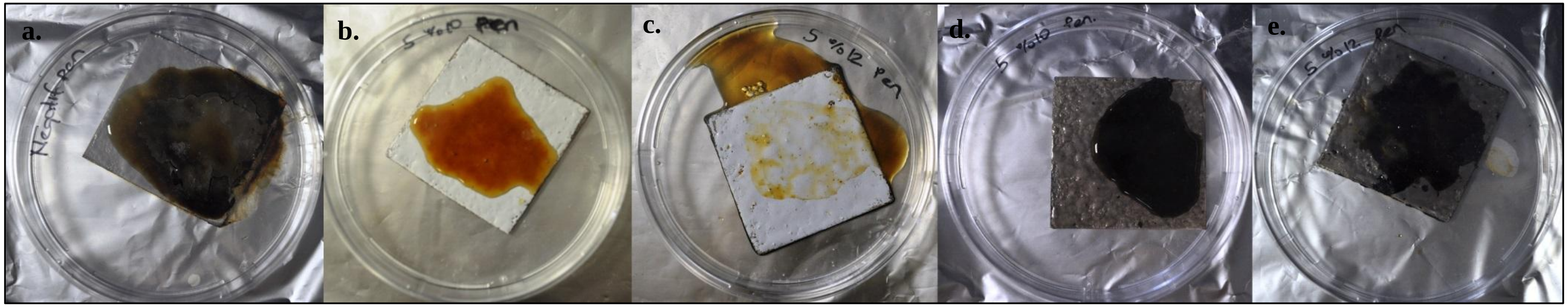


Figure 5.22. Growth of *Penicillium expansum* on metal plates painted with a. commercial dust paint, b. 10% zinc zeolite 5 dust paint mixture, c. 12% zinc zeolite 5 dust paint mixture, d. 10% silver zeolite 5 dust paint mixture, e. 12% silver zeolite 5 dust paint mixture

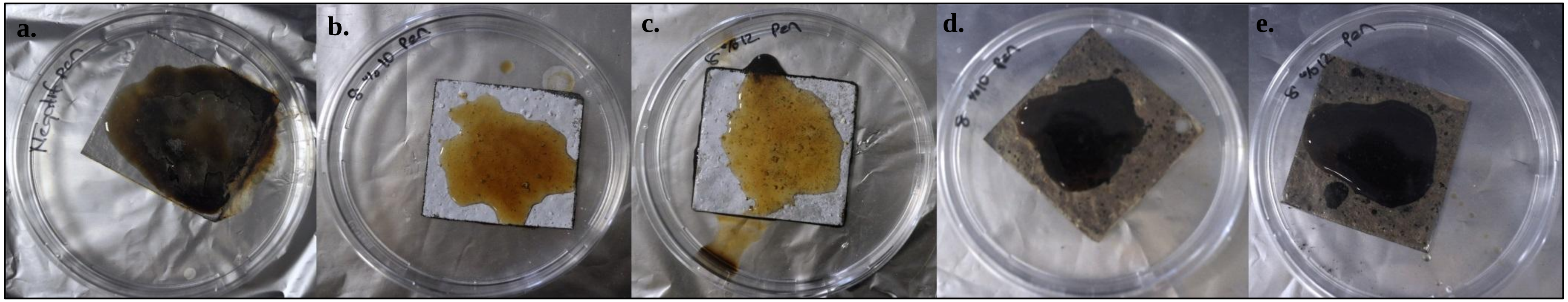


Figure 5.23. Growth of *Penicillium expansum* on metal plates painted with a. commercial dust paint, b. 10% zinc zeolite 8 dust paint mixture, c. 12% zinc zeolite 8 dust paint mixture, d. 10% silver zeolite 8 dust paint mixture, e. 12% silver zeolite 8 dust paint mixture

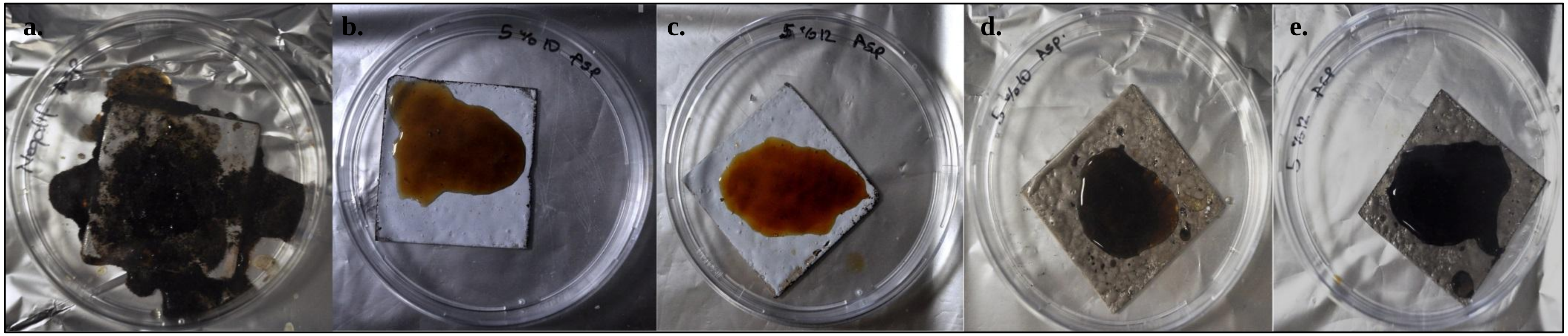


Figure 5.24. Growth of *Aspergillus niger* on metal plates painted with a. commercial dust paint, b. 10% zinc zeolite 5 dust paint mixture, c. 12% zinc zeolite 5 dust paint mixture, d. 10% silver zeolite 5 dust paint mixture, e. 12% silver zeolite 5 dust paint mixture

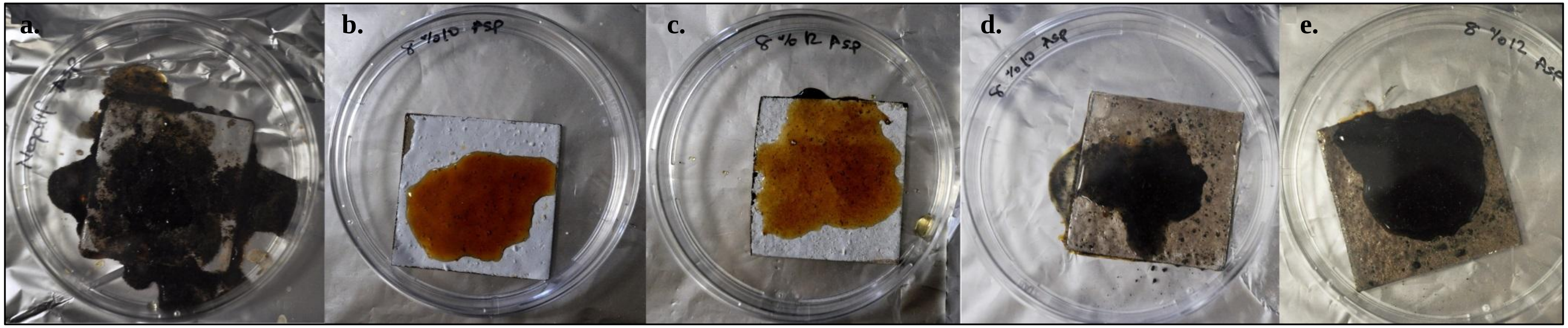


Figure 5.25. Growth of *Aspergillus niger* on metal plates painted with a. commercial dust paint, b. 10% zinc zeolite 8 dust paint mixture, c. 12% zinc zeolite 8 dust paint mixture, d. 10% silver zeolite 8 dust paint mixture, e. 12% silver zeolite 8 dust paint mixture

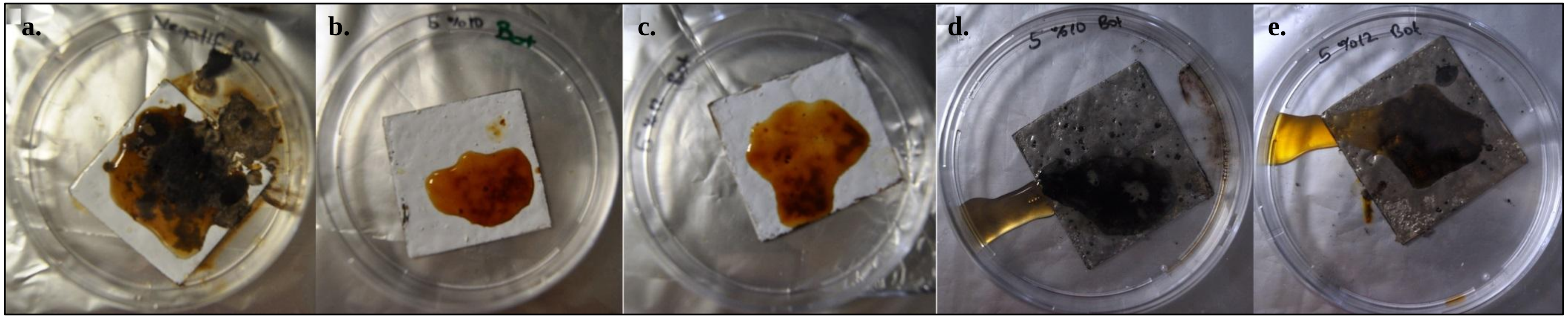


Figure 5.26. Growth of *Botrytis cinerea* on metal plates painted with a. commercial dust paint, b. 10% zinc zeolite 5 dust paint mixture, c. 12% zinc zeolite 5 dust paint mixture, d. 10% silver zeolite 5 dust paint mixture, e. 12% silver zeolite 5 dust paint mixture

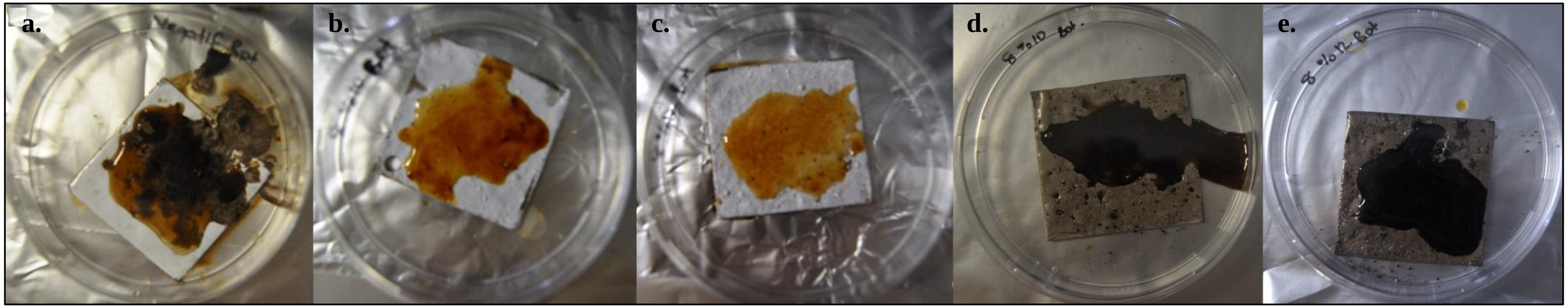


Figure 5.27. Growth of *Botrytis cinerea* on metal plates painted with a. commercial dust paint, b. 10% zinc zeolite 8 dust paint mixture, c. 12% zinc zeolite 8 dust paint mixture, d. 10% silver zeolite 8 dust paint mixture, e. 12% silver zeolite 8 dust paint mixture

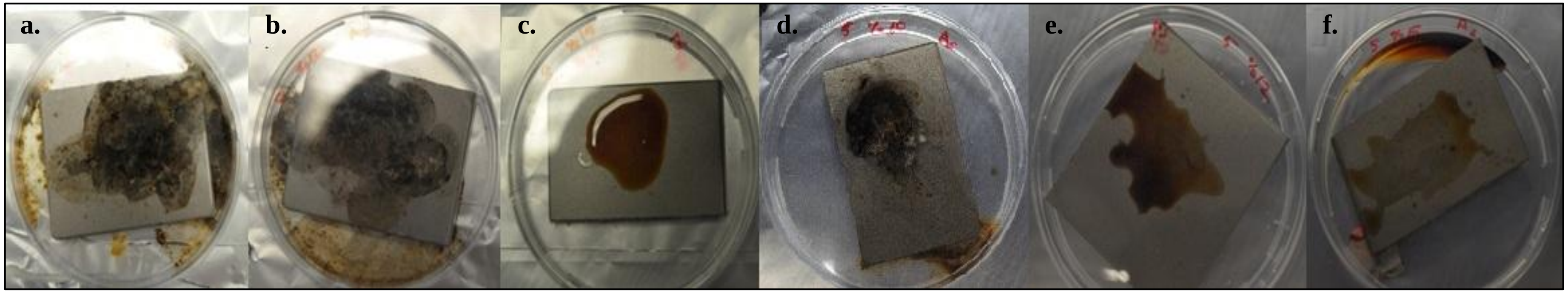


Figure 5.28. Growth of *Aspergillus niger* on polypropylene plates painted with acrylic paint a. 10% silver zeolite 8 plastic paint mixture b. 12% silver zeolite 8 plastic paint mixture c. 15% silver zeolite 8 plastic paint mixture d. 10% silver zeolite 5 plastic paint mixture e. 12% silver zeolite 5 plastic paint mixture f. 15% silver zeolite 5 plastic paint mixture

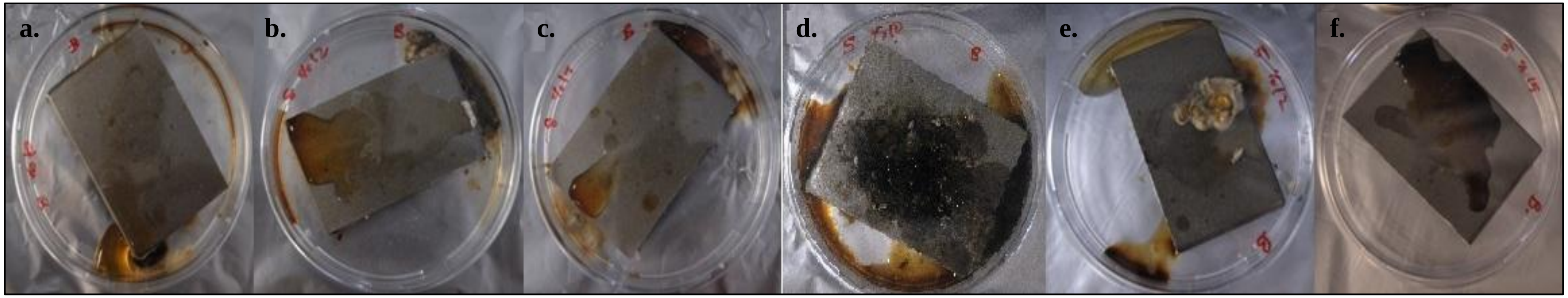


Figure 5.29. Growth of *Botrytis cinerea* on polypropylene plates painted with acrylic paint a. 10% silver zeolite 8 plastic paint mixture b. 12% silver zeolite 8 plastic paint mixture c. 15% silver zeolite 8 plastic paint mixture d. 10% silver zeolite 5 plastic paint mixture e. 12% silver zeolite 5 plastic paint mixture f. 15% silver zeolite 5 plastic paint mixture

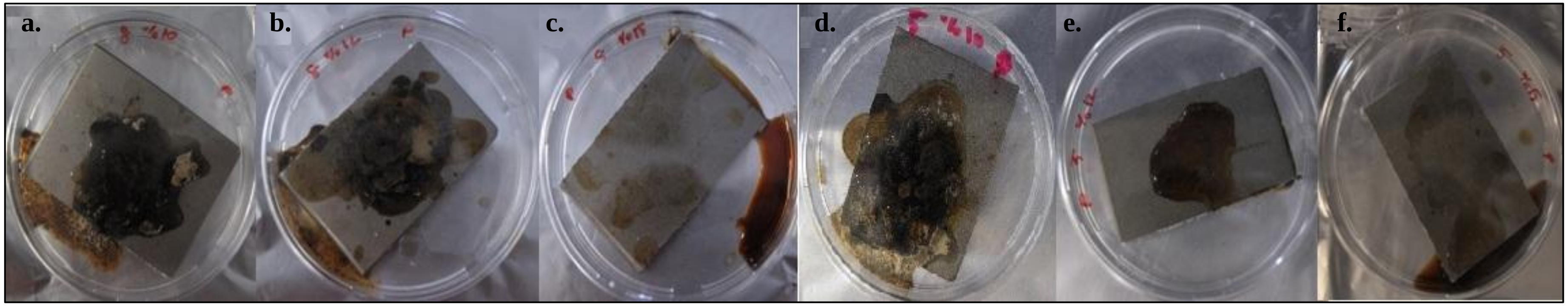


Figure 5.30. Growth of *Penicillium expansum* on polypropylene plates painted with acrylic paint a. 10% silver zeolite 8 plastic paint mixture b. 12% silver zeolite 8 plastic paint mixture c. 15% silver zeolite 8 plastic paint mixture d. 10% silver zeolite 5 plastic paint mixture e. 12% silver zeolite 5 plastic paint mixture f. 15% silver zeolite 5 plastic paint mixture

5.4.4. Composite Surfaces Prepared by Mixing Polypropylene Raw Material and Zeolite

The next step to make the parts of the washing machine antimicrobial; was making polymeric surfaces hygienic. For this purpose liquid detergent level plate which was made from polypropylene was mixed with ion exchanged zeolites. Polypropylene (pp.) beads were mixed with silver ion exchanged zeolites at 10% weight fraction and fed to the extruder. 90% of the plate was emerged from polypropylene while 10% was emerging from ion exchanged zeolite. Polypropylene surfaces; that are commercially used; were mixed with ion exchanged zeolites were contaminated with *Penicillium expansum, Aspergillus niger* and *Fusarium oxysporum*.

Contamination process and tests were performed as it was explained in previous sections 5.4.1 and 5.4.2. Figure 5.31 represents 30th day of the polypropylene surfaces contaminated with *Penicillium expansum*. Figure 5.31.a shows the commercial polypropylene liquid detergent level plate. As it can obviously be seen from the figure *Penicillium expansum* spores cover the surface of the plate. Figure 5.31.b shows the polypropylene polymer mixed with ion exchanged silver zeolite 5 at 10% ratio. Polypropylene mixed with antimicrobial zeolite kept its antimicrobial effect for 30 days which is 2 years in daily life. The only disadvantage of the mixture was silver ion exchanged zeolite changed the color of the plate. But this new color can be used at silver colored washing machines.

Figure 5.32 represents 30th day of the pp. plates contaminated with *Aspergillus niger*. Figure 5.32.a shows the commercial polypropylene liquid detergent level plate was covered by the *Aspergillus niger* spores. Figure 5.32.b shows the polypropylene polymer mixed with ion exchanged silver zeolite 5 at 10% ratio. Polypropylene mixed with silver ion exchanged zeolite kept its antimicrobial effect for 30 days during fastened aging tests.

Figure 5.33 represents 30th day of the pp. surfaces contaminated with *Fusarium oxysporum*. Figure 5.33.a shows the commercially used polypropylene was covered with *Fusarium oxysporum* spores. Figure 5.33.b shows the polypropylene mixed with silver ion exchanged zeolite 5 at 10% ratio. Polypropylene mixed with silver ion exchanged zeolite kept its antimicrobial effect for 30 days during fastened aging tests.

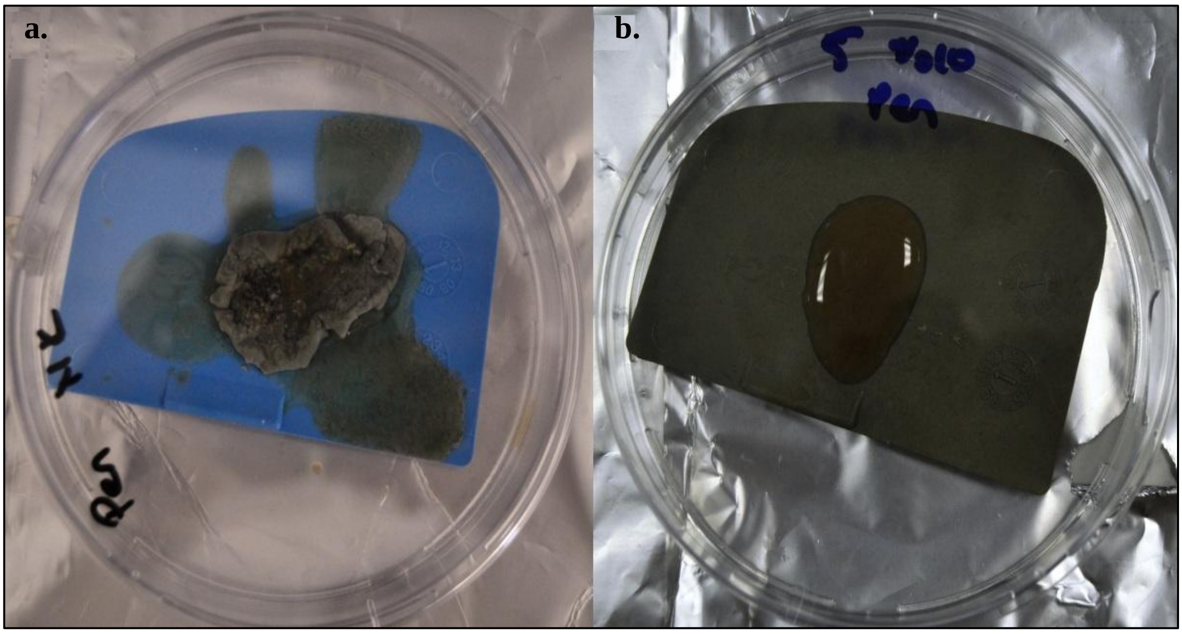


Figure 5.31. Growth of *Penicillium expansum* on polypropylene plates a. commercial plate b. plate mixed with 10% silver ion exchanges zeolite 5

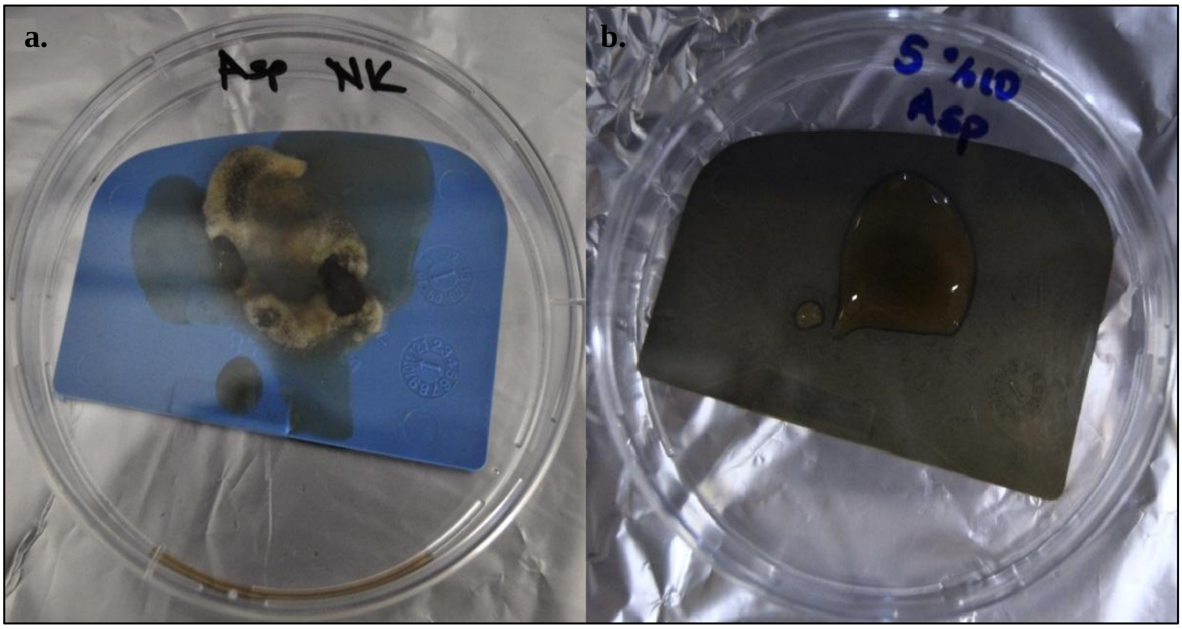


Figure 5.32. Growth of *Aspergillus niger* on polypropylene plates a. commercial plate b. plate mixed with 10% silver ion exchanges zeolite

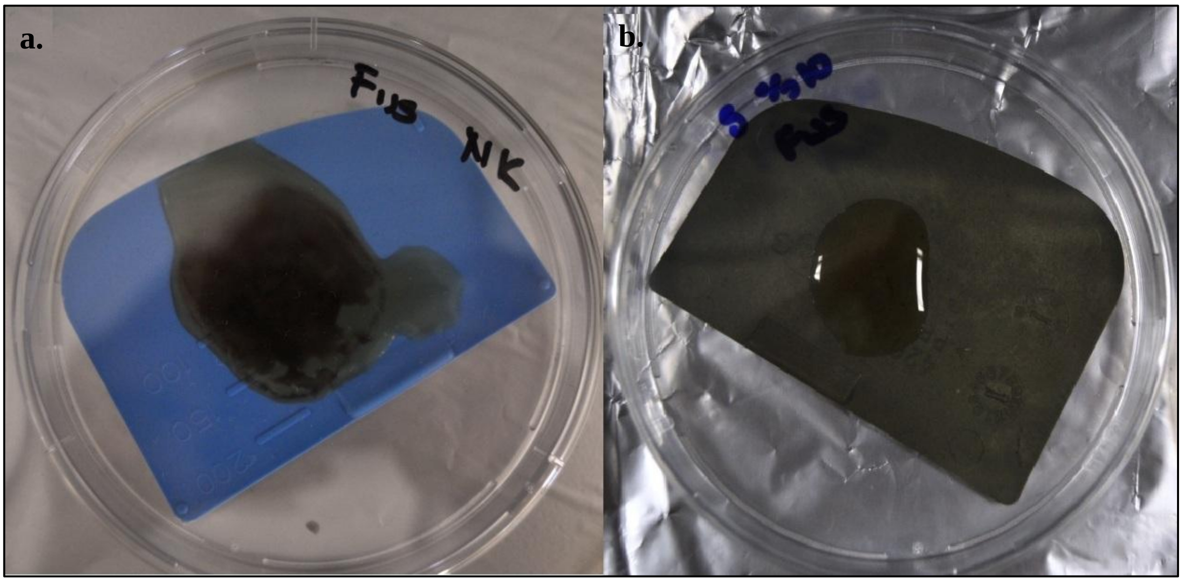


Figure 5.33. Growth of *Fusarium oxysporum* on polypropylene plates a. commercial plate b. plate mixed with 10% silver ion exchanges zeolite

5.4.5. Plaster of Paris Prepared by Mixing Zinc Ion Exchanged Zeolite

This part of this project is not related with washing machines. Plaster of Paris is usually used as a construction material to cover walls or ceilings. It is in a white powder form; in order to process it is mixed with water, applied to the surface and let to dry. Since plaster is white, zinc zeolite was the proper choice to mix with this material. Zinc zeolite 5 and 8 were mixed with plaster at different ratios and poured into 6 well plates. Then plasters contaminated with different microorganisms. A pure plaster surface was prepared and poured into another well plate; it was also contaminated with same microorganisms. After 120 days microbial growth on the plaster surfaces was like in Figure 5.34. All wells were contaminated with *Aspergillus niger, Botrytis cinerea, Penicillium expansum, Candida albicans* and *Escherichia coli*. As it can be seen from the Figure 5.34.d, e, and f; at the end of the 120 days microorganisms grew on the plaster surfaces that were mixed with zinc ion exchanged zeolite 8 for all three cases which were 10%, 12% and 15%. Figure 5.34.a represents the plaster surfaces prepared by mixing zinc ion exchanged zeolite 5 added to plaster at 10% weight fraction where no *Penicillium expansum* grew until the end of the 120th day. Figure 5.34.b and 5.34.c represent plaster surfaces prepared by mixing zinc zeolites 5 added to the plaster at 12% and 15% respectively. As it can be seen from the figures there is no microbial growth on the surfaces at the end of the 120th day while negative control; which is showed at Figure 5.34.g; was all covered by fungus spores. Negative control surfaces were prepared by commercial plaster. At the end of the 6th day fungus spores started to appear. The reason why all wells were covered by spores is; well plate shutter does not completely fit to all wells. There are gaps between the upper part of the wells and shutter. Therefore fungus spores can leak to another well and contaminate that well. That's why spores are emerged in two wells, which were contaminated with *Candida albicans* and *Escherichia coli* that are yeast and bacteria respectively, that actually not supposed to be.

As a result plaster mixed with 12 weight per cent zinc ion exchanged zeolite sample 5 keeps its antimicrobial activity for a long time. Also its stiffness is better than the commercial plaster. Zinc ion exchanged zeolite hardens plaster and gives it strength.

5.5. REISOLATION OF MICROORGANISM FROM THE ANTIMICROBIAL SURFACES

On the control surfaces prepared from different materials including paints, polymers and plasters, microbial (bacteria, fungi and yeast) contamination and growth were determined after 2-4 days after incubation. However, no microbial growth was detected on metal surfaces painted with liquid paint and dust paints mixed with ion exchanged zeolite (7-15%) up to 45th and 60th day of incubation, respectively. Similarly polymeric surfaces painted with acrylic paint and silver ion exchanged zeolite 8 with 15 weight per cent mixture and zeolite 5 with 15 weight per cent mixtures have kept its antimicrobial property for 7.5 month. The surfaces of composite prepared by mixing polypropylene raw material and silver ion exchanged zeolite 5 at 10 weight per cent, and paris plaster prepared by mixing zinc ion exchanged zeolite 5 with 12 and 15 weight per cent had antimicrobial activities against all possible microbial contaminations for 30 and 120 days, respectively.

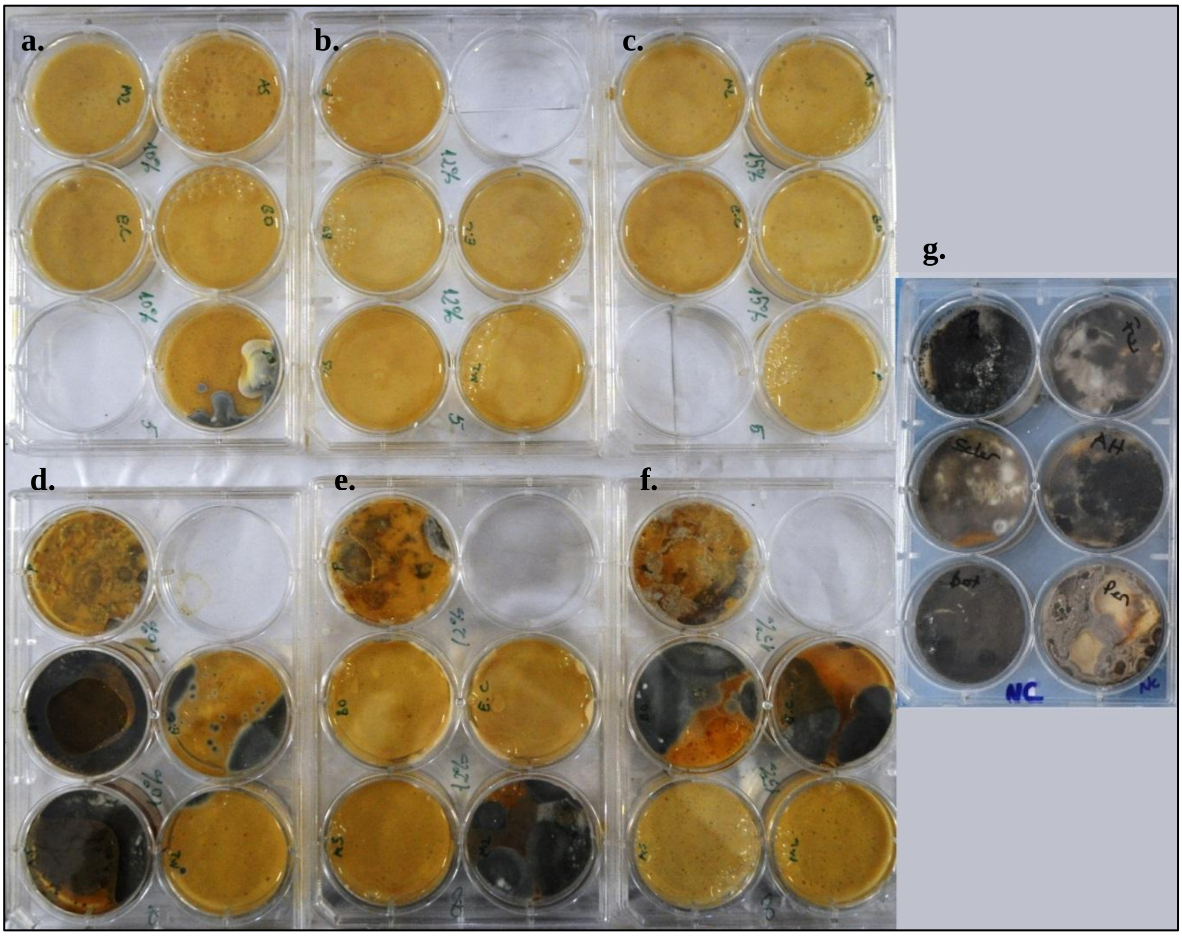


Figure 5.34. Growth of different fungi spicies 4 month after contamination of plaster surfaces a. Plaster with 10% zinc ion exchanged zeolite 5, b. Plaster with 12% zinc ion exchanged zeolite 5 c. Plaster with 15% zinc ion exchanged zeolite 5, d. Plaster with 10% zinc ion exchanged zeolite 8, e. Plaster with 12% zinc ion exchanged zeolite 8, f. Plaster with 15% zinc ion exchanged zeolite 8, g) plaster surface prepared with commercial plaster.

6. DISCUSSION

Total 32 different bacteria and 3 fungi species were isolated from washing machine parts which were operated with and without heating system. Isolated bacterial and fungal species are different from each other, except *Pseudoxanthomonas* spp. and *Nocardia brasiliensis*. This difference may occur because of the temperature difference during the washing process. One washing machine was operated between 30-60 ^{o}C and the other one was operated with tap water (15-25^{o}C). As it can be seen from the Table 5.2 the washing machine which was operated with heating system hosts less number of microbial species compared with washing machine with no heating system. Even though there has been no study to determine microbial flora on the surfaces of washing machines with different heating programs up to now, presence of some bacterial strains in the genera of *Pseudomonas*, *Bacillus*, *Micrococcus, Staphylococcus, Enterobacter* and *Streptomces*, and fungal species belonged to *Rhizopus*, *Penicillium, Cladosporium, Aspergillus, Alternaria, Fusarium* and *Curvularia* spp. have been reported from the normal and deteriorated painted surfaces [157]. According to the study of Neely and Maley vancomycin resistant *Enterococci* spp. and methicillin resistant *Staphylococci* spp. can live on the polyester surface for 16 days and 51 days on polyethylene [158]. It has been also reported that *Chaetomium globosum, Stachybotrys chartarum, Trichoderma harzianum, Alternaria tenuissima, Aspergillus versicolor, Penicillium chrysogenum, Penicillium brevicompactum* and *Aspergillus ustus* were isolated form the gypsum (raw material of plaster of paris) [158]. Therefore, this is the first study investigated total microbial flora of washing machines with cold and hot washing programs.

For the antimicrobial studies, three bacteria and fungi, and six different yeast species have been taken from the stock culture and used in this study in addition to newly isolated microorganisms.

Then zeolites with 11 different gel formulas were synthesized and ion exchanged with silver, copper and zinc ions at different concentrations. These ion exchanged zeolites were investigated for antimicrobial properties with modified disc diffusion assay against 48 different microorganisms which were isolated from washing machine parts and obtained from the culture collection of Department of Genetics and Bioengineering at Yeditepe University. 0.6M silver ion exchanged zeolite 5 and zeolite 8, 1M copper ion exchanged zeolite 5 and zeolite 8 and 2M zinc ion exchanged zeolite 5 and zeolite 8 showed antimicrobial properties against microorganisms. However, 0.6M silver ion exchanged zeolite mixed with paints and tested against microorganisms results were not satisfactory. Therefore, silver content of the silver nitrate solution was increased to 1M and tests were repeated. When 1M silver ion exchanged zeolite mixed with paints they showed antimicrobial effect against microorganisms. According to the modified disc diffusion test results, silver ion exchanged zeolite 5 and 8 showed maximum inhibition zones against microorganisms however silver effects the color of the material that is added in. Thus, during the study zinc ion exchanged zeolite 5 and 8 were also added into materials.

Antimicrobial paints are obtained as the result of the study in which ion exchanged zeolites were mixed with three different types of paints known as liquid paint, dust paint and acrylic paint. Holtz and his collegues added nanostructured silver vanadate to water-based paints and tested it against methicillin-resistant *Staphylococcus aureas* (MRSA), *Enterococcus faecalis*, *Escherichia coli*, and *Salmonella enterica* [160]. Developed paint inhibited the growth of those bacteria strains. However, the developed paint is only effective against bacteria. They do not inhibit the growth of yeast and fungus species. Also Zielecka et. al. were reported that addition of copper and silver nanoparticles into architectural paint brings antimicrobial properties effective against fungi including *Aspergillus niger*, *Paecilomyces varioti*, *Penicillium funiculosum*, *Chaetomium globosum* mixture, and bacteria *Pseudomonas aeruginosa* [161].

In the literature, there are no studies which combine ion exchanged zeolites with paints enabling antimicrobial paints. In our study, 1M silver ion exchanged zeolites and 2M zinc ion exchanged zeolites mixed with liquid paint at 10 weight per cent ratio and those paints kept their antimicrobial property for 45 days. Also 1M silver ion exchanged zeolites and 2M zinc ion exchanged zeolites mixed with dust paint at 10 and 12 weight per cent ratio and those paints kept their antimicrobial property for 60. Finally 1M silver ion exchanged zeolites mixed with acrylic paint at 15 weight per cent ratio and those paints kept their antimicrobial property for 7.5 months.

Another part of the study was preparing a composite surface by mixing polypropylene raw material with ion exchanged zeolite. Silver, copper and zinc ion-exchanged zeolite-polyurethane composites were reported to be effective against Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida tropicalis (*with antimicrobial tests) in previous studies[162]. They are only tested against those three type of bacteria and yeast species, they did not tested the effectiveness of composites against fungi speceis. Also Fox et. al. reported that Nitric oxide loaded Zn^{2+}-exchanged zeolite materials combination with polytetrafluoroethylene polymer showed antibacterial effect against three bacterial species, *Pseudomonas aeruginosa* and methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* [91].

Finally antimicrobial activity of plaster of paris prepared by mixing zinc ion exchanged zeolite was investigated. 2M zinc ion exchanged zeolite 5 which was mixed with plaster at 12 and 15 weight per cent ratio and those plasters kept their antimicrobial property for 120 days. Chen et. al. developed a supramolecular plaster which has an antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, *Bacillus megaterium*, *Bacillus thuringiensis*, and *Bacillus pumilus* [163].

In the literature, there has been no study showed development of antimicrobial surfaces from different materials with ion-exchanged zeolites. Therefore, this is the first study demonstrated that plasters, plastics and paints can easily have antimicrobial properties by adding 10-15% of ion-exchanged zeolites of silver, zinc and copper. This approach can be applicable for a number of polymers and materials.

7. CONCLUSION AND RECOMMENDATION

7.1. CONCLUSION

Microorganisms such as fungi, yeast, and bacteria can exist almost everywhere on earth and some of them are primary and opportunistic pathogens causing clinically important diseases on human beings, animals or plants. In order to control those opportunistic or pathogenic microorganisms, antimicrobial agents such as; antibiotics, antiseptics, disinfectants and synthetic drugs were developed. However these agents can be toxic to living organisms or adverse drug reactions can occur. Moreover microorganisms gain resistance against these drugs and they lose their efficiency. All these situations forced scientists to develop novel and safer antimicrobial agents that have broad spectrum antimicrobial activity. Furthermore scientists try to develop antimicrobial surfaces in order to reduce contamination.

Especially nosocomial infections increase the morbidity, mortality of patients. Therefore, scientist developed many biomedical materials such as; catheters, surgical masks, prosthetic heart valve sewing cuffs. Moreover paints, plastics, concrete, water filters, paints, food packaging materials, cleaning gels, dental materials, ceramics, and napkins are developed. However these developed materials are only bactericidal that is responsible for 5 per cent of infections. They have effect against neither molds nor yeasts.

Therefore we aimed to develop novel antimicrobial agents which can be combined with different materials and produce antimicrobial surfaces. In order to achieve this aim we used zeolites. Low silica zeolites are known to be good ion exchangers and they can be ion-exchanged with heavy metal ions which are known to be antimicrobial. During the study we tried a lot of combinations of zeolite samples and various concentrations of metal solutions. We decide to use solutions of $AgNO_3$, $ZnCl_2$ and $CuSO_4$ as metal ion supplier. There have been dozens of concentration trials during the study. Concentrations varied from 0.1M to 2M for 11 types of zeolites. In order to optimize the results modified disc diffusion tests were performed for almost 50 different microorganisms. Modified disc diffusion tests were begun with testing ion-exchanged zeolites as itself, ion-exchange zeolites mixed with dust paint at different weight per centages and ion-exchange zeolites mixed with liquid paint at different weight per centages. According to the preliminary results 0.6M silver ion-exchanged sample 5, which has a chemical composition of 4.64 Na_2O : Al_2O_3: 3.2 SiO_2:400 H_2O, and silver ion-exchanged sample 8, which has a chemical composition of 2 Na_2O : Al_2O_3: 1.6 SiO_2: 200 H_2O, showed maximum inhibition zones. Thus, 0.6M silver ion-exchanged sample 5 and sample 8 were mixed with dust and liquid paints at different concentrations and modified disc diffusion tests were performed. However, results were not satisfactory therefore concentration of $AgNO_3$ was increased to 1M and tests were repeated. Silver ion-exchanged zeolite samples 5 and 8 in 1M $AgNO_3$ inhibited the microbial growth.

The next step was combining these zeolites with various surfaces. Silver ion-exchanged zeolites were mixed with variant paints and metal and plastic surfaces are painted. The optimum mixing concentration for liquid paint is 10 per cent (wt/wt) for both zeolite 5 and 8. Liquid paint mixed with silver ion-exchanged zeolites inhibit microbial growth for 45 days which is about 3 years in daily life while microbial contamination of commercial paint was started in the first 3 days. Also for dust paint optimum mixing concentration is 10 per cent (wt/wt) for both zeolite 5 and 8. Dust paint mixed with silver ion-exchanged zeolites inhibit microbial growth for 60 days which is about 4 years in daily life while microbial contamination of commercial paint was started in the first 3 days. Another surface painted with plastic paints at different concentrations. Both for zeolite 5 and 8 silver ion-exchanged zeolites optimum amounts were 15 per cent (wt/wt) and they inhibit the microbial growth for 7.5 months which is about 15 years in daily life.

Moreover, polypropylene surfaces were prepared by mixing the polypropylene beads with ion-exchanged zeolites at various weight percentages. Silver ion-exchanged zeolite 5 with 10 per cent (wt/wt) inhibits the microbial growth for 30 days which is about 1 year in daily life.

Finally, plaster of paris was mixed with zinc ion exchanged zeolites. 12 per cent (wt/wt) zinc ion-exchanged zeolite inhibits the microbial growth for 4 months (about 8 years) and also the stiffness of the plaster was improved.

As a conclusion, the represented work involves the development of a novel antimicrobial material with full broad spectrum that effect on bacterial strains, yeast and mold species, that is easy applicable and producible. The material is very versatile, since it can be tailored into different composites used for use in manufacturing of antimicrobial surfaces.

7.2. RECOMMENDATIONS

In this study ion-exchanged zeolites were combined with various paints, polypropylene and plaster of paris. These ion-exchanged zeolites with antimicrobial effect can combine with different materials such as ceramics, metals, other polymers etc. Moreover it can be used as additive to disinfectants, antiseptic gels, soaps, detergents, shampoos etc.

During the study we used two characterization techniques which are X-Ray Spectroscopy and SEM. However we assumed equilibrium during three days of ion exchange, so we did not determine the metal amount after ion-exchange process. We could use inductively coupled plasma mass spectrometry (ICP-MS) to determine the metal content of ion-exchanged zeolites.

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APPENDIX A: SEM IMAGES OF ZEOLITES

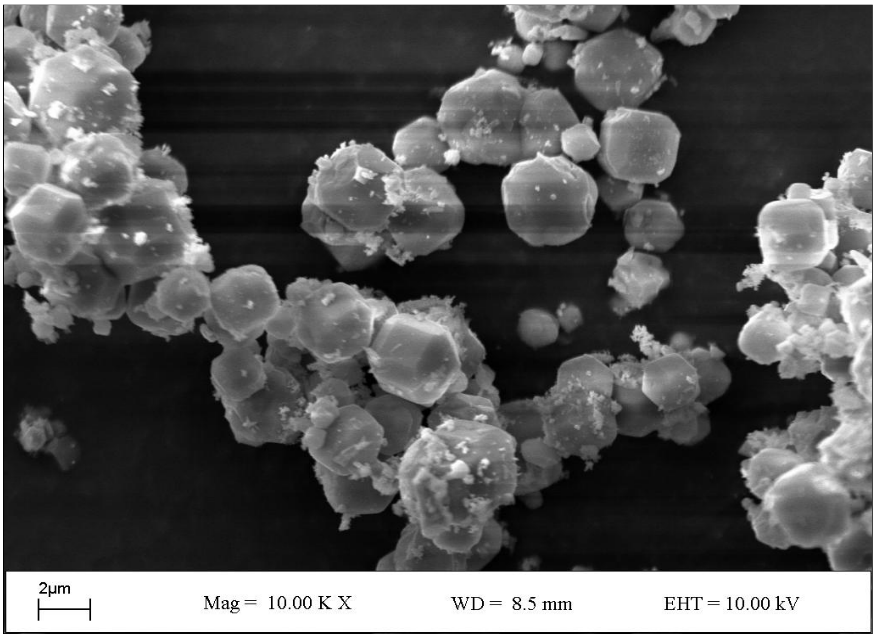

Figure A.1. SEM Images of 0.6 M Ag^{+} Ion-exchanged Zeolite Sample 1

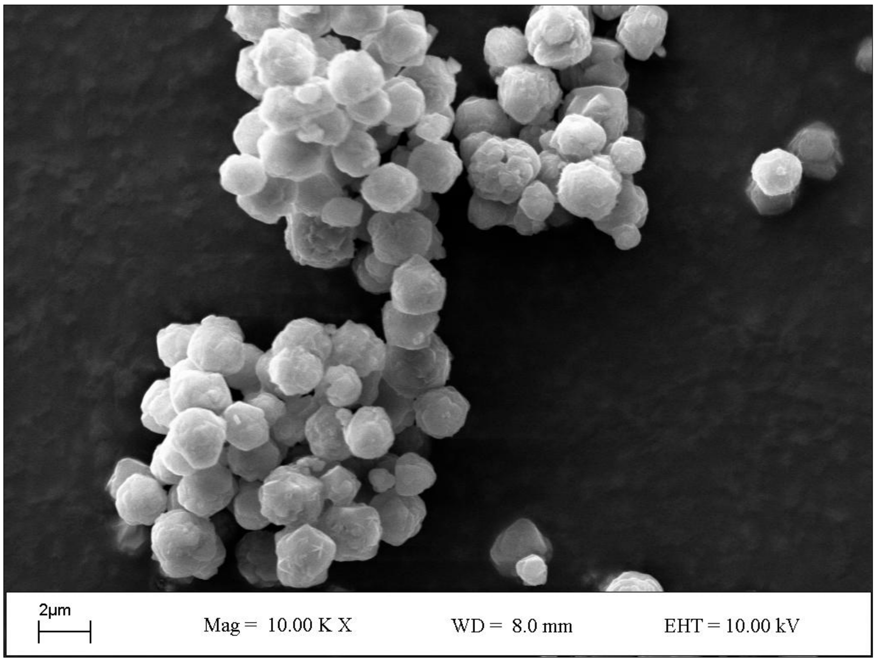

Figure A.2. SEM Images of 0.6 M Ag^{+} Ion-exchanged Zeolite Sample 3

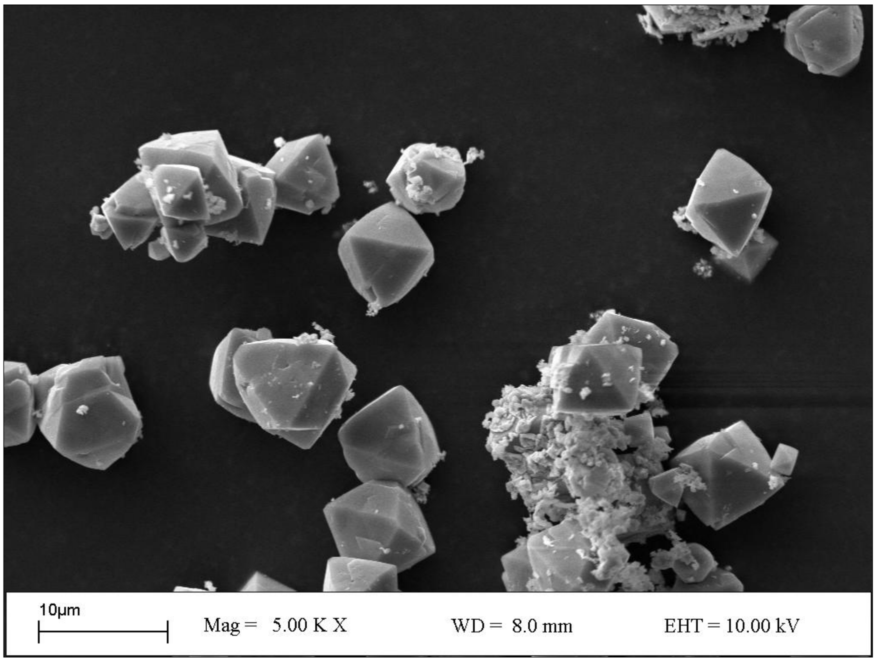

Figure A.3. SEM Images of 0.6 M Ag^{+} Ion-exchanged Zeolite Sample 5

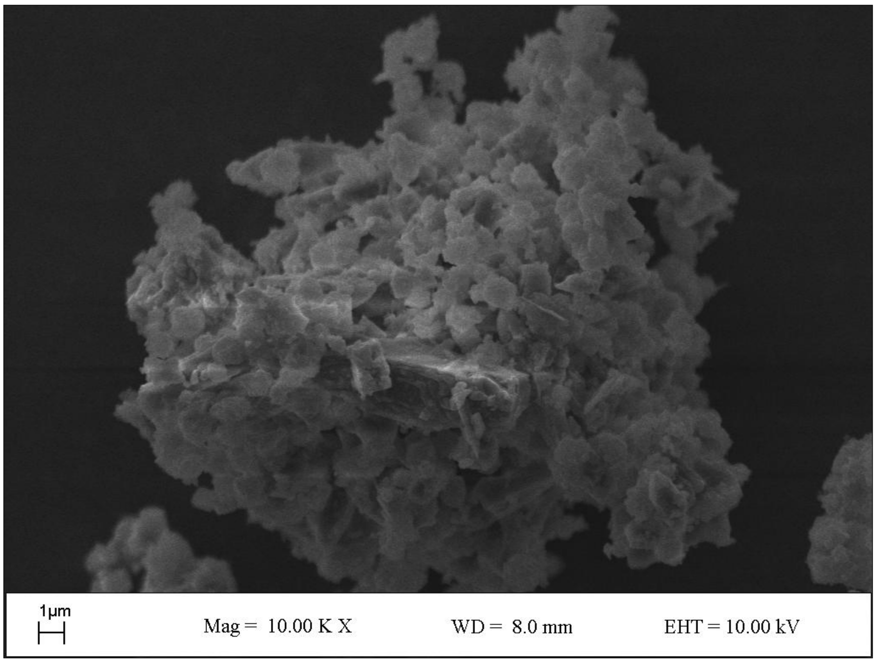

Figure A.4. SEM Images of 0.6 M Ag^{+} Ion-exchanged Zeolite Sample 7

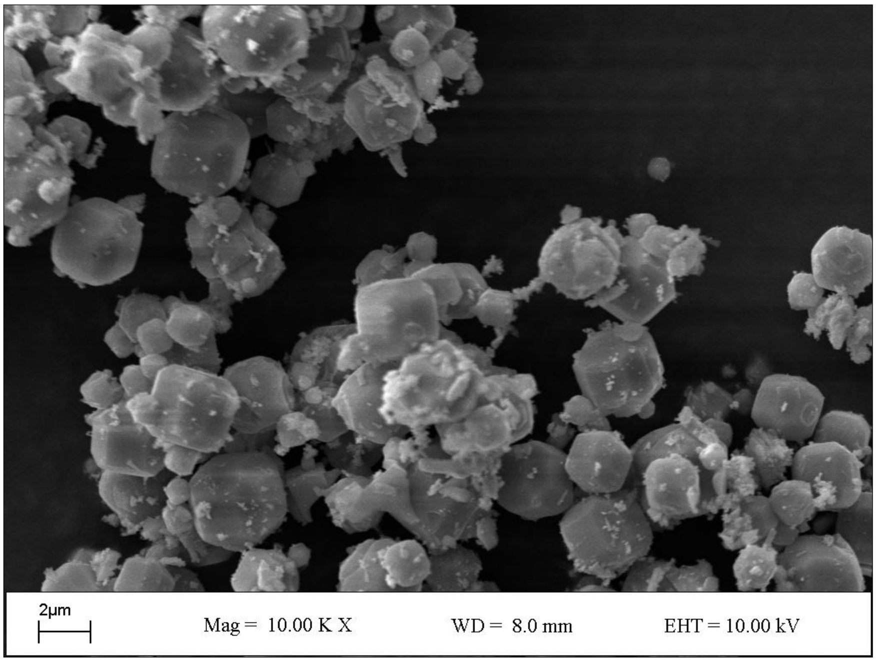

Figure A.5. SEM Images of 0.6 M Ag^+ Ion-exchanged Zeolite Sample 8

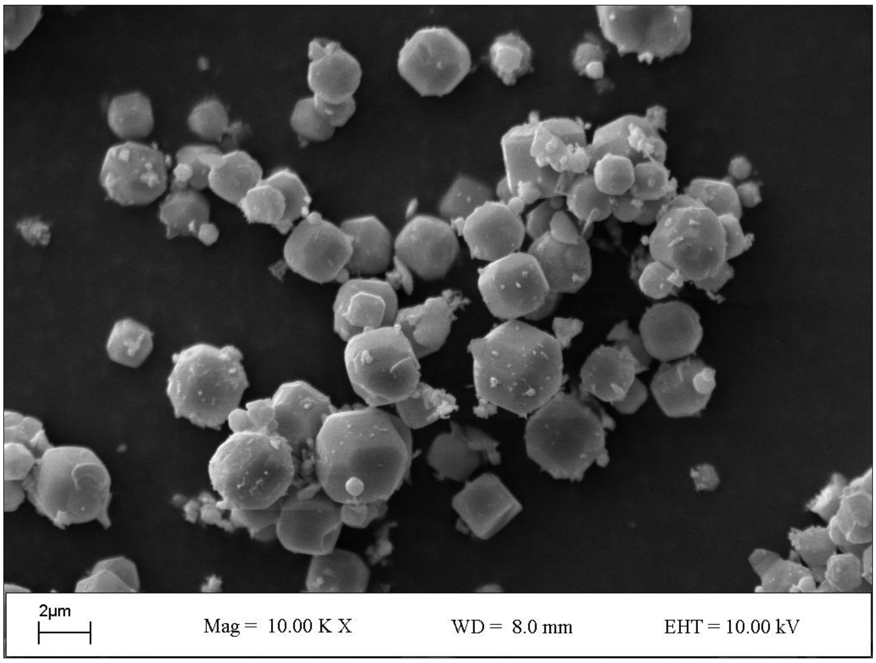

Figure A.6. SEM Images of 0.6 M Ag^+ Ion-exchanged Zeolite Sample 10

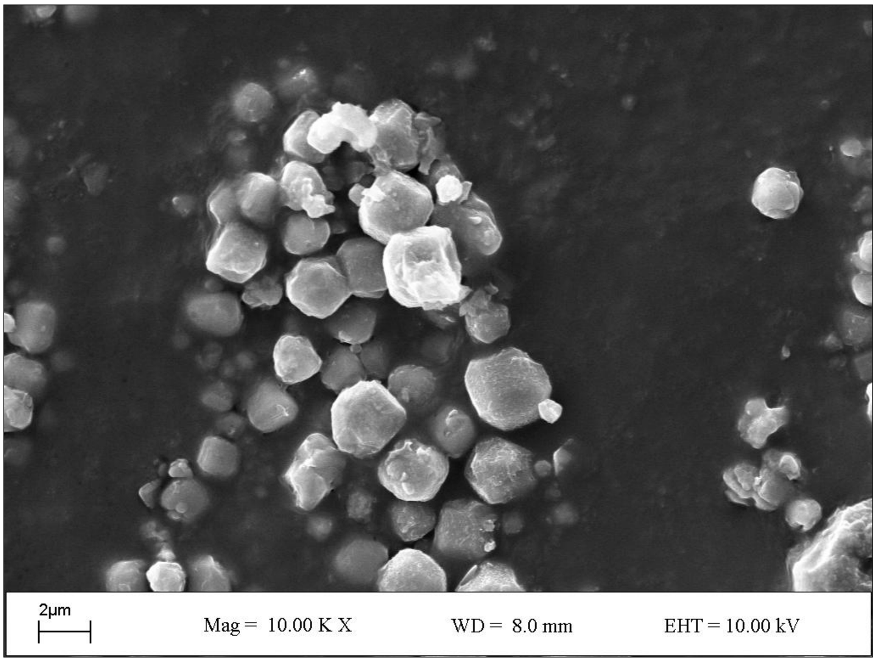

Figure A.7. SEM Images of 0.6 M Ag^{+} Ion-exchanged Zeolite Sample 11

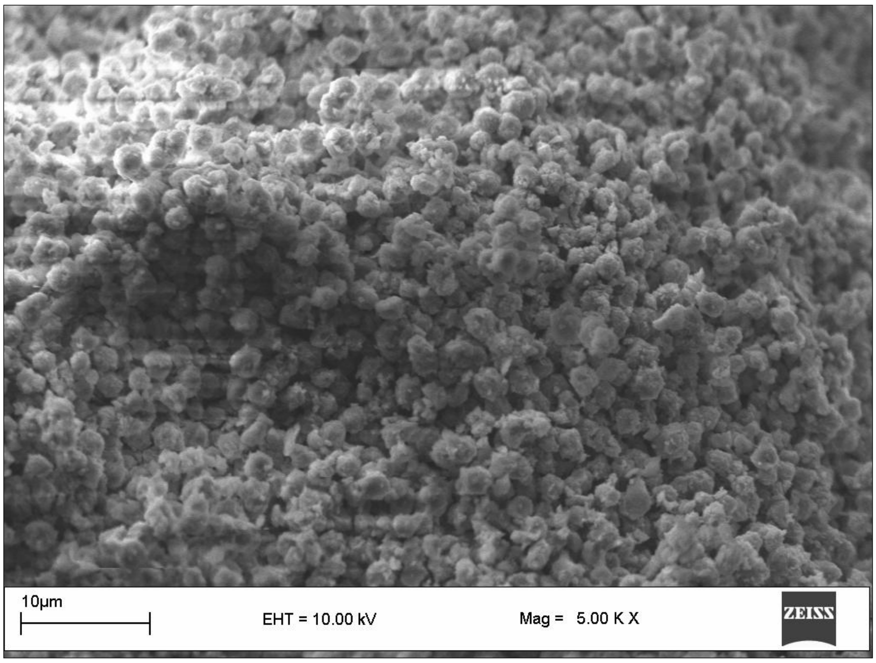

Figure A.8. SEM Images of 1 M Cu^{2+} Ion-exchanged Zeolite Sample 1

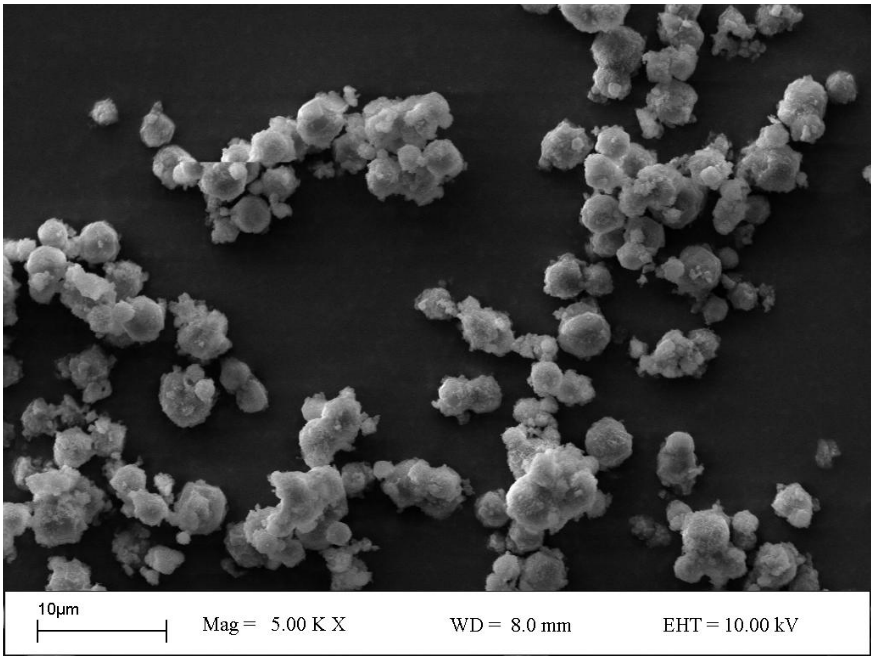

Figure A.9. SEM Images of 2 M Zn^{2+} Ion-exchanged Zeolite Sample 8

APPENDIX B: MODIFIED DISK DIFFUSION TEST RESULTS

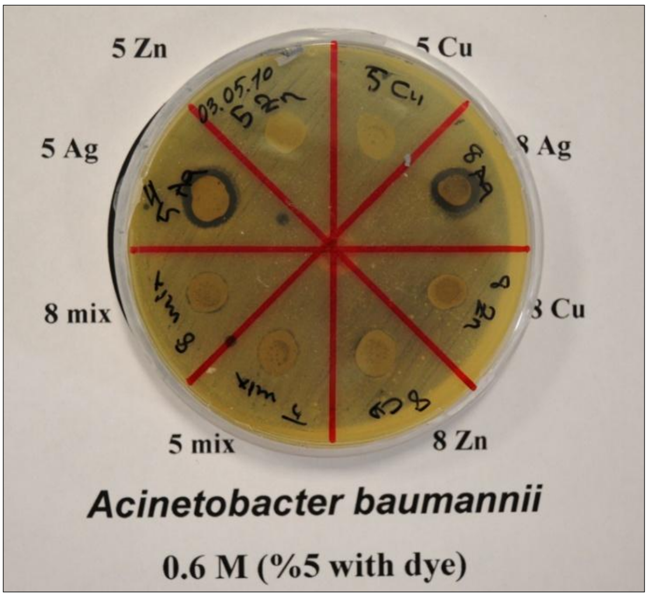

Figure B.1. Liquid paint mixed with Ag^{+}, Cu^{2+} and Zn^{2+} ion exchanged zeolites (0.6 M) in 5 % (w/w) and tested against *Acinetobacter baumannii*

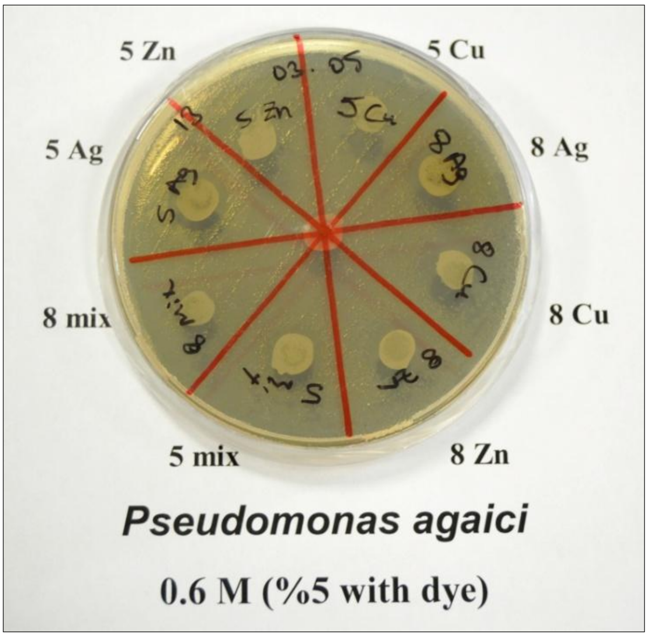

Figure B.2. Liquid paint mixed with Ag^{+}, Cu^{2+} and Zn^{2+} ion exchanged zeolites (0.6 M) in 5 % (w/w) and tested against *Pseudomonas agaici*

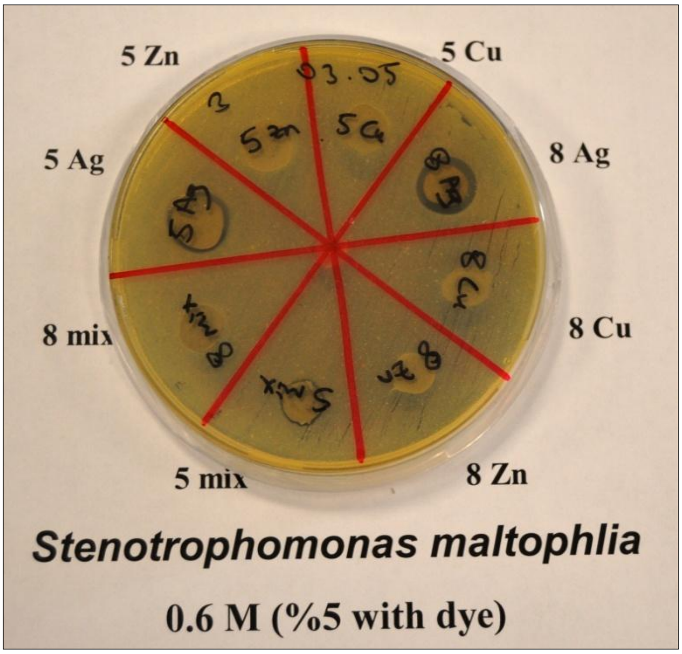

Figure B.3. Liquid paint mixed with Ag^{+}, Cu^{2+} and Zn^{2+} ion exchanged zeolites (0.6 M) in 5 % (w/w) and tested against *Stenotrophomonas maltophilia*

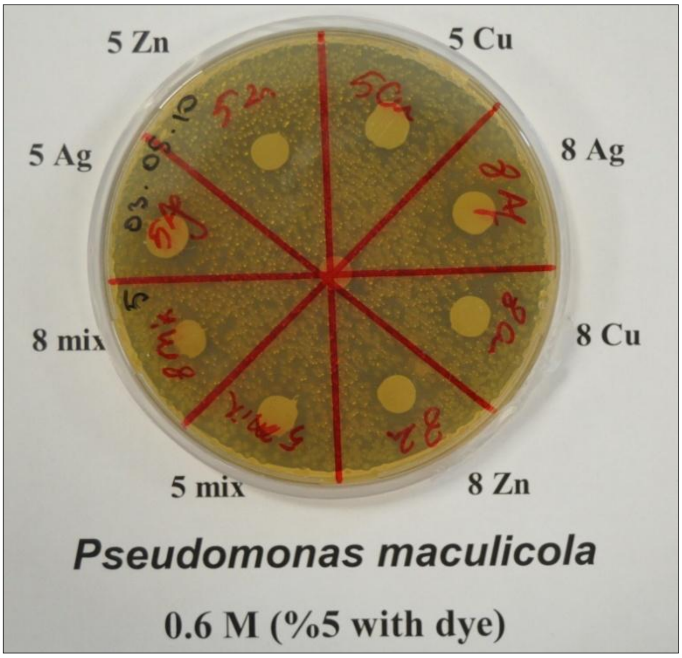

Figure B.4. Liquid paint mixed with Ag^{+}, Cu^{2+} and Zn^{2+} ion exchanged zeolites (0.6 M) in 5 % (w/w) and tested against *Pseudomanas maculicola*

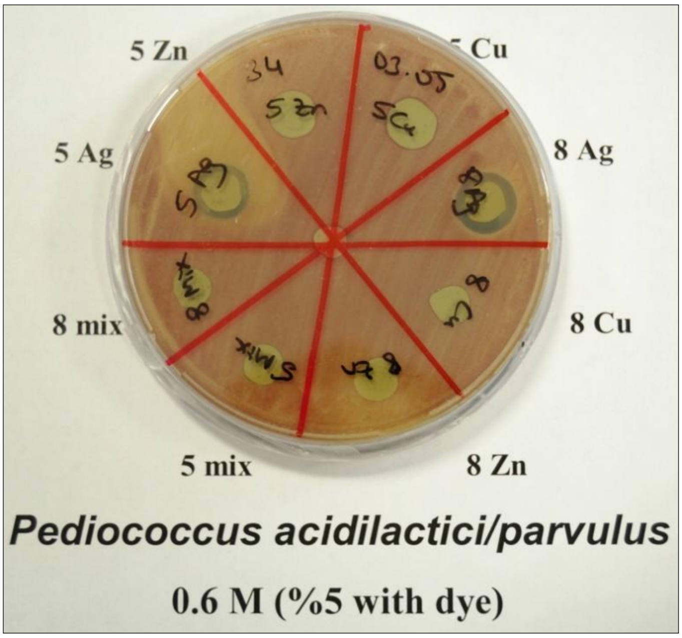

Figure B.5. Liquid paint mixed with Ag^{+}, Cu^{2+} and Zn^{2+} ion exchanged zeolites (0.6 M) in 5 % (w/w) and tested against *Pediococcus acidilactici / parvulus*