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Antibacterial Activity with Zeolitic Nano-Particles Activated by Microwave Plasma

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Abstract

Human wellbeing is jeopardized by microbial pollution. Antimicrobial compounds produced from a number of nanoparticles (NPs) were shown to have strong antimicrobial efficacy in studies. There are possible effects of nanostructured metal oxides on microbial contamination reduction are well established. This research aimed to look at the antimicrobial properties of zeolite (nanoparticles in zeolite) against E. coli (negative bacteria) and Staphylococcus aureus (positive bacteria). Real or synthetic crystalline Aluminum silicates with ion-exchanging properties are known as zeolites. The current search focuses on zeolites' uses in biomedical applications, Normal zeolites have been used in a variety of applications. Normal zeolites have been used in the pharmaceutical industry and medicine for many years. Since several biomedical processes are linked to ion exchange, adsorption, and catalysis, natural zeolites are poised to make major advances in these fields in the near future. Natural silicate compounds, such as zeolite, have been found to have a wide range of biological activities and have been used to treat diarrhea and as a vaccine is adjuvant. The search includes the usage of microwave plasma in enhancing the result of (NPS) solution of zeolite in three concentrations. The result of the Atomic Force Microscope (AFM) shows that the diameters of the particles become smaller with solution exposed to plasma and with increasing the time expose and the energy gap decrease over time exposing so the diameter of the inhibitions zone increase with increasing the time exposing to plasma.

Keyword: Microwave plasma, nano- particles, zeolite, Atomic Force Microscope, E. coli, Staphylococcus aureus;

1. Introduction

Antibacterial issues are becoming more prevalent, largely due to bacterial tolerance and environmental factors. The construction of a pollution control system is needed due to the high release of antibacterial agents in water. Antibacterial agents that are both new and powerful One of the methods for dealing with these issues is to using zeolite Malek. N.A. et al. (2018). The development of microbial resistance to antibiotics, as well as the diseases associated with it, has become a major concern for global health in recent years. There must be new unidentified signs in the future.

Advanced diseases associated with this issue Aside from tracking antibiotic use and keeping an eye on local antibiotic resistance outbreaks, To combat this issue, it is critical to develop a new and efficient antimicrobial agent Molstad. S. et al. (2017. A recent advancement is by using zeolite as a micro carrier system containing antibacterial agents such as zinc, copper, etc Alswat A. (2016). Swedish mineralogist Baron Alex Frederick Cronstedt, who had the distinction of discovering the so-called stilbite, coined the name zeolite in 1756. He discovered that rapidly heating produced a significant amount of steam. After their discovery, this material was given the name "zeolite" after a classical Greek term .For hundreds of years, zeolites have been considered a distinct category of minerals found in volcanic rocks. Natural zeolites are the result of thousands of years of chemical reactions between volcanic ash and alkaline water. For decades, they have been used in different parts of the world. Zeolites are micro porous crystalline minerals. Alkali and alkaline earth metals are found in hydrated alum-silicate minerals. Their frameworks are made up of tetrahedral $[SiO_4]_4$ and $[AlO_4]_5$ that share corners to form different open structures. The tetrahedral are linked to form cages that are bound by pore openings of varying sizes Bacakova. L. et al (2018) .

2. Cold plasma

In physics, “plasma” indicates the fourth state of matter Danil. D.et al. (2009). It is an ionized gas containing a similar number of positive and negative lading Hopwood J.A. (2000) and neutrals (atoms, molecules, radicals) Lieberman A.& Lichtenberg A.

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J.(2005) It is the fourth state of matter after the most common status of solids, liquids, and gaseous forms more than 99% of the matter in the universe and its electrified gas Nehra V. (2018). It can be distinguished into two main groups, namely high temperature (above 2000K) and low temperatures (less than 2000K). The plasma heating means that all species (electrons, ions, and neutral species) are in a state of thermal equilibrium Tony. Sch.et al (2013). The low-temperature plasma is divided into thermal plasma, also called semi-balanced plasma, in the case of local thermal balance (LTE) and non-thermal plasma (NTP), also called cold plasma Nehra V. (2018). Irving Langmuir, the Nobel laureate who developed the scientific study of ionized gases gave this new case of matter Goldston R. J& Rutherford P.H. (2018). It is necessary to identify the plasma used in the biological field and to induce inhibition of bacteria and the interaction of the plasma with the natural zeolite sample due to the effected against bacteria.

3. Microwave Plasma

Microwave plasma is operated by the electromagnetic wave at a frequency greater than 30 MHZ and wavelength at (mm to cm), generating non-equilibrium (non-thermal) plasma with a continued wave of energy (watt to kilowatt) and it has operation pressure ranging (10^{-5}torr up to atmospheric pressure), used in many medical and biological applications Emmanuel N. et al., (2019). The microwave plasma used in this search has voltage "175v" and the gas flow at " 2liter /min" and frequency (2.45 GHz) at room temperature for two and three minutes.

4. Materials and Methods

The zeolite sample was collected from tuff and volcanic glass areas in the Mafraq governorate in Jordan. The chemical synthesis of the Zeolite sample has been obtained via the technique of XRF examination. The primary metal oxides present in the sample are SiO_2(0.529%), Fe_2O_3(18.67%), Al_2O_3(40.7%), CaO(11.62%), MgO(7.649%),Na_2O (1.64%) and TiO_2(4.401%) . There are a number of minerals that did not show any effect on the bacteria, which constitute 25%, and each mineral has a small percentage represents the remaining percentage of the full concentration of 100% .

Experimental setup

The zeolite was crushed with a nano powder mill, 1.61 grams of the powder was taken and it was dissolved with 100 ml of de-ionized water. Then it was placed under a device (hot engine) to dissolve it for an hour, where the concentration of the natural zeolite solution was obtained, which was considered 100%, and it was taken Half-dilutions of the concentrated solution (50%, 25%). The solution was exposed to microwave plasma at different exposure times (2,3) minutes to study the effect of the plasma on the properties and activation of the solution as shown in fig.1

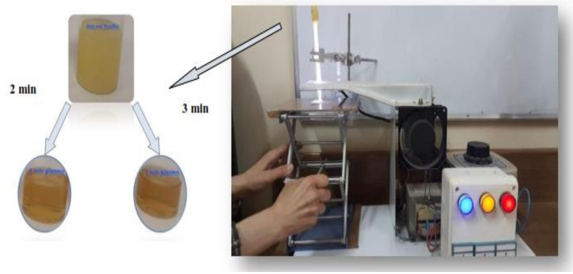


Fig .1. Plasma microwave system

Mueller-Hinton (MH) agar

It is prepared by dissolving 38g of media in 1L D.W., After autoclaving, the media was cooled to 45_50°C., then the media was poured into plates at a depth of 4 mm. To check their sterility, all plates were incubated at 37ºC for 24 hours.

Inoculums reparation

To prepare the inoculums, young culture colonies (18- 24 h) of the isolates were transferred to a 5ml tube of normal saline to provide an inoculums with density 1.5*10^8 cell/ml culture equal to No. 0.5 McFarland standard solution measure at 600 nm. Where we taking a ready and sterling bacteria and was diagnosed by the Faculty of Science / Biotechnology Section / Laboratory

Antibacterial analysis using zeolite.

The antibacterial activity of the zeolite sample was evaluated against E. coli and Staphylococcus aureus by the good diffusion method. -Mueller-Hinton (MH) agar media were used for the growth of bacterial. After solidification of (MH) media, 5 mm diameter wells were punched with the help of a cork borer. Uniform Spreading of bacterial culture was done on each agar plate. In every well, Different dilution of the zeolite solution was placed%100, %50, %25,and here was divided the zeolite solution to three sections:

1-The first section of zeolite solution was placed directly in the well.

2-As for the second Section of the zeolite solution, we exposed it to a plasma microwave for 2 minutes and then put it in the well.

3-In the third part of the Zeolite solution we have exposed to a plasma microwave for 3 minutes and then put in the well and plates were incubated at 37 °C for 24 hours. After incubation, the zone of inhibition was measured.

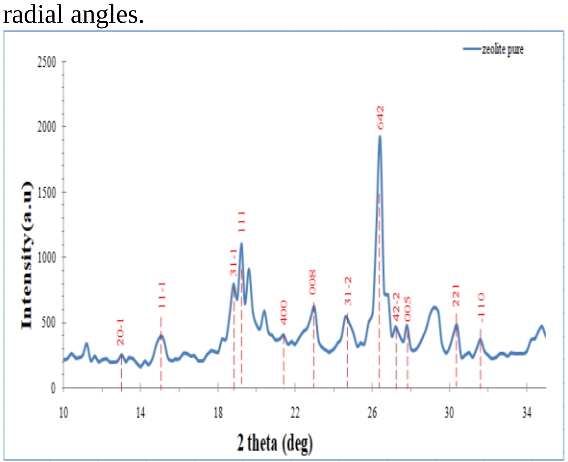
Results

Characterization of prepared nanostructures

XRD analysis: Fig. (2.a and b) shows the results of the X-ray diffraction assay for powder zeolite and zeolite exposure to plasma, which shows that the peaks of the zeolites representing the crystalline levels represented by the plot Ayaa.H. et al (2018). Fig.2 shows the X-ray diffraction pattern performed on normal zeolite and natural zeolite exposed to the plasma where different mineral phases were observed. These phases of minerals appear due to an interaction between the oxides of the elements that make up the zeolite as a result of exposure to plasma which gives energy to the elements in zeolite. These are distinguished by their respective Miller indices. The crystal size was calculated using equation (1). Table 1 represents the crystallite size (D) of crystalline material that can be easily estimated from the X-ray spectrum using the full width at half-maximum peak (FWHM).It can be calculated by the Scherrer's formula Nsreen. K.et al (2020) .

$$D = \frac{0.9\,\lambda}{\beta\ cos\theta} \ldots\ldots\ldots (1)$$

where λ represents the X-ray wavelength=(0.15406) nm, Ө represents Bragg's angle and represents the full width at half-maximum peak (FWHM) in units of radial angles.



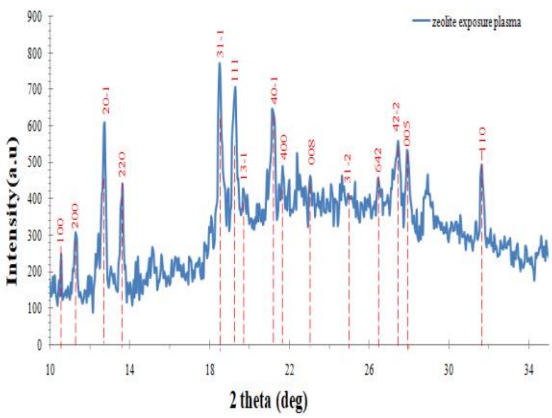


Fig.2. The X-RD spectra of a. pureZeolite and b. Zeolite exposure to microwave plasma

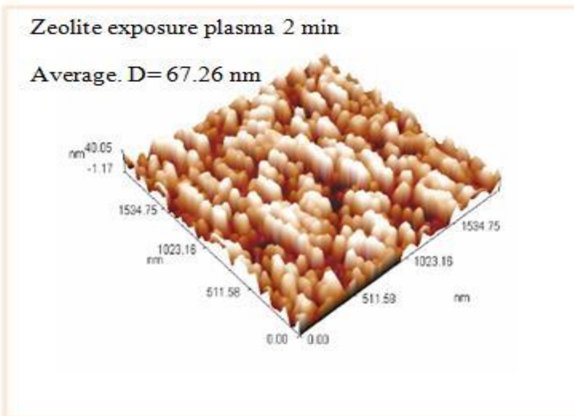
Table 1: Structural properties of Zeolite exposure to plasma (Nano particles).

FWHM	2THETA	hkl	Crystalline size (nm)
0.1476	11.34	200	54.23063
0.1968	12.98	201-	40.73464
0.2952	13.6	220	27.17345
0.1476	18.56	311-	54.67819
0.1968	19.54	111	41.06717
0.1968	19.89	131-	41.08884
0.2952	21.45	401-	27.46023
0.1968	21.78	400	41.21285
0.1968	23.21	008	41.31462
0.2952	25.12	312-	27.64092
0.1476	26.65	642	55.45068
0.5904	27.65	422-	13.89173
0.5904	28.01	005	13.90248
0.1476	31.54	110	56.06437

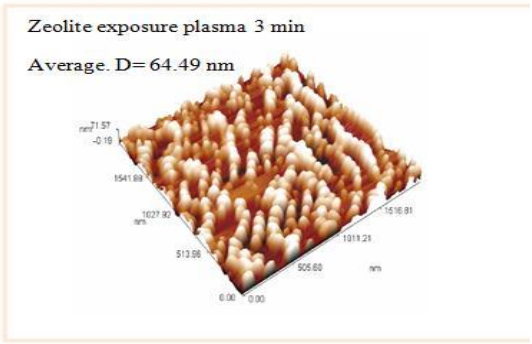
Atomic Force Microscope (AFM) analysis

Fig.3(a, b, c) shows atomic force microscopy (AFM) images of Natural Zeolites and Zeolites exposed to plasma for 2 and 3 min time. Where a smooth surface and high-quality adhesion were found to the glass base, in addition to the formation of semi-spherical clusters with similar grain sizes. Where it was observed that the granular diameter decreases by increasing the exposure to plasma with the increase the plasma exposure.

a



b



c

Fig.3. Topographical images of (a) Natural Zeolite (b) Natural Zeolite expose to plasma for 2 min (c) expose to plasma for 3 min.

Optical properties

Optical Energy Gap (Eng)

The optical energy gap is one of the most important optical constants in semiconductor physics. The use of semiconductors in optical and electronic applications depends on the value of this constant (Eng) Tony. Sch.et al (2013), Alyaa.H. et al (2018). The resulting spectrum obtained on Zeolite is shown in fig. 4(a, b, c). The spectral data recorded showed the strong cut-off at 420 nm; where the absorbance value is minimum. The data is corroborated in the % Reflectance mode as shown in table 2. The result shows that the energy gap decrease with increasing the time exposure to plasma.

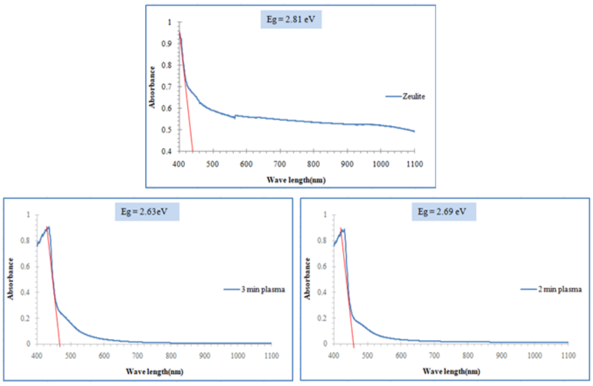


Fig. 4. UV/Vis Absorption measurements for(a)Natural Zeolite, (b) Natural Zeolite exposure plasma for 2min, (c) Natural Zeolite exposure plasma for 3min.

Table 2

Calculations of the energy gap of Natural Zeolite

Band Gap Energy (E) = h*C/λ

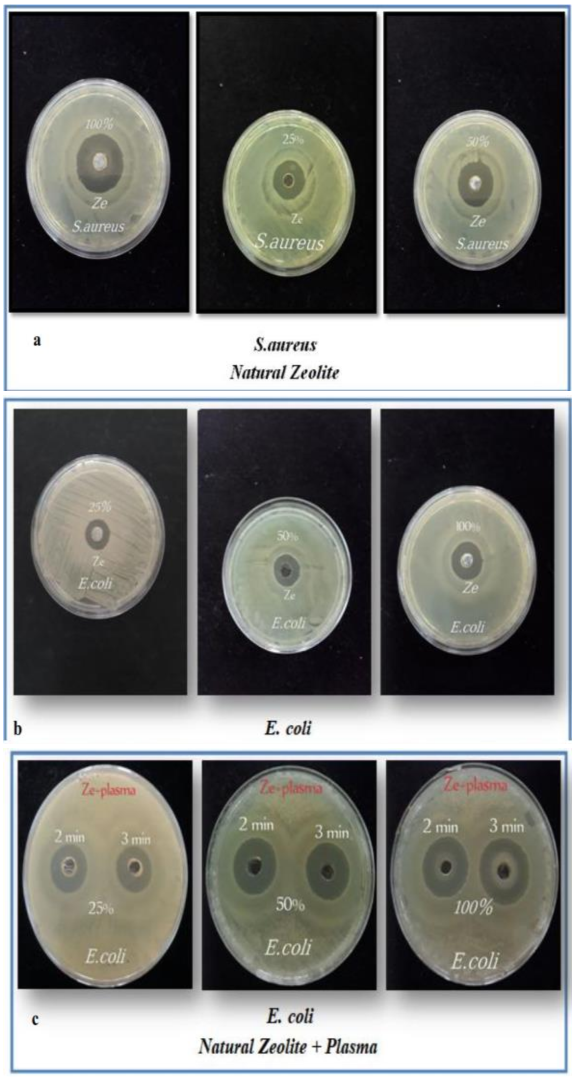
h = planks constant = 6.626×10^{-34} Joules sec

C= Speed of light $=3\times10^{8}$ meter /sec

Sample	λ= Cut off wave length (nm)	ng(eV)E
zeolite	420	2.81
Zeolite +Plasma 2 min	460	2.69
Plasma 3 min+zeolite	464.78	2.63

Discussion

Antibacterial Activity: The antibacterial effect of the natural zeolite solution and the natural zeolite exposed to the plasma for different times(2 and 3 mints) against human pathogens is measured based on the inhibition zone where the inhibition was very pronounced at 100% concentration and dilution as well as it was observed that the damping diameter decreases as the concentration of the solution decrease.In fig.5(a, b, c, d)the case of the natural solution, it was observed that the diameter of the flagellation was higher in the case of Gram-positive *staph. aureus*bacteria, while the solution exposed to the plasma at 3 minutes had the highest inhibition of the solution exposed to the plasma at 2 minutes for the two types of gram-positive(*staph. Aureus*) and gram-negative (*E.coli*)bacteria, its behavior was similar to the behavior of the normal solution not exposed to plasma in terms of concentrations, table.3 shows the damping diameters for all samples. The activity of zeoliteas as an antibacterial effect comes from the number of minerals.



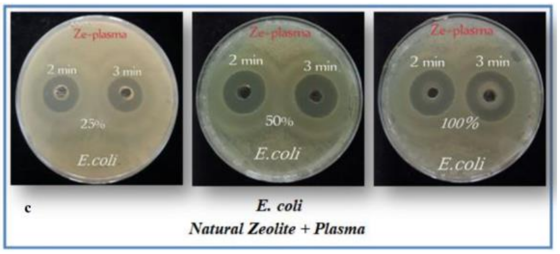


Fig .5. The inhibition effect of solutions onbacteriatypes: (a) S.aureus for Natural Zeolite (b)E.coli for Natural Zeolite (c)E.coli for Natural Zeolite+plasma (d) S.aureus for Natural Zeolite+plasma

Table 3 Diameter of inhibition of solutions for different types of bacteria

Concentration%	Bacteria	Inhibition zone without plasma microwave mm	Inhibition zone with plasma microwave at 2 min, mm	Inhibition zone with plasma microwave at 3 min, mm
100	S. aureus	25	27	30
50		22	26	27
25		19	22	24
100	E.coli	18	25	26
50		17	23	24
25		15	19	19.5

The reason for the high effectiveness of the solution is due to the size and surface area of these particles, which enable them to reach the DNA of the microbial cell and communicate with it. The zeolite can play an important role in increasing surface interaction with microbes and preventing clumping the nanoparticles (NPs),this can be attributed to the shape and size of NPs, bearing in mind that the properties of bacterial cells differ from Gram-positive bacteria (S. aureus) and Gram-negative bacteria (E. coli) which affect cell permeability and sensitivity of NPs towards bacteria. Cell physiology, metabolism, and degree of contact can contribute to other factors that make a difference in the zeolite activity of the plasma exposed to different bacteria.

Conclusions

The antibacterial function of zeolite is linked to alkalinity and activated oxygen species in its minerals. The surface area of the minerals in the zeolite increased by converting the solution to nano particles. Nano-particles of Al_2O_3,MgO, SiO_2 Fe_2O_3,CaO, and TiO_2 destroy the cell membrane, allowing intracellular contents to leak out, resulting in bacterial cell death. Any of the compounds in the nano composite has diffraction peaks, according to the XRD data. The average diameter of the NPs was measured using an Atomic Force Microscope (AFM), which revealed that the solution with plasma effect has a smaller diameter than the solution without plasma and that the diameter decreases over time exposing to plasma. The role of plasma is to increase the effectiveness of metal oxides present in different amounts in the zeolite since the effectiveness of solutions without exposure to plasma is less than the effectiveness of solutions exposed to plasma. It was observed that the zeolite solutions exposed to the plasma for three minutes were more effective by observing the diameter of the inhibition zone, the diameter of inhibition zone increase with time exposure to plasma, and also the energy gab decrease by increasing time exposing, the result also shows that the 100% concentration has a large diameter of the inhibition compare the other concentration 25% and 50%.

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