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Green Synthesis of Zinc Oxide Nanoparticles from *Lyngbya sp*. and Their Antibacterial Activity

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Abstract

In this study, the antibacterial activity of zinc oxide (ZNo NPs) was evaluated. ZnO NPs are considered as one of the many multifunctional inorganic nanoparticles with potent antibacterial properties. ZnO NPs were synthesized from the *Lyngbya sp*. extract.Following the green synthesis method,several other methods were used to characterize the biologically produced ZnO nanoparticles, including X-ray diffraction (XRD), field emission scanning electron microscope (FE-SEM), atomic force microscopy (AFM), and UV–visible spectrophotometry (UV-Vis).The AFM results showed 52.09 nm diameter as an averagesize of ZnO NPs, while the shapes of NPs were irregular as revealed through the FE-SEM image.Also, using UV-visible a cut-off phenomenon of the biological synthesized ZnO was found to be around 265 nm. The antibacterial activity of ZnO NPs at 0.05, 0.025, 0.0125, 0.0062, 0.0031 and 0.0015 gm/mlconcentrations were also tested on both *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria). The results demonstrated the inhibitory effects of ZnO NPs on *S. aureus* at0.05,0.025,0.0125, and 0.0062 gm /ml with inhibition zone of 10 ,9 ,8 and 6 mm respectively.While the *E. coli* results showed a growth inhibition at 0.05, 0.025, 0.0125 and 0.0062 gm/ml concentration treatments of ZnO NPs, represented by 22, 20, 18 and 10 mm of inhibition zone respectively.

Keywords: green synthesis**,** ZnO NPs, *S. aureus.* *E.coli* , *Lyngbya sp.*

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التخليق الأخضر لدقائق اوكسيد الزنك النانوية من طحلب ال*Lyngbya sp.* وفعاليتها المضادة للبكتريا

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الخلاصة

قُيمت جزيئات أكسيد الزنك النانوية ZnO NPs في هذه الدراسة كمضاد للمكروبات، كونها تعتبر واحدة من العديد من الجسيمات النانوية غير العضوية متعددة الوظائف ذات الخصائص القوية المضادة للبكتيريا . تم تصنيع جسيمات الزنك النانوية من ***Lyngbya sp.*** ولتشخيص الجسيمات النانوية للزنك تم استخدام العديد من الطرق بما في ذلك مقياس الطيف الضوئي بالأشعة المرئية فوق البنفسجية UV–Vis ، مجهر القوة الذرية AFM، حيود الاشعة السينية XRDو المجهر الإلكتروني الماسح FE–SEM.فقد كشفت نتائج AFM

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عن متوسط قطر يبلغ 52.09 نانومتر لجزئيات الزنك النانوية، في حين اظهرت صور FE–SEM الشكل غير المنتظم للجزيئات، وبإستخدام فحص UV–Vis ظهرت النتيجة عند 265 نانومتر.

لقد تم اختبار الفعالية المضادة للبكتيريا لجزيئات أكسيد الزنك النانوية على بكترياالموجبة لصبغة غرام الممثلة بـ*Staphylococcus aureus* والسالبة لصبغة كرام ممثلةبـ*Escherichia coli* باستخدام تركيزات مختلفة .أظهرت النتائج أن جزيئات ZnO NPs تثبط نمو بكتريا *S. aureus* بتراكيز 0.0125،0.025،0.05 و 0.0062 غم/مل مع مناطق تثبيط بلغت 10، 9، 8 , 6 ملم على التوالي ، بينما بلغت مناطق التثبيط الخاصة بـ *E. coli* 22، 20، 18 و 10 ملم عند ذات التركيزات من ZnO NPs .

Introduction

Nanoscale technology has emerged as a new field of study, synthesizing nanoparticles for various applications, including catalysis, electrochemistry, biomedicine, pharmaceutics and food technology [1]. Physical and chemical processes such as photochemical, radiation and chemical precipitation approaches are being utilized to synthesize nanoparticles. However, the employment of these procedures is not eco-friendly, is expensive and hazardous [2, 3].Due to their distinctive and fascinating features, as well as uses that are superior to those of their bulk counterparts, nanoparticles, particles with one or more dimensions in the order of 100 nm or less, have received a great deal of attention [4, 5, 6]. Many kinds of nanoparticles can be created using a wide range of physical, chemical, biological and hybrid techniques [7].In contrast, NPs can be produced using a biosynthetic methods utilizing plant extracts and microbial communities (bacteria, fungi, algae, etc.).To increase the use of microorganisms in the synthesis of nanoparticles for biomedical applications, it is crucial to create dependable, non-toxic and environmentally friendly technologies. Sustainable resources are used in the biosynthesis of metal nanoparticles to produce green metal nanoparticles using naturally biodegradable substances such polysaccharides, biopolymers, vitamins, plant extracts and microbes **[8,**9]. Cyanobacteria are of particular relevance in the biosynthesis of nanoparticles among all other microorganisms as they may be a source of new chemicals with significant biotechnological utility**[**10**]**.Henceforth, a lot of research has been done on cyanobacteria which also known as blue-green algaedue of their capacity to produce nanoparticles [11, 12, 13].This is due to their high biomass productivity as well as their capacity to bioremediate dangerous metals and then transform them into forms that are easier to be utilised [14]. Organic and inorganic metal and metal oxide NPs can all be produced by cyanobacteria (platinum,gold, zinc oxide, silver, aluminum oxide, copper oxide, etc.). Among metal oxide nanoparticles, ZnO NPs, due to their outstanding biocompatibility, affordability and low toxicity,have emerged as one of the most widely used metal oxide nanoparticles in biology.They have robust ability to stimulate excessive reactive oxygen species (ROS) formation, liberate zinc ions, and induce cell apoptosis, ZnO NPs and, hence, have shown great promise in biomedicine, particularly in the disciplines of anticancer and antibacterial sectors [15]. According to several studies [16, 17,18], the U.S. Food and Drug Administration (US FDA) has designated ZnO as a GRAS (generally regarded as safe) metal oxide. The current work used the cyanobacterium *Lyngbyasp*. to synthesize ZnO-NPs and test the antibacterial activity against the clinical bacterial pathogen *Staphylococcus aureus* and *Escherichia coli.*

Materials and Methods

*Culture and Cultivation of Cyanobacteria***:**

Algae were obtained from Ecology and Pollution Laboratory for Postgraduate Studies of the Department of Biolog, College of Science, University of Baghdad. The obtained samples were cultivated in big docks with BG 11 growth medium at 30 – 35°C under 14/10 hours of light/ dark and harvested two weeks later.

***The Extraction of Lyngbya sp*.:**

*Lyngbya sp.*algae were fully dried and milled into a fine powder after being cleansed with distilled water and freed from any other residues. Ten grams of powder was soaked in 50 ml of 95% ethanol for 24 hours with continuous stirring to create the extract. The suspended extract was then centrifugated for 20 minutes at 4000 rpm at room temperature. The supernatant was collected and was later used to produce ZnO NPs [19].

***Synthesize of ZnO-NPs*:**

Zinc sulfate ($ZnSo_4.7H_2O$) was used to produce ZnO NPs, following the biological (green) synthesis method. ZnO nanoparticles were synthesized as described by a previous study [20] with some modifications.Method of synthesis was done by adding 10 g of $ZnSo_4.7H_2O$ in 100 ml of algal extract.This was the first study for synthesis ofZnO nanoparticles from cyanobacteria(***Lyngbya sp)***.The mixture was incubated for 24 h at (30± 2)° C and 120 rpm shaking condition. The resultant solution was then centrifuged and washed using deionized water several times. Hereinafter, the obtained weight residuals were dried at 60°C and then kept in a dark condition for further use.

***NPs Characterization Techniques*:** Figure 1revealsspectroscopic analysis (UV-visible)which is used to measure the maximum absorbance of the particle in a certain wavelength. It is an absorption spectroscopy where light gets absorbed by the particles which shows excitation of the electrons from the ground state to excitation state [21,22] **.**WhileFigure 2 demonstrates the synthesized ZnO NPs 2D and 3D topologies as revealed by atomic force microscopy (AFM) measurements of the average crystalline size. Figure 3 displays the field emission scanning electron microscopy (FE-SEM)which works with electron rather than light. The electron is discharged from the field emission source and are scanned in a zig-zag pattern [23]. Figure 4 showsX-ray diffraction patterns which is a powerful non-destructive technique that gives information about characteristics of crystalline nature, structureand average size [24].These techniques are employed by putting 0.001 gm of nanoparticles on a slide and inserting it into the equipment except UV-visible test which is done by two phase blank and sample.

Examining the Antimicrobial Effectiveness

***Bacterial isolate Preparation*:**

Staphylococcus aureus and ***Escherichia coli*** bacteria were obtained from the Department of Biology laboratories at the College of Science,University of Baghdad. Nutrient broth growth medium was used for bacteria activation.To prepare the bacteria for the test of ZnO NPs effectiveness as a suspected antibiotic, the bacteria were cultured on a solid nutrient medium in 10cm/ diameter peter plates and incubated for 24 hours at 37°C. The diffusion of agar wells method was used to evaluate the antibacterial effectiveness of ZnO NPs. To accomplish that sterilized nutrient agar medium was poured in petri plates (25ml/plate) and was then allowed to solidify at room temperature. A sterile spreader was used to apply the active bacteria to the agar. Wells were drilled onto each plate to assess each ZnO NPs antibacterial potency. The plates were incubated for 24 hours at 37°C and the inhibition zones around the wells were measured after incubation [25].

Results and Discussion:

The synthesis of metal nanoparticles was first visibly confirmed by a change in color. Furthermore, UV-visible spectra were used to describe nanoparticles produced biologically. The produced ZnO NPs had significant UV absorption at 265 nm (Figure 1).This result is similar to that achieved by previous studies[26, 27].

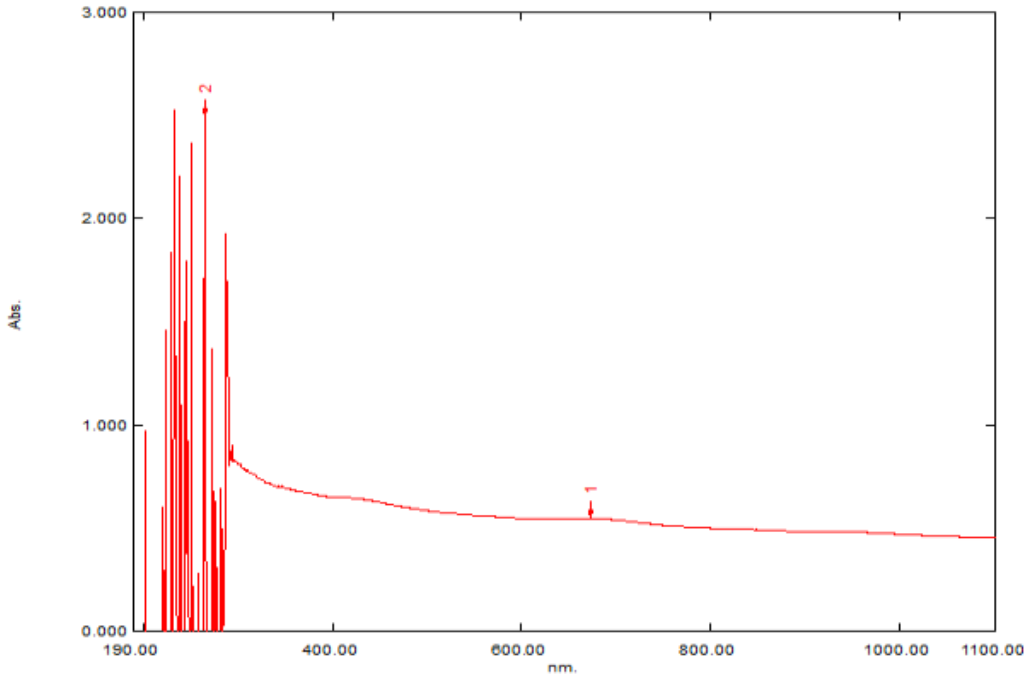

Figure 1: UV-Vis spectrum of the biosynthesized ZnO NPs

To measure the dimensions of nanoparticles, which was considered as a critical subject, AFM made it simple to calculate their size. The average diameter read in this study was 52.09 nm… AFM has a number of advantages for identifying nanomolecular structures [28]. The objective assessment of the nanoparticles dimensions is made possible by the three-dimensional information provided by the AFM image(Figure 2).

Sample: Sample Name	**Code:**Sample Code
Line No.: LineNo.	**Grain No.:**3303
Instrument:SPM	**Date:**2022-10-31
Avg. Diameter:52.09 nm	**<=10% Diameter:25.00 nm**
<=50% Diameter:45.00 nm	**<=90% Diameter:75.00 nm**

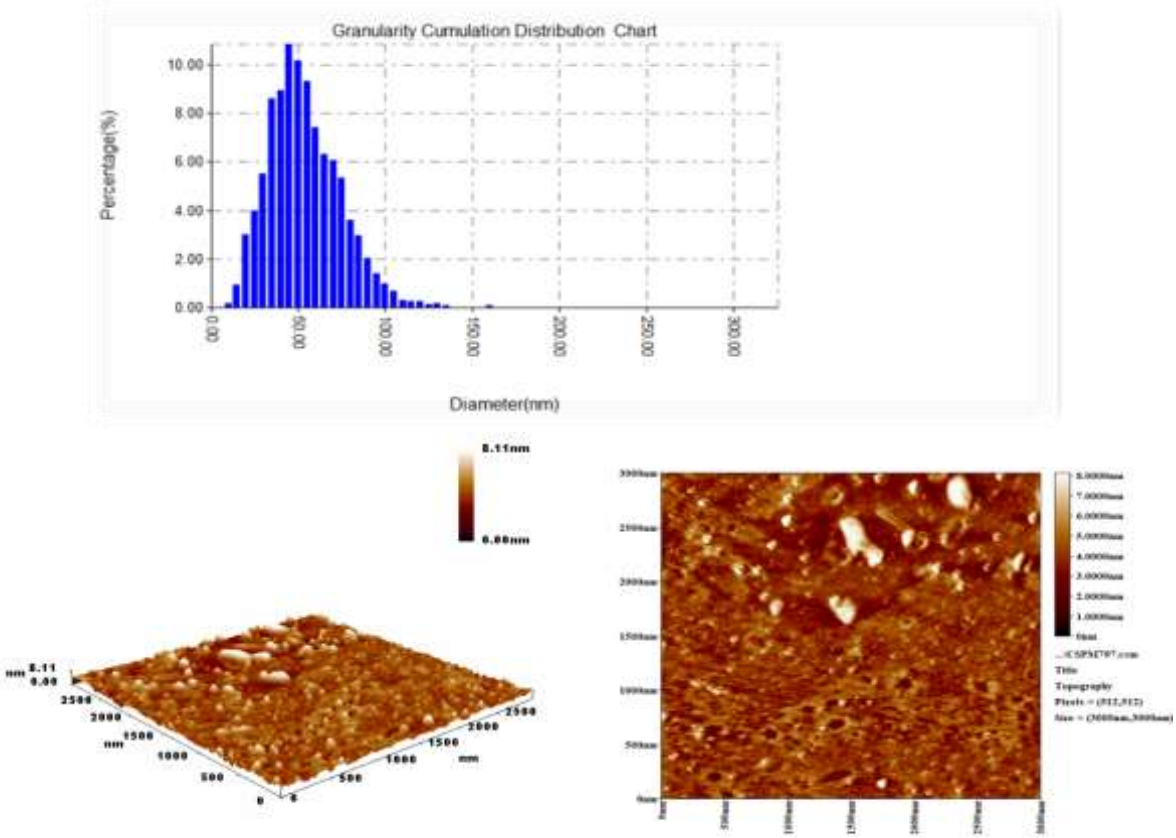

Figure 2: Image of bio-synthesized ZnO NPs.

The generated ZnO appeared irregular in shape when FE-SEM was used to analyze the morphological characteristics (Figure 3).

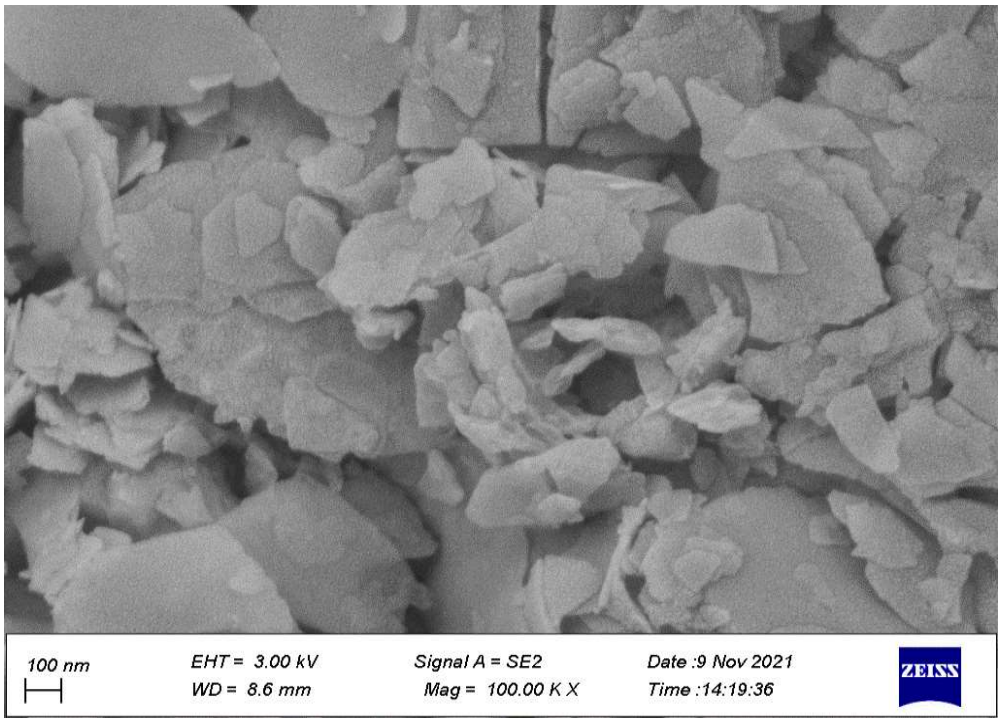

Figure 3: Field emission scanning electron microscopy of ZnO nanoparticles

The XRD pattern of ZnO nanoparticles produced through green synthesis, revealed peaks at 9.1, 18.7, 26.2, 30.16 and 35.72 degrees (Figure 4). Pronounced peaks were observed between 2θ -30 to 40 degrees. Such peaks correspond to the basal planes of 100 and 002. The mentioned peaks are matched with the diffraction data standard of ZnO nanoparticles (JCPDS file no. 89-1397) which can be indexed to the occurrence of metal oxide ZnO nanoparticles hexagonal phase wurtzite structure [29].

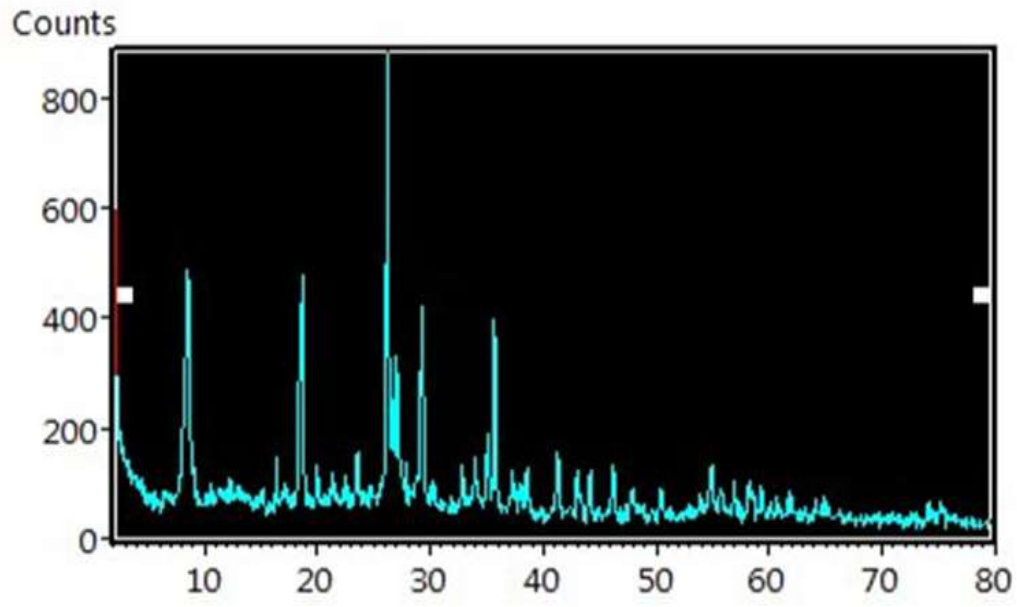

Figure 4: X-ray diffraction (XRD) of ZnO NPs

The antibacterial activity of the bio-synthesized ZnO nanoparticles at different concentrations(0.05, 0.025, 0.0125, 0.0062, 0.0031 and 0.0015 gm/ml) on *S. aureus*, indicated in Table 1 and Figure 5, is quite obvious. While ZnO NPs' antibacterial action on *E. coli* is indicated in Table 2 and Figure 6.

Table 1: Antibacterial activity of ZnO NPs against *S. aureus.*

ZnO Concentration (mg/mL)	Inhibition Zone (mm)
0.05	**10**
0.025	**9**
0.0125	**8**
0.0062	**6**
0.0031	**-**
0.0015	**-**

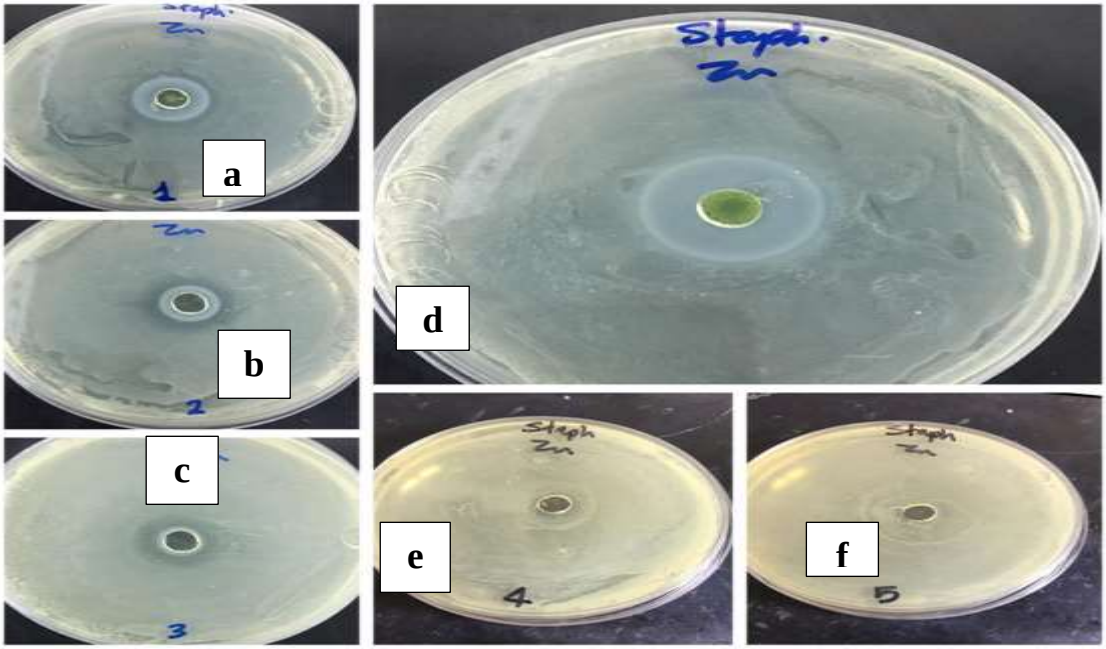

Figure 5: Antibacterial activity of the bio-synthesized ZnO nanoparticles against *S. aureus* at concentrations: a. 0.05, b. 0.025, c.0.0125, d. 0.0062, e. 0.0031, f. 0.0015 gm/ml of ZnO NPs.

Table 2: Antibacterial activity of ZnO NPs against *E. coli*

ZnO Concentration (gm/mL)	Inhibition Zone (mm)
0.05	**22**
0.025	**20**
0.0125	**18**
0.0062	**10**
0.0031	-
0.0015	-

The antibacterial activity was affected by ZnO NPs as the results demonstrated that concentrations as low as 0.0031 and 0.0015gm/ml did not display any zone of inhibition. Whereas 0.05, 0.025, 0.0125 and 0.0062 gm/ml of ZnO NPs concentrations obtained inhibition zones of 10, 9, 8 and 6 mm respectively on *S. aureus* (Figure 5). While the results of inhibition zones on *E. coli* were 22, 20, 18 and 10 mm respectively (Figure 6). The findings showed that ZnO NPs had larger surface area to volume ratio which increased their surface reactivity and the release of more ions. Their smaller particle size promoted their antibacterial activity [30]. Direct interaction between ZnO-NPs and bacterial cell walls damages the integrity of the cells [31, 32] and releases antimicrobial ions, primarily Zn_{2+} ions [33, 34] also generates reactive oxygen species (ROS) [35, 36, 37].

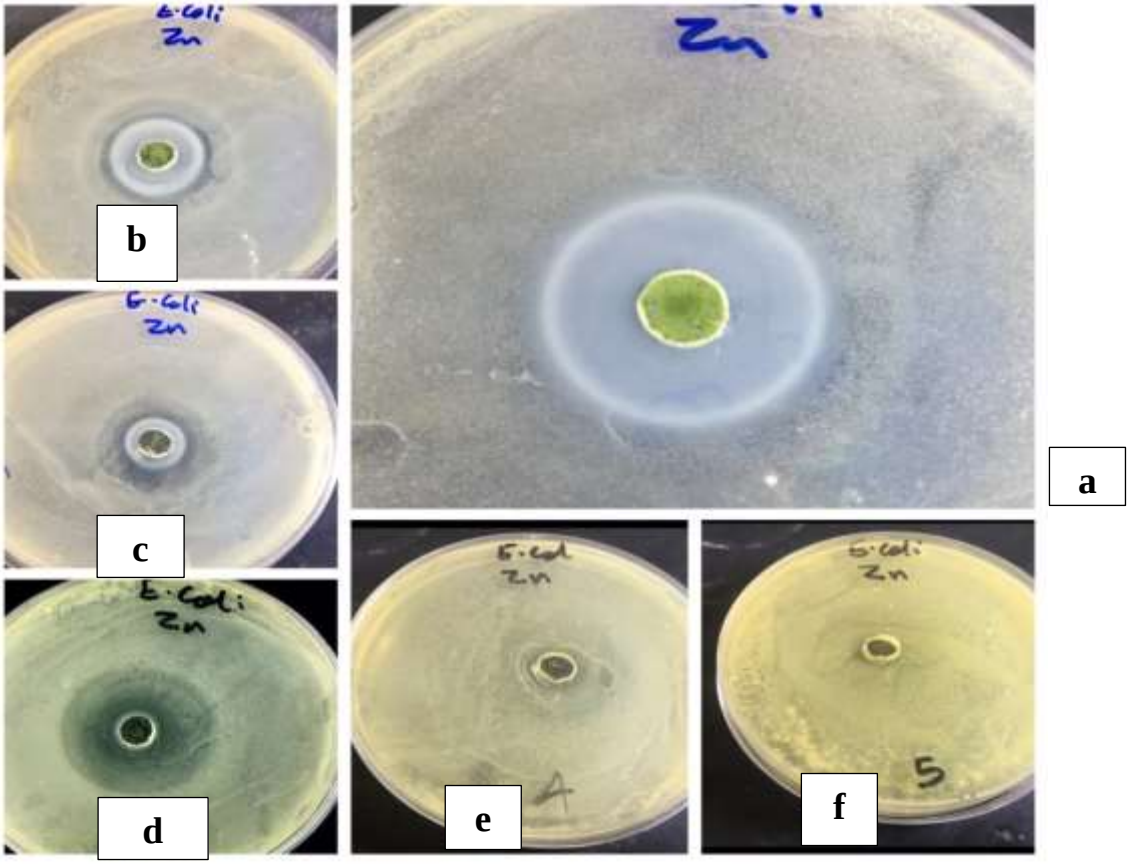

Figure 6: Antibacterial action of the bio-synthesized ZnO (NPs) against *E. coli* at concentrations: a. 0.05, b. 0.025, c.0.0125, d. 0.0062, e. 0.0031, f. 0.0015 gm/ml of ZnO NPs.

Conclusion

In this study, which is considered the first study in the green synthesis of zinc oxide nanoparticles from *Lyngbya sp*., biosynthesized ZnO NPs using *Lyngbya sp*. extract as a reducing agent was demonstrated successfully. Additionally, the attained ZnO NPs were characterized using UV-Vis, AFM, XRD and FE-SEM techniques. In particular, the UV-Vis showed the successful ZnO NPs phase formation, while the FE-SEM demonstrated that the prepared ZnO NPs exhibited irregularity in shapes. The AFM revealed an average diameter of 52.09nm. In the antibacterial activity test, it was found that the bio-synthesis has an antibacterial activity against the introduced bacteria *E. coli* and *S. aureus.*

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