Journal University of Kerbala , Vol. 16 No.2 Scientific . 2018

Characterization of ZnO nanoparticles produced by *Streptomyces* spp. isolated from Iraqi soil samples

وصيف جزيئات اوكسيد الزنك النانوية المنتجة بواسطة الستربتومايسس المعزولة من عينات تربه عراقيه

Samer M. Al-Hulu
Al-Qasim Green University/ Environmental Sciences college

Abstract:

Thirty two soil samples were collected from Hilla city, Babylon Province, Iraq. Ten isolates of *Actinomyctes* were isolated. Four *Streptomyces* spp. isolate were diagnosed, and all of its were gram positive, grey aerial mycelium and yellowish- green substrate mycelium after culturing on Yeast-Malt extract medium. All *Streptomyces* spp. isolates screened for production Zno nanoparticles. *Streptomyces* spp.4 was able for producing of nanoparticle. Ultra violet (UV) spectrum for ZnO particles showed that maximum absorption at 404 nm. FT-IR spectrum of Zno nanoparticles represented absorption peak at 3319.49 cm-1 indicate to produce presence of O-H group. While 2245.14 cm-1 indicate to produce presence of CΞC. Results of 1670.35 cm-1 indicate to C=C group, 1614.42 cm-1 refered to C=O group. As well as,1101.35 cm-1 indicated to C-O bending, 983.70 cm-1 indicated to C-O stretching,744.52 cm-1 indication to C-H, and 613.36 cm-1 indicate to C-X stretching. The SEM for Zno particles showed that its a spherical in shape and size range (60.40-67.04) nm. EDXA spectrum detected a strong signal for Zn with purity 42%.

Key words: *Streptomyces*, Zno nanoparticles, soil.

ألخلاصه:

جمعت ثلاث وعشرون نموذج تربه من مدينه الحله، محافظه بابل، العراق.عزلت عشر عزلات من الاكتينومايستات.شخصت أربعه عزلات من الستربتومايسس.كانت العزلات جميعها موجبه لصبغه غرام وتمتلك مايسليم هوائي رصاصي اللون ومايسليم اساس اصفر-اخضر عندما زرعت على وسط مستخلص الخميره والشعير.فحصت العزلات لانتاج جزيئات اكسيد الزنك النانويه .كانت عزله الستربتومايسس 4 قادره على انتاج جزيئات النانو.اظهر طيف امتصاص الاشعه فوق البنفسجيه لجزيئات اوكسيد الزنك اقصى امتصاص عند 404 نانوميتر .اظهر طيف امتصاص الاشعه تحت الحمراء لجزيئات اوكسيد الزنك النانويه قمه امتصاص عند3319.49 سم -1 والذي يشير الى وجود مجموعه هيدروكسيل (O-H) ، قمه امتصاص في 2245.14 سم -1 والتي تشير الى وجود مجموعه C≡C ، و1614.42سم -1 يشير الى وجود مجموعه كاربونيل (C=O) ،1101.35سم -1 تشير الى وجود مجموعه C-O bending،983.70سم -1 تشير الى وجود مجموعه C-H stretching، 744.52 سم -1 يشير الى مجموعه C-H،613.36 سم -1 يشير الى وجود C-X stretching.اظهر المجهر الالكتروني الماسح ان جزيئات اوكسيد الزنك النانويه امتلكت شكلا كرويا وبحجم يترواح بين (60.4-67.04) نانوميتر ووجود اشاره قويه لتواجد الزنك وبنقاوة 42%.

الكلمات ألمفتاحيه: ستربتومايسس،جزيئات اوكسيد الزنك النانويه ، التربه.

Introduction:

Nanotechnology is a gradual step for production of new materials scientific. It's able for preparing of new usage in the sciences and other technologies at nanoscale level [1].This technology important for synthesis, character, and exploring of materials with (1–100 nm) within nanometer state. Zinc oxide (ZnO) particles having antibacterial action. Its achieved by rule of reactive oxygen species (ROS) that produced on particles surface. It's also action on zinc ion release, membrane dysfunction, and nanoparticles internalization which considered the causing for swelling cells [2].These particles having many applications. It's acts as an invisible obstacle which scatters UV radiation away from the skin which having to important using in sunscreens, paints, varnishes, plastics and cosmetics, especially for broad UV-A and UV-B blocking [19; 11], using as preservative for many materials and products such as plastics, ceramics, glass, pigments, foods, etc. Zinc nanoparticles have also been explored for use as quantum dots for use in photoconduction devices [9]. ZnO is nontoxic particle which having antibacterial and antimicrobial activity that increases with decreasing in particle size which demonstrated and confirmed by various researches [17; 22; 7]. *Streptomyces* are gram-positive bacteria located at Actinobacteria phylum. It having life style closely related to filamentous fungi and, like those, most streptomycetes live as saprophytes in the soil [10]**.** Zinc NPs produced by actinomycetes are recorded by many researchers [13, 20].and showed antimicrobial activity against *S. aureus, E. coli, and Salmonella sp*. Scanning Electron Microscopy and TEM used for morphologically characterizing at nano- micrometer scale estimating [15].Elemental composition for metal nanoparticles is determined by Energy Dispersive Spectroscopy (EDS) [15]. FTIR spectroscopy is very important for surface chemistry characterizing [4].

This study aimed to isolation of *Streptomyces* spp. having ability for producing of ZnO nano particles and study the characterization of these particle.

Materials and Methods:

Isolation of *Streptomyces* spp. from soil samples:

Soil samples gathered from the Hilla City, CaCo3 treated and dried by oven at 45^{o}C for one hr. to reduce bacteria and mold occurrence. Soil dilution plate technique was used for isolating of *Steptomyces* spp. on (YMD) agar media. pH was made to 7.2. The plates incubate at 30^{o}C (10 days) [16].

ZnO nanoparticles synthesis :

Preparation of Biomass :

Streptomyces spp.4 were grow in Erlenmeyer flask (500 ml) containing (Malt extract glucose yeast extract peptone (MGYP) broth (100 ml) provided by griseofulvin 50 µg/ml on shaker (200 rpm) at 35 ^{o}C for (4) days. Flasks taken away from shaker and kept at 5 to 10 ^{o}C, so that mycelial biomass could get settled. *Streptomyces* supernatant was removed , 100 ml of sterile distilled water was added for washing the cells. Flasks were kept at 5 to 10 ^{o}C to settle the biomass, for 30 minutes. Supernatant was removed slowly, 100 ml of (D.W) was again added in the flask, and its made for 3 times. Mycelial mass was then separated from the sterile distilled water by centrifugation (1500 rpm) for 10 minutes, mycelial pellets were weighed and used for synthesis of zinc nanoparticles. (5) gram of Streptomyces wet biomass treated with 50 ml znso4 in 250 ml Erlenmeyer flaks and it kept on shaker at 200 rpm and 35^{O}C for four days. Color change was observed after 12, 24, 48 and 72 h. Formation of yellow color indicates the formation zinc nanoparticles indicate to positive result [21].

Characters of ZnO nanoparticles :

UV–Visible Spectroscopy (UV–Vis):

Ultraviolet (UV) spectrum was checked by UV–Vis spectrophotometer (Double Beam Spectrophotometer T80 UV/Vis spectrometer) it measured at wave length (200 to 700) nm [21].

Fourier Transform Infrared Spectroscopy (FT-IR):

FT-IR for Zno nanoparticles tested by using (Shimadzu IR-470 model) apparatus at chemical department, college of Science at Babylon University. Sample preparing by nanoparticles dispersing in matrix of dry KBr pressed to disc formation. The spectrum for measuring (500- 4000)cm^{-10} [18].

Scanning Electron Microscopy (SEM):

Nanoparticles morphology, shape and size was checked by SEM. SEM measurements was worked at Babylon university College of Pharmacy, by using SEM (FEI QUANTA 450) apparatus, at (10,000 V) [8].

Energy dispersive X-ray analysis (EDX):

EDX was done by X-ray microanalysis system coupled with scanning electron microscope (SEM). It was checked at the Babylon university, College of Pharmacy. (EDX) analysis was achieved by same instrument to confirming nanoparticles presence in particles and to detecting for other sample elementary compositions [14].

Results and Discussion:

Streptomyces spp. isolation:

(23) Soil Samples collecting from Hilla city. Ten *Actinomyctes* isolates were isolated. Four *Streptomyces* spp. isolate were diagnosed (Table 1).All *Streptomyces* spp. isolates was gram positive and having grey Aerial mycelium and yellow- green substrate mycelium after culturing on Yeast-Malt Extract medium. *Streptomyces*, Gram-positive bacteria, Actinobacteria phylum. It having similar lifestyle to filamentous fungi. *Streptomycetes* live as soil saprophytes [5].It produce branching substrate and aerial mycelium. *Streptomyces* having ability for producing different of secondary metabolites [12].

Screening for biosynthesis of Zno nanoparticles:

All *Streptomyces* spp. Isolates were screened for Zno nanoparticles production. the result found *Streptomyces* spp.4 was able for producing of nanoparticle. The changing color after 72 hr indicator for nanaoprticle production. (Table 1).

Table (2): Screening of *Streptomyces* spp. Isolate for zno nanoparticle production

Streptomyces spp. isolates	Results
Streptomyces spp.8	-
Streptomyces spp.4	+(yellowish-brown)
Streptomyces spp.11	-
Streptomyces spp.17	-

Dark yellow color for streptomyces spp. HBUM171191 produced after treated with ZnSO4(aqueous solution) 10^{-3} Mm for 72 hr. [21].

UV–Visible spectroscopy:

UV spectra for *Streptomyces* spp.4 particles showed that maximum absorption at 404 nm Figure (1). Maximum absorbance for *Streptomyces* sp. HBUM171191 after treated with ZnSO4 For 72 hr. is 380 nm.[21].

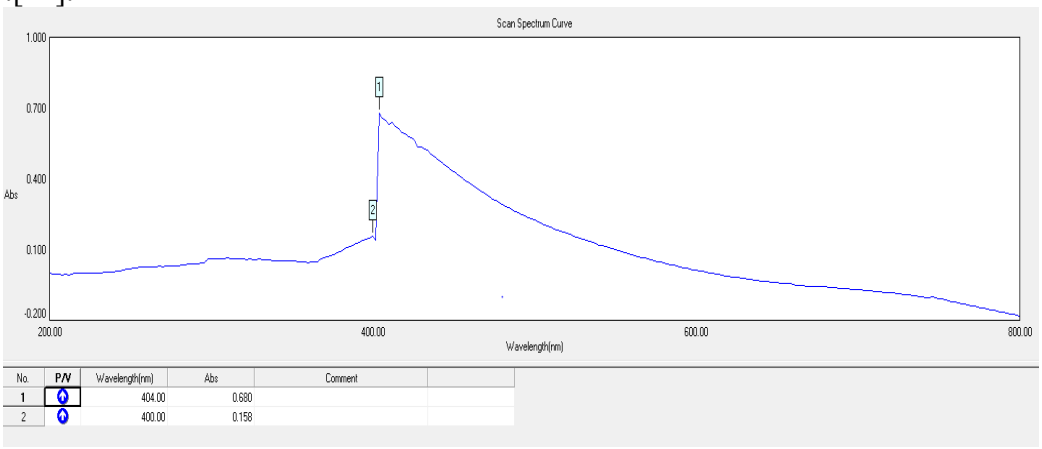


Figure (1) :UV–Vis absorption spectrum of Zno NPS synthesized by *Streptomyces* spp.4

Fourier Transform Infrared Spectroscopy (FT-IR):

FT-IR spectrum for Zno nanoparticles represented absorption peak at 3319.49 cm-1 indicate to produce presence of O-H group, 2245.14 cm-1 indicate to produce presence of CΞC , 1670.35 cm-1 indicate to C=C group, 1614.42 cm-1 refer to C=O group,1101.35 cm-1 indicate to C-O bending, 983.70 cm-1 indicating to C-O stretching,744.52 cm-1 indication to C-H, and 613.36 cm-1 indicate to C-X stretching.

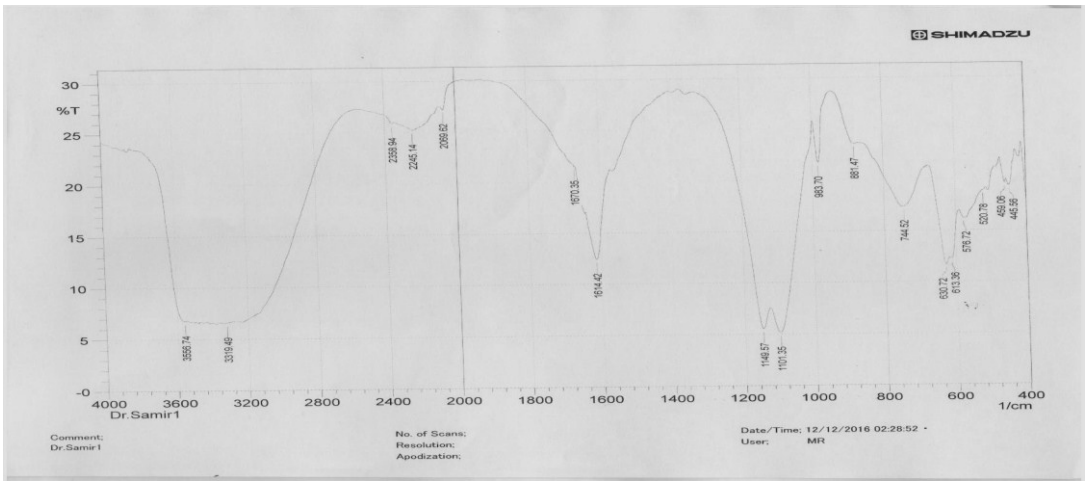
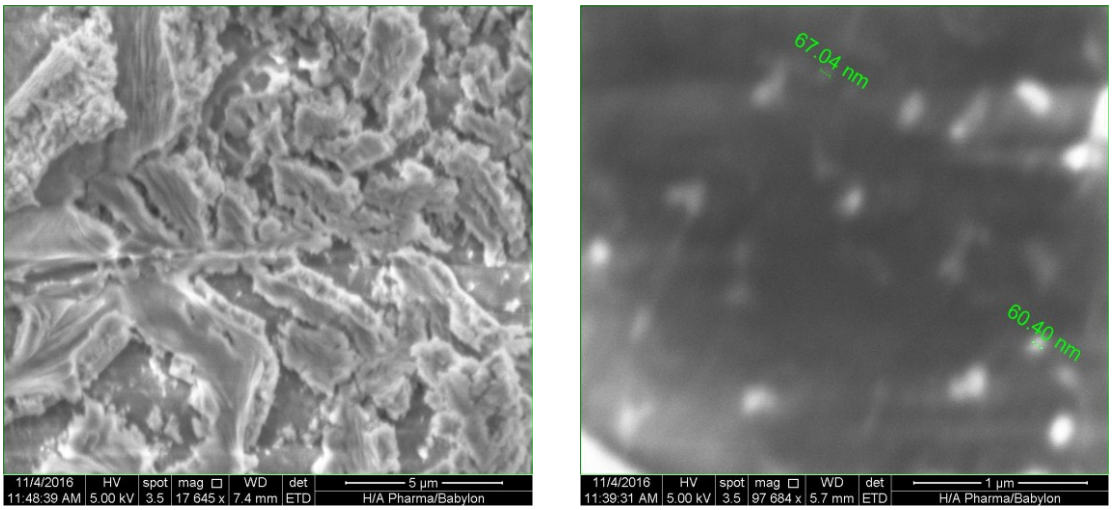


Figure (2): FT-IR analysis of Zno nanoprticles synthesized by *Streptomyces* spp.4

The FT-IR spectrum of synthesized ZnO-NPs showed strong absorption peaks at 3915.47, 3883.65, 3740.43, 3780.91, 1569.56, 1026.12, 951.66, 830.56, 691.10, and 617.83 cm_1, which were assigned to O-H stretching of alcohols and phenols, N-O asymmetric stretching of nitro compounds, -C-H bending of alkenes, respectively. The weaker bands at 3365.71, 2497.85, 2284.07, and 1414.80 cm_1, correspond to 1_, 2_ amines, amides (N-H stretching), aldehydes (C-H stretching), nitriles (C-N stretching), and alkanes (C-H bending). This results confirms that the bioactive metabolites of actinobacteria involve formation and stabilization of ZnO-NPs [3].

SEM analysis of ZnO nanoprticles synthesized by *Streptomyces* spp.4:

Scanning electron microscope analysis (SEM) was executed to characterizing *Streptomyces* spp.4 (ZnO nanoprticles) shape and size .The SEM shows that the bacterium has ability for synthesizing of ZnO nanoprticles and its spherical in shape. Obtained nanoparticles having size ranging (60.40-67.04) nm (Figure 3). UV-vis spectrophotometer, FTIR, XRD, SEM and TEM used for nanopaticle characterizing and its have spherical shape arranged between 10-75 nm [8].The toxicity of the nanoparticles increased with (size<100 nm) on the bacteria with decreasing of their size which showed by toxicological studies [6].Another researcher, tested the effects of ZnO-NPs size (100–800 nm) on antibacterial activity against *S.aureus* and *E.coli* by changing the electrical conductivity with bacterial growing ; the found the particle size diminution which due to antibacterial activity rising [22].



Figure(3) : SEM analysis of ZnO nanoprticles synthesized by *Streptomyces* spp.4

Energy dispersive X-ray analysis (EDX):

ZnO nanoprticles purity was recorded by EDXA (Figure 4) cooperating with FE-SEM. EDXA spectrum detected a strong signal for Zn with purity 42%.Other peaks observed includes O, S, and C .Metal nanoparticles elemental content established by using EDS [18].

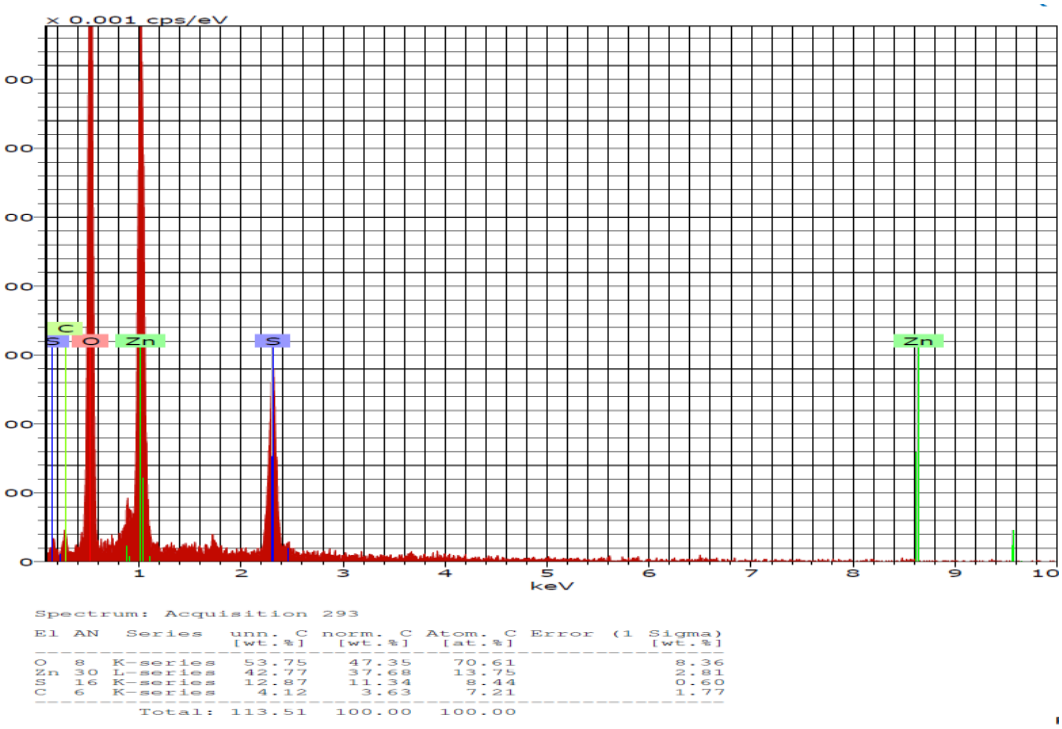


Figure (4) : EDX analysis of Zno nanoprticles synthesized by secondary metabolites of *Streptomyces* spp.4.

References:

[1] Albrecht MA, Evans CW, Raston CL. (2006). Green chemistry and the health implications of nanoparticles. Green Chem 8:417–32.

[2] Ashe B. A (2011).Detail investigation to observe the effect of zinc oxide and Silver nanoparticles in biological system [dissertation]. NIT, Orissa, India;

[3] Balagurunathan R, Radhakrishnan M, Rajendran RB, Velmurugan D.(2011). Biosynthesis of gold nanoparticles by actinomycete Streptomyces viridogens strain HM10. Indian J Biochem Biophys 48:331–5.

[4] Chithrani BD, Ghazani AA, Chan WC. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells.Nano Lett 6:662–8.

[5] Flärdh, K. (2003). Growth polarity and cell division in *Streptomyces*. Curr. Opin. Microbiol. **6**: 564–571.

[6] Gajjar P, Pettee B, Britt DW, Huang W, Johnson WP, et al. (2009) Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, Pseudomonas putida KT2440. J Biol Eng 3: 9.

[7] Gordon T, Perlstein B, Houbara O, Felner I, Banin E, Margel S. (2011). Synthesis and characterization of zinc/iron oxide composite nanoparticles and their antibacterial properties. Colloids and Surfaces A: Physicochem Eng Aspects.374: 1–8.

[8] Isaa, Z,I, Kadhim, J,F, Asimuddin,M, Nagarjun,A, and Jamil,K,P (2015).Biosynthesis of Zinc oxide nanoparticle and its characterization for antibacterial and anticancer properties.V4(11). World journal of pharmacy and pharmaceuticals sciences.

[9] Kim S, Fisher B, Eisler HJ, Bawendi M. (2003). Type-II quantum dots: CdTe/CdSe (core/shell) and CdSe/ZnTe(core/shell) hetero structures. Journal of American Chemical Society., 125: 11466–11467.

[10]Labeda,D.P., Goodfellow,M., Brown,R., Ward, A.C., Lanoot,B.,Vanncanneyt,M., Swings,J., Kim,S.B., Liu,Z., Chun,J. et al. (2012) Phylogenetic study of the species within the family Streptomycetaceae. Antonie Van Leeuwenhoek, 101, 73–104.

[11] Lu PJ, Huang SC, Chen YP, Chiueh LC, Shih DYC. (2015). Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics. Journal of Food and Drug Analysis . 23: 587–594.

[12] Nandhini, S. U. and Selvam, M. M. (2013). Bioactive compounds produced by *Streptomyces* strain. *International Journal of Pharmacy and Pharmaceuti-cal Sciences, 5* (1), 176-178.

[13] Rajamanickam U, Mylsamy P, Viswanathan S, Muthusamy P (2012).Biosynthesis of zinc nanoparticles using actinomycetes for antibacterial food packaging. International Conference on Nutrition and Food Sciences IPCBEE vol 39 IACSIT.

[14] Saha S, Sarkar J, Chattopadhyay D, Patra S, Chakraborty A, Acharya K.(2010). Production of silver nanoparticles by phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. Digest Journal of Nanomaterials and Biostructures., 5(4), 887-895.

[15] Schaffer B, Hohenester U, Trugler A, Hofer F. (2009). High-resolution surface plasmon imaging of gold nanoparticles by energy-filtered transmission electron microscopy. Phys Rev B 79:1–10.

[16] Shirling EB, and Gottlieb D (1966).Methods for characterization of *Streptomyces* species. Int. J. Syst. Baceriol., 16: 313-340.

[17] Söderberg TA, Sunzel B, Holm S, Elmros T, Hallmans G, Sjöberg S. (1990). Antibacterial effect of zinc oxide in vitro. Scand J Plast Reconstr Surg Hand Surg., 24: 193–197.

[18] Strasser P, Koh S, Anniyev T, et al. (2010). Lattice-strain control of the activity in dealloyed core–shell fuel cell catalysts. Nat Chem 2: 454–60.

[19] Stypuła B , Starowicz M, Hajos M, Olejnik E. (2011).Electrochemical Synthesis of ZnO Nanoparticles During Anodic Dissolution of Zinc in Alcohols Solvents. Archives of Metallurgy and Materials.56: 286–292.

[20] Usha R, Prabu E, Palaniswamy M, et al. (2010). Synthesis of metal oxide nano particles by Streptomyces Sp for development of antimicrobial textiles. Global J Biotechnol Biochem 5:153–60.

[21] Waghmare SS, Deshmukh AM, Kulkarni W, Oswaldo LA (2011).Biosynthesis and characterization of manganese and zinc nanoparticles.Univ J Environ Res Technol 1:64–69.

[22] Yamamoto O.(2001). Influence of particle size on the antibacterial activity of zinc oxide. Int J Inorg Mater3: 643–646.