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Nano-Bioremediation of Municipal Wastewater Using Myco-Synthesized Iron Nanoparticles

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Abstract

Metal nanostructures have potential effective impacts with many arenas like catalysis, sensors, optics, electronics, functional materials, environment and medicine. Thus, iron nanoparticles (FeNPs) were mycogenic synthesized in this study as effective antimicrobial agent and heavy metals removers. The isolation of 37 fungi were done from contaminated 10 soil (with iron ions or metals) samples and they screened. The most powerful fungal isolate was *Fusarium oxysporum*. Biosynthesized FeNPs size were 0.7 to 3 nm (high small size) with spherical shape based on TEM analysis. However, XRD, FT-IR analyses confirmed that characterization and led to form iron at zero valent. The obtained FeNPs were used as antibacterial agent against broad spectrum of environmental pathogens with minimum amounts (about 20 µg/ml) compared with reference antibiotics. This drive for using it to control microbial pathogens through municipal wastewater. For its high adsorption activity, it is used for removing heavy metals from their contaminated wastewater.

Keywords: Nano-bioremediation; Municipal wastewater; Antibacterial activity; Heavy metals; FeNPs.

1. Introduction

Metal Nanoparticles are the most dominate between inorganic nanoparticles and defined as particles with a diameter between 1 and 100 nm. Smaller nanoparticles less than 10 nm in diameter are creating a new category of materials whose properties are quite unique and different from the corresponding bulk or from atoms [1]. Many methods are applied for synthesis of metals nanoforms such physical, chemical and biological actions. The produced nanomaterials forms, size and action may change from method to other depend on many parameters as capping agents, reducing system, stabilizing materials and environmental conditions [2,3].

Fungi has a significant role in nanobiotechnology research. They have more attention concerning the investigation on biogenic production of metallic nanoparticles due to their toleration and metal bioaccumulation ability [4]. Also, Fungi have effectual extracellular enzyme secretion, so achieving substantial enzyme production [5]. Heavy metals are toxic to Microorganisms because they inhibit the activity of metal enzymes which contribute to reactive oxygen species being produced. Fungi are used different techniques for detoxification of heavy metals e.g., exclusion from cells using transporters, intracellular sequestration in vacuoles and enzymatic detoxification using redox enzymes [6]. The detoxified metals are minorally precipitated or maintained as metal ions into cells [7]. It is important to know nanomaterials properties before using. There are many methods used to characterize such materials, among them, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Fourier Transform Infrared spectroscopy (FTIR), Powder X-ray Diffraction (XRD) Technique, Atomic Absorption Spectroscopy (AAS), Gel Permeation Chromatography (GPC), Differential Scanning Calorimetry (DSC), Thermo gravimetric Analysis (TGA), Electrical Conductivity Measurement [8,9]. Nanoparticles have a lot of applications in environmental science including

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environmental remediation, hazardous waste management, metal biosensors, antimicrobial, removal of heavy metals and decolorization of dyes. Because of nanomaterials have high surface/volume ratio, it has been explored to detect and treat pollutants in various environmental matrixes like wastewater, soil and sediment by means of adsorption, oxidation–reduction, surface complication and other mechanisms [1]. Modern technology for pathogens disinfectant means to produce and apply new substances have antimicrobial activity such as plant or microbial agents [10-13], new chemical materials or derivatives [14,15] and new composites [16-18]. Also, many researchers introduced new adsorbents like lignin derivatives and low-cost lignocellulosic substances for wastewater remediation from heavy metals and toxic dyes [19-20]. Due to varying concentrations of reactive surface sites and disordered surface regions, metal nanoparticles have unusual adsorption properties and particle sizes that make these particles have far broader surface areas than bulk particles, with efficient effect on the adsorption of dissolved heavy metals. Iron oxide and titanium dioxide are good sorbents for metal contaminants [21]. Thus, the present study focused on mycogenic synthesis of FeNPs and their significant applications for pathogens disinfection and heavy metals removal. As well as environment protection from pollution and introduce non-traditional water resource.

2. Materials and Methods

2.1. Isolation and identification of nanostructure iron producing fungi

Ten soil samples were collected from El Gharbyia and Menoufia governorates; 3 samples from agricultural soil at El-Santa city and 2 from metal work shop in El Gharbyia governorate and other five samples from Maady steel factory at Menoufia governorate. The samples were collected from the areas contaminated with iron and transferred to lab under standard septic conditions [22]. For enrichment and isolation of fungi, 10 g soil sample was added to 100 ml distilled tap water and shacked for 1 h. After settling the soil particles, 10 ml from the supernatant was added to enrichment medium (Czapeks-Dox medium containing 1 g/l $FeCl_3$) and incubated under shaking conditions (150 rpm) for 2 weeks. One ml of enriched cultures was spread onto Czapeks-Dox agar plates [23] supplemented with ampicillin (50 µg/ml) as antibacterial and $FeCl_3$ (0.1 % w/v) as a source of iron ions. The inoculated plates were incubated at 28 °C for 7 days. The different fungal colonies grown onto the inoculated plates were selected and transferred to new plates for purification. The purified fungi were transferred to slant culture tubes of potato dextrose agar medium, stored at 4°C and sub-cultured every six months.

The isolated fungi were checked for their ability to grow into potato dextrose broth medium supplemented with iron ions and the ability of fungal isolates on iron nanoparticles production was tested. The inoculation size of 10^6 spores ml^{-1} was prepared in physiological saline and inoculated into 250 ml Erlenmeyer flasks containing 100 ml of potato dextrose broth medium (pH 5.0), and then incubated at 150 rpm and 25 ˚C for 3 days. The grown fungal biomass at the end of incubation period was filtered through filter paper grade 4 and washed thoroughly with sterilized deionized water and dried. An equal volume of $FeCl_3$ solution with concentration of 1000 ppm was mixed with the obtained supernatants for each isolate separately and incubated again on a rotary shaker at 150 rpm and 28 ˚C for 48 h and checked continuously. For checking of FeNPs formation, the incubated mixtures were scanned and read its optical density at 420 nm using Perkin-Elmer LS 50B spectrophotometer. To decrease the number of isolates, the selected fungi from previous step were tested under serial up concentrations of iron. The different concentrations of iron salt ($FeCl_3$) ranged between 1000: 5000 ppm was added to 100 ml of supernatants for each isolate and incubated on a rotary shaker at 150 rpm and 28 ˚C for 7 days. The optical density was measured at 420 nm. The most active isolate was identified by morphological and microscopic observations. The Morphological characteristics were examined using light microscope (Olympus cx41) after 3 days of growing on PDA agar plates. For molecular Identification, fungal mycelium from a 3 days old culture in potatoes dextrose broth (PDB) was harvested using Whatman No. 1 filter Paper. The total genomic DNA was extracted using CTAB protocol [24]. DNA of the fungal isolates was amplified using polymerase chain reaction (PCR) by ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) designed for sequencing. The identification was achieved by comparing the contiguous DNA sequence with data from the reference and type

strains available in public databases GenBank using the BLAST program (National Centre for Biotechnology Information) (http://www.ncbi.nlm.nih.Gov/BLAST). The obtained sequences were aligned using Jukes Cantor Model.

2.2. Morphological characterization of FeNPs using transmission electron microscopy (TEM)

Characterizations of the produced biosynthesized FeNPs were done using High-resolution transmission electron microscopy (HRTEM) (JEOL 2100 Japan, at National Research Centre (NRC), Cairo, Egypt) to define the size and shape of the produced biosynthesized nanostructures [25,26]. The samples were prepared by drop-coating of FeNPs solutions onto the carbon-coated copper grid which loaded onto a specimen holder after drying. The water suspended nanostructures were subjected to visualization under HRTEM and the sizes and shape of FeNPs were recorded.

Fourier transforms infrared spectroscopy (FTIR) characterization of FeNPs

The probable biomolecules responsible for reduction, capping and effective stabilization of the biosynthesized FeNPs were noted using FTIR spectrophotometer at diffuse reflectance mode. A known weight of sample (1 mg) was taken in a mortar and pestle with 2.5 mg of dry potassium bromide (KBr). The obtained powder was filled in a 2 mm internal diameter micro cup and loaded onto FTIR set at 26 ± 1˚C. The samples were scanned using infrared in the range of 4000: 400 cm^{-1} using FTIR spectrometer (Agilent system Cary 630 FTIR model - Chemical Department, at National Research Centre, Cairo, Egypt). The obtained spectral data were compared with the reference chart to identify the functional groups present in the sample.

2.3. Crystalline structure characterization of FeNPs by X-ray diffraction (XRD)

The crystalline structure of the biosynthesized iron nanoparticles was characterized by X-Ray Diffractometer. X-Ray Diffraction patterns were obtained with the XRD- 6000 series by Shimadzu apparatus using nickel-filter and Cu-K_α X-ray target, PANalytical Xpert PRO Instruments, Holand, Central lab at Agricultural Research Center, Cairo, Egypt. Under condition of 2θ scan range (10- 80), step side (0.02), scan rate (0.5 sec) and anode source copper.

2.4. Application of the biosynthesized FeNPs for municipal wastewater remediation

Two main hazardous substances found in municipal wastewater are heavy metals and pathogenic microbes. The produced biosynthesized FeNPs were applied for pathogens controlling and heavy metals removing from their contaminated wastewater. In case of pathogenic bacteria controlling, 7 different pathogens were used. *Bacillus cereus, Listeria monocytogenes, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella typhi, E. coli* and *Candida albicans* as reference strains were obtained from American Type Culture Collection [18,27]. The stock cultures of microorganisms maintained on plate count agar slants at 4 ˚C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient agar broth and incubated at 37 ˚C for 24 h. About 60 µl of bacterial suspensions adjusted to 10^6-10^7 CFU/ml were taken and poured into Petri plates containing 25 ml sterilized nutrient agar medium. Method of well diffusion was applied in this procedure according to the previous illustrated by Abdelhameed et al. [25]. Different concentrations (12.50, 14.28, 16.67, 20.00, 25.00, 33.30, 50, 10, 133.33, 200.00 and 266.60 mg/ml) of biosynthesized FeNPs suspended into sterilized ddH_2O were prepared and 50 µl of them were added individually to the wells. The plates were allowed to stand at 4ºC for 2 h before incubation to make diffusion of tested samples. The plates were incubated at 37 ˚C for 24 h and measured the diameter of inhibition zone expressed in millimeter. Amoxicillin ($C_{16}H_{19}N_3O_5S$) as an antibiotic with concentration of 250 mg/ml was applied as reference disinfectant. All samples were performed in triplicate and expressed as average values ± SD. Minimum inhibitory concentration (MIC) also determined [28]. For heavy metal removal, 5 common heavy metals (Chromium, lead, Nickel, Cadmium and Zinc) were used as module for heavy metals contaminated wastewater. A 100 ppm of the tested heavy metals ions was prepared using standard of these heavy metals. FeNPs (produced by *Fusarium oxysporum*) at concentration of 100 mg/ 10 ml were applied to remove these ions from their solutions [29]. Wastewater sample was collected from domestic treatment station. A 500 mg of FeNPs was added to 10 ml of the collected wastewater sample and incubated with shaking (100 rpm) for overnight. After that, the total microbial counts were examined before and after treatment and the percentage of

decreasing was recorded. Also, the heavy metals (Pb, Cd, Zn) were determined and the percentage of removal was recorded.

3. Results and Discussion

3.1. Isolation and identification of fungi reducing iron

The objective of the present study is to obtain active fungal isolates capable for reducing iron ions and converting to iron nanostructure. To achieve that, 10 soil samples were collected from different soil locations contaminated with iron (metal or ion). The sample source is a key to success of targeted microbes isolation [29]. In order to increase the ability of fungi to synthesize nanostructure of iron, fungi found in the collected soil samples were enriched into enrichment medium contained iron chloride as a source of iron ions. The observation to find different fungal colonies indicating that these fungi had resistant system against iron were isolated. After purification, 37 different fungal isolates were recorded. To confirm the ability of isolated fungi for iron reducing, the spectrophotometer reading was applied. The initial results illustrated that 17 different fungi were had ability to reduce the iron ions more than others and coded as F4, F8, F9, F11, F13, F17, F19, F22, F23, F26, F27, F30, F32, F34, F35, F36 and F37. In case of obtaining the most active isolates in reducing iron, fungal isolates were advanced screened using increased concentrations of iron chloride. Results illustrated in Fig. (1) indicated that the fungal isolate, F23 showed the best trends for iron nanostructures biosynthesize from the screening step at concentration of 1 % and continued to concentration of 5 % w/v. These isolate may be had reduction system more than others. Also, the results showed some isolates had minus adsorption, this is may be return to decreasing the colour of tested medium but not producing iron nanoforms.

The isolate number F23 showed the highest activity for iron nanostructure synthesis. The morphological identification of these isolate showed that the colonies exhibit elliptical to kidney-shaped microconidia; thin-walled, sickle-shaped and delicate macroconidia, the microconidia produced in false heads on short monophialides and a single, fatal chlamydo spore. This isolate was initially identified as *Fusarium oxysporum* [8]. The molecular biology techniques were applied to confirm morphological and cultural characterizations. The genomic DNA was isolated and purified using isopropyl method [30,31]. The Internal transcribed spacer (ITS) genes were amplified by PCR technology and sequenced. The obtained sequence was compared with sequences available in Gene Bank using BLAST program (http://www.ncbi.nlm.nih.Gov/BLAST). The result of comparing introduced the isolate F23 had similarity percentage accounted for 98 % with other related strains of *Fusarium oxysporum* in gene bank.

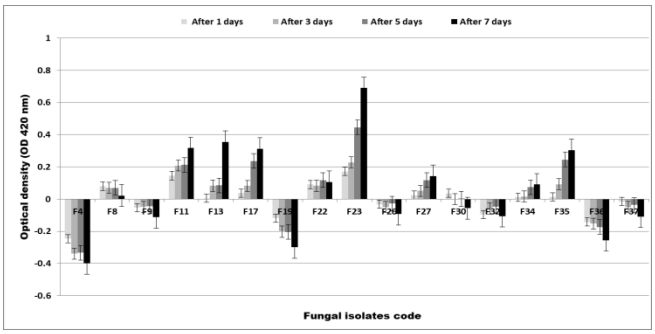

Fig. 1. Spectrophotometric analyses of iron ions reducing and nanostructure formation by fungal isolates at 5 % of iron chloride

3.2. Nanoparticles production and characterization

Iron nanoform was produced by fungal strain, *Fusarium oxysporum* and the produced nanoparticles were characterized using transmission electron microscopy, fourier transform infrared spectroscopy and X-ray diffraction spectrophotometer. Transmission electron microscopy (TEM) is considered the main technique for nano-size materials characterization (especial of morphological characterization), because it illustrates the size and morphology of nanoforms [17]. In this study, high-resolution transmission electron microscopic (HRTEM) was applied for analysis of biosynthesized FeNPs morphology. The micrographs obtained indicated production of nanoparticles using supernatant of *Fusarium oxysporum* culture growth was had the size ranged between 1 and 3 nm. In case of shape, the FeNPs had spherical shape with marginal variation with some aggregates as represented in Fig. (2). Also, found film surrounding the particles indicated found proteins as protective agents. These proteins are useful for protection the produced particles from aggregation [26].

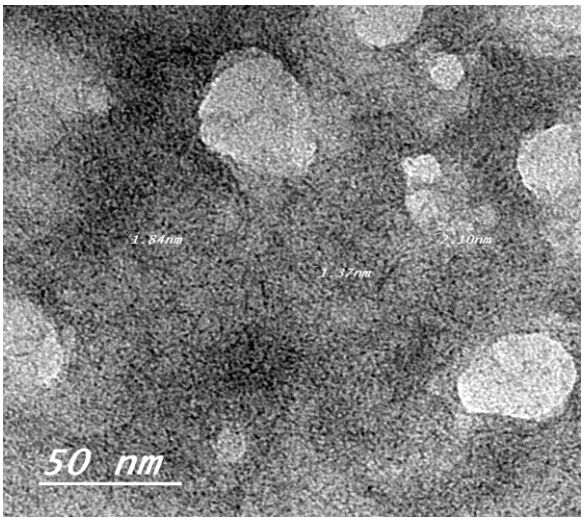

Fig. 2. Transmission electron microscopy pictures of FeNPs biosynthesized by the supernatant of *Fusarium oxysporum*.

3.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrum of biosynthesized FeNPs prepared by supernatant of *Fusarium oxysporum* was performed to give insights about the presence of functional groups in the synthesized FeNPs in order to understand how they transform from simple inorganic iron ($FeCl_3$) to elemental iron due to the effect of different photo-chemicals that might act in a simultaneous way as reducing, stabilizing and capping agent [26]. The FTIR spectrum of iron biosynthesized by *Fusarium oxysporum* at array of absorbance bands from 400 to 4000 cm^{-1}. Fig. (3) represents IR spectrum of iron nanoparticles with some vibration bands at different wavelengths. The peak displayed around the range of 600 cm^{-1} refers to the band of alkyl halides (C-Cl) [32] as in position of 9, while O-H group stretching due to the proposed presence of alcohols, flavonoids and phenols was viewed at 3426 and 3460 cm^{-1} [33,34], as in position of 2. Whereas C=O bond stretching peak was detected at 2362 cm^{-1} [35] as in position of 5. Based on the detected peaks from FTIR spectroscopy, we can conclude found the protein agents like reductase enzymes in order to mediate the FeNPs biosynthesis, as well as inactive proteins as capping agent.

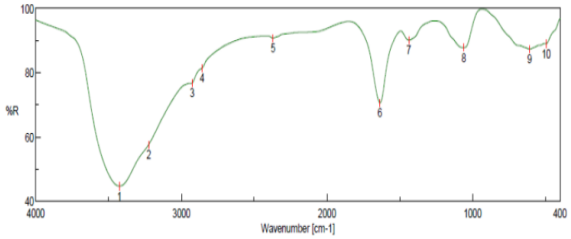

Fig. 3. FTIR analyses of iron nanoparticles biosynthesized by the supernatant of *Fusarium oxysporum.*

3.4. Metal crystallinity of FeNPs using XRD spectroscopy

The specific peaks of metallic crystalline iron in biosynthesized FeNPs were identified in XRD spectrum. The XRD analysis at 2θ of FeNPs which biosynthesized by the supernatant of *Fusarium oxysporum*, the peak position with 2θ values was represented in Fig. (4) and Table (1). The results appeared in Table (1) illustrated that production of some iron oxides nanoparticles like Fe_2O_3, Fe_3O_4 and FeO_2, as well as iron zero valent. These iron forms were matched and fabricated in crystalline structure.

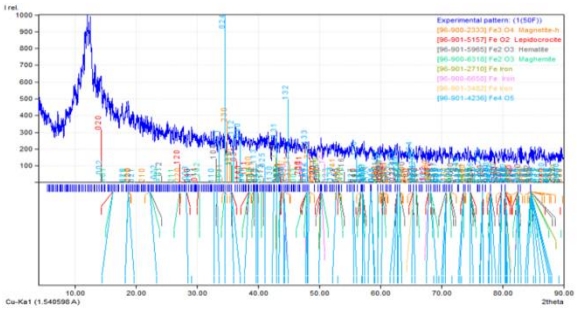

Fig. 4. XRD of iron Nano particles biosynthesized by the supernatant of *Fusarium oxysporum.*

Table 1. Matching XRD peaks of FeNPs biosynthesized by the supernatant of *Fusarium oxysporum*

Fe derivatives	Matching
Lepidocrocite FeO_2	14.15 – 27.31 – 28.50 – 30.49 – 36.52 – 37.17 – 38.03 – 43.53 – 43.97 - 46.99 – 49.25 – 50.13 – 53.03 – 56.70 – 59.67 – 60.05 – 60.64 - 60.99 – 62.83 – 64.78 – 65.55 – 66.14 – 67.69 – 68.75 – 72.82 – 75.19 – 75.49 – 76.68 – 77.89 – 79.29 – 79.90 – 80.81 – 81.36 – 81.84 – 82.32 – 84.06
Magnetite Fe_3O_4	18.82 – 27.31 – 34.00 – 34.91 – 35.32- 39.01 - 39.30 – 40.24 - 43.53 – 43.97 – 44.10 – 45.18 – 48.70 – 49.25 – 52.77 - 53.03 – 53.84 – 55.98 – 59.67 – 60.05 – 60.64 – 60.99 – 61.30- 63.24 – 63.82 – 64.49 – 67.69 – 68.31 - 70.95 – 71.61 – 72.82 – 73.91 - 74.84 -77.24 – 78.85 – 80.81 - 81.36 – 83.28- 84.06 – 87.18
Maghemite Fe_2O_3	15.06 – 18.62 – 23.78 – 26.05 – 30.49 – 34.00- 35.47 – 37.40 – 39.01 - 40.54 – 43.53 – 46.26 - 47.28 – 47.73 – 50.13 – 51. 08- 53.84 - 56.42 - 57.41 – 59.67 - 60.99 – 63.24 – 65.12 – 66.14 – 68.31 - 69.60 - 71.61 – 73.47 – 75.49 - 76.68 – 77.89 - 79.90 – 81.84 – 82.68 – 84.06.
Goethite FeO_2	17.85 – 26.05 – 33.22 - 34.54 – 35.32 – 36.23 – 36.52 – 39.01 - 39.75 - 40.24 – 41.25 – 43.15 – 44.93 – 48.07 – 49.85 – 50.56 – 51.08 – 53.03 – 54.10 – 55.50- 57.41 – 58.89 – 59.12 – 60.99 – 61.30 62 .83 – 63.24 – 63.82 – 64.49 - 65.55 – 66.83 – 67.69 - 68.31 - 69.04 – 69.35 – 69.60 - 71.35 – 71.61 – 72.10 – 73.47 – 74. 83 - 75.19 – 76.08 – 76.68 – 77.24 – 77.89 - 78.85 - 79.90 – 81.36 – 82.68 – 84.06 – 87.18
iron oxide hematite α-Fe_2O_3	24.00 – 33.22 – 35.74 – 39.30 – 43.53 – 49.57 – 54. 10 - 57.41 62.83 – 63.82 – 69.60 – 72.10 – 75.49 – 77.89 – 80.81 - 82.68 – 84.06.

3.5. Antimicrobial activity

The inhibition zone obtained against the gram positive and gram negative bacteria, as well as yeast

markedly illustrated the effective nature of the biosynthesized FeNPs. A considerable diameter of inhibition zone was detected with the concentration of 20 mg/ml as presented in Fig. (5). The results obtained also indicated a good antimicrobial activity against the seven tested species of microorganisms. FeNPs exhibited its effect even at the lowest applied concentration (20 µg/mL) which reflects its good attitude as antimicrobial agent which resemble to the FeNPs biosynthesized by numerous mechanisms of antimicrobial action have been reported to state that the FeNPs has been shown in multiple studies focusing on the optimal size range of about 1 to 10 nm have antimicrobial activity [36,37], and have been promising for medicine and environmental fields due to their properties, specially their interaction with pathogens [38].

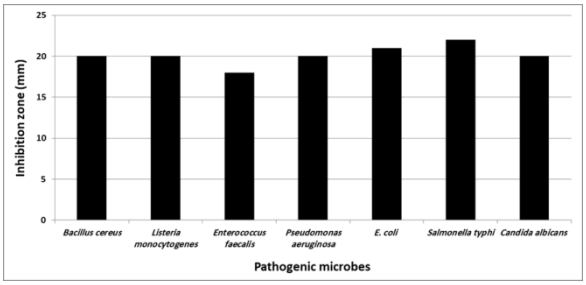

Fig. 5. Antimicrobial activity of FeNPs (20 mg/ml) produced by the supernatant of *Fusarium oxysporum*

To obtain the minimal concentration makes microbial growth inhibitory, gradually concentrations were examined on the tested pathogenic microbes and the obtained values were represented in Table (2). The results showed that the produced FeNPs by *Fusarium oxysporum* recorded antibacterial activity more effective because it made antimicrobial activity at low concentration around 20 µg/ml. These results led to apply the produced FeNPs as antimicrobial agent especially with municipal wastewater treatment.

Table 2. Minimal Inhibitory Concentration (MIC) of the produced FeNPs

Pathogens	**MIC (µg/ml)**
Bacillus cereus	20
Listeria monocytogenes	20
Enterococcus faecalis	25
Pseudomonas aeruginosa	20
E. coli	20
Salmonella typhi	20
Candida albicans	20

3.6. Heavy metals removal from contaminated synthetic wastewater

Synthetic wastewater polluted by 3 dangerous heavy metals was prepared by completely dissolved Cd, Pb and Zn ions with concentration of 100 ppm using their salts. FeNPs produced by the supernatant of *Fusarium oxysporum* at concentration of 100 mg/ 10 ml were added and the results were noted in Table (3). The results indicated that the Pb was removed by high capacity reached to 95.4 %, followed to Zn (25.37 %) and Cd (14.62 %). From these results, FeNPs can be used for treatment of wastewater contaminated by the dangerous lead ions. also, it can be used for removing of zinc and cadmium from their contaminated sites after increasing its concentration.

Table 3. Application of FeNPs produced by *Fusarium oxysporum* (0.1 g / 10 ml) to adsorb some heavy metal

Heavy metals	Control (ppm)	Treated sample by FeNPs (ppm)	Percentage (%) of decreasing
Lead (Pb)	16.88	4.60	95.4
Cadmium (Cd)	132.32	85.38	14.62
Zinc (Zn)	114.62	74.63	25.37

3.7. Application of the biosynthesized FeNPs for municipal wastewater remediation

The amount of municipal wastewater is increased daily and becomes big problems due to its containing from heavy metals and pathogenic microbes. Thus, it is important to treat it using efficient and environmental materials [39]. In the current research, the myco-synthesized FeNPs were used for remediation of municipal wastewater collected from El Gharbyia wastewater treatment station. Data tabulated and figured in Table (4) and Figure (6) indicated that the ability of FeNPs to decrease microbial load of municipal wastewater by 89.23 and 100 % for total bacterial and total fungal counts, respectively, compared with the standard antibiotic. In case of heavy metals removal, FeNPs had capability for treatment of such wastewater nearly to 95 % for Pb and percentages between 20 – 50 % with Cd, Cr, Ni and Zn heavy metals.

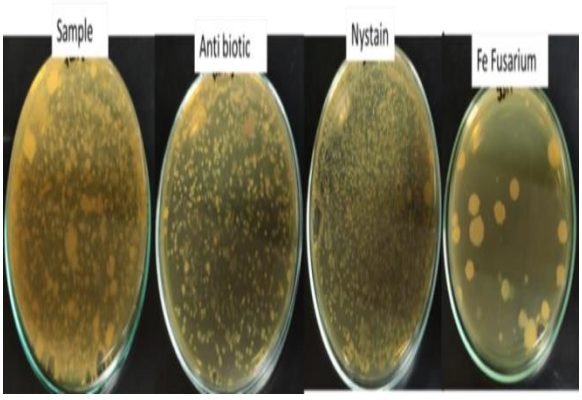

Fig. 6. Total microbial counts of municipal wastewater sample treated with different antimicrobial agents

Table 4. Total bacteria and fungi counts of municipal wastewater sample treated with different antimicrobial agents

Sample	Count of bacteria		Count of fungi	
	Total count (CFU)	Percentage of decreasing %	Total count (CFU)	Percentage of decreasing %
Municipal wastewater (control)	116 x 10^7	-	7 x 10^5	-
Treated by Antibiotic (Amoxicillin)	52.5 x 10^5	54.8	16 x 10^3	97.72
Treated by anti-fungi (Nystatin)	175 x10^5	84.91	10 x 10^3	98.57
Treated by FeNPs	12500	89.23	No growth	100

4. Conclusions

In summary, iron nanoparticles were myco-synthesized using *Fusarium oxysporum* as most active fungal isolate. Characterization studies confirmed the produced FeNPs had irregular spherical morphology with size ranged from 1 to 3 nm. This very small size was significant effective as an antimicrobial and adsorbed agent. Thus, it is used for remediation of municipal wastewater as eco-friendly, cost-effective and easy-to-handle synthesis process with large scale. Consequently, biogenic-synthesized FeNPs can be a good candidate for environmental protection.

5. Acknowledgments

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6. Conflicts of Interest

The authors declare no conflict of interest.

7. References

[1] Wang L., Hu C., Shao L., The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Internat J Nanomed, 12, 1227–1249 (2017).

[2] Hussein H.A., Darwesh O.M., Mekki B.B., Environmentally friendly nano-selenium to improve antioxidant system and growth of groundnut cultivars under sandy soil conditions. Biocatal Agr Biotechnol, 18, 101080, (2019), https://doi.org/10.1016/j.bcab.2019.101080.

[3] Khan I., Saeed K., Khan I., Nanoparticles: Properties, applications and toxicities. Arab J Chem, 12, 908–931 (2019). http://dx.doi.org/10.1016/j.arabjc.2017.05.011.

[4] Sastry M., Ahmad A., Khan M., Kumar R., Microbial nanoparticle production. in Chemosphere. eds. C. M. Niemeyer and C. A. Mirkin, 126–135 (2005), doi: 10.1002/3527602453.ch9.

[5] Castro-Longoria E., Vilchis-Nestor A.R., Avalos B.M., Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus Neurospora crassa. Coll Surf B: Biointerf, 83(1), 42–48 (2011).

[6] Bruins M.R., Kapil S., Oehme F.W., Microbial Resistance to Metals in the Environment. Ecotoxicol Envir Safety, 45(3), 198-207 (2000), DOI: 10.1006/eesa.1999.1860.

[7] Jan A.T., Azam M., Siddiqui K., Ali A., Choi I., Haq Q., Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. Intern J Mol Sci, 16(12), 29592-29630 (2015). https://doi.org/10.3390/ijms161226183.

[8] Elshahawy I., Abouelnasr H.M., Lashin S.M., Darwesh O.M., First report of Pythium aphanidermatum infecting tomato in Egypt and its control using biogenic silver nanoparticles. J Plant Prot Res, 15(2), 137–151 (2018). DOI: 110.24425/122929.

[9] Kato Y., Suzuki M., Synthesis of Metal Nanoparticles by Microorganisms. Crystals, 10(7), 589 (2020). https://doi.org/10.3390/cryst10070589.

[10] Ali S.I., Mohamed A.A., Sameeh M.Y., Darwesh O.M., Abd El-Razik T.M., Gamma-Irradiation Affects Volatile Oil Constituents, Fatty Acid Composition and Antimicrobial Activity of Fennel (Foeniculum vulgare) Seeds Extract. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7, 524-532 (2016).

[11] Kheiralla Z.H., Hewedy M.A., Mohammed H.R., Darwesh O.M., Isolation of Pigment Producing Actinomycetes from Rhizosphere Soil and Application It in Textiles Dyeing. Res J Pharm, Biol Chem Sci, 7, 2128-2136 (2016).

[12] Sultan Y.Y., Ali M.A., Darwesh O.M., Embaby M.A., Marrez D.A., Influence of Nitrogen Source in Culture Media on Antimicrobial Activity of Microcoleus lacustris and Oscillatoria rubescens. Res J Pharm, Biol Chem Sci, 7(2), 1444-1452 (2016).

[13] Darwesh O.M., Barakat K.M., Mattar M.Z., Sabae S.Z., Hassan S.H., Production of antimicrobial blue green pigment pyocyanin by marine Pseudomonas aeruginosa. Biointerf Res Appl Chem, 9, 4334-4339, (2019) https://doi.org/10.33263/BRIAC95.334339.

[14] Abdelhameed R.M., El-Sayed H.A., El-Shahat M., El-Sayed A.A., Darwesh O.M., Novel Triazolothiadiazole and Triazolothiadiazine Derivatives Containing Pyridine Moiety: Design, Synthesis, Bactericidal and Fungicidal Activities. Curr Bioact Comp, 14(2), 169-179 (2018). https://doi.org/10.2174/1573407213666170127095158.

[15] Abdelhameed R.M., Darwesh O.M., Rocha J., Silva A.M., IRMOF-3 Biological Activity Enhancement by Post-Synthetic Modification. Europ J Inorg Chem, 1243-1249 (2019). https://doi.org/10.1002/ejic.201801442.
[16] Abdel-Monem R.A., Khalil A.M., Darwesh O.M., Hashim A.I., Rabie S.T., Antibacterial properties of carboxymethyl chitosan Schiff-base nanocomposites loaded with silver nanoparticles. J Macromol Sci, Part A, 57(2), 145-155 (2020), https://doi.org/10.1080/10601325.2019.1674666.
[17] Mourad R., Helaly F., Darwesh O.M., Sawy S.E., Antimicrobial and physicomechanical natures of silver nanoparticles incorporated into silicone-hydrogel films. Contact Lens and Anterior Eye, 42, 325–333 (2019). DOI:10.1016/j.clae.2019.02.007.
[18] Mourad R.M., Darwesh O.M., Abdel-Hakim A., Enhancing physico-mechanical and antibacterial properties of natural rubber using synthesized Ag-SiO2 nanoparticles. Internat J Biolog Macromol, 164, 3243–3249 (2020), https://doi.org/10.1016/j.ijbiomac.2020.08.063.
[19] Darwesh O.M., Abd El-Latief A.H., Abuarab M.E., Kasem M.A., Enhancing the efficiency of some agricultural wastes as low-cost absorbents to remove textile dyes from their contaminated solutions. Biomass Conv Biorefin (2021), https://doi.org/10.1007/s13399-020-01142-w.
[20] Li H., Yuan Z., Shang X., Shang H., Liu J., Darwesh O.M., Li C., Fang J., Application of gradient acid fractionation protocol to improve decolorization technology by lignin-based adsorbent. Internat J Biolog Macromol, 172, 10–18 (2021), https://doi.org/10.1016/j.ijbiomac.2020.12.206.
[21] Tiwari D.K., Behari J., Sen P., Application of Nanoparticles in Waste Water Treatment. World Appl Sci J, 3(3), 417-433 (2008).
[22] Hasanin M.S., Darwesh O.M., Matter I.A., El-Saied H., Isolation and characterization of non-cellulolytic Aspergillus flavus EGYPTA5 exhibiting selective ligninolytic potential. Biocatal Agr Biotechnol, 17, 160–167 (2019). https://doi.org/10.1016/j.bcab.2018.11.012.
[23] Darwesh O.M., El-Maraghy S.H., Abdel-Rahman H.M., Zaghloul R.A., Improvement of paper wastes conversion to bioethanol using novel cellulose degrading fungal isolate. Fuel, 262, 116518 (2020), https://doi.org/10.1016/j.fuel.2019.116518.
[24] Eida M.F., Darwesh O.M., Matter I.A., Cultivation of oleaginous microalgae Scenedesmus obliquus on secondary treated municipal wastewater as growth medium for biodiesel production. J Ecolog Engin, 19, 38–51 (2018). https://doi.org/10.12911/22998993/91274.
[25] Abdelhameed R.M., Darwesh O.M., El-Shahat M., Synthesis of arylidene hydrazinylpyrido[2,3-d]pyrimidin-4-ones as potent anti-microbial agents. Heliyon, 6, e04956 (2020), https://doi.org/10.1016/j.heliyon.2020.e04956.
[26] El-Shanshoury A.R., Darwesh O.M., Sabae S.Z., Awadallah O.A., Hassan, S.H., Bio-manufacturing of selenium nanoparticles by Bacillus subtilis isolated from Qarun Lake and evaluation their activity for water remediation. Biointerf Res Appl Chem, 10(4), 5834 – 5842 (2020). https://doi.org/10.33263/BRIAC104.834842.
[27] Emam H.E., Darwesh O.M., Abdelhameed R.M., Protective Cotton Textiles via Amalgamation of Cross-Linked Zeolitic Imidazole Frameworks. Industr Engin Chem Res, 59(23), 10931-10944 (2020). https://doi.org/10.1021/acs.iecr.0c01384.
[28] Darwesh O.M., El-Hawary A.S., El Kelany U.S., El-Sherbiny G.M., Nematicidal Activity of Thermostable Alkaline Protease Produced by Saccharomonospora viridis strain Hw G550. Biotechnol Rep, 24, e00386 (2019), https://doi.org/10.1016/j.btre.2019.e00386.
[29] Powara N.S., Patelb V.J., Pagarec P.K., Pandavd R.S., Cu Nanoparticle: Synthesis, Characterization and Application. Chem Methodol, 457-480 (2019), DOI:10.22034/chemm.2019.154075.1112.
[30] Sadek Z.I., Abdel-Rahman M.A., Azab M.S., Darwesh O.M., Hassan M.S., Microbiological evaluation of infant foods quality and molecular detection of Bacillus cereus toxins relating genes. Toxicol Rep, 5, 871-877 (2018). DOI:10.1016/j.toxrep.2018.08.013.
[31] Darwesh O.M., Eida M.F., Matter I.A., Isolation, screening and optimization of L-asparaginase producing bacterial strains inhabiting agricultural soils. Biosci Res, 15, 2802-2812 (2018).
[32] Bagherzade G., Tavakoli M.M., Namaei M.H., Green synthesis of silver nanoparticles using aqueous extract of saffron (Crocus sativus L.) wastages and its antibacterial activity against six bacteria. Asian Pacific J Trop Biomed, 7(3), 227-233 (2017), https://doi.org/10.1016/j.apjtb.2016.12.014.
[33] Tungmunnithum D., Thongboonyou A., Pholboon A., Yangsabai A., Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines (Basel), 5(3), 93 (2018). doi: 10.3390/medicines5030093.
[34] Oliveira Renata N., Mancini M.C., Oliveira F.C., Passos et al., FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. Matéria (Rio de Janeiro), 21(3), 767-779 (2016) https://dx.doi.org/10.1590/S1517-707620160003.0072
[35] Misra A., Tyagi P.K., Singh M.K., Misra D., FTIR studies of nitrogen doped carbon nanotubes. Diamond Related Mater, 15(2), 385-388 (2006). DOI: 10.1016/j.diamond.2005.08.013.
[36] Arakha M., Pal S., Samantarrai D., et al. Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Scientific Rep, 5, 14813 (2015). https://doi.org/10.1038/srep14813.
[37] Vitta Y., Figueroa M., Calderon M. Ciangherotti C., Synthesis of iron nanoparticles from aqueous extract of Eucalyptus robusta Sm and evaluation of antioxidant and antimicrobial activity. Mater

Sci Energy Technol, 3, 97-103 (2020). https://doi.org/10.1016/j.mset.2019.10.014.
[38] Thukkaram M., Sitaram S., Kannaiyan S.k., Subbiahdoss G., Antibacterial Efficacy of Iron-Oxide Nanoparticles against Biofilms on Different Biomaterial Surfaces. Internat J Biomater, 716080, (2014) https://doi.org/10.1155/2014/716080.
[39] Hosseini M., Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts: Technologies and Approaches for Scale-Up and Commercialization. Elsevier Inc., ISBN 978-0-08-101031-0. 500pp (2019).