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Application of ZnO nanoparticles for wastewater treatment and antimicrobial activity

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Abstract

The present work used ZnO-NPs from the aqueous extracts *of Moringa Oleifera*, *Ocimum Tenuiflorum*, and Neem (*Azadirachta indica*) leaves as adsorption for the removal of heavy metals such as Cu+2 and Ni+2 were studied to investigate the effect of zinc oxide nanoparticles ZnO-NPs versus contact time and different concentrations. The maximum percentages of Cu (II) and Ni (II) versus contact time removal were found to be (99.94%) from the Neem (A*zadirachta indica*) leaves and *Ocimum Tenuiflorum* leaves for Cu (II) and (75.70%) from Neem (*Azadirachta indica*) leaves for Ni (II), respectively at the same time (50 min). In cases of versus the difference concentration percentages of Cu (II) and Ni (II) of ZnO-NPs, the highest produced from Neem (*Azadirachta indica*) leaves was (99.99 and 97.60 respectively) at the same dose (0.01g/100 ml). Also, the ZnO-NPs exhibited strong antibacterial activity was evaluated toward were tested for *Bacillus subtilis* (gram-positive) and *E. coli* (gram-negative) bacteria at a concentration (75, 150, 300, 450, and 600 mg/ml), the highest produced at the concentration (600 mg/ml) the antibacterial activity towards varied extensively with the synthesis parameters. This study presents an efficient green synthesis of ZnO nanoparticles that have potential applications in antibacterial activity.

Key words: wastewater; ZnO nanoparticles; heavy metals; antibacterial.

1. Introduction

Two crucial characteristics of nanoparticles make them particularly desirable as sorbents. First off, compared to bulk particles, they have substantially bigger surface areas on a mass basis. Second, to boost their affinity for the target chemicals, they can also be functionalized with different chemical groups. Our partnership with other research teams allowed us to create high-capacity and selective sorbents for metal ions and anions by taking use of the special features of nanoparticles **[1].**

Water was an essential compound for the survival and sustenance of life on planet Earth. The wastewater thrown out from industries was either used for irrigation purposes or it runs off to natural sources of water. If these effluents were not treated before their disposal, they can be harmful for human consumption as well as for other uses too **[2].**

Presently, industrial waste dyes represent one of the main problems of water contamination; therefore, their disposal before discharge has become a priority **[14].** Wastewater treatments with photocatalysts were an ecological and inexpensive method and represent a good alternative to solve this problem **[23].**

Wastewater management has become a great concern recently due to industrial and domestic discharges containing metals, organic pollutants and pathogenic microbes that contaminate water bodies and cause waterborne diseases. Although there have been multiple conventional processes for removing contaminants, nanoparticles have recently gained importance for wastewater treatments **[22].**

Green synthesized NPs are being used in water and wastewater treatment due to their high efficiency and biocompatible nature. Green synthesized NPs were highly proficient in recycling and removal of heavy metals from wastewater without loss of their stability and degradation of variety of organic pollutants from wastewater and, thus, purify the wastewater for reuse and recycling and could solve various water quality **[7]** and **[18].**

Removal of Cu (II) ions from water using nanoscale zinc oxide (ZnO) particles as an adsorbent. ZnO's ability to adsorb Cu (II) ions from aqueous solutions was studied at varying pH levels, with respect to contact time, metal ion concentration, and adsorbent quantity. ZnO particles were able to remove a high percentage (98.71%) of Cu (II) from aqueous solutions at pH 5.0 and an initial heavy metal ion concentration of 300 mg/l **[13].**

Water contamination was one of the major problems which the world was facing today. Water contamination not only affects the environment and human health, but it has also impacted economic and social costs. There are various ways used commercially and non-commercially to fight this problem which was advancing day by day due to technological progress. Nanotechnology has also proved to be one of the finest and most advanced ways for wastewater treatment. Nanoparticles have very high absorbing, interacting, and reacting capabilities due to their small size with a high proportion of atoms the at surface. It can even be mixed with aqueous suspensions and thus can behave as a colloid. Nanoparticles can achieve energy conservation due to their small size which can ultimately lead to cost savings. Nanoparticles have the great advantage of treating water and antimicrobial **[16]** and **[20].**

Heavy metals and other toxins in wastewater have become a global crisis that requires immediate attention. Many technological solutions have been created to address this issue. Nanotechnology is a relatively new field of study, but it has already garnered considerable attention, and numerous nanomaterials have been designed to filter out toxic substances from water pollution. Applications of nanoparticles for the purification of wastewater, such as carbon-based nanomaterials, zero-valent metal nanomaterials, metal-oxide-based nanomaterials, and nanocomposites **[25].**

Surendra et al., (2016) prepared the ZnO-NPs from Moringa oleifera (*M. Oleifera*) and evaluated the degradation of crystal violet (CV) dye. The synthesized ZnO-NPs were determined for effectiveness on biological activities such as antifungal, hemolytic, and antibacterial activity. ZnO-NPs showed good antifungal activity against Alternaria Saloni and Sclerrotium rolfii strains.

Goutam *et al.*, (2017) synthesized zinc oxide nanoparticles for antibacterial properties. ZnO nanoparticles have significantly better antibacterial activity, which may be the result of a confluence of factors, as shown by their effectiveness against the harmful bacteria *E. coli* during the preparation process.

Zinc oxide (ZnO) nanocomposites were tested for their ability to remove dyes from synthetic and textile industry wastewater, and the results were analyzed by **Nakkeeran *et al.*, (2018).** The ZnO nanocomposite was found to be 99% effective at removing dye from synthetic and textile industry wastewater.

The solid precipitation approach was used by **[7]** to manufacture the ZnO particles. With only 1 hour of UV light exposure, these particles were able to remove >85% of heavy metal ions such as Cu (II), Ag (I), and Pb (II). It was found, however, that Cr (VI), Mn (II), Cd (II), and Ni (II) ions had a poor removal effectiveness of 15%.

Primo et al., (2020) used synthesized zinc oxide using *Aloe vera* (green synthesis) for the removal of copper in wastewater. The results showed that at low Cu+2 ion concentration (~40 mg/L) the nanoparticles synthesized have the same removal efficiency. However, increasing the absorbate concentration (> 80 mg/L), the ZnO nanoparticles synthesized using *Aloe Vera* has higher removal efficiency.

To remove the toxicity of methylene blue (MB) dye, **[21]** developed a nanoparticle production method that was inexpensive, non-toxic, and quick using an extract from Syzygium Cumini leaves. Degradation of methylene blue (MB, a poisonous dye) in the presence of produced ZnO-NPs under sunlight was monitored, demonstrating their photo catalysis with pseudo-first-order kinetics. At pH 7 and 180 min of exposure time, and in direct sunlight, synthesized nanoparticles aided in the degradation of the MB dye by about 91.4%.

Mendes *et al.*, (2022) evaluated the ZnO NPs at concentrations from 0.2 to 1.4 mM for antimicrobial activity of clinical bacteria Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa. They found that the ZnO-NPs growth inhibition against *E. coli* and *P. aeruginosa* in inhibitory concentrations IC100 values at 0.6 mM for both strains. The inhibitory concentrations IC100 values for *B. subtilis* and *S. aureus* were reached at concentrations of 0.8 and 1.0 mM, respectively.

The aim of this investigation is to study the ability of zinc oxide nanoparticles (ZnO-NPs) using *Moringa Oleifera, Ocimum Tenuiflorum and Neem (Azadirachta Indicia)* leaves processes for treatment to removal of a heavy metal ions such as copper (Cu^{+2}) and nickel (Ni^{+2}) in wastewater using zinc oxide nanoparticles (ZnO-NPs) at different contact time such as (10,20,30,40, and 50 min) and at different concentration of ZnO-NPs such as (0.02,0.04,0.06,0.08 and 0.10 g/L). Also, studied the antibacterial activity using *Bacillus subtilis* as a gram-positive and *Escherichia coli* as gram-negative bacteria at concentrations (75, 150, 300, 450, and 600 mg/ml).

Materials And Methods

Standard solutions of Nickel (II) Ni $(NO_3)_2.6H_2O$ and copper (II) $CuSO_4.5H_2O$ were prepared of 1000 mg/L of and by deionized water, at the concentrations (0.02,0.04,0.06,0.08 and 0.10 g/L) of standard stock solutions were prepared for each metal ion. Then, determines the concentration of metals by inductively coupled plasma mass spectrometry ICP: perkin Elmer® Model: Optima ™ 7000 DV **[3].**

In vitro assessment of antibacterial activities of ZnO-NPs:

Bacterial strains:

The pathogenic bacterial strains were collected from the Department of microbiology Lab at Benha University: Two test microorganisms *Bacillus subtilis* (gram-positive) *and E. coli* (gram-negative) were used in this study.

Study of the adsorption:

Several volumetric flasks containing 50 ml of nickel (II) or Copper (II) ions solution containing (100 mg/L) for each one, then (0.1g) of synthesized zinc oxide from green leaves such as *Moringa Oleifera, Ocimum Tenuiflorum* and Neem (*Azadirachta Indica*) leaves was added to each flask. These materials were placed in a water bath shaker device at speed (185 rpm) at 30 min and at room temperature. Solid/liquid phases were then separated by centrifugation (6000 rpm) for 15 min. The concentration of heavy metals Copper (Cu^{+2}) and Nickel (Ni^{+2}) were measured by inductively coupled plasma mass spectrometry ICP, and the percentage removal of metals under investigation by using the equation as following described by **[3].**

$$R\,\% = ((Co-Ce))/Co*100$$

Where:

(R%) is the percentage removal of the two metals.

(Co) is the initial concentration (in a single system) of metal ions (mg/L).

(Ce) is the concentration of copper or nickel ions after removal (mg/L).

Effect of contact time:

Batch experimental procedures were conducted with different shaking times of (10, 20, 30, 40, and 50 min), while other conditions remained the same as the adsorption studies **[3].**

Effect of initial concentrations of ZnO-NPs:

To determine the effect of concentration of ZnO-NPs to removal of heavy metals on the adsorption of the metals from an aqueous solution onto zinc oxide ZnO-NPs using green leaves were studied first at optimum conditions, using different initial concentrations of an aqueous solution of (0.02, 0.04, 0.06, 0.08 and 0.1 g/L) for all metal ions **[3].**

Antimicrobial activity using of the synthesized zinc oxide nanoparticles (ZnO-NPs):

Gram-positive *Bacillus subtilis* and gram-negative *Escherichia coli* were used to test the antibacterial efficacy of ZnO-NPs. both gram-positive and gram-negative bacteria can be grown at 37°C culture in the right medium. The primary step in culturing bacteria is to grow the cells in a liquid medium until they reach 600 nm. To test the effects of ZnO-NPs (5 mg/ml), both untreated and treated bacterial cell cultures were inoculated at 37°C for 12 hours. A control group consists of bacteria cells without ZnO-NPs. Measurements of the diameter (in millimeters) of bacterial colonies developed in the presence and absence of ZnO-NPs after 12 hours of incubation were used to determine antibacterial activity **[19].**

Results and Discussion

The present investigation showed the zinc oxide nanoparticles (ZnO-NPs) which produced from *Moringa Oleifera*, *Ocimum tenuiflorum*, and Neem (*Azadirachta indica*) leaves extract were used for the removal of heavy metal ions in stander solutions of Cu^{+2} and Ni^{+2} and the ability

Effect of ZnO-NPs from different green leaves on the removal of heavy metal ions:

Heavy metal ions, including Cu^{+2} and Ni^{+2}, were assessed for their removal efficiencies (R%) from the aforementioned samples. Zinc oxide nanoparticles had their adsorption factors for the two metals under study assessed.

Effect of contact time:

The effect of producing ZnO-NPs from different green leaves Prepared from *Moringa Oleifera* leaves, *Ocimum tenuiflorum* leaves, and Neem (*Azadirachta indica*). on the removal of heavy metals Cu^{+2} and Ni^{+2} versus contact time i.e. (10, 20, 30, 40, and 50 min) of prepared an aqueous solutions of Cu^{+2} and Ni^{+2} were measured and the obtained results are presented in **Table (1 and 2).**

From these results, it can be generally noticed that the removal percentage of Cu^{+2} and Ni^{+2} ions under different contact times from single metals from aqueous solution was increased with increasing time.

From the obtained data it can be observed that the removal percentage of Cu^{+2} and Ni^{+2} as heavy metals on synthesized zinc oxide nanoparticles as a surface was reached in a contact time at 50 min.

In the case of productive ZnO-NPs from the *Moringa Oleifera* leaves, the maximum removal percentage of Copper (Cu^{+2}) was found to be (99.91%) and *Ocimum tenuiflorumm* leaves were found to be (99.93%) at 50 min. Which, with Neem (*Azadirachta indica*) leaves they have been found to be significantly higher (99.94%) at the same time 50 min**, Table (1).**

By using ZnO-NPs from *Moringa Oleifera* leaves, the obtained results inducted that the maximum removal percentage of Nickel (Ni^{+2}) was found to be (65.80%) at the time of 50 min. However, the highest level of removal percentage of Nickel (Ni^{+2}) in the case of ZnO-NPs produced from Neem (*Azadirachta indica*) leaves under investigation was (75.70%) at the same time. On the other have the highest level of removal percentage of Ni^{+2} in the case of ZnO-NPs produced from leaves of *Ocimum tenuiflorum* leaves by ZnO-NPs was found to be (68.30%) at the same time 50 min **Table (2).**

Cu (II) and Ni (II) ion removal percentages (vcommercial zinc oxide) were found to drop before gradually increasing, suggesting that a saturation point had been reached. In later stages, the availability of surplus adsorption sites on the adsorbents acts as a modifier. The high rate of early absorption in sample may be due to ion exchange followed by the sluggish chemical reaction of the active groups of metal ions, and the remaining empty surface sites are difficult to fill due to repulsive force. Metal ions have a longer and more difficult journey via the pores **[19].**

These results are different to those reported by **[4] and [19]** they found that the maximum removal percentage was found to be increase with increasing the contact time to reach an equilibrium adsorption established at maximum value after 30 min, this is due to a large number of vacant surface sites available for adsorption. After that, the percent removal efficiency becomes unchanged by a further increase in contact time; because the number of available adsorption sites saturated with the amounts two heavy metals under investigation of Cu^{+2} and Ni^{+2} adsorbed on zinc oxide and no significant increase in removal efficiency will occur, and then the equilibrium will be established.

Table (1) Effect of ZnO-NPs from different green leaves on the removal percentage of Cu^{+2} ion versus contact time of an aqueous solutions

Time	Moringa Oleifera leaves			Neem (Azadirachta indicia) leaves			Ocimum tenuiflorum leaves		
	Initial concentration	Residue concentration (mg/L)	Removal (%)	Initial concentration (100mg/L)	Residue concentration (mg/L)	Removal (%)	Initial concentration (100mg/L)	Residue concentration (mg/L)	Removal (%)
10	0.10	0.00034	99.66	0.10	0.00030	99.70	0.10	0.00016	99.84
20	0.10	0.00022	99.78	0.10	0.00017	99.83	0.10	0.00015	99.85
30	0.10	0.00017	99.83	0.10	0.00013	99.87	0.10	0.00011	99.89
40	0.10	0.00011	99.89	0.10	0.00008	99.92	0.10	0.00009	99.91
50	0.10	0.00009	99.91	0.10	0.00006	99.94	0.10	0.00007	99.93

Table (2) Effect of ZnO-NPs from different green leaves on the removal percentage of Ni^{+2} ion versus contacts time of an aqueous solutions.

Time	Ni^{+2} Concentration								
	Moringa oleifera leaves			Neem (Azadirachta indicia) leaves			Ocimum tenuiflorum leaves		
	Initial concentration	Residue concentration (mg/L)	Removal %	Initial concentration (100mg/L)	Residue concentration (mg/L)	Removal %	Initial concentration (100mg/L)	Residue concentration (mg/L)	Removal (%)
10	0.10	0.0370	63.00	0.10	0.0257	74.30	0.10	0.0330	67.00
20	0.10	0.0362	63.80	0.10	0.0255	74.50	0.10	0.0326	67.40
30	0.10	0.0349	65.10	0.10	0.0249	75.10	0.10	0.0324	67.60
40	0.10	0.0343	65.70	0.10	0.0247	75.30	0.10	0.0320	68.00
50	0.10	0.0342	65.80	0.10	0.0243	75.70	0.10	0.0317	68.30

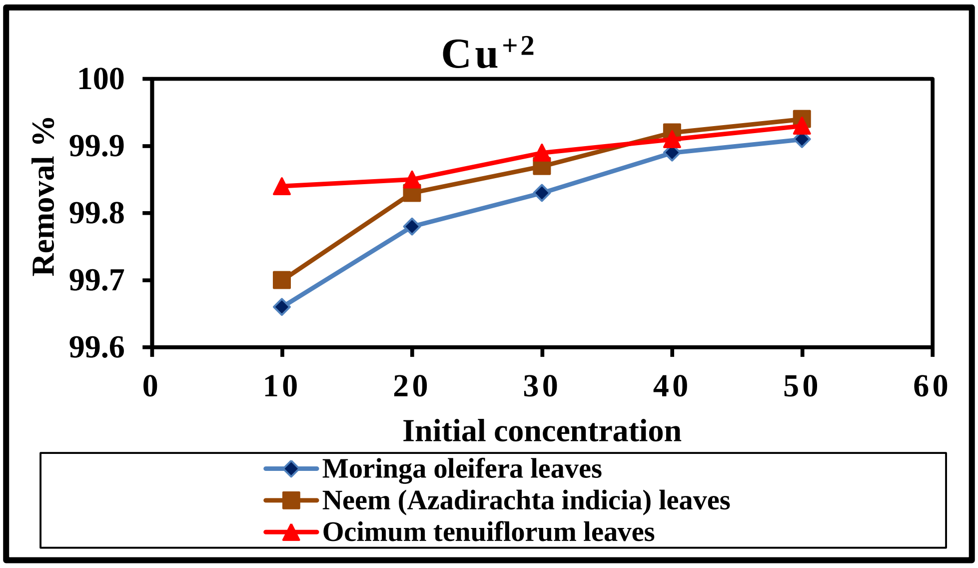


Fig. (1) Removal percentage of Cu^{+2} using ZnO-NPs from different green leaves.

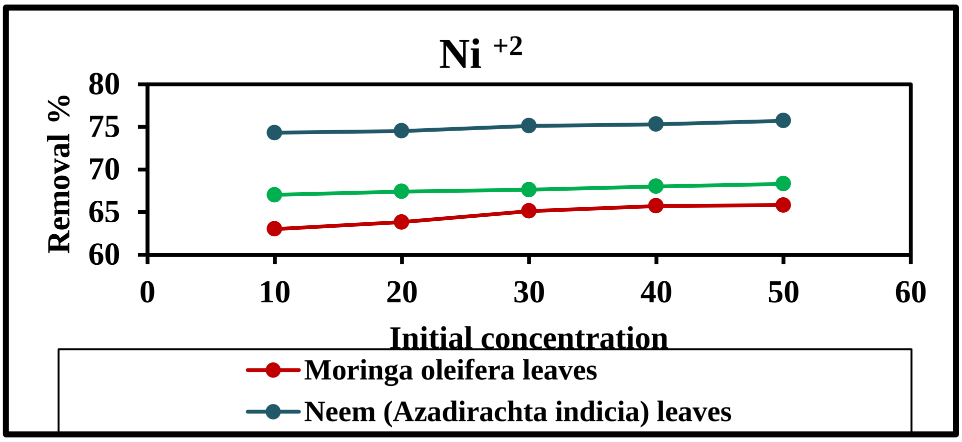


Fig. (2) Removal percentage of Ni^{+2} using ZnO-NPs from different green leaves.

Adsorbent quantity effect

The present investigation shows the removal percentages (R%) of Copper Cu^{+2} and Ni^{+2} versus the adsorbent quantity effect in an aqueous solution by ZnO-NPs produced from the above-mentioned samples. The obtained results are illustrated in **Fig (3 and 4).**

The removal of Cu (II) increases with increasing higher dosages of zinc oxide nanoparticals **(Fig 3).** This is attributed to the increased adsorbent surface area and more available adsorption sites or functional groups because of the increase in adsorbent quantity.

The maximum removal of copper Cu^{+2} in the case of ZnO-NPs from the Moringa Oleifera leaves was 99.91%, but with Ocimum Tenuiflorum leaves, this value was 99.98%. However, the highest level of removal percentage of Cu^{+2} in the case of ZnO-NPs produced from Neem (*Azadirachta indica*) leaves was significantly higher 99.99% at the same dose 0.01g\100 ml.

The affectivity of these plants' biomass on the removal of heavy metals is attributed to the biochemical constitution of their cell wall which contains the amino, carboxyl, and hydroxyl functional groups that have a significant role in the adsorption process **[10].**

Nalwa (2017) examined the effect of the amount of ZnO nanoparticles on the adsorption of Cu (II) ions from their aqueous solutions by varying the amount of adsorbent from 20 to 120 mg per 10 ml for a contact time of 50 min. It was observed that Cu (II) removal efficiency increased with increasing adsorbent dose, up to 105 mg after which it became almost constant.

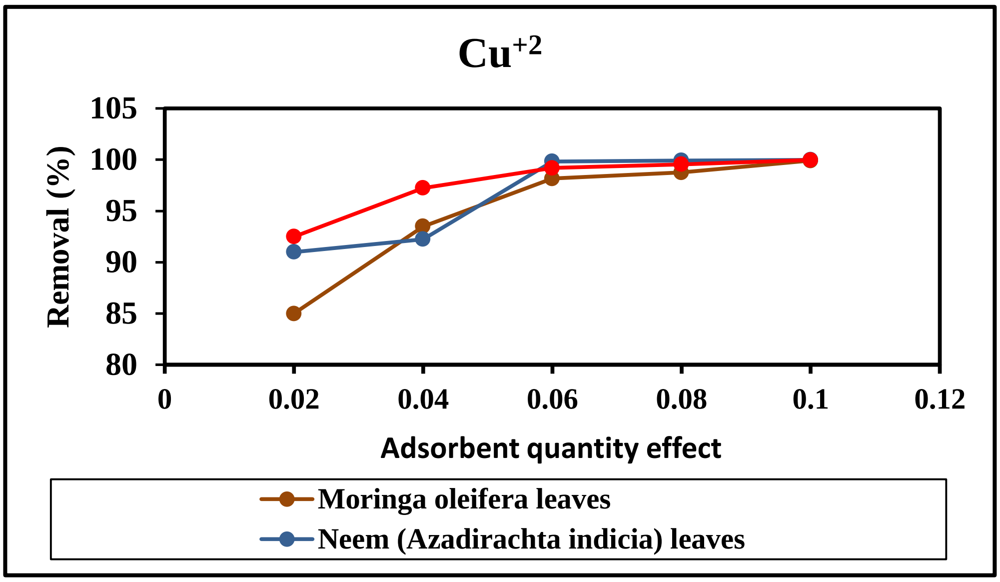


Fig. (3) Removal percentage of Cu+2 using ZnO-NPs from different green leaves.

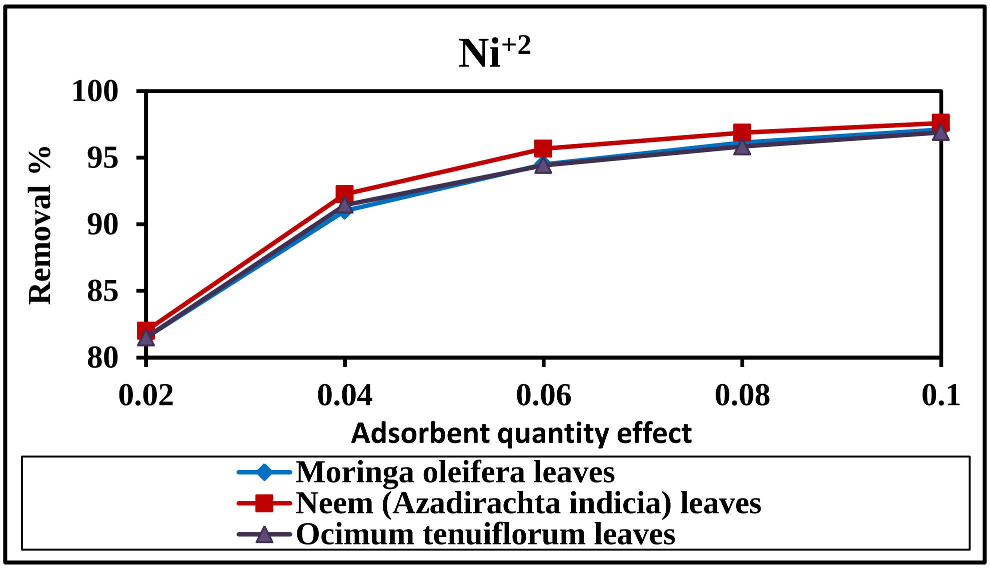


Fig. (4) Removal percentage of Ni^{+2} using ZnO-NPs from different green leaves

While, the effect of Zinc oxide nonpractical (ZnO-NPs) on removal percentage (R%) of Nickel (Ni^{+2}) of an aqueous solution was evaluated. The obtained results are recorded in **Fig (4).** From these results, it can be generally noticed that the removal percentages of ions from wastewater increased with increasing the adsorbent dosage of the Zinc oxide nonpractical.

On the other hand, in the case of Zinc oxide nonpractical (ZnO-NPs) from green leaves the maximum removal percentage of Nickel (Ni^{+2}) was found to be (99.71%) for Moringa Oleifera leaves, but ZnO-NPs with Neem (*Azadirachta indica*) leaves were significantly higher (99.76%) at doses 0.01g/100ml. However, the level of removal percentage of Ni^{+2} in the case of produced from *Ocimum Tenuiflorum* leaves equaled to (99.69%) at the same dose of 0.01g/100ml.

These results are close to their reported by Ali and Hassan (2022) showed that an increase in the removal rate with an increase in the amount of absorbent. Due to the results of the experiment showed an increase in the removal rate with an increase in the amount of absorbent. Due to the increased surface area, the percentage of desorption increases as adsorption occurs, and the optimum amount of adsorption that can be used to remove nickel (II) and copper (II) ions is (0.1 g). After that, the removal percentage will increase a little.

The antibacterial activity of ZnO-NPs nanoparticles was studied against *E.coli* (gram-negative) and *Bacillus subtilis* (gram-positive) bacterial pathogens by the disc and well diffusion agar methods were evaluated.

The antibacterial activaties of biosynthesized ZnO-NPs Produced from green leaves extract (Moringa Oleifera, Ocimum tenuiflorum, and Neem (Azadirachta indicia) leaves. were investigated with five different concentrations (75, 150, 300, 450, and 600 mg/ml) with using Bacillus subtilis (gram-positive) and E.coli (gram negative) and the obtamined results are presented Table (3) and illustrated in Fig (5,a,b,c). The diameters of zone inhibition from Moringa oleifera leaves Fig (5,a). Clearly represent the excellent anti-bacterial activity of ZnO nanoparticles against Bacillus subtilis (Gram-positive) and E.coli (Gram-negative). The experimental outcomes undeniably suggest an effective growth inhibitory activity of the nanoparticles upon both the microorganisms and strong activity alongside *Bacillus subtilis* and *E.coli* **[15].**

From the obtained results, the maximum inhibition zone of ZnO-NPs against two bacterial strains from Ocimum tenuiflorum leaves **Table (3), Fig (5)** and 6) produced the highest inhibition was found at a concentration 450 g/ml of Bacillus subtilis (Gram-positive and the inhibition zone was (1.3) cm. While, in *E. coli* (Gram-negative) they found that the inhibition zone at (1.1) cm at the same a concentration of 600 mg/ml.

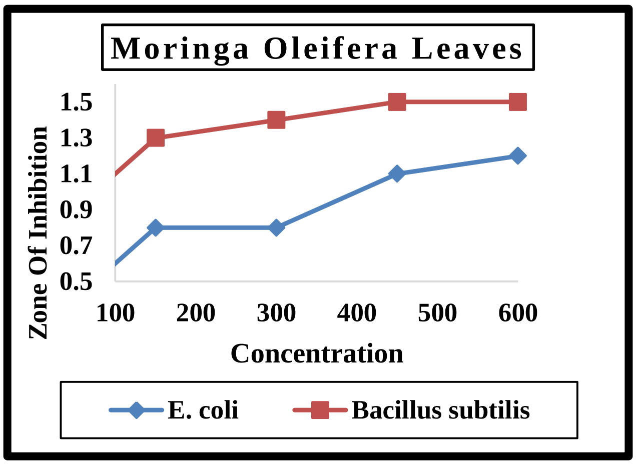
In the case of inhibition ZnO-NPs which synthesized from Neem (*Azadirachta indica*) leaves of antibacterial Gram-positive and Gram-negative Table (3), Fig (5c). The highest inhibition zoon at 600 mg/ml concentration at Bacillus subtilis (Gram-positive) and the inhibition zone was (0.8) cm. While, in E. coli (Gram-negative) they found that the inhibition zone was (0.6) cm at the same concentration (600 mg/ ml).

The antibacterial properties of nanoparticles, where the zone of inhibition increases with an increase in the concentration of nanoparticles. In both Gram-positive and Gram-negative bacteria, the cell wall and cell membrane are crucial in maintaining the osmotic balance and integrity of the cell **[6]** Also, the physical and chemical properties, including the size, shape and surface charge of the NPs, can have a great influence on their antimicrobial activity **[5].**

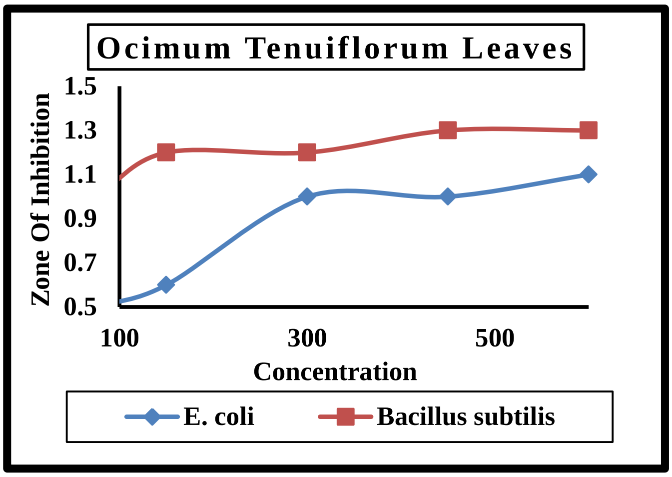
Various possible mechanisms involved in the antimicrobial activity of ZnO-NPs. It results due to the production of ROS from ZnO-NPs. Such species lead to destruct the of cellular components. There was internalization within the membrane. Superoxides and hydroxyl radicals carry negative charges and due to this, they can't penetrate into the membrane and always remain at the outer surface of the bacteria. However, H2O2 molecules pass through the cell wall. It injures and damages as well as destroys the cell. In the present case, ZnO-NPs are present in the growth media, it releases peroxides and covers the surface of bacteria. The greater the adsorption of H_2O_2 at the bacteria's surface, the more antimicrobial efficiency. The gram-negative bacteria have double cell membranes as compared to the gram-positive bacterial strain. The outer membrane in gram-negative bacteria restricts the permeability of various molecules37 which results in less growth reduction. Whereas single-cell membranes in gram-positive bacterial strains can easily be damaged, this reflects its superiority for antimicrobial activity **[19].**

Table (3) Diameters of inhibition zone of ZnO-NPs product from different leaves against two bacterial strains

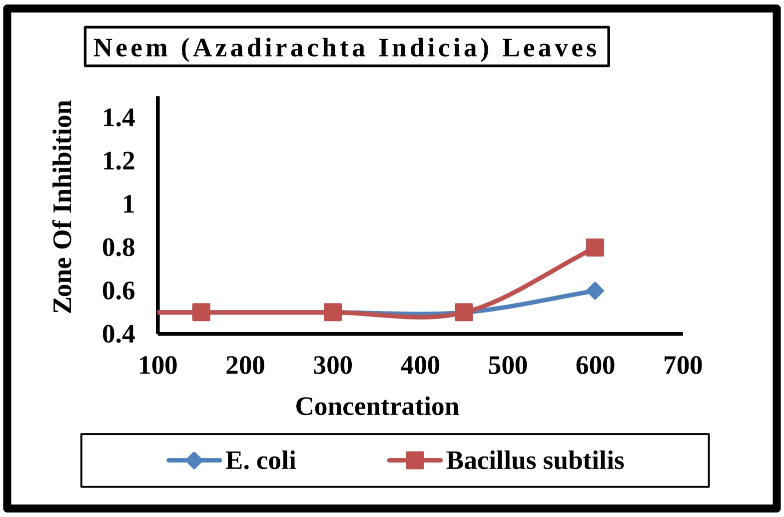
Bioactive Agent of ZnO-NPs concentrations (mg\mL)	Zone of Inhibition (cm)					
	Moringa oleifera		Ocimum tenuiflorum		Neem (Azadirachta indica)	
	E. coli (Gram negative)	Bacillus subtilis (Gram positive)	E. coli (Gram negative)	Bacillus subtilis (Gram positive)	E. coli (Gram negative)	Bacillus subtilis (Gram positive)
75	**0.5**	**1.0**	**0.5**	**1.0**	**0.5**	**0.5**
150	**0.8**	**1.3**	**0.6**	**1.2**	**0.5**	**0.5**
300	**0.8**	**1.4**	**1.0**	**1.2**	**0.5**	**0.5**
450	**1.1**	**1.5**	**1.0**	**1.3**	**0.5**	**0.5**
600	**1.2**	**1.5**	**1.1**	**1.3**	**0.6**	**0.8**



(a) Moringa Oleifera Leaves



(b) Ocimum Tenuiflorum Leaves



(c) Neem (Azadirachta Indicia) Leaves

Fig. (5$_{,a,b,c}$) Inhibition zone of ZnO-NPs produced from different leaves against two bacterial strains

Conclusions

The present work also demonstrates that ZnO-NPs can be considered a promising photocatalyst in the removal of the contaminant of wastewater such as Cu^{+2} and Ni^{+2} antimicrobial studies show that ZnO-NPs exhibit better results with *Bacillus subtilis* gram-positive bacterial strains as compared to *E.Coli* gram-negative bacterial strains.

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