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An Evaluation of the Activity of Prepared Zinc Nanoparticles with Extracted Alfalfa Plant in the Treatment of Heavy Metals

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Abstract:

The alfalfa plant, after harvesting, was washed, dried, and grinded to get fine powder used in water treatment. We used the alfalfa plant with ethanol to make the alcoholic extract characterized by using (GC-Mass, FTIR, and UV) spectroscopy to determine active compounds. Alcoholic extract was used to prepare zinc nanoparticles. We characterized Zinc nanoparticles using (FTIR, UV, SEM, EDX Zeta potential, XRD, AFM). Zinc nanoparticle with Alfalfa extract and alfalfa powder were used in the treatment of water polluted with inorganic elements such as Cr, Mn, Fe, Cu, Cd, Ag by (Batch processing). The batch process with using alfalfa powder gets treated with Pb (51.45%), which is the highest percentage of treatment. Mn (13.18%), which is the lowest percentage of treatment. The batch process with using Zinc nanoparticles gets the result treated with Pb(98.822%), which is the highest percentage of treatment, and Mn (10.31%), which is the lowest percentage of treatment. When comparing alfalfa powder and zinc nanoparticle, it has been found that the treatment with zinc nanoparticle is more efficient in the removal of inorganic pollutants.

Keywords: Alfalfa plant, Inorganic elements, Water pollutants, Water treatment, Zinc Nanoparticles.

Introduction:

Over the decades, the sources of clean water began to decrease with the increase in the need for it, so there was an urgent need to reuse water, especially in the field of industry or irrigation. Due to the increase in the need to use pure and industrial water, which, at the same time, causes increases in water pollution¹ while, water sources suffer from chemical pollution (organic and inorganic), it was important to find scientific, commercial, and efficient methods to water treatment. There are many methods of water treatment and purification². The choice of treatment method must be preceded by a qualitative and quantitative study of the pollutants present in the water, after which the best method of treatment is determined. There are treatments that rely on physical methods and others that rely on chemical or biological methods. But the important reason to distinguish between these methods is cost and efficiency³. Therefore, scientists searched for

efficient and economical treatment methods that do not affect the environmental balance. Many studies indicate the use of plant residues in water treatment because of their potential in absorbing chemical pollutants. Water hyacinth, water lettuce, duckweed, and vetiver grass have been used for the treatment of wastewater⁴. *Eichhornia crassipes* has been considered a problematic aquatic free-floating weed because of its uncontrolled growth in water bodies, and it is difficult to control and eradicate this plant from water bodies. However, its ability to uptake heavy metals from the aquatic ecosystem has been considered a bio indicator. Limited research has been done on the phytoremediation technique for the treatment of kitchen wastewater using *Eichhornia crassipes*. In recent years, chemists have focused on nano techniques especially nano-related with plants which are referred to as a "Green Nano"⁵. In this work, we will study the treatment of polluted water

because of pesticides and negative ions by employing Zinc Nanoparticles with alcoholic alfalfa extract by applying two methods, batch processing, and special columns processing. Silver nanoparticles have previously won the use of extracts using special capsules to treat water contaminated with phenols and aromatic compounds⁶. Another study used Zinc nanoparticles with cumin leaf extract to treat water from inorganic pollutant elements⁷.

Materials and Methods:

1. Materials and instrumentation

The following chemical materials (Methanol, Ethanol, Zinc sulphate, Sodium hydroxide, Ascorbic acid, Polyvinyl alcohol) were used, and the (FTIR) in the rang (4000-400) cm^{-1} on a Shimadzu-3800 Spectro-meter was applied as a detector. The electronic spectral data were detected by using Shimadzu160 Spectro-photometer. Mass indication analysis of compounds was carried out with GC Mass100P Shimadzu. In this study, Scanning Electron Microscopy (SEM) was used to describe the size and morphology of nanoparticles, and the analysis was managed to this method. A tiny drop of nanoparticles was put on a carbon-coated copper grid and allowed to dry by using a mercury lamp for 5 min. Finally, readings were taken at magnifications of 5000x, 10000x, 20000, and 50000x and with steady voltage. In Atomic force microscopy (AFM), a dropping of 100 μL of the sample on the slide to make made a thin film of nanoparticles, put on a glass slide and allowed to dry for 5 min., then the slides were scanned with AFM. While the EDX measurements were carried out using a high-resolution spectrophotometer in a transmission electron microscope to confirm the Zinc nanoparticle. The evaluation of Zinc-nano particles Constants was estimated by using zeta-potential analysis of the flow from -160 to +160 ml volt.

2. Plant harvest and preparation for grinding and extraction

In this study, the used Alfalfa plants were obtained from the Youssefia District south of Baghdad Governate, \ Iraq. It was washed several times with deionized water to remove any particles then dried at room temperature, after that grinded by special laboratory grinder for 20 seconds to get fine powder. Alcoholic Alfalfa extract was produced by adding a mixture of 65% methanol, 20% ethanol and 15% distilled water Alfalfa fine powder, left for a period of time then heated up to 50C. At the same time, it was condensated by using laboratory condenser for 8 hours. The extraction process followed by evaporating and condensation to

increase the enrichment is accomplished at 50°C⁸. Show Fig. 1.



A-



B-



C-

Figure 1. Alfalfa plant: A. Alfalfa plant after wash B. Alfalfa plant after drying, C. Alcoholic alfalfa plant extract

3. Preparation of zinc nanoparticles

Zinc nanocomposites with Alfalfa extract were prepared according to the method of Elumalai with modifications via the following steps: putting 10 ml:20% of alfalfa extract in a round flask and supplying 1000 ml distilled water to it. The flask was left for stirring and heating at various temperatures. After 1 min, Zinc sulfate was added gradually with keeping on continuous stirring to perform the 17.5 g and mixing process. Then pure sodium hydroxide was added to equivalence the acidity of mixture⁹. Then, the mixture was filled into a glass can and left in a furnace at 200°C for 2h. To accomplish the separation, the filtered solution separates out of the precipitate then precipitate is collected and dried from the remnant of water in a furnace at 70°C to perform the dryness, here it will be crashed, finally the gradual addition of 17.5g from Zinc sulfate occurs to accomplish mixing of components.

resultant green powder stored in airtight container for characterization¹⁰. Show Fig.2.



Figure 2. zinc nanoparticle: A. zinc nanoparticle after drying B. zinc nanoparticle after prepared

4. Determining the activated compounds of alfalfa plant extract.

The activated compounds in alcoholic alfalfa plant extract were identified by using the gas chromatography- mass spectroscopy technique (GC-Mass), an analytical technique for a qualitative and

quantitative group of a broad domain of compounds; in addition, it is cleaner, faster, and less expensive than the traditional extraction methods¹¹. Show Fig. 3, Table.1 compounds detected in the alfalfa extract by GC-Mass.

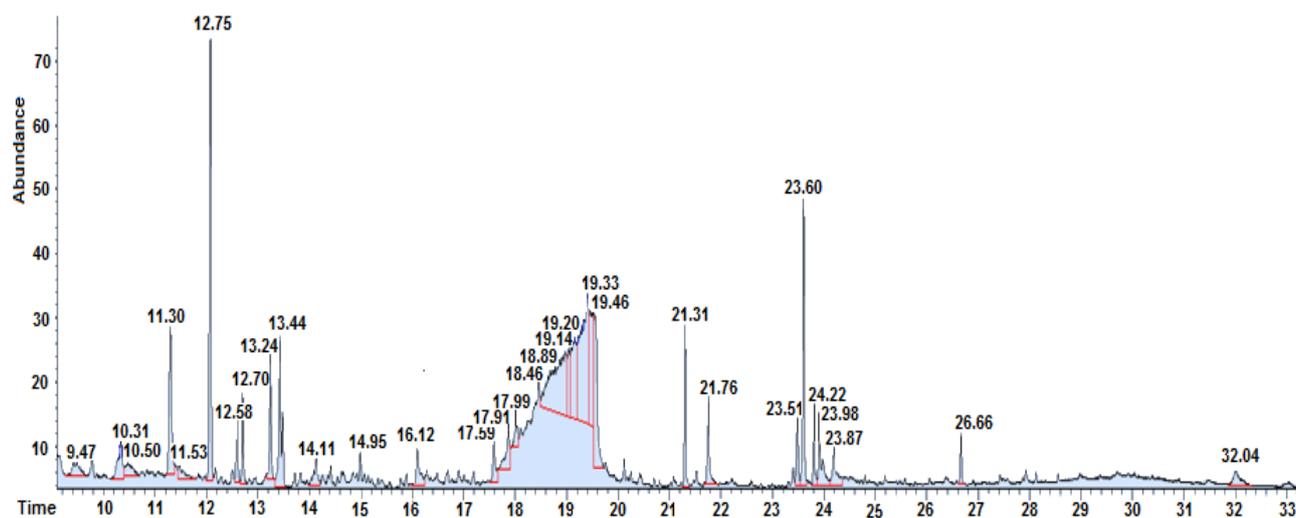


Figure 3. GC-Mass for the alfalfa extract

Table 1. Compounds detected in the alfalfa extract by GC-Mass

Compound	Activity	Group
Amino-1,2,4-triazole-5-carboxylic acid	antiparasitic	Amino acid amid
Caprolactam	antibacterial	Amino acid amid
cis-Crotyl alcohol, trimethylsilyl ether		Ether
1-Benzoyl-2-(pyrrolidinomethyl)pyridine	anti-inflammatory agents	Amines
Methotrexate	anti-cancer	Amino acid
Eugenol	Antimicrobial	Organic Acid
Isoquinolin-3(2H)-one, 6,7-dimethoxy-1-methyl-	Anticancer	Amines
Benzoic acid N'-(4,4,5,5,6,6,6-heptafluoro-3-oxo-1-phenyl-hex-1-enyl)-hydrazide	Antifungal	Organic Acid
N,N'-Dimethyl-5-pyrrolidinone-3-carboxamide	antimicrobial	Amides
Caryophyllene	anti-inflammatory, antibiotic, antioxidant, anticarcinogenic	Alkenes
(5-Methylenebicyclo[2.2.1]hept-2-en-7-ylidene)acetic acid, methyl ester		Esters
Quinoline, 2-ethyl-	antibiotic, antioxidant, anticancer	Amines
7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl		Alcohol
2-O-Methyl-D-mannopyranosa	sugars	
2-Trimethylsilyl-1,3-dithiane	sugars	
alpha.-Methyl mannofuranoside	sugars	
-Methyl mannofuranoside	sugars	
4,6-Di-O-methyl-.alpha.-d-galactos	sugars	
3-O-Methyl-d-glucose	sugars	
Methyl (methyl 4-O-methyl-.alpha.-d-mannopyranoside) urinate	sugars	
3-O-Methyl-d-glucose	sugars	
Hexadecenoic acid, methyl ester	Antimicrobial	Esters
n-Hexadecenoic acid	antimicrobial	Fatty Acid
11-Octadecenoic acid, methyl ester	Antimicrobial	Fatty Acid
Phytol	Anticancer	Phenols
Methyl stearate	antioxidant, antimicrobial, antitumor, and anticancer	Esters
cis-5-Dodecenoic acid	antioxidant activities	Fatty Acid
Octadecanoic acid	Anticancer	Fatty Acid
Phytol acetate	antimycobacterial	phenols esters
Stigmasterol	Antimicrobial	Steroids

Results and Discussion:

This part includes the characterization and result of water polluted with inorganic elements such as Cd, Ag, Fe, Mn, Cr, Cu treated either by Alfalfa plant or of Zinc nanoparticle and determining the treatment ability. The two used water treatment methods were 1- column processing and 2- batch processing. Then, the statistical study was used to determine efficiency first to plant, second to zinc nanoparticle with extract and finally, comparing treated water and standard water specifications adopted by WHO.

1. Ultraviolet-Visible Spectroscopy for alcoholic extract and zinc nanoparticles and compare between them

Ultraviolet-visible spectroscopy is an important part to characterize active compounds in

the Alfalfa plant by studying the absorptions that appear in spectra due to the colors of the Alfalfa plant, which has been screened as an alcoholic extract and zinc nanoparticle with Alfalfa extract then absorbed peaks appear at 278, 662 and 234nm and the last two peaks refers to organic compounds¹². These peaks belong to ($n \rightarrow \pi^*$) and ($\pi \rightarrow \pi^*$) in the ultraviolet area, 662 nm refers to green plant plus organic and inorganic compounds. When comparing alcoholic extract to green alfalfa plant and zinc nanoparticle with the alcoholic extract, new peaks were observed on (478,513,591,638,729 and 268) nm in ultraviolet close with alcoholic extract peak shifted towards a shorter wavelength 10 nm blue shift, and another peak in visible area refers to zinc nanoparticle^{13,14}

2. Fourier Transform Infrared spectroscopy for alcoholic extract and zinc nanoparticles and comparing between them

Fourier transform infrared is an important technique for characterizing organic compounds to determine active compounds and check if they are associated with some elements or compounds to form new compound, which refers to form nanoparticles when comparing zinc nanoparticles

with alcoholic extract and alcoholic extract with Alfalfa plant, in alcoholic extract, a peak appears on 3388 cm^{-1} and zinc nanoparticles appear on 3424 cm^{-1} these peaks refer to the (OH) group has changed in two compounds around 35 cm^{-1} ,¹⁵ then other peaks appear on 2928 cm^{-1} in alcoholic extract and 2924 cm^{-1} in zinc nanoparticle. These peaks refer to C-H aliphatic group, 1624 cm^{-1} and 1629 cm^{-1} to the carbonyl group¹⁶⁻¹⁸ show Figs.4, 5.

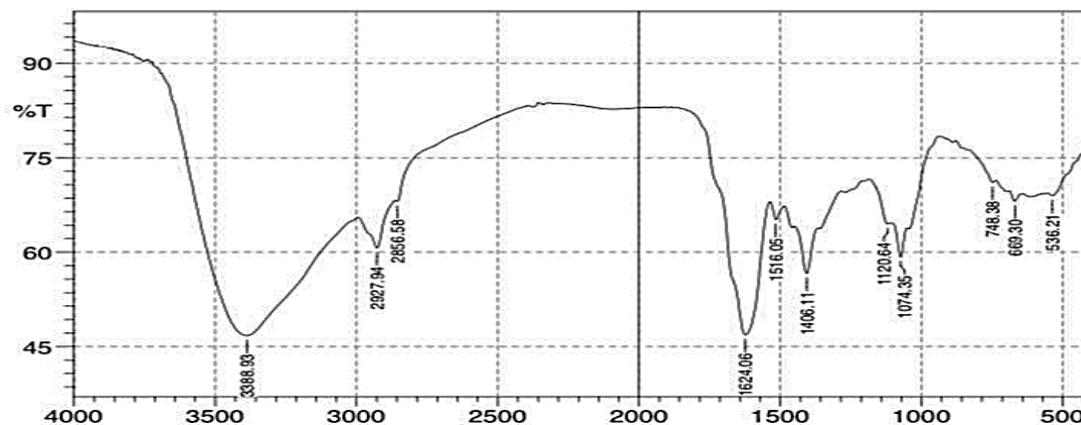


Figure 4. FT-IR Spectrum of alcoholic extract

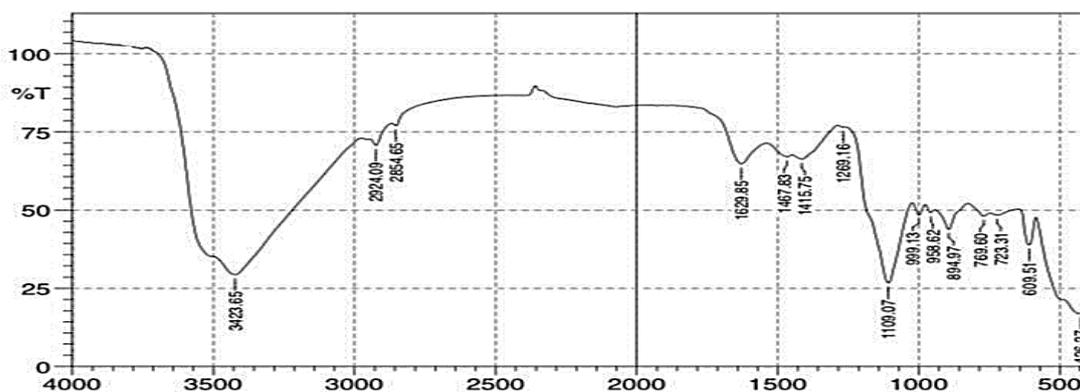


Figure 5. FT-IR Spectrum of zinc nanoparticle

3. Scanning Electron Microscope (SEM)

Scanning Electron Microscope for zinc nanoparticles shows spherical crystal shape in average particles (77.7 nm), which refers to zinc element, this indicates that it has granules size

includes a range of nano, this result is consisted¹⁹. Other nanoparticles appear on (64.29, 63.15, 55.47, 54.06) nm, which refer to hydrocarbon group range of nano except hydrogen because the small size of it^{20,21}. Show Fig. 6.

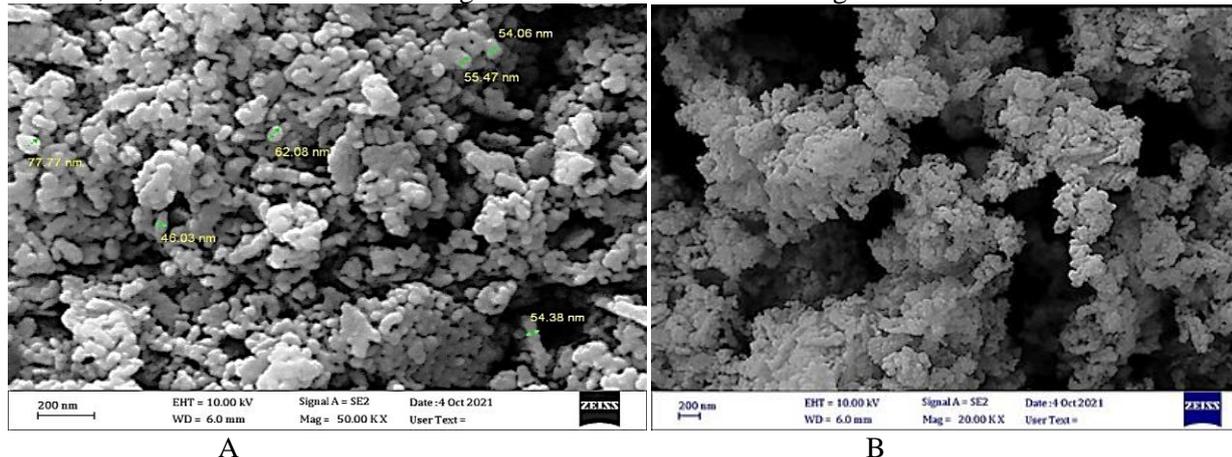


Figure 6. Show SEM for zinc nanoparticle (A), B. enlarged zinc nanoparticle image

4. Atomic Force Microscopy (AFM)

AFM is a technique used to map surfaces with nano particles and even micron dimensions and used to measure the flexibility of nanoparticles. Besides, it is used to measure the energy of adhesion between chemical molecule and nano-particles. As a result, AFM shows both 2D and 3D zinc nanoparticle with Alfalfa extract, which was spherical in shape

and 31.9 nm in height. This result refers to zinc element with average particle sizes of 6-9, 10-13, 14-18, 19-25, 26-31.9 nm show the nano-particles which included hydrocarbons, alcohol, sodium hydroxide. as a result, the nano-Zinc are spherical shape or grouped balls with each other's in irregular or random diameters between 6 to 31.9 nano-meters²², (Fig.7).

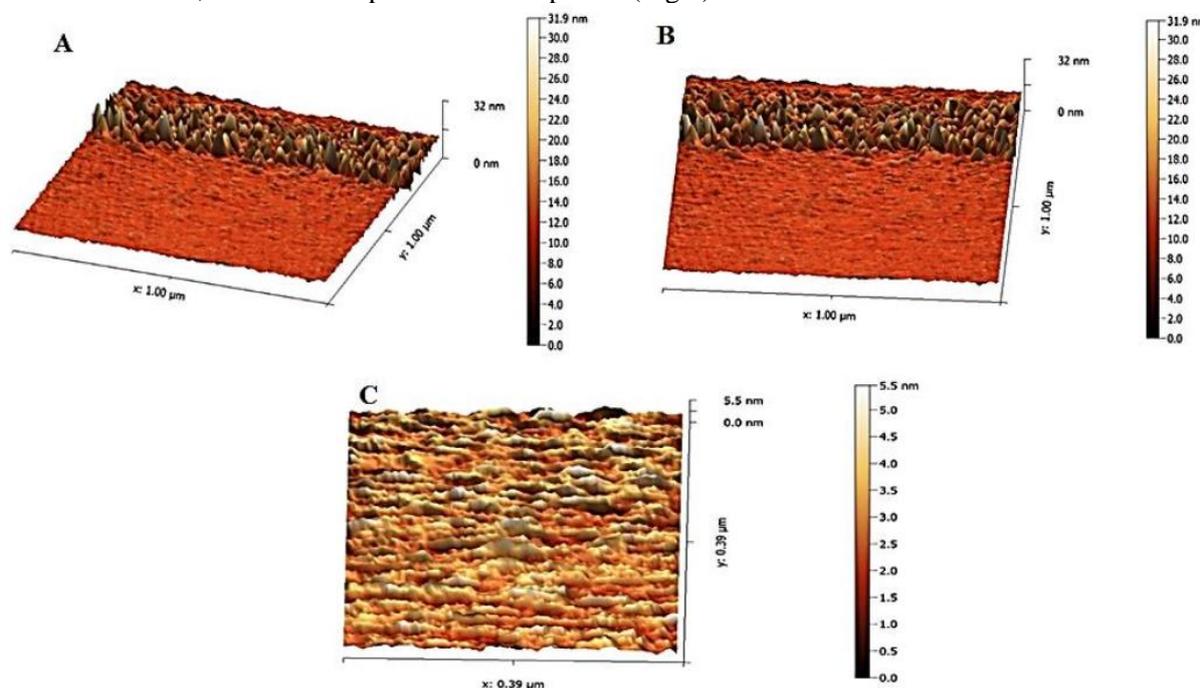


Figure 7. A. shows 3D zinc nanoparticles, B. shows 2D zinc nanoparticles, C. shows small nanoparticles

5. Energy dispersive X-Ray spectroscopy

EDX is a type of X-ray emission used to identify the chemical properties of the samples; on another mean, each element has its atomic structure

and specific values in x-ray spectroscopy²³. When examining a zinc nanoparticle, it was confirmed with three peaks (1,7.6, 8.6) keV and they were crystal spherical structure. Show Fig. 8.

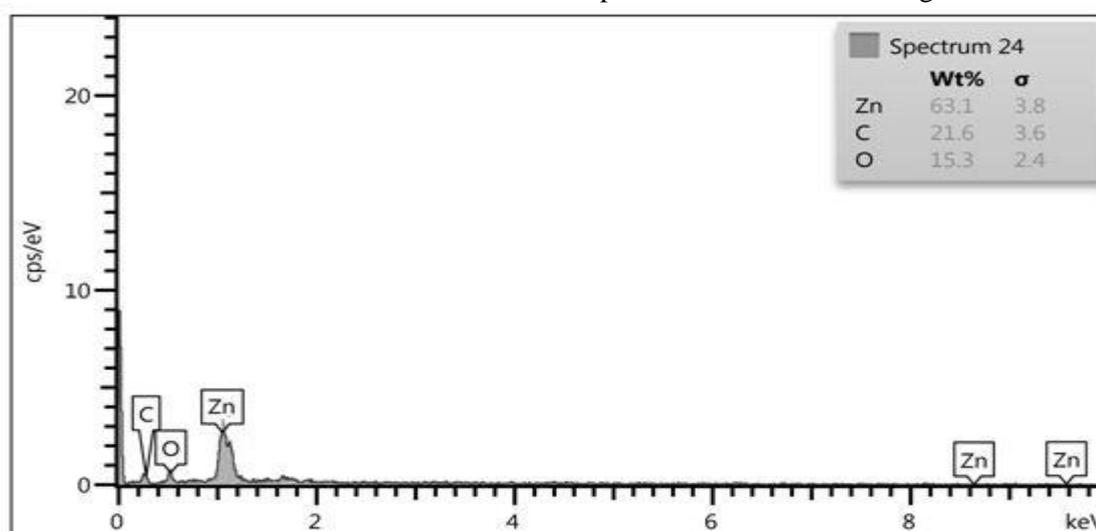


Figure 8. Energy-dispersive X-ray spectroscopy (EDX)

6. X-Ray diffraction spectroscopy

Is a technique used to identify the arrangement of the crystal atoms, where the X-RAY hit the crystal to show certain directions according to the angles and strength of reflected rays, where 3D images are formed for the Electrons density inside the crystal. To measure the diffraction of X-RAY, the nano-crystals inserted on the angle meter and transformed while targeting the X-RAY on it, which shall result in random diffraction called measurements. All directions image of angles is

taken for 2D dimensions in order to be transformed to 3D images representing the electronic density inside the crystal. The study of X-RAY diffraction used for identifying the crystal molecular weight. Fig. 9 shows the result of X-ray testing, where the three readings between were 30-40 due to Zinc elements presence in more readings due to the size of nano-molecule with Zinc elements, while the remaining values show the presence of Hydrocarbon compounds except the Hydrogen which does not appear because of its small size^{24,25}.

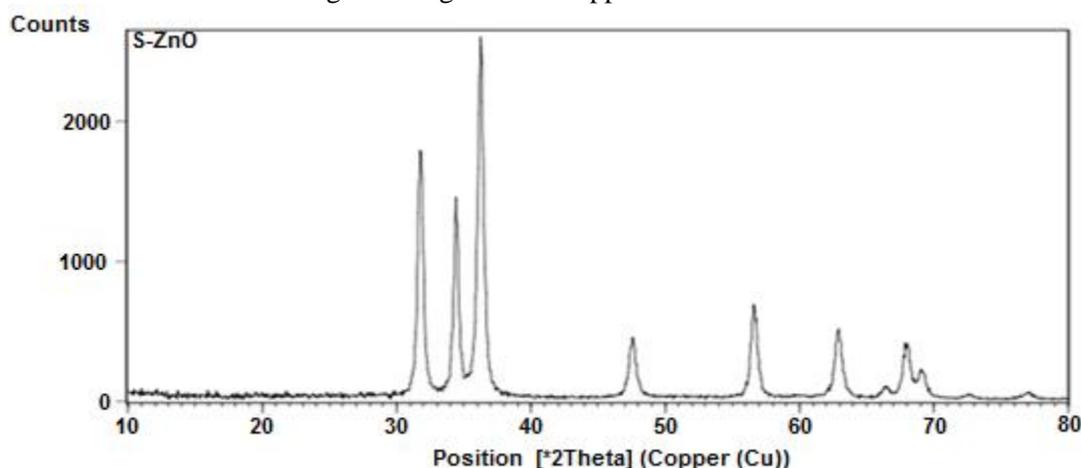


Figure 9. XRD-diffraction of Zinc nano-particles.

7. Zeta potential analysis

The results of zeta potential values of the synthesized nanoparticles were -24.78mV for cur ZnO NPs (Fig. 10), Zeta potential values are a key indicator of the stability of colloidal dispersions. Zeta potential values indicate the extent of electrostatic repulsion between adjacent particles similarly charged in dispersion. For molecules and particles small enough, the high zeta potential will provide stability, that is, the solution or dispersion will resist

agglomeration. When the voltage values are small, the attractive forces may exceed this repulsion and the dispersion may be broken and sintered, and therefore, the high potential colloids of zeta (negative or positive) are fixed electrically while colloids with lower potentials tend to coagulate or flocculate. Generally, the zeta potential of the nanoparticles should be either higher than +30 mV or lower than -30 mV²⁶.

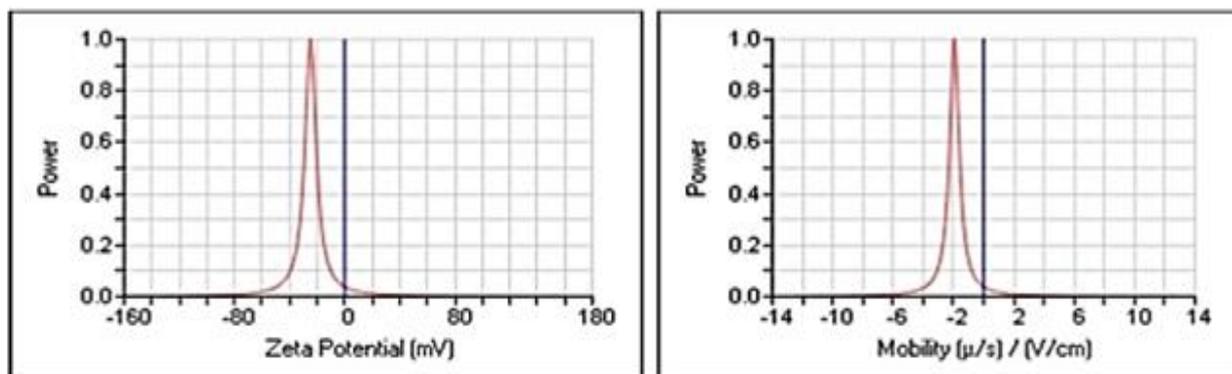


Figure 10. Zeta potential for zinc nanoparticles

Treatment of heavy metals with alfalfa plant

The polluted water sample was synthesized in a definite ratio from (Ag, Cd, Cr, Mn, Fe, Pb) 10 ppm to each element, then the treatment was carried out in two ways; the first was **batch method** which

5 g of Alfalfa powder were employed adding 50 ml polluted water. Then they were put onto a magnetic stirrer to accomplish the mixing and shaking process. After one hour, a part of the sample was pulled out, and the treatment percentage was measured. The next

percentages, 49.31, 51.45, 27.23, 13.18, 21.56 for elements were discovered respectively²⁶. Re measuring operation was carried out in several periods as tabulated below: it has been noticed that the optimal treatment was at 5 h with no vary in

treatment percentages along the spent hours²⁷. The sample treatment was measured by UV apparatus to view the processing concentration²⁸. Show result some inorganic elements after purification by two methods batch and continuous in Tables. 2, 3.

Table 2. Treatment percentages from heavy metals by the batch method using alfalfa powder

Time hours	Element 10ppm	Concentration in batch method	% batch method	Time hours	Element 10ppm	Concentration in batch method	% batch method
1	Ag	5.1	49.31	4	Ag	1.8	82.32
	Pb	4.9	51.45		Pb	1.3	87.12
	Cr	7.3	27.23		Cr	2.3	67.32
	Mn	8.7	13.18		Mn	5.7	43.21
	Fe	7.9	21.56		Fe	4.2	58.96
	Cd	8.3	17.34		Cd	5.1	49.21
2	Ag	4.4	65.92	5	Ag	1.6	84.9
	Pb	4.1	59.37		Pb	1.1	89.12
	Cr	6.4	36.29		Cr	2.8	72.03
	Mn	8.8	22.67		Mn	5.2	48.04
	Fe	7.1	29.41		Fe	3.4	66.49
	Cd	7.2	28.41		Cd	4.7	53.88
3	Ag	3.9	71.35	10	Ag	1.6	84.9
	Pb	3.2	68.23		Pb	1.1	89.12
	Cr	5.7	43.28		Cr	2.8	72.03
	Mn	6.5	35.65		Mn	5.2	48.04
	Fe	6.3	37.89		Fe	3.4	66.49
	Cd	6.8	32.84		Cd	4.7	53.88

Table 3. Treatment percentages from heavy metals by batch method using zinc nanoparticles

Time hours	Element 10ppm	Concentration in batch method	% batch method	Time hours	Element 10ppm	Concentration in batch method	% batch method
1	Ag	1.447	85.225	5	Ag	0.6599	93.401
	Pb	1.2469	87.531		Pb	0.1178	98.822
	Cr	3.0958	69.042		Cr	1.4292	85.706
	Mn	8.969	10.311		Mn	4.8179	85.706
	Fe	6.856	31.437		Fe	2.7987	72.013
	Cd	7.7969	22.031		Cd	3.5705	64.295

1. Results of inorganic pollutant measurements in wastewater

In the recent work, the atomic absorption spectroscopy - graphite furnace technique has been employed in measuring the concentrations of heavy metals after schematic the standard curve for each

element. For more accuracy in obtaining the returns, the conductively coupled plasma technique was employed in measurements of inorganic elements in standard solutions. These results are shown in Table. 4.

Table 4. Result concentrations inorganic pollutant in water

R	Absorbance	Conc.	Element
R ² = 0.9992	0.018	0.25	Pb
	0.034	0.50	
	0.065	1	
	0.135	2	
	0.015	0.2	
R ² = 0.9976	0.031	0.4	Cr
	0.044	0.6	
	0.061	0.8	
	0.014	0.1	
	0.068	0.5	
R ² = 0.9996	0.134	1	Mn
	0.206	1.5	
	0.016	0.25	
	0.035	0.5	
	0.065	1	
R ² = 0.9992	0.126	2	Fe
	0.013	0.1	
	0.029	0.2	
	0.06	0.4	
	0.126	0.8	
R ² = 0.9998	0.017	0.1	Cd
	0.081	0.5	
	0.165	1	
	0.249	1.5	
R ² = 0.9999			Ag

Conclusions:

In this study, water has been treated via environmental safety methods; such method was accomplished successfully by obeying zinc nanoparticles which is in turn prepared by zinc sulphate as starting material, and the formation of which is proved by the following techniques (SEM, EDX, FTIR, UV, Zeta potential, AFM). Then, the contaminated water has been treated from inorganic elements by batch processing using zinc nanoparticles with alcoholic Alfalfa extract and alfalfa powder alone. It has been noticed that zinc nanoparticles are preferred compared with plant powder because if the plant is left inside the beaker for more than 24 h, it gets rotted with a bad stink. The treatment by nanoparticle is the best, more accurate and more efficient, because when using Alfalfa in treatment, water discolorations have been observed.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the

Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' contributions statement:

A. A. presented the idea, analysis, discussion of the results and writing of the manuscript. Z. A. contributed to the design and implementation of the research, laboratory work, F. A. R., L. A. A., S. M. I., verified the analytical methods and discussed the results and contributed to the final manuscript.

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تقييم فعالية الزنك النانوية مع مستخلص نبات الجت الكحولي في معالجة العناصر اللاعضوية في المياه

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الخلاصة:

نبات الجت بعد حصاده تم غسله وتجفيفه وطحنه للحصول على مسحوق جيد ليستخدم في معالجة المياه. تم استخدام نبات الجت مع الايثانول للحصول على المستخلص الكحولي وتم تشخيصه بالتقنيات الطيفية (UV, FTIR, GC-Mass) لتحديد المركبات الفعالة للمستخلص. تم استخدام المستخلص الكحولي في تحضير مركب الزنك النانوي وتشخيصه بالتقنيات الطيفية (UV, FTIR, SEM, EDX, XRD Zeta potential). استخدم مسحوق نبات الجت ومركب الزنك النانوي مع مستخلص الجت الكحولي لمعالجة المياه الملوثة من العناصر اللاعضوية (Cr, Mn, Fe, Cu, Cd, Ag) بطريقة (المعالجة بالدفع). أظهرت نتائج المعالجة بمسحوق نبات الجت معالجة عنصر الرصاص (pb) بنسبة 51.45% وهي اعلى نسبة معالجة من باقي العناصر، واقل نسبة معالجة كانت لعنصر المنغنيز (Mn) بنسبة 13.18%. وعند استخدام المعالجة بمركب الزنك النانوي مع مستخلص الجت الكحولي كانت اعلى نسبة معالجة لعنصر الرصاص (Pb) بنسبة 98.822% واقل نسبة معالجة لعنصر المنغنيز. 10.31% (Mn) عند المقارنة بين المعالجتين وجدنا ان معالجة المركب النانوي اكثر كفاءة واكثر نسبة ازالة للملوثات.

الكلمات المفتاحية: نبات الجت، المياه الملوثة، معالجة المياه، مركب الزنك النانوي، العناصر اللاعضوية.