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An Evaluation of the Activity of Prepared Zinc Nano-Particles with Extract Alfalfa Plant in the Treatments of Peptidase and Ions in Water

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ABSTRACT

After harvesting, Alfalfa plant was washed, dried and ground to get fine powder used in treatment water. We used alfalfa plant with ethanol to made alcoholic extract and characterized it applying (GC-Mass, FTIR, UV) spectroscopy to determine active compounds. Alcoholic extract was used to prepare zinc nanoparticle. We characterized Zinc nanoparticles by using FTIR, UV, SEM, EDX Zeta potential and AFM. Zinc nanoparticle with Alfalfa extract and alfalfa powder was used to treat pollutant water with pesticides and negative ions by two methods, namely Batch and continuous processing. Batch process was used two times firstly, with Alfalfa plant to treat water affected by pesticides and negative ions, after 1h pesticides (glyphosate 44.76%, sulfon 49.21%) Negative ions (NO_3 33.8%, NO_2 46.8%, 17.2%) and when left it 5h to get treated off pesticides (glyphosate 64.52%, sulfon 69.38%), Negative ions (NO_3 71%, NO_2 80%, SO_4 70%). Secondly, we used with Zinc nanoparticles to treat water after 1h pesticides (glyphosate 71.45%, sulfon 52.6%) Negative ions (NO_3 72.13%, NO_2 14.50% SO_4 78.30%) and when left 5h (glyphosate 81.26% sulfon 60.11%) Negative ions (NO_3 79.55%, NO_2 32.45%, SO_4 86.80%), followed by continuous processing flowrate pertaining to pesticides (glyphosate 57.44%, sulfon 59.50%), Negative ions (NO_3 32.24%, NO_2 6.28%, SO_4 65.57%). Zinc nanoparticles were treated in continuous process at concentration 10 ppm only for pesticides for 1h to get treated (glyphosate 77.22%, sulfon 100%) and concentration 50 ppm for treating pesticides (glyphosate 64.52%, sulfon 69.38%), Negative ions (75.41%, 18.69%, 90.70%). Comparing the two process, we found the continuous one more efficient than batch process. Further, comparing alfalfa powder and zinc nanoparticle, we found treatment with zinc nanoparticle more efficient and most removal for organic and inorganic pollutant.

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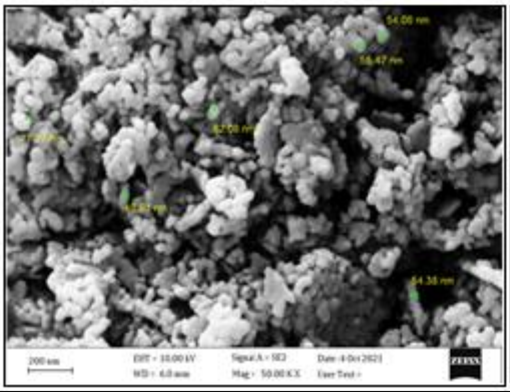
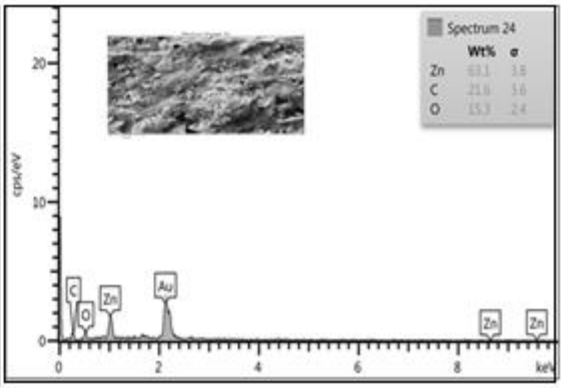
GRAPHICAL ABSTRACT





Introduction

Over decade's years, 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 pollution [1] while water sources suffer chemical pollution (organic and inorganic), it is important to find scientific, commercial, and efficient methods to water treatment. There are many methods of water treatment and purification [2]. 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 should be 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 efficiency [3]. Therefore, scientists have 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, such as water hyacinth, water lettuce, duckweed, and vetiver grass have been used for the treatment of wastewater [4]. Eichhornia crassipes has been considered a problematic aquatic free-floating weed because of its uncontrolled growth in water bodies, also 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]. Silver nanoparticles have previously won the use of extracts using special capsules to treat water contaminated with phenols and aromatic compounds [6]. Another study used Zinc nanoparticles with cumin leaf extract to treat water from inorganic pollutant elements [7-11]. The subject intended by the current study has not been addressed before; we accordingly studied the treatment of polluted water caused by pesticides and negative ions by employing Zinc Nanoparticles with alcoholic alfalfa extract via applying two methods, batch processing, and special columns processing.

Material and Methods

The chemical materials were obtainable trading (methanol, ethanol, zinc sulphate, sodium hydroxide, Ascorbic acid polyvinyl alcohol). The devices were used (FTIR) were detected in the range 4000-400 cm^{-1} on a Shimadzu-3800 Spectro-meter. The electronic spectral data were detected by using Shimadzu160 Spectro-photometer. Mass indication analysis of compounds was carried out with GC Mass100P Shimadzu, Scanning Electron Microscopy, and Atomic Florescent Microscopy. In this study, we used SEM to describe the size and morphology of nanoparticles and the analysis was managed to this method. A tiny drop of nanoparticle was put on carbon coated copper grid and allowed to dry by using mercury lamp for 5 min. finally, readings were taken at magnification of 5000x, 10000x, 20000, 50000x and with steady voltage. In Atomic force microscopy (AFM), dropping

100micro liter of the sample on the slide was to make a thin film of nanoparticles put on glass slide and was allowed to dry for 5min. The slides were scanned with AFM, while the EDX measurements were carried out using a high-resolution spectrophotometer in transmission electron microscope to confirm the zinc nanoparticle. The evaluation of Zinc nanoparticles constants was estimated by using zeta-potential analysis the flow from -160 to +160 Ml.

Plant Collection and preparation of extraction

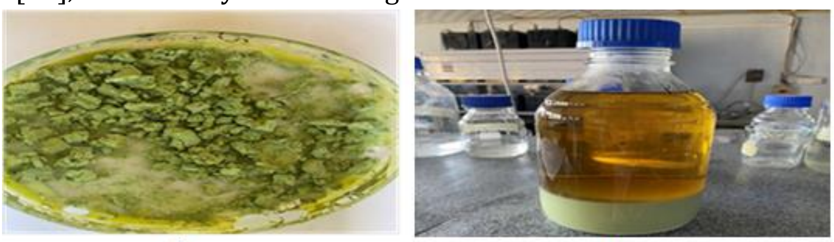
In this study, the plant of alfalfa was obtained from Youssefia in Baghdad. The plant was washed several times with double distilled water to remove any particles then dried at room temperature. After that the plant was ground in a special laboratory grinder for 20 seconds to give fine powder. Alcoholic alfalfa extract was gained by adding a mixture consisting of 65% methanol, 25% ethyl alcohol and 15% distilled water into appropriate alfalfa, followed by its being crashed into powder, left for a period of time and heated up to 50 °C with respect to the distillation of vapor for 8 hours. When the extraction process was accomplished, the extract was concentrated via dissector at 50 °C. The concentrated extract was put into well closed container and left in refrigerator in order to be fit for nano particles synthesis [12] (Figure 1).

A B C

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

Preparation of zinc nanoparticle

Zinc nanoparticles with alfalfa extract are prepared according to the method of Elumalai with modifications via the following steps: Putting 10 ml:20% of alfalfa extract in round flask and supplying it by 1000ml distilled water. The flask is left for stirring and heating at various temperature; after 1 min., zinc sulfate is added gradually with keeping on continuous stirring to preform 17.5 g and mixing process, then pure sodium hydroxide is added to equivalize the acidity of mixture [13], followed by transferring the mixture into glass can and leaving it in furnace at 200 °C for 2h (Daneshvar *et al.,* 2008). To accomplish the separation in which the filtered solution separates out of the precipitate, the 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, and finally the gradual addition of 17.5 g from zinc sulfate occurs to accomplish mixing of components. The resultant green powder is stored in airtight container for characterization [14] (Figure 2).



A B

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

Determining activated compounds of alfalfa plant extract

The activated compounds in alcoholic alfalfa plant extract were identified by using gas chromatography-mass spectroscopy technique (GC-MASS). GC-MS can be used to study liquid, gaseous or solid samples. Analysis begins with the gas chromatograph, where the sample is effectively vaporized into the gas phase and separated into its various components using a capillary column coated with a stationary (liquid or solid). In addition, it is cleaner, faster and less expensive than the traditional extraction methods [15] (Figure 3).

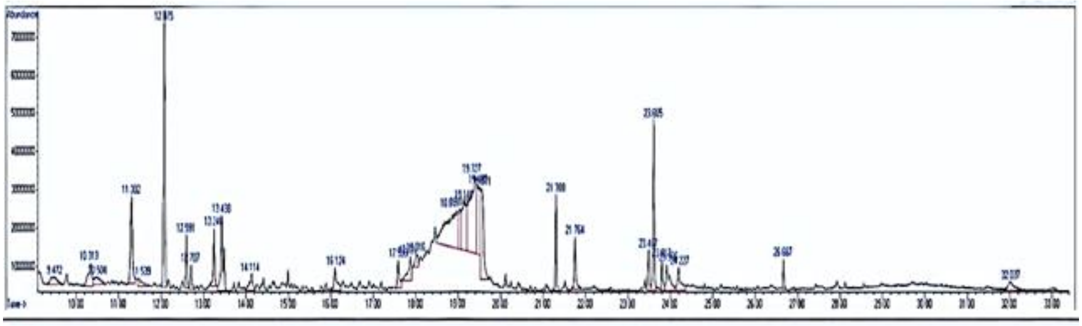


Figure 3: GC-Mass for alfalfa extract

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)pip uridine	Anti-inflammatory agents	Amines
Methotrexate	Anti-cancer	Amino acid
Eugenol	Antimicrobial	Organic Acid
Isoquinolin-3(2H)-one, 6,7-dimetho xy-1-methyl-	Anticancer	Amines
Benzoic acid *N′*-(4,4,5,5,6,6,6-hep tafluoro-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	
α-Methyl mannofuranoside	Sugars	
-Methyl mannofuranoside	Sugars	
4,6-Di-*o*-methyl-α-d-galactos	Sugars	
3-*o*-Methyl-d-glucose	Sugars	
Methyl(methyl 4-o-methyl- α-d-mannopyranoside)	Sugars	

urinate		
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 included characterization and result of polluted water with pesticides and inorganic elements such as Cd, Ag, Fe, Mn, Cr, Cu and some ions such as NO_3, NO_2, SO_4 by using alfalfa plant and determining the ability of treatment, followed by applying zinc nanoparticle and determining the ability of treatment. We used two methods of treatment water 1- column treatment 2- batch treatment. In addition, statistical study was done to determine the efficiency of the plant, and that of zinc nanoparticle with extract. Finally, we compared water treatment and environmental determinants water of World Health Organization (WHO).

Ultraviolet-Visible Spectroscopy for alcoholic extract and zinc nanoparticles

Ultraviolet-visible spectroscopy is important to characterize active compounds in alfalfa plant by studying the absorptions that appear in spectra due to the colors of alfalfa plant, screening 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 compounds [16]. These peaks belong to (n→π*) and (π→π*) in ultraviolet area, and 662nm refers to green plant plus organic and inorganic compounds. When comparing alcoholic extract with green alfalfa plant and zinc nanoparticle with alcoholic extract, we observed new peaks on (478, 513, 591, 638, 729 and 268 nm), 268 in ultraviolet close with alcoholic extract peak shifting towards a shorter wavelength by 10nm blue shift, and other peaks in visible area pertaining to zinc nanoparticle [17,18] (Figure 4).

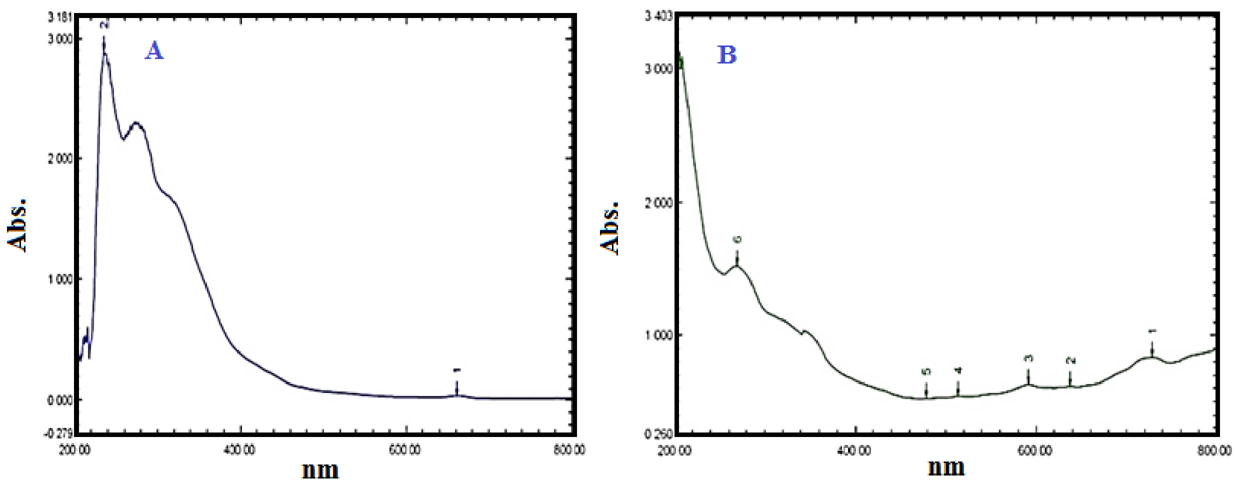


Figure 4: A. Uv-Visible spectroscopy for alcoholic extract, B. Uv-Visible spectroscopy for zinc nanoparticle

Fourier Transform Infrared spectroscopy for alcoholic extract and zinc nanoparticles

Fourier transform is an important technique for characterizing organic compounds to determine active compounds and check whether they are associated with some elements or compounds to form new compound when comparing between compounds in Zinc nanoparticles with alcoholic extract and compounds in alcoholic extract with alfalfa plant. In alcoholic extract, the peak appears on 3388cm^{-1} and zinc nanoparticle appear on 3424cm^{-1}, which pertain to (OH) group's change in two compounds around 35 cm^{-1} [19]. Then, other peaks appear on 2927.9 cm^{-1} in alcoholic extract and 2924 cm^{-1} in zinc nanoparticle, which refer to C-H aliphatic group, 1624 cm^{-1} and 1629 cm^{-1} to carbonyl group [20-22] (Figure 5).

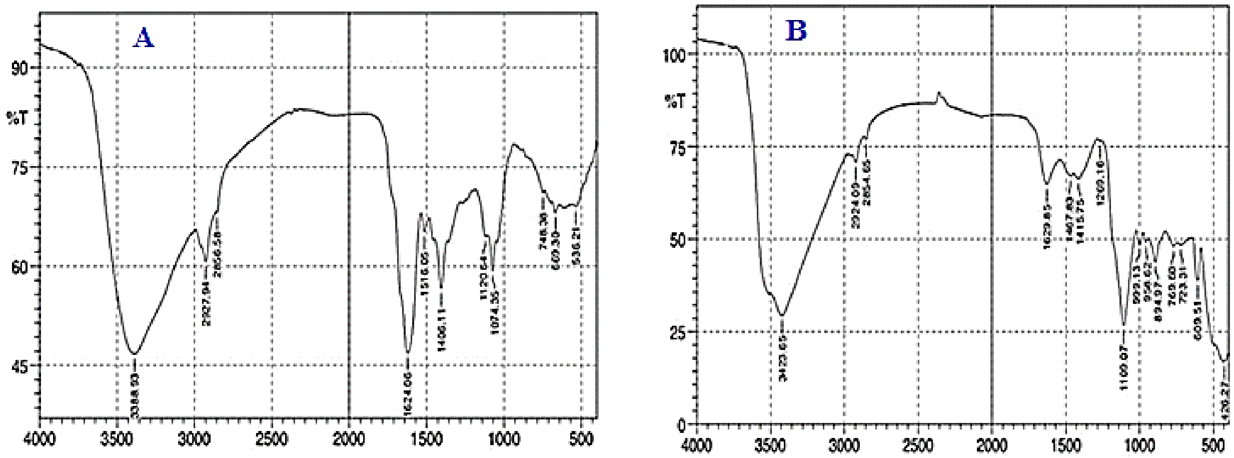


Figure 5: A. FT-IR spectroscopy for alcoholic extract, B. FT-IR spectroscopy for zinc nanoparticle

Scanning Electron Microscope (SEM)

SEM for zinc nanoparticle explains crystal spherical shape in average particles (77.7 nm) in association with zinc element, indicating the size of the granules includes range of zinc nanoparticles [23]. Other nano particles appear on 64.29, 63.15, 55.47, 54.06 nm in relation to hydrocarbon group range of nano except for hydrogen because of its small size [24, 25] (Figure 6).

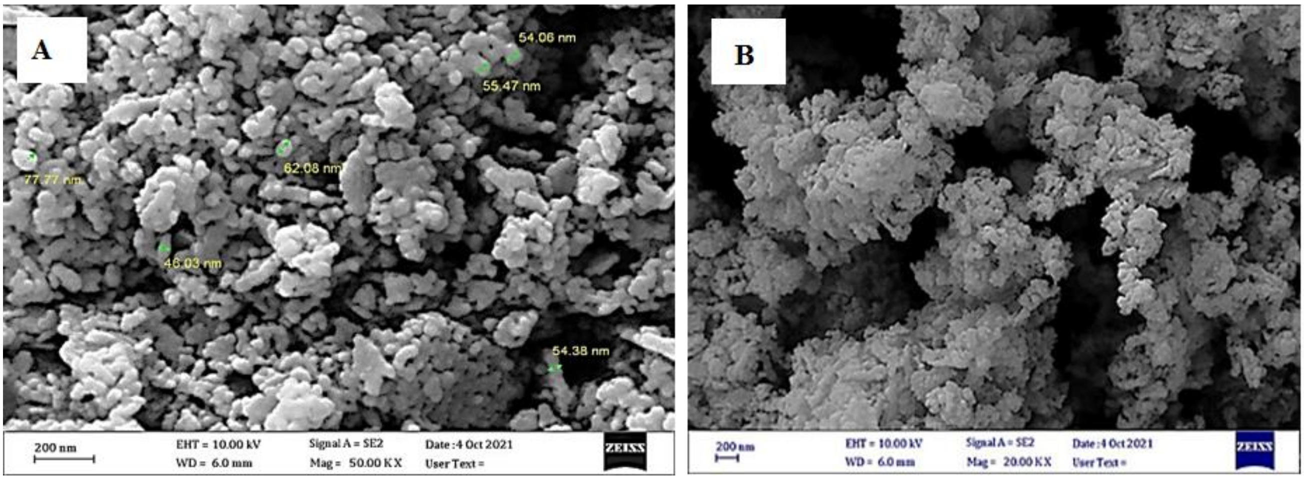


Figure 6: A. SEM for Zinc nanoparticle, B. Enlarged Zinc nanoparticle image

Atomic Florissant Microscopy (AFM)

AFM is a technique used to map surface with nano and even micron dimension and to measure the flexibility of nanoparticle, as result of both 2D and 3D of zinc nanoparticle with alfalfa extract, the shape of nanoparticle was spherical shape, 31.9 nm is high result referring to zinc element and average size particles (6-9, 10-13, 14-18, 19-25, 26-31.9) nm; as a result, spherical shapes combined together irregularly random between 6-31.9 nm explain 3D and 0.1-5.5 nm [26] (Figure 7).

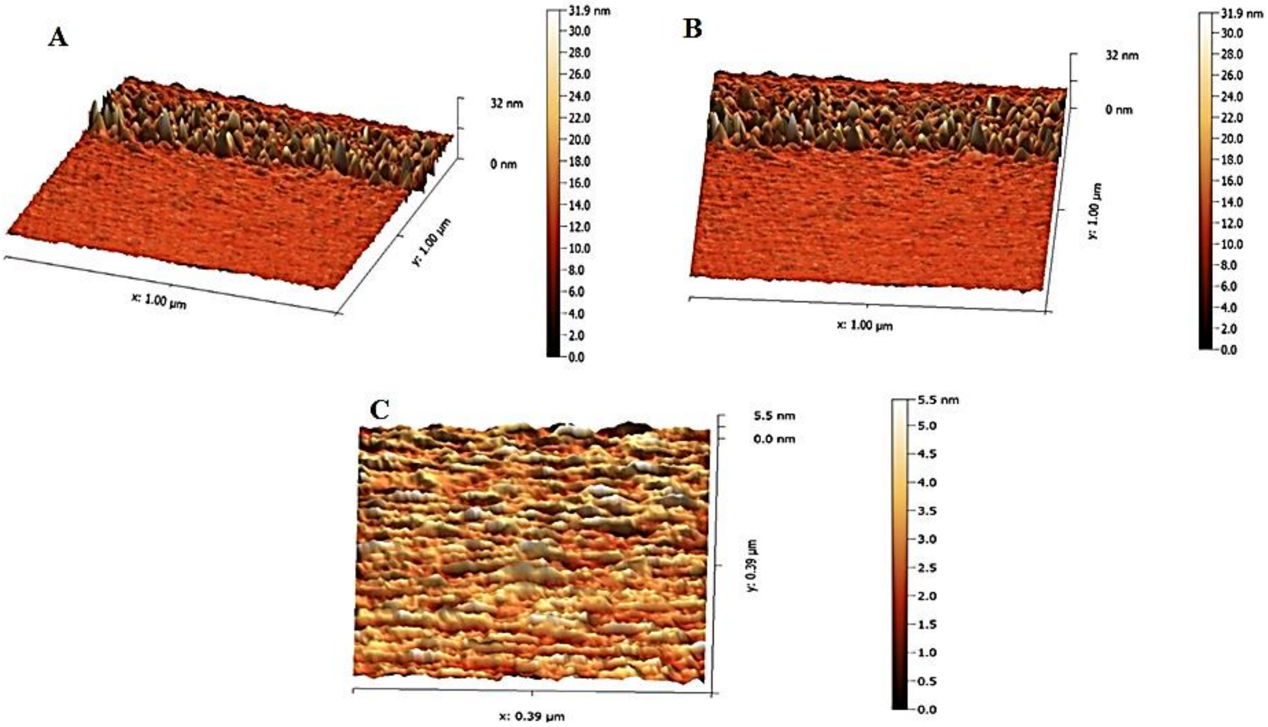


Figure 7: A. 3D Zinc nanoparticles, B. 2D Zinc nanoparticles, C. show small Zinc nano particles

Energy dispersive X-Ray spectroscopy

X-ray diffraction was used to know chemical properties of the samples; in other word, each element has its atomic structure and specific values in x-ray spectroscopy [27]. Examining a zinc nanoparticle was confirmed with three peaks (1, 7.6, 8.6) kev, having a crystal spherical structure (Figure 8).

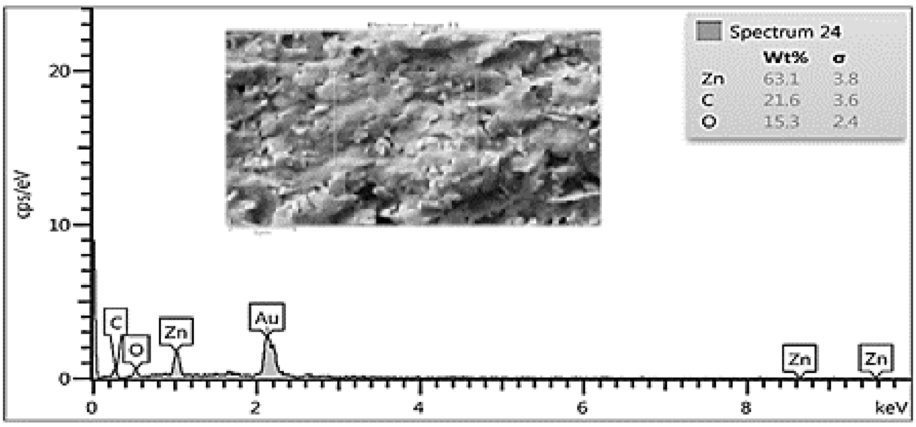


Figure 8: energy dispersive x-ray spectroscopy (EDX)

Zeta potential analysis

The results of zeta potential values of the synthesized nanoparticles were -24.78 mV for cur ZnO NPs, Zeta potential values as 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 to be 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; 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 mV [28] (Figure 9).

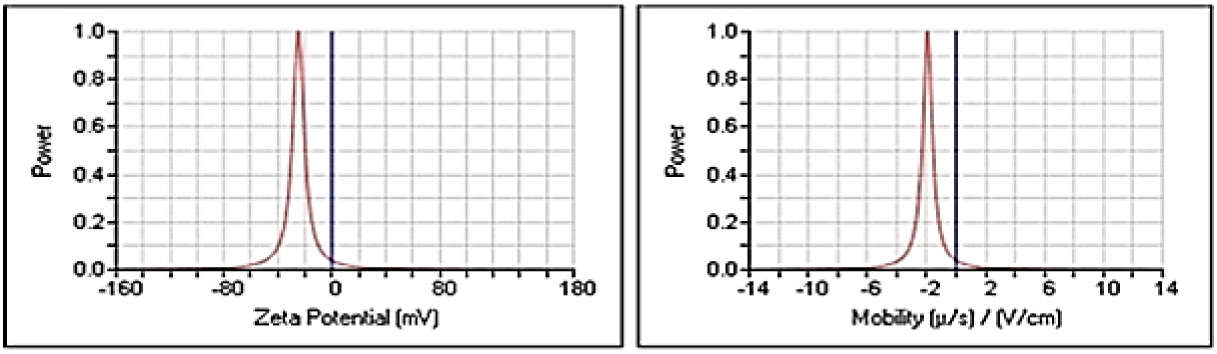


Figure 9: Zeta potential for zinc nanoparticles

Treatment methods

Pesticide's treatment by alfalfa plant

The polluted water sample was synthesized in definite ratios from sulfon and glyphosate pesticides in 50ppm, then the treatment was carried out in two ways, the first was batch method at which 5 g of alfalfa powder was employed by adding it to 50ml polluted water. Then, we put it onto magnetic stirrer to accomplish mixing and shaking process, after one hour ago. A part of the sample was pulled out and the treatment percentage was measured. 57.44% and 59.5% for each of glyphosate and sulfon were respectively found. Remeasuring operation was carried out in several periods as tabulated below. We noticed that the optimal treatment was at 5 hours with no variation in treatment percentages along the spent hours [29].

The second way was continuous flowing method at which 5 g of alfalfa leaves powder was added in column with glass wool because the wool cannot absorb water leading to simple flowing and powder particles retained avoiding its downward direction, followed by adding 50ml of polluted water. After one hour, the flowing of water was accomplished and the treatment of sample was measured by UV apparatus resulting in 57.44% and 59.5 % for each of Glyphosate and Sulfon, respectively [30]. After five hours of adding the powder into column, the powder started to be rotted and when left for 20 hours it was noticed that the powder was entirely rotted, which denoted that the effectiveness of such a method was for only 1 hour by employing the plant powder, then its tended to be ineffective compared with the previous method.

Treatment by zinc nanoparticles

The water polluted sample was synthesized in definite ratios of pesticides (sulfon and glyphosate) in 10 ppm by adopting column method. A specific glass column was employed at which, 5 g of nano powder with wool was added, then 50 ml of polluted water was added; after 1hour, the flowing was completed and the treatment percentage was measured by UV-Vis apparatus. The following results were obtained: 100% glyphosate and 77% sulfon [30].

Table 2: Treatment percentages by batch method and flowing method in various concentrations

Time (h)	Pesticides	Concentration batch method	% batch method	Conc. flowing method	% flowing method	Conc. flowing method 10 ppm and 50 ppm	% Flowing method 10 and 50 ppm
1	Glyphosate	21.28	57.44	21.28	57.44	10 ppm 0, 50 ppm 10.69	10 ppm 100 50 ppm 78.63
	Sulfone	20.23	59.55	20.23	59.55	10 ppm 7.7, 50 ppm 16.21	10 ppm 77 50 ppm 67.59
2	Glyphosate	20.09	59.83	--	--	--	--
	Sulfone	19.02	61.67	--	--	--	--
3	Glyphosate	19.2	60.087	--	--	--	--
	Sulfone	19.06	64.44	--	--	--	--

Continues of Table 1

4	Glyphosate	17.88	62.75	--	--	--	--
	Sulfone	18.63	66.91	--	--	--	--
5	Glyphosate	16.55	64.52	--	--	--	--
	Sulfone	17.74	69.38	--	--	--	--
10	Glyphosate	16.55	64.52	--	--	--	--
	Sulfone	17.74	69.38	--	--	--	--
20	Glyphosate	16.55	64.52	--	--	--	--

Table 3: Concentration of treatments from negative ions by alfalfa powder

Time (h)	Negative ion/ 50 ppm	Conc. of treatment by batch method
1	NO_3	--
	NO_2	--
	SO_4	--
2	NO_3	12.51
	NO_2	12.05
	SO_4	8.88
3	NO_3	19.15
	NO_2	23.14
	SO_4	14.06
4	NO_3	30.995
	NO_2	28.04
	SO_4	24.77
20	NO_3	10
	NO_2	14.52
	SO_4	15.09

Table 4: Concentration of treatments from negative ions by zinc nanoparticles

Time (h)	Negative ion/ 50 ppm	Conc. of treatment by batch method
1	NO_3	33.88
	NO_2	46.85
	SO_4	17.21
2	NO_3	22.03
	NO_2	35.32
	SO_4	12.61
3	NO_3	12.09
	NO_2	24.03
	SO_4	8.65
4	NO_3	4.22
	NO_2	17.54
	SO_4	3.54
20	NO_3	2.61
	NO_2	11.07
	SO_4	0.44

Conclusion

In this study, we aimed to treat water via environmentally friendly methods, accomplished successfully by embracing zinc nanocomposites which were in turn prepared by zinc sulfate as starting material and the formation of which was proved by the following techniques: SEM, EDX, FTIR, UV, Zeta potential, AFM. Then, we treated the contaminated water due to negative ions and pesticides using two methods, namely batch method and column method and each method was used with each of zinc nanocomposites with alcoholic alfalfa extract and alfalfa powder alone. We noticed that batch was preferred compared with column method in both strategies because if plant was left inside the column for more than one day, it would be rotted with bad odder and the treatment by zinc nanoparticles was best and more accurate, because when we used alfalfa in treatment, we observed a light color was left behind.

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Authors' contributions

All authors contributed toward data analysis, drafting, and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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