Evaluation of the efficiency of biologically synthesized zinc oxide nanoparticles using the fungus Trametes sp. in removing heavy metals and salts from well water

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Abstract

White Root Fungi are a group of basidiomycetous fungus, including many species, such as *Trametes* sp. The study aimed to evaluate the efficiency of biologically synthesized zinc oxide nanoparticles using the fungus *Trametes* sp. in removing heavy metals and salts from well water. The macrofungal sample was collected from dead trunks of willow trees (*Salix* sp.) in the Hurman area, Sulaymaniyah Province, Iraq, during April 2025. The selected fungus was identified based on morphological, microscopic, and molecular characteristics. ITS sequence analysis confirmed the fungus as *Trametes trogii*, and the sequence was deposited in GenBank under accession number PZ427876.1.The fungal extract was used for the green synthesis of zinc oxide nanoparticles (ZnO-NPs). The biosynthesized ZnO-NPs from *Trametes* extract were applied at concentrations of 0.5 gram, 1 gram and 2 gram for the treatment of contaminated well water with heavy metals and salts. The results showed that *Trametes trogii* extract in combination with ZnO nanoparticles was very effective in the enhancement of the physicochemical quality of heavy metal contaminated water as well as saline water. As the concentration of the extracts increased, the electrical conductivity (EC), total dissolved solids (TDS) and chemical oxygen demand (COD) all decreased, suggesting that the extracts were effective in removing dissolved ions and organic pollutants. The removal of the heavy metals such as Pb, Cd, As and Cr also decreased significantly, with the highest removal being observed at 2 g treatment level. The acid–base balance of the water was not negatively impacted by the treatment process, as the pH values did not drop into the acidic range

Keywords: zinc oxide, *Trametes trogii*, heavy metals, well water.

Introduction

Groundwater is considered a major source of fresh water for the world's population and is used for domestic, agricultural, and industrial purposes. Approximately one-third of the world's population relies on groundwater as a source of drinking water[1]. Urban expansion, agricultural practices, industrial activities, and climate change pose significant threats to groundwater quality[2]. Pollutants such as toxic metals, hydrocarbons, trace organic pollutants, pesticides, nanoparticles, microplastics, and other emerging contaminants represent a danger to human health, environmental services, and sustainable social and economic development[3].

Recently, researchers have shown their interest in synthesizing different nanomaterials in order to meet the growing demand for efficient wastewater

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treatment[4]. Nanoparticles (NPs) are materials that typically have a size of 100 nm and display a wide range of shapes, such as spheres, rods, tubes, plates, stars, dots, wires, and prisms [5]. Due to their high reactivity and a larger surface-to-volume ratio, NPs possess special chemical, physical, optical, magnetic, and biological properties [6]. Metal oxide nanoparticles, including zinc oxide nanoparticles, have proven to be excellent adsorbents with high efficiency against various contaminants [7]. Zinc oxide exists in different crystalline forms, such as rock salt, wurtzite, and zinc blende, with a white or yellowish color. Researchers have shown keen interest in using zinc oxide nanoparticles for wastewater treatment due to their ability to dissolve in both acidic and basic solutions, as well as their higher stability in terms of charge and chemical bonding [8].

WRF are a group of basidiomycetous fungus, including many species, such as *Trametes versicolor, Ganoderma lucidum, Pleurotus ostreatus, Stropharia rugosoannulata, Irpex lacteus* etc. WRF, as is known to all, can aerobically decompose lignin and other organic pollutants using its non-specific intracellular and extracellular enzymes, such as ligninolytic enzymes, cytochrome P450 enzyme system, laccases [9]. Trametes sp. is a common species of WRF, and it has been verified to biodegrade various of organic pollutants, such as cyclophosphamide, bentazone, etoposide, diuron, chlorpyrifos, dicofol, cypermethrin and fluoroquinolone, etc [10].The study aimed to evalutae of the efficiency of biologically synthesized zinc oxide nanoparticles using the fungus Trametes sp. in removing heavy metals and salts from well water

Material and Methods

Collection and Cultivation Samples

The macrofungal sample was collected from dead trunks of willow trees (*Salix* sp.) in the Hurman area, Sulaymaniyah Province, Iraq, during April 2025, and taken to the laboratory for morphological and molecular identification and nanoparticle biosynthesis.

The collected macrofungal samples were initially washed under running tap water to remove dirt and debris. The fruiting bodies were then cut into small sections and surface-sterilized with 1% sodium hypochlorite for 2 min, followed by washing three times with sterile distilled water. The sterilized sections were dried on sterile filter paper and subsequently cultured on Potato Dextrose Agar (PDA) medium. The plates were incubated for 7 days to obtain fungal growth.

Morphological Identifucation

The fungus was identified morphologically based on both macroscopic and microscopic characteristics. The fruiting bodies were examined for their color, texture, shape, and hymenial surface. Microscopic observations were carried out using samples prepared in the laboratory to examine important structures, including spores. features. The identification process was performed with the aid of published taxonomic descriptions and identification keys, particularly those reported by[11,12,13].

Preparation of Fungal Filtrate

Seven fungal discs (6 mm in diameter) obtained from the actively growing margins of 7-day-old fungal cultures were transferred into flask containing 200 mL of Potato Dextrose Broth (PDB) and incubated in a shaker incubator at 25–28 °C for 20 days . After mycelial formation, the biomass was separated and washed with non-ionic water, then soaked again in non-ionic water and returned to the shaker incubator for 5 days. The obtained fungal

filtrate was collected and filtered using Whatman filter paper (0.22 µm), then stored under refrigeration until further use[14]

Biosynthesis of Zinc oxide Nanoparticles Using Fungal Filtrate

A 0.2 M zinc acetate solution was heated on a hot plate at 65 °C with continuous stirring for 10 min. Subsequently, 25 mL of fungal filtrate was added dropwise to 500 mL of the zinc acetate solution and mixed for 5 min. The pH of the mixture was adjusted to 12. The reaction mixture was stirred for 30 min under heating, followed by continuous stirring for an additional 1 h without heating. The flask was then transferred to an ultrasonic bath for 1 h. Formation of a precipitate or cloudy solution indicated nanoparticle synthesis. The resulting mixture was centrifuged at 6000 rpm for 30 min. The obtained precipitate was separated, washed, recentrifuged, and finally dried in an oven at 60 °C for 24 h. The zinc oxide nanoparticles characterization were explained and showed in details in Scopus manuscript of the authors.

Molecular identification of fungal isolates

For confirm the identification of *T. trogii*, the isolates were purified on Potato Dextrose Agar, then sent to Macrogen company in south Korea

Treatment of contaminated well water

the two most polluted wells out of a total of six were selected. Well No. (1), contaminated with organic matter and heavy metals, is in the Al-Ishaqi district (Watermelon Wells area). The second well, No. (6), is in the Al-Kayan area and is contaminated with salt. The pollution was confirmed by laboratory tests conducted at the College of Engineering (Department of Environmental Engineering) at Tikrit University. An aerial survey was conducted to identify the sources of the contaminated wells, revealing that the most polluted wells were located far from the Tigris River. The contaminated water was treated using three concentrations of nano-zinc extract (0.5 g, 1 g, 2 g).

Method for determining heavy metals and salts in well water

First: Estimation of heavy metals:

Well water samples were collected before and after treatment with nano-zinc oxide extract, then the samples were filtered and preserved according to approved laboratory procedures. Concentrations of heavy metals, including lead (Pb), cadmium (Cd), and chromium (Cr), were measured using an atomic absorption spectrophotometer (AAS), and the results were expressed in mg/L or ppm depending on how the results were presented.

Second: Estimation of salts and salinity:

The salinity of well water before and after treatment was estimated by measuring electrical conductivity (EC), which reflects the amount of ions and salts dissolved in the water, using an electrical conductivity meter. Total dissolved solids (TDS) were also measured, and results were usually expressed in µS/cm or dS/m for EC, and mg/L for TDS.

AAS atomic absorption device

The device was calibrated for each element separately using a certified standard solution for the element to be estimated, namely Pb, Cd, and Cr, with known concentrations. Then a calibration curve was created for each element, and the curve was used to determine its concentration in well water samples.

EC electrical conductivity device

The EC device was calibrated using a standard solution of known electrical conductivity, often KCl standard solution, then the electrode was rinsed with distilled water and measurements were made.

Results and Discussion

Identification of the Fungus *Trametes trogii*

Basidiocarp broad 10.0 – 12.5 x 2.5 – 5.0 cm, sessile, semicircular or fan-shaped, with a prominent downy upper surface and coloration ranging from creamy white to light brown, fleshy when young, becoming leathery to corky with age, the lower surface was porous, creamy white, with finely angular, evenly distributed, round to angular pores. 4-sopred, Habit and habitat: gregarious or cluster on decaying wood, particularly dead tree trunks in willow, fig and pomegranate forests environments, indicating its role as a wood decomposer causing white rot [15]. The results of macroscopic and microscopic analysis confirmed by molecular analysis of fungal isolates in Macrogen company/South Korea.

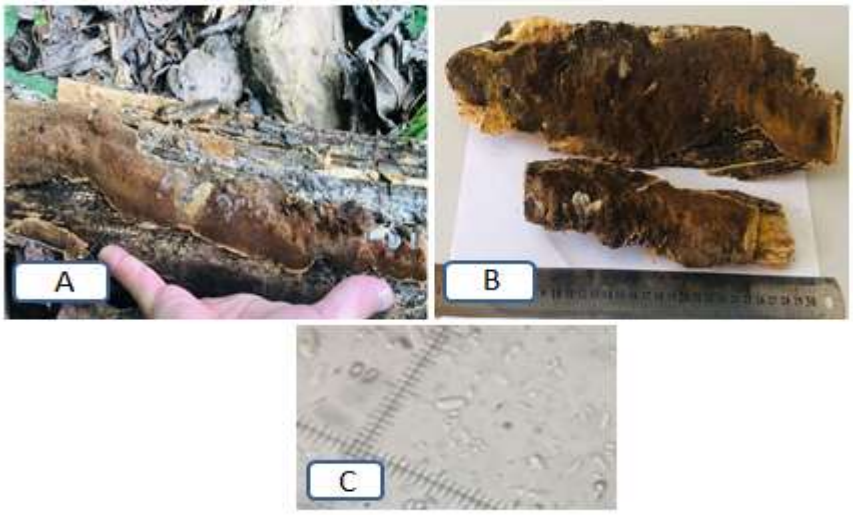


Figure (1): Morphological and microscopical characteristics of the fungus *Trametes trogii* on wood. A. fruiting body in nature; B. fruiting body in lab; C. basidiospores(400x).

Molecular Identification

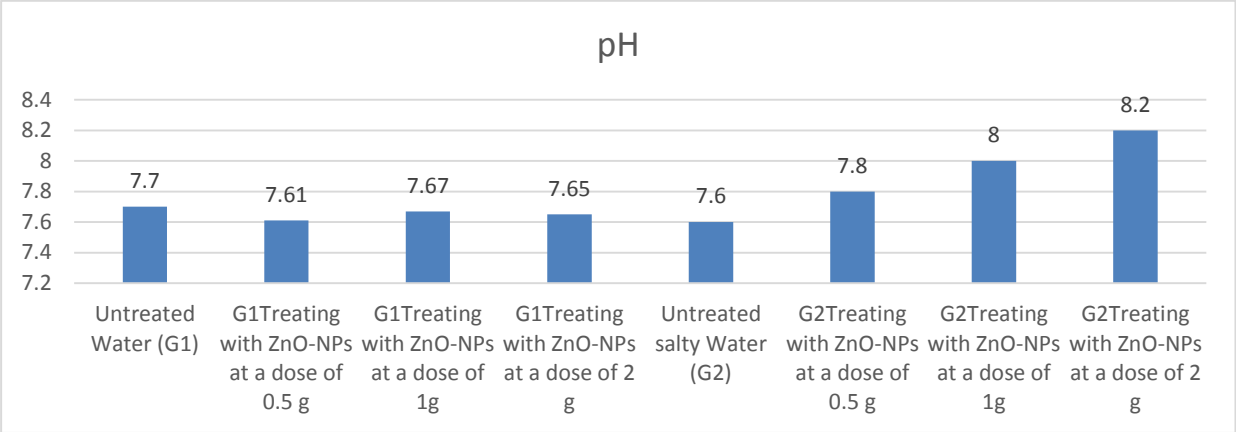
The nucleotide sequence of the ITS region showed 100% identity with reference sequences of *Trametes trogii* available in the GenBank database. This result confirmed the molecular identification of the isolate as *Trametes trogii*. The sequence was deposited in GenBank under accession number PZ427876.1

Mean level of pH in contaminated well waters before and after treatments

The results in table(1) and figure(2) showed that the pH values of all samples were within the neutral to slightly alkaline range, indicating that treatment with the extract did not cause significant changes in the nature of the aqueous medium.

In water contaminated with heavy metals and organic pollutants (G1), the pH value before treatment was 7.7. After adding the extract at concentrations of 0.5, 1, and 2 g, the values decreased slightly to 7.61, 7.67, and 7.65, respectively. In saline water (G2), the pH value gradually increased from 7.6 before treatment to 7.8, 8.0, and

8.2 after using 0.5, 1, and 2 g of the extract, respectively.

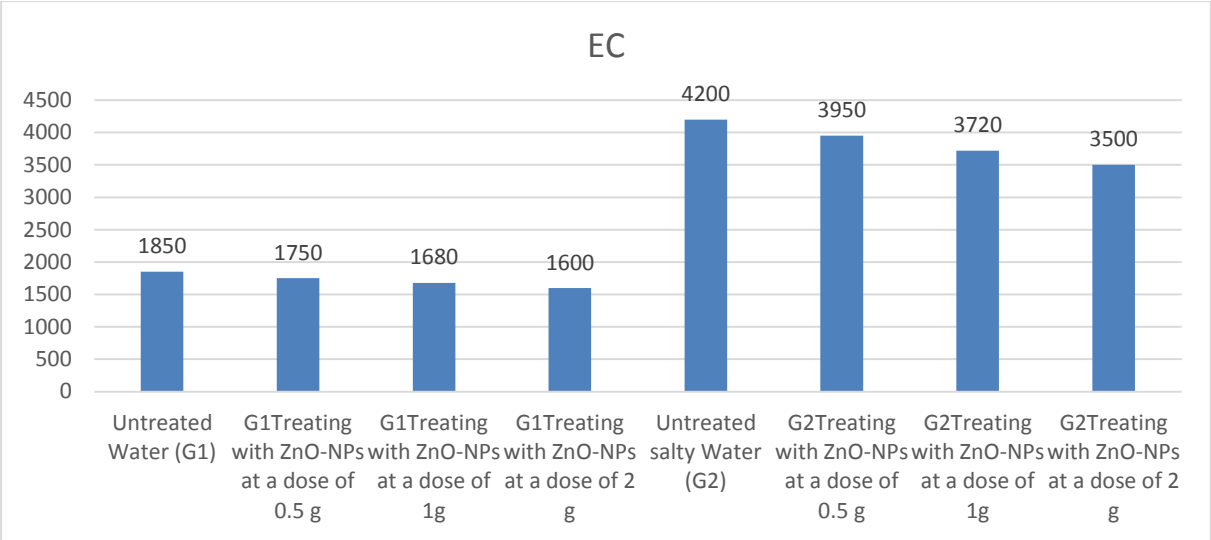


Figure(2):Mean level of pH in contaminated well waters before and after treatments

Mean level of electrical conductivity in contaminated well waters before and after treatments

The results in table(1) and figure(3) showed a gradual decrease in electrical conductivity values after treatment with the extract in both water types, indicating the extract's effectiveness in reducing dissolved ion concentrations. In water contaminated with heavy metals and organic pollutants (G1), the EC value decreased from 1850 µS/cm before treatment to 1750, 1680, and 1600 µS/cm when using 0.5, 1, and 2 g of the extract, respectively.

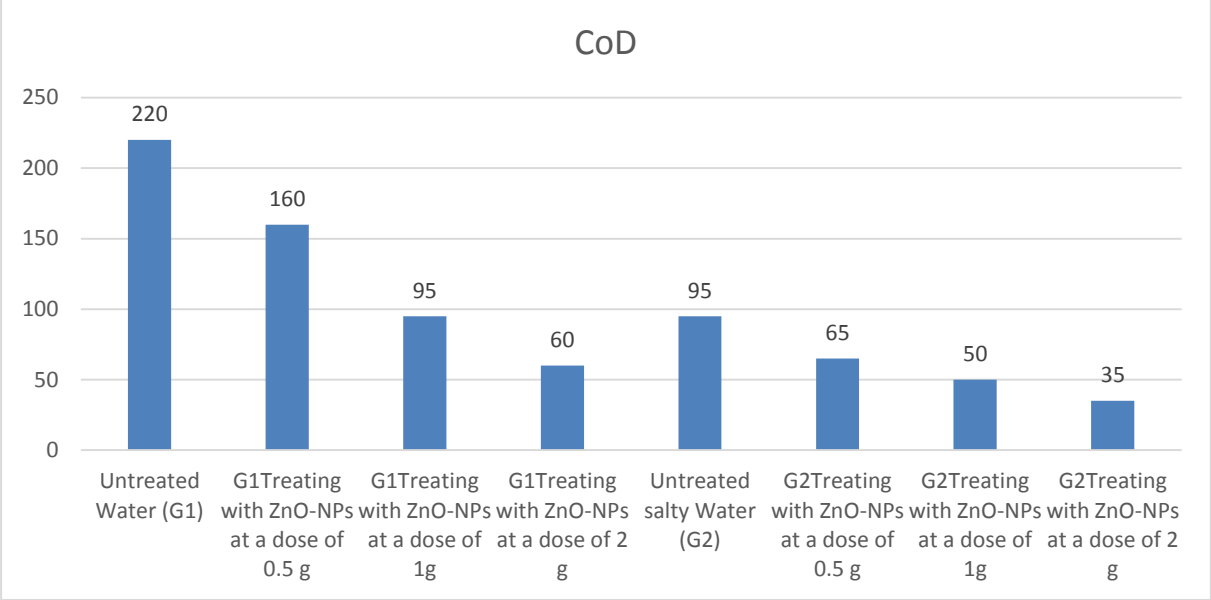
In saline water (G2), the EC value decreased from 4200 µS/cm to 3950, 3720, and 3500 µS/cm with increasing extract dosage, indicating the extract's ability to reduce a portion of the dissolved salts. Although the values remained relatively high due to the water's salinity, the gradual decrease confirms the treatment's effectiveness in improving water quality.



Figure(3):Mean level of electrical conductivity in contaminated well waters before and after treatments

Mean level of Chemical Demand for Oxygen (COD)in contaminated well waters before and after treatments

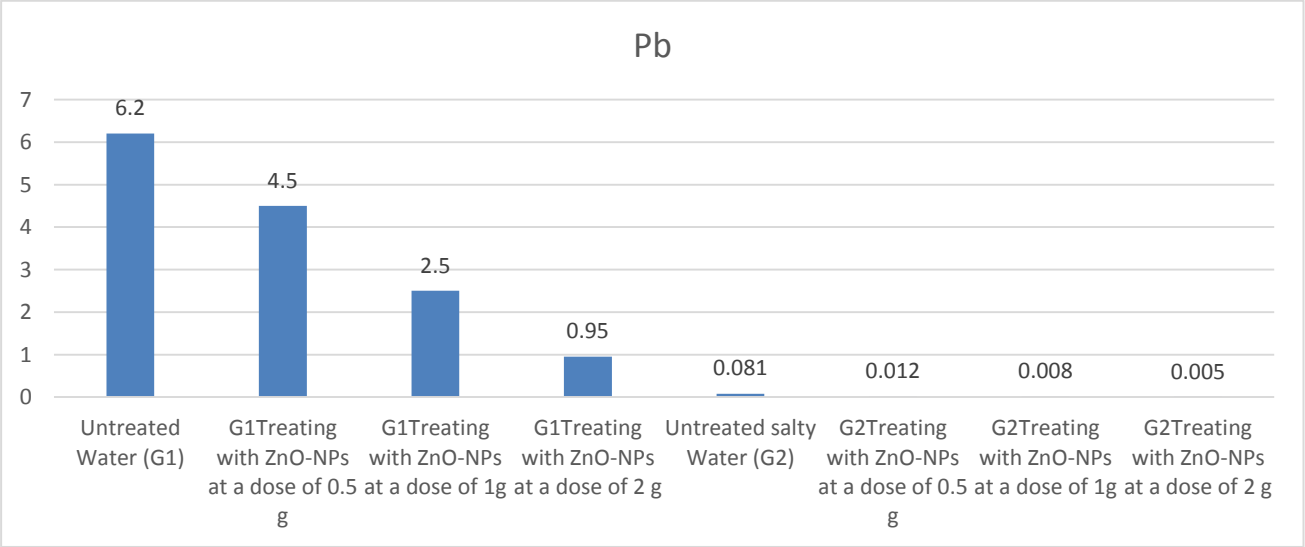
The results in table(1) and figure(4) showed a clear decrease in COD values after treatment, indicating the removal of a large proportion of oxidizable organic compounds. In water type G1, the COD decreased from 220 mg/L before treatment to 160, 95, and 60 mg/L with increasing extract dosage. In water type G2, it decreased from 95 mg/L to 65, 50, and 35 mg/L. This decrease reflects the extract's ability to remove organic compounds that contribute to high organic loads in water, thus improving water quality and reducing the harmful environmental impacts of organic pollutants.



Figure(4): Mean level of COD in contaminated well waters before and after treatments

Mean level of Lead (Pb) in contaminated well waters before and after treatments

The results in table(1) and figure(5)showed a significant decrease in lead concentration after treatment. In G1, the concentration decreased from 6.2 mg/L to 4.5, 2.5, and 0.95 mg/L with increasing extract dosage. In G2, the concentration decreased from 0.081 mg/L to 0.012, 0.008, and 0.005 mg/L.

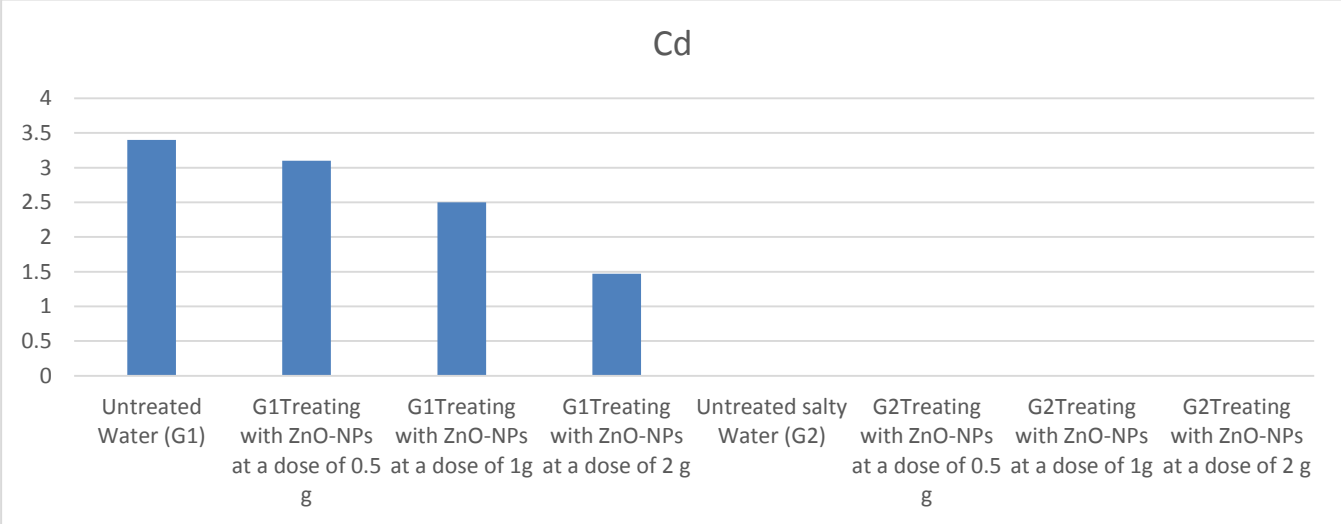


Figure(5): Mean level of Pb in contaminated well waters before and after treatments

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Mean level of Cadmium(Cd) in contaminated well waters before and after treatments

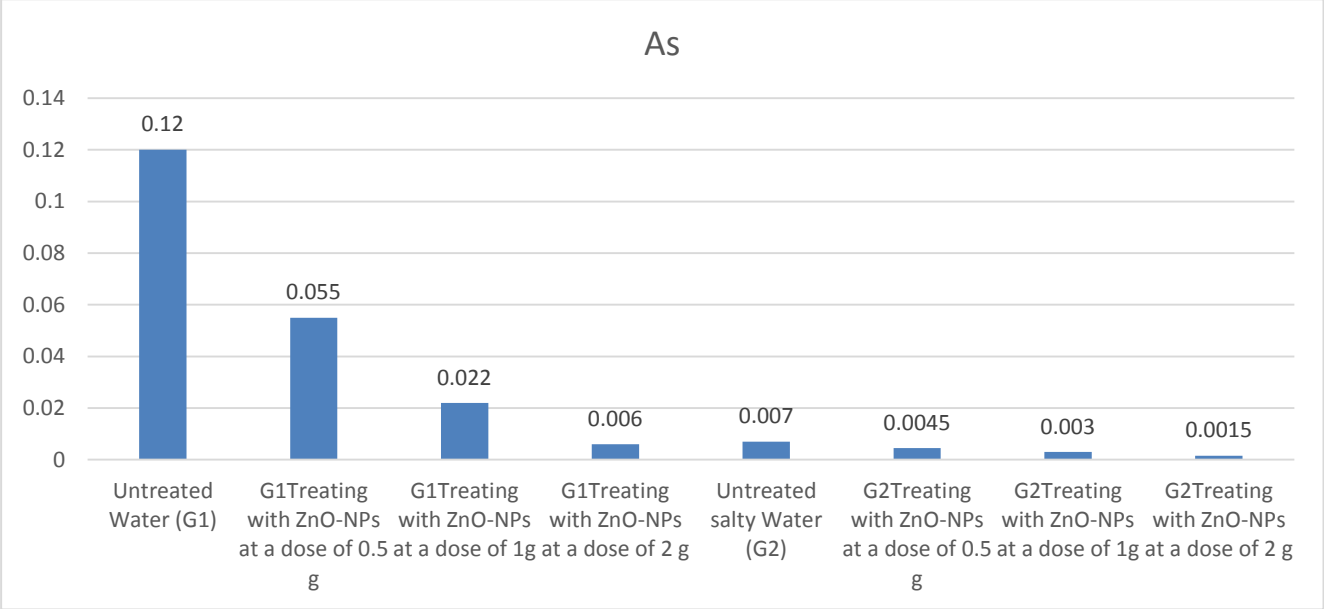
The results in table(1) and figure(6)showed a gradual decrease in cadmium concentration after treatment. In water G1, the concentration decreased from 3.4 mg/L to 3.1, 2.5, and 1.47 mg/L, while in water G2, it decreased from 0.0045 mg/L to 0.003, 0.002, and 0.001 mg/L.



Figure(6): Mean level of Cd in contaminated well waters before and after treatments

Mean level of Arsenic in contaminated well waters before and after treatments

Arsenic levels showed a significant decrease after treatment. In water G1, the concentration decreased from 0.12 mg/L to 0.055, 0.022, and 0.006 mg/L, while in water G2, it decreased from 0.007 mg/L to 0.0045, 0.003, and 0.0015 mg/L(Table2, figure7).

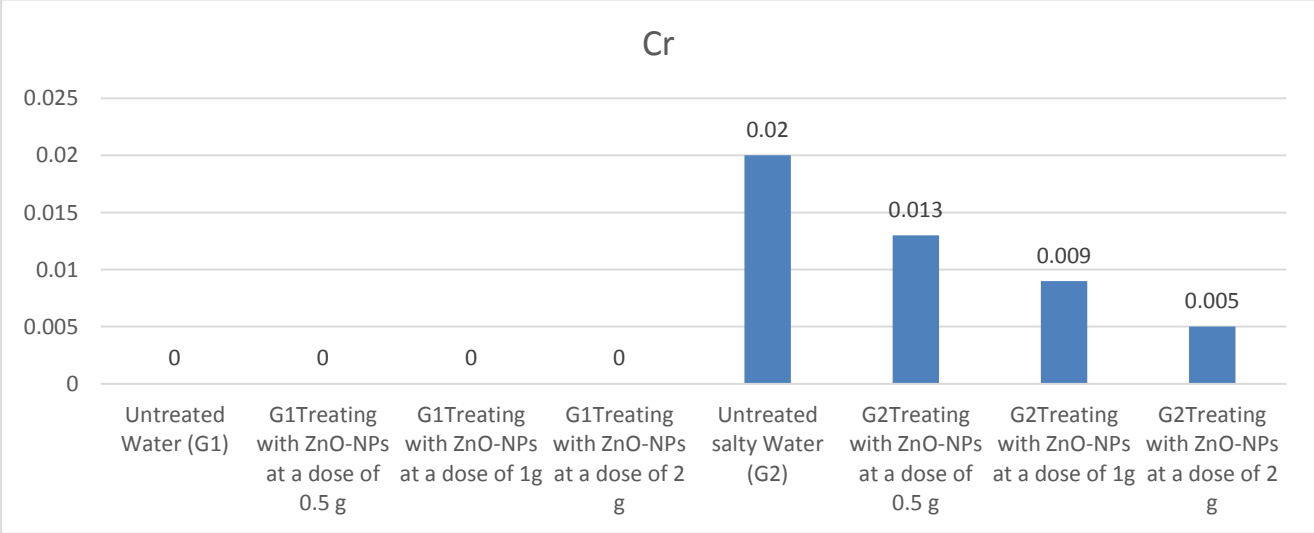


Figure(7): Mean level of As in contaminated well waters before and after treatments

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Mean level of chromium in contaminated well waters before and after treatments

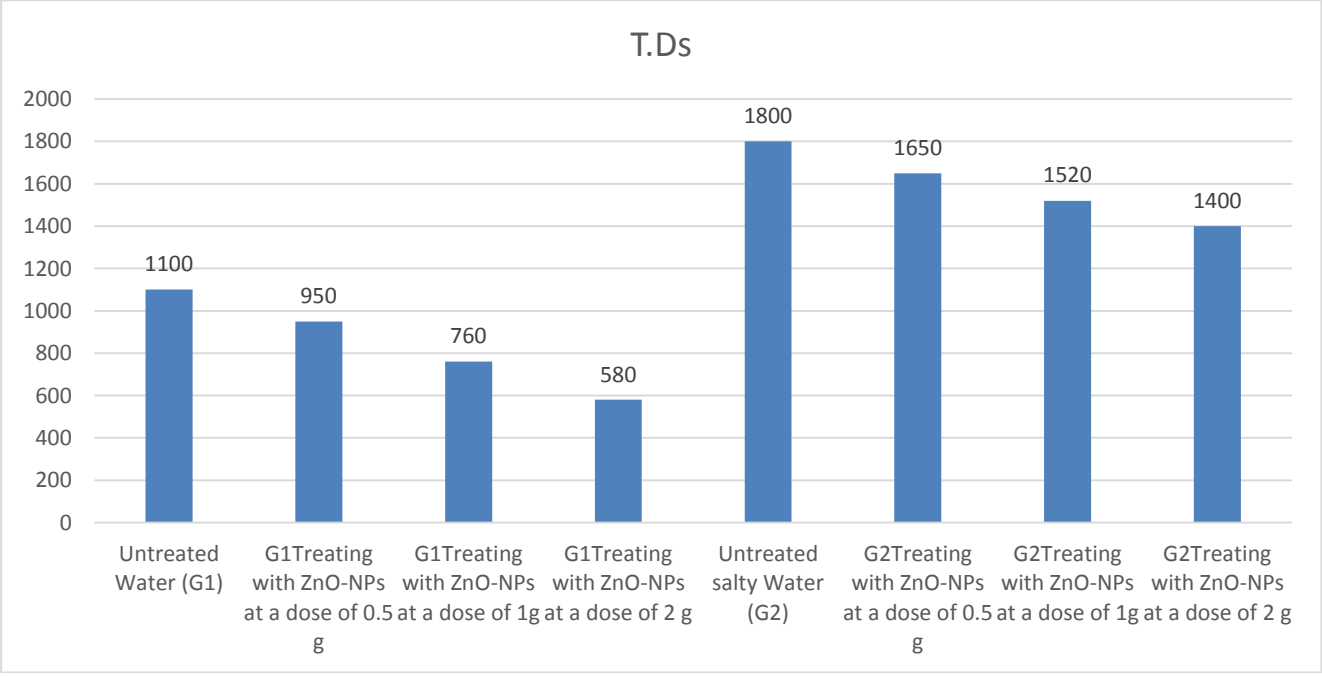
A gradual decrease in chromium concentration was observed in water G2 from 0.02 mg/L to 0.013, 0.009, and 0.005 mg/L after treatment. This indicates the extract's ability to remove chromium from the solution through chemical reactions with active sites(Table2, figure8).



Figure(8): Mean level of Cr in contaminated well waters before and after treatments

Mean level of Total dissolved solids (TDS) in contaminated well waters before and after treatments

Total dissolved solids values showed a significant decrease after treatment. In water G1, TDS decreased from 1100 ppm before treatment to 950, 760, and 580 ppm upon the addition of 0.5, 1, and 2 g of the extract, respectively. In water G2, TDS values decreased from 1800 ppm to 1650, 1520, and 1400 ppm, demonstrating that the extract contributed to reducing the concentration of dissolved substances even in highly saline water.



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Figure(9): Mean level of T.Ds in contaminated well waters before and after treatments

Table(1): mean level of salts, organic matter and heavy elements in contaminated well waters before and after treatments

Treatments	pH	EC	COD	Pb	Cd	As	Cr	T.Ds
Untreated Water (G1)	7.7	1850	220	6.2	3.4	0.12	ND	1100 ppm
G1Treating with ZnO-NPs at a dose of 0.5 g	7.61	1750	160	4.5	3.1	0.055	ND	950 ppm
G1Treating with ZnO-NPs at a dose of 1g	7.67	1680	95	2.5	2.5	0.022	0	760 ppm
G1Treating with ZnO-NPs at a dose of 2 g	7.65	1600	60	0.95	1.47	0.006	0	580 ppm
Untreated salty Water (G2)	7.6	4200	95	0.081	0.0045	0.007	0.02	1800 ppm
G2Treating with ZnO-NPs at a dose of 0.5 g	7.8	3950	65	0.012	0.003	0.0045	0.013	1650 ppm
G2Treating with ZnO-NPs at a dose of 1g	8	3720	50	0.008	0.002	0.003	0.009	1520 ppm
G2Treating with ZnO-NPs at a dose of 2 g	8.2	3500	35	0.005	0.001	0.0015	0.005	1400 ppm

***G1: water contaminated with organic and heavy metal, G2; salty well water; ND: Not detected**

Discussion

In this current study, the synthesized zinc oxide nanoparticles (ZnO-NPs) using Trametes trogii fungal extract was observed to be promising in the treatment of contaminated well water. The use of ZnO-NPs led to a gradual decrease in the concentration of Pb, Cd, As, Cr, COD, EC and TDS along with an increase in dissolved oxygen (DO). The greater changes at higher doses of nanoparticles could be linked to the increase in the surface area of the reactive surface of the ZnO nanoparticles and its interaction with contaminants in solution[16].

The decrease in the level of heavy metal (such as Pb, Cd, As, and Cr) can be mainly attributed to the physicochemical interactions between the surface of ZnO-NPs and the metal ions. The excellent ratio of surface area to volume and the high number of potentially reactive surface sites of ZnO make it a promising material for nanoscale applications [17]. These properties can help to facilitate interactions between the dissolved metal ions and the surface of the nanoparticles by adsorption, electrostatic interactions and surface complexation [18]. Other processes, like ion exchange or surface precipitation or immobilization, also can be involved depending on the chemical composition of the water. The reduction of heavy metals in the present study is therefore a result of their removal from the aqueous phase and not biodegradation.

The surface properties of the obtained ZnO-NPs can also be modified by using the extract of T. trogii in the synthesis process of green synthesis. Biomolecules, such as proteins, polysaccharides, phenolic

compounds and other metabolites found in fungal extracts can be involved in nanoparticle reduction, stabilization and capping during biosynthesis. These compounds can stay on the surface of the nanoparticles and impart functional groups such as hydroxyl (–OH), carboxyl (–COOH), amino ($–NH_2$), and phosphate. These functional groups can coordinate, electrostatically interact with metal ions, and form surface complexation [17]. The removal efficiency observed in the present study could be attributed to the combined effect of the chemically obtained surface of the ZnO and the bio-degradable surface coating that forms during the fungal mediated method of formation[19].

The decrease of COD after the treatment with ZnO-NP is due to the decrease of the oxygen demanding substances present in the contaminated well water. The reduction could be related to physicochemical interactions between the oxidizable dissolved compound with the reactive surface of the nanoparticle. This further decrease with increase in ZnO-NP dosage could be attributed to the more available surface sites with increasing concentration of NP[20].

Besides the removal of heavy metals, treatment with ZnO-NPs has also led to decreases in EC and TDS, in addition to saline water group (G2). The EC value was reduced from 4200 in untreated G2 to 3500 after treatment of 2 g ZnO-NPs, and TDS value was reduced from 1800 ppm to 1400 ppm. The overall decrease in the concentration of dissolved ionic constituents is suggested by these changes. The observed decrease could stem from the interaction of some of the dissolved ionic species with the surface of the ZnO-NPs, such as adsorption/association, ion exchange, and even precipitation. The biologically derived compounds present on the surface of the nanoparticles can also offer extra functional sites which can interact with charged species, which can improve treatment performance [21].

This decrease in EC and TDS does not necessarily mean degradation/destruction of any salt. Inorganic salts are not biodegradable as such. Changes in EC however, do not represent changes in TDS, but instead, represent changes in the concentration of dissolved ionic constituents, which can be caused by physicochemical removal or immobilization of some of these ions. In addition, the parameters, EC and TDS, are general and cannot distinguish the type of ions removed. The results therefore should be considered as a decrease in the dissolved ionic content, and not as desalination of the well water.

The rise in DO after the treatment could be correlated to the decrease in oxygen demanding substances, which was seen in COD. A lower concentration of oxidizable substances will lower the water's oxygen demand and thus help to increase the DO levels [22]. However, dissolved oxygen is also influenced by a number of environmental factors such as temperature, mixing and aeration, and the observed rise should be viewed as evidence of the improvement of water quality rather than of a specific removal mechanism.

After the treatment with ZnO-NP, the pH values were relatively stable and mostly neutral to slightly alkaline. The stability is a sign that the treatment with the nanoparticles did not greatly affect the acid–base conditions of the well water. Interactions between the ions in solution and the surface functional groups formed during the biological synthesis of the ZnO-NPs can be associated with minor changes in the pH [23]. The relative stability of ZnO could also have been due to the influence of pH on metal speciation and the surface charge of the ZnO, which could

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have kept the metal's interactions with dissolved metal contaminants favorable[24].

The overall results indicated that the ZnO-NPs synthesized with the extract of T. trogii could be used as a biologically mediated nanomaterial for the treatment of the contaminated well water. The efficacy of the treatment might be due to the high surface reactivity and abundant surface sites of ZnO-NPs and further functional groups found on the surface of the fungal derived compounds[25]. This interaction with heavy metals and other dissolved contaminants could be improved with this combination. The dose-dependent decreases shown for heavy metals, EC, TDS and COD also indicate the prospective potential of the water treatment, using increasing ZnO-NP dosage.

Conclusion

ZnO nanoparticles were successfully synthesized using *Trametes trogii* fungal extract and evaluated for the treatment of heavy-metal-contaminated and saline well water. The treatment efficiency increased with increasing ZnO-NP dosage, with the 2 g dose producing the greatest reductions in the investigated parameters. In heavy-metal-contaminated water (G1), the highest removal was observed for As (95.0%), followed by Pb (84.68%) and COD (72.73%). In saline water (G2), Pb showed the highest removal (93.83%), followed by As (78.57%), Cd (77.78%), and Cr (75.0%), while COD, EC, and TDS decreased by 63.16%, 16.67%, and 22.22%, respectively. These quantitative findings indicate that the fungal-mediated ZnO-NPs have potential for reducing heavy-metal concentrations and selected physicochemical parameters in contaminated well water. Further studies are warranted to optimize treatment conditions and elucidate the physicochemical mechanisms underlying contaminant removal.

Acknowledgment: non.

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