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# Heavy Metals Removal by Using Chlorella Vulgaris Microalgae/ Zinc Oxide Nanocomposite for Wastewater Treatment in Menyet El-Nasr, Egypt

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ABSTRACT

**Background**: Microalgae are a highly competitive candidate for the role of a potent nanofactory since they are abundant and genetically diverse microorganisms capable of accumulating harmful pollutants and heavy metals from wastewater. Microalgae are as significant as fungi, yeast, or bacteria in the synthesis of nanoparticles, and this fact has led to the development of a new field of study, phytonanotechnology, which focuses on the biosynthesis of nanometals via the mediation of algae. **Aim**: In order to remove heavy metals from water, we constructed nanocomposite from a chitosan, gelatin, and Chlorella vulgaris freshwater microalgae composite impregnated with zinc oxide nanoparticles (ZnO-NPs). The created composite includes Chlorella vulgaris as a component due to the plant's exceptional phytoremediation capabilities. **Methods:** Transmission electron microscope (TEM), Dynamic Light Scattering (DLS), Zeta-Potential, and polydispersity index (PDI) were used to characterize the shape and structure of nanocomposite beads. **Results**: Removal efficiency of heavy metals in wastewater were observed higher in the nanocomposite treated water than microalgae treated water respectively. Reduction % of NH4+, PO43-, NO3-, and SO42- in nanocomposite treated water were observed to be 87.3%, 90%, 82.6% and 83.6%, respectively. Reduction % of NH4+, PO43-, NO3-, and SO42- in microalgae treated water were observed to be 68.9%, 53.8%, 52.1% and 62.4%, respectively. **Conclusion**: Results of this study well demonstrate the efficiency of the synthetized nanocomposite treatment of wastewater than microalgae treated water.

## Introduction:

Domestic, industrial, and agricultural wastes are all contributing to the worrisome rise in water pollution that is being driven mostly by human activity. Despite the widespread adoption of sustainable water management practices, there is a serious threat to the world's water supplies as a result of the widespread dispersal of a wide variety of contaminants. Therefore, it is crucial to investigate newer, more complex wastewater treatment methods and to make sure that the right standards are being used. Purified wastewater is the end aim of wastewater treatment systems, and purification requires the elimination of key contaminants such suspended particles, biochemical oxygen demand

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(BOD), nutrients (organic and inorganic), toxicity, and coliform bacteria (1).

The application of aquaculture systems as constructed systems for the treatment and recycling of wastewater from either households or industries has grown substantially during the past few years. One or more kinds of water-tolerant vascular plants, such duckweed or water hyacinth, are grown in shallow ponds as part of an aquatic treatment system. Many species of marine algae remain to be discovered, despite the fact that they are the most diverse group of organisms on Earth's surface. Microalgae biotreatment is appealing because of the algae's photosynthetic properties, which allow them to transform solar energy into biomasses with high calorific values while also absorbing eutrophication-causing pollutants like nitrogen and phosphorus (2). Microalgal nanoparticles are a promising area of study for improving removal efficiency, which would be useful in avoiding these unintended outcomes. The term "nanotechnology" is used to describe an enabling technology that investigates items on the nanoscale scale in order to create, implement, and comprehend systems, devices, and materials with fundamentally new features and functions (3). Microalgae have attracted a great deal of attention because of their ability to bioremediate harmful metals, transforming them into more manageable forms (4).

Because of zinc oxide nanoparticles (ZnO-NPs) distinct physical, chemical, and biological properties including its biocompatibility, environmental friendliness, low cost, and non-toxic nature— ZnO-NPs have found extensive use in the field of materials research. ZnO-NPs have been used as a functional advanced material in a variety of contexts, including catalysis for wastewater treatment, cosmetics, and antibacterial compounds, and other areas (5). In comparison to other metal oxides like $TiO_2$, $WO_3$, $SiO_2$, and $Fe_2O_3$, ZnO-NPs have various benefits, including exceptional chemical and thermal stability, toughness, and long shelf life making them a promising candidate for use in wastewater purification. Because of these features, ZnO-NPs' adsorptive potentials for the removal of heavy metals from industrial wastewater have attracted the attention of numerous researchers in recent years It has been found that ZnO-NPs of varying shapes are highly efficient at removing heavy metals. Lead, cadmium, and mercury were all eliminated from an aqueous solution by spherical ZnO-NPs (6).

Preparation of novel compounds by novel technical methods with the potential to remove various contaminants from contaminated wastewater by great reduction efficiency is the focus of the present investigation

## Material and Methods:

### Chemicals:

Chlorella vulgaris microalgae in the form of dried green powder was purchased from the Institute of National Research Center, Cairo. Gelatin (GT, isoelectric point of 5, Mw 40–50 kDa), chitosan (CT, degree of deacetylation (DD) 88%), and zinc acetate ($Zn(CH_3COO)_2·2H_2O$, 99.5%) were purchased from Sigma-Aldrich Chemicals Ltd. (Schnelldorf, Germany).

### Preparation of Zinc Oxide Nanoparticles (ZnO-NPs)

A solution of 1 M zinc acetate was slowly diluted with 2 M sodium hydroxide while being continuously stirred to create a white slurry. The white precipitate that formed after 20 hours of stirring was filtered off and washed, then dried in an oven, ground into a powder, and calcined at 400 °C (7).

**Fabrication of Sorbent Beads:** 2 g of chitosan was dissolved in 100 mL of 2% acetic acid at room temperature to make a 2% chitosan solution. 0.25 mL of gelatin was dissolved in 50 mL of water to make a 0.25% gelatin solution at 50 °C. Combine the two solutions and whisk them together for an hour at 50 °C to create a uniform mixture. After that, beakers were loaded with either ZnO-NPs and microalgae combination (1 mg of microalgae was added to the chitosan/gelatin solution). Beads were prepared by adding 10 mL of the most recent solution through a 100 L spray nozzle into a salt solution consisting of 100 mL of 3% (w/v) NaOH as a cross-linker and stirring for 30 minutes. The resulting particles were processed through a filtration system and rinsed with pure water (8).

### Characterization of Chitosan/Gelatin Beads Loaded with Chlorella vulgaris Microalgae/ Zinc Oxide Nanoparticles:

#### Transmission electron microscope:

The morphology of the resultant nanoparticles was studied using a transmission electron microscope (JEOL-JEM-2100). The samples were homogenized in a 15-minute ultrasonic bath in which they were suspended in distilled water. Under a microscope, a few drops of the suspension were placed on grids coated with evaporated carbon (9).

#### Dynamic Light Scattering and Z-Potential:

Dynamic Light Scattering (DLS) of the synthesized nanocomposite in solution were physically characterized using a Malvern ZetaSizer Nano Series (4 mW, 632.8 nm laser) in low volume disposable DLS cuvettes at 25 ◦C, equipped at a scattering angle of 90◦. Analyses were performed in water (viscosity: 0.8872 Cp, refractive index: 1.33). The size measurements were averaged from at least three repeated measurements (10).

### Preparation of wastewater samples:

All wastewater samples were collected from Menyet El-Nasr / Dakahlia Province.

### Treatment of Wastewater:

The Microalgae alone and/or the synthesized nanocomposite were used to investigate their efficacy in the treatment of wastewater under the determined optimum conditions of the catalytic experiment. The experiment was carried out in 250 mL conical flasks containing 100 mL wastewater mixed with the optimum concentration of Microalgae alone and/or the synthesized nanocomposite under light irradiation conditions. The mixture was stirred for 30 min to confirm the absorption/desorption equilibrium.

At the end of the experiment, the biological oxygen demand (BOD), total suspended solids (TSS), and total dissolved solids (TDS) as indicators for the successful treatment were assessed according to the standard protocols. Physico-chemical parameters of water such as the minor, trace and soluble heavy metals and non-metals are NH4+, PO43-, NO3-, SO42-, Boron, Cadmium, Zinc, Copper, Aluminum, Iron, Chromium, Manganese, Nickel and Lead were analyzed before and after the growth period using standard methods (11).

### Statistical Analysis:

Data represented in the current study are the means of three independent replicates. Data were analyzed using the statistical package SPSS v17 and Excel 2013.

## Results

### Characterization of Chitosan/Gelatin Beads Loaded with Chlorella vulgaris Microalgae/ Zinc Oxide Nanoparticles:

As shown, the synthesized nanocomposite was well-dispersed without aggregation and have a spherical shape (Figure 1). Moreover, the size of the nanocomposite was with an average size of 35.3 ± 0.08 nm (Table 1).

We used Dynamic Light Scattering (DLS) to learn about the synthesized nanocomposite' particle size, polydispersity distribution, aggregation, and stability. Table 1 summarizes the measured values of the particle size, polydispersity index (PDI), and Zeta-potential. The dominant symmetric peak of high intensity is located at approximately 25 nm in diameter for the synthesized nanocomposite as seen in the DLS plot (Figure 2). We found that the synthesized nanocomposite had a measured 58.4 mV negative potential (Table 1).

**Effect of Microalgae alone and/or the nanocomposite Chitosan/Gelatin Beads Loaded with Chlorella vulgaris Microalgae/ Zinc Oxide particles on polluted wastewater:**

Table 2 and 3 showed the measured parameters in the microalgae treated water and the nanocomposite treated water during the experiment and its reduction %. TSS, TDS, BOD, and pH parameters were decreased in the nanocomposite treated water than the microalgae treated water, the values in the nanocomposite treated water were 7.4, 2.9±0.17, 45±3, and 64.3±2.08, while in the microalgae treated water the values were 7.6, 3.7±0.18, 57.6±2.5, and 76.6±1.5. Also, NH4+, PO43-, NO3-, and SO42- were 1.10±0.10, 0.13±0.06, 0.40±0.20 and 0.67±0.24 at the nanocomposite treated water and were 2.70±0.51, 0.60±0.25, 1.10±0.36, and 1.54±0.14 at the microalgae treated water, respectively. According to the results of calculated parameters within the experiment, the most reduction observed and the highest reduction percentages belonged to the nanocomposite treated water as follow NH4+ (87.3%), PO43- (90%), NO3- (82.6%), SO42- (83.6%), Boron (92.11%), Copper (92.8%), Aluminum (98.2%), Iron (93.9%), Chromium (99.1%), Manganese (96.6%) and Nickel (95%), respectively than other groups.

## Discussion

Green chlorella vulgaris is one of the most widely utilized microalgae in industry. Reasons for this include its high production rates, simple cultivation needs, and approval by the Food and Drug Administration for use in food. Proteins, peptides, omega-3 polyunsaturated fatty acids, polysaccharides, vitamins, minerals, and other trace elements are just some of the useful macro- and micro-nutrients found in this microalga. Antioxidant, anticancer, antihypertensive, and antibacterial activity are just some of the many favourable health impacts and possible therapeutic applications associated with the bioactive peptides found in Chlorella vulgaris (12).

Microalgal nanoparticles are a promising area of study for improving removal efficiency, with the goal of avoiding these negative outcomes. Nanotechnology is an excellent research field because nanoparticles have useful physiochemical and crystallographic properties. Due to their unique thermal, electrical, biological, optical, chemical, and physical features compared to their bulk-scale counterparts, nanoparticles have attracted the attention of researchers (3).

Metal nanoparticles can be produced in living microalgal cells with one step technique including the addition of an aqueous solution of metallic salts to the cells while they are still in their culture conditions. Furthermore, microalgae preserved their nanoparticle biosynthetic ability when encapsulated within organic vesicles. Silver nanoparticles have been reported to be biosynthesized by a number of microalgal species, including Chlorophyta, Haptophyta, and Ochrophyta these include Merin et al. (13), Mohseniazar et al. (14), and Dahoumane et al. (15).

Transmission electron microscopy confirmed the presence of gold nanoparticles produced intracellularly by Cholera vulgaris, as described by Luangpipat et al. (16). The Size, aggregation, shape, surface charge, and stability are only few of the factors that affect a nanoparticle's activity (17). Therefore, it is essential to determine the morphological characteristics, especially the size, shape, and aggregation of the synthesized nanocomposite. In order to evaluate these factors, the TEM instrument is a helpful tool. In our results the synthesized nanocomposite was well-dispersed without aggregation and have a spherical shape. The samples showed a smaller peak at a higher value, suggesting particle aggregation rather than the existence of larger particles. This is supported by the PDI values, which are close to 0.3 for the samples. For a sample to be deemed monodispersed, the PDI must be less than 0.1 (18), which is an estimate of the degree to which the particles in the solution are uniform. However, if the PDI value is more than 0.7, the sample has a wide distribution and is unfit for DLS measurements (19). Smaller Zeta-potential implies less negative surface due to shielding effect of strong contact (20). Our synthesized nanocomposite measured 58.4 mV negative potential. Dispersions of colloids with Zeta-potentials greater than 30 mV are often quite stable.

According to our results of calculated parameters within the experiment, the highest reduction percentages belonged to the nanocomposite treated water than other groups. These results were in constituent with the following data.

Multiple contaminants can be eliminated by the employment of algae in treatment processes, including coliform bacteria, chemical and biochemical oxygen demand, nitrogen and phosphorus, and even heavy metals (21).

Algae can be used as a biological treatment to remove nutrients and produce biomass. During the course of the trial, a general downward trend was seen in all metrics, with NH4 showing the greatest percentage decrease. Through the photosynthetic process, microalgae are able to absorb the nutrients from wastewaters, which may then be filtered out and used to create biomass (22). Because of microalgae's metabolic flexibility, they can be grown in a variety of water types. Complete removal of phosphorus and ammonia from aquaculture wastewater is critical due to their central roles in the eutrophication of aquatic ecosystems (23). Chlorella vulgaris has the potential of nitrogen-phosphorus mediums. Algae was utilized to remove ammonia, demonstrating that micro-algae like Scenedesmus can be beneficial in wastewater treatment due to their rapid growth, resilience to manipulation in culturing systems, straightforward technology, and low production costs (24). Hammoda et al. found that Chlorella vulgaris and Scenedesmus quadricauda eliminated nearly all of the nitrogen, phosphorus, and $NH_3$ (25). Wang et al. found that Chlorella was commonly utilized to treat wastewater, removing nitrogen, phosphorus, BOD, and chemical oxygen demand (COD) very successfully with retention times varying from 10 hours to 42 days (26). The use of Chlorella vulgaris resulted in the greatest decrease in COD, BOD, $NO_3^-$, $PO_4^{2-}$, and TC, as measured by Ahmad et al. (27). Phosphorus removal by Chlorella vulgaris was reported to be greater than 99% additionally, 71% of COD was decreased by Salgueiro et al. (28).

Mineral NPs with an oxide base can be generated by both metallic and non-metallic sources. Wastewater treatment plants rely heavily on these NPs for the removal of harmful pollutants (29). Also, the chemical inertness, low toxicity, and biocompatibility of iron oxide nanoparticles suggest that they have extraordinary promise when combined with biotechnology. There is great promise for phosphorus removal using magnetic NPs, as demonstrated by De Vicente et al. (30). The COD and total organic carbon removal rates in urban secondary wastewater treated with ferrate (VI) by Gombos et al. (31) were reduced by roughly 40% and 20%, respectively, during the exposure time. Nitrate and phosphate removal by Iron and Copper NPs was amazing, according to Baharvand et al. (32), by NPs complex in the waste water. Comparatively, 93% of Pb(II) was removed from an aqueous solution by using green-synthesized ZnO-NPs. According to the study by Pandey et al. (33), ZnO-NPs were able to remove approximately 26.7 mg $g^{-1}$ of Cr (VI) from an aqueous solution with an initial chromium concentration of 30 mg $L^{-1}$. Heavy metal ions in wastewater can be effectively removed using ZnO-NPs, as has been previously documented (34).

Nanomaterials may be managed externally and provide a number of benefits, including the ability to adjust their size and increase contrast in magnetic resonance imaging. In order to purify wastewater, Liu et al. created magnetic chitosan nanocomposites. They have unique features that make them easy to extract from water with the aid of a magnet. When nanotechnology is applied to biological systems, a new field of study known as nanobiotechnology emerges. The dawn of the nanobiotechnology era is a major present for the progress of science all across the world. The new approach is in line with the present scientific drive to develop and refine novel methods of biosynthesizing nanoparticles. Wastewater treatment can benefit from algae in many ways, including lowering BOD, getting rid of nitrogen and phosphorus, and getting rid of metals (35).

## Conclusion:

As a result, microalgal treatment of wastewater, via biological and physicochemical mechanisms, may be a desirable complement to the currently employed biological treatment for the purification of wastewater. Understanding the mechanism by which nanoparticles are formed by microalgae and identifying the molecules responsible both require more study. Given the relative infancy of the area, many questions remain unanswered about the biotechnological potential of nanoparticles created using environmentally friendly processes. An ideal technique would be one that could choose a particular strain of algae or cyanobacteria to produce particles of a desired size and form. Overall, result showed that nutrients and other parameters were significantly reduced in the nanocomposite treated water which $NH_4$, $NO_3$, $PO_4$, and $SO_4$ had the highest reduction percentage in comparison with The microalgae treated water. This study concludes that it is possible to use simultaneous Chitosan/Gelatin Beads Loaded with Microalgae/ ZnO-NPs for the treatment of wastewaters. Regard to the limitations of this study, thus future research should analyses the findings more thoroughly by using a different biological and chemical system. Our findings serve as a template for further research and development into microalgae- nanocomposite systems, which may prove useful in a variety of fields including biology, industry, and the sensory realm.

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**Table 1.** The characterization of Chitosan/Gelatin Beads Loaded with Chlorella vulgaris Microalgae/ Zinc Oxide nanocomposite.

| | **Particle Size (PS) (nm)** | **Polydispersity Index (PDI)** | **Zeta Potential (ZP) (mV)** |
|---|---|---|---|
| **The synthesized nanocomposite** | 35.3±0.08 | 0.3±0.01 | -58.4±0.03 |

**Table 2.** The amounts of measured parameters (Mean±SE) in the untreated water, the microalgae treated water and the nanocomposite treated water

| **Biosolid** | **Untreated water** | **The Microalgae treated water** | **The nanocomposite treated water** |
|---|---|---|---|
| **pH** | 7.8 | 7.6 | 7.4 |
| **$NH_4^+$ (ppm)** | 8.70±0.20 | 2.70±0.51 | 1.10±0.10 |
| **$PO_4^{3-}$ (ppm)** | 1.30±0.20 | 0.60±0.25 | 0.13±0.06 |
| **$NO_3^-$ (ppm)** | 2.30±0.52 | 1.10±0.36 | 0.40±0.20 |
| **$SO_4^{2-}$ (ppm)** | 4.10±0.30 | 1.54±0.14 | 0.67±0.24 |
| **Boron (ppm)** | 0.203±0.002 | 0.15±0.03 | 0.016±0.009 |
| **Cadmium (ppm)** | 0.004±0.002 | 0.0025±0.001 | 0.0013±0.001 |
| **Zinc (ppm)** | 1.46±0.30 | 0.93±0.30 | 0.30±0.14 |
| **Copper (ppm)** | 0.07±0.02 | 0.03±0.02 | 0.005±0.003 |
| **Aluminum (ppm)** | 0.23±0.04 | 0.14±0.04 | 0.004±0.002 |
| **Iron (ppm)** | 0.83±0.25 | 0.25±0.12 | 0.05±0.03 |
| **Chromium (ppm)** | 0.08±0.02 | 0.03±0.04 | 0.0007±0.001 |
| **Manganese (ppm)** | 0.33±0.07 | 0.07±0.02 | 0.01±0.008 |
| **Nickel (ppm)** | 0.02±0.03 | 0.006±0.002 | 0.001±0.0008 |
| **Lead (ppm)** | 0.04±0.05 | 0.009±0.002 | 0.005±0.003 |
| **BOD (mg $l^{-1}$)** | 6.3±1.5 | 3.7±0.18 | 2.9±0.17 |
| **TSS (mg $l^{-1}$)** | 74.3±4 | 57.6±2.5 | 45±3 |
| **TDS (mg $l^{-1}$)** | 85±4 | 76.6±1.5 | 64.3±2.08 |

Values are mean ± standard error of three measurements.

**Table 3.** The reduction percentage (%) of measured parameters in the untreated water, the microalgae treated water and the nanocomposite treated water

| **Biosolid reduction percentage (%)** | **The Microalgae treated water** | **The nanocomposite treated water** |
|---|---|---|
| **$NH_4^+$** | 68.9% | 87.3% |
| **$PO_4^{3-}$** | 53.8% | 90% |
| **$NO_3^-$** | 52.1% | 82.6% |
| **$SO_4^{2-}$** | 62.4% | 83.6% |
| **Boron** | 26.1% | 92.11% |
| **Cadmium** | 37.5% | 67.5% |
| **Zinc** | 36.3% | 79.4% |
| **Copper** | 57.1% | 92.8% |
| **Aluminum** | 39.1% | 98.2% |
| **Iron** | 69.8% | 93.9% |
| **Chromium** | 62.5% | 99.1% |
| **Manganese** | 78.7% | 96.6% |
| **Nickel** | 70% | 95% |
| **Lead** | 77.5% | 87.5% |
| **BOD** | 41.2% | 53.9% |
| **TSS** | 22.4% | 39.4% |
| **TDS** | 9.88% | 24.3% |

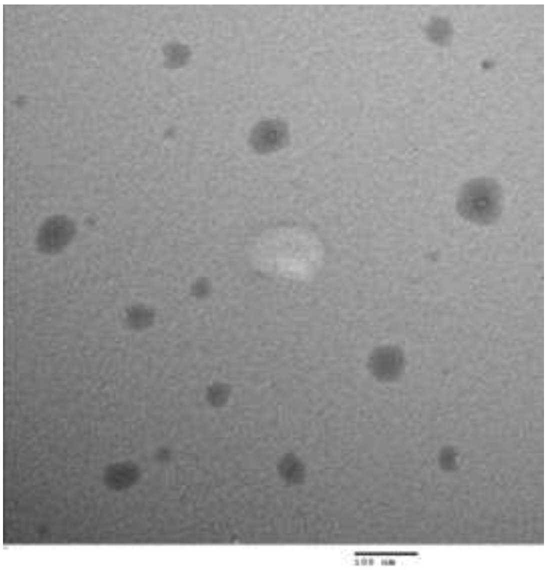

**Figure (1):** Transmission electron microscope image of the nanocomposite.

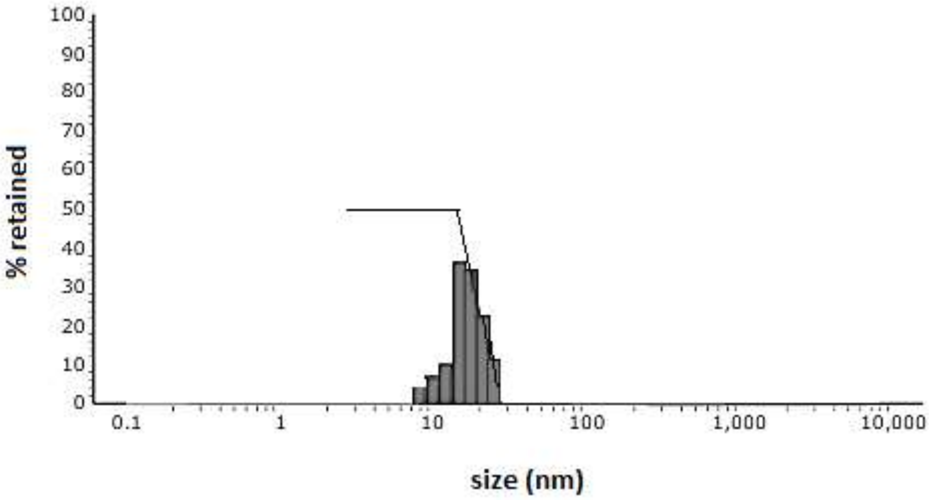

**Figure (2):** Dynamic light scattering size measurement of the nanocomposite.