

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES (AGNPS) FROM BASIL (*OCIMUM BASILICUM*) SEEDS AND ITS EFFECTS ON WASTEWATER NUTRIENT REMOVAL BY CONSTRUCTED WETLANDS.

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Abstract.

Background: In industrial and commercial items, silver nanoparticles (AgNPs) are widely used, which inevitably increases the release of AgNPs into wastewater which could have a negative impact on the sewage treatment plan. Objective: the experiments were carried out to assess the impact of AgNPs on nutrient removal in constructed wetlands. Materials and Methods: AgNPs were synthesized from seeds of basil (*Ocimum basilicum*) and characterized by the UV-Vis Spectrometer, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD). Results: NH_3 , NO_2 , NO_3 , P and PO_4 , were similar to the control group during the first week of the treatment period. The mean removal efficiencies of NH_3 , NO_2 , and P were 90.64%, 13.68%, and 13.09%, respectively. AgNPs had no impact on removing ammonia, nitrite and phosphorous at the level of $0.1 \mu\text{g.L}^{-1}$. The removal of NH_3 , NO_2 and P showed no differences at the three levels of AgNPs in the first seven days of treatment, while the inhibition of NH_3 removal occurred after two weeks of exposure to 0.1, 5 and $10 \mu\text{g.L}^{-1}$ each. After exposure for 4 weeks, NH_3 and P concentrations were significantly increased, as well as a significant reduce was recorded in removal efficiency. Conclusion: High AgNPs' levels can affect the nutrients' removal after exposure for a long period of time, as the a positive relationship was recorded between the AgNPs' concentration with the removal efficiency.

Keywords: AgNPs, biosynthesis, *Ocimum basilicum*, Wastewater, Nutrient, Removal, Constructed Wetlands

Introduction

In recent years, scientists have taken a special interest in the development of environmentally friendly products made from renewable and biodegradable resources [1,2]. Parallel to this, due to the nanomaterials distinctive qualities that make them appropriate for a variety of applications in various industries, it have drawn particular interest [3,4]. The use of nanotechnology in various approaches to environmental rehabilitation is one of its most notable approach. Industries include those in food, medicine, cosmetics, paint, plastics, paper, textiles, and even agriculture discharge into the environment toxic organic pollutants and harmful biological pollutants [5]. Nanotechnology is currently at the forefront of scientific research and approaches because of its wide-range use in the rehabilitation of environmental issued caused by the ever-evolving industrialization and expanding today's society urbanization [6,7].

When the active ingredient is provided as a crude extract, its bioavailability is significantly decreased; however, when a modified form of the crude extract is provided, such as nanomaterial, this bioavailability can be increased.[8]. A nanoparticle (NP) could be defined as a very small particle with a large surface area. The most recent attention has been on the synthesis of NPs as a result for the wide range of NPs' applications in fields like catalysis, optics, and electronics. [9, 10, 11], in addition to antibacterial, and antimicrobial activities [12, 13, 14]. Metallic nanoparticles' physical and chemical characteristics are noticeably different from those of their corresponding bulk forms, and they can also be utilized as an anti-bacterial agent [15]. Metal can be reduced by using plants and plant parts to synthesis the appropriate metal nanoparticles [16, 17]. AgNPs is one of the several metallic NPs, which have enormous applications in the field of the environment [18]. AgNPs synthesis could be achieved both physically and chemically. On the other hand, physicochemical methods have disadvantages such high costs for operation, as well as rising energy constraints and utilizing hazardous chemicals. Physical processes are intricate actions those fall short of controlling particles sizes at nanoscale level. The greatest limitations are that they produce particles of varying sizes and have a high cost of synthesis [19]. The chemical synthesis of nanoparticles are energy intensive, inefficient, and environmentally hazardous [20]. At this point, biological methods and as AgNPs precursors, less expensive materials are used. Due to the use of non-toxic phytochemicals and the avoidance of potentially hazardous components, the green production of nanoparticles has become quite popular [21, 22]. Green synthesis techniques are those that make use of various plant extracts, microbial cells, and biopolymers. Metallic NPs could be biologically synthesized by using a variety of plants and their easily accessible, abundant extracts. The plants and their extracts are non-toxic, eco-friendly, and safe to handle [23, 24].

Several plant extracts, have already been used to synthesis AgNPs, including olive and green tea [25], *Canna albica* [26], *Cinnamomum camphora* [27] and more. Although, for the synthesis of AgNPs from the basil seeds and any of its ecological applications there is no such information available. in consequence, this study aimed to synthesize AgNPs from the basil seed extract and evaluate the potential impacts of AgNPs on the removal of nutrient from sewage wastewater.

In a study conducted by [28] to test the efficiency of a constructed wetland system in removing nitrogen in the presence of AgNPs, it was found that concentration of AgNPs at a $0.5 \mu\text{g.L}^{-1}$ didn't show any impact on removing ammonia, while the concentration of AgNPs at $2 \mu\text{g.L}^{-1}$ reduced the removal of nitrates. [29] concluded that the addition of AgNPs in constructed wetlands affects the efficiency of denitrification of constructed wetlands in the short-term and long-term, also found that the addition of AgNPs to constructed wetlands as a wastewater treatment system did not cause obvious negative effects in COD removal, while nitrogen and phosphorous removal was slightly activated and the ability to efficiently remove AgNPs from waste water accumulated in soil and plant tissues. The results showed that the highest AgNPs' accumulation was in the constructed wetland soil. The presence of AgNPs also changed the relative abundance of bacteria.

Materials and Methods

Synthesis of AgNPs by Basil seeds extract

Preparation of the Plant Extract A Synthesis of AgNPs by Basil seeds extract

Dried basil (*Ocimum basilicum*) seeds were washed thoroughly in D.I. water several times with to get rid of any potential dirt or dust. Later, the seeds were grounded and 10 g of the seed powder were dissolved in 1 L of D.I. and left for 12 h, by using Whatman No. 1 filter paper the extract was then filtered and kept at a temperature of 4 °C for further use [30].

Preparation of (1M) of Silver Nitrate (AgNO₃)

In order to prepare 1M of AgNO₃ solution, 0.169 g of AgNO₃ (molecular weight = 169.87 g.mol⁻¹) were dissolved in 100 mL D.I. [31]

Green Synthesis of AgNPs.

The pH for the seeds extract was adjusting to 10.5, and then filtered by a 0.45 µm syringe filter. About 25 mL of AgNO₃ (1M) were added to 50 mL of the seeds extract under constant stirring rate at a speed of 500 rpm for 30 minutes and at a temperature of 80 °C. After 30 minutes, the colour changed from colourless into a reddish-brown as a result of the synthesis of the AgNPs according to [32]. The synthesized AgNPs were dried in the oven at 80 °C for 24 hours, after which the sample was cooled at room temperature it was collected and stored in an opaque tube for later use for research purposes.

Characterization of green AgNPs

Some properties of biologically synthesized AgNPs were determined by using the characterization methods where the first indication for AgNPs was the colour change from colourless into a reddish-brown in the plant mixture [33]. To confirm the biosynthesis of AgNPs, the spectras were taken in the visible range between 400 nm and 800 nm after AgNO₃ was added to the plant extract and the reduction of AgNPs optical property was determined using T80 UV\VIS Spectrometer PG Instruments Ltd.

To detect the functional groups present in the synthesized AgNPs and basil seed extract that could be responsible for the reduction of nanoparticles and efficient capping and stability, the Fourier transform infrared (FTIR) spectroscopy was used [34] and the dried synthesized AgNPs was examined within the range of 400-4000 cm⁻¹ by using Hitachi Ltd., Tokyo, Japan.

By Using SEMTESCAN-Vega3 scanning electron microscope (SEM), the morphology, size and synthesized AgNPs' image were detected [35].

X-ray diffraction was performed using the Powder X-ray diffraction system STOE STADI P System device with an $\lambda = 1.5405$ nm radiation at an angle 2θ from 20 to 90 degrees [36].

Setup of Constructed Wetlands

A total of 12 artificial wetlands were constructed according to a previous research done by [37] with dimensions of 40 cm (height) x 60 cm (width) x 80 cm (length). The media used in the

constructed wetlands were gravel and soil. The bottom was filled with a gravel to the height of 20 cm over layered (10 cm) with soil and planted with *Canna indica*. The constructed wetland systems were flooded from the top and for each system, a controlled outlet valve was placed at the bottom of it.

Analytical Methods

Standard procedures were used to determine NH_3 , NO_2 , NO_3 , P and PO_4 [38].

Ethics statement

The authors did not perform and studies or experiment with animals or human participant in this article..

Results

UV

The UV-visible spectroscopy confirmed the biosynthesis of AgNPs using basil seed by scanning the synthesized AgNPs at wavelengths ranging from 400nm to 800nm. A peak at 420nm, as shown in Figure 1, of the synthesized AgNPs.

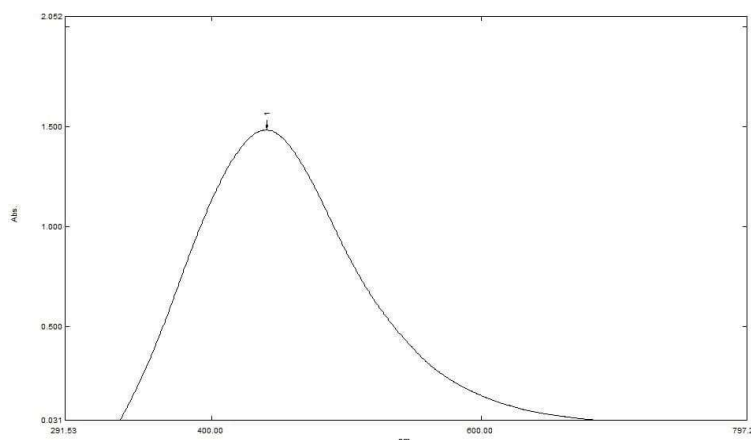


Figure (1): UV-Visible Spectroscopy of synthesized AgNPs from Basil Seed Extract

Fourier transform infrared (FTIR)

FTIR spectra of the biosynthesized AgNPs extract revealed the presence of 7 distinct different peaks as shown in Figure 2 at $3,352.85\text{ cm}^{-1}$, 2350 cm^{-1} , 2114 cm^{-1} , 1923.24 cm^{-1} , 1283.69 cm^{-1} , 789.50 cm^{-1} and 723.93 cm^{-1} , due to the presence of many functional biomolecules. The peak at 3352.85 cm^{-1} refers to the secondary amine group (N-H), the peak at 2350 cm^{-1} refers to the carboxylic acid group (O=H), and the peak of 2114 cm^{-1} ($\text{C}\equiv\text{C}$) refers to the terminal alkenes and acetylene, and either The peak cm^{-1} 1923.24 refers to the isothiocyanate group ($\text{N}=\text{C}=\text{S}$), the peak cm^{-1} 1283.69 refers to the group of aromatic ethers and esters (C-O), and the peak cm^{-1} 789.50 refers to the aromatic rings of the monosubstituted benzene group (C-H), The peak 723.93 cm^{-1} refers to alkenes (C=C).

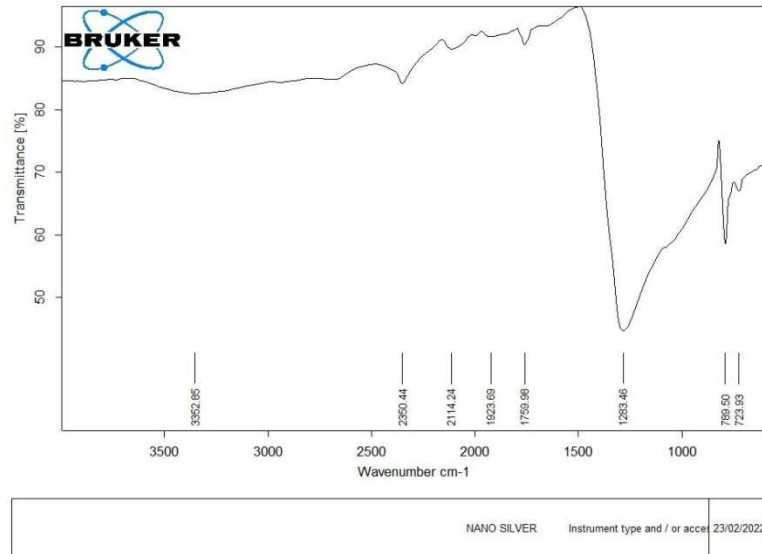
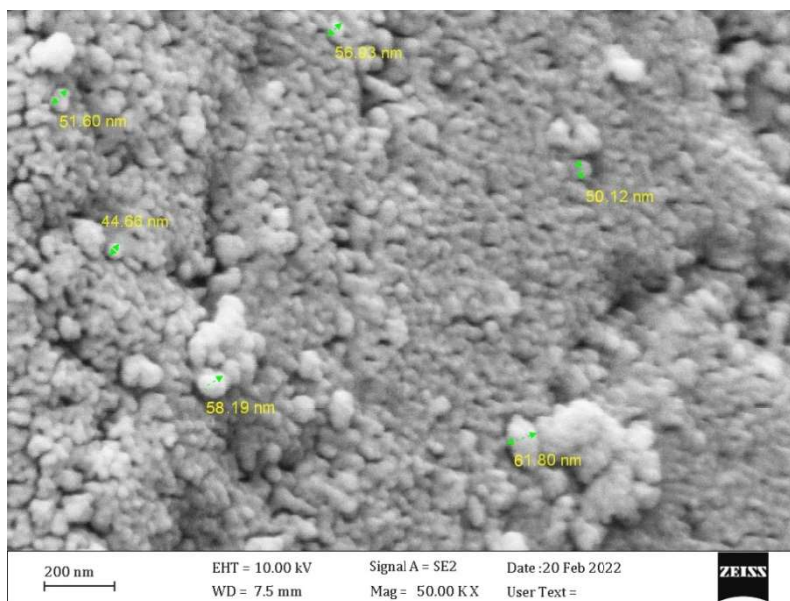


Figure 2 FTIR analysis of synthesized AgNPs from basil seed extract

Scanning electron microscope analysis (SEM)

Results from SEM (Figure 3) substantiates that the size of synthesised AgNPs at 200 were ranged from 44.66 to 91.80 nm and at 10 μm their sizes were between 20 -30 μm . It also clearly shows that the shape of the particles is cubic and there are no agglomerations in the NPs. The SEM results also illustrated that the particles formed are of nanosized.



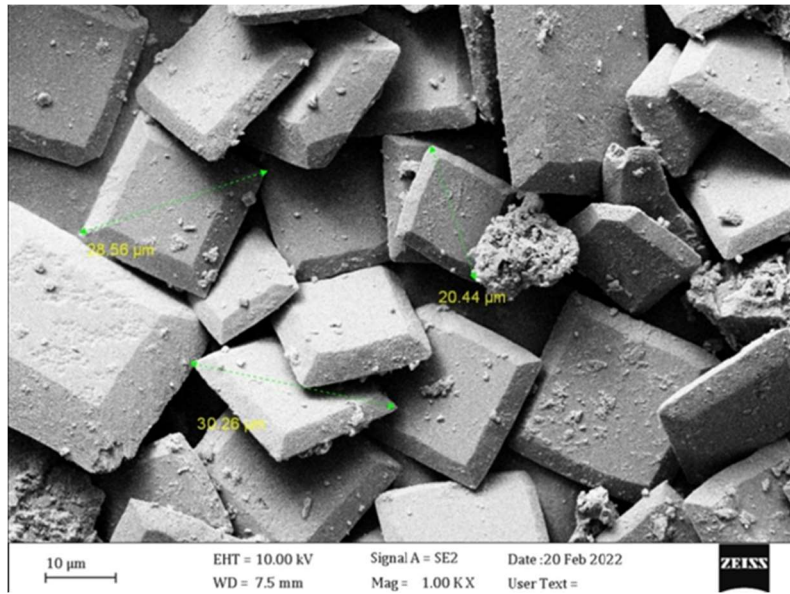


Figure (3) SEM examination of synthesized AgNPs

X-Ray Diffraction (XRD) pattern

The technique of XRD was used for further investigation and to diagnose the crystallization state of AgNPs and measure the particles' size by determining the X-ray diffraction peaks as illustrated in Figure (4) the results clarify that the particles were cubic crystal with diffraction angles of 25.691, 35.672, 64.667, 68.944, 69.453. The diffraction peaks were compared with the standard data (01-087-0718), and the crystal size was estimated 33.733 nm according to the Scherrer equation.

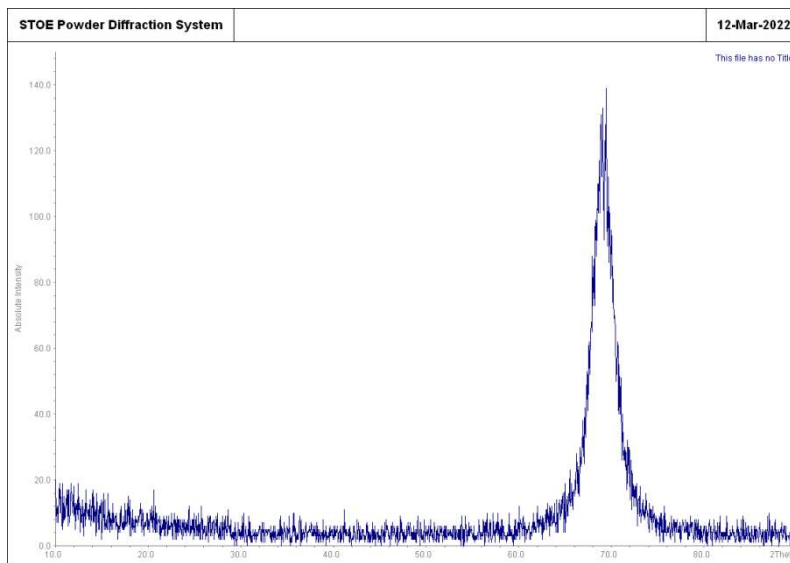


Figure (4) XRD pattern of synthesized AgNPs from basil seed extract

Ammonia NH₃

The values of ammonia in the first week ranged between the lowest value of 85.45 $\mu\text{g.L}^{-1}$ at 0.1 $\mu\text{g.L}^{-1}$ AgNPs and the highest recorded value was 481.70 $\mu\text{g.L}^{-1}$ at 10 $\mu\text{g.L}^{-1}$ AgNPs, and in the second week ranged between the lowest value of 12.7 $\mu\text{g.L}^{-1}$ at 0 $\mu\text{g.L}^{-1}$ AgNPs and the highest value is 150 $\mu\text{g.L}^{-1}$ at 5 $\mu\text{g.L}^{-1}$ AgNPs. As for the third week, the ammonia values ranged between the lowest value of 20.42 $\mu\text{g.L}^{-1}$ at 10 $\mu\text{g.L}^{-1}$ AgNPS concentration and the highest value being 55.88 $\mu\text{g.L}^{-1}$ at 5 $\mu\text{g.L}^{-1}$ AgNPS concentration. In the fourth week, the ammonia values ranged between the lowest value of 682.30 $\mu\text{g.L}^{-1}$ at 10 $\mu\text{g.L}^{-1}$ AgNPS concentration and the highest value being 702.79 $\mu\text{g.L}^{-1}$ at 0 $\mu\text{g.L}^{-1}$ of AgNPs.

There was a significant difference ($p \leq 0.05$) indicated by the statistical analysis for ammonia between the concentrations of AgNPs for all weeks of the experiment. Also, there was a very strong negative correlation between ammonia and orthophosphate during the third week of the experiment

Nitrite NO₂

The nitrite values ranged between the lowest value of 0.011 mg.L^{-1} at 0 $\mu\text{g.L}^{-1}$ AgNPs, and the highest value of 0.018 mg.L^{-1} at 0.1 $\mu\text{g.L}^{-1}$ AgNPs during the first week of the experiment, while in the second week the nitrite values ranged between the lowest value of 0.01098 mg.L^{-1} at 0 $\mu\text{g.L}^{-1}$ AgNPs, and the highest value was 0.01784 mg.L^{-1} at 10 $\mu\text{g.L}^{-1}$ AgNPs. In the third week, the nitrite values ranged between the lowest value of 0.01098 mg.L^{-1} at 0 $\mu\text{g.L}^{-1}$ AgNPs, and the highest value of 0.01784 mg.L^{-1} at 10 $\mu\text{g.L}^{-1}$ AgNPs. The nitrite values ranged between the lowest value of 0.01239 mg.L^{-1} at 10 $\mu\text{g.L}^{-1}$ AgNPs, and the highest value of 0.02435 mg.L^{-1} at 0 $\mu\text{g.L}^{-1}$ AgNPs in the fourth week.

There was a significant difference ($p \leq 0.05$) indicated by the statistical analysis between different concentrations of AgNPs during the study period. Also, it was found that there was a strong significant positive correlation between NO₂, P, and PO₄ in the fourth week.

Nitrate NO₃

The nitrate values ranged between the lowest value of 0.818 mg.L^{-1} at 0.1 $\mu\text{g.L}^{-1}$ AgNPs and the highest value was 0.954 mg.L^{-1} at 5 $\mu\text{g.L}^{-1}$ AgNPs for the first week. The nitrate values ranged between the lowest value of 0.77 mg.L^{-1} at the concentration of 0 $\mu\text{g.L}^{-1}$ AgNPs and the highest value was 2.78 mg.L^{-1} at 0.1 $\mu\text{g.L}^{-1}$ AgNPs for the second week, and the nitrate values ranged between the lowest value of 0.7067 mg.L^{-1} at 5 $\mu\text{g.L}^{-1}$ AgNPs and the highest value was 0.8023 mg.L^{-1} at 0 $\mu\text{g.L}^{-1}$ AgNPs for the third week. The values of nitrate ranged between the lowest value of 178.29 mg.L^{-1} at 10 $\mu\text{g.L}^{-1}$ AgNPs and the highest value was 182.91 mg.L^{-1} at 0 $\mu\text{g.L}^{-1}$ AgNPs for the fourth week.

There was a significant difference ($p \leq 0.05$) indicated by the statistical analysis between the AgNPs' concentrations for the first, third and fourth weeks. While there was no significant difference ($p \geq 0.05$) between the AgNPs concentrations for the second week.

Also, it was found that there was a very strong correlation between the NO_3 , P and PO_4 in the fourth week of the experiment

Phosphorous P

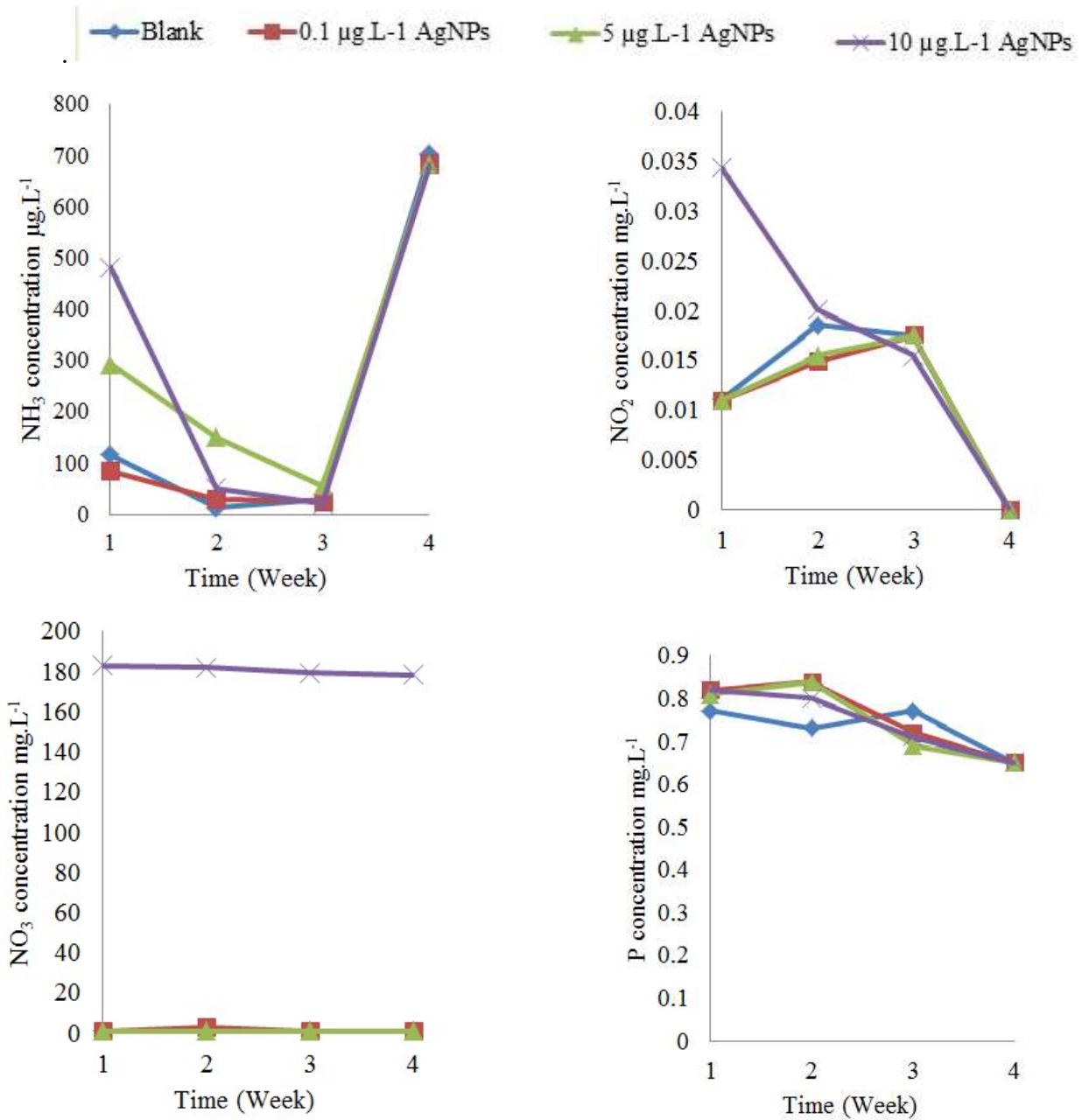
Phosphorous values for the first week ranged between the lowest value of 0.65 mg.L^{-1} at $10 \text{ }\mu\text{g.L}^{-1}$ AgNPs and where the highest value reached 0.77 mg.L^{-1} at $0.5 \text{ }\mu\text{g.L}^{-1}$ of AgNPs, and during the second week the phosphorous values ranged between the lowest value of 0.65 mg.L^{-1} at $10 \text{ }\mu\text{g.L}^{-1}$ AgNPs, and the highest value was 0.84 mg.L^{-1} at $0.1 \text{ }\mu\text{g.L}^{-1}$ AgNPs. In the third week, the phosphorous values ranged between the lowest value of 0.65 mg.L^{-1} at the concentration of $10 \text{ }\mu\text{g.L}^{-1}$ AgNPs and the highest value of 0.84 mg.L^{-1} at the concentration of $0.1 \text{ }\mu\text{g.L}^{-1}$ of AgNPs, and during the fourth week of the experiment the phosphorous values ranged between the lowest value of 0.65 mg.L^{-1} at the concentration of $10 \text{ }\mu\text{g.L}^{-1}$ AgNPs and the highest value of 0.82 mg.L^{-1} at $0 \text{ }\mu\text{g.L}^{-1}$ of AgNPs.

There was a significant difference ($p \leq 0.05$) indicated by the statistical analysis between the AgNPs' concentrations for the four weeks of the experiment, as well as a strong positive correlation between phosphorous and orthophosphate during the second and fourth weeks.

Orthophosphate PO_4

Orthophosphate values ranged between the lowest value of 0.97 mg.L^{-1} at $5 \text{ }\mu\text{g.L}^{-1}$ AgNPs and the highest value of 2.84 mg.L^{-1} at $0.1 \text{ }\mu\text{g.L}^{-1}$ of AgNPs for the first week. The values of orthophosphate ranged between the lowest value of 0.643 mg.L^{-1} at the concentration $5 \text{ }\mu\text{g.L}^{-1}$ of AgNPs and where the highest value reached 1.470 mg.L^{-1} at $0 \text{ }\mu\text{g.L}^{-1}$ of AgNPs in the second week. In the third week, the orthophosphate values ranged between the lowest value of 1.280 mg.L^{-1} at $5 \text{ }\mu\text{g.L}^{-1}$ of AgNPs and where the highest value reached 1.713 mg.L^{-1} at $0.1 \text{ }\mu\text{g.L}^{-1}$ of AgNPs, and the values of the orthophosphate ranged in the fourth week between the lowest value 1.04 mg.L^{-1} at $10 \text{ }\mu\text{g.L}^{-1}$ AgNPs, and the highest value was 1.4 mg.L^{-1} at $0 \text{ }\mu\text{g.L}^{-1}$ AgNPs.

There was no significant difference ($p \geq 0.05$) indicated by the statistical analysis between the concentrations of AgNPs in the first week, while a significant difference ($p \leq 0.05$) indicated between the concentrations of AgNPs for the second, third and fourth week.



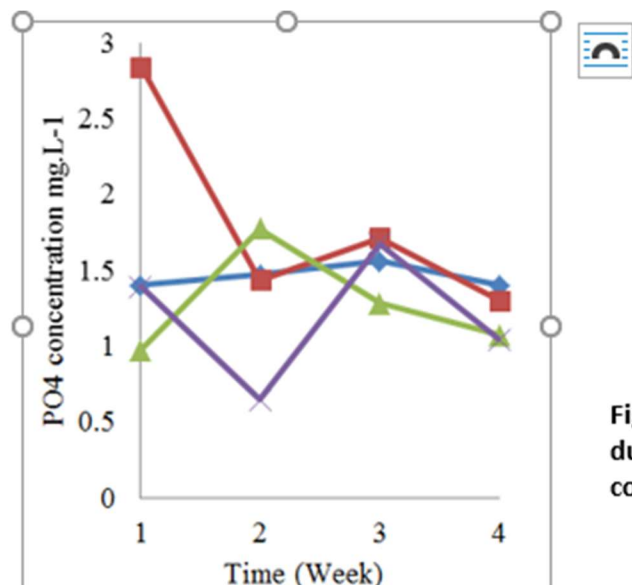


Figure (1) Variation of NH₃, NO₂, NO₃, P, and PO₄ during four weeks exposure at different concentrations of AgNPs

Discussion

The concentrations of NH₃, NO₂, NO₃, P and PO₄, as seen in figure (1) in the presence of AgNPs at 0.1 μg.L⁻¹ of is relatively volatile during the treatment period and it is similar to the control group during the first week of the treatment period. The mean removal efficiencies of NH₃, NO₂, and P were 90.64%, 13.68%, and 13.09%, respectively. These results indicated that the 0.1 μg.L⁻¹ AgNPs had no effect on removing ammonia, nitrite and phosphorous. The accumulation of NO₂ was approximately the same low values of 0.019 mg.L⁻¹, while NO₃ remained slightly higher at 0.82 μg.L⁻¹, demonstrating that 0.1 g.L⁻¹ of AgNPs may have a weak inhibitory effect on the denitrification process. It is possible that AgNPs addition inhibits the nitrate reductase activity, although causing less effects on nitrite reductase activity [39], so the AgNPs' low level may prevent the removal of NO₃ while neither affecting nor increasing NO₂ concentration. High concentrations of AgNPs may further inhibit the reduction activity of nitrite and result in accumulation of NO₂ in constructed wetlands, which increased as the concentration of AgNPs increase.

The removal of NH₃, NO₂ and P showed no differences at the three levels of AgNPs in the first seven days of treatment, while the inhibition of NH₃ removal occurred after two weeks of exposure to 0.1, 5 and 10 μg.L⁻¹ each. 1 liter of AgNPs and after three weeks of exposure to 5 μg.L⁻¹ of AgNPs, and P was clearly inhibited after two weeks of exposure to 5 μg.L⁻¹ of AgNPs. After exposure for 4 weeks, the concentrations of NH₃ and P were increased significantly in comparison with the (no added AgNPs) control group, and the removal efficiency was significantly reduced. At the same time, the results clearly determined that high AgNPs' levels can affect the nutrients' removal after exposure for a long period of time, as the removal had a positive relationship with the AgNPs' concentration. Meanwhile, the concentration of NO₂ in constructed wetlands increased from 0.02 mg.L⁻¹ to 0.026 mg.L⁻¹ with increasing concentrations of AgNPs, while the concentration of NO₃ gradually decreased to 0.71 μg.L⁻¹, indicating that AgNPs' higher levels of inhibit the process of nitrification during lengthy periods of exposure. On the other hand, the current study's results differed from earlier studies, and when adding different concentrations of

AgNPs to wastewater, the impacts ranged from non inhibition to inhibition with varying degrees [28; 40] where the variability of the effects of inhibition was connected to the changes in the studied parameters, that are well-known to affect the role and fate of the nanoparticles, along with the type of AgNPs and their properties in terms of size, the type of particles used as well as the concentration of silver, the method of dosing and the duration of the treatments. Furthermore, in activated sludge, AgNPs can be converted into Ag-sulfhydryl complexes, and Ag₂S, and Ag⁺ can interact with ligands in wastewater (for instance PO₄³⁻, S²⁻, Cl⁻, etc.) to produce silver-complexes, that lessen the toxicity of AgNPs. [16].

Conclusion

In the current study, basil seeds (*Ocimum basilicum*) were used to synthesize and characterize AgNPs. Constructed wetlands were exposed to various AgNPs, in an effort to study their interaction. The results clearly showed that that nutrient removal at AgNPs concentration of 0.1 mg.L⁻¹ showed no difference from the control after one week of exposure. However, high levels of AgNPs can affect the removal of nutrients after exposure for four weeks period of time, as the removal had a positive relationship with the AgNPs concentration. As AgNPs concentrations increased, the concentration of NO₂ in constructed wetlands increased from 0.02 mg.L⁻¹ to 0.026 mg.L⁻¹, but NO₃ gradually decreased to 0.71 mg.L⁻¹. These findings will be a helpful resource for studying the impacts of manmade nano particles on the environment and ecological safety.

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