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Green Synthesis of Nanoparticles Using Aqueous Leaves Extract of *Capparis spinosa* L. and Evaluation of their Resistance to Salt Stress of some Aquatic Plants

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Abstract. Salinity is one of the major biological stressors that has an impact on the growth and development of plants. Because of AgNPs distinct physical and chemical characteristics as well as their huge surface area in relation to their size. Green synthesized of AgNPs was used to reduce the salt stress aquatic plant *Ceratophyllum demersum*. AgNPs were biosynthesized by *Capparis spinosa* L. extract and characterized using UV-Vis, FT-IR, XRD, SEM and TEM techniques which gave spherical shape of crystal size 44.33 nm. Three concentrations of AgNPs were prepared (0.05 , 0.1 and 0.15 g) and used to study the salt stress. The effect on the properties of the aquatic environment was carried out at four weeks for salt pressure. As a result, the study showed that these particles improved the properties of the aquatic environment by reducing the amount of dissolved salt ions. In addition, the growth rate of the *C. demersum* plant increased, the amount of chlorophyll and the total organic carbon content increased and the proline content decreased So, we conclude from this study that AgNPs is a useful, ecofriendly and interesting application for use in environmental treatments, especially in water treatment.

Keywords. AgNPs, Salinity, Plant Extracts, Water physicochemical parameter, *Ceratophyllum demersum, Capparis spinosa* L.

1. Introduction

The greatest abiotic factor that significantly affects plant development and productivity is salt stress (salinity). Because of the buildup of sodium (Na) and chloride (Cl) ions, saline water can have a significant impact on plant physiological and biochemical responses, including overall disruption in the nutritional status of plants, osmotic stress, and ion-specific toxicity [1]. The main cause of the salinity of the water in many nations is the dry climate. The majority of plants are sensitive to the effects of salt stress, which ultimately reduce production , Numerous physiological and structural characteristics are used by plants as adaptations to deal with salinity in order to lessen the impact of salt stress [2].

Aquatic creatures are exposed to a variety of anabiotic stressors, especially freshwater salinity, which can have a variety of consequences on an organism's characteristics (such as survival, fertility, photosynthetic rates, growth, etc.) [3] .A rise in salinity causes the extinction of some aquatic plant

species and has a significant impact on the quantity and quality of aquatic plant life in the aquatic environment [4].

A nanomaterial, according to the European Union, is any material that contains particles in an unbound state, as an aggregate, or as an agglomeration. The size range of 1 to 100 nm is an additional exterior dimension" [5]. Due to their special physical and chemical characteristics, silver nanoparticles (AgNPs) are being employed more and more in a variety of industries, including medicine, food, and health care [6]. Various physical, chemical, or biological processes can be used to create nanoparticles [7]. Silver nanoparticles (AgNPs) have received the most attention among all forms of NPs because of their environmentally acceptable applications in biological research, unrivaled physiochemical properties, and sizable specific surfaces [8].

In this study, AgNPs were biosynthesized using extract *C. spinosa* and characterized using spectroscopic methods. The AgNPs and the plant extracts were tested to show their activity to resist the salinity for aquatic ecosystem of plant *C. demersum.*

2. Materials and Methods

2.1. Plant Sample Collection

C. spinosa was collected from the Abu Al-Khasaib area during July, August and November 2022.

2.2. Preparation of the Aqueous Extract of Capparis spinosa L.

The water extract was prepared for a *C. spinosa* according to method [9]. Plant leaves were washed by distilled water to remove pollutants from the surface, dried in an oven at 105°C for an hour, and then grinded. Amount of plant 25 g was placed in a 500 ml conical flask containing 150 ml of deionized distilled water. The mixture was then heated with continuous stirring using the magnetic bar for 30 minutes. The extract was then filtered using a Whatman filter paper and the extract was preserved in the refrigerator at 4°C until later use.

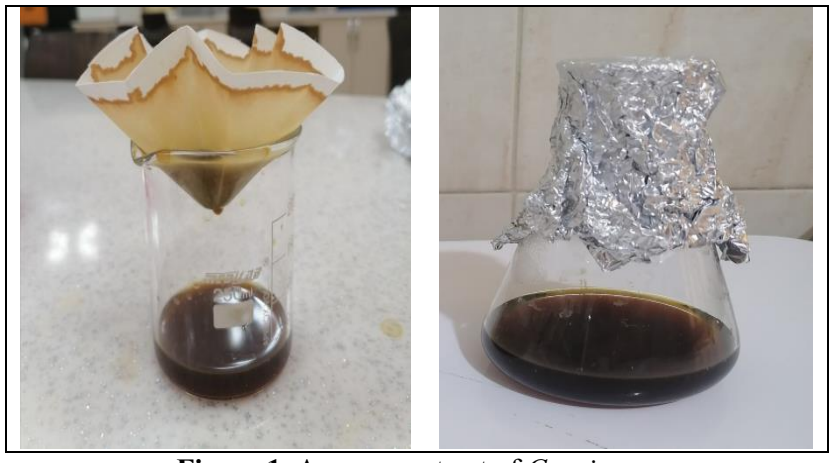

Figure 1. Aqueous extract of *C. spinosa*.

2.3. Preparation of $AgNO_3$ Solution

The concentration of 1 mM of silver nitrate solution was prepared by dissolving 0.016 g of silver nitrate in 100 ml of deionized distilled water.

2.4. Biosynthesis of Silver Nanoparticles (Green Synthesis)

AgNPs were synthesized biologically using of the aqueous extract of *C. spinosa* according to the method [10]. Volume of 100mL solution of 1 mM AgNO3 was added to 10 ml of the plant extract in a ratio of 1:10. The mixture was subjected to heat at 60 ^{o}C until its color changed from faint yellow to dark brown, indicating the initial AgNPs formation.

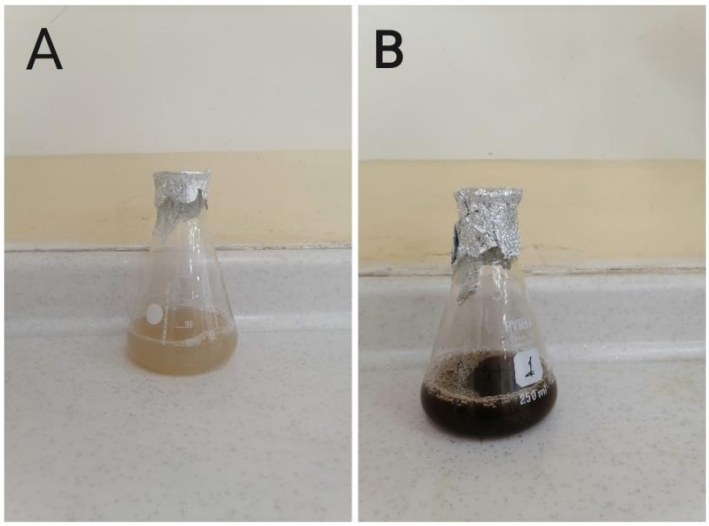

Figure 2. The final coloring of the aquatic extract of the *C. spinosa* after adding it to the silver nitrate and forming the nano-silver particles. A: Color change 30 minutes after the reaction. B: Color change 48 hours after the reaction.

2.5. Characterization of Green Synthesized Silver Nanoparticles

UV-Vis spectroscopy, a powerful analytical tool that was used to characterize the greenly produced AgNPs and confirm the reduction procedure for AgNP synthesis, was applied. To check for the presence of possible biomolecules and functional groups, Fourier transform infrared spectroscopy was carried out using a Termo Scientific Nicolet 6700 FT-IR spectrometer (Waltham, MA, USA). X-ray diffraction (Rigaku Ultima IV, Neu-Isenburg, Germany XRD) was used to analyze the structure of the produced silver nanoparticles. Data were gathered in two ranges. D. Scherrer's equation was used to determine the crystallite domain size. Transmission electron microscopy (TEM; JEM-1011; JEOL Ltd., Tokyo, Japan) was used to take images of the particle morphology and size of the phytomediated AgNPs.

$$D= K\lambda/\beta\cos\theta$$

2.6. Description of Experience

C. demersum was collected from the Qurnah region, taking into account that it was close to age and was washed well to remove the suspended pollutants and the remains of the living, and then placed in the tubs of its retrofit before experimenting.

The experiment was conducted in the laboratories of the Department of Environmental Science at the College of Sciences from 23 January 2023 to 27 February 2023 to determine the success of biosynthesis silver nanoparticels from *C. spinosa* and the plant's own raw aquatic extract in improving the various aquatic environmental properties, resisting salt stress and also studying its impact on the plant *C. demersum*.

It weighs 20 grams of plant. *C. demersum* was placed in plastic basins plus 5 L of water taken from the area near Najibiya, and the experiment consisted of 7 basins (3 treatment basins with 3 concentrations of AgNPs (0.05, 0.1, 0.15 g) and 3 treatment basins with 3 concentrations of raw plant extract (0.05,0.1,0.15 g) and a control basin

2.7. Measurement of Physical and Chemical Properties in Water

- Hydrogen ion was measured using the pH-meter system after calibrating it with standard solutions.
- The dissolved oxygen in the water was measured using the water dissolved oxygen meter type Extech and the output was expressed in a mg/l unit.

- Total soluble solids, electrical conductivity and temperature were measured using HANA HI9813-6 and expressed as total solvents in ppm, electrical conductivity in µS/cm and temperature in Celsius.
- Salinity was measured by the product times the electrical conductivity values of 0.64 and expressed as a unit mg/L.

2.8. Measurement of Plant Characteristics

2.8.1. Measurement of Relative Growth Rate

The relative growth rate in plants was measured using the equation described by [11]:

Growth rate % = final soft plant weight in g/first soft plant weight in g

2.8.2. Measurement of Proline

Took 0.5 grams of *C. demersum* dried and grinded leaves. Then 5ml of Ethyl alcohol were added at 95% concentration., Then the centrifuge was carried out for 30 minutes. And let it dry in the air, and then add 2 ml of distilled water, do the centrifuge. and then took 1 ml , and the absorption was read using the spectrometer and at the 520 nm wavelength, then estimated the sample content of the proline [12].

2.8.3. Measurement The chlorophyll a, b, Total, Carotene

In accordance with the method described in Arnon [13], one gram of *C. demersum* leaves was added to 20 ml of acetone 80% , stored the sample for 24 hours and crushed by ceramic mortar to get the green material of plant. The mixture was filtered and the filtrate was used to measure the chlorophyll a at 663 nm and chlorophyll b at 645 nm using the spectrophotometer using equations.

Chlorophyll a (mg/g) =[12.7(A663)-2.69(A645)]*V/1000 *W

Chlorophyll b (mg/g) = [22.9 (A645) - 4.68(A663)]*V/1000 *W

Total Chlorophyll (mg/g) =[20.2(A645)+8.02 (A663)]*V/1000*W

In the same way, the concentration of the Carotenoid was measured at 480 nm according to [14], according to the following equation:

Carotenoid (mg/g) =[A480+(0.114*A663)-(0.638*A645)]*V/1000*W

2.8.4. Measurement of Total Organic Carbon TOC%

Organic carbon was measured according to Ball [15], which 0.5 g of dry plant was placed in a concrete cranium with a known weight, then placed in a Fernace oven at 550^{o}C for three hours, and then weighed. TOC% was calculated according to equation:

Total organic carbon %TOC = the weight of the burrow before burn - the weight of the burrow after burn

2.9. Gas Chromatography - Mass Spectrometer

Effective chemicals have been estimated in the water extract of plant *C. spinosa* [16] was analyzed at the Basrah oil company, Nehran Ben Omar laboratories using the GC-MS device manufactured by Shimadzu.

2.10. Statistical Analyses

In SPSS v. 20 for Windows, two-way ANOVA was used for statistical analysis, and Duncan's new multiple range test ($\rho \leq 0.05$) was used to determine the mean separation between treatments.

3. Results and Discussion

3.1. Characterization of AgNPs Nanoparticles

3.1.1. UV-Visible Spectrum

The reacted mixture of *C. spinosa* leaf extract and AgNO3 solution showed a color change in the extract from light yellow to dark brown after adding 1 mM AgNO3. This change in color could be an initial indicator of AgNPs formation. However, But in order to do additional research, a UV-Vis spectrophotometer was used to find the silver ion's surface plasmon resonance (SPR). The highest absorption point at wavelength is 430 nm, and this is for a peak within the diagnostic limits of nano-silver particles, which range from 400 to 450 nm, because of the plasma surface absorption phenomenon [17] as in shape.

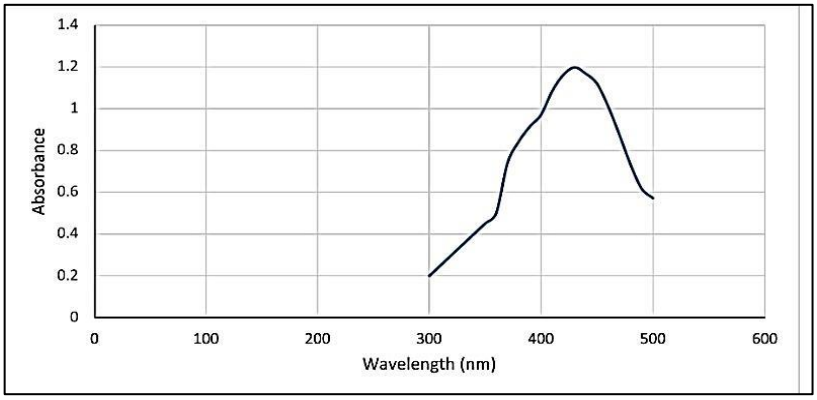

Figure 3. UV-visible spectrum of the silver nanoparticles (AgNPs).

3.1.2. FT-IR Spectrum

FTIR spectroscopy was used in order to detect the functional groups involved in AgNPs synthesis, where the FT-IR showed demonstrated six peaks located at about 3294, 2920, 1651, 1519, 1458, and 1072 cm^{-1}. The apparent bands are representative of functional groups of various compounds as in Figure 2. Phenol groups act as important reduction agents, which play an important role in the production of AgNPs; this was confirmed through package 3294 cm-1 which was responsible for stretching vibration of the O-H extension of carboxyl acid and phenol [18]. The 2920 cm^{-1} peak referred to stretching vibration of aliphatic C-H, the peak at 1651 cm^{-1} as well as 1519 cm-1 as a attributed to vibration of Aromatic C=C [19] [20]. Finally, packages 1072 cm^{-1} and 1458 cm^{-1} referred to C-N and C-O of aromatic Amine groups and organic acid or alcohol [21].

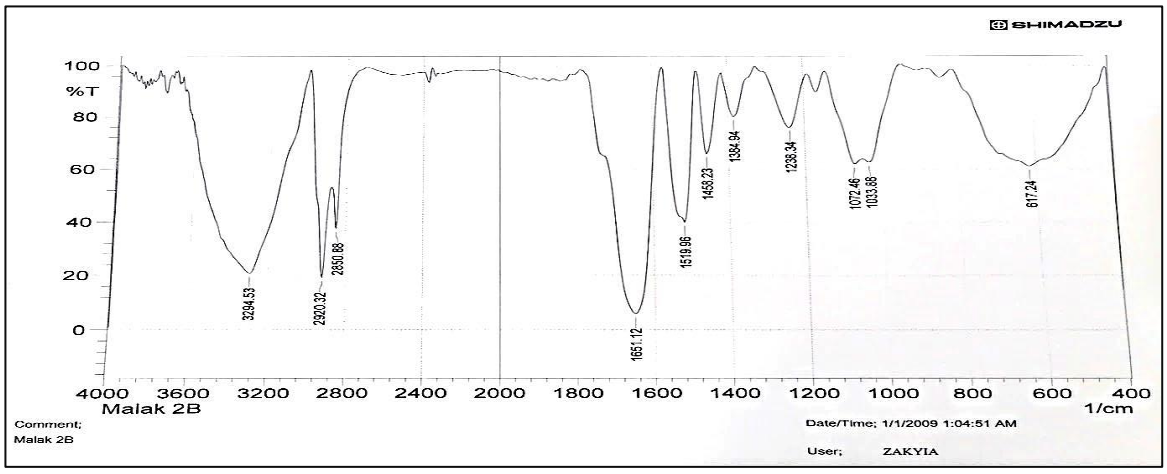

Figure 4. FT-IR spectrum of synthesized AgNPs.

3.1.3. X-ray Diffraction Analysis

X-ray Diffraction spectrometer showed the size and the crystal nature of AgNPs, the AgNPs crystal size rate was measured at 100% peak and using the Debbye Scherrer equation, where the crystal volume rate was 44.33nm.

Table 1. X-ray spectrometer data for nano-silver particles synthesis from *C. spinosa.*

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
12.2417	3.71	2.5190	7.23031	63.37
38.0203	5.86	1.2595	2.36676	100.00
44.3269	1.76	3.1488	2.04357	30.04
77.2959	1.19	1.5360	1.23340	20.29

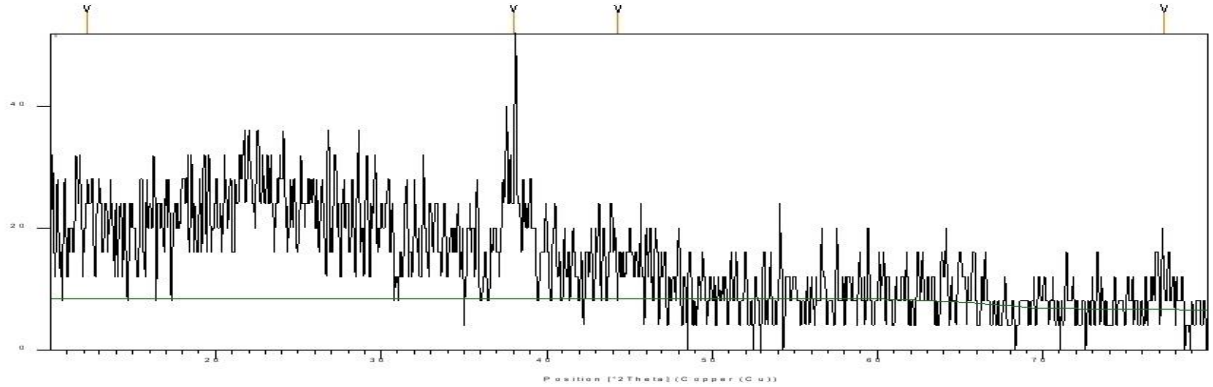

Figure 5. XRD patterns.

3.1.4. SEM Analysis

Figure 6 displayed a SEM image of synthesized AgNPs. The SEM picture showed that the produced AgNPs had a limited size range, falling between 26.09 and 70.70 nm, and were spherical in shape. The scanning electron microscope (SEM) showed nanoparticle silver, spherically shaped, with a difference in size, with a AgNPs size rate of 48.31 nm. This study is consistent with Shaikhaldein *et al* [22] if the particles formed are spherical.

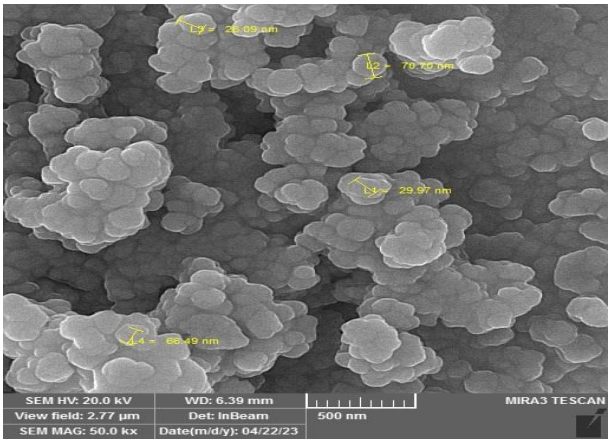

Figure 6. SEM image of synthesized AgNPs.

3.2. Measurement of Plant Characteristics

3.2.1. Measurement of Relative Growth Rate

The relative growth rate of *C. demersum* was the highest relative growth rate in the aquatic extraction of *C. spinosa L.* basin at 0.1 g concentration of 1.085% and lesser 0.745% in the aquatic extraction

basin compared to the control sample of 0.8225% . The high growth rate may be due to the ability of AgNPs to promote plant growth, increase living mass and increase plant tolerance for salt stress [23], which he has demonstrated. [22]. The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles and the concentrations at $\rho \leq 0.05$.

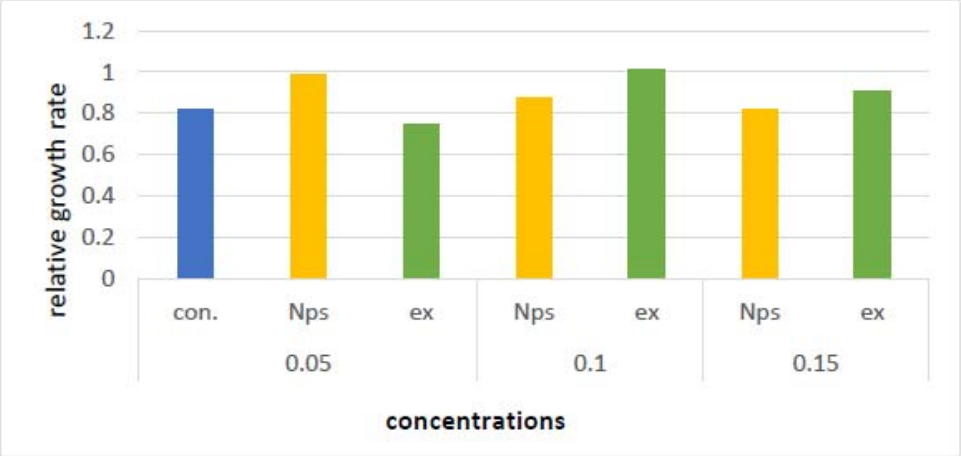

Figure7. Impact of the change in plant extract concentrations and AgNPs on the relative growth rate.

3.2.2. Measurement of Proline

The figure shows the low proline content of *C. demersum* with the increased amounts added from AgNPs and the plant extract, the highest amount of proline was 0.457 µg/g in the basins with nanoparticle silver at 0.05g concentration and lesser 0.151 µg/g in the basin with the aquatic extract of *C. spinosa* plant compared to the control sample. The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles and the concentrations at $\rho \leq 0.05$. The reason for the high proline content of the *C. demersum* plant in the control basin may be due to salt stress, as plants increase the production of proline in cytosol to cope with the adverse effect of sodium chloride toxicity, which causes extra osmosis, so adding AgNPs to the water will play an important role in helping plants reduce proline production, which results are similar to Shaikhaldein *et al* [22]. And the increase in proline in plant leaves is the organization of Osmosis cells to resist harsh conditions, including salinity [24].

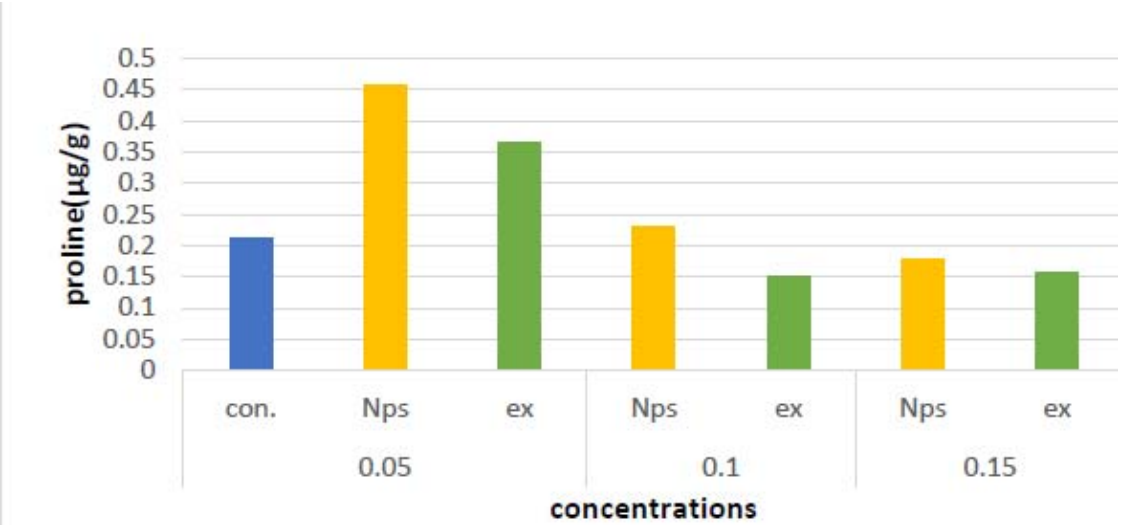

Figure 8. Impact of the change in plant extract concentrations and AgNPs on proline content.

3.2.3. Measurement The chlorophyll a, b, Total, Carotene

The results showed that the value of chlorophyll a,b,c, and total in aquatic extract treated basins and AgNPs treated basins were higher than control basins, were the highest value of chlorophyll a 0.33 mg/g at 0.05g in the AgNPs treated basins and the lowest value of 0.126 mg/g in the aquatic extract treated basins at 0.1g , While, the highest value of chlorophyll b 0.294 mg/g at 0.05 in the AgNPs treated basins and the lowest value of 0.126 mg/g bin the aquatic extract treated basins at 0.1g . the highest value of chlorophyll total 0.605 mg/g at 0.05g in the AgNPs treated basins and the lowest value of 0.252 mg/g bin the aquatic extract treated basins at 0.1g . and also the highest value of carotenoid 0.0129 mg/g at 0.05g in the AgNPs treated basins and the lowest value 0.0049 mg/g in the aquatic extract treated basins at 0.1g. The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles, weeks and the concentrations at $\rho \leq 0.05$ for The chlorophyll a, b, total , and indicated moral differences between plant extracts and the concentrations while there are no moral differences between the nanoparticles for carotene .

According to the study's findings, compared to the control sample, the *C. demersum* in AgNPs and plant extract basins included more chlorophyll. According to Wu [25], the decrease in chlorophyll in the control basin is brought on by salt stress, which also harms green polymers. Additionally, salt stress inhibits the absorption of nutrients required for the synthesis of the chlorophyll molecule. According to [26] [27], salt stress inhibits plant growth by reducing chlorophyll, iron absorption, and iron ion transfer to plant leaves, all of which are caused by oxidative stress. This has also been demonstrated by [28] [29]. The usage of AgNPs, which effectively promotes chlorophyll dye under salt stress circumstances, is the cause of the rise in chlorophyll in treated basins. This work is compatible with Shaikhaldein *et al* [22] because AgNPs have accelerated growth by boosting chlorophyll levels and enhancing optical system performance.

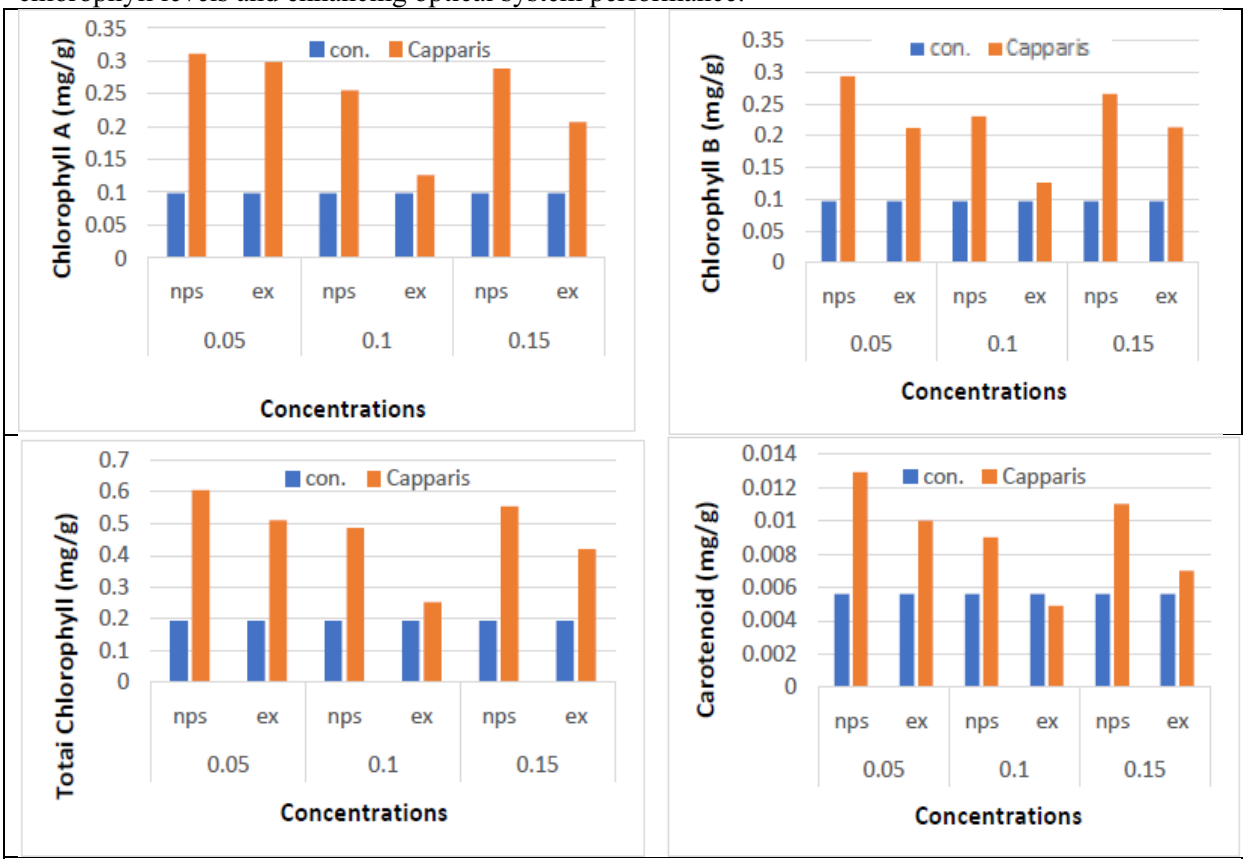

Figure 9. The effect of the change in plant extrace and AgNPs on the amount of chlorophyll A, B, total and carotenoid.

3.2.4. Measurement of Total Organic Carbon

The results of this study showed an increase in the total organic carbon at the end of the experiment and in all treatments compared to the control sample, the highest organic carbon in the aquatic extract basin of 0.21 g and the lowest in the control basin of 0.12 g. The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles, while there are no moral differences between the concentrations at $\rho \leq 0.05$. The significant increase may be due to the increase in the biomass of the *C. demersum* plant as a result of the enhancement of the photography process when processing plants with AgNPs, as it reduces salt stress and facilitates light construction.

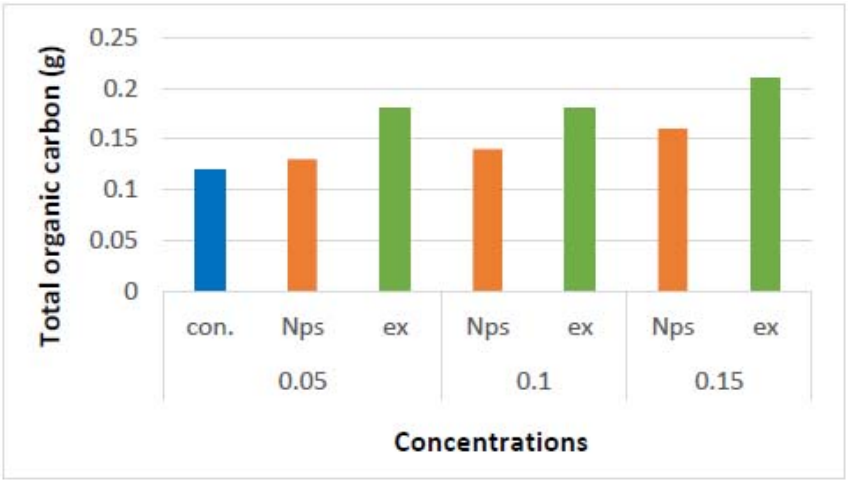

Figure 10. The effect of the change in plant extrace and AgNPs on the total organic carbon.

3.3. Water Physiochemical Parameters

3.3.1. pH

The study showed a higher value of pH in AgNPs and water extracts compared to the control basin, and the decrease of pH in the control basin may be caused by sodium chloride ions and thus a lower rate of light construction, making the basin environment acidic, while the increase in AgNPs and plant extracts is due to the photosynthesis process carried out by aquatic plants, resulting in the consumption of CO2 gas, which makes PH's value lean towards the base [30] [31]. The results of the statistical analysis indicated moral differences between plant extracts and weeks, while there are no moral differences between nanoparticles and the concentrations at $\rho \leq 0.05$.

3.3.2. The Dissolved Oxygen (DO)

The results of this study showed that dissolved oxygen values were higher in AgNPs and aquatic extracts from the control basin in the week of 1,2,4, and close in the third week of the experiment and all the basins. Dissolved oxygen is essential for living [32]. The reason for its decline in the basins was the death of submerged plants, which led to a decline in the photosynthesis process, which is a source of dissolved oxygen, but its height is due to the lighting of the aquatic plants. The results of the statistical analysis indicated moral differences between plant extracts and weeks, while there are no moral differences between nanoparticles and the concentrations at $\rho \leq 0.05$.

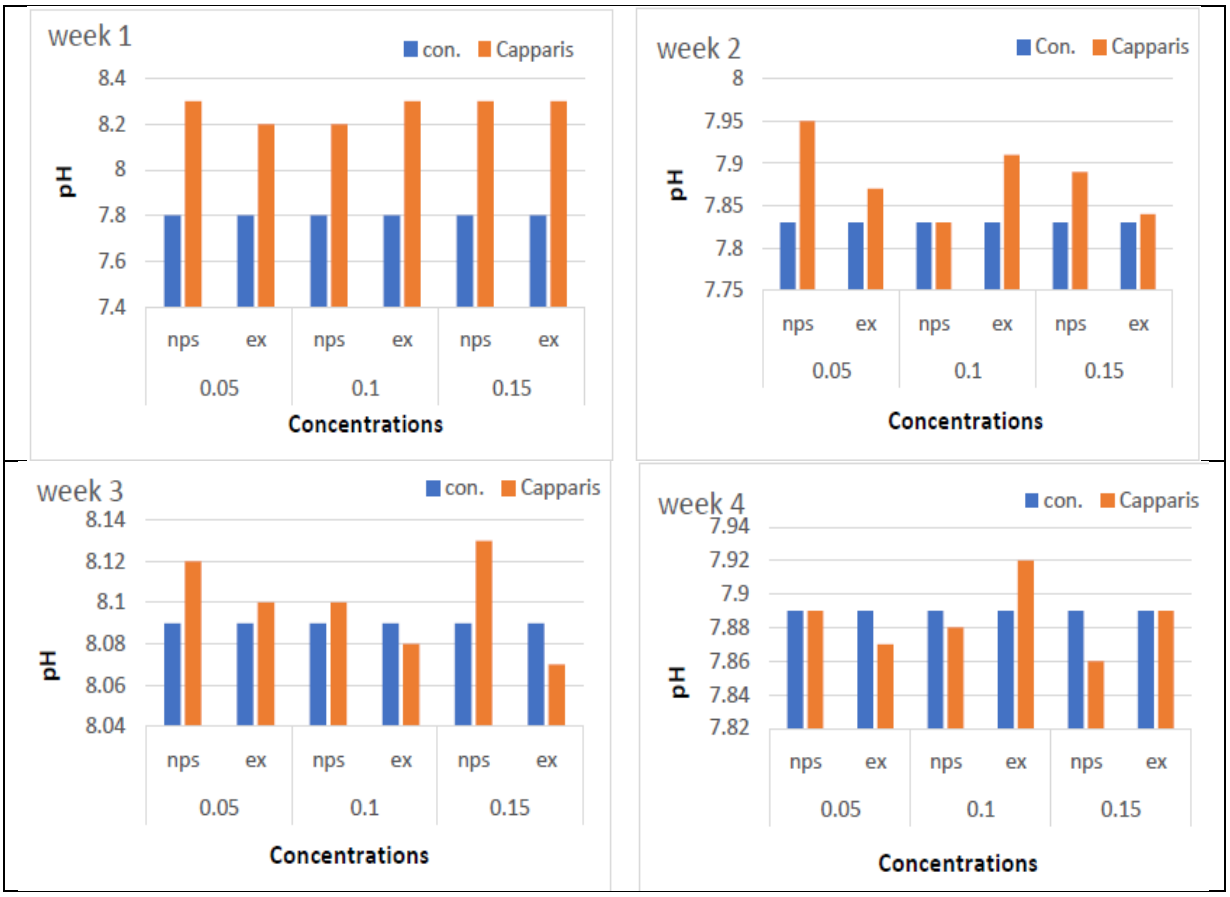

Figure 11. Impact of the change in plant extract concentrations and AgNPs on the values of pH during 4 weeks.

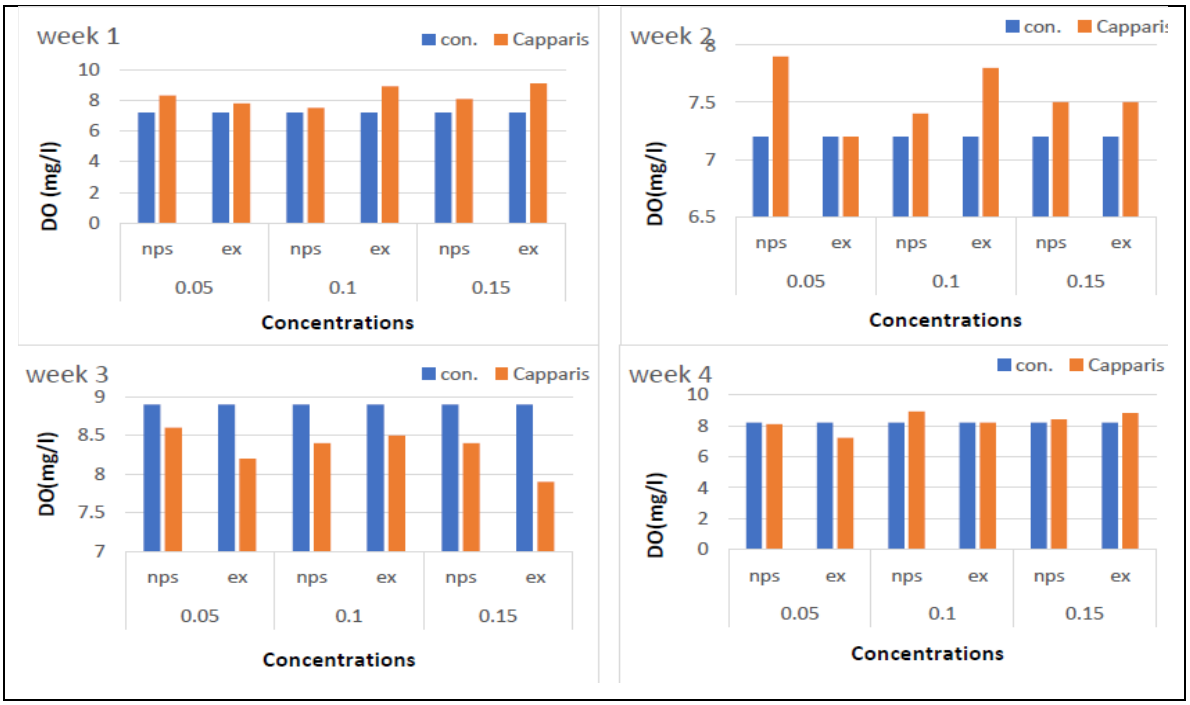

Figure 12. Impact of the change in plant extract concentrations and AgNPs on the values of DO during 4 weeks.

3.3.3. Dissolved Solids

The study showed that the values of dissolved solids in AgNPs and biosynthesis from *C. spinosa* and aquatic extracts in all concentrations (0.05, 0.1, 0.15)g were declining compared to the control sample. At the end of the study period, soluble solids in the control basin rose to 2730 ppm , and in the AgNPs treated basins decreased to (2366, 2265 and 2220) ppm for concentrations (0.05, 0.1,0.15)g and in the aquatic extract treated basins dropped to (2216, 2248 and 2215) ppm for concentration (0.05, 0.1 , 0.15)g . The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles, weeks and the concentrations at $\rho \leq 0.05$.

In a row, the reason for the rise in dissolved solids at the end of the experiment compared to the beginning of the experiment is the increase in the amount of ions and substances from metabolic processes performed by plants, the decrease in treatment basins and the absorption of these substances on the surfaces of these particles.

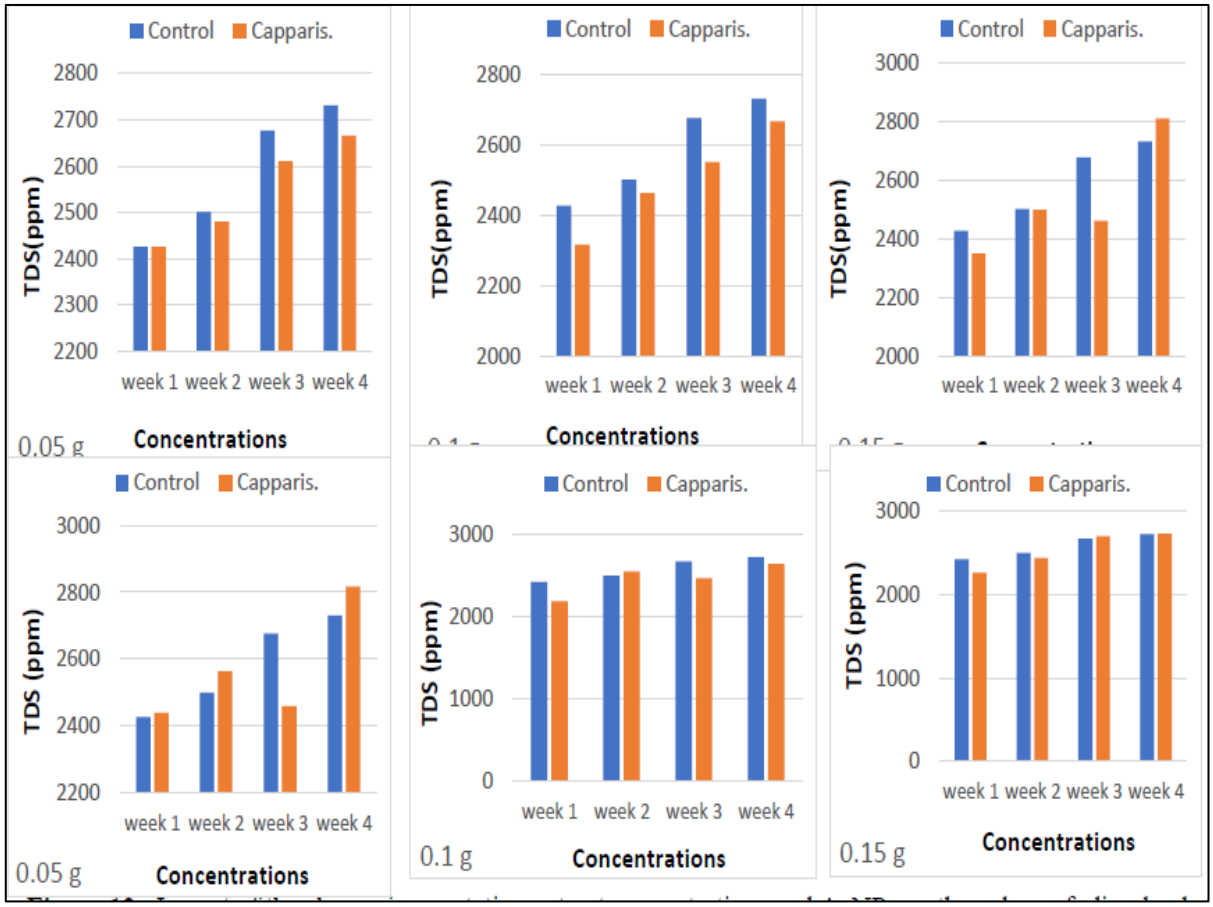

Figure 13. Impact of the change in vegetation extract concentrations and AgNPs on the values of dissolved solids.

3.3.4. EC

The study showed that the values of EC in AgNPs biosynthesis from *C. spinosa* and aquatic extracts in all concentrations (0.05, 0.1, 0.15)g were declining compared to the control sample. At the end of the study period, EC in the control basin rose to 5264 µs/cm , and in the AgNPs treated basins decreased to (4692 , 4006, 4480)µs/cm for concentrations (0.05, 0.1,0.15)g and in the aquatic extract treated basins dropped to (4432 , 4336 ,4290)µs/cm for concentration (0.05, 0.1 , 0.15) g . The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles, weeks and the concentrations at $\rho \leq 0.05$. EC rose in the control basins because of sodium chloride ion, which caused salt stress, and its decline in treated basins was due to the absorption of dissolved ions on the surface of biosynthesis nanoparticles from *C. spinose.*

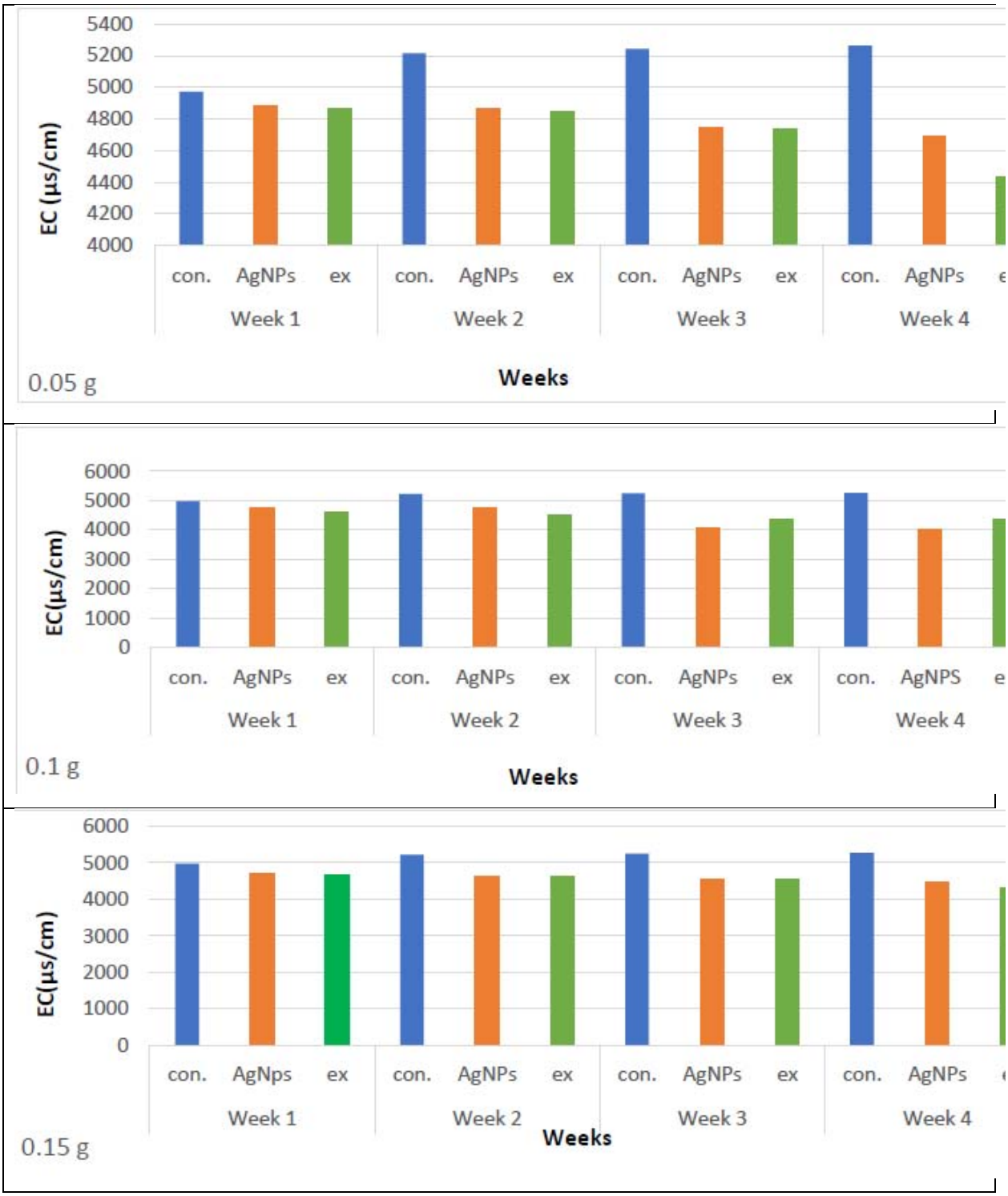

Figure 14. Impact of the change in plant extract concentrations and AgNPs on the values of EC during (0.05, 0.1 ,0.15) g.

3.3.5. Salinity

The investigation demonstrated that, in comparison to the control sample, the salinity values in the biosynthesis of AgNPs from *C. spinosa* and aquatic extracts decreased at all doses (0.05, 0.1, and 0.15) g . At the conclusion of the study period, salinity in the control basin increased to 3368.9 mg/L , while salinity in basins treated with AgNPs decreased to (3002.8, 2563.8, 2867.2) mg/L for concentrations (0.05, 0.1,0.15)g and decreased to (2836.4, 2775,2745.6) mg/L for concentrations

(0.05, 0.1,0.15)g in basins treated with aquatic extract. The results of the statistical analysis indicated moral differences between plant extracts, nanoparticles, weeks and the concentrations at $\rho \leq 0.05$. Salinity is a significant aquatic environmental component that has an impact on the dispersion and spread of submerged plants, which has an impact on the viability of aquatic plants [33]. and also [34] has shown that high salinity concentrations have an adverse effect on the survival and growth of aquatic plants. The adsorption of salt ions on the surface of these particles is what causes the decrease in salinity in basins treated with AgNPs. According to Shaikhaldein *et al*. [22], salt stress slows down the evolution of oblong-folia M, which in turn slows down plant growth. The use of AgNPs promotes the growth of aquatic plants, increases biomass, and improves the plants' tolerance for salt stress. The low growth of the plant is likely caused by the plants' inability to absorb enough nutrients or by the increased absorption of sodium ions [35,36].

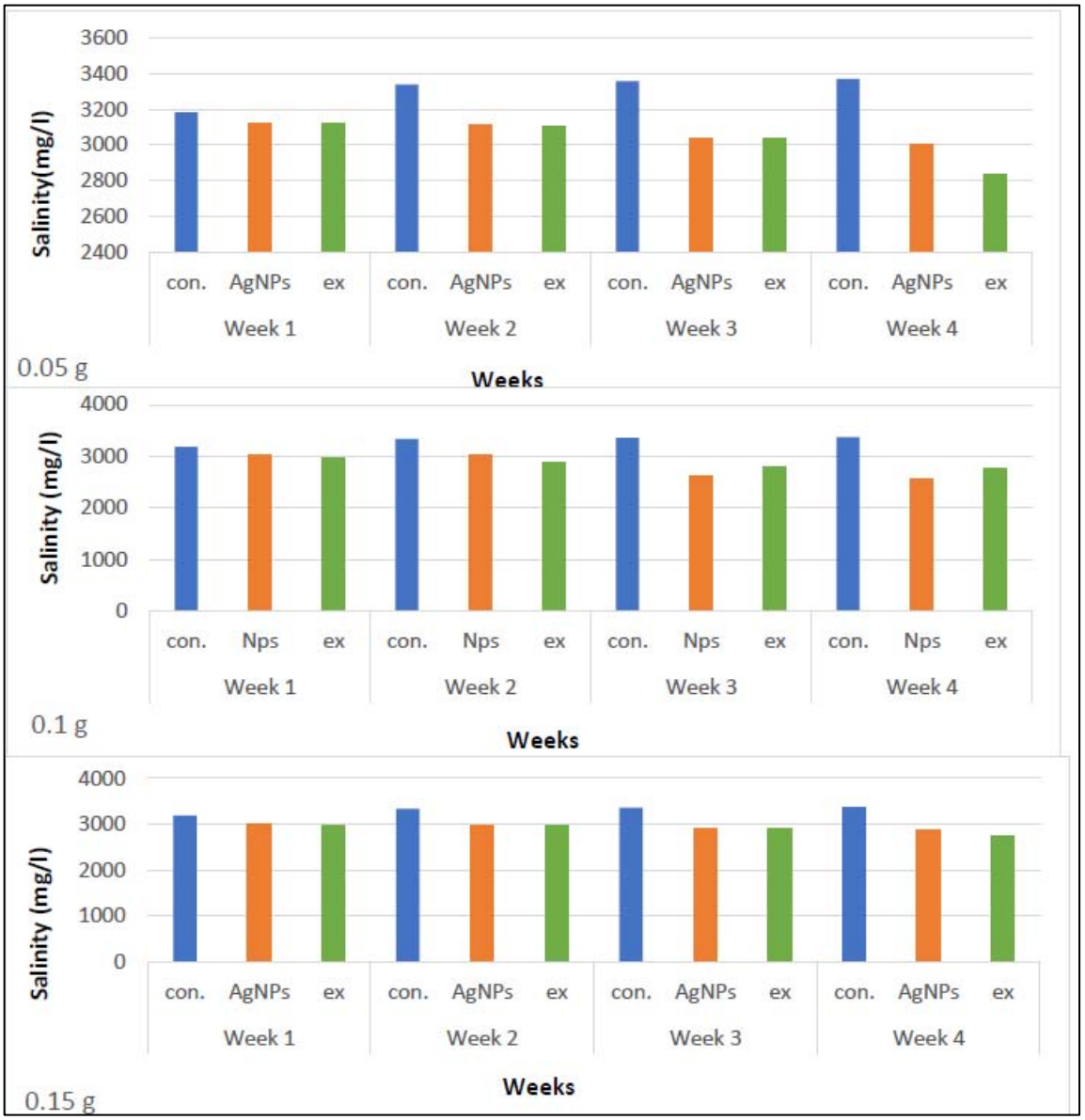

Figure 15. Impact of the change in plant extract concentrations and AgNPs on the values of salinity during (0.05, 0.1 ,0.15) g.

Conclusions

The result of the present study revealed that AgNPs have an effective and important role to play in changing the characteristics of the aquatic environment towards better direction, increasing the tolerance of aquatic plants for salt stress, and improving the anatomy and visual properties of plants under the influence of salt stress. He also suggested that the use of AgNPs was an environmentally friendly, useful and cheap way of reducing salt stress in the aquatic environment and increasing plant tolerance and growth against salt stress.

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