Impact of gamma-irradiated silver nanoparticles biosynthesized from Pseudomonas aeruginosa on growth, lipid, and carbohydrates of Chlorella vulgaris and Dictyochloropsis splendida.

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\textbf{ABSTRACT}
In this study, the biosynthesis of extracellular silver nanoparticles (AgNPs) was obtained using Pseudomonas aeruginosaisupernatant integrated with gamma radiation. The biosynthesis was confirmed by UV-Vis Spectrophotometer. The Atomic Force Microscopy (AFM) recorded the spherical nanoparticle sizes of 10 nm, while the Dynamic Light Scattering (DLS) recorded the size range of 6.7 to 12.1 nm (84.2\%) and the particles were monodispersed. The Gas Chromatography/Mass Spectroscopic analysis of the bacterial filtrate suggested the presence of ethylene glycol derivatives, which may act as a reducing agent. In addition, the impact of synthesized AgNPs on the cell growth and metabolite contents of Chlorella vulgaris and Dictyochloropsis splendida was evaluated. The low AgNPs concentrations (1, 3, and 5 mg L\textsuperscript{-1}) enhanced the lipid production but at the expense of cell growth. All AgNPs concentrations however displayed negative effects on the carbohydrates content. The fatty acid profile of the microalgal species studied was significantly affected by the addition of AgNPs. The saturated fatty acids represented the highest composition (61–67\%) of the total fatty acids, and palmitic acids (16:0) were dominant (43.06–46.57\%). Lipids of this composition could withstand autoxidation during storage and are perfect feedstock for biodiesel and other lipid-based applications.

1. Introduction
Microalgal cell growth and secondary metabolite production can be enhanced by manipulating the cell growth and culture conditions. Excess macro/micronutrients could elevate cell growth while limited nutrient levels could promote lipid content of green and brown microalgae in photobioreactor and open-tank systems (Shah & Abdullah, 2018; Abo-State et al., 2019). The elicitation of compounds in different microalgal and cyanobacterial species has also been affected by metal and metal oxide nanoparticles (NPs) (such as Zn, Mg, Se, Cu, TiO\textsubscript{2}, Fe\textsubscript{3}O\textsubscript{4}, MgO). Metal NPs have unique physicochemical characteristics including catalytic and antimicrobial activities and the incorporation of nanomaterials are increasing in a number of commercial products such as cosmetics, medicine, food packaging, odor-resistant textile, household applications, and medical devices (Chaudhari et al., 2012). With their nano-sizes and highly precise surface areas, the metal NPs can easily penetrate the cell wall and interact with internal cellular biomolecules (Razack et al., 2016). The use of trace or low NPs concentrations has reportedly induced stimulation of algal biomass and pigment content with enhanced lipid production (Kang et al., 2014; He et al., 2017; Sibi et al., 2017; González-Fernández et al., 2020; Tayemeh et al., 2020).

Silver is known to exhibit inhibitory effects on micro-organisms and is commonly used in medical and industrial processes (Jiang et al., 2009). Silver nanoparticles (AgNPs) are one of the most widely synthesized particles which have effects on the growth of plant, algae, and microorganisms (Alshehdi & Bokhari, 2020; Bakht-Dalir et al., 2020; Khoshnamvand et al., 2020). The wide range and potent antimicrobial properties of silver and AgNPs have found broad applications in the biomedical sector, food, and household products, cosmetics, clothing, electrical gadgets, and equipments, toys, and the environmental sector such as in water and air purification (Marambio-Jones & Hoek, 2010). The AgNPs have exhibited different degrees of in vitro cytotoxicity (Hussein et al., 2020a) and antimicrobial activities (Hussein et al., 2020b). The impacts of AgNPs on diatom, Skeletonema costatum (J. Huang et al., 2016), and the eukaryotic green algae (Dash et al., 2012) have been well documented. The AgNPs have reportedly disrupted the cell wall and promoted the release of intracellular components such as carbohydrate and lipid from C. vulgaris for biofuel (Razack et al., 2016). The AgNP–Algae interactions may actually...
cause oxidative stress from excessive Reactive Oxygen Species (ROS) (D. He et al., 2012), which ultimately diverts the algal metabolic pathway away from the growth pathways into the production of hydrocarbon (lipids or carbohydrates) as storage compounds (Sibi et al., 2017).

The synthesis of AgNPs has been performed using the chemical and physical processes (Irvani et al., 2014). These routes nevertheless have led to the generation of harmful and toxic by-products that the inclination has now shifted toward the biosynthesis of AgNPs (D. Tripathi et al., 2019). Gamma radiation-induced synthesis of NPs may have unique advantages, as compared to the conventional chemical methods, such as providing control of the size, shape, and scalability with minimal steps to follow; use of non- or low toxic precursors with fewer chemical reagents or nontoxic solvents; low reaction by-product formation, and low hazardous waste generation, leading to a more environmental friendly route (Flores-Rojas et al., 2020). The NPs, nevertheless, are not thermodynamically stable in the solution due to their high surface energy and tend to aggregate to form larger particles, thus losing their special characteristics. In order to avoid aggregation during the radiation process, suitable capping agents or stabilizing agents can be used. However, many organic capping agents are themselves toxic and pollute the environment (N. M. Huang et al., 2009).

The biosynthesis from biological sources such as microorganisms, algae, and plants has evolved as a green alternative to the traditional chemical synthesis (Biswal & Misra, 2020). The biosynthesis may occur intracellularly or the NPs may be extracellularly dispersed to the reaction solution from which the NPs can be separated out by physical means (Razack et al., 2016). Several compounds such as the hydroxyl or carbonyl groups, terpenoids, phenolics, alkaloids, proteins, amines, and pigments, in the biological filtrate may act as reducing agents to trigger the metal NPs biosynthesis (Asmathunisha & Kathiresan, 2013). Some green capping agents such as starch, chitosan, laurate, and natural compounds in the biological extracts can be used as stabilizing agents to negate the aggregation of NPs (N. M. Huang et al., 2009). The biosynthesis of AgNPs, in certain conditions, is also attributed to the presence of enzyme nitrate reductase (Anthony et al., 2014). There are still limited studies that have investigated the effects of high gamma radiation on the biosynthesis of NPs (El-Batal et al., 2020; El-Sayyad et al., 2020).

The objectives of this study were to biosynthesize the AgNPs using bacterial supernatant of Pseudomonas aeuruginosa strain (Accession no. 3NPO614) where the reaction solution was exposed to gamma irradiation. The UV-Vis spectroscopic, morphological, size, and dispersion characteristics of the produced AgNPs were analyzed. The effects of the biosynthesized AgNPs on the cell growth, lipid, carbohydrate, and free fatty acids of the green microalgae, Chlorella vulgaris, and Dictyochloropsis splendid, were investigated.

2. Materials and methods

2.1. Bacterial strain cultivation and extracellular biosynthesis of AgNPs

The bacterial strain for AgNPs biosyntheses was isolated from petroleum-polluted soil at Suez Canal, Egypt. It was identified by 16S rRNA as Pseudomonas aeruginosa with Accession no. 3NPO 614.

It was grown on LB broth medium (Martin et al., 1981) for 48 h at 37°C. The cell-free suspension after centrifugation (8000 rpm for 10 min, Hettich1635, Germany) was mixed with 3 mM AgNO₃ (Sigma Chemical Co., USA). The solution was incubated overnight at 35°C and then exposed to the gamma irradiation of 100 Gy (Co-60 unit, 4000-A-India) for 5 min. at 1.2 kGy/h, at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The bacterial supernatant (control) was subjected to the same dose and duration. The color change of the reaction mixture from pale yellow to brown indicated the formation of AgNPs. To evaluate the effect of gamma radiation on AgNPs biosynthesis, 3 mM AgNO₃ was separately irradiated by 100 Gy.

2.2. Characterization of AgNPs

UV-Vis spectrophotometer (T60, UK) was utilized to confirm the successful formation of AgNPs. The morphology and size of AgNPs were analyzed by Atomic Force Microscopy (AFM) (Agilent 5500 AFM Scanning Probe Microscopy, USA). The images were recorded at different magnification ranges using a silicon cantilever with a force constant of 42 Nm⁻¹. Dynamic Scattering Light (DSL) was used to determine the size and dispersion of biosynthesized AgNPs. The size of AgNPs was determined using the Nano ZS Zetasizer system (Malvern Instruments). Before DSL measurement, the supernatant was passed through a 0.2 μm polyvinylene fluoride (PVDF) membrane. The sample was loaded into a quartz microcuvette and five measurements were performed and the mean was recorded. A laser wavelength of 633 nm (He-Ne), a scattering angle of 173° (fixed without any possibility for changes), a measurement temperature of 25°C, a medium viscosity of 0.8872 mPAs, and a medium refractive index of 1.330 and material refractive index of 0.200.

2.3. Determination of the bioreductant in the bacterial filtrate

The bacterial filtrate before the reaction with AgNO₃ was analysed by Trace GC1300-TSQ Mass Spectrometer(GC/
8.2.4.1. Determination of lipid content

Lipid was extracted using a mixture of chloroform: methanol: deionized water (1:1:0.9) ratios according to Bligh and Dyer (1959). The lipid content was expressed as the percentage of the cell dry weight:

\[ L = \frac{W_L}{W_B} \times 100 \]  

where \( L \) is the lipid content (% DW), \( W_L \) and \( W_B \) are the weights of the lipid and the dry biomass, respectively.

The lipid productivity \((LP, \text{g L}^{-1}\text{d}^{-1})\) was calculated as follows (Hempel et al., 2012):

\[ LP = BP \times L \]  

where \( BP \) (g L\(^{-1}\) d\(^{-1}\)) is the biomass productivity and \( L \) (% DW) is the lipid content.

Lipid yield \((LY, \text{mg L}^{-1})\) was calculated according to Yang et al. (2014):

\[ LY = BY \times L \]  

2.4.3. Fatty acids analyses

The extracted lipid was transesterified by methanol and catalyst (2% H\(_2\)SO\(_4\)) according to Christie (1993). The Fatty Acids Methyl Esters (FAME) mixture (99.9% pure, Sigma–Aldrich) were used as a standard to analyze the FAME content. The fatty acid analysis was performed by Gas Chromatography (Perkin Elmer Auto System XL) equipped with a flame ionization detector and a DB5 silica capillary column (60 m x 0.32 mm i.d.). The oven temperature was maintained initially at 45°C and programmed to 60°C at a rate 1°C min\(^{-1}\), and later programmed from 60°C to 240°C at a rate of 3°C min\(^{-1}\). Helium was used as the carrier gas at a flow rate of 1 mL min\(^{-1}\). The injector and the detector temperatures were set at 230°C and 250°C, respectively. The FAME external standard gave rise to well-individual peaks that allow the identification of the fatty acid composition by comparing their retention times and peaks with those of the standards.

2.4.4. Determination of carbohydrates

The phenol-sulfuric acid method (Liu et al., 1973) was used for carbohydrate determination. The carbohydrate

2.4. Effects of AgNPs on microalgal growth, lipid and carbohydrates

The green microalgal species, Chlorella vulgaris, and Dictyochloropsis splendida were cultured on BG-11 medium (Stanier et al., 1971), consisting of (g L\(^{-1}\)) 1.50 NaNO\(_3\), 0.04 K\(_2\)HPO\(_4\), 0.075 MgSO\(_4\)\(\cdot\)7H\(_2\)O, 0.036 CaCl\(_2\)\(\cdot\)2H\(_2\)O, 0.006 Citric acid, 0.006 Fe ammonium citrate, 0.02 Na\(_2\)CO\(_3\), 0.001 Na-EDTA, and 1 ml of trace metal solution per liter of double-distilled water. The trace metal solution contained (g L\(^{-1}\)): 2.86 H\(_3\)BO\(_3\), 1.81 MnCl\(_2\)\(\cdot\)4H\(_2\)O, 0.222 ZnSO\(_4\)\(\cdot\)7H\(_2\)O, 0.39 Na\(_3\)MoO\(_4\)\(\cdot\)2H\(_2\)O, 0.079 CuSO\(_4\)\(\cdot\)5H\(_2\)O, 0.0494 Co (NO\(_3\))\(_2\)\(\cdot\)6H\(_2\)O. The cultures were grown for 25 days under continuous illumination of 40 \(\mu\)E m\(^{-2}\) s\(^{-1}\) at 25 ± 1°C, with the constant bubbling of air filtered through a microporous filter of 0.22 \(\mu\)m pore size.

The effects of AgNPs concentrations (1, 3, 5, 15, 25, and 50 mg L\(^{-1}\)) on microalgal cell growth, lipid and carbohydrate contents were studied in 500 mL Erlenmeyer flasks under the previously described growth conditions.

2.4.1. Determination of cell growth and kinetics parameters

The growth was determined by optical density (OD) spectrophotometrically at 680 nm using 3 mL of culture at a regular interval of 5 days in triplicates. The dry weight (DW) was determined gravimetrically, 20 mL of culture suspensions were filtered through filter paper (0.45 \(\mu\)m), dried at 60°C for 24 h, and the DW (g L\(^{-1}\)) was estimated.

The maximum specific growth rate, \(\mu_{\text{max}}\) (d\(^{-1}\)), was calculated as

\[ \mu_{\text{max}} = \frac{1}{t} \times \ln \frac{x_t}{x_0} \]  

where \( x_t \) and \( x_0 \) are the DW (g L\(^{-1}\)) at the final and starting of the batch culture, respectively, and \( t \) is the duration of the experiment (day).

The biomass productivity \((BP, \text{g L}^{-1}\text{d}^{-1})\) and the biomass yield \((BY, \text{g L}^{-1})\) were calculated as follows (Vidyashankar et al., 2015):

\[ BP = \frac{(X_t - X_0)}{(T_2 - T_1)} \]  

\[ BY = \frac{(X_t - X_0)}{t} \]  

where \( T_1 \) and \( T_2 \) (day) represents the incubation period of an experiment at the initial time (day 0) and the final day of incubation, respectively.
concentration was estimated from the calibration curve of glucose at 10–150 µg mL⁻¹.

2.5. Statistical analyses

The one-way ANOVA and the Tukey’s test at a reliability level of p < 0.05 were used to estimate the significance. Statistical analyses were carried out using Minitab software (V18, Minitab Inc., State College, PA, USA). All the experiments were conducted in triplicate.

3. Results and discussion

3.1. Biosynthesis and characterizations of AgNPs

The AgNPs formation from silver nitrate solution incubated with *P. aeruginosa* supernatant overnight at 35°C was demonstrated by the color change of the reaction mixture to brown. The UV-Vis spectrometry exhibited the maximum absorption of the AgNPs at 455 nm Figure 1(a), which was consistent with the recorded range at 400–475 nm of AgNPs synthesized by various bioreductant such as bacterial supernatant (Abo-State & Partila, 2015, 2018; Razack et al., 2016; Abo-State et al., 2017; Hussein et al., 2020a), fungal filtrates (Gudikandula et al., 2017), algal extracts (Abdel-Raouf et al., 2017; Arévalo-Gallegos et al., 2018) or plant extracts (Tripathi et al., 2017; Tahir et al., 2018; Ahmad et al., 2020). The AFM image suggested the spherical shape of the AgNPs with a size range of 6–12 nm, monodispersed Figure 1(b).

Table 1 shows the size of AgNPs synthesized from irradiation of 100 Gy and supernatant of *P. aeruginosa* irradiated by 100 Gy and Figure 2 (a) exhibits the AgNPs size distribution based on DLS analysis. The AgNO₃ solutions singly irradiated by gamma rays at 100 Gy dose produced AgNPs of 29.39–70.89 nm in which 90.9% were in the 34–52 nm range Table 1 and Figure 2 (a). The exposure of the bacterial supernatant reaction mixture to the same dose of gamma rays produced the AgNPs of 6–12 nm, with 84.2% in the 6.77–12.18 nm size range, 14.8% at 14.4–21.18 nm; and 1% in 25.37–39.40 nm range Table 1 and Figure 2(b). In comparison, the AgNPs sizes were 5–35 nm when *P. aeruginosa* filtrate is mixed with AgNO₃ solution and incubated at 85°C, pH 7, and 30 min (Abo-State &

![Figure 1](image_url)

Figure 1. (a) The UV--Vis spectrum, (b) AFM images of mono-dispersed AgNPs synthesized by *P. aeruginosa* supernatant irradiated by 100 Gy.
This means that the combination of the biological method and gamma irradiation (100 Gy) produced the AgNPs of smaller nano sizes than those produced by the biological method only. A similar finding has been reported that the property of the grown crystals, production properties, and function of copper oxide NPs (CuO NPs) synthesized by Penicillium chrysogenum, is improved after being exposed to 50 K Gy gamma irradiation (El-Batal et al., 2020). The gamma-rays interact with the matters in aqueous solution, and through a photoelectric absorption, and as a result of radiolysis of water, free electrons such as hydrated electrons and hydrogen radicals are generated which act as reducing agents to convert the metal ions (M$^+$) to zero-valent state (M$^0$) (Chen et al., 2007).

### 3.2. Determination of reducing agent

The GC/MS analyses of the bacterial filtrate revealed the predominant presence of ethylene glycol derivatives Table 2 that may have a role in the reduction of Ag$^+$ to Ag$^0$, as well as acting as a capping agent with other compounds in the filtrate to prevent the NPs aggregation Figure 3. Some bacteria such as *Pseudomonas aeruginosa* Table 1. The size of the AgNPs synthesized from 100 Gy irradiation of AgNO$_3$ and the supernatant of *P. aeruginosa* irradiated by 100 Gy.

| AgNO$_3$ Irradiated by 100 Gy | Supernatant of *P. aeruginosa* irradiated by 100 Gy |
|-----------------------------|---------------------------------------------|
| Size of AgNPs (nm) | Volume (%) | Size of AgNPs (nm) | Volume (%) |
| 29.39 | 5.5 | 6.78 | 5.60 |
| 34.03 | 21.0 | 7.84 | 18.40 |
| 39.41 | 32.2 | 9.08 | 25.40 |
| 45.64 | 25.8 | 10.52 | 21.20 |
| 52.85 | 11.9 | 12.18 | 13.60 |
| 61.2 | 13.2 | 14.11 | 7.60 |
| 70.89 | 0.4 | 16.34 | 4.00 |
| 82.09 | 0.0 | 18.92 | 2.10 |
| 21.91 | 1.10 |
| 25.37 | 0.50 |
| 29.39 | 0.30 |
| 34.03 | 0.10 |
| 39.41 | 0.10 |

Figure 2. Size distribution of AgNPs synthesized from (a) irradiation by 100 Gy, (b) supernatant of *P. aeruginosa* irradiated by 100 Gy.
may excrete reducing enzymes and reducing agents such as ethylene glycol derivatives or nitrate reductase (Anthony et al., 2014) into the culture media which may be utilized in the formation of nanoparticles. Also, the presence of capping agents in the medium such as the hydroxyl or carbonyl groups, terpenoids, phenolics, alkaloids, proteins, amines, and pigments (Asmathunisha & Kathiresan, 2013) could play a role in the stability of AgNPs. The obtained results were in agreement and conformity with previous reports (Sun & Xia, 2002; Sun et al., 2002). The cubic AgNPs have been reportedly synthesized by the reduction of AgNO₃ using ethylene glycol at 140°C in the presence of polyvinylpyrrolidone (PVP) and HCl (Im et al., 2005). The morphology of the produced AgNPs is suggested to be highly affected by the reaction conditions (temperature, AgNO₃ level, the molar ratio of the units PVP and AgNO₃). Polyol (alcohol-containing hydroxyl group) such as ethylene glycol could act both as the solvent and reducing agent. Three different shapes of AgNPs have been synthesized in the presence of ethylene glycol as a reducing agent (Wiley et al., 2007).

### Table 2. Major compounds in the supernatant of P. aeruginosa as analyzed by the GC/MS.

| Compound name       | R.T.         | Area (%) | Molecular Formula  |
|---------------------|--------------|----------|--------------------|
| Octaethylene glycol | 12.09 – 18.77| 30.40    | C₂₉H₄₂O₈            |
| monododecyl ether   | 22.53 – 31.48|          |                    |
|                     | 32.48– 34.18 |          |                    |
|                     | 34.41– 34.72 |          |                    |
| Heptaethylene glycol| 22.53– 26.95 | 0.48     | C₁₄H₉O₂             |

Figure 3. Schematic diagram illustrating the capping agents attached on the surface of AgNP.

### 3.3. Impacts of AgNPs on microalgal cell growth

Investigations carried out on the AgNPs have focussed mainly on the synthesis, characteristics, antimicrobial activities, and applications in different fields, including the AgNPs toxicity to living organisms, and the inhibition on the aquatic and terrestrial environments. Table 3 and Figure 4 show that the maximum specific growth rates, biomass productivity, and biomass yield of both C. vulgaris and D. splendida progressively decreased with increasing AgNPs concentrations, as compared to the control. These reductions with AgNPs concentrations were in agreement with the growth inhibition of *Parachlorella kessleri* (by 30% and 60%) when exposed to bio- or chemo-synthesized AgNPs (Marzikova et al., 2017). The adverse influence of the AgNPs on the filamentous freshwater green microalgae, *Pithophora oedogonium*, and *Chara vulgaris*, is exhibited in the progressive depletion of chlorophyll content and the mitotic disturbance and accompanied by the morphological malformation (Dash et al., 2012).

The autotrophic algae *Chlamydomonas reinhardii* in the waterbodies, which receive effluents contaminated with various nanoparticles, has been found to experience initial toxic effects leading to the damage of ATP and photosynthesis due to the oxidative stress-induced as a result of exposure to AgNPs (Pillai et al., 2014). Algal cell structure, membrane permeability, and the size of AgNPs affect nanoparticle uptake, translocation, and accumulation (Li et al., 2015). The smaller size and the larger surface area to volume enable AgNPs to pass through the pores of the cell wall and reach the plasma membrane (Samberg et al., 2011). The AgNPs may enter the cell...
Table 3. Kinetics of cell growth and lipid of C. vulgaris and D. splendida.

| Microalgal species | AgNPs (mg L⁻¹) | Max specific growth rate, \( \mu_{\text{max}} \) (d⁻¹) | Biomass productivity, \( BP \) (g L⁻¹) | Biomass yield, \( BY \) (g L⁻¹) | Lipid productivity, \( LP \) (g L⁻¹ d⁻¹) | Lipid yield, \( LY \) (mg L⁻¹) |
|--------------------|----------------|-----------------------------------|----------------------------------|---------------------------------|--------------------------------|--------------------------------|
| C. vulgaris        | 0              | 0.137 ± 0.002*                     | 0.064 ± 0.003*                   | 1.58 ± 0.047*                   | 0.63 ± 0.026*                   | 156.90 ± 5.14*                 |
|                    | 1              | 0.132 ± 0.003*                     | 0.056 ± 0.001*                   | 1.38 ± 0.036*                   | 0.39 ± 0.029*                   | 97.81 ± 4.44*                  |
|                    | 3              | 0.121 ± 0.003*                     | 0.052 ± 0.001*                   | 1.28 ± 0.050*                   | 0.31 ± 0.008*                   | 77.70 ± 2.25*                  |
|                    | 5              | 0.111 ± 0.003*                     | 0.039 ± 0.003*                   | 1.11 ± 0.116 ¹                  | 0.57 ± 0.061 ²                 | 73.76 ± 15.5î                 |
|                    | 15             | 0.092 ± 0.003               ³       | 0.031 ± 0.003                    | 0.76 ± 0.033                   | 0.27 ± 0.031 ³                 | 66.55 ± 3.34                 |
|                    | 25             | 0.088 ± 0.005                     | 0.021 ± 0.002               ³       | 0.62 ± 0.018 ³                 | 0.19 ± 0.026 ³                 | 55.52 ± 1.95                 |
|                    | 50             | 0.071 ± 0.003                ³       | 0.016 ± 0.002                ³       | 0.42 ± 0.022 ³                 | 0.11 ± 0.018 ³                 | 29.95 ± 3.34                 |
| D. splendida       | 0              | 0.121 ± 0.011*                    | 0.041 ± 0.002                   | 1.03 ± 0.063 ³                 | 0.38 ± 0.016 ³                 | 98.71 ± 5.55                 |
|                    | 1              | 0.112 ± 0.011            ³       | 0.031 ± 0.002                   | 0.82 ± 0.030 ³                 | 0.43 ± 0.024 ³                 | 111.20 ± 3.23                 |
|                    | 3              | 0.110 ± 0.007                ³       | 0.029 ± 0.001                ³       | 0.69 ± 0.042 ³                 | 0.39 ± 0.019 ³                 | 96.62 ± 6.60                 |
|                    | 5              | 0.097 ± 0.006                ³       | 0.024 ± 0.003                ³       | 0.59 ± 0.035 ³                 | 0.34 ± 0.039 ³                 | 82.34 ± 5.57                 |
|                    | 15             | 0.088 ± 0.001                ³       | 0.018 ± 0.003                ³       | 0.52 ± 0.031 ³                 | 0.16 ± 0.026 ³                 | 46.61 ± 3.46                 |
|                    | 25             | 0.074 ± 0.003                ³       | 0.015 ± 0.002                ³       | 0.42 ± 0.030 ³                 | 0.13 ± 0.019 ³                 | 36.70 ± 3.23                 |
|                    | 50             | 0.070 ± 0.004                ³       | 0.014 ± 0.003                ³       | 0.35 ± 0.026 ³                 | 0.11 ± 0.016 ³                 | 28.90 ± 2.23                 |

All cultures were incubated under continuous illumination of 40 μE m⁻² s⁻¹ and temperature of 25 ± 1 °C, with constant bubbling of air filtered through a microporous filter of 0.22 μm pore size. Different superscript letters within the same column for each microalgal species indicate a significant difference at \( p < 0.05 \). The data are presented as mean ± standard deviation of three replicates.

Figure 4. Growth curves of (a) C. vulgaris, (b) D. splendida, under the influence of different AgNPs concentrations synthesized by P. aeruginosa supernatant irradiated by 100 Gy. Error bars represent ± standard deviation of three replicates.

membrane (Mueller & Nowack, 2008) and get adsorbed to the different cell organelles and enhance the reactive oxygen species (ROS) that interfere with the cellular biochemical reactions (Miao et al., 2010). From adhesion to the cell membrane, as an alternative to permeability or ion transport properties, the AgNPs may disturb the cellular phosphate management. This is followed by the inhibition of the DNA synthesis by breaking the hydrogen bonding and induces the denaturation of ribosomes and inactivation of the enzymes and proteins by occupying the active sites (Taylor et al., 2016).

Algae have specific mechanisms to tolerate and reduce the toxic effects of nanoparticles in general.
On the entry of nanoparticles, algae may release secrete certain compounds (metal-chelators) to increase the nanoparticle flocculation and decrease its toxicity or availability (Taylor et al., 2016). Also, the algal defense system generates low molecular weight antioxidant substances and enzymes to combat and scavenge the excess ROS generated. The enhancement of generation of antioxidant enzymes has been reported in *Chattonella marina* as a defense mechanism against the adverse effect of AgNPs on PS II, which may involve the inhibition of electron transport activity and the alteration of oxygen evolution (D. He et al., 2012; J. Huang et al., 2016). Silver ions and AgNPs have reportedly altered the cell division and gene expression (cd2 gene) in *Allium cepa* (onion) (Fouad & Hafez, 2018). The AgNPs or silver ions foliar application to 4-week old cucumber (*Cucumis sativus*) plant significantly alters the metabolite profile with the activation of the antioxidant defense system, and consequently inhibits respiration, alters membrane properties, and reduces inorganic nitrogen fixation (Zhang et al., 2018).

### 3.4. Effects of AgNPs on lipids and carbohydrates

Table 3 and Figure 5 (a) show that 5 mg L\(^{-1}\) of AgNPs in *C. vulgaris* promoted the lipid content to be elevated to 14.3% while 1–5 mg L\(^{-1}\) of AgNPs in *D. splendida* had the lipid content remained high at 14%. Further increase in AgNPs level resulted in growth retardation and reduced lipid content compared to control in both species. In general, *D. splendida* showed lower biomass growth and lipid productivity, yield, and content than the *C. vulgaris*. This result suggests that the effects of AgNPs may be species-dependent or may suggest that *D. splendida* was more susceptible to the oxidative stress than *C. vulgaris*. Figure 5(b) illustrates that increased AgNPs concentrations induced a significant and progressive decrease in carbohydrate yield in *C. vulgaris* and *D. splendida*.

Improved lipid production has also been reported in *C. vulgaris* treated with TiO\(_2\) and MgO nanoparticles which is attributable to induced oxidative stress (Kang et al., 2014). *C. vulgaris* has been cultivated in growth media containing different concentrations of metal nanoparticles (Cu, Zn, Mg,

![Figure 5](image_url)

**Figure 5.** (a) Lipid contents, (b) Carbohydrate contents, of *C. vulgaris* and *D. splendida* cultured at different treatment of AgNPs concentrations synthesized by *P. aeruginosa* supernatant irradiated by 100 Gy. Different small letters on the bars indicate significant difference (p < 0.05). Error bars represent ± standard deviation of three replicates.
Pb) to induce firstly the metal resistance capacity, before being cultivated in second media containing the metal salts of the corresponding nanoparticles under the same-controlled culture conditions. As a result, the growth rate, biomass, algal pigments, protein, carbohydrates, and lipid production have increased depending on the salt concentration as compared to the control and wild strain (Sibi et al., 2017). In a study on the effect of carbon, ferric oxide, and magnesium oxide NPs on the green alga Scenedesmus obliquus, it is suggested that the algal metabolism modifies its normal pathways toward, in most cases, lipid production (M. He et al., 2017). The AgNPs may break open the cell wall of C. vulgaris for the excretion of biomolecules such as proteins or lipids for biodiesel production (Razack et al., 2016).

### 3.5. Effects of AgNPs on fatty acid profile

Table 4 shows the lipid profile of C. vulgaris and D. splendida before and after AgNPs treatment. The fatty acid profile of both studied algae were affected by the treatment with biosynthesized AgNPs. The nanoparticles may alter the metabolism of many algal species toward hydrocarbon production (lipids and/or carbohydrates) (Kang et al., 2014; He et al., 2017; Sibi et al., 2017).

In addition, the carbon chain lengths were from C12 to C24. Generally, the increasing chain length will lead to an improvement in the heat of combustion, viscosity, and cetane number, which means that C16–18 fatty acids are desirable for biodiesel (Francisco et al., 2010). The saturated fatty acids (SFAs) were increased in C. vulgaris and D. splendida (61.47% and 67.27%, respectively) as compared to the controls (54.88% and 52.81%, respectively). In contrast, the unsaturated fatty acids (UFAs) were decreased from 45.11% to 39% and from 47.18% to 32.73%, and the monounsaturated fatty acids (MUFA) from 30.99% to 14.33% and from 33.57% to 15.75%, respectively, in the AgNPs-treated algae. The fatty acids, lauric, linolenic, behenic, and lignoceric acids were recorded after the AgNPs treatment. However, heptadecanoic and eicosadienoic acids disappeared after the AgNPs treatment. Fatty acids production such as penta-decanoic, linoleic, and linolenic acids were elevated, while stearic, oleic, and arachidic acids were reduced upon the AgNPs treatment as compared to the control. Palmitic acid (16:0) at 43.06% and 46.57% represented the highest composition, respectively, in the investigated algae, and linoleic acid was the second-highest fatty acid content at 20.62% and 20.12%, respectively. The high composition of SFAs C. vulgaris and D. splendida, after AgNPs treatment as compared to the UFAs, suggests that the produced lipids tend to be more stable and not susceptible to autoxidation (peroxidation) during storage. Also, the FAMEs profiles of AgNPs-treated algae contain a remarkable percentage of polyunsaturated fatty acids (PUFAs). Based on the European standard (EN 14,214) for biodiesel, the polyunsaturated fatty acids (≥3 double bonds) should be 1%, which could affect the properties of biodiesel (Branco-Vieira et al., 2017). These lipids are a promising feedstock for biodiesel production and can be used blended with petroleum oil for transportation and other applications.

### 4. Conclusion

The biosynthesis of AgNPs was successfully achieved from the reaction of AgNO₃ and the P. aeruginosa supernatant, and the reaction mixture exposure to 100 Gy irradiation. The AgNPs biosynthesis was confirmed and the shape was spherical with 84.2% were within 6–12 nm sizes. The combination of the biological method and gamma irradiation (100 Gy) therefore had produced the AgNPs of smaller nano sizes than those produced by the biological method only. The introduction of the AgNPs at low concentrations of AgNPs (1, 3, and 5 mg L⁻¹) had elevated the lipid levels and improved the fatty acid profile, but at the expense of the cell growth and carbohydrate contents. The palmitic acid (16:0), as well as the saturated fatty acids, were predominant, suggesting the excellent composition that could withstand the autoxidation during storage, and suitability for use as feedstocks for biodiesel production and other lipid applications.

### Disclosure statement

The authors declare that they have no conflict of interest.
References

Abdel-Raouf, N., Hozayen, W. G. M., El Neem, M. A., & Ibraheem, I. (2017). Potentiality of silver nanoparticles prepared by ulvafasciata as anti-nephrotoxicity in albino rats. *Egypt. Journal of Botany*, 57(3), 479–494. https://doi.org/10.21608/ejbo.2017.9131070

Abo-State, M. A. M., & Partila, A. M. (2015). Microbial production of silver nanoparticles by *Pseudomonas aeruginosa* cell free extract. *Journal of Ecological Health and Environment*, 3(3), 91–98. http://www.naturalspublishing.com/files/published/3tg77106bx5kb.pdf

Abo-State, M. A. M., & Partila, A. M. (2018). Production of silver nanoparticles (AgNPs) by certain bacterial strains and their characterization. *Novel Research in Microbiology Journal*, 2(1), 19–32. https://doi.org/10.21608/nrmj.2018.58384

Abo-State, M. A. M., Partila, A. M., & Fathy, S. (2017). The bactericidal activities of silver nanoparticles (AgNPs) produced by cell-free supernatant of *Pseudomonas aeruginosa* and sterilization by the effect of radiation. *Journal of Ecological Health and Environment*, 5(2), 49-56. http://www.naturalspublishing.com/files/published/2344b3e2h9tol2.pdf

Abo-State, M. A. M., Shanab, S. M. M., & Ali, H. E. A. (2019). Effect of nutrients and gamma radiation on growth and lipid accumulation of *Chlorella vulgaris* for biodiesel production. *Journal of Radiation Research and Applied Sciences*, 12(1), 332–342. https://doi.org/10.1080/16878507.2019.1662216

Ahmad, F., Taj, M. B., Ramzan, M., Raheel, A., Shabbir, S., Imran, M., & Iqbal, H. M. (2020). *Flacourtia indica* based biogenic nanoparticles: Development, characterization, and bioactivity against wound associated pathogens. *Materials Research Express*, 7, 015026. https://doi.org/10.1088/2053-1591/ab6123

Alshehddi, L. A. A., & Bokhari, N. (2020). Influence of gold and silver nanoparticles on the germination and growth of *Mimusops laurifolia* seeds in the South-Western regions in Saudi Arabia. *Saudi Journal of Biological Sciences*, 27(1), 574–580. https://doi.org/10.1016/j.sjbs.2019.11.013

Anthony, K. J. P., Murugan, M., & Gurunathan, S. (2014). Biosynthesis of silver nanoparticles from the culture supernatant of *Bacillus marisflavi* and their potential antibacterial activity. *Journal of Industrial and Engineering Chemistry*, 20(4), 1505–1510. https://doi.org/10.1016/j.jiec.2013.07.039

Arévalo-Gallegos, A., García-Perez, J. S., Carrillo-Nieves, D., Ramirez-Mendoza, R. A., Ibqal, H. M. A., & Parra-Saldívar, R. (2018). *Botryococcus braunii* as a bioreactor for the production of nanoparticles with antimicrobial potentialities. *International Journal of Nanomedicine*, 13, 5591–5604. https://doi.org/10.2147/IJN.S174205

Asmathunisha, N., & Kathiresan, K. (2013). A review on biosynthesis of nanoparticles by marine organisms. *Colloids and Surfaces B: Biointerfaces*, 103, 283–287. https://doi.org/10.1016/j.colsurfb.2012.10.030

Bakht-Dalir, S. J., Djahaniian, H., Nabati, F., & Hekmati, M. (2020). Characterization and the evaluation of antimicrobial activities of silver nanoparticles biosynthesized from *Carya illinoinensis* leaf extract. *Helyon Journal*, 6, e03624. https://doi.org/10.1002/heol.2020.e03624

Biswal, A. K., & Misra, P. K. (2020). Biosynthesis and characterization of silver nanoparticles for prospective application in food packaging and biomedical applications. *Materials Chemistry and Physics*, 250, 123014. https://doi.org/10.1016/j.matchemphys.2020.123014

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917. https://doi.org/10.1139/o59-099

Branco-Vieira, M., Martin, S. S., Agurto, C., Santos, M. A., Freitas, M. A. V., & Caetano, N. S. (2017). Analyzing phaeo-dactylum tricornutum lipid profile for biodiesel production. *Energy Procedia*, 136, 369–373. https://doi.org/10.1016/j.egypro.2017.10.251

Chaudhari, P. R., Masurkar, S. A., Shidore, V. B., & Kamble, S. P. (2012). Antimicrobial activity of extracellularly synthesized silver nanoparticles using *lactobacillus* species obtained from VIZYLAC capsule. *Journal of Applied Pharmaceutical Science*, 02(3), 25–29. https://www.japsonline.com/abstract.php?article_id=3929tsst=2

Chen, Y., Munechika, K., & Ginger, D. S. (2007). Dependence of fluorescence intensity on the spectral overlap between fluorophores and plasmon resonant single silver nanoparticles. *Nano Letters*, 7(3), 690–696. https://doi.org/10.1021/nl062795z

Christie, W. (1993). Preparation of ester derivatives of fatty acids for chromatographic analysis. In W. Christie (Ed.), *Advances in Lipid Methodology - Two* (pp. 69–111). Oily Press.

Dash, A., Singh, A. P., Chaudhary, B. R., Singh, S. K., & Dash, D. (2012). Effect of silver nanoparticles on growth of eukar-yotic green algae. *Nano-Micro Letters*, 4(3), 158–165. https://doi.org/10.1007/s13579-012-0147-1

El-Batal, A. I., El-Sayyad, G. S., Mosallam, F. M., & Fathy, R. M. (2020). *Penicillium chrysogenum*-mediated mycogenic synthesis of copper oxide nanoparticles using gamma rays for in vitro antimicrobial activity against some plant pathogens. *Journal of Cluster Science*, 31(1), 79–90. https://doi.org/10.1007/s10876-019-01619-3

El-Sayyad, G. S., Mosallam, F. M., El-Sayed, S. S., & El-Batal, A. I. (2020). Facile biosynthesis of tellurium dioxide nanoparticles by *Streptomyces cyaneus* melanin pigment and gamma radiation for repressing some *Aspergillus* pathogens and bacterial wound cultures. *Journal of Cluster Science*, 31(1), 147–159. https://doi.org/10.1007/s10876-019-01629-1

Flores-Rojas, G. G., López-Saucedo, F., & Bucio, E. (2020). Gamma-irradiation applied in the synthesis of metallic and organic nanoparticles: A short review. *Radiation Physics and Chemistry*, 169, 107962. https://doi.org/10.1016/j.radphyschem.2018.08.011

Foud, A. S., & Hafez, R. M. (2018). Effect of silver ions and silver nanoparticles on cell division of onion (*Allium cepa*) and expression of cdcalc2 gene. *Biologia Plantarum*, 62(1), 166–172. https://doi.org/10.1007/s10535-017-0751-6

Francisco, E. C., Neves, D. B., Jacob-Lopes, E., & Franco, T. T. (2010). Microalgae as feedstock for biodiesel production: Carbon dioxide sequestration, lipid production and biofuel quality. *Journal of Chemical Technology and Biotechnology*, 85(3), 395–403. https://doi.org/10.1002/jctb.2338

González-Fernández, C., Le Grand, F., Bideau, A., Huvet, A., Paul-Pont, I., & Soudant, P. (2020). Nanoplastics exposure modulate lipid and pigment compositions in diatoms. *Environmental Pollution*, 262, 114274. https://doi.org/10.1016/j.envpol.2020.114274

Gudikandula, K., Vadapally, P., & Charya, M. S. (2017). Biogenic synthesis of silver nanoparticles from white rot fungi: Their
characterization and antibacterial studies. OpenNano, 2, 64–78. https://doi.org/10.1016/j.onano.2017.07.002
He, D., Dorantes-Aranda, J. J., & Waite, T. D. (2012). Silver nanoparticle algae interactions: Oxidative dissolution, reactive oxygen species generation and synergistic toxic effects. Environmental Science & Technology, 46(16), 8731–8738. https://doi.org/10.1021/es300588a
He, M., Yan, Y., Pei, F., Wu, M., Gebreluelu, T., Zou, S., & Wang, C. (2017). Improvement on lipid production by Scenedesmus obliquus triggered by low dose exposure to nanoparticles. Scientific Reports, 7(1), 1–12. https://doi.org/10.1038/s41598-017-15667-0
Hemapel, N., Petrick, I., & Behrendt, F. (2012). Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. Journal of Applied Phycology, 24(6), 1407–1418. https://doi.org/10.1007/s10811-012-9795-3
Huang, J., Cheng, J., & Yi, J. (2016). Impact of silver nanoparticles on marine diatom Skeletonema costatum. Skeletonema Costatum. Journal of Applied Phycology, 36 (10), 1343–1354. https://doi.org/10.1007/s10811-015-0232-7
Huang, N. M., Radiman, S., Lim, H. N., Yeong, S. K., Khiew, P. S., Chiu, W. S., Mohamed Saeed, G. S., & Nadarajah, K. (2009). γ-Ray assisted synthesis of Ni2Se3 nanoparticles stabilized by natural polymer. Chemical Engineering Journal, 147(2–3), 399–404. https://doi.org/10.1016/j.cej.2008.12.018
Hussein, H. A., Mohamad, H., Ghazaly, M. M., Laith, A. A., & Abdullah, M. A. (2020a). Cytotoxic effects of Tetraselmis suecica chlorofluor extracts with silver nanoparticle co-application on MCF-7, 4 T1, and Vero cell lines. Journal of Applied Phycology, 32, 127–143. https://doi.org/10.1007/s10811-019-01005-7
Hussein, H. A., Syamsumir, D. F., Mohd Radzi, S. A., Siong, J. Y. F., Mohamed Zin, N. A., & Abdullah, M. A. (2020b). Physicochemical screening, metabolite profiling and enhanced anti-microbial activities of microalgae crude extracts in co-application with silver nanoparticle. Bioresources and Bioprocessing, 7, 39. https://doi.org/10.1186/s40643-020-00322-w
Im, S. H., Lee, Y. T., Wiley, B., & Xia, Y. (2005). Large-scale synthesis of silver nanocubes: The role of hcl in promoting cube perfection and monodispersity. Angewandte Chemie International Edition, 44(14), 2154–2157. https://doi.org/10.1002/anie.200462208
Irvani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: Chemical, physical and biological methods. Research in Pharmaceutical Sciences, 9(6), 385–406. https://pubmed.ncbi.nlm.nih.gov/26339255/
Jiang, W., Mashayekhi, H., & Xing, B. (2009). Bacterial toxicity comparison between nano- and micro-scale oxide particles. Environmental Pollution, 157(5), 1619–1625. https://doi.org/10.1016/j.envpol.2008.12.025
Kang, N. K., Lee, B., Choi, G. G., Moon, M., Park, M. S., Lim, J., & Yang, J. W. (2014). Enhancing lipid productivity of Chlorella vulgaris using oxidative stress by TiO2 nanoparticles. Korean Journal of Chemical Engineering, 31(5), 861–867. https://doi.org/10.1007/s11814-013-0258-6
Khoshnamvand, M., Ashtiani, S., Chen, Y., & Liu, J. (2020). Impacts of organic matter on the toxicity of biosynthesized silver nanoparticles to green microalgae. Chlorella Vulgaris. Environmental Research, 185, 109433. https://doi.org/10.1016/j.envres.2020.109433
Li, X., Schirmer, K., Bernard, L., Sigg, L., Pillai, S., & Behra, R. (2015). Silver nanoparticle toxicity and association with the alga Euglena gracilis. Environmental Science: Nano, 2 (6), 594–602. https://doi.org/10.1039/CSEN00093A
Liu, D., Wong, P. T. S., & Dutka, B. J. (1973). Determination of carbohydrate in lake sediment by a modified phenol-sulfuric acid method. Water Research, 7(5), 741–746. https://doi.org/10.1016/0043-1354(73)90090-0
Marambio-Jones, C., & Hoek, E. M. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. Journal of Nanoparticle Research, 12(5), 1531–1551. https://doi.org/10.1007/s11051-010-9900-y
Martin, P. A., Lohr, J. R., & Dean, D. H. (1981). Transformation of Bacillus thuringiensis protoplasms by plasmid deoxyribonucleic acid. Journal of Bacteriology, 145(2), 980–983. https://doi.org/10.1128/JB.145.2.980-983.1981
Marzikova, A., Velgosova, O., & Kavulicova, J. (2017). Effect of chemically and biologically synthesized Ag nanoparticles on the algal growth inhibition. Recent advances on environ- onment, chemical & engineering and materials. AIP Conference of Proceedings, 1918. https://doi.org/10.1063/1.5018503
Miao, A. J., Luo, Z., Chen, C. S., Chin, W. C., Santschi, P. H., Quigg, A., & Johnson, S. J. (2010). Intracellular uptake: A possible mechanism for silver engineered nanoparticle toxicity to a freshwater alga Ochloosansadna. PLoS One, 5(12), e15196. https://doi.org/10.1371/journal.pone.0015196
Mueller, N. C., & Nowack, B. (2008). Exposure modeling of engineered nanoparticles in the environment. Environmental Science & Technology, 42(12), 4447–4453. https://doi.org/10.1021/es7029637
Pillai, S., Behra, R., Nestler, H., Suter, M. J. F., Sigg, L., & Schirmer, K. (2014). Linking toxicity and adaptive responses across the transcriptome, proteome, and phenotype of Chlamydomonas reinhardtii exposed to silver. Proceedings of the National Academy of Sciences, 111(9), 3490–3495. https://doi.org/10.1073/pnas.131938811
Razack, S. A., Duraiarasan, S., & Mani, V. (2016). Biosynthesis of silver nanoparticle and its application in cell wall disruption to release carbohydrate and lipid from C. vulgaris for biofuel production. Biotechnology Reports, 11, 70–76. https://doi.org/10.1016/j.btre.2016.07.001
Samberg, M. E., Orndorff, P. E., & Monteiro-Riviere, N. A. (2011). Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. Nanotoxicology, 5(2), 244–253. https://doi.org/10.3109/17435390.2010.525669
Shah, S. M. U., & Abdullah, M. A. (2018). Effects of macro/micronutrients on green and brown microalgal cell growth and fatty acids in photobioreactor and open-tank systems. Biocatalysis and Agricultural Biotechnology, 14, 10–17. https://doi.org/10.1016/j.bact.2018.01.011
Sibi, G. A. K. D., Ananda Kumar, D., Gopal, T., Harinath, K., Banupriya, S., & Chaitra, S. (2017). Metal nanoparticle triggered growth and lipid production in Chlorella vulgaris. International Journal of Scientific Research, Environmental Science Toxicology, 2(1), 1–8. https://symbiosisonlinepublishing.com/toxicology/toxicology-environmental-science12.php
Stanier, R. Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chrococcales). Bacteriological Reviews, 35(2), 171. https://doi.org/10.1128/MMBR.35.2.171-205.1971
Sun, Y., & Xia, Y. (2002). Shape controlled synthesis of gold and silver nanoparticles. Science, 298(5601), 2176–2179. https://doi.org/10.1126/science.1077229
Sun, Y., Yin, Y., Mayers, B., Herricks, T., & Xia, Y. (2002). Uniform silver nanowires synthesis by reducing AgNO3 with
ethylene glycol in the presence of seeds and poly(vinyl pyrrolidone). *Chemistry of Materials*, 14(11), 4736–4745. https://doi.org/10.1021/cm020587b

Rasheed, T., Bilal, M., Li, C., Nabee, F., Khalid, M., & Iqbal, H. M. (2018). Catalytic activity of bio-synthesized silver nanoparticles using *Convolvulus arvensis* extract for the degradation of environmental pollutants. *Journal of Photochemistry and Photobiology B: Biology*, 181, 44-52. https://doi.org/10.1016/j.jphotobiol.2018.02.024

Tayemeh, M. B., Esmailbeigi, M., Shirdel, I., Joo, H. S., Johari, S. A., Banan, A., Nourani, H., Mashhadi, H., Jami, M. J., & Tabarrok, M. (2020). Perturbation of fatty acid composition, pigments, and growth indices of *Chlorella vulgaris* in response to silver ions and nanoparticles: A new holistic understanding of hidden ecotoxicological aspect of pollutants. *Chemosphere*, 238, 124576. https://doi.org/10.1016/j.chemosphere.2019.124576

Taylor, C., Matzke, M., Kroll, A., Read, D. S., Svendsen, C., & Crossley, A. (2016). Toxic interactions of different silver forms with freshwater green algae and cyanobacteria and their effects on mechanistic endpoints and the production of extracellular polymeric substances. *Environmental Science: Nano*, 3(2), 396–408. https://doi.org/10.1039/C5EN00183H

Tripathi, D., Modi, A., Narayan, G., & Rai, S. P. (2019). Green and cost effective synthesis of silver nanoparticles from endangered medicinal plant *Withania coagulans* and their potential biomedical properties. *Materials Science and Engineering: C*, 100, 152–164. https://doi.org/10.1016/j.msec.2019.02.113

Tripathi, D. K., Tripathi, A., Singh, S., Singh, S., Singh, Y., Vishwakarma, K., Yadav, G., Sharma, S., Singh, V. K., Mishra, R. K., Upadhyay, R. G., Dubey, N. K., Lee, Y., & Chauhan, D. K. (2017). Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: A concentric review. *Frontiers in Microbiology*, 8, 1–16. https://doi.org/10.3389/fmicb.2017.00007

Vidyashankar, S., VenuGopal, K. S., Swarnalatha, G. V., Kavitha, M. D., Chauhan, V. S., Ravi, R., Bansal, A. K., Singh, R., Pande, A., Ravishankar, G. A., & Sarada, R. (2015). Characterization of fatty acids and hydrocarbons of Chlorophycean microalgae towards their use as biofuel source. *Biomass & Bioenergy*, 77, 75–79. https://doi.org/10.1016/j.biombioe.2015.03.001

Wiley, B. J., Chen, Y., McLellan, J. M., Xiong, Y., Li, Z. Y., Ginger, D., & Xia, Y. (2007). Synthesis and optical properties of silver nanobars and nanorice. *Nano Letters*, 7(4), 1032–1036. https://doi.org/10.1021/nl070214f

Yang, F., Long, L., Sun, X., Wu, H., Li, T., & Xiang, W. (2014). Optimization of medium using response surface methodology for lipid production by *Scenedesmus* sp. *Marine Drugs*, 12(3), 1245–1257. https://doi.org/10.3390/md12031245

Zhang, H., Du, W., Peralta-Videa, J. R., Gardea-Torresdey, J. L., White, J. C., Keller, A. A., Guo, H., Ji, R., & Zhao, L. (2018). Metabolomics reveals how cucumber (*cucumis sativus*) reprograms metabolites to cope with silver ions and silver nanoparticle-induced oxidative stress. *Environmental Science & Technology*, 52(14), 8016–8026. https://doi.org/10.1021/acs.est.8b02440