The effects of silver nanoparticles and ions on *Chlorella vulgaris*

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INTRODUCTION

Silver nanoparticles (AgNPs) belong to a group of frequently used materials in medicine and science due to their antimicrobial properties, but are also used in the textile industry and agriculture [1]. They can be found in cosmetics, textiles, and a variety of dyes [2]. They are also used as antibiotics due to their improved catalytic properties, reactivity, and appropriate size-to-surface ratio [3]. Due to their tendency to agglomerate in various media, they are stabilised with various surface coatings like polyvinylpyrrolidone (PVP) [4]. Given their increased production, it is necessary to examine their effect on the natural ecosystem, the processes of biodistribution of environmental substances and accumulation in food chains. Since *Chlorella vulgaris* is one of the most ubiquitous microalgae inhabiting aquatic ecosystems, it is widely used as a model organism for assessing the impact of materials of anthropogenic origin, e.g. AgNPs, on aquatic habitats [5].

MATERIALS & METHODS

To evaluate the effect of AgNP-PVP and silver ions ($AgNO_3$) on biota, the growth, lipid peroxidation, and catalase activity in microalga *C. vulgaris* were measured upon exposure to 0.5, 1.0, 1.5 and 2.0 mg L^{-1} concentrations after 5, 24 and 48 hours in modified liquid BBM nutrient medium. Additionally, the changes of pH, levels of dissolved oxygen and absorbance spectra in the range 300-800 nm in the algae cultures were analysed after the same periods of treatment. Since stress conditions negatively affect ribulose bisphosphate carboxylase (Rubisco) protein synthesis, we used immunoblotting assay to analyse the expression levels of the large Rubisco chain to evaluate the fitness of algae upon treatment.

RESULTS

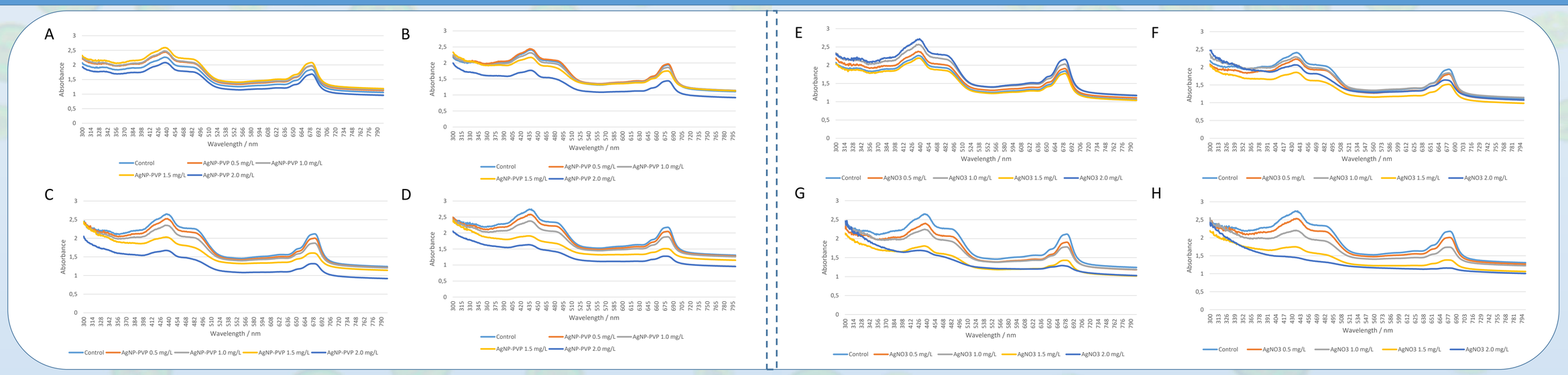

Figure 1. Absorbance spectra in the range of 300-800 nm of the algae cultures. Control samples and cultures treated with 0.5, 1.0, 1.5 and 2.0 mg L^{-1} concentrations of AgNP-PVP (A-D) and $AgNO_3$ (E-F) immediately after treatment (A and E), after 5 hours (B and F), 24 hours (C and G), and after 48 hours (D and H) of treatment.

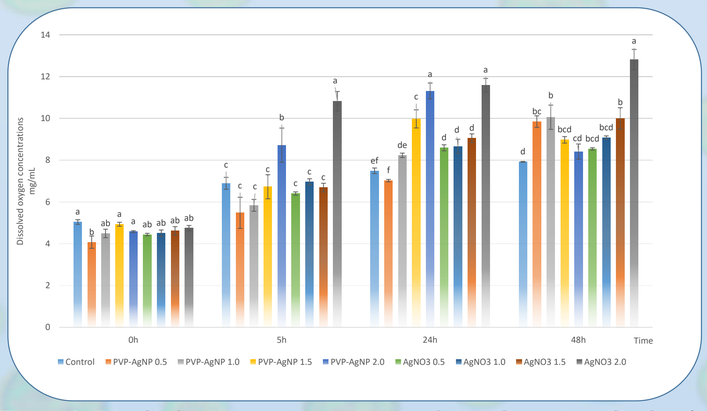

Figure 2. Dissolved oxygen concentrations in algae cultures immediately after treatments and after 5, 24, and 48 hours of treatment with AgNP-PVP or $AgNO_3$ in final concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. The presented results show mean values of 3 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Newman-Keuls test.

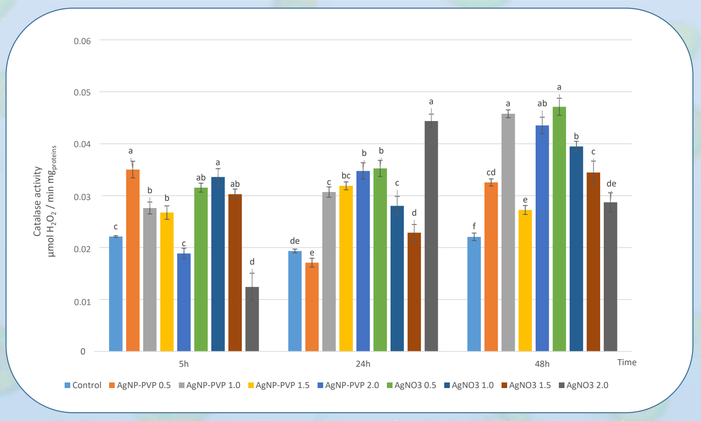

Figure 3. Catalase activity in algae cultures after 5, 24, and 48 hours of treatment with AgNP-PVP or $AgNO_3$ in final concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. The presented results show mean values of 3 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Newman-Keuls test.

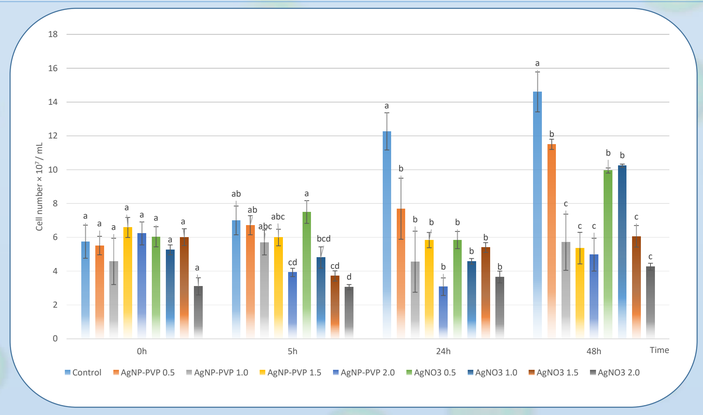

Figure 4. Algae cell number measured immediately after treatments and after 5, 24, and 48 hours of treatment with AgNP-PVP or $AgNO_3$ in final concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. The presented results show mean values of 3 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Newman-Keuls test.

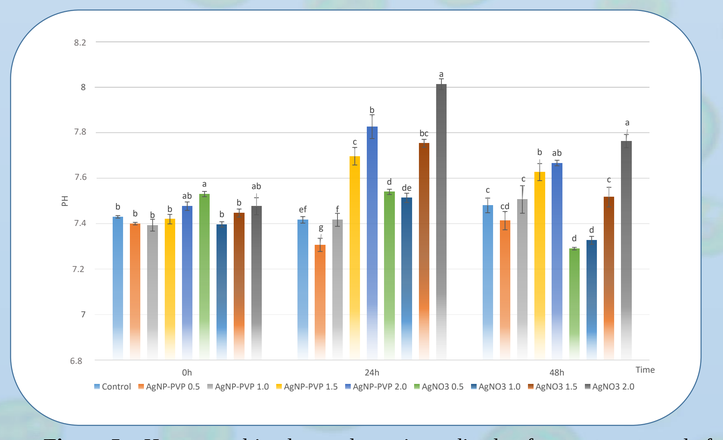

Figure 5. pH measured in algae cultures immediately after treatments and after 24 and 48 hours of treatment with AgNP-PVP or $AgNO_3$ in final concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. The presented results show mean values of 3 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Newman-Keuls test.

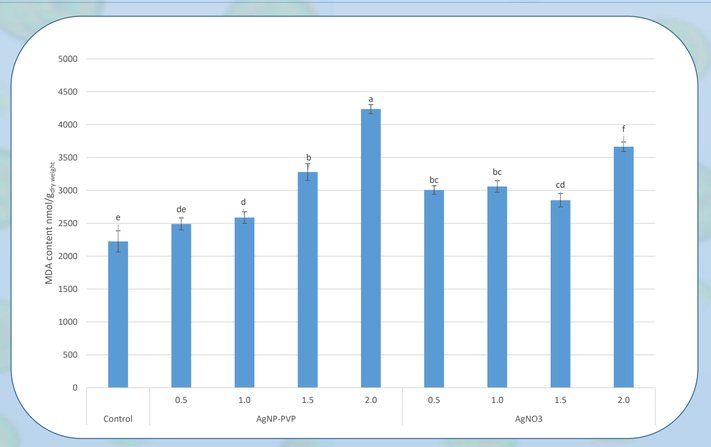

Figure 6. Lipid peroxidation of algae cultures after 24 hour treatment with AgNP-PVP or $AgNO_3$ in final concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. The presented results show mean values of 3 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Newman-Keuls test.

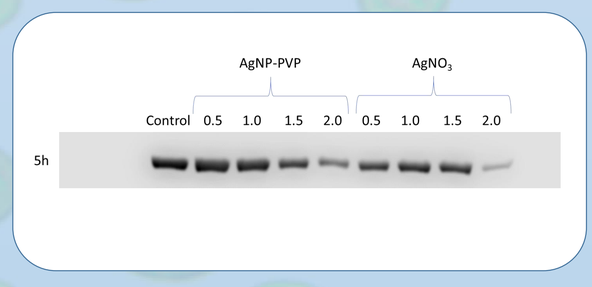

Figure 7. Immunobloting assay for large Rubisco subunit after 5 hours treatment with AgNP-PVP or $AgNO_3$ in final concentrations of 0.5, 1.0, 1.5 and 2.0 mg L^{-1}

CONCLUSIONS

- After the treatment with AgNP-PVP or $AgNO_3$, a significant dose-dependent decrease in cell number and an increase in dissolved oxygen and lipid peroxidation were observed.
- Increase in catalase activity was measured after all of the treatments.
- Increase in pH was observed after 24 and 48 hours of treatments with 1.5 and 2.0 mg L^{-1} of both AgNP-PVP and $AgNO_3$.
- Visible light absorbance significantly decreased after the treatment with both AgNP-PVP and $AgNO_3$, with the decrease most noticeable in the characteristic chlorophyll *a* and *b* absorbance ranges.
- Immunoblotting revealed significant dose-dependent decreases in the levels of Rubisco large subunit after treatments with both AgNP-PVP and $AgNO_3$.
- Decrease in visible light absorbance and in the levels of Rubisco large subunit after both treatments indicate a negative influence of AgNP-PVP and $AgNO_3$ on the photosynthetic apparatus of algae.

References:
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