Silver nanoparticles – *chlorella* interaction: effect on metabolites

Zaidi S, Maurya C, Shankhadarwar S and Pius J

Department of Botany, Ramnarain Ruia College, Matunga, Mumbai-19, India
shama.zaidi@rediffmail.com

Keywords:

Chlorella, secondary metabolites, silver nanoparticles.

Abstract

The field of nanotechnology has received a great impetus owing to the wide applicability of nanoparticles in diverse fields. In recent years , the use of silver nanoparticles (Agnps) have increased, however, the short term and long term effects of these nanoparticles on the environment has not been investigated in depth. The present work attempts to understand the effect of silver nanoparticles on the freshwater micro alga *Chlorella sp*. The effect of Agnps was induced by growing *Chlorella* in two different concentrations of Agnps (5ppm and 10 ppm). The amount of chlorophyll, protein and secondary metabolites like tannins, phenolics and flavonoids were estimated. Results indicated a significant difference in the level of chlorophyll, protein and secondary metabolites compared to control, suggesting that the Agnps may have a detrimental effect on the growth of algal cells. These results seem to indicate that the Agnps released in the environment may pose a potential risk to the aquatic macro and microflora.

INTRODUCTION

Noble metal nanoparticles are gaining a lot of importance in recent years owing to their diverse uses in the field of medicine, biology, material science, physics and chemistry (Yokohama and Welchons, 2007). Silver nanoparticles, in particular, have received special focus due to their distinctive properties like good electrical conductivity, chemical stability, catalytic and antibacterial activity (Sharma *et.al*., 2009). Due to their high anti-microbial activity, silver nanoparticles have also been used in clothing (Vigneshwaran *et al.*, 2007) and some cases as powder for use in shoes (Luoma 2008), food industry(Chaudhry and Castle, 2011), sunscreens and cosmetics (Martinez-Gutierrez *et al*., 2010; Kokura *et al*., 2010) . Agnps are also commonly used as coating for many products such as medical devices, food storage containers, handrails etc. The optical and physical properties of Agnps make it also very useful in medical applications (Winjhoven *et al*., 2009).

Like many industrial products that are used in bulk quantities, silver is most likely to end up in the aquatic environment (Navarro *et al*., 2008; Blaser *et al.*, 2008; Fabrega *et al.*,2011). Silver ions have been found to be toxic to several organisms such as bacteria, algae and fungi amongst many others (Wood *et al*., 1996; Ratte, 1999; Moore 2006; Choi *et al*., 2008; Navarro *et al*., 2008). Silver ions also inhibit different important cycles (S, N and P), of nitrifying bacteria, disturb DNA transcription, destroy cell wall of bacteria and thus its membrane permeability which could cause cell lysis (Ratte 1999).

Chlorella is a unique, single-celled fresh water micro alga with grass like odour belonging to the Division Chlorophyta. Its emerald green colour and odour is due to the presence of high amount of chlorophyll which is higher than any known plant. The alga is a popular food supplement in Asia and has been used as energy-producing food for centuries. It is used to prevent the spread of cancer, enhance immunity, promote a good balance of bacteria in the gut, and lower blood cholesterol. It is traditionally used as a treatment for duodenal ulcers, gastritis, hypertension, diabetes, hypoglycemia, asthma, and constipation. *Chlorella* provides all of the dietary-essential amino acids in excellent ratios.

The present attempt aims to understand the effect of Agnps on the aquatic microflora. *Chlorella*, being a unicellular alga, having a simple cell structure, can be a model system to understand the Agnp-alga interaction.

MATERIALS AND METHODS:

Materials: Aqueous plant extract, 1mM AgNO3, BG-11 Medium,

Preparation and Culturing of *Chlorella sp*

The alga was collected from the Powai lake, Mumbai and cultured in conical flasks on BG11 medium at room temperature to study its growth pattern. The cell growth pattern of the cultured algal

cells was studied for 20 days by taking OD at regular intervals.

Phytosynthesis and Characterization of silver nanoparticles:

Silver nanoparticles (Agnps) were fabricated using plant extract with 1mM AgNO3 solution. The change in colour from colourless to yellow indicated the formation of Agnps. The nanoparticles were characterized using UV-Visible Spectroscopy (Shimadzu UV 1800) and Photon Correlation Spectroscopy (PCS). Hydrodynamic diameter was measured using zetasizer (ZS-90 Malvern Instruments UK) with laser angle 90°. Particles with an average of 1ess than 100nm size were used for the study.

Co-culturing of algal cells with the synthesized Agnps:

The algal cells were co cultured in conical flasks in BG-11 medium containing 5ppm and 10 ppm of Agnps and allowed to grow for 20 days. A control flask was also kept in a similar manner. After 20 days, the algal culture from the control flask and the flasks containing different concentrations of Agnps was tested for chlorophyll, protein and secondary metabolites.

Study of metabolites:

Chlorophyll, protein and various secondary metabolites like total phenol, tannin and flavonoid from co -cultured and control algal cells were estimated by using standard procedures (Singleton and Rossi, 1965; Killedar *et al*.,2010; Woisky and Salatino, 1998 respectively).

RESULTS AND DISCUSSION:

Cell growth was studied which showed maximum growth on 20th day (Fig 1).

Characterization of phytosynthesized nanoparticles:

Synthesized nanoparticles were characterized using UV-Visible Spectroscopy and Photon Correlation Spectroscopy. The particles showed absorption maxima at 439 nm and the average size was found to be in the range of 10 to 40 nm.

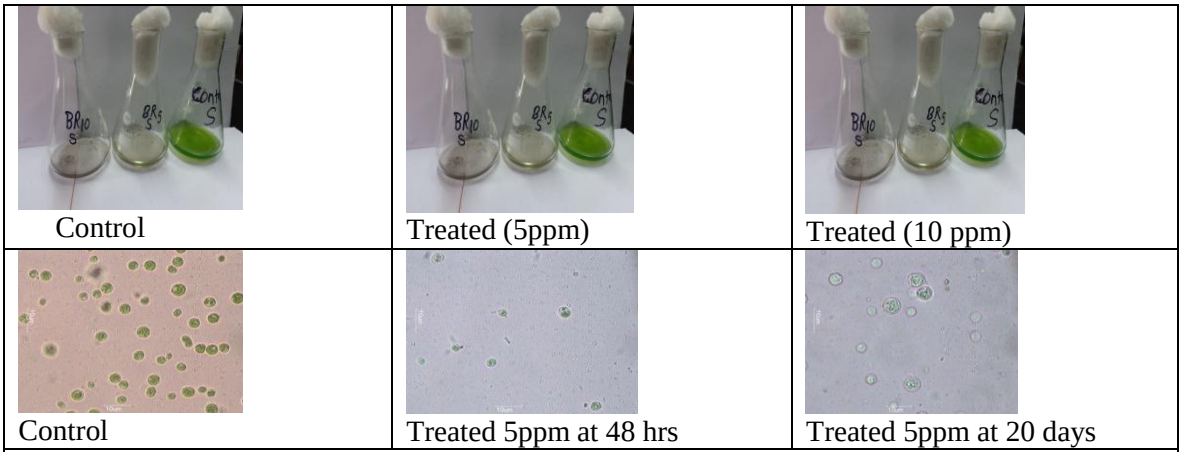
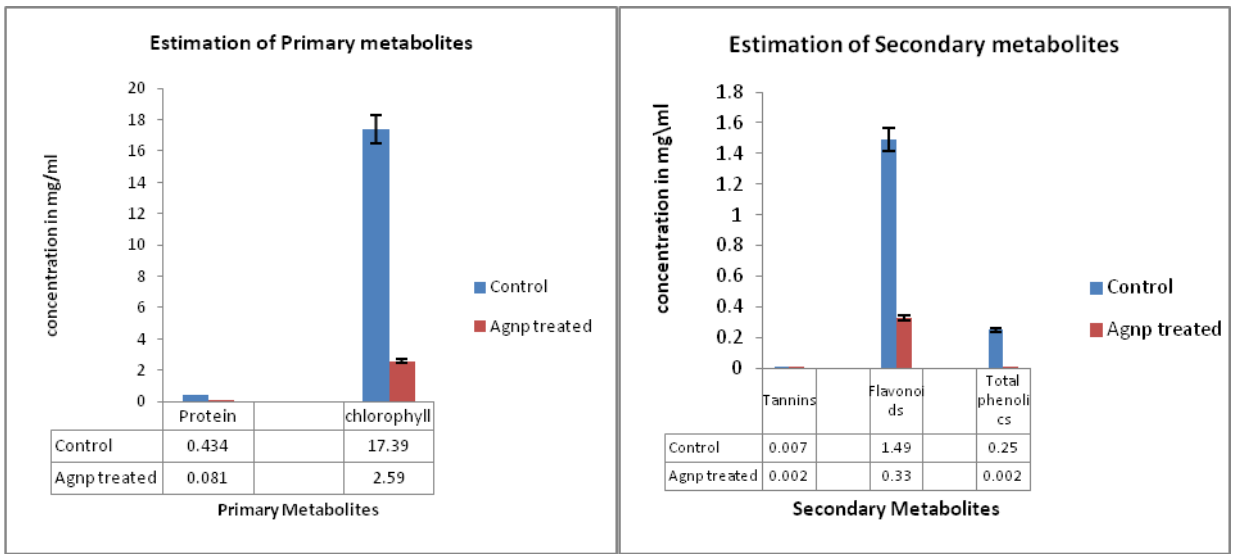


Figure 1 a-c: Algal cells grown in control and 5 ppm and 10 ppm Agnps d-f: Morphological variation of Chlorella cells in control and in 5ppm Agnps at 48 hrs and 20 days.

Estimation of chlorophyll, protein and secondary metabolites:

A comparative analysis of the primary and secondary metabolites showed a sharp decrease in the amount of metabolites in cells co-cultured with 5 ppm Agnps as compared to the control (Fig 2). *Chlorella* is known to contain maximum amount of chlorophyll and this can be observed in the control cells (17.39mg/ml). Among the secondary metabolites estimated in control algal cells, flavonoids were found to be in maximum concentration followed by total phenolics and tannins. Cells treated with Agnps showed a similar trend but with a drastic decline in the concentrations of these metabolites.

Cells co-cultured in 10 ppm of Agnps were unable to survive. Morphologic observation of the treated and control cells after 48 hours and 20 days showed an adverse effect on the chloroplast, with treated cells showing gradual decrease in the amount of chloroplast (Fig 1d-f)



B

Figure 2: A:Estimation of Primary and B: Secondary Metabolites from Control and Treated Algal cells (Error bars on the line graph denote standard deviation).

An in depth knowledge about the fate of Agnps as well as biotic and abiotic factors affecting Agnp behavior in the aquatic environment is necessary for complete understanding of Agnp-algae interaction. Initial results demonstrated that Agnps have a detrimental effect on the algal cells. This effect may be dependent on the size of Agnp and reactivity of silver with other components of the aquatic ecosystem. It could be due to the interaction of Agnp with certain enzymes thus disturbing cellular function (Choi *et al*., 2010)

Acknowledgement

The authors are sincerely grateful to Principal and Staff of Department of Botany, R. Ruia College for their whole hearted support and cooperation. We, also express our gratitude to the Department of Chemical Engineering, Institute of Chemical Technology, Mumbai for their help in assisting us in the PCS and TEM analysis.

REFERENCES

Blaser SA, Scheringer M, MacLeod M and Hungerbuhler K, 2008. Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. *Sci Tot Env*., **390**:396-409.

Chaudhry Q and Castle L, 2011. Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries.*Trends Food Sci.Technol*.,**22**: 595–603.

Choi O, Kanjun deng, K, Kim, NJ, Ross Jr L, Surampalli RY and Hu Z, 2008. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res*., **42**:3066-3074.

Fabrega J, Luoma SN, Tyler CR, Galloway TS and Lead JR, 2011. Silver nanoparticles: Behaviour and effects in the aquatic environment. *Env. Intl*., **37**: 517-531.

Killedar S and More H, 2010. Estimation of Tannins in different parts of Memecylon Umbellatum Burm. *J. of Phar. Res*., **3**(3):554-556.

Kokura S, Handa O, Takagi T, Ishikawa T, Naito Y and Yoshikawa T, 2010. Silver nanoparticles as a safe preservative for use in cosmetics. *Nanomed* .,**6**:570–574.

Luoma NS, 2008. Silver nanotechnologies and the environment: Old problems or new challenges?. *The Project on Emerging Nanotechnologies* 15.

Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N and Sanchez E, 2010. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. *Nanomed.*, **6**:681–688.

Moore MN, 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?. *Env. Intl*.,**32**: 967-976. **Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N, Sigg L and Behra R, 2008.** Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Env. Sc and Tech.*,**42**:8959-8964.

Ratte HT,1999. Bioaccumulation and toxicity of silver compounds: a review. *Env. Tox and Chem.*,**18**(1): 89-108.

Sharma VK, Yngard RA and Lin Y, 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv. Colloid Interface Sci*., **145**:83–96.

Singleton VL and Rossi A, 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Viticult.*, **16**:144-158.

Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP and Balasubramanya RJ, 2007. Functional finishing of cotton fabrics using silver nanoparticles. *J. Nanosci.Nanotechnol*., **7**:1893–1897.

Winjhoven WPS, Peinenburg JGMW, Herberts AC, Hagens IW, Oomen GA, Heugens HWE, Roszek B, Bisshops J, Gosens I, Van De Meent D, Dekkers S, De Jong HW, Van Zijverden M, Sips JAMA and Geersma ER, 2009. Nano-Silver-a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotox*., **3** (2):109-138.

Woisky RG and Salatino A, 1998. Analysis of propolis: some parameters and procedures for chemical quality control. *J.Apicult. Res*., **37** (2):99-105.

Wood MC, Hogstrand C, Galvez F and Munger RS, 1996. The physiology of waterborne silver toxicity in freshwater rainbow trout (Oncorhynchus mykiss)-The effect of ionic Ag+. *Aqu. Toxicology*., **35**: 93-109.

Yokohama K and Welchons DR, 2007. The conjugation of amyloid beta protein on the gold colloidal nanoparticles surfaces. *Nanotech.*, **18**:105101–105107.