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Toxicity evaluation based on particle size, contact angle and zeta potential of SiO_2 and Al_2O_3 on the growth of green algae

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Abstract. In this investigation, ecotoxicity of nano and micro metal oxides, namely silica (SiO_2) and alumina (Al_2O_3), on the growth of green algae (*Porphyridium aerugineum Geitler*) is discussed. Effects of nano and micro particles on the growth, chlorophyll content and protein content of algae are analysed using standard protocols. Results indicate that SiO_2 nano and micro SiO_2 particles are non-toxic to *P. aerugineum Geitler* up to a concentration of 1000 mg/L. In addition, Al_2O_3 microparticles are less toxic to *P. aerugineum Geitler*, whereas Al_2O_3 nanoparticles are found to be highly toxic at 1000 mg/L. Moreover, Al_2O_3 nanoparticles decrease the growth, chlorophyll content, and protein content of tested algae. In addition, zeta potential and contact angle are also important in enhancing the toxicity of metal oxide nanoparticles in aquatic environment. This study highlights a new insight into toxicity evaluation of nanoparticles on beneficial aquatic organisms such as algae.

Keywords: nano metal oxides; Porphyridium aerugineum Geitler; chlorophyll content; zeta potential; contact angle; protein content

1. Introduction

The rapid development in the field of nanotechnology enhances the potential applications of nano materials in all fields of science and technology due to their excellent physicochemical properties (Lin 2010). Metal oxide nanoparticles are the most widely used materials in the areas such as thermal barrier coatings, catalysts and biomedical implantations (Mueller and Nowack 2008). Thus, with the increased use, these nanoparticles are certainly released in the environment and may affect aquatic environments and agricultural lands (Klaine 2008). Silica (SiO_2) is the most widely used metal oxide nanoparticle for different applications such as cosmetics, photocatalytic treatment, cancer therapy, bioimaging, and plant growth (Cheng 2010, Suriyaprabha 2012). An increase in the level of silica may lead to an increase in its content in the environment, which

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causes metal toxicity.

Alumina (Al_2O_3) is one of the very common metal oxides used in different applications such as biosensors, textiles, electronics, drug delivery systems, and tissue engineering (Jeng and Swanson 2006, Handy 2008a, Handy 2008b). Thus, its immense use causes alumina accumulation in the ecosystem and in the food chain. The ecotoxicity of metal oxide nanoparticles such as TiO_2, CuO, and ZnO is extensively studied against bacteria (Jiang 2009), soil earthworm (*Eisenia fetida*) (Hu 2010), nematodes (*Caenorhabditis elegans*) (Wang 2003), zebrafish (*Danio rerio*) (Xiong 2011), and *Daphnia similis* (Marcone 2012). It is inferred from the above studies that the toxicological properties of metal oxide nanoparticles depend on the surface property and their contact with the environment. Interestingly, a few of the nanoparticles are nontoxic to *Vibrio fischeri*, *Daphnia magna* and *Thamnocephalus platyurus* (Heinlaan 2008). Moreover, the surface properties play a key role in determining the toxic nature of nanoparticles, in addition to the type of nanoparticles (Jiang 2009).

Algae are important autotrophic organisms that contribute to aquatic ecosystem by taking part in food web, water recycling, and biomass production. Thus, algae are chosen as an appropriate model organism for the assessment of toxicity. The previous investigation reveals that metal oxide particles such as ZnO, TiO_2 and CuO (Aruoja 2008) show significant toxicological responses in aquatic ecosystem, particularly in algae. The toxicity mechanisms show differences based on the type of particles and algal species. The above studies expand knowledge in the field of nanotoxicity, but still detailed studies are required to understand the fate of algae during the exposure of nanoparticles.

Water is the main component on the earth, covering 75% of the total surface. Currently, there is a high possibility of interaction of nanoparticles with the living systems in the aquatic environment. In aquatic ecosystem, green algae have a major role in recycling process. Thus, it is necessary to evaluate the toxicity of nanoparticles on green algae. Although the studies are focused on the toxicity of green algae, but there is lack of information about the actual behavior of metal oxide nanoparticles based on the concentration, size, zeta potential and hydrophobicity of the material.

This study is mainly aimed to analyse the mechanism of toxicity by relating different growth parameters on algae. The study is conducted based on the type of particles correspond to the variations in size. The dose dependent applications of metal oxide nanoparticles such as SiO_2 and Al_2O_3 on the variation in growth, chlorophyll content, and protein content of algae are studied to determine their hazardous effects. In addition, the role of zeta potential and hydrophobicity is considered to relate the mechanism of toxicity among metal oxides.

2. Materials and methods

2.1 Sources of metal oxide microparticles

Al_2O_3 (99% purity) and SiO_2 (99% purity) micro particles were procured from Merck and Loba Chemie (India), respectively and used without any further purification.

Synthesis and characterisation of metal oxide nanoparticles

Al_2O_3 and SiO_2 nanoparticles were synthesised using low-cost chemical precipitation methods in our laboratory using bauxite and rice husk as precursor (Yuvakkumar 2012, Manivasakan 2011) and were characterised comprehensively. The characteristics of Al_2O_3 and SiO_2 nanoparticles were

described in our earlier studies (Yuvakkumar 2012, Manivasakan 2011). Contact angle (model no. DCAT 11EC; DataPhysics, Germany) and Zetasizer (MAL1037088; Malvern Instruments Ltd., Malvern, UK) instruments were used to explore respectively, the hydrophobic potential and charge of metal oxide particles.

2.2 Algal source

The green algae (*Porphyridium aerugineum Geitler*) used in this study was obtained from the Department of Botany, University of Madras, India. The standard OECD (Organisation for Economic Cooperation and Development) TG 201 medium was used for culturing. Similarly, the analysis of algal toxicity was carried out as per the OECD guideline 201 (Organisation for Economic Cooperation and Development 1984). The OECD TG 201 medium was prepared and made to 1000 mL. The 100 mL of medium was dispensed in the sterile 250 mL flasks. Then the cultures were inoculated into the medium. The cultures were maintained at 21-25°C with 13:11 h light/dark cycle for 3 days. The algal culture was subcultured and monitored up to fifth cycle to attain a well uniform growth. After completing the fifth cycle, the algal cells were counted using hemocytometer under optical microscope (Labomed TCM400 inverted microscope; OEM-OPTICAL, Roseville, CA, USA). Thus, the well grown algae culture with the cell density of 10^4 cells per milliliter was used for further experiments.

2.3 Algae treatment with metal oxides and growth inhibition analysis

The algal growth tests were designed to investigate the dose-dependent response between the SiO_2 and Al_2O_3 particles. The 100 mL of OECD TG 201 medium (100 mL) containing different concentrations of metal oxide particles namely 0, 1, 10, 100, 500 and 1000 mg/L was dispersed through ultrasonication (VC 505; Sonics, Newtown, CT, USA) at 30 kHz for 30 min. *P. aerugineum Geitler* was inoculated in the prepared media with a same density of cultures followed by incubation in a shaker with 200 rpm at 21-25°C in 13:11 h light/dark cycle for 6 days. The growth of *P. aerugineum Geitler* exposed to different suspensions of nano- and microparticles was measured in different days of incubation, namely zero, second, fourth, and sixth days. The growth pattern was analysed at 21–25 °C at 200 rpm for 6 days. The algal growth was measured at 540 nm on the zero, second, fourth, and sixth days using UV-Vis spectrophotometer (U-2900/2910; Hitachi, Japan). The effect of metal oxide nano- and microparticles on algae propagation was evaluated using the turbidity measurement. The turbidity method is a standard method by which we can easily conclude the growth of algae using this method. The growth inhibition percentage was related to control, which was obtained for the algal samples under metal oxide treatments.

The effect of metal oxide nano and microparticles on the algal metabolic activity was monitored by analysing the total chlorophyll content and the protein content. The algal culture was collected at 2 day intervals up to sixth day. Then it was washed thrice using sterile ultrapure water. The culture was then centrifuged at 5000 rpm to obtain the algal biomass pellet for the estimation of total chlorophyll and total protein contents. The total chlorophyll content of algae was extracted by hot methanol extraction technique (Fargasova 2001) and estimated using UV-Vis spectrophotometer at 650 and 665 nm. The variations in total chlorophyll content were calculated using the following formula

$$\text{Total chlorophyll (in } \mu\text{g/mL)} = (2.55 \times 10^{-2} \times OD_{650}) + (0.4 \times 10^{-2} \times OD_{665}) \quad \text{(i)}$$

The protein content from the extracted algal biomass was measured using Lowry's method

(Lowry 1951). The algae biomass was homogenised and then centrifuged at 3000 rpm for 30 min to form a suspension. Bovine serum albumin (BSA) of different dilutions was prepared in the ratio of 1 mg/mL in water and then, the final volume was made up to 5 mL. Protein solution (0.2 mL) was pipetted out to each tube. Similarly, 2 mL alkaline copper sulfate solution was added to the solution. Then, the solution was mixed well and incubated for 10 min at room temperature. Folin's Ciocalteau solution (0.2 mL) was added to the each tube followed by incubation in water bath for 30 min. The optical density of each tube was measured at 660 nm. Then the absorbance of the unknown concentration of the control and the treated algae cultures was determined with the help of standard curve of BSA.

2.4 Metal oxide adsorption analysis in algae

The dried algae culture was subjected to Fourier transform infrared spectroscopy (FTIR) (Spectrum 100; PerkinElmer, USA) and X-ray fluorescence spectrometry (XRF) (EDX-720; Shimadzu, Japan) to explore the adsorption or absorption of metal oxide nano and microparticles from the growth medium. During the FTIR analysis, the dried algae cultures from the treated and untreated samples were mixed with KBr in the ratio of 1:10. The mixed powder was ground well using mortar and pestle. Then the powders were kept under a pressure of 126 kg/cm^2 in a pellet maker. The obtained pellets were screened through FTIR instrument in the wavelength ranges from 400 to 4000 cm^{-1}. The XRF analysis was performed on the dried algae pellets and the corresponding elemental composition was determined.

2.5 Statistical analysis

Statistical Package for the Social Sciences (SPSS, version 16.0) was used to interpret the observed results statistically for all the treatments. All the experiments were performed in triplicates. The differences in the algae growth and chlorophyll contents among the control and treatments were analysed by one-way analysis of variance, followed by multiple comparisons among the groups using Tukey's *post hoc* test at a significance level of 5% ($p < 0.05$). The error bars were defined in the respective figures.

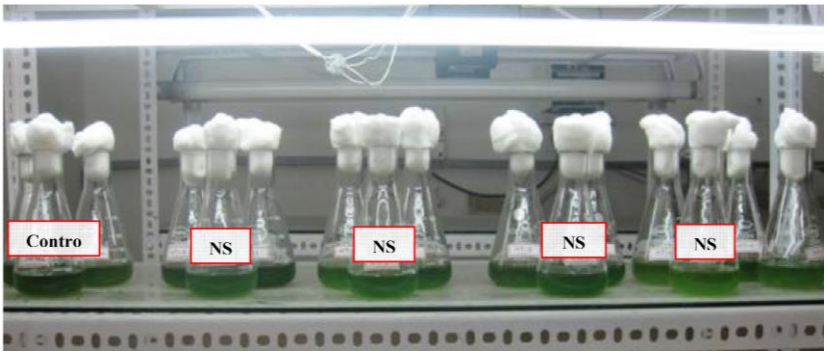
3. Results

The measured particle size distribution of metal oxides is shown in Table 1. The hydrodynamic size of Al_2O_3 and SiO_2 nanoparticles increases with the medium used. The initial size of Al_2O_3 nanoparticles is 58 nm in water and 68 nm in OECD medium. Similarly, the size of SiO_2 nanoparticles is 50 nm in water and 70 nm in OECD medium.

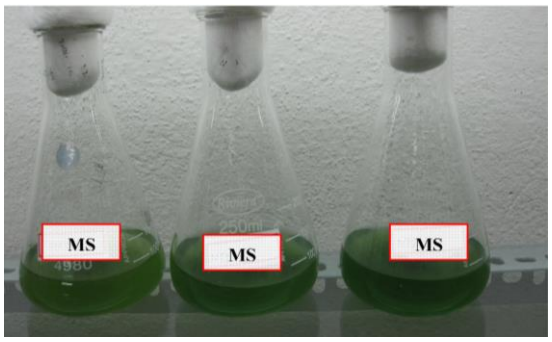
The algae culture treated with different concentrations of Al_2O_3 nano and micro particles are shown respectively in Figs. 1 and 2. The algae growth is found to be varied with the concentration of particles used. Least toxicity is observed at 1 mg/L, whereas highest toxicity is observed at 1000 mg/L on algal propagation for Al_2O_3. The EC_{50} value of Al_2O_3 micro particles is in the range from 500 to 1000 mg/L, whereas that for Al_2O_3 nanoparticles is from 100 to 300 mg/L. The growth of algal culture at different dosage of SiO_2 nano and micro particles is graphically represented in Fig. 2. SiO_2 micro particles do not show significant toxicity to algae from 1 to 1000 mg/L. However, a

Table 1 Characterisation of nano and micro metal oxide particles

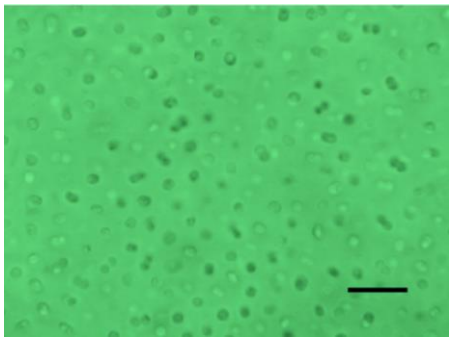
Particles	Crystalline phase	Purity	Zeta potential	Contact angle	Particle size distribution (mean size)		BET	References
		(%)	(mV)	(°)	Water (nm)	Medium (nm)	($m^2 g^{-1}$)	
Nano SiO_2	Amorphous	≥ 99	-25.8	54.65	50	70	361	(Yuvakkumar 2012)
Micro SiO_2	-	≥ 99	-25.6	50.31	-	-	-	Lobachemie (14808-60-7)
Nano Al_2O_3	Cubic	≥ 99	+49	35.67	58	68	190	(Manivasakan 2011)
Micro Al_2O_3	-	≥ 99	-21.8	17.37	-		-	Merck (1344-28-1)



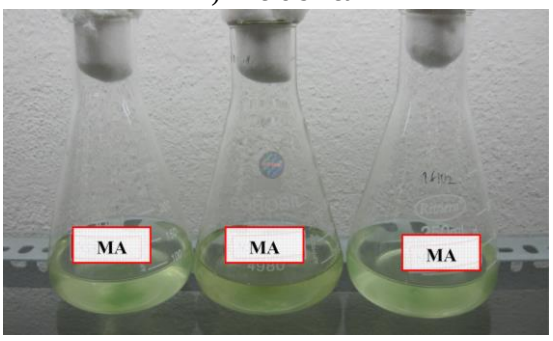
i) Nano silica



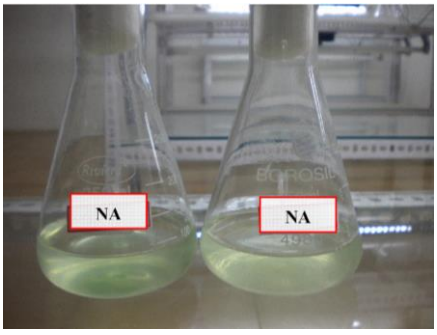
ii) Micro silica



iii) Culture image



iv) Micro alumina



iv) Nano alumina

Fig. 1 Culture flasks containing different concentrations of metal oxide particles

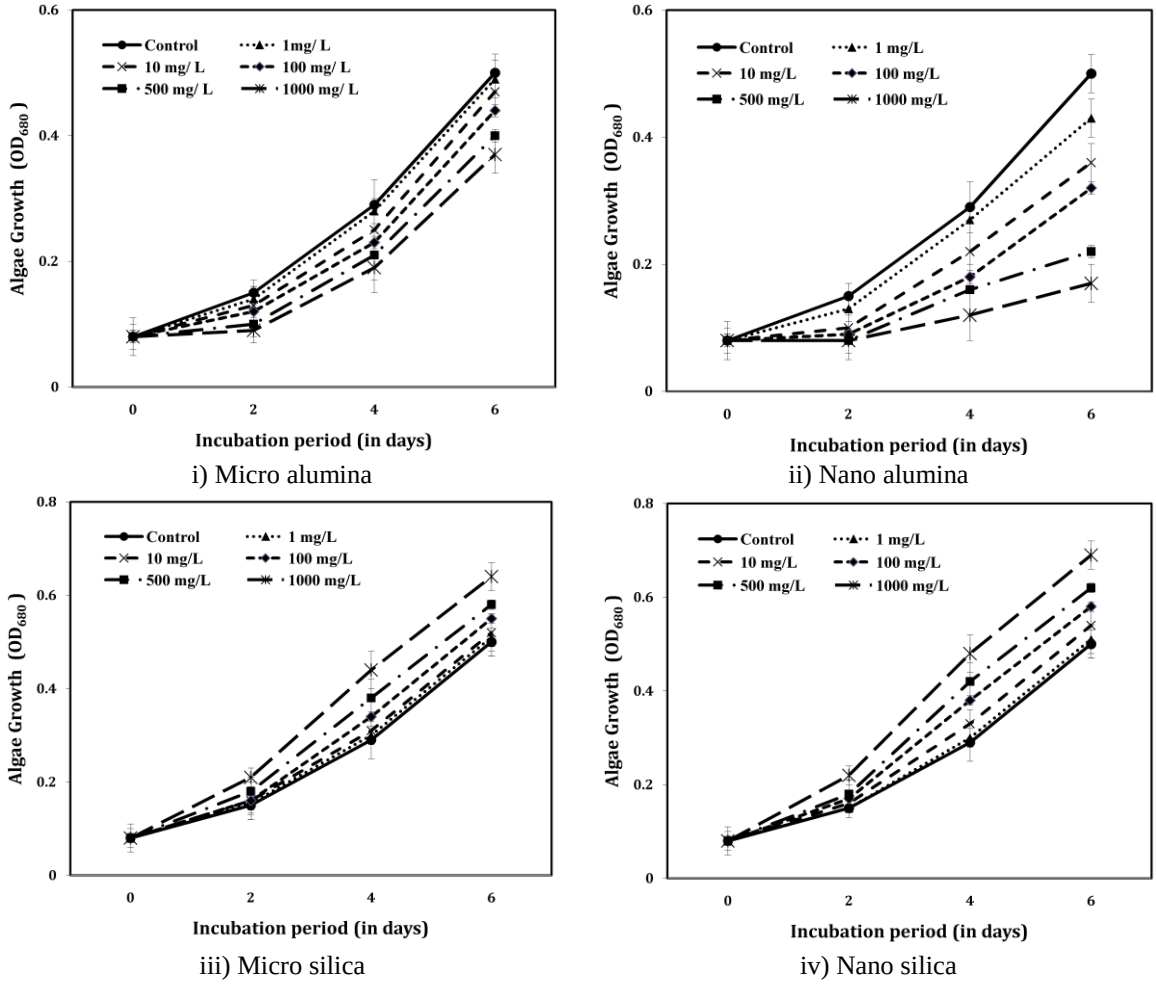


Fig. 2 Growth curve of *Porphyridium aerugineum Geitler* at different concentrations of metal oxide nanoparticles at two days interval

similar result is observed for SiO_2 nanoparticles.

The effect of Al_2O_3 nano and micro particles on the total chlorophyll and protein content is shown respectively in Fig. 3 and Table 2. The effective concentration for the reduction in chlorophyll content in Al_2O_3 is found to be between 500 and 1000 mg L^{-1} while for nano Al_2O_3 nanoparticles is between 100 and 500 mg/L. Similarly, the reduction in protein content in Al_2O_3 is found respectively, to be 151 and 158 µg/mL at 500 mg/L when compared with control (169.6 µg/mL). The variation in total chlorophyll and protein content of algae to SiO_2 treatment is represented in Fig. 3 and Table 2, respectively. The maximum increase in chlorophyll content observed for SiO_2 particles is from 500 to 1000 mg/L, whereas that for SiO_2 nanoparticles is from 100 to 500 mg/L. For the protein enhancement of 232.5 and 210 μg/mL, the most effective concentration for SiO_2 nano- and microparticles is found to be at 500 mg/L.

FTIR spectra of the dried algae cells treated with Al_2O_3 nanoparticles are shown in Fig. 4. The stretching vibrations at 715 and 1013 cm^{-1} show the characteristic peaks of AlO_4 and Al–OH,

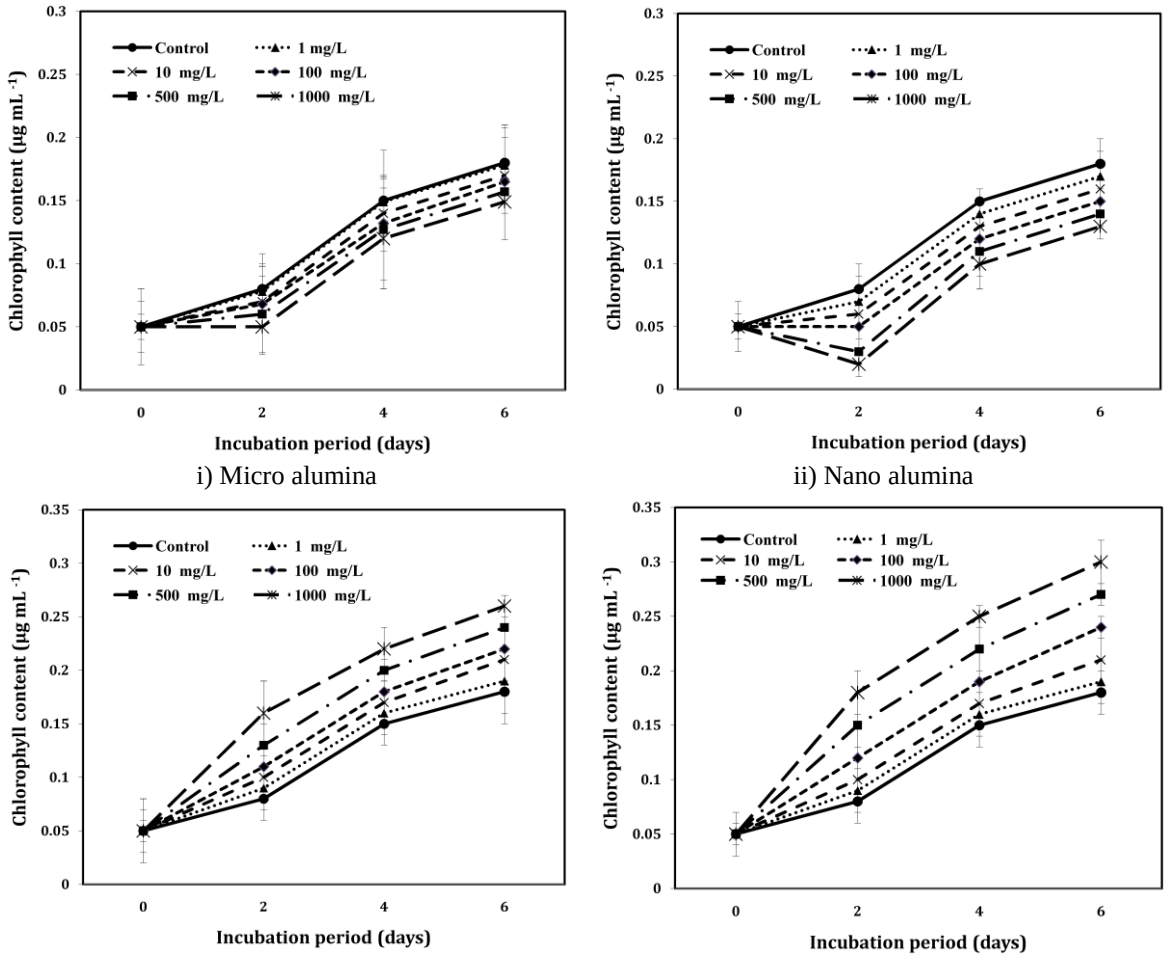


Fig. 3 Total chlorophyll content as a function of different concentrations of metal oxide particles

Table 2 Effect of metal oxides on total protein content in *Porphyridium aerugineum Geitler*

Metal oxides	Protein content (in μg/ml) in different days			
	0 day	2 day	4 day	6 day
Control	151.10± 0.03	158.05± 0.01	165.90± 0.04 [a]	169.65± 0.01 [a]
Nano Al_2O_3	150.30± 0.01	150.33± 0.04	151.68± 0.03	151.02± 0.04
Micro Al_2O_3	151.14± 0.04	153.14± 0.03	156.14± 0.02	158.05± 0.02
Nano SiO_2	152.90± 0.01	189.24± 0.02	214.70± 0.01[a]	232.50± 0.03[a]
Micro SiO_2	152.05± 0.04	165.90± 0.03	184.22± 0.06	210.00± 0.01 [a]

[a] represents the level of significance at $p<0.05$

respectively. The peaks observed at 1403 and 1634 cm^{-1} represent C–C and –OH, H_2O bonds. The peak observed at 2095 cm^{-1} is assigned for the bands of protein in the algae. The wide region between 3700 and 3300 cm^{-1} corresponds to O–H and N–H vibrations. The effects of SiO_2

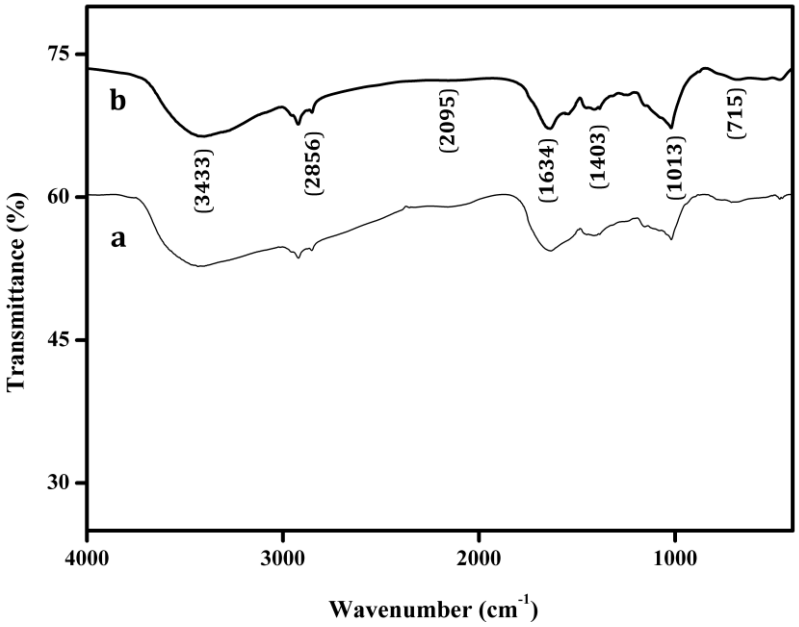


Fig. 4 FTIR spectrum of Porphyridium aerugineum Geitler a) before and b) After treatment with Al_2O_3 nanoparticles

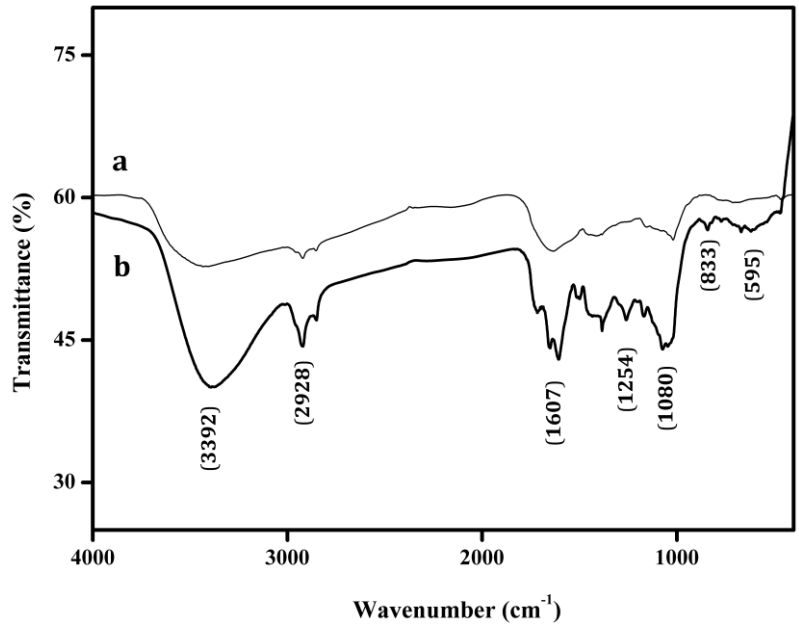


Fig. 5 FTIR spectrum of Porphyridium aerugineum Geitler a) before and b) After treatment with SiO_2 nanoparticles

nanoparticles in algal cells were screened through FTIR spectra, as represented in Fig. 5. The vibration bands at 595 and 833 cm^{-1} show the characteristic peaks of Si–OH and Si–O–Si. The

Table 3 Elemental compositions of *Porphyridium aerugineum Geitler* treated with nano and micro Al_2O_3 and SiO_2 particles at 500 mg L^{-1} concentration

Analyte (%)	Control	Nano Al_2O_3	Micro Al_2O_3	Nano SiO_2	Micro SiO_2
SiO_2	89.3	88.1	87.35	96.35	92.05
K_2O	5.93	6.05	7.92	1.51	4.06
Fe_2O_3	0.28	0.49	0.29	0.21	0.3
SO_3	0.22	0.48	0.5	0.21	0.21
MnO	0.18	0.3	0.2	0.1	0.19
Al_2O_3	0	2.25	0.98	0	0
CuO	0.05	0.01	0.04	0.06	0.09
P_2O_5	4.01	2.3	2.68	1.52	3.05
ZnO	0.03	0.02	0.04	0.04	0.05

peaks observed at 2928 and 3392 cm^{-1} represent CH_2 and –OH, H_2O bonds. The stretching peak observed between 800 and 1254 cm^{-1} indicates the superimposition of different SiO_2 peaks of Si–OH. The peak observed at 2928 cm^{-1} corresponds to proteins. Corresponding peaks for C–O functional groups are observed in green algae at 1080 cm^{-1}.

Elemental analyses of control and metal oxide treated algal samples through XRF studies are given in Table 3. The results show that the adsorption of Al_2O_3 nanoparticles is found to be 2.25% while that of micro Al_2O_3 is 0.98%. Similarly, SiO_2 adsorption in algae is found to be 7.05% and 2.75%, respectively, for SiO_2 nano and micro particles when compared with control at a concentration of 500 mg/L.

4. Discussion

SiO_2 and Al_2O_3 nanoparticles used in this study are in the form of suspensions, so it is necessary to analyse the difference in particle size in ultrapure water and in OECD medium. As the medium composition varies, the particle size varies. In the water, the initial size of SiO_2 and Al_2O_3 nanoparticles is found to be 50 and 58 nm, respectively. In OECD medium, the size of SiO_2 and Al_2O_3 nanoparticles is increased from 50 and 58 nm to 70 and 68 nm, respectively. An increase in particle size by 1/4th folds is observed for both particles in the medium. The nature of the particle in suspension varies due to the chemical or physical disturbances (Filella and Buffle 1993). The continuous shaking may lead to an increase in the aggregation of the particles, as well as an increase in the particle size. The medium of the particle mainly depends on ionic strength and surface charge (Handy 2008a, Handy 2008b).

Generally higher hydrophobic potential of nanoparticles imparts lesser solubility and lesser dispersion due to the formation of aggregates. The electrostatic force and contact angle of nano and micro particles are essential to contribute the adhesion of charged particles on the cell surfaces (Jiang 2009). Hence, zeta potential of a material is necessary for the interaction with cell surface. Al_2O_3 nanoparticles have a contact angle of 35.67° with positively charged zeta potential (+49 mV) that leads to the adhesion of particles on the surface of algae which is toxic to algae. In contrast, Al_2O_3 microparticles show a decrease in contact angle of 17.37° with negative zeta

potential (–21.8 mV). However, SiO_2 microparticles show an increase in contact angle of 50.31° with negative zeta potential (–25.8 mV) whereas SiO_2 nanoparticles also show an increase in contact angle of 54.65° with negative zeta potential (–25.6 mV).

Thus, it is clear from the observations that toxicity mainly depends on zeta potential. Because Al_2O_3 nanoparticles have positive zeta potential, they have the highest toxicity on algae than other particles. Owing to the negative charge on the surface of algae, Al_2O_3 nanoparticles with positive zeta potential tend to attract on the surface of algae to neutralise the charge which directly influences its surface and leads to cell death. These results convey the biological interaction mechanism of nano and micro particles with algae cells.

The important parameters such as no observed effect concentration (NOEC) and half maximal effective concentration (EC_{50}) are determined from the growth curve in the toxicity evaluation. The results show that Al_2O_3 is found to be toxic in both particle sizes. For Al_2O_3 microparticles, the EC_{50} value is found to be in the ranges from 500 to 1000 mg/L whereas that for the NOEC is found to be in the ranges from 1 to 100 mg/L. Similarly, for Al_2O_3 nanoparticles, the EC_{50} value is found to be in the ranges from 100 to 300 mg/L whereas that for the NOEC is found to be in the ranges from 1 to 10 mg/L when compared with control. The change in chlorophyll content of algae treated with Al_2O_3 nano and micro particles is observed. The decrease in chlorophyll content as a function of particle concentration and particle size is observed. The effect of Al_2O_3 nanoparticles on chlorophyll content is found to be more than that of Al_2O_3 microparticles. SiO_2 are nontoxic to algae at a concentration of 1000 mg/L. After 6 days, the EC_{50} values of bulk and nanoparticles are not determined because of the nontoxic nature. It also indicates that an increase in the chlorophyll content of 0.3 μg/mL for nano SiO_2 and 0.26 µg/mL for micro SiO_2 particles is observed at a concentration of 1000 mg/L. The increase in the growth is due to the uptake of SiO_2 particles which is required for the cellular metabolic activities.

Interaction of algae cells with nanoparticles is identified by observing the color intensity of culture in OECD medium (Fig. 1). The algae interaction with the treated particles is observed from the culture flasks. As shown in flask images, the color intensity increases for SiO_2 nano- and micro particles, whereas a decrease is observed for Al_2O_3 nano and micro alumina particles. The algae cells settled down at the bottom of the flask and no growth is observed further. From the growth curve, results of algae cells are compared with the color intensity and it was found that Al_2O_3 nanoparticles are more toxic than their bulk counterparts. It is mainly due to the difference in particle size. It is well known that differences in the reactivity of nanomaterials also rely on the difference in surface area to the change in biological activity (Oberdorster 2005). Similarly, a study on green algae (*Desmodesmus subspicatus*) shows a difference in activity with the surface area (Hund-Rinke and Simon 2006). The change in chlorophyll content is due to the breakdown of organic molecules, which may result in the deactivation of the active receptor sites (Zhang 2003).

The cell wall is the primary site for the attraction of any material for the reaction. The major cell wall components are protein, lipid and carbohydrate chains (Knox 1995). The main active sites for the attraction are amine, phosphate, imidazole, carboxylate, sulfhydryl and hydroxyl in the biomolecules of the cell wall. To understand intake of nanoparticles by algae, FTIR study is performed for control and the algae cells treated with particles. The study confirms the attachment of Al_2O_3 and SiO_2 on the surface of the algae cells. The importance of the condensed or isolated state of AlO_6 and AlO_4 coordination groups in c-$A1_2O_3$ structure are reviewed to understand the characteristic infrared absorption band frequencies (Tarte 1967). On the basis of the experimental results, it is shown that for condensed AlO_6 octahedra and isolated AlO_4 tetrahedra, vibrational frequencies are found to be in the range of 680–500 cm^{-1} and 800–700 cm^{-1}, respectively. The

significance of localised vibrations of AlO_4 and AlO_6 coordination groups in c-$A1_2O_3$ vibrational spectra has previously been revealed (Saniger 1995). The effect of SiO_2 nanoparticles in algal cells is screened through FTIR analysis. The observed peaks for the presence of SiO_2 content are in line with the previous reports (Yee 2004, Beganskiene 2004). A strong band observed near 753 cm^{-1} lies in the middle of the expected vibration range of isolated AlO_4 coordination groups, as reported earlier (Chandradass and Balasubramanian 2006, Naskar 2002).

Toxicity of algae depends on the physicochemical factors such as size, ionic strength, chemical composition, and concentration. The shading effect plays a key role in toxicity of nanoparticles by retarding the light energy (Navarro 2008). The opacity of nanoparticle suspension indirectly plays a role in inhibition of growth by decreasing the solution intensity. The physical restraint is one of the indirect mechanisms of nanoparticles toxicity toward algae (Navarro 2008). The accumulation of nanoparticles on the surface of algae causes shading effects that inhibit the photosynthetic activity. The earlier study (Hoeckel 2008) suggested that there is no evidence for the uptake of SiO_2 nanoparticles (12.5 and 27 nm) into the cells of *Pseudokirchneriella subcapitata* from electron microscopic images.

Sorption of nanoparticles to the cell walls of algae is reported to be a function of aggregation tendency and interaction with other organics present in the system (Chen and Elimelech 2007). Similarly, an increase in cellular weight is reported owing to TiO_2 nanoparticles adsorbed onto the surface of algae (Huang 2005). The FTIR studies (Figs. 4 and 5) correlating an active participation of the surface groups in the interaction confirms the adsorption of the aggregated nanoparticles onto the surface. Decreased light availability (shading effect) owing to surface adsorption of the particles on the cell wall of algae is one of the factors for the observed growth inhibitory effects. An increase in surface area of the nanoparticles compared to the micron sized particles results in enhanced adsorption. Thus, it leads to more growth inhibitory effect.

5. Conclusions

A systematic study on the evaluation of toxicities of SiO_2 and Al_2O_3 nano and micro particles on the algae is performed. Al_2O_3 nanoparticles were remarkably more toxic to algae than their micro counterparts. The toxicity of Al_2O_3 nanoparticles was explained by correlating the zeta potential and hydrophobicity in water and in OECD medium. SiO_2 nano and micro particles were found to be nontoxic. The nanoparticles formed an aggregate in the culture media that entrapped the algal cells and caused algal growth inhibition. This study confirms that the particle size, zeta potential and hydrophobicity play a key role in the toxicity mechanism. The ecological effects of SiO_2 and Al_2O_3 particles observed in this study elucidate the toxicity of nanoparticles, particularly in the aquatic environment.

Acknowledgments

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