CHAPTER 3
RESULTS AND DISCUSSION
This chapter describes the results obtained from the experimental methods used for the synthesis and characterisation of metal oxide nanoparticles (SiO$_2$, Al$_2$O$_3$, TiO$_2$, and ZrO$_2$) and the evaluation of the toxicological behaviour of metal oxide nano- and microparticles on soil, animal cell line, plant and algae.

3.1 SYNTHESIS AND CHARACTERISATION OF METAL OXIDE NANOPARTICLES
In this section, metal oxide nanoparticles such as SiO$_2$, Al$_2$O$_3$, TiO$_2$, and ZrO$_2$ are synthesised using natural sources such as biomass rice husk, bauxite, ilmenite and zircon sand. The synthesised powders are characterised through different techniques such as X-ray diffraction (XRD), FTIR, particle size distribution (PSD), transmission electron microscopy (TEM), zetasizer and contact angle studies.

3.1.1 Nano-Silica
Silica (SiO$_2$) nanoparticles are synthesised from a low-cost natural source (biomass rice husk) using precipitation method (Yuvakkumar et al 2012). The SiO$_2$ powder is obtained at pH 2. The XRD pattern of the prepared powder is shown in Figure 3.1a. The diffraction pattern of the sample observed at 22° (2θ) confirms the amorphous nature of the synthesised powder (Yuvakkumar et al 2012). The obtained XRD pattern of the sample agrees well with the standard powder diffraction data for SiO$_2$ nanoparticles (JCPDS file no. 29-0085).

Figure 3.1 Characterisation of SiO$_2$ nanoparticles
a) X-Ray Diffraction Pattern
b) FTIR Spectrum
c) Particle Size Distribution
d) TEM image

The FTIR spectrum for the SiO$_2$ nanoparticles is shown in Figure 3.1b. The bands observed at 470, 1108 and 1644 cm$^{-1}$ are assigned, respectively, to Si–O bond rocking, Si–O–Si stretching vibration and –OH bonding (Yuvakkumar et al 2012). The observed result confirms the presence of silanol group, siloxane linkage and adsorbed water group as found in SiO$_2$ microparticles. PSD shown in Figure 3.1c confirms the average size ($d_{50}$) of the powder to be 50 nm when dispersed in water whereas PSD of the powder in dispersed medium (saline/DMEM) shows 70 nm. The TEM image shown in Figure 3.1d confirms the spherical nature of SiO$_2$ nanoparticles. The specific surface area (SSA) of the prepared sample is shown in Table 3.1, which is found to be 361 m$^2$ g$^{-1}$.

SiO$_2$ microparticles show a negative zeta potential of 25.6 mV with a contact angle of 50.31°. On the other hand, SiO$_2$ nanoparticles show a negative zeta potential of 25.8 mV with a high contact angle of 54.65°. Hydrophobic potentials for SiO$_2$ nano- and microparticles in terms of contact angles are 54.65° and 50.31°, respectively, which confirm the hydrophobic nature of the particles. Thus, contact angle is one of the factors for hydrophobicity.

3.1.2 Nano-Alumina
The XRD pattern of the synthesised alumina (Al$_2$O$_3$) nanoparticles shown in Figure 3.2a confirms the cubic nature (Manivasakan et al 2011a) of the particles. The XRD pattern of the synthesised powder is also found to be cubic in nature, which agrees well with the standard powder diffraction data (JCPDS file no. 79-1558). Functional groups present in the Al$_2$O$_3$ particles are found through FTIR spectrum (Figure 3.2b). The bands observed at 1100 and 1633 cm$^{-1}$ are assigned, respectively, to Al–OH and –OH, H$_2$O (Manivasakan et al 2011a). The band observed at 1410 cm$^{-1}$ corresponds to the bending vibration of C–C bond. The band observed at 502 cm$^{-1}$ corresponds to the
bending and stretching vibrations of Al–O bond. PSD shown in Figure 3.2c confirms the average particle size \((d_{50})\) of the prepared powder to be 58 nm when dispersed in water and 68 nm when dispersed in medium (saline/DMEM).

a) X-Ray Diffraction Pattern  
b) FTIR Spectrum  
Figure 3.2 Characterisation of Al\(_2\)O\(_3\) nanoparticles  
69  
c) Particle Size Distribution  
d) TEM image  
Figure 3.2 (Continued)  
70

The TEM image of the prepared powder shown in Figure 3.2d confirms the almost spherical shape of the particles. The SSA of the synthesised powder samples is 190 mg \(^{-1}\), as given in Table 3.1. The zeta potentials of Al\(_2\)O\(_3\) micro- and nanoparticles are found to be 21.8 and +49 mV, respectively. The measured contact angles of the Al\(_2\)O\(_3\) nano- and microparticles are 35.67° and 17.37°, respectively.

3.1.3 Nano-Titania

The crystalline structure of the prepared titania (TiO\(_2\)) nanoparticles is analysed from the XRD pattern wherein the full width at the half maximum value shows the presence of tetragonal structure (JCPDS file no. 21-1276) (Figure 3.3a). The FTIR band (Figure 3.3b) observed at 834 cm \(^{-1}\) is assigned for Ti–O–Ti vibration. The peak observed at 1055 cm \(^{-1}\) is attributed to stretching form of Ti–OH groups. At 1630 cm \(^{-1}\), the bending vibration of Ti–OH group is observed. The band observed at 3392 cm \(^{-1}\) corresponds to the stretching and bending vibrations of –OH bond. PSD (Figure 3.3c) shows the average size of the prepared powder to be 50 nm in water and 65 nm in medium (saline/DMEM).

The TEM image of the synthesised powder (Figure 3.3d) shows the almost spherical shape of the particles. The SSA (Table 3.1) of the as-prepared powder samples is found to be 112 mg \(^{-1}\). The zeta potential for both TiO\(_2\) nano- and microparticles is found to be 22.0 mV. The hydrophobic potentials of TiO\(_2\) nano- and microparticles in terms of contact angles are 47.82° and 23.12°, respectively.

3.1.4 Nano-Zirconia

The prepared ZrO\(_2\) powder is characterised comprehensively. The XRD pattern of the prepared ZrO\(_2\) powder shown in Figure 3.4a confirms the cubic nature of the prepared powder (JCPDS file no. 65-0461). The FTIR spectral bands observed at 585, 1126, 1643 and 2047 cm \(^{-1}\) are assigned, respectively, to Zr–O bond rocking, Zr–OH stretching vibration, Zr–OH bond linking and OH bonding (Figure 3.4b).

The above result confirms the presence of zircon group, zircoxane linkage and adsorbed water group as found in ZrO\(_2\) microparticles. The average PSD of the synthesised powder is 39 nm (Figure 3.4c) in water whereas it is 50 nm when dispersed in medium (saline/DMEM). Figure 3.4d shows the TEM image where the particles are observed to be almost spherical in shape. The SSA of the as-prepared powder (Table 3.1) is found to be 227 mg \(^{-1}\), which is calculated using the Brunauer–Emmett–Teller (BET) plot.

The zeta potentials of ZrO\(_2\) nano- and microparticles are found to be, respectively, +11.8 and 13.4 mV. The hydrophobic potentials of ZrO\(_2\) nanoand microparticles in terms of contact angles are, respectively, 73.37° and 33.20°.
3.2.1 Effect of Metal Oxide Particles on Plant Growth-Promoting Rhizobacteria

The effect of SiO$_2$, Al$_2$O$_3$, TiO$_2$ and ZrO$_2$ nano- and microparticles on PGPR is evaluated and the results are shown in Table 3.2. Evaluation of toxicity using the disc diffusion method shows 14 ± 0.3 mm (Bacillus megaterium), 13 ± 0.2 mm (Bacillus brevis), 16 ± 0.2 mm (Azotobacter vinelandii) and 15 ± 0.2 mm (Pseudomonas fluorescens) diameter zones of inhibition for Al$_2$O$_3$ nanoparticles. This shows that Al$_2$O$_3$ nanoparticles exhibit higher toxicity towards all four tested bacterial species. Al$_2$O$_3$ microparticles does not show any zone of inhibition (Table 3.2). In a previous study, Al$_2$O$_3$ nanoparticles exhibited toxic response over Bacillus licheniformis, an isolate from freshwater (Pakrashi et al 2011). SiO$_2$ and ZrO$_2$ particles found to have no toxicity towards PGPR at both nano and micro levels (Table 3.2) as there is no visible zone of inhibition.

Nano-TiO$_2$ treatment results in 13 ± 0.9 mm (B. megaterium), 12 ± 0.3 mm (B. brevis), 16 ± 0.2 mm (A. vinelandii) and 15 ± 0.6 mm (P. fluorescens) diameter zone of inhibition. TiO$_2$ microparticles shows inhibition zones of 13 ± 0.7 mm (P. fluorescens), 11 ± 0.4 mm (B. megaterium), 10 ± 0.2 mm (B. brevis) and 14 ± 0.5 mm (A. vinelandii). The observed results reveal that the size of the particles plays a key role in bacterial toxicity. According to a previous report, TiO$_2$ nanoparticles greatly affect the nitrogen-fixing bacteria, which directly influences the sludge treatment process (Zheng et al 2011). Thus, the toxicity potential assessment of particles using disc diffusion method shows that SiO$_2$ and ZrO$_2$ particles (Table 3.2) are non-toxic to PGPR in both micro and nano forms, whereas TiO$_2$ nanoparticles are highly toxic for all the tested bacterial species.

3.2.2 Influence of Metal Oxide Nano- and Microparticles on PGPR Growth in Different Media

PGPR exposed to SiO$_2$, Al$_2$O$_3$, TiO$_2$ and ZrO$_2$ nano- and microparticles at 20 mg L$^{-1}$ concentration in water and in saline medium shows difference in the percentage of viable colony-forming units (CFU) formed. In water (Figure 3.5), micro-Al$_2$O$_3$ treatment shows a mean decrease in bacterial colony of 97% (A. vinelandii), 96% (B. megaterium), 98% (B. brevis) and 96% (P. fluorescens), whereas nano-Al$_2$O$_3$ shows a decrease in cell viability of 45% (A. vinelandii), 53% (B. megaterium), 56% (B. brevis) and 48% (P. fluorescens) in treated bacterial cells compared with control ($p < 0.05$; Figure 3.5). SiO$_2$ microparticles shows an increase in CFU, i.e., of 101% for A. vinelandii, 103% for B. megaterium, 111% for B. brevis and 104% for P. fluorescens. Similarly, nano-SiO$_2$ treatment also shows an increase in the percentage of bacterial colonies formed, 104% for A. vinelandii, 106% for B. megaterium, 114% for B. brevis and 105% for P. fluorescens, which are significant ($p < 0.05$).

In saline medium (Figure 3.6), micro-Al$_2$O$_3$ treatment shows a mean decrease in bacterial colonies to 98% (A. vinelandii), 97% (B. megaterium), 96% (B. brevis) and 97% (P. fluorescens). Nano-Al$_2$O$_3$ treatment shows a decrease in bacterial colonies to 63% (A. vinelandii), 71% (B. megaterium), 75% (B. brevis) and 65% (P. fluorescens) in treated...
bacterial cells when compared with control ($p < 0.05$; Figure 3.6).

Both SiO$_2$ micro- and nanoparticles enhanced the percentage of viable bacterial colonies. In case of SiO$_2$ microparticles, an increase in bacterial colonies to about 101% (A. vinelandii), 105% (B. megaterium), 115% (B. brevis) and 108% (P. fluorescens) is observed. For SiO$_2$ nanoparticles, the increase in bacterial colonies is about 105% (A. vinelandii), 110% (B. megaterium), 121% (B. brevis) and 123% (P. fluorescens), which are significant ($p < 0.05$).

Thus, the toxicity evaluation of metal oxides against bacterial suspension in deionized water and 0.01% NaCl (saline) followed by plating shows difference in the growth of PGPR. SiO$_2$ in both nano and micro forms does not show any toxicity in either water or 0.01% NaCl (saline) bacterial suspensions. However, this result differs from the earlier results (Adams et al 2006) in which SiO$_2$ nanoparticles water suspension is found to have antimicrobial activity against Gram-positive B. subtilis and Gram-negative Escherichia coli.

The electrostatic force of attraction and contact angle of the nanoand microparticles are important, which contribute in the adhesion of particles on the surface of the bacterial cell (Jiang et al 2009). The greater the hydrophobic potential of nanoparticles, the lesser the solubility and dispersion due to the formation of aggregates. Hence, zeta potential of a material is an important phenomenon that determines the extent of their interaction with cell surface.

A contact angle of $35.67^\circ$ for Al$_2$O$_3$ nanoparticles with positively charged zeta potential (+49 mV) has rendered it hydrophilic. Since bacterial surface is negatively charged, Al$_2$O$_3$ nanoparticles are attracted towards the surface and adhere onto it. This could be the reason for reduction in bacterial viability. Al$_2$O$_3$ microparticles have a small contact angle of $17.37^\circ$ as compared to Al$_2$O$_3$ nanoparticles but possess negative zeta potential (21.8 mV). The negative charge of Al$_2$O$_3$ microparticles makes them to repel from the bacterial surface, and hence, the percentage of bacterial viability is high.

SiO$_2$ microparticles show a contact angle of $50.31^\circ$ with a negative zeta potential (25.8 mV) whereas SiO$_2$ nanoparticles show a contact angle of $54.6^\circ$ with a negative zeta potential (25.6 mV). The observed contact angle is high for both SiO$_2$ micro and nanoparticles as compared to Al$_2$O$_3$ micro- and nanoparticles.

Figures 3.7 and 3.8 depict the results of bacterial response to TiO$_2$ and ZrO$_2$ micro- and nanoparticles dispersed in water and in saline medium at 20 mg/L concentration. A steep decrease in bacterial growth is observed for TiO$_2$ particles after incubation. In water (Figure 3.7), a decrease in bacterial cell viability is observed for TiO$_2$ nanoparticles, viz., 45% (A. vinelandii), 31% (B. megaterium), 37% (B. brevis) and 40% (P. fluorescens). In addition, micro-TiO$_2$ treatment also causes a decrease in bacterial colony formation of about 58% (A. vinelandii), 51% (B. megaterium), 45% (B. brevis) and 56% (P. fluorescens), compared with control ($p < 0.05$; Figure 3.7).

In contrast to TiO$_2$ nano- and microparticles, ZrO$_2$ both at nano and micro-levels promoted bacterial growth comparable to control. The percentage of viable CFU for ZrO$_2$ nanoparticles is 98% (A. vinelandii), 99% (B. megaterium), 97% (B. brevis) and 98% (P. fluorescens) whereas that for ZrO$_2$ microparticles, it is 98% (A. vinelandii), 97% (B. megaterium), 97% (B. brevis) and 98% (P. fluorescens), which are significant at $p < 0.05$.

Figure 3.5 Average viable PGPR CFU with respect to SiO$_2$ and Al$_2$O$_3$ particles in deionised water suspension. *, **and *** indicates the corresponding significance in their homogenous subset by Tukey’s test at 5% level

In saline medium (Figure 3.8), TiO$_2$ nanoparticles have reduced the percentage of bacterial colonies to 75% (A. vinelandii), 60% (B. megaterium), 55% (B. brevis) and 70% (P. fluorescens). For TiO$_2$ microparticles, it is 85%
(A. vinelandii), 80% (B. megaterium), 76% (B. brevis) and 82% (P. fluorescens), which is found to be significant ($p < 0.05$; Figure 3.8). In both ZrO$_2$ nano- and microparticle treatment, the growth is almost comparable to that of control. For ZrO$_2$ nanoparticle, it is 99% (A. vinelandii), 98% (B. megaterium), 97% (B. brevis) and 98% (P. fluorescens) whereas for ZrO$_2$ microparticle, it is 99% (A. vinelandii), 98% (B. megaterium), 97% (B. brevis) and 98% (P. fluorescens), which are found to be significant ($p < 0.05$). Figure 3.6 Average viable CFU with respect to SiO$_2$ and Al$_2$O$_3$ particles in saline (0.01% NaCl) solution. *, ** and *** indicates the corresponding significance in their homogenous subset by Tukey’s test at 5% level
The growth inhibition under water suspension may be due to the interaction of particles with water molecules, which leads to the formation of corresponding hydroxides (Jassby et al 2012) or it might be due to the release of ions in water (Brunner et al 2006). A similar kind of result is obtained when the nano-sized TiO$_2$, silicon dioxide (SiO$_2$), and ZnO particles react with B. subtilis and E. coli (Adams et al 2006).
ZrO$_2$ nanoparticles possess a contact angle of 73.37° with a positive zeta potential of +11.8 mV. As it is having a less positive charge, it may get attracted strongly towards bacterial surface. ZrO$_2$ microparticles shows a contact angle of 33.20° with a negative zeta potential of -13.4 mV. The negative charge of ZrO$_2$ microparticles makes it unable to react with bacterial cells. TiO$_2$ micro- and nanoparticles show contact angles of 23.12° and 47.82°, respectively. It is interesting to note that both TiO$_2$ particles possess same negative potential of 22.0 mV but differ in their hydrophobicity. Even though TiO$_2$ particles have negative charge, a considerable reduction in bacterial viability is observed. In other words, they suppressed the bacterial growth and multiplication. TiO$_2$ nanoparticles are found to be more toxic than TiO$_2$ microparticles. It is clearly pictured from these results that zeta and hydrophobic potentials play key roles in rendering bacterial toxicity.
Figure 3.7 Average viable PGPR CFU with respect to TiO$_2$ and ZrO$_2$ particles in deionised water suspension. *, ** and *** indicates the corresponding significance in their homogenous subset by Tukey’s test at 5% level
Figure 3.8 Average viable CFU with respect to TiO$_2$ and ZrO$_2$ particles in saline (0.01% NaCl) solution. *, ** and *** indicates the corresponding significance in their homogenous subset by Tukey’s test at 5% level
3.2.3 Effect of Metal Oxide Nanoparticles and Micro Particles on Soil Bacterial Population and Soil Nutrients
The metal oxide nano- and microparticles show a dose-dependent difference in total CFU. The SiO$_2$-nanoparticle-incorporated soil (Figure 3.9) dramatically increases the soil bacterial population from $4.1 \times 10^5$ to $4.8 \times 10^5$ CFU per gram of soil at a concentration of 0.1 and 1000 mg kg$^{-1}$. Similarly, SiO$_2$ microparticle-incorporated soil (Figure 3.9) shows a steep increase in the soil bacterial population from $3.9 \times 10^5$ to $4.4 \times 10^5$ CFU per gram of soil at a concentration of 0.1 and 1000 mg kg$^{-1}$. In contrast, in Al$_2$O$_3$ nanoparticle- and microparticle-incorporated soil (Figure 3.9), there is a decrease in the soil bacterial population from $2.8 \times 10^5$ to $1.6 \times 10^5$ CFU g$^{-1}$ of soil and $3.7 \times 10^5$ to $3.4 \times 10^5$ CFU g$^{-1}$ of soil at a concentration of 0.1 and 1000 mg kg$^{-1}$, respectively.
The initial and final pH measurements (Table 3.3) of the soil clearly show the influence of metal oxide nano- and microparticles on soil pH. SiO$_2$ nanoparticle-incorporated soil shows a decrease in pH from 8.25 to 6.5 from initial day to 30th day, whereas SiO$_2$ microparticle-incorporated soil shows a decrease in pH from 8.7 to 7.0 on 30th day. However, Al$_2$O$_3$ nanoparticle- and microparticle-incorporated soil shows an increase in pH from 8.35 to 9.86
and 7.04 to 8.05, respectively, at 30th day. The change in pH of the soil does not affect the texture of the soil. But the colour of the soil changed from black to milky white when the concentration of the nano- and microparticles reaches 1000 mg kg⁻¹ of soil.

In addition, the effect of SiO₂ and Al₂O₃ nano- and microparticles on total soil organic carbon, total nitrogen, phosphorus and available potassium content is analysed and compared with control. As shown in Table 3.3, after 30th day of incubation, addition of SiO₂ nanoparticle increases the total organic carbon content from 2.01 to 4.8 g kg⁻¹, nitrogen content from 0.14 to 0.28 g kg⁻¹, phosphorus content from 7.6 to 11.3 mg kg⁻¹ and potassium content from 504 to 804 kg ha⁻¹ when compared to control. Similarly, addition of SiO₂ microparticle increases the total organic carbon content from 2.0 to 3.2 g kg⁻¹, nitrogen content from 0.12 to 0.20 g kg⁻¹, phosphorus content from 7.3 to 10.1 mg kg⁻¹ and potassium content from 503 to 704 kg ha⁻¹ when compared to control.

Addition of Al₂O₃ nanoparticles shows minor change in the nutrient content for total organic carbon content from 1.89 to 1.9 g kg⁻¹, nitrogen content from 0.13 to 0.12 g kg⁻¹, phosphorus content from 7.4 to 6.5 mg kg⁻¹ and potassium content from 506 to 498 kg ha⁻¹ after incubation. But addition of Al₂O₃ microparticle shows an increase in nutrient content at 30th day of incubation as follows: total organic carbon content from 2.1 to 2.2 g kg⁻¹, nitrogen content from 0.12 to 0.14 g kg⁻¹, phosphorus content from 7.5 to 9.2 mg kg⁻¹ and potassium content from 503 to 597 kg ha⁻¹.

The toxicity of metal oxide nano- and microparticles in soil is also evaluated by counting the number of CFU after treatment. The total number of CFU (Figure 3.9) shows that SiO₂ nano- and microparticles enhance microbial population. It is being reported (Wainwright et al. 2003) that silicic acid stimulates bacterial growth of both aerobic and facultative anaerobic bacteria in ultra-pure water with soil inoculant at short oligotrophic condition. Also, addition of silicon in soil enhances the microbial population. In addition, it is also being proposed from the same study that there is a possibility of first bacteria being evolved on earth due to silicon. Thus, the incorporation of SiO₂ nanoparticles into soil enhances the soil nutrient value. In contrast, a reduction in total C, N, P and available K content is observed in soil incorporated with Al₂O₃.

The results of soil nutrient analysis (Table 3.3) reveal the influence of Al₂O₃ nano- and microparticles on total carbon, nitrogen, phosphorus and available potassium levels in soil. The variation in available nutrient after the addition of nano- and microparticles might be due to their effect on total soil microbial population.

Figure 3.9 Average viable soil bacterial CFU with respect to metal oxide particles at different concentrations. *, ** and *** indicates the corresponding significance in their homogenous subset by Tukey’s test at 5% level

ZrO₂ microparticle-incorporated soil (Figure 3.10) shows a decrease in the bacterial population from 3.7 × 10⁵ to 3.6 × 10⁵ CFU per gram of soil whereas ZrO₂ nanoparticle-incorporated soil shows increase in the bacterial population from 3.6 × 10⁵ to 3.8 × 10⁵ CFU per gram of soil at a concentration of 0.1 and 1000 mg kg⁻¹. Similar to ZrO₂ microparticle-, TiO₂-microparticle and TiO₂-nanoparticle-incorporated soil (Figure 3.10) shows a significant decrease in the bacterial population from 3.1 × 10⁵ to 2.4 × 10⁵ CFU per gram of soil and 2.8 × 10⁵ to 1.8 × 10⁵ CFU per gram of soil at a concentration of 0.1 and 1000 mg kg⁻¹.

ZrO₂ microparticle-incorporated soil (Table 3.4) shows an increase in pH from 7.04 to 7.82 whereas a slight change in pH from 8.35 to 8.37 is observed for ZrO₂ nanoparticle-incorporated soil from 0th day to 30th day. TiO₂ microparticle incorporation (Table 3.4) shows a significant increase in pH from 7.05 to 8.73. Similarly, TiO₂ nanoparticle incorporation also shows a
steep increase in pH from 7.04 to 9.86 between 0th and 30th days. The variation in pH may be due to reaction of particles with the soil. The increase in pH may be because of the formation of salts, which in turn enhances the soil pH. Since nano-sized ZrO$_2$ does not interfere much with the soil pH, it may be considered to be inert in soil. The results of bacterial population count and pH analysis of treated soil show that nano- and microparticles affect soil pH, which in turn affects the CFU. Thus, a steep increase in pH of soil on addition of ZrO$_2$ microparticles, TiO$_2$ microparticles and TiO$_2$ nanoparticles has reduced the bacterial population. The micro- and nanoparticles does not interfere much with soil texture but the colour of the soil changes from black to creamy white, when the concentration reaches to 1000 mg kg$^{-1}$. The drastic reduction in bacterial population on addition of TiO$_2$ nano- and microparticles shows their toxic nature. It is reported that a lower range of TiO$_2$ nanoparticles from 0.5 to 2 mg g$^{-1}$ results in a significant shift in bacterial communities (Ge et al 2011). The toxic nature of TiO$_2$ nanoparticles might be due to production of ROS when it comes in contact with the cell surface (Long et al 2006). The proposed toxicity of metal nanoparticles on soil in terms of CFU is investigated and it has been concluded that the impact of metal nanoparticles on bacteria is measurable (Shah and Belozerova 2009). It is essential to understand the effect of a particle, its charge and its hydrophobic potential (Jiang et al 2009). The higher hydrophobic potential results in less solubility and stability, and hence, it is found to be less reactive. 

In addition to pH, TiO$_2$ and ZrO$_2$: micro- and nanoparticles affect the soil nutrient contents, i.e., total organic carbon, total phosphorus, nitrogen and potassium. After incubation, ZrO$_2$: microparticles increase the total organic carbon from 2.1 to 2.45 g kg$^{-1}$, nitrogen content from 0.12 to 0.18 g kg$^{-1}$, phosphorus content from 7.5 to 9.8 mg kg$^{-1}$ and potassium content from 504 to 612 kg ha$^{-1}$. Similarly, ZrO$_2$: nanoparticles also enhance total organic carbon from 1.89 to 1.9 g kg$^{-1}$, nitrogen content from 0.12 to 0.20 g kg$^{-1}$, phosphorus content from 6.5 to 10.8 mg kg$^{-1}$ and potassium content from 501 to 615 kg ha$^{-1}$. In contrast, TiO$_2$: microparticles decrease the nutrient contents. For TiO$_2$: microparticles, total organic carbon content decreased from 2.01 to 1.7 g kg$^{-1}$, nitrogen content from 0.14 to 0.12 g kg$^{-1}$, phosphorus content from 7.6 to 7.2 mg kg$^{-1}$ and potassium content from 503 to 473 kg ha$^{-1}$. TiO$_2$: nanoparticles show the change in nutrient content for total organic carbon from 2.0 to 1.4 g kg$^{-1}$, nitrogen content from 0.12 to 0.10 g kg$^{-1}$, phosphorus content from 7.3 to 5.2 mg kg$^{-1}$ and potassium content from 504 to 390 kg ha$^{-1}$ when compared to control. 

Thus, it is clear from the above observations that the incorporation of ZrO$_2$: nanoparticles into soil enhances the soil nutrient value. In contrast, a reduction in total C, N, P and K contents is observed in TiO$_2$: incorporated soil. The soil nutrient analysis (Table 3.4) discloses the impact of TiO$_2$: nano and microparticles on total carbon, total nitrogen, phosphorus and potassium levels in soil. The change in nutrient content on incorporation of nano- and microparticles may be due to their impact on microbial population, which has been discussed previously. 

It is well known that microbes such as Azotobacter, B. mucilaginous and B. megaterium improve soil properties and nutrient content by recovering the soil N, P and K values (Wu et al 2005b). Nanoparticles that possess antimicrobial activity kills the bacteria and decrease the soil nutrient value. The result of the current investigation reveals the importance of particle size and the correlation between soil microbial populations and soil nutrient values. Therefore, it is essential to consider safety measures for the usage and disposal of such engineered metal oxide nanoparticles.

**Figure 3.10** Average viable soil bacterial CFU with respect to metal oxide particles at different concentrations. *, ** and *** indicates the corresponding significance in their homogenous subset by Tukey’s test at 5% level.
3.3 IN VITRO TOXICITY STUDIES

This section deals with the *in vitro* toxicity analysis of metal oxide nano- and microparticles such as SiO$_2$, Al$_2$O$_3$, TiO$_2$ and ZrO$_2$ on animal cell line and SBF and checking their antioxidant potential using DPPH.

### 3.3.1 Cytotoxicity Evaluation in NIH 3T3 Cell Lines

To evaluate the dosage effect on cell morphology, cultured NIH 3T3 cells were exposed to metal oxide nano- and microparticles for about 48 h. Phase-contrast microscopic images of NIH 3T3 cells exposed to metal oxide nano- and microparticles are shown in Figures 3.11–3.18. The control NIH 3T3 cell appears spindle-shaped, remains intact and adheres to the culture plate (Figure 3.11a). NIH 3T3 cells treated with Al$_2$O$_3$ microparticles have lost their adhesion property and hence, the extent of which depends on the concentration of the particles. Hence, the cells become spherical and float freely in the medium, as shown in Figure 3.11d. Cells treated with Al$_2$O$_3$ nanoparticles do not show any significant change with respect to cell number and morphology (Figure 3.12d). The influence of SiO$_2$ micro- and nanoparticles on NIH 3T3 cells is shown in Figures 3.13 and 3.14. SiO$_2$ microparticles show more impact on cell morphology and cell proliferation when compared to nanoparticles.

TiO$_2$ microparticle-treated NIH 3T3 cells shows visible morphological change, which is evident from phase-contrast microscopic image (Figure 3.15) whereas ZrO$_2$ micro- and nanoparticles (Figures 3.17 and 3.18) and TiO$_2$ nanoparticles (Figure 3.16) have hardly shown changes even 1. NIH 3T3 cells treated with TiO$_2$ microparticles have lost their adhesion and have become spherical in the medium (Figure 3.15d). Cells treated with ZrO$_2$ and TiO$_2$ nanoparticles do not show any significant changes with respect to cell numbers (Figures 3.18d and 3.16d) whereas ZrO$_2$ microparticles have significantly influenced the cell count.

### 3.3.2 Cytotoxicity Assay

The cytotoxicity study using murine embryonic fibroblast (NIH 3T3) cells is performed to evaluate the toxicological difference under laboratory conditions. The NIH 3T3 cells exposed to different concentrations of metal oxide nano- and microparticles show difference in mitochondrial damage, which is estimated using MTT assay. After 48 h of incubation, a decrease in the percentage of cell viability is observed as a result of dosedependent
treatment (Figure 3.19). Al₂O₃ microparticle treatment shows a mean decrease in cell viability from 87% to 62.3% as the concentration is increased. Al₂O₃ nanoparticle treatment shows a considerable decrease in cell viability from 99% to 73.3% at a concentration of 1. With SiO₂ microparticle treatment, a significant decrease in cell viability from 98.3% to 60.5% is observed as the concentration is increased. In addition, SiO₂ nanoparticle treatment also shows a considerable decrease in cell viability from 99% to 73.3% at a concentration of 1, when compared with control (p < 0.05; Figure 3.19).

With SiO₂ microparticle treatment, a significant decrease in cell viability from 98.3% to 60.5% is observed as the concentration is increased. In addition, SiO₂ nanoparticle treatment also shows a considerable decrease in cell viability from 99% to 73.3% at a concentration of 1, which is significant (p < 0.05). Thus, the observed order of cytotoxicity is as follows: micro-SiO₂ > micro-Al₂O₃ > nano-SiO₂ > nano-Al₂O₃ (Figure 3.19). Therefore, SiO₂ microparticles are considered to be more toxic whereas Al₂O₃ nanoparticles are considered to be least toxic to NIH 3T3 cells.

Previous observation (Sha et al 2011) shows the difference in toxicity with respect to cell type (cancer or normal). Cancer cells are found to be more tolerant to nanoparticles than normal cells. Thus, in the present study, a naturally occurring totipotent cell—NIH 3T3—is taken as a model system to analyse the toxicity difference for comparing the already existing reviews.

Figure 3.19 Percentage cell viability of NIH 3T3 cell lines with respect to nano/micro Al₂O₃ and SiO₂ metal oxides treatment. * and ** indicate the corresponding significance in their homogenous subset by Tukey’s test at 5% level

When nano- and microparticles are compared, microparticles render more toxicity towards the cells. This is observed for both Al₂O₃ and SiO₂ particles. The higher toxic effect of microparticles may be due to the gravitational force exhibited by them, leading to adhesion onto the surface of cells (Teeguarden et al 2007). As the microparticles are larger, they exhibit a high force of precipitation and adhesion than nanoparticles in the medium. Thus, the toxicity of microparticle is higher than that of the nanoparticles.

However, a study on human skin fibroblast cell line (BJ) concludes that Al₂O₃ nanoparticles range between 16 and 80 nm show less toxicity on human umbilical vein endothelial cells (HUVECs). On the other hand, an increase in cytotoxicity is observed at 212 nm. An earlier study (Lin et al 2006) on toxicity of nanoparticles shows that SiO₂ nanoparticle of size 46 and 15 nm induces damage to lung cancer cells. It is also found that 60 nm SiO₂ particle causes low cytotoxicity to human umbilical vein cell line (EAHY926) (Napierska et al 2009). Hence, it is revealed that in addition to mass and volume, the surface area is also crucial in determining the toxicity.

In addition to size, electrostatic force and the contact angles of both nano- and microparticles are essential to contribute to adhesion of charged particles on the cell surfaces (Jiang et al 2009). Al₂O₃ nanoparticles have a positively charged zeta potential (+49 mV) with a contact angle of 35.6°, which leads to greater adhesion of particles on the cell surface and hence, a greater toxicity whereas Al₂O₃ microparticles show negative zeta potential (~21.8 mV) and a lesser contact angle of 17.37°. Contrary to Al₂O₃ particles, SiO₂ micro and nanoparticles show a greater contact angle of 50.31° and 54.65°, respectively. Similar to Al₂O₃ microparticles, SiO₂ nano and microparticles shows negative zeta potential with minor difference. The result of zeta potential measurement seems to coincide with cytotoxicity result. Thus, the surface charge of particles plays a major role in determining the toxicity.
A dose-dependent decrease in the cell viability percentage is observed for ZrO$_2$ and TiO$_2$ nano- and microparticles (Figure 3.20). Similar to Al$_2$O$_3$ and SiO$_2$ particles, ZrO$_2$ microparticle-treated cells show a decrease in cell viability from 96.7% to 62.3% and ZrO$_2$ nanoparticle-treated cells show a decrease from 97.0% to 73.3% as the concentration is increased from 12.5 to 200 against control ($p < 0.05$; Figure 3.20). Similarly, TiO$_2$ microparticle and TiO$_2$ nanoparticle-treated cells also show a reduction in cell viability. TiO$_2$ microparticle-treated cells shows cell viability reduction from 87.16% to 54.3% and TiO$_2$ nanoparticle-treated cells from 99.7% to 68.9% when tried with 1 concentrations, which differ significantly ($p < 0.05$). Figure 3.20 Percentage cell viability of NIH 3T3 cell lines with respect to nano/micro ZrO$_2$ and TiO$_2$ metal oxides treatment. * and ** indicate the corresponding significance in their homogenous subset by Tukey's test at 5% level

Thus, TiO$_2$ microparticles have shown higher toxicity than TiO$_2$ nanoparticles. Few studies have reported particle size influence on cell viability i.e., microparticles killed more number of cells compared to nanoparticles (Bauer et al 2011). However, our study result differs with that of Dechsakulthorn et al (2007) who have reported that a concentration of 2696 ± 667 ppm TiO$_2$ nanoparticles is required to cause toxicity to human skin fibroblast cell line. Hence, micro-sized TiO$_2$ is considered to be the most toxic metal oxide among the tested materials whereas nano-sized ZrO$_2$ to be the least toxic. The order of cytotoxicity observed is micro-TiO$_2$ > micro-ZrO$_2$ > nano-TiO$_2$ > nano-ZrO$_2$ (Figure 3.20).

The hydrophobic nature of a particle relies on their contact angle with the medium. In this study, it is suggested that an increase in contact angle with positively charged zeta potential makes a particle inert. Thus, nano-sized ZrO$_2$: with its increased contact angle (73.37°) and positively charged zeta potential (+11.8 mV) makes it biologically inert. Hence, nanosized ZrO$_2$: is biologically compatible, and it could be used for medical applications such as implants and dental fillings. On the other hand, micro-sized TiO$_2$: shows a reduced contact angle of 23.12 with a negative zeta potential of 22 mV. Thus, it is evident but both the nanoparticles (ZrO$_2$: and TiO$_2$:) have higher hydrophobic potential than microparticles. The above results have made it clear that high-contact-angle material renders less toxicity whereas the low-contact-angle material shows higher toxicity in NIH 3T3 cell lines.

### 3.3.3 Antioxidant Activity of Metal Oxide Nano- and Microparticles

To establish the free radical scavenging potential of the tested particles, DPPH assay is carried out. The metal oxide nano- and microparticles show a dose-dependent difference in the percentage of DPPH scavenging activity from 1 to 100 mg. The radical scavenging activity of Al$_2$O$_3$ microparticles and Al$_2$O$_3$ nanoparticles (Figure 3.21) gradually increases, respectively, from 38.9% to 59.1% and from 53.4% to 72.1% when added at a mass of 1–100 mg. Also, the percentage of radicals scavenged by SiO$_2$: microand SiO$_2$: nanoparticles (Figure 3.21) increases from 52.5% to 67.2% and from 61.0% to 81.0%. The results of radical scavenging activity of ZrO$_2$: and TiO$_2$: micro and nanoparticles are depicted in Figure 3.22. Of these, ZrO$_2$: nanoparticles scavenge most of the free radicals followed by TiO$_2$: nanoparticles, ZrO$_2$: microparticles and TiO$_2$: microparticles. Furthermore, on increasing the concentration of the particles, the percentage of free radical scavenging activity also increased. ZrO$_2$: microparticles scavenge about 57.4% of free radicals at 1 mg whereas it is 69.4% at 100 mg. Similarly, ZrO$_2$: nanoparticles scavenge about 71.4% and 76.9% at 1 and 100 mg, respectively. TiO$_2$: microparticles scavenge free radicals of 23.1% and 41.0% between the mass range 1 and 100 mg. TiO$_2$: nanoparticles show an increase in scavenging percentage from 66.52% to 73.3% at a mass of 1 and 100 mg. The percentage of free radical scavenging activity of particles is in
the following order: nano-SiO$_2$ > nano-ZrO$_2$ > nano-TiO$_2$ > nano-Al$_2$O$_3$ > micro-ZrO$_2$ > micro-SiO$_2$ > micro-Al$_2$O$_3$ > micro-TiO$_2$. Thus, nanoparticles seem to possess more scavenging potential than microparticles. A gradual increase in antioxidant activity is observed when the mass of the particle is increased from 1 to 100 mg. The highest antioxidant activity is shown by SiO$_2$ nanoparticles whereas the least activity is evidenced in TiO$_2$ microparticles. The result of this study reveals that the radical scavenging activity of the tested particles depends on dose as well as their size.

Figure 3.21 Percentage DPPH radical scavenging activity with respect to nano/micro Al$_2$O$_3$ and SiO$_2$ metal oxides. * and ** indicate the corresponding significance in their homogenous subset by Tukey’s test at 5% level.

Figure 3.22 Percentage DPPH radical scavenging activity with respect to nano/micro ZrO$_2$ and TiO$_2$ metal oxides. * and ** indicate the corresponding significance in their homogenous subset by Tukey’s test at 5% level.

Identifying the antioxidant potential of nanoparticles is a key area that may open up new venue in cancer research and treatment. Nanoparticles show higher antioxidant activity than microparticles, which might be due to the difference in surface area and colloidal nature. It is well known that as the size of the particle decreases, the surface area of the particle increases. Thus, a direct correlation exists between the surface area and antioxidant potential of the particles. Enhanced antioxidant activity on increasing the size from 0.8–5 to 0.25–10 mg is reported earlier (Paul et al 2009, Saikia et al 2010). This observation correlates with our result, revealing lesser antioxidant potential of microparticles.

3.3.4 In Vitro Biocompatibility Analysis in Simulated Body Fluid

Biocompatibility analysis of particles in SBF mimics the in vivo system. The in vitro biocompatibility of nano- and microparticles is analysed by SBF study (Kukubo and Takadam 2006). Biocompatibility is confirmed through SBF analysis in terms of changes in pH, FTIR, and XRF studies. The changes in pH of the SBF after immersion of Al$_2$O$_3$ and SiO$_2$ nano and microparticles for 21 days are shown in Figure 3.23. There is a decrease in pH value of all the samples up to third day. After that, no significant change is observed. At 15th day, the solution shows a marked decrease in pH value in all the samples. However, from 15 to 21 days, no significant variation in the pH value in any of the samples is seen. The observed marked decrease in the pH value in all samples on third day may be due to the release of ions from the samples into SBF and deposition or precipitation of calcium phosphates and carbonates on metal oxide pellet. However, metal oxide maintains the stationary phase between 15 and 21 days, which is due to the saturation of ion exchange after the hydroxyapatite formation.

Figure 3.23 pH change with respect to the soaking period of nano and micro metal oxides

It is observed that the evolution of carbonate band is higher in Al$_2$O$_3$ nano- and microparticle samples when compared with other samples after 21 days. Similarly, a report (Martinez et al 2000) on the presence of carbonate and phosphate bands indicates the formation of an apatite layer on the surface of bioactive glass samples. The higher biocompatibility of the metal oxide may be due to fast dissolution of Ca$^{2+}$ from bioactive glass as
well as fast nucleation of Ca^{2+} and P^{5+} ions on its surface (Misra et al 2008).

**Figure 3.24 Transmittance versus wavenumber of nano/micro Al_2O_3 particles, before and after SBF studies**

**Figure 3.25 Transmittance versus wavenumber of nano/micro SiO_2 particles, before and after SBF studies**

The elements present in the samples are studied through XRF analyses; the results are given in Table 3.5. From the observed results, the percentage of calcium content for Al_2O_3 nanoparticle, Al_2O_3 microparticle, SiO_2 nanoparticle and SiO_2 microparticle is, respectively, 0.924, 0.251, 0.618 and 0.251. The XRF analysis confirms the presence of calcium deposition over metal oxide powders. It also shows the existence of calcium deposition in different percentages.

The changes in pH value of the SBF after immersion of individual powders of ZrO_2 and TiO_2 nano- and microparticles for 21 days are observed and depicted in Figure 3.26. A marked decrease in pH value in all the samples is observed till 15th day after which none of the samples shows significant change in the pH value except TiO_2 nanoparticle sample.

The FTIR spectra of the ZrO_2 and TiO_2 samples soaked in SBF for 21 days are shown in Figures 3.27 and 3.28. The absorption of the phosphate band observed at 572, 724 and 834 cm\(^{-1}\) confirms the formation of calcium phosphate layer in the crystalline phase. This result is in accordance with that of Ohtsuki et al (1992). In addition, carbonate absorption peaks are observed at 961 and 1050 cm\(^{-1}\), whereas phosphate vibration peaks are observed at 1123, 1267, 1623 and 1657 cm\(^{-1}\). In addition, the evolution of carbonate band is higher in TiO_2 nano and microparticle samples when compared with ZrO_2 samples after 21 days of incubation.

The calcium content of the SBF-treated nano- and microparticles are studied by XRF analysis and the values are presented in Table 3.5. From the results, the percentage of calcium content in ZrO_2 nano- and microparticles is, respectively, 0.094% and 0.066%, whereas in TiO_2 nano and microparticle, it is 0.251% and 0.112%, respectively. The XRF analysis confirms the presence of calcium deposition over metal oxide powders in different percentages. Higher calcium and phosphorus deposition in the nanoparticles indicates the formation of hydroxyapatite layer as well as enhanced biocompatibility. Earlier studies on nanomaterials (Misra et al 2008, Misra et al 2010) show that nanoparticles have higher biocompatibility than microparticles, which supports our present study.

**Figure 3.26 Change in pH as a function of soaking period of nano/micro ZrO_2 and TiO_2 particles**

**Table 3.5 Calcium content of metal oxide particles after SBF study**

<table>
<thead>
<tr>
<th>Test particles</th>
<th>Percentage (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro SiO_2</td>
<td>0.251 ± 0.04</td>
</tr>
<tr>
<td>Nano SiO_2</td>
<td>0.618 ± 0.06</td>
</tr>
<tr>
<td>Micro Al_2O_3</td>
<td>0.251 ± 0.02</td>
</tr>
<tr>
<td>Nano Al_2O_3</td>
<td>0.251 ± 0.08</td>
</tr>
<tr>
<td>Micro ZrO_2</td>
<td>0.066± 0.03</td>
</tr>
<tr>
<td>Nano ZrO_2</td>
<td>0.094± 0.06</td>
</tr>
<tr>
<td>Micro TiO_2</td>
<td>0.112± 0.02</td>
</tr>
<tr>
<td>Nano TiO_2</td>
<td>0.251± 0.08</td>
</tr>
</tbody>
</table>

**Figure 3.27 Transmittance versus wavenumber of nano/micro ZrO_2 particles**
particles, before and after SBF studies

Figure 3.28 Transmittance versus wavenumber of nano/ micro TiO$_2$ particles, before and after SBF studies

3.4 ALGAE TOXICITY ANALYSIS

3.4.1 Effect of Particle Size, Zeta Potential and Contact Angle of Metal Oxides on Algal Growth

*P. aeruginae* treated with different concentrations of Al$_2$O$_3$, SiO$_2$, ZrO$_2$ and TiO$_2$: nano- and microparticles is shown in Figures 3.29 and 3.31. The growth curves of treated *P. aeruginae* are depicted in Figures 3.30 (Al$_2$O$_3$ and SiO$_2$) and 3.32 (ZrO$_2$ and TiO$_2$). Algal growth is found to differ with the concentration and the type of the particles used. Al$_2$O$_3$ particles reduced algal growth, as evidenced from the density of the OECD medium (Figure 3.29) after incubation in contrast to SiO$_2$ particles. Algal cells treated with Al$_2$O$_3$ particles settled down at the bottom of the flask and no growth is observed further. The intensity of the medium reflects the fact that Al$_2$O$_3$ nanoparticles are more toxic when compared to their micro counterparts.

Growth of algal culture as a function of OD at different dosages of Al$_2$O$_3$ and SiO$_2$: nano and microparticles is graphically represented in Figure 3.30. The least toxicity is observed at 1 mg L$^{-1}$, whereas the highest toxicity is observed at 1000 mg L$^{-1}$ for Al$_2$O$_3$ microparticles. The EC$_{50}$ (half maximal effective concentration) value of Al$_2$O$_3$ microparticles is in the range from 500 to 1000 mg L$^{-1}$, whereas that of Al$_2$O$_3$ nanoparticles is in the range from 100 to 300 mg L$^{-1}$. SiO$_2$: microparticles do not show significant toxicity on algae from 1 to 1000 mg L$^{-1}$. Surprisingly, a similar result is observed for SiO$_2$: nanoparticles. After 6 days, the EC$_{50}$ values of SiO$_2$: nano and microparticles are not determined because of non-toxicity.

Higher the hydrophobic potential of nanoparticles, lesser the solubility and dispersion due to the formation of aggregates. Electrostatic force and contact angle of nano- and microparticles are essential to contribute to the adhesion of charged particles on the cell surfaces (Hu et al 2010).

Hence, zeta potential of a material is necessary for the interaction with cell surface. Al$_2$O$_3$: nanoparticles have a contact angle of 35.67° with positive zeta potential (+49 mV). In contrast, Al$_2$O$_3$: microparticles show a decrease in contact angle of 17.37° with a negative zeta potential (-21.8 mV). However, SiO$_2$: microparticles show an increase in contact angle of 50.31° with a negative zeta potential (-25.6 mV), whereas SiO$_2$: nanoparticles show a contact angle of 54.65° with a negative zeta potential (-25.8 mV).

From the above results, it is clearly known that only Al$_2$O$_3$: nanoparticles possess positive zeta potential and also render toxicity to algal cells at low concentration. This positive charge may cause adhesion of particles on the surface of algae and may interfere with cellular metabolism, which in turn may result in cell death. Thus, it is clear from the observations that zeta potential of the tested particles contributes more to algal toxicity. These results convey the biological interaction of nano- and microparticles with algal cells.

The important parameters such as NOEC (No Observed Effect Concentration) and EC$_{50}$ (half maximal effective concentration) values are determined from the growth curve in toxicity evaluation. The result shows that Al$_2$O$_3$ is found to be toxic in both particle sizes. For Al$_2$O$_3$: microparticles, the EC$_{50}$ value is found to be in the range from 500 to 1000 mg L$^{-1}$ whereas the NOEC is found to be in the range from 1 to 100 mg L$^{-1}$. For Al$_2$O$_3$: nanoparticles, the EC$_{50}$ value is found to be in the range from 100 to 300 mg L$^{-1}$ whereas the NOEC is found to be in the range from 1 to 10 mg L$^{-1}$ when compared with control.

Figure 3.29 Culture flasks containing different concentrations of metal oxide particles.

a) Micro alumina
b) Nano alumina

Figure 3.30 Growth curve of *Porphyridium aerugineum* at different concentrations of metal oxide particles at two days interval

122
c) Micro silica
d) Nano silica

Figure 3.30 (Continued)

Effect of ZrO$_2$ and TiO$_2$ micro and nanoparticles on the growth of *P. aerugineum* is illustrated in Figure 3.31, which shows the culture in OECD medium after incubation. The OD of the medium increases for ZrO$_2$ particles whereas it decreases for TiO$_2$ particles, compared to that of control. This reveals that ZrO$_2$ particles enhance algal growth whereas TiO$_2$ particles suppress the growth. It could be clearly evidenced from Figure 3.32. Similar to Al$_2$O$_3$ particles, TiO$_2$ particles also result in settling down of algal cells at the bottom of the flask and inhibition of growth.

Algal growth increases for ZrO$_2$ nano- and microparticles when the concentration is increased from 1 to 1000 mg L$^{-1}$ in contrast to TiO$_2$ particles. Of these, TiO$_2$ microparticles show higher toxicity than TiO$_2$ nanoparticles. According to a previous report, microparticles lead to more number of cell deaths as compared to nanoparticles (Bauer et al 2011), which is in accordance with our result. The greater density of microparticles may result in their deposition on algal cell surfaces and hence reduced the viability.

The order of cytotoxicity observed is micro-TiO$_2$ > nano-TiO$_2$ > micro-ZrO$_2$ > nano-ZrO$_2$ as evident from Figure 3.32. TiO$_2$ nanoparticles form characteristic aggregates and entrap the cells of the microalgae *P. subcapitata* (Aruoja et al 2009). Ji et al (2011) have reported that nanoparticulates Al$_2$O$_3$, SiO$_2$ and TiO$_2$ (DJ3, rutile) had no significant toxicity on green algae *Chlorella* sp., whereas nano-ZnO and nano-TiO$_2$ (HR3, anatase) greatly inhibited the algal growth.

Figure 3.31 Culture flasks containing different concentrations of metal oxide particles

a) Micro zirconia
b) Nano zirconia

Figure 3.32 Growth curve of *Porphyridium aerugineum* at different concentrations of metal oxide particles at two days interval

126
c) Micro titania
d) Nano titania

Figure 3.32 (Continued)

For TiO$_2$ microparticles, the EC$_{50}$ value is found to be in the range from 10 to 100 mg L$^{-1}$ whereas the NOEC is not observed because it is found to be toxic even at the lowest concentration of 1 mg L$^{-1}$. For TiO$_2$ nanoparticles, the EC$_{50}$ value is found to be in the range from 100 to 300 mg L$^{-1}$ whereas the NOEC is found to be in the range from 1 to 10 mg L$^{-1}$ when compared to control. This shows that TiO$_2$ microparticles render more toxicity to algal cells than its nano counterpart. Interestingly, ZrO$_2$ nano- and microparticles are non-toxic to algae even at 1000 mg L$^{-1}$ concentration. Instead, they enhanced the algal growth. After 6 days, the EC$_{50}$ values of ZrO$_2$ nano- and microparticles are not determined as it is found to be non-toxic.

3.4.2 Influence of Metal Oxides on Chlorophyll and Protein Contents of Algal Cells

The tested nano and microparticles found to influence the total chlorophyll and protein contents in algal cells. The variation in total chlorophyll and protein contents for Al$_2$O$_3$ and SiO$_2$ nano- and microparticles are represented, respectively, in Figure 3.33 and Table 3.6. Both chlorophyll and protein contents seem to be reduced when algal cells are treated with Al$_2$O$_3$ micro- and nanoparticles. The effective concentration of Al$_2$O$_3$ microparticles for the reduction in chlorophyll content is found to be between
500 and 1000 mg L\(^{-1}\) whereas it is from 100 to 500 mg L\(^{-1}\) for Al\(_2\)O\(_3\) nanoparticles.

In contrast to the effect of Al\(_2\)O\(_3\) particles, treatment with SiO\(_2\) particles has enhanced the chlorophyll and protein contents in algal cells. The maximum increase in chlorophyll content observed for SiO\(_2\) microparticles is from 500 to 1000 mg L\(^{-1}\), whereas for SiO\(_2\) nanoparticles it is from 100 to 500 mg L\(^{-1}\). SiO\(_2\) nanoparticles have m\L\(^{-1}\) whereas SiO\(_2\) microparticles have enhanced it when the concentration was at 500 mg L\(^{-1}\). In contrast to our result, SiO\(_2\) nanoparticles have decreased the chlorophyll content significantly in Scenedesmus obliquus at 50, 100 and 200 mg L\(^{-1}\) concentrations after 96-h exposure. But bulk particles of SiO\(_2\) are non-toxic up to 200 mg L\(^{-1}\). Toxicity of SiO\(_2\) nanoparticles probably might be due to their sorption to algal cell surfaces (Wei et al 2010).

A reduction in chlorophyll content in algae treated with Al\(_2\)O\(_3\) nano- and microparticles is observed. The influence of Al\(_2\)O\(_3\) nanoparticles on chlorophyll content is found to be more than that of Al\(_2\)O\(_3\) microparticles. Typically, the biological activity of particles increases as the particle size decreases. Smaller particles occupy less volume, resulting in a larger number of particles with a greater surface area per unit mass and increased potential for biological interaction (Oberdorster 1996, Cassee et al 2002, Huang et al 2004, Warheit 2004). Thus, the reduction in chlorophyll content may be a function of particle concentration and particle size. In addition, reduction may also be due to the breakdown of organic molecules, which may result in the deactivation of the active receptor sites (Zhang 2003).

In contrast to the effect of Al\(_2\)O\(_3\) particles, an increase in the chlorophyll content is observed on increasing the concentration of SiO\(_2\) nanoand microparticles. Similarly, a study on green algae (Desmodesmus subspicatus) shows a difference in activity with the surface area (Hund-Rinke and Simon 2006).

The variation in total chlorophyll and protein contents for ZrO\(_2\) and TiO\(_2\) nano- and microparticles is represented in Figure 3.34 and Table 3.7, respectively. Similar to SiO\(_2\) particles, ZrO\(_2\) particles enhance the chlorophyll and protein contents. The effective concentration of ZrO\(_2\) microparticles to increase the chlorophyll content is from 500 to 1000 mg L\(^{-1}\), whereas for ZrO\(_2\) nanoparticles it is from 100 to 500 mg L\(^{-1}\). Protein content is enhanced (nano-ZrO\(_2\)) at 500 mg L\(^{-1}\) concentration when compared to control, whereas with ZrO\(_2\) microparticles a slight reduction is noticed. It is also observed that chlorophyll content increases with increase in the concentration of ZrO\(_2\) nano- and microparticles. The effective concentration of TiO\(_2\) microparticles for the reduction in chlorophyll content is found to be between 10 and 100 mg L\(^{-1}\) whereas that for TiO\(_2\) nanoparticles is from 100 to 500 mg L\(^{-1}\). Protein contents are reduced to mL\(^{-1}\) for TiO\(_2\) nano- and microparticles, respectively, at 500 mg L\(^{-1}\) concentration when compared to control (169.6 mg mL\(^{-1}\)). The influence of TiO\(_2\) microparticles on chlorophyll content is found to be more than that of TiO\(_2\) nanoparticles.
Figure 3.34 Total chlorophyll content after treatment with different concentrations (mg/L) of zirconia and titania metal oxide particles

c) Micro titania
d) Nano titania

Figure 3.34 (Continued)

Table 3.6 Effect of alumina and silica metal oxide particles on total protein content in Porphyridium aerugineum

<table>
<thead>
<tr>
<th>Metal Oxides</th>
<th>Protein content (µg) in different days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th day 2nd day 4th day 6th day</td>
</tr>
<tr>
<td>Control</td>
<td>151.10±0.03 158.05±0.01 165.90±0.04 a 169.65±0.01 a</td>
</tr>
<tr>
<td>Nano Al₂O₃</td>
<td>150.30±0.01 150.33±0.04 151.68±0.03 151.02±0.04</td>
</tr>
<tr>
<td>Micro Al₂O₃</td>
<td>151.14±0.04 153.14±0.03 156.14±0.02 158.05±0.02</td>
</tr>
<tr>
<td>Nano SiO₂</td>
<td>152.90±0.01 159.24±0.02 164.70±0.01 232.50±0.03</td>
</tr>
<tr>
<td>Micro SiO₂</td>
<td>152.05±0.04 156.90±0.03 184.22±0.06 210.00±0.01 a</td>
</tr>
</tbody>
</table>

a represents the level of significance at p<0.05

Table 3.7 Effect of zirconia and titania metal oxide particles on total protein content in Porphyridium aerugineum

<table>
<thead>
<tr>
<th>Metal Oxides</th>
<th>Protein content (µg) in different days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th day 2nd day 4th day 6th day</td>
</tr>
<tr>
<td>Control</td>
<td>151.10±0.03 158.05±0.01 165.90±0.04 a 169.65±0.01 a</td>
</tr>
<tr>
<td>Nano ZrO₂</td>
<td>152.05±0.04 157.90±0.03 163.22±0.06 168.00±0.01 a</td>
</tr>
<tr>
<td>Micro ZrO₂</td>
<td>152.90±0.01 166.24±0.02 169.70±0.01 232.50±0.03 a</td>
</tr>
<tr>
<td>Nano TiO₂</td>
<td>150.30±0.01 152.33±0.04 153.68±0.03 154.02±0.04</td>
</tr>
<tr>
<td>Micro TiO₂</td>
<td>150.30±0.01 152.33±0.04 153.68±0.03 154.02±0.04</td>
</tr>
</tbody>
</table>

a represents the level of significance at p<0.05

3.4.3 Surface Chemical Change Analysis through FTIR and XRF

FTIR spectrum of the dried algal cells treated with Al₂O₃ nanoparticles is shown in Figure 3.35. The peaks observed at 715 and 1013 cm⁻¹ show the stretching vibrations of AlO₄ and Al–OH, respectively. The peaks observed at 1403 and 1634 cm⁻¹ indicate the specific peaks for the C–C bond and OH, H₂O bonds. The peak observed at 2095 cm⁻¹ is assigned for the bands of protein in the algae (Dumas and Miller 2003). The peaks observed in the wide region between 3700 and 3300 cm⁻¹ indicate the characteristic bonds for O–H and N–H vibrations.

The effect of SiO₂ nanoparticles in algal cells is screened through FTIR spectrum and is represented in Figure 3.36. The characteristic peaks observed at 595 and 833 cm⁻¹ show, respectively, the vibration bands of Si–OH and Si–O–Si. The peaks observed at 2928 and 3392 cm⁻¹ represent, respectively, the CH₂ and OH, H₂O bonds. The stretching peaks observed between 800 and 1254 cm⁻¹ indicate the super-imposition of different SiO₂ peaks of Si–OH. The peak observed at 2928 cm⁻¹ is assigned for proteins (Dumas and Miller 2003). Corresponding peak for C–O functional groups is observed in green algae at 1080 cm⁻¹.

Elemental analysis of control and nano-Al₂O₃- and SiO₂-treated algal samples through XRF study is tabulated in Table 3.8. The observed result shows that the Al₂O₃ nanoparticle adsorption is found to be 2.25% whereas Al₂O₃ microparticle adsorption is 0.98%. Similarly, adsorptions of 7.05% and 2.75% are obtained, respectively, for SiO₂ nano- and microparticles when compared to control at 500 mg L⁻¹ concentration.

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 groups in the biomolecules of the cell wall. To understand the uptake of nanoparticles...
by algae, FTIR study is carried out for the control and particle-treated algal cells. The FTIR study confirms the attachment of Al₂O₃ and SiO₂ on algal cell surface.

**Figure 3.35** FTIR spectrum of *Porphyridium aerugineum* a) Before and b) After treatment with Al₂O₃ nanoparticles

**Figure 3.36** FTIR spectrum of *Porphyridium aerugineum* a) Before and b) After treatment with SiO₂ nanoparticles

The importance of the condensed or isolated state of the AlO₆ and AlO₄ co-ordination groups in c-Al₂O₃ structure is reviewed to understand the characteristic infrared absorption band frequencies (Tarte 1967). Based on experimental results, it is shown that for AlO₆ condensed octahedral and AlO₄ isolated tetrahedral, the 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 AlO₆ and AlO₄ co-ordination groups in c-Al₂O₃ vibrational spectra are revealed previously (Saniger 1995). The influence of SiO₂ nanoparticles in algal cells is screened through FTIR analysis (Figure 3.36). The observed peaks for the presence of SiO₂ content are in line with the previous reports (Dumas and Miller 2003, Beganskiene et al 2004, Guo and Zhang 2004, Yee et al 2004).

A strong band observed near 753 cm⁻¹ lies in the middle of expected vibration range of isolated AlO₄ co-ordination groups as reported earlier (Saniger 1995, Chandradass and Balasubramanian 2006, Naskar et al 2002). Toxicity of algae depends on the physico-chemical 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. The opacity of nanoparticles suspension indirectly plays a role in growth inhibition by decreasing the solution intensity. The physical restraint is one of the indirect mechanisms of nanoparticle toxicity towards algae (Navarro et al 2008). The accumulation of nanoparticles on the algal surface causes shading effects that inhibit the photosynthetic activity. An earlier study (Hoeckel et al 2008) suggests that there is no evidence for SiO₂ nanoparticles (12.5 and 27 nm) uptake into the cells of *P. subcapitata* from electron microscopic images.

Sorption of nanoparticles to the algal cell walls is reported to be a function of aggregation tendency and interaction with other organics present in the system (Chen and Elimelech 2007). The FTIR studies (Figures 3.35 and 3.36) correlate an active participation of the surface groups in the interaction and adsorption of the aggregated nanoparticles onto the surface. Thus, the presence of nanoparticles in the algal medium interferes with algal photosynthesis by decreasing the light availability (shading effect) and hence, becomes toxic to the algal cells.

The size of nanoparticles alone may not be the critical factor in determining their toxicity; the overall number and the total surface area may also be important. As a particle decreases in size, the surface area increases and a greater proportion of atoms/molecules is found at the surface compared to those inside. Thus, nanoparticles have a much larger surface area per unit mass compared with larger particles. The increase in the surface-to-volume ratio results in an increase in particle surface energy that may render them more biologically reactive (Oberdorster et al 2005b).

**Table 3.8** Elemental composition of *Porphyridium aerugineum* treated with nano and micro Al₂O₃ and SiO₂ particles at 500 mg L⁻¹ concentration

<table>
<thead>
<tr>
<th>Analyte</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Nano Al₂O₃</td>
<td></td>
</tr>
<tr>
<td>Micro Al₂O₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The FTIR spectra obtained for ZrO$_2$ and TiO$_2$ nanoparticles are shown, respectively, in Figures 3.37 and 3.38. The observed stretching vibrations at 2095 and 1634 cm$^{-1}$ show the characteristic peaks of $\text{Zr-OH}$ and $\text{Zr(OH)}_2$ bonds, respectively. The peaks observed at 1013 and 715 cm$^{-1}$ correspond to $\text{Zr-OH}$ and $\text{Zr-O}$ bonds. The vibration bands at 3392 and 1607 cm$^{-1}$ show the characteristic peaks of $\text{Ti-OH}$ and $\text{Ti(OH)}_2$ bonds. The peaks observed at 1080 and 833 cm$^{-1}$ represent Ti-O-Ti bonds.

Figure 3.37 FTIR spectrum of Porphyridium aerugineum a) Before and b) After treatment with ZrO$_2$ nanoparticles

Figure 3.38 FTIR spectrum of Porphyridium aerugineum a) Before and b) After treatment with TiO$_2$ nanoparticles

Table 3.9 Elemental composition of Porphyridium aerugineum treated with nano and micro ZrO$_2$ and TiO$_2$ particles at 500 mg L$^{-1}$ concentration

<table>
<thead>
<tr>
<th>Analyte (%)</th>
<th>Control Micro ZrO$_2$ Nano ZrO$_2$ Micro TiO$_2$ Nano TiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>89.3 89.2 89.1 87.35 88.2</td>
</tr>
<tr>
<td>K$\text{O}$</td>
<td>5.93 6.05 6.04 6.04 6.05</td>
</tr>
<tr>
<td>Fe$\text{O}_3$</td>
<td>0.28 0.49 0.48 0.48 0.48</td>
</tr>
<tr>
<td>SO$_3$</td>
<td>0.22 0.48 0.5 0.21 0.21</td>
</tr>
<tr>
<td>MnO</td>
<td>0.18 0.3 0.3 0.3 0.3</td>
</tr>
<tr>
<td>ZrO$_2$</td>
<td>0 0.45 0.25 0 0</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>0 0 0 2.35 1.25</td>
</tr>
<tr>
<td>CuO</td>
<td>0.05 0.01 0.01 0.01 0.02</td>
</tr>
<tr>
<td>P$_2$O$_5$</td>
<td>4.01 3.8 3.3 3.2 3.3</td>
</tr>
<tr>
<td>ZnO</td>
<td>0.03 0.02 0.02 0.02 0.02</td>
</tr>
</tbody>
</table>

3.5 PHYTOTOXICITY ANALYSIS

This section deals with the phytotoxicity analysis of metal oxide nano- and microparticles such as SiO$_2$, Al$_2$O$_3$, TiO$_2$, and ZrO$_2$ on maize seed germination and root elongation. Results of three different growth substrates (metal oxide addition in Petri dish, cotton, and soil) used in this investigation are discussed in detail.
3.5.1 Petri Dish Method
A difference in percentage of seed germination is noticed with respect to the type of metal oxide. Germination results of maize seeds treated with both nano- and microparticles in Petri dish method are shown in Figure 3.39. Control seeds show 95% of germination. On the other hand, both TiO$_2$ nano- and microparticles show, respectively, 60% and 55% of germination. Similarly, seeds treated with Al$_2$O$_3$ nano- and microparticles reveal, respectively, 50% and 45% of germination. Thus, the exposure of seeds to Al$_2$O$_3$ and TiO$_2$ nanoparticles significantly reduces germination percentage when compared to control (95%). However, a recent study shows that Al$_2$O$_3$ nanoparticles promote the growth of *Lemna minor* by enhancing their morphology and photosynthetic parameters. It also enhances the biomass accumulation, increases root length, increases number of fronts per colony and increases photosynthetic efficiency (Juhel et al 2011).

However, it is interesting to note that ZrO$_2$ and SiO$_2$ nano- and microparticles enhance the seed germination percentage. The seeds treated with ZrO$_2$ nano- and microparticles result, respectively, in 98% and 97% of germination whereas those treated with SiO$_2$ nano- and microparticles show, respectively, 105% and 100% at 1000 mg L$^{-1}$ concentration. The above results are in close agreement with the phytotoxicity study on *Arabidopsis thaliana* (Slomberg and Schoenfisch 2012) in which SiO$_2$ nanoparticles (14, 50 and 200 nm) are non-toxic up to 1000 ppm.

The result of root elongation experiment is shown in Figure 3.40. The observed result reveals that ZrO$_2$ and SiO$_2$ do not have any negative impact in both nano and micro forms at 10 to 1000 mg L$^{-1}$ concentrations. Exposure of maize seeds to ZrO$_2$ nano- and microparticles at 1000 mg L$^{-1}$ results in root elongation to about 27 and 28 mm, whereas SiO$_2$ nano- and microparticles enhance root elongation up to 30 and 29 mm compared to control (28 mm). Hence, no significant change is observed in root elongation with respect to size. In contrast, the addition of TiO$_2$ nano- and microparticles drastically decreases root elongation when the concentration lies between 500 and 1000 mg L$^{-1}$. The root elongations for TiO$_2$ nano- and microparticles are, respectively, 20 and 18 mm, whereas those for Al$_2$O$_3$ nano- and microparticles are, respectively, 15 and 10 mm. Similarly, a study by Yang and Watts (2005) reports that coated and uncoated Al$_2$O$_3$ nanoparticles are toxic to soya bean, carrot and cabbage, which agree well with our present study.

3.5.2 Cotton Method
The difference in germination percentage of seeds treated with nano- and microparticles through cotton method is depicted in Figure 3.41. ZrO$_2$ nano- and microparticles show the seed germination percentage, respectively, as 98% and 97% at 1000 mg L$^{-1}$, which is greater than that of control (96%). Similarly, the seed germination percentage of SiO$_2$ nano- and microparticles is observed, respectively, as 104% and 98%. However, Al$_2$O$_3$ nano- and microparticles inhibited seed germination as, respectively, 60% and 55%. The germination percentage of seeds treated with TiO$_2$ nano- and microparticles reduces, respectively, to 80% and 75% at 1000 mg L$^{-1}$ concentration. In contrast to our result, an earlier study (Zheng et al 2005) shows positive effect of TiO$_2$ nanoparticles on *Spinacia oleracea* L., where it promotes seed germination. Interestingly, TiO$_2$ improves nitrogen assimilation in spinach (Yang et al 2006). Phytotoxicity study of nano and bulk TiO$_2$ (Feizi et al 2013) on fennel seeds shows a positive effect on shoot dry weight and germination rate at 60 ppm. However, bulk TiO$_2$ shows a negative effect, which in turn decreases the shoot biomass up to 50% at 40 ppm. In addition, nano and bulk TiO$_2$ were found to be non-toxic to bacterium *V. fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus* (Hienlaan et al 2008). Treatment of canola seeds with TiO$_2$ nanoparticles (20 nm mean particle size) at 2000 mg L$^{-1}$ concentration promoted both seed germination and seedling vigour. Higher concentrations of TiO$_2$ nanoparticles (1200 and 1500 mg L$^{-1}$)
showed large radicle and plumule growth of seedling compared to other concentrations and control (Mahmoodzadeh et al. 2013). The result of root elongation study by cotton method is depicted in Figure 3.42. Root elongation observed in control is 28 mm. SiO$_2$ nano- and microparticles show positive effect on root elongation as they increase the root lengths to 30 and 29 mm, respectively, at 1000 mg L$^{-1}$. ZrO$_2$ nano- and microparticles show neither positive nor negative effect as the root length is closer to that of control, 27 and 28 mm, respectively. But Al$_2$O$_3$ and TiO$_2$ show significant negative effect on root elongation in both forms. Al$_2$O$_3$ nano and microparticles inhibit root elongation and reduce the root length to 19 and 17 mm, respectively. TiO$_2$ nano- and microparticles stunt root length to 22 and 20 mm, respectively, when compared with control. Boonyanitipong et al. (2011) investigated the effects of ZnO-NPs (nano-ZnO) and nano-TiO$_2$ on rice (Oryza sativa L.) roots and concluded that TiO$_2$ nanoparticles have no effect on root length.

3.5.3 Soil Method
The results of the effect of metal oxide particles on maize seed germination by soil method are shown in Figure 3.43. The obtained results explore that ZrO$_2$ nano- and microparticles (98% and 97%, respectively) elicit no toxic response to maize seed germination even at higher concentration (1000 mg L$^{-1}$). Al$_2$O$_3$ nano- and microparticles (75% and 70%, respectively) inhibit seed germination whereas TiO$_2$ nano- and microparticles slightly reduce the percentage to 90% and 85%, respectively, when compared with control (98%). A recent study on phytotoxicity based on soil type such as GL1 and GL2 (Josko and Oleszczuk 2013) shows that TiO$_2$ is non-toxic to Lepidium sativum in soil medium. However, an enhancement in seed germination is observed for SiO$_2$ nano- and microparticles (106% and 98%, respectively). In soil treatment, root elongation is not inhibited much by the tested metal oxides except Al$_2$O$_3$ and TiO$_2$ microparticles as shown in Figure 3.44. The results of ZrO$_2$ particles are comparable to that of control (28 mm) as they have not exhibited any toxic response. SiO$_2$ nano- and microparticles enhance root elongation to 30 and 29 mm, respectively. Al$_2$O$_3$ and TiO$_2$ nanoparticles result, respectively, in 24 and 26 mm root elongation, whereas their micro forms reduce the root length to 23 and 25 mm, respectively, when compared with control.

3.5.4 Metal Oxide Uptake Analysis
The XRF data of the treated and untreated seed samples are shown in Table 3.10. Seeds treated with ZrO$_2$ nano- and microparticles show ZrO$_2$ uptake of, respectively, 0.64% and 0.16% whereas those treated with Al$_2$O$_3$ nano- and microparticles show, respectively, 2.26% and 1.47% of uptake at 1000 mg L$^{-1}$ concentration. The uptake of TiO$_2$ nano- and microparticles is found to be 3.07% and 2.13%, respectively. The highest uptake is noticed for SiO$_2$ particles, which is about 4.79% (nano-SiO$_2$) and 2.36% (micro-SiO$_2$) when compared with control. A recent report on nanoparticles shows that nanoparticles can penetrate through the wall of the seed and thus can interact with the seed cells (Zheng et al 2005). Thus, the uptake of nanoparticles is more compared to microparticles because microparticles are unable to penetrate due to their large size. This statement supports the results of our study, in which all the tested metal oxides penetrate better into seeds in nano forms than in micro forms. In addition, multi walled carbon nanotube at concentration between 10 and 40 mg L$^{-1}$ enhances seed germination in tomato plants. The penetration of porous carbon nanotubes improved water uptake by seed, which is the primary need for seed germination and hence leads to an increase in seed germination (Khodakovskaya et al 2009).

<p>| Table 3.10 Percentage of bio-uptake of metal oxides after 3 days treatment through XRF analysis |</p>
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Metal oxides</th>
<th>nano-SiO$_2$</th>
<th>micro-SiO$_2$</th>
<th>nano-ZnO</th>
<th>micro-ZnO</th>
<th>nano-TiO$_2$</th>
<th>micro-TiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Al$_2$O$_3$</td>
<td>2.26%</td>
<td>1.47%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ZrO$_2$</td>
<td>0.64%</td>
<td>0.16%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TiO$_2$</td>
<td>3.07%</td>
<td>2.13%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>SiO$_2$</td>
<td>4.79%</td>
<td>2.36%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
% uptake by seed after 3 days
1 Nano ZrO\(_2\): 0.64±0.03
2 Nano TiO\(_2\): 3.07±0.04 *
3 Nano SiO\(_2\): 4.79±0.01
4 Nano Al\(_2\)O\(_3\): 2.26±0.02
5 Micro ZrO\(_2\): 0.16±0.04 *
6 Micro TiO\(_2\): 2.13±0.01
7 Micro SiO\(_2\): 2.36±0.03
8 Micro Al\(_2\)O\(_3\): 1.47±0.02 *
* Represents level of significant at \(p<0.05\)

Figure 3.39 Incubation of maize seeds in petridish containing different concentrations of metal oxides

Figure 3.40 Root elongation of maize seeds in petridish containing different concentrations of metal oxides

Figure 3.41 Incubation of maize seeds on cotton incorporated with different concentrations of metal oxides

Figure 3.42 Root elongation of maize seeds on cotton incorporated with different concentrations of metal oxides

Figure 3.43 Incubation of maize seeds in soil incorporated with different concentrations of metal oxides

Figure 3.44 Root elongation in soil incorporated with different concentrations of metal oxides