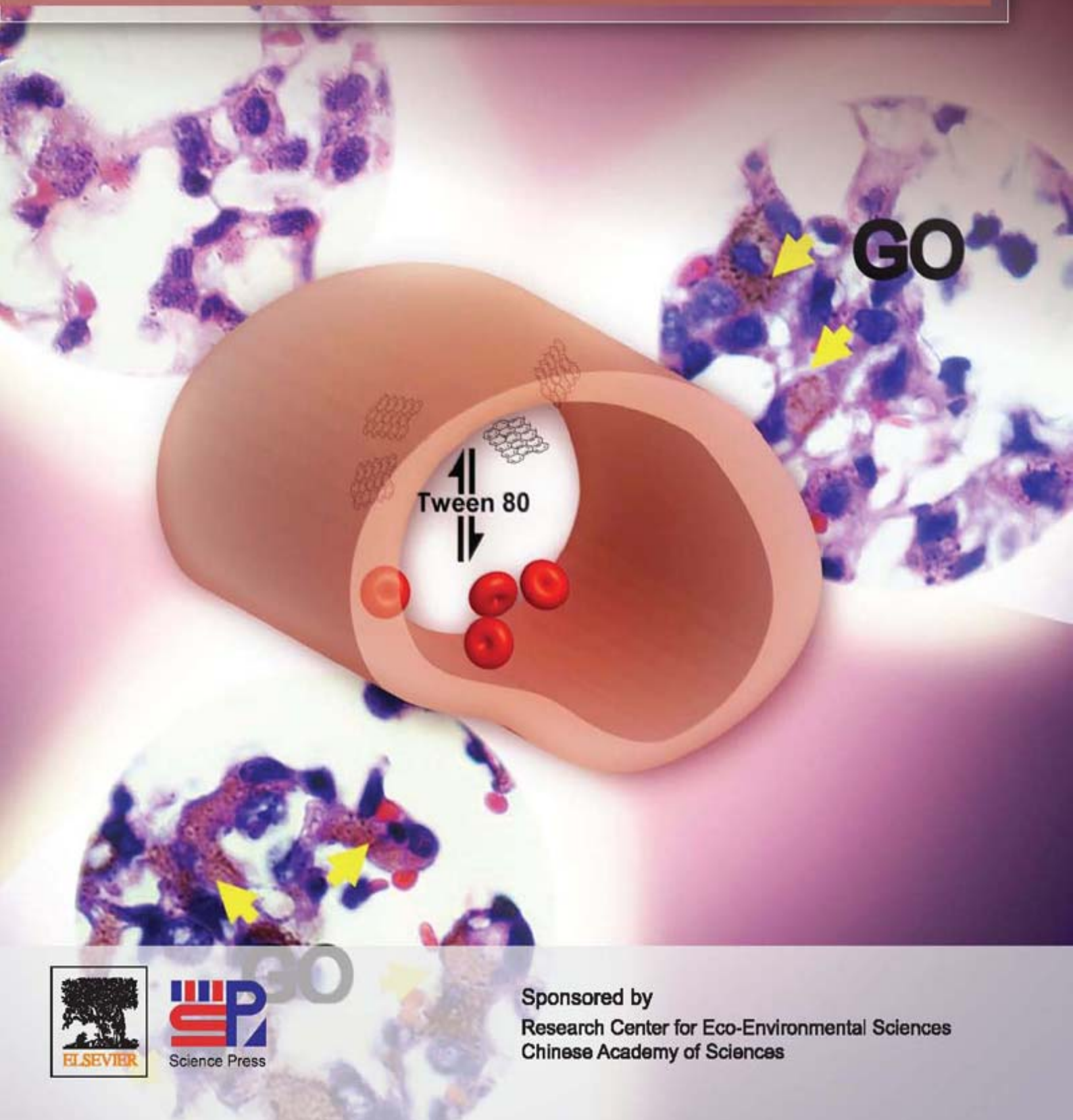
JES
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
May 1, 2013 Volume 25 Number 5
www.jesc.ac.cn



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Chinese Academy of Sciences

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 25 No. 5 2013

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Serial parameter: CN 11-2629/X*1989*m*205*en*P*24*2013-5

Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2013, 25(5) 882–888
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Determination of the mechanism of photoinduced toxicity of selected metal oxide nanoparticles (ZnO, CuO, Co_3O_4 and TiO_2) to *E. coli* bacteria

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Received 15 December 2012; revised 27 February 2013; accepted 05 March 2013

Abstract

Cytotoxicity of selected metal oxide nanoparticles (MNPs) (ZnO, CuO, Co_3O_4 and TiO_2) was investigated in *Escherichia coli* both under light and dark conditions. Cytotoxicity experiments were conducted with spread plate counting and the LC_{50} values were calculated. We determined the mechanism of toxicity via measurements of oxidative stress, reduced glutathione, lipid peroxidation, and metal ions. The overall ranking of the LC_{50} values was in the order of ZnO < CuO < Co_3O_4 < TiO_2 under dark condition and ZnO < CuO < TiO_2 < Co_3O_4 under light condition. ZnO MNPs were the most toxic among the tested nanoparticles. Our results indicate depletion of reduced glutathione level and elevation of malondialdehyde level correlated with the increase in oxidative stress. Released metal ions were found to have partial effect on the toxicity of MNPs to *E. coli*. In summary, the dynamic interactions of multiple mechanisms lead to the toxicity of the tested MNPs to *E. coli*.

Key words: metal oxide nanoparticles; reactive oxygen species; median lethal concentration; reduced glutathione; lipid peroxidation
DOI: 10.1016/S1001-0742(12)60152-1

Introduction

As the global nanotechnology market is growing, the engineered nanoparticles (ENPs) are being produced rapidly because of their diverse applications in various personal and commercial products. In addition, ENPs are widely used in industries, research, wastewater treatment and in the generation of energy (Wang et al., 2010). Among ENPs, metal oxide nanoparticles (MNPs) are of economic importance because of steady increase in their application in nanotechnology. MNPs possess unique physicochemical characteristics because of their dissolution properties, electronic charges, small size and large-surface to mass ratio (Wang et al., 2010). ZnO and TiO_2 NPs are known as ingredients in cosmetics, skin care products, semiconductors and UV detectors. TiO_2 is a useful photocatalyst for degrading many organic contaminants in water and air (Keller et al., 2005; Armon et al., 2004; Dillert et al., 1998). CuO NPs are used as pigments in ceramics, semiconductors, and optical equipments, and in disposal of hazardous materials (Huang et al., 2010). Co_3O_4 is a promising material for a wide range of applications, including catalyst, gas sensor, electrochromic device, solar energy absorber, and magnetic material (Li et al., 2011). The large-scale production of ENPs will eventually increase their release into natural environments via manufacturing effluents or spills during handling and shipping processes (Oberdörster et al., 2005; Wiesner et al., 2006). The major sinks for the released ENPs may include air, water and soil. Evaluation of ENPs hazard includes detection, determination of environmental fate, and development of risk assessment of ENPs. After all, assessing the hazards of MNPs is vital for protecting human and environmental health. For the development of regulatory standard the toxicity data of MNPs is required before they are allowed to be released into the environment (Kahru et al., 2008). Microorganisms were used as a surrogate to predict the nanotoxicity to humans and ecosystems due to their role in biogeochemical cycling of elements (Heinlaan et al., 2008). Among the microorganisms, *Escherichia coli* bacteria were adopted as a good test model for studying the toxicity of chemical contaminants including MNPs (Hu et al., 2009).

In the publications, very few provided comparative study on the phototoxicity of nanomaterials. Among the MNPs, SiO_2, TiO_2, and ZnO NPs had been shown to be phototoxic to *Caenorhabditis elegans*, *E. coli* and *Bacillus subtilis* (Adams et al., 2006; Ma et al., 2011). Armon et al. (2004) reported that photocatalytic inactivation of

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the spores of *B. subtilis* and *Bacillus cereus* by TiO_2 in the disinfection process of water. Sapota et al. (2011) studied the photocatalytic inactivation of *E. coli* and *B. subtilis* by ZnO nanocrystals and nanorods. Compared to the laboratory conditions, the phototoxicity experiments conducted under natural sunlight have greater implications in assessing the realistic ecotoxicity of MNPs.

ZnO NPs were reported to be toxic to natural bacterial communities (Adams et al., 2006), *Saccharomyces cerevisiae* (Kasmetes et al., 2009) and to the aquatic organisms *Vibrio fischeri*, *Daphnia magna* and *T. platyurus* (Heinlaan et al., 2008). Moreover, ZnO and TiO_2 NPs were shown to be toxic to the aquatic organisms *V. fischeri*, *D. magna* and *T. platyurus* and the toxicity could be mediated by the contact between cell wall of bacteria or crustacean gut environment rather than entry of metal oxide inside the cell (Heinlaan et al., 2008). In addition, the antibacterial activity of ZnO NPs was more geared toward the Gram-negative bacteria and the mechanism of antibacterial activity was due to membrane dysfunction (Nair et al., 2009). In our previous cell line study, ZnO, TiO_2, and CuO NPs were found to be more toxic to the human HepG2 cells than the catfish primary hepatocytes (Wang et al., 2011). The difference in the sensitivity was attributed to the intact metabolic capability of the primary hepatocytes.

The main objective of this study was to evaluate the mechanisms of photoinduced toxicity of MNPs (ZnO, TiO_2, CuO and Co_3O_4) to *E. coli*. Since oxidative stress and bioavailability of metal ions were reported as the common mechanisms of the nanotoxicity of photocatalysts (Li et al., 2012; Hwang et al., 2012), therefore this research was conducted with measurements of reactive oxygen species (ROS), reduced glutathione (GSH), lipid peroxidation (LPO) and metal ions.

1 Materials and methods

1.1 Chemicals

All the MNPs used in this study were purchased from Sigma-Aldrich (Milwaukee, USA) and Sky Springs with chemical purity of 98% or higher. MNPs were prepared to reach the final concentrations in Millipore water. Aminophenyl fluorescein (APF) was purchased from Sigma Aldrich (St. Louis, USA). The Gram negative *E. coli* (ATCC#25254) bacterium was purchased from ATCC (Manassas, USA).

1.2 Transmission electron microscopy analysis

Transmission electron microscopy (TEM) measurements were performed on a JEM 1011 electron microscope (Jeol Inc., USA) operating at 100 kV and equipped with a Gatan camera. For each sample, 10 μL of the sample was placed on the TEM grid (Ted Pella, CA) and dried overnight at room temperature.

1.3 Cytotoxicity assays

E. coli (Migula) Castellani & Chalmers strain was prepared at 37°C overnight using Luria-Bertani broth. The cultures were centrifuged at 3220 ×*g* for 10 min and resuspended in sterilized physiological saline. Bacterial density was adjusted to $0.5 \times 10^9 - 1.66 \times 10^9$ bacteria/mL as determined by plate counting on Luria-Berlani petri dishes. To ensure dispersal, the stock solutions of NPs were prepared at a concentration of 1.2 g/L with sonication treatment (FS30 ultrasonic system, Fisher Scientific) at 25°C for 20 min. They were sonicated again for 10 min right before starting the exposure experiments. ZnO NPs were prepared to reach the final concentrations of 0.01, 0.05 and 0.1 ppm and 0.5, 2.5, 5 ppm under light and dark conditions respectively. TiO_2 NPs were prepared to reach the final concentrations of 1, 2.5, 5 ppm and 250, 500, 750 ppm under light and dark conditions respectively. Co_3O_4 NPs were prepared to reach the final concentrations of 20, 40, 60 ppm and 40, 60, 80 ppm under light and dark conditions respectively. CuO NPs were prepared to reach the final concentrations of 0.1, 0.25, 0.5 ppm and 2.5, 5.0, 7.5 ppm under light and dark conditions respectively. The control and experimental groups were incubated outdoors in a tub under the natural sunlight at noon time during sunny days at Jackson, Mississippi (32°19′N; 90°5′W), USA. The incubation temperature for all the test tubes was maintained with enclosed tap water. The samples were incubated for 30 min.

1.4 Measurement of ROS dependent oxidative stress assay

APF dye can only detect the hydroxyl radicals and it is highly resistant to auto oxidation (Setsukinai et al., 2003). Intracellular ROS dependent oxidative stress was determined with a fluorescence-based method using APF solution. The bacterial samples were exposed to various concentrations of NPs and incubated with 10 μmol/L APF solution for 30 min under sunlight irradiation. The fluorescence intensity was measured before and after exposure at 485 nm excitation and 535 nm emission filters. The percent increase in fluorescence was calculated (Choi and Hu, 2009).

1.5 Measurement of metal ions

To investigate the role of metal ions in causing the nanotoxicity, sub-samples for the assay were separated from atomic ZnO-NPs, TiO_2-NPs, Co_3O_4-NPs and CuO-NPs in the samples (two replicates for each treatment) by centrifugation at 19,000 ×*g* for 20 min to pellet the NPs. To protect the analytical instrument, the supernatant was collected and the samples were filtered through 0.2 μm membrane filters (Corning Incorporated, Germany) (Dimkpa et al., 2011). The filtered samples containing Zn, Cu, and Co ions were digested with 5% nitric acid and Ti ion samples with 5% hydrofluoric acid. Concentration of zinc, titanium,

cobalt and copper ions in the samples was measured by inductively coupled plasma mass spectroscopy (ICP-MS, Varian Model No. 820-MS). The values are calculated as mg/L of ions released supernatant in the respective samples. We did not analyze the amount of Zn, Cu, and Co and Ti ions present in the pellet.

1.6 Reduced glutathione assay

GSH was estimated by the method previously described by Ellman (1959). Treated and untreated bacterial cells were disrupted by sonication on ice-cooled water and the supernatants were collected after centrifugation at 4000 ×*g* for 20 min. Briefly, 50 μL of the supernatant was added to 0.2 mL of trichloroacetic acid (5%) and then 0.25 mL of Ellman reagent (0.0198% DTNB in 1% sodium citrate) and the final assay volume was made up to 1 mL with 0.2 mmol/L phosphate buffer. The absorbance was measured at 412 nm using micro plate reader (Triad series, Dynex Technologies, Chantilly, VA, USA), and the amount of GSH was calculated using a GSH standard curve and expressed as nano molar of GSH formed per mg protein. Data was normalized with respect to controls and expressed as percent decrease in GSH formed. Protein abundance was estimated by using BSA (bovine serum albumin) as the standard (Bradford, 1976).

1.7 Lipid peroxidation assay

LPO was determined by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) to form a MDA-TBA adduct after peroxidation of lipids (Esterbauer and Cheeseman, 1990). One milliliter of treated bacterial culture was mixed with 1 mL of 20% (*W*/*V*) trichloroacetic acid and kept at room temperature for 10 min. The samples were centrifuged at 5000 ×*g* for 40 min to precipitate proteins. Then 2 mL of 0.67% TBA was added to the supernatants and incubated in a boiling water bath for 10 min. The samples were cooled and the absorbance was read at 532 nm in a micro plate reader (Triad series, Dynex Technologies, Chantilly, VA, USA). A standard curve was constructed by extrapolating the amount of commercially bought MDA to the measured absorbance. Values are calculated as nanomoles of MDA formed per milligram (wet weight) of cells and the data was expressed as percent increase in MDA formed with respect to controls.

The data from measurements of ROS, GSH and LPO above lethal concentrations (LC_{50}) showed little activity or no activity (data not shown). Therefore, all the experiments of ROS, GSH, LPO and metal ion release experiments were based on the lethal (LC_{50}) and sublethal concentrations (LC_{25} and LC_{10}).

1.8 Statistical analysis

Unless specifically indicated, all data are expressed as mean ± standard deviation (SD) of three independent experiments. Statistical analysis was performed with student's *t*-test and the data was considered statistically significant at $p < 0.05$ (Hwang et al., 2004).

2 Results and discussion

2.1 Nanoparticle characterization

Data of the average size and specific surface area of the studied MNPs were provided by the vendor. Size was also measured with TEM in our lab. The average particles size were 47–106 nm for ZnO, 17–64 nm for TiO_2, 17–45 nm for CuO, and 51–132 nm for Co_3O_4 (**Table 1**). **Figure 1** shows the TEM images of TiO_2, ZnO, Co_3O_4 and CuO metal oxide NPs. The TEM images confirm that spherical/rhomboid/rod, spherical, rhomboid/quadrate and rhomboid/spherical shapes were observed in the aqueous solutions of ZnO, CuO, Co_3O_4 and TiO_2 MNPs respectively.

2.2 Cytotoxicity of ZnO, TiO_2 CuO and Co_3O_4

E. coli bacteria treated with four MNPs showed concentration dependent toxicity under light and dark conditions. ZnO, TiO_2, CuO and Co_3O_4 NPs significantly decreased viability of *E. coli* as indicated by the result of plate counting. The LC_{50} concentrations of MNPs are tabulated

Table 1 Average particles size

Metal oxide nanoparticles	Size range (nm) (TEM measurement)	Average size (nm) Vendor	Specific surface area (m^2/g)
ZnO	47–106	71	15
TiO_2	17–64	42.3	35.5
CuO	17–45	28	33
Co_3O_4	51–132	78.3	Unavailable

Information from the vendor and transmission electron microscopy (TEM) measurements in our lab.

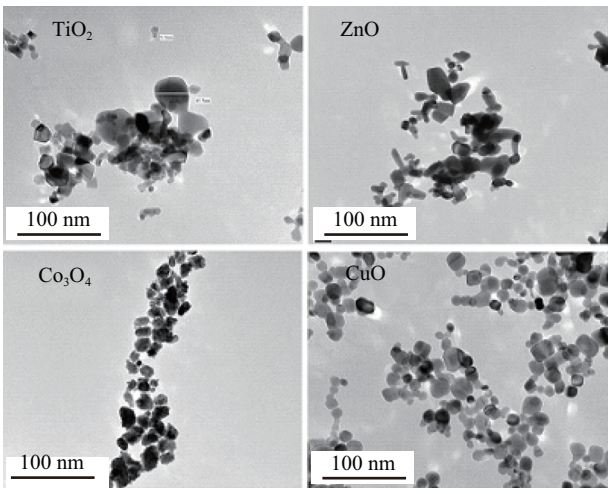

Fig. 1 TEM images of TiO_2 (200,000×), ZnO (80,000×), Co_3O_4 (100,000×) and CuO (250,000×) NPs in aqueous solutions.

Table 2 LC_{50} concentrations for the *E. coli* exposed to MNPs under light and dark conditions for 30 min

Metal oxide NPs	LC_{50} (mg/L) (light)	LC_{50} (mg/L) (dark)
ZnO	0.048 (0.99)	0.13 (0.92)
CuO	0.16 (0.99)	4.63 (0.90)
TiO_2	1.68 (0.98)	583 (0.99)
Co_3O_4	35.06 (0.95)	55.56 (0.87)

* Values of correlation coefficient (R^2) of linear regressions are given in the parentheses.

in **Table 2**. The overall ranking of the LC_{50} values in the presence of sunlight is ZnO < CuO < TiO_2 < Co_3O_4. Under dark exposure, the ranking of LC_{50} values is in the order of ZnO < CuO < Co_3O_4 < TiO_2. Under light exposure, among the four MNPs ZnO had the lowest LC_{50} value (0.048 mg/L) and Co_3O_4 had the highest LC_{50} value (35.06 mg/L). Under dark exposure conditions, ZnO had the lowest LC_{50} value (0.13 mg/L) and TiO_2 had the highest LC_{50} value (583 mg/L). Therefore, ZnO exhibited the highest toxicity under the light and dark conditions whereas TiO_2 exhibited extremely low toxicity to *E. coli* bacteria under dark condition.

The mitigation of viability of *E. coli* cells was more in light exposure groups than that in dark exposure groups. TiO_2 NPs showed the greatest difference in viability reduction between the light and dark exposure groups. In the presence of light *E. coli* bacteria suffered more toxicity compared to the dark exposure groups ($p < 0.05$). The light induced toxicity by exposure to ZnO, TiO_2, and CuO NPs could be due to the production of ROS by being the semiconductors (Li et al., 2012). The toxicity of MNPs to *E. coli* in dark may be due to the mechanisms other than ROS production.

2.3 Role of ROS in exerting the toxicity of MNPs

The major postulated toxicity mechanisms of MNPs include production of ROS and release of metal ions (Kahru et al., 2008). ZnO and TiO_2 NPs are photo-reactive and they can produce ROS in the presence of light irradiation. In addition, numerous abiotic and biotic environmental factors can also influence NPs in the production of ROS. Overall, the toxicity of MNPs depends on chemical stability and aggregation of particles, and chemical speciation (Kahru et al., 2008; Auffan et al., 2009).

Figure 2 shows the ROS production in the studied MNPs at various concentrations under light condition. Among the studied MNPs the highest amount of ROS was produced in *E. coli* cells treated with TiO_2 at LC_{50} (1.68 mg/L) and LC_{25} (0.85 mg/L) compared to the control. The production of ROS in TiO_2 treated *E. coli* cells at LC_{50} (1.68 mg/L) is 822% higher than the control. Additionally, significant increase in ROS production was also observed in ZnO treated *E. coli* cells at LC_{50} (0.048 mg/L), LC_{25} (0.024 mg/L) and LC_{10} (0.009 mg/L) concentrations. The production of ROS at LC_{50} (0.048 mg/L) is 187.08% higher than the control. The data indicated that production of ROS does not seem to account for the photo-induced toxicity of *E. coli* bacteria treated with CuO and Co_3O_4 MNPs. Comparatively ROS production was higher in TiO_2 treated *E. coli* cells. The ROS production was greater in TiO_2 treated *E. coli* but ZnO was more toxic to *E. coli*. However, exposure at concentrations higher than LC_{50} did not result in any further increase in the production of ROS production in *E. coli* treated with MNPs (data not shown).

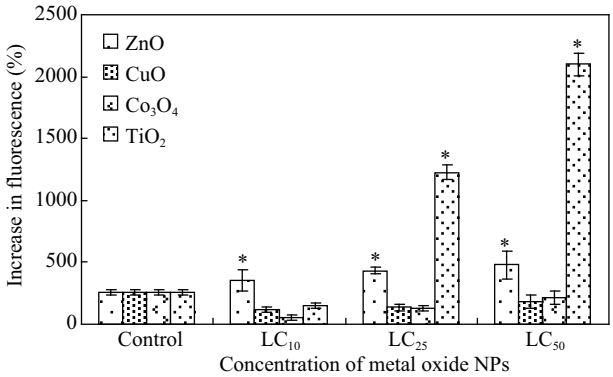

Fig. 2 Increase of fluorescence levels in *E. coli* exposed (30 min) to ZnO, CuO, Co_3O_4 and TiO_2 NPs under light condition. * Statistically different from control at $p < 0.05$.

There was insignificant production of ROS under dark condition compared to the light exposure groups. Therefore, the occurrence of ROS production in *E. coli* treated with MNPs was mainly caused by light mediated processes. The production of ROS was concentration dependent in *E. coli* treated with TiO_2 and ZnO NPs. This confirms the correlation between toxicity of TiO_2 and ZnO NPs and ROS production in *E. coli*. Our finding is in agreement with selected reports in the literature. For example, the ROS production was found to be high in the light control groups, whereas no ROS was generated in dark exposures (Dalai et al., 2012). ZnO and TiO_2 NPs were toxic to Zebrafish via oxidative damage (Xiong et al., 2011). Additionally, ZnO and TiO_2 NPs were found to induce oxidative stress and DNA damage in *E. coli* cells (Kumar et al., 2011).

In our study, there was no significant increase in ROS production observed in the CuO and Co_3O_4 treatment groups. Therefore, the nanotoxicity to *E. coli* cells may also be caused by mechanisms other than the oxidative stress via production of ROS. Limbach et al. (2007) demonstrated that the Co_3O_4 NPs elevated ROS levels by dissolving in cell-free culture system. Co_3O_4 MNPs, due to their strong oxidative properties, could initiate cytotoxic and genotoxic effects via *in vitro* route to the biological targets (Auffan et al., 2009). In summary, the ROS production under light condition was in the order of TiO_2 > ZnO > CuO > Co_3O_4 in *E. coli* treated with metal oxide NPs. On the contrary the ROS production under dark condition was negligible.

2.4 Correlation between GSH and LPO levels and toxicity of metal oxide NPs

GSH has a central role in antioxidant defense processes as a free-radical scavenger. Since GSH is an antioxidant, depletion of GSH leads to increased levels of ROS, nitrogen species, mitochondrial dysfunction and ATP depletion (Lu, 2009). In our study, significant decrease in GSH values was observed in *E. coli* cells treated with the test MNPs in concentration dependent manner. The ranking of percent decrease of GSH in the four studied MNPs was Co_3O_4 > CuO > TiO_2 > ZnO and CuO > Co_3O_4 > TiO_2 > ZnO at LC_{50} concentrations under light and dark conditions, respectively (**Fig. 3**). The similar trend in GSH values was not observed at LC_{25} and LC_{10} concentrations but significant decrease in values was observed. Under the light and dark conditions exposure to ZnO NPs significantly decreased the level of GSH. The greatest decrease in GSH levels seem to correlate with the highest toxicity observed with ZnO NPs exposure under light and dark conditions. Similar results were observed in other studies conducted on ZnO and TiO_2 NPs (Kumar et al., 2011).

LPO is defined as the oxidative degradation of membrane lipids leading to the cell damage, thereby a key indicator of oxidative stress (Cornejo-Garrido et al., 2011). Pigeot-Rémy et al. (2012) reported that the LPO levels were elevated during the photocatalytic treatment with the generation of ROS. Our data also indicated that increased levels of LPO were correlated with the increased production of ROS. There was a clear increase of MDA after treatment of *E. coli* with the four MNPs under light and dark conditions. The order of LPO increase was TiO_2 < CuO < Co_3O_4 < ZnO and TiO_2 < Co_3O_4 < CuO < ZnO (**Fig. 3**) at LC_{50} concentrations under light and dark conditions, respectively. The similar trend in LPO values was not observed at LC_{25} and LC_{10} concentrations but significant increase in values was observed. Highest amounts of LPO were produced in ZnO, CuO and Co_3O_4 under light and dark conditions. This confirms that the toxicity of ZnO, CuO and Co_3O_4 in *E. coli* may be due to the induction of LPO, the consequence of oxidative stress.

2.5 Role of ions in exerting the toxicity of metal oxide NPs

Aruoja et al. (2009) reported that ZnO and TiO_2 NPs were toxic to *Pseudokirchneriella subcapitata* algae due to the release of soluble metal ions from the MNPs. Baek and An (2011) investigated the toxicity of MNPs (CuO, ZnO, NiO, Sb_2O_3) to *E. coli*, *B. subtilis* and *Streptococcus aureus*. They indicated the induction of toxicity from dissolved ions was negligible. To determine the toxicity mechanism of MNPs in *E. coli*, ion analysis was performed to verify if dissolved ions account for the toxicity of MNPs in our *E. coli* study. **Table 3** shows the amount of free ions derived from MNPs in the treated *E. coli* after exposure at the respective lethal and sublethal concentrations under light or dark conditions. This data indicates samples of ZnO and CuO NPs exposure groups released higher percentages of ions under light conditions than in dark condition, when compared to other two studied MNPs. On the contrary, the level of respective ions was far less in the treatment groups containing MNPs Co_3O_4 and TiO_2 under light conditions, whereas the greatest difference in the level

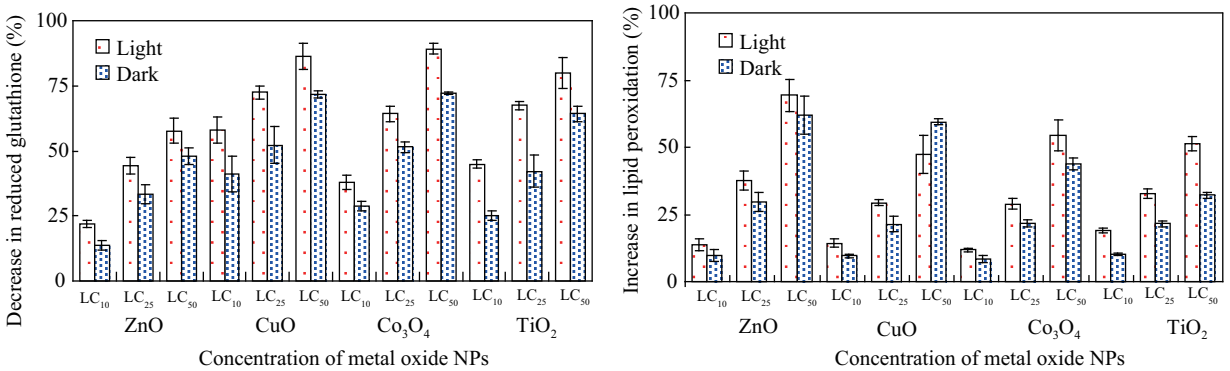

Fig. 3 Increase of intracellular reduced GSH levels and LPO levels in *E. coli* cells after exposure (30 min) to ZnO, CuO, Co_3O_4 and TiO_2 NPs under light and dark conditions. * Statistically different from control at $p < 0.05$.

Table 3 Bioavailability of Zn^{2+}, Cu^{2+}, Co^{2+} and Ti^{2+} ions in samples containing *E. coli* that were exposed to MNPs at the respective lethal and sublethal concentrations

	LC_{50}		LC_{25}		LC_{10}	
	Light	Dark	Light	Dark	Light	Dark
Zn^{2+}	79.64 (0.07)	15.63 (0.03)	16.21 (0.56)	10.10 (0.06)	21.70 (0.48)	9.49 (0.17)
Cu^{2+}	75.77 (1.72)	9.12 (0.24)	66.03 (7.25)	15.01 (0.43)	42.39 (2.38)	18.03 (0.90)
Co^{2+}	1.07 (0.02)	0.66 (0.01)	1.48 (0.01)	0.71 (0.00)	2.55 (0.13)	0.43 (0.01)
Ti^{2+}	9.21 (0.25)	2.39 (0.00)	13.95 (0.01)	0.56 (0.00)	31.53 (0.19)	0.52 (0.00)

Values of standard deviations (SD) are given in the parentheses.

of respective ions was observed in the treatment groups containing MNPs ZnO and CuO under light or dark conditions. Therefore, metal ion was also one of the factors contributing to the toxicity of ZnO and CuO NPs under light exposure conditions. This finding is in agreement with what was reported by Jiang et al. (2009) and Heinlaan et al. (2008) that nanoparticles toxicity was attributed to dissolved ions rather than the particles themselves.

Although release of ions could be one of the mechanisms that account for the photoinduced toxicity observed in the *E. coli* cells treated with ZnO and CuO NPs, amount of the released ions were not significant in the Co_3O_4 and TiO_2 treatment groups; hence release of ions does not seem to be a major mechanism of causing nanotoxicity to *E. coli* in that case. It is noteworthy that toxicity could also be affected by the attachment of nanoparticles to the cell walls of bacteria (Jiang et al., 2009). Based on the study with TEM images, Jiang et al. (2009) suggested that toxicity of nanoparticles was not only from dissolved metal ions, but also from their greater tendency to attach to the cell walls. Additionally, cytotoxicity could also occur when the nanoparticles should, be associated with cell membrane or in near vicinity to cells (George et al., 2011). Upon photo-activation, ROS could cause direct damage to cell membrane components to disrupt the membrane integrity or some of the ROS could enter into the membrane to induce oxidative stress and cellular damage subsequently.

3 Conclusions

To assess the mechanism of toxicity we assessed ROS dependent oxidative stress and measured the amount of released ions, GSH, and LPO. These data give valuable information on the mechanisms leading to the toxicity of the studied MNPs to *E. coli*. Among the measured variables, released ions were found to have partial effect on the toxicity of MNPs to *E. coli*. Therefore, the dynamic interactions of multiple mechanisms lead to the toxicity of the tested MNPs to *E. coli*. In summary, we found that under light condition the tested MNPs induced ROS production, decrease in GSH and elevation of LPO levels in *E. coli* cells in a concentration dependent manner. This confirms that the light is an important environmental factor that affects the toxicity of MNPs. The ROS production was negligible in dark exposure groups. In dark exposure treatment groups the toxicity might be due to ion release and decreased GSH and elevation of LPO levels. Overall the data indicated that the toxicity appears to be much more intense under light exposure conditions than that under dark exposure conditions.

Acknowledgments

This research was supported by (1) NSF-SBIR grant # IIP-0823040, (2) NSF-CREST program with grant # HRD-0833178, and (3) Strengthening the Environmental Science Ph.D program in instruction, grant # P031B090210-11. We thank Dr. Zikri Arslan physical chemistry lab at Jackson State University for providing assistance in ICP-MS analysis.

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Journal of Environmental Sciences (Established in 1989)

Vol. 25 No. 5 2013

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
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	E-mail: jesc@263.net, jesc@rcees.ac.cn		http://www.elsevier.com/locate/jes
Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742