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Toxicology Letters 158 (2005) 122–132
Toxicology Letters
www.elsevier.com/locate/toxlet

Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles

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Received 5 January 2005; received in revised form 7 March 2005; accepted 7 March 2005
Available online 29 March 2005

Abstract

The phytotoxicity of alumina nanoparticles loaded with and without phenanthrene (Phen) was investigated by means of root elongation (RE) experiments in this study. Five plant species, *Zea mays* (corn), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot) were used in our study of phytotoxicity by root elongation experiments. The surface characteristics of Phen-loaded and Phen-nonloaded nanoparticles were investigated using the Fourier transformed infrared (FTIR) spectroscopy technique. It was found that when loaded with 10.0%, 100.0%, or 432.4% monomolecular layer (MML) of Phen, the degree of the root elongation inhibition caused by the particles was reduced. The loading of Phen leads to the appearance of a vibrational mode in the region of 850–1050 cm^{-1}, which was assigned to the surface characteristics of the particles and arises from the disappearance of free hydroxyl groups according to an earlier study. When mixed with a known free hydroxyl radical scavenger, DMSO (0.5% and 1.0%), the non-loaded particles also showed decreased inhibition of root elongations. We supposed that the surface characteristics of the particles play an important role in the phytotoxicity of alumina nanoparticles.

Keywords: Phytotoxicity; Alumina nanoparticles; Phenanthrene; FTIR; DMSO

1. Introduction

The developing nanotechnology has brought enormous amount of manufactured nanoparticles into the work place as well as into the ambient air (Oberdorster, 2003). To protect the individuals in the work place as well as the public in the ambient environment from the possible harmful effect of the industrial particles, it is necessary to determine the toxicity and toxicological mechanisms of the manufactured nanoparticles.

Evidence from epidemiological studies suggest that fine particles (particles with aerodynamic diameter smaller than 2.5 μm, $PM_{2.5}$) have an association with various adverse human health effects including premature mortality, exacerbation of asthma

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doi:10.1016/j.toxlet.2005.03.003

and other respiratory-tract diseases, decreased lung function (Dockery and Pope, 1994; Schwartz et al., 1996; Pope, 1999, 2000), and cardiovascular diseases (Dockery, 2001; Donaldson et al., 2001). Studies are currently ongoing to investigate the human health related issues of particles with nanoscale sizes (Spurny, 1998), including the tracheal uptake of 21-nm titania (Churg et al., 1998), the impairment of alveolar macrophage phagocytosis by 29-nm titania and 14.3-nm carbon black (Renwick et al., 2001), and the endocytosis of 50-nm titania particles by human airway cells (Stearns et al., 2001).

Nanometer-sized particles have special toxicity and are usually more toxic than the same material of larger size (Donaldson et al., 1999). When inhaled as single particles, particles with diameter smaller than 50 nm can be highly toxic (Oberdorster, 1996). These particles can be internalized by type II lung epithelium cells (Stearns et al., 2001) because of their nanometer sizes. They have higher deposition efficiency in the lower respiratory tract and slower clearance rates (Spurny, 1998). Intrinsically toxic nanoparticles may damage macrophages that are essential to clear inhaled particles (Donaldson et al., 1998; Renwick et al., 2001), thus, leads to failed clearance of these particles. The failure clearance of these particles may also come from the overload of the particle clearance system, which is caused by the large numbers per unit mass of nanoparticles (Donaldson et al., 1998). The characteristic large specific surface area (surface area per unit mass) of nanoparticles would allow increased interaction between particles and epithelium cells (Donaldson et al., 1998), and results in greater toxicity of the particles (Oberdorster et al., 1992, 2000).

Based on the evidence from the studies published so far, it is typically proposed that the toxicity of particles increases with the decrease of the particle size, because smaller particles have smaller sizes, larger particle numbers and larger surface area per unit mass. However, Oberdorster (Oberdorster et al., 1994) found in their studies that 800-nm SiO_2 particles are as toxic as 20-nm TiO_2 particles. The more intensive and extensive toxicity of nanoparticles, thus, cannot only be explained by their larger specific surface areas, larger number/mass ratios, and smaller sizes.

The surface characteristics of nanoparticles are much different than the large particles (Roco, 1999). Few works up to date have been contributed to the study on the roles of the surface characteristics of nanoparticles taken in their toxicity. To study the surface characteristics of nanoparticles, vibrational spectroscopy is a powerful technique, which has often been used in catalysis studies to determine the surface nature of catalyst carriers using suitable probe molecules (Lavalley and Benaissa, 1985).

In this work, we studied the phytotoxicity of alumina nanoparticles, which is currently one of the two US market leaders for nano-sized materials according to a recent report (Abraham, 2000). The phytotoxicity of pure particles was investigated by means of root elongation (RE) tests. Phenanthrene (Phen), a major constituent of polycyclic aromatic hydrocarbons (PAHs) associated with the atmospheric particulate matters, was loaded onto the pure particles, and the phytotoxicity of Phen-loaded particles were investigated by root elongation tests. The surface characteristics of particles were determined by a Fourier transformed infrared (FTIR) spectroscopy.

2. Materials and methods

2.1. Chemicals

Alumina (purity >99.6%, average particle individual size as reported by the manufacturer: 13 nm, average size of particle aggregates as determined by a Coulter N4+: 201.0 nm, specific surface area determined by the BET method: 103.0 m^2/g) was provided by Degussa. Phenanthrene (purity: 98%) was purchased from Fisher Scientific Inc.

2.2. Loading of Phen to alumina particles

Phen was dissolved in acetone. Particles were then added and homogenized by continuous stirring into 4 ml of the solution. The slurries were left under vacuum at $38 \pm 1\,^{\circ}C$ overnight, and kept in vacuum for 24 h to ensure the removal of residue acetone. At the same time, equivalent amount of particles were treated with 4 ml of acetone following the similar procedure to make the “non-loaded particles”. The nonloaded and the loaded particles were then transferred to sample vials, sealed and stored in dark at room temperature. The loading amounts of Phen are

1.6×10^{-5} mol/g [2.83 mg/g, 10.0% monomolecular layer (MML)], 1.6×10^{-4} mol/g (28.40 mg/g, 100.0% MML), and 6.9×10^{-4} mol/g (122.81 mg/g, 432.4% MML). The phytotoxicity of the three Phen-loaded particles and the non-loaded particles was tested by the root elongation test using *Cucumis sativus*. The toxic effect of the 10.0% MML Phen-loaded particles and non-loaded particles was investigated using five plant species including *C. sativus*, *Zea mays*, *Glycine max*, *Brassica oleracea*, and *Avena sativa*.

2.3. Seeds

Seeds of five plant species: *Z. mays* (corn), *C. sativus* (cucumber), *G. max* (soybean), *B. oleracea* (cabbage), and *A. sativa* (oat), were purchased from Territorial Seed Company (Oregon, USA). These five species are among the 10 recommended species by USEPA (1996) for the determination of ecological effects of pesticides and toxic substances. The average germination rates of the seeds are larger than 85% according to a preliminary study. Seeds are stored in a dry place in the dark under room temperature.

2.4. Seedling culture

Seeds were soaked in a 10% sodium hypochlorite solution for 10 min to ensure sterility (USEPA, 1996). After rinsing three times with Milli-Q water, they were soaked overnight in Milli-Q water in an incubator at $25 \pm 1\,^{\circ}C$ in the dark except for *C. sativus* seeds, which were allowed to germinate immediately after the rinse. Seeds of the other plant species were then transferred to filter papers placed in 100 mm × 15 mm Petri dishes that contain 5 ml of Milli-Q water per dish, with 10 seeds per dish and 1 cm or larger distance between each seed, and allowed to germinate at $25 \pm 1\,^{\circ}C$ in the dark for 24–48 h (depends on the species) prior to the particle exposure.

2.5. Preparation of particle suspensions, Phen–water mixture, and controls

Particles were suspended in Milli-Q water. The concentration of particle suspensions was determined in a preliminary study using the plant species of *Z. mays*, *C. sativus*, *B. oleracea*, and *Daucus carota*. The tested concentrations in the preliminary study were 20 μg/ml, 200 μg/ml, and 2 mg/ml. Only 2 mg/ml of the particle suspension showed statistically significant inhibition effect on root growth (Table 1). The 2 mg/ml was, thus, used as testing concentration of the particle suspensions. Milli-Q water was used as the negative control. The positive control was 0.05 M $CdCl_2$.

For the test of the phytotoxicity of Phen, the compound was dissolved in acetone (HPLC, Fisher Scientific) at the concentration of 28 mg/ml. Fifty microlitres of this solution was added to 5 ml of Milli-Q water to make a concentration of 0.28 mg/ml, which was the highest amount of Phen that had been loaded onto the particles in 2 mg/ml of particle suspensions. The mixture was left overnight under the hood at room temperature to remove the residual acetone, and then tested. The negative control was Milli-Q water plus the equiv-

Table 1
Relative root growth (RRG) of plant seedlings exposed to alumina nanoparticles for 24 h in the dark at $25 \pm 1\,^{\circ}C$—the preliminary study for the determination of the testing concentration

Concentration	*Z. mays*	*C. sativus*	*B. oleracea*	*D. carota*
2 mg/ml	0.79 ± 0.24 (0.68–0.90) $p = 0.001$	0.80 ± 0.14 (0.76–0.85) $p = 0.001$	0.78 ± 0.13 (0.73–0.84) $p = 0.000$	0.83 ± 0.30 (0.72–0.93) $p = 0.009$
200 μg/ml	0.92 ± 0.33 (0.78–1.06) $p = 0.206$	0.94 ± 0.16 (0.89–1.00) $p = 0.081$	0.87 ± 0.30 (0.75–0.98) $p = 0.106$	0.93 ± 0.42 (0.77–1.09) $p = 0.426$
20 μg/ml	1.03 ± 0.32 (0.90–1.17) $p = 0.618$	0.96 ± 0.13 (0.92–1.01) $p = 0.255$	1.05 ± 0.43 (0.91–1.19) $p = 0.625$	1.13 ± 0.43 (0.97–1.28) $p = 0.072$

The results are reported as mean ± S.D., with 95% confidence level. Statistical analysis was performed between the results for the particle suspensions and the negative controls. Significant difference is reported when the $p < 0.05$.

alent volume of acetone (50 μl of acetone to 5 ml of Milli-Q water), which was also left overnight under hood at room temperature, together with the samples. The positive control was 0.05 M $CdCl_2$.

2.6. Seedling exposure

For seedling exposure, 5 ml per Petri dish of each particle suspensions, Phen–water mixture, negative controls, or positive controls was used. Three replicates, with 10 seedlings per dish, were tested for each concentration and control. The dishes were marked at the outside bottom to ensure the exact site of each seedling. One piece of filter paper was put into each dish, and 5 ml of each test medium was added. Seedlings were then transferred to the filter paper, and placed on the marked sites. The exposure process was done at $25 \pm 1\,^{\circ}C$ in the dark for 24 h. The length of the primary root of each seedling was measured before and after exposure. Root elongation during exposure period was calculated using the Eq. (1). Relative root growth (RRG) was calculated based on what was proposed by Schildknecht (Schildknecht and de Campos Vidal, 2002), using the Eq. (2).

$$\mathrm{RE} = L_{\mathrm{after}} - L_{\mathrm{before}} \quad (1)$$

$$\mathrm{RRG} = \frac{\mathrm{RE}_{\mathrm{sample}}}{\mathrm{RE}_{\mathrm{control}}} \quad (2)$$

where L_{after} and L_{before} refer to the measured root lengths after and before exposure, respectively.

2.7. FTIR study of particles and particles loaded with Phen

About 10 mg of particle samples were mixed in ~200 mg of ground KBr (IR grade, >99%, Fisher Science). The powder was pressed into pellets (Ø = 10 mm) with low pressure (~1.5 psi). Each pellet contains 3–5 mg of particles. Twenty-five hundred scans were recorded on each pellet. The spectrometer used was a Mattson Research Series FT-IR.

2.8. Statistical analysis

The results are expressed as mean values ± standard deviations (S.D.). The statistical analysis was performed using the procedure of one-way analysis of variance (ANOVA). Statistically significant difference was reported when the probability of the result assuming the null hypothesis (p) is less than 0.05. The coefficient of determination (R^2) was calculated to evaluate the relation between the two values that were being compared.

3. Results

3.1. Effects of non-loaded/loaded particles on root elongation

Fig. 1 gives the average RE values and the RRG of *C. sativus* seedlings exposed to 2 mg/ml of non-loaded particles, 0.28 mg/ml of Phen–water mixture, as well as the 2 mg/ml of 10.0% MML, 100.0% MML, and 432.4% MML–Phen loaded particles, along with their respective RE results for the negative controls.

The RE values of *C. sativus* seedlings exposed to 2 mg/ml suspensions of 10.0% MML and 100.0% MML of Phen-loaded particles were very similar to those of the seedlings in the blank control (p is 0.341 and 0.632, R^2 is 0.9870 and 0.9960), but it was significantly different than the RE values of the seedlings exposed to the 2 mg/ml of the non-loaded particles (p is 0.000 and 0.0067, R^2 is 0.8200 and 0.8907).

The RRG values of *C. sativus* seedlings exposed to 2 mg/ml of suspensions of Phen-loaded particles were found to be 1.05 (10.0% MML) and 0.97 (100.0% MML), which were 1.24-fold (10.0%, $p = 0.0001$) and 1.21-fold (100.0%, $p = 0.0067$) to that of the seedlings exposed to 2 mg/ml of non-loaded particles (RRG = 0.83). This result suggests the toxicity of the particles was reduced significantly after being loaded with 10.0% MML and 100.0% MML of Phen.

The situation of the 432.4% MML of Phen-loaded particles was complicated. The average RE of the seedlings grown in the presence of the 2 mg/ml of the 432.4% MML Phen-loaded particles was comparable to of the average RE of the seedlings grown in the negative control with $p = 0.228$, at the same time it was also comparable to the average RE of the seedlings exposed to the 2 mg/ml of the non-loaded particles with $p = 0.068$. It can, however, still be stated that the 432.4% MML of Phen-loaded particles were not toxic to the root growth, because whether the sample was toxic or not was concluded referring to the negative control.

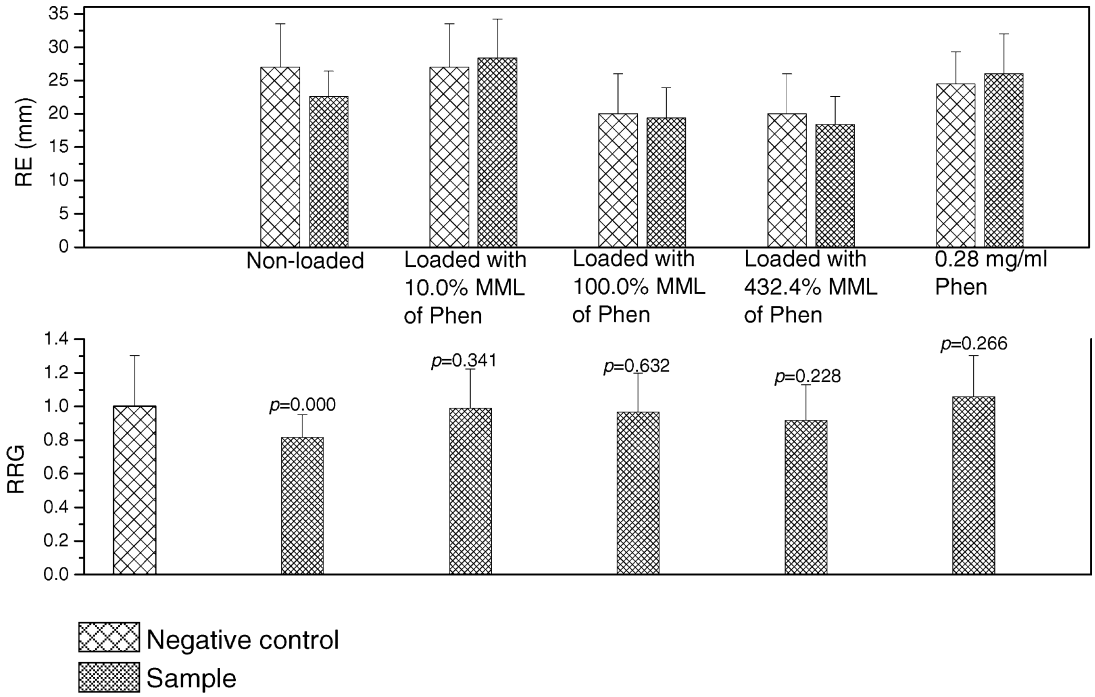

Fig. 1. The root elongation (RE), and the relative root growth (RRG) of *C. sativus* seedlings after their exposure for 24 h to 2 mg/ml of nonloaded alumina nanoparticles, 2 mg/ml of alumina nanoparticles loaded with 10.0%, 100.0%, and 432.4% monomolecular layer (MML) of phenanthrene (Phen), as well as 0.28 mg/ml of Phen. The values are expressed as mean ± S.D. of triplicate samples with 10 seedlings each. Statistical analysis was performed by the procedure of one-way ANOVA between a sample and its respective negative control. Significant difference is reported when the $p < 0.05$.

The three levels of loaded particles have increasing R^2 compared to the non-loaded particles (from 0.8200 for the 10.0% MML of Phen-loaded particles, 0.8907 for the 100.0% MML of Phen-loaded particles, to 0.9489 for the 432.4% MML of Phen-loaded particles). This increase in R^2 indicates that the inhibition effect of the Phen-loaded particles increased with the loading amount of the Phen, and approaches the inhibitory effect of the non-loaded alumina particles. This phenomenon was assigned to the toxicity of the particle-loaded Phen, not the particles, because the only thing that changed in these three particle suspensions was the amount of the Phen that had been loaded. The 0.28 mg/ml of Phen, which is the same amount of Phen as contained in the 2 mg/ml of the 432.4% MML of Phen-loaded particle suspension, when tested separately, was found to be nontoxic to the root growth. The particles loaded with the same amount of the Phen however, were significantly increased in the inhibitory effect on root growth than the Phen alone ($p = 0.0165$). A possible explanation for the observation is that the bioavailability to the root of Phen may be increased after it becomes attached to the particle surfaces.

The influence on the toxicity of particles with the 10.0% MML of Phen loading was further investigated using additional four plant species: *Z. mays*, *G. max*, *B. oleracea*, and *D. carota*. Fig. 2 shows the average RE values of the seedlings exposed to2 mg/ml non-loaded particles or 2 mg/ml 10.0% MML of Phen-loaded particles.

The non-loaded particles can inhibit the root growth in a statistically significant manner: the average p value is about 0.015 when compared to the control. However, the effect on root growth of the 10.0% MML Phen-loaded particles is significantly different than the non-loaded particles: the average p value is about 0.018 when compared to the RE results for the non-loaded particles. The loaded particles at the same time have no detectable effect on root growth: the average p value is about 0.84 when compared to the control. This result,

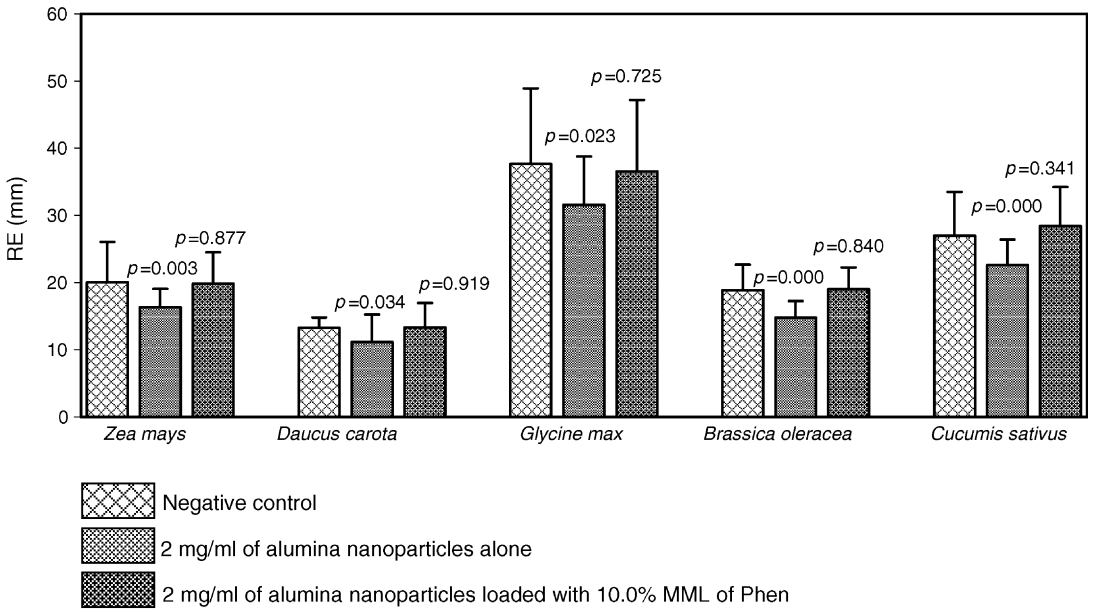

Fig. 2. The average root elongation of seedlings of five plant species after their exposure for 24 h to 2 mg/ml of nonloaded alumina nanoparticles or 2 mg/ml of alumina nanoparticles loaded with 10.0% MML of Phen. The values are expressed as mean ± S.D. The statistical analysis was performed using the procedure of one-way ANOVA. Statistically significant difference is reported when the $p < 0.05$.

along with that found in the study discussed above, demonstrates that the Phen-loading using the method described in this study reduces the inhibitory effect of the alumina nanoparticles significantly.

3.2. Interaction between particle surface and surface-loaded Phen, as determined by FTIR

The FTIR spectra of non-loaded or loaded particles are given in Fig. 3, which also shows the difference spectra of loaded particles to non-loaded particles. After subtraction of spectrum of non-loaded particles from spectra of loaded particles, a band in the 850–1050 cm^{-1} range appears. This band was assigned to surface vibrational modes of alumina nanoparticles, and its appearance arises from the disappearance of free alumina hydroxyl groups (M–OH) (Lavalley and Benaissa, 1985).

3.3. Effects of DMSO treated particles on root elongation

DMSO is a known free hydroxyl radical scavenger, and in water it can react with free hydroxyl groups on the surface of the alumina nanoparticles, forming stable products (Tai et al., 2002; Zhang et al., 2002).

$$^{\bullet}OH + (CH_3)_2SO \rightarrow CH_3SO(OH) + {}^{\bullet}CH_3$$

$$^{\bullet}CH_3 + O_2 \rightarrow CH_3OO^{\bullet}$$

$$2CH_3OO^{\bullet} \rightarrow HCHO + CH_2OH + O_2$$

In this study, the 13-nm alumina particles were dispersed in 5 ml of Milli-Q water at 2 mg/ml, in which 25 μl [0.5% (v/v)] and 50 μl [1.0% (v/v)] of DMSO had been added to make DMSO-treated particle suspensions. The toxicity of the DMSO-treated particle suspensions was then tested by the root elongation of *Z. mays* seedlings in triplicates. The same concentrations of DMSO in Milli-Q water were tested as the solvent blank. Milli-Q water was used as negative control. The test species was *Z. mays*. The test procedure and conditions were similar as described in Section 2.6. Fig. 4 presents root elongation of seedlings exposed to the 2 mg/ml of particle suspensions that had been added with DMSO.

The adding of 0.5% or 1.0% of DMSO increased the root elongation of seedlings compared to the un-

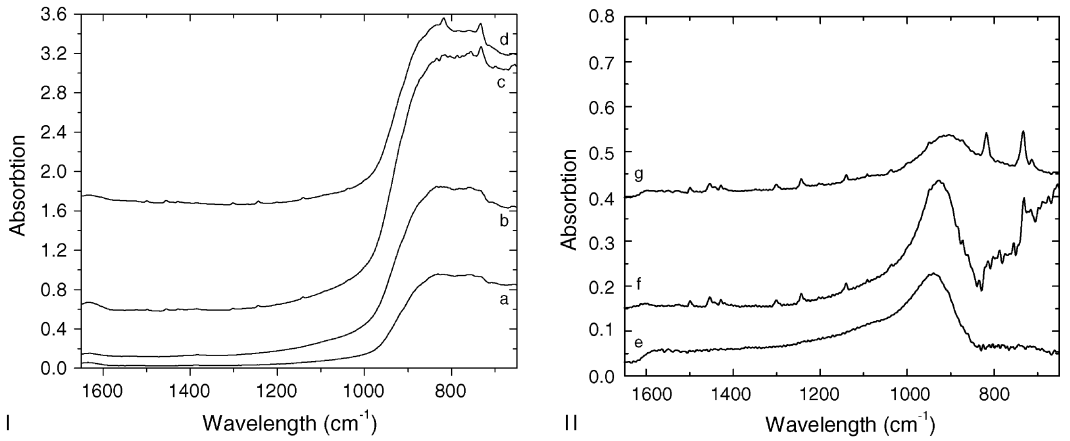

Fig. 3. FTIR spectra I: loaded particles (a); particles loaded with 10.0% (b); 100.0% (c); and 432.4% (d) monomolecular layer of phenanthrene; and FTIR spectra II that are obtained by subtraction of spectrum a from b, c, and d, resulting in e, f, and g, respectively. The band near 950 cm^{-1} is related to the surface characteristics of alumina nanoparticles (Lavalley and Benaissa, 1985). The absorbance of phenanthrene can be distinguished in both spectrum f and spectrum g.

treated particle suspensions to 1.39-fold ($p = 0.0001$) or 1.24-fold ($p = 0.002$), respectively. The root elongation of the *Z. mays* seedlings was not statistically different than that of the seedlings in the solvent blanks and the negative controls (0.5%, $p = 0.301$ compared to the negative controls, and 0.9111 compared to the solvent blanks; 1.0%, $p = 0.656$ compared to the negative controls, and 0.1252 compared to the solvent blanks). The result suggests that the adding of DMSO was causing the reduction in OH groups, and significantly reduced the phytotoxicity of the alumina nanoparticles under these conditions.

3.4. Effects of submicron alumina particles on seedling root growth

To determine whether the phytotoxicity of the alumina nanoparticles was induced by the material of Al_2O_3, we investigate the effects of submicron alumina particles on seedling root growth.

The submicron alumina particles were purchased from Atlantic Equipment Engineers (AEE) Inc. The individual size of the particles are 200–300 nm, as reported by the commercial agent. The size of the particle aggregates, as determined by a Coulter LS-230, was 1.00 ± 0.056 μm.

The particles were non-loaded or loaded with Phen following the procedures described in Section 2.2. The loading amount of Phen was 2.9×10^{-6} mol/g (0.52 mg/g, 92.6% MML).

Non-loaded or 92.6% MML of Phen-loaded particles were suspended in Milli-Q water to make the concentrations of 20 mg/ml and 2 mg/ml, which were chosen from the results of a preliminary study of 20 mg/ml, 2 mg/ml, 200 μg/ml, and 20 μg/ml.

For the non-loaded particles, effects on seedling root growth were investigated with two plant species: *B. oleracea*, and *C. sativus*, following the procedures described in Section 2.6. The 24-h exposure of *B. oleracea* seedlings presented a RRG of 0.95 ± 0.26 for 20 mg/ml, and 0.98 ± 0.28 for 2 mg/ml. They were

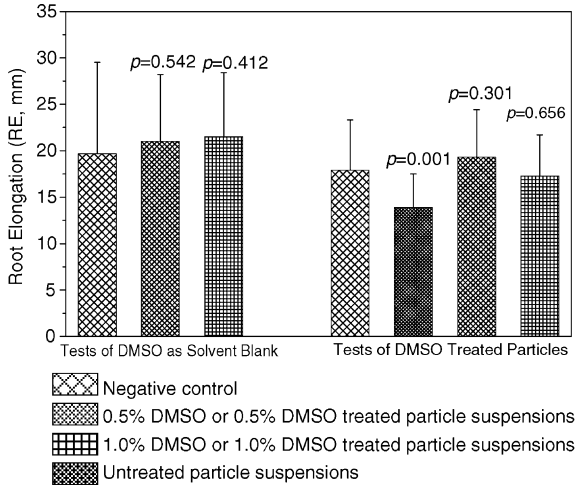

Fig. 4. Root elongation (RE) of *Z. mays* seedlings exposed to 0.5% DMSO, 1.0% DMSO, 2 mg/ml 0.5% DMSO-treated alumina nanoparticle suspensions, 2 mg/ml 1.0% DMSO-treated alumina nanoparticle suspensions, and 2 mg/ml untreated alumina nanoparticle suspensions. The result is reported as the mean value ± S.D. The statistical analysis was performed for the comparison between the blank and the sample, using the procedure of one-way ANOVA. Statistically significant difference is reported as the $p < 0.05$.

not significantly different than the root elongation of seedlings in negative controls, with $p = 0.538$ and 0.815 for 20 mg/ml and 2 mg/ml, respectively. The 24-h exposure of *C. sativus* seedlings showed a similar result with RRG of 0.98 ± 0.094 for 20 mg/ml, 0.99 ± 0.10 for 2 mg/ml, and the p values obtained from the comparison to the negative controls was 0.424 and 0.697 for the 20 mg/ml and the 2 mg/ml, respectively.

Effects of Phen-loaded submicron alumina particles were investigated by seedlings of *B. oleracea* at 20 mg/ml, following the procedure described in Section 2.6. The 24-h exposure to the Phen-loaded particles gave a RRG result of 0.98 ± 0.28, which was neither statistically different than the RRG result for the negative controls ($p = 0.835$) nor than the RRG result for the non-loaded particles ($p = 0.707$).

4. Discussion

It is well known that epidemiological evidence suggested there is an association between fine particulate matters ($PM_{2.5}$) and adverse health effects (Dockery and Pope, 1994; Dockery, 2001). But the mechanisms of effect are not determined. A number of experimental studies support that the particle surface-associated chemicals contribute to the adverse health effect of particles (Tsien et al., 1997; Kawasaki et al., 2001; Carter et al., 1997; Hannigan et al., 1998). There is also evidence at the same time supporting the postulation that not a single major or trace chemical component of the particles is responsible for the adverse effects (Harrison and Yin, 2000; Obot et al., 2002).

This study was carried out to investigate the phytotoxicity and underlying mechanisms of toxic effects of alumina nanoparticles. Phytotoxic effects of "clean" (i.e., nonloaded or pure) alumina nanoparticles were tested with five plant species. Simultaneously, we loaded the alumina nanoparticles with Phen, a major constituent of polycyclic aromatic hydrocarbons in the atmosphere, and the toxicity (inhibition of root growth in this case) of the particles loaded with 2.83 mg/g (10.0% MML, which is more than the Phen present on most of the atmospheric particles), 28.40 mg/g (100.0% MML), or 122.81 mg/g (432.4% MML) was tested with *C. sativus* seeds. All of the loaded particles were found to be less toxic than the non-loaded particles. We, then further, compared the phytotoxicity of non-loaded particles to that of the 10.0% MML of Phen loaded particles by the means of the root elongation examination using seeds of other four plant species: *Z. mays* (corn), *G. max* (soybean), *B. oleracea* (cabbage), and *D. carota* (carrot). The similar results for the five plant species proves that phytotoxicity of alumina nanoparticles was reduced significantly by the loading of 10.0% MML of Phen.

Effects of submicron alumina particles loaded with or without Phen were investigated to evaluate if the chemical material of Al_2O_3 can impose toxic effect on the seedling root growth, and if the phenomenon of decreased toxicity due to Phen-loading was size specific. Results demonstrated that the submicron alumina particles, either nonloaded or loaded with Phen, did not induce any detectable effects on the seedling root growth under the experimental circumstances in this study, and no difference was found between the root growth effects induced by nonloaded and loaded particles. The possibility that the material of Al_2O_3 can induce toxic effects on seedling root growth was, thus, excluded. And the decreased toxicity due to Phen-loading observed in this study was specific to the alumina nanoparticles.

Because the phytotoxicity of the particles was changed after the particles had been loaded with Phen, it was surmised that certain particle properties have been changed after the Phen loading process. The size of the particle aggregates after being loaded with Phen was one suspect. As a typical case of nanoparticles, the alumina nanoparticles we studied exist as particle aggregates instead of individual particles in the particle suspensions that were tested as seedling culture media, meaning that the seedlings may take them as particle aggregates and/or individual particles. This is normal because these particles were not manufactured specifically to be monodispersed, and disaggregating would be extremely difficult so that no such procedures were performed in our study. Phen-loading may hold the particles together more strongly than the original status of the particles. As a result, the size of particle aggregates would increase after being loaded with Phen, which possibly may induce the reduction of the toxic effect of the particles. To investigate this possibility, we analyzed the size of the nonloaded and Phen-loaded particle aggregates by the instrumentation of a Coulter N4+. However, loading of Phen did not significantly change the size of particle aggregates. Actually, the size of the particle aggregates remained almost unchanged after the Phen-loading: the average size of the original particle aggregates is 201.0 nm; the average size of the nonloaded particle aggregates is 217.9 nm; and those of the 10.0% MML, 100.0% MML, and 432.4% MML Phen-loaded particle aggregates are 210.9 nm, 214.0 nm, and 225.7 nm, respectively. Compared to the size of nonloaded particle aggregates, the sizes of Phen-loaded particle aggregates were either smaller or larger. The mean value of the sizes of Phen-loaded particle aggregates was 216.9 nm. The three numbers, 210.9 nm, 214.0 nm, and 225.7 nm, were within the 95% confidence level of the mean value, which indicates that statistically, the size of the particle aggregates remained after being loaded with Phen. The result suggests the possibility that the change in the particle size induces the change in the inhibition effect of the particles does not exist.

We then investigated the nonloaded or loaded alumina nanoparticles by the means of FTIR spectroscopy technique, which has been proven to be a powerful tool for studies on surface characteristics in material science. The difference spectra of loaded particles to nonloaded particles showed that an infrared band appears in the region of 850–1050 cm^{-1}. A band in this region was first reported by Lavalley and Benaissa (Lavalley and Benaissa, 1985). According to the authors, it was assigned to the surface vibrational mode of alumina nanoparticles, which arises from the losing of free alumina hydroxyl groups.

In order to examine whether the free alumina hydroxyl groups may contribute to the phytotoxic effect of the particles, we treated the particle suspensions with 0.5% and 1.0% (v/v) DMSO, which is a known free hydroxyl radical scavenger and can react with free hydroxyl groups on the surface of alumina nanoparticles. The phytotoxicity of the DMSO treated particle suspensions were tested using seedlings of *Z. mays*. After the comparison to the untreated particles, we found the treatment of both concentrations of DMSO lowered phytotoxicity of alumina nanoparticles.

Based on the results from this study, we supposed that (1) the loading of 10.0% MML, 100.0% MML, and 432.4% MML of Phen did not change the size of the alumina nanoparticle aggregates; however, it may change the surface characteristics of the alumina nanoparticles through interaction with the free hydroxyl groups on the particle surface; (2) the change of the surface characteristics of the alumina nanoparticles changes the root growth inhibition effect of the particles; and (3) the surface characteristics of alumina nanoparticles are important to the phytotoxicity of the particles.

The mechanism of how alumina nanoparticles inhibit root growth of the seedlings needs further work, which helps to elucidate the influence of surface characteristics of the particles on their toxicity, and can provide scientific basis for the control of the manmade nanoparticle pollution.

In this study, Phen, a PAH, was chosen as the chemical species to be adsorbed on the particle surfaces. PAHs are known to be unstable under UV radiation. Though Phen is much less photochemical reactive and to some extent resistant to photodecomposition than other PAHs, such as benzo[a]pyrene, pyrene, and anthracene (Korfmacher et al., 1980; Teinemaa and Kirso, 1999), it is possible that in the field the particle surface associated Phen may receive UV radiation and decompose into some toxic intermediate products. As a result, the Phen-loaded particles in the field may impose adverse effects on plant growth. Future studies on the photochemical stability of nanoparticle surface asso-

ciated PAHs, the change of the health effects as well as environmental consequences induced by the photochemical reactions of nanoparticle surface associated PAHs are needed.

Acknowledgements

This work was funded by the project of "Smart Coating", and supported by Otto H. York Center for Environmental Engineering and Science, New Jersey Institute of Technology, Newark, NJ, USA.

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