Influence of Sediment Grain Size on Elutriate Toxicity of Inorganic Nanomaterials

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Knowledge concerning the ecotoxic effects of nanomaterials, chemical structures with novel properties owing to their small sizes (1 to 100 nm), is wanting and deserves to be documented more fully. In this study we conducted testing with the MARA (microbial array for risk assessment) assay—an 11 microbial species 96-well microplate toxicity test measuring growth inhibition—to determine the toxic potential of four metallic nanopowders (MNPs): copper zinc iron oxide, samarium (III) oxide, erbium (III) oxide, and holmium (III) oxide. MTC (microbial toxicity concentration) endpoint values showed a range of toxicity responses generated by individual strains that was MNP-specific. Cluster analysis undertaken with the (n = 11) MTC values of the four MNPs, reflecting a toxic fingerprint proper to each nanochemical, indicated that their modes of action may be different. Experiments were also conducted with an artificial sediment, composed of varying concentrations of silica sand and kaolin (fine particles < 0.004 mm), spiked with each MNP to assess the contribution of fine particles on the resulting elutriate toxicity. The latter was shown to increase as fines contents decreased, except for CuZnFeO where no particular trends were observed. Toxicity testing was then undertaken with each MNP spiked into natural Saint Lawrence River freshwater sediments displaying low, medium, and high fines contents. Once again, analogous results to those obtained with the artificial sediment experiments were observed for MNP elutriate toxicity. Overall, MARA bioassay data indicate that MNP toxicity can be modulated by sediment grain size and that resulting adverse effects on aquatic biota will in part depend on such sediment characteristics.

Key words: metallic nanomaterials, aquatic toxicity, MARA assay, sediments, grain size

Introduction

Nanotechnology implies research and development conducted with particle sizes in the 1 to 100 nm range. Owing to their composition, small size, and shape, nanomaterials display novel properties that have varying applications in the biomedical, electronics, and environmental fields (e.g., Aitken et al. 2006). Nano Erbium (III) oxide, for instance, is presently used in the industrial (as a superconductor and as a colouring agent for glass and ceramics), optical (as an UV absorbant in glass and in optic fibers), and medical (as a dopant for laser surgery) fields. While distinct benefits are expected from nanotechnology products, there are preoccupations about the hazards that nanoparticles (NPs) can have on human and environmental health. Indeed, recent studies suggest that adverse effects can be linked to some nanoproducts (Lovern and Klaper 2006; Chen et al. 2007; Handy and Shaw 2007; Zurita et al. 2007).

While the presence of NPs in the aquatic environment is still largely undocumented (Moore 2006), their future release via industrial wastewaters or municipal treatment plant effluents, for example, is expected to occur. Already, uptake and ecotoxic effects of some (in)organic NPs have been shown for several taxa (e.g., bacteria, microalgae, microinvertebrates, fish) of aquatic organisms (Nowack and Bucheli 2007; Blaise et al. 2008). Noted adverse effects of NPs are perhaps not surprising in view of their intrinsic (colloidal, size/shape, surface charges, and vectorization) properties that confer remarkable reactivity with other (a)biotic materials (Gagné et al. 2008). Yet, proper interpretation of hazard/risk for NPs will require comprehensive information on their characteristics that is not readily available owing to present shortcomings in analytical science. This is clearly an outstanding issue that remains to be addressed.

In a recent study, we explored the potential ecotoxicity that NPs might have on aquatic organisms by conducting bioassays representing different taxa on several nanopowders already in use commercially (Blaise et al. 2008). Among other findings, this investigation showed that four metallic nanopowders (SmO [samarium (III) oxide], ErO [erbium (III) oxide], HoO [holmium (III) oxide], CuZnFeO [copper zinc iron oxide]), when spiked into a reference sediment, could augment elutriate toxicity. Since grain size has been recognized as an important physicochemical characteristic of sediments that can modulate the bioavailability of contaminants...
(Chapman and Wang 2001), we sought to determine how this factor might influence the toxicity of these four metallic nanopowders (MNPs) when mixed in sediment with differing fines contents. In fact, grain size distribution in sediment is reported to be a crucial factor controlling sediment metal concentrations with correlations commonly shown between decreasing grain size and increasing metal concentrations (Fürstner 1989; Horowitz 1991; Wang et al. 2007). Therefore, how MNPs might behave in sediment of differing grain sizes (i.e., low fines versus high fines sediments) is important to ascertain whether sediments will behave as a sink (remove from the water column) or a source (release to the water column) for such materials, as this may have implications for benthic and/or water column organisms.

Material and Methods

The following four MNPs were purchased from Sigma-Aldrich: copper zinc iron oxide (# 641650: CuZnFe$_2$O$_4$, <100 nm particle size with an average size of 29.0 nm and a surface area of 17.8 m$^2$/g, 98.3% purity), samarium (III) oxide (# 637319: Sm$_2$O$_3$, <100 nm particle size with an average size of 14.8 nm and a surface area of 9.5 m$^2$/g, 99.9% purity), erbium (III) oxide (# 637343: Er$_2$O$_3$, <100 nm particle size with an average size of 41 to 53 nm and a surface area between 13 to 17 m$^2$/g, 99.9% purity), holmium (III) oxide (# 637327: <100 nm particle size with an average size of 36.6 nm and a surface area of 19.5 m$^2$/g, 99.9% purity). No further information on MNP features (e.g., aggregation, surface coating, or crystal structure characteristics) was available from the manufacturer.

MARA Assay Description

The MARA (microbial array for risk assessment) assay is a multispecies 96-well microplate-based toxicity test that measures concurrent cell growth (via reduction of tetrazolium red dye) of 11 different microorganisms (individually lyophilized in microplate wells) after a 24 h exposure to chemical solutions or liquid samples (Gabrielson et al. 2003). The strains have been selected for their wide genetic diversity to favour a wide span of sensitivity to chemicals (Gabrielson et al. 2003). Although the identity of strains is proprietary, we do know that strains 1 to 10 are bacteria (1, 5, 7, 8 are Gr+ and 2, 3, 4, 6, 9, 10 are Gr-), and that strain 11 is a yeast species. After exposure to a dilution series of test sample, the resulting growth in microplate wells for each species was captured with a computer-connected flatbed scanner (HP 7400c) and a copy of each scanned microplate was saved on an Excel spreadsheet. The size of bacterial pellets recorded in each well (diameter and density indicative of growth) is then measured with the MARA software. In our study, we undertook testing with MARA products (reagents, growth medium, microplates, scanner, software) available commercially from NCIMB Limited, Aberdeen, Scotland, U.K. (NCIMB 2008). After completion of the MARA procedure, MTC (microbial toxic concentration) values for each of the 11 strains, corresponding to the mean pellet size of each microbial strain, were calculated with the software according to the following formula:

$$MTC = c^{min}d^{[P_{total}P_{0}]^{0.5}}$$ (1)

where $c^{min}$ is the lowest concentration tested, $P$-0 is the pellet size in the control well of each strain, $d$ is the dilution factor (e.g., 1/2 or 1/3), and $P_{total}$ is the sum of the pellet sizes in all wells exposed to the sample dilution series.

Because the selected microbial species represent a wide genetic diversity, their individual toxicity responses (i.e., differential growth after exposure) yield 11 MTC values that reflect a unique profile that has been termed the “toxic fingerprint” of the test sample (Gabrielson et al. 2003). MTC values and toxic fingerprints of the NPs investigated are discussed further on.

MARA Testing of the Four NPs

In preparation for toxicity testing, each MNP was first processed as previously described (Blaise et al. 2008). Briefly, 75 mg of MNP were weighed into a small culture tube (VWR, catalogue # 89001-476) and 150 mL of purified water (bidistilled) were added to give a starting concentration of 500 mg/L. Once capped, each tube was rotated (VELP Scientifica, Overhead mixer, Rotax 6.8) and the contents were allowed to mix at room temperature (12 rpm; 24 h). Afterwards, 100 mL of tube contents were filtered through a preweighed (and predried) 0.22-$\mu$m cellulose (white plain, 47 mm) membrane to remove any unsolubilized nanopowder. The membrane was then dried for 24 h (desiccator with Drierite at 60°C) and reweighed in order to determine, by difference, the initial concentration of MNP present in the filtrate that would be bioassayed with the liquid phase toxicity tests.

Preparation of Artificial Sediment (Sand/Kaolin Mixtures) Elutriates for Subsequent MARA Testing

To determine whether elutriate toxicity responses of MNPs could be influenced by sediment particle size, MARA assay testing was first undertaken with a mixture of silica sand (Allwhite Silica Sand, medium fine grade #0, grain size 0.125 to 0.25 mm; Shaw Resources, Milford, Nova Scotia, Canada) and kaolin clay (Fisher Scientific, catalogue # K2-500, lot # 9502120; grain size < 0.004 mm). In this experiment, selected kaolin concentrations were prepared between 0 and 100% sediment (i.e., sand/kaolin) content. Each MNP was thus mixed in a 1:10 ratio with a sand/kaolin sediment mixture to later determine whether it could increase the latter’s elutriate toxicity (as measured with the MARA assay). For example, a 40%
kaolin mixture (0.5 g of kaolin + 0.75 g of silica sand = a total of 1.25 g) was placed into a 15-mL plastic tube with screw cap (Sarstedt, PP GWB) filled with 10 mL of purified water, to which was added 0.125 g of MNP. Each such solution of sand/kaolin percentage mixes, with and without MNP addition, was vertically rotated (Caframo Reax 2 rotating mixer) for 1 h at 12 RPM, followed by centrifugation (3,000 RPM; 15 min). Because MARA is an 11-species microbial array requiring sterile conditions, each elutriate was then filtered (0.45-μm cellulose membrane) and the resulting filtrate (= filtered elutriate) was recovered to determine its toxicity.

**MARA Testing with Natural Sediments of Different Grain Sizes**

To demonstrate whether elutriate toxicity responses of MNPs could be influenced by different grain sizes under more realistic conditions, grab samples of several natural Saint Lawrence River freshwater sediments (located in the vicinity of the Island of Montreal) were collected in August, 2007, with the help of an Ekman dredge. The sediments came from frequently monitored sites and their grain size characteristics are reported in Table 1. For ensuing MARA testing, sediment elutriates were prepared from 1:10 mixes of sediment and MNP, as described above for the sand/kaolin and MNP experiments.

**Statistical Analyses**

MARA assay MTC values were determined with statistical methods and software recommended for this procedure (NCIMB 2008, version 1.1). Each MARA assay provides 11 MTC values that reflect a specific toxicity profile or toxic fingerprint. Their (dis)similarities were determined for each sample (i.e., individual MNPs, MNPs with sand/kaolin clay mixtures, and MNPs with natural sediments) using cluster tree analysis. The classification thresholds were based on the Pearson-moment correlation between each subsample response (1 - r). Differences between mean MTC responses (obtained from each 11-species sediment mixture test expressed in percent vol/vol of elutriate) were analyzed using ANOVA (analysis of variance) procedure, and critical differences from controls (i.e., 100% silica sand) were determined by the least square difference test. All analyses were performed using Statistica version 7 software with significance set at p < 0.05.

**Results and Discussion**

The MARA assay comprises an array of 11 microbial species in a 96-well microplate exposed to a concentration gradient of the sample under investigation. After exposure, MARA plates are read with a flatbed scanner and each plate image is computer-stored. As an example, the MARA plate scanned following the SmO toxicity test is illustrated in Fig. 1. Visibly, different strains display varying sensitivity toward SmO. Microbial pellet size (diameter and density) in each well is then measured with MARA software, from which individual MTCs generated for each of the 11 microbial species can be calculated. MTCs for each of the four MNPs studied are reported in Table 2. As can be seen, the MTCs displayed by each of the 11 strains and for each MNP differ, thereby indicating a particular toxic profile. For example, strain 1 exhibits the most sensitive toxicity response toward SmO (MTC = 7 mg/L), while strains 3, 4, 6, and 9 are the least sensitive (MTC = 243 mg/L). Although the four MNPs yield average MTC values ranging between 69 and 114 mg/L, suggesting that toxic effects are generally similar, individual strain responses show that sensitivities can markedly differ. Again, this is especially noticeable for SmO where microbial species toxicity can differ by more than two orders of magnitude (MTC of 243 mg/L versus 7 mg/L).

In Fig. 2, the four bar graphs produced from the MNP MTC data notably demonstrate a distinct pattern known as a “toxic fingerprint” (Gabrielson et al. 2003). The dendrogram resulting from cluster tree analysis of MTC values determined for each MNP is further able to discriminate their (dis)similarities based on this toxic fingerprinting concept (Fig. 2). Accordingly, MNPs with matching toxic fingerprints are likely to exhibit similarities in their toxic mode of action. Indeed,

**TABLE 1. Grain size characteristics of sediments collected in the St. Lawrence River**

<table>
<thead>
<tr>
<th>Site</th>
<th>GPS location</th>
<th>Respective percentages of fines sizes</th>
<th>Total % of fines *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>63 μm (sand)</td>
<td>33.1 μm or less (silt)</td>
</tr>
<tr>
<td>Ile Robinet</td>
<td>45°44′511″N 73°25′687″W</td>
<td>12.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Ile Beauregard</td>
<td>45°45′451″N 73°24′489″W</td>
<td>30.9</td>
<td>7</td>
</tr>
<tr>
<td>Islet vert</td>
<td>45°42′439″N 73°27′244″W</td>
<td>3.6</td>
<td>13.9</td>
</tr>
<tr>
<td>Ile de Grâce</td>
<td>46°06′214″N 73°00′524″W</td>
<td>2.8</td>
<td>81.6</td>
</tr>
</tbody>
</table>

*Fines are defined as sediment particles ≤ 63 μm in size (Environment Canada 2002).
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CuZnFeO and ErO, closely clustered, also show similar MTC fingerprints suggesting modes of toxic action that are linked, while SmO and HoO, in contrast, have distinct toxic fingerprints altogether, which indicates that their toxicity manifests itself differently. As previously reported, MARA toxic fingerprinting was able to demonstrate, for example, close clustering of two cytochrome P450 inhibitors (orphenadrin HCl and quinidine sulphate) while two oxidizing agents (sodium fluoride and hydrogen peroxide), also closely clustered, were well demarcated (i.e., clustered far off) from the former two substances (Gabrielson et al. 2003). Hence, MARA cluster analysis based on the toxic fingerprinting concept demonstrates that the MNPs studied can differ in terms of their toxic mode of action. Overall, other studies have also highlighted the adverse effects that various metal nanopowders could have on aquatic biota in the event of their discharge to aquatic systems: ZnO, TiO$_2$, and Al$_2$O$_3$ on the zebrafish *Danio rerio* (Zhu et al. 2008); ZnO, CuO and TiO$_2$ on bacteria, *Vibrio fischeri*, and crustaceans, *Daphnia magna* and *Thamnocephalus platyurus* (Heinlaan et al. 2008); and ZnO on the microalgae, *Pseudokirchneriella subcapitata* (Franklin et al. 2007).

![Fig. 1. Scan of a MARA microplate following a 24 h exposure to a concentration gradient of SmO. Highest test concentration is in row G (243 mg/L) with a 1/3 dilution factor applied in subsequent rows up to row B (1 mg/L). Row A indicates control growth. Wells in Row H contain the lyophilized microbial strains (strain 1 is in well H-1, strain 2 is in well H-2, etc.) from which inocula in other wells are made, and wells in column 12 are uninoculated. Black circles in each well result from precipitated tetrazolium dye indicative of microbial growth.](https://iwaponline.com/wqrj/article-pdf/44/3/201/229585/wqrjc0440201.pdf)

![Fig. 2. Cluster analysis of nanopowders based on their toxic fingerprint (*n* = 11 MTC values of the microbial strains generated with the MARA assay for each nanopowder). The bar graphs illustrate the toxic fingerprints of the four MNPs investigated. SmO was replicated twice with the MARA assay (SmO-1 and SmO-2) as a spot check for reproducibility.](https://iwaponline.com/wqrj/article-pdf/44/3/201/229585/wqrjc0440201.pdf)
To determine the influence of sediment particle size, if any, on elutriate toxicity, we undertook MARA bioassays to assess microbial toxicity after MNPs were mixed in a 1:10 ratio with sand/kaolin mixes (see Materials and Methods section). Prior to treatments involving any of the MNPs, MARA testing of elutriates resulting only from gradients of prepared 20, 40, 60, 80, and 100% sand/kaolin clay mixtures invariably demonstrated an absence of toxicity responses by all microbial strains (i.e., MTCs >100% elutriate). ANOVA testing comparing the 11 MTC values generated for each treatment (MNP + 0% kaolin mix and MNP + different gradients of kaolin mixes) was undertaken to observe the influence of fines. The presence of increasing amounts of fines (kaolin clay) clearly decreases the elutriate toxicity of SmO (Table 3), as average MTCs increase when 20% or more fines are present in the experimental mixes. Cluster analysis of MTC values obtained following each treatment further illustrates this effect (Fig. 3). In particular, 60, 80, and 100% kaolin mixes with SmO are more closely clustered and more distant from the 0, 20, and 40% mixes with SmO. For HoO and ErO, similar effects generally displaying a decrease in elutriate toxicity with an increase in fines (kaolin) were observed, although ANOVA analysis did not show significant differences between treatments. Cluster analysis, however, did suggest increasing dissimilarities in their elutriate toxicity responses with fines contents augmenting, as the HoO dendrogram illustrates (Fig. 4). No fines effects on elutriate toxicity of CuZnFeO were seen after MARA testing with varying degrees (0, 20, 40, 60, 80, and 100%) of kaolin mixes (results not shown), indicating that aqueous release of this MNP adsorbed to a sediment matrix does not seem influenced by sediment grain size.

To further document the influence that fines could have on MNP elutriate toxicity under more realistic environmental conditions, experiments were conducted on MNPs mixed in a 1:10 ratio with four natural sediments exhibiting differing grain sizes (Table 1). MARA testing first conducted on elutriates prepared from each of these sediments showed them to be nontoxic to all microbial strains (i.e., MTCs >100% elutriate). In a manner similar to the kaolin experiments reported above, increasing amounts of fines in sediment again contributed to a decrease in elutriate toxicity of SmO (Table 4), as average MTCs increase when 20% or more fines are present. Additionally, cluster analysis of the MTC values obtained following each sediment treatment generally illustrates the growing dissimilarity of elutriate toxicity as sediment fines content is augmented (Fig. 5). As Table 4 shows, the IB (Ile Beauregard) sediment, whose average MTC is not significantly different from that of the SmO + 100% silica sand and 0% kaolin treatment, and characterized by 46.9% of total fines made up of small sand, silt, and clay (Table 1), has the highest proportion of fines in the 0.063 mm sand category (66%), followed by the IR (Ile Robinet) sediment (59%), IV (Islet Vert) sediment (6%), and IG (Ile de Grâce) sediment (2.8%). This may explain its closer cluster with the 0% kaolin clay treatment and also with the IR sediment (Fig. 5). Paradoxically, the latter sediment mixed with SmO displays an average MTC significantly higher than the 0% kaolin treatment.

### Table 2. MTCs of the four nanomaterials determined with the MARA assay

<table>
<thead>
<tr>
<th>Nanopowders</th>
<th>MTCs in mg/L for each MARA strain (#1–11)</th>
<th>Avg. MTC *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Samarium oxide</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>Copper zinc iron oxide</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Erbium oxide</td>
<td>58</td>
<td>70.9</td>
</tr>
<tr>
<td>Holmium oxide</td>
<td>16</td>
<td>83.2</td>
</tr>
</tbody>
</table>

* Average MTC of the 11 microbial strains calculated with MARA software.

### Table 3. Influence of fines (kaolin clay) on MARA elutriate toxicity of SmO as shown by ANOVA

<table>
<thead>
<tr>
<th>Treatments *b</th>
<th>MTC (%) elutriate</th>
<th>Level of statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmO + 100% silica sand and 0% kaolin:</td>
<td>22.3</td>
<td>—</td>
</tr>
<tr>
<td>versus SmO + 80% silica sand and 20% kaolin</td>
<td>26.9</td>
<td>NS</td>
</tr>
<tr>
<td>versus SmO + 60% silica sand and 40% kaolin</td>
<td>29.5</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>versus SmO + 40% silica sand and 60% kaolin</td>
<td>37.4</td>
<td>p &lt; 0.1</td>
</tr>
<tr>
<td>versus SmO + 20% silica sand and 80% kaolin</td>
<td>30</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>versus SmO + 0% silica sand and 100% kaolin</td>
<td>30.8</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

* Fines are defined as sediment particles ≤ 0.063 mm in size (Environment Canada 2002). Kaolin clay has a grain size ≤ 0.004 mm.

* Treatments compared in 1:10 SmO to kaolin clay mixes.

* Average MTC (% elutriate) of the 11 MARA microbial strains.

* Level of statistical significance: NS (not significant); p < 0.05; p ≤ 0.1.
Fig. 3. Cluster analysis of SmO and K (kaolin clay/sand) mixes (e.g., “40% K” is composed of 40% kaolin clay and 60% silica sand) after MARA testing.

Fig. 4. Cluster analysis of HoO and K (kaolin clay/sand) mixes (e.g., “20% K” is composed of 20% kaolin clay and 80% silica sand) after MARA testing.
Elutriate Sediment Toxicity of Nanomaterials

TABLE 4. Influence of fines content of natural sediments on MARA elutriate toxicity of SmO as shown by ANOVA

<table>
<thead>
<tr>
<th>Treatments b</th>
<th>Avg. MTC e (% elutriate)</th>
<th>Level of d statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmO + 100% silica sand and 0% kaolin:</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>versus SmO + IR sediment containing 20.9% fines e</td>
<td>47.2</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>versus SmO + IB sediment containing 46.9% fines e</td>
<td>27.5</td>
<td>NS</td>
</tr>
<tr>
<td>versus SmO + IV sediment containing 59.1% fines e</td>
<td>62.7</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>versus SmO + IG sediment containing 99.6% fines e</td>
<td>47.2</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

a Fines are defined as sediment particles ≤ 0.063 mm in size (Environment Canada 2002).
b Treatments compared in 1:10 SmO to natural sediment mixes.
c Average MTC (% elutriate) of the 11 MARA microbial strains.
d Level of statistical significance: NS (not significant); p < 0.05; p ≤ 0.1.
e IR (Ile Robinet), IB (Ile Beauregard), IV (Islet Vert), IG (Ile de Grâce); see Table 1.

Despite its lower fines content (20.9%). Notably, the IR sediment has 41% of its fines as silt and clay whereas the IB sediment has somewhat less (34%) in comparison (as can be calculated from Table 1). This might thus confer more SmO adsorption potential to IR sediment and possibly explain the resulting significant increase in average MTC. The higher proportion of fines in the 0.063-mm sand category (66%) seen in the IB sediment may also suggest that sediment grain sizes close to the limit of fines dimensions (i.e., near 0.063 mm) are less able to retain SmO trapped into a solid matrix. Larger grain size implies reduced surface area and hence reduced interaction with the SmO MNP. Finally, one cannot exclude other confounding factor(s) unlinked to fines content that may also have influenced the resulting elutriate toxicity of the IR and IB sediments, as shown by their dissimilar average MTC values (Table 4).

Fig. 5. Cluster analysis of SmO + 0% K (0% kaolin clay and 100% silica sand) and natural sediment F (fines) mixes (see Table 1 for sites and fines’ contents characteristics e.g., “IB 47%” is site Ile Beauregard sediment with 47% fines) after MARA testing. IdG was replicated twice with the MARA assay (IdG-1 and IdG-2) as a spot check for reproducibility.
Experiments conducted for HoO and ErO with natural sediment mixes showed similar trends in that effects displayed a general decrease in elutriate toxicity with an increase in sediment fines, although ANOVA analysis again did not show significant differences between treatments. Cluster analysis, however, once again suggests increasing dissimilarities in elutriate toxicity responses as fines contents increase, as the HoO dendrogram illustrates (Fig. 6). As with the kaolin experimental mixes, no natural sediment fines effects on elutriate toxicity were observed for CuZnFeO (results not shown), indicating that it is impervious to sediment grain size features.

MNPs may well behave akin to metallic compounds in that the latter’s uptake by sediments are closely linked to organic content (Lin and Chen 1998) and grain size characteristics (Soares et al. 1999; Kominkova and Nabelkova 2007). Because of high surface area reactivity, clay components play important roles in metal-solid interactions as compared with coarser sediment particles (Tessier et al. 1982). Several investigations have indeed demonstrated the improved retention capacity of metals by sediments with higher fines contents. Humic and fulvic acids, intrinsic colloids found in natural sediments, were shown to mitigate copper and zinc toxicity of lake sediment, thereby permitting less impeded growth of the cyanobacteria *Microcystis aeruginosa* (Ohkubo et al. 1998). After amending sediments collected from two U.S.A. freshwater lakes with AgNO₃, lethal effects toward *Chironomus tentans* were markedly more pronounced in pore waters extracted from sandy sediment in comparison with those extracted from a silt/clay sediment (Call et al. 1999). Metal contamination (Cu, Cr, Zn, Cd, and Pb) reported in estuarial sediments were shown to more adversely affect denitrifying microbial communities in muddy sediments (high fines contents) than in sandy ones (lower fines contents), owing to higher retention of metals by the former (Magalhaes et al. 2007). In two marine areas similarly contaminated by copper, pore waters of a fine-grained sediment site displayed less toxicity in the sea urchin embryological development test conducted with *Schizopera knabeni* than pore waters of a sandy sediment site, indicating stronger metal retention by sediment fines (Wauhob et al. 2007). In artificially prepared sediments, clay content increased Hg concentrations in the sediment matrix (Zhong and Wang 2008).

Overall, these studies demonstrate that high fines sediments retain metals more than lower fines sediments thereby making them less extractable in pore water or elutriate fractions. Our study shows that nano metal oxides, barring CuZnFeO, are similarly sequestered by sediment fines and that this characteristic is important in determining the potential threat that MNPs released to aquatic environments could pose toward water column organisms, if sediments become displaced owing to natural (e.g., flood scouring) or anthropogenic (e.g., dredging activities) events. In contrast, MNPs bound to high fines sediments will likely have greater toxic impact on benthic organisms because of their increased presence and retention in whole sediment, but further studies will be required to verify this situation.

**Fig. 6.** Cluster analysis of HoO + 0% K (0% kaolin clay and 100% silica sand) and natural sediment F (fines) mixes (see Table 1 for sites and fines’ contents characteristics e.g., “IB 47%” is site Ile Beauregard sediment with 47% fines) after MARA testing.
Of the four metallic nano oxides investigated, only CuZnFe\textsubscript{2}O\textsubscript{4} appeared unaffected by fines contents, as its elutriate toxicity was not altered under differing grain size regimes. Based on the crystal structure of kaolinite (kaolin clay), the presence of aluminum hydroxide groups permits the formation of hydrogen bonds which can, theoretically, bind oxide particles (kaolin-O-H + metal oxides-O \rightarrow kaolin-O-H-O-metal oxides). For example, a ferrite oxide usually contains calcium bound to the external layer of oxygen atoms, which could impede the formation of hydrogen bonds (Aubert et al. 1978). With CuZnFe oxide, whose toxicity appeared independent of kaolin clay or natural sediment fines proportions, metals (Cu, Zn) would thus be associated with the oxygen atom in place of calcium, usually associated to ferrites, thereby blocking the formation of hydrogen bonds. Formation of hydrogen bonds may explain the basis of kaolin clay-MNP interactions in aquatic environments.

Conclusions

In this work, we first showed that four MNPs were toxic when subjected to MARA testing, and that cluster analysis based on the toxic fingerprinting concept suggests that some MNPs may have different modes of toxic action (e.g., contrasting samarium oxide and holmium oxide toxic fingerprints, Fig. 2). When amended with different gradients of fines in artificial sediment (silica + kaolin clay) experiments, MNP elutriate toxicity increased as fines contents decreased, except for CuZnFeO\textsubscript{4} where no particular trends were observed. In similar experiments conducted with natural freshwater sediments collected from the Saint Lawrence River, we noted analogous results. Hence, sediment contamination by some nanomaterials may be a future issue should these ever be discharged into aquatic systems. In this event, SmO, ErO, and HoO are likely to partition more easily into the water column from low fines sediments and less so from high fines sediments, with potentially greater toxic impact on either water column or benthic organisms. Adverse effects on selected aquatic biota will likely be mediated by sediment grain size, as our results indicate. Uncertainty related to ecotoxic assessment of nanomaterials will remain, however, until their unique features (size, shape, dispersion, surface area/charge, etc.) are more fully elucidated and their behaviour investigated under varying environmental compartments and conditions. In this respect, future studies seeking to better comprehend the potential adverse effects of nanomaterials will likely profit from breakthroughs in analytical expertise capable of shedding new knowledge on their characteristics.

Acknowledgments

The first two authors wish to thank the Brazilian government for a CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) grant that allowed the first author to undertake a stay (September 2007 to end of December 2007) in the Saint-Lawrence Centre to contribute to this study as part of her doctorate thesis. Authors Blaise and Gagné are grateful to the Chemical Management Plan under CEPA (Canadian Environmental Protection Act) for financial assistance supporting this research initiative into nanomaterial toxicity.

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Received: 17 March 2008; accepted: 20 March 2009.