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Environ. Sci. Technol., **2008**, 42 (24), 9424-9430 • Publication Date (Web): 14 November 2008

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Quantum Dot Weathering Results in Microbial Toxicity

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Received August 21, 2008. Revised manuscript received
October 17, 2008. Accepted October 21, 2008.

Quantum dots (QDs) are increasingly being used for electronics, solar energy generation, and medical imaging applications. Most QDs consist of a heavy metal core/shell coated with amphiphilic organics that stabilize the nanoparticles and allow conjugation with biological molecules. In this study, QDs were evaluated for their effects on bacterial pure cultures, which serve as models of cell toxicity and indicators of potential impact to ecosystem health. QDs with intact surface coatings decreased growth rates of Gram positive *Bacillus subtilis* and Gram negative *Escherichia coli* but were not bactericidal. In contrast, weathering of various types of QDs under acidic (pH \leq 4) or alkaline (pH \geq 10) conditions significantly increased bactericidal activity due to the rapid (<1 min) release of cadmium and selenite ions following QD destabilization upon loss of the organic coating. Toxicity was mitigated by humic acids, proteins, and other organic ligands that reduced metal bioavailability. The best available science, which is limited, suggests that QDs are potentially safe materials when used in their intended applications at near-neutral pH. These results forewarn us that even moderately acidic or alkaline conditions could lead to significant and localized organism effects due to toxic exposure to dissolved heavy metals. Thus, biocompatibility and ecotoxicity tests for QDs should consider *in vivo* and/or *in situ* transformations to fully characterize the potential risks to environmental health.

Introduction

Quantum dots (QDs) are semiconductor nanocrystals that hold great promise for diverse technologies including biomedical imaging, targeted gene and drug delivery, solid state lighting, and solar cells (1–5). They are available in various sizes and compositions, and typically consist of a CdSe, CdTe, ZnSe, or PbSe core surrounded by a zinc or cadmium sulfide shell (6). These highly uniform QDs are initially formed at high temperatures in nonpolar solvents; they are stable in these solutions because of the association of fatty acids at their surface. To create water soluble materials suitable for various applications, the hydrophobic QDs are encapsulated with amphiphilic polymers that permit their transfer into relatively concentrated aqueous suspensions (7–10). Much of the technological success of QDs depends

on the ability of these organic coatings to protect the inorganic core of the material from the solution environment.

QD surface coatings should also be central for characterizing the environmental impacts of these substances in various use and disposal scenarios. Weathering, defined here as degradation of the core/shell material, will be a significant outcome if the integrity of the surface is compromised. Some of the chemical conditions encountered during extended use of these materials could lead to such degradation. For example, QDs may be exposed to acidic pH in the stomach (pH 2) during *in vivo* imaging, in landfill leachates, or while incorporated into solar cells if applied in regions affected by acid rain and pollution. After disposal, QD weathering will depend on product packaging and site-specific environmental conditions. Solid waste disposal sites, for example, are a likely end point for many of the proposed QD products and may be sites for QD release scenarios into local watersheds. The environmental fate of QDs may be similar to that of Ni–Cd batteries. While consumers do not encounter the effects of their toxic components, once the batteries enter a landfill, their casings eventually degrade causing heavy metals and other toxins to leach into the environment (11). QD surface coatings have been engineered to protect the material against degradation on relatively short and well-defined use conditions. Whether these same coatings will significantly prevent the release of toxic components of these materials over a wide range of environmental conditions is an outstanding question.

To probe this question, this work evaluates artificial weathering of QDs and the consequences of these processes for bacterial growth. Microorganisms are at the foundation of all ecosystems and play key roles in primary productivity and global biogeochemical cycles. Consequently, understanding their interactions with engineered nanomaterials such as QDs is important to enable their sustainable use in medical, electronic, and environmental applications.

QD-microbial interactions were considered by Kloepfer and co-workers, who reported tunable uptake of transferrin-conjugated, mercaptoacetic acid (MAA)-coated QD into specific strains of bacteria and fungi under certain metabolic conditions (12). In another study by Kloepfer et al., QD impacts on microorganisms were linked to purine-assisted transport of adenine- or AMP conjugated, MAA-coated QDs smaller than 5 nm size into *E. coli* and *B. subtilis* (13). The proposed mechanism was enzyme-assisted QD binding with cell walls, followed by photooxidative membrane damage to permeabilize cells and facilitate QD uptake. In that study, intracellular adenine-conjugated QDs produced bacteriostatic but not bactericidal effects (13).

Comparatively more is known about the short-term interactions of water soluble QDs on human cell lines and laboratory test animals, and these data suggest the central role that surface coatings play in acute QD toxicity (14–22). Mammalian studies with QDs that usually contain CdSe cores and organic functional coatings suggest that QD uptake may lead to accumulation of toxic metals in the cells (17–20, 23, 24). Moreover, the surface coating of the QDs has been established as a critical factor for determining the extent and time scale of cytotoxicity (17, 25, 26). Cadmium and selenium can cause oxidative damage (27–30) and are bactericidal at concentrations above 30 mg/L (31, 32). QD coatings were thought to mitigate cadmium release and reduce cytotoxicity (17). While no evidence of acute (<8 h) QD toxicity at doses <10 mg/L was found with human, pig, or mice cells (16, 18, 21, 22), long-term (~days) QD toxicity was reported at >1 g/L for frog and rat cells (7, 14, 20, 33). Thus, the current literature

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on QD toxicity reports wide ranges of QDs, their doses, exposure conditions, target organisms or cells, and toxic effects. In light of the observations from mammalian systems, the distinction between toxicity caused by the QD constituents or by the intact particles is critical. However, none of the published studies have measured the release of components from QDs and categorically attributed their role in causing toxicity to bacteria.

In this study, we address QD weathering and its potential impact to bacteria. We show that the release of toxic constituents during QD weathering instead of QD uptake causes microbial toxicity and that organic ligands that reduce metal bioavailability mitigate toxicity. Release of core constituents and total organic carbon due to QD weathering were quantified, and the effects of intact and weathered QDs on the growth and survival of model bacteria were investigated. The effects of QDs were compared to those found from exposure to salts of cadmium and selenite, which was determined to be the dominant form of released selenium.

Materials and Methods

QD Preparation, Surface Modification, and Characterization. Three distinct types of QDs were used in this work to establish the generality of the findings as well as probe how coating composition influences weathering. We used QDs provided by a major commercial supplier (Invitrogen Inc., Eugene, OR) as well as more custom designed materials formed via comparable routes. The purchased QDs were 655 ITK-carboxyl systems (QD655, with CdSe/ZnS ellipsoid core 6×12 nm; 18 nm with coating) referred to herein as QD655-carboxyl. These materials were shipped as $8 \mu\text{M}$ QD solutions in 50 mM borate buffer, and after dilution the solution pH was approximately 7.1 with QD concentration of 80 nM. Since Invitrogen does not disclose the detailed composition of its proprietary surface coating, we also produced our own QDs so as to accurately define the surface character and provide an avenue to tune its core/shell/coating composition. These custom-made materials were produced via established methods and intentionally produced without a ZnS or CdS shell (34). The size of custom-made CdSe cores was 3.99 ± 0.40 nm. Surface modification was accomplished using two polymeric materials: polyanionic polymaleic anhydride-alt-1-octadecene (PMAO, $M_n=30,000-50,000$), referred to as QD557-PMAO, and polycationic polyethylenimine (PEI, $M_n=10,000$), referred to as QD559-PEI henceforth. QD concentrations, estimated using UV-visible absorption peak and extinction coefficient based on Beer's Law, were $18.3 \mu\text{M}$ and $15.2 \mu\text{M}$ for QD557-PMAO and QD559-PEI, respectively. Supernatant concentrations of Cd and Se in QD preparations were measured by ICP-OES analyses after serial dilution and filtration with $0.02 \mu\text{m}$ syringe filters, followed by ultracentrifugation at 30,000 rpm for 2–5 h.

Bacterial Strains. *Escherichia coli* K-12 (ATCC 25404), *Bacillus subtilis* 168 (ATCC 31578), and *Pseudomonas aeruginosa* (NCTC 50076) were used in this study. All strains were grown overnight in Luria–Bertani broth at 37°C to high cell densities, centrifuged, and resuspended in modified Davis (MD) medium (0.7 g/L K_2HPO_4 , 0.2 g/L KH_2PO_4 , 1 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L Na-citrate, 0.1 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), with 1 g/L glucose. Assays with Gram positive *B. subtilis* versus Gram negative *E. coli* were used to test the hypothesis that the thicker cell wall of the former offered added protection against the bactericidal effect of QDs. *P. aeruginosa* harboring metal resistance *czc* genes was tested for its extent of resisting QD-derived cadmium toxicity (35).

Experimental Design. QDs were artificially weathered in the laboratory by exposure to acidic or alkaline pH in the range of 2 to 12 for 30 min, and changes in particle size, suspension stability in water, and supernatant concentrations of QD constituents were measured. Weathering experiments

were carried out at room temperature ($\sim 23^\circ\text{C}$) under ambient lighting using unstirred QD suspensions (80 nM in 2.5 mM borate buffer). After exposure to various pH conditions, all QD samples were carefully neutralized by adding 1 N HNO_3 or 1 N NaOH , as needed, and incubated with bacterial strains at 37°C . For dose–response analyses, the samples were serially diluted before incubating with bacteria. Bacterial cultures not exposed to QDs were used as controls. Experiments were carried out at QD-derived Cd and Se concentrations ranging from 10 mg/L to 1 g/L and assay-appropriate cell culture densities. Each QD material was tested with its coating intact as well as after being degraded by exposure to various pH conditions. The effects of QDs on bacterial growth were determined by spectrophotometric analysis (absorbance at 600 nm corrected for QD background) and viable plate counts. To discern cell death from lack of replication, the ability of the bacteria to grow after 48-h QD exposure was measured by diluting and plating the cells. In parallel experiments, bacterial toxicity was also evaluated for bulk cadmium salts ($\text{Cd}(\text{NO}_3)_2$ and CdSO_4) and selenium compounds (Na_2SeO_4 and SeO_2) to compare growth responses due to aqueous ions and those derived from QDs. Twenty-four different combinations of cadmium, selenite, and selenate species spanning a range of three log scales were tested.

Analytical Methods. Cd, Se, and Zn concentrations were quantified using a Perkin-Elmer (Norwalk, CT) Optima 4300 DV Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES).

Nonpurgeable organic carbon (NPOC) was measured using a total organic carbon analyzer (ASI-V, Shimadzu Scientific Instruments, Japan).

Selenite and selenate were analyzed by Dionex DX-600 ion chromatograph equipped with a suppressed anion conductivity detector and Dionex IonPac AS20 column (35 mM KOH eluent, 1.0 mL/min flow rate, 25 μL sample injection). The detection limits for selenite and selenate were 3 mg/L and 0.1 mg/L, respectively.

Small angle X-ray scattering (SAXS) analysis was performed to analyze the particle size distribution using a Rigaku SmartLab X-ray Diffractometer (The Woodlands, TX).

Particle sizes were also determined using a noninvasive backscatter (NIBS) instrument (Zetasizer Nano, Malvern Instruments, U.K.).

Results and Discussion

Coated QDs. At neutral pH, QDs coatings were expected to be intact. However, some free metals were present. Baseline levels of non-QD associated cadmium and selenium at pH near 7 were 14–30 mg/L total Cd and 5–23 mg/L Se as measured by ICP-OES. Zinc concentrations ranged from nondetectable for the materials made in our laboratory to 5 mg/L for QD655-carboxyl. NPOC ranged from 9.5 to 21.5 mg/L. QD655-carboxyl core sizes, as measured by SAXS analyses, were 6.07 ± 0.99 nm (aspect ratio 2.50).

Weathering of QDs Due to pH Variation. To simulate a range of possible weathering conditions, QDs were exposed in the laboratory to conditions ranging from pH 2 to 12. Several different processes could be initiated under extreme acidic or alkaline conditions, including core/shell leaching, QD aggregation, and precipitation of metal oxides. Low pH was expected to readily solubilize core/shell metals, while high pH could be relevant for Cd or Zn speciation, precipitation, and bioavailability. Polymer coatings themselves are unlikely to be stable at very acidic (pH < 3) or very basic conditions (pH > 10) due to hydrolysis of the ether bonds (PMAO) or decrease in the number of amine linkages (PEI). Additionally, both coatings have residual functional groups that are both basic (amines) and acidic (carboxylic acids); once these sites are neutralized, the contribution of charge

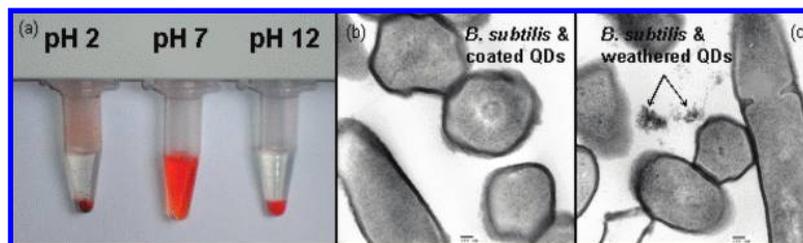


FIGURE 1. Effect of pH on stability and uptake of QDs. (a) Water-stable QD557-PMAO QDs at pH 7 and large, hydrophobic aggregates at pH 2 and 12 (b) and (c) no morphological changes were observed in *Bacillus subtilis* exposed to QD655 carboxyl coated and weathered QDs. Individual coated QDs could not be seen at 75000 \times but clusters of weathered QDs were visible.

TABLE 1. Supernatant Concentrations of QD Constituents Measured at Various pH Values^a

QD	pH treatment	total Cd (mg/L)	total Se (mg/L)	NPOC (mg/L)
QD557-PMAO	coated (pH 7)	29.2 \pm 5.3	23 \pm 3.8	9.5 \pm 0.4
	weathered (pH 2)	2853 \pm 93.3	2760 \pm 129	119.4 \pm 6.2
	weathered (pH 12)	1511 \pm 97.6	1617 \pm 94.5	230.2 \pm 14.4
QD559-PEI	coated (pH 7)	28.0 \pm 7.3	21.5 \pm 5.6	21.5 \pm 3
	weathered (pH 2)	3362 \pm 207.4	3029 \pm 42.5	261.8 \pm 8.8
	weathered (pH 12)	3123 \pm 101.9	2819 \pm 103.8	171.2 \pm 15.6
QD655-carboxyl	coated (pH 7)	14.9 \pm 1.2	5.3 \pm 0.8	nd
	weathered (pH 2)	3528 \pm 74.5	934 \pm 106.7	611.2 \pm 13.5
	weathered (pH 12)	3729 \pm 99.0	1052 \pm 88.3	751.8 \pm 49.0

^a An increase in cadmium and selenium ions and nonpurgeable organic carbon indicated weathering of organic coating and core metals. For QD655-carboxyl, zinc was nondetectable at pH 7, while Zn concentrations released at other pH values ranged from 20.9 \pm 0.6 to 157.4 \pm 8.3 mg/L. The metals were analyzed by ICP-OES, and NPOC was measured by total organic carbon analyzer. Values represent the average \pm the range of 3 observations. nd, not detected.

to the particle stabilization will be lost and could lead to aggregation.

Upon exposure to acidic or alkaline conditions, monodisperse QD557-PMAOs immediately flocculated and settled out of solution (Figure 1a). QD aggregation was visually observed at pH values lower than pH 6 or higher than pH 8. Measured zeta potential values at pH 7 indicated stable dispersion of coated QDs (-56.7 ± 7.1 mV for QD655-carboxyl, -29.4 ± 7.3 mV for QD557-PMAO, and 89.5 ± 5.5 mV for QD559-PEI) (Table S1). At low pH, the negative charges of anionic QDs (PMAO and carboxyl) were neutralized, and particle-particle interactions resulted in precipitation of aggregates. The positive surface charges of PEI-coated QDs decreased in magnitude with increasing pH, resulting in unstable suspensions. While the size of intact QD655-carboxyls was nearly 6 nm, the sizes of the weathered aggregates at pH 2 and 10 were measured by SAXS as 238 ± 162 nm and 63 ± 32 nm, respectively. The hydrodynamic diameters measured for particle aggregates at pH 2, 4, 10, and 12 were in the 0.1–60 μ m range (Table S2).

Release of QD Constituents. Along with QD aggregation and sedimentation, at pH conditions away from neutral, high concentrations of total Cd, Se, and NPOC were rapidly (<1 min) released into the supernatant (Table 1). It was not clear whether QD weathering under acidic or basic conditions was initiated by dissociation of polymer coating or by dissolution of QD cores. However, regardless of the initial destabilizing mechanisms, rapid release of toxic elements ensued. Low pH conditions resulted in the highest dissolved concentration of cadmium and selenium. Chemical equilibrium modeling indicated that free Cd²⁺ ions were the most abundant form of cadmium at pH < 6 , whereas dissolved cadmium phosphate or hydroxide species were dominant at pH > 6 (Figure S1). Selenium released from weathered cores, as measured by ion chromatography at pH 7, was 99% selenite (SeO₃²⁻) and 1% selenate (SeO₄²⁻). At pH 10 and 12, the dissolved concentrations remained high, and no precipitation of metal salts was observed.

Table 1 also indicates that concurrent with the release of the QD constituents is an increase in the NPOC concentrations. This is consistent with our interpretation that the artificial weathering used here led to removal of the organic coating of QDs. The total dissolved concentrations of Cd and Se from all three weathered QDs were approximately 100-fold higher than those in intact QDs (Table 1). In general, larger amounts of byproduct were found at pH 2 than at pH 12. One sample, the QD655-carboxyl, was formed with a ZnS shell surrounding CdSe cores, and this shell did not impede the release of cadmium and selenite. Furthermore, the increase in dissolved QD constituent concentrations was not significant even after 7-day incubation, suggesting that the equilibrium between particles and dissolved species was reached quickly (Figure S2). QDs coated with PMAO or PEI contained Cd and Se in equal ratio, while the Cd:Se ratio in QD655-carboxyl was 4:1 (36). The ratios of measured Cd and Se concentrations were close to expected ratios of elemental composition of these QDs (Table 1).

An interesting finding in Table 1 is that the presence or absence of a ZnS shell as well as the details of the QD surface coatings had only a minor effect on measured cadmium concentrations after weathering. The three samples had approximately comparable molar concentrations of QDs, and the resulting weathering yielded similar cadmium levels. These various barriers are not effective at protecting the QD cores even at relatively gentle pH values. The data also illustrate that the PMAO materials may provide some benefit in terms of minimizing cadmium release; the overall weathered concentrations of free cadmium are 30% lower than the comparative PEI sample. This may be due to the fact that these samples have a much thicker layer of organic coatings. The mechanism of PMAO attachment to the core/shell utilizes its amphiphilic properties, so that a hydrophobic end penetrates the hydrophobic coating (TOPO/TOP) on the CdSe surface and a hydrophilic block imparts water solubility. Overall hydrodynamic diameters for these systems range from 30 to 40 nm. In contrast, PEI sample was produced via ligand-

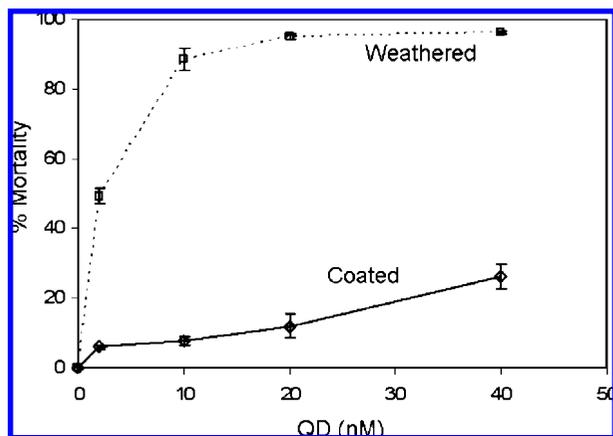


FIGURE 2. Dose–response of *Bacillus subtilis* exposed to QD655-coxyl coated and weathered QDs. Percent cell mortality was calculated after 48 h with respect to unexposed bacteria. Weathered QDs were bactericidal at concentrations higher than 20 nM, while QDs with intact surface coatings were significantly less toxic. The error bars represent the range of duplicate measurements.

exchange. The TOPO/TOP molecules, which constituted an additional layer in PMAO-coated QDs, were replaced by molecules containing thiol or amine groups. This led to a smaller hydrodynamic diameter and, consequently, a more unstable barrier against dissolution at acidic and alkaline conditions.

Toxicity of QDs to Bacteria. Dose–response analysis at various QD concentrations found that weathered QDs had much greater effects on bacteria than similar systems with intact coatings. Percent cell mortality was calculated after 48 h with respect to unexposed bacteria. Representative data for *B. subtilis* and QD655-coxyl are shown in Figure 2. Weathered QDs killed nearly 100% bacteria at QD concentrations above 20 nM, while coated QDs were significantly less toxic. Gram positive bacterium, *B. subtilis*, exhibited slightly lower growth rate and yield in the presence of coated QDs as compared to unexposed controls. QDs with intact surface coatings induced longer lag periods in Gram negative *E. coli* and *P. aeruginosa*. However, there was no statistically significant decrease in the growth yields ($p > 0.05$), suggesting a bacteriostatic rather than bactericidal effect that was more pronounced for *E. coli* than *P. aeruginosa* (Figure 3).

No growth of *B. subtilis* and *E. coli* was observed in the presence of weathered QDs. In contrast, metal-resistant *P. aeruginosa* could tolerate weathered QDs but the growth rates and yields were lower, and lag phase was further elongated. The extent of growth inhibition was in strong agreement with the measured total Cd and Se concentrations associated with coated QDs. *P. aeruginosa*, which contains a cation-antiporter Cd and Zn efflux pump (35), was able to tolerate Cd and Se concentrations up to 150 mg/L (Figure 3). Thus, this strain was able to grow in the presence of weathered QDs too. Weathered QDs releasing higher than 40 mg/L Cd were bactericidal to *E. coli* and *B. subtilis* (Figure 4).

The bacterial growth inhibition and mortality found with QDs, and its association with weathering conditions, strongly suggests that molecular or ionic forms of Cd, Se, and Zn may be responsible for the effects on bacteria. In effect, QDs can act as agents that deliver toxic metals and other elements to bacteria. To evaluate whether the constituents once released are in a form as lethal as inorganic salts, QD observations were benchmarked against exposure to dissolved Cd and Se compounds.

The mortality of bacteria in the presence of equivalent concentrations of individual Cd and Se salts was actually

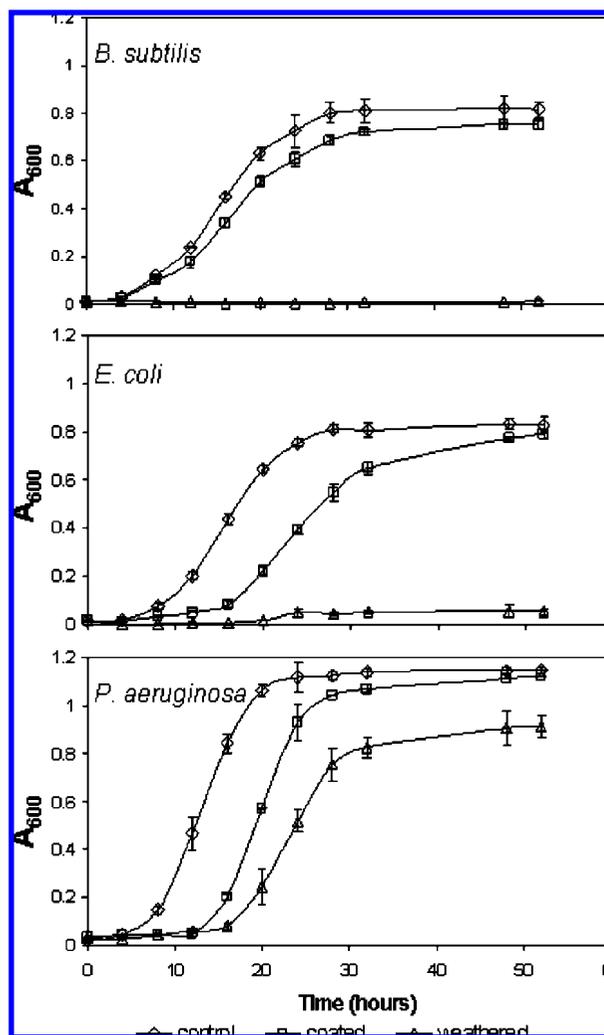


FIGURE 3. Growth of bacteria in the presence of PMAO-coated and weathered QD557s. Gram positive *Bacillus subtilis* exhibited slightly lower growth rate and yield in the presence of coated QDs. Gram negative *Escherichia coli* and *Pseudomonas aeruginosa* had elongated lag phase, but growth rate and yield were not affected. *B. subtilis* and *E. coli* were unable to grow in the presence of weathered QDs. Heavy metal resistant *P. aeruginosa* tolerated weathered QDs but the growth rates and yields were lower, and lag phase was further elongated. Cell growth was measured spectrophotometrically as absorbance at 600 nm. The error bars represent the range of measurements ($n = 3$).

lower than that found in the presence of QD-derived Cd and Se (Figure 4). Cadmium added as a salt, either CdSO_4 or $\text{Cd}(\text{NO}_3)_2$, was not sufficient to explain bacterial toxicity of weathered QDs. Sodium selenate added together with $\text{Cd}(\text{NO}_3)_2$ did not significantly increase mortality due to $\text{Cd}(\text{NO}_3)_2$ alone. Only by including selenite (added as aqueous SeO_2) with the cadmium could the bactericidal effects of equivalent amounts of QDs be obtained (Figure 4). Selenite is reported to be more toxic than selenate (37–40). This suggests that both cadmium and selenite ions participate in mechanisms that promote bacterial mortality. Although the release of selenite from MAA coated CdSe QDs has been previously suggested (14), we experimentally confirmed in our study that it was indeed the dominant form of selenium released and report it for the first time. These data illustrate the importance of both the metallic and nonmetallic constituents of QDs in determining their toxicity toward bacteria.

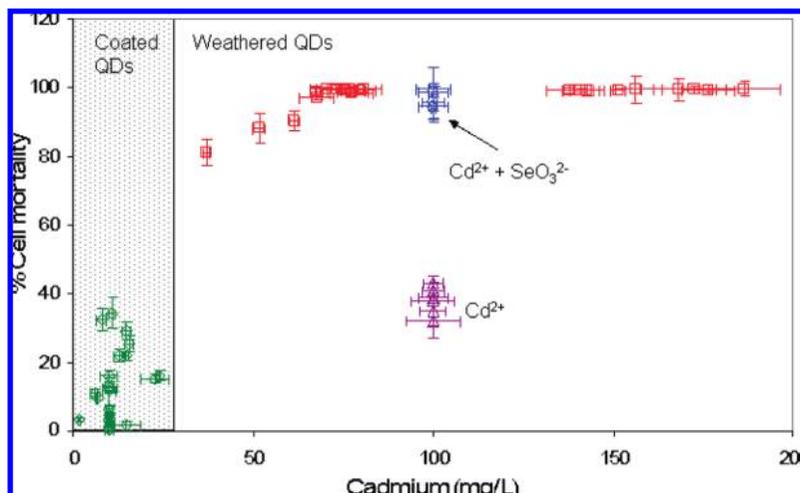


FIGURE 4. Core constituents released from QDs due to weathering are toxic to bacteria. For simplicity, only Cd is shown on the x-axis. Although toxic effects could not be explained by Cd²⁺ ions alone (see 40% mortality for 100 mg/L Cd(NO₃)₂ salt versus 100% mortality for 100 mg/L total Cd and Se from weathered QDs or 100 mg/L Cd(NO₃)₂ salt plus 100 mg/L SeO₂. The error bars represent the range of measurements (*n* = 3).

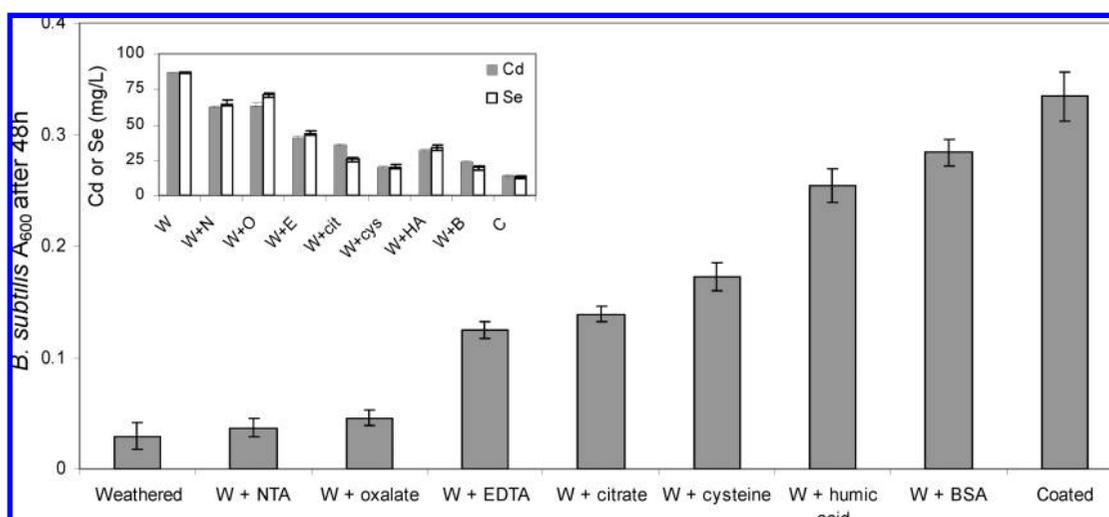


FIGURE 5. Bacterial toxicity due to weathered QDs is alleviated by organic ligands. Compounds that chelate metal ions or coat particles reduce the bioavailable Cd and Se concentrations released from weathered QD559-PEIs and consequently enhance growth. Large organic molecules were most efficient in diminishing toxicity. Tetra- and hexadentate ligands significantly decreased Cd and Se concentrations released from weathered QDs and subsequently increased *B. subtilis* growth. Bi- and tridentate ligands removed about 30% Cd from the supernatant, but the residual concentrations were still too high to allow bacterial growth. The errors indicate the range of measurements (*n* = 3).

The data in Figure 4 suggest that the bactericidal effects of QDs can be accounted for by the biological effects of their constituent inorganic components. Organic coatings in this case are unlikely to contribute to the observed data. Some QD coatings such as MUA and cysteamine have been noted to cause some biological impacts in mammalian systems (14, 25). While the PMAO coating used in this study contains carboxylic acid groups like MUA, it has a higher molecular weight (30,000–50,000) than 218 of MUA. PEI and its derivatives were previously determined to be noncytotoxic and successfully applied as gene-delivery materials (41–43). We tested that PMAO and PEI exposed to various pH values used in this study did not affect the growth of bacteria (Table S3), and the bactericidal effects of weathered QDs could be primarily attributed to Cd and Se released from the QD cores. Similarly, residual solvents, such as TOP/TOPO and chloroform, could be present at trace levels in spite of the repeated sedimentation and resuspension purification procedures. These molecules were not toxic at the relevant concentrations tested.

In eukaryotic cells, toxicity caused by QDs is related to their uptake into the cells (22, 23, 26). QD uptake into bacteria, which are smaller than eukaryotic cells, has been previously reported (44). In that work, cytotoxicity toward *E. coli* and *B. subtilis* was observed and was correlated to intracellular QD concentrations. This is in contrast to our results where the observed bactericidal effect, which was correlated with the release of QD core constituents (Figure 4), was independent from whether bacteria took up the QDs. We did not observe uptake of coated QDs (4–6 nm) or weathered QD aggregates (>200 nm) into *B. subtilis* cells (Figure 1b,c).

Effect of Metal-Binding Substances. Weathered QDs were exposed to a range of concentrations of Aldrich humic acids, proteins (bovine serum albumin, BSA), cysteine, and other environmentally relevant substances. On one hand, these compounds could accelerate the weathering rate by chelating dissolved cadmium and zinc and increase the driving force for dissolution. However, given the high surface area of the QDs and their active surface chemistry we anticipated that

these additives could protect QDs from weathering by blocking surface sites from etching and dissolution processes.

QD toxicity was generally reduced in the presence of these organic molecules and metal ion chelators. PEI coated QD559s were weathered by exposure to pH 2, neutralized, exposed to the additives listed above for 4 h, and then incubated overnight with bacteria. We observed that amounts as low as 5 mg/L (1 mM) humic acid and 1 mg/L (15 nM) BSA significantly decreased the Cd and Se released from these QDs (Figure 5 inset and Figure S2). Similarly, 20 mM metal-chelating ligands, such as oxalate (bidentate), nitrilotriacetic acid (NTA, tridentate), ethylenediamine tetraacetic acid (EDTA, tetradentate), and citrate (hexadentate), reduced dissolved concentrations of Cd and Se. Cysteine, which forms metal sulfides and other sulfur-containing species, also decreased bioavailable Cd and Se species from the supernatants (Figure 5 inset). Consequently, bacteria incubated with weathered QDs in the presence of the above substances received much lower doses of Cd and Se. Although bi- and tridentate ligands removed nearly 30% Cd and 20% Se from the supernatant, bacterial growth was not significant, possibly because high dissolved Cd and SeO_3^{2-} concentrations still remained in the supernatant. Tetra- and hexadentate ligands significantly lowered weathered QD-derived Cd and Se concentrations and subsequently enhanced bacterial growth. Large organic molecules (e.g., BSA) were most efficient in reducing toxicity. Due to reduced availability of toxic ions, 48-h bacterial growth was comparable to coated QD and unexposed controls (Figure 5).

This is the first report of QD weathering and release of toxic core components following the degradation of surface coatings after exposure to moderate acidic and alkaline conditions. Previous studies have reported metal release from QDs by photooxidation (14) or degradation inside the cells (17). In our study, microbial toxicity increased as a consequence of QD weathering. The likely mechanism of cytotoxicity was oxidative stress exerted by dissolved cadmium and selenium compounds (15, 45–47). The role of metal toxicity was further confirmed by significant decrease in toxicity in the presence of metal ion-chelating compounds. The relative biological effect of cadmium in a QD form subjected to weathering was greater than that of cadmium provided in a soluble salt; this discrepancy could largely be accounted for by the biological activity of selenite.

Overall, this research suggests that QDs may be safely used in a variety of applications at circum neutral pH. However, the release of toxic inorganic constituents during their weathering under acidic or alkaline conditions in the human body or the environment may cause unintended harm that might be difficult to predict with short-term toxicity tests. Thus, long-term toxicity and biocompatibility tests that include QD transformations under various environmental conditions are essential for safe use in their intended applications without compromising risks to public or environmental health.

Acknowledgments

The authors thank Dong Li, Arjun Prakash, Alison Harris, and Xuejiao Tang for assistance with analytical methods. This research was supported by Center for Biological and Environmental Nanotechnology (NSF Award EEC-0647452) and the EPA STAR Grant R833858.

Supporting Information Available

Detailed descriptions of QD preparation and analytical methods, measurements of particle size and zeta potentials of coated QDs and weathered aggregates, toxicity assessment of QD coatings, equilibrium modeling of cadmium ions, and evidence of rapid QD weathering. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ES8023385