Defense Mechanisms of *Pseudomonas aeruginosa* PAO1 against Quantum Dots and Their Released Heavy Metals

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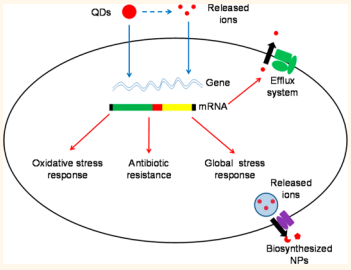
ABSTRACT The growing use of quantum dots (QDs) in numerous applications increases the possibility of their release to the environment. Bacteria provide critical ecosystem services, and understanding their response to QDs is important to assess the potential environmental impacts of such releases. Here, we analyze the microbial response to sublethal exposure to commercial QDs, and investigate potential defense and adaptation mechanisms in the model bacterium *Pseudomonas aeruginosa* PAO1. Both intact and weathered QDs, as well as dissolved metal constituents, up-regulated *czcABC* metal efflux transporters. Weathered QDs also induced superoxide dismutase gene *sodM*, which likely served as a defense against oxidative stress. Interestingly, QDs also induced antibiotic resistance (ABR) genes and increased antibiotic minimum inhibitory concentrations by 50 to 100%, which suggests up-regulation of global stress defense mechanisms. Extracellular synthesis of nanoparticles (NPs) was observed after exposure to dissolved $Cd(NO_3)_2$ and SeO_2. With extended X-ray absorption fine structure (EXAFS), we discerned biogenic NPs such as CdO, CdS, CdSe, and selenium sulfides. These results show that bacteria can mitigate QD toxicity by turning on energy-dependent heavy-metal ion efflux systems and by mediating the precipitation of dissolved metal ions as less toxic and less bioavailable insoluble NPs.



KEYWORDS: quantum dots · nanoparticles · gene expression · extracellular nanoparticle biosynthesis · extended X-ray absorption fine structure · *Pseudomonas aeruginosa*

The growing production and use of manufactured nanomaterials (MNMs) increases the potential for their release to the environment. Several studies have broadly addressed the mechanisms by which some MNMs can harm bacteria and their potential ecosystem services.[1−4] However, little is known about the bacterial response at the molecular level and the associated adaptation and defense mechanisms.

Quantum dots (QDs) are semiconductor nanocrystals that consist of a metalloid crystalline core, an encapsulating shell, and an organic coating for enhanced stability and biocompatibility.[5] QDs display unique optical properties, such as wide absorption and narrow emission spectra, and high extinction coefficients,[6] while their uniform sizes and strong stable fluorescence make QDs easy to track and detect in various matrices.[7] These properties, coupled with QDs ability to conjugate many biomolecules, offer biologists and physicists new capabilities in the fields of bioimaging, solar cells and drug delivery.[8−11]

Weathering, defined as degradation of the QD organic coating and core/shell components, could jeopardize the integrity of QDs. Our previous studies have demonstrated weathering of QDs and the release of QD components under different pH conditions.[10] In particular, we found that at pH 6 or lower, free Cd^{2+} ions are the most abundant cadmium species released from weathered QDs, while at a higher pH the most dominant species are dissolved cadmium phosphate or hydroxide. Selenite is the most abundant selenium species (99%), and a small amount of selenate was detected. At alkaline pH, the dissolved metal concentrations remained high, with no precipitation occurring. Therefore, weathered QDs could result in release of toxic heavy metal components, particularly under acidic, basic, or high salinity conditions,[10,12,13] which poses a risk to environmental health.[5,14−18] Thus, QDs are both convenient and relevant nanoparticles to study how bacteria respond to potentially toxic MNMs.

Gram-negative *Pseudomonas aeruginosa* PAO1, one of the most studied indigenous bacteria, is a heavy metal-tolerant strain.[19] Previous studies have shown that *P. aeruginosa* has a greater capacity to resist weathered

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QDs than *Escherichia coli* and *Bacillus subtilis*,[10] making it an appropriate model to investigate bacterial adaptation and defense mechanisms against QD toxicity.

This study considers the cellular and molecular response of *P. aeruginosa* PAO1 exposed to intact QDs, weathered QDs, and dissolved heavy metal salts (cadmium and selenium). Low (sublethal) concentrations were used because MNMs are expected to be dispersed at relatively low concentrations in the environment.[20] Transmission electron microscopy (TEM), energy dispersive spectrometry (EDS), and extended X-ray absorption fine structure (EXAFS) were applied to detect the interaction between heavy metals and PAO1 cells, and quantitative PCR was used to monitor changes in gene expression. Overall, we demonstrate the up-regulation of heavy metal efflux and stress response genes. We also observed extracellular biosynthesis of metallic nanoparticles (NPs) following exposure to dissolved heavy metal salts, which is postulated to be a defense mechanism to decrease metal bioavailability and toxicity.

RESULTS AND DISCUSSION

Transcriptomic Analysis Following Sublethal Exposure to QDs. Two known bacterial defense mechanisms against heavy metal toxicity are increased expression of heavy metal efflux pumps and up-regulation of antioxidant enzymes. However, it is not known how these defense systems respond to heavy metals in MNMs. PAO1 was exposed to sublethal concentrations of either intact QDs, weathered QDs, or an equivalent concentration of Cd and Se (dissolved as $Cd(NO_3)_2$ or SeO_2 salts), and the expression of several transport- and stress-related genes was monitored.

The transcriptional unit *czcABC* (encoding a resistance-nodulation-cell division (RND) divalent metal cation efflux transporter (CzcA), a RND divalent metal cation efflux membrane fusion protein (CzcB) and an outer membrane protein (CzcC)) was induced by all three treatments to varying degrees (Figure 1). Together, the *czcABC* proteins comprise a three-component transporter that spans the entire cell wall, resulting in efflux of heavy metal ions, including cadmium, from both the cytoplasm and the periplasmic space.[21] Intact QDs (which exhibit the lowest release of metals, if any) had the least effect on *czcABC* expression (increased 1.1- to 3.7-fold). Surprisingly, weathered QDs exerted a greater transcriptional response (increased 65- to 404-fold) than Cd and Se salts at similar concentrations (increased 13- to 74-fold).

Cadmium exposure has been shown to promote oxidative stress in bacteria, plants, and animals.[22,23] Therefore, we also evaluated differences in oxidative stress responses to the three treatments by measuring changes in the expression of iron-dependent superoxide dismutase (*sodB*), manganese-dependent superoxide dismutase (*sodM*), and a putative DNA-binding

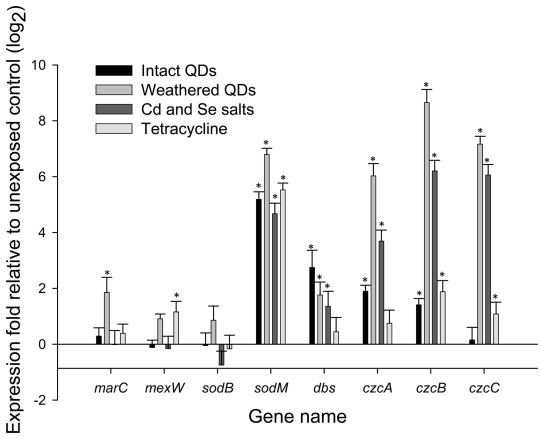

Figure 1. Effect of intact QDs, weathered QDs, heavy metal salts, and tetracycline on gene expression. Asterisk indicates significant up-regulation compared to housekeeping gene *gapA* ($p < 0.05$). Error bars represent ±1 standard deviation from the mean of triplicate measurements.

stress protein (*dbs*). No significant change occurred in *sodB* expression relative to unexposed controls, while *sodM* was highly induced by all treatments (Figure 1), probably due to the lack of iron and availability of manganese in M9-glucose growth medium. This result suggests that the bacteria could have experienced oxidative stress and the induction of *sodM* was a response to repair oxidative damage caused by QD exposure.[24] Similar to that of *czcABC*, *sodM* upregulation was significantly greater in cells exposed to weathered QDs (111-fold) than a similar equivalent concentration of heavy metal salts (26-fold). Up-regulation of DNA binding stress proteins may also suggest that PAO1 required DNA repair, possibly as a result of oxidative stress. While this could indicate QDs can be genotoxic, it could also imply induction of a global stress response as a result of QD exposure.[25]

Intact QDs (<500 nM) did not inhibit microbial growth (Supporting Information, Figure S1) and had a lower transcriptomic effect than weathered QDs (Figure 1) in seven of eight genes assayed. The exception was *dbs,* which was up-regulated 6.7-fold by intact QDs but only 3.4-fold by weathered QDs; however, this difference was not statistically significant. The effect of weathered QDs on efflux pump expression (*czcABC*) was similar to that of Cd and Se salts, but differed greatly from intact QDs, suggesting that released QD components were critical effectors of the observed response. A possible explanation for the higher effect of weathered QDs than the metal salts is that QDs might serve as an efficient delivery vehicle for metal constituents subsequently released as they encountered low localized pH at the cell membrane (caused by proton motive force) or higher salinity if internalized into the cytoplasm. Our previous study demonstrated the attachment of QDs to the *Pseudomonas* specie.[17] On the other hand, released (dissolved) metals within close proximity to the bacteria can be chelated by

many common ligands (*e.g.*, chloride, phosphate, sulfides, and organic matter) to a greater extent than QDs, which reduces their bioavailability and toxicity. On the basis of the broth composition and equilibrium speciation modeling (Supporting Information, Table S4), Cd^{2+} ions were likely associated with an organic carbon source, and Se existed in the form of $HSeO_3^-$ and SeO_3^{2-}. No Cd or Se precipitation was predicted. Accordingly, dissolved metals would experience a higher decrease in bioavailability than NPs[26,27] and exert a lower effect on gene expression.

Since CBA transporters are known to be involved in the export of several classes of organic compounds as well as heavy metal ions, we also monitored *czcABC* expression during exposure to an antibiotic (tetracycline) to determine whether another chemical stressor (which could co-occur with QDs in hospital settings due to their use in medical imaging) could elicit a similar transcriptional response. This experiment also provides insight into whether the observed responses were specific to QDs or were part of a more global stress response. Similar to QD exposure, tetracycline induced *czcB* and *czcC* (2.1- to 3.4-fold) as well as the superoxide dismutase gene *sodM* (46-fold), but not *czcA, dbs* or *sodB* (Figure 1).

The observed transcriptomic response to tetracycline was similar to that for intact QDs or weathered QDs (*i.e.*, oxidative stress protection and metal efflux systems were induced), suggesting that sublethal exposure to one chemical stressor may increase PAO1 adaptation and fitness against the other. Concurrent addition of sublethal concentrations of tetracycline and weathered QDs, however, attenuated the effect of QDs on the expression of *czcABC* and *sodM* (Supporting Information, Figure S2). For instance, expression of *czcC* was up-regulated 144-fold by the weathered QDs, while only 14-fold in the presence of tetracycline (Supporting Information, Figure S2). Tetracycline can inhibit protein biosynthesis,[28] and low levels of cellular enzymes might reduce general gene expression.

To determine whether QDs could also increase expression of antibiotic resistance (ABR) genes, we monitored induction of two chromosomally located multidrug resistance efflux pump genes, *mexW* and *marC,* which have been associated with tetracycline resistance.[29,30] In the *P. aeruginosa* genome, these two genes are located distal from *czcABC*,[19] and their expression, independent of the *czcABC* operon, should be regulated by antibiotics rather than heavy metals or QDs. Accordingly, *mexW* was significantly up-regulated by tetracycline, but not QD exposure (Figure 1). Interestingly, however, weathered QDs significantly induced *marC* (3.6-fold) while tetracycline, intact QDs,

TABLE 1. MIC of Test Antibiotics to *P. aeruginosa* PAO1 Exposed to Intact QDs and Weathered QDs[a]

	MIC (mg/L)		
antibiotics	control	intact QDs	weathered QDs
ampicillin	40	55	60
chloramphenicol	3	6	6
kanamycin	300	300	450
tetracycline	20	26	44

[a] Serial dilutions of antibiotics were prepared and aliquoted into 96-well plates where growth of control and QD-exposed PAO1 was determined using absorption at 600 nm. Six replicates were prepared for each antibiotic concentration.

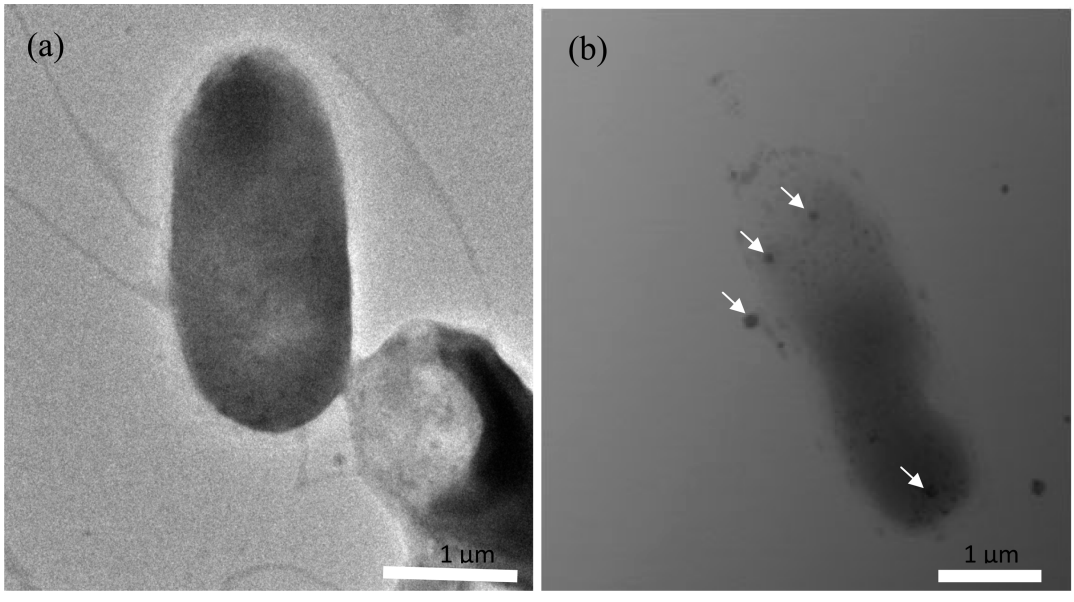

Figure 2. TEM images of strain PAO1 unexposed control (a) and those exposed to dissolved Cd and Se salts (b). Thin-section TEM images of unexposed control and treated samples are shown in the Supporting Information, Figure S3. White arrows point to the biosynthesized NPs. Duplicate samples were used for TEM analysis.

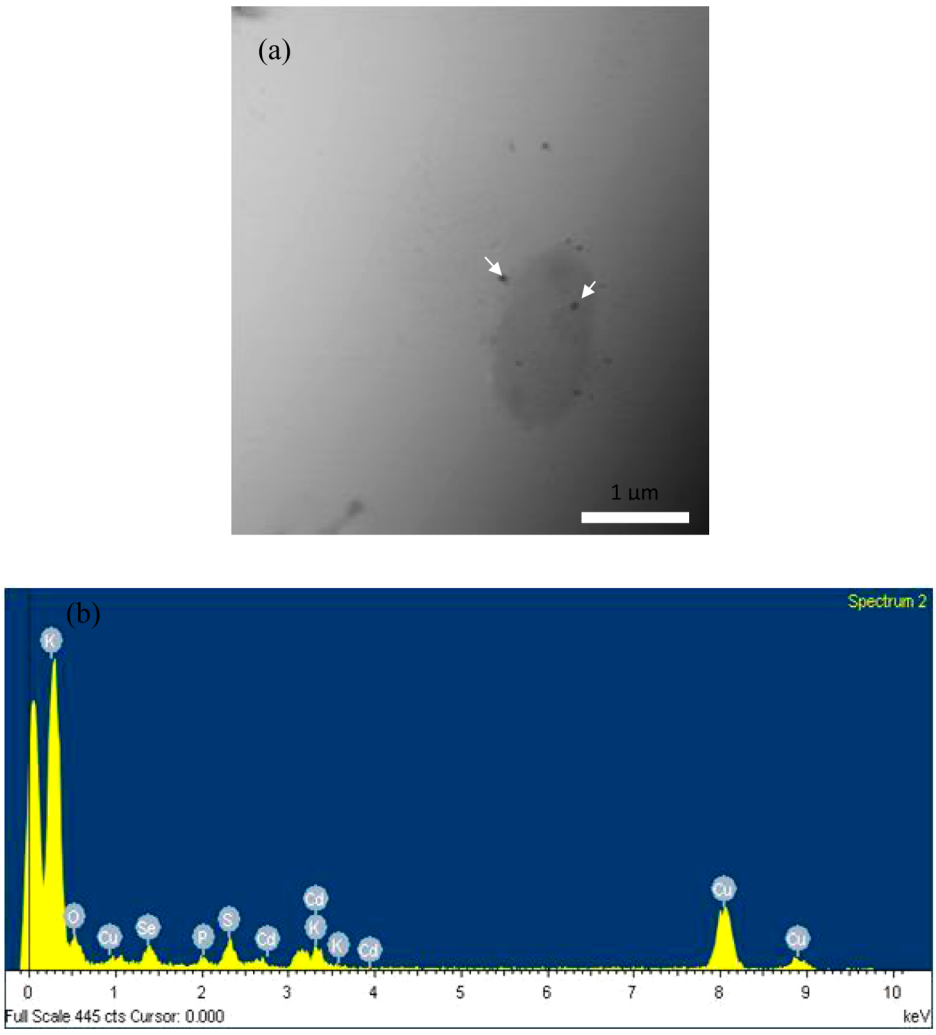

Figure 3. Synthesis of NPs by *P. aeruginosa* PAO1 during exposure to $Cd(NO_3)_2$ and SeO_2 (0.1 mM each). Panel a shows a TEM image of PAO1 and the biogenic NPs, pointed by white arrows. Panel b depicts the EDS spectrum of one of the NPs in the image.

and heavy metal salts did not (1.0- to 1.2-fold). This raises the possibility that weathered QDs could be inducing a global stress response in PAO1 that enhances tolerance not only to heavy metals but also to antibiotics, as discussed above. Induction of a global stress response by metal containing NPs (*i.e.,* Cu) has been previously observed using microarray studies with *Enchytraeus albidus*.[31]

Effect of QD Exposure on Antibiotic Resistance. We exposed PAO1 to weathered or intact QDs prior to inoculation in antibiotic-amended media, and determined whether cell fitness against antibiotics had been increased due to QD exposure. Ampicillin is known to inhibit cell wall synthesis,[32] and the mechanisms of chloramphenicol, kanamycin, and tetracycline involve, either directly or indirectly, inhibition of translation and protein synthesis.[28,33,34] PAO1 was sensitive to chloramphenicol, but exhibited resistance to ampicillin, kanamycin, and tetracycline. Exposing PAO1 to intact QDs enhanced their resistance to ampicillin, chloramphenicol, and tetracycline, and weathered QDs increased the microbial resistance to all four antibiotics, with MIC values increasing significantly by 50% to 100% (Table 1). Furthermore, in all but one case, weathered QDs increased antibiotic MIC to a greater degree than intact QDs. The observed up-regulation of ARGs by weathered QDs could be one reason for the observed increase in antibiotic resistance. Previous work has shown that environmental stressors, such as osmotic stress and pH extremes, are able to increase microbial ABR,[35] and weathered QDs seem to exert a similar effect on ABR as these environmental stressors. Efflux pump inactivation in *P. aeruginosa* is known to increase cell membrane permeability and susceptibility to antibiotics.[36] Conversely, the observed up-regulation of membrane efflux pumps by QDs likely contributed to antibiotic excretion and increased tolerance.[37,38] The enhanced ABR we observed could also be due to changes in microbial membrane composition associated with altered expression of transporters or efflux systems.[39,40] In general, these data support our hypothesis that a global stress response

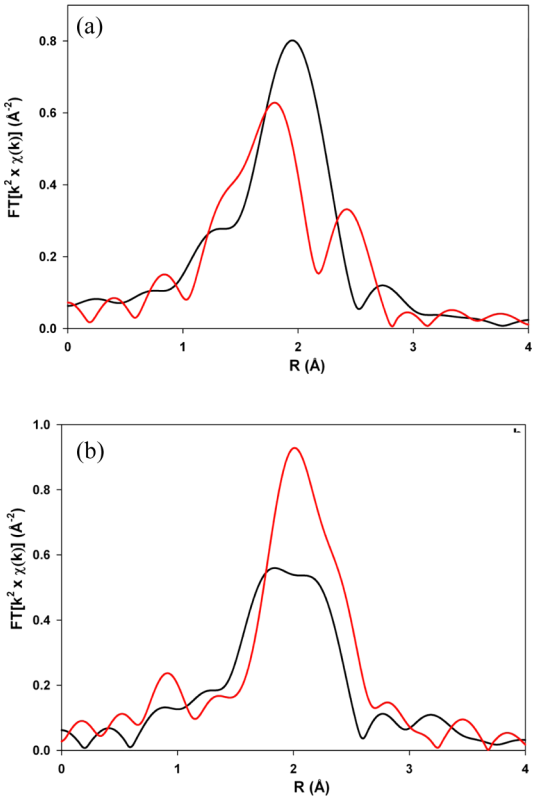

Figure 4. The magnitude of k^2-weighted Fourier transforms (FT) of EXAFS spectra. Panel a shows PAO1 exposed to Cd plus Se (red) and Cd alone (black) at Cd K edge (Δk = 2.5−9.8 Å^{-1} and ΔR = 1.1−3.2 Å), and panel b shows PAO1 exposed to Cd plus Se (red) and Se alone (black) at Se K edge (Δk = 2.54−12.0 Å^{-1} and ΔR = 1.2−3.0 Å).

was induced in PAO1 after QD exposure. Putatively, this could also be part of a hormetic response.[41]

Extracellular Biosynthesis of Metallic NPs. The Cd and Se solution inoculated with PAO1 changed color from white-yellowish to orange-reddish (Supporting Information, Figure S3), likely due to the reduction of Se(IV), while the abiotic control solution did not change color. TEM images showed that NPs (ranging from 30 to 100 nm) were formed only in the PAO1-inoculated solution (Figure 2b). No NPs were observed in similar treatments with dead bacteria (autoclaved 10 min at 121 °C) or without bacteria, proving that these NPs were biogenic. Thin-section TEM analysis did not detect intracellular NPs (Supporting Information, Figure S4a,b), suggesting that they were synthesized extracellularly. Previous studies have shown that extracellular synthesis of Se and CdS NPs by membrane-bound reductive enzymes or soluble secreted enzymes,[42−44] and *P. aeruginosa* has also been able to synthesize silver NPs extracellularly.[45] However, our study demonstrates the possibility that a metal-containing nanoparticle could be dissolved and reformed (as nanoparticle of different composition) in the presence of bacteria.

EDS analysis showed that the biogenic NPs contained Se and Cd (Figure 3b), as well as O, P, S, and K (possibly as bacterial components) and Cu, which is the material of the supporting grid. These NPs exist in mixed phase or nanophase clusters, and they are not large enough to characterize their crystal quality. We postulate that the formation of these NPs is likely a detoxification mechanism to precipitate dissolved metals and thus reduce their bioavailability and toxicity.[46−50]

X-ray Absorption Spectroscopy. EXAFS was used to study the structure of biogenic NPs and confirmed the Cd and Se content in the NPs. The Fourier transforms (FT) of the Cd and Se K edge EXAFS spectra are shown in Figure 4 panels a and b, respectively, for PAO1 suspension exposed to Cd plus Se and PAO1 exposed to Cd or Se alone. By comparing the FT magnitude with the spectra of references CdO, CdS, CdSe, and SeS_2 at Cd or Se edge (as shown in Supporting Information, Figure S6), it is suggested that there are contributions from scatterings of Cd−O, Cd−S, Se−S, and Cd−Se in PAO1 exposed to Cd plus Se, Cd−O, and Cd−S in PAO1 exposed to Cd alone, and Se−S in the PAO1 exposed to Se alone. At the Cd edge, X-ray absorption near edge structure (XANES) of PAO1 samples were compared with reference compounds CdO, CdS, and CdSe, and the linear combination of the PAO1 XANES with reference compound XANES was consistent with the relative contributions suggested by the coordination numbers of Cd−O, Cd−S, and Cd−Se paths in EXAFS data fitting (Table S2). The XANES results indicated that there are ∼37% CdO, ∼19% CdS, and ∼44% CdSe for NPs in PAO1 exposed to Cd plus Se, while for PAO1 exposed to Cd alone, the NPs contain ∼80% CdS and ∼20% CdO. Moreover, we cannot rule out the possibility that some CdO was formed abiotically by heating during the autoclaving process. The attempt to quantify CdSe and SeS_2 in PAO1 exposed to Cd plus Se and Se alone at the Se edge was unsuccessful, probably due to the presence of multiple types of Se sulfide with different phases and crystal structures in our samples.

The EXAFS analysis showed that the biogenic NPs formed during concurrent exposure to dissolved Cd and Se contained CdO, CdS, CdSe, and selenium sulfides. Similarly, NPs formed during exposure to dissolved Cd alone contained significantly more CdS than CdO, and those formed during exposure to dissolved Se alone consisted of dominant selenium sulfides. This indicates that selenium (+IV) dioxide as the source of Se is reduced to selenium (−II) in CdSe by PAO1. Sulfur-containing amino acids, such as cysteine and glutathione, are likely sources of sulfur in the NPs,[51] and enzymes involved in sulfide production (*e.g.*, cysteine desulfhydrase) might participate in this process.[46] EXAFS also confirmed that no NPs were formed in unexposed bacterial suspensions or metal solutions without bacteria, corroborating that these NPs were biosynthesized from dissolved metals that are commonly released from QDs.

CONCLUSION

In a natural environment, NPs may be coated by organic matter, including humic acids, proteins, or polysaccharides. The composition of this biomolecular corona on the surface of NPs plays a key role in the interaction of NPs and bacteria, and could determine the fate and acute toxicity of NPs.[52−54] The cellular response to NPs, including the defense mechanisms, could also be influenced by this nanobio interface.[55,56] However, the corona is unstable and weathering eventually results in exposure of the NP core. Thus, this study considered the molecular response of one model specie of bacteria to weathered QDs in comparison to intact QDs. Overall, this work shows that sublethal exposure to QDs induces several defense mechanisms in PAO1, including energy-dependent heavy-metal ion efflux systems and oxidative stress defense genes. Importantly, these responses were particularly strong in cultures exposed to weathered QDs. ABR and general stress response were also linked to microbial defense against QDs, suggesting induction of a global stress response after sublethal exposure. Detection of extracellular, biogenic Cd and Se NPs also suggests that cell-mediated precipitation of released metals is another adaptation mechanism, although further research is needed to determine what metabolic pathways and enzymes are involved in NP biosynthesis. Further research is also needed to test the hypothesis that the observed enhanced tolerance to antibiotics was related to a general stress response induced by weathered QDs, which increased the overall fitness of PAO1, and to determine whether the observed transcriptomic responses are common among indigenous microorganisms. This is important given the potential diversity of bacterial responses and reciprocal effects following exposure to nanomaterials.[57−59]

MATERIALS AND METHODS

Bacterial Strain and Reagents. *P. aeruginosa* strain PAO1 (ATCC 15692) was purchased from the American Type Culture Collection (Manassas, VA) and was grown at 37 °C overnight in Difco M9 minimal salt broth from BD (Franklin Lakes, NJ) supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.4% glucose (W/V). RNaseOUT, Random primers, dNTP set, and Superscript II reverse transcriptase were supplied by Invitrogen Inc. (Carlsbad, CA). RNAprotect bacteria reagent, QIAquick PCR Purification Kit and RNeasy Mini Kit were obtained from Qiagen Inc. (Valencia, CA). SYBR Green Master Mix was purchased from Applied Biosystems (Carlsbad, CA), and PCR primers were synthesized by Integrated DNA Technologies, Inc. (San Diego, CA). Lysozyme, ethylenediamine−tetraacetic acid (EDTA) buffer, Tris-EDTA (TE) buffer, phosphate buffered saline (PBS), multielement standard solution, carbon powder, acetone, osmium tetroxide, and other chemicals were provided by Sigma-Aldrich (St. Louis, MO). Glutaraldehyde (2.5%) and paraformaldehyde (2.5%) in 0.1 M cacodylate buffer, and Poly/Bed 812 buffer were purchased from Electron Microscope Science (Hatfield, PA) when copper grids were obtained from Ted Pella, Inc. (Redding, CA).

QD Characterization and Preparation. Qdot 655 ITK carboxyl QDs (8 μM in 50 mM borate buffer) were purchased from Invitrogen Inc. (Carlsbad, CA), and their size and zeta-potential are 7.4 ± 1.1 nm (Supporting Information, Figure S5) and −10.2 ± 1.2 mV, respectively, measured by Zetasizer Nano (Malvern Instruments, U.K.). The metal content of carboxyl QDs was 0.87 ± 0.01 mg/L/nM-QD for Cd and 0.26 ± 0.01 mg/L/nM-QD for Zn. No antibacterial activity was observed for QDs concentrations lower than 500 nM. Described as our previous study,[10] weathered QDd were prepared by exposure to acidic conditions (pH = 2) for 30 min, and the QD sample was neutralized with NaOH (1 M). For weathered QDs, the released Cd concentration was 2100.0 ± 100.0 mg/L, 510.2 ± 24.5 mg/L for Zn, and 879.9 ± 21.0 mg/L for Se.

Transcriptomic Analysis. *P. earuginosa* strain PAO1 was exposed to sublethal levels of intact QDs (20 nM), weathered QDs (20 nM), and heavy metal salts (40 mg/L $Cd(NO_3)_2$ and 40 mg/L SeO_2), and microbes were harvested around midlog phase (A_{600} = 0.2−0.5). RNA was extracted using RNeasy Mini Kit according to the manufacturer's protocol, and their concentrations were determined by Nanodrop ND-1000 from Nanodrop products Inc. (Wilmington, NE). cDNA was synthesized overnight at 42 °C by reverse transcription polymerase chain reaction (PCR) using random primers RNaseOUT, dNTPs, and Superscript II reverse transcriptase. Quantitative real-time polymerase chain reaction (q-rt-PCR) was performed in 15 μL of reaction mixture composed of 1 ng of cDNA, SYBR Green Master Mix (7.5 μL), 0.3 μM of each primer and water, to quantify the expression of target chromosomal genes, including metal resistance genes (*czcABC*), tetracycline resistance genes (*argE* and *mexW*), peroxide dismutase genes (*sodB* and *sodM*), and stress gene (*dbs*). Each PCR sample was prepared in triplicate. The $2^{-\Delta\Delta CT}$ method was used to quantify differential gene expression, and the results were analyzed with SDS 1.3.1.[60] Speciation modeling was performed using Visual MINTEQ version 3.0 to determine the likelihood of metal precipitation in M9-glucose broth and identify the chemical species that would prevail in solution at equilibrium. Stockholm Humic Model was used to assess potential organic carbon−metal interactions.[61]

Effect of QD Exposure on Antibiotic Resistance. *P. aeruginosa* strain PAO1 was grown in LB media overnight at 37 °C, and then the bacteria (2 mL of broth) were harvested by centrifugation (5000 rpm for 5 min). After washing three times with 0.85% NaCl, the bacteria were treated with 160 nM of either intact QDs or weathered QDs for 4 h at 37 °C. The control sample (no QDs or metals) was treated similarly. Unstressed control, intact-QD-treated PAO1, and weathered-treated PAO1 were inoculated on a 96-well plate and exposed to serial concentrations of ampicillin, chloramphenicol, kanamycin, and tetracycline to determine their MIC. A total volume of 0.2 mL was used in each well, and at least six repeats were performed for each concentration.

Detection of NP Biosynthesis. The bacterial samples were exposed to 0.1 mM $Cd(NO_3)_2$ and 0.1 mM SeO_2 for approximately 48 h, and the microbial controls (no salts) were treated similarly. The microbes were collected by centrifugation at 5000 ppm for 10 min, and then suspended in water. The samples (10 μL) were added on copper grids and dried overnight. The samples were detected with JEOL 2100 field emission gun transmission electron microscope from Jeol USA, Inc. (Peabody, MA). For thin-section TEM, collected samples were fixed with 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer and then 1% osmium tetroxide in cacodylate buffer, dehydrated in serial acetone buffer (from 30% to 100%), and infiltrated with acetone/Poly/Bed 812 buffer. The samples were cut by MTX ultramicrotome into 70−100 nm thick sections. All the sections were collected on copper grids, and a JEOL 2100 field emission gun transmission electron microscope was used for the EDS−TEM detection.

EXAFS. Strain PAO1 was exposed to 0.1 mM $Cd(NO_3)_2$, 0.1 mM SeO_2, or both for 2−3 days. PAO1 cultures without heavy metal amendment and Cd−Se solution without PAO1 were used as controls and treated similarly. All samples were centrifuged (5000 ppm for 10 min), autoclaved (10 min at 121 °C),

dried (70 °C overnight), and then mixed with carbon powder (0.1 g). The Cd k-edge (26 711 eV) and Se k-edge (12 658 eV) EXAFS measurements were performed at MRCAT 10ID beamline at the Advanced Photon Source, Argonne National Laboratory.[62] Details about the EXAFS experiment and data analysis are available in the Supporting Information.

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: Characterization and toxicity of carboxyl QDs, equilibrium speciation in broth, gene expression under exposure to tetracycline, thin-section TEM and EXAFS. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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