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Remediation of Cadmium Toxicity by Sulfidized Nano-Iron: The Importance of Organic Material

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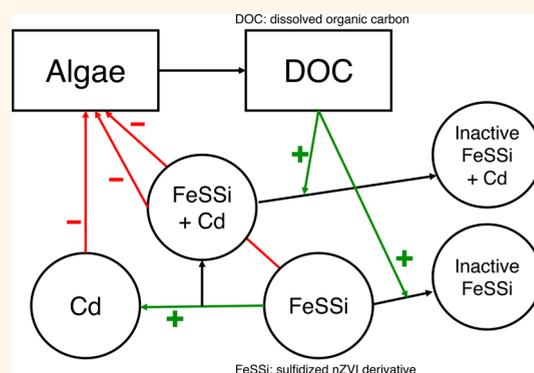
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Supporting Information

ABSTRACT: Nanozerovalent iron (nZVI) is widely used for its ability to remove or degrade environmental contaminants. However, the effect of nZVI-pollutant complexes on organisms has not been tested. We demonstrate the ability of a sulfidized derivative of nZVI (FeSSi) to sorb cadmium (Cd) from aqueous media and alleviate Cd toxicity to a freshwater alga for 32 days. FeSSi particles removed over 80% of the aqueous Cd in the first hour and nearly the same concentration of free Cd remained unbound at the end of the experiment. We found that FeSSi particles with Cd sorbed onto them are an order of magnitude *more* toxic than FeSSi alone. Further, algal-produced organic material facilitates safer remediation of Cd by FeSSi by decreasing the toxicity of FeSSi itself. We developed a dynamic model to predict the maximum Cd concentration FeSSi can remediate without replacing Cd toxicity with its own. FeSSi can remediate four times as much Cd to phytoplankton populations when organic material is present compared to the absence of organic material. We demonstrate the effectiveness of FeSSi as an environmental remediator and the strength of our quantitative model of the mitigation of nanoparticle toxicity by algal-produced organic material.

KEYWORDS: sulfidized nano-iron, remediation, nanotoxicity, algae, ecological modeling



Nanozerovalent iron (nZVI) and its derivatives are currently the most commonly applied technology for nano-enabled remediation¹⁻⁴ and can remove a wide array of metals and organic compounds in the laboratory^{5,6} and in the field.⁷⁻⁹ nZVI-based nanoremediation technology is appealing both because of its high reactivity and because it can be applied *in situ*, without the need for excavation and/or waste disposal.^{1,4,7,8} Recently, a sulfide-modified derivative of nZVI has attracted growing attention due to its enhanced reactivity and selectivity.¹⁰⁻¹² However, it is unclear how the direct introduction of nZVI and derivatives for environmental remediation will affect natural ecosystems. Many studies have investigated the potential fate and transport of nZVI in natural systems,^{8,13-16} and others have found that nZVI and derivatives alone can be toxic to bacteria,^{2,17-19} phyto- and zooplankton,²⁰ fish,²¹⁻²³ and earthworms²⁴ through mechanisms such as oxidative stress^{17-19,21-23,25} and membrane damage.^{18,25} Recent studies have focused on ways to decrease the toxicity

of nZVI and derivatives, such as the addition of sulfur that has been found to decrease the toxicity of silver nanoparticles.^{26,27}

In all of the existing studies investigating the toxicity of nZVI, the nanoparticles are exposed to test organisms as pristine nZVI.¹¹ However, these nanoparticles are released into the environment for remediation purposes and will ideally be bound to the contaminants^{28,29} or will have reacted with other environmental constituents.^{8,13,16,30,31} Our study investigates the effects of nZVI or its derivatives on an organism when the nanoremediator is bound to its target contaminant.

Cadmium (Cd) is a potent environmental contaminant and second in algal toxicity only to mercury.³² Here we test the toxicity of a highly sulfidized derivative of nZVI²⁸ named FeSSi

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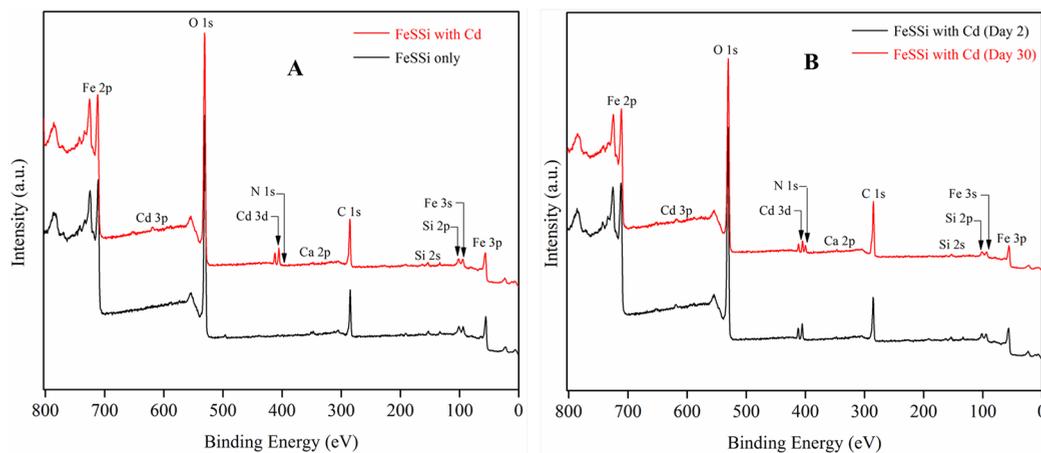


Figure 1. XPS analyses of FeSSi. (A) Surveys of FeSSi with and without cadmium (Cd) obtained after 2 days in 1 day culture, while (B) compares the survey spectra of FeSSi with Cd obtained after 2 and 30 days.

used to remove Cd to cultures of *Chlamydomonas reinhardtii*. We exposed algal cultures with different amounts of accumulated dissolved organic carbon (DOC) to FeSSi bound with Cd (“FeSSi+Cd experiments”). We monitored algal growth and changes in the nanoparticle-contaminant composites over time for up to 32 days utilizing an array of analytical methods. Using these data, we expanded our model that describes the mitigating effect of algal DOC on FeSSi toxicity when algal cultures are exposed to FeSSi alone³³ to include the effect of Cd (characterized by experiments and a model of Cd toxicity) and the effect of FeSSi with Cd sorbed onto it.

RESULTS/DISCUSSION

In this section we present and discuss our main findings. First, we measured the toxicity of Cd to *C. reinhardtii* and parametrized a model of this toxicity. Through analytical measurements such as X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and others, we demonstrated that FeSSi removes Cd from aqueous media, and this removal reduces the toxicity of Cd to the alga. We found that algal-produced DOC mitigates the toxicity of FeSSi, facilitating remediation of Cd. We discovered that FeSSi with Cd sorbed onto it is more toxic than FeSSi alone. Lastly, we used all of this information to develop a quantitative model to predict the amount of FeSSi needed to remediate concentrations of Cd and the consequences of these exposures.

Cd Toxicity to *C. reinhardtii*. Dissolved Cd is toxic to *Chlamydomonas reinhardtii* at low mg Cd/L concentrations (Figure S1). 0.1 and 1.0 mg Cd/L has no effect on algal cultures, however 5 and 10 mg Cd/L cause complete mortality of new batch cultures and reduce populations of 11-day old cultures to a very low population size (0.36 and 0.24 μg chlorophyll a/L at 5 and 10 mg Cd/L, respectively; Figure S1). We resuspended in fresh media 11-day old algal cultures exposed to 5 and 10 mg Cd/L to see if the populations were dead or at low cell numbers and found that cultures exposed to 5 mg Cd/L grew to population sizes similar to controls after 25 days in new, Cd-free media. Cultures exposed to 10 mg Cd/L did not grow even after being moved to the new media (Figure S2). This may indicate that 11-day old cultures were able to either survive or even acclimate to 5 mg Cd/L. Our finding that 5 and 10 mg Cd/L is toxic to *C. reinhardtii* is in agreement with some studies in the literature,^{34–37} while other studies have

found that lower concentrations are toxic^{32,38} or that the concentration must be much higher to exert toxicity to *C. reinhardtii*.^{39,40} These discrepancies may be due to differences in experimental conditions, such as media composition and pH, which can have a large effect on the speciation, bioavailability, and thus, toxicity of Cd to algae.³⁸ Cd exerts toxicity through oxidative stress,^{37,40} disruption of photosynthesis,^{32,40,41} and/or nitrate uptake inhibition.³⁵ Further, numerous studies have found that *C. reinhardtii* accumulates Cd,^{39,42} primarily in the chloroplast.⁴¹

We fit a model of Cd toxicity to the data (see Figure S3) from the Cd-only experiment (Cd Model). The estimated NEC value (0.47 ± 0.33 mg Cd/L) is in agreement with several studies,^{36,37} but note variation in outcome described above. We also fit a model in which algal-produced DOC decreased the toxicity of Cd, however the addition of the multiple parameters to describe DOC binding and mitigating Cd did not improve the fit of the model (data not shown). This makes sense, as the highest concentrations of Cd have similar effects on new and 11-day old cultures (Figure S2) even though older cultures have accumulated significantly more DOC. In addition to allowing comparison to other studies, the development and parametrization of this Cd model allows us to predict the effect of Cd concentrations beyond the concentrations used in our experiments, which is crucial in evaluating whether or not FeSSi is able to mitigate the toxicity of Cd to algae.

FeSSi Removes Cd from Aqueous Media. Prior to dosing the algal cultures, we added 180 mg FeSSi/L and 4.5 mg Cd/L to new algal media or media from 11-day old cultures with the algal cells removed (with DOC present), and FeSSi and Cd interacted for an hour before dosing three concentrations of the FeSSi+Cd mixtures to *C. reinhardtii*. In the absence of algae, FeSSi sorbed Cd within the first hour and then retained Cd for the duration of the algal exposure.

FeSSi removed most of the dosed Cd in the hour prealgal exposure. The Cd removed was mainly adsorbed on the surface of FeSSi given that bulk Fe/Cd ratio (45.7 and 49.3 in new and day 11 cultures, respectively) was much larger than surface Fe/Cd ratio (7.8 and 5.8 in new and day 11 cultures, respectively). The FeSSi particles bound $87.6 \pm 1.0\%$ and $81.2 \pm 0.7\%$ (averages \pm standard error of 3 treatments) of the Cd introduced in new media and media from 11-day old cultures, respectively, in an hour pre-exposure (inductively coupled plasma atomic emission spectroscopy [ICP-AES] data; Figure

S4). These data indicate that FeSSi bound significantly more Cd in new cultures than 11-day old cultures ($t(4) = -5.11$, $p = 0.009$). XPS analysis of FeSSi-Cd composites on day 2 of the experiment also demonstrated that FeSSi particles removed Cd from the aqueous phase in both new and 11-day old algal cultures (Figure 1a). Since XPS typically probes the top ~ 10 nm of samples, these data reflect the chemical status of Cd immobilized on the surface of the nanoparticles. We then dosed new algal cultures with FeSSi and Cd that had interacted for an hour in new media and dosed 11-day old cultures with FeSSi and Cd suspended in media from 11-day old cultures.

Additional XPS analyses were done on FeSSi-Cd composites 30 days after they were introduced to algal cultures (Figure 1b and see SI Section 1.1 for further discussion of XPS results). As can be seen in Figure 1b, peaks for Cd were present in the spectra obtained from the undissolved FeSSi-Cd composites analyzed after 30 days, which shows that Cd mostly remained strongly adsorbed to the surface of FeSSi particles. Further, ESEM imaging of samples of the algal cultures at the end of the FeSSi+Cd experiment qualitatively confirms that Cd is bound onto the FeSSi particles (Figure 2). However, it is likely that

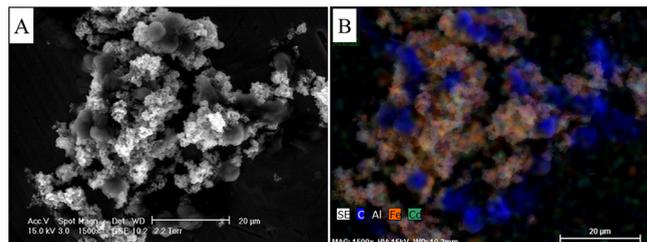


Figure 2. A representative ESEM micrograph with EDS hypermaps showing interactions between FeSSi-Cd composites and 11-day old culture algal cells. (A) Heteroaggregation between FeSSi-Cd aggregates and *C. reinhardtii* cells, with (B) showing an overlay of carbon (C, from algae), iron (Fe, from FeSSi), cadmium (Cd), and aluminum (Al, from the specimen mount) distribution on micrograph. The hypermap shows that Cd (green color) was mostly adsorbed to FeSSi (orange color) and not algae (blue color).

the small concentration of Cd not bound by FeSSi is taken up by the algal cells themselves, as has been shown in the literature,^{39,41,42} and may explain why Cd concentrations decrease in some treatments throughout the incubation (Figure S4 and discussion in SI Section 2.2.2).

Transformation of Fe and Cd Throughout Algal Incubation. High-resolution spectra of Cd 3d were collected in order to understand the chemical state of Cd adsorbed on the surface of FeSSi nanoparticles (Figure 3). In the XPS data collected on days 2 and 10, the peak for Cd $3d_{5/2}$ in new cultures was found ~ 405.5 eV (Figure 3, Table S3), which may be assigned to CdS or Cd^{2+} .^{43,44} FeSSi particles contain sulfur (mostly as SO_4^{2-} , S_n^{2-} , and S^{2-} as shown in ref 33), and it is reasonable to expect the existence of CdS due to the high affinity between Cd^{2+} and S^{2-} (we also observed a considerable amount of S in aqueous media, Figure S10). However, XPS survey showed that the sulfur atoms present were insufficient to form pure CdS (atomic Cd:S ratio > 2). As a result, the peak for Cd $3d_{5/2}$ at 405.5 eV suggests the formation of a mixture of CdS and Cd^{2+} , which also agrees with previous studies (e.g., refs 43 and 45) that showed that zerovalent iron (Fe^0) removed Cd from aqueous phase as Cd^{2+} ions via adsorption.

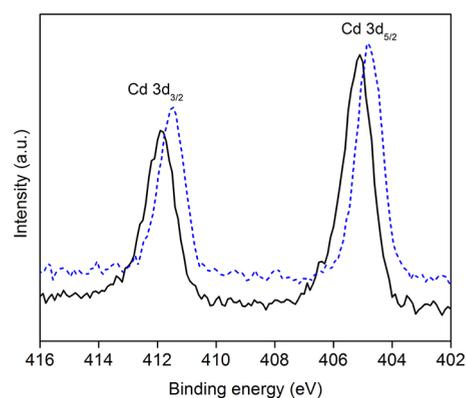


Figure 3. Cd 3d high-resolution spectra of samples obtained from new culture (black solid line) and 11-day old culture (dashed blue line) after 2 days.

In contrast, the peaks for Cd $3d_{5/2}$ in the XPS data collected on day 30 from new cultures and those collected on days 2 and 10 from 11-day old cultures were found at 404.8 eV (Figure 3, Table S3) and assigned to $Cd(OH)_2$. We previously showed that the pH of COMBO media (which contains no buffer) increases over time when *C. reinhardtii* cells are cultured in it.³³ The prevalent species of Cd at pH > 10 is $Cd(OH)_2$,⁴³ which explains the XPS finding in 11-day old cultures and that of day 30 sample from new algal cultures. These results show that FeSSi is able to bind strongly to Cd in a variety of media chemistry, which makes it a good candidate for adsorption of very toxic environmental pollutants such as Cd.

The high-resolution XPS spectra for Fe ($Fe\ 2p_{3/2}$ and $Fe\ 2p_{1/2}$ peaks located at 711.1 and 724.6, respectively) were assigned to $FeOOH$ (Figure 4a),³⁰ and no peaks for Fe^0 were observed in the XPS analyses of samples from both new and 11 days old cultures throughout the experiments. The assignment of $FeOOH$ was supported by the high-resolution data for O 1s (Figure 4b), which showed the presence of O^{2-} (~ 529.9 eV) and OH^- (~ 531.2 eV) with the ratio of O^{2-}/OH^- varying between 0.77 and 0.99.⁴⁶ The expected stoichiometric ratio of O^{2-}/OH^- in $FeOOH$ is 1.0, but some of the O^{2-} and OH^- are potentially associated with Cd and other trace elements present in the algal media. The peaks at 532.2 and 533.2 eV were assigned to water or organic C–O and organic C=O groups, respectively. The organic groups originated from algae and/or associated organic materials and may have played a role in toxicity mitigating reported in the next section.

In summary, XPS and XRD analyses (discussed in SI Section 1.2) show that Fe^0 in FeSSi was rapidly transformed to higher Fe oxidation states in algal cultures, except in the presence of high amount of DOC (in 11 days old cultures) when Fe^0 was detected in the core of FeSSi up to 30 days after exposure to algal cultures. The chemical state of Fe determines its environmental effects (e.g., reactive oxygen species production). Our study shows that algal DOC moderates the transformation of FeSSi in freshwater media, which is an important feedback between biota and the engineered nanomaterial.

FeSSi Reduces Cd Toxicity to *C. reinhardtii*. We dosed three nominal concentrations of FeSSi+Cd to both new and 11-day old cultures: 180 mg FeSSi/L+4.5 mg Cd/L (full concentration), 18 mg FeSSi/L+0.45 mg Cd/L (10-fold dilution), and 1.8 mg FeSSi/L+0.045 mg Cd/L (100-fold dilution). We chose 4.5 mg Cd/L as the highest concentration because we knew this concentration is toxic to the algae in the

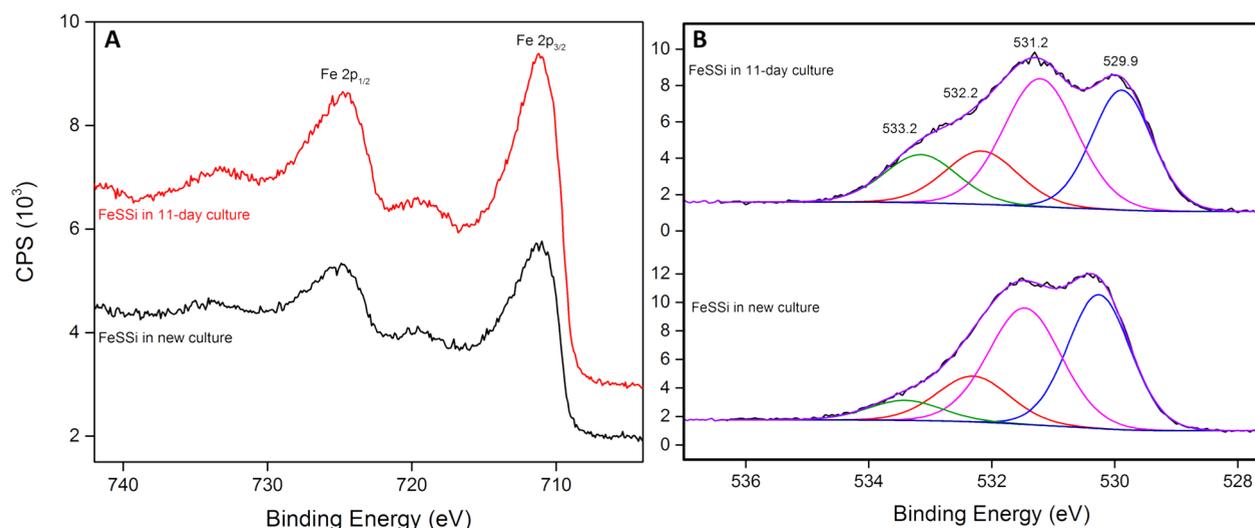


Figure 4. (A) Fe 2p and (B) O 1s high-resolution spectra of samples obtained from new and 11-day old cultures after 2 days.

absence of remediation by FeSSi, and this concentration is similar to concentrations of Cd found in some contaminated natural waters from industrial runoff.⁴⁷ We found that none of these concentrations had any noticeable effect on new or 11-day old cultures, except that 180 mg FeSSi/L+4.5 mg Cd/L was completely toxic to new cultures (Figure 5).

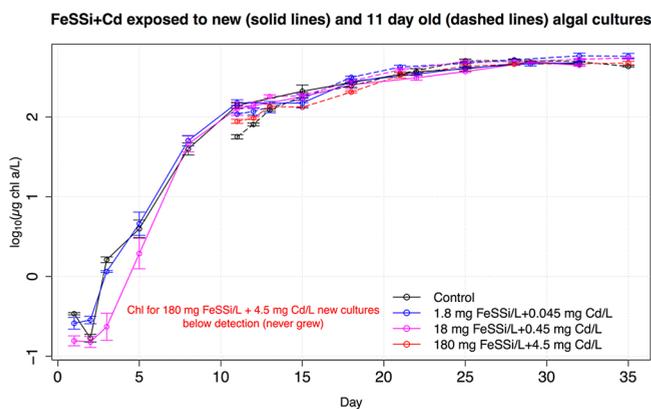


Figure 5. FeSSi removes the toxicity of Cd to all cultures except new cultures at the highest concentrations of FeSSi and Cd, which never grow. The data points are averages of that treatment's replicates ($n = 3$ for all treatments), and the error bars reflect their standard error.

Based on our estimated NEC value of 0.47 mg Cd/L, exposure to the two lowest Cd concentrations (0.045 and 0.45 mg Cd/L) would have no effect on the algae, while exposure to 4.5 mg Cd/L would be toxic. However, we found that 180 mg FeSSi/L+4.5 mg Cd/L has no effect on 11-day old cultures, indicating that immobilization of Cd by FeSSi removes Cd toxicity in this treatment (Figure 5). This result is consistent with measurements of the extracellular concentration of Cd throughout the algal experiment (Figure S4) and XRD and XPS analyses. However, nearly the same amount of unbound Cd is present in new cultures as 11-day old cultures at the start of the experiment (Figure S4), but 11-day old cultures are able to grow almost indistinguishably from unexposed 11-day old cultures, while new cultures die. Through the development of a quantitative model, we found that this is due to the presence of

algal-produced DOC in 11-day old cultures and the absence of DOC in new cultures, which we will discuss in the next section.

Algal-Produced DOC Mitigates FeSSi Toxicity. High concentrations (180 mg FeSSi/L) of pristine FeSSi are toxic to *C. reinhardtii*, however we found in a past study that FeSSi coated with DOC produced by the algae themselves can mitigate this toxicity,³³ in agreement with other studies that found that natural organic material can mitigate nZVI toxicity.^{23,48} In exposures to FeSSi alone, 180 mg FeSSi/L delayed the growth of new cultures for over a week while not having an effect on 11-day old cultures.³³ This is a similar result to our past work that identified a feedback in which algal-produced organic material mitigates ionic and nano-specific toxicity of silver nanoparticles to the algae themselves.⁴⁹ We adapted the model developed in ref 49 and fit it to the response of *C. reinhardtii* to FeSSi exposures in ref 33. We found that DOC again explained the patterns of toxicity observed: 11-day old cultures had produced enough DOC to mitigate FeSSi's toxicity, however FeSSi delayed growth of new cultures until enough FeSSi particles had been inactivated by DOC that the cultures were able to grow.³³

We expanded the model (hereafter called the Combined Model) to explain how FeSSi and Cd exposure has no effect on 11-day old cultures but is toxic to new cultures at the highest exposure concentration. The Combined Model includes Cd toxicity (Cd Model), the sorption of Cd onto FeSSi, and the binding of DOC to FeSSi and FeSSi particles with Cd sorbed (see Table 1 for model equations and Table 2 for parameter estimates).

FeSSi with Cd Sorbed onto It Is More Toxic Than FeSSi Alone. In the algal exposures to FeSSi and Cd, algal cultures experience toxicity from three possible sources: FeSSi with (F_C in the Combined Model) and without Cd (F in the Combined Model) and from free Cd (not sorbed to FeSSi). Parameterization of the Combined Model allows the estimation of the relative strength of these three forms of toxicity. The Cd Model gives a good fit to the effects of free Cd toxicity to *C. reinhardtii* in batch cultures (Figure S3). Both F and F_C are toxic to the algae—our past work³³ identified the toxicity of FeSSi alone, and we assume that FeSSi with Cd sorbed to the surface of the particle is at least as toxic as FeSSi alone. Our first hypothesis is that the toxicity of F is equivalent to F_C (Tables 1

Table 1. State Variables, Functions, And Balance Equations for Combined Model–FeSSi-Cd-DOC Model

State Variables	
N	Algal biomass ($\mu\text{g chl a/L}$)
D	DOC concentration (mg C/L)
C	Unbound (free and toxic) Cd (mg Cd/L)
F	Bioavailable FeSSi (FeSSi particles/L)
F_D	FeSSi inactivated by DOC (FeSSi particles/L)
F_C	FeSSi+Cd (FeSSi particles/L)
F_{DC}	FeSSi+Cd+DOC (FeSSi particles/L)
Functions	
$\mu = k_F F + k_{FC} F_C + k_C(C - NEC)$	Mortality due to FeSSi and Cd exposure
$P_D = j_{DN} N + h_{DN} \frac{dN}{dt}$	DOC production
$J_{FD} = \gamma_{UN} F D$	Inactivation of FeSSi by DOC
$J_{FCD} = \gamma_{UN} F_C D$	Inactivation of FeSSi+Cd by DOC
$B_{FC} = b F C$	Binding of Cd by FeSSi
$B_{FDC} = b F_D C$	Binding of Cd by FeSSi+DOC
Balance equations	
$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) - \mu N$	Algal biomass
$\frac{dD}{dt} = P_D - \beta(J_{FD} + J_{FCD})$	DOC
$\frac{dC}{dt} = -\alpha_{CF}(B_{FC} + B_{FDC})$	Unbound Cd
$\frac{dF}{dt} = -B_{FC} - J_{FD}$	Bioavailable FeSSi
$\frac{dF_C}{dt} = B_{FC} - J_{FCD}$	FeSSi with Cd bound to it
$\frac{dF_D}{dt} = J_{FD} - B_{FDC}$	FeSSi inactivated by DOC
$\frac{dF_{DC}}{dt} = J_{FCD} + B_{FDC}$	FeSSi with Cd bound inactivated by DOC

and 2, $k_F = k_{FC}$), and FeSSi particle-specific toxicity combined with the concentration of Cd that is not bound by FeSSi is great enough to cause new cultures to never grow, while 11-day old cultures have produced enough DOC to mitigate the toxic effect of FeSSi particles. We can simulate this scenario by setting $k_F = k_{FC}$ in the Combined Model, and the model predicts that new cultures would be able to grow after 2 weeks of exposure (Figure S5), which is an incorrect prediction; new cultures never recovered from FeSSi+Cd exposure at the highest concentration. The model correctly predicts that there is no effect on 11-day old cultures due to DOC binding and

inactivating FeSSi, but the mismatch between our empirical results and the model's prediction for new cultures indicates that we are underestimating toxicity in cultures without DOC.

FeSSi particles with Cd sorbed onto the particles' surface could also be more toxic than FeSSi particles alone ($k_{FC} > k_F$). Allowing this in the model gives a good fit to the data (Figure 6) when the toxicity of FeSSi particles with Cd is over 10 times greater than FeSSi particles alone (see Table 2 for parameter values). The model still correctly predicts that this concentration does not have an effect on 11-day old cultures due to DOC inactivation of the FeSSi particles for the hour prior to dosing the algal cultures. We found a similar result in ref 33 in which we exposed algal cultures to FeSSi alone (without Cd). In the hour before dosing, the model predicts that almost 30% of the FeSSi particles had been inactivated by DOC before dosing the 11-day old cultures (FeSSi was introduced into DOC-rich media in the same manner in ref 33 as the experiments reported in this paper). The model in this paper predicts a greater initial inactivation by DOC when FeSSi interacts with Cd for an hour pre-exposure in the presence of DOC. A little more than half of the total amount of FeSSi particles are inactivated by DOC (F_D), and all of the Cd is sorbed onto FeSSi particles that are also inactivated by DOC (F_{DC}) after the hour of pre-exposure.

Implications for *in Situ* Remediation. Using the Combined Model, we are able to predict the toxicity of mixtures of FeSSi and Cd concentrations, thereby suggesting that this model could contribute to environmental remediation decisions. Since we have characterized the toxicity of Cd, FeSSi alone, and FeSSi with Cd sorbed onto it (FeSSi+Cd), we can use the model to predict the FeSSi concentration needed to successfully remediate an environmental concentration of Cd (remove enough Cd such that it is no longer toxic to the alga) and test to see if the necessary concentration of FeSSi is toxic in and of itself (both as FeSSi and FeSSi+Cd). This allows us to predict the Cd concentrations that FeSSi can remediate without replacing Cd toxicity with FeSSi and FeSSi+Cd toxicity (Figure 7). Our model simulates a simple ecological community of a single algal species and involves assuming arbitrary, though defensible, criteria for remediation and for the boundary between the three forms of toxicity the algae may experience (see SI Section 3 for discussion of the rationale for these

Table 2. Parameters for Combined Model–FeSSi-Cd-DOC Model^a

Parameters			Data used for parametrization
r	algal intrinsic growth rate	0.44 1/day	Control cultures during FeSSi exposure
K	algal carrying capacity	453 $\mu\text{g chlorophyll a/L}$	Control cultures during FeSSi exposure
j_{DN}	parameter in DOC production rate	0.003484 $\text{mgC}/(\mu\text{g chlorophyll a}\cdot\text{day})$	Control cultures during FeSSi exposure
h_{DN}	parameter in DOC production rate	0.0072 $\text{mgC}/\mu\text{g chlorophyll a}$	Control cultures during FeSSi exposure
k_F	FeSSi toxicity parameter	$5.57 \times 10^{-14} \text{ L}/(\text{FeSSi particles}\cdot\text{day})$	FeSSi-only exposure
γ_{UN}	FeSSi inactivation rate	0.2484 $\text{L}/(\text{mgC}\cdot\text{day})$	FeSSi-only exposure
β	Loss of DOC due to heteroaggregation with FeSSi	$4.069 \times 10^{-15} \text{ mgC}/\text{FeSSi particles}$	FeSSi-only exposure
k_C	Cd-specific toxicity	0.1873 $\text{L}/(\text{mg Cd}\cdot\text{day})$	Cd-only expt
NEC	No effect concentration of Cd	0.4687 mg Cd/L	Cd-only expt
k_{FC}	Toxicity of FeSSi particles with Cd bound	$7 \times 10^{-13} \text{ L}/(\text{FeSSi particles}\cdot\text{day})$	Fit from FeSSi+Cd exposures
b	Rate of FeSSi binding Cd	0.4211 $\text{L}/(\text{mg Cd}\cdot\text{day})$	Fit from FeSSi+Cd exposures
α_{CF}	Maximum mg Cd bound per FeSSi particle	$1.005 \times 10^{-14} \text{ mg Cd}/\text{FeSSi particle}$	Fit from FeSSi+Cd exposures

^aAlgal growth and DOC production parameters were fit to control cultures.³³ FeSSi toxicity parameters, rate of DOC inactivation of FeSSi, and loss of DOC due to heteroaggregation with FeSSi and binding rates were fit previously from FeSSi-only exposures.³³ Parameters pertaining to Cd toxicity are from the fit of the Cd-only experiment and are identical to the values in Table S5. Parameters pertaining to FeSSi and Cd toxicity, FeSSi binding Cd rate, toxicity of FeSSi with Cd bound, and the maximum amount of Cd bound per particle were fit to data on FeSSi+Cd exposure (empirical results in Figure 5).

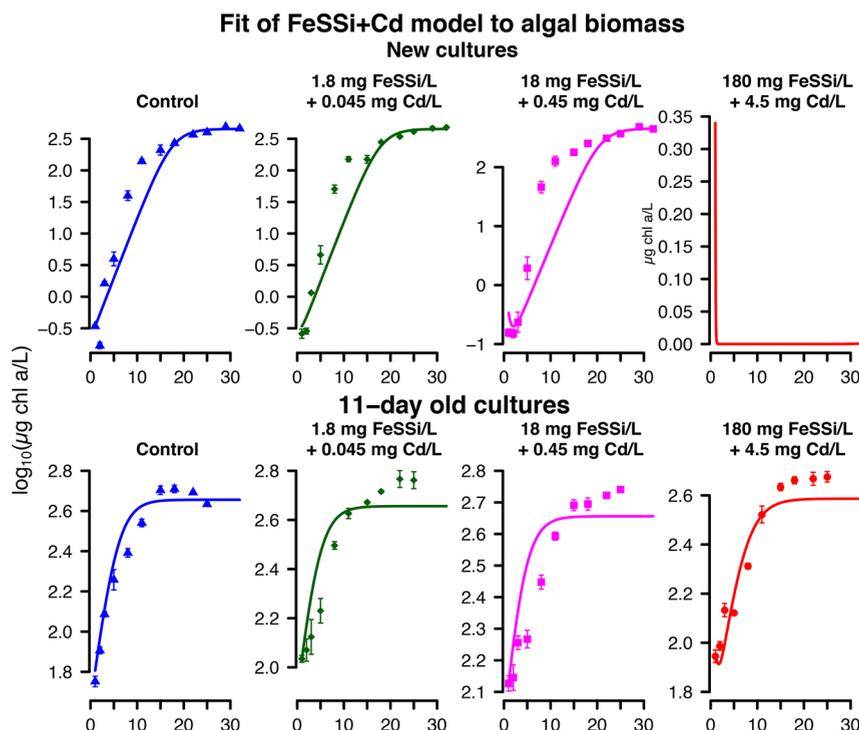


Figure 6. Fit of Combined Model (lines) to data from FeSSi+Cd exposures (data points) when FeSSi particles that have sorbed Cd (F_C) are more toxic than FeSSi particles alone (F). Note that new cultures never grew at the highest FeSSi and Cd concentrations (top right panel) so that model simulation is not shown on a log scale to demonstrate that the correctly model predicts complete toxicity to these cultures. The data points are averages of that treatment's replicates ($n = 3$ for all treatments), and the error bars reflect their standard error.

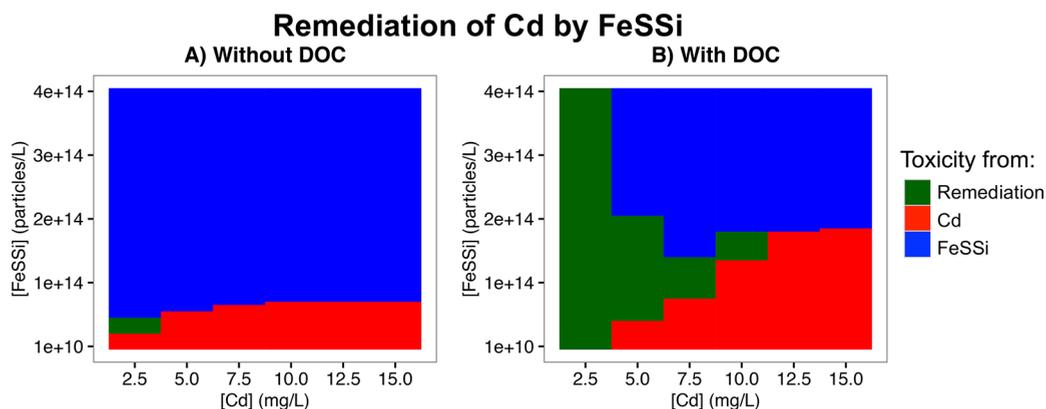


Figure 7. Presence of DOC significantly increases the ability of FeSSi to remediate Cd. We compared the ability of FeSSi to remediate Cd from algae without any initial DOC present (A) and algae with an initial concentration of 8.8 mg C/L DOC (B), equivalent to conditions in our lab studies of 11-day old cultures. We simulated remediation of Cd concentrations 2.5–15 mg Cd/L with 1×10^{10} – 4×10^{14} particles/L of FeSSi (Cd is not toxic below 2.5 mg Cd/L by the assumed definition of “remediation”). We assumed that the FeSSi and Cd interacted with (B) and without (A) DOC for an hour prior to algal exposure, which is identical to our experiments and simulates runoff of FeSSi into a freshwater body from FeSSi’s application for the remediation of contaminated soils. We then coded the output as either “remediation” (either no effect or causing a growth delay of 30 days or less), “Cd”, or “FeSSi” toxicity. We distinguished between Cd and FeSSi toxicity by simulating the same Cd and FeSSi concentrations without any Cd toxicity (parameter k_C set to 0). In reality, many of the observed toxic responses are the result of a combination of FeSSi and Cd toxicity, however we distinguished between Cd and FeSSi toxicity by choosing which toxicant contributed to the majority of the toxic response (see details behind the calculations and simulations conducted for this figure in SI Section 3). We simulated the Cd concentrations displayed (2.5, 5, 7.5, 10, 12.5, and 15 mg Cd/L) and FeSSi concentrations in the range 1×10^{10} – 4×10^{14} particles/L in increments of 5×10^{12} particles/L.

assumptions). In particular, we characterize remediation in terms of duration of the delay in algal response (described above) following exposure on the grounds that a sustained delay in response of an important phytoplankton species would correspond in any natural system to significant ecological impact. More precise prediction of impact for any particular

water body would require extending the model to incorporate context-specific ecological information.

We used the model to mimic FeSSi runoff into a freshwater system from application to soils contaminated with Cd. We assumed that the Cd and FeSSi particles interacted for an hour prior to algal exposure and used the Combined Model to

simulate the effect of a range of Cd (2.5–15 mg Cd/L) and FeSSi (1×10^{10} – 4×10^{14} particles/L) concentrations for algae growing in environments initially without DOC and for algae with an initial DOC concentration equal to that in our 11-day old algal cultures (Table 1, see SI Section 3 for more details on this analysis). Although our data are from experiments in which we only considered the mitigating ability of algal-produced DOC, studies have shown that algal-produced DOC and natural organic matter share a lot of similarities in their composition and functional groups.^{50,51} We defined “remediation” as the Cd and FeSSi concentrations either having no effect on the algae (exposure growth pattern was similar to the controls) or causing a “growth delay” ≤ 30 days. We explored the sensitivity of these remediation boundaries to model parameters by simulating the model 600 times with variations of parameters $\pm 15\%$ of the best fit parameter value and recorded the outcome (see SI Section 4 for details and Figures S16 and S17). The predicted remediation boundaries are most sensitive to the value of k_{FC} , the toxicity of FeSSi with Cd sorbed onto it (Figure S17).

The amount of Cd that FeSSi can remediate depends on the initial DOC concentration: In algal cultures with no DOC present at the start of algal growth, FeSSi can only remediate up to 3 mg Cd/L (Figure 7a), however FeSSi can remediate up to 12 mg Cd/L with some DOC present (Figure 7b). We could have chosen a more or less strict definition of remediation by deciding that FeSSi successfully removed Cd toxicity if the exposed cultures grew the same as controls or allowing a longer growth delay to still be considered successful remediation, and the implications of this choice are discussed in SI Section 3.2. This analysis emphasizes the importance of considering natural organic material when estimating the remediation potential of nZVI and derivatives.

The model predictions are sensitive to our determination that FeSSi+Cd is more toxic than FeSSi, though less toxic than Cd alone. If FeSSi and FeSSi+Cd have identical toxicity parameters, the model predicts that FeSSi could be applied to mitigate up to 15 mg Cd/L in the absence of DOC and up to 30 mg Cd/L with DOC initially present. These numerical values of course depend on the choice of environmental scenario, but the model’s predictions are qualitatively robust. This result emphasizes the importance of determining the toxicity of nZVI when its contaminant of interest (here Cd) is sorbed onto it when considering the nanoremediator for an environmental application. The information from studies measuring the toxicity of pristine nZVI^{2,17–24} or a derivative to biological organisms is insufficient for predicting the outcome of proposed remediation.

CONCLUSION

We have demonstrated the ability of FeSSi to mitigate the toxicity of its target contaminant through the systematic exposure of organisms to the contaminant (Cd), FeSSi alone, and FeSSi with the contaminant. Our study found that FeSSi with Cd sorbed onto it is more toxic than FeSSi alone, which is an important finding when considering the implications of using an nZVI derivative as an environmental remediator. While FeSSi does exert toxicity to the algae,³³ our study confirms that it is able to remove the toxicity of Cd to all algal cultures when DOC is present. Since all freshwater bodies contain some amount of both autochthonous (produced by autotrophs and heterotrophs, such as algal-produced DOC) and allochthonous (from the watershed) DOC, our findings

demonstrate that FeSSi can be a useful environmental remediator. Finally, the development of a model to quantify the strength of FeSSi, Cd, and FeSSi with Cd toxicity to algae emphasizes the importance of the mitigating effect of DOC on FeSSi toxicity. This adds to the growing body of work that has identified the detoxifying effect of organic material, especially algal-produced organic material,^{33,49} on nanoparticle toxicity,^{25,52–54} indicating that this feedback may be a generalizable mechanism that influences nanotoxicity.

METHODS

We performed three sets of experiments exposing new and 11-day old cultures to (1) Cd only (“Cd only experiments”), (2) FeSSi with and without Cd (FeSSi-only exposures are reported in ref 33 and denoted as “FeSSi only experiments”; FeSSi with Cd exposures are reported here and denoted as “FeSSi+Cd experiments”), and (3) additional exposures to FeSSi and FeSSi+Cd for XRD and XPS (“XRD and XPS exposures”). In all of these experiments, algal cultures were exposed to the toxicants as new and 11-day old cultures in order to test for the effect of algal-produced DOC on toxicity. New cultures were inoculated on the first day of the experiment, and 11-day old cultures were grown for 11 days prior to the experiment’s start such that exposures and sampling of both ages occurred at the same time.

Synthesis and Characterization of Nanoparticles. We synthesized sulfide-modified nZVI seeded with silica (FeSSi) according to the method described in ref 28. Briefly, we mixed 7.6 g of sodium borohydride (NaBH_4) purchased from Oakwood Chemical (Estill, SC), 1.5 g of sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) purchased from Sigma-Aldrich (St. Louis, MO), and 0.2 mL of colloidal silica (30 wt %) together in an Erlenmeyer flask and made up the volume to 250 mL with deionized water (DI, 18.2 $\text{M}\Omega\text{-cm}$, Barnstead Nanopure Diamond). This solution was titrated into another 250 mL solution containing 4.9 g of FeCl_3 (Fisher Scientific). After the reduction reaction, we collected the FeSSi nanoparticles and triple-washed them with nitrogen-purged DI water. We then separated FeSSi from the aqueous phase with a neodymium-iron-boron magnet and stored them in 30% ethanol at 4 °C until use.

The physicochemical properties of FeSSi are published in previous studies,^{28,33} and we present the major ones here and are also summarized in Table 3: FeSSi is mainly spherical with an average

Table 3. Characterization Data for FeSSi Particles, Published in Refs 28 and 33^a

Property	Value
Primary particle size	150 nm
Hydrodynamic diameter	340 nm a
ζ potential	−38 mV a
Isoelectric point (IEP)	pH 7.4
Main composition	Fe0 (68%), FeS (13%), FeOOH (11%), Fe-O (8%)

^aMeasurement was done in deionized water at pH 7 (adjusted with 5 mM phosphate buffer).

particle size of 90 nm (Figure S6). Mössbauer analyses revealed that FeSSi is made up of 55.6% Fe(0), 9.9% Fe(II), and 34.5% Fe(III). The hydrodynamic diameter of FeSSi was determined as 340 nm in DI water (adjusted to pH 7.5 using phosphate buffer), while its zeta (ζ) potential in the same media was −38 mV. The hydrodynamic diameter of FeSSi increased to 369 nm in new algal COMBO media⁵⁵ (ζ potential of FeSSi in media = −17 mV) and 390 nm in media from 11-day old cultures with the algal cells removed (ζ potential of FeSSi in media = −11.4 mV). According to ref 33, increase in hydrodynamic diameter of FeSSi in new algal COMBO media was due to slight aggregation but the increase in size in media from 11-day old cultures was due to coating of FeSSi by DOC released into the media by algae.

Toxicity of Cd to *Chlamydomonas*. We exposed new and 11-day old cultures to 0.1, 1, 5, and 10 mg/L Cd as Cd chloride (CdCl_2 , “Cd only experiment”). Algal cultures were 250 mL in COMBO media,⁵⁵ and all glassware and sampling apparatus were autoclaved prior to the experiment. We measured the concentration of chlorophyll a fluorometrically on a microplate reader and converted the relative fluorescent units to chlorophyll concentrations using a standard curve developed in our lab using chlorophyll a standard (see Methods in ref 49). We also measured the concentration of Cd throughout the Cd only experiment by taking samples from the cultures at a few time points, filtering (0.45 μm) them to remove cells, digesting the samples with trace-metal grade HNO_3 (Fisher Scientific), and analyzing them for Cd via ICP-AES (Thermo Scientific iCAP 6300). To confirm whether or not 5 and 10 mg Cd/L was toxic to 11-day old cultures or if the populations were just at low cell densities, we spun down all 11-day old cultures after 19 days of exposure and resuspended them in fresh, Cd-free COMBO media. To resuspend the cells, we concentrated the cells by centrifuging them for 4 min at 7000 rpm (Eppendorf 5430R Centrifuge), resuspended the pelleted cells in COMBO media, and centrifuged them for another 4 min at the same speed. We then resuspended the pelleted cells and added them to media to dilute the cultures to 250 mL and measured the chlorophyll a concentrations of these cultures for another 25 days to see if the algal cultures could recover from Cd toxicity.

Influence of FeSSi on Toxicity of Cd to *Chlamydomonas*. To investigate the effect of FeSSi-Cd composites in freshwater systems (“FeSSi+Cd experiment”), we added 180 mg/L of FeSSi and 4.5 mg/L Cd (as CdCl_2) to new algal COMBO media or media from 11-day old cultures with the algal cells removed (with DOC present) and allowed them to interact for 1 h by shaking on a Dayton-6Z412A roller-mixer (80 rpm). A previous study showed that adsorption of Cd onto FeSSi from the aqueous phase mostly occurs within 1 h.²⁸ We then dosed three dilutions (dilution factors of 1, 10, and 100) of this FeSSi-Cd composite to both new and 11-day old cultures to obtain (1) 180 mg/L FeSSi+4.5 mg/L Cd, (2) 18 mg/L FeSSi+0.45 mg/L Cd, and (3) 1.8 mg/L FeSSi+0.045 mg/L Cd, assuming that Cd was homogeneously distributed on the surface of FeSSi in suspension. We incubated algal cultures at 20 °C on a diurnal light cycle (12:12 light:dark) under fluorescent growing lights. All glassware was autoclaved prior to the experiment, and all algal cultures were in 250 mL of COMBO media.⁵⁵ We monitored the effect of FeSSi on algal populations through time by measuring chlorophyll a concentrations (see methods in ref 49). We also measured dissolution of FeSSi and availability of Cd by taking aliquots from the supernatant of cultures at time points, filtered (0.45 μm) them to remove cells and undissolved particles, then digested the samples with trace-metal grade HNO_3 (Fisher Scientific), and analyzed them for iron (Fe), silicon (Si), sulfur (S), and Cd via ICP-AES (Thermo Scientific iCAP 6300). As we reported previously, FeSSi aggregates in COMBO media to sizes $>0.45 \mu\text{m}$ within 3 min so it is not expected to pass through the filter.³³ In addition, we visualized the interactions between FeSSi-Cd composites and algal cells using a Phillips FEI XL30 FEG environmental scanning electron microscope (ESEM) equipped with a Bruker XFlash 6160 energy dispersive spectrometer (EDS). Imaging was done in wet mode at 2.2 Torr, 4 °C, and an accelerating voltage of 10 kV. ESEM images were taken at the end of the experiments by fixing 1 mL of cultures (dosed with 180 mg/L FeSSi + 4.5 mg/L Cd) with formalin (5%). The fixed cultures were deposited on a JEOL aluminum specimen mount and then imaged directly (without gold sputtering) using the ESEM.

Transformation of FeSSi in Culture. We performed X-ray diffraction and X-ray photoelectron spectroscopy (XRD and XPS), the two most common techniques for determining the chemical state of solids and powders, to investigate the transformation of FeSSi and the fate of Cd during these experiments. We conducted an additional algal exposure experiment (“XRD and XPS exposures”) so that we could sacrifice entire algal cultures at various time points to collect a large enough mass of FeSSi particles for XRD and XPS. We prepared stocks of FeSSi-Cd composites (180 mg/L of FeSSi and 4.5 mg/L Cd) in new algal COMBO media and media from 11-day old cultures and dosed new and 11-day old cultures as described in the previous

section. The response of these algal cultures (chlorophyll a concentrations measured fluorometrically) was comparable to the results of our FeSSi+Cd experiment (Figure S7), so we are confident that the XRD results reflect the transformation of FeSSi during our the FeSSi+Cd experiments. At certain time points (2, 10, and 30 days), we sacrificed an entire 250 mL culture and separated the solid fractions from suspension *via* centrifugation (10,000 g, 30 min; Sorvall RC 5B Plus). After centrifugation, we decanted the supernatant and immediately vacuum-dried the particles (Yamato ADP-21). The solid fractions obtained thereafter were then analyzed *via* XRD (fluorescence mode; Bruker D8 Advance) and XPS. For XRD analyses, step scans were performed from 10 to 90° 2θ and a step size of 0.02°. XPS analyses were carried out with a Kratos Axis Ultra DLD spectrometer using a monochromatic Al $K\alpha$ source at 150 W. Samples were spread over double-sided tape, and an analysis area of 300 $\mu\text{m} \times 700 \mu\text{m}$ was used. Instrument base pressure was below 10^{-8} Torr. All survey spectra were collected at 160 eV pass energy, 0.5 eV step and 120 ms dwell time per sample. High-resolution spectra were collected using 20 eV pass energy. Step and dwell time for each element are shown in Table S1. All spectra were calibrated using the adventitious C 1s peak at 284.8 eV.

Description of Models. To interpret our results, we first developed a model of the effect of Cd on *C. reinhardtii* (Cd Model, see SI Section 2.1 for details). We then incorporated this model of Cd toxicity into a mechanistic model based on concepts from ref 49 and developed for FeSSi-only exposure in ref 33 (Combined Model, see SI Section 2.2 for details). The state variables of the model are algal biomass, DOC concentration, Cd concentration, and the various forms of FeSSi: F (unbound FeSSi), F_D (FeSSi inactivated by DOC), F_C (FeSSi with Cd sorbed onto it), and F_{DC} (FeSSi with Cd sorbed onto it that is inactivated by DOC) (see Table 1 for model equations and Table 2 for parameter values). Algal growth is modeled using a logistic growth curve, and DOC production is assumed to be proportional to algal biomass and growth rate. FeSSi (with and without DOC; F and F_D) sorbs Cd (turning F into F_C and F_D into F_{DC}) at the same rate (b) (Table 2). Cd, F and F_C are all toxic at different rates (described by k_C , k_F , and k_{F_C} , respectively) and DOC inactivates F and F_C (turning F into F_D and F_C into F_{DC}) at the same rate (γ_{UN}) (Table 2). We fit parameters specific to Cd exposure by fitting the Cd-only model to the Cd only experimental data (Cd Model) and used parameter values from ref 33 for parameters related to FeSSi only toxicity (Table 2). All other parameters (k_{F_C} , b , and α_{CF}) were fit to the FeSSi+Cd data: algal biomass, Cd concentration, and DOC concentration (Table 2). We fit parameters using BYOM (“Bring Your Own Model”) platform for parameter estimation, developed by Tjalling Jager for Matlab (<http://debttox.info>). Further details on both models are in the SI Section 2.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.7b05970.

Further information on Cd and FeSSi characterization from samples taken during the algal exposure experiment, model details for the Cd and Combined Model, details on the methods used to predict the ability of FeSSi to remediate other Cd concentrations than those tested in the exposure, details on the methods used to characterize model uncertainty, and ICP QA/QC (PDF)

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Notes

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REFERENCES

- (1) Adeleye, A. S.; Conway, J. R.; Garner, K.; Huang, Y.; Su, Y.; Keller, A. A. Engineered Nanomaterials for Water Treatment and Remediation: Costs, Benefits, and Applicability. *Chem. Eng. J.* **2016**, *286*, 640–662.
- (2) Lefevre, E.; Bossa, N.; Wiesner, M. R.; Gunsch, C. K. A Review of the Environmental Implications of in Situ Remediation by Nanoscale Zero Valent Iron (nZVI): Behavior, Transport and Impacts on Microbial Communities. *Sci. Total Environ.* **2016**, *565*, 889–901.
- (3) Stefaniuk, M.; Oleszczuk, P.; Ok, Y. S. Review on Nano Zerovalent Iron (nZVI): From Synthesis to Environmental Applications. *Chem. Eng. J.* **2016**, *287*, 618–632.
- (4) Karn, B.; Kuiken, T.; Otto, M. Nanotechnology and in Situ Remediation: A Review of the Benefits and Potential Risks. *Environ. Health Perspect.* **2009**, *117*, 1823–1831.
- (5) Su, Y.; Adeleye, A. S.; Huang, Y.; Sun, X.; Dai, C.; Zhou, X.; Zhang, Y.; Keller, A. A. Simultaneous Removal of Cadmium and Nitrate in Aqueous Media by Nanoscale Zerovalent Iron (nZVI) and Au Doped nZVI Particles. *Water Res.* **2014**, *63*, 102–111.
- (6) Phenrat, T.; Thongboot, T.; Lowry, G. V. Electromagnetic Induction of Zerovalent Iron (Zvi) Powder and Nanoscale Zerovalent Iron (nZVI) Particles Enhances Dechlorination of Trichloroethylene in Contaminated Groundwater and Soil: Proof of Concept. *Environ. Sci. Technol.* **2016**, *50*, 872–880.
- (7) Su, C.; Puls, R. W.; Krug, T. A.; Watling, M. T.; O'Hara, S. K.; Quinn, J. W.; Ruiz, N. E. A Two and Half-Year-Performance Evaluation of a Field Test on Treatment of Source Zone Tetrachloroethene and Its Chlorinated Daughter Products Using Emulsified Zero Valent Iron Nanoparticles. *Water Res.* **2012**, *46*, 5071–5084.
- (8) Su, C.; Puls, R. W.; Krug, T. A.; Watling, M. T.; O'Hara, S. K.; Quinn, J. W.; Ruiz, N. E. Travel Distance and Transformation of Injected Emulsified Zerovalent Iron Nanoparticles in the Subsurface During Two and Half Years. *Water Res.* **2013**, *47*, 4095–106.
- (9) Wei, Y.-T.; Wu, S.-C.; Chou, C.-M.; Che, C.-H.; Tsai, S.-M.; Lien, H.-L. Influence of Nanoscale Zero-Valent Iron on Geochemical Properties of Groundwater and Vinyl Chloride Degradation: A Field Case Study. *Water Res.* **2010**, *44*, 131–140.
- (10) Song, S.; Su, Y.; Adeleye, A. S.; Zhang, Y.; Zhou, X. Optimal Design and Characterization of Sulfide-Modified Nanoscale Zerovalent Iron for Diclofenac Removal. *Catal. B* **2017**, *201*, 211–220.
- (11) Fan, D.; O'Brien Johnson, G.; Tratnyek, P. G.; Johnson, R. L. Sulfidation of Nano Zerovalent Iron (nZVI) for Improved Selectivity During in-Situ Chemical Reduction (ISCR). *Environ. Sci. Technol.* **2016**, *50*, 9558–65.
- (12) Han, Y.; Yan, W. Reductive Dechlorination of Trichloroethene by Zero-Valent Iron Nanoparticles: Reactivity Enhancement through Sulfidation Treatment. *Environ. Sci. Technol.* **2016**, *50*, 12992.
- (13) Crane, R. A.; Scott, T. B. Nanoscale Zero-Valent Iron: Future Prospects for an Emerging Water Treatment Technology. *J. Hazard. Mater.* **2012**, *211–212*, 112–125.
- (14) Shi, Z.; Fan, D.; Johnson, R. L.; Tratnyek, P. G.; Nurmi, J. T.; Wu, Y.; Williams, K. H. Methods for Characterizing the Fate and Effects of Nano Zerovalent Iron During Groundwater Remediation. *J. Contam. Hydrol.* **2015**, *181*, 17–35.
- (15) Phenrat, T.; Cihan, A.; Kim, H.-J.; Mital, M.; Illangasekare, T.; Lowry, G. V. Transport and Deposition of Polymer-Modified Fe0 Nanoparticles in 2-D Heterogeneous Porous Media: Effects of Particle Concentration, Fe0 Content, and Coatings. *Environ. Sci. Technol.* **2010**, *44*, 9086–9093.
- (16) Adeleye, A. S.; Keller, A. A.; Miller, R. J.; Lenihan, H. S. Persistence of Commercial Nanoscaled Zero-Valent Iron (nZVI) and by-Products. *J. Nanopart. Res.* **2013**, *15*, 1418.
- (17) Sevcu, A.; El-Temsah, Y. S.; Joner, E. J.; Cernik, M. Oxidative Stress Induced in Microorganisms by Zero-Valent Iron Nanoparticles. *Microbes Environ.* **2011**, *26*, 271–81.
- (18) Diao, M.; Yao, M. Use of Zero-Valent Iron Nanoparticles in Inactivating Microbes. *Water Res.* **2009**, *43*, 5243–51.
- (19) Auffan, M. I.; Achouak, W.; Rose, J. r.; Roncato, M.-A.; Chanéac, C.; Waite, D. T.; Masion, A.; Woicik, J. C.; Wiesner, M. R.; Bottero, J.-Y. Relation between the Redox State of Iron-Based Nanoparticles and Their Cytotoxicity Towards *Escherichia Coli*. *Environ. Sci. Technol.* **2008**, *42*, 6730–6735.
- (20) Keller, A. A.; Garner, K.; Miller, R. J.; Lenihan, H. S. Toxicity of Nano-Zero Valent Iron to Freshwater and Marine Organisms. *PLoS One* **2012**, *7*, e43983.
- (21) Li, H.; Zhou, Q.; Wu, Y.; Fu, J.; Wang, T.; Jiang, G. Effects of Waterborne Nano-Iron on Medaka (*Oryzias Latipes*): Antioxidant Enzymatic Activity, Lipid Peroxidation and Histopathology. *Ecotoxicol. Environ. Saf.* **2009**, *72*, 684–92.
- (22) Chen, P. J.; Wu, W. L.; Wu, K. C. The Zerovalent Iron Nanoparticle Causes Higher Developmental Toxicity Than Its Oxidation Products in Early Life Stages of Medaka Fish. *Water Res.* **2013**, *47*, 3899–909.
- (23) Chen, P. J.; Su, C. H.; Tseng, C. Y.; Tan, S. W.; Cheng, C. H. Toxicity Assessments of Nanoscale Zerovalent Iron and Its Oxidation Products in Medaka (*Oryzias Latipes*) Fish. *Mar. Pollut. Bull.* **2011**, *63*, 339–46.
- (24) El-Temsah, Y. S.; Joner, E. J. Ecotoxicological Effects on Earthworms of Fresh and Aged Nano-Sized Zero-Valent Iron (nZVI) in Soil. *Chemosphere* **2012**, *89*, 76–82.
- (25) Lee, S.; Kim, K.; Shon, H. K.; Kim, S. D.; Cho, J. Biototoxicity of Nanoparticles: Effect of Natural Organic Matter. *J. Nanopart. Res.* **2011**, *13*, 3051–3061.
- (26) Levard, C.; Hotze, E. M.; Colman, B. P.; Dale, A. L.; Truong, L.; Yang, X. Y.; Bone, A. J.; Brown, G. E., Jr.; Tanguay, R. L.; Di Giulio, R. T.; Bernhardt, E. S.; Meyer, J. N.; Wiesner, M. R.; Lowry, G. V. Sulfidation of Silver Nanoparticles: Natural Antidote to Their Toxicity. *Environ. Sci. Technol.* **2013**, *47*, 13440–8.
- (27) Reinsch, B. C.; Levard, C.; Li, Z.; Ma, R.; Wise, A.; Gregory, K. B.; Brown, G. E., Jr.; Lowry, G. V. Sulfidation of Silver Nanoparticles Decreases *Escherichia Coli* Growth Inhibition. *Environ. Sci. Technol.* **2012**, *46*, 6992–7000.
- (28) Su, Y.; Adeleye, A. S.; Huang, Y.; Zhou, X.; Keller, A. A.; Zhang, Y. Direct Synthesis of Novel and Reactive Sulfide-Modified Nano Iron through Nanoparticle Seeding for Improved Cadmium-Contaminated Water Treatment. *Sci. Rep.* **2016**, *6*, 24358.
- (29) Su, Y.; Adeleye, A. S.; Keller, A. A.; Huang, Y.; Dai, C.; Zhou, X.; Zhang, Y. Magnetic Sulfide-Modified Nanoscale Zerovalent Iron (S-nZVI) for Dissolved Metal Ion Removal. *Water Res.* **2015**, *74*, 47–57.
- (30) Liu, A. R.; Liu, J.; Pan, B. C.; Zhang, W. X. Formation of Lepidocrocite (γ -Fe₂O₃) from Oxidation of Nanoscale Zero-Valent Iron (nZVI) in Oxygenated Water. *RSC Adv.* **2014**, *4*, 57377–57382.
- (31) Liu, A.; Liu, J.; Zhang, W.-x. Transformation and Composition Evolution of Nanoscale Zero Valent Iron (nZVI) Synthesized by

- Borohydride Reduction in Static Water. *Chemosphere* **2015**, *119*, 1068–1074.
- (32) Trevors, J. T.; Stratton, G. W.; Gadd, G. M. Cadmium Transport, Resistance, and Toxicity in Bacteria, Algae, and Fungi. *Can. J. Microbiol.* **1986**, *32*, 447–64.
- (33) Adeleye, A. S.; Stevenson, L. M.; Su, Y.; Nisbet, R. M.; Zhang, Y.; Keller, A. A. Influence of Phytoplankton on Fate and Effects of Modified Zerovalent Iron Nanoparticles. *Environ. Sci. Technol.* **2016**, *50*, 5597–605.
- (34) Prasad, M. N.; Drej, K.; Skawinska, A.; Strzalka, K. Toxicity of Cadmium and Copper in *Chlamydomonas Reinhardtii* Wild-Type (Wt 2137) and Cell Wall Deficient Mutant Strain (Cw 15). *Bull. Environ. Contam. Toxicol.* **1998**, *60*, 306–11.
- (35) Mosulen, S.; Dominguez, M. J.; Vigar, J.; Vilchez, C.; Guiraum, A.; Vega, J. M. Metal Toxicity in *Chlamydomonas Reinhardtii*. Effect on Sulfate and Nitrate Assimilation. *Biomol. Eng.* **2003**, *20*, 199–203.
- (36) Cain, J. R.; Allen, R. K. Use of a Cell Wall-Less Mutant Strain to Assess the Role of the Cell Wall in Cadmium and Mercury Tolerance by *Chlamydomonas Reinhardtii*. *Bull. Environ. Contam. Toxicol.* **1980**, *25*, 797–801.
- (37) Jammers, A.; Blust, R.; De Coen, W.; Griffin, J. L.; Jones, O. A. An Omics Based Assessment of Cadmium Toxicity in the Green Alga *Chlamydomonas Reinhardtii*. *Aquat. Toxicol.* **2013**, *126*, 355–64.
- (38) Macfie, S. M.; Tarmohamed, Y.; Welbourn, P. M. Effects of Cadmium, Cobalt, Copper, and Nickel on Growth of the Green-Alga *Chlamydomonas Reinhardtii* - the Influences of the Cell-Wall and Ph. *Arch. Environ. Contam. Toxicol.* **1994**, *27*, 454–458.
- (39) Aguilera, A.; Amils, R. Tolerance to Cadmium in *Chlamydomonas* Sp. (Chlorophyta) Strains Isolated from an Extreme Acidic Environment, the Tinto River (Sw, Spain). *Aquat. Toxicol.* **2005**, *75*, 316–29.
- (40) Gillet, S.; Decottignies, P.; Chardonnet, S.; Le Marechal, P. Cadmium Response and Redoxin Targets in *Chlamydomonas Reinhardtii*: A Proteomic Approach. *Photosynth. Res.* **2006**, *89*, 201–11.
- (41) Nagel, K.; Adelmeier, U.; Voigt, J. Subcellular Distribution of Cadmium in the Unicellular Green Alga *Chlamydomonas Reinhardtii*. *J. Plant Physiol.* **1996**, *149*, 86–90.
- (42) Hu, S.; Lau, K. W.; Wu, M. Cadmium Sequestration in *Chlamydomonas Reinhardtii*. *Plant Sci.* **2001**, *161*, 987–996.
- (43) Boparai, H. K.; Joseph, M.; O'Carroll, D. M. Cadmium (Cd²⁺) Removal by Nano Zerovalent Iron: Surface Analysis, Effects of Solution Chemistry and Surface Complexation Modeling. *Environ. Sci. Pollut. Res.* **2013**, *20*, 6210–6221.
- (44) Moulder, J.; Stickle, W.; Sobol, P. E.; Bomben, K. *Handbook of X-Ray Photoelectron Spectroscopy. A Reference Book of Standard Spectra for Identification and Interpretation of Xps Data*; Physical Electronics: Chanhassen, MN, 1995.
- (45) Li, X. Q.; Zhang, W. X. Sequestration of Metal Cations with Zerovalent Iron Nanoparticles - a Study with High Resolution X-Ray Photoelectron Spectroscopy (Hr-Xps). *J. Phys. Chem. C* **2007**, *111*, 6939–6946.
- (46) Baltrusaitis, J.; Cwiertny, D. M.; Grassian, V. H. Adsorption of Sulfur Dioxide on Hematite and Goethite Particle Surfaces. *Phys. Chem. Chem. Phys.* **2007**, *9*, 5542–54.
- (47) Zhai, L.; Liao, X.; Chen, T.; Yan, X.; Xie, H.; Wu, B.; Wang, L. Regional Assessment of Cadmium Pollution in Agricultural Lands and the Potential Health Risk Related to Intensive Mining Activities: A Case Study in Chenzhou City, China. *J. Environ. Sci.* **2008**, *20*, 696–703.
- (48) Li, Z.; Greden, K.; Alvarez, P. J.; Gregory, K. B.; Lowry, G. V. Adsorbed Polymer and Nom Limits Adhesion and Toxicity of Nano Scale Zerovalent Iron to *E. Coli*. *Environ. Sci. Technol.* **2010**, *44*, 3462–7.
- (49) Stevenson, L. M.; Dickson, H.; Klanjscek, T.; Keller, A. A.; McCauley, E.; Nisbet, R. M. Environmental Feedbacks and Engineered Nanoparticles: Mitigation of Silver Nanoparticle Toxicity to *Chlamydomonas Reinhardtii* by Algal-Produced Organic Compounds. *PLoS One* **2013**, *8*, e74456.
- (50) Adeleye, A. S.; Keller, A. A. Interactions between Algal Extracellular Polymeric Substances and Commercial TiO₂ Nanoparticles in Aqueous Media. *Environ. Sci. Technol.* **2016**, *50*, 12258–12265.
- (51) Adeleye, A. S.; Conway, J. R.; Perez, T.; Rutten, P.; Keller, A. A. Influence of Extracellular Polymeric Substances on the Long-Term Fate, Dissolution, and Speciation of Copper-Based Nanoparticles. *Environ. Sci. Technol.* **2014**, *48*, 12561–8.
- (52) Miao, A. J.; Schwehr, K. A.; Xu, C.; Zhang, S. J.; Luo, Z.; Quigg, A.; Santschi, P. H. The Algal Toxicity of Silver Engineered Nanoparticles and Detoxification by Exopolymeric Substances. *Environ. Pollut.* **2009**, *157*, 3034–41.
- (53) Kim, J. Y.; Kim, K. T.; Lee, B. G.; Lim, B. J.; Kim, S. D. Developmental Toxicity of Japanese Medaka Embryos by Silver Nanoparticles and Released Ions in the Presence of Humic Acid. *Ecotoxicol. Environ. Saf.* **2013**, *92*, 57–63.
- (54) Hall, S.; Bradley, T.; Moore, J. T.; Kuykindall, T.; Minella, L. Acute and Chronic Toxicity of Nano-Scale TiO₂ particles to Freshwater Fish, Cladocerans, and Green Algae, and Effects of Organic and Inorganic Substrate on TiO₂ toxicity. *Nanotoxicology* **2009**, *3*, 91–97.
- (55) Kilham, S. S.; Kreeger, D. A.; Lynn, S. G.; Goulden, C. E. Combo: A Defined Freshwater Culture Medium for Algae and Zooplankton. *Hydrobiologia* **1998**, *377*, 147.