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In situ remediation-released zero-valent iron nanoparticles impair soil ecosystems health: A *C. elegans* biomarker-based risk assessment

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HIGHLIGHTS

- Fe^0 NPs induced infertility risk in *C. elegans*.
- A *C.elegans*-based probabilistic risk assessment model is developed.
- *In situ* remediation-released Fe^0 NPs impair soil ecosystems health.

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ABSTRACT

There is considerable concern over the potential ecotoxicity to soil ecosystems posed by zero-valent iron nanoparticles (Fe^0 NPs) released from *in situ* environmental remediation. However, a lack of quantitative risk assessment has hampered the development of appropriate testing methods used in environmental applications. Here we present a novel, empirical approach to assess Fe^0 NPs-associated soil ecosystems health risk using the nematode *Caenorhabditis elegans* as a model organism. A Hill-based dose-response model describing the concentration–fertility inhibition relationships was constructed. A Weibull model was used to estimate thresholds as a guideline to protect *C. elegans* from infertility when exposed to waterborne or foodborne Fe^0 NPs. Finally, the risk metrics, exceedance risk (ER) and risk quotient (RQ) of Fe^0 NPs in various depths and distances from remediation sites can then be predicted. We showed that under 50% risk probability (ER = 0.5), upper soil layer had the highest infertility risk (95% confidence interval: 13.18–57.40%). The margins of safety and acceptable criteria for soil ecosystems health for using Fe^0 NPs in field scale applications were also recommended. Results showed that *RQs* are larger than 1 in all soil layers when setting a stricter threshold of ~1.02 mg L^{-1} of Fe^0 NPs. This *C. elegans* biomarker-based risk model affords new insights into the links between widespread use of Fe^0 NPs and environmental risk assessment and offers potential environmental implications of metal-based NPs for *in situ* remediation.

1. Introduction

Concerns have been raised about potential impacts on human health and soil ecosystems posed by metal-based nanoparticles (NPs) owing to rapid development and widespread use of nanomaterials [1,2]. Among the metal-based NPs being widely used in environmental nanotechnologies, zero-valent iron (Fe^0) NPs have been extensively applied for *in situ* remediation worldwide [3–8]. The consumption of Fe^0 NPs is approximately 70% of all remediation sites [9]. The large amounts of Fe^0 NPs that released into environments have caused the ecotoxicity impacts on soil ecosystems [9–12]. Fe^0 NPs have high efficiency in reducing hardly removed pollutants e.g., poly-aromatic hydrocarbons, trichloroethylene, arsenic, cadmium, radioactive, and virus and have the advantages for replacing traditional methods [13–15]. The concentrations of Fe^0 NPs used for *in situ* remediation could be as high as 300,000 mg L^{-1} [4].

Investigations on the toxicity of Fe^0 NPs are growing yet limited. It was found that Fe^0 NPs caused developmental and reproductive toxicity, phytotoxicity, cytotoxicity, increase of oxidative stress, and disruption of microbial community in aquatic and terrestrial ecosystems [16–24]. In aquatic ecosystems, Fe^0 NPs were found to exhibit toxicity to phytoplankton, water flea, and microalgae [20,25,26]. Moreover, increase of mortality, higher bioaccumulation, and developmental toxicity were also observed in medaka exposed to Fe^0 NPs [19,21].

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Toxicity of Fe^0 NPs in soil ecosystems has been found in both earthworms and the nematode *Caenorhabditis elegans* [11,24,27]. Fe^0 NPs with concentrations ranged from 500–1500 mg kg^{-1} were most likely to cause a decrease of reproduction, an increase of mortality and avoidances in earthworms [11]. A concentration-dependent DNA damage was also observed in earthworms [27]. Moreover, Fe^0 NPs in the range of 500–10,000 mg L^{-1} were likely to pose the reproductive and developmental toxicity in *C. elegans* [24]. Inhibition of rice seedlings growth and induction of chlorosis by Fe^0 NPs were also observed in plants [28].

Nematode is a large population accounting for approximately 80% of all earth animals in various trophic levels in many ecosystems [29]. They effectively regulate bacterial population, influence nitrogen cycle by nitrogen mineralization and can be found as deep as 3.6 km on earth [30,31]. *C. elegans* are nematodes that freely reside in the soil and have been commonly used for assessing ecological risk in pore water, soil, and sediments [32–37]. Therefore, it is worthwhile to use *C. elegans* as a model organism to assess the potential risks posed by Fe^0 NPs in soil ecosystems.

Although there are increasing studies investigating the potential toxicity of Fe^0 NPs, information of predicting and evaluating the hazards of environmental concentrations of Fe^0 NPs in soil ecosystems is scarce and limited. Therefore, we intended to develop a probabilistic risk-based framework for assessing Fe^0 NPs-associated soil ecosystems health risk by using biomarker derived from *C. elegans*. The purposes of this study were fourfold: (1) to obtain dose-response profiles based on laboratory *C. elegans* exposure experiments, (2) to analyze acceptable levels of Fe^0 NPs based on data of inhibition concentrations yielding fertility to *C. elegans*, (3) to estimate exceedance risks and risk quotients of Fe^0 NPs in various depths and distances from the injection sites under the waterborne- and foodborne-based thresholds, and (4) to implicate the potential environmental risk management of Fe^0 NPs for *in situ* remediation.

2. Materials and methods

2.1. Fe^0 NPs characterization

Fe^0 NPs was synthesized by the method of borohydride reduction in the presence of carboxymethyl cellulose (CMC, MW = 90,000) as described in previous studies [38,39]. The size distribution of freshly prepared Fe^0 NPs was analyzed by dynamic light scattering (DLS) machine after sonication for 30 min (Delsa Nano C; Beckman Coulter, CA, USA). The morphological feature of Fe^0 NPs was captured by transmission electron microscopy (TEM; JEM1200EXII; Jeol Ltd., Tokyo, Japan). The time-dependent dynamic behaviors of Fe^0 NPs in dosing solutions such as pH, dissolved oxygen (DO), oxidation reduction potential (ORP), and iron speciation were all described in the previous study [19].

2.2. Study data and experimental design

Study data related to relationships between concentrations of waterborne Fe^0 NPs and inhibition of fertility (%) of *C. elegans* were obtained from the published study of Yang et al. [39] with new experimental data introduced from the present waterborne and foodborne reproduction bioassay. The experimental assays of the effects of waterborne and foodborne Fe^0 NPs on fertility of *C. elegans* were performed based on Boyd et al. [40].

Briefly, for the reproduction bioassay of waterborne Fe^0 NPs in *C. elegans*, synchronized wild-type L4 larvae were exposed to various concentrations of 5, 25, 50, 100, 250, 500 mg L^{-1} of Fe^0 NPs for 48 h in the presence of *Escherichia coli* OP50 (optical density (O.D.) = 0.4). Subsequently, the offspring of each nematode were scored. For the reproduction bioassay of foodborne Fe^0 NPs in *C. elegans*, *E. coli* OP50 (O.D. = 0.4) were exposed to Fe^0 NPs with various concentrations of 5, 25, 50, 100, 250, and 500 mg L^{-1} for 16 h and washed 5–6 times with distilled water to eliminate the residual irons in the medium.

However, some adhering irons out of the bacteria cells could not be removed completely by repeating washing steps. Synchronized wild-type L4 larvae of *C. elegans* were then fed with Fe^0 NPs-exposed *E. coli* OP50 for 48 h and offspring of each nematode were scored. At least 6 independent experiments were performed and approximately 6 worms were examined per condition in each trial.

2.3. Effect modeling

Concentration–inhibition of fertility profiles was constructed by fitting the Hill model to published and present experimental data based on reproduction bioassays of *C. elegans* exposed to waterborne and foodborne Fe^0 NPs,

$$I(C) = \frac{100}{1 + \left(\frac{IC50}{C}\right)^n}, \quad (1)$$

where $I(C)$ is the inhibition of fertility (%) to a specific exposure concentration of Fe^0 NPs, C (mg L^{-1}), IC50 is the concentration of Fe^0 NPs that cause 50% inhibition of fertility (mg L^{-1}), and n is the fitted Hill coefficient in that $n = 1$ represents a linear response fashioned as the Michaelis-Menton mode at low concentrations, and $n > 1$ represents a superlinear (sigmoidal) response that is ultrasensitive to the toxicants.

Both IC10 and IC50 values representing 10% and 50% inhibition of fertility were selected to compare and were considered as the potential risk baselines of waterborne and foodborne Fe^0 NPs to *C. elegans*. The IC10 and IC50 data were adopted from the model fitted to Eq. (1) probabilistically. The IC10 and IC50 cumulative distribution functions (CDFs) were obtained and can be expressed as a conditional probability function,

$$P(I|C) = \Phi\left(\frac{100}{1 + \left(\frac{IC50}{C}\right)^n}\right), \quad (2)$$

where $\Phi(\bullet)$ is the cumulative standard normal distribution.

2.4. Predictive risk threshold modeling

A three-parameter Weibull threshold model was employed to best fit IC10 and IC50 CDF toxicity data to estimate threshold concentrations that can be used as a guideline to protect *C. elegans* from infertility when exposed to waterborne or foodborne Fe^0 NPs. The toxicity data were obtained from estimated IC10 and IC50 CDFs (Eq. (2)). The Weibull threshold model can be written as

$$F(C) = 1 - \exp\left[-\left(\frac{C-\gamma}{\alpha}\right)^{\beta}\right], C > \gamma > 0, \alpha > 0, \beta > 0, \quad (3)$$

where $F(C)$ represents IC10 or IC50 CDF data in correspondence to specific Fe^0 NPs concentrations, α is the scale parameter that has the effect on distribution as a change of the abscissa scale, β is the shape parameter that equals to the slope of the line in the CDF, and γ represents the fitted threshold (mg L^{-1}).

The Weibull threshold model was used to fit to the extracted 2.5th, 5th, 50th, 95th, and 97.5th percentiles of IC10 and IC50 CDF data.

2.5. Risk characterization

To characterize the exposure risk of Fe^0 NPs in soil ecosystem, a probabilistic risk assessment model linking the Fe^0 NPs exposure model with the Hill-based dose-response model was implemented. Following the Bayesian inference, the cumulative infertility risk $R(C_E)$ for *C. elegans* under certain environmental relevant concentration C_E (i.e., the posterior probability) were the products of a prior probability $P(C_E)$ and a likelihood $P(I|C_E)$ describing the conditional probability of the infertility effect I on *C. elegans* given certain C_E,

$$R(C_E) = P(C_E) \times P(I|C_E). \quad (4)$$

The likelihood can be obtained by incorporating the Hill model-based dose-response relationships. The exceedance risk profiles can then be derived as $1 - R(C_E)$.

To evaluate the adverse effects posed by waterborne and foodborne Fe^0 NPs on soil ecosystem, the risk quotient (RQ) model was employed for assessing the potential environmental risk as,

$$RQ = \frac{C_E}{\gamma}, \quad (5)$$

where γ is the predicted threshold value estimated by Eq. (3) and denoted as a predicted no-effect concentration (PNEC).

Here C_E and γ are estimated probabilistically. $RQ > 1$ implies Fe^0 NPs pose a potential hazard to the fertility of *C. elegans* by waterborne or foodborne Fe^0 NPs, whereas $RQ < 1$ indicates that no significant risk is posed by environmental relevant concentrations of Fe^0 NPs.

2.6. Uncertainty analysis

TableCurve 2D (Version 5.01, AISN Software, Mapleton, OR, USA) was used to perform all model fittings. A Monte Carlo analysis was used to perform the uncertainties in the concentration–infertility relationships and predicted risks. We generated 2.5th and 97.5th percentiles as the 95% confidence interval (CI) for all fitted models. The Monte Carlo simulation was implemented using Crystal Ball software (Version 2000.2, Decisioneering, Denver, CO, USA). The results showed that 10,000 iterations are sufficient to ensure the stability of outcomes. The overall probability risk assessment framework of this study is illustrated in Fig. 1.

3. Results

3.1. Concentration–infertility assessment

The constructed Hill model-based relationships between waterborne and foodborne Fe^0 NPs concentrations and fertility inhibition in *C. elegans* are shown in Fig. 2A and C (see Supplementary Table S1 for reproduction bioassay data). The size distribution of Fe^0 NPs was well fitted ($r^2 = 0.99$) to a lognormal (LN) function with a geometric mean (gm) of 10.46 nm and a geometric standard deviation (gsd) of 1.14, denoted as LN(10.46, 1.14) (Fig. 2B). The fitted result is also agreed well with the original size distribution measured by a dynamic light scattering (DLS) machine (Fig. 2B).

The fitted IC10 and IC50 of waterborne Fe^0 NPs were 11.79 (95% CI: 4.20–33.20) and 69.30 (37.42–126.26) mg L^{-1}, respectively, with a Hill coefficient n of 1.24 (Fig. 2A, Supplementary Table S2). On the other hand, the fitted IC10 and IC50 of foodborne Fe^0 NPs were 11.77 (4.70–29.70) and 81.50 (50.51–131.31) mg L^{-1}, respectively, with a Hill coefficient n of 1.14 (Fig. 2C, Supplementary Table S2).

3.2. Threshold estimation

Thresholds under the exposure of waterborne or foodborne Fe^0 NPs were estimated by using IC10 and IC50 values extracted from the Hill model-based concentration–infertility profiles (Fig. 2A and C). Both IC10 and IC50 were appropriately estimated probabilistically with distribution profiles shown in Fig. 3A and D (Supplementary Table S3). The relationships between probability of causing 10% or 50% infertility and concentrations of waterborne or foodborne Fe^0 NPs can be constructed by fitting the Weibull model to data points extracted from IC10 and IC50 CDFs (Fig. 3B,C,E and F, Supplementary Table S4).

The median threshold estimates for waterborne and foodborne Fe^0 NPs in IC10 CDF (γ_{10}) were 7.32 and 7.92 mg L^{-1} ($r^2 = 0.99$, $p < 0.001$), respectively (Fig. 3C and F, Supplementary Table S4). On the other hand, the median threshold estimates of waterborne and foodborne Fe^0 NPs in IC50 CDF (γ_{50}) were 58.78 and 72.87 mg L^{-1} ($r^2 = 0.99$, $p < 0.001$), respectively (Fig. 3B and E, Supplementary Table S4).

3.3. Environmental relevant concentration determinations

There is limited and scarce information on environmental concentrations of Fe^0 NPs. However, Wei et al. [6] provided the suitable data allowing us to estimate C_E of Fe^0 NPs in soil ecosystems.

Briefly, Fe^0 NPs were injected into three wells in the depth of 18 m, denoted as IW-1, IW-2 and IW-3 (Fig. 4A). Among these three wells, only two wells (IW-1 and IW-2) were continuously monitored for environmental concentrations of Fe^0 NPs. The concentrations of total iron were measured in nested monitoring wells in the distances of 1–3, and 5 m from injection wells in different layers, denoted as #S1-1, #S2-1, #S3-1, and #S5-1 from IW-1 and #S1-2, #S2-2, #S3-2, and #S5-2 from IW-2 (Fig. 4A). Each nested well was embedded with three separate wells with different descending levels of 6, 12, and 18 m, referred to as upper, middle, and bottom layers (Table 1). We collated and reanalyzed monitoring data from Wei et al. [6] as C_E estimates, denoted as S1–S3, and S5 (Table 1). Here S1–3, and S5 represent average total iron concentrations in two adjacent monitoring wells with distances of 1–3, and 5 m from injection site (Fig. 4A).

After obtaining environmental concentrations of Fe^0 NPs, the 10,000 iterative Monte Carlo simulations were performed to evaluate the possible C_Es in the multiple distances with different layers in soil. The results showed that maximum C_E was 89.17 (95% CI: 21.22–365.30) mg L^{-1} S3 in upper layer of soil, whereas minimum C_E was 4.18 (1.44–11.93) mg L^{-1} in S2 in middle layer of soil (Table 1). Overall, the C_E estimates in the remediation site ranged from 4.18–89.17 mg L^{-1}.

3.4. Risk estimates

The exceedance risks (ERs) in fertility inhibition of both waterborne and foodborne Fe^0 NPs in multiple distances from injection sites (S1–S3, and S5) within three layers (upper, middle, and bottom) were estimated (Fig. 4B–G). The waterborne Fe^0 NPs had slightly higher ERs than that of foodborne Fe^0 NPs in same distance from injection site in same layer of soil. Specifically, in upper layer of soil, both waterborne and foodborne Fe^0 NPs of S2 and S3 had higher ERs than that of S1 and S5 (Fig. 4B and C). In middle layer, both waterborne and foodborne Fe^0 NPs in S3 and S5 had higher ERs than S1 and S2 (Fig. 4D and E). Whereas in bottom layer, the ERs of both waterborne and foodborne Fe^0 NPs are highest in S5 (Fig. 4F and G). Overall, under 50% risk probability (ER = 0.5), the infertility risks in different layers of soil are in the following order of upper > bottom > middle layer with risk estimates

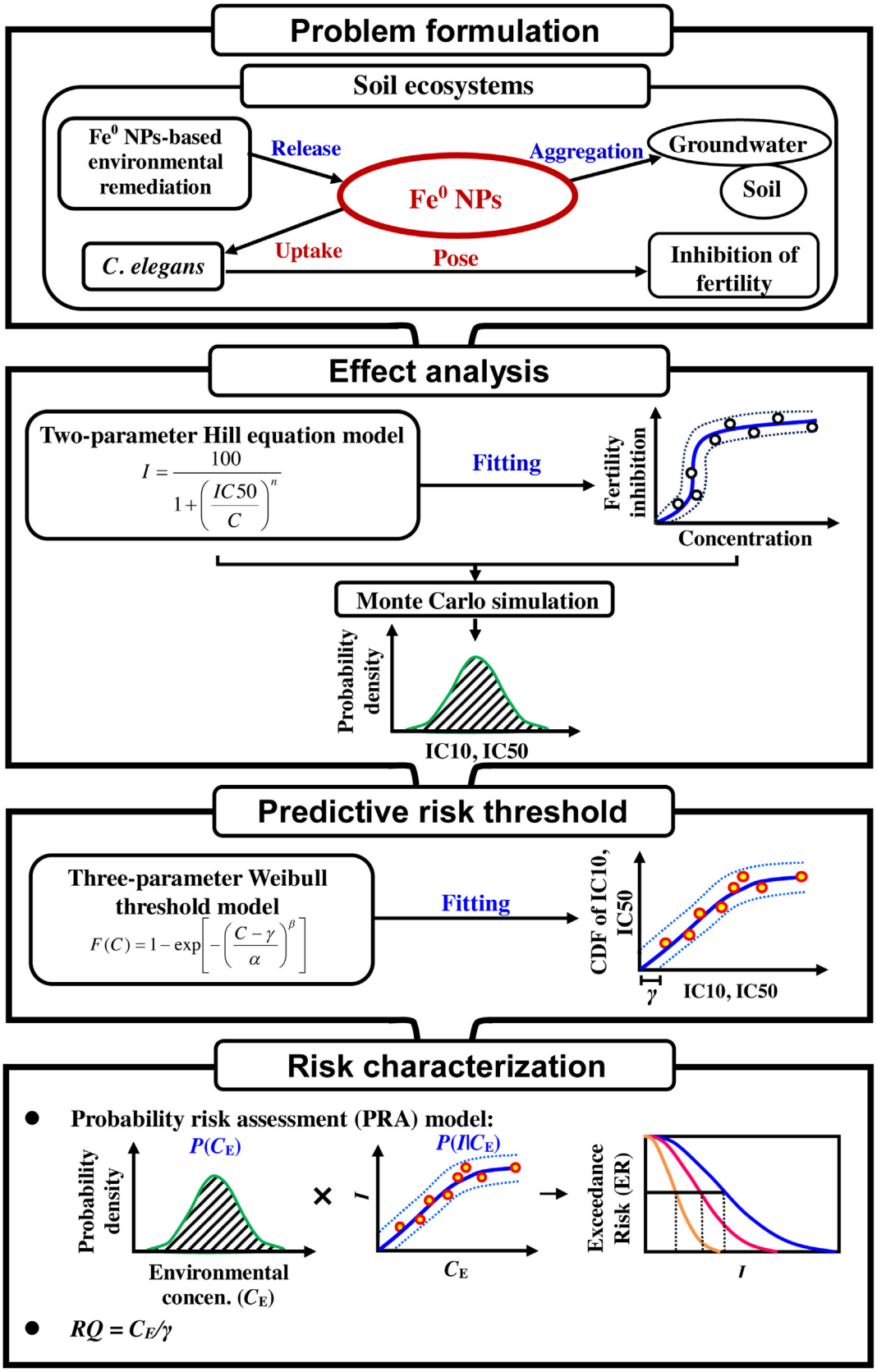

Fig. 1. *C. elegans*-based probabilistic risk assessment framework and computational algorithms applied in this study.

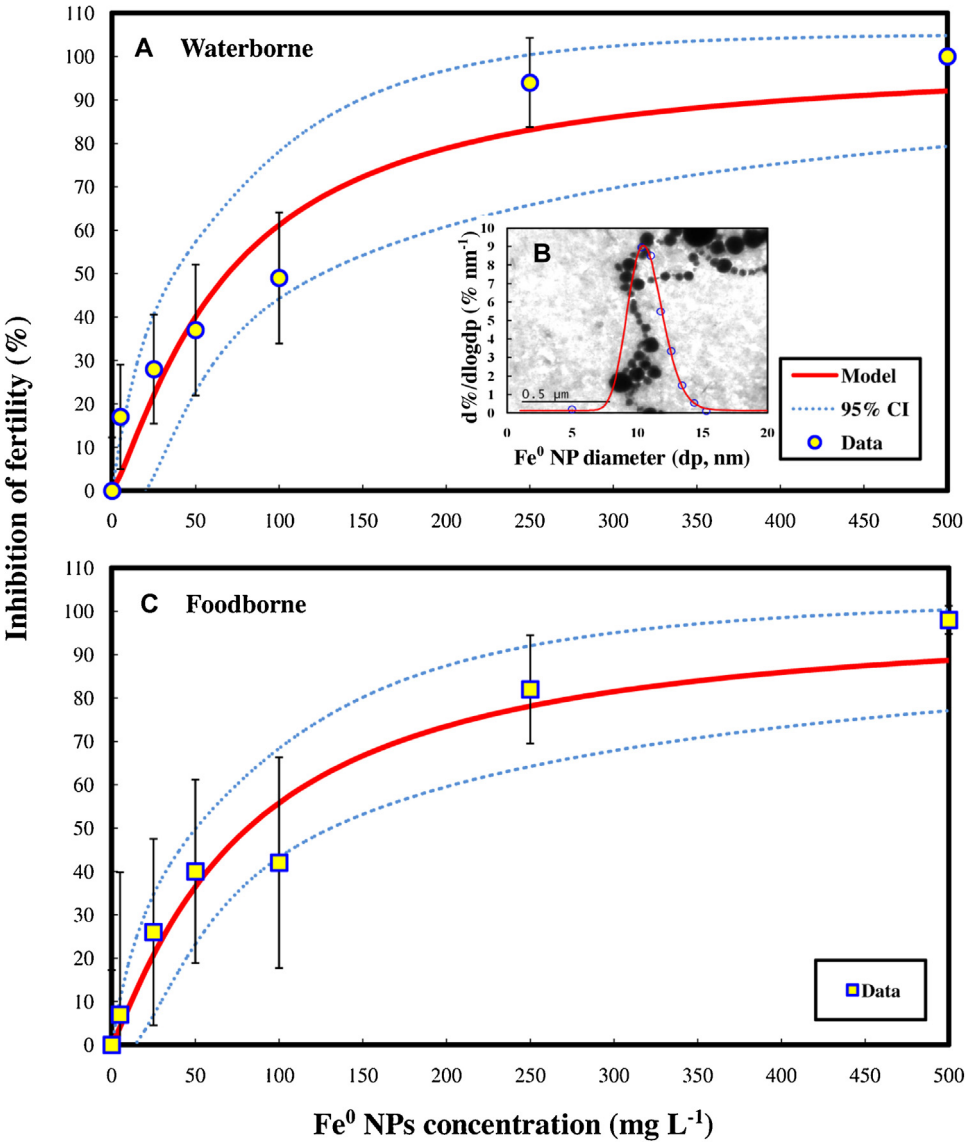

Fig. 2. (A, C) Constructed waterborne and foodborne Fe^0 NPs concentration-inhibition of fertility profiles and (B) Size distributions of Fe^0 NPs suspension. Open circles are size distribution of Fe^0 NPs suspension data, whereas solid circles and squares are concentration-fertility inhibition data of waterborne and foodborne Fe^0 NPs in the model scenarios, respectively. Error bars are standard deviation from the mean.

Table 1
Environmental concentrations of Fe^0 NPs (mg L^{-1}) in the study pumping wells in Taiwan[a].

	Locations of sampling point[b]			
	S1	S2	S3	S5
Upper layer	29.79 ± 18.21[c]	100.91 ± 74.68	117.54 ± 99.68	18.70 ± 11.38
	25.31[d]	80.78	89.17	15.93
	(8.42–76.69)	(22.41–302.99)	(21.22–365.30)	(5.32–49.40)
Middle layer	4.84 ± 2.82	5.32 ± 2.29	7.53 ± 2.88	7.44 ± 3.77
	4.18	4.90	7.01	6.66
	(1.44–11.93)	(2.19–11.10)	(3.40–14.23)	(2.61–16.84)
Bottom layer	23.80 ± 15.93	13.46 ± 5.80	19.39 ± 9.47	33.95 ± 12.93
	20.10	12.47	17.26	31.57
	(6.14–65.99)	(5.46–28.35)	(7.01–42.95)	(15.17–65.98)

[a] Estimated from Wei et al. [6].
[b] See Fig. 4A for the geometric locations of sampling point: *S*1 = (#*S*1–1 + #*S*1-2)/2, *S*2 = (#*S*2–1 + #*S*2-2)/2, *S*3 = (#*S*3–1 + #*S*3-2)/2, and *S*5 = (#*S*5–1 + #*S*5-2)/2.
[c] Mean ± S.D.
[d] Median (95% CI).

of 13.18–57.40, 10.34–27.02, and 2.93–5.68%, respectively, for both waterborne and foodborne Fe^0 NPs.

Waterborne Fe^0 NPs had higher *RQ*s compared with that of foodborne Fe^0 NPs in the same distance from injection site of same layer of soil (Fig. 5A and B). The *RQ*s are highest in upper followed

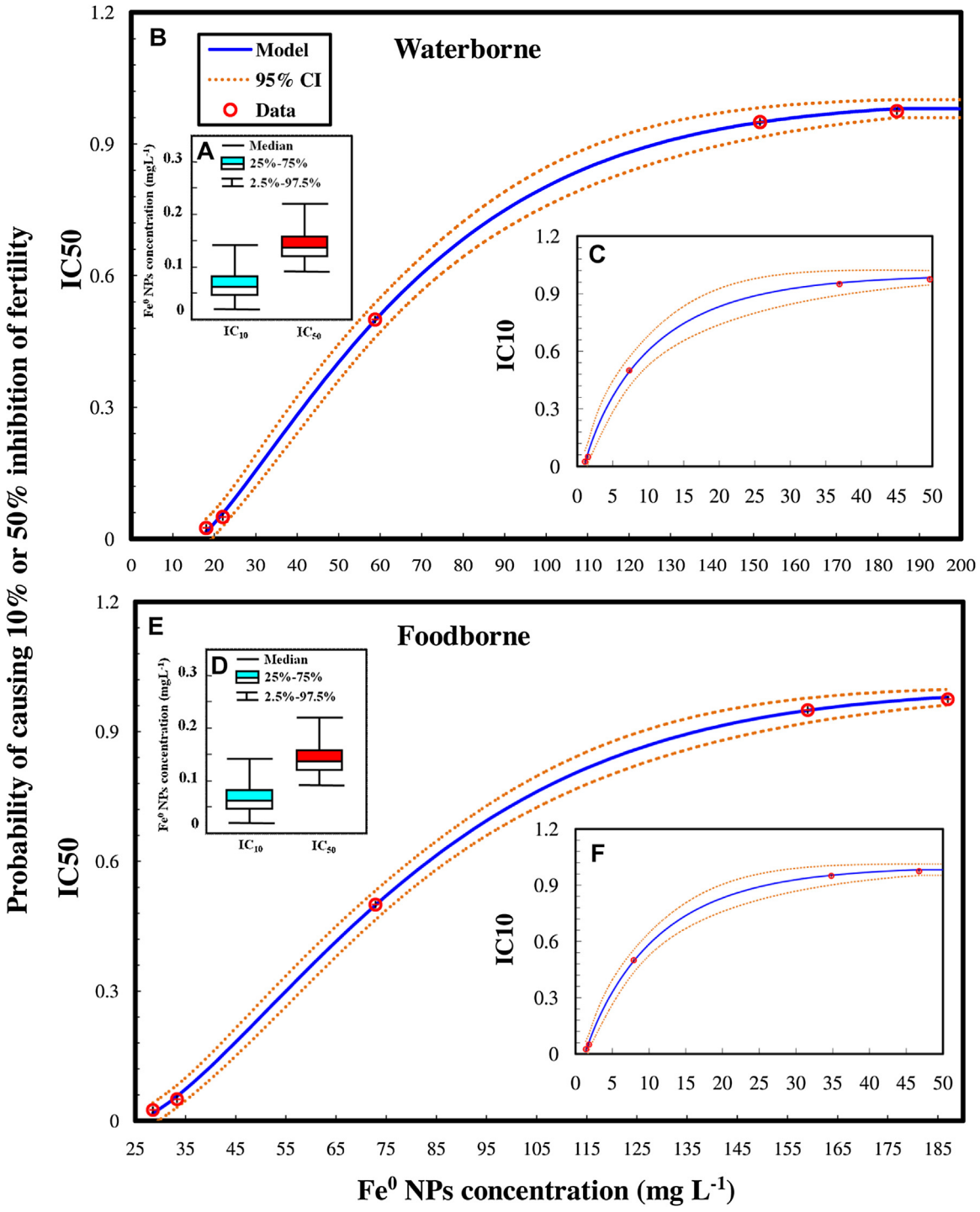

Fig. 3. (A, D) Box and whisker plots of Fe^0 NPs concentrations corresponding to percentiles of data points extracted from 10% and 50% inhibition concentrations (IC10 and IC50)-derived cumulative distribution functions (CDFs). (B, C) Best fit of the Weibull threshold models to the CDFs of IC50 and IC10 for *C. elegans* exposed to waterborne and to (E, F) foodborne Fe^0 NPs.

by bottom and middle layers with estimates of 0.34–358.14 and 0.21–278.86, 0.35–64.69 and 0.22–50.37, as well as 0.09–16.51and 0.06–12.86, respectively, for waterborne and foodborne Fe^0 NPs (Fig. 5A and B). Results also showed that all *RQs* are larger than 1 in three layers of soil when setting a stricter threshold, i.e., γ_{10} (Fig. 5A), whereas *RQs* are lower in three layers while adopting γ_{50} (Fig. 5B). All *RQs* are larger than 1 except for some *RQs* smaller than 1 in middle and bottom layers of soil while taking γ_{50} as thresholds (Fig. 5B).

4. Discussion

4.1. Exposure and dose-response modeling

Our study indicates that the large amounts in use of Fe^0 NPs with high concentration are highly likely to pose an ecotoxicity threat to soil ecosystems. However, concentrations used in the previous studies [11,24] were not environmental relevant to soil ecosystems. They also pointed out that both the exposure stage and endpoints

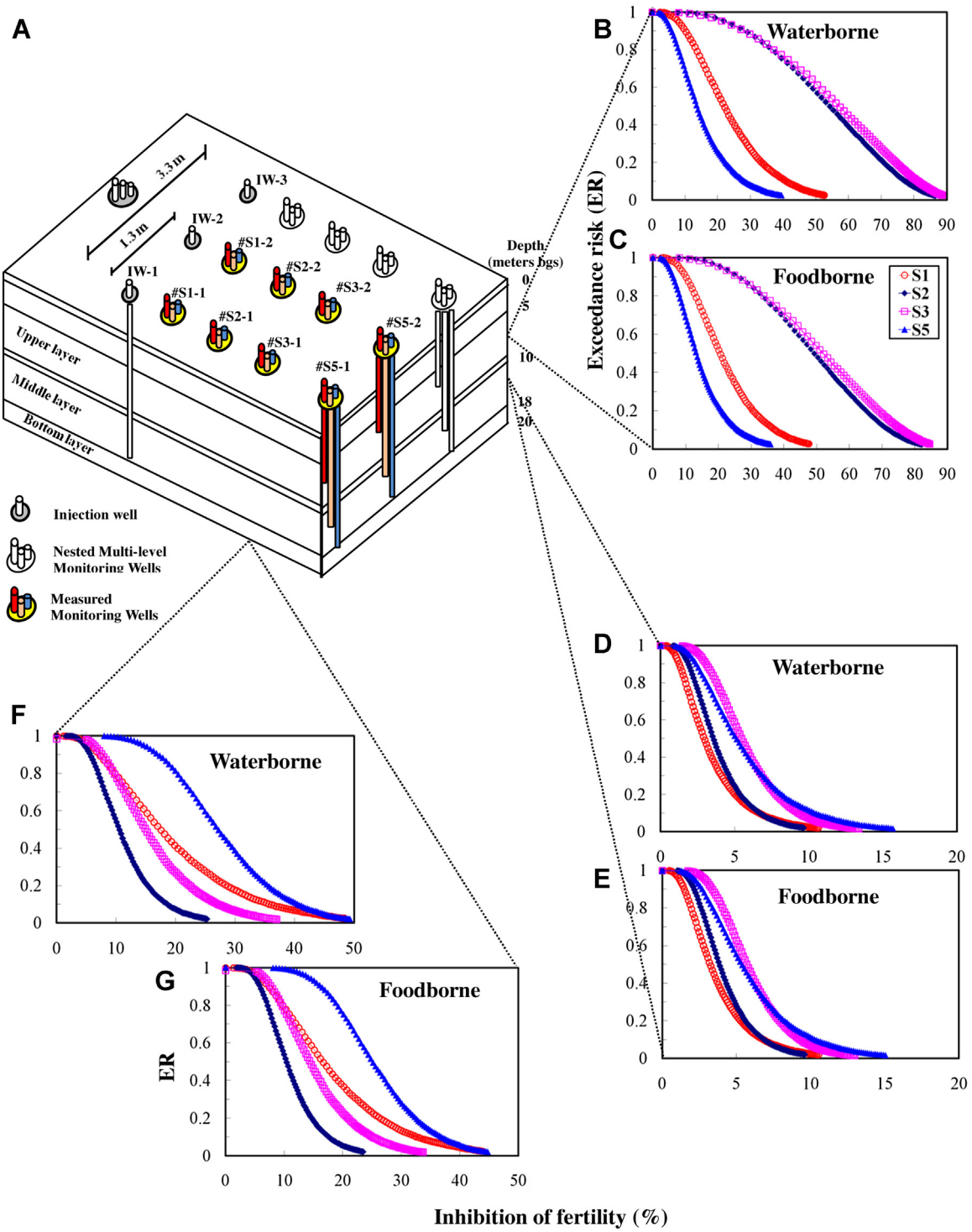

Fig. 4. (A) Schematic of the injection and monitoring wells of Fe^0 NPs test sites. Estimated exceedance risks (ERs) curves for *C. elegans* exposed to waterborne or foodborne Fe^0 NPs in (B, C) upper, (D, E) middle, and (F, G) bottom layers of the nested wells. Depth unit is the meter below ground surface (bgs).

may not reflect the most sensitive effects posed by Fe^0 NPs. Due to the limited knowledge of effects posed by Fe^0 NPs in low doses, we used both waterborne and foodborne Fe^0 NPs in environmental relevant concentrations to treat *C. elegans*.

Concentrations of Fe^0 NPs used for *in situ* remediation are approximately 1000–300,000 mg L^{-1} [4,12], which is much higher than environmental relevant concentrations ranging from 4.18–89.17 mg L^{-1} estimated in this study. In the field-scale trials, Fe^0 NPs are injected directly into the contaminated sites or through permeable reactive barriers (PRBs) [9]. The Fe^0 NPs can be transported and detected in the distances of approximately 1–5 m [6,41–43]. The concentrations used in this study for dose-response modeling are environmental relevant based on C_E estimations.

The inhibition of fertility was adopted as the most sensitive endpoint among all biological responses posed by Fe^0 NPs for the dose-response modeling. We have compared the toxicity of waterborne and foodborne Fe^0 NPs and found that there are similar fashions presenting in dose-response modeling, suggesting that

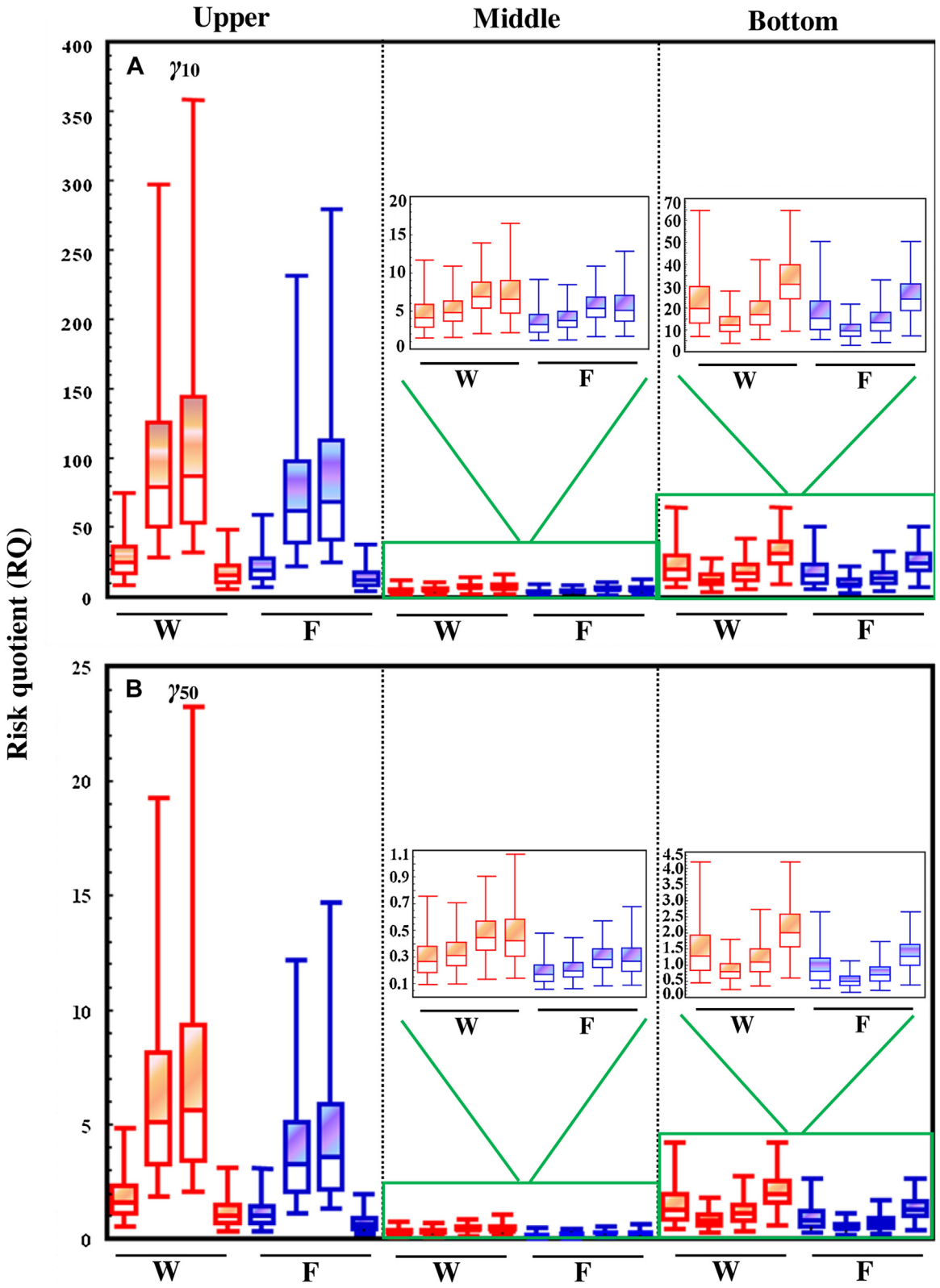

Fig. 5. Box and whisker plots of risk quotients (*RQs*) for *C. elegans* exposed to waterborne and foodborne Fe^0 NPs in different layers of soil in the criteria of (A) γ_{10}-, and (B) γ_{50}-based thresholds. The capitals W and F denote *C. elegans* exposed to waterborne and foodborne Fe^0 NPs, respectively. Box represents median with 25–75%-tile and whisker represents 2.5–97.5%-tile.

reproductive toxicity of Fe^0 NPs is attributable to both waterborne and foodborne exposure routes. In addition, the inhibition of fertility in *C. elegans* is slightly lower in the exposure of foodborne Fe^0 NPs than that of waterborne Fe^0 NPs, implicating that the toxicity of Fe^0 NPs in *C. elegans* could be mainly deduced from dietary exposure.

Our result is consistent with Yu et al. [44] that foodborne toxicants exhibited slightly lower toxicity at growth in *C. elegans* than

that of waterborne toxicants with clean bacteria. Kim et al. [45] also found that reproductive toxicity posed by Au NPs could be transferred from different trophic level to *C. elegans*, which is in agreement with our result. *C. elegans* is a filter feeder that assimilates both liquid and suspended particles and excretes most of the liquid through pharyngeal pumping, supporting the similar influences in reproductive toxicity posed by waterborne and foodborne Fe^0 NPs.

Another factor contributing to similar effects of foodborne and waterborne Fe^0 NPs exhibited in *C. elegans* is the interaction between oxidized-Fe NPs and cell membrane of *E. coli* OP50 that makes it difficult, if not impossible, to eliminate the residual Fe^0 NPs on cell membrane by washing of the medium [46].

Although similar trends in infertility were observed, IC50 of foodborne was higher than that of waterborne due in part to the differences in actual exposed total iron concentrations of waterborne and foodborne Fe^0 NPs. The actual exposed concentrations of total iron in waterborne and foodborne assays could be different because some Fe^0 NPs were excreted in the trophic level of *E. coli* OP50 and most of Fe^0 NPs were removed during washing of the medium.

Our result is consistent with Yu et al. [44] that actual concentrations of waterborne toxicants added with clean foods were higher than that of foodborne toxicants, revealing that waterborne toxicants pose higher toxicity in the growth of *C. elegans* than that of foodborne exposure. Previous study also evidenced that metal ions in aqueous state caused higher body burden and toxicity in *C. elegans*, supporting our results and indicating that the different routes of toxicant exposure could lead to differences in the extent of toxicity in organisms [47].

4.2. Threshold estimates and risk assessment

Although there is no appropriate regulation and risk management for the *in situ* use of Fe^0 NPs based on the risk assessment framework, two kinds of thresholds were estimated and adopted as the criteria to compare the infertility risks between waterborne and foodborne Fe^0 NPs exposures. The waterborne-based threshold estimates of γ_{10} and γ_{50} were close to but to some extent stricter than those of foodborne due to the slight difference in fertility inhibition.

Our threshold estimates were much stricter and lower compared to *in situ* concentrations compiled in the previous studies partly due to the sensitive biological responses exhibited by *C. elegans* [12,48]. On the other hand, most of the remediation sites that used Fe^0 NPs were located in USA, whereas only a small number of pilot tests and remediation have been conducted in Europe [9,12].

In Europe, most of the Fe^0 NPs concentrations ranged from 1000 to 10,000 mg L^{-1}, which were 980–9800 and 64–640 times higher than waterborne-based threshold estimates, whereas 763–7634 and 40–400 times higher than foodborne-based thresholds [12]. Although the thresholds are difficult to follow, our threshold estimates could be able to set as the most stringent criteria for long-term safety in soil ecosystems.

We found that the ERs of waterborne Fe^0 NPs in three layers of soil remained almost the same as foodborne Fe^0 NPs. Moreover, upper and middle layers of soil were found to exhibit the highest and lowest ERs, respectively, suggesting that total iron concentrations in soil were not dependent on depths of soil. There is limited information of transport and fate of Fe^0 NPs in soil or groundwater. The environmental concentrations and transported distances of Fe^0 NPs could be varied due to the complexities of injected velocity, hydraulic gradient and conductivity, aquifer heterogeneity, composition of soil matrix, and aggregation of Fe^0 NPs [9,49,50].

In this study, the longest distance that Fe^0 NPs could be delivered was 5 m and estimated concentrations of total iron were in the range of 4.18–89.17 mg L^{-1} [6]. The results of our study are also supported by Johnson et al. [50], demonstrating that Fe^0 NPs in the distance of 1 m from injection sites were almost oxidized and total iron concentrations were decreased from 900 to approximately 25 mg L^{-1} in the field trial, close to our estimated environmental concentrations.

Consistent in the results of ERs, our study revealed *RQ*s of waterborne or foodborne Fe^0 NPs in upper and middle layers of soil exhibited the highest and lowest *RQ*s either in the criteria of γ_{10}- or γ_{50}-based thresholds. The *RQ*s of waterborne are slightly higher than that of foodborne Fe^0 NPs in their corresponding delivery distances due to the little differences in the threshold levels of γ_{10} or γ_{50} between waterborne and foodborne Fe^0 NPs. Considering the information of threshold levels and toxicity of Fe^0 NPs from different trophic levels are scarce and limited, the *RQ*s of both waterborne and foodborne Fe^0 NPs could provide new insights into the links between widespread use of Fe^0 NPs and environmental risk management.

4.3. Limitations and implications

In this paper, we have quantified the ecological risks posed by Fe^0 NPs released from an *in situ* remediation site and have recommended the margins of safety and acceptable criteria. The regulation of *in situ* remediation by using Fe^0 NPs requires evaluation of the potential hazards for human and soil environment. To date, soil ecological risk assessment of metal-based NPs is taken into account as an essential for making these decisions on a scientifically sound basis [2].

Yet there are large data and conceptual gaps, which our study is attempting to overcome. However, numerous obstacles remain before such *C. elegans* biomarker-based risk assessment is routine. Specifically, the major points requiring further attention are the validation of model estimations with real monitoring data and the development of a holistic approach for risk characterization. We believe that the *C. elegans* biomarker-based risk model for soil ecosystems proposed in this study can be used to assess the risks to humans and other species of exposure to environmental released NPs on remediation and other managed systems.

Previous study indicated that soil biodiversity is increasing recognized as providing multiple benefits to human health [51]. Thus, soil ecosystems are of particular concern. Our paper then provides such perspectives to speed up the assessment process. Instead of traditional risk assessment approach by USEPA [52], our *C. elegans* biomarker-based risk model provides a new tool to assist difficult ecological risk assessment for soil environments and to offer a protocol that can be referred to specific release or use patterns from *in situ* remediation tasks, particular exposure routes or specific ecological receptors, all of which offer a large potential for covering regulatory needs.

Overall, this *C. elegans* biomarker-based risk model affords a new insight into standardization of metal-based NPs risk assessment in soil and offers considerable opportunities for prompting soil ecological assessment through improved management practices that may help mitigate the impacts of external drivers of soil ecosystems' functioning. Most importantly, our research implicates that soil ecosystems health can be maintained and restored if managed properly after Fe^0 NPs releasing from an *in situ* remediation task.

5. Conclusions

We have present a novel, empirical approach based on a *C. elegans* biomarker-based probabilistic risk assessment model to quantitatively assess the potential environmental risks using field scale applications. Two risk metrics of ER and *RQ* for Fe^0 NPs

in various depths and distances from remediation sites were estimated. The margins of safety and acceptable criteria for soil ecosystems health in using *in situ* field scale applications were also recommended. Our risk assessment reveals that *in situ* remediation-released Fe^0 NPs impair soil ecosystems health. We suggest that it is necessary to take both waterborne and foodborne routes into accounts when assessing the risks posed by metal-based NPs in soil ecosystems. We implicate that the *C. elegan* biomarker-based probabilistic risk framework proposed in this study could be employed in assessing the risks to humans and other species of exposure to metal-based NPs and be implemented to the terrestrial ecological risk assessment in the future.

Conflicts of interest

The authors declare that no competing interests exist.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2016.05.070.

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