Synthesis and Characterization of Zero-Valent Iron Nanoparticles, and the Study of Their Effect against the Degradation of DDT in Soil and Assessment of Their Toxicity against Collembola and Ostracods

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ABSTRACT: Of late, novel magnetic nanomaterials have drawn worldwide attention because of the uniqueness in their properties and uses. In our studies, we have prepared nearly monodisperse zero-valent iron nanoparticles (nZVIs) of diameter of less than 60 nm in aqueous medium by a reductive precipitation process and pectin as stabilizing agent. The characterization of these nanoparticles was done by dynamic light scattering and transmission electron microscopy (TEM) techniques. The TEM images confirmed that the average size of the nZVIs was about 25 nm. The resultant nZVIs were then employed to degrade DDT (dichlorodiphenyltrichloroethane) in spiked soil, and their toxicity toward Collembola (Folsomia candida) and Ostracods (Heterocypris incongruens) was measured. The fabricated nZVIs degraded DDT in soil quite effectively. Further, the effects of nZVIs on Collembola and Ostracods were found to be negative. This was due to the oxidation of nZVIs and creation of anoxic conditions thereupon, and the generation of excess Fe(II) in soil. In addition, the negative effects of DDT on ostracod development and Collembola reproduction were found to be quite weak.

INTRODUCTION

DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane], an organochlorine pesticide, is in use extensively worldwide since the 1940s to control agricultural pests and vector-borne diseases like typhus, malaria, yellow fever, encephalitis, and so forth.1−3 It is a persistent organic pollutant, a potent carcinogen, a known teratogen, and an endocrine disruptor.4 DDT and its metabolites have also been shown to cause chronic effects on the nervous system, the liver, the kidneys, and the immune system. Although it has been regulated under the Stockholm convention4 and banned by many western countries in the 1970s owing to environmental concern and toxicity to humans and wildlife, DDT and its metabolites are still found in many places in the world because of their persistency, bioaccumulation, and long-range atmospheric transport.5,6−10 Their persistency in the environment is attributed to their long half-life, which stretches from 4 to 30 years.11 India is yet to ban the production and use of this compound.

Several physical, chemical, and biological methods have been employed to mitigate the menace of DDT and its metabolites from the environment. Some of them are soil excavation,12 incineration,13 washing the contaminated soil with surfactants,14 oxidation processes like chemical oxidation,15 as well as advanced oxidation processes (AOPs) using semiconductor photocatalysts,16 catalytic reactions using metal catalysts like Pd/C,17,18 and bioremediation treatments.3 Among these, AOPs using photocatalysts and metal-catalyzed reactions have been found promising but their prohibitive cost and cumbersome process make them less attractive. More recently, the use of zero-valent iron (ZVI) to degrade DDT in water and soil has become an attractive process because of its ability to remove all chlorines much faster than natural processes under...
moderate conditions. Moreover, ZVI is inexpensive and environmentally friendly. Compared to bulk or macro-scale ZVI, nanoscale ZVI offers larger surface area with high surface activity. Because of their small size, zero-valent iron nanoparticles (nZVIs) can be injected and transported easily in porous media. nZVIs have been successfully used for transformation and detoxification of common environmental pollutants including chlorinated organic compounds (COCs) and pesticides. Of late, some researchers have shown that nZVIs can be used to degrade DDT and its metabolites effectively. In one such study, the concentrations of DDT residues in a contaminated soil were reduced by about 40 percent in 28 h through nZVI addition. Similarly, El-Temesh et al. reported 45 percent reduction in DDT concentration when treating columns containing DDT-contaminated soil with nZVI suspensions. It was also reported that DDT degradation was slower with micron-sized ZVI particles compared to nZVIs. Such studies have affirmed that DDT degradation by nZVIs in a contaminated soil is indeed possible.

ZVI is an effective reductant (E° = −0.44 V) and is capable of reducing COCs like DDT by reductive dehalogenation. The mechanism of this reaction involves the transfer of electrons from metallic iron (Fe⁰) to chlorinated organics which undergo reductive dechlorination.

\[ \text{Fe}^0 \rightarrow \text{Fe}^{2+} + 2e^- \]  
\[ \text{RCl} + \text{H}^+ + 2e^- \rightarrow \text{RH} + \text{Cl}^- \]  
\[ \text{RCl} + \text{Fe}^0 + \text{H}^+ \rightarrow \text{RH} + \text{Fe}^{2+} + \text{Cl}^- \]

Dechlorination is also possible when there is transformation of Fe²⁺ to Fe³⁺.

Several synthetic approaches to fabricate nZVI particles have been reported in the literature. In this study, nZVIs, however, have been synthesized by the reductive precipitation of iron(III) salts, a conventional wet chemical method. The colloidal chemistry of these particles is such that they tend to agglomerate. Therefore, various types of water-soluble organic polymeric coatings like starch, sodium carboxymethyl cellulose, or pectin are used nowadays to prevent their agglomeration and enhance stability. These polymeric surfactants provide long-range steric repulsion forces to particles and eventually achieve their stabilization.

nZVIs are considered as the single largest variety of engineered nanomaterials entering the environment. Despite their use in environmental protection processes for the last 2 decades, the ecotoxicological studies of nZVIs are limited. As a result, their large-scale use in environmental remediation is hampered. However, they are reportedly toxic to pure bacterial cultures of Escherichia coli, Pseudomonas fluorescens, and bacillus subtilis var. niger. The lack of their proper ecotoxicological data and hitherto unknown harmful effects on organisms has hampered the large-scale use of this technology around the world.

The objectives of the present work were to (i) synthesize nZVIs, (ii) identify a good stabilizing agent which prevents the agglomeration of synthesized nZVIs, and (iii) use the synthesized nZVIs against DDT in soil slurries and assessment of their toxicity toward Collemmbola and Ostracods as they represent the key soil organisms in their respective environment.
temperature was 300 °C. The total DDT recovery from the soil was 90.1 ± 5.2%.

**Ecotoxicity Studies. Ostracod Toxicity.** A 6 day direct contact microbio test for mortality/growth-inhibition of Ostracods (Heterocypris incongruens) was performed according to the standard test protocol of Ostracodtoxkit F (Micro-Bio Tests, Nazareth, Belgium), using 24-well trays. Each Ostracodtoxkit F is equipped with all the disposable materials to carry out 3–5 bioassays. Other equipment, in addition to Ostracodtoxkit F, needed for the test were an incubator [25 °C (±1 °C)], a dissecting microscope (magnification 10–12x), and normal laboratory glassware. Three replicates of each soil treatment were taken into account in this study. The study was conducted by taking 0.4 g of soil (dry weight) in each well and mixing it thoroughly with 1 mL of medium-hard EPA water (Ostracodtoxkit F). The soil was then left to settle. Live algae suspension (1 mL) (food for Ostracods) and five neonate Ostracods were added to each well. Parafilm was used to seal the test tray. It was then covered by a lid and kept under darkness in an incubator, maintained at 25 °C (±1 °C). After 6 days of incubation, microsieving of the contents of each well was carried out to separate the Ostracods. Mortality and growth of the surviving Ostracods were then determined. Length measurements of surviving Ostracods were carried out by unique micrometric slips provided with each Ostracodtoxkit. % growth inhibition (GI) of Ostracods was calculated as:

\[ \text{GI} = 100 - \left( \frac{A}{B} \times 100 \right) \]

where A is growth of Ostracods in the reference sediment, B is growth of Ostracods in the treatment.

**Collembola Tests.** We followed the standard protocol and accordingly 10 Collembola were added to treated and untreated soil following the incubation of 7, 15, 21, and 28 days. About 30 g of soil samples from each category were taken in plastic cylinders of height 6 cm and inner diameter 4.2 cm. A small space in the cylinder cover was left for respiration of the test species Collembola. Dried baker's yeast weighing about 15 mg was added onto the top soil to serve as food for Collembola. All soil samples from each category were treated with Triton X-100.

**Fe(II) Extract from Soil.** Following the incubation period of 7, 15, 21 and 28 days, about 0.5 g of soil was taken in a glass vial to which 5 mL of 0.5 M HCl was added and the entire content was mixed by swirling for 30 s. The whole mixture was then left for 1 h at room temperature. Then, 0.1 mL extract of the above mixture was added to 5 mL of ferrozine (1 g/L) in 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) and the solution was maintained at pH 7 by adding NaOH. The determination of Fe(II) content was done spectrophotometrically by measuring the absorbance of the supernatant at 562 nm. During the extraction process, Fe(II) was neither oxidized nor Fe(III) was reduced. Similarly, another soil sample of the same quantity was extracted by the above-described method with the exception that the extractant was 5 mL of hydroxylamine hydrochloride (0.25 M) in HCl (0.25 M). Fe(III) is reduced to Fe(II) under acidic conditions by hydroxylamine. The calculated difference between the Fe(II) obtained in the hydroxylamine and HCl extractions was taken as the amount of hydroxylamine-reducible Fe(III).

A wide range of concentrations of Fe(II) (FeSO₄), Fe(III) (FeCl₃), nZVIs, and DDT were used to establish standard toxicity curves for Ostracods to determine LC5₀ and EC5₀. Ostracods were exposed to soil spiked with DDT, nZVIs, Fe(II), and Fe(III).

**In Vitro Study. Hemolysis Assay.** Blood sample (5 mL) was collected from an anonymous human donor. It was drawn directly into K₂-EDTA-coated Vacutainer tubes to prevent coagulation and centrifuged at 3000 rpm for 10 min and the supernatant was discarded. RBCs were washed thrice with 1× phosphate-buffered saline (PBS), pH 7.4, for 5 min. and washed erythrocytes were finally suspended in 1× PBS (1:9 dilution). To study hemolysis, 950 µL of the above suspension was mixed with a 50 µL solution of nanoparticles of different concentrations to obtain 0.1, 0.2, 0.3, 0.4, 0.5, and 1 g/mL of nanoparticles with RBC suspension, and also the 1:9 diluted RBC suspension was mixed with Triton X-100 to make 1% solution for complete lysis of the erythrocytes. RBCs suspended in 1 mL of PBS were used as negative control to assess background lysis, if any. Each suspension was incubated at 37 °C for 15, 30, 60, 120, and 180 min. After the predetermined time of incubation, the suspension was centrifuged, and the supernatant was analyzed using a spectrophotometer at 540 nm. The percentage of hemolysis was determined by the following equation:

\[ \text{% Hemolysis} = \left( \frac{\text{Abs}_s - \text{Abs}_b}{\text{Abs}_{100} - \text{Abs}_b} \right) \times 100 \]

where \( \text{Abs}_b \) is the absorption of the blank sample, \( \text{Abs}_s \) is the absorption of the sample, and \( \text{Abs}_{100} \) is the absorption of the sample treated with Triton X-100.

**MTT Assay.** It is used to measure the in vitro cytotoxicity of the synthesized nanoparticles at different concentrations on specimen cells. Cell viability, as opposed to cytotoxicity, is measured by this method to assess the cytotoxicity. Cell viability can be determined on the basis of their mitochondrial dehydrogenase activities. For metabolically active cells, the mitochondrial activities remain constant and any variations in them are reflected in the numbers of active cells. The mitochondrial activity of cells is assessed by the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan crystals by mitochondrial enzymes. Therefore, any changes in mitochondrial activity will be reflected on the formation of formazan. By measuring the concentration of formazan formed, one can estimate the number of viable cells. For this purpose, macrophages were collected from sodium thioglycolate-stimulated mice, which were then put into a growth medium, RPMI 1640 (incomplete). It was then centrifuged at 1400 × g for 10 min. The 24-well tissue culture plates at 0.1 mL per well, and were then exposed to various concentrations of nZVIs (0.1, 0.2, 0.3, 0.4, 0.5, and 1 g/mL) for 12 h in an incubator with 5% CO₂ at 37 °C. After incubation, the formazan crystals formed were made soluble by dissolving in DMSO solvent and their absorbance measurements were done later at 492 nm. The absorbance at 492 nm represented the total number of viable cells. LD₅₀ values for different concentrations of nZVIs were calculated from the dose–response curve. The mean percentage of viable cells was calculated as follows.

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RESULTS AND DISCUSSION

DLS Analysis. DLS, also known as photon correlation spectroscopy (PCS), is a technique used to determine the size and size distribution profile of submicron particles in suspension. The particles’ size distribution is calculated from their erratic motion or diffusion using the Stokes–Einstein equation,

\[ d = \frac{k_B T}{3 \pi \eta D} \]

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \eta \) is the solvent viscosity, \( D \) is the diffusion coefficient, and \( d \) is the hydrodynamic diameter of the particle. In this DLS technique, a vertically polarized He−Ne laser of wavelength 632.8 nm was passed through a dilute nanoparticle suspension and the intensity of the scattered light was collected on an autocorrelator. CONTIN algorithms were used in the Laplace inversion of the autocorrelation function to obtain the size distribution.41 The particles size analysis of the nZVIs was performed by DLS at 25 °C (DLS/Malvern Instrument, PCS). The number average mean hydrodynamic sizes of iron nanoparticles at 0.05 M aqueous solution of FeCl₃ was 29 nm (Figure 1), with a narrow polydispersity index (0.11−0.16).

TEM Analysis. TEM micrographs of nZVIs synthesized by borohydride reduction method (reduction of aqueous solution of FeCl₃ by NaBH₄) are shown in Figure 2. The mean diameter of the synthesized nZVIs, obtained from the TEM image, was found to be 25 nm with spherical shape and low polydispersity.

DDT Degradation Study. The degradation of DDT in soil with 1, 5, and 10 g nZVI/kg soil after 7, 15, 21, and 28 days of incubation is shown in Figure 3. The amounts of DDT degraded in the nZVIs’ treatments were significant compared to controls without nanoparticles. It was observed that after 7 days of incubation, the concentration of DDT in treatments was lowest for the lowest concentration of added nZVI. Addition of 1 and 5 g of nZVI/kg soil thus resulted in 58.3 and 29.62% degradation of DDT (as the sum of DDT and its degradation products), respectively, after 7 days, whereas it was only 18.87% with 10 g nZVI/kg soil (comparable to control). At the highest dose of nZVIs, the DDT degradation continued for the entire incubation period of 28 days, whereas no additional degradation was observed for the lowest dose of nZVIs during the incubation period spanning from 7 to 28 days.

The results of the above study showed that nZVIs are capable of degrading DDT. In our control treatments, it was observed that in the absence of nZVIs, there were no significant changes in concentrations of DDT in spiked soil during the entire incubation period of 28 days. This was attributed to the recalcitrant and persistent nature of DDT. Thus, in treated soil, nZVIs were clearly responsible for the degradation of DDT. It was also observed from the data that after an incubation period of 7 days, a dose of 1 g nZVI/kg soil reduced the level of DDT faster than the dose at 10 g nZVI/kg soil. This was probably because of increased reduction of organochlorine compounds like DDT in the presence of excess electrons released by the complete and extensive oxidation of nZVIs. However, the degradation capability of 1 g nZVI/kg soil did not find any improvement when the incubation period was extended from 7 to 28 days. This could be due to the oxidation process and consequent formation of hydroxide substances on particles’ surface, which might have retarded the reactivity of nZVIs. On the other hand, when similar experiments were carried out using macrosized ZVIs, the

| time (day) | control (mg/kg) | 1 g nZVI (mg/kg) | 5 g nZVI (mg/kg) | 10 g nZVI (mg/kg) |
|-----------|----------------|------------------|-----------------|---------------|
| 7         | 16.74          | 6.98             | 11.78           | 13.58         |
| 15        | 15.61          | 6.14             | 12.18           | 13.54         |
| 21        | 14.94          | 5.99             | 10.2            | 11.75         |
| 28        | 14.25          | 5.83             | 7.39            | 8.91          |

Table 1. Residual Concentrations of DDT in Spiked Soil (40 mg DDT/kg) after Incubation with 1, 5, and 10 g of nZVIs/kg for 7, 15, 21, and 28 Days in Soil Slurries
degradation efficiency was quite low. Yao et al., 200642 found that DDT degradation in spiked soil was very low even after adding high concentrations of macrosized ZVIs (50 g ZVIs/kg soil for 1 week resulted in 70% degradation of DDT). nZVIs offer larger surface area compared to ZVIs and the reductive interactions of chlorinated contaminants like DDT with iron particles is proportional to latter’s surface area (Table 1).20

Toxicity Study. Collembola Mortality Study. Collembola (Folsomia candida) are one of the most abundant groups of soil arthropods. Toxicity of nZVIs in soil can be assessed by studying its toxic effects on soil arthropods like Collembola as they are abundant on the top soil. Mortality and reproduction of juveniles have been used as the basis to measure the toxicity of nZVIs toward Collembola. The results of our study when Collembola were exposed to 1, 5, and 10 g nZVIs/kg soil for 28 days are shown in Figure 4. Negative effects on both adult and juvenile Collembola were quite significant (p < 0.05) in test soil compared to control soil (without nZVIs and DDT). It was also observed that toxicity of nZVIs toward Collembola after an incubation period of 28 days was significantly lower than that after 7, 15, and 21 days of incubation when the test species were exposed to nZVIs at 1, 5, and 10 g/kg soil concentrations. After an incubation period of 7 days, there was

![Figure 4](image-url)

Table 2. Effects of DDT with and without nZVIs on Collembolan Mortality (Adult Survival) in Spiked Soil after Incubation with 1, 5, and 10 g of nZVIs/kg for 7, 15, 21, and 28 Days

| time (day) | control | DDT | 1 g nZVI | 1 g nZVI + DDT | 5 g nZVI | 5 g nZVI + DDT | 10 g nZVI | 10 g nZVI + DDT |
|-----------|---------|-----|---------|---------------|--------|---------------|---------|---------------|
| 7         | 8 ± 1.20| 10 ± 0.57 | 0 ± 0 | 0 ± 0.00 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 15        | 9 ± 0.80| 9 ± 1.23 | 2 ± 0 | 2 ± 0.40 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 21        | 9 ± 0.91| 9 ± 1.08 | 3 ± 0 | 2 ± 0.32 | 0 ± 0 | 1 ± 0 | 0 ± 0 | 0 ± 0 |
| 28        | 10 ± 0.85| 8 ± 1.21 | 5 ± 0 | 4 ± 0.17 | 3 ± 0.5 | 2 ± 0.18 | 1 ± 0.04 | 3 ± 0.04 |

![Figure 5](image-url)

Figure 5. Iron in the solid phases from slurries of DDT-spiked soils after 28 days of incubation with nZVIs (mean ± SD, n = 2).

![Figure 6](image-url)

Figure 6. EC50 and LC50 values of components from nZVI contributing to ostracod toxicity.

![Figure 7](image-url)

Figure 7. Hemolysis assay of pectin-capped iron nanoparticles.

Table 3. Collembolan Mortality Studies on Juveniles

| time (day) | control | DDT | 1 g nZVI | 1 g nZVI + DDT | 5 g nZVI | 5 g nZVI + DDT | 10 g nZVI | 10 g nZVI + DDT |
|-----------|---------|-----|---------|---------------|--------|---------------|---------|---------------|
| 7         | 64 ± 10.87| 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 15        | 72 ± 9.89 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 21        | 102 ± 10.83 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 28        | 142 ± 14.86 | 4 ± 0.9 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
100% mortality when adult Collembola were exposed to any of the above nZVI concentrations in soil, whereas mortality reduced to 65, 75, and 89% for the dose of 1, 5, and 10 g nZVIs/kg soil, respectively, after an incubation of 28 days because of reduced toxicity. In addition, the negative effect of fresh nZVI suspension on adult and juvenile Collembola was quite high. On the contrary, there were no significant negative effects on adults by DDT alone. However, in soil spiked with DDT alone, no juveniles were observed. Irrespective of the length of the incubation period, no juveniles were found on any of the soils treated with three different concentrations of nZVIs (Table 2).

**Iron Concentrations in DDT-Contaminated Soil.** The solid phase iron concentrations in DDT-spiked soil slurry after 28 days of incubation are shown in Figure 5. Addition of both 1 phase iron concentrations in DDT-spiked soil slurry after 28 nZVIs (Table 2).

**Toxicity Effects on Ostracods.** Ostracods are sediment dwellers and are found in abundance in top soil. The toxicity studies of nZVIs in the ostracod population reflect the toxicity of the whole sample soil matrix. Ostracods (H. incongruens) as test organisms have been successfully used earlier to assess the toxicity of the soil.27 Ostracods are very sensitive to nZVIs in both water and soil. This could be due to the creation of anoxic conditions following initial oxidation of nZVIs. In support of this assumption, it was observed that the mortality of Ostracods decreased rapidly as the slurry was subjected to prolonged incubation before test. The longer incubation period was able to supply enough oxygen to end the anoxic conditions developed at the early stage of incubation. Further, mortality remained high in treatments which received higher doses of nZVIs. At a higher dose, nZVIs consume more oxygen because of their oxidation and create a state of anoxia, which cause death among ostracods. From EC₅₀ thresholds of the individual components, as shown in Figure 6, it was observed that toxicity of nZVIs is lower than that of either DDT or Fe(II). Therefore, the ostracod mortality by nZVIs could be indirect. When studying the toxic effects of nZVIs on medaka fish larvae, Chen et al.45 concluded that hypoxia was caused by nZVIs because of consumption of oxygen, and the Fe(II) released in the process caused toxicity because of generation of reactive oxygen species. Toxicity of nanoparticles is also explained on the basis of their indirect link to food depletion,46 which seems irrelevant in this case (Table 3).

**In Vitro Study. Hemolysis Assay of nZVIs.** The results of the hemolytic experiments indicate that the pectin-capped iron nanoparticles greatly increase the blood compatibility and remain nontoxic up to 1 mg/mL to erythrocytes. The results significantly emphasize the usefulness of iron nanoparticles for the soil and water treatment processes apart from their use against many diseases (Figure 7), (Table 4).

**MTT Assay.** From the MTT assay, a dose-dependent response was observed and LD₅₀ values were calculated. Cytotoxicity data for the pectin-capped iron nanoparticles and the macrophages are presented (Figure 8). The LD₅₀ values presented for different concentrations of iron nanoparticles were found to be nontoxic after 12 h of exposure time. The LD₅₀ values calculated from the MTT assay are 0.1 g/mL (confidence interval ± 1.09), 0.2 g/mL (confidence interval ± 1.09), 0.3 g/mL (confidence interval ± 1.09), and 0.4 g/mL (confidence interval ± 1.09), and 0.5 g/mL (confidence interval ± 1.09), and 1 g/mL (confidence interval ± 2.97), for the 25 nm nZVIs. This shows that the particles have negligible cytotoxicity in comparison to Triton X-100 (Table 5).

**Table 4. Iron Concentrations in Solid Phases of DDT-Spiked Soil after 28 Days of Incubation with nZVIs**

| iron conc. | control | 1 g nZVI | 5 g nZVI | 10 g nZVI |
|------------|---------|----------|----------|----------|
| Fe(II) (mg/kg) | 63 ± 6.50 | 104 ± 14.80 | 147 ± 13.23 | 226.73 ± 8.39 |
| Fe(III) (mg/kg) | 238 ± 16.00 | 369 ± 18.00 | 716 ± 42.00 | 1549.1 ± 102.00 |

**Figure 8.** Plots of cell viability MTT assay studied for iron nanoparticles (nZVI) compared with negative control (Triton X-100) after a 12 h incubation period at various concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 1 g/mL).

**Table 5. EC₅₀ and LC₅₀ Threshold Values of Components from nZVIs Contributing toward Ostracod Toxicity**

| Test | DDT (mg/kg) | nZVI (mg/L) | Fe²⁺ (mg/L) |
|------|-------------|-------------|-------------|
| EC₅₀ | 10.78       | 47          | 17          |
| LC₅₀ | 0           | 97          | 10          |

**CONCLUSIONS**

In this study, we synthesized well-defined monodisperse pectin-capped nZVIs, which were used to study the degradation of DDT in spiked soil. Pectin-capped nZVIs are generally used in the field of biomedical sciences and are rarely used in environmental remediation. However, our study showed that the degradation rates of DDT in spiked soil by pectin-capped nZVIs were as good as that of earlier studies taken up by nZVIs capped with other polymers. Hence, pectin-capped nZVIs could be a promising and effective material against soil pollutants like DDT. The ecotoxicity of nZVIs on soil biota was studied by taking Collembola and Ostracods as test species as these are predominantly sediment dwellers. The negative effects of nZVIs on mortality and growth of both Collembola and Ostracods were quite severe, whereas the negative effects of DDT on reproduction of Collembola and development of Ostracods were very weak. It was also observed that prolonged incubation period led to diminishing toxicity of nZVI on Collembola and Ostracods. Concentrations of Fe(II) and Fe(III) were increased in the soil after addition of nZVIs followed by incubation. Fe(II), particularly, was found to be more toxic than nZVIs toward Ostracods. More
studies are required to optimize the effectiveness of nZVIs against DDT in different soil categories and to assess their ecotoxicity with respect to severity and impact period by covering a wide spectrum of soil biota.

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**Notes**
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