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Review Article

ECOTOXICITY CONCERT OF NANO ZERO-VALENT IRON PARTICLES- A REVIEW

M. I. NIYAS AHAMED*

Centre for Nanobiotechnology, VIT University, Vellore 632014, Tamil Nadu, India.
Email: iniyasahamed@vit.ac.in

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ABSTRACT

Nanotechnology is based on the idea that, by engineering the size and shape of materials at the scale of atoms, i. e. nanometers (nm), distinct optical, electronic, or magnetic properties can be tuned to produce novel properties of commercial value. However, there is an obvious concern that such novel properties may also lead to novel behavior when interacting with biological organisms, and thus to potentially novel toxic effects. Nano zero-valent iron particle has been used for site remediation by creating permeable reactive barriers (PRBs) by filling trenches with ZVI designed to allow groundwater to pass through while "filtering" out the contaminants. Research indicates that using the nanoscale ZVI in place of the macroscale ZVI will accomplish the same remedial work more efficiently and with less cost. The full acceptance of NZVIs as a remediation agent depends on several issues. One of the most important factors relates to the fate, impact and toxicity of these nanomaterials on the ecosystems to which they are applied.

Keywords: Nanotechnology, Nano zero-valent iron, Ecotoxicity.

INTRODUCTION

In recent decades, the use of nanomaterials has become increasingly significant in industrial processes, consumer and medical products [1] and more recently, in environmental remediation [2]. This has led to the introduction of significant amounts of distinct types of nanomaterials into all the environmental categories: soils, aquatic systems and air. In soils, nanoparticles can be introduced either directly, through fertilizers and products used for plant protection or liquid suspensions used in contaminated sites, or indirectly, through the land application of sludge or biosolids. The presence of nanoparticles in aquatic systems is mainly due to the disposal of wastewater treatment plant effluents, industrial discharges, and surface runoff from soils. Volcanic eruptions, combustion processes and industrial emissions are some of the sources of nanoparticles release in the air [3].

After the introduction of nanoparticles into the environment, the particles undergo several changes involving biological, physical and chemical processes, which make it difficult to quantify their prevalence and evaluate their degree of ecotoxicity. In particular, these changes include chemical interactions (e. g. redox reactions) and agglomeration effects. The type and extent of these processes depend on the properties of both the nanoparticles and the receiving medium [1]. For example, in aqueous systems, hardness, biochemical oxygen demand, pH, alkalinity and organic matter content are some of the parameters that influence the behavior of the nanoparticles.

Difficulty relates to the quantification of trace amounts of nanoparticles in the environment. Knowledge of this subject remains scarce mostly because there are neither any specific standardized methods nor protocols nor any certified reference materials for the testing of nanomaterials [4, 5]. Nevertheless, the scientific community is trying to adopt the best methodologies for conducting such studies. All these facts contribute to the growing concerns about the fate and effects of these materials in the environment. This encourages and puts pressure on the scientific community to answer these issues and to evaluate the real impact of nanomaterial usage, nanomaterial ecotoxicity and the need for more information on the proper handling of these materials in order to prevent environmental and human health effects after long-term exposure [4]. Macroscale zero-valent iron particle (ZVI) has long been recognized as an excellent electron donor with a tendency to release electrons in aquatic environments regardless of its particle size [5]. ZVI has been used for site remediation since the early 1990s by creating permeable reactive barriers (PRBs) by filling trenches with ZVI designed to allow groundwater to pass through while "filtering" out the contaminants [6, 7]. Research indicates that using the nanoscale ZVI (NZVI) in place of the macroscale ZVI will accomplish the same remedial work more efficiently and with less cost. A benefit of using NZVI is being able to inject it directly into a contaminated aquifer, which avoids the need to dig a trench for installation of the PRB. Using this injection technique is believed to be faster and more effective for groundwater treatment than either pump-and-treat or PRB methods [6].

ZVI, or elemental iron, is a moderate reducing reagent that can react with dissolved oxygen and water resulting in electrochemical/corrosion reactions that oxidize the iron [8]. As shown below, the Fe^0 becomes oxidized to ferrous iron (Fe^{2+}) ions and the electrons that are released become available to reduce other compounds. This process can be accelerated or inhibited by changing the solution chemistry and/or solid composition by adding coatings or catalysts [8].

$$2Fe^0_{(s)} + 4H^+_{(aq)} + O_{2\,(aq)} \rightarrow 2Fe^{2+}_{(aq)} + 2H_2O_{(l)} \quad (1)$$

$$Fe^0_{(s)} + 2H_2O_{(aq)} \rightarrow Fe^{2+}_{(aq)} + H_{2\,(g)} + 2OH^-_{(aq)} \quad (2)$$

The chemistry behind NZVI is much the same as with macroscale ZVI. NZVI is highly reducing and generates reactive oxygen species (ROS) through Fenton chemistry. In aqueous environments, NZVI oxidizes over time (i. e., ages) to iron oxides such as magnetite, maghemite, lepidocrocite, and goethite [9, 10].

Research has shown the NZVI can react not only with dissolved oxygen and water, but also with a variety of environmental contaminants, and may prove to be a more effective and less costly remediation alternative. NZVI can remove contaminants through reduction or adsorption. The treatment of chlorinated solvents, for example, primarily uses reduction, and the removal of arsenic is done by adsorption to iron oxides and hydroxides formed during oxidation. An example that has received a lot of attention is the process of degrading trichloroethene (TCE) and perchloroethylene (PCE), TCE and PCE were used historically as industrial cleaning and degreasing solvents, as paint removal solvents, and as dry-cleaning solvents [11, 12]. Chlorinated solvents can be degraded through reductive dechlorination or beta elimination. Under the reducing conditions presented by the introduction of NZVI in groundwater, reductive dechlorination follows the pathway of PCE → TCE → DCE → VC → ethane, where DCE is dichloroethene and VC is vinyl chloride [7].

Although NZVI particles can exist naturally, remediation techniques generally use engineered nanoparticles. NZVI can be created in a variety of different ways. Researchers discovered three distinct methods for producing NZVI through the reduction and precipitation of ZVI from an aqueous solution using sodium borohydride for the chemical reduction of ferrous (Fe^{2+}) or ferric (Fe^{3+}) ions. NZVI also can be produced by hydrogen reduction of iron oxides or hydroxides.

The following equations illustrate some of the reactions that can take place to produce NZVI [14, 15]:

$Fe\ (H_2O)_6{}^{3+} + 3BH_4 - + 3H_2O \rightarrow Fe^0\downarrow + 3B(OH)_3 + 10.5H_2$ (3)

$4Fe^{3+}{}_{(aq)} + 3BH^-{}_{4(aq)} + 9H_20(l) \rightarrow 4Fe^0{}_{(s)} + 3H_2BO^-{}_{3(aq)} + 12H^+{}_{(aq)} + 6H_{2(g)}$ (4)

$2Fe^{2+}{}_{(aq)} + BH\text{-}4(aq) + 3\ H_20\ (l) \rightarrow 2Fe^0{}_{(s)} + H_2BO^-{}_{3(aq)} + 4H^+{}_{(aq)} + 2H_{2(g)}$ (4)

It is important to know how the nanoparticles are produced. Even particles with the same chemical composition will have different reactivity, mobility and active life due to the process or vendor used to obtain the nanoparticles [13]. Contamination also can be an issue for reactivity. The greater the percentage of ZVI present in the resulting nanoscale powder, the more reactive it will be. When Fe^{2+} or Fe^{3+} ions is present in the NZVI powder it will be less reactive due to the decreased availability of electrons [16].

There are three main forms of nanoscale iron that can be used during remediation efforts – NZVI, bimetallic nanoscale particles (BNP) and emulsified zero-valent iron (EZVI). BNPs are particles of NZVI that have been coated with a catalyst, such as platinum, gold, nickel, or palladium, to increase mobility and enhance reduction reaction. EZVI are NZVI particles that have been coated with a membrane made from biodegradable oil and water to facilitate the treatment of chlorinated hydrocarbons by making the particles more hydrophobic. This allows the particles to mix directly with dense non-aqueous phase liquids (DNAPL), such as TCE, to increase mass transfer between the DNAPL and the NZVI through the emulsion membrane.

According to the Environmental Protection Agency (EPA), NZVI is the most common nanoparticles that has been tested and field applied. It constitute one of the most common materials used in nano-remediation because of their high superficial area and reactivity with distinct contaminants such as metals, halogenated hydrocarbons [18], polychlorinated biphenyls (PCBs) [19] and pharmaceutical products [20].

The high efficiencies obtained in recent tests in the laboratory and in pilot studies indicate that the use of NZVIs is extremely promising in terms of environmental remediation [20-26]. However, as with the other nanomaterials, several concerns are being raised about the impact and ecotoxicity of NZVIs.

This is largely due to the formation of iron oxides, which are already present in the ground as rust, during remediation [27]. The toxicity of iron is based on its ability to catalyze the formation of hydroxyl radicals ($OH^{\cdot}$) from superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). Free radicals are highly reactive, unstable molecules that are in need of an additional electron for stabilization. Because of this, they can "affect antioxidant enzymatic activities, per oxidation of membrane lipids, modification of nucleic acids, and eventually cause cell death and tissue injury" [27].

Iron toxicity studies have primarily focused on Fe^{2+} and its oxides and little is known about the toxicity specific to NZVI or macroscale ZVI. However, ZVI produces Fe^{2+} and iron oxides through oxidation and ZVI/NZVI can produce free radicals through this transformation process.

Toxicity performance of NZVIs against soil and aquatic organisms

Bacteria, along with algae, are at the bottom of the aquatic food chain, being the food of aquatic crustaceans such as *Daphnia*, which are in turn consumed by fish. In vitro tests have shown that NZVIs are bactericidal to certain aqueous cultures of *Escherichia coli* or *Bacillus nealsonii* [29-31]. In contaminated environments, the native microbial consortia are generally already inhibited by the presence of significant concentrations of the contaminants.

Certain studies in environmental matrices showed opposite results. Fajardo et al. observed that NZVIs had a reduced impact on microbial cellular viability and on biological activity in soils of *Klebsiella planticola* and *Bacillus nealsonii* and concluded that the ecotoxicity of NZVIs could be highly dose- and species dependent [29]. Kirschling et al. observed that NZVIs had no effect on the bacterial abundance in the soil and that the bacterial populations increased when the NZVIs were coated with a biodegradable poly aspartate [32]. This could indicate that the use of coated NZVIs may reduce their toxicity. On the other hand, Barnes et al. [33] observed a negative impact of NZVIs on the capacity of an indigenous dechlorinating bacterial community to degrade trichloroethylene (TCE). This impact was dose dependent: the biological degradation rate started to decrease at NZVI concentrations above 0.01 g/L and ceased at concentrations above 0.3 g/L.

Studies indicate that there is a possible impact of NZVIs on bacterial communities and that this impact is dose dependent. Therefore, NZVIs can be applied to soils, but their dosage should not exceed the level that is detrimental to bacteria. On the other hand, the use of coated NZVIs can enhance environmental remediation. However, it is not certain that these coatings can allow for the use of higher NZVI dosages without causing a negative effect on bacteria. This is a field of research that should be explored further in order to widen the applicability of NZVIs.

Aquatic invertebrates are commonly affected by most of the contaminants released into the environment. This is one of the reasons that these organisms are important and appropriate for ecotoxicity tests [34]. Behavior and bioavailability of nanoparticles in aquatic environments, invertebrate testing, used to increase knowledge of their toxicology. *Daphnia magna* is used in the majority of tests to evaluate the ecotoxicity of nanoparticles [35].

Several studies show that *Daphia magna* is generally very sensitive to the presence of distinct nanoparticles; continuous exposure leads to the immobilization or death of the organisms [36]. However, only a few works have focused on the ecotoxicity of NZVIs. Marsalek et al. Studied the possible application of NZVIs to destroy and prevent, in a simple and environmentally benign way, the formation of cyanobacterial water blooms. They observed an NZVI EC_{50} of 50 mg/L against cyanobacteria, while for *Daphnia magna* they observed an EC_{50} higher than 1000 mg/L [37]. Keller et al. Registered that *Daphnia magna* survival was drastically influenced by commercial NZVIs (Nanofer 25S and Nanofer STAR) [38].

These few studies indicate that NZVIs significantly affect the *Daphnia magna* communities and can even lead to their death. Considering that the use of NZVIs for environmental remediation is mainly focused on contaminated waters, these results are very important and reinforce the need for more detailed and structured studies. These studies consider the impact of NZVIs on *Daphnia magna* in the absence of contaminants. However, the NZVIs are applied to contaminated environments, and therefore future research should evaluate the impact of contaminants on *Daphnia magna*/other species as well as the additional impact of NZVIs.

Earthworms are common soil organisms that play an important and distinct role in the soil ecosystem and for this reason; they are used as test organisms in soil ecotoxicity studies [39] and to assess the bioavailability of contaminants in soils [40]. On account of the limitations in forming reliable conclusions about the validity of these tests, NZVIs are generally only applied to specific situations in which contaminations have occurred and where evaluation is required. However, some studies have examined the ecotoxicity of different nanoparticles {e. g. Aluminum oxide [41], silver [42] or titanium oxide [43]} in soils, but, as far as is known; only one study has focused on the ecotoxicity of NZVIs in earthworms.

The eco-toxicological effects of NZVIs coated with carboxymethyl cellulose on *Eisenia fetida* and *Lumbricus rubellus*. This work proved the negative impact of NZVIs on both of these earthworm species, affecting reproduction when the NZVI concentration reached 100 mg/kg and leading to decreased weight and an increased mortality rate in concentrations above 500 mg/kg [44].

The present insufficiencies of information delay a supported assessment of the impact of NZVIs on terrestrial organisms. However, research indicates that, above specific NZVI concentrations, the organisms' reproduction, weight and mortality rates are affected.

It is clear that further research is needed and such research should evaluate the relative impact of NZVI application on contaminated environments. Research to be carried out in other types of earthworms to assess at which concentrations, the organism's reproduction, weight and mortality rates are affected.

Lactuca sativa, the common lettuce, is probably the plant that is more often used for germination test on account of its high sensitivity to distinct contaminants. Other plants are also used, such as cabbage (*Brassica oleracea L.*), corn (*Zea mays*) or soybean (*Glycine max*), but there is still no consensus on the most appropriate plant for such a test [45]. These tests are commonly used for soils contaminated with distinct contaminants such as metals [46], petroleum hydrocarbons [47] or pharmaceutical products [48]. A few studies have been performed with nanomaterials [49, 50]. Barrena et al. studied the toxicity of gold, silver and iron oxide (Fe_3O_4) nanoparticles on cucumber (*Cucumis sativus*) and lettuce (*Lactuca sativa*) [49].

Ravindran et al. performed germination tests with *Lycopersicum esculentum* and *Zea mays* to evaluate the ecotoxicity of silver nanoparticles and silver ions.

This study carried out in our laboratory, showed a higher toxic effect with silver nanoparticles than with silver ions; however, when the nanoparticles were supplemented with bovine serum albumin, there was a reduction in adverse effects [50].

The ecotoxicity of NZVIs and three types of silver nanoparticles in germination tests with ryegrass, barley and flax was conducted. In aquatic systems, inhibitory effects were observed for NZVI concentrations of 250 mg/L, while concentrations of 1000–2000 mg/L completely inhibited germination [51]. The tolerance of *Panicum maximum* (purple guinea grass) and *Helianthus annuus* (common sunflower) in a TNT-contaminated soil and in an NZVI-contaminated soil was studied. *Panicum maximum* showed more tolerance than *Helianthus annuus* to the presence of NZVIs [52].

This type of test not only indicates the impact of NZVIs on the germination process; it can also provide information on the uptake of NZVIs by the plant's roots and leaves. This knowledge allows a more complete and thorough evaluation of the impact of NZVIs on plants. Nevertheless, there is some evidence that indicates that the germination of some plants is affected by the presence of NZVIs. Therefore, in order to protect superficial plants, NZVI suspensions supposed to be applied in soils via deep slurry injections.

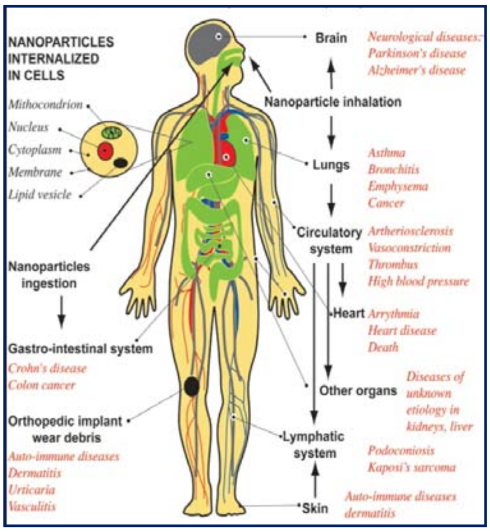

Fig. 1: Diseases associated to nanoparticles exposure

Source: Ecotoxicity of nanoscale zero-valent iron particles – a review. http: //www. visaemdebate. incqs. fiocruz. br/

CONCLUSION

As per the available literature, not sufficient work was carried out in ecotoxicity behavior of NZVIs with special reference to India. Few studies have been completed; several research groups are currently in the process of analyzing data pertaining specifically to the toxicity of NZVI. The full acceptance of NZVIs as a remediation agent depends on several issues. One of the most important factors relates to the fate and impact of these nanomaterials on the ecosystems to which they are applied. The existing literatures are clearly insufficient. Nevertheless, the majority of the studies point toward the toxic effects of NZVIs on all the tested organisms.

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CONFLICT OF INTEREST

None declared

REFERENCES

1. Batley GE, Kirby JK, McLaughlin MJ. Fate and risks of nanomaterials in aquatic and terrestrial environments. Accounts Chem Res 2013;46(3):854-62.
2. Sanchez A, recillas S, Font X, Casals E, gonzalez E, Puntes V. Ecotoxicity of, and remediation with, engineered inorganic nanoparticles in the environment. Trac-trends Anal Chem 2011;30(3):507-16.
3. Farre M, Sanchis J, Barcelo D. Analysis and assessment of the occurrence, the fate and the behavior of nanomaterials in the environment. Trac-trends Anal Chem 2011;30(3):517-27.
4. Peralta-Videa JR, Zhao LJ, Lopez-Moreno ML, de la Rosa G, Hong J, Gardea-Torresdey JL. Nanomaterials and the environment: a review for the biennium 2008-2010. J Hazard Mater 2011;186(1):1-15.
5. Zhang X-W, Elliott DW. Applications of iron nanoparticles for groundwater remediation. Remediation J 2006;16(2):7-21.
6. ITRC. Interstate Technology and Regulatory Council. Permeable Reactive Barriers: Lessons Learned/New Directions. PRB-4. Available at: www.itrcweb. org; 2005.
7. U S EPA. Office of Solid Waste and Emergency Response. Nanotechnology for Site Remediation Fact Sheet. Report number: EPA 542-F-08-009. Available at: http://www.epa.gov/ tio/ download/remed/542-f-08-009.pdf; 2008c.
8. Zhang W-X. Nanoscale iron particles for environmental remediation: an overview. J Nanopart Res. 2003;5:323-32.
9. You Y, Han J, Chiu PC, Jin Y. Removal and inactivation of waterborne viruses using zerovalent iron. Environ Sci Technol 2005;39:9263-9.
10. Phenrat T, Long TC, Lowry GV, Veronesi B. Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ Sci Technol 2009c;43:195-200.
11. Doherty RE. A History of the production and use of carbon tetrachloride, tetrachloroethylene, trichloroethylene and 1, 1, 1-trichloroethane in the United States: Part 1 – historical background; carbon tetrachloride and tetrachloroethylene. Environ Forensics 2000a;1(2):69-81.
12. Doherty RE. A History of the production and use of carbon tetrachloride, tetrachloroethylene, trichloroethylene and 1, 1, 1-trichloroethane in the United States: Part 2 – n trichloroethylene and 1,1,1-trichloroethane. Environ Forensics 2000b;1(2):83-93.
13. Jose TA, Hendrikus PAN, Cristina MDM. Ecotoxicity of nanoscale zero-valent iron particles – a review. Vigilancia Sanitaria em Debate 2013;1(4):38-42.
14. Wang C-B, Zhang W-X. Synthesizing nanoscale iron particles for rapid and complete dechlorination of TCE and PCBs. Environ Sci Technol 1997;31(7):2154-6.
15. Zhang X-W, Elliott DW. Applications of iron nanoparticles for groundwater remediation. Remediation J 2006;16(2):7-21.

16. Mace C, Desrocher S, Gheorghiu F, Kane A, Pupeza M, Cernik M, *et al*. Nanotechnology and groundwater remediation: a step forward in technology understanding. Remediation J 2006;6(2):23-33.
17. Zhu HJ, Jia YF, Wu X, Wang H. Removal of arsenic from water by supported nano zero-valent iron on activated carbon. J Hazard Mater 2009;172(2-3):1591-6.
18. Wang Q, Jeong SW, Choi H. Removal of trichloroethylene DNAPL trapped in porous media using nanoscale zerovalent iron and bimetallic nanoparticles: Direct observation and quantification. J Hazard Mater 2012;213:299-310.
19. Petersen EJ, Pinto RA, Shi XY, Huang QG. Impact of size and sorption on degradation of trichloroethylene and polychlorinated biphenyls by nano-scale zerovalent iron. J Hazard Mater 2012;243:73-9.
20. Machado S, Stawinski W, Slonina P, Pinto AR, Grosso JP, Nouws HPA, Albergaria JT, *et al*. Application of green zero-valent iron nanoparticles to the remediation of soils contaminated with ibuprofen. Sci Total Environment 2013;461-462:323-9.
21. Kirschling TL, Gregory KB, Minkley EG, Lowry GV, Tilton RD. Impact of nanoscale zero valent iron on geochemistry and microbial populations in trichloroethylene contaminated aquifer materials. Environ Sci Technol 2010;44(9):3474-80.
22. Fang ZQ, Qiu XQ, Huang RX, Qiu XH, Li MY. Removal of chromium in electroplating wastewater by nanoscale zero-valent metal with synergistic effect of reduction and immobilization. Desalination 2011;280(1-3):224-31.
23. Cao J, Zhang W. Stabilization of chromium ore processing residue (COPR) with nanoscale iron particles. J Hazard Mater 2006;132:213–9.
24. Chrysochoou M, Johnston CP, Dahal G. A comparative evaluation of hexavalent chromium treatment in contaminated soil by calcium polysulfide and green-tea nanoscale zero-valent iron. J Hazard Mater 2012;201:33–42.
25. Du J, Lu J, Wu Q, Jing C. Reduction and immobilization of chromate in chromite ore processing residue with nanoscale zero-valent iron. J Hazard Mater 2012;215:152–8.
26. Franco DV, Da Silva LM, Jardim WF. Reduction of hexavalent chromium in soil and ground water using zero-valent iron under batch and semi-batch conditions. Water Air Soil Pollut 2009;197:49–60.
27. Watlington K. Emerging Nanotechnologies for Site Remediation and Wastewater Treatment. Available at: http://www.cluin.org/download/studentpapers/K_Watlington Nanotech.pdf; 2005.
28. Li H, Zhou Q, Wu Y, F 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(3):684-92.
29. Kirschling TL, Gregory KB, Minkley EG, Lowry GV, Tilton RD. Impact of nanoscale zero valent iron on geochemistry and microbial populations in trichloroethylene contaminated aquifer materials. Environ Sci Technol 2010;44(9):3474-80.
30. Fajardo C, Ortiz LT, Rodriguez-Membibre ML, Nande M, Lobo MC, Martin M. Assessing the impact of zero-valent iron (ZVI) nanotechnology on soil microbial structure and functionality: a molecular approach. Chemosphere 2012;86(8):802-8.
31. Auffan M, Achouak W, Rose J, Roncato MA, Chaneac C, Waite DT, *et al*. Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward Escherichia coli. Environ Sci Technol 2008;42(17):6730-5.
32. Lee C, Kim JY, Lee WI, Nelson KL, Yoon J, Sedlak DL. Bactericidal effect of zero-valent iron nanoparticles on Escherichia coli. Environ Sci Technol 2008;42(13):4927-33.
33. Barnes RJ, Riba O, Gardner MN, Singer AC, Jackman SA, thompson IP. Inhibition of biological tCE and sulphate reduction in the presence of iron nanoparticles. Chemosphere 2010;80(5):554-62.
34. Baun A, Hartmann NB, grieger K, Kusk Ko. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 2008;17(5):387-95.
35. la Farre M, Perez S, Kantiani L, Barcelo D. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. Trac-Trends Anal Chem 2008;27(11):991-1007.
36. Zhu XS, Zhu L, Chen YS, Tian SY. Acute toxicities of six manufactured nanomaterial suspensions to Daphnia magna. J Nanopart Res 2009;11(1):67-75.
37. Marsalek B, Jancula D, Marsalkova E, Mashlan M, Safarova K, tucek J, Zboril r. Multimodal action and selective toxicity of zerovalent iron nanoparticles against cyanobacteria. Environ Sci Technol 2012;46(4):2316-23.
38. Keller AA, Garner K, Miller RJ, Lenihan HS. Toxicity of nano-zero valent iron to freshwater and marine organisms. PLoS One 2012;7(8):e43983.
39. Spurgeon DJ, Weeks JM, Van Gestel CAM. A summary of eleven years progress in earthworm ecotoxicology. Pedobiologia 2003;47(5-6):588-606.
40. Ma WCW. Critical body residues (CBrs) for ecotoxicological soil quality assessment: copper in earthworms. Soil Biol Biochem 2005;37(3):561-8.
41. Coleman JG, Johnson DR, Stanley JK, Bednar AJ, Weiss CA Jr, Boyd rE, Steevens JA. Assessing the fate and effects of nano aluminum oxide in the terrestrial earthworm, eisenia fetida. Environ Toxicol Chem 2010;29(7):1575-80.
42. Lapied E, Moudilou E, Exbrayat JM, Oughton DH, Joner EJ. Silver nanoparticle exposure causes apoptotic response in the earthworm Lumbricus terrestris (Oligochaeta). Nanomedicine 2010;5(6):975-84.
43. Lapied E, Nahmani JY, Moudilou E, Chaurand P, Labille J, Rose J, *et al*. Ecotoxicological effects of an aged tio2 nanocomposite measured as apoptosis in the anecic earthworm Lumbricus terrestris after exposure through water, food and soil. Environ Int 2011;37(6):1105-10.
44. El-temsah YS, Joner EJ. Ecotoxicological effects on earthworms of fresh and aged nano sized zero-valent iron (NZVI) in soil. Chemosphere 2012;89(1):76-82.
45. Banks MK, Schultz KE. Comparison of plants for germination toxicity tests in petroleum-contaminated soils. Water Air Soil Pollut 2005;167(1-4):211-9.
46. Martí E, Sierra J, Cáliz J, Montserrat g, Vila X, garau MA, *et al*. Ecotoxicity of Cr, Cd, and Pb on two mediterranean soils. Arch Environ Contam Toxicol 2013;64(3):377-87.
47. Masakorala K, Yao J, guo H, Chandankere r, Wang J, Cai M, *et al*. Phytotoxicity of long-term total petroleum hydrocarbon-contaminated soil-A comparative and Ccombined approach. Water Air Soil Pollut 2013;224(5):1553.
48. Hillis DG, Fletcher J, Solomon KR, Sibley PK. Effects of ten Antibiotics on Seed germination and root Elongation in three Plant Species. Arch Environ Contam Toxicol 2011;60(2):220-32.
49. Barrena R, Casals E, Colon J, Font X, Sanchez A, Puntes V. Evaluation of the ecotoxicity of model nanoparticles. Chemosphere 2009;75(7):850-7.
50. Ravindran A, Prathna TC, Verma VK, Chandrasekaran N, Mukherjee A. Bovine serum albumin mediated decrease in silver nanoparticle phytotoxicity: root elongation and seed germination assay. Toxicol Environ Chem 2012;94(1):91-8.
51. El-temsah YS, Joner EJ. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environ Toxicol 2012;27(1):42-9.
52. Jiamjitrpanich W, Parkpian P, Polprasert C, Laurent F, Kosanlavit R. The tolerance efficiency of Panicum maximum and Helianthus annuus in TNT-contaminated soil and NZVI-contaminated soil. J Environ Sci Health A Tox Hazard Subst Environ Eng 2012;47(11):1506-13.