Doi: 10.3395/vd.v1i4.62en

ARTIGO

Ecotoxicity of nanoscale zero-valent iron particles – a review

Ecotoxicidade de nanopartículas de ferro zerovalente – Uma revisão

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ABSTRACT

The use of nanoscale zero-valent iron particles (nZVIs) in the environmental remediation of water and soil is increasing. This increase is related to the higher reactivity and mobility of nZVIs compared with that of macro- or micro-sized iron particles. The introduction of nZVIs into the environment raises concerns related to their fate and effect on aquatic and terrestrial biota. Knowledge of these issues will allow a better understanding not only of the remediation process but also of the long-term effects and impact of nZVIs on ecosystems, leading to a safer and more efficient application of these particles.

This paper presents the current state of play concerning the toxic effects of nZVIs on organisms at different stages of the food chain. The majority of studies show that nZVIs have a negative impact on bacteria, aquatic invertebrates, such as *Daphnia magna*, terrestrial organisms, such as *Eisenia fetida*, and seed germination. However, the number of published studies related to this issue is clearly insufficient. This reinforces the need for further research in order to specify the toxic concentrations of nZVIs that affect the most important target organisms. Furthermore, an evaluation of the effects of the coating of nanoparticles should also be pursued.

KEYWORDS: nanoscale zero-valent iron; bacteria, *Daphnia magna*, earthworms, germination tests

RESUMO

A utilização de nanopartículas de ferro zerovalente na remediação ambiental de águas e solos tem vindo a aumentar, suportado na maior reatividade e mobilidade destas partículas quando comparadas com outras de tamanho macro e micrométrico. A introdução destas partículas no ambiente tem levantado preocupações relativas ao destino e ao efeito em ambientes aquáticos e terrestes. Um maior conhecimento destas questões permitirá uma melhor compreensão do processo de remediação e das transformações a longo prazo e o impacto das nanopartículas de ferro zerovalente nos diferentes ecossistemas, permitindo uma aplicação mais segura e eficiente.

Este trabalho apresenta o estado atual do conhecimento sobre os efeitos tóxicos das nanopartículas de ferro zerovalente em diferentes organismos nas várias fases da cadeia alimentar. Concluiu-se que os estudos realizados são insuficientes e que na sua maioria apontam no sentido do impacto negativo daquelas nanopartículas em bactérias, invertebrados aquáticos, organismos terrestes e em testes de germinação. Esta informação reforça a necessidade da realização de estudos complementares que especifiquem a concentração das nanopartículas de ferro zerovalente que se tornam tóxicas para organismos-alvo importantes; e a avaliação dos efeitos de nanopartículas revestidas.

PALAVRAS-CHAVE: Nanopartículas de ferro zerovalente, bactérias, *Daphnia magna*, minhocas, testes de germinação

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[1]. Volcanic eruptions, combustion processes and industrial emissions are some of the sources of nanoparticle 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[4]. 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. Another 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 or 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 the 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. Peralta-Videa et al.[4] reinforced this idea by conducting several studies, between 2008 and 2010, to address the concerns about 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.

Nanoscale zero-valent iron particles (nZVIs) 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[6], halogenated hydrocarbons[7], polychlorinated biphenyls (PCBs)[8] and pharmaceutical products[9]. 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[9-11]. However, as with the other nanomaterials, several concerns are being raised about the impact and ecotoxicity of nZVIs.

The objective of this review is to present the current state of play concerning the ecotoxicity of nZVIs on bacteria, aquatic invertebrates, terrestrial organisms, and germination tests.

Bacteria

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*[12,13,14]. In contaminated environments the native microbial consortia are generally already inhibited by the presence of significant concentrations of the contaminants. Therefore, care should be taken regarding the application of nZVIs so as not to increase the ecotoxicity of this medium any further because this could jeopardize the medium's capacity to biodegrade the remaining contaminant after chemical remediation.

Certain studies in environmental matrices showed opposite results. Fajardo et al.[12] 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. Kirschling et al.[10] 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 polyaspartate. This could indicate that the use of coated nZVIs may reduce their toxicity. On the other hand, Barnes et al.[15] 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 $g \cdot L^{-1}$ and ceased at concentrations above 0.3 $g \cdot L^{-1}$.

These 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

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[16]. For future investigation of the behavior and bioavailability of nanoparticles in aquatic environments, the same author recommends that invertebrate testing should be used to increase knowledge of their toxicology. According to Sanchez et al.[2] and La Farre et al.[17], *Daphnia magna* is used in the majority of tests to evaluate the ecotoxicity of nanoparticles.

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[18]. However, only a few works have focused on the ecotoxicity of nZVIs. Marsalek et al.[19] 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 $mg{\cdot}L^{-1}$ against cyanobacteria, while for *Daphnia magna* they observed an EC_{50} higher than 1000 mg L^{-1}. Keller et al.[20] registered that *Daphnia magna* survival was drastically influenced by commercial nZVIs (Nanofer 25S and Nanofer STAR).

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* as well as the additional impact of nZVIs.

Terrestrial organisms

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[21] and to assess the bioavailability of contaminants in soils[22]. 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[2]. However, some studies have examined the ecotoxicity of different nanoparticles [e.g. aluminum oxide[23], silver[24] or titanium oxide[25]] in soils, but, as far as is known, only one study has focused on the ecotoxicity of nZVIs in earhworms. El-Temsah and Joner[26] evaluated the ecotoxicological 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 $mg{\cdot}kg^{-1}$ and leading to decreased weight and an increased mortality rate in concentrations above 500 $mg{\cdot}kg^{-1}$.

The present scarcity of information hinders a supported evaluation 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, as previously stated, such research should evaluate the relative impact of nZVI application on contaminated environments.

Germination tests

Germination tests are short-term ecotoxicity assays that involve plant processes in the assessment of acute toxicity effects. In these tests, seeds are planted in a small portion of a representative contaminated soil and, after a defined incubation period, the number of successful seed germinations is counted. The results are compared with those obtained in a non-contaminated soil of similar composition and properties. This "germination index" is commonly used as an indicator of phytotoxicity in soils[27].

Lactuca sativa, the common lettuce, is probably the plant that is more often used for this type of 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[28].

These tests are commonly used for soils contaminated with distinct contaminants such as metals[29], petroleum hydrocarbons[30] or pharmaceutical products[31]. A few studies have been performed with nanomaterials[32,33,34]. Barrena et al.[32] studied the toxicity of gold, silver and iron oxide (Fe_3O_4) nanoparticles on cucumber (*Cucumis sativus*) and lettuce (*Lactuca sativa*). Ravindran et al.[33] performed germination tests with *Lycopersicum esculentum* and *Zea mays* to evaluate the ecotoxicity of silver nanoparticles and silver ions. The results of this study 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.

El-Temsah et al.[35] studied the ecotoxicity of nZVIs and three types of silver nanoparticles in germination tests with ryegrass, barley and flax. In aquatic systems, inhibitory effects were observed for nZVI concentrations of 250 $mg{\cdot}L^{-1}$, while concentrations of 1000–2000 $mg{\cdot}L^{-1}$ completely inhibited germination. Jiamjitrpanich et al.[36] studied the tolerance of *Panicum maximum* (purple guinea grass) and *Helianthus annuus* (common sunflower) in a TNT-contaminated soil and in an nZVI-contaminated soil. *Panicum maximum* showed more tolerance than *Helianthus annuus* to the presence of nZVIs.

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 should be applied in soils via deep slurry injections.

Conclusions

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 literature is clearly insufficient and, in some cases, opposite results are reported. Nevertheless, the majority of the studies point toward the toxic effects of nZVIs on all the tested organisms. Based on research it is also possible to conclude that the ecotoxicity of nZVIs is concentration dependent and that when coated nZVIs are used, their ecotoxicity decreases. However, these, and other, conclusions can only be supported by further studies.

Acknowledgements

This work has been financially supported by the "Fundação para a Ciência e a Tecnologia (FCT)" through projects PEst-C/EQB/LA0006/2013, PTDC/ECM/103141/2008 and PTDC/AAG-TEC/2692/2012.

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Data de recebimento: 30/7/2013
Data de aceite: 05/11/2013