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AVALIAÇÃO DA TOXICIDADE DE NANOPARTÍCULAS DE FERRO NA VALÊNCIA ZERO NO SOLO: APLICAÇÃO EM ESCALA DE CAMPO

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RESUMO

A aplicação de nanopartículas de ferro na valência zero é uma das tecnologias de remediação mais utilizadas na atualidade, entretanto, os potencias riscos ambientais da aplicação são em grande parte desconhecidos. Neste sentido, realizou-se uma pesquisa bibliométrica apresentando a distribuição temporal das publicações, artigos mais citados, os autores, as instituições, os países e as revistas científicas que mais publicam sobre o assunto. Ainda, avaliou-se as unidades formadores de colônias de pseudômonas e bacillus expostos a nanopartículas de ferro na valência zero em um solo residual de basalto após a nanorremediação de local contaminado com cromo hexavalente e pentaclorofenol em escala de campo. E, foi avaliado a eficiência da injeção de nZVI de local *in situ* contaminado com cromo hexavalente e pentaclorofenol. A bibliometria possibilitou a visualização de um panorama sobre as publicações permitindo direcionar a pesquisa para uma contribuição efetiva com a ciência. A avaliação das unidades formadoras de colônias de pseudômonas e bacillus expressou o potencial tóxico das substâncias no solo. O processo de injeção de suspensões de nanoferro em corpos de prova com contaminantes orgânicos e inorgânicos mostrou-se complexo, sendo necessário desenvolver e montar equipamentos para aplicação da técnica de *jet grouting* com suspensões de nanoferro em escala de campo, simulando uma contaminação em zona não saturada; A remediação de solos com injeção de nZVI apresentou mais de 90% de eficiência na degradação dos contaminantes Cr^{6+} e PCP. A degradação de Cr^{6+} mostrou-se rápida e constante, o contaminante PCP mostrou-se mais persistente no ambiente ao apresentar maior degradação a partir de 30 dias. Observamos a migração de ambos os contaminantes e também de nZVI para camadas mais profundas do solo, entretanto não foram encontrados contaminantes no lixiviado. Avaliamos o efeito tóxico sobre o crescimento de unidades formadoras de colônias (UFCs) de *Bacillus cereus* e *Pseudomona aeruginosa*, foram observadas oscilações temporárias na abundância da comunidade microbiológica, caracterizando a adaptação das bactérias aos contaminantes. As bactérias apresentaram comportamento semelhante, noventa dias após a injeção de nZVI as médias de UFCs foram estatisticamente iguais, ocorrendo o menor coeficiente de variação e a maior concentração de UFCs. As cepas de *B. cereus* e *P. aeruginosa* foram resistentes às concentrações de nZVI, Cr^{6+} e PCP. A nanorremediação de nZVI em solo contaminado por Cr^{6+} e PCP não caracterizou efeito tóxico sobre a população de bactérias nativas do solo e não apresenta grandes distúrbios de temperatura, condutividade elétrica, pH e umidade ao longo do tempo.

Palavras Chave: Contaminação; Cromo hexavalente; nZVI; Nanorremediação; Pentaclorophenol.

ABSTRACT

The application of zero valence iron nanoparticles is one of the most used remediation technologies today, however, the potential environmental risks of the application are largely unknown. In this sense, bibliometric research was carried out by presenting the temporal distribution of publications, most cited articles, authors, institutions, countries, and scientific journals that most publications on the subject. Furthermore, we evaluated the colony-forming units of pseudomonas and bacillus exposed to zero valence iron nanoparticles in a residual basalt soil after nanoremediation of a site contaminated with hexavalent chromium and pentachlorophenol on a field scale. And, the efficiency of the injection of nZVI in situ site contaminated with hexavalent chromium and pentachlorophenol was evaluated. Bibliometrics made it possible to visualize an overview of the publications, allowing directing research towards an effective contribution to science. The evaluation of the colony-forming units of pseudomonas and bacillus expressed the toxic potential of the substances in the soil. The process of injecting nano-iron suspensions into specimens with organic and inorganic contaminants proved to be complex, and it was necessary to develop and assemble the equipment for the application of the jet grouting technique with nano-iron suspensions on a field scale, simulating contamination of in a non-iron zone. saturated; Soil remediation with nZVI injection showed more than 90% efficiency in the degradation of Cr^{6+} and PCP contaminants. The degradation of Cr^{6+} was fast and constant, the PCP contaminant showed to be more persistent in the environment when showing greater degradation after 30 days. We observed the migration of both contaminants and also of nZVI to deeper soil layers, however, no contaminants were found in the leachate. We evaluated the toxic effect on the growth of colony-forming units (CFUs) of *Bacillus cereus* and *Pseudomonas aeruginosa*. Temporary oscillations in the abundance of the microbiological community were observed, characterizing the adaptation of bacteria to contaminants. The bacteria showed similar behavior, ninety days after the injection of nZVI the averages of CFUs were statistically equal, with the lowest coefficient of variation and the highest concentration of CFUs. The strains of *B. cereus* and *P. aeruginosa* were resistant to the concentrations of nZVI, Cr^{6+}, and PCP. The nanoremediation of nZVI in soil contaminated by Cr^{6+} and PCP did not have a toxic effect on the population of native soil bacteria and did not present major disturbances in temperature, electrical conductivity, pH, and humidity over time.

Keywords: Contamination; Hexavalent Chromium; nZVI; Nanoremediation; Pentachlorophenol.

SUMÁRIO

INTRODUÇÃO GERAL

A contaminação de solos é um assunto bem difundido no meio acadêmico, a grande quantidade de áreas contaminadas vem despertando a preocupação dos órgãos ambientais tanto no exterior como no Brasil (HU et al., 2006; THOMÉ et al., 2015). Existem muitas tecnologias disponíveis para a remediação de solos e algumas utilizam materiais em escala nanométrica (REDDY, 2013; USEPA 2012).

Embora tais técnicas sejam aplicadas para a melhoria da qualidade de vida e do ambiente, transformando contaminantes nocivos em substâncias menos agressivas, a utilização de nanopartículas para remediação do solo tem gerado questionamentos e preocupações com relação à toxicidade (THOMÉ et al., 2015). As reações que ocorrem com as nanopartículas sofrem influência de muitos fatores característicos do local de estudo, tornando necessária a realização de estudos para se observar o real comportamento reativo com os contaminantes *in situ* (THOMÉ, 2015).

A aplicação de nanopartículas de ferro na valência zero (nZVI) é uma das tecnologias de remediação mais utilizadas da atualidade, devido a suas características, como baixa solubilidade e biodegradabilidade, baixa toxicidade do ferro (Fe), e menor custo de produção com relação a outras nanopartículas (KHARISOV et al., 2012; YAN et al., 2013). Entretanto, os potenciais riscos ambientais da aplicação de nZVI são em grande parte desconhecidos, as abordagens tradicionais de avaliação de risco ambiental ainda não estão concluídas, sendo esta uma área de estudos de interesse (KHARISOV et al., 2012; TOSCO et al., 2014). Pesquisas em escala laboratorial vêm sendo desenvolvidas, sendo já estudada a toxicidade de nanopartículas de ferro em diversos organismos. Entretanto, o comportamento destas partículas com o contaminante em um ambiente *in situ* ainda é uma grande incógnita. Neste sentido escolhemos dois contaminantes para avaliar a reatividade do nanoferro e a toxicidade deste com o meio.

O Cromo hexavalente Cr^{6+} é um contaminante inorgânico, tóxico para humanos, animais e plantas e está relacionado a diversos danos clínicos, como câncer no pulmão, irritação e ulceração nasal, reações de hipersensibilidade e dermatite por contato. As propriedades tóxicas do Cr^{6+} resultam da livre difusão de compostos dessa espécie de cromo através da membrana celular (COTTON; WILKINSON, 1999, MATOS, 2006). A efetividade da remediação de áreas contaminadas com Cr^{6+} por injeção de nanoferro já foi avaliada (REGINATTO, 2017). Entretanto a interação com o meio ambiente neste processo e o potencial efeito tóxico do residual precisa ser estudada, assim como para contaminantes orgânicos.

Neste ponto de vista, escolhemos o pentaclorofenol (PCF), um organoclorado persistente que foi amplamente utilizado como pesticida, herbicida e conservante de madeira. Este composto foi escolhido, pois apresenta toxicidade aguda, carcinogenicidade e mutagenicidade para diversos organismos USEPA (1998). A estabilidade da molécula deste composto resulta na sua permanência no solo, fundamentando o desenvolvimento de processos que visam sua retirada do ambiente e inibam o contato com os seres vivos.

A biodegradação é o principal processo para a transformação de poluentes orgânicos, no qual os microrganismos do solo são os responsáveis pela degradação dessas substâncias (MCALLISTER; LEE; TREVORS, 1996, BARBEAU et al., 1997). Segundo Mohn e Tiedje (1992), sob condições anaeróbias, as bactérias promovem a desalogenação redutiva do PCF, de modo que os átomos de cloro são sequencialmente substituídos por hidrogênio até a transformação completa em compostos menos tóxicos. A nanobiorremediação de pentaclorofenol foi estudada por Tessaro (2018), a efetividade e a toxicidade em escala laboratorial foram avaliadas e descritas, porém as implicações pertinentes aos fatores abióticos e a escala real *in situ* são desconhecidas.

Escolhemos duas bactérias que apresentam sensibilidade ao nZVI, *Bacillus cereus* e *Pseudomona aeruginosa*, e avaliamos o potencial efeito tóxico sobre o crescimento dos microrganismos em solo quando expostos a diferentes contaminantes no solo, também avaliamos a eficiência do processo de nanorremediação por nZVI em solos contaminados com Cr^{6+} e PCP.

No atual cenário o assunto abordado por esta proposta é inovador, nenhum trabalho semelhante foi encontrado nos principais bancos de dados, tais quais CAPES, Web of Science, Scopus e Google Acadêmico. Em relação à toxicidade de nanopartículas nos solos espera-se um desenvolvimento de pesquisas nesta área sendo bem-vindos estudos básicos em laboratórios como estudos em campos experimentais. Aplicações *in situ* já foram abordadas, entretanto não objetivaram avaliar a toxicidade das nanopartículas. A relação toxicológica de nanopartículas *in situ* é mais complexa quando comparada a testes em laboratório, estudos avaliando a toxicidade em escala de campo ainda são desconhecidos, para tal, se faz necessário o monitoramento sistemático e de longo prazo, tornando este trabalho desafiador.

A qualidade do solo é diretamente relacionada à saúde humana principalmente em função da contaminação da água e alimentos. Desta forma, muitos pesquisadores têm mostrado relações entre o estado do solo e a saúde humana (BREVIK; SAUER, 2015). Neste sentido, a recuperação de áreas contaminadas é atraente porque reduz os riscos para a saúde humana e os impactos causados ao meio ambiente, ocasionando uma série de benefícios sociais e econômicos (ALBERINI et al., 2006).

Essas características tornam o trabalho de relevante interesse para a comunidade científica, pois é capaz de proporcionar um conhecimento maior sobre a toxicidade de nZVI. Descrevendo a interação das nanopartículas de ferro com os contaminantes em ambiente natural e a influência dos fatores abióticos no processo, algo até hoje desconhecido sendo pioneiro na aplicação em escala de campo. Ainda, caso a toxicidade não seja comprovada o trabalho pode ajudar a viabilizar estas técnicas de remediação no solo com nZVI, intensificando a sua aplicação em escala no futuro.

O trabalho foi dividido em capítulos para publicação. O capítulo 1 apresenta um estudo bibliométrico publicado em 2019 sobre a toxicologia de nanoferro zero valente utilizado na remediação de solos, ao final do capítulo apresentamos a atualização dos dados. O capítulo 2 descreve a eficiência da aplicação *in situ* de nanorremediação por nZVI em solo contaminado com cromo hexavalente e pentaclorophenol. E por fim, o capítulo 3, publicado em 2022, aponta os efeitos tóxicos do nZVI em unidades formadoras de colônias de *Bacillus cereus* e *Pseudomona aeruginosa*, duas bactérias nativas do solo.

CAPÍTULO 1

O capítulo 1 apresenta um estudo bibliométrico sobre a toxicologia de nanoferro zero valente utilizado na remediação de solos. O artigo encontra-se em inglês pois foi publicado em 2019 na revista Environmental Pollution, fator de impacto 8,071, atualmente o artigo possui 19 citações.

Vanzetto, G. V. Thomé, A. Bibliometric study of nanoescale zero valent iron used in soil remediation. **Environmental Pollution**. 252, 74-83, 2019.
DOI:10.1016/j.envpol.2019.05.092

Highlights

- Provided the visualization of a panorama of the publications through indicators.
- Presented the temporal distribution, most cited articles and authors.
- Showed the institutions, countries and magazines that have published the most.

BIBLIOMETRIC STUDY OF THE TOXICOLOGY OF NANOESCALE ZERO VALENT IRON USED IN SOIL REMEDIATION

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Abstract: The application of nanoscale zero-valent iron is one of the most widely used remediation technologies; however, the potential environmental risks of this technology are largely unknown. In order to broaden the knowledge on this subject, the present work consists of a bibliometric study of all of publications related to the toxicity of zero-valent iron nanoparticles used in soil remediation available from the Scopus (Elsevier) and Web of Science (Thompson Reuters) databases. This study presents a temporal distribution of the publications, the most cited articles, the authors who have made the greatest contribution to the theme, and the institutions, countries, and scientific journals that have published the most on this subject. The use of bibliometrics has allowed for the visualization of a panorama of the publications, providing an appropriate analysis to guide new research towards an effective contribution to science by filling the existing gaps. In particular, the lack of studies in several countries reveals a promising area for the development of further research on this topic.

Keywords: Indicators, nZVI, Review, Scopus, Web of Science.

INTRODUCTION

Soil contamination is a well-publicized issue in the academic world, and a large number of contaminated areas have been raising environmental concerns in many different countries (Hu et al. 2006; Thomé et al. 2015). Research on soil remediation has shown remarkable growth over the past two decades. Today, the use of nanometer-scale materials is among the many technologies available for soil remediation (United States Environmental Protection Agency 2012; Reddy 2014; Niu et al. 2014).

Although remediation techniques are used to improve the quality of life and the environment by transforming harmful contaminants into less aggressive substances, the use of nanoparticles for soil remediation has generated questions and concerns regarding their toxicity (Thomé et al. 2015; Lefevre et al. 2016). The reactions that occur with nanoparticles are influenced by many factors characteristic of the site where they are being used, making it necessary to carry out studies that observe their actual reactive behavior with contaminants in situ (Thomé, 2015). Nanotechnology has been intensively investigated with bibliometric methods due to its technological importance and expected impact on economic activity (Takeda et al. 2009).

The application of nanoscale zero-valent iron (nZVI) is one of the most widely used remediation technologies due to its characteristics, such as low solubility and biodegradability, low iron (Fe) toxicity, and lower production costs compared to other nanoparticles (Kharisov et al. 2012; Yan et al. 2013; Cai et al. 2019). However, the potential environmental risks of the use of nZVI are largely unknown as the traditional approaches to assessing these risks have not yet been completed, thus making this a highly desirable area for study (Kharisov et al. 2012; Tosco et al. 2014; Mukherjee et al. 2015).

It is known that the main environmental investigations of the application of nZVI are based on its behavior in the soil, especially in its potential to migrate long distances, changing the conditions of the environment, making it alkaline or reducing (Uyusur; Unlu, 2009) reducing the hydraulic conductivity and altering the soil microbiota (Kirschling et al., 2010). Affecting several organisms such as bacteria, protozoa, fungi, algae, as well as dissimilar, methanogenic and homoacetogenic reducers (Lowry et al., 2009). Causing disruption of cell membrane integrity (Nel et al, 2009; Fang et al, 2007), interfering with respiration (Lyon et al, 2008), promoting damage to DNA proteins or enzymes due to the release of nanoparticle ions (Gogoi et al., 2006).

Several authors have been working on relative articles, attempting to present the potential environmental risks of nZVI (Garner; Kelle, 2014, Mukherjee et al., 2015, Pease; Rucker; Birk, 2016, Pettibone; Louie, 2016). In this sense, the importance of the elaboration of the information contained in this article is clear along with the need for the dissemination of the most important bibliometric data available. The present work consists of a bibliometric study of the articles on the toxicity of nZVI used in soil remediation available from the Scopus (Elsevier) and Web of Science (WoS; Thompson Reuters) databases and presents the search results according to a temporal distribution of the publications, the most cited articles, the authors who have made the greatest contribution to the theme, and the institutions, countries, and scientific journals that have made the greatest contribution to this subject.

METHODOLOGY

Bibliometrics is a method used to analyze and construct indicators on the dynamics and evolution of scientific information. This technique allows researchers to select and analyze the studies covering their subject of interest and to elaborate on the characteristics of the studies that have been produced. This then more precisely clarifies the direction for new research and reduces the margin of error in decision making (Macedo et al. 2010). Understanding these indicators can help researchers when initiating research in a field or help others who want to know the current state of the art of a topic.

With this in mind, a quantitative-descriptive temporal bibliometric study was performed with the objective of increasing the knowledge of the studies related to the toxicity of nZVI used in soil remediation that are available in the Scopus and WoS databases.

The choice of these databases was based on their relevance. Scopus is the largest database of abstracts and citations from peer-reviewed literature, scientific journals, books, and other scholarly works. The WoS is a database of bibliographical references that contains information about scientific research produced since 1945 (Scopus 2018; Web of Science 2018).

The procedures that were adopted for this study followed the methodology found in other bibliometric studies but with some adaptations (Macedo et al. 2010; Trentin et al. 2017). A search was performed of the documents in the databases that contained the words nanoiron, toxicity, soil, and remediation in the title, abstract, or keywords.

However, words like those used in this study may appear in several variations. For example, the word "toxic" is the root of several terms, such as toxicity, toxicology, toxicological, ecotoxicological, ecotoxicity, among others. To find these different variations among the words in the databases, a wildcard (*) was used. For example * toxic * would be able to find all the above-mentioned terms because the root of the word remains the same despite the variations in the affixes.

The same does not apply to the word "nanoiron," however, as it can be written without a space, with a hyphen as in "nano-iron," with the abbreviation of nanoscale zero-valent iron of "nZVI," or as two words as in "nano iron." In this case, it is necessary to use quotation marks ("") to find phrases or exact phrases and to deactivate the lemmatization and synonym features of these platforms in addition to not implying operators or the spaces between words. Due to this characteristics of this study, it was necessary to find different expressions, and it was thus necessary to use the Boolean operator "or." This operator allows for at least one term to appear. These were connected to the operator "and," which ensured that the words "soil" and "remediation" appeared somewhere in each article. Fig. 1 shows the search pattern used for the Scopus database.

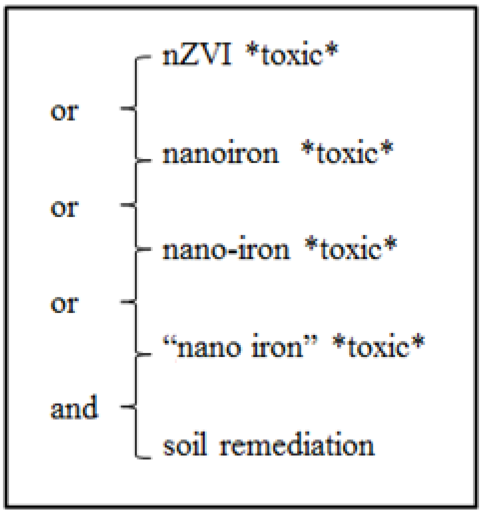

Fig. 1: Search pattern used on the databases.

For the WoS database, the procedure was the same as with the Scopus database since both platforms use the same operators, and the words and Boolean operators were the same as shown in Fig. 1. However, in addition to searching the title, abstract, and keywords, the WoS database covers topics and includes a search of the “keywords plus” of a work. The keywords plus consist of words or phrases that have been extracted from the titles of the articles cited by an article and are retrieved by a search in the topic field. This is the only difference in the search of the two databases.

RESULTS AND DISCUSSION

The following are the general characteristics of the publications that were found according to the following categories: the time distribution of the articles, the authors of the studies, the articles most cited, and the countries, institutions, and scientific journals that have contributed the most to the subject of study.

Temporal distribution of the scientific production

The first step in the bibliometric research provided a total of 343 articles, 20.4% (70) found in the Scopus database and 79.6% (273) in the WoS database. The temporal distribution of these studies covered 19 years. The Scopus database provided articles from 1999 to 2018 and the WoS database from 2002 to 2018. However, some works found at this stage contained the terms that were used in the word search but still did not match the research theme. The articles that presented only the application of nZVI and not the toxicity of nZVI to the environment or to human health were not chosen for the bibliometric study. For example, some papers contained the search terms but addressed the reduction of the toxic effects of contaminants by nZVI, soil remediation by iron nanoparticles, or dealt with the decontamination of water and sediments by nZVI, and these were thus not selected.

Therefore, it was necessary to carry out a second search stage in which we sought to exclude those works that were not connected with the proposed theme. For each article, a reading of the abstract was performed and the subject of a work was evaluated to see if the theme was the toxicity of nZVI used in soil remediation. Of the 70 papers found in the Scopus database, 50.0% (35) of them were selected, and of the 273 papers found in the WoS database, 33.3% (91) of them were selected. In this way, the temporal distribution changed to 12 years prior, as depicted in Fig. 2, with the first publications related to the theme appearing in 2006.

It should be noted that the 35 publications found in the Scopus database were repeated because they were also indexed in the WoS database, so it can be said that the total number of articles that resulted in the bibliometric search was 91 publications. Table 1 below shows the total number of articles found. When comparing the databases used for keyword searches, we noticed that WoS was more efficient at representing the results, providing 61.5% (56) more articles than the Scopus database. Therefore, it is recommended that researchers working in this field use WoS to search for references and journals.

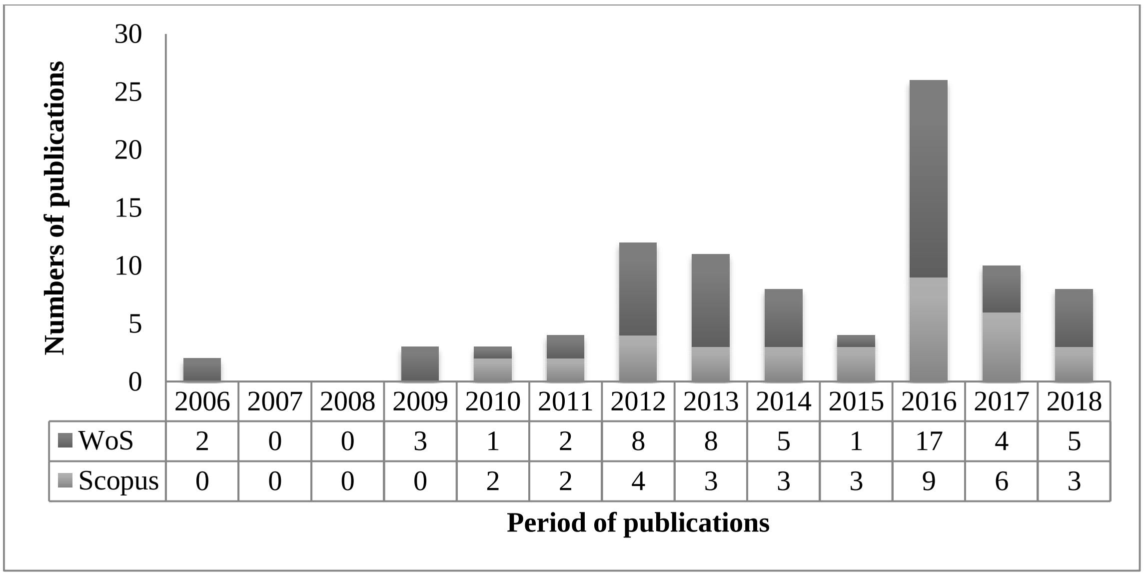

Fig. 2: Temporal distribution of the production of scientific studies related to the toxicity of nZVI used in soil remediation and a comparison between the Scopus and WoS databases.

Table 1: Articles used in the bibliometric study presented per year.

Year	Reference
2006	Cheng et al., 2006; Li et al., 2006.
2009	Phenrat et al., 2009; Li et al., 2009; Keenan et al., 2009.
2010	Sevcu; Cernik, 2010; Li et al., 2010; Grieger et al., 2010.
2011	Chen et al., 2011; Chen et al., 2011; Kadar et al., 2011; Grieger et al., 2011.
2012	Dong et al., 2012; Kharisov et al., 2012; Bobcikova et al., 2012; Fajardo et al., 2012; Marsalek et al., 2012; Ban et al., 2012; Chen et al., 2012; Keller et al., 2012; El-Temsah; Joner, 2012; Zanaroli et al., 2012; Kadar et al., 2012; El-Temsah; Joner, 2012.
2013	Yang et al., 2013; Chen et al., 2013; Ma et al., 2013; Kadar et al., 2013; Qiu et al., 2013; Tang; Lo, 2013; El-Temsah; Joner, 2013; El-Temsah et al., 2013; Chen et al., 2013; Fajardo et al., 2013; Pawlett et al., 2013.
2014	Predescu et al., 2014; Nemecek et al., 2014; Saccà et al., 2014; Barzan et al., 2014; Zhou et al., 2014; Saccà et al., 2014; Garner; Keller, 2014; Gonzalo et al., 2014.
2015	Velimirovic et al., 2015; Le et al., 2015; Fajardo et al., 2015; Jiang et al., 2015.
2016	Libralato et al., 2016; Rede et al., 2016; Padrova et al., 2016; Pettibone et al., 2016; Sun et al., 2016; Parvathi et al., 2016; Xie et al., 2016; Padrova et al., 2016; Chaithawiwat et al., 2016; Dong et al., 2016; El-Temsah et al., 2016; Stefaniuk et al., 2016; Pease et al., 2016; Yang et al., 2016; Yirsaw et al., 2016; Fajardo et al., 2016; Ortega-Calvo et al., 2016; Yirsaw et al., 2016; Wang et al., 2016; Schivvy et al., 2016; Chaithawiwat et al., 2016; Lefreve et al., 2016; Lei et al., 2016; Yang et al., 2016; Semerad et al., 2016; Wang et al., 2016.
2017	Liang et al., 2017; Bhuvaneshwari et al., 2017; Kumar et al., 2017; Gosh et al., 2017; Gonzales-Andres et al., 2017; He et al., 2017; Hsueh et al., 2017; Hjorth et al., 2017; Kotchaplai et al., 2017; Lv et al., 2017.
2018	Nguyen et al., 2018; Corsi et al., 2018; Liang et al., 2018; Huang et al., 2018; Zhang et al., 2018; Murugan et al., 2018; Mohammadi et al., 2018; Lei et al., 2018.

The first works that addressed the topic of the potential impact of the use of nZVI used in soil remediation questioned the perspectives of the technology and called attention to needed future research. After two years without additional publications, the appearance of new articles began again in 2009 and studied other characteristics and began to investigate the toxicity of nanoparticles in several indicator organisms, such as epithelial cells, bronchial cells, and soil microorganisms.

During the period from 2006 to 2011, there was a stable publication rate, but in the year 2012 there were 12 articles published, which was the exact sum of all articles from 2006 to 2011. From 2012 to 2015, the number of published studies decreased, but the research profile expanded and the number of indicator organisms studied increased, thus increasing the knowledge of the toxicological potential of nZVI. During this period, researchers investigated the germination toxicity shown in soil microbial structure and function, soil bacteria activity, cyanobacteria metabolism, the lung cells of mice, and the population rates of phytoplankton, zooplankton, microalgae, earthworms, and fish larvae.

When comparing the periods of 2006–2011 to 2012–2015, the average annual rate of published articles was two publications per year for 2006–2011 and 8.75 publications per year for 2012–2015, a 22.8% increase in the rate of publications per year, indicating that there is a growing trend of publications on this subject.

The number of publications in 2016 increased to 26, which was 28.6% of the total and corroborates this increasing trend. For the periods 2006–2011 and 2012–2015, the percentage of publications was 13.2% and 38.4%, respectively. It is worth noting that in 2016 there were a high number of bibliographical reviews related to the topic and a further dissemination of the research on several more indicator organisms. During the period 2017 to 2018, the number of publications reduced, demonstrating the need for studies on the toxicity of nZVI used in soil remediation.

Several organisms have been studied in the laboratory to estimate the toxic effect of nZVI, Table 2 shows concentrations toxic to some organisms. Although iron occurs naturally in the soil, its nanometer scale may result in patterns of behavior and fate different from conventional (Boxall et al., 2007). Studies have shown that the effect of nZVI on microorganisms from aquifer sediments can occur up to 250 days (Kirschling et al., 2010) and 130 days (Kumar et al., 2014) after its application, suggesting that nZVI causes a long-term impact on native microorganisms of aquifers.

In the soil microbiota the nZVI also has a decisive effect (Tilston et al., 2013) which varies according to the type of soil (Saccà et al., 2014). The number of cells of some types of bacteria and fungi decreased with the addition of nanoferro, more intensely in sandy soils than in clayey soils, probably due to the effect of organic matter and particle texture (Pawlett et al., 2013).

Table 2: Toxicity of zero-valent iron nanoparticles in different organisms.

Organism tested	nZVI	Reference
Microorganisms		
Dehalococcoides culture	100 mg/L	Xiu et al., 2010
Artemia salina	100 mg/L	Kumar et al., 2017
Escherichia coli	100 mg/L	Li et al., 2010
Daphinia magna	14.22 mM	Bhuvaneshwari, et al., 2017
Erwinia amylovora	625 ppm	Barzan et al., 2014
Xanthomonas oryzae	550 ppm	Barzan et al., 2014
Bacillus cereus	1250 ppm	Barzan et al., 2014
Pseudomonas putida	5.0 g/L	Kotchaplai et al., 2017
Soil and water organisms		
Folsomia candida	1.0-10 g/L	Chen et al., 2011
Heterocypris incongruens	1.0-100 mg/L	El-Temsah; Joner, 2012
Larvaes *Oryzias latipes*	1.0-100 mg/L	El-Temsah; Joner, 2012
Medaka *Oryzias latipes*	0.5-50 µg/ml	Li et al., 2009
Eisenia fetida	100-1000 mg/kg	Liang et al., 2017
Plants		
Allium cepa	250 mg/L	Gosh et al., 2017
Lolium perrene	100-500 mg/kg	Huang et al., 2018
Oryza sativa	250-1000 mg/kg	Wang et al., 2016
Linum usitatissimum	300-5000 mg/L	El-Temsah; Joner, 2012
Hordeum vulgare	1 g/kg	El-Temsah; Joner, 2013
Typha latifolia	0-1000 mg/L	Ma; Gurung; Deng, 2013
Populous deltoids	0-1000 mg/L	Ma; Gurung; Deng, 2013
Barley	n.a.	El-Temsah; Oughton; Joner, 2013
Cells of mammals		
Lung lymph node	n.a.	Ban et al., 2012
Neurons	n.a.	Phenrat et al., 2009
Rodent Cells	n.a.	Phenrat et al., 2009
Human bronchial epithelial	n.a.	Keenan et al., 2009

*n.a.: don’t show concentration.

Articles that have been cited the most often

Among the bibliometric characteristics of an article, perhaps the number of times that a study has been cited in other works is the most important, because it expresses the relevance of this in the academic world, allowing the reference to something, providing the credit to idea or discovery, facilitating the consultation of the information. Therefore, we have listed the works that have been cited the greatest number of times for the topic being researched. Table 3 below shows the top 10 papers that have been used as citations.

Table 3: Articles on the toxicity of nZVI used in soil remediation that have been used the greatest number of time as citations.

AUTHORS	TITLE	CITATION
Li et al.	Zero-valent iron nanoparticles for abatement of environmental pollutants: Materials and engineering aspects	507
Tang et al.	Magnetic nanoparticles: Essential factors for sustainable environmental applications	278
Grieger et al.	Environmental benefits and risks of zero-valent iron nanoparticles (nZVI) for in situ remediation: Risk mitigation or trade-off?	157
Li et al.	Adsorbed polymer and NOM limits adhesion and toxicity of nano-scale zerovalent Iron to *E. coli*	151
Chen et al.	Effect of natural organic matter on toxicity and reactivity of nanoscale zero-valent iron	136
Phenrat et al.	Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron	135
El-Temsah et al.	Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil	112
Kharisov et al.	Iron-containing nanomaterials: Synthesis, properties, and environmental applications	112
Keenan et al.	Oxidative stress induced by zero-valent iron nanoparticles and Fe (II) in human bronchial epithelial cells	110
Li et al.	Effects of waterborne nano-iron on medaka (*Oryzias latipes*): Antioxidant enzymatic activity, lipid peroxidation and histopathology	101

The work of Li et al. (2006) is considered as one of the pioneers in the field and deals with the use of nZVI for the reduction of organic and inorganic pollutants and presents a synthesis and characterization of nZVI. It also provides an overview of the mechanisms of the transport, degradation, and remediation for organic and inorganic contaminants. Another point of the study is the concern with the possible environmental impact caused by nZVI, indicating the need for research to determine the toxicity of these nanoparticles.

Tang et al. (2013) composed a literature review that provided a holistic view of magnetic nanoparticles and discussed their toxicological effects. For Grieger et al., (2010) the potential environmental risks of nZVI in in-field field-scale applications were largely unknown and traditional approaches to environmental risk assessment could not yet be considered. In their analysis, they found that most of the reported benefits of the use of nZVI were based on short-term considerations and that its characteristics, such as its persistence, bioaccumulation, and toxicity, need to be known, recommending continuous monitoring at sites using nZVI.

At the same time, Li et al. (2010) found that the coating of nanoparticles may reduce their toxicity to some organisms. In this study, they reported that polyelectrolyte and natural organic matter (NOM) coatings mitigated the toxicity of nZVI in *Escherichia coli* bacteria. A year later, Chen et al. (2011) reported the mitigation of the bactericidal activity of nZVI for Gram-negative *E. coli* and Gram-positive *Bacillus subtilis* in the presence of humic acids from a river that was used as its NOM. These results suggest that the presence of NOM offers a compensation for nZVI-based remediation with a greater potential for simultaneous or sequential bioremediation over abiotic reactivity partially inhibited with the target contaminant.

The sixth most cited article was written by Phenrat et al. (2009) and reports that oxidation and surface modification reduce redox activity, sedimentation, and the in vitro neurotoxicity of nZVI. The results of this study were based on the results obtained by the use of nZVI in the treatment of ryegrass, barley, and flax seeds. In a literature review, Kharisov et al. (2012) discussed the synthesis, properties, and environmental applications of nZVI and also their toxicity and the risks of the application of iron nanomaterials.

The synopsis of the work of Keenan et al. (2009) exposed the oxidative damage caused to bronchial epithelial cells when exposed to nZVI attributable to iron oxidation. Finally, Li et al. (2009) evaluated the harmful effects of nZVI in aqueous solution with the bioindicator *Oryzias latipes*. The biomarkers evaluated were enzymatic activity, antioxidant, lipid peroxidation, and histopathology.

Given these data, it is possible to make an analysis of the approaches of the most cited articles and search for ways to elaborate further with new research by presenting a similar point of view or line of thought. Work that is innovative for any organization, scale, or analysis is recommended.

Authors who have made the greatest contribution to the theme

Based on the 91 publications found, it was possible to identify the authors who had more publications on the toxicity of nZVI used in soil remediation, and this is shown in Fig. 3. In total, 282 authors were found, 2.1% with six publications, 0.7% with five publications, 0.3% with four publications, 3.2% with three publications, and 11% with two publications, and 82.7 % with one publication.

The authors Mar Nande, Margarita Martin, Gonzalo Costa, and Carmen Fajardo are researchers at the Complutense University of Madrid in Spain and are members of the Department of Biochemistry and Molecular Biology. Nande and Fajardo are researchers with an *h*-index of 10 on ResearchGate, while the indexes for Martin and Costa were not found. Another author is Norwegian researcher Yehia Sayed El-Temsah, with a Ph.D. in Molecular Biology. Linked to the Norwegian Institute of Bioeconomics Research in the Department of Environment and Climate, El-Temsah has six publications on the subject and has an *h*-index of 7 on ResearchGate.

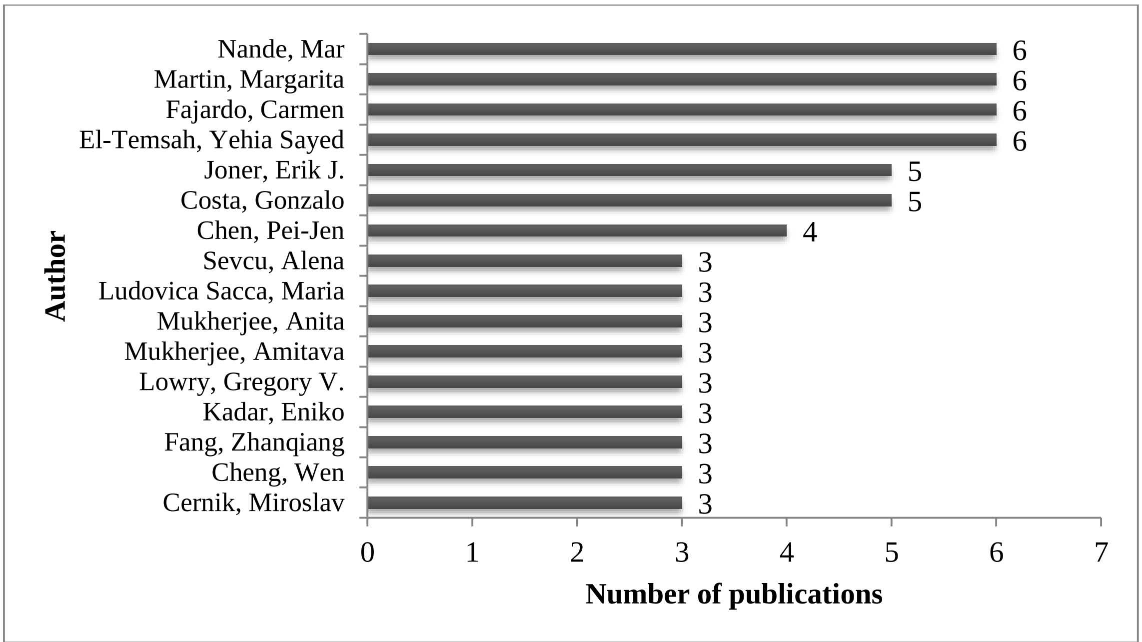

Fig. 3: Authors with the largest contributions to the study of the toxicity of iron nZVI in soil remediation.

Erik J. Joner has a Ph.D. in soil microbiology (Norwegian University of Life Sciences 1994), and one of his specializations is in the environmental impact of nanoparticles. Joner has an *h*-index of 40 and is linked to the Norwegian Institute of Research and Bioeconomics in the Department of Soil Quality and Climate. Other authors are highlighted and are listed in Figs. 3 and 4. It should be noted that there is a tendency to produce articles with groups of authors, and besides the authors with greater prominence being bound to the same institutions, many publications occur among the same authors. When we take into account only the first author of each article, as in Fig. 4, we observe this effect better.

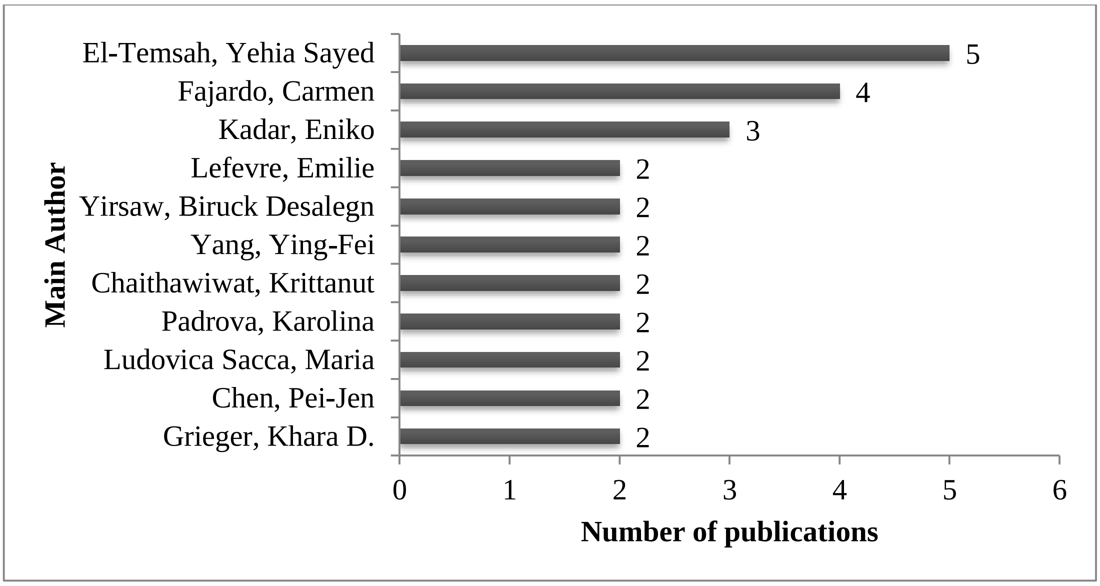

Fig. 4: The main authors with the largest contributions to the study of the toxicity of nZVI used in soil remediation.

Based on this analysis, the author El-Temsah stands out as the researcher with the highest number of publications as the main author, followed by Fajardo. Third is a researcher specializing in the ecotoxicology of legacy pollutants and emerging contaminants, Eniko Kadar, who has a Ph.D. in Environmental Toxicology, an *h-index* of 14 on ResearchGate, and is linked to the Faculty of Science Engineering and Medicine at the University of Manchester in the United Kingdom.

Another point to be observed is the multidisciplinarity of the authors involved with this subject; they are experts from several different areas of science. This fact encourages research on the subject because it is not dominated by a single discipline, allowing for a freer exchange of information, research, and professionals.

Countries that have published the most on the theme

An analysis was made of the countries with the highest number of publications. As shown in Fig. 5, the United States is the most prominent country, having presented 17.5% of articles related to the toxicity of nZVI used in soil remediation. China follows with 14.3% of publications, and tied in third place with 9.8% of publications are the Czech

Republic and Spain. Other countries with a high number of works are England (8.8%), Taiwan (7.7%), Norway (7.7%), India (4.3%), Denmark (3.3%), Italy (2.2%), South Korea (2.2%), and Australia (2.2%). Another nine countries have only one article each, representing 1.1% of the publications related to the topic.

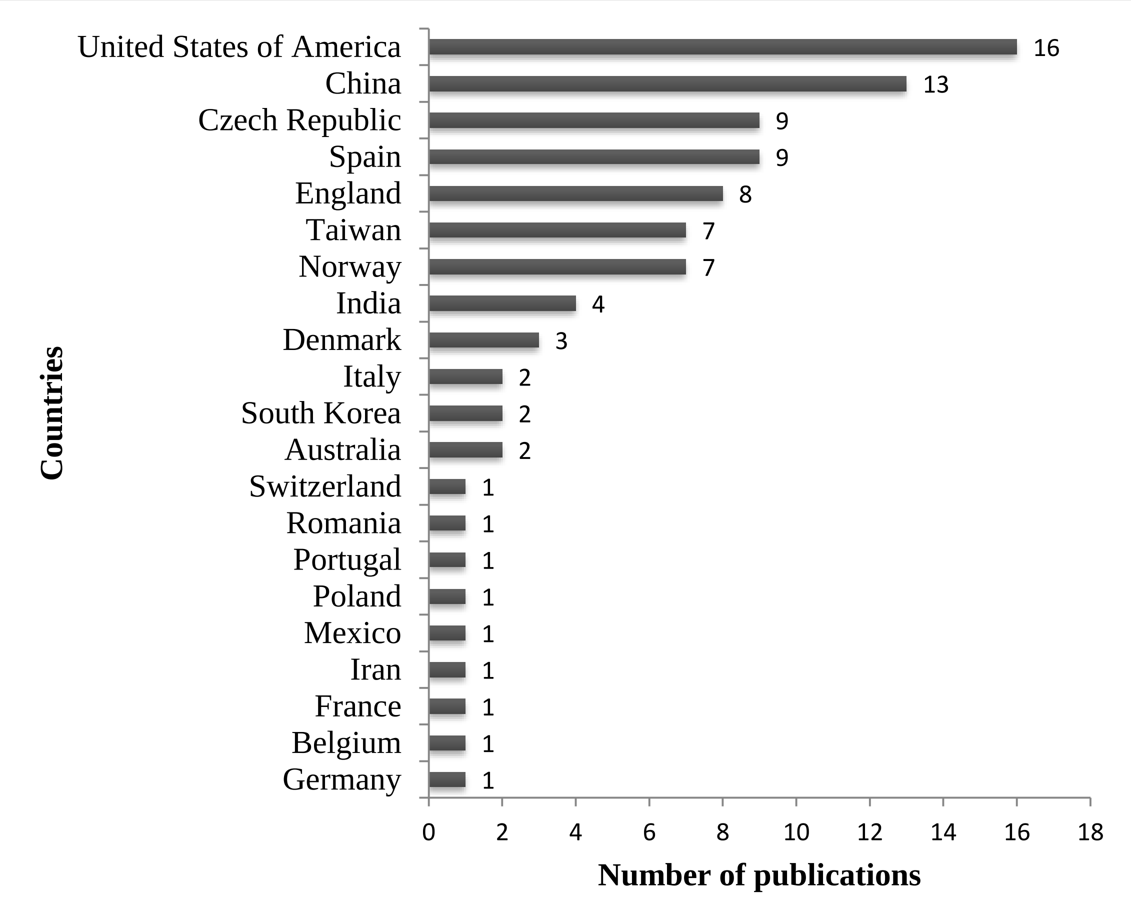

Fig. 5: Countries that have made the greatest to the study of the toxicity of nZVI used in soil remediation.

Some countries that have a strong tradition of research stand out in the indicators, such as the United States and China. Recent and influential reports highlight that China, India, and other emerging economies can compete with the advanced countries of the Organization for Economic Cooperation and Development (OECD) in the study of nanotechnology and can assert their position on the global stage (Bhattacharya; Bhati 2012; Nassi-Calò 2015).

While the countries mentioned above performed well in producing research, Latin American, such as Brazil, African, and several OECD countries do not appear in the indicators, revealing the need for further worldwide studies to demonstrate the potential environmental effects of nZVI use. Environmental risk assessments still need to be completed, and the particularities of each application site need to be published as there are many site-related influencing factors.

The microorganisms present in the soil are different for each site, and the sensitivities of these organisms to nZVI are still unknown. In this sense, publications related to the application of nZVI and its toxicity, mainly studies with in situ applications, are strongly recommended.

Institutions that have published the most on the theme

Fifty-five research institutions that have produced research related to the subject were identified, and among them, Complutense University of Madrid stands out with 7.8% of the publications. This is followed by National Taiwan University with 6.5% and South China Normal University with 3.9% of the publications. Another 11 institutions with two publications each are listed in Fig. 6 and represent 28.6% of the publications, while an additional 41 institutions produced one article each, accounting for 53.2% of the works found.

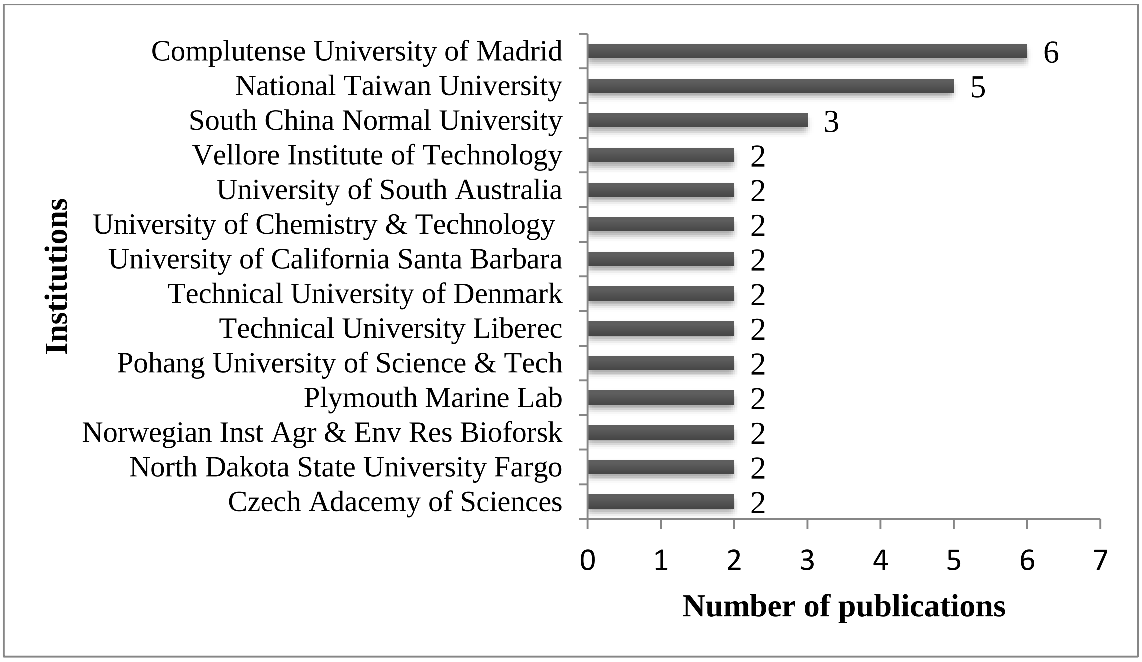

Fig. 6: Institutions with the greatest contribution to the study of the toxicity of nZVI used in soil remediation.

Complutense University Madrid, which is linked to researcher Carmen Fajardo Ph.D., was the institution that made the greatest contribution to the theme. Founded in 1949 in the University City of Madrid, this public university has approximately 80,000 students and is one of the most prestigious in Europe (Complutense University of Madrid 2017). National Taiwan University appears next, and this university was founded in 1928, currently has 33,000 students, and several of the university's undergraduate and graduate courses contribute to making this school the most influential institution in Taiwan (National Taiwan University 2017).

Founded in 1933, the South China Normal University also stood out in the indicators. With approximately 33,000 students and several postgraduate programs, the institution has about 1,500 international students (South China Normal University 2017). These characteristics make all of these research centers great resources for an exchange of researchers and joint project development, providing an international perspective on the toxicity of nZVI used in soil remediation.

The main purpose of highlighting this data is to enable researchers to view the institutions that are best suited for an exchange of experiences, research, postdoctoral studies, study trips, and other activities that involve an exchange of knowledge among the institutions that dominate knowledge in this area.

Scientific journals that have published the most on the theme

In order to assist researchers with an interest in the subject of this study, an analysis of the main scientific journals that have published on the topic was carried out and is seen in Fig. 7. The scientific journal *Chemosphere* (Elsevier) stands out with 16.8% of the publications, which is followed by 10.4% in *Science of the Total Environment* and 7.8% in *Environmental Science and Technology*.

The journals *Environmental Pollution*, *Nanotoxicology*, *Environmental Science and Pollution Research*, *PLOS One*, and *Water Research* published a combined total of 19.5% of the articles that were found. *Journal of Hazardous Materials*, *Chemical Engineering Journal*, *RSC Advances*, *Environmental Toxicology and Ecotoxicology*, and *Environmental Safety* published a combined total of 13% of the articles. Another 22 journals, while no less important, were less expressive in their number of publications, each with only one article on the subject. An additional three papers were found that were presented at conferences and indexed in the WoS database.

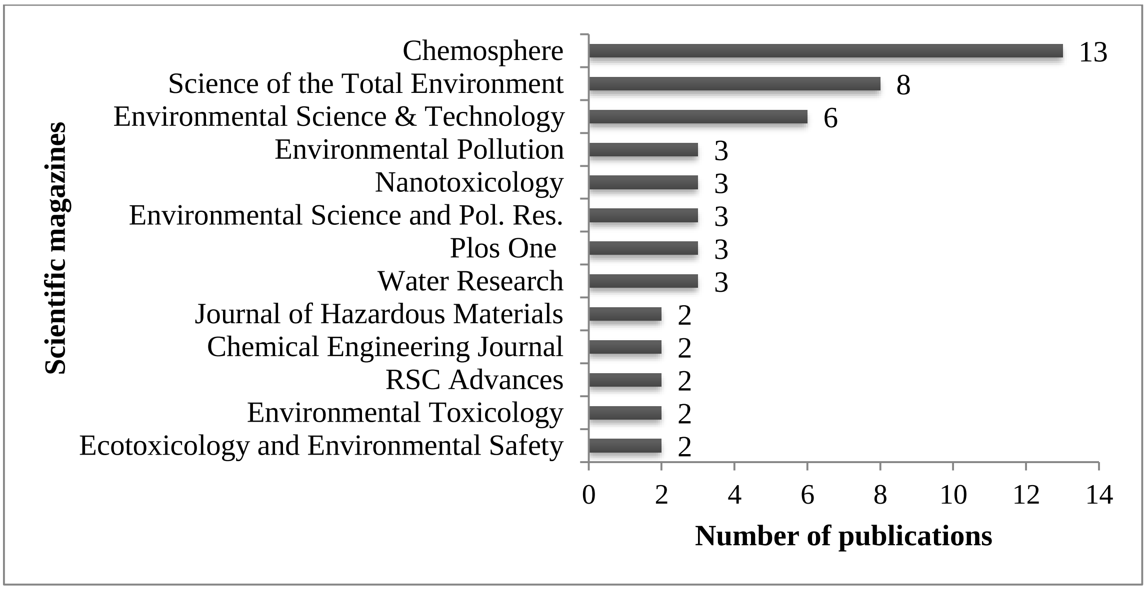

Fig. 7: Scientific journals that have published the most articles on the toxicity of nZVI used in soil remediation.

Considering the dissemination and visibility of the research on the subject, some journals stood out. This was the case with *Chemosphere*, an international and multidisciplinary journal that provides a wide and influential dissemination of information. This journal has impact factor of 4.2 and has an exclusive section on environmental toxicology and risk assessment covering all aspects of toxicology.

Following is the journal entitled *Science of the Total Environment*, an international journal for the publication of original research on the environment as a whole, including the atmosphere, hydrosphere, biosphere, lithosphere, and anthroposphere. This journal is indexed by Elsevier and has an impact factor of 4.1. *Environmental Science and Technology*, which features an impact factor of 6.1 and is indexed by ACS Publications, is an authoritative source of information for practitioners in a wide range of environmental disciplines.

CONCLUSION

This quantitative-descriptive temporal bibliometric study has brought together and clarified the knowledge on the publications related to the toxicity of nZVI used in soil remediation that are available from the Scopus and WoS databases. It enables the visualization of a panorama of publications, authors, countries, institutions, and journals through indicators that allow researchers to select and analyze the existing literature, directing their research for a better scientific contribution.

Considering the large number of publications in 2016, this study has also shown that the toxicity of nZVI used in soil remediation is an extremely new and developing topic for study. According to the data analyzed, the main authors with more publications were Yehia Sayed El-Temsah, Carmen Fajardo, and Eniko Kadar.

The United States and China were shown as the leading countries in the ranking of countries with publications on the subject. Other countries were cited according to their contributions, highlighting the absence of countries in South America and Africa. This issue reveals a promising area for the development of research and articles on the toxicity of nZVI used in the remediation of soils of these regions.

With regard to the institutions producing research, large research centers have stood out as enabling the exchange of researchers, development of joint projects, and the possibility of an international experience. Regarding the articles themselves, it is evident that the theme is of relevant scientific interest because they are published in journals with high impact factors, including *Chemosphere*, *Science of the Total Environment*, and *Environmental Science and Technology*.

Finally, the objective of this study was to provide an analysis to broaden knowledge on the publications related to this subject and enable researchers to focus their research on filling the gaps in the subject and contribute more effectively to science. We need to remedy the lack of research carried out in situ and in underdeveloped countries, the need to investigate new environmental quality indicators, especially non-target species that have a relevant interest in ecosystem balance, which will vary according to the region of study. Pointing to acute toxicity of nZVI, presenting lethal contractions, effective risk concentrations and concentrations of non-effective risk. The substance's classification as to the potential for hazard and risk analysis. We also need to observe the need for research that best points the nanoparticle migration system and better follow-up by long-term studies.

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ATUALIZAÇÃO DA REVISÃO BIBLIOMÉTRICA

O monitoramento das publicações foi mantido com o uso de um alerta, as publicações indexadas nas bases de dados com as mesmas palavras chave da revisão bibliométrica disparam um e-mail automático de “artigo recomendado”. Isto permitiu a constante atualização do assunto e o direcionamento adequado para a pesquisa.

Desde a publicação do artigo até maio de 2022 foram indexadas 64 novas publicações abordando a toxicidade de nanopartículas de ferro na valência zero (nZVI) em remediação do solo, uma publicação em 2018; 20 registros em 2019; 23 em 2020; 16 artigos em 2021 e 4 trabalhos em 2022. Todos os trabalhos foram indexados na Web of Science (WoS) e 12 artigos foram encontrados também na base de dados Scopus, ou seja 81,25% menos publicações, reforçando a ideia de que a base de dados WoS é mais adequada para a busca de informações. A tabela 1 abaixo, apresenta os artigos publicados.

Tabela 1: Artigos indexados após a revisão bibliométrica apresentados por ano.

Ano	Referência
2018	Amen et al., 2018.
2019	Gosh et al., 2019; Sun et al., 2019; Semerad et al., 2019; Dong et al., 2019; Kim et al., 2019; Vanzetto; Thome, 2019; Yang; Kung; Chen, 2019; Liang et al., 2019; Horvathova; Laszlova; Dercova, 2019; Weil et al., 2019; Ghosh; Mukherjee; Mukherjee, 2019; Hu et al., 2019; Zhu et al., 2019; Zhu et al., 2019; Han et al., 2019; Zhou et al., 2019; Cheng et al., 2019; Crampon et al., 2019; Fajardo et al., 2019; Cecchin et al., 2019.
2020	Shariati; Poordeljoo; Zanjanchi, 2020; Cheng et al., 2020; Gura et al., 2020; Semerad et al., 2020; Zhang et al., 2020; Fajardo et al., 2020; Latif et al., 2020; Li et al., 2020; Teodoro et al., 2020; Sun et al., 2020; Xia et al., 2020; Semerad et al., 2020; Sun et al., 2020; Li et al., 2020; Pasinszki; Krebsz, 2020; Liu et al., 2020; Zhang et al., 2020; Fajardo et al., 2020; Brasili et al., 2020; Wang et al., 2020.
2021	Flores-Rojas et al., 2021; Canção et al., 2021; Sim et al., 2021; Zand; Tabrizi, 2021; Li et al., 2021; Xie et al., 2021; Wang et al., 2021; Cheng et al., 2021; Lin et al., 2021; Sun et al., 2021; Albarano et al., 2021; Mielcarz-Skalska; Smolins-

Ano	Referência
2021	ka; Szynkowska-Jozwik, 2021; Guha; Mukherjee; kundu, 2021; Liu et al., 2021; Zhang et al., 2021; Sim et al., 2021.
2022	Krawczyk et al., 2022; Wang et al., 2022; Shakoor et al., 2022; Wu et al., 2022.

Ao analisar os trabalhos, observa-se a evolução na investigação da toxicidade de nZVI para diferentes organismos (Cheng et al., 2019; Li et al., 2020) diversas publicações investigando a toxicidade em plantas (Kim et al., 2019; Brasili et al., 2020; Li et al., 2020; Guha; Mukherjee; Kundu, 2021), outras avaliando o efeito combinado de contaminantes (Fajardo et al., 2020), até mesmo de nanopartículas combinadas (Fajardo et al., 2019; Sun et al., 2021). A tabela 2 a seguir apresenta alguns organismos avaliados.

Tabela 2: Novos dados de toxicidade de nanopartículas de ferro de valência zero.

Organismo testado	nZVI	Referência
Plantas		
Medicago sativa	Não toxico	Kim et al., 2019
Solanum lycopersicum	Não toxico	Brasili et al., 2020
Vigna radiata	600 mg/L	Sun et al., 2019
Brassica napus	Não toxico	Li et al., 2020
Cucumis Sativus	Não toxico	Li et al., 2020
Zea mays	30 g/kg	Thomé et al., 2020
Glycine max	30g/kg	Thomé et al., 2020
Oryza sativa	Não tóxico	Guha; Mukherjee; Kundu, 2021
Fungos		
Acaulospora mellea	Não toxico	Cheng et al., 2021
Micorriza arbuscular	Não toxico	Sun et al., 2021
Microorganismos		
Caenorhabditis elegans	n.a.	Li et al., 2021

*n.a. – não apresenta concentração.

Quanto aos artigos mais citados, o trabalho de Li; Elliot; Zhang (2006) continua no topo com 770 citações, 263 a mais que a primeira busca. Entre os novos artigos destaca-se o artigo de Zhu et al (2019) com 53 citações, apontando o comportamento e o efeito da remediação e da toxicidade de materiais em ambientes aquáticos, e o estudo bibliométrico realizado por Vanzetto; Thome (2019) sendo o 14° mais citado com 19 citações.

Diversos autores aparecem entre as novas publicações, salienta-se a participação de Amitava Mukherjee, Professor Senior e Diretor do Centro de Nanobiotecnologia da Universidade de Vellore na India, o Pesquisador Yuhuan Sun da Universidade de Finanças e Economia de Lanzhou na China, o Pesquisador Fayuan Wang da Universidade de Ciências e Tecnologia de Qingdao na China, Wenjie Wang da Universidade Farmacêutica da China e Shu-Wu Zhang da Universidade Agrícola da China, todos com 4 publicações cada no período de 2018 a 2022. Os autores Mar Nande, Margarita Martin e Carmen Fajardo, pesquisadores da Universidade Complutense de Madri, na Espanha, e membros do Departamento de Bioquímica e Biologia Molecular continuam sendo os maiores contribuintes somando 3 publicações, atingindo o total de 9 artigos sobre o tema.

Como podemos observar pelo vínculo dos autores a China vem ganhando destaque entre os países que mais publicam, no período avaliado foram 33 artigos vinculados ao país, 51,5% do total das publicações. Com isto, a China passa os Estados Unidos totalizando 46 publicações contra 23 dos norte-americanos, 7 a mais que o período anterior.

Entre as instituições a Universidade Complutense de Madrid continua sendo a principal contribuinte com 8 publicações, 2 novas. Ainda, destaca-se a Universidade Chinesa de Ciências que não estava na lista anterior e aparece com 6 novas publicações, a Academia de Ciências da República Checa que alcança 6 publicações, 4 novas, e a Universidade de Passo Fundo que aparece com 3 novas contribuições neste período, sendo a primeira Universidade Brasileira a contribuir com o tema, colocando o Brasil na 6ª colocação, empatado com Alemanha e Coreia do Sul.

As publicações permanecem sendo publicadas em revistas de alto fator de impacto, destaque para Chemosphere e Environmental Pollution com 12,5% das novas publicações, Science of the total environment com 10,9% e nanomaterials com 7,8%. Observa-se uma evolução na área com novos países e pesquisadores ingressando na temática e principalmente com a inclusão de novos trabalhos avaliando a toxicidade em plantas e efeitos tóxicos de contaminantes combinados.

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CAPÍTULO 2

O capítulo 2 apresenta a eficiência da aplicação *in situ* de nanorremediação por nZVI em solo contaminado com cromo hexavalente e pentaclorophenol. O artigo encontra-se em português pois ainda está em revisão para posterior tradução e publicação.

Highlights

- A aplicação *in situ* de nZVI em solo contaminado com Cr^{6+} e PCP apresentou mais de 90% de eficiência na degradação dos contaminantes;
- Comprovamos a persistência de PCP e a migração de contaminantes pelo perfil de solo;
- O ambiente nanoremediado com nZVI não sofre grandes perturbações quanto a temperatura, condutividade elétrica e umidade ao longo do tempo.

APLICAÇÃO *IN SITU* DE NANORREMEDIAÇÃO POR NZVI EM SOLO CONTAMINADO COM CROMO HEXAVALENTE E PENTACLOROPHENOL

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Resumo: A contaminação de solos é preocupante, a qualidade do solo está diretamente relacionada à saúde humana principalmente em função da contaminação da água e de alimentos. Neste sentido, a aplicação de técnicas de remediação de solos faz-se necessária para garantir a qualidade dos ambientes. Desta forma, escolhemos dois contaminantes, Cromo hexavalente (Cr^{6+}) e Pentaclorophenol (PCP), para avaliar a eficiência da nanorremediação de nZVI em uma aplicação *in situ*. A técnica de remediação de solos com injeção de nZVI apresentou mais de 90% de eficiência na degradação dos contaminantes Cr^{6+} e PCP. A degradação de Cr^{6+} mostrou-se rápida e constante, o contaminante PCP mostrou-se mais persistente no ambiente ao apresentar maior degradação a partir de 30 dias. Foi registrada a migração de ambos os contaminantes e também de nZVI para camadas mais profundas do solo, entretanto não foram encontrados contaminantes no lixiviado. O monitoramento do ensaio possibilitou a compreensão de que o ambiente nanoremediado com nZVI não sofre grandes perturbações quanto a temperatura, condutividade elétrica e umidade ao longo do tempo. Os procedimentos de extração de contaminantes mostraram-se complexos e a influência de fatores externos dificultam a interpretação das iterações entre os indicadores.

Palavras-chave: Contaminantes; Metais pesados; Nanopartícula; Organoclorados; Remediação de solo;

INTRODUÇÃO

A qualidade do solo é diretamente relacionada à saúde humana principalmente em função da contaminação da água e alimentos. Desta forma, pesquisadores têm mostrado relações entre o estado do solo e a saúde humana (BREVIK; SAUER, 2015). Neste sentido, a recuperação de áreas contaminadas é atraente porque reduz os riscos para a saúde humana e os impactos causados ao meio ambiente, ocasionando uma série de benefícios sociais e econômicos (ALBERINI et al., 2006).

A contaminação de solos é um assunto bem difundido no meio acadêmico, a grande quantidade de áreas contaminadas vem despertando a preocupação dos órgãos ambientais tanto no exterior como no Brasil (HU et al., 2006; THOMÉ et al., 2015).

Existem muitas tecnologias disponíveis para a remediação de solos e algumas utilizam materiais em escala nanométrica (REDDY, 2013; USEPA 2012).

A aplicação de nanopartículas de ferro na valência zero (nZVI) é uma das tecnologias de remediação mais utilizadas da atualidade devido a suas características (KHARISOV et al., 2012; YAN et al., 2013). Diversas pesquisas *ex situ* relatam a efetividade do nZVI em remediar solos contaminados (REGINATTO, 2017; TESSARO, 2018; CECCHIN, 2018), sendo utilizados nesta pesquisa o Cromo hexavalente (Cr^{6+}) e Pentaclorophenol (PCP) a fim de avaliar a eficiência da nanorremediação de nZVI em uma aplicação em escala de campo.

METODOLOGIA

Caracterização do Solo

Para a realização do experimento, foi utilizado um solo argiloso da região sul do Brasil, o solo foi coletado em uma trincheira aberta no local a uma profundidade de 1,2 metros (horizonte B). As amostras de solo foram coletadas em estado deformado, sendo estas utilizadas para a caracterização físico-química do material, bem como a moldagem dos corpos de prova e posterior esterilização. Para a caracterização do solo foram realizadas análises de granulometria, limites de Atterberg (liquidez e plasticidade), massa específica, curva característica de sucção, condutividade hidráulica, densidade dos grãos e porosidade, seguindo as orientações propostas pelas NBRs 6457/1986; 6459/1984; 7180/1984; 7181/1984 e 6508/1984.

Para caracterização físico-química do solo determinou-se o pH em água; o teor de argila; a concentração de matéria orgânica; macrosais existentes (N, P, K, S, Ca, Mg); microsais (Mn, Zn, Cu, Fe, Mo, B, Cl); cátions trocáveis (Al, Na); acidez potencial (Al+H); capacidade de troca de cátions (CTC); saturação por bases; saturação por alumínio e; saturação por potássio.

O solo foi classificado pedologicamente como um Latossolo Vermelho Distrófico Húmico (STRECK et al. 2008). Quanto a geotécnica o mesmo é classificação como CH, ou argila de alta plasticidade. A Tabela 1 apresenta a caracterização geotécnica e física do solo em estudo.

Tabela 1: Caracterização geotécnica, física e química do solo em estudo.

Parâmetro	Valor
Argila (%)	72
Silte (%)	15
Areia (%)	13
Limite de Liquidez (%)	53
Limite de Plasticidade (%)	42
Peso Específico das Partículas (kN/m³)	26,7
Índice de Vazios	1,19
Peso Especifico Natural (kN/m³)	16,3
Grau de Saturação (%)	75,7
Porosidade (%)	54
pH	5,1
Matéria Orgânica (%)	< 0,8
Condutividade Hidráulica (cm/s)	$1,39 \times 10^{-3}$
Fósforo (mg/dm³)	0,7
Potássio (mg/dm³)	23
Alumínio (cmolc/dm³)	2,7
Ferro (g/dm³)	4,73
Cálcio (cmolc/dm³)	1,1
Magnésio (cmolc/dm³)	0,7
H+Al (cmolc/dm³)	10,9
CTC (cmolc/dm³)	12,7
Saturação de Bases (%)	15
Saturação de Alumínio (%)	60

Como pode se observar na Tabela 1, o solo em estudo possui pH ácido, elevado teor de argila e baixa Capacidade de Troca de Cátions (CTC). Segundo Streck et al. (2008), a CTC é uma característica típica e preponderante em solos com predominância do argilomineral caulinita, haja vista a conformação estrutural apresentada por esta. O teor de matéria orgânica presente no solo é inferior a 0,8%, tal resultado se deve a profundidade de coleta de solo adotada, sendo esta uma característica de solos de maior profundidade (MOREIRA; SIQUEIRA, 2006).

Nanopartículas de Ferro de Valência Zero

O pó de nanoferro utilizado foi adquirido com a empresa NANO IRON s.r.o., originária da República Tcheca. O nanoferro possui estabilização coloidal, sendo, consequentemente, mais estável depois de ativado. A Tabela 2 apresenta as características do nanoferro e a Figura 1 apresenta a morfologia das nanopartículas Nanofer Star, todas obtidas através de microscopia eletrônica de transmissão.

Tabela 2: Características do nanoferro Nanofer Star em pó da Nano Iron s. r. o.

NanoFer Star	
Composição	**Conteúdo em massa (%)**
Ferro (Fe)	65 a 80
Magnetita (Fe_3O_4) e óxido de ferro	20 a 30

Fonte: NANOIRON s.r.o, (2017).

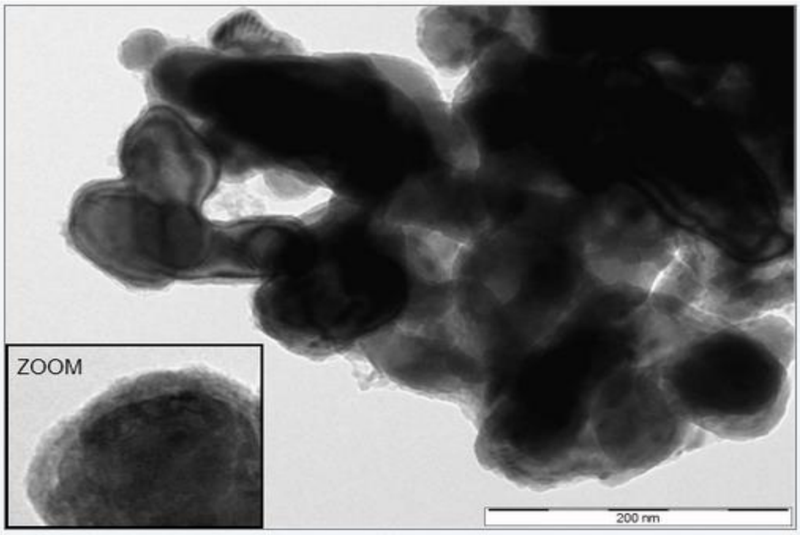

Figura 1: Morfologia das nanopartículas de Nanofer Star em microscopia eletrônica de transmissão.
Fonte: NANOIRON s.r.o, (2017).

Processo de Ativação do nZVI

O processo de ativação do nanoferro foi realizado de acordo com a metodologia do fabricante. O procedimento consiste na ativação ou quebra dos grumos, utilizando a agitação das partículas para atingir a escala nanométrica. Deste modo, foram utilizados 12,83 litros de água e adicionados 4,39 kg de nZVI em um recipiente fechado e agitou-se a mistura a uma velocidade constante.

O recipiente foi construído em acrílico extrudado para suportar a pressão de injeção e possibilitar a visualização do conteúdo, em uma das tampas foram instalados orifícios para instalação da haste agitadora e recarga do equipamento. A concentração de 40 g/kg de nanoferro utilizada para o experimento foi proveniente de ensaios *ex situ* e recomendada por Cecchin, (2018).

Equipamento de injeção de nZVI

O equipamento utilizado consiste na adaptação de escala do modelo desenvolvido por Siveris (2018). A Figura 2 apresenta o modelo esquemático do equipamento construído.

Figura 2: Equipamento para injeção de nZVI no solo.

O sistema apresenta um controle rotacional manual, que coordena a velocidade de subida da haste de injeção, bem como o número de giros realizados pelo sistema. A vazão de injeção é controlada por um sistema pressurizado, que proporciona uma dispersão uniforme da suspensão por todo o corpo de prova.

A haste de injeção projetada apresenta uma altura de 0,90 metros de altura e 1 centímetro de diâmetro interno. Ainda, apresenta uma ponta cônica para facilitar a penetração no solo e 12 orifícios de 1,0 mm de diâmetro para a injeção da suspensão, conforme mostra a Figura 3. A haste de injeção desceu 15 cm/min, cerca de 80 segundos por cm de profundidade, a pressão de injeção foi de 600 Kpa.

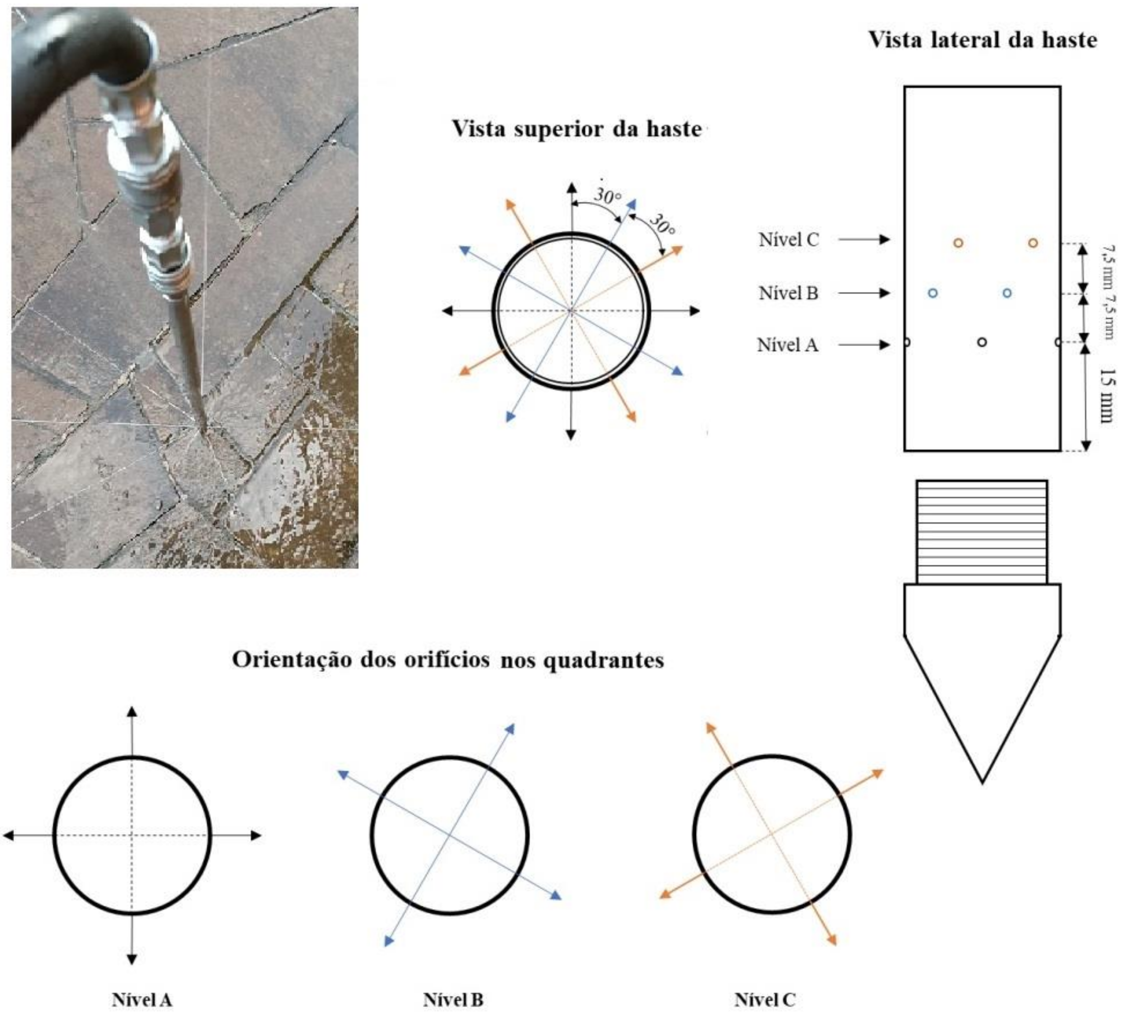

Figura 3: Haste do equipamento de injeção. Fonte: Adaptado de (Cecchin, 2018).

Montagem dos reatores *in situ*

Para a montagem dos reatores, foram utilizados oito tambores de polietileno de alta densidade e alto peso molecular reciclado (pead-apm) com capacidade para 200 L (60 x 90 cm). Visando simular uma contaminação de superfície, os reatores foram instalados ao nível do solo (Figura 4A). Ao fundo de cada reator, foi implantado um sistema de drenagem do percolado com a utilização de uma camada de esferas de vidro estéreis, (Figura 4B), seguido de uma tubulação, proporcionando a coleta do material lixiviado. Para evitar a colmatação do sistema de drenagem, foi inserida uma camada de geotêxtil sobre o sistema, (Figura 4C), evitando que partículas de solo prejudiquem a passagem do percolado.

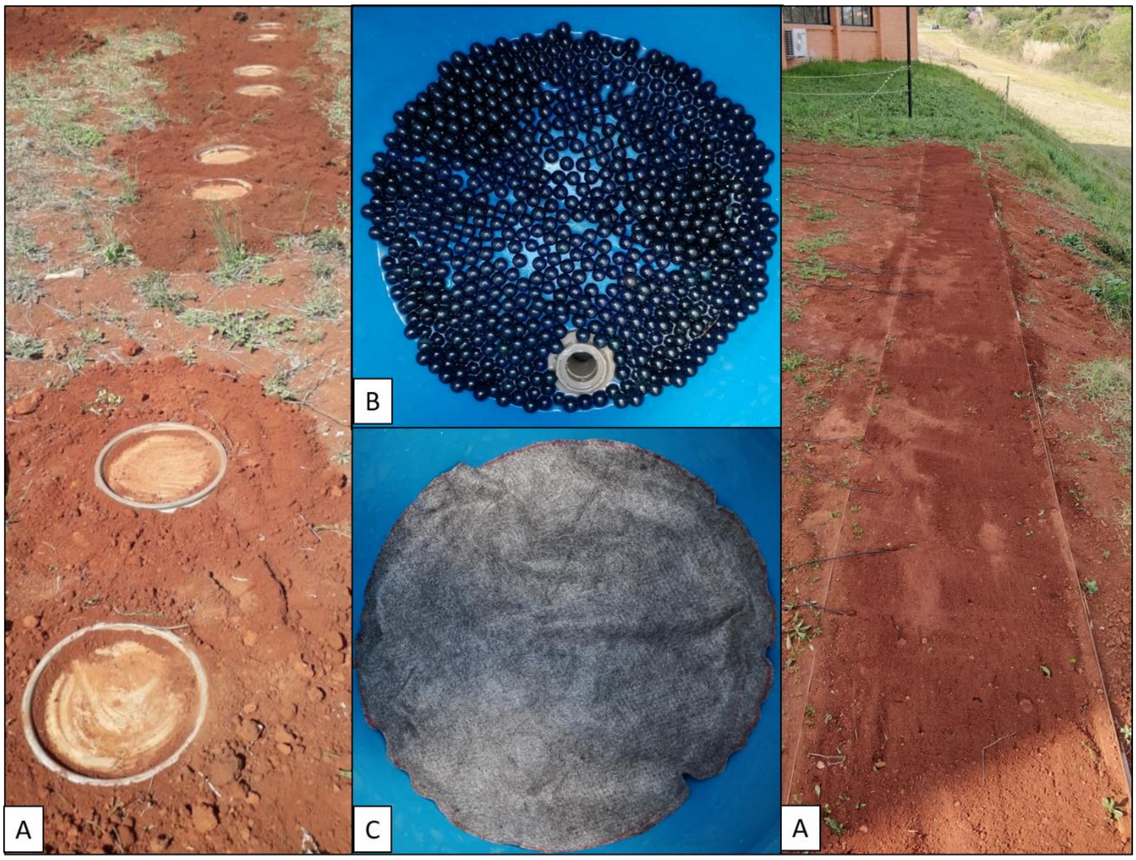

Figura 4: Reatores do experimento *in situ.* A. Tambores dispostos no nível do solo. B. Esperas de vidro que compõem o sistema de drenagem. C. Geotêxtil utilizada para evitar colmatação.

O solo foi compactado em densidade natural de 16 kN/m³. Para uma maior homogeneidade de compactação, o processo de montagem do reator ocorreu em camadas de 10 cm, fazendo com que a densidade do solo se torne a mais equivalente possível em todo o reator. A umidade de moldagem adotada foi de 34%, sendo o valor do contaminante dependente das análises de caracterização laboratorial, conforme descrito por CECCHIN (2018).

Processo de Amostragem

O processo de amostragem foi realizado com o auxílio de um trado (10 cm de diâmetro) especialmente desenvolvido para coletas em reatores. A amostragem ocorreu em raios aleatorizados e em 2 profundidades, visando deste modo abranger a maior área possível do reator, bem como possibilitar a modelagem de dispersão do sistema de injeção utilizado. O lixiviado foi coletado pelo sistema de drenagem e armazenado em um tambor de 20 L.

As amostras de solo foram coletadas e analisadas nos tempos 7, 15, 21, 30, 60 e

90 dias após o processo de injeção de nZVI, totalizando seis coletas. Para cada tempo procedeu-se duas coletas em 25 e 50 cm de profundidade e raios aleatorizados.

Contaminantes utilizados

Para este experimento foi utilizado 45 cm de solo livre de contaminante na parte inferior do corpo de prova e 45 cm de solo contaminado na parte superior. A haste de injeção foi penetrada até a profundidade de 45 cm fazendo a remediação do solo contaminado nesta profundidade.

Os contaminantes utilizados neste experimento foram Cr^{6+} e PCP. A contaminação do solo por Cromo Hexavalente ocorreu a partir da dissolução em água de Dicromato de Potássio ($K_2Cr_2O_7$), formando uma solução de 2000 $mg.L^{-1}$. A concentração utilizada para os ensaios é a mesma descrita por Cecchin (2018), utilizada para ensaios em escala laboratorial, 100 $mg.Kg^{-1}$.

O processo de contaminação por Pentaclorophenol seguiu o método descrito por (DARKO-KAGYA et al., 2010; WANG et al., 2010; CAO et al., 2015). O PCP foi pesado e solubilizado em um volume de acetona e então conduzido ao agitador magnético, até haver o completo desaparecimento dos sólidos. As amostras de solo contaminado com PCP foram preparadas adicionando 100 $mg.L^{-1}$ de solução de acetona/PCP no solo, mesma concentração utilizada por Cecchin (2018) em ensaios de laboratório, posteriormente o solo foi homogeneizado com auxílio de uma betoneira e disposto em exaustor até a completa evaporação da acetona. Também ocorreu um ensaio com a mistura dos contaminantes utilizando as mesmas concentrações.

Monitoramento dos Fatores Abióticos

Por se tratar de um experimento *in situ* foi monitorada a temperatura, umidade, condutividade elétrica e pH de cada reator. Para tanto, foram inseridos dois sensores modelo 5TE da Decagon®, em cada um dos reatores, sendo instalados em duas profundidades, visando monitorar possíveis variações de saturação no interior do experimento (25 e 50 cm).

O sensor escolhido determina o conteúdo volumétrico da água (CVA) medindo a constante dielétrica do meio usando de tecnologia de domínio capacitância/frequência. A frequência emitida é de 70 MHz, o que minimiza os efeitos da salinidade e textura do solo. A temperatura é mensurada por um termistor onboard, a condutividade elétrica (CE) é estimada por um conjunto de eletrodos de aço inoxidável e o pH é medido por um pHmetro incluso no eletrodo central.

Análise do Teor Residual

Para a determinação dos valores de degradação dos contaminantes foram utilizadas amostras homogeneizadas de 10 gramas de solo, sendo adotado acetona como solvente. Para a extração de Cr^{6+} foi utilizado o método USEPA 3060A (1996) e para a leitura do teor residual procedeu-se o método colorimétrico USEPA 7196A (1992). Como recomendado pelo método foi elaborada uma curva padrão de calibração com 8 pontos de concentração conhecidos, o comprimento de onda (λ) utilizado para leitura da absorbância foi de 540 nm, obtendo regressão linear acima de 0,99, conforme Figura 5.

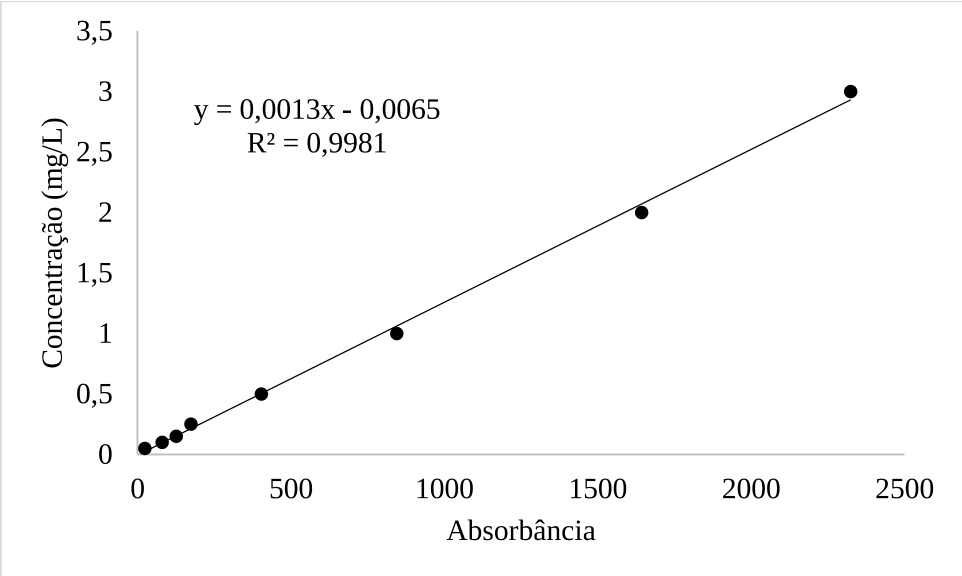

Figura 5: Curva de calibração utilizada na extração de Cr6+ em espectrometria de luz UV e comprimento de onda de 540 nm.

Para a determinação de Pentaclorophenol seguiu-se o método de espectrometria proposto por Cecchin (2018) adaptado de Alves (1998). O comprimento de onda escolhido para leitura foi de 230 nm, segundo os autores supracitados a identificação de PCP através da radiação UV (espectro não visível) no intervalo escolhido proporciona uma melhor adaptação dos dados para a concentração de contaminante utilizada. Elaborou-se uma curva padrão com 8 pontos de concentração conhecidos e obteve-se uma regressão linear satisfatória acima de 0,99, Figura 6.

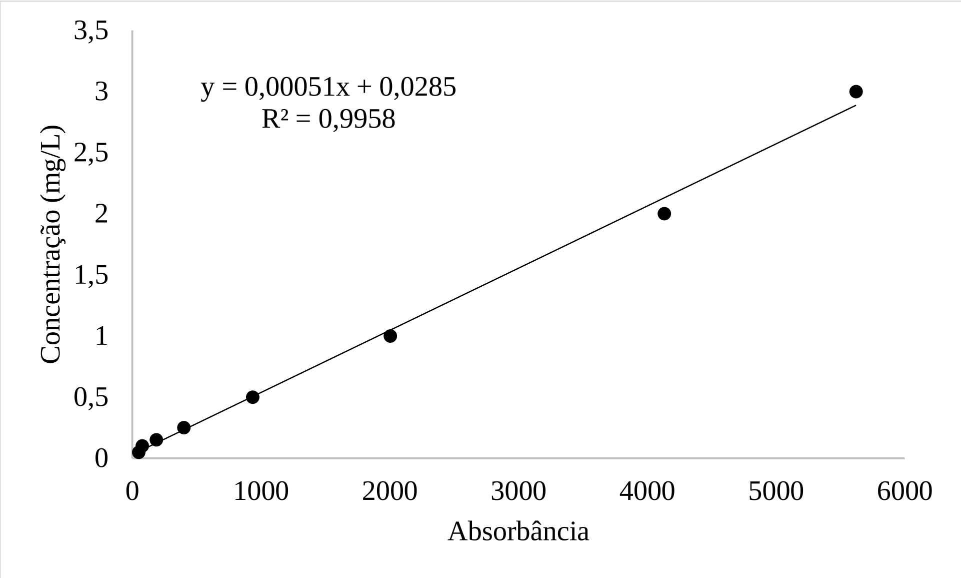

Figura 6: Curva de calibração utilizada na extração de PCP em espectrometria de luz UV e comprimento de onda de 230 nm.

Análise do Lixiviado

A caracterização do lixiviado ocorreu a cada 30 dias, o líquido coletado pelo sistema de drenagem foi analisado em espectrofotometria de luz UV. O equipamento utilizado para análise foi um PRO-TOOLS, modelo UV-1600, os procedimentos para leitura de Cr^{6+} e PCP foram os mesmos utilizados para avaliação de teor residual no solo.

RESULTADOS E DISCUSSÃO

Fatores Abióticos

Foi monitorada a umidade, temperatura, condutividade elétrica e pH de cada reator. Os parâmetros de cada data foram coletados no datalogger e uma média dos dados foi realizada para as duas profundidades do ensaio. Desta forma, a Tabela 3 apresenta a média dos fatores abióticos monitorados na profundidade inferior do tambor (25 cm) e na parte superficial (50 cm) local que recebeu injeção de nZVI.

Tabela 3: Parâmetros de umidade, temperatura, condutividade elétrica e pH do experimento.

Fatores Abióticos	Datas	Controle		Controle + nZVI		Cr^{6+}		Cr^{6+} + nZVI		PCP		PCP + nZVI		Cr^{6+} + PCP		Cr^{6+} + PCP + nZVI	
		Inf.	**Sup**	**Inf.**	**Sup.**	**Inf.**	**Sup.**	**Inf.**	**Sup**	**Inf.**	**Sup.**	**Inf.**	**Sup.**	**Inf.**	**Sup**	**Inf.**	**Sup.**
Umidade (m^3/m^3)	7 dias	0,27	0,29	0,30	0,23	0,29	0,44	0,34	0,31	0,42	0,38	0,32	0,29	0,36	0,30	0,30	0,32
	15 dias	0,31	0,31	0,23	0,31	0,31	0,44	0,34	0,31	0,42	0,38	0,32	0,30	0,36	0,32	0,30	0,32
	21 dias	0,30	0,31	0,23	0,31	0,31	0,45	0,34	0,31	0,42	0,38	0,32	0,30	0,35	0,32	0,30	0,33
	30 dias	0,30	0,31	0,23	0,31	0,39	0,45	0,34	0,31	0,42	0,38	0,31	0,30	0,35	0,32	0,30	0,33
	60 dias	0,31	0,33	0,32	0,28	0,31	0,45	0,33	0,31	0,41	0,38	0,32	0,30	0,34	0,32	0,30	0,32
	90 dias	0,31	0,34	0,33	0,28	0,31	0,44	0,33	0,31	0,41	0,38	0,32	0,30	0,34	0,33	0,30	0,33
Temperatura °C	7 dias	23,9	25,9	25,3	23,9	25,8	25,1	25,4	25,1	25,3	25,0	24,0	25,0	23,7	25,3	23,6	25,3
	15 dias	22,5	22,6	22,8	22,2	22,8	21,6	22,8	22,2	22,7	22,9	22,7	22,8	22,9	22,4	22,5	22,7
	21 dias	24,3	25,2	23,0	23,9	23,5	23,5	23,8	23,7	23,2	23,9	22,9	24,0	23,0	24,0	22,6	24,0
	30 dias	25,6	25,8	25,0	25,3	25,1	25,6	24,8	24,9	24,7	24,6	24,0	23,8	24,0	23,4	23,7	23,8
	60 dias	24,0	24,1	24,0	23,6	23,9	23,8	23,6	23,9	23,6	23,7	23,9	24,0	24,2	23,8	23,7	24,1
	90 dias	24,5	24,6	24,7	24,4	24,8	24,8	23,9	24,7	23,5	24,1	24,4	24,4	24,5	24,3	24,2	24,6
Condutividade Elétrica (dsm/cm)	7 dias	0,05	0,02	0,08	0,02	0,03	0,02	0,04	0,08	0,03	0,06	0,05	0,01	0,16	0,03	0,05	0,08
	15 dias	0,03	0,07	0,08	0,02	0,04	0,02	0,04	0,08	0,03	0,06	0,05	0,01	0,15	0,04	0,01	0,08
	21 dias	0,03	0,07	0,08	0,02	0,04	0,02	0,05	0,08	0,04	0,06	0,04	0,01	0,15	0,04	0,01	0,09
	30 dias	0,03	0,07	0,08	0,02	0,05	0,02	0,04	0,08	0,05	0,06	0,04	0,01	0,15	0,04	0,02	0,08
	60 dias	0,03	0,07	0,05	0,01	0,04	0,02	0,04	0,16	0,04	0,07	0,05	0,01	0,14	0,04	0,03	0,07
	90 dias	0,03	0,06	0,04	0,01	0,05	0,02	0,05	0,19	0,05	0,07	0,06	0,01	0,14	0,05	0,03	0,07
pH	7 dias	5,25	5,21	5,19	5,21	5,21	5,22	5,19	5,13	5,13	5,14	5,28	5,18	5,20	5,22	5,25	5,25
	15 dias	5,14	5,18	5,18	5,28	5,11	5,33	5,24	5,18	5,18	5,28	5,28	5,16	5,24	5,28	5,12	5,30
	21 dias	5,17	5,21	5,24	5,10	5,19	5,14	5,18	5,18	5,14	5,19	5,13	5,20	5,15	5,20	5,16	5,24
	30 dias	5,11	5,05	5,16	5,03	5,08	5,14	5,06	5,07	5,11	5,06	5,14	5,10	5,00	5,10	5,13	5,02
	60 dias	5,19	5,12	5,03	5,04	5,07	5,10	5,03	5,13	5,19	5,15	5,17	5,13	5,07	5,10	5,04	5,07
	90 dias	5,15	5,07	5,05	5,09	5,15	5,07	5,09	5,16	5,19	5,05	5,10	5,09	5,10	5,15	5,06	5,09

Após 7 dias de injeção de nZVI o ambiente manteve-se estável, a temperatura média nos tambores foi de 24,8 °C, pH de 5,2, a condutividade elétrica média foi de 0,05 dsm/cm e a umidade do solo manteve-se na faixa de 32%. Aos 15 dias a temperatura esteve entre 21,6 °C e 22,9 °C, pH manteve-se em 5,2, a condutividade elétrica em 0,05 dsm/cm e a umidade do solo próxima a 32%. Com 21 dias o ambiente manteve-se estável, a temperatura variou entre 22,6 °C e 24,3 °C, o pH foi de 5,2, a condutividade elétrica manteve-se em 0,05 dsm/cm e a umidade do solo em 32%.

Um mês após a nanorremediação por nZVI a temperatura média foi de 24,6 °C, a condutividade elétrica esteve inalterada em 0,05 dsm/cm e a umidade do solo em 33%. Aos 60 dias, os parâmetros do solo registraram média de 23,8 °C, pH de 5,1, condutividade média de 0,05 dsm/cm e umidade do solo de 33%. Na fase final das avaliações, aos 90 dias, a temperatura variou entre 23,5 °C e 24,8 °C, o pH manteve-se em 5,1, a condutividade elétrica permaneceu em 0,05 dsm/cm e a umidade do solo em 33%.

Teor Residual de Cromo Hexavalente

Para todos os tratamentos com presença de Cromo hexavalente foram realizadas análises de teor residual. Salienta-se que as amostras foram analisadas em duas profundidades, uma que recebeu contaminante e injeção de nZVI e outra com solo natural. A Figura 7, apresenta o residual de Cr6+ na profundidade de 25 cm entre os tratamentos ao longo do tempo.

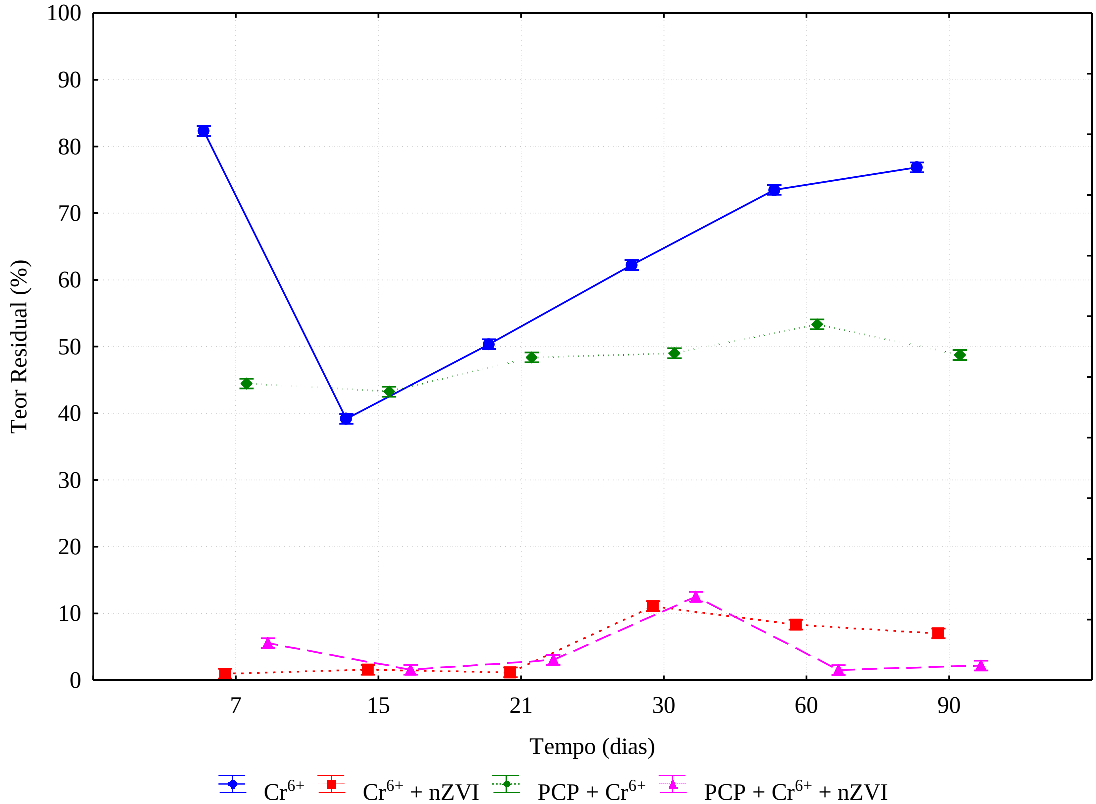

Figura 7: Teor residual de Cromo hexavalente na profundidade de 25 cm em diferentes tratamentos.

Observa-se que o teor residual dos ensaios sem injeção de nZVI não apresentam um comportamento linear próximo a 100% conforme esperado. Ao avaliarmos os tratamentos individualmente identificamos que após 7 dias foi possível extrair 82,32% de Cr^{6+} da amostra de solo avaliada. Aos 15 dias o teor residual mensurado cai para 44,45% de Cr^{6+} e ao longo dos 90 dias obtemos resultados entre este intervalo. De fato, o processo de extração de Cr^{6+} do solo é complexo e possibilita erros amostrais, entretanto, não podemos eliminar o papel da microbiota na biorredução e biorremediação do solo contaminado.

A mesma correlação pode ser realizada para o tratamento com PCP + Cr^{6+} que apresentou comportamento constante de teor residual extraído do solo, todavia, menor que o esperado, em média de 47,8% de Cr^{6+}. A influência de fatores externos não mensurados por este trabalho pode ter contribuído para menor eficiência do processo de extração.

Ao avaliarmos os tratamentos com injeção de nZVI observa-se uma considerável redução no teor residual do contaminante demonstrando a eficiência da nanorremediação. Logo aos 7 dias o teor residual de Cr^{6+} obtido é de 1,28%, levando-se em consideração o teor residual extraído no mesmo tratamento sem injeção de nZVI e na mesma data, os resultados apontam

98,44% de eficiência de degradação do contaminante. Fazendo a mesma análise para o tratamento com a combinação dos contaminantes a eficiência é de 87,8%.

Também avaliamos a possível migração dos contaminantes, a (Figura 8) apresenta os resultados de teor residual de Cr^{6+} para a profundidade de 50 cm. Salienta-se que nesta profundidade o solo não foi contaminado e também não ocorreu injeção de nZVI.

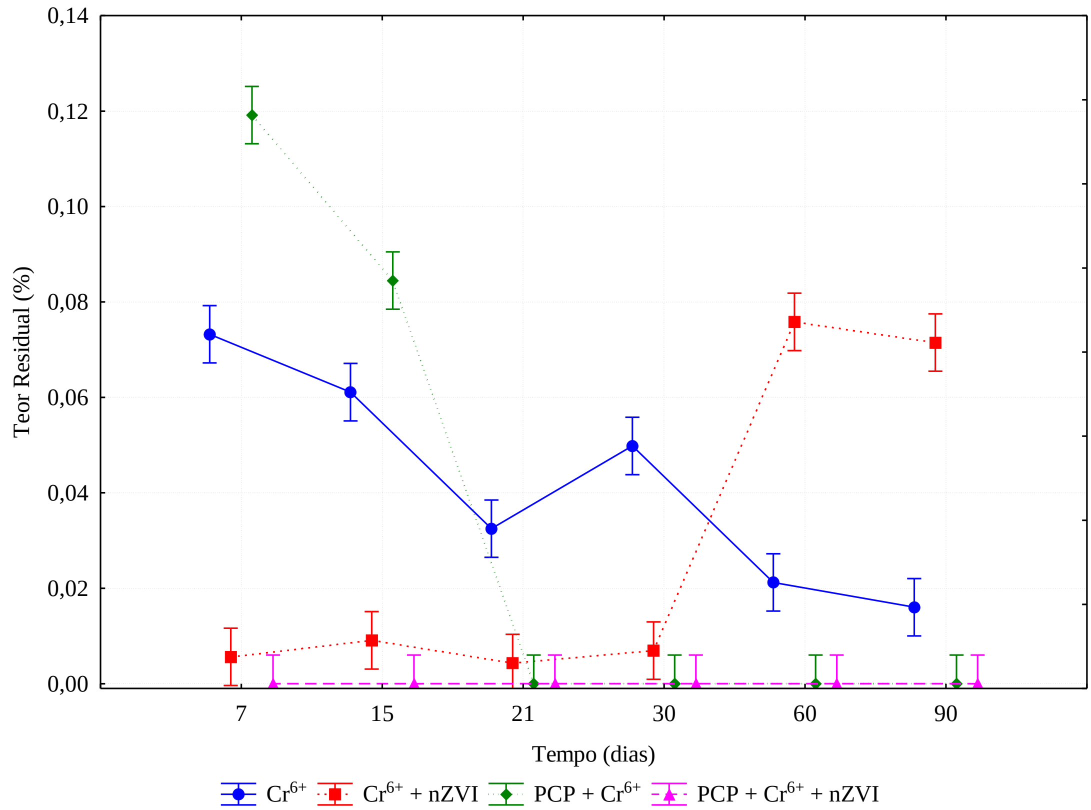

Figura 8: Teor residual de Cromo hexavalente na profundidade de 50 cm em diferentes tratamentos.

Como podemos observar na Figura 8, identificamos a migração de Cr^{6+} para camadas mais profundas do solo. A maior concentração de contaminante foi observada no tratamento PCP + Cr^{6+} no período de 7 dia após a injeção de nZVI. Destaca-se que os tratamentos sem injeção de nZVI apresentaram maior teor residual de Cr^{6+}, apontando para uma migração relacionada precipitação. Não podemos descartar a possibilidade de migração dos contaminantes relacionada pelo procedimento de injeção do nZVI, todavia o menor teor residual obtido nestes tratamentos indica uma migração conjunta da nanopartícula, promovendo nanorremediação nestes pontos.

Teor Residual de Pentaclorophenol

As análises de teor residual de PCP foram realizadas em duas profundidades, uma que recebeu contaminante e injeção de nZVI e outra com solo natural. A Figura 9, apresenta o residual de PCP na profundidade de 25 cm entre os tratamentos ao longo do tempo.

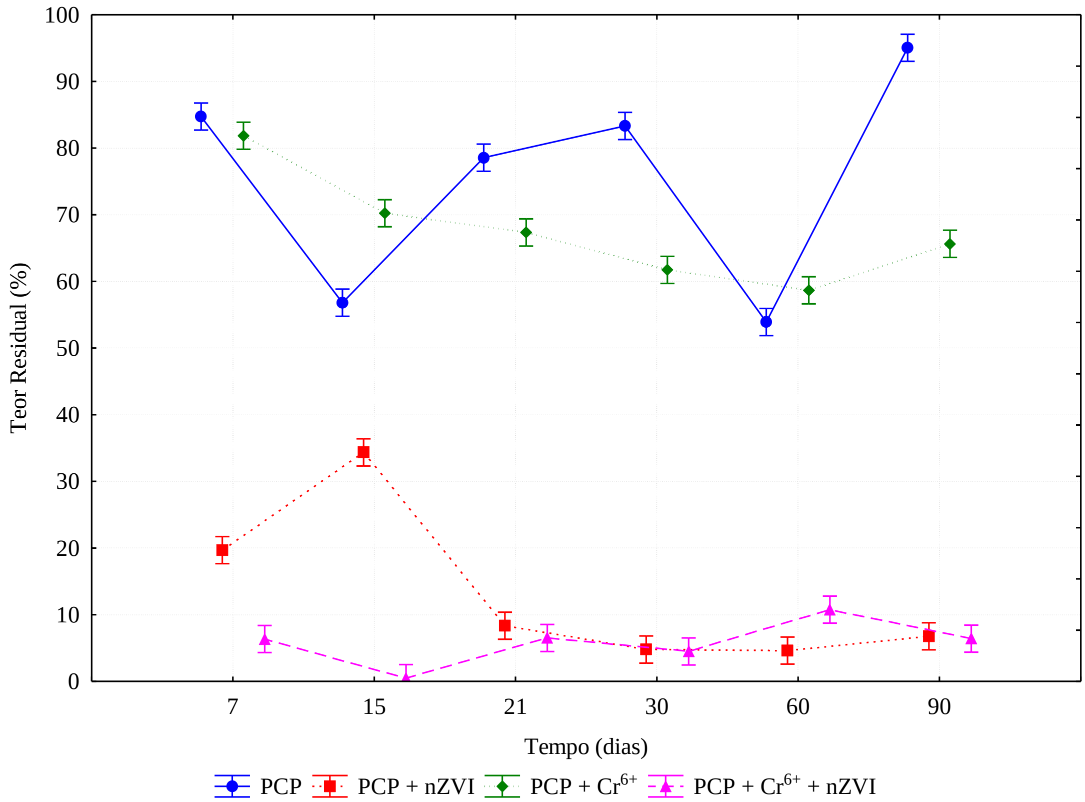

Figura 9: Teor residual de Pentaclorophenol na profundidade de 25 cm em diferentes tratamentos.

O teor residual dos ensaios sem injeção de nZVI não apresentaram um comportamento linear próximo a 100% conforme esperado. Ao avaliarmos os tratamentos individualmente identificamos que após 7 dias foi possível extrair 83,16% de PCP da amostra de solo avaliada. Aos 60 dias obtemos o menor teor residual, 54,09% de PCP e ao longo dos 90 dias obtivemos resultados entre este intervalo. Os resultados foram abaixo do esperado, o processo de extração de PCP do solo é complexo e possibilita erros amostrais, entretanto, não podemos eliminar o papel da microbiota na degradação do contaminante.

O tratamento com PCP + Cr^{6+} apresentou uma linha de decaimento de teor residual extraído do solo, todavia, menor que o esperado, a extração de PCP foi maior aos 7 dias, 81,53% e menor 59,19% aos 60 dias. A complexidade dos métodos de extração somados a influência de fatores externos podem ter contribuído para menor eficiência do processo de extração.

Ao avaliarmos os tratamentos com injeção de nZVI observa-se uma considerável redução no teor residual do contaminante comprovando a eficiência da nanorremediação. O PCP mostrou-se mais persistente ao ambiente, apresentando maior decaimento aos 30 dias, 4,62%, logo aos 7 dias o teor residual de PCP obtido é de 20,53%. A maior eficiência comparando-se o teor residual extraído de PCP com ou sem a presença de nZVI ocorre aos 90 dias, 93,08% de eficiência na degradação do contaminante. Fazendo a mesma análise para o tratamento com a combinação dos contaminantes a maior eficiência foi de 92,3% aos 7 dias.

Também avaliamos a possível migração do contaminante, a (Figura 10) apresenta os resultados de teor residual de PCP para a profundidade de 50 cm. Salienta-se que nesta profundidade o solo não foi contaminado e também não ocorreu injeção de nZVI.

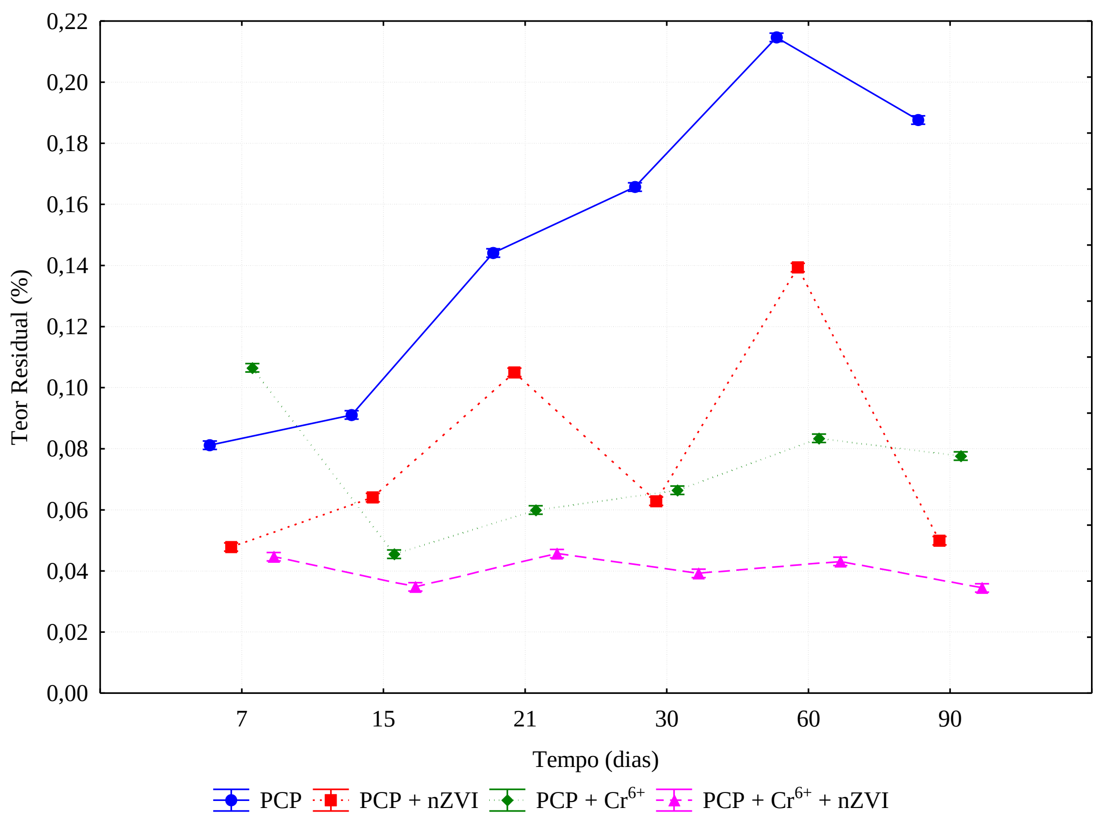

Figura 10: Teor residual de Pentaclorophenol na profundidade de 50 cm em diferentes tratamentos.

Identificamos a migração de PCP para camadas mais profundas do solo. A maior concentração de contaminante, 0,21%, foi observada no tratamento PCP no período de 60 dias após a injeção de nZVI. A migração de PCP pela água é improvável devido a insolubilidade do mesmo com está molécula. Não podemos descartar a possibilidade de migração do contaminante relacionada ao procedimento de injeção do nZVI, todavia os resultados apontam uma migração conjunta da nanopartícula, promovendo nanorremediação nestes pontos.

Teor Residual de Ferro

De acordo com a caracterização química do solo, estima-se 4,73 g/dm^3 de ferro nos tratamentos. A Figura 11, apresenta o teor residual de ferro total encontrada em 25 cm de profundidade, 90 dias após a injeção de nZVI.

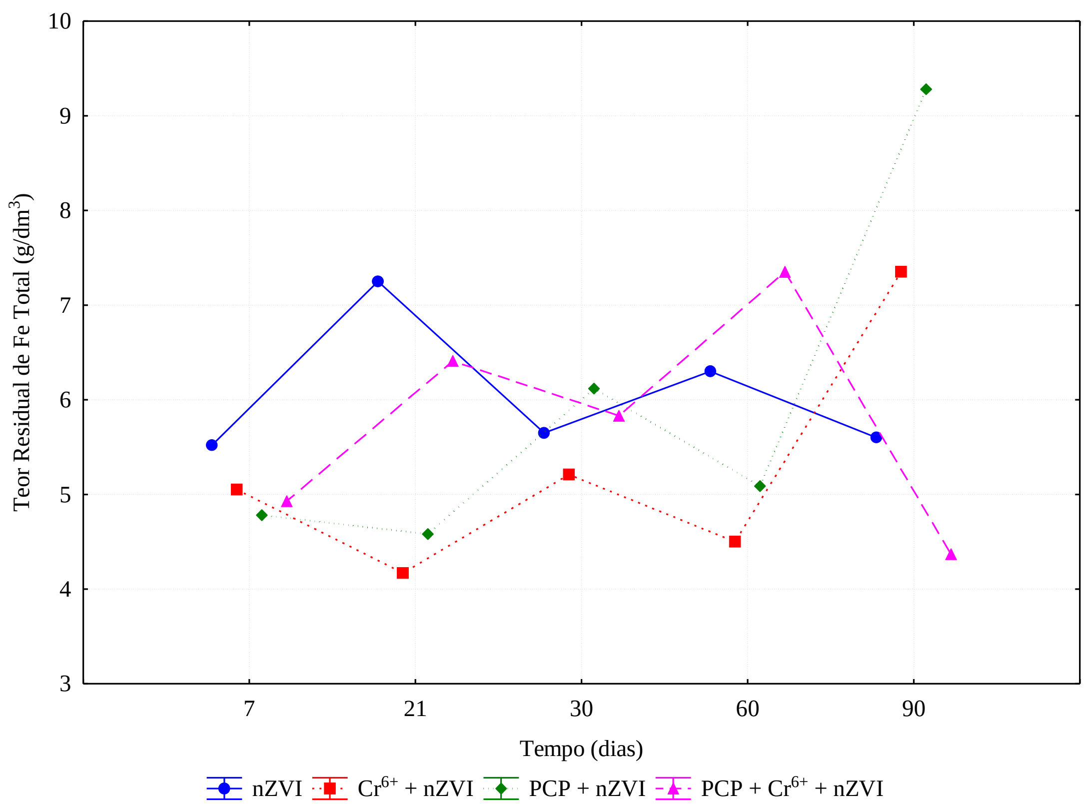

Figura 11: Teor residual de Ferro total na profundidade de 25 cm em diferentes tratamentos.

Observa-se o aumento no teor residual de ferro total em todos os tratamentos. A maior concentração, 9,28 g/dm^3, foi obtida 90 dias após a injeção de nZVI no tratamento com PCP, um dos tratamentos com maior degradação de contaminante observada, esse acréscimo representa 96,19% a mais que o teor inicial. O tratamento Cr^{6+} apresentou o menor valor de ferro total residual, 4,51 g/dm^3 no 21º dia. A Figura 12, apresenta o teor residual de ferro total encontrada em 50 cm de profundidade.

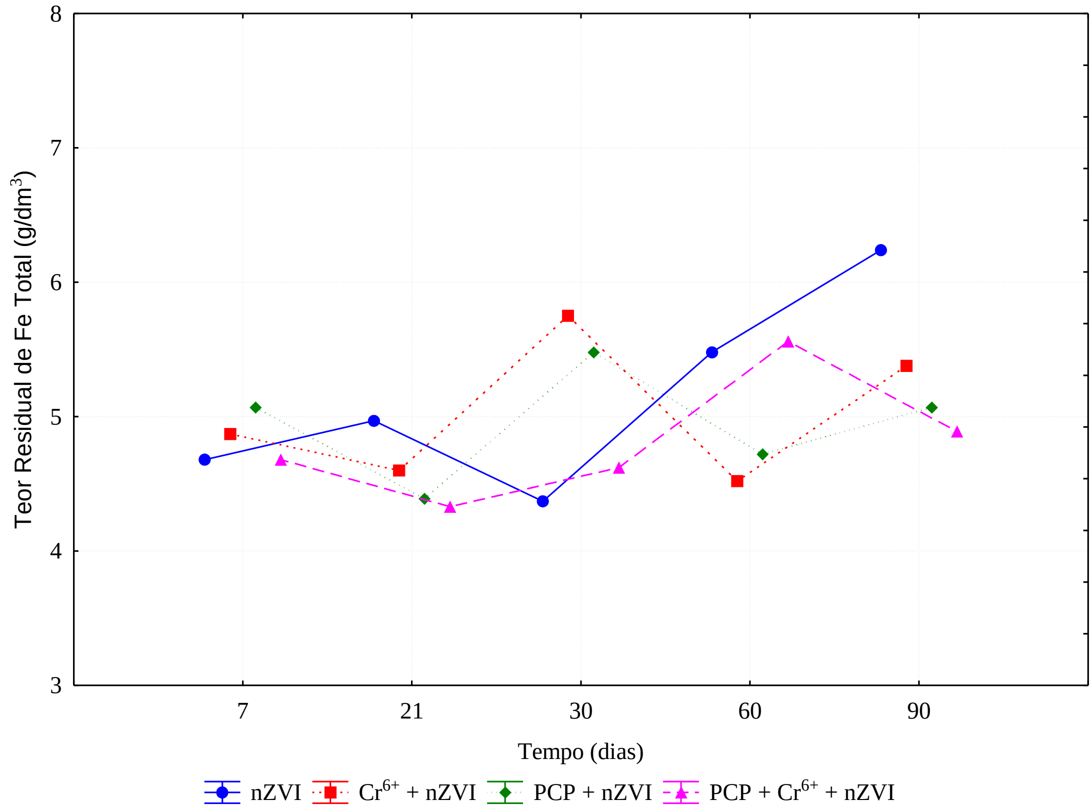

Figura 12: Teor residual de Ferro total na profundidade de 50 cm em diferentes tratamentos.

Analisando os dados da camada inferir, observa-se a elevação do teor residual de ferro total em alguns picos. Destaca-se o tratamento com injeção de nZVI em solo natural, com 6,24 g/dm^3 sendo o ensaio com maior elevação nesta profundidade. Os demais tratamentos apresentam elevações abaixo de 15 %. Desta forma, podemos afirmar que o nZVI também alcançou camadas mais profundas do solo.

Lixiviação

No período do ensaio a precipitação acumulada foi de 322,1 mm, no total foram coletados em média 50,6 Litros de água em cada tratamento. Considerando a área de abertura dos tambores, em média 14,16 litros de água passaram pelos ensaios ao longo dos 90 dias. As análises do lixiviado não apontaram concentrações de Cr^{6+} e PCP. Neste sentido, podemos afirmar que não ocorreu lixiviação dos contaminantes na profundidade de 90 cm, ponto de instalação do sistema de drenagem.

CONCLUSÃO

A técnica de remediação de solos com injeção de nZVI apresentou mais de 90% de eficiência na degradação dos contaminantes Cr^{6+} e PCP. A degradação de Cr^{6+} mostrou-se rápida e constante, o contaminante PCP mostrou-se mais persistente no ambiente ao apresentar maior degradação a partir de 30 dias.

Foi registrada a migração de ambos os contaminantes e também de nZVI para camadas mais profundas do solo, entretanto não foram encontrados contaminantes no lixiviado. O monitoramento do ensaio possibilitou a compreensão de que o ambiente nanorremediado com nZVI não sofre grandes perturbações quanto a temperatura, condutividade elétrica e umidade ao longo do tempo.

Os procedimentos de extração de contaminantes mostraram-se complexos e a influência de fatores externos dificultam a assertividade das análises. Recomenda-se trabalhos com avaliações ainda mais longas e detalhadas sobre esses ambientes.

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CAPÍTULO 3

O capítulo 3 apresenta os efeitos tóxicos do nZVI em unidades formadoras de colônias de *Bacillus cereus* e *Pseudomona aeruginosa*, duas bactérias nativas do solo. O artigo encontra-se em inglês pois foi publicado em 2022 na revista Chemosphere, fator de impacto 7,086.

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Highlights

- The nanoremediation of nZVI has no toxic effect on the bacteria population.
- The bacteria showed similar behaviors and were resistant to contaminant concentrations.
- The nanoremediated environment does not suffer major disturbances.

TOXICITY OF nZVI IN THE GROWTH OF BACTERIA PRESENT IN CONTAMINATED SOIL

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Abstract: The use of nano zero-valent iron (nZVI) for the remediation of degraded areas is a consolidated practice. However, the long-term reactions that occur in the environment remain unknown. This study aimed to evaluate the potential toxic effects on the growth of colony-forming units (CFUs) of *Bacillus cereus* and *Pseudomona aeruginosa* present in soil contaminated with hexavalent chromium (Cr^{6+}) and pentachlorophenol (PCP) nanoremediated with nZVI. The treatments were natural soil (control), soil contaminated by Cr^{6+}, soil contaminated by PCP, and soil contaminated by Cr^{6+} and PCP (Cr^{6+} and PCP), all in duplicate. The concentration of contaminants used was 100 mg/kg of soil. One of the drums of the duplicate received an injection of nZVI solution with a concentration of 50 g/kg. Analysis was performed 7, 15, 21, 30, 60, and 90 days after the nZVI injection. Temporary oscillations in the abundance of the microbiological community were observed, characterizing the adaptation of bacteria to the contaminants. The bacteria showed similar behavior. Ninety days after the injection of nZVI, the averages of the CFUs were statistically equal, with the lowest coefficient of variation and the highest concentration of CFUs occurring. The strains of *B. cereus* and *P. aeruginosa* were resistant to the concentrations of nZVI, Cr^{6+}, and PCP. The nanoremediation of nZVI in soil contaminated by Cr^{6+} and PCP had no toxic effects on the population of the bacteria evaluated and did not present major disturbances in temperature, electrical conductivity, pH, and humidity over time.

Key-words: bacillus; nZVI; population; pseudomonas; toxicity.

INTRODUCTION

Although the use of nano zero-valent iron (nZVI) in contaminated areas is a remediation technique used to improve the quality of life and the environment, the use of nanoparticles for soil remediation has raised doubts and concerns about their toxicity (Thomé et al., 2015, Lefevre et al., 2016). The reactions that occur with nanoparticles are affected by several factors characteristic of the place where they are used, thus making it necessary to conduct studies that observe their real reactive behaviors with in situ contaminants (Thomé et al., 2015, Crampon et al., 2019).

The application of nZVI is a widely used remediation technology because of its characteristics, such as low solubility and biodegradability, low iron toxicity, and lower production costs compared to other nanoparticles (Kharisov et al., 2012, Yan et al., 2013, Cai et al., 2019). Although the potential benefits of using nZVI are considerable, it is necessary to understand the possible risks to the environment, and their effects on organisms and ecosystems and so environmental protection and sustainable development need to be aimed for after exposure to the product (Xue et al., 2018; Luo et al., 2021a). Therefore, we need to address the lack of research carried out in situ and in developing countries (Vanzetto and Thomé, 2019).

The main environmental investigations on the application of nZVI have been based on its reactivity, surface area, soil pH, and reaction to organic matter (Xue et al., 2018), its behavior in the soil, and especially its potential to migrate to long distances, changing the conditions of the medium by making it alkaline or reducing it (Uyusur and Unlu, 2009), and reducing hydraulic conductivity and altering the microbiota (Kirschling et al., 2010). Some studies have pointed out the negative effects of nZVI (Gogoi et al., 2006, Fang et al., 2007, Nel et al., 2008, Lyon et al., 2008, Lowry et al., 2009, Fajardo et al., 2013, Saccà et al., 2014). However, only the short-term toxic effects are known, and it is necessary to determine the existing interactions with the soil microbiota in field-scale applications (Huang et al., 2018, Vanzetto and Thomé, 2019, Li et al., 2020a, 2020b).

nZVI particles differ in reactivity and synthesis methods (Oprckal et al., 2017), the reaction of iron nanoparticles with the contaminants consists of the reduction, oxidation, and immobilization of the contaminants (Cecchin et al., 2016; Gil- Díaz et al., 2017; Reginatto et al., 2020a). In microbiological communities, the effects are diverse, such as oxidative stress (Fajardo et al., 2013; Saccà et al., 2014), bactericidal effects (Gogoi et al., 2006), and adaptive effects (Fang et al., 2007; Li et al., 2020a, 2020b).

Specifically, bacteria are known for their benefits in soil, surface, and groundwater and are capable of degrading contaminants and balancing chemical elements that can be negatively affected by nZVI (Barnes et al., 2010, Tilston et al., 2013, Kumar et al., 2014, Velimirovic et al., 2015, Zabetakis et al., 2015). The behavior of gram-positive bacteria is different from that of gram-negative bacteria (Fajardo et al., 2012, Fajardo et al., 2013, Pawlett et al., 2013). *Bacillus cereus*, a gram-positive bacterium, and *Pseudomona aeruginosa*, a gram-negative bacterium, are common in soil (Fajardo et al., 2013, Schobert and Jahn, 2010). Both bacteria are known to have defense mechanisms in response to nZVI exposure (Fajardo et al., 2013, Saccà et al., 2014, Chaithawiwat et al., 2016, Kotchaplai, Khan, and Vangnai, 2017). However, most studies on the toxicity of nZVI to bacteria have evaluated only the growth stages, limiting the understanding of their behavior in the environment (Chaithawiwat et al., 2016).

Contaminants such as hexavalent chromium (Cr^{6+}) are easily found in industrial activities such as metal processing, tanning, and steel production (Zhitkovich, 2011). Different techniques for reducing this contaminant have already been evaluated and proven, such as the use of photocatalysts (Luo et al., 2021b) or the nZVI itself (Reginatto et al., 2020a). However, the interaction with the environment in this process and the potential toxic effect of the residue need to be studied, as well as organic contaminants.

Pentachlorophenol (PCP) is a persistent organochlorine that has been widely used as a pesticide, herbicide, and wood preservative. This compound was chosen because it presents acute toxicity, carcinogenicity, and mutagenicity for several organisms (USEPA, 1998). Nanobioremediation of PCP contaminated soil was studied by (Cecchin et al., 2016) the effectiveness and toxicity at the laboratory scale were evaluated, but the implications relevant to abiotic factors and field-scale are unknown.

In this sense, we evaluated the potential toxic effects on the growth of colony-forming units (CFUs) of *B. cereus* and *P. aeruginosa* present in soil contaminated with hexavalent chromium (Cr^{6+}) and pentachlorophenol (PCP) nanoremediated with nZVI in field-scale tests.

METHODOLOGY

B. cereus is an abundant bacterium in soil. The optimum temperature for its growth is 30°C, but it supports temperatures between 4°C and 55°C. A neutral pH (pH 7) is ideal, but it can tolerate variations of pH 4.5–9.5. Its spores can resist high acidity (pH 1–5.2) (Bergey et al., 1957, Clavel et al., 2004, Li et al., 2020a). *P. aeruginosa* is a gram-negative, aerobic *bacillus* that is freely found in soil. It is a species that does not adapt well to acidic environments (pH levels lower than 4.5). Its optimal growth temperature is 37°C, but it can tolerate temperatures of 4°C–42°C (Bergey et al., 1957, Schobert and Jahn, 2010).

The samples containing the bacteria were collected through an in situ nZVI nanoremediation experiment (Figure 1). Drums of 200 L were implanted, and the following treatments were placed: natural soil (control), soil contaminated by Cr^{6+}, soil contaminated by PCP, and soil contaminated by Cr^{6+} and PCP (Cr^{6+} and PCP), all in duplicate. The concentration of contaminants used was 100 mg/kg of soil. One of the drums of the duplicate was injected with a solution of Nanofer Star nZVI (Table 1) at a concentration of 50 g/kg of soil, this concentration was recommended by (REGINATTO et al., 2020a) in similar works carried out on a laboratory scale and tests bench.

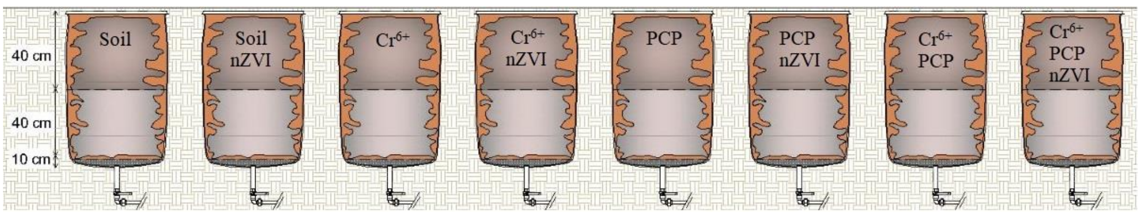

Figure 1: Experimental design of the research on the injection of nZVI for the nanoremediation of soil contaminated by Cr^{6+} and PCP.

Table 1: Characterization of NanoFer Star powder from NANO IRON s. r. o., Czech Republic.

NanoFer Star	
Composition	**Contents (%)**
Iron (Fe)	65 a 80
Magnetite (Fe_3O_4) and iron oxide (FeO)	20 a 35

As shown in Figure 1, the drums contained two layers. The first layer was 0–40 cm with contaminated and/or nanoremediated soil, and the second layer was 40–80 cm with natural soil free of contaminants. The soil used was classified as an Oxisol, however, it is formed by kaolinite and has little retention due to its permeability similar to sand, its characterization is presented in (Table 2), and the behavior of nZVI in this soil was described by (Reginatto et al., 2020b). The collection was conducted with an auger in both layers at depths of 25 and 50 cm.

Table 2: Geotechnical, physical, and chemical characterizations of the soil under study.

Parameter	Value	Parameter	Value
Clay (%)	72	Porosity (%)	54
Silt (%)	15	pH	5.1
Sand (%)	13	Organic matter (%)	< 0.8
Liquidity Limit (%)	53.0	Hidraulic Conductivity (cm/s)	1.39×10^{-3}
Plasticity Limit (%)	42.0	H+Al ($cmolc/dm^3$)	10.9
Specific Weight of Particles (kN/m^3)	26.7	CEC ($cmolc/dm^3$)	12.7
Void Index	1.19	Base Saturation (%)	15
Natural Specific Weight (kN/m^3)	16.3	Aluminum Saturation (%)	60
Saturation Degree (%)	75.7	Iron Total (g/dm^3)	4.73

The analyzes were performed at 7, 15, 21, 30, 60, and 90 days after the nZVI injection, at the end of the experiment, chemical characterization was carried out with the residual content of contaminants, according to (Table 3). In each layer of the drum, ECH20 5TE sensors (Decagon) were installed to monitor the environment, and the sensors were connected to the EM50 datalogger (Decagon). The temperature, pH, electrical conductivity, and humidity were evaluated at each collection.

Table 3: Residual chemical characterization of soil contaminants.

Contaminants	Treatments							
	Soil	Soil nZVI	Cr^{6+}	Cr^{6+} nZVI	PCP	PCP nZVI	Cr^{6+} PCP	Cr^{6+} PCP nZVI
Hexavalent Chromium (%)	-	-	76.86	7.01	-	-	48.74	2.17
Pentachlorophenol (%)	-	-	-	-	95.06	6.77	65.65	6.42
Iron Total (g/dm^3)	4.48	5.61	4.68	7.35	4.33	9.28	4.85	4.37

The toxicity of nZVI was tested using growth on an agar medium, and assessed after the incubation period (24 h) by counting the number of CFUs. The plates were contaminated with 0.5 ml of soil solution and left in an oven for bacteriological growth. Cell viability was determined using the plate-counting method. The culture medium *B. cereus* agar base (Mossel) (Kasvi) was used for the isolation of *B. cereus*, and the agar produced by ACU was used for *P.*

aeruginosa. The procedures for preparing the culture medium were recommended by the manufacturers. The identification of the strains was based on Bergey's Manual of Systematic Bacteriology (Bergey et al., 1957). CFU tests have been performed since the 1990s to improve the pattern of responses in toxicity assays (Deldar and Stevens, 1993, Parchment et al., 1993, Deldar, 1994, Fajardo et al., 2013).

All treatments were prepared in triplicate, and each set of experiments was repeated three times to ensure the reproducibility of the data. A statistically significant difference was calculated by one and two factors. The normality of the samples was tested using the Shapiro–Wilk test. The differences between treatments and the sample depth were determined using Tukey's multiple comparison test. The values were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

B. cereus

The results of the CFUs of *B. cereus* after the injection of nZVI are shown in Figure 2. After 7 days, we found a coefficient of variation (CV) of 55.93%. According to the F-test result, the means were statistically equal. The means were 29.5 and 47.5 CFUs for the PCP + Cr^{6+} + nZVI treatment and the PCP + nZVI treatment, respectively. The bacterial population remained the same between treatments without interference from the chemical compounds. The environment remained stable. The average temperature in the drums was 24.8°C, the pH was 5.2, the average electrical conductivity was 0.05 dsm/cm, and the soil moisture remained at 32%.

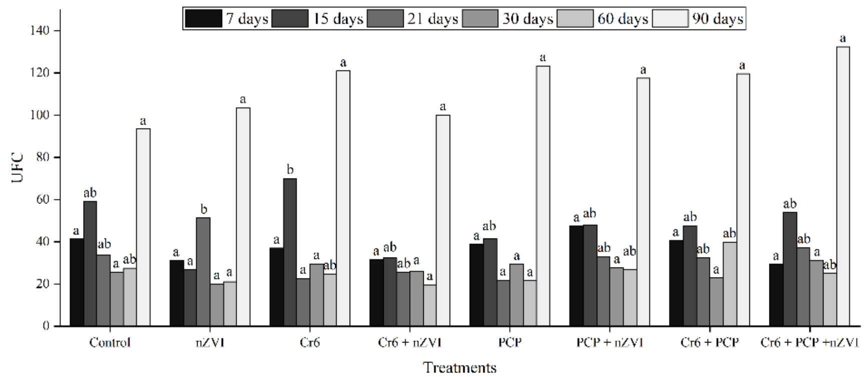

Figure 2: Comparison of the CFUs of *B. cereus* in different treatments on the evaluation dates. * Lowercase letters compare the CFUs evaluated within each treatment type. The means followed by the same letter do not differ statistically using Tukey's test ($p \leq 0.05$).

After 15 days, the treatment with nZVI presented the lowest mean CFUs (27). The highest mean occurred in the Cr^{6+} treatment (70), and the other treatments did not differ statistically, with the CV at 46.65%. The temperature was 21.6°C–22.9°C, the pH remained at 5.2, the electrical conductivity was 0.05 dsm/cm, and the soil moisture was almost 32%.

Consistent with this result, Fajardo et al. (2013) performed in vitro assays with *B. cereus* and reported a negative effect of nZVI on the development of cells at an early stage of sporulation, pointing to changes at the protein level in the bacterial cell wall. In another study, Fajardo et al. (2012) found this bacterium to be sensitive to the presence of nZVI when subjected to fluorescence in situ hybridization.

The increase in the number of CFUs in the treatment with Cr^{6+} can be explained by the tolerance to chromium of *B. cereus* and its efficiency in reducing the contaminant presented by the bacteria. The *B. cereus* strains tested by Li et al. (2020a) survived at small concentrations of Cr^{6+} and even helped in the reduction to Cr^{3+}. The bioreduction capacity and resistance of *B. cereus* to Cr^{6+} were also described by Zhao et al. (2012) and Murugavelh and Mohanty (2013).

By contrast, after 21 days, the treatment with nZVI had the highest mean of CFUs (51.3), and the treatment with Cr^{6+} (22.6) had the lowest mean, together with the PCP treatment (21.6). The other treatments did not statistically differ, with the CV being 40.54%. The environment remained stable, the temperature varied from 22.6°C–24.3°C, the pH was 5.2, the electrical conductivity remained at 0.05 dsm/cm, and the soil moisture was 32%.

These results demonstrate the need for further investigation into the use of nZVI in field studies and long-term evaluations. Several environmental factors are ignored in laboratory research in a controlled environment, and they can mask or misestimate the real toxic effects of a substance in nature. According to Elliott and Zhang (2001), the environment resulting from remediation by nZVI could lead to an increase in the population of bacteria and also help in the reduction of contaminants. The presence of a thick peptidoglycan layer on the cell walls of gram-positive bacteria makes them more resistant to nanoparticles (Barzan, Mehrabian, and Irian, 2014).

After 30 days, the treatments did not show statistical differences, with the CV at 51.93%. The pH was 5.1, the average temperature was 24.6°C, the electrical conductivity was unchanged at 0.05 dsm/cm, and the soil moisture was 33%. After 60 days, treatments with nZVI (21), PCP (21.5), and Cr^{6+} + nZVI (19.5) showed the lowest average CFUs. The treatment with PCP + Cr^{6+} had the highest mean (39.8), and the other treatments did not statistically differ, with the CV being 35.54%. The average temperature was 23.8°C, the pH was 5.1, the average conductivity was 0.05 dsm/cm, and the soil moisture was 33%.

The reduction in the number of CFUs overtime was expected due to the effect of nutritional parameters, such as carbon and nitrogen consumption, on the bioremediation process (Tripathi and Garg, 2013). The increase in CFUs in the PCP + Cr^{6+} treatment is explained by the combined biodegradation capacity of PCP and the Cr^{6+} bioreduction shown by *B. cereus* (Chandra et al., 2009, Singh et al., 2009, Tripathi and Garg, 2013).

Ninety days after the injection of nZVI, the means were statistically equal. The lowest CV was 21.82%, a characteristic that rules out the hypothesis of a toxic effect by one or more of the components tested when evaluated in the long term. An increase in the average of CFUs found in all treatments was observed, with 93.5 CFUs for the control and 132.5 CFUs for the PCP + Cr^{6+} + nZVI treatment. The temperature varied from 23.5°C–24.8°C, the pH remained at 5.1, the electrical conductivity remained at 0.05 dsm/cm, and the soil moisture was 33%.

A two-factor analysis was performed to verify the interaction between the treatments and the depth of soil collection for further evaluation of the CFUs. No significant iterations were found at 7, 15, 30, 60, and 90 days of evaluation of *B. cereus* (Figure 3). However, at 21 days, the control, nZVI, and PCP + Cr^{6+} + nZVI treatments showed a significant difference between the collection points of 25 cm deep and 50 cm deep.

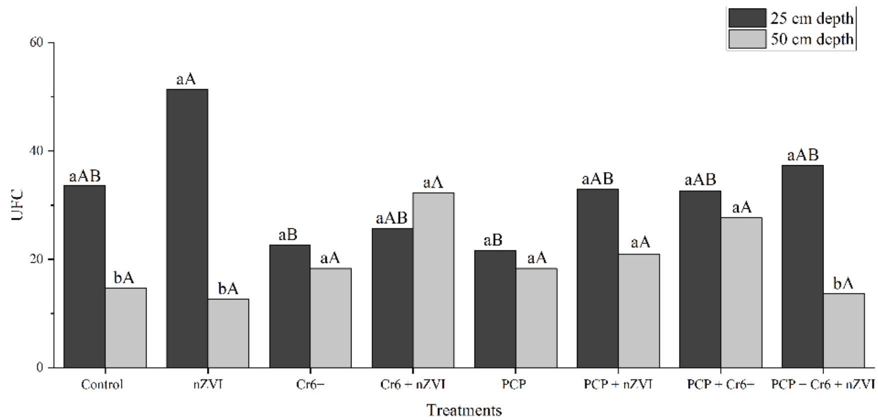

Figure 3: Comparison of the CFUs of *B. cereus* between treatments and depths 21 days after the nZVI injection. * Lowercase letters compare the depths assessed within each treatment type. Capital letters compare the treatment types within each depth. The means followed by the same letter do not differ statistically using Tukey's test ($p \leq 0.05$).

The control, nZVI, and PCP + Cr^{6+} + nZVI treatments showed the highest number of CFUs in the surface layer of the soil (i.e., 25 cm depth), which is a contaminated and nanoremediated site. Moreover, on this evaluation date, the Cr^{6+} and PCP treatments showed average CFUs that statistically differed from those expected for the evaluated depths, in contrast to the expected toxic effect discussed by other authors (Fajardo et al., 2012, Tilston et al., 2013, Fajardo et al., 2013, Kumar et al., 2014, Velimirovic et al., 2015, Li et al., 2020a).

Even with the temporary effects on the abundance of the microbiota community, the resilience and adaptation of the evaluated bacteria to the altered environment stand out. For Elliott and Zhang (2001), the reducing and anaerobic environment provided by the remeasurement of nZVI stipulates specific conditions that benefit the growth of some bacteria. The toxicity of nZVI in the bacterial population is dose and species-dependent (Fajardo et al., 2012). *B. cereus* shows greater resistance to nZVI than gram-negative bacteria and has no harmful effects from exposure to these contaminants (Barzan, Mehrabian, and Irian, 2014).

P. aeruginosa

The gram-positive and gram-negative bacteria exhibit different behaviors (Fajardo et al., 2012, Fajardo et al., 2013, Pawlett et al., 2013). However, the behaviors of *P. aeruginosa* and *B. cereus* were similar. The short-term responses of bacteria are important for population maintenance after contact with contaminants. The gram-negative bacteria, such as *P.*

aeruginosa, have mechanisms that can lead from cell wall stiffening to the creation of cell aggregates or biofilms (Saccà et al., 2014, Eberlein et al., 2018).

The analysis for *P. aeruginosa* showed that at seven days, the averages of CFUs did not differ statistically and presented a low average CV occurrence of 45.67% (Figure 4). The environment in the tests remained stable. The average temperature was 24.8°C, the pH was 5.2, the average electrical conductivity was 0.05 dsm/cm, and the soil moisture remained at 32%.

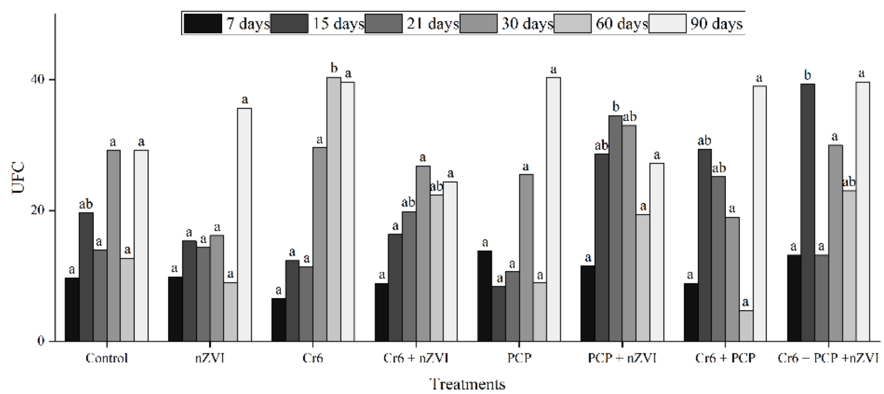

Figure 4: Comparison of the CFUs of *P. aeruginosa* in different treatments on the evaluation dates. * Lowercase letters compare the CFUs evaluated within each treatment type. The means followed by the same letter do not differ statistically using Tukey's test ($p \leq 0.05$).

At 15 days, the lowest average CFUs were 8.3 for PCP, 12.3 for Cr^{6+}, 15.3 for nZVI, and 16.3 for Cr^{6+} + nZVI. The highest incidence was in the PCP + Cr^{6+} + nZVI treatment (39.3), and the other treatments did not statistically differ, with the CV at 54.73%. The temperature was 21.6°C–22.9°C, the pH was 5.2, the electrical conductivity was 0.05 dsm/cm, and the soil moisture was close to 32%.

The results indicate an adaptation of the bacteriological community. Low CFU values indicate the occurrence of migration, adaptation, or improper conditions for the development of communities. An analysis of *Pseudomona stutzeri*, a bacterium of the same genus, showed the occurrence of defense mechanisms in response to exposure to nZVI. The cell membrane was affected by nanoparticles fixed on the bacterial wall, revealing oxidative stress (Saccà et al., 2014).

At 21 days, the PCP + nZVI treatment had the highest mean (34.5), followed by the PCP + Cr^{6+} (25.1) and Cr^{6+} + nZVI (19.8) treatments. The other treatments had lower means and did not statistically differ, with the CV at 50.02%. The temperature was 22.6°C–24.3°C, the pH was 5.2, the electrical conductivity was 0.05 dsm/cm, and the soil moisture was 32%. The tolerance and reduction capacity of Cr^{6+} by *P. aeruginosa* (Kiliç and Donmez, 2008, Oves, Khan and Zaidi, 2013, Li et al., 2020b), combined with the ability to degrade PCP (Premalatha and Rajakumar, 1994, Ammeri et al., 2017), justified the results.

At 30 days, the means were statistically equal, with a CV of 45.48%. For the nanoremediated environment, no disturbances were observed. The average temperature was 24.6°C, the pH was 5.1, the electrical conductivity was unchanged at 0.05 dsm/cm, and the soil moisture was 33%. This behavior points to the adaptation of the population to the nanoremediated environment.

The largest data discrepancy occurred at 60 days, with a mean of 40.3 CFUs for Cr^{6+} and low occurrences in the control (12.6), nZVI (9), PCP (9), and PCP + Cr^{6+} (4.6) treatments. The other treatments did not statistically differ, with a CV of 49.7%. The parameters recorded an average temperature of 23.8°C, pH of 5.1, average conductivity of 0.05 dsm/cm, and soil moisture of 33%. The addition of nZVI has the potential to inhibit microbial functions (Barnes et al., 2010, Tilston et al., 2013, Kumar et al., 2014, Velimirovic et al., 2015, Zabetakis et al., 2015). However, the treatments that showed greater oscillation in the number of CFUs did not have nZVI. These interactions are difficult to interpret and indicate that the effects of nZVI are highly context-dependent (Pawlett et al., 2013).

Pseudomonas has been described as having the ability to reduce Cr^{6+} (Kiliç and Donmez, 2008, Oves, Khan and Zaidi, 2013, Li et al., 2020b), survive exposure to high doses of this contaminant (Mat Arisah et al., 2021), and present several resistance mechanisms against metal ions (Kiliç et al., 2010). For example, the bacterium can use the nutritional and energy resources intended for growth to manage protection from the stress caused by PCP (Ray and Peters, 2008). Research has shown that bacterial responses depend on the concentration, morphology, and constitution of the species being evaluated (Fang et al., 2007).

Corroborating the hypothesis of adaptation of *P. aeruginosa* and discarding the idea of toxicity to the compounds tested 90 days after the injection of nZVI, the averages of CFUs were the same, and the lowest CV of 34.39% was verified. The environment remained stable, with a temperature of 23.5°C–24.8°C, pH of 5.1, the electrical conductivity of 0.05 dsm/cm, and soil moisture of 33%.

Taking into account the treatments and collection depths, the bifactorial analysis for *P.*

aeruginosa showed a significant interaction between depths only at 60 days of collection (Figure 5). Only the PCP + Cr^{6+} treatment differed statistically between depths. It should be noted that the greatest fluctuation in the population occurred on this date and that the possibility of migration was not ruled out.

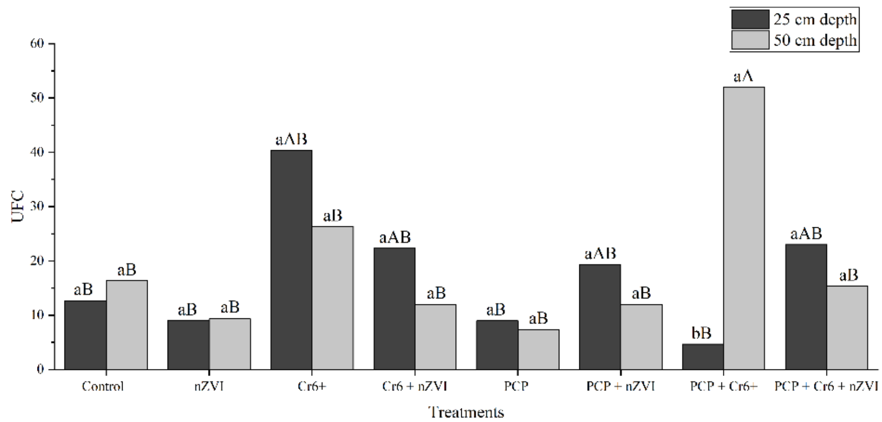

Figure 5: Comparison of *P. aeruginosa* CFUs between treatments and depths 60 days after the nZVI injection. * Lowercase letters compare the depths assessed within each treatment type. Capital letters compare the treatment types within each depth. The means followed by the same letter do not differ statistically using Tukey's test ($p \leq 0.05$).

Our findings corroborate the results of other researchers (Nel et al., 2008, Kirschling et al., 2010, Xue et al., 2018). The lack of a broad bactericidal effect has positive implications for field applications of nZVI (Kirschling et al., 2010). As most toxicity studies have been performed in vitro, it is not possible to directly link the effect of nZVI on microbial populations (Xue et al., 2018). Furthermore, the interface between nanoparticles and microbiological systems comprises an extensive and dynamic range of iterations that can determine whether the material is bioavailable, whether it can participate in iterations with bacteria, or whether it will have an adverse effect (Nel et al., 2008). It is imperative to demonstrate the feasibility of nZVI applications on a large scale rather than in laboratory conditions and to show that they are effective, safe, and cost-effective (Xue et al., 2018).

CONCLUSION

The toxic effects of nZVI on *B. cereus* and *P. aeruginosa* were evaluated for colony formation. Oscillations were observed in the behavior of the microbiological population over time. However, this behavior was explained by the bacteria's adaptation to the contaminated environment, resilience, defense mechanisms, tolerance to chemical components, and ability to biodegrade and/or bioreduce compounds.

The nanoremediation of nZVI in soil contaminated by Cr^{6+} and PCP had no toxic effect on the population of native soil bacteria. At 90 days after the nZVI injection, the populations did not differ statistically between treatments and reached the highest levels of CFUs. The strains of *B. cereus* and *P. aeruginosa* showed similar behavior and were resistant to concentrations of nZVI, Cr^{6+}, and PCP.

Monitoring the test made it possible to understand that the nanoremediated environment does not suffer major disturbances in terms of temperature, electrical conductivity, and humidity over time. However, research with even longer and more detailed assessments of these environments is recommended.

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CONCLUSÕES GERAIS

O estudo bibliométrico temporal quantitativo-descritivo reuniu e esclareceu o conhecimento sobre as publicações relacionadas à toxicidade do nZVI utilizado na remediação de solos disponíveis nas bases de dados Scopus e WoS. Possibilitou a visualização de um panorama de publicações, autores, países, instituições e periódicos por meio de indicadores que permitem aos pesquisadores selecionar e analisar a literatura existente, direcionando suas pesquisas para uma melhor contribuição científica.

Considerando o grande número de publicações em 2016, este estudo também mostrou que a toxicidade do nZVI usado na remediação do solo é um tema de estudo extremamente novo e em desenvolvimento. Fornecemos uma análise para ampliar o conhecimento sobre as publicações relacionadas a esse assunto e possibilitar que os pesquisadores foquem suas pesquisas no preenchimento das lacunas do assunto e contribuam de forma mais efetiva para a ciência.

Precisamos suprir a carência de pesquisas realizadas in situ e em países subdesenvolvidos, a necessidade de investigar novos indicadores de qualidade ambiental, principalmente espécies não-alvo que tenham relevante interesse no equilíbrio ecossistêmico, que irá variar de acordo com a região de estudo. Apontando para toxicidade aguda do nZVI, apresentando contrações letais, concentrações de risco efetivas e concentrações de risco não efetivas. A classificação da substância quanto ao potencial de perigo e análise de risco. Também precisamos observar a necessidade de pesquisas que melhor apontem o sistema de migração das nanopartículas e melhor acompanhamento por estudos de longo prazo.

A técnica de remediação de solos com injeção de nZVI apresentou mais de 90% de eficiência na degradação dos contaminantes Cr^{6+} e PCP. A degradação de Cr^{6+} mostrou-se rápida e constante, o contaminante PCP mostrou-se mais persistente no ambiente ao apresentar maior degradação a partir de 30 dias.

Foi registrada a migração de ambos os contaminantes e também de nZVI para camadas mais profundas do solo, entretanto não foram encontrados contaminantes no lixiviado. O monitoramento do ensaio possibilitou a compreensão de que o ambiente nanorremediado com nZVI não sofre grandes perturbações quanto a temperatura, condutividade elétrica e umidade ao longo do tempo.

Os procedimentos de extração de contaminantes mostraram-se complexos e a influência de fatores externos dificultam a assertividade das análises. Recomenda-se trabalhos com avaliações ainda mais longas e detalhadas sobre esses ambientes, as iterações entre os contaminantes e a microbiota precisa ser explicada e quantificada.

Avaliamos os efeitos tóxicos do nZVI sobre *Bacillus cereus* e *Pseudomonas aeruginosa*. Foram observadas oscilações no comportamento da população microbiológica ao longo do tempo. No entanto, esse comportamento é explicado pela adaptação da bactéria ao ambiente contaminado, resiliência, mecanismos de defesa, tolerância a componentes químicos, capacidade de biodegradar e/ou biorreduzir compostos.

A nanorremediação de nZVI em solo contaminado por Cr^{6+} e PCP não teve efeito tóxico sobre a população de bactérias nativas do solo. Aos noventa dias após a injeção de nZVI, as populações não diferiram estatisticamente entre os tratamentos e atingiram os níveis mais altos de UFCs. As cepas de *B. cereus* e *P. aeruginosa* apresentaram comportamento semelhante e foram resistentes às concentrações de nZVI, Cr^{6+} e PCP.

O acompanhamento do teste permitiu entender que o ambiente nanorremediado não sofre grandes perturbações em termos de temperatura, condutividade elétrica e umidade ao longo do tempo. No entanto, é recomendável trabalhar com avaliações ainda mais longas e detalhadas desses ambientes.

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