**Charles University, Faculty of Science**

Institute for Environmental Studies

Ph.D. study program: Environmental Sciences

Summary of the Ph.D. Thesis

# Toxicity and environmental fate of nanoscale zerovalent iron

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**The presented thesis is based on the following publications:**

**Publication 1**

Semerád, J., Cajthaml, T. (2016). Ecotoxicity and environmental safety related to nano-scale zerovalent iron remediation applications. *Applied Microbiology and Biotechnology*, 100 (23), 9809–9819.

**Publication 2**

Semerád, J., Čvančarová, M., Filip, J., Kašlík, J., Zlotá, J., Soukupová, J., Cajthaml, T. (2018). Novel assay for the toxicity evaluation of nanoscale zero-valent iron and derived nanomaterials based on lipid peroxidation in bacterial species. *Chemosphere*, 213, 568–577.

**Publication 3**

Semerád, J., Moeder, M., Filip, J., Pivokonský, M., Cajthaml, T. (2019). Oxidative stress in microbes after exposition to iron nanoparticles: the analysis of aldehydes as oxidative damage products of lipids and proteins. *Environmental Science and Pollution Research* (submitted).

**Publication 4**

Semerád, J., Filip, J., Ševců, A., Brumovský, M., Nguyen, N., Mikšíček, J., Lederer, T., Filipová, A., Holecová, J., Pivokonský, M., Cajthaml, T. (2019). Environmental fate of sulfidated nZVI particles: interplay of nanoparticle corrosion and toxicity during the aging. *Environmental Science: Nano* (submitted).

**Publication 5**

Wu, S.-L., Cajthaml, T., Semerád, J., Filipová, A., Klementová, M., Skála, R., Vítková, M., Michálková, Z., Teodoro, M., Wu, Z., Martínez-Fernández, D., Komárek, M. (2019). Nano zero-valent iron aging interacts with soil microbial community: a microcosm study. *Environmental Science: Nano.* 6, 1189–1206.

**Publication 6**

Semerád, J., Pivokonský, M., Cajthaml, T. (2019). Nano-bioremediation: nZVI for inorganic and organic contamination. *Springer* (book chapter in: "Advanced Nano-Bio Technologies for Water and Soil Treatment"; submitted).

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## Abstract

Nowadays, nanoscale zerovalent iron (nZVI) is a nanomaterial commonly used in remediation practice. Although worldwide applications of nZVI have shown its effectiveness in degradation and immobilization of a wide range of organic and inorganic pollutants, potential negative effects of nZVI on exposed organisms have not been sufficiently explored. To avoid possible environmental risks, understanding of the mechanism of nZVI toxicity and its overall effects on microbial populations indigenous to remediation sites is needed. The presented thesis summarizes current knowledge of nZVI toxicity, and, moreover, deals with the development and application of a new test for *in vitro* evaluation of acute toxicity caused by commonly used as well as newly developed nZVI-based materials. Additionally, in this thesis, the risk associated with changes in the toxicity of the aforementioned materials during the aging process was examined. In the last part, the effect of several nZVI-based materials on microbial communities of a real contaminated soil was monitored and evaluated using artificial microcosms. In addition, in this part, the potential of nZVI and its derived materials in combination with a biostimulation step during nanobioremediation is outlined.

# 1. Introduction

In recent years, an increase in the application of reactive nanomaterials based on nanoscale zerovalent iron (nZVI) has been observed in remediation practice (Jang et al., 2014). Results of pilot and full-scale applications document high efficiency of nZVI-based decontamination technologies (Mueller et al., 2012; Zou et al., 2016). However, injections of reactive nanomaterials in high doses during the applications associate these materials with potential environmental risks. For this reason, it is necessary to study this subject and extend existing knowledge concerning potential negative effects of these materials on exposed organisms.

Given the nature of the application, mainly resident microbes from contaminated localities will be exposed (Ševců et al., 2011). Although nZVI-based nanomaterials are currently widely introduced into the environment, the mechanism of the toxic effect of nZVI has not yet been described adequately and appropriate attention to the study of the effect on microbial populations has not been paid, especially to those from real contaminated sites (Lefevre et al., 2016). Moreover, many new modifications of nZVI are currently being developed in order to achieve higher reactivity and mobility in the geosphere. These novel modifications of nZVI also represent new risks and the evaluation of their potential negative effect on the environment is needed.

The first part of this thesis summarizes current knowledge about *in vitro* and *in situ* effects of nZVI on microorganisms. Furthermore, in this section, the potential of nZVI-based

materials for use in nanobioremediation is evaluated in detail. In the second part of the presented thesis, newly developed and optimized tests are presented and used for toxicity determination of nZVI-based nanomaterials to study the mechanisms of the toxic effects. Using one of the developed tests, changes in toxicity during aging of new sulfidic modifications of nZVI was also studied. In the last part of this thesis, the effect of other modifications of nZVI on microbial populations in artificially created microcosms containing real contaminated soil was investigated together with material changes during aging.

## 2. Aims of the study

The main objectives of the presented thesis are summarized below:

1. A systematic review of literature on nZVI toxicity
2. The development and optimization of a new protocol for testing nanomaterials based on nZVI using bacterial species
3. The development of a test for the evaluation of oxidative damage of biomolecules in various microorganisms after exposure to a nanomaterial (nZVI)
4. The study of changes in toxicity and structure of sulfidic modifications of nZVI during their 2-month aging
5. The evaluation of the use of the combination of nanoremediation and biostimulation in remediation applications
6. The study of the effect of nZVI on soil microorganisms in artificially created microcosms

## 3. Materials and methods

The experimental methods are described in detail in the correspondent publications. This is only a brief overview:

- The acute toxicity test developed in this work was based on the cultivation of bacteria, their subsequent exposure to nZVI, extraction and derivatization of malondialdehyde and quantification by HPLC-FLD.
- The second test presented in this work was based on the quantification of carbonyls as markers of oxidative damage to biomolecules via derivatization and subsequent analysis by HS-SPME-GC-MS.
- Next generation sequencing was used to evaluate microbial communities in experiments with engineered microcosms.
- The course of the toxicity of nZVI sulfide modifications over time was measured using the acute toxicity test developed in this work, as well as the activated sludge respiration inhibition test and other microbiological methods based on cultivation.
- In order to monitor the structural changes of nZVI-based materials during aging, X-ray diffraction, transmission electron microscopy and disk centrifugation were used.

# 4. Results and discussion

## 4.1. Ecotoxicity and environmental safety related to nano-scale zerovalent iron remediation applications (publication 1)

This publication provides an overview of the existing knowledge about the negative effects of nZVI-based nanomaterials on microorganisms. In addition to the toxic effect, studies observing a positive, biostimulating effect of these nanoparticles, often overlooked by current literature, are highlighted.

## 4.2. Novel assay for the toxicity evaluation of nanoscale zero-valent iron and derived nanomaterials based on lipid peroxidation in bacterial species (publication 2)

This publication presents a newly developed and optimized acute toxicity test for nZVI-based materials using cultivable bacteria. Furthermore, the influence of passivating (oxide layer modification) on the toxicity of nZVI was observed in this publication. This trend of decreasing toxicity with increasing thickness of the oxide shell was observed in three Gram positive and three Gram negative bacteria.

## 4.3. Oxidative stress in microbes after exposition to iron nanoparticles: the analysis of aldehydes as oxidative damage products of lipids and proteins (publication 3)

A test for the detection of carbonyl compounds as markers of oxidative damage to biomolecules has been developed using advanced methods of analytical chemistry HS-SPME-GC-MS. Degradation of cellular proteins or lipids was subsequently observed in representatives of algae, fungi and bacteria after

exposure to various concentrations of nZVI. The dose-dependent increase in carbonyl fractions confirmed the oxidative damage caused by this nanomaterial.

### 4.4. Environmental fate of sulfidated nZVI particles: interplay of nanoparticle corrosion and toxicity during the aging (publication 4)

The subject of this publication was to study the aging of nZVI and its sulfide modifications in terms of material and toxicity development over several months. In this experiment, a decreasing trend of acute toxicity was observed for newly prepared materials, depending on the weight ratio of added sulfur. After two months of aging, all tested materials showed lesser toxicity than at the start of the experiment.

### 4.5. Nano zero-valent iron aging interacts with soil microbial community: a microcosm study (publication 5)

The influence of nZVI and its derived modifications on microbial population structure in artificially created microcosms was discovered in this work. Using γ-radiation, microcosms with different microflora were artificially created. In this study, disruption of microbial communities by nanomaterials has been observed and potential involvement of microorganisms (iron reducing/oxidizing bacteria and some fungi) in the oxidization (aging) of nZVI-based materials has been reported.

### 4.6. Nano-bioremediation: nZVI for inorganic and organic contamination (publication 6)

This book chapter documents and evaluates the potential of nZVI-based nanomaterials for use in nanobioremediation applications in the Czech Republic. In both studied localities, a site with inorganic contamination by Cr(VI) and a site with combined contamination of Cr(VI) and chlorinated solvents, a positive effect of nZVI on the development of the subsequent biostimulation phase was demonstrated. The application of nZVI reduced the level of contamination and created suitable conditions for the growth of natural microorganisms, which, after subsequent addition of substrate, managed to continue decontaminating the site.

## 5. Conclusions

This work summarizes existing knowledge of positive and negative aspects associated with nZVI and its application. In this work, new protocols for the evaluation of nZVI-based nanomaterial toxicity with potential use in remedial practice were developed. These tests were used to study the development of toxicity of individual materials during their aging, representing their natural fate in the environment. Furthermore, damage to biomolecules caused by oxidative stress, one of the major toxic effects of nZVI, was discovered. In microbial cultures of yeast, fungi and bacteria, markers of protein or lipid oxidation after exposure to various concentrations of this nanomaterial were detected, and thus confirmed damage to these biomolecules. Moreover, the influence on microbial populations in artificially created soil microcosms after exposure to various materials approximated the effect of nZVI in real conditions.

Despite the toxicity of nZVI to microorganisms which has been demonstrated in this work, the ability of complete nZVI oxidation and its low mobility in soil reduce its environmental risk. What's more, the high efficiency of this decontamination technology, suitably complemented by a biostimulation step, can significantly reduce the environmental risk on contaminated sites with certain types of pollutants.

## 6. References

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## Curriculum vitae

- **Personal information:**

Name: Jaroslav Semerád
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- **Education:**
  - Charles University, Faculty of Science, Ph.D. study program Environmental sciences, 2015 – present
  - Charles University, Faculty of Science, master´s degree in Clinical and toxicological analysis, 2013–2015
  - Charles University, Faculty of Science, bachelor´s degree in Clinical and toxicological analysis, 2009–2013
- **Work experience:**
  - Academy of sciences of the Czech Republic, Institute of Microbiology, Laboratory of environmental biotechnology, 2014 – present
- **Research interests:**
  - Toxicological and ecotoxicological tests
  - Toxicology of nanomaterials
  - Environmental chemistry and analysis
  - Separation methods
  - Development of extraction procedures (ASE and SPE)
  - Methods of abiotic degradation

- **Achievements:**
  - STARS – program supporting talented PhD students at the Faculty of Science, Charles University 2015 – present
  - Foundation for internationalization support, Charles University – scholarship for an internship in Sweden
  - GAUK 522218 – Biodegradation of polychlorinated biphenyls by the oyster mushroom – co-investigator
- **Foreign activities:**
  - Helmholtz center for environmental research, Germany, Development and optimization of GC-MS methods, 2/2017
  - University of Gothenburg, Sweden, Advanced methods in ecotoxicology, 1/2018 – 3/2018
- **Language skills:**
  - English a French
- **Courses:**
  - Modern trends in liquid chromatography HPLC/UPLC
  - Modern trends in gas chromatography
  - Chromatography – a useful tool in science and industry
  - 17. school of mass spectrometry
  - 16. school of mass spectrometry

# List of scientific publications and conference contributions

## Publications:

Semerád, J., Cajthaml, T. (2016). Ecotoxicity and environmental safety related to nano-scale zerovalent iron remediation applications. *Applied Microbiology and Biotechnology*, 100 (23), 9809–9819.

Roubalová, R., Dvořák, J., Procházková, P., Škanta, F., Navarro Pacheco Natividad, I., Semerád, J., Cajthaml, T., Bilej, M. (2018). The role of CuZn- and Mn-superoxide dismutases in earthworm Eisenia andrei kept in two distinct field-contaminated soils. *Ecotoxicology and Environmental Safety*, 159, 363–371.

Svobodová, K., Semerád, J., Petráčková, D., Novotný, A. (2018). Antibiotic Resistance in Czech Urban Wastewater Treatment Plants: Microbial and Molecular Genetic Characterization. *Microbial Drug Resistance*, 24 (6), 830–838.

Semerád, J., Čvančarová, M., Filip, J., Kašlík, J., Zlotá, J., Soukupová, J., Cajthaml, T. (2018). Novel assay for the toxicity evaluation of nanoscale zero-valent iron and derived nanomaterials based on lipid peroxidation in bacterial species. *Chemosphere*, 213, 568–577.

Semerád, J., Moeder, M., Filip, J., Pivokonský, M., Cajthaml, T. (2019). Oxidative stress in microbes after exposition to iron nanoparticles: the analysis of aldehydes as oxidative damage

products of lipids and proteins. *Environmental Science and Pollution Research* (submitted).

Semerád, J., Filip, J., Ševců, A., Brumovský, M., Nguyen, N., Mikšíček, J., Lederer, T., Filipová, A., Holecová, J., Pivokonský, M., Cajthaml, T. (2019). Environmental fate of sulfidated nZVI particles: interplay of nanoparticle corrosion and toxicity during the aging. *Environmental Science: Nano* (Submitted).

Wu, S.-L., Cajthaml, T., Semerád, J., Filipová, A., Klementová, M., Skála, R., Vítková, M., Michálková, Z., Teodoro, M., Wu, Z., Martínez-Fernández, D., Komárek, M. (2019). Nano zero-valent iron aging interacts with soil microbial community: a microcosm study. *Environmental Science: Nano*, 6, 1189–1206.

Semerád, J., Pivokonský, M., Cajthaml, T. (2019) Nano-bioremediation: nZVI for inorganic and organic contamination. Springer (book chapter in: "Advanced Nano-Bio Technologies for Water and Soil Treatment"; submitted).

Semerád, J., Ševců, A., Nguyen, N., Mikšíček, J., Hrnčířová, K., Bobčíková, K., Pospišková K. Filip, J., Medřík, I., Kašlík, J., Šafařík, I., Filipová, A., Špánek, R., Pivokonský, M., Cajthaml, T. (2019). Discovering the potential of a new nZVI-biochar composite material for the nanobioremediation of chlorinated solvents: Degradation efficiency and the effect on resident microbial species. (manuscript prepared for submission).

**Conference contributions:**

- PRVOUK 02, Prague, Czech Republic, 23. – 24. 9. 2015

  Oral presentation: Novel assay for nanoparticles ecotoxicity determination

- French-Czech „Vltava" Biosciences meeting, Valence, France, 15. – 16. 10. 2015

  Oral presentation: Novel assay for nanoparticles ecotoxicity determination

- 7. PGS conference, Prague, Czech Republic, 19. 2. 2016

  Oral presentation: Development and application of novel ecotoxicity tests to assess environmental safety of nanomaterials

- 5. Conference of CSMS, České Budějovice, Czech Republic, 13. – 15. 4. 2016

  Poster: Use of quantification of aldehydes in biological samples as a marker of cellular damage caused by nZVI.

- 17$^{th}$ European Congress on Biotechnology, 3. – 6. 7. 2016, Krakow, Poland

  Poster: New method for *in vitro* toxicity testing of nanomaterials based on nZVI used in bionanoremediations

- French-Czech „Vltava" Biosciences meeting, Liblice, Czech Republic, 28. – 30. 9. 2016

  Oral presentation: Novel approaches for toxicity determination of nanoscale zero valent iron

- 8. PGS conference, Chotěboř, Czech Republic, 3. 11. 2016

  Oral presentation: Novel approaches for toxicity determination of nanoscale zero valent iron

- Eurotox 2017, Bratislava, Slovensko, 10. – 13. 9. 2017

  Poster: Assessment of nanoscale zero-valent iron toxicity towards several bacterial species by specific marker of oxidative stress monitoring

- 9. PGS conference, Chotěboř, Czech Republic, 14. 10. 2017

  Oral presentation: Novel approaches in toxicity determination of nano-scale zero valent iron

- 7th European Bioremediation Conference, Crete, Greece, 25. – 28. 6. 2018

  Poster: Novel approach for *in situ* monitoring of nanoscale zero-valent iron effects during nanobioremediation

- 10. PGS conference, Chotěboř, Czech Republic, 1. 10. 2018

Oral presentation: Development and application of novel ecotoxicity tests to assess environmental safety of nanomaterials

- Seminar of Center for geosphere dynamics (UNCE/SCI/006), Prague, Czech Republic, 15. 11. 2018

  Oral presentation: Ecotoxicity and environmental safety related to nano-scale zerovalent iron remediation applications

- Progres Q16, Prague, Czech Republic, 6.2. 2019

  Oral presentation: Environmental risks of nanoscale zero-valent iron

- Aquaconsoil 2019, Antwerp, Belgium, 20. – 24. 5. 2019

  Poster: Screening for 21 perfluorinated organic compounds in Czech waste-water treatment plants

- Nanotech 2019, Paris, France, 26. – 28. 6. 2019

  Poster: Evaluation of novel composit material based on Nanoscale Zero-Valent Iron for nanobioremediation of chlorinated ethenes: Degradation efficiency, microbial populations and material changes during ageing process