



**Taking Nanotechnological Remediation Processes
from Lab Scale to End User Applications
for the Restoration of a Clean Environment**

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**WP6: Analytical Methods for *In situ* Determination of
Nanoparticles Fate.**

**DL 6.2: Analytical Toolbox for *in situ* and on-site
Monitoring**

Deborah Oughton, Philip Kozin (NMBU), Steffen Bleyl (UFZ),
Jan Filip, Petra Skácelová (UPOL), Norbert Klaas (USTUTT),
Frank von der Kammer, Andreas Gondikas (UNIVIE).

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List of co-authors:

Name, First Name	Partner Organisation	
Hans-Christian Teien, Ole Christian Lind, Merethe Kleiven, Deborah Oughton, Philip Kozin, Pablo Lebed	Norwegian University of Life Sciences	
Frank von der Kammer, Stephan Wagner, Doris Schmid, Andreas Gondikas, Milica Velimirovic	University of Vienna	
Norbert Klaas	University of Stuttgart, VEGAS	
Anett Georgi, Glenn Gillies, Katrin Mackenzie, Steffen Bleyl	UFZ	
Jonathan Lloyd, Vicky Coker, Mat Watts, James Byrne, Richard Patrick, Nimisha Joshi	University of Manchester	
Beate Agnes Krok, Rainer Meckenstock, Julian Bosch	Universität Duisburg-Essen	
Jan Filip, Petra Skácelová	Palacky University Olomouc	
Melanie Auffan, Armand Maison	Centre National de la Recherche Scientifique	

Reviewed by PAG member(s):

Name, First Name	Partner Organisation	
Gerhardt, Rolf	Deutsche Bahn AG	
Matz, Pierre	Solvay	

Reviewed and agreed by PMG

Executive Summary

One of the strengths of the NanoRem project has been the opportunity to test a wide variety of methods for field measurement and detection of NPs, from measurement of simple chemical parameters to high-end sophisticated techniques. The work carried out has covered applications in simple laboratory experiments, through to large-scale tank experiments, and finally testing methods in field applications during the injection of Fe-based NPs. This has enabled an evaluation of the applicability of different methods for Fe-based NPs, as well as providing insight into specific challenges, advantages and factors influencing detection limits for field measurements.

This deliverable describes the development and application of a range of analytical methods for *in situ* measurement and detection of NPs. It builds on DL6.1, which covered both laboratory and field methods for characterizing and monitoring Fe-based NPs, but focuses primarily on field and at site measurements for tracking nanoparticles, as tested at the various NanoRem pilot studies. This includes measurements that actually monitor particles within the aquifer, and methods that combine *in situ* or at site sampling with subsequent at site or laboratory measurement.

The first section provides an overview of the monitoring requirements for different remediation phases: why are we monitoring and what information is needed. This is followed by a short section on sampling protocols and strategies for all types of remediation nanoparticles, including what, when and how to sample, and how this would fit in with other remediation monitoring. The main part of the report details the different analytical methods tested and developed in NanoRem for various Fe-based NPs (e.g., nZVI, milled Fe, Carbo-Iron®, Trap-Box zeolites) and their applicability and field performance at the different remediation stages. This includes an overview of what information can be provided, the main factors influencing monitoring performance, and an estimate of costs. The final section summaries results, detection limits and recommendations for which methods work best at which stage of remediation.

To conclude, the NanoRem project has demonstrated that there are a number of techniques that can be applied for monitoring Fe-based NPs during remediation, and that determination of concentrations at levels below those linked to ecotoxicological effects is relatively straightforward within the remediation area. Monitoring of transport of NPs outside the treatment area (e.g. for so called fine or “renegade” particles) is more challenging, since increased Fe concentrations do not necessarily mean movement of NP. However, the methods tested to date, suggest this type of monitoring is possible, and tests to date show low or no mobility of nZVI and milled-Fe NPs outside of the injection area.

A summary of the results according to remediation phase:

Monitoring of particle dispersion during injection phase: Results from tanks and field applications show that the detection of particle loads 0.5-5 mg/L during the injection process is relatively straightforward, with a combination of at site sampling, and analysis of suspensions (turbidity, conductivity, redox, temperature and Fe content). This is sufficient to follow the distribution of particles during injection, but follow-up data analysis is required for a quantitative assessment and design of standard operating protocols. On-site measurements of turbidity, conductivity and Fe concentrations using spectrophotometry are all relatively fast and cheap methods. The instrumentation required for on site measurements is portable and not expensive. Of all methods, magnetic susceptibility has the best potential, but has relatively high detection limits (ca 500 mg/L). While instrumentation costs for the magnetic array sensors are higher than those for the above methods (ca. 1000 Euro for the hardware and 1000 Euro for the electronics), they are one of the few truly *in situ* methods and have the advantage of giving continuous logging data.

Post injection monitoring. Monitoring during the post injection phase needs to provide information not only the concentrations of Fe, but also its speciation in order to understand the fate and reactivity of the injected particles. For total Fe concentration, measurements on suspensions/liquids and soils/sediments can either be carried out after acid digestion and measurement using standard chemical analysis (e.g., ICP-OES, or spectrophotometry). For low particle densities, pre-concentration by centrifugation or filtration can be applied to improve detection limits. Specific protocols for acid digestion need to be developed for the different particles to ensure complete dissolution. The detection limits of all methods will be site specific, depending largely on the background levels, and, for Fe-based NPs, dissolved iron concentrations. Field applications have demonstrated that Mössbauer (for nZVI) can give useful additional information on the time dependent changes in particle state and reactivity, in both water and solid phases. These can be supported by other methods for measurements of structure and oxidation state (e.g. X-ray photoelectron spectroscopy, transmission/scanning electron microscopies, X-ray powder diffraction, X-ray fluorescent spectroscopy).

Monitoring of renegade particles More sensitive methods are required to distinguish lower concentrations of Fe-based NPs from background matrix, such as for controlling for the transport of fine or so-called “renegade” particles outside the injection area. Tests during NanoRem pilot studies indicate that ICP-MS multielement fingerprinting was able to identify NPs at all sites, with positive controls shown for wells close to injection, and little or no migration of particles outside the application area. The detection limits are dependent on site specific parameters, and the number of wells that require such monitoring, but tests indicate the potential for measurability down to a sub-mg levels, at costs of between 1000-3000 EURO per remediation site.

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1. Introduction and aim of the deliverable

DL6.2 builds on DL6.1 and consolidates information on the applicability of methods as tested in the various NanoRem field experiments, with a focus on *in situ* and at site measurements for tracking nanoparticles. An update on the laboratory characterization methods provided in D6.1 is provided in the Annex. Although the main subject of the deliverable, and the work package, is on monitoring of the nanoparticles, and not monitoring for remediation performance (i.e., levels of contaminants) sampling requirements for these types of goals will often concur. This has been taken into account when addressing the recommendations, protocols and available methods for different phases of remediation.

The first section provides an overview of the monitoring requirements for different remediation phases: why are we monitoring and what information is needed. This is followed by a short section on sampling protocols and strategies for all types of remediation nanoparticles, including what, when and how to sample, and how this would fit in with other remediation monitoring. The main part of the report details the different analytical methods tested and developed in NanoRem for various Fe-based NPs (e.g., nZVI, milled Fe, Carbo-Iron®, Trap-Box zeolites) and their applicability and field performance at the different remediation stages. This includes an overview of what information can be provided, the main factors influencing monitoring performance, and an estimate of costs. The final section summaries results, detection limits and recommendations for which methods work best at which stage of remediation.

2. Monitoring and characterisation requirements at different remediation phases.

This section focuses on the different types of information required for different remediation phases. Following DL6.1, goal of the monitoring as well as the corresponding techniques can be divided into four main areas, all having specific analytical requirements and issues.

- (1) Field characterization studies prior to NP remediation
- (2) Monitoring the movement and distribution of NPs during injection. The main question at this point is whether the NP suspension reaches the required location, at the required concentration and state. During this phase the NP concentrations are relatively high, which makes detection more straightforward, but there is a need for rapid feedback at relatively high resolution
- (3) Monitoring for transport of “fine” or “renegade” particles out of the core application area during and after injection. Low NP concentrations give rise to challenges with detection against background levels of colloids. But monitoring can be carried out with a lower spatial resolution, and less urgency for a rapid feedback.
- (4) Post injection behaviour. Transformation and reactivity of the particles. Need for reinjection.

The links between these four objectives and the different remediation phases is sketched in Figure 1 below. In addition to monitoring for nanoparticle fate, there will be a number of other monitoring and measurement requirements at the different phases, such as site characterization and contaminant monitoring. These generic measurement requirements are described in the following sections, and the relationship to nanoparticle characterization outlined.

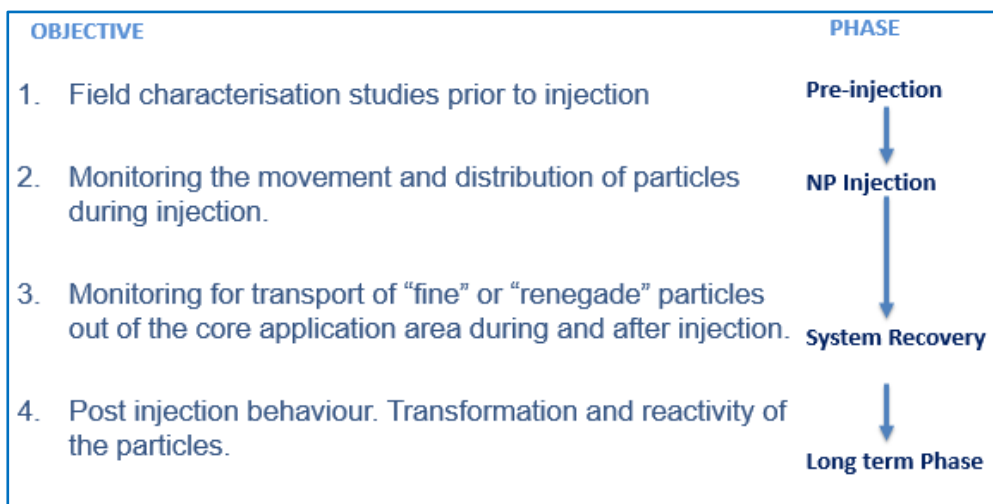


Figure 1: Objectives and remediation phases

2.1. Pre-Injection

The measurement techniques applied in this phase are mostly standard methods used in chemical or hydraulic engineering. Measurement systems such as sampling and injection wells, or *in situ* sensors (if needed) have to be installed, if they are not already available from assessment of the conceptual site model. If new installations are required, disturbances of the system and a corresponding time requirement for the system to stabilize again need to be taken into consideration. The main task is to describe the temporal and spatial concentration profiles of contaminants, but sampling can also provide background and baseline data of relevance for NP tracking. The duration of this phase and frequency of sampling should be sufficiently long to provide a sound background for quantification of the remediation success and to identify impacts of the particle injection. Ideally this would be over a similar time scale to the post remediation monitoring phase in order to identify natural seasonal and temporal fluctuations. Since contaminant monitoring, in many cases, contains volatile substances, a more frequent sampling or a longer duration of the phase is advisable in order to obtain sound background information.

2.2. NP Injection Phase

The requirements in this phase are very different from the previous and also from the following phases. While this phase has only a duration of hours to days, acute changes within minutes have to be detected. The main focus is the behaviour of the particles, namely the radius of influence (ROI), the travel distance and the homogeneity of the distribution around an injection point of well, and acute changes within minutes have to be detected. This requires not only much higher measurement frequencies, but also the application of different measurement techniques.

Since particles are injected as a suspension, the liquid and the solid phases may behave differently, and methods need to address both phases in order to provide information about the overall efficiency of the injection and potential deviations from the planned behaviour. For the liquid phase relatively simple methods are available such as temperature measurements (usually the temperature of the injected fluid differs from the ground water temperature) or the addition of tracer substances (dyes or tracer ions) to the suspension. For the particles, unfortunately, only a few *in situ* methods are available. Thus, in most cases the particle detection will have to be based on sampling and on-site or laboratory methods (see section 4).

2.3. System Recovering Phase

This phase is a relatively short, intermediate phase between injection and a return to the natural groundwater flow. The volume of injected fluid will cause considerable disturbance of the hydraulics in the aquifer and intensive analytical activities for both NPs and contaminants are usually not required during this phase. Thus a reduced monitoring programme is advisable where only some main chemical parameters are monitored in order to follow the overall changes and determine when natural groundwater flow conditions have re-established, taking into account the modifications to be expected by the injected NPs. Depending on the expected rate of groundwater movement, monitoring for the potential transport of NPs outside of the treatment area could be started.

2.4. Long Term Steady State Phase (monitoring of success, reinjection)

During this phase contaminants, reaction products, metabolites and general milieu parameters of the ground water are monitored on a regular (monthly) basis, in order to verify the success of the remediation. The main focus of the monitoring is to ensure the efficiency of the desired reaction in terms of reduction of concentrations of contaminants in the ground water, reduction of emissions or contaminant masses. The criteria for the decision on the success of a nanoremediation have to be defined beforehand and a monitoring program chosen accordingly. All monitoring results should be compared to the status defined during the pre-injection phase. The long-term phase should also include monitoring for potential transport of NPs out of the site. The programme should be designed so that decisions can be made about the need for a reinjection, if a single injection does not reach the remediation goals.

3. Sampling Protocols

This section deals with sampling procedures for determining changes in the groundwater prior, during, and after injection of particle suspension. Sampling frequency, sample collection and storage protocols are described for the analytical methods that have been tested in the framework of this project.

3.1. Sampling areas and Frequency

Two sampling areas need to be identified during the planning stage: the injection and the remote areas. The former is the area where remediation is required, while the latter is not expected to be impacted significantly by the remediation effort, but needs to be sampled in order to conclude on an eventual presence of renegade particles. The limits of these two areas are site specific and depend on the hydrogeological conditions of the aquifer. Although sample collection, storage, and analysis needs to be identical for the three phases in order to facilitate comparison, sampling frequency should differ. Background levels and natural variability of groundwater composition need to be determined prior to injection. Therefore, weekly or monthly samples need to be collected from the vicinity of the injection area for at least three months; this period should be shortened in case of emergency situations, such as accidental spills or extreme hydrogeological conditions (extremely high precipitation, floods, etc).

Sampling frequency should increase during the injection due to the sharp increase of particle content in the aquifer and fast physicochemical changes incurred by the injected suspension. But the actual frequency will depend on the monitoring method employed and expected movement of the particles deployed. For example, hourly sampling and analysis is attainable for on-site observations of color, chemical changes, Fe spectroscopy and turbidity in near-by wells. A lower frequency, e.g. every 2-6 hours, would be sufficient for sampling for off-site monitoring of total Fe concentrations. The advantage of on-site monitoring means that sampling frequencies can be adapted to field observations.

Post- injection sampling frequency should be lower: on a daily basis for the first week, on a weekly

basis for the following month, and on a monthly basis if monitoring is required further on. Sample collection should be carried out directly from a groundwater well, after rinsing the well volume for at least three times. The groundwater samples should be collected in acid cleaned plastic bottles (preferably NALGENE). The bottles should also be rinsed three times with the sample and filled in a way as to minimize the amount of air after capping. If not immediately analyzed, Parafilm can be used to wrap the bottle caps and samples should be kept at low oxygen environment and low temperature (approximately 5 °C) for as long as practically possible. The low oxygen environment is not necessary for most analytical techniques, such as turbidity, ICP-MS, and spICPMS, but is important for light scattering and SedFFF analysis. Table 1 summarizes the above instructions.

Table 1: Summary of sampling protocols at various stages of remediation

	Pre-injection	During-injection	Post-injection
Sampling frequency	Weekly or monthly (duration: > 3 months)	Up to hourly, depending on the duration of injection and monitoring method	Daily (first week) Weekly (following month) Monthly (further on)
Sampling method	Pump and hose in pre-cleaned plastic bottles	Pump and hose in pre-cleaned plastic bottles	Pump and hose in pre-cleaned plastic bottles
Sample storage	5 °C until analysis; anoxic conditions if to be analyzed with SedFFF or light scattering. Low temperature storage for Mössbauer	5 °C until analysis; anoxic conditions if to be analyzed with SedFFF or light scattering. Low temperature storage for Mössbauer	5 °C until analysis; anoxic conditions if to be analyzed with SedFFF or light scattering- Low temperature storage for Mössbauer

3.2. Co-ordination with other site characterization and monitoring.

Wherever possible, monitoring should be coordinated with ongoing site characterization and remediation monitoring. Prior to injection, this should be in conjunction with, for example, contaminant mapping and selection of modelling and monitoring wells. During injection it is very important to collect samples of the injected particles and solvents. Sampling during recovery and late phases should be coordinated with other sampling regimes, e.g. to follow changes in contaminant concentration. Where possible, it is advisable to collect and store “archive” samples, in case of unexpected changes in groundwater properties. Monitoring to control for off site migration of “renegades”, or transport of fine particles outside of the injection area can usually be carried in a few pre-selected monitoring wells with relatively low frequency.

4. Analytical Toolbox

NanoRem has evaluated and developed a range of methods, covering application of general laboratory methods for characterisation, “truly” *in situ* measurements that actually monitor NPs within the aquifer, and methods that combine *in situ* or at site sampling with subsequent at site or laboratory measurement (Table 2). The results presented in this deliverable re focused predominantly on those *in situ* and at site methods that are applicable for monitoring of Fe NPs injected into groundwater during NanoRem field studies.

Table 2: Overview of Nanoparticle Monitoring Methods tested in NanoRem

Type of Method	Examples	Applications	Comments
Laboratory Particle characterisation	Field flow fractionation (FFF), Inductively coupled plasma-mass spectrometry (ICP-MS), transmission electron microscopy (TEM), dynamic light scattering (DLS), synchrotron techniques, isotope tracing techniques,	All particle characteristics: size, structure, composition, aggregation, mineralogy.	Required to understand fundamental particle behaviour in laboratory and field experiments
<i>In situ</i> measurement and characterisation	Ferro-magnetic methods; redox measurement; H ₂ production	Particle concentration, particle reactivity	High data resolution over time and space is possible
On site applications: sampling combined with on site or laboratory measurement techniques	Turbidity, Fe spectrometry, ultrafiltration; stable isotope and REE ratios; Mössbauer, Temperature programmed oxidation (TPO)	Particle size and concentration, Fe concentration	Turbidity, spectrometry and ultrafiltration can be carried out on site. Mossbauer, TPO, Isotope and REE ratios are laboratory measurements that can provide more detailed information on field behaviour, and/or particle reactivity

The various methods developed and tested in NanoRem are complementary and depend on the remediation phase, the NP utilised and the question to be asked. The monitoring measurements made in the pre-injection phase are relatively straightforward, with the objective of providing background and baseline data for the choice of monitoring method post injection. Hence this section focuses on the methods available for following NPs during the injection and post injection phases.

4.1. Monitoring of NP dispersion during injection phase:

Results from NanoRem field measurements during the injection of nZVI (NANOFER 25S, NANOFER STAR), Nano-Goethite and milled Fe (FerMEG12) show that the detection of NP suspension loads is relatively straightforward, and can be easily carried out at the site. The methods include a combination of on-site sampling and analysis of suspensions (turbidity, conductivity, redox, temperature and Fe content), or *in situ* methods such as magnetic susceptibility, redox (ORP) and H₂ measurements. The detection limits, from sub mg/L for total Fe to ca 500 mg/L for magnetic susceptibility, are sufficient to follow the dispersion of injection liquids and NPs during injection, both within and outside the injection area. Of the various methods tested, magnetic susceptibility, turbidity and total Fe measurements are most appropriate for monitoring during injection.

4.1.1. Magnetic susceptibility

Magnetic susceptibility is one of the very few *in situ* methods that can be used to detect Fe NPs, and has the advantage of allowing for continuous monitoring. It can be combined with other sampling and monitoring arrays. The sensor arrays developed by the University of Stuttgart can be installed in the subsurface and use the magnetic properties of Fe to detect changes in magnetic properties in the vicinity of the susceptibility probe. The probe itself consists of two intertwined inductors, wherein an alternating electromagnetic field produced through the outer (primary) inductor induces a voltage in the inner (secondary) inductor that is proportional to the magnetic susceptibility of the environment around the probe (Figure 2).

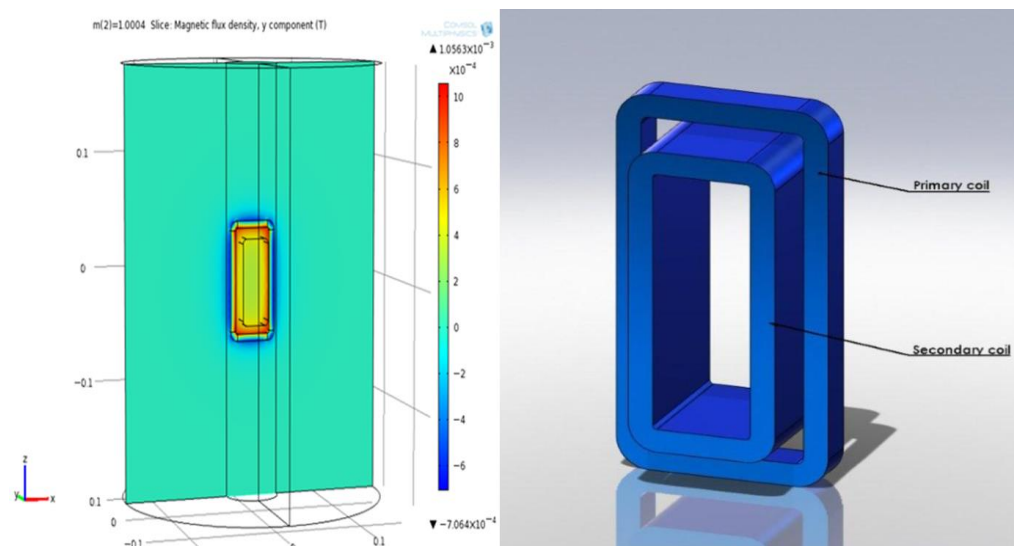


Figure 2: Sketch showing the susceptibility probe, electromagnetic field (left), position and shape of the inductors (right).

Figure 3 shows two arrays before the installation in the subsurface. Such arrays have been installed at the Czech Republic (nZVI - NANOFER 25S, NANOFER STAR) and Solvay, Switzerland (milled Fe FerMEG12) field sites together with a temperature sensor and sampling ports. The field results from the two sites are presented below.

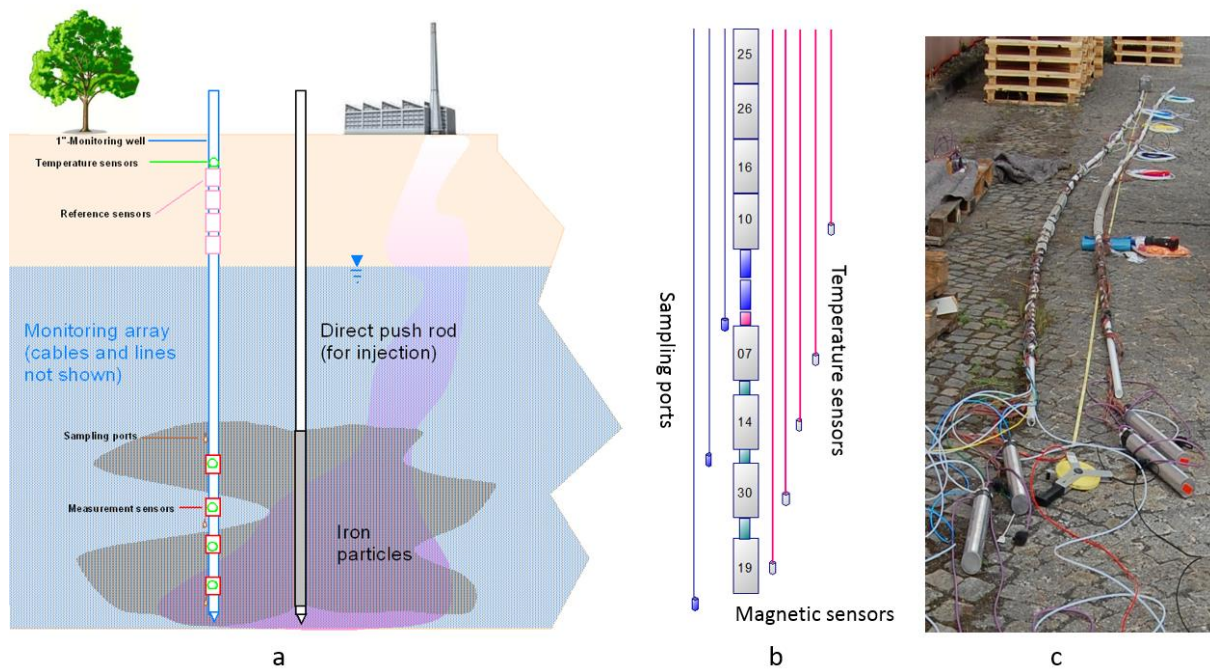


Figure 3: Magnetic susceptibility arrays: use in nano-remediation (a), components (b), photo of finishing on site before installation (c)

Solvay, Switzerland

The pilot studies showed the arrays were successful in detecting the iron particles during injection at both sites. Figure 4 shows the site layout at Solvay, Switzerland, and the location of the existing monitoring and sampling installations (left side on top), the geology and the location of the measurement systems in the aquifer (right) and the availability of the installations (lower left hand side). Monitoring points B153 and B154 are located inside the injection area and equipped with magnetic susceptibility sensors, temperature sensors and mini pressure pumps for sampling. B155 is only equipped with sampling systems, since it is outside the injection area and no particles had been expected there.

The injection was performed from March 24 to March 26 2014. The measured sensor data is shown in Figure 5. The signals show clear temperature changes in accordance with the injection times and positions. The susceptibility signals are, however, less distinct. The deepest sensor 154D shows neither a temperature nor a susceptibility signal, since it is embedded in the very low permeable marl layer. Thus, a direct effect of the iron injection on the signals cannot be expected. Sensor 154M shows clear temperature signals on March 25 and 26, which correspond to injections in the vicinity of the sensor (distance < 2 m).

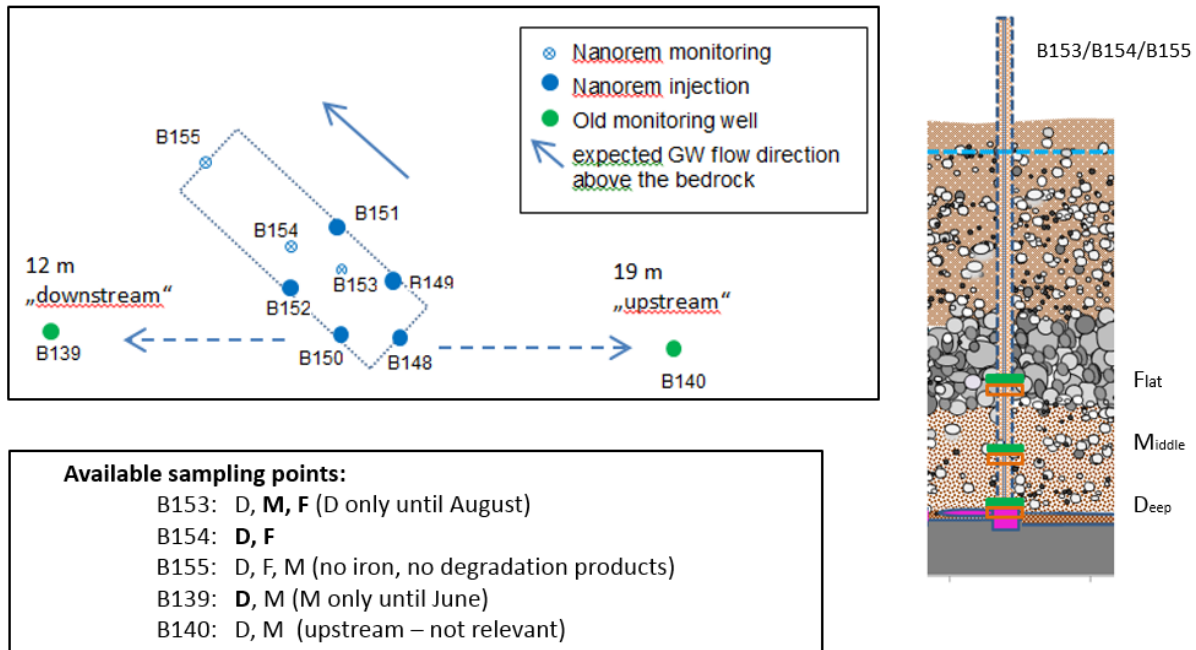


Figure 4: Site layout at Solvay

The susceptibility signals show a reduction of the voltage which goes back to the original value. This is typical for a reaction of the sensor to changes in the electric conductivity of the fluid around the sensor. On March 26 (second change in signal) this reduction is followed by a positive signal which can be contributed to a deposit of particles. Sensor 153M does not show a corresponding behaviour. This could be due to heterogeneities directly around the sensor. The injection fluid should have reached the sensor, but even the temperature does only change gradually within a day supporting this assumption. The upper sensors do not show susceptibility signals which cannot be expected, since the injection was only targeted to the aquifer base by the use of packers. The signals reflect more or less the findings in the samples taken from the mini pressure pumps close to the sensors.

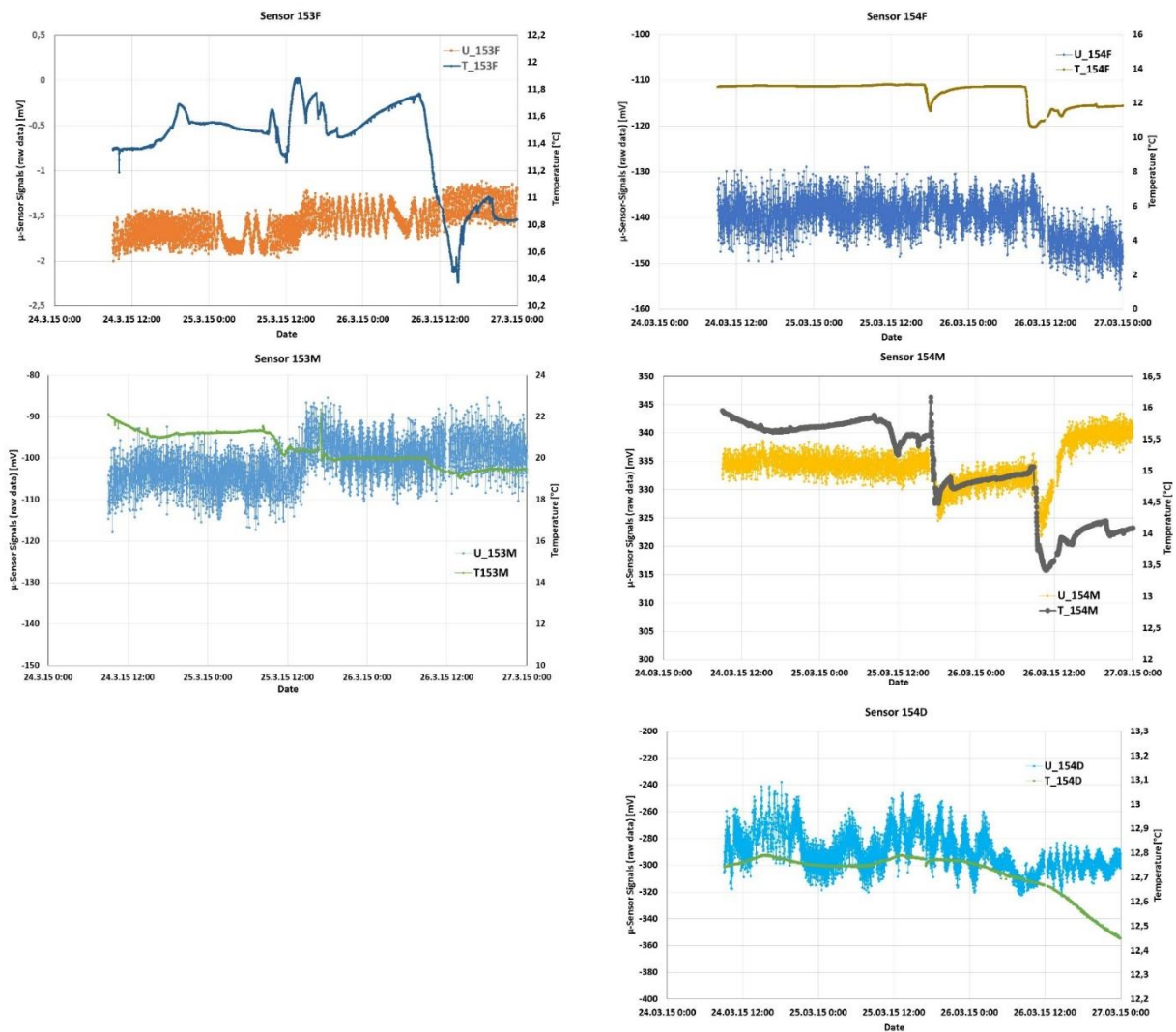


Figure 5: Sensor signals (susceptibility and temperatures) during injection, left sensor 153, right sensor 154 (sensor 153D broken)

Spolchemie, Czech Republic

The site layout of the DNAPL-site Spolchemie is shown in Figure 6. There were three arrays with susceptibility sensors, temperature sensors and sampling ports (mini pressure pumps) installed (array 9, array 10 and array 11). Array 10 had been equipped with 5 sensors of each type (depths: 5 m, 6.5 m, 8 m, 9.5 m and 11 m), array 9 and 11 had only 3 sensors (depths: 6.5 m, 8 m, 9.5 m). Since the ground water level in 2015 was very low, the sensor in 5 m depth was dry.

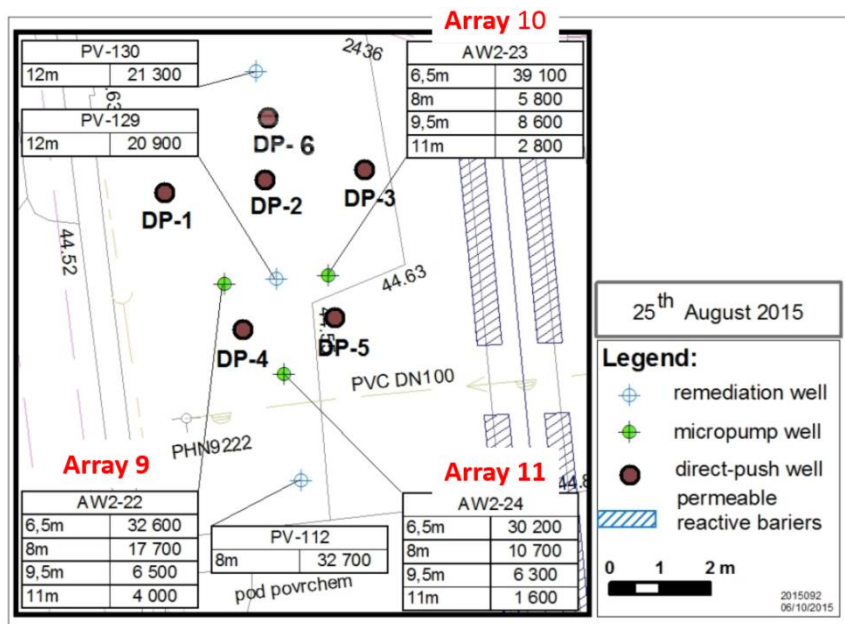


Figure 6: Site layout at Spolchemie

The results for array 10 are shown in Figure 7¹. The upper graph shows the temperature on the 5 sensors, the middle graph shows the susceptibility signals and the lower graph shows the ongoing of the injections. As already mentioned, the upper sensor was dry and therefore shows strong temperature signals, since the water table was raised during the injection and the sensor came in contact with the ground water. The other temperature signals are less pronounced, but correspond to the injection locations and times. The signals of the susceptibility sensors are very small except for CZ23 located in 9.5 m depth. Here a clear deposition of iron was detected during the injections in DP2, DP3 and DP5. This is evident since the signal raises and stays at the elevated value. These findings are in accordance with samples taken during the injection containing high concentrations of particulate iron.

The results for Array 9 are shown in Figure 8. The upper two sensors show sharp temperature signals during the injection in DP-1 and weak signals during injection in DP-4, the closest injection points. The lowest sensor shows little reaction. The susceptibility signals are in line with the temperature signals, but go back to the original values, indicating that no large amounts of particles have been deposited in the vicinity of the sensors.

Array 11 shows pronounced susceptibility signals at 6.5 and 9.5 m (see Figure 9) during injection in DP-5 and weaker signals at DP-4. Since the signals go back and reach almost the original levels, only little amounts of particles remain around the sensors. The sensor at 8 m shows almost no reaction as well with respect to the temperature as to the susceptibility indicating hydraulic heterogeneities with lower conductivities around the sensor location.

¹ In the night of Oct 16 a power failure caused the susceptibility sensors to stop, but also the injection activities paused during this period

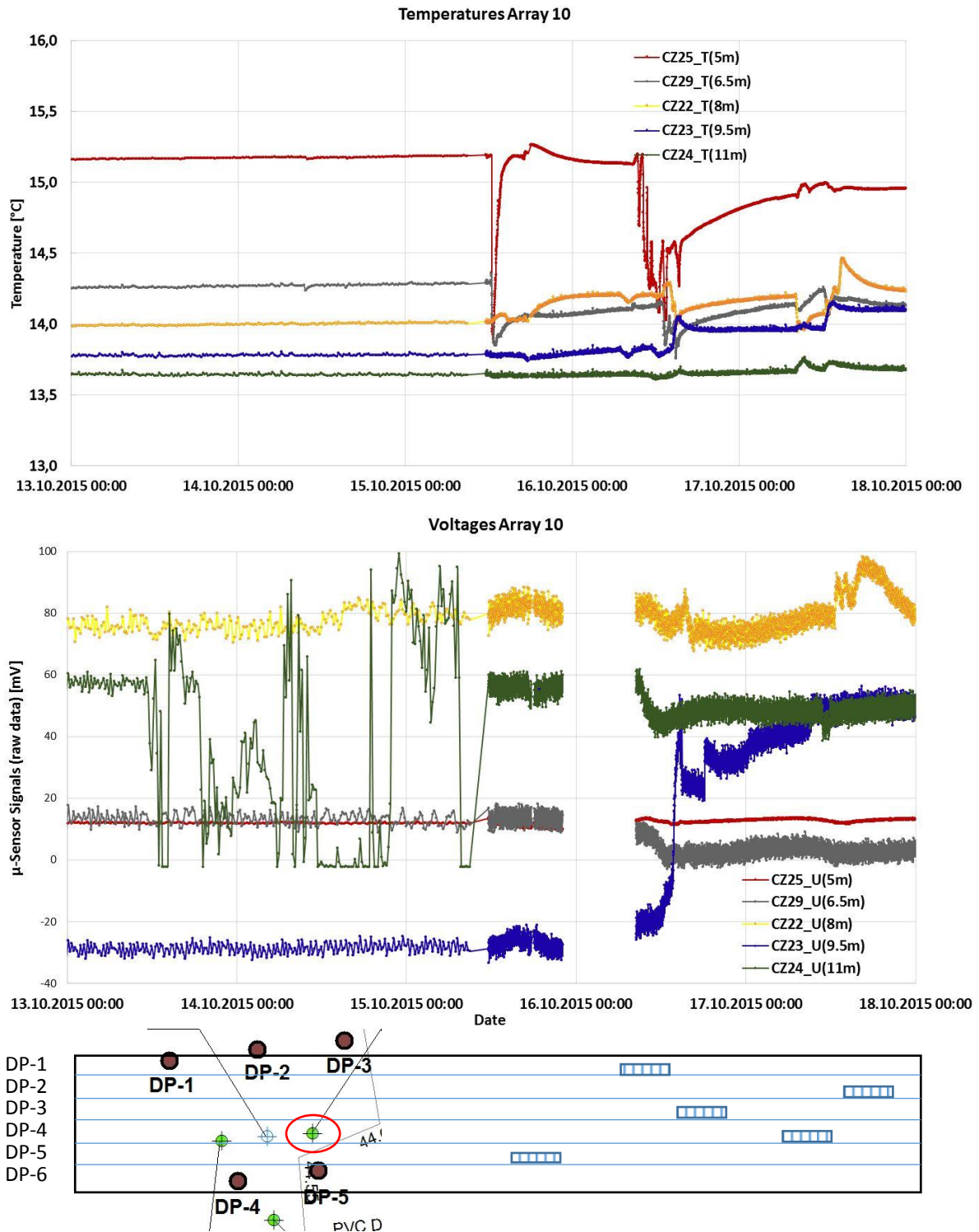


Figure 7: Temperature and Susceptibility results for Array 10

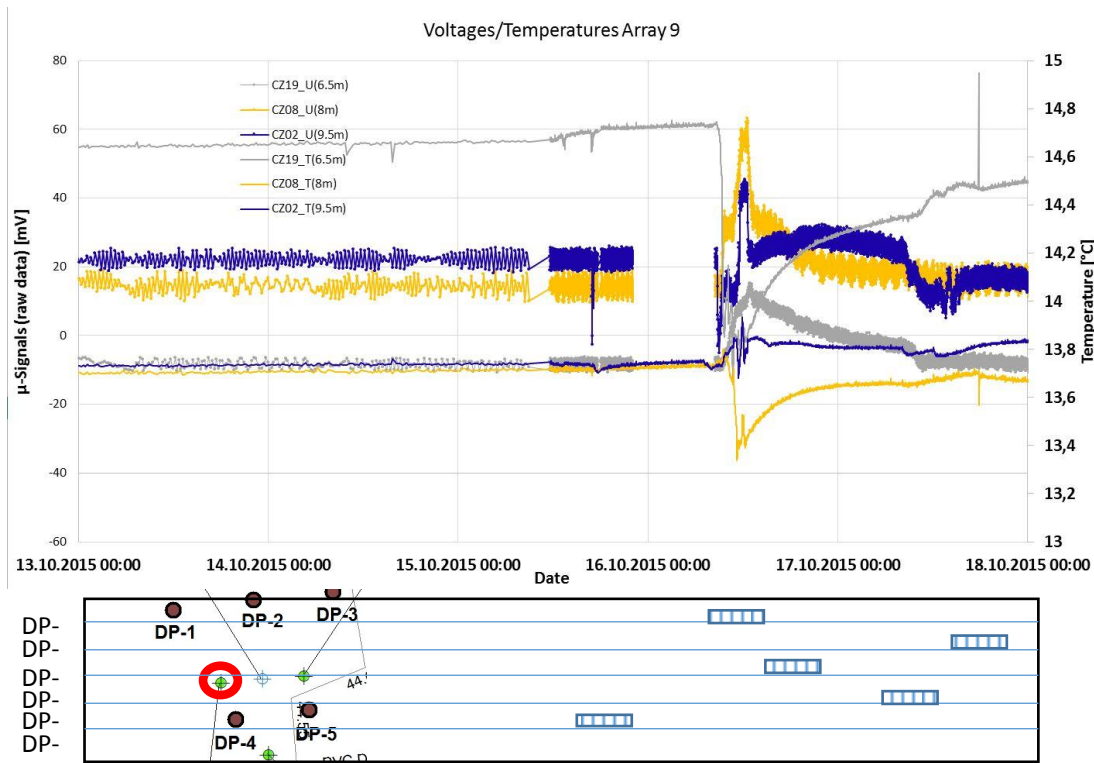


Figure 8: Temperature and Susceptibility results for Array 9

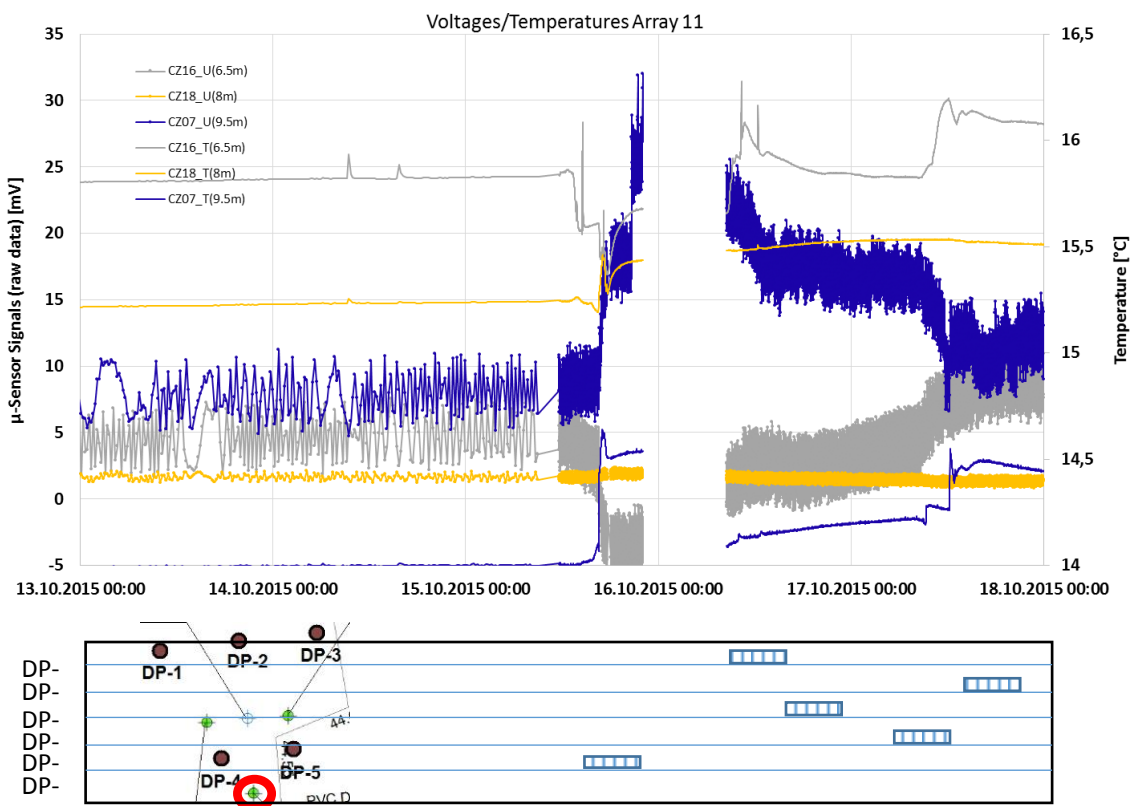


Figure 9: Temperature and Susceptibility results for Array 11

Advantages/ disadvantages of the method

The pilot tests at Spolchemie and Solvay demonstrate the support that the arrays can provide for monitoring the injection of nZVI particles. Several susceptibility sensors can be lined up in arrays allowing the detection of iron particles and sampling of ground water. The systems can provide readily available information on the distribution of the fluid injected (i.e. temperature signals) as well as the distribution of the particles. Detection limits are about 50 mg/L (for the laboratory device) and of 500 mg/L (for the field device). Although initially developed for measurement of nZVI NPs, laboratory tests showed that it can be used for other particles (e.g. Carbo-Iron®), albeit with higher detection limits. Despite the fact that detection limits are slightly higher and instrumentation costs for the magnetic array sensors are greater than those for on site sampling and measurement, (ca. 1000 Euro per array and 1000 Euro for the electronics), it is one of the truly *in situ* methods and has the advantage of giving continuous logging data.

4.1.2. Chemical Parameters: Temperature, pH and redox

Changes in Fe concentration, pH, temperature, and conductivity can provide a relatively rapid assessment of the spatial and temporal status of the NP suspensions. Providing the chemical properties of injection suspensions are significantly different from those in the groundwater, a number of standard chemical techniques can be applied at site, and can give results within a few minutes of sampling. These include temperature, redox, pH, conductivity as well as turbidity and total Fe content, for which the instrumentation required for on-site measurements is portable and not expensive. Cheap and portable instrumentation for on-site measurements of temperature, redox, pH, conductivity is available. *In situ* measurement of the temperature, Eh, redox potential and pH levels during the injection is a simple and efficient way of distinguishing between the liquid phase of the suspension and the particles, and can be measured either on samples collected at site or with *in situ* sensors. For example, during the tank experiment using NanoGoethite, the injected suspension had an elevated electrical conductivity (>1 mS/cm), while the LSC groundwater only had an EC of ~ 320 μ S/cm, enabling a good overview of the movement of the liquid fraction (Figure 10).

It should be stressed that neither redox, pH nor temperature changes would precisely indicate the presence of particles. For example, solution conditions can be reducing without particles (Shi et al., 2011). Furthermore, the temperature and pH of injected nanoparticle dispersion should be significantly different from the temperature and pH of remediated groundwater, to be able to detect the difference. However, when combined, and with suitable calibration and background measurements, these sensors could provide important *in situ* information on changes in groundwater conditions. Tests indicated that other *in situ* sensors for detecting milieu parameters such as pH or the oxygen content in the ground water were not stable enough.

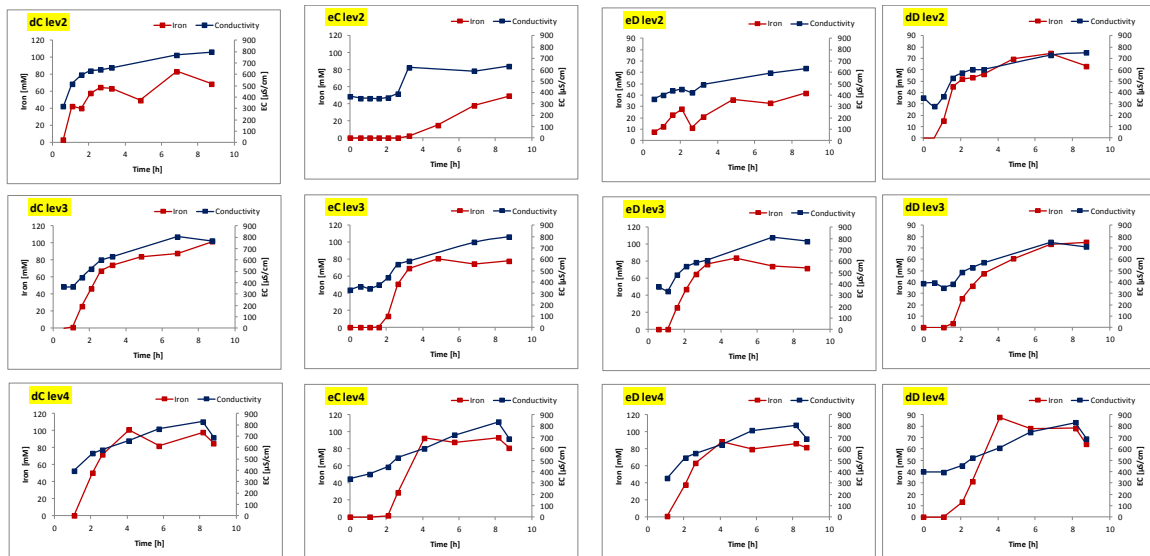


Figure 10: Iron total and electrical conductivity measured in the four monitoring wells around the injection well over 3 depth layers (level2 – level4) and over the timeframe of the FeOx nanoparticle injection at the VEGAS tank experiment. For more Information see D6.1, Oughton *et al.* 2015

4.1.3. Turbidity and Fe Concentrations

Turbidity and spectrophotometry measurement of total Fe concentrations both provide a direct analysis of NP concentrations, and are relatively fast and cheap methods for which portable equipment is available for on site measurement.

Turbidity measurements can be applied at site and over a relatively large particle concentration range. On site turbidity measurements were tested on Nano-Goethite during tank and field injections using a Turbidimeter (2100N IS, ISO Method 7027). The required sample volume (undiluted) is 2 to 20 ml, the time of measurement about 1 to 2 minutes per sample, and the method can be applied over a concentration range of 0.5 mg L^{-1} to 1.0 g L^{-1} (depending on the water quality). Figure 11 shows turbidity measurements indicating the distribution of Nano-Goethite particles 20 hours after injection in tank experiments at the VEGAS facility, University of Stuttgart.

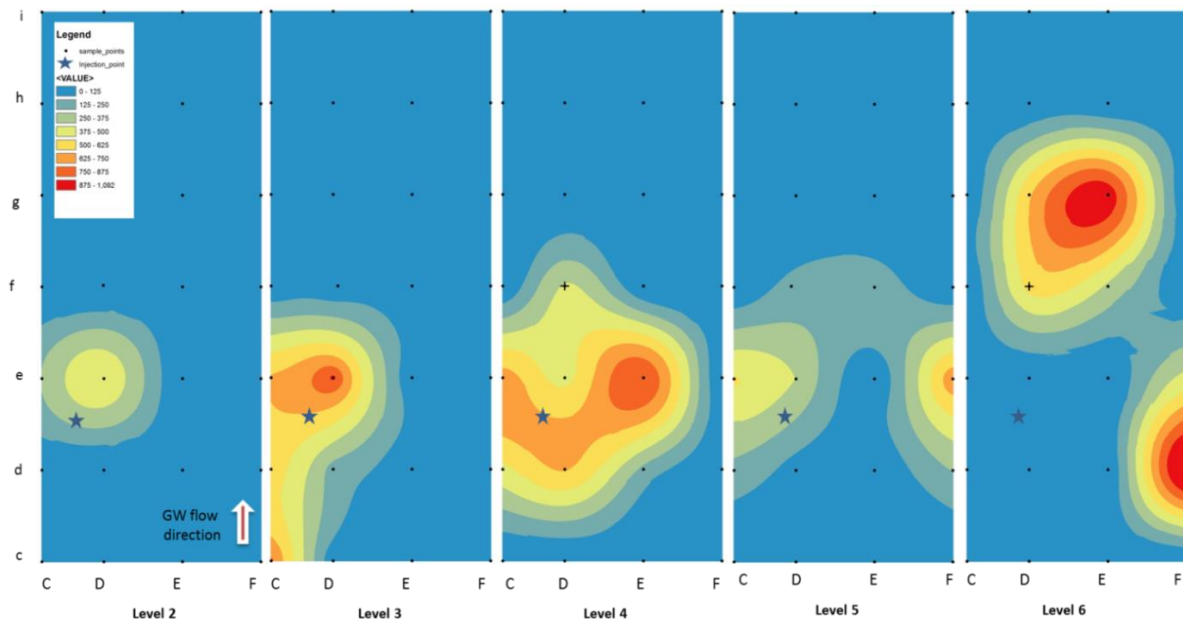


Figure 11: Turbidity data indicating particle distribution at different depths (Level 2-6) 20 hours after injection of Nano-Goethite at the VEGAS facility, University of Stuttgart. The red colour indicates the highest turbidity measurements.

Analysis of total Fe content provides a good overview of NP distribution, and was used at all NanoRem field sites. Whereas acid digestion followed by inductively coupled plasma optical emission spectrometry (ICP-OES) gives a quantitative measurement of Fe content, a rapid on site assessment can be provided with spectrophotometry. Portable spectrophotometers (e.g., Hach DR 2000) can provide measurement of Fe content in 25 ml samples within 15 mins. Information on both total Fe and Fe^{2+} , based on complexation with FerroZine[®] and 1,10-phenanthroline, respectively, can be obtained for concentration ranges of $0.2\text{-}200\text{ mgL}^{-1}$ (or greater with dilution). As for all methods, detection limits will depend on background concentrations, and the total Fe can be underestimated with large particle sizes due to incomplete dissolution. Tests during injections of nZVI (NANOFER 25S and NANOFER STAR) and Nano-Goethite at the Spolchemie field site showed that the method gave sufficient sensitivity to track NP distribution at the monitoring sites.

4.1.4. H₂ production

For a successful field application of milled ZVI particles, along with optimal mobility, reactivity and lifetime (longevity) of these particles play a decisive role. Hydrogen gas measurement can be applied for tracking ZVI particles mobility, reactivity and life time by H₂ gas measurement directly in wells, as well as for characterization of either nZVI slurry or nZVI-containing sediment collected from wells following acid digestion. The direct measurement method exploits the basic property of iron to reduce water accompanied by the release of H₂ gas. Measurement of the H₂ concentration increase in monitoring or applications wells (namely just above the groundwater level) can be used to trace nZVI. Portable GS1 gas sniffer (Wöhler GmbH, Germany) instruments are available for on-site measurement, or even more sophisticated portable gas detectors. The method works well in

application wells, where concentrations of nZVI (typically 1 – 2 g/L) are high enough to permit measurable concentrations of H₂ (i.e., above 10 ppm) over background H₂ content (being up to 5 ppm in most cases). Table 4 shows some representative results of measurements conducted in 3 monitoring wells at Spolchemie, Ústí nad Labem, Czech Republic, before the application of nZVI.

Table 3: Hydrogen gas measurement: 'A' refers to the surface of the wellhead and 'B' to the exact point of the measurement that was inside the well, above the water level.

Well number		CH ₄	CO ₂	O ₂	LeI	H ₂ S	H ₂
		%	%	%	%	Ppm	Ppm
PV-129	A	0	0,1	20,7	0	0	0-5
	B	0	1,9	18,7	0	0	0-6
PV-130	A	0	0,1	20,1	0	0	0-6
	B	0	2,2	17,2	0	0	0-6
PV-112	A	0	0	20,4	0	0	0-6
	B	0	0,7	19,4	0	0	0-6

In addition to field site studies, the H₂ production method was also used to study the reactivity and the longevity of particles under conditions that are characteristic for the two NanoRem field sites: Solvay (Switzerland) and Balassagyarmat site (Hungary). The longevity of milled ZVI particles was studied via recording the H₂ production during in the course of the experiment and used to calculate Fe₀ corrosion rates applying the zero-order model of H₂ (mL) production as suggested by Liu and Lowry (2006).

A limitation of this method is that it cannot trace the oxide/oxohydroxides reaction products resulting from nZVI as these common reaction products do not produce hydrogen gas when in contact with water. Moreover, H₂ presence in wells can be also influenced by changes in microbial activity, which can in turn change upon introduction of nZVI, and thus cannot be easily subtracted from background measurement. Nevertheless, provided levels of nZVI are high enough, it can give useful information on the presence of, and changes in levels of reactive Fe.

4.2. Post-injection monitoring.

Monitoring during the post-injection phase needs to provide information on not only the concentrations of Fe, but also its speciation in order to understand the fate and reactivity of the injected NPs. For total Fe concentration, measurements on suspensions/liquids and soils/sediments can be carried out directly after acid digestion and measurement using standard chemical analysis (e.g., ICP-OES, or spectrophotometry). Alternatively, for low particle densities, pre-concentration by centrifugation or filtration can be applied to improve detection limits. Specific protocols for acid digestion need to be developed for the different NPs to ensure complete dissolution. The detection limits of all methods will be site specific, depending largely on background concentrations of metals and colloids, and, for Fe-based NPs, dissolved Fe concentrations. Field applications have demonstrated that Mössbauer (for nZVI) can give useful additional information on the time dependent changes in particle state and reactivity, in both water and solid phases. These can be supported by other methods for measurements of structure and oxidation state (e.g. X-ray photoelectron spectroscopy, transmission/scanning electron microscopies, X-ray powder diffraction, X-ray fluorescent spectroscopy).

4.2.1. Mössbauer Spectroscopy

Within the NanoRem project, transmission ^{57}Fe Mössbauer spectroscopy has proved to be a useful tool for the characterisation of nZVI NPs deployed in remediation and it represents a unique technique for probing the Fe⁰/Fe^{tot} ratio in field samples, including the identification of nZVI (NANOFER 25S and NANOFER STAR) NPs in complex environmental and geological matrices.

The method can provide a detailed characterization of nZVI (in both dry powders and aqueous slurries) with respect to iron speciation (i.e., iron valence state, form of iron oxides/hydroxides and their magnetic ordering). ^{57}Fe Mössbauer spectra can be collected at a constant acceleration mode with a $^{57}\text{Co}(\text{Rh})$ source (1.85 GBq). For environmental samples, slurries (typically magnetically pre-concentrated in order to measure more iron-containing particles) are fast frozen in a liquid-nitrogen bath. Measurements are carried out at 250 K and in an external magnetic field of 0T for a time period of >1 day per sample. For measurement of nZVI-reaction products, the measurement temperature above magnetite Verwey temperature is crucial, as the magnetite spectra below Verwey transition are quite complicated. Alternatively, fast-dried (e.g., lyophilized) powder samples can be prepared under protective N₂ atmosphere into a conventional absorber (~5 mg Fe cm⁻²) and measured at RT using a spectrometer located directly in the glove-box. In both cases, the isomer shift values are calibrated against an α -Fe foil at RT, and spectra fitted with Lorentz functions. The effects of non-ideal absorber thickness and variable recoil-free fractions for iron atoms in non-equivalent structural sites of different phases are expected to be within experimental errors (hyperfine parameters ± 0.02 mm s⁻¹, relative spectral area ± 3 %).

While the Mössbauer spectrometer is compact and portable, the main disadvantage lies in the relatively long counting times. These are typically about 1 day per sample of nZVI, but can be up to 1 week for environmental samples with a low Fe content (i.e., below 1% Fe atoms in the sample),

during which time the measured sample could further oxidise. Therefore, samples need to be analysed in a protective atmosphere (e.g., a glove box under nitrogen, see Filip et al., 2014) or pre-concentrated samples are frozen and measured at low temperatures (optimally at 150 K or simply at liquid nitrogen temperature, see Filip et al. 2007). The method has been successfully tested during nZVI injections at Spolchemie I, and measurements taken for suspensions, sediments and soil samples showed both the formation of nZVI reaction products and extent of nZVI migration in groundwater conditions (Figure 12 and Figure 13).

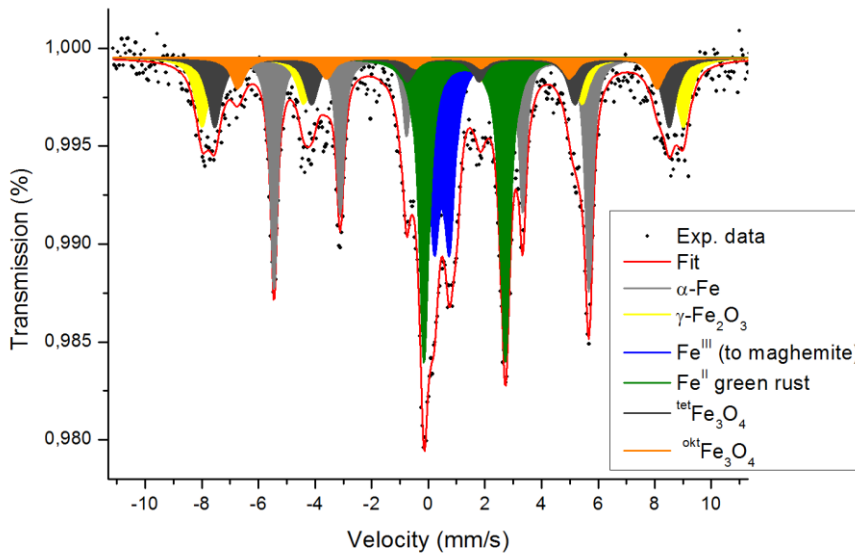


Figure 12: Mössbauer spectrum of sediment from well PV-129, Spolchemie I site, Czech Republic, collected June 2015, 9 months after application of NANO FER STAR. Black dots represent the measured values and the red line a fitted curve. The other colours are reference measurements of different oxidation/crystal states of iron

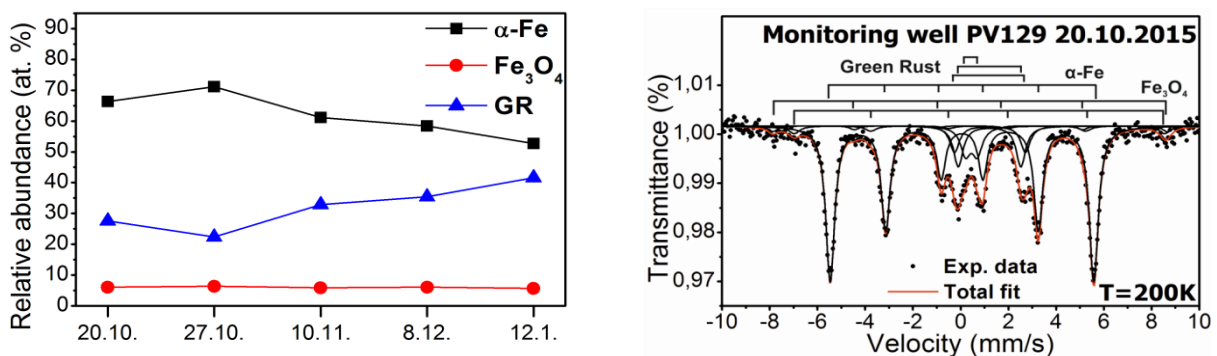


Figure 13: Left: Representative room-temperature Mössbauer spectrum of a sample collected from Spolchemie, PV-129, 20.10.15; Right: Changes of iron atoms proportions during 4 months after injection of NANO FER STAR (2015) investigated using Mössbauer spectroscopy; GR = green rust.

Advantages/ disadvantages of the method

While the Mössbauer spectrometer is compact and portable, the main disadvantage lies in the relatively long counting times (typically about 1 day per sample of nZVI, but can be up to 1 week for environmental samples with low iron content) during which the measured sample could further oxidize or transform in terms of its crystal structure. This drawback could be overcome by measuring samples under protective atmosphere, as the whole spectrometer could be installed e.g., in a glove box (Filip et al., 2014). Alternatively, pre-concentrated nZVI-containing samples could be fast frozen and measured at low temperatures (optimally at 150 K or LN₂ temperature) utilizing a special cryogenic system which slows oxidation of nZVI (Filip et al. 2007). The low-temperature measurements are further beneficial in the case when reaction products of nZVI are amorphous (therefore hard to detect by XRD). The other very important benefit of this method is its iron-selectivity (Sharma et al. 2014). Therefore, ⁵⁷Fe Mössbauer spectroscopy could be applied for complicated samples like the direct observation of nZVI oxidation in soil samples collected from either laboratory column experiments or field tests.

4.2.2. X-ray powder diffraction: nZVI

X-ray powder diffraction is an accepted and widespread tool for characterization of the phase composition (i.e. the crystal structure and quantitative phase analysis) of crystalline solids, including nZVI and their reaction products (Clearfield et al., 2008). It also provides estimation on particle size (or mean size of X-ray coherent domains) according to the broadening of diffraction peaks or when combined with small-angle X-ray scattering (SAXS) (Williams et al., 2005). Sample preparation procedures for measurement, data collection and processing have been reviewed in detail many times (Buhrke et al., 1998). Specific challenges linked to nZVI sample preparation and measurement arise from the fast oxidation of nZVI in air (Filip et al., 2014). In order to test method reproducibility, two protocols were followed for either wet nZVI samples (i.e., magnetically pre-concentrated slurries) or dry nZVI powders (see below) – similar as for transmission ⁵⁷Fe Mössbauer spectroscopy to ensure complementarity of both methods. Moreover, successive short scans using of fast solid-state detector provide a unique insight into the possible process of nZVI oxidation during sample measurement (typically acquired within less than 1 hour) accompanied by spontaneous drying of the slurry. For the subsequent quantitative phase analysis utilizing full-profile fitting (i.e., Rietveld refinement) one can use either the first fast scan collected at the beginning of nZVI drying/oxidation or sum up all collected scans when no phase changes took place in the course of successive measurements.

This methodological approach has been demonstrated on samples collected at Spolchemie Site 1 (Czech Republic – see section “Mössbauer: nZVI”). X-ray diffraction patterns were recorded on a X’Pert PRO (PANalytical, The Netherlands) instrument in Bragg-Brentano geometry with iron-filtered CoKa radiation ($\lambda = 0.178901$ nm; 40 kV and 30 mA) equipped with a fast X’Celerator detector and programmable divergence and diffracted beam anti-scatter slits. Figure 14 shows the representative XRD pattern recorded with the above-described experimental setup and the results of quantitative phase analysis of samples collected at the Spolchemie Site 1 over >300 days after nZVI injection. The

main crystalline phases identified include: α -Fe (non-oxidized iron from injected nZVI particles), magnetite (Fe_3O_4), green rust (mineral name fougérite, theoretically close to $[\text{Fe}^{2+}_4\text{Fe}^{3+}_2(\text{HO}^-)_{12}]^{2+} \cdot [\text{CO}_3^{2-}, 2\text{H}_2\text{O}]^{2-}$), ferrous hydroxide ($\text{Fe}(\text{OH})_2$) and quartz (SiO_2).

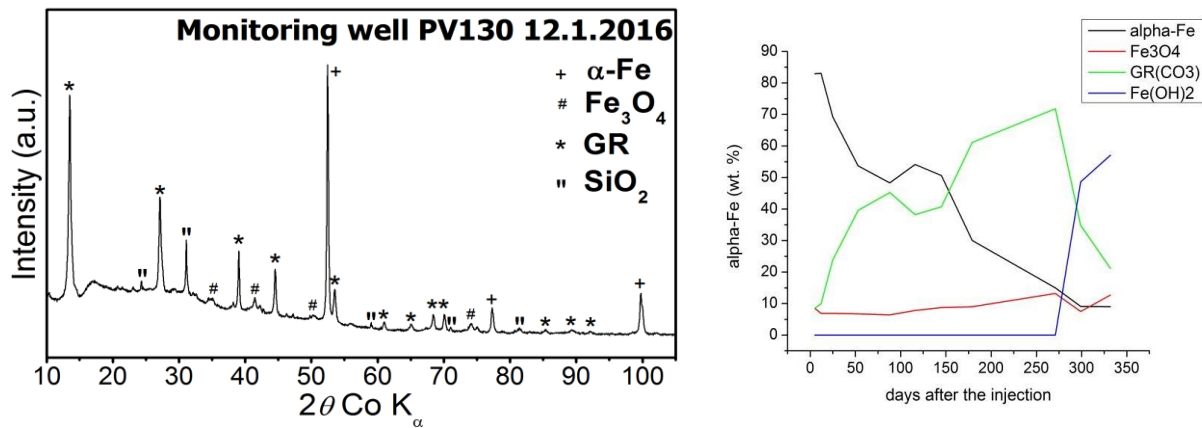


Figure 14: Left: Representative XRD pattern of sediment sample from the monitoring well PV130 (α -iron, green rust, magnetite and silica were detected); Right: Phase changes during >300 days after injection NANOFE STAR (2015) investigated using XRD; GR = green rust

Advantages/ disadvantages of the method

XRD is one of the main techniques for characterisation of crystallographic phases and crystal structure. It requires minimal sample preparation and widely available. The data interpretation is relatively straight forward.

4.2.3. Methods for Carbo-Iron[®] and Trap-Ox Fe-zeolites

Methods for tracing Carbo-Iron[®] and Trap-Ox Fe-zeolites are still at the laboratory development state, although preliminary results are promising. A combination of Temperature-programmed oxidation (TPO) with parallel CO_2 -analysis seems to be the best approach to distinguish Carbo-Iron[®] remaining attached in the long-term to the sediment from other carbon-containing sediment background. With detection limits of 0.1 wt % (1 mg particles per g sediment), this method can trace the particle fate within the reaction zone.

Temperature-programmed oxidation (TPO) of solid sediment samples utilises the fact that the immediate vicinity of the carbon has a fingerprint-like effect on the carbon-specific oxidation temperature. In the case of Carbo-Iron[®], the embedment of Fe decreases the incineration temperature significantly in comparison to pure powdered activated carbon (AC) (Bleyl et al. 2012). One can take advantage of this temperature shift to detect engineered carbon-based NPs within a complex matrix containing a natural carbon background. **Figure 15** shows the specific incineration patterns in air atmosphere for Carbo-Iron[®] colloids, aged Carbo-Iron (was obtained by oxidation of fresh Carbo-Iron particles in aqueous media to generate an iron-oxide/AC composite, which

represents the transformation product of injected Carbo-Iron suspension), the raw material AC, the colloid stabiliser carboxymethyl cellulose and a coal-derived humic acid as a model compound for natural carbon background.

The main challenges are sample preparation of natural heterogeneous aquifer sediment to achieve representative results for the sediment loading with carbon species and the detection of low-concentrated particle fractions ($\ll 0.1$ wt-%) in complex matrices. To quantify Carbo-Iron[®] particles immobilised on sediment grains, predefined loadings of aged Carbo-Iron on the NanoRem standard material M.I (Dorsilit[®]) in a typical expected range of 0.1 wt% up to several wt% have been studied.

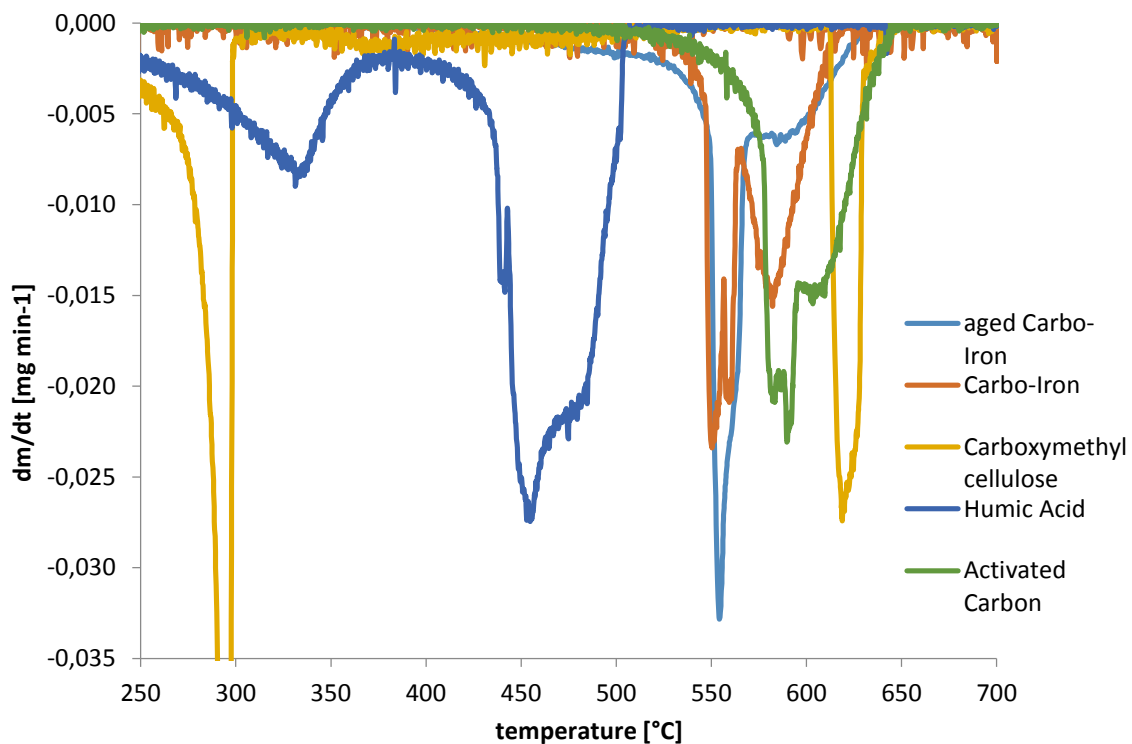


Figure 15: Temperature-programmed oxidation (TPO) of carbonaceous materials in a thermogravimetric balance (TGA-50 Shimadzu: air flow rate 50 mL/min; $m_{\text{sample}} = 6\text{...}16$ mg; $\Delta T: 10$ K/min; $T_{\text{max}} = 700^\circ\text{C}$). Aged Carbo-Iron[®] was obtained by oxidation of fresh Carbo-Iron particles in aqueous media to generate an iron-oxide/activated carbon composite, which represents the transformation product of injected Carbo-Iron suspension. Carboxymethyl cellulose is the colloid stabiliser and coal-derived humic acid a model compound for natural carbon background.

The combination of TPO with gas analysis (formation of CO_2 and CO) is an additional promising tool to unequivocally trace particles in natural matrices and increase the sensitivity, reliability and applicability for real sediment samples from field sites. As an off-site method the TPO approach can be understood as complementary tool, which contributes to existing on-site methods. **Figure 16**

shows a typical CO₂-formation profile during combustion of a model soil sample, which contains distinct carbon species like carbonates, carboxymethylcellulose and the target Carbo-Iron colloids. Carboxymethylcellulose shows two decomposition peaks at 350°C and at higher temperatures above 600°C, which will be superposed by the carbonate decomposition showing a pronounced peak at 680°C.

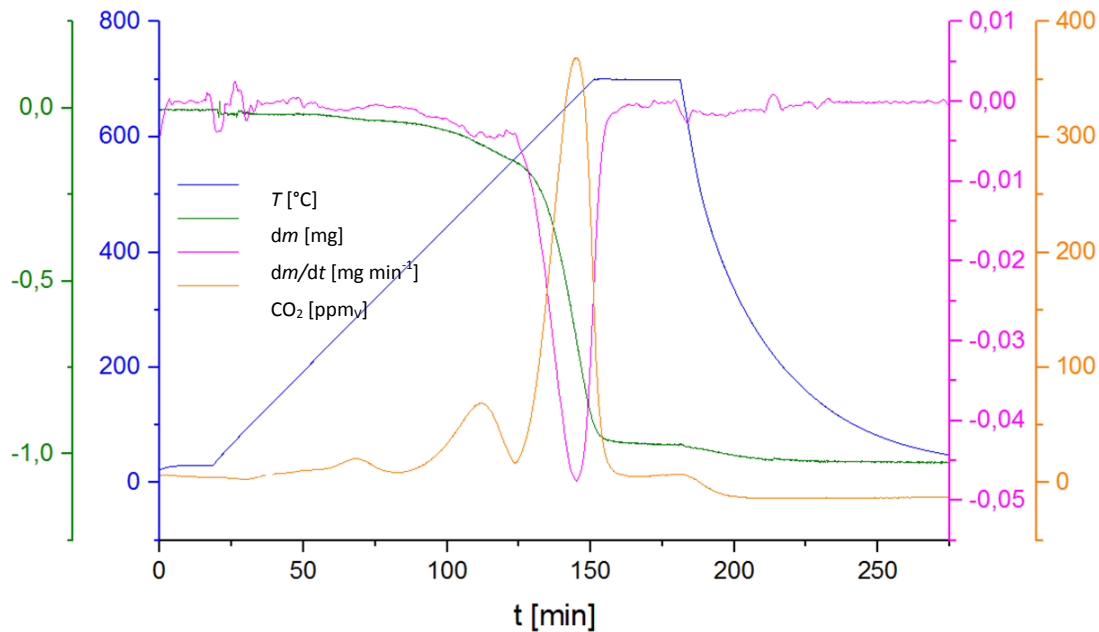


Figure 16: Temperature-programmed oxidation coupled with IR gas analysis (TPO-IR) of a model quartz sand loaded with Carbo-Iron in a thermogravimetric balance (TGA-50 Shimadzu: air flow rate 50 mL/min; sample = 47.02 mg; ΔT : 10 K/min; T_{\max} = 700°C).

In order to test the concept of TPO, real sediment samples from the large scale flume experiment and the field-test site (Balassagyarmat, Hungary) will be subject to further investigations (Figure 17). Preliminary studies using TOC-analyses showed the presence of carbon-rich zones in the vicinity to the injection well at the Hungarian field site, which will be of interest for further TGA-TPO investigations.

Two tracing methods (based on invasive sampling) have been developed which successfully proved to detect and distinguish Carbo-Iron from other particle types and background carbonaceous materials in the sediment. The quantification of particle loading on standard quartz sand could be shown for a range of 0.1 to 5 wt% for Carbo-Iron on standard material M.I and in IR-coupled TPO investigations the sensitivity could be increased significantly using the CO₂-signal in the Carbo-Iron specific incineration window (LOD: ~ 0.03 wt-%).

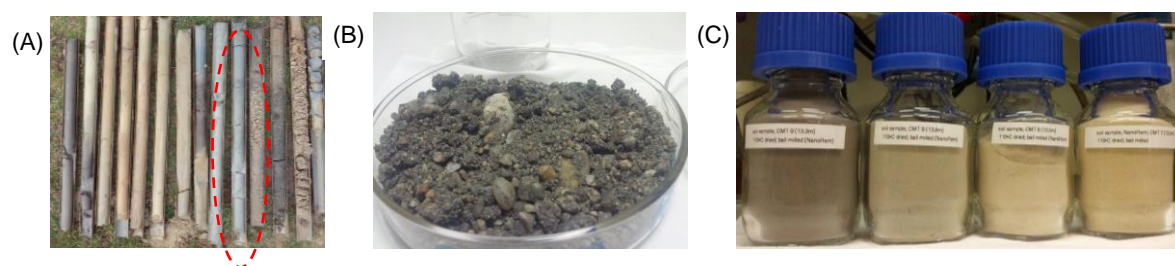


Figure 17: Soil samples from hungarian field site (Balassagyarmat): core samples taken at CMT 9 in a distance of 0.5m downstream to injection well (A – from left to right going downwards from ground level, marked core samples represent the depth 12 – 14 m below ground level, where particles have been injected and obviously are present); sandy and gravel raw material from well CMT 9 (B); homogenized soil samples (ball milled and dried at 110°C) particle analytic as TOC and TGA-TPO (C).

Advantages/ Disadvantages of the method

CMC does not disturb the analysis and is well separated from the specific Carbo-Iron-CO₂-peak. The carbonates can be removed by acid pre-treatment to increase the accuracy of analysis of Carbo-Iron sediment loading. The method allows detection of a mass loading of Carbo-Iron as low as 0.03 wt-% (compared to 0.3 wt-% with the conventional TGA measurement) and allows application of the method for complex matrices. The main challenges that remain are sample preparation of natural heterogeneous aquifer sediment to achieve representative results for the sediment loading with carbon species and the detection of low-concentrated particle fractions (<<0.1 wt-%) in complex matrices. Finally, the applicability and feasibility of monitoring tools for Carbo-Iron detection are currently based on off-site lab tests and will be currently intensified and extended to field applications, however, will not be able to provide a true *in situ* method.

Trap-Ox Fe-Zeolites

The application of colloidal Trap-Ox Fe-zeolites for *in situ* remediation is still in the lab-scale research stage, however a number of detection methods have been initiated. Since Trap-Ox Fe-zeolites basically consist of the elements silicon, aluminium and iron, which are ubiquitous in the environment, element detection techniques can only be applied at relatively high particle concentrations. As for other Fe-based particles, the main standard methods are turbidity (measuring absorbance at 860 nm) and total iron measurement after acid digestion.

Figure 18 shows the calibration curves for absorbance measurements over the concentration range of 0.1 to 5 g/L of the Trap-Ox Fe-zeolite particle type Fe-BEA35. With respect to total Fe measurements, to completely dissolve the zeolite-bound iron from Fe-BEA35, a suspension with 4 M HCl has to be shaken overnight (≥ 18 h). Dynamic light scattering (DLS), nanotracking analysis (NTA) and laser diffraction were tested for analysis of particle size distribution of zeolite suspensions. Laser diffraction analysis and DLS were identified as suitable methods for particle size analysis of Trap-Ox Fe-zeolite suspensions.

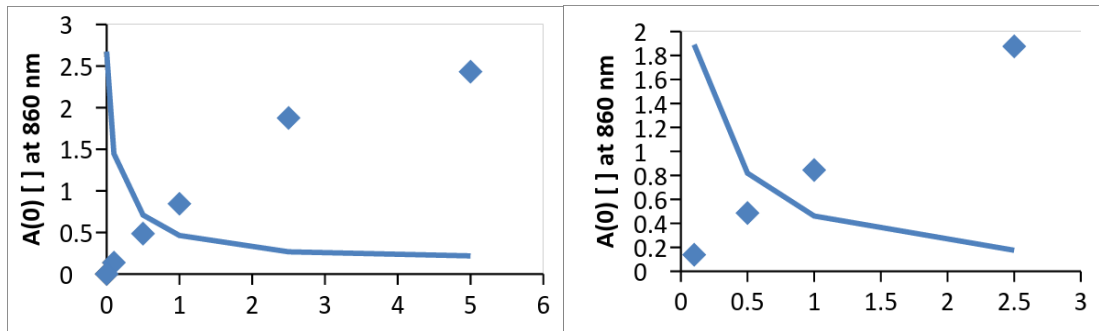


Figure 18: Calibration curves for determination of the concentration of Trap-Ox Fe-BEA35 in aqueous suspension by means of measurement of absorbance at 860 nm (UV-Vis instrument Varian Cary 300) (right figure for concentration range 0.1 – 2.5 g/L).

Other characteristic features of the native Fe-zeolites which could be utilized for indirect quantitative determination of their content in sediments are: I) high specific surface area, II) activity for H_2O_2 decomposition and III) high adsorption affinity towards small organic molecules. Approach I) relies on determination of BET area by N_2 adsorption experiments. For acid washed Ottawa quartz sand (0.59 a limit of detection for this method of 0.03 wt% Fe-BEA-35 was determined, with this mass fraction of Fe-BEA-35 causing a twofold increase in BET area compared to the original sand ($0.08 \pm 0.02 \text{ m}^2/\text{g}$, BET surface area of Fe-BEA35: $612 \text{ m}^2/\text{g}$). For sediments with a larger fraction of fines the DL is expected to be higher.

With respect to H_2O_2 decomposition (approach II) catalytic activities of the Trap-Ox Fe-zeolites were compared with those of the porous media used in NanoRem, i.e. M.I (Dorsilit® 8 – acid washed) and M.II (middle sand) as well as untreated Dorsilit 8. In case of Fe-BEA35 a mass fraction of only 0.01 wt% of Fe-zeolite on M.I causes already a two-fold increase in the rate constant for H_2O_2 decomposition, set as detection limit. However, untreated Dorsilit 8 as well as M.II show relatively high activities for H_2O_2 decomposition, so that high Trap-Ox Fe-zeolite concentrations are needed to cause a significant increase.

For testing approach (III) suspensions of M.I with various amounts of Fe-BEA35 in 10 mM KNO_3 solution containing 20 mg/L of MTBE were prepared and the concentration of MTBE in the aqueous phase was analysed after 24 h. A linear correlation between C_{total}/C_e (ratio of concentration of total MTBE added and equilibrium aqueous phase concentration) and mass fraction of Fe-BEA35 in relation to porous medium M.I was obtained within the relevant range of 0.1 to 1 wt%, showing a nearly constant sorption coefficient for MTBE on the zeolite ($c_{e,\text{MTBE}} = 4 - 20 \text{ mg/L}$). In general, the DL of this approach depends on the difference in K_d for the original sediment and the zeolites. Results showed a K_d for Fe-BEA35 of 1500 L/kg, compared to the K_d for sorption of MTBE to the original porous medium M.I (and also untreated Dorsilit 8) of 0.17 L/kg. For Fe-BEA35 on M.I the DL is 0.05 wt%. In general, the DL of this approach depends on the difference in K_d for the original sediment and the zeolite. The latter can reach values in the order of 105 L/kg for hydrophobic zeolites so that the DL in this case can be even lower. Sediment properties such as high content of fines or natural organic matter (NOM) can increase background adsorption by the sediment to some extent and thus

slightly increase the detection limit for this approach. Nevertheless, sorption coefficients of MTBE on NOM are expected to be lower by two orders of magnitude than $K_{d,Fe-BEA35}$ based on the low hydrophobicity of MTBE ($\log K_{ow,MTBE} = 0.94$). In addition, before conducting the adsorption experiment, sediment samples could be calcined in air to remove any organic material and treated with dilute acid to remove inorganic carbonate precipitates which could interfere with the analysis. Target contaminant adsorption appears most suitable among the indirect methods for determination of Trap-Ox Fe-zeolite content in sediments with respect to handling and DL.

Finally, with respect to direct methods for Trap-Ox Fe-zeolite detection a fluorescence labelling approach was studied. A fluorescence-labelled zeolite should overcome the disadvantages of turbidity measurement as means of quantifying zeolite content in suspensions. Furthermore, it can be used to investigate zeolite loaded sediment by confocal fluorescence microscopy and obtain insight into filtration details and transport pathways of injected zeolite particles. A 'ship-in-a-bottle' synthesis approach was used to produce a fluorescent BEA-zeolite, whereby fluorescein is synthesized inside the zeolite framework (Figure 19) from inexpensive educts by a simple solid phase reaction at elevated temperature and reduced pressure (Gillies et al., 2016). The resulting product shows a stable fluorescence which is non-extractable by various organic solvents and water. The zeolite-trapped product acts similar to fluorescein in solution, but with a slight blue-shift of the absorption and emission maxima and shift in the occurrence of maximum fluorescence as function of pH towards more alkaline conditions, so that a plateau is reached at about pH 11.5. When the fluorescent labelled zeolite is added at a concentration of 1 wt.% referring to the total zeolite mass, a very low detection limit of 1 mg/L of total zeolite is obtained. Compared to commonly applied turbidity measurements, detection via fluorescence labelling is much more specific and sensitive. Transport properties of fluorescent labelled and non-labelled Fe-zeolite particles are in agreement as determined in a column study with quartz sand and synthetic groundwater (classified as very hard). H_2O_2 degrades the fluorescein inside the zeolite channel system. Particle detection by fluorescence analysis can thus be applied for aqueous samples collected at field sites during infiltration and installation of the Fe-zeolite barrier. By this means information on travel distances and particle spreading can be obtained. The tracer function will be lost after the first regeneration of the *in situ* sorption zone by H_2O_2 injection.

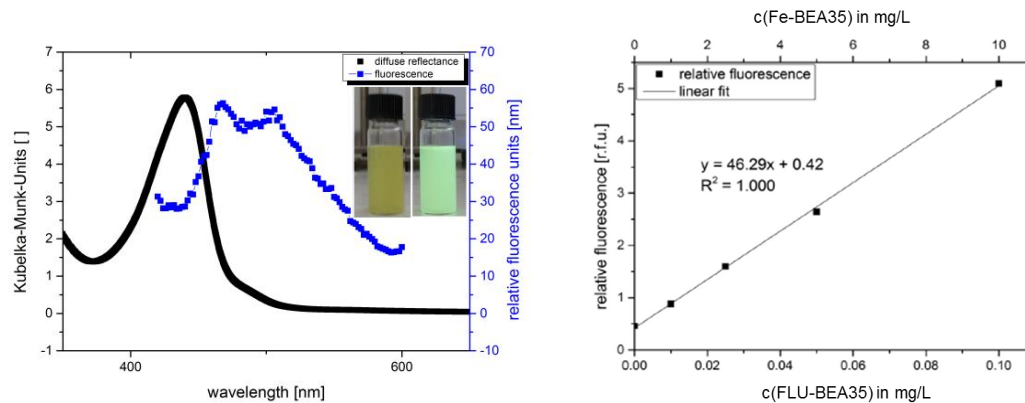


Figure 19: UV-Vis spectrum in diffuse reflectance mode for fluorescent zeolite (FLU-BEA-35); its fluorescence spectrum ($\lambda_{\text{ex}} = 385$ nm, suspension in deionized water) and photo of suspensions under daylight (photo left) and UV light (photo right) (left figure); Calibration curves based on fluorescence intensity of a mixture of Fe-BEA35 and 1 wt.% FLU-BEA35 in 10 mM KNO_3 , pH 11.5, $\lambda_{\text{ex}}=485$ nm, $\lambda_{\text{em}}=535$ nm; lower and upper x-axes show concentrations of fluorescent and catalyst particles, respectively (right figure).

Advantages/ disadvantages of the methods

A disadvantage of the method (I) involving determination of BET surface area is the low sample throughput (2 samples/day). Considering H_2O_2 decomposition (approach 2), even though it can be applied for lab experiments with cleaned sand, is not a suitable quantitative parameter for determination of Trap-Ox Fe-zeolite concentration in real aquifer sediments. Approach (III) is not a trace method but only applicable close to the targeted range of zeolite concentration on sediment of 0.1 to 1 wt%. Finally, as for fluorescence labelling approach, the pH of the samples need to be adjusted to $\text{pH} \geq 11.5$ in order to obtain a stable sensitivity. Compared to commonly applied turbidity measurements, detection via fluorescence labelling is much more specific and sensitive.

4.3. Monitoring for transport of NPs out of the treatment area

Total Fe content and other chemical parameters can give a reliable picture of the behaviour of injected suspensions in the application area, but more sensitive methods are needed to control for the possible transport of NPs outside the treatment area, often termed “renegade” NPs. Demarcation of low concentrations of Fe-based NPs from background matrix requires greater sensitivity, but tests and developments of a variety of methods within NanoRem have been promising.

4.3.1. ICP-MS and PCA Fingerprinting

By ICP-MS analysis of lanthanides (rare earth elements, RREs) and other trace elements in particles and background groundwater site samples, a group of elements can be selected to “fingerprint” the injected NPs. By applying Multivariate Statistics tools such as Principal Components Analysis, it is then possible to discriminate injected NPs from the background with a much greater degree of sensitivity than by measuring Fe concentrations alone. Detection limits for these methods are extremely low (ng/L levels) in clean media (as tested in laboratory column experiments); but, as for all methods, the performance and applicability in the field is highly dependent on site-specific parameters. Nevertheless, field tests carried out at various NanoRem field injections (Negev, Solvay, Spolchemie 1 and 2) on nZVI and Nanogoethite show good separation of NPs from background components at most sites, with the potential for detection down to sub mg/L levels (inset, **Figure 20**). Although the analytical costs are higher than for total Fe measurement (ca. 1-3000 EUR per remediation site), by targeting selected monitoring wells, measurements can be carried out over a lower spatial and temporal frequency.

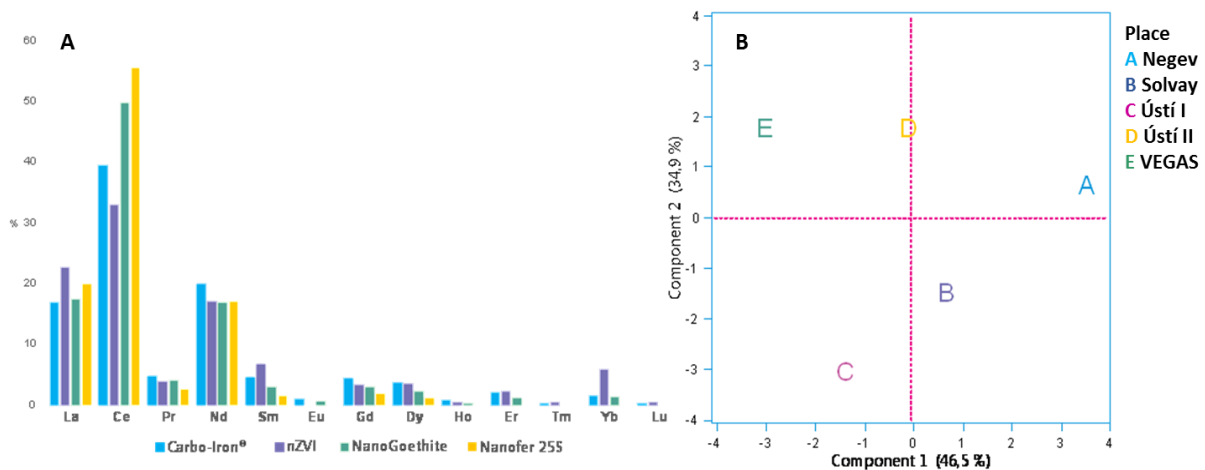


Figure 20: Lanthanide profiles of different NPs applied at NanoRem sites (A) and PCA of Lanthanide profiles of groundwater samples taken at these NanoRem sites before NPs application (B)

Figures 21-23 show the PCA analysis and signal comparisons for different monitoring regimes and NanoRem remediation sites. Figure 21 shows “positive controls” at the Spolchemie and Solvay site, showing a clear shift of the PCA signal towards NP profiles at close in wells. Signals at more distant wells showed lower, or no shift towards the NP profile, confirming no or low transport of NP outside the treatment area (Figure 22). Increasing the number of elements included in the measurement

(Figure 23) increases the variables and the scatter, and for the three sites tested. But this did not increase sensitivity over analysis of the rare earth elements, thus reducing analysis costs. However, including more elements can provide a better understanding of the environmental changes at the site after its remediation, but this does not increase the sensitivity for monitoring renegades.

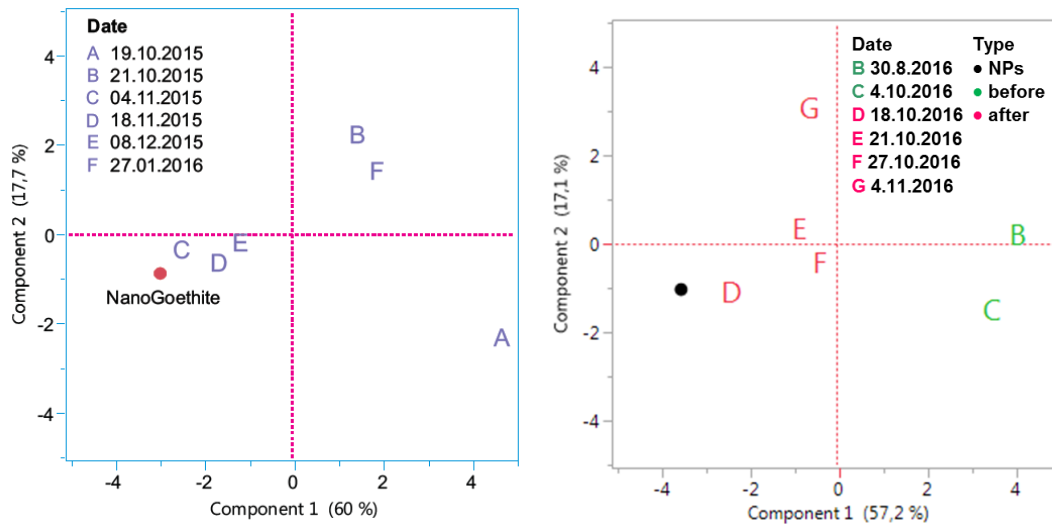


Figure 21: PCA of Lanthanide profiles of groundwater samples taken at Spolchemie and injected NanoGoethite NPs and Solvay and injected micro- and nano-iron milled Fe. A significant shift towards the NP profile was seen at close in wells, but none was seen at more distant wells.

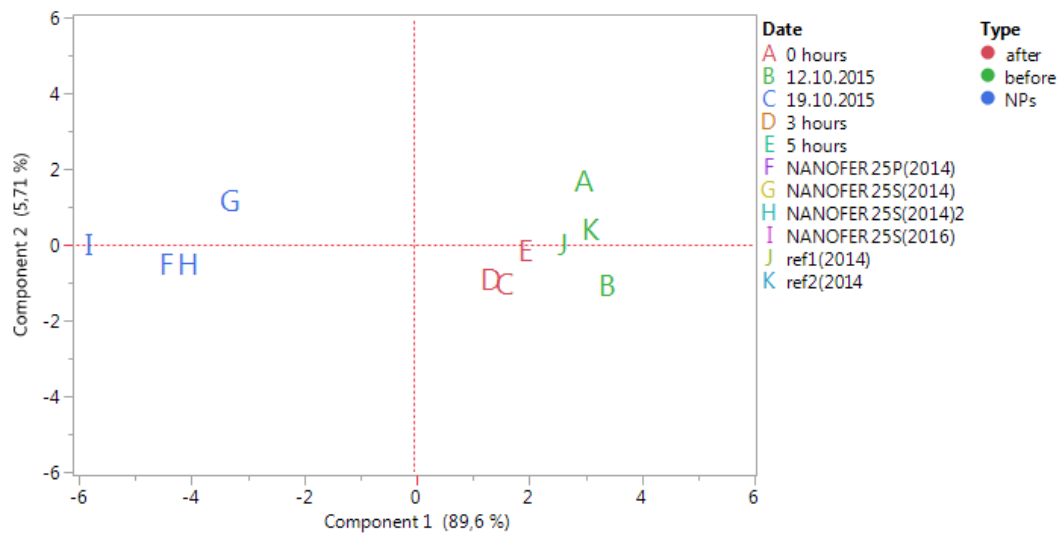


Figure 22: PCA of Lanthanide profiles of groundwater samples taken at Spolchemie (site 1, monitoring well PV-129) and all the NPs used there during last two years – the samples taken after application of NPs show only a slight shift towards NPs profiles

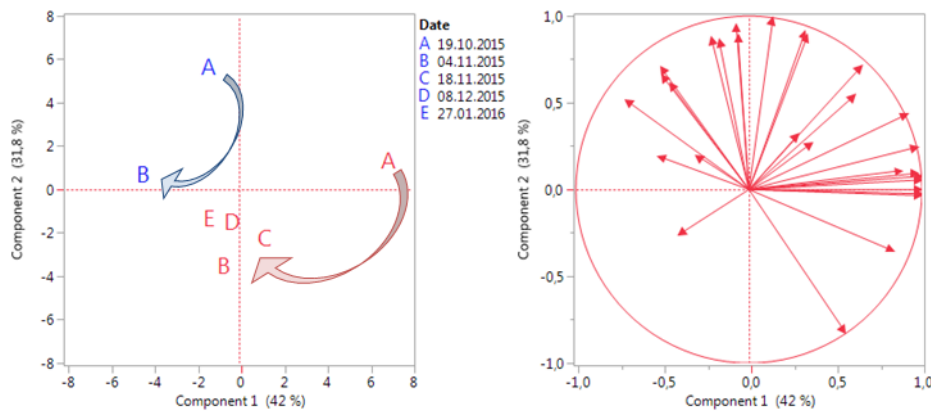


Figure 23: PCA results for monitoring wells 42 (blue labels) and 43 (red labels) at Spolchemie, Site 2. Score plot for components 1 and 2 (left side) shows the changes in environment before (samples A) and after (samples B, C, D, E) the NPs injection. The direction of change is marked with arrows. Scatter plot for components 1 and 2 (right side) shows the spread of variables in the directions of individual components.

Advantages/ disadvantages of the method

Although extremely low detection limits (ng/L levels) are achievable in clean media (as tested in laboratory column experiments); but the performance and applicability in the field is highly dependent on site-specific parameters. Analytical costs are higher than for total Fe measurement, by targeting selected monitoring sites, measurements can be carried out over a lower spatial and temporal frequency. Main costs are related to the multielement ICP-MS measurements, but by selective targeting of relevant sampling wells, monitoring for renegades should be possible at a cost of 1000-5000 EUR for a remediation site.

4.3.2. Single particle ICP-MS

Single particle inductively coupled plasma mass spectrometry is an emergent ICP-MS method for detecting, characterizing, and quantifying nanoparticles. To be able to detect particles the instrument has to be operated in a different way versus measuring dissolved samples. Samples containing nanoparticles has to be introduced at a low flow rate and the number of particles in the sample has to be quite low. Operating Single particle ICP-MS allows collecting the intensity for a single particle as it is ionized in the plasma. Obtained signal can be correlated to the size and mass fraction of analysed nanoparticles.

In November, 2014 UNIVIE participated in the sampling campaign at the Spolchemie (Usti nad Labem, Písečná, Czech Republic) injection site. Samples were collected from wells AW6A-4 and AW6A-20, as shown in Figure 24, left. The former well is located approximately one meter away from the injection point, while the latter well is at approximately five meters distance from the injection point. Samples were collected from these two wells before and after injection with iron oxide

particles to test the transport of the injected suspension in the groundwater. Three samples were collected for each location and time: one sample with no addition treatment, one sample with the addition of 0.38 ppm Humic Acid (HA), and one sample with the addition of 3.8 ppm HA. The addition of HA was done to test for the possibility of particle instability between the time of sampling and analysis.

Samples were stored at 7 °C upon return to the laboratory. In order to remove large structures that may have been present, groundwater samples were lightly centrifuged for 5 minutes at 200 rpm. This step would remove particles larger than approximately 10 µm, which may have adverse effects on the ICP-MS instrumentation. Total iron was determined after a microwave assisted digestion of the samples with a mix of hydrogen peroxide, hydrochloric acid, and nitric acid, using an ICP-OES. Control tests showed that 90% of total iron remained in the supernatant, after the centrifugation step, indicating that no significant losses of iron occurred. The supernatants were analyzed with an Agilent 8800 Series ICP-MS for single particle ICP-MS analysis.

ICP-OES analysis revealed a strong increase of iron concentration after the injection for sampling well AW6A-4, while no such difference was observed for sampling well AW6A-20 (figure 36). In addition to iron, major elements were measured: Ca, Mg, K, Na, Al, and Si. However, no significant differences were measured before and after injection for both sampling wells.

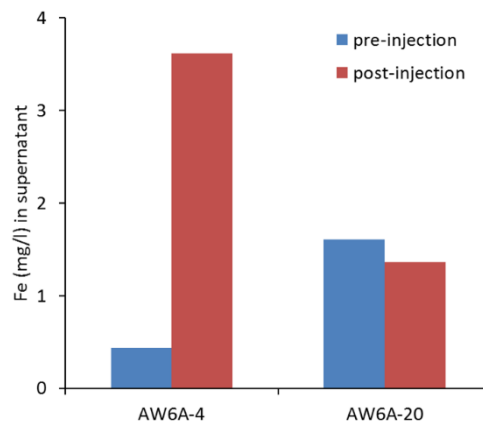


Figure 24: Total iron (Fe) concentration in groundwater taken from wells AW6A-4 and AW6A-20 before and after injection of iron oxide particles.

The particle number concentrations of iron-bearing particles in the samples were measured with single particle ICP-MS and the results are shown in Figure 25. The method used has been developed at UNIVIE and is capable of detecting particles larger than 40 nm (calculated for spherical hematite particles). There is a clear increase of iron-bearing particles measured in location AW6A-4 after injection. The number concentration is 28 times higher compared to pre-injection. No such increase was observed for samples collected at location AW6A-20. These data are in agreement with the ICP-OES and DLS data and indicate that the injected particles were transported quickly at a distance of one meter and not longer than five meters.

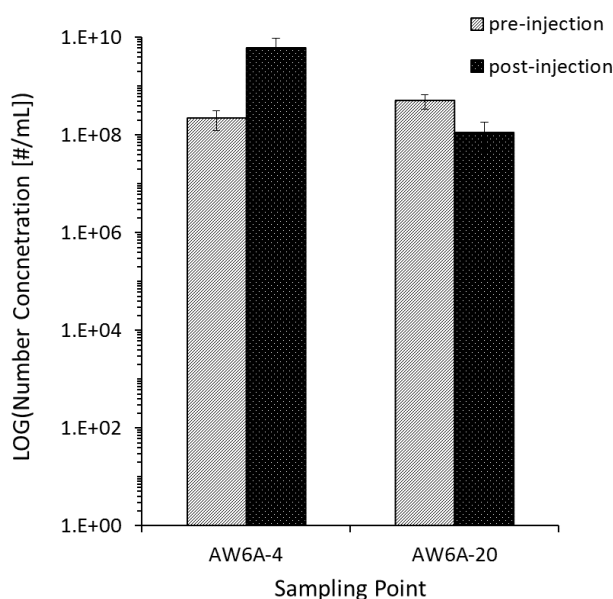


Figure 25: Particle number concentrations of iron-bearing particles before and after injection in sampling points AW6A-4 and AW6A-20.

5. Summary: Applicability of the Methods

A wide variety of methods is available for monitoring and characterising nanoparticles in laboratory and field experiments. The methods are complementary and applicability depends on both the particle utilised and the question to be asked. The NanoRem project has demonstrated that there are a number of techniques that can be applied for monitoring Fe-based NPs during remediation, and that determination of concentrations at levels below those linked to ecotoxicological effects should be straightforward both within and outside the remediation area. Existing challenges include the discrimination of intact Fe particles from dissolved Fe, since increased Fe concentrations outside the treatment area do not necessarily mean movement of NP. However, fingerprinting techniques using trace element and lanthanides analysis look promising.

A summary of the various methods, detection limits and limitations is given in Table 6.

Monitoring of particle dispersion during injection phase: Results from tanks and field applications show that the detection of particle loads 0.5-5 mg/L during the injection process is relatively straightforward, with a combination of at site sampling, and analysis of suspensions (turbidity, conductivity, redox, temperature and Fe content). This is sufficient to follow the distribution of particles during injection, but follow-up data analysis is required for a quantitative assessment and design of standard operating protocols. On-site measurements of turbidity, conductivity and Fe concentrations using spectrophotometry are all relatively fast and cheap methods. The instrumentation required for on site measurements is portable and not expensive. Of all methods, magnetic susceptibility has the best potential, but has relatively high detection limits (ca 500 mg/L). While instrumentation costs for the magnetic array sensors are higher than those for the above

methods (ca. 1000 Euro for the hardware and 1000 Euro for the electronics), they are one of the few truly *in situ* methods and have the advantage of giving continuous logging data.

Post injection monitoring. Monitoring during the post injection phase needs to provide information not only the concentrations of Fe, but also its speciation in order to understand the fate and reactivity of the injected particles. For total Fe concentration, measurements on suspensions/liquids and soils/sediments can either be carried out after acid digestion and measurement using standard chemical analysis (e.g., ICP-OES, or spectrophotometry). For low particle densities, pre-concentration by centrifugation or filtration can be applied to improve detection limits. Specific protocols for acid digestion need to be developed for the different particles to ensure complete dissolution. The detection limits of all methods will be site specific, depending largely on the background levels, and, for Fe-based NPs, dissolved iron concentrations. Field applications have demonstrated that Mössbauer (for nZVI) can give useful additional information on the time dependent changes in particle state and reactivity, in both water and solid phases. These can be supported by other methods for measurements of structure and oxidation state (e.g. X-ray photoelectron spectroscopy, transmission/scanning electron microscopies, X-ray powder diffraction, X-ray fluorescent spectroscopy).

Monitoring of renegade particles More sensitive methods are required to distinguish lower concentrations of Fe-based NPs from background matrix. Tests during NanoRem pilot studies indicate that ICP-MS multi-element fingerprinting were able to identify NPs at all sites. While detection limits of these methods are known to be extremely low in clean media, the performance is dependent on site specific parameters, but indicate measurability down to a sub-mg levels.

Table 4: Summary of Applicability of Selected Nanoparticle Characterisation Methods

Method	Concentration range	Size Range				Comments/Limitations
Tested for at site or <i>in situ</i> applicability						
Turbidity (TurbidScan)	0.5 mg – 1 g/L (up to 1000 NTU)					Tested for FeOx and Trap-Ox Fe-zeolites. Applicable in the field but detection limits depend on background turbidity measurements.
Fe-content (ICP-OES, spectrophotometry)	1 µg – 1 g/L	NR				Applicable for all particles. Applicable in the field (spectrophotometer), but detection limits are dependent on site background concentrations. Restricted to water samples, solid samples are affected by the ground content.
Magnetic susceptibility	50 mg/L – 1 g/L					Primarily nZVI. <i>In situ</i> measurements, but low mobility of nZVI means that detection will be contingent on the sensor being placed in a location where the particles will migrate. Some limitations in placement, eg under buildings.

Method	Concentration range	Size Range				Comments/Limitations
Direct H ₂ Measurement	>10 mg/L of H ₂ 1-2 g/L nZVI	NR				nZVI only. Cannot trace oxide/hydroxide reaction products. On site applications dependent on background measurements and redox
Ultrafiltration	1 µg – 1 g/L	ca 10 – 450 nm				All particles. Field sampling technique. Used in combination with other techniques for separating dissolved and particulate Fe, and for insight into size distribution measurements and changes over time. Avoids problems with particle aggregation and dissolution between sampling and measurement.
Tracing Renegades						
ICP-MS and RRE fingerprinting	1 µg – 1 g/L	All sizes				Site and situation specific. Detection limits depend on the difference in profile between remediation NPs and background samples.
Single particle ICP-OES	1 µg – 1 g/L	<10 µm				Site and situation specific.
Laboratory analysis						
Mossbauer	>100 mg/L					nZVI only. Lab technique, but can be applied to field samples that are frozen after collection.
H ₂ Acid digestion		<500 nm				nZVI only. When combined with total Fe measurements, Fe ⁰ /Fe _{tot} ratios can be used to follow reactivity.
Laser diffraction (Mastersizer)	>500 mg/L	0.6 – 1 µm				Applicable for most particles. FeOx below the limit of detection for size
Time of Transition (EyeTech)	<500 mg/L	0.6 – 600 µm				Sedimentation problems with nZVI (Nanofer) and milled Fe. FeOx below the limit of detection for size
Dynamic light scattering (Zetasizer)	<500 mg/l	1 nm - 10 µm				Applicable for most particles. Sedimentation problems with nZVI (Nanofer) and milled Fe.
SEDFFF		0.05-100 µm				
Carbo-Iron and Trap-Ox Zeolites						
Carbo-Iron: Particulate organic carbon	>5 mg/L	NR				Filtration of water sample.
Carbo-Iron: Fe/C ratios		NR				Preliminary <i>in situ</i> test

Method	Concentration range	Size Range				Comments/Limitations
Carbo-Iron: TPO	ca 0.3 % wt					Sediments. Test on NanoRem porous media
Carbo-Iron: TPO-IR	ca. 0.03 % wt					Sediments. Test on NanoRem porous media
Trap-Ox Fe-Zeolites: DLS, Fe-content, laser diffraction	0.1-2.5 g/L					As above
Trap-Ox Fe-Zeolites: BET	0.05 % wt					Test on acid washed sand, not suitable for field samples
Trap-Ox Fe-Zeolites: target adsorption	0.1 % wt					Test on M:1 (Dorsilit)
Trap-Ox Fe-Zeolites: Fluorescence labelling	1.1 mg/L					Test on suspensions and column effluents with standard-type water (F.I.h)

NA – Not relevant

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ANNEX: Update on Laboratory and Pilot Methods

Sedimentation Field flow Fractionation (SedFFF)

The developed method comprises of two parts: the particle fractionation process and their detection with a series of detectors, both providing a wide array of information. Sedimentation field flow fractionation (SedFFF) is the method of choice in this case, as it separates particles based on their size and density. This technique is characterized by high separation ability and a working range between 0.05 and 100 μm . The retention parameter λ , which describes the time it takes for a particle of certain size and density to elute from the SedFFF channel is calculated from the following equation:

$$\lambda = \frac{6kT}{\pi d^3 |\rho_p - \rho| \omega^2 r w}$$

where k is the Boltzmann constant, T is temperature, d is the particle diameter, ρ_p and ρ are the densities of the particle and the solvent, ω is the angular rotation frequency, r is the radius of curvature of the rotor, and w is the channel thickness. The high separation ability of SedFFF, compared to other separation methods, lies in the fact that the retention parameter is a function of diameter cubed. It is also a function of density, which allows for the separation of particles with different chemical composition or crystallinity. In addition, there is a lot of flexibility for optimizing operation parameters to maximize separation efficiency. Figure A1 shows a theoretical calculation of the retention time for nZVI (reference material Nanofer 25S) and hematite nanoparticles under different operational conditions.

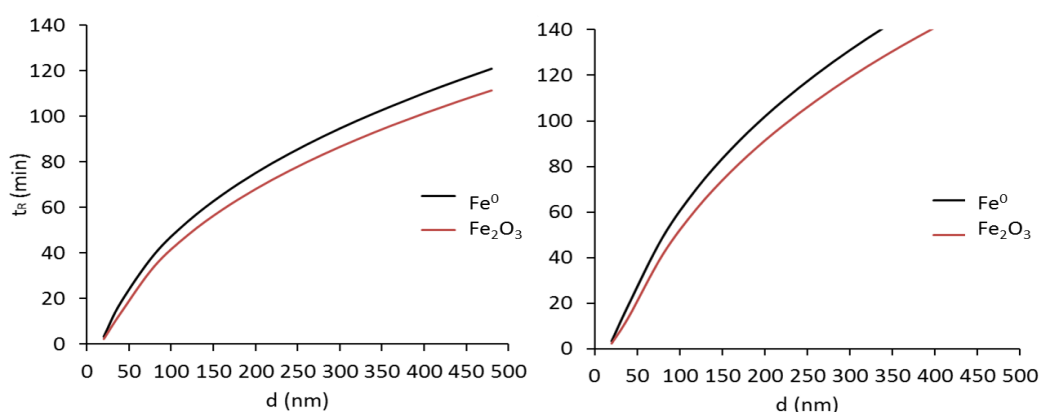


Figure A1: Retention time (t_R) dependence on particle diameter (d) for nZVI (Fe^0) and hematite (Fe_2O_3) nanoparticles under two different operational conditions.

Hyphenated with the SedFFF we connected a series of detectors: Multi angle laser light scattering (MALLS), ultra violet - visible absorbance (UV-Vis), fluorescence (FLD), and inductively coupled plasma mass spectrometry (ICP-MS). The MALLS detector provides online information about the diameter of eluting particle sizes. The scattered intensity is measured at several angles and can be used to calculate particle size using appropriate models. UV-Vis and FLD detectors may provide information on both the eluting particles as well as organic matter that may be present in the sample. Finally, the ICP-MS offers high-sensitivity detection of the elemental composition of the eluting particles. In order to minimize the risk of nZVI oxidation, the sample introduction system and SedFFF have been placed in an anaerobic glove box, under nitrogen/hydrogen atmosphere. A catalyst has been put in place to facilitate the reaction of oxygen traces with hydrogen and an oxygen/hydrogen detector is used for verifying anaerobic conditions.

Application within NanoRem project

The instrumentation setup has been completed and checks of the hyphenated systems' electronics and hydraulics have been made. Preliminary results of runs using in-house prepared hematite nanoparticles of 50 and 100 nm are shown in figure A2. The retention times of 50 and 100 nm particles are in good agreement with the theoretically calculated ones, under the specific operational conditions (figure 1).

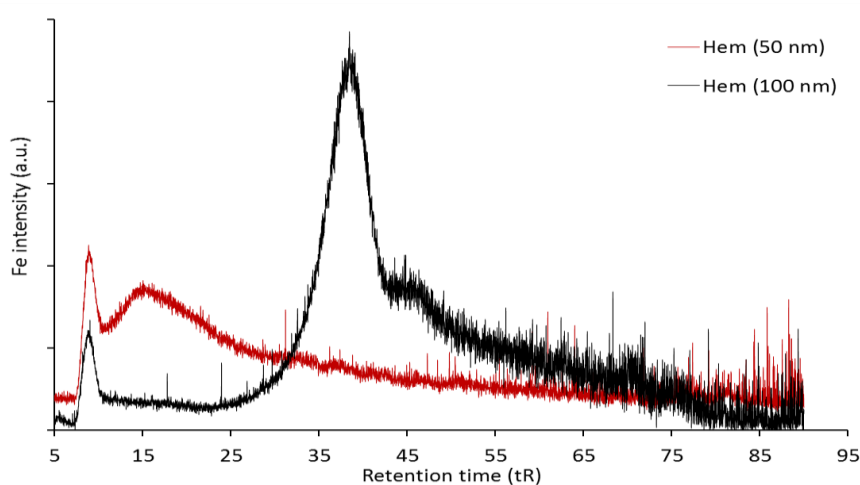


Figure A2: Iron ICP-MS intensity signal of SedFFF runs using 50 and 100 nm hematite (hem) nanoparticles.

Hyphenated with the SedFFF we connected a series of detectors: MALLS (DAWN Helios II, Wyatt Technology), UV-Vis (Agilent Technologies), fluorescence (FLD: Agilent Technologies), and ICP-MS (7900, Agilent Technologies). The MALLS detector provides online information about the diameter of eluting particle sizes. The scattered intensity is measured at several angles and can be used to calculate particle size using appropriate models. UV-Vis and FLD detectors may provide information on both the eluting particles as well as organic matter that may be present in the sample. Finally, the ICP-MS offers high-sensitivity detection of the elemental composition of the eluting particles. In

order to minimize the risk of nZVI oxidation, the sample introduction system and SedFFF have been placed in an anaerobic glove box, under nitrogen/hydrogen atmosphere.

NanoRem standard particles, Nanofer 25S were used as an example for nZVI particles. Four samples were prepared by diluting Nanofer 25S suspension in moderately hard EPA water (F.I.m); two samples were kept in the anoxic chamber and two under ambient laboratory conditions. Additionally, one sample in F.I.m was kept in the dark by covering it with Aluminum foil, one sample was prepared in ultrapure water (MilliQ Water), and one sample was prepared in 1 mM Na_2HPO_4 at pH 9.5–10, which was the eluent (SPP) used for particle separation with the SedFFF. The original material consisted of large aggregates and only a fraction of the particles was in the truly nano-range; here we focus on this fraction, assuming that large aggregates will be mostly immobilized under realistic conditions and act as bulk ZVI. Large flocks in the suspensions were therefore allowed to settle for 48 h and the supernatant was decanted in separate vials and characterized immediately. Characterization was repeated after 6 days and again after 35 days. A spacer with 250 μm thickness was used in the SedFFF channel, initial rotation speed was set to 4,500 rpm, and flow was 2 mL/min. An injection volume of 10 μl was used and samples were diluted 10x for the recovery measurements.

Samples that were kept under anoxic conditions contained particles with lower size and density, which increased with time, while samples kept under ambient laboratory conditions contained particles of larger size and density that was constant for the duration of our experiments (figure5). In both cases, the majority of the iron eluted in the first 5 min of fractionation, which indicates that Fe is either in the form of very small particles, or dissolved species. To test the latter assumption, samples were filtered through a 20 nm pore size membrane and 25% of the iron was able to pass through the filter. It can be therefore concluded that the majority of the Fe eluting from the SedFFF is not in the form of dissolved species, but very small particles (<70 nm). In this size range the ability of the SedFFF separation efficiency is reduced and therefore these particles are eluting in the first 5 min of fractionation. However, it is clear that a fraction of Fe is forming larger particles if left under ambient laboratory conditions (figure A3).

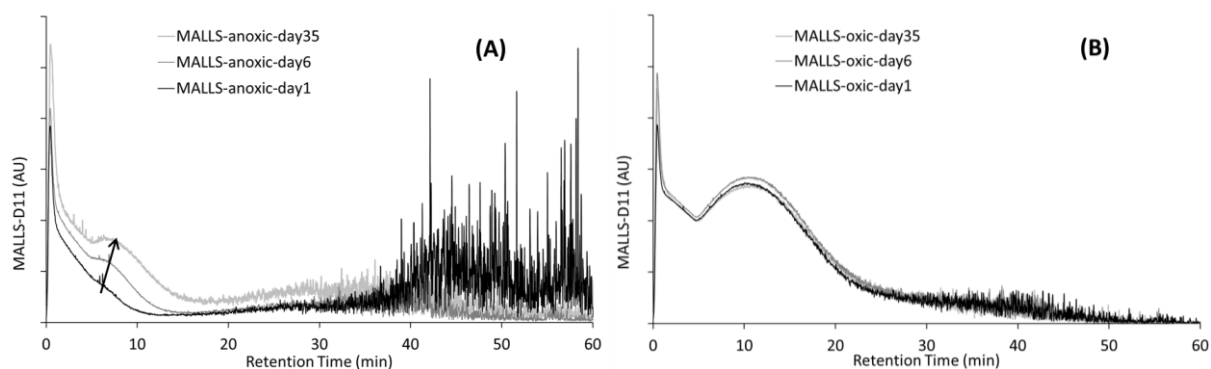


Figure A3: MALLS signal of SedFFF fractograms for samples that were kept under (A) anoxic conditions and (B) under ambient laboratory conditions. The appearance of a peak at approximately 8 mins of retention time is marked with a black arrow.

Due to the very large volume of data produced from the various detectors, analysis and interpretation of the results is an ongoing process. There are, however, indications that the oxidation

of nZVI is leading to the formation of larger particles and that this oxidation occurs within the first few hours in oxic waters. In anoxic waters oxidation also takes place, but at a much slower rate. In figure A4 the theoretical elution patterns of various particles are plotted over retention time and can be compared to the geometric and hydrodynamic radius of our nZVI particles under oxic conditions. It can be observed that both the geometric and hydrodynamic radius lie between the several Fe-containing particles and polystyrene particles, indicating that the overall particle density is lower than in pure Fe-containing particles, most likely due to the organic stabilizer that is coating the particles. It is possible that as the nZVI particles are oxidized, the organic stabilizer is reduced and possibly removed from the particles surface. Such reactions would lead to a simultaneous increase of particle size (due to the formation of Fe oxides) and of the overall particle density (due to the removal or degradation of the low-density organic coating). Both of these processes would increase the retention time of particles during the SedFFF separation.

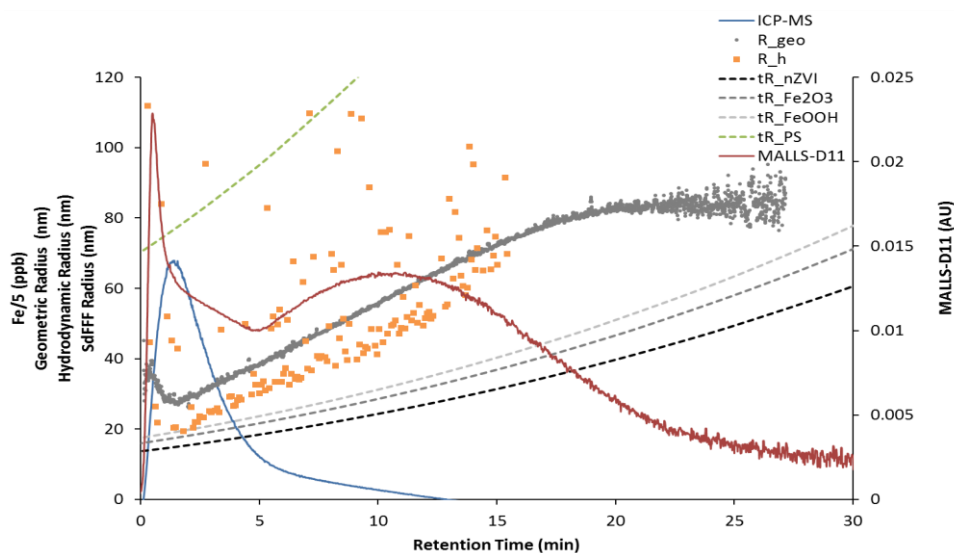


Figure A4: Signals of the ICP-MS and MALLS (detector 11, at 90°) are plotted over retention time (tR). In addition, the geometric radius (R_geo) and hydrodynamic radius (R_h) are shown after analysis with the MALLS data analysis software, ASTRA (Version 5).

In addition to the effect of O₂ during sample storage, the possibility of nZVI oxidation during sample measurement was tested. For that purpose, samples analyzed after 35 days of storage were measured first with anoxic eluent and subsequently with oxic eluent. No major differences were observed for the particle size distribution, however samples measured with oxic eluent exhibited higher recoveries (figure A5). Given that the SedFFF channel is comprised of stainless steel, one possible explanation for the improved recoveries is that a layer of oxidized Fe species is formed on the channel walls, which are more effective in repelling nZVI particles. This finding suggests that the use of oxic eluent is advantageous and the risk of sample alteration during measurement is minimal.

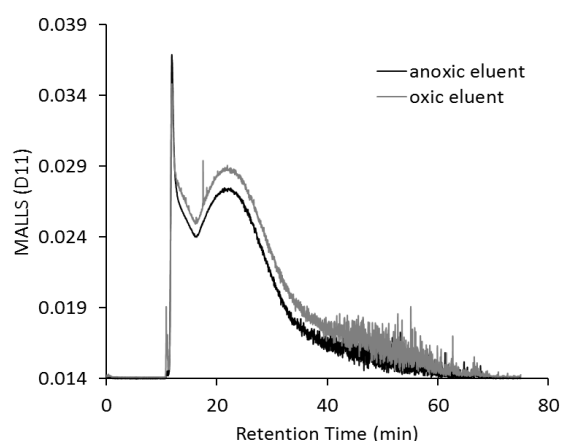


Figure A5: MALLS signal of SedFFF fractograms for samples stored under anoxic conditions for 35 days and analyzed under anoxic and oxic eluents.

Advantages/disadvantages of the method

One of the main disadvantages of the method is that method optimization can be time and effort consuming; however, the excellent separation performance of the SedFFF compensates for the optimization effort.

Dynamic Light Scattering (DLS)

The Dynamic Light Scattering (DLS) technique measures diffusion of the particles and calculates the particles hydrodynamic radius from 1 nm up to about a micrometer using dynamic light scattering, using Mastersizer (Malvern, Mastersizer 2000). The performance of the DLS technique was first tested on various Fe-nanoparticles, as described in DL6.1 and summarized in Table A1.

Table A1: Performance of Zetasizer for Nanoparticle DLS Characterisation.

Particles (producer)	Method	Limitation
Nanofer products (Nanofer 25S, Nanofer Star, Modified Nanofer Star) (Nanoiron)	measured concentration 200 mg L ⁻¹ (1g L ⁻¹ diluted 1:5)	Fast sedimentation of the particles
Milled Iron (UVR-FIA)	measured concentration 200 mg L ⁻¹ (1g L ⁻¹ diluted 1:5)	Size above limit of detection Fast sedimentation of the particles
Carbo-Iron (UFZ)	measured concentration 200 mg L ⁻¹ (1g L ⁻¹ diluted 1:5)	
Trap-Ox Fe-Zeolites (UFZ)	measured concentration 200 mg L ⁻¹ (1g L ⁻¹ diluted 1:5)	

Bio-Fe-oxides (UMAN)	measured concentration 200 mg L ⁻¹ (1g L ⁻¹ diluted 1:5)	High size variation due to different batches
Fe-oxides (HGMU)	measured concentration 200 mg L ⁻¹ (1g L ⁻¹ diluted 1:5)	

In November 2014, the DLS method was tested during the injection of NanoGoethite at Spolchemie (Usti nad Labem, Czech Republic). Samples were collected from wells AW6A-4 and AW6A-20. The former well is located approximately one meter away from the injection point, while the latter well is at approximately five-meter distance from the injection point. Samples were collected from these two wells before and after injection with iron oxide particles to test the transport of the injected suspension in the groundwater. Three samples were collected for each location and time: one sample with no addition treatment, one sample with the addition of 0.38 ppm Humic Acid (HA), and one sample with the addition of 3.8 ppm HA. The addition of HA was done to test for the possibility of particle instability between the time of sampling and analysis.

Samples were stored at 7 °C upon return to the laboratory. In order to remove large structures that may have been present, groundwater samples were lightly centrifuged for 5 minutes at 200 rpm. This step would remove particles larger than approximately 10 µm, which may interfere with the DLS analysis

The average hydrodynamic diameter of centrifuged groundwater samples collected from the two wells was analyzed with DLS; the pH of the samples was also measured and the results are shown in table 2.

Table A2: pH and average hydrodynamic diameter (Z-Average) of groundwater samples.

Sample	pH	Z-Average (d.nm)	Notes
AW6A-4 (pre inj., no HA)	7.5	1500	DLS out of range (>1 µm)
AW6A-4 (pre inj., 0.38 ppm HA)	7.5	1500	DLS out of range (>1 µm)
AW6A-4 (pre inj., 3.8 ppm HA)	7.5	1300	DLS out of range (>1 µm)
AW6A-4 (post inj., no HA)	7.8	300	Bimodal; Peak 1= 100 nm, Peak 2= 400 nm
AW6A-4 (post inj., 0.38 ppm HA)	7.7	310	Bimodal; Peak 1= 80 nm, Peak 2= 350 nm
AW6A-4 (post inj., 3.8 ppm HA)	7.8	300	Bimodal; Peak 1= 70 nm, Peak 2= 300 nm
AW6A-20 (pre inj., no HA)	7.6	1200	DLS out of range (>1 µm)

AW6A-20 (pre inj., 0.38 ppm HA)	7.4	1200	DLS out of range (>1 μm)
AW6A-20 (pre inj., 3.8 ppm HA)	7.7	1200	DLS out of range (>1 μm)
AW6A-20 (post inj., no HA)	7.3	1500	DLS out of range (>1 μm)
AW6A-20 (post inj., 0.38 ppm HA)	7.3	1100	DLS out of range (>1 μm)
AW6A-20 (post inj., 3.8 ppm HA)	7.3	1100	DLS out of range (>1 μm)
Injected Stock (diluted 1:100)		174	

DLS is highly biased towards larger particles; a suspension containing particles of a few micrometers and particles of some tens of nanometers would appear as only containing the larger particles, after DLS analysis. In such a sample, where exist particles of several sizes, it is impossible to draw quantitative results using DLS. However, a few qualitative conclusions can be drawn. The results show that prior to injection, there exist large particles in the groundwater, but smaller particles are likely overshadowed by the larger ones in the DLS. After injection, and for sampling location AW6A-4, a distinct bimodal distribution can be measured, which lies in the same size range as the injected stock. This change was not observed for sampling location AW6A-20, which is further away from the injection point. Overall, the ICP-OES and DLS data indicate that the injected particles were transported within a short time at a distance of one meter (AW6A-4), but not further than five meters (AW6A-20).

Stable Fe isotope ratios

Measurements of stable Fe isotopes have the potential for tracking NPs in the field, either by making use of the natural variation of Fe-isotopes in different Fe sources, or by labelling NPs with enriched stable Fe isotopes. As a pilot study, samples of Nano Goethite and Nanofer Star NPs were compared with the signatures in field samples from the Solchemie Usti site, as well as the Vegas tank (ratios varied between batches, but showed good reproducibility). Results showed significantly enriched $\delta^{56}\text{Fe}$ and $\delta^{57}/^{54}\text{Fe}$ signals for both NP types with $\delta^{56}\text{Fe}$ ratios of up to 0.8 for the NPs compared to ratios down to 0.04 for the site samples (Figure A6). Ratios varied between batches, but showed good reproducibility for the same batch. Signals from close in wells showed variable increases in the isotope ratios following injection. For these relative ratios, it should be possible to determine changes in the order of 20% of background Fe concentrations, which for the Spolchemie site amounted to a few mg/L Fe. Since the method requires measurement by high resolution multiple-collector ICP-MS, the analysis costs are greater than for ICP-MS fingerprinting (ca. 5000-10000 EURO) and also suffer from the fact that an change in Fe isotope ratios is not necessarily correlated with movement of NPs, since the dissolved fraction would also show a similar change in isotope ratio. Work is continuing on data processing and follow up samples from the sites, but suggest that it has the potential as a support for ICP-MS fingerprinting.

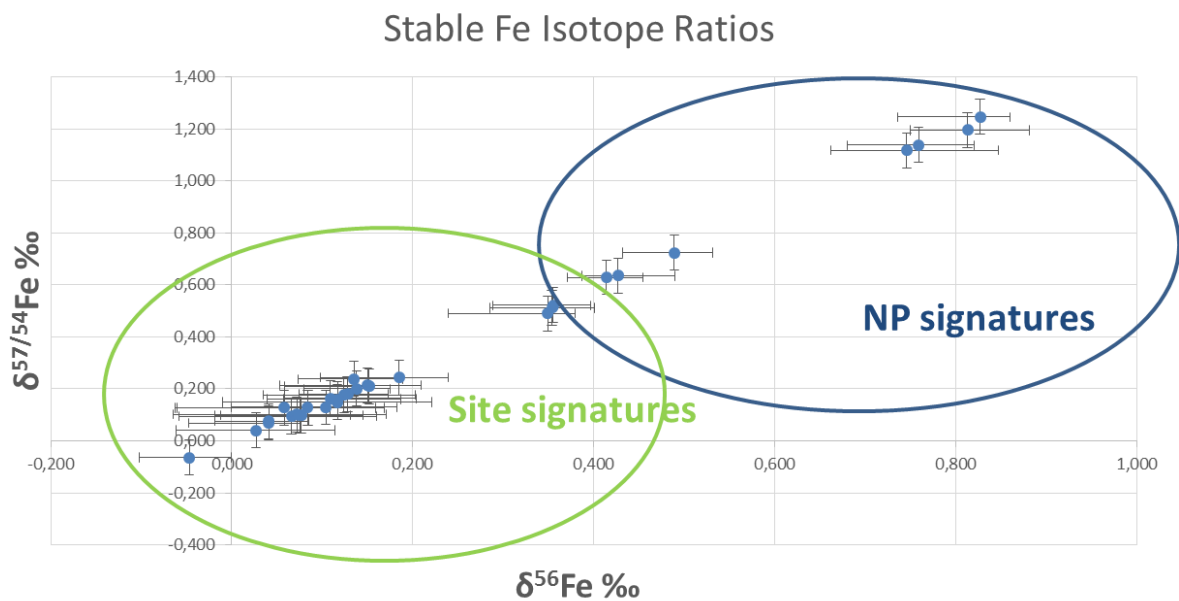


Figure A6: $\delta^{56}\text{Fe}$ and $\delta^{57}/^{54}\text{Fe}$ (‰) signals for NPs and site samples: preliminary data.