FCT Project Reference

PTDC/AGR-AAM/101643/2008

FCT Project Name

nanoDC

Electrokinetic remediation of soil contaminated with persistent organic pollutants using iron nanoparticles

Project Data

Project Location:	Coimbra (PT), Lyngby (DK), Utrecht (NL)	
Project start date:	15-03-2011	
Project end date:	14-03-2014	**Extension until**: 14-12-2014
Total project duration	36 months	**Including extension**: 45 months
Total budget	167.138,00 €	
Principal contractor	IPC – Polytechnic Institute of Coimbra, Portugal	
Partner Institutions	PRODEQ – Associação para o Desenvolvimento da Engenharia Quimica, Coimbra, Portugal	
	Department of Earth Sciences, Utrecht University, The Netherlands	
	DTU - Technical University of Denmark, Denmark	
Investigadora Principal	Celia Maria Dias Ferreira	
e-mail	celia@esac.pt	
Project website	www.nanodc.org	

FCT Fundação para a Ciência e a Tecnologia

QREN Quadro de Referência Estratégico Nacional

COMPETE

União Europeia

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1. NanoDC Framework

NanoDC (Electrokinetic remediation of soil contaminated with persistent organic pollutants using iron nanoparticles) was a project funded by the FCT – Portuguese Foundation for Science and Technology. The Consortium consisted of 4 partners:

- **IPC – Polytechnic Institute of Coimbra, School of Agriculture**: coordinated the project and has considerably experience in electrokinetic technique, on soil characterization and on chemical analysis of organic pollutants.
- **PRODEQ**: this team from the University of Coimbra has experience in pollutant degradation
- **DTU – Technical University of Denmark**: leads the research in electrodialytic remediation, and has been working on electrokinetics since the early 1990.
- **Utrecht University, Department of Earth Sciences**, is highly experienced in electro-osmotic transport processes in clayey soils and on the modeling of mass transfer processes in the subsurface.

The general objective of the NanoDC Project was to develop a deeper understanding regarding a new remediation process to recover contaminated sites, by coupling nanotechnology and electrokinetics. More specifically NanoDC addressed two scientific problems:

(i) Degradation of pollutants using iron nanoparticles

(ii) Transport of nanoparticles using electric fields

The NanoDC project was divided in the following tasks:

- **Task 1** – Management (Task manager: Célia Ferreira, IPC)
- **Task 2** – Sample collection (Task manager: Rosinda Pato, IPC)
- **Task 3** - Degradation studies (Task manager: Rosa Quinta-Ferreira, PRODEQ)
- **Task 4** - Electrokinetic transport (Task manager: Lisbeth Ottosen, DTU)
- **Task 5** – Transport and degradation model (Task manager: Gustav Loch, DES-UU)
- **Task 6** – Dissemination (Task manager: Célia Ferreira, IPC)

This is the Final Report for the NanoDC Project and presents the activities carried out and the result obtained.

2. Progress and Results

The planning and execution of the tasks is given in Table 1. As indicated, there were delays in some task (explained below) but the overall plan was followed.

Although the official starting date for the project was the 15th of March 2011, the PI was on maternity leave until the 28th of April 2011. After this date, and until the end of August, the PI was further in and out of work for family support. This meant the effective start of the project was only possible at the end of August, with the kick-off meeting taking place on the 29th of August 2011.

At the 31st of December 2011 all the funds available to run the project during the first part of 2012 and which were already at the Principal Contractor were frozen, as the net revenue of the institution was retained by the State, as the result of the heavy financial constraints imposed by the Portuguese Government due to the economic crisis succeeding in the Country. In addition, more restrictions aiming at deficit control were imposed by a new Portuguese Law known as "Lei dos Compromissos", throughout 2012, which affected all public bodies in Portugal and limited their capacity to advance funding. Therefore, although human resources hired by the project continued to receive monthly payments, all other acquisitions were practically halted, and the project entered in slow motion.

The funds retained at 31st of December 2011 were liberated more than one year after, in March 2013. At this point all the work was severely delayed, and approximately half of the human resources had their grant ending in 2012, meaning that the project had now funds for the necessary reagents and laboratory acquisitions but few human resources to actually carry out the work. Nevertheless, during the second half of 2013 the project gained full speed again and the recovery continued throughout 2014. Two extensions were requested to FCT and granted, so the official ending date for the project moved first 6 months from 14-03-2014 to 14-09-2014, and then an additional 3-month period, till 14-12-2014.

A detailed description of the activities and results for each task is presented next.

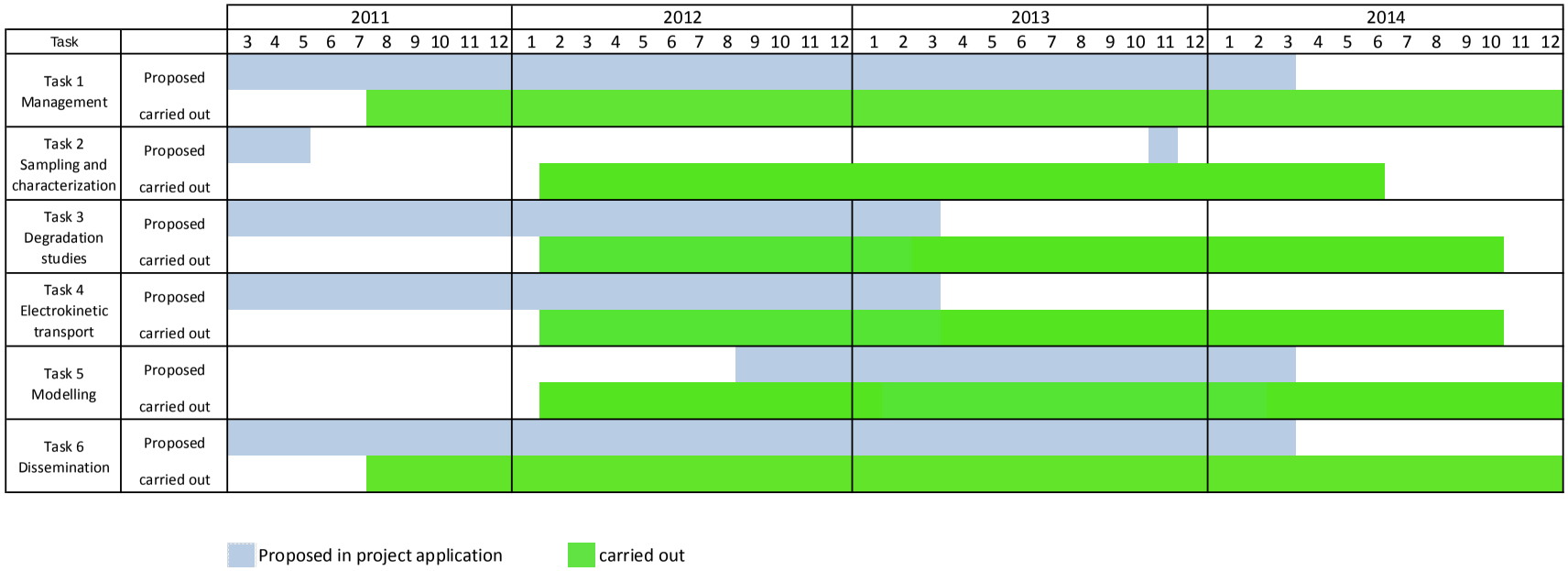


Table 1 – Overview and timeframe of the activities proposed and carried out in Project NanoDC

2.1 Management (Task 1)

The objectives proposed for this task were the overall coordination of the project and the expansion of the international network.

One additional objective was set in the initial stage of the project, which was not in the original application. This consisted in the development and implementation of a document management system for the project.

2.1.1. Overall coordination

This comprises the scientific, technical, financial and staff coordination and was in charge of the Principal Investigator (PI). The activities carried out were:

i.1) **Project kick-off meeting** took place on the 29-08-2011 at Coimbra School of Agriculture. During this meeting some suggestions were made to improve the project development, namely it was suggested to start the modeling task earlier than planned, so that it would be developed alongside tasks 3 and 4. Another suggestion was to reduce the number of soil samples to work within the project from three to two. It was then suggested also that if no contamination was found in agricultural soils than samples could be collected at more contaminated industrial sites.

i.2) **Reviewing the status of the work** of different partners and making sure objectives, milestones and deliverables were timely reached. This was done through multiple meetings with the partners. Meetings between the PI (Principal Investigator) and the PRODEQ team were mostly in presence and were carried out regularly at PRODEQ facilities. The follow up was also carried out through e-mail and telephone. As for the other international partners, meetings were carried out using skype videoconferencing and by e-mail. Some meetings also took place in person, usually taking advantage of other events, such as Conferences and MSc. Public presentation. Hal-way through the project a general Assembly took place in Coimbra School of Agriculture, and a final one also took place(together with the final workshop) on the 19-12-2014, a few days after the official ending of the project.

i.3) **Management of the human resources of the project.** The list of the human resources hired for the project is shown in table 2. Due to late start of the project and to an institutional change in the formalities related to the contracts, the research grants could only be started in January 2012. These delays were reported to FCT together with a corrective plan for two additional temporary grants during 8 months, to strengthen the capacity of the research team and speed up the work, counteracting the delay.

The team originally proposed of two grant holders was segmented into 5 different people. In February 2012 the team was further reinforced by inclusion of three master students and in August 2012 of one PhD student.

The permanent staff of partner institutions that were team members in NanoDC are presented in table 3. Only one change needs reporting: team member Gustav Loch from the University of Utrecht retired in March 2012, making the selection of another member essential on order to keep the University of Utrecht as Partner. An agreement was attained with Dr. Ruud Schotting, from the same University and an experienced

hydraulic modeler. Dr. Gustav Loch nevertheless maintained his participation in the project, being till the end a key element and very committed to the project.

Table 2 – Young Researchers participating in the project (note: GH - Grant Holder)

GH 1	Emilio R. Villanueva	36 months grant, proposed for a PhD student who would do his/her Thesis on the topic. However, Dr. Emilio applied already holding a PhD degree. Being the most qualified and suited person for the task, he was the selected candidate. Dr. Emilio terminated the contract after 15 months, after receiving a Pos-doc grant by the Galiza government. Dr. Emilio remained in the project for 6 more months, as a visiting pos-doc, being replaced by GH5. (15-01-2012 to 14-10-2013)
GH 2	Helena Silva	Originally thought to be a 24 month grant, the grant was reduced to 21 months, due to team reinforcement GH4 (15-01-2012 to 14-10-2013)
GH 3	Bruno Guimarães	8 month temporary grant, agreed with FCT to counteract the delay in the start of GH 1 (01-03-2012 to 31-10-2012)
GH 4	Daniela Lopes	8 month temporary grant, agreed with FCT to counteract the delay in the start of GH 2 (28-02-2012 to 28-10-2012)
GH 5	Daniel Pôças	9,5 m. This grant holder was hired to replace GH1 (01-06-2013 to 14-03-2014
PhD student	Helena Gomes	Holder of a FCT grant on the topic of the project (grant not paid by the project)
MSc. Student	Elsa Neto	Student at PRODEQ/UC (no grant)
MSc. Student	Mariana Serra	Student at ESAC/IPC (no grant).
MSc. Student	João Salvador	Student at ESAC/IPC (no grant).

The activities of managing young researcher and the team were carried out through regular meetings and continuous support of the work developed, serving the very important function of empowering and motivating all young researchers to the work developed for the project. One of the ways to achieve this was encouraging the participation of young researchers at conferences and workshops and exchange visits to other institutions relevant for the project topic, either funded by the project, when possible, by providing other financial support and by encouraging applications to alternative funding schemes. Table 4 shows the overall missions carried out during the project, where the role of young researcher is clear, as well as the use of other funding instruments. The missions carried out by the young researchers contributed to their education and scientific development. Their contribution to the project is highly relevant in the different tasks were they participated, translated into the overall indicators of 1 PhD Thesis and 3 MSc. Dissertations.

i.4) **Project representation**. The PI was the contact person for the project before the funding agency (FCT), the Principal Contractor and other parties. In this respect the PI did multiple contacts with FCT in order to request the necessary changes to bring the project to successful completion. The PI was also invited to speak about the project at

some occasions, such as at a workshop for Future of Science that took place at ESAC/IPC in April 2012 and was targeted at motivating students to carry out research, and at the International Workshop for Nanotechnologies for Environmental Geotechnics, in Braga (Portugal) in December 2014. Several meetings with team members and other institutions also took place regularly to discuss the project so far and prepare the work ahead (table 4). In addition several participations at international meetings/conferences were also carried out, further described in task 6, below.

Table 3 – Permanent staff in the project

Team Member	Partner Institution	% time
Celia Maria Dias Ferreira (Principal Investigator)	IPC	35
Jorge Varejão	IPC	10
Micaela Soares	IPC	20
Rosinda Pato	IPC	15
José Azevedo	IPC	10
Rosa Quinta Ferreira	PRODEQ	5
Margarida Quina	PRODEQ	5
Licínio Ferreira	PRODEQ	5
Lisbeth Ottosen	DTU	20
Johan Peter Gustav Loch	Utrecht University	20
Ruud Schotting (entered the project on 30-08-2012)	Utrecht University	20

i.5) **Managing the funds received from FCT** in an appropriate and transparent manner. This implied continuous monitoring of budget together with the Project Office (contact person: Mª José Gouveia) to ensure consistency between project expenses and the work. Efforts were taken also in the form of internal meeting with the accounting office and with the director of the Institute in an attempt to overcome the financial constrains originating from the change of accounting rules for public bodies that occurred in Portugal during the project (as explained above).

i.6) **Reporting**. Collecting and analyzing reports from the participants and writing the overall reports for the project were carried out. Three progress reports (April 2012; April 2013; September 2014) and one final report (January 2015) were written to fulfill the reporting obligations imposed by the funding entity (FCT). In addition, several internal reports were produced by the research grantees and by the task coordinators (annex A)

2.1.2 Expansion of the international network

Aiming at the expansion of international network of contacts and internationalization of the team the following long term exchange visits took place (see also table 4):

Jan 2012 – Aug 2012 (8 month): Research fellow Emilio Rosales from IPC did an exchange period at Univ. of Utrecht (the Netherlands) where he developed a new laboratory prototype to measure the electroosmotic transport of nanoparticles (task 4), under the supervision of Professor Gustav Loch and the collaboration of Dr Pieter Kleingeld. During this period regular meetings also took place with Professor Ruud Schotting related to model development (task 5).

Feb 2012 – July 2012 (6 month): 2 young researchers from IPC conducted their master thesis at partner Technical University of Denmark on the electrokinetic transport of iron nanoparticles in contaminated soils, under the supervision from Professor Lisbeth Ottosen.

Sep 2011 – August 2012 (12 month): PhD student Helena Gomes went to Lehigh University (USA), to learn about the synthesis of iron nanoparticles and conduct some experiments related to their transport under electric fields. This work was carried out under the supervision from Professor Sibel Pamukcu.

Mar 2013 – April 2013 (1 month): Pos-doc researcher Emilio Rosales went to the Technical University of Denmark to produce and conduct degradation tests of green tea nanoparticles, under the supervision from Professor Lisbeth Ottosen

Sept 2013 – Dec 2013 (3,5 month): PhD student Helena Gomes went to the Technical University of Denmark to conduct experimental work on the electrokinetic transport of iron nanoparticles (task 4) and on PCB degradation (task 3). This work was carried out under the supervision from Professor Lisbeth Ottosen.

Jan 2014 – March 2014 (3 month): PhD student Helena Gomes went to Malaga University in order to use the experimental data obtained so far as the basis of a model for the electrokinetic transport of nanoparticles, under the supervision from Professor José Miguel Rodriguez-Maroto

These exchange and networking activities resulted in new and stronger ties between the institutions involved. Expansion of international network was also pursued by participation at international conferences (25 presentations) and national conferences (2 presentations) and through short term visits (table 4). These are also related to dissemination of the project and are detailed in section 2.6 of the current report.

2.1.3 Development and implementation of a document management system

A document management system was implemented. This system is described in document DM-01-2012 (annex B) and all documents related to the project are included, comprising external and internal documents (produced within the project) such as analytical procedures, experimental results, reports, budget, exchanged mail, and others. Although not originally predicted in the project's proposal this system allows the systematization of all the information related to the project, and regulates access, providing additional support to project management and to the team, increasing scientific productivity.

2.1.4 Conclusion for task 1

The management of the NanoDC project was not smooth at all, due to the innumerous outside changes and some internal changes. Nevertheless, all the problems arising were dealt with and a solution was found for each. So, all the objectives proposed initially for the management task were fully achieved, and even surpassed at some points.

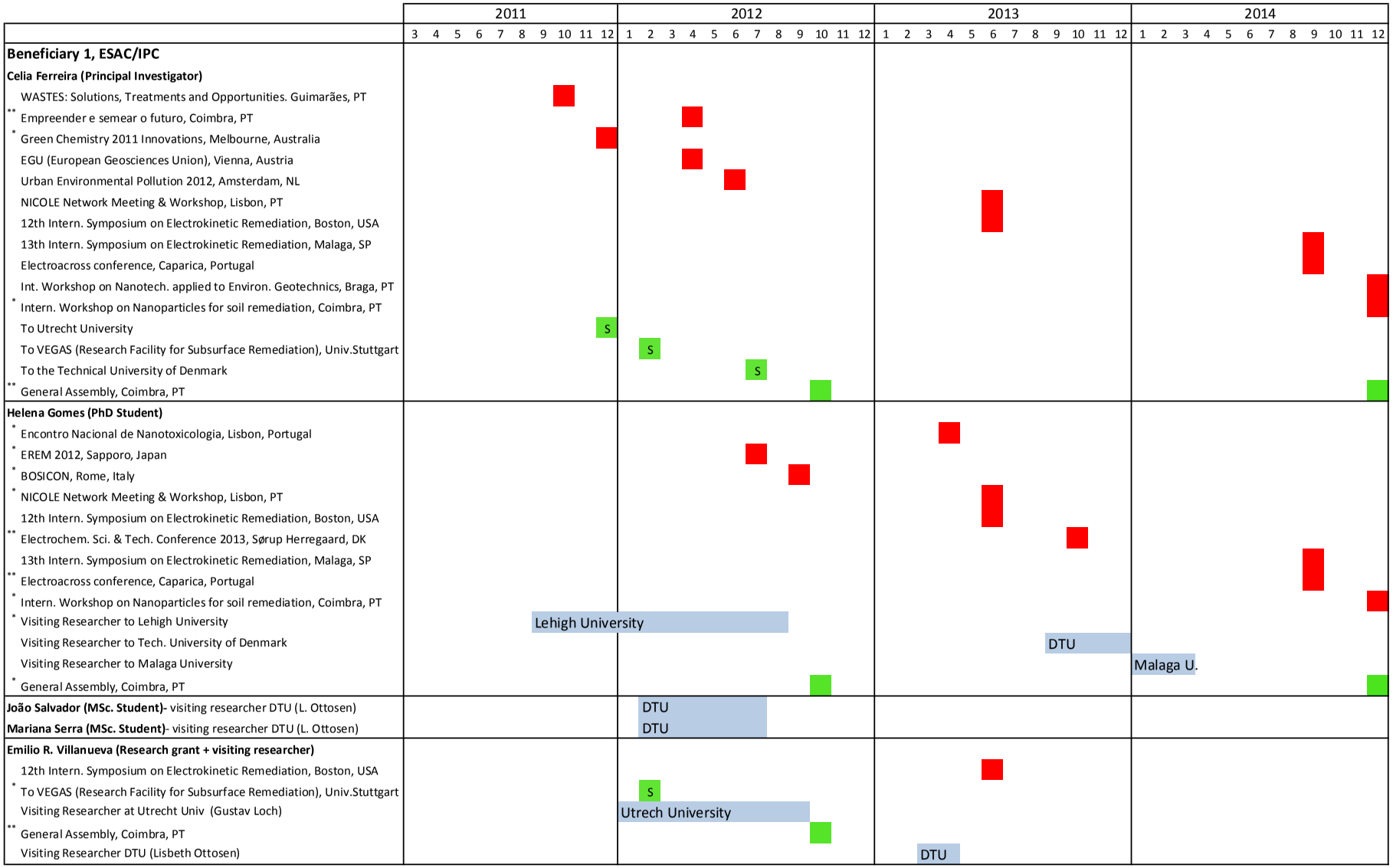


Table 4a: Gantt chart of secondments, exchange visits and dissemination activities (continues on the next page)

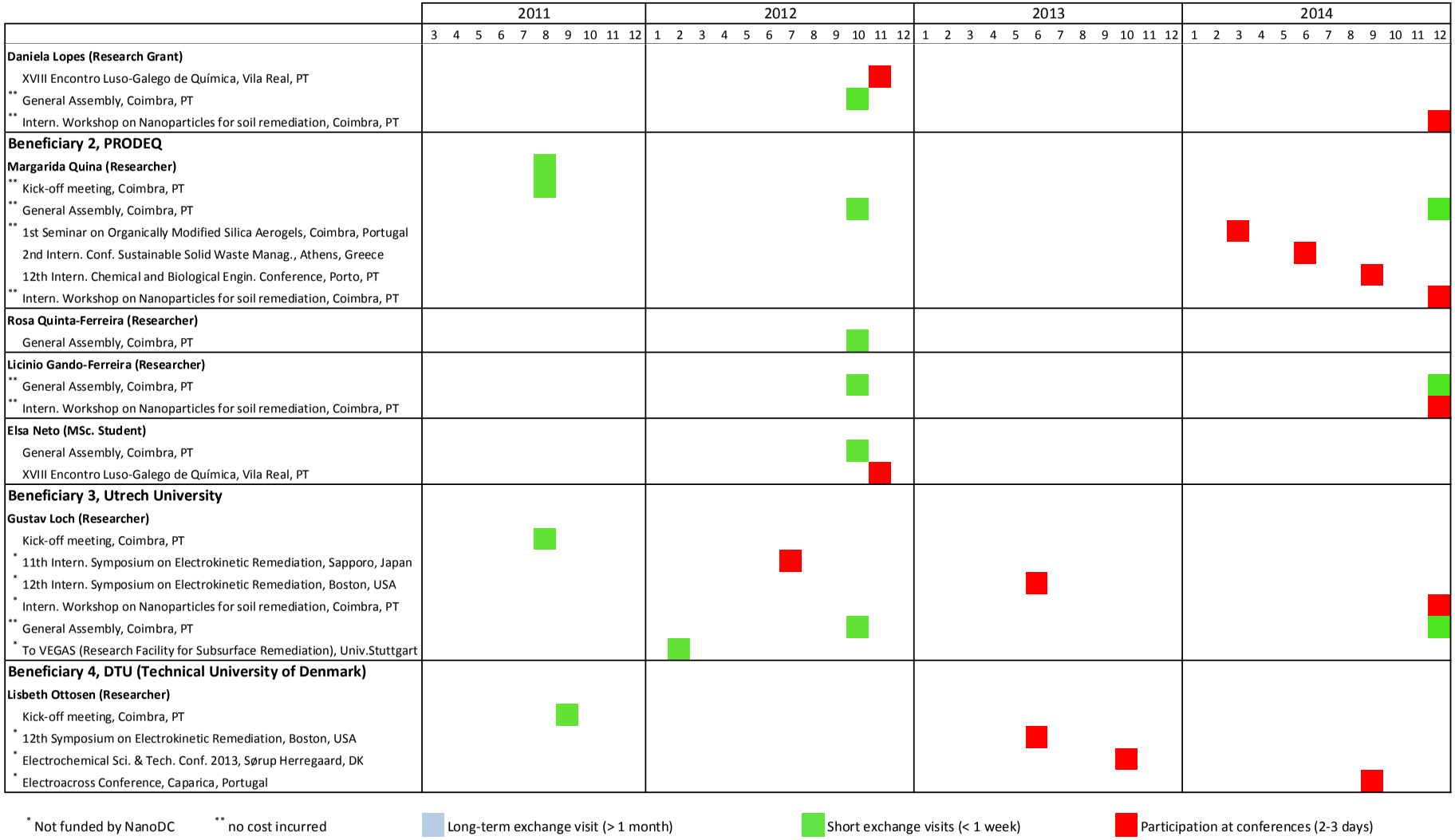


Table 4b: Gantt chart of secondments, exchange visits and dissemination activities (continuation from previous page)

2.2 Sample collection and analysis (Task 2)

This task started at January 2012, as soon as Grant Holder (GH) 2 was hired. The activities carried out were: Development of analytical procedures for PCB analysis; Sample collection and analysis; and, PCB analysis in samples obtained during tasks 3 and 4. These activities are detailed below.

2.2.1 Implementation within the laboratory of analytical procedures to analyze PCBs

This activity took place during the first months and comprised first the extraction PCB from soil using a solvent, then the clean up of the extract, and finally the analysis by Gas Chromatography (GC).

At first, two extraction techniques of PCBs from soils were compared: (a) solvent extraction and (b) QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe). The results indicated that the solvent extraction method is better for extraction of PCBs, and therefore although this technique is more time consuming, it was selected for all subsequent extractions.

Following, an initial simplified procedure for PCB analysis by GC was tried, based on a method proposed on a published paper. However, the recovery rates were low raising doubts about the quality of the results obtained at that point, so a more robust methodology was pursued based on reference procedures from EPA. This second analytical procedure for PCB analysis was subsequently fully implemented. All the procedures developed were fully documented and the following written documents give the necessary operational instructions:

- Experimental procedure for setting calibration curves from PCB analysis in liquid extracts by GC-ECD (document: **OP-02-2012**)
- Experimental calibration curves obtained (document: **RG-06-2012**)
- Experimental procedure for ultrasound extraction and cleanup of PCB's from soil samples (document: **OP-04-2012**)
- Experimental procedure for PCB analysis in liquid extracts by GC-ECD (document: **OP-05-2012**) and associated laboratory procedures (document: **OP-14-2012**)
- Quality manual for PCB analysis (document: **OP-16-2012**)

Some of the supporting documents are presented in annexes C to F.

2.2.2 Soil screening

After establishing the analytical procedures the tasks activities consisted in analyzing soil samples from different locations and land uses with the objective of finding samples with high concentrations of PCB, to be further used in project tasks 3 and 4.

The procedure for sample collection and storage was defined (document: OP-01-2012). Soil samples were collected in vineyards, cornfields and streams and a summary characterization was made regarding pH, water content, organic matter and in some cases, heavymetals. PCB concentrations for several congeners (PCB 52, PCB101, PCB138, PCB153

and PCB 209) were analyzed using the previously established procedures.

It was found that samples of agricultural soils had less than 300 ng/kg of total PCB, while sediments collected at the bottom of an urban stream contained 10 times higher. One sediment sample even reached 21 thousand ng/kg. Nevertheless, the concentrations found for PCBs in the screened samples were very low, as all values were well below the threshold value for contaminated soils, which ranges between 10 and 50 mg kg^{-1} total PCB, depending on the country (UK, Australia, USA, Denmark). Additionally, the soil quality criteria in Denmark is 0.02 mg kg^{-1} total PCB, and all the samples were around or below this quality criteria.

This is a good result, environmentally speaking, as it meant that agricultural soils collected in the region were not contaminated with PCB. But on the other hand it also meant that the project had no real contaminated soils to work with. From the start the team had decided that it would rather not work with spiked soils, as these represent poorly the real historically contaminated soils. So, following what had been decided at the kick-off meeting if such a situation occurred, we moved to industrial sites to get more contaminated samples. In order to prevent additional delays (and costs) instead of screening industrial sites in search for PCB-contaminated soils we followed two approaches: the first was to contact a waste hazardous operator and ask for a sample of contaminated soil; and the second was to collect a soil sample in a location previously identified as contaminated by PCBs. In the first case we got a sample of a mixture of soils from different locations in Portugal were contamination by PCB was suspected; in the second case we collected soil around a decommissioned school in Denmark, where PCB contamination had been identified as a result of the window sealants used in the seventies. In this way we obtained the two samples that were subsequently used in the project in tasks 3 and 4. Table 5 presents the physical and chemical characteristics of the soils.

2.2.3 PCB analysis in experimental samples from tasks 3 and 4

This activity was carried out mostly during 2013 and early 2014, and followed the experimental work carried out in tasks 3 and 4. A large amount of samples have been analyzed and the results were used in the discussion of the results. Calibration curves for PCB analysis were re-made half way.

2.2.4 Conclusion for task 2

Procedures were set for PCB analysis by Gas Chromatography, sample collection and storage, extraction of PCBs from solid samples, and clean up of extracts.

Agricultural soils had less than 300 ng $kg^{-1}$of total PCB, while sediments collected at the bottom of an urban stream contained 10 times higher. Nevertheless, the concentrations found for PCBs in the screened samples were very low, as all values were well below the threshold value for contaminated soils, which ranges between 10 and 50 mg kg^{-1} total PCB. So two additional soil samples were collected from contaminate sites, with PCB concentration of 258 ± 24 µg kg^{-1} and 156±2 µg kg^{-1} and these samples were further used in subsequent tasks.

Table 5 – Physical and chemical characteristics of the soils

Parameter	Soil 1	Soil 2
Particles distribution (%)		
Coarse sand (200 < Ø < 2000 µm)	19.1	3.2
Fine sand (20 < Ø < 200 µm)	67.3	69.6
Silt (2 < Ø < 20 µm)	12.7	23.6
Clay (Ø < 2 µm)	0.9	3.6
Textural classification	Loamy sand	Silty loam
pH (H_2O)	12.2	8.20
Conductivity (mS cm^{-1})	18.76	0.221
Exchangeable cations ($cmol_{(c)}$ kg^{-1})		
Ca^{2+}	83.75	259.14
Mg^{2+}	3.2	9.75
K^{+}	26.88	7.36
Na^{+}	9.37	8.34
Sum of exchangeable cations ($cmol_{(c)}$ kg^{-1})	123.2	284.59
Calcium carbonate (%)	18.0	1.3
Organic matter (%)	16.46	0.57
PCBs[a] (µg kg^{-1})	258 ± 24	156 ± 2
Metals[b] (mg kg^{-1})		
Al	20980 ± 590	4952± 71
As	9 ± 2	0.6 ± 0.97
Cd	0.7 ± 0.1	0.4 ± 0.04
Cr	52 ± 3	2.5 ± 0.04
Cu	142± 95	10 ± 0.3
Fe	13162 ± 301	6773± 97
Ni	32± 1	6 ± 0.3
Pb	45± 3	25 ± 0.9
Zn	2155 ± 40	135 ± 0.1

[a] Sum of PCB 52, 65, 101, 138, 153, 180, 204 and 209

[b] Acid digestion with HNO_3 according to the Danish Standard DS259.

2.3 Degradation Studies (Task 3)

This task aimed at understanding the degradation of organic pollutants, mostly PCB, by iron nanoparticles. The underlying reaction is a reductive dechlorination, in which the iron nanoparticles remove chlorine atoms from the molecule of chlorinated organic pollutants, reducing it, and in turn becoming turn oxidized. In general, the dechlorination can be expressed by the following reaction equation

$$C_xH_yCl_z + zH^+ + zFe^0 \rightarrow C_xH_{y+z} + zFe^{2+} + zCl^-$$

in which iron acts as a reductant (electron donor) for the removal of chlorine.

At the initial stage of this task the team made an overview of the types of iron nanoparticles that could be used to achieve reductive dechlorination and established their synthesis procedure. This was followed by the characterization of the synthesized nanoparticles. At the last stage the nanoparticles were used in degradation studies of PCBs, and other pollutants such molinate (pesticide) and dyes. These activities are detailed next. The work carried out throughout this task is quite extensive and was the object of the six following papers (the first 4 already published or accepted for publication):

1) Assessment of combined electro-nano remediation of molinate contaminated soil. Science of the total environment, 493, 178–184
10.1016/j.scitotenv.2014.05.112

2) Electrodialytic remediation of PCB contaminated soil with iron nanoparticles and two different surfactants. Journal of Colloid and Interface Science, vol 433, 189-195 10.1016/j.jcis.2014.07.022

3) Treatment of a suspension of PCB contaminated soil using iron nanoparticles and electric current. Journal of Environmental Management, 1-6 http://dx.doi.org/10.1016/j.jenvman.2015.01.015

4) Electroremediation of PCB contaminated soil combined with iron nanoparticles: Effect of the soil type (accepted Chemosphere)

5) Emilio Rosales, Celia Dias-Ferreira, M. Pazos, M.A. Sanromán. Green zero-valent iron nanoparticles from aromatic herbal extracts as catalyst in Fenton degradation of dyes (to be submitted Feb 2015).

6) Emilio Rosales, M. Ángeles Sanromán, Celia Dias-Ferreira. Green zero-valent iron nanoparticles synthesized using herbal tisanes (to be submitted Feb 2015).

2.3.1 Nanoparticle synthesis

Different types of iron nanoparticles (Fe^0) were synthetized in aqueous solution through iron salts reduction. The first is based on the reduction by sodium borohydride in which iron nanoparticles without coating are produced (Fe^{BARE}). The second is also based on the

reduction by sodium borohydride, but in which nanoparticles are no longer bare but have polymeric coating of PAA (polyacrylic acid), Fe^{PAA}. This second type of nanoparticles is more stable, as the coating provides a repulsive layer that prevents agglomeration.

An alternative synthesis method based on green tea extracts was also performed, in which green tea polyphenols are both the reducing and capping agent, leading to a third type of nanoparticels Fe^{GREEN} . The extract of green tee has lesser toxicity that the previously considered (sodium borohydride) and is more environmentally friendly.

Operational conditions of nanoparticle synthesis have been tuned, aiming at obtaining a stabilised suspension that does not precipitate. In this respect we have selected the molecular weight of the PAA that leads to a more stabilized suspension of iron nanoparticles. The speed and duration of agitation during synthesis are examples of other parameters that have been optimized. The final procedure used for the synthesis of three types of iron nanoparticles are described in the respective operational procedures documents that are part of the document management structure. Some pictures of the synthesis procedure are shown in Figure 1.

Later on, the synthesis of new "Green" nanoparticle particles was carried out using other extracts in addition to Green Tea (*Camellia sinensis*), such as Rooibos (*Aspalathus linearis*), Lemon verbena (*Aloysia citrodora*) and Camphora leaves (*Cinnamomum camphora*).

The extraction of the desired compounds (polyphenols) present in the leaves has been performed using different configurations: extraction using boiling water (decoction) and extraction using infusion. The best results were obtained when the extraction using boiling water was employed, so this was the selected method.

The properties of the leaf extracts have been analyzed and different parameters that affect to the synthesis processes have been determined (Table 6). The leaves' extracts tested contain significant values of polyphenols. The quantity of polyphenols decreased from Green tea > Rooibos > Lemon verbena > Camphora extracts. Additionally, two commercial samples were also obtained (From NanoIron, s.r.l.) and used in the trials, for comparison purposes.

Table 6 - Total phenolic content of the extracts used in the nanoparticle synthesis (source: Rosales et al. 2015a)

Sample		pH	Phenol index (280 nm)	Total phenolic content [a]	Syn-thesis	Reactivity, H_2 generation (g nanoparticle/g)
Fe^{Green}	Green Tea (*Camellia sinensis*)	5.27	75.36	1,003.61 ±19.22	+	32.41 %
$Fe^{Rooibos}$	Rooibos (*Aspalathus linearis*)	4.87	49.44	234.36 ±43.49	+	43.47 %
Fe^{Lemon}	Lemon verbena (*Aloysia citrodora*)	7.14	43.08	191.57 ±16.50	+	21.52 %
$Fe^{Camphora}$	Camphora (*Cinnamomum camphora*)	5.26	15.6	44.39 ±8.25	-	

[a] mg Gallic Acid equivalent/L + successful synthesis - failed synthesis

Figure 1 – different aspect of the laboratory synthesis of zero-valent iron nanoparticles.

2.3.2 Nanoparticle characterization

After synthesis, the nanoparticles were characterized regarding the most relevant properties affecting iron nanoparticle's behavior in the presence of pollutants. Namely, size distribution (using dynamic light scattering - DLS), morphology (using transmission electron microscopy - TEM), surface area (BET) and reactivity (using the hydrogen generation method) were determined.

The results show that conventional bare Fe^{BARE} nanoparticles were aggregated due to their magnetic properties, forming big chain-like structures, whereas each nanoparticle is the base unit of the bulk structure attached to others nanoparticles (Fig. 2a).

Fe^{GREEN} presented a dispersed, spherical shape (Fig. 2b), which means that the tea

polyphenols avoid the aggregation. This fact reveals an important characteristic of these environmentally green nanoparticles, since they seem to be able to overpass the difficult aggregative character of the conventional magnetic iron particles. Spherical Fe^{PAA} are also dispersed, suggesting that the polyacrylic acid polymer coating helped to stabilize the suspension, preventing nanoparticle agglomeration (Fig. 2c).

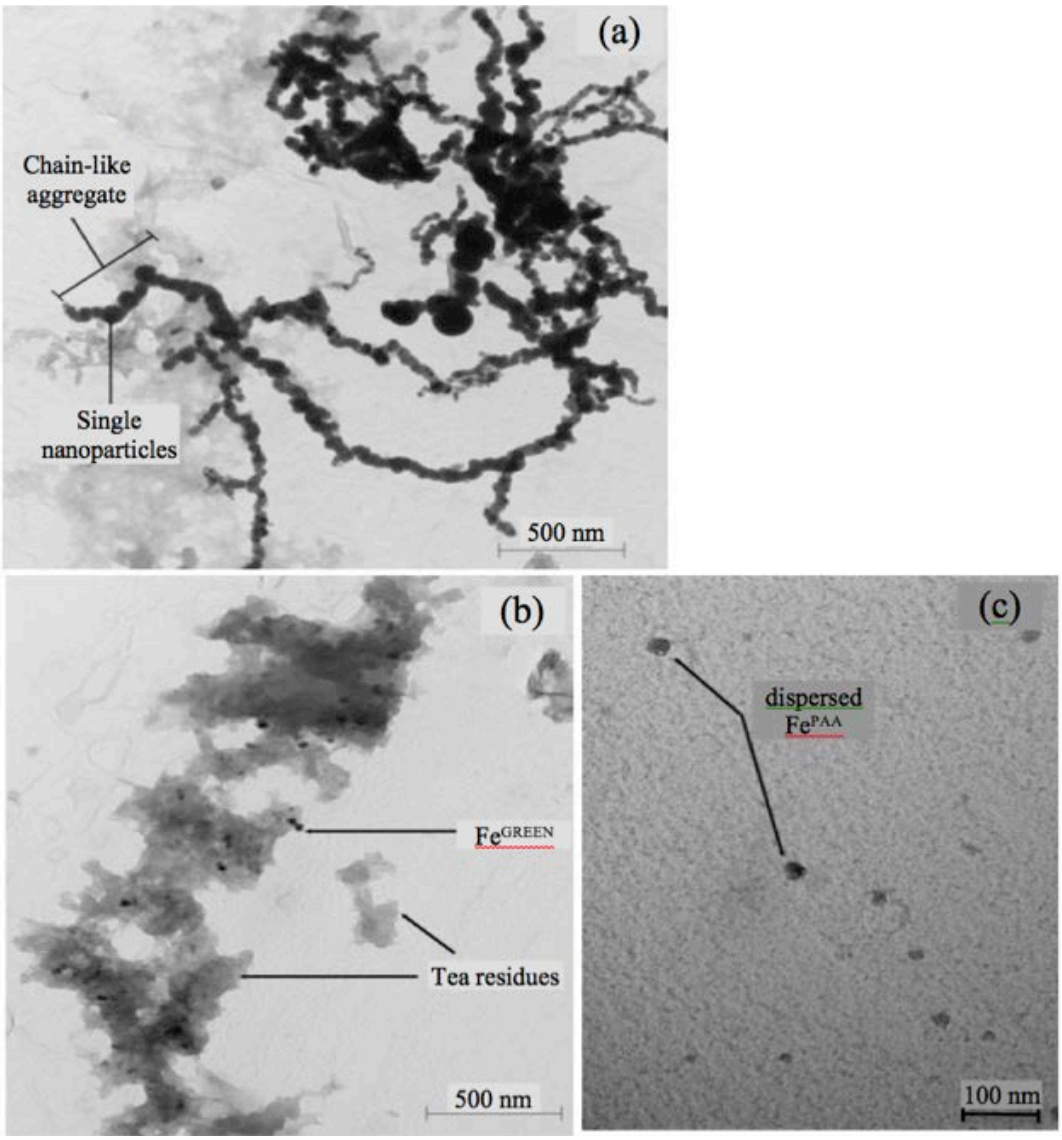


Figure 2 - TEM images of the three types of Fe^0 produced: (a) Fe^{BARE}, (b) Fe^{GREEN} and (c) Fe^{PAA}.

Average particle size and surface areas are shown in table 7. All the Fe^{PAA} synthesized were in the nano-domain and approximately 90 % of the nanoparticles showed to be smaller than 31 nm (Fig. 3c). 90 % of Fe^{BARE} have diameters smaller than 102.5 nm (Fig. 3a) and the mean particle size diameter is 51.9 ± 33.2 nm. On the other hand, Fe^{GREEN} presented a smaller range of size distribution, where all particles are lower than 100 nm and around 90 % are smaller than 59.7 nm (Fig. 3b).

Table 7 - Particle characterization

	TEM		S_{BET} (m^2/g)	pH	average zeta potential (mV)
	Particles counted	average diameter (nm)			
Fe^{BARE}	155	51.9 ± 33.2	46.94	5.7-6.8	32.1± 0.2
Fe^{GREEN}	90	33.8 ± 18.4	0.939	4.1-4.2	24.0± 1.7
Fe^{PAA}	104	25.7 ± 9.7	29.78	5.7-6.0	13.2± 0.1

The average zeta-potential and pH of Fe^{BARE}, Fe^{GREEN} and Fe^{PAA} suspensions are shown in table 7. Zeta-potential (ξ) is a crucial parameter because it relates to the level of aggregation between particles. As a rule of thumb, zeta-potential values should be higher than ± 30 mV to guarantee stable suspensions. In pH ranges close to the point of zero charge (PZC) the electrostatic repulsion decreases and there is a higher aggregation tendency. To measure the zeta potential it is important that mixing conditions are adequate so that consecutive samples of the same suspension are representative and the results consistent. Too much stirring can promote aggregation of particles and not enough stirring makes the sample subject to zeta potential measurements highly variable. Different mechanical stirring velocities were tested with the Fe^{BARE} particles. Fig. 4a shows how the stirring velocity of the suspension during sampling can influence the results of four consecutive measurements of the zeta-potential value. The higher reproducibility of results obtained at 80 rpm lead to the selection of this stirring velocity to carry out zeta-potential measurements for the remaining Fe^0 (Fig. 4b).
Considering only the electrostatic stabilization, Fe^{BARE} nanoparticles seem to be more stable than the other nanoparticles, because the ξ value is greater than +30 mV. However, Fe^{PAA} and Fe^{GREEN} might additionally present steric stabilization due to the coating and therefor the zeta potential is not the only parameter to consider when assessing nanoparticle stability. Figure 5 shows the evolution of the zeta-potential for Fe^{Bare}.

DLS measurements revealed to be difficult to carry out with these iron nanoparticles due to their strong tendency to agglomerate and the use of TEM was found to be indispensable to scrutinize the nanoparticles size distribution and morphology, preferable to DLS.

As nanoparticles age, a layer of oxides is formed on the surface. The smaller the amount of oxides, the more reactive nanoparticles are because more Fe^0 is available at the surface to reduce pollutants. When the metallic iron reacts with HCl, the H_2 released is directly proportional with the zero-valent iron content in the nanoparticles. Hence by using the amount of hydrogen gas generated it is possible to quantify the reactivity of a suspension of Fe^0 and its evolution over time, and estimate nanoparticle's behaviour during environmental applications. Figure 6 shows the aging of Fe^{BARE} over time. A 40% reduction in the reactivity of Fe^{BARE} was seen after 1.5 month.

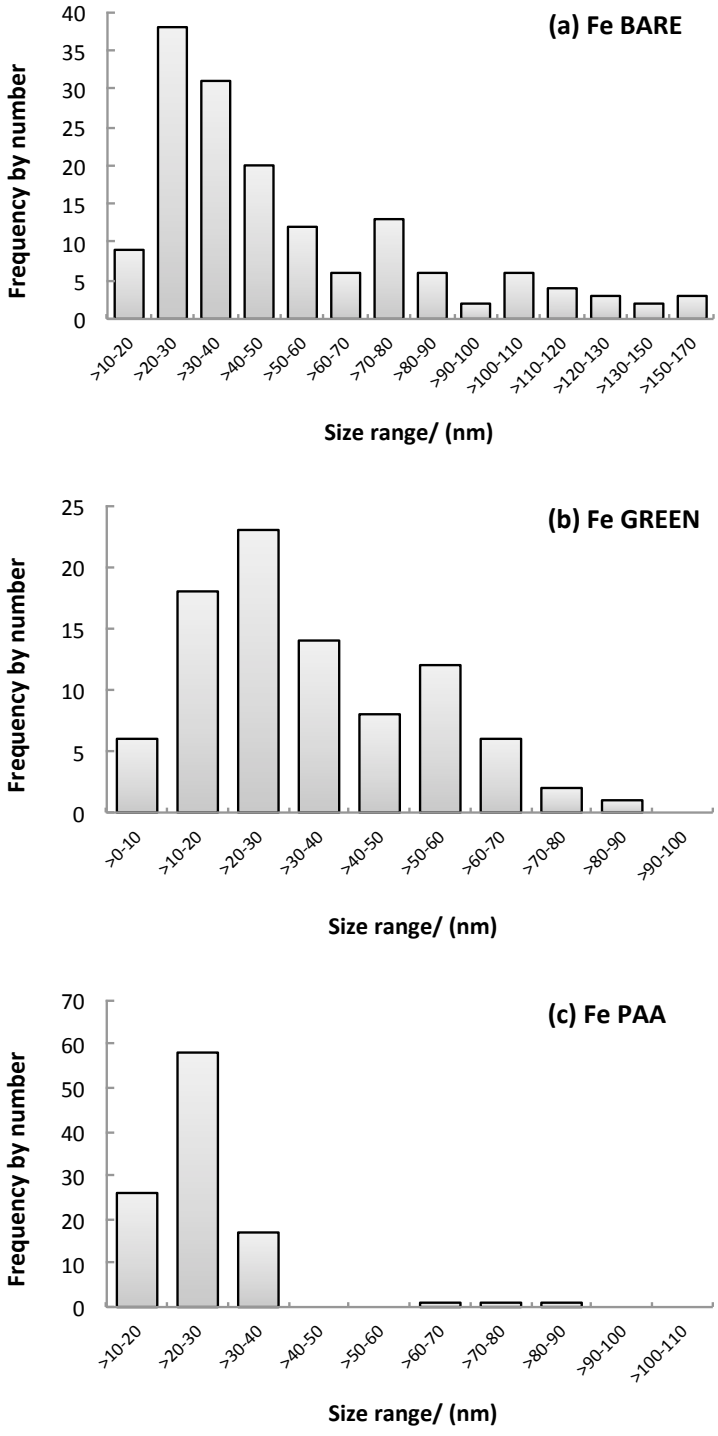


Figure 3 - Size distribution histogram according to size measurements by TEM for: (a) Fe^{BARE} by counting 155 particles, (b) Fe^{GREEN} by counting 90 particles and (c) Fe^{PAA} by counting 104 particles.

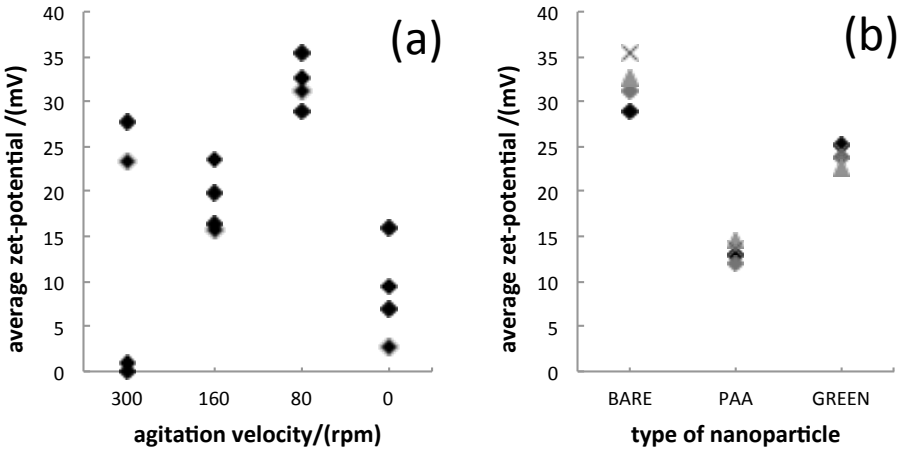


Figure 4 - Zeta-potential reproducibility measurements for: (a) Fe^{BARE} at 300 rpm, 160 rpm, 80 rpm and 0 rpm; (b) Fe^{BARE}, Fe^{GREEN} and Fe^{PAA}, at 80 rpm.

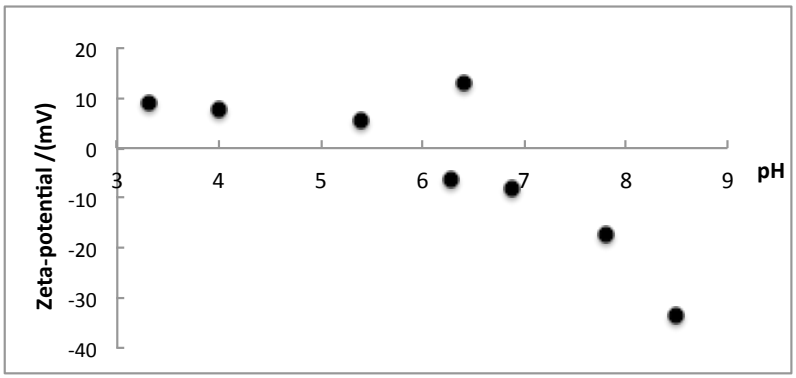


Figure 5 - Variation of zeta-potential with pH for Fe^{BARE}

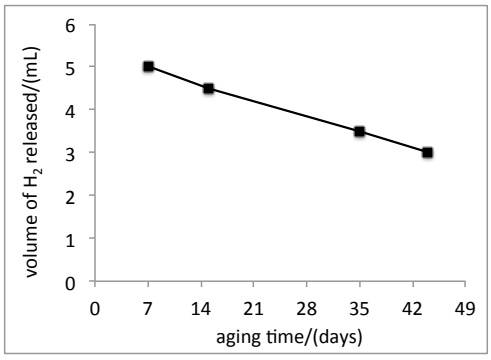


Figure 6 - Reactivity test: evolution of H_2 gas released and iron oxides content for Fe^{BARE} during 44 days.

The initial ratio of H_2 released per mass of nanoparticles to Fe^{GREEN} and Fe^{PAA} were, respectively, 0.26 mL/mg and 0.18 mL/mg, which means that for the same mass of nanoparticles, Fe^{BARE} are the ones with higher amount of zero-valent iron, and are therefore the most reactive. This is because the coatings in Fe^{GREEN} and Fe^{PAA} contribute to the total weight of these nanoparticles and it might also be that the coating protects the inner Fe^0 from corrosion. The hydrogen generation taken with freshly synthesised nanoparticles provided therefore a relative measure of the weight percentage of iron versus coating. Used along time and can be used to assess nanoparticle aging.

The same testing procedure was used when assessing the reactivity of different "green" nanoparticles, such as $Fe^{Rooibos}$, Fe^{Lemon}, $Fe^{Camphora}$, and the results are shown in table 6 above. The nanoparticle obtained from Rooibos extract showed the best reactivity.

2.3.3 Degradation of pollutants by iron nanoparticles

Several experiments were conducted to assess the degradation of PCB in contaminated soils by iron nanoparticles under different experimental conditions:
- With/without surfactants (saponin, Tween 80)
- With different voltage gradients
- With different concentrations of nanoparticles
- With different setups (in suspension, 2-chamber, 3-chamber)
- Different remediation times.
- With different contaminated soils
- With different pollutants (molinate and PCB and dyes)
- With "green" nanoparticles and conventional nanoparticles

Due to the large extent of data produced during this task (and presented in the articles/manuscripts referred above) this section is divided into the subsections "degradation of molinate", "degradation of PCB", and "degradation of dyes", which are detailed below.

2.3.3.1 Degradation of molinate

The first degradation experiments were carried out with molinate, which is a pesticide widely used for weed control in rice paddies. Due to its water solubility and affinity to organic matter, it is a contaminant of concern in ground and surface waters, soils and sediments. In NanoDC the matrix under study was soil, and it was the first time that molinate degradation by zero valent iron nanoparticles was done in soils. Soil is a highly complex matrix, and pollutant partitioning between soil and water and its degradation rates in different matrices is quite challenging.

A system combining iron nanoparticles and electrokinetics (EK) was set up and the experimental cell is shown in figure 7. The cell is divided into three compartments, consisting of two electrode compartments (L = 7.46 cm, internal diameter = 8 cm) and a central one (L = 4 cm, internal diameter = 8 cm), in which the soil, saturated with deionized water, is placed. This central compartment, made of Plexiglas, was equipped with an injection reservoir (L = 1 cm) for the iron nanoparticles, separated with a 1 mm nylon mesh and a low speed filter paper. A set of five cellulose filters, previously tested and known to work as passive membranes (Whatman filter paper), were used to separate the soil from the electrolytes. The soil section near the cathode is a non-spiked S1 soil in order to assess molinate transport towards the cathode.
Five different laboratory experiments (A–E) were carried out and the variables considered were: i) the type of soil (two different soils with different textures, cation exchange capacities and organic matter contents), ii) pH control as an EK enhancement method, and iii) control experiment, without electric current.

Only residual amounts were found in the soil at the end of the experiments. Results show that molinate can be degraded by nanoparticles in soils, even though the process is more time demanding. With electrokinetics, molinate can be removed from soil to an aqueous solution, and with iron nanoparticles, molinate can be further degraded in situ. The major advantage of the simultaneous use of both methods is the molinate degradation instead of its accumulation in the catholyte.

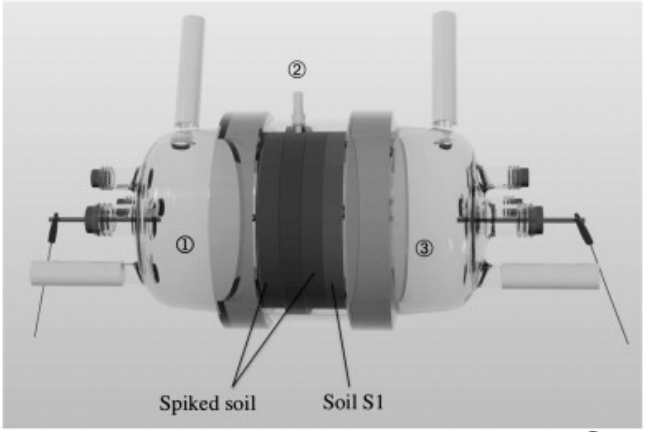
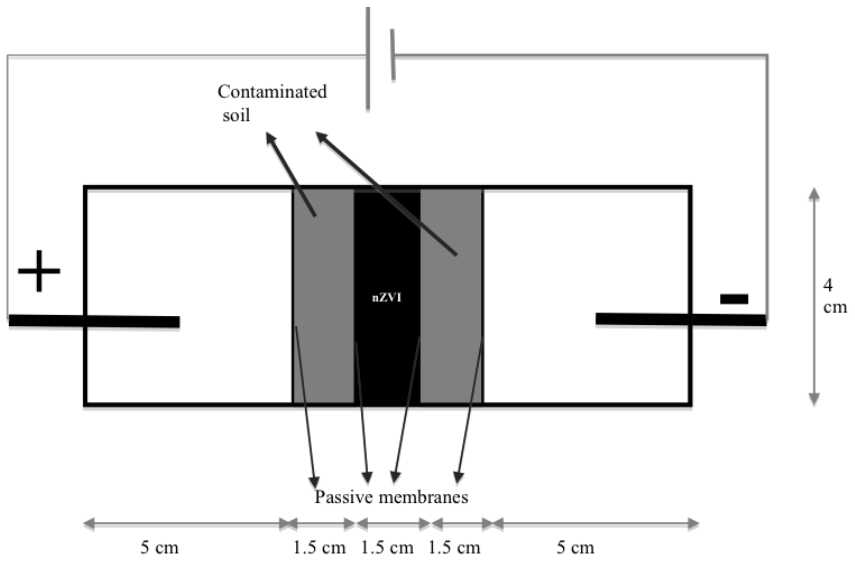


Figure 7 - Schematic representation of the laboratory cell. Legend: ① Anode compartment; ② reservoir for the iron nanoparticles injection; ③ cathode compartment. The separation between the soil and the compartments containing liquids was made through passive membranes (filter paper) (source: Gomes et al 2014a)

2.3.3.2 Degradation of PCB using iron nanoparticles

The first experiments with the pollutant PCB were carried out using a new laboratory cell design (A) proposed for electrodialytic remediation and iron nanoparticles. This new cell was compared to the conventional electrokinetic cell design (B) (figure 8). An historically contaminated soil (soil 1, described in task 2) with an initial PCB concentration of 258 μg kg^{-1} was treated during 5, 10, 20 and 45 d using different amounts of iron nanoparticles in both cells. The results are shown in figure 9. A PCB removal of 83% was obtained in setup A compared with 58% of setup B. Setup A also showed additional advantages, such as a higher PCB dechlorination, in a shorter time, with lower nanoparticle consumption, and with the use of half of the voltage gradient when compared with the traditional setup (B). Energy and nanoparticle costs for a full-scale reactor were estimated at 72 € for each cubic meter of PCB contaminated soil treated on-site, making this technology competitive when compared with average off-site incineration (885 € m^{-3}) or landfilling (231 € m^{-3}) cost in Europe and in the USA (327 USD m^{-3})

(a)



(b)

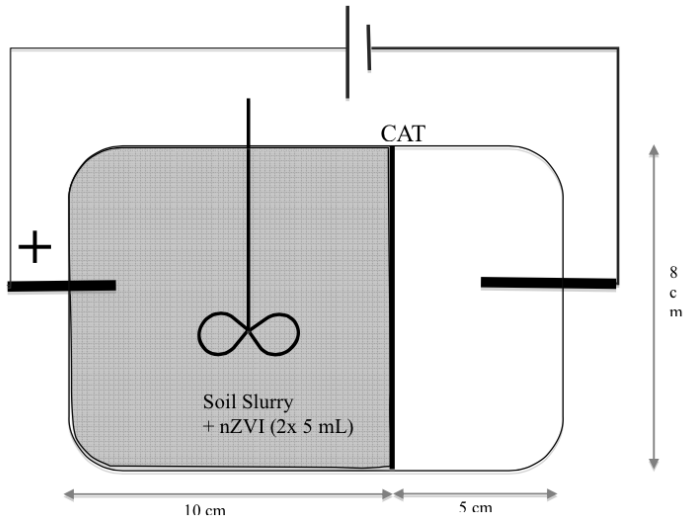


Figure 8 – Schematic representation of the experimental setups used in the experiments: a) three-compartment electrokinetic cell and b) two-compartment electrodialytic cell (CAT = cation exchange membrane) (source: Gomes et al. 2015b)

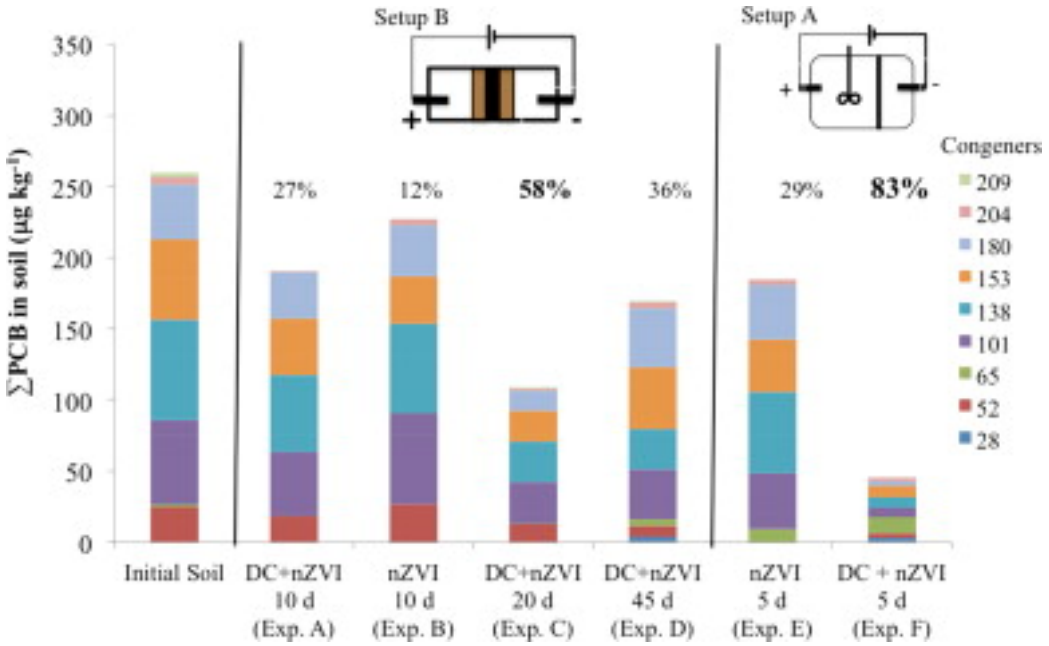


Figure 9 - Average concentration of the sum of PCB congeners (PCB28, 52, 65, 101, 138, 153, 180, 204 and 209) in soil before and after the experiments using conventional electrokinetics (setup B) and the new electrodialytic setup (A). Percentages on the top of each column represent PCB removal regarding the sum of congeners analyzed in the initial soil. PCB – polychlorinated biphenyls, DC – Direct Current, nZVI – zero valent iron nanoparticles (source: Gomes et al. 2014c)

Another set of experiments similar were carried out with a superficial soil from a decommissioned school where PCB were used as windows sealants (soil 2, described in task 2) aiming at comparing the PCB degradation by iron nanoparticles in both soils.
The results show higher PCB removal for Soil 1 (loamy sand with highest pH, carbonate content, organic matter and PCB concentrations) with the electrodialytic two-compartment cell, while for Soil 2 (silty loam with highest sum of exchangeable cations) only the nanoparticle addition in the three compartments cell allowed to obtain the highest removal percentage. The use of the two-compartment cell allowed a uniform distribution of iron nanoparticles through the soil, while in the three-compartments cell, there was accumulation in the injection reservoir.

The results show that the soil characteristics, like pH and buffer capacity, are important and affect the reaction between nanoparticle and the target contaminant. The effects of soil composition are relevant, especially the soil buffer capacity and the carbonate content, which can neutralize the acid front generated at the anode. Soil texture is also relevant for electroremediation and nanoparticle transport in EKR/nanoparticle, as the soil particle distribution and their charge affect the transport mechanisms. The soil cation exchange capacity allows the soil to immobilize significant quantities of heavy metal ions while the organic matter content can strongly influence the sorption/desorption of contaminants

and it was also shown to affect the electroosmotic properties and ionic modification of soils.

Based on the results obtained the degradation pathways of PCB 138 and PCB180 were proposed (figure 10)

The final set of experiments aimed at testing the effect of two different surfactants (saponin and Tween 80) to enhance PCB desorption and removal from soils.

Higher chlorinated PCB congeners (penta, hexa, hepta and octachlorobiphenyl) showed removal percentages between 9% and 96%, and the congeners with highest removal were PCB138, PCB153 and PCB180. The use of low-level direct current enhanced PCB removal, especially with saponin.

2.3.3.3 Degradation of dyes using green-synthesis nanoparticles

The degradation experiments described so far in sections iii-1 and iii-2 were carried out using conventional iron nanoparticles. This section shows the results of the performance evaluaiton of the nanoparticles obtained through a green-synthesis process based on plant extracts, which were developed during the Project NanoDC.

The degradation studies were carried out using the RB5 dye (Reactive black 5), which represented a model pollutant. The advantage of using this dye is the facility of analysis compared to PCBs, as it can be easily determined by spectrophotometry (in the visible range)

Experiments were carried out in a cylindrical reactor with a working volume of 0.1 L. and with three types of green nanoparticles: Fe^{Green} (Green Tea - *Camellia sinensis)*, $Fe^{Rooibos}$ (Rooibos - *Aspalathus linearis*); Fe^{Lemon} (Lemon verbena - *Aloysia citrodora*). Nanoparticles synthesized using Camphora extracts (*Cinnamomum camphora*) were not used due to the low synthesis results obtained (see section i, above).

The RB5 dye content was measured spectrophotometrically (based on the constructed calibration curves at maximum absorption wavelength). The decolourisation kinetics were studied and the regression coefficients for zero, first and second-order reactions were calculated. According to the results obtained the highest performance was obtained with $Fe^{Rooibos}$, reaching values near 80%. Both Fe^{Lemon} and Fe^{Green} showed a decolourization degree of approximately 60%. Following, the degradation of RB5 dye using $Fe^{Rooibos}$ was tested at different pH values. As can be seen in figure 11, there is a marked increase of the dye degradation at lower pH values. Working at pH 4.5, the decolourisation efficiency reached values near of 90% after 60 minutes.

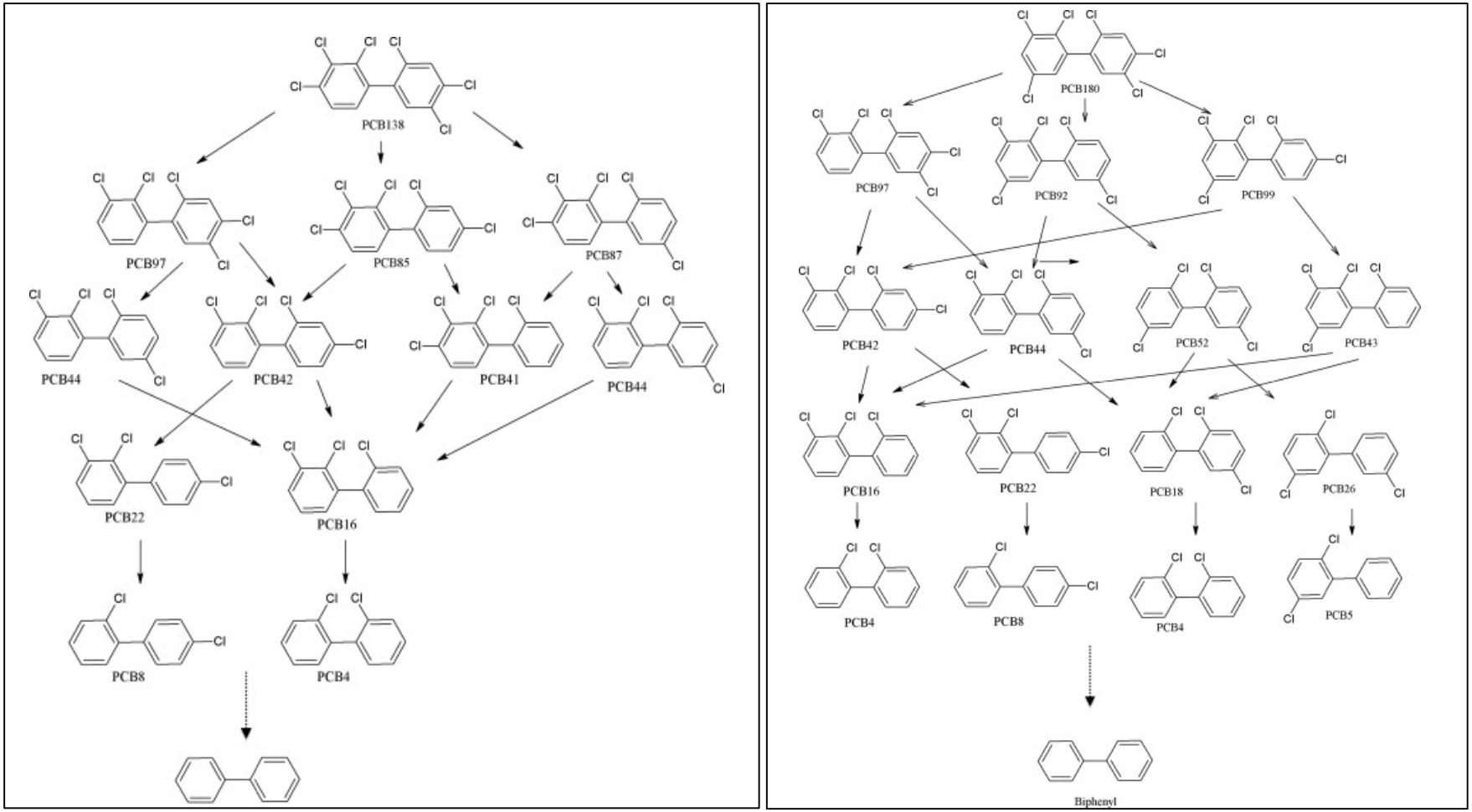


Figure 10 – Degradation pathways through dechlorination proposed for (a) PCB138; (b) PCB180 (source: Gomes et al. 2014b)

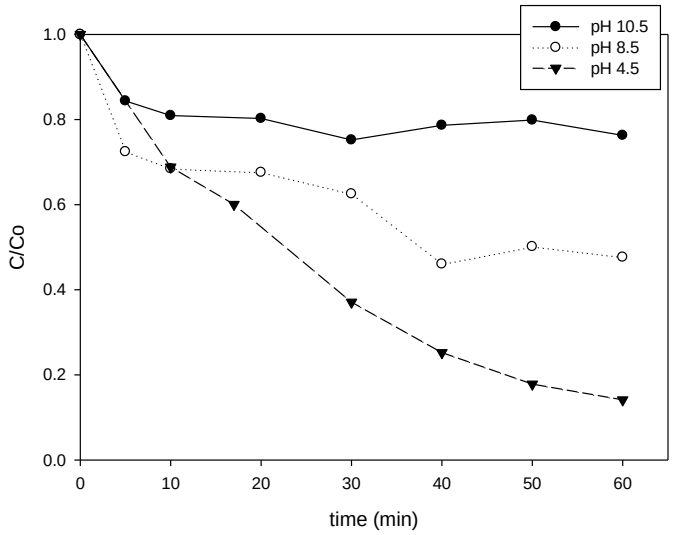


Figure 11 - Decolourisation of RB5 (0.05 g/L) at different pH using $Fe^{Rooibos}$ nanoparticles (source: Rosales et al. 2015a)

The decolourisation kinetic parameters were studied working at the optimum pH values. The rate constant values and the statistical correlation parameters are shown in Table 8. The results indicated that the decolourisation of RB5 at pH 4.5 could be quantitatively described by a first-order kinetic equation (eq. 1) with respect to the dye concentration.

$$dC/dt = - k \cdot C \qquad \text{(eq. 1)}$$

where C, concentration of dye (mg L^{-1}); t, reaction time (min); k, kinetic coefficient for the first order reaction (min^{-1}).

Table 8 – kinetic parameters for the degradation of RB5 dye (source: Rosales et al. 2015a)

pH	Kinetics	K	r^2
4.5	Zero order	0.0177	0.9624
	First order	0.0315	0.9934
	Second order	0.0105	0.8259

This study was afterwards extended to other plant extracts, and the results obtained are shown in figure 12, indicating removal efficiencies higher than 90% for some green nanoparticles.

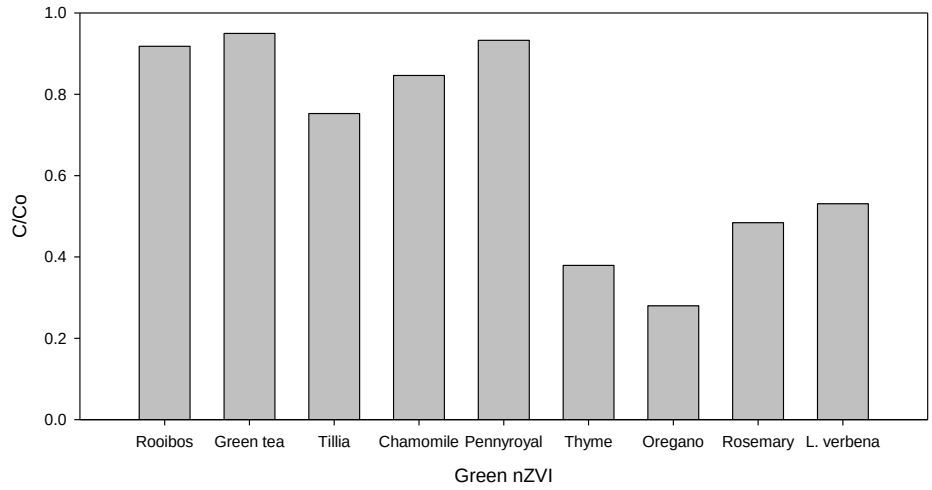


Figure 12 - Degradation of Reactive black 5 using different synthesized green nanoparticles after 30 min (source: Rosales et al 2015b)

2.3.4 Conclusions for task 3

Degradation of the 3 groups of pollutants using iron nanoparticles carried out throughout task 3 showed promising results. Degradation of pollutants by iron nanoparticles prior to the NanoDC Project had been reported only in solution. During NanoDC, degradation of the soil-sorbed fraction of molinate and PCB was carried out for the first time. The results show that these pollutants can be degraded by iron nanoparticles in soils, even though the process is more time demanding than in aqueous solution. An overall PCB removal of 83% from soils could be achieved in 5 days and for molinate only residual amounts were found in soil after treatment. Electrodechlorination of PCB with surfactants and nanoparticle showed encouraging tendencies, especially when using saponin, and as a result of NanoDC project a base is formed towards a new method for remediation of PCB polluted soils.

In addition, Rooibos and other plant extract showed potential to be applied to the "green" synthesis of iron nanoparticles for environmental remediation. The new synthesis process of iron nanoparticles using plant extracts is more environmentally friendly and, when applied to the removal of dyes in solution, decolourisarion efficiency reached values above 90% after 30 minutes.

2.4 Electrokinetic transport (Task 4)

Electrokinetic transport involves two different transport mechanisms, namely electroosmosis and electrophoresis.

For the study of the electro-osmosis transport mechanism a small-scale laboratory prototype was developed where experiments were carried out (figure 13). For the study of electrophoretic transport we have used pre-existing laboratory cells from the different partners involved as shown in figure 14. Following, a large-scale prototype was also developed (figure 15), for additional experiments.

2.4.1 Small scale prototype

The laboratory prototype to measure electroosmotic transport of nanoparticles was developed based on previous apparatus existing at partner Utrecht University, but which was not adequate for use with nanoparticles.

To develop the laboratory prototype three different designs were proposed and tested, labeled A, B and C (see figure 13).

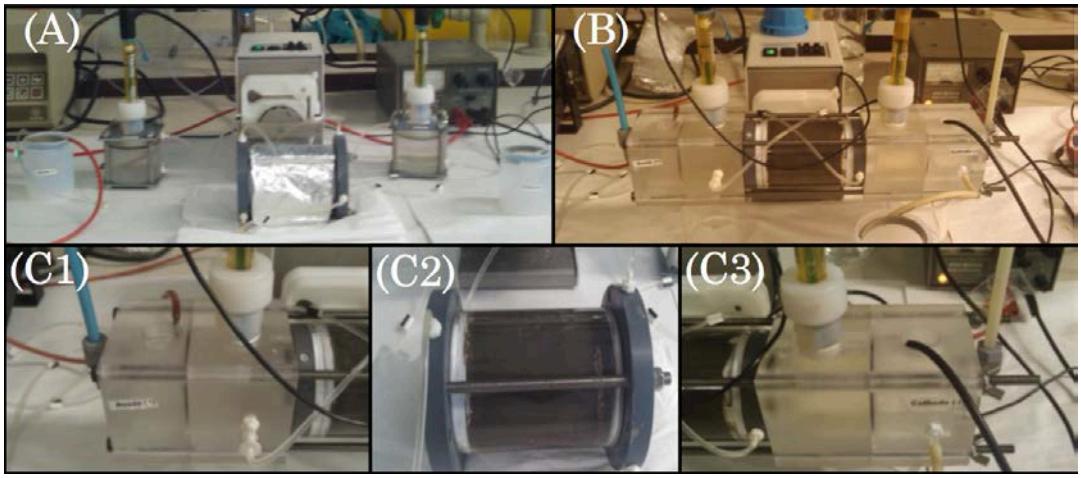


Figure 13 – Experimental designs (A, B and C) for the laboratory prototype to measure electroosmotic transport of nanoparticles in soils. For prototype C, C1 represents the anode compartment, C2 the middle compartment and C3 the cathode compartment.

Prototype C was selected the best suited to study the transport mechanism of nanoparticles by electroosmosis. In the design of this prototype several factors were considered, such as pH control, generation of O_2 and H_2 at the electrodes, side reactions, and capacity to measure and control electric and hydraulic parameters. This new prototype designs include two new reservoirs: one for injecting and and other for sampling the nanoparticles. Control of voltage applied to the prototype and the system to measure operational parameters (voltage, pH, current, volume of water transported due to the electroosmotic transport) have been considered and included. The

distinguishing features of this prototype are described below:

- The pumps and the reservoir that are connected at each side of the cell allow to keep the hydraulic level constant and moreover, to control pH in anode and cathode.

- Anode: Electrode Pt. Open system to allow exit of generated oxygen and prevent its movement to the clay.

- Reference electrodes (Ag/AgCl) were included, to allow measuring the real potential difference across the clay sample by excluding the effect due to the polarization. Reference electrodes are located in the extra reservoirs adjacent to the soil.

- Nanoparticle's reservoir: separated from the clay using a porous stone and a paper filter (D < 500 nm). One of the reference electrodes is located here. Between this reservoir and the clay, a paper filter is placed, to prevent transport of soil particles into the reservoir.

- Sampling reservoir: separated from clay by porous stone. Here is placed the other reference electrode. A filter paper separates this compartment from the cathode.

2.4.2 Existing laboratory apparatus

Figure 14 represents the laboratory apparatus available already at the partners and at the collaborating institutions used during this task.

Figure 14-a depicts a modified commercial electrophoretic (EP) cell (Econo-SubmarineGel Unit, model SGE-020), used in the experiments carried out at Lehigh University (USA). The cell is a rectangular translucent box with a square (20 cm x 20 cm) sample tray. There are two liquid chambers on each side of the sample tray (to hold the electrolyte) and a lid that covers the whole apparatus. The standard cell is equipped with platinum working electrodes and both auxiliary electrodes and a reference electrode were added for NanoDC experiment. This modified EP cell allowed direct measurement of the redox potential (ORP) in the soil by use of 0.25 mm diameter platinum wire electrodes fixed in the base plate of the sample tray at equal intervals (3 cm) with conductive glue. ORP measurements were made in the wire electrodes, using a Ag/AgCl reference electrode and a device attached to a low resistance multimeter to facilitate the accurate measurement of soil redox potential (Rabenhorst, 2009). Compressed fiberglass wool pads were used on both sides to help transport the migrating ions from the electrolyte into the clay and vice versa. The levels of the liquids in the anode and cathode chambers were kept slightly below that of the clay in the sample tray to avoid flooding of the soil cell with excess liquid and any preferential transport of nZVI through water pool at the top.

Figure 14-b depicts the laboratorial cell used at the New University of Lisbon. The cell is divided into three compartments, consisting of two electrode compartments (L=7.46cm, internal diameter=8 cm) and a central one (L=4 cm, internal diameter= 8 cm), in which the saturated soil is placed. This central compartment, made of Plexiglas, was equipped with an injection reservoir (L=1 cm) for the iron nanoparticles, separated

with a 1mm nylon mesh and a low speed filter paper. A set of five cellulose filters, previously tested and known to work as passive membranes (Whatman filter paper), were used to separate the soil from the electrolytes. A power supply (Hewlett Packard E3612A, Palo Alto, USA) was used to maintain a constant DC and the voltage drop was monitored (Kiotto KT 1000H multimeter). The electrodes were platinized titanium bars, with L =5 cm and a diameter of 3 mm (Bergsøe Anti Corrosion A/S, Herfoelge, Denmark).

Figure 14-c shows the cylindrical 3-chamber electrokinetic cell used at the Technical University of Denmark (DTU). This cell consists of two electrode compartments (L = 5 cm, internal diameter Ø = 4 cm) and a central compartment. The central compartment subdivided in three parts (L = 1.5 cm each, total of 4.5 cm, Ø = 4 cm). The nanoparticles are placed in the middle part and saturated soil in the other two. Cellulose filters (passive membranes) are used to assure the separation between the soil and electrolytes, and the soil and the iron nanoparticles. A power supply (Hewlett Packard E3612A, Palo Alto, USA) is used to maintain a constant voltage and the current was monitored (Fluke 179 multimeter). The electrodes are platinized titanium bars, with a diameter of 3 mm and a length of 5 cm (Permascand®).

Figure 14-d shows the electrodialytic cell (ED) developed at DTU. It consists of a cylindrical Plexiglas-cell with one compartment (L = 10 cm, Ø = 8 cm) where the anode, the soil slurry and the plastic-flaps attached to a glass-stick stirrer (Lab-egg Bie&Bernsten, Denmark, 350 rpm) are placed. A cation exchange membrane (CAT, GE Water & Process Technologies Bvba – ED, Cation, CR67, MKIII, Blank) separates this compartment from the one (L = 5 cm, Ø = 8 cm) where the cathode is placed. Catholytes are recirculated by mechanical pumps (Plastomec magnet pump, model P05) between the chamber and glass bottle. A power supply (Hewlett Packard E3612A, Palo Alto, USA) was used to maintain a constant voltage and the current was monitored (Fluke 179 multimeter). The working electrodes were platinized titanium bars, with a 3mm diameter and a length of 10 cm (Permascand®).

2.4.3 Large-scale prototype

A large-scale prototype was also built (figure 15) to measure the transport of nanoparticles. It consists of a 60 degree section of a 1,8m diameter test domain that considers radial symmetry and is consistent with the larger scale use of an electro-kinetic treatment of contaminated soils proposed within this project. It consists of a polymer wedge (90cm radius, 60 degrees) built from polyester resin reinforced with glass fibre and a vinyl ester surface layer (called a “gel-coat”) for improved chemical resistance. One significant innovation was the introduction of non-metallic electrodes to reduce contamination and electrode cost. The developed electrodes consist of carbon fibre (7µm diameter filaments in groups of 12 000) textile that combines chemical resistance with high electrical conductivity and a very high specific surface area.

In figure 15 a circular configuration is shown whereby the test domain can be used to validate 1D radial models: assuming a central electrode (Figures 15 b and 15 c) and a periphery (circular) electrode (Figures 15 f and 15 g). In a different configuration the system can be used to simulate a hexagonal configuration – with three “point”

electrodes at centre, 0deg and 60deg – which is the design configuration for field deployment of the technology: a hexagonal array of electrodes throughout a contaminated site.

As the electrodes are made from exposed carbon fibre there is a risk of damage of the electrode materials by the soil sample. Therefore the prototype implementation of the electrodes encased them in a protective PVC tube for the center and hexagonal configurations (Figure 15b) and rectangular PVC shield for the peripheral flat electrode (Figure 15d).

2.4.4 Experiments and results

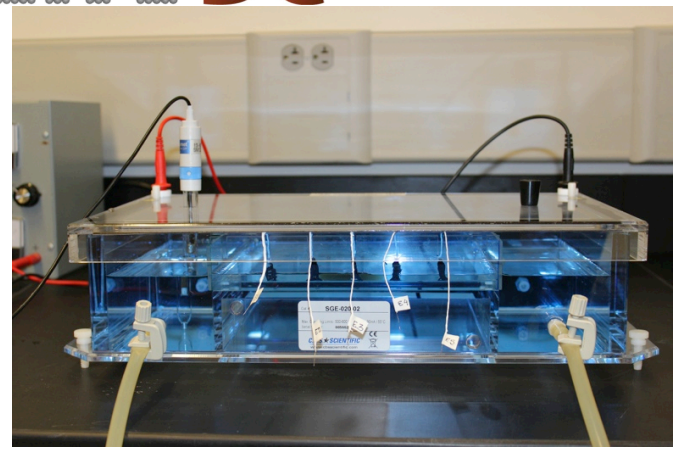
The first experiments using direct current to promote the transport of iron nanoparticles were conducted with model soils to provide a first insight on the transport processes. Then gradually the experiments moved to real soils.

Four model soils of different porosity were used: (i) 100% glass beads, (ii) 50% glass beads and 50% kaolin, (iii) 75% glass beads and 25% kaolin; and, (iv) 100% kaolin. Kaolin represents a low permeability medium, while glass beads a high permeability one.

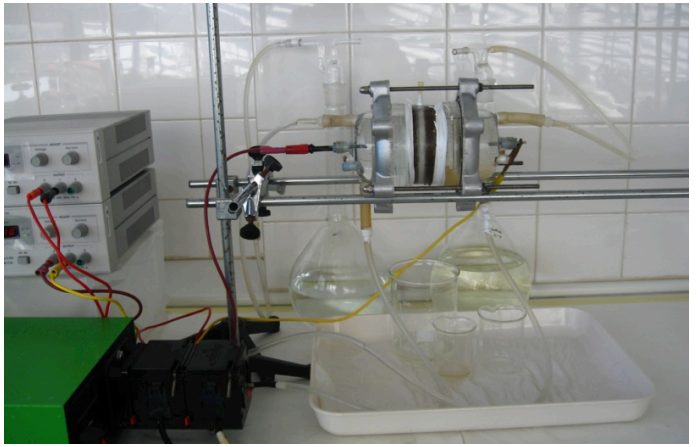
Transport using electric fields was compared with diffusion. In general, higher concentrations of iron across the test bed were measured when a direct current was applied, indicating nanoparticle transport was enhanced. Nanoparticles have reached across the soil to the electrode chambers in transport tests with 100% kaolin, where nanoparticles were transported towards the cathode, and on the test with 100% glass beads, in which nanoparticles were moved towards the anode. This indicates that the electroosmotic flow was the dominant mechanism in transporting nanoparticles in pure clay, whereas electrophoresis was the main mechanism in the surface neutral glass beads. In mixed samples, it appears that both fluxes occur, in opposite direction counteracting each other, resulting in the prolonged presence of iron in the pores and potential capture on the clay surfaces.

The next experiments were carried out to assess if direct current can enhance the nanoparticle transport at concentrations typical of field applications, in clay rich soils. Different electrolytes of varying ionic strengths and pH were tested: 0.05 M $CaCl_2$, 0.1M Na_2SO_3, 1 mM NaOH and 1 mM NaCl. The voltage were 10V, 5V and 0V (diffusion). As in the previous experiments, kaolin clay represented a low permeability medium.

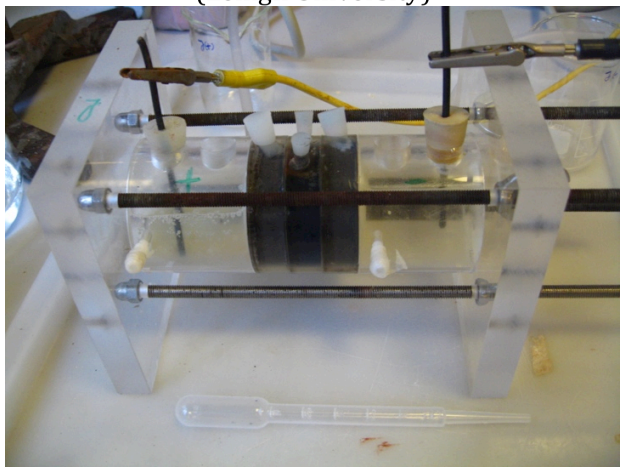
Similarly to the previous experiments, higher iron concentrations were measured when a direct current was applied (figure 16), indicating an enhancement of iron nanoparticle transport over diffusion in kaolin clay. A 25% increase in the average concentration was observed. The iron concentrations obtained in the enhanced transport tests were statistically different from the diffusion tests at a 0.05 level of significance [one-way ANOVA, $F(1,38) = 5.04$, $p = 0.03$]. Simultaneously, statistic models with variables pH, oxidation-reduction potential (ORP), electrode location and voltage did not return any of these variables to be significant to explain this variance.



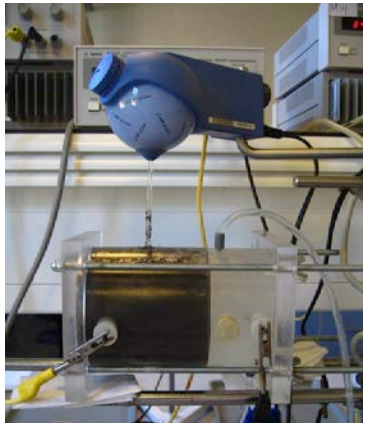
a) Electrophoretic cell used in the nanoparticle transport experiments (Lehigh University)



b) Modified EK cell for the molinate experiments with nanoparticle injection reservoir (Universidade Nova de Lisboa).



c) Three compartment electrokinetic setup used with PCB contaminated soil (DTU)



d) Two compartment Electrodialytic setup used with PCB contaminated soil (DTU).

Figure 14 – Different apparatus from the different partners and colaborators used in project NanoDC to study the electrokinetic transport of iron nanoparticles (source: Helena Gomes, PhD Thesis, Universidade Nova de Lisboa, Set 2014).

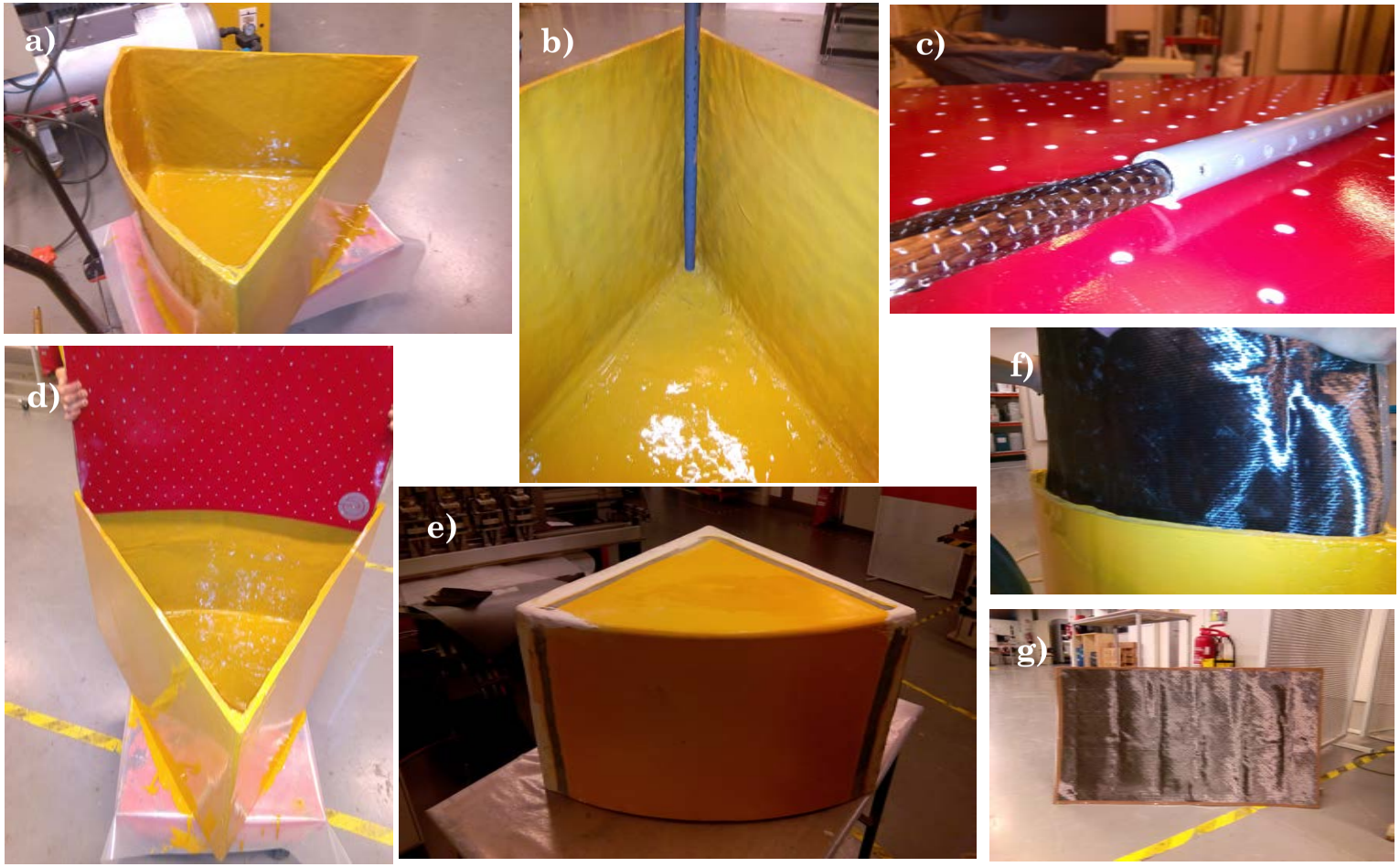


Figure 15 – Large scale prototype to study electrokinetic transport of nanoparticles built for Project NanoDC: a) topview, b) electrode (anode) at corner; c) detail of anode before positioning inside the prototype; d) insertion of carbon protective board to make the cathode compartment; e) prototype during painting; f) insertion of cathode; g) carbon fiber cathode prior to being positioned inside the prototype

The electrolyte 1 mM NaOH presented the highest differences in the experiments with and without direct current, as well as higher iron concentrations near the cathode in relation with the other electrolytes. The experiments using Na_2SO_3 and $CaCl_2$ showed limited enhancement in nanoparticle transport when compared with diffusion. The higher ionic strength of these electrolytes may have contributed to lower nanoparticles stability, increasing their agglomeration and limiting the transport. Recent studies by other researchers in columns showed that the addition of salts (more than 0.5 mM L^{-1} $CaCl_2$), can decrease nanoparticle mobility, and changes in pH to values below six can inhibit mobility at all. Also, the higher ionic strength and the divalent cation Ca^{2+} can have affected the kaolin by reducing the diffuse double layer of the clay particles, and consequently reducing the electroosmotic transport.

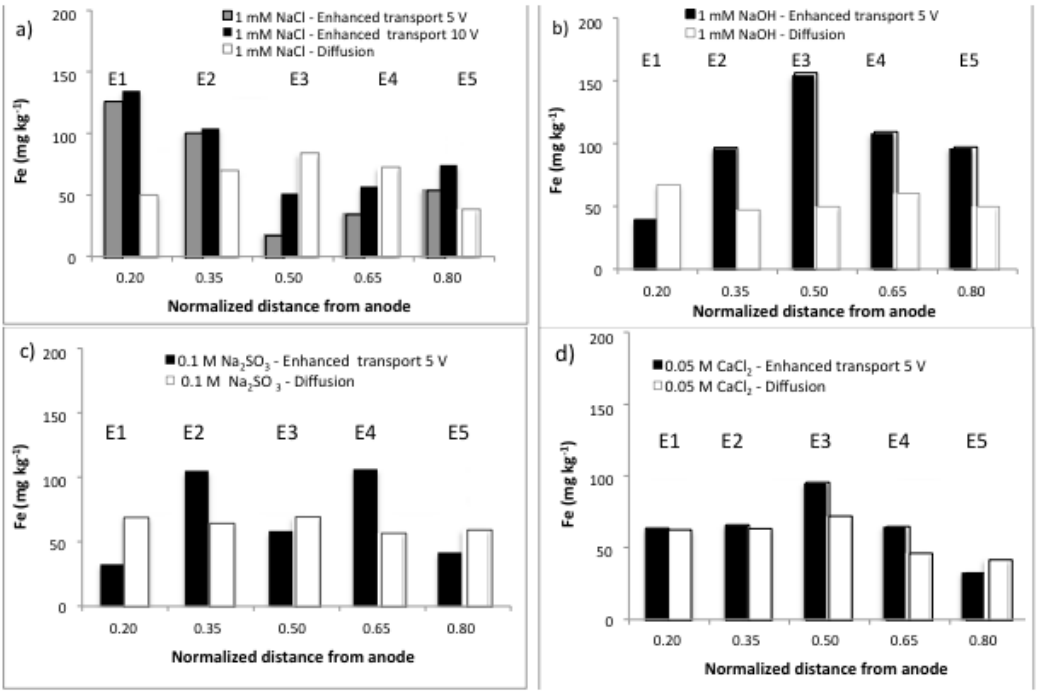


Figure 16 - Additional total iron (mg kg^{-1}) in soil sections compared with the initial soil concentration using different electrolytes and voltages in the enhanced transport and diffusion experiments: a) 1 mM NaCl with 0.5 and 10V; b) 1 mM NaOH using 0 and 5V; c) 0.1M Na_2SO_3 using 0 and 5V; and d) 0.05 M $CaCl_2$ using 0 and 5 V (source: Gomes, 2014)

The increase in the applied voltage from 5V to 10V resulted in an increase in the transport of nanoparticle towards the cathode for the NaCl electrolyte clay sample, as well as in an even larger increase in transport towards the anode. This transport is mainly due to electrophoresis, due to the negative charge of the nanoparticle polymer coating (polyacrylic acid), whereas the movement towards the cathode is due to electro-

osmosis. The concentrations of total Fe measured in the 10 V test near the cathode are around 1.5 times higher than in the 5 V test, possibly due to an increase in the electro-osmotic advection with voltage. Electro-osmotic flow measurements were not made in these experiments, but the results showed the need for additional research to assess the electro-osmotic advection of iron nanoparticles in clays, so the next experiments were made with dense clay.

In total, 6 experiments on electro-osmotic transport were carried out using dense clay. Experiment 0 assessed polarization effects and allowed selection of the working voltage. Experiments I and II tested two opposite injection points for nanoparticle, one near the cathode (exp. I) and the other near the anode (exp. II). The following experiments (III and IV) tested electro-osmotic flow in the presence and absence of iron nanoparticles, during 160 hours and experiment V replicated experiment IV, but extended time to 360 hours. During the experiments, daily measurements of current and pH at the anode and cathode reservoirs were and the electrolyte flow due to the electrokinetic transport was measured

Electro-osmotic transport was detected. Flow of solution from the anode to the cathode was measured in all the experiments and the cumulative flow collected at the cathode is shown in figure 17 for two of the experiments. Based on these data the electro-osmotic permeability coefficients were calculated: between $0.489 \cdot 10^{-10}$ and $1.0 \cdot 10^{-10}$ $m^2V^{-1}s^{-1}$ in the presence of iron nanoparticles and between $1.2 \cdot 10^{-10}$ and $1.93 \cdot 10^{-10}$ $m^2V^{-1}s^{-1}$ in the absence of iron nanoparticles. The values obtained in the absence of nanoparticles are in accordance with those shown in the literature for clays. So the presence of iron nanoparticles affects the electro-osmotic flow, decreasing it by 50%.

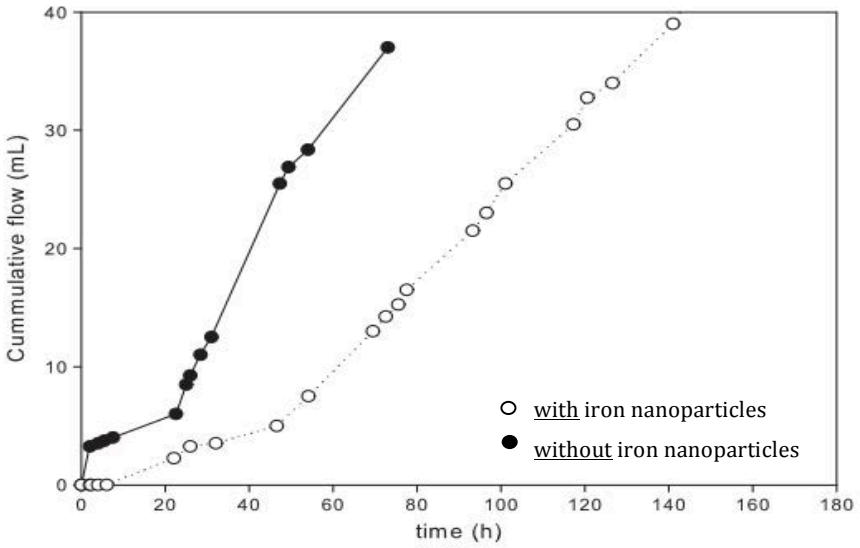


Figure 17 - Electro-osmotic flow obtained with and without iron nanoparticles (source: Rosales et al, 2014)

Next, the geochemical changes in the clay were studied. The cation distribution in the Boom clay was determined using micro-XRF (figure 18). Initially, the effect of electrokinetic transport without nanoparticle in the Boom clay was studied for 160 hours (experiment III). As observed in figure 18, in absence of iron nanoparticles, potassium (K) is uniformly distributed in the soil and some native iron spots are seen in the clay and a small amount of calcium appears in the soil nearest to the cathode. When a new assay was developed under the same conditions but with iron nanoparticles injection at the anode side (experiment IV), calcium presence at the cathode side becomes more evident, probably because of the higher electrical conductivity and current densities in this experiment. Iron content in soil also increases, when compared to experiment III, suggesting that iron nanoparticles is transported into the soil from the reservoir at the anode side, and moves towards the cathode by electro-osmosis. Finally, conducting the experiment with iron nanoparticles for the longer period of 360 hours (experiment V) enhances calcium presence at the cathode side. The presence of iron was also strongly visible, in a front in slice number 5, most probably in precipitated form (figure 18). This observation may be justified because of the change in the pH profile. It is known than the acidic front mobility due to the H+ is higher than the basic front and according to the obtained data, the point where both fronts meet is in soil section 5, where there are sharp pH changes (4.72 to 9.86).

For this particular system, as well as in other systems where electro-osmosis towards the cathode is the leading transport mechanism, the best location to inject iron nanoparticles is therefore at the anode side of the electrokinetic cell. However, due to the low pH values and the presence of oxygen care should be taken to prevent nanoparticle corrosion. With the new prototype used in our work, nanoparticles are injected in a chamber adjacent to the anode compartment, thus avoiding the presence of oxygen by the physical separation between compartments and by allowing oxygen generated at the anode to escape to the atmosphere through a hole located at the top of the chamber. So the new prototype has proven to be a successful design, in preventing iron nanoparticle corrosion due to the oxygen generated at the anode.

2.4.5 Conclusions for task 4

The developed studies demonstrate that an increase in transport of iron nanoparticles occurs in low permeability media, with advection by electro-osmosis as the main transport mechanism towards the cathode.

In case of coarser grained soil, or sandy soils, the main transport mechanism is electrophoresis, and in this case the movement is towards the anode. However, in sandy soils electrokinetic enhancement is probably small compared to hydraulic transport processes. It is in low permeability soils that transport enhancement by electrokinetics has greater potential, and can deliver iron nanoparticles to locations of difficult access.

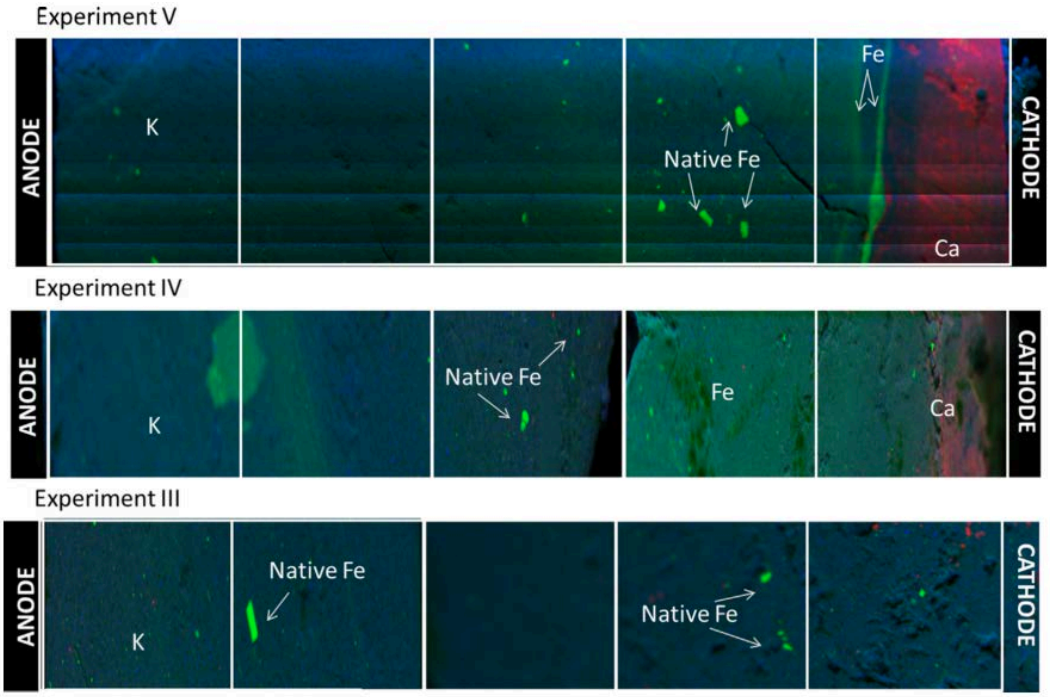


Figure 18 - Micro-XRF images of Experiments III, IV & V (from bottom to top) using Boom clay: iron, green; calcium, red; potassium, blue (source: Rosales et al, 2014)

2.5 Model of transport and degradation (Task 5)

A generalized physicochemical model was developed and solved numerically to describe the nanoparticle transport through porous media, and with different electrolytes (with different ionic strengths), under electric field.

The model basically consists of the Nernst-Planck coupled system of equations, which accounts for the mass balance of ionic species in a fluid medium, when both the diffusion and electromigration of the ions are considered in the transport process. The diffusion and electrophoretic transport of the negatively charged nanoparticle particles were also considered in the system. The contribution of electroosmotic flow to the overall mass transport was included in the model for all cases.

The analytical model operates in two steps: first the kinetic process is simulated by integrating forward in time the one- dimensional transport equations, including the electrochemical reactions at the electrodes; then the chemical equilibriums are reestablished before the next step of integration. This is done because chemical equilibriums are considered instantaneous when compared with the transport time.

The governing equations and electrochemicals reactions considered are described in detail in Gomes et al (2015a).

Model validation was performed against experimental data obtained previously in tasks 3 and 4. It was found that the model reproduces satisfactorily the nanoparticle concentration profiles in the porous media, as well as the anodic and cathodic pH values over time. Model and experimental results for nanoparticle concentrations profiles in the various porous specimens at the end of the 48h diffusion control tests are presented in figure 19.

It was detected that, in some cases, an important fraction of the nanoparticles tends to aggregate when the concentration is high relative to the available pore volume, becoming immobile. In fact, in experiments 2 and 4 only about 19% and 8% of the injected nanoparticles remained mobile over the experiment, respectively. At high iron nanoparticle concentration (1–6 gL^{-1}) there is higher agglomeration. Also, when iron nanoparticles aggregate they become larger than the pores, restricting their transport through the matrix.

The model result shows that using an electric current enhances nanoparticle transport by preventing or hindering the nanoparticle aggregation at the injection location. However, the model also predicts very low effective mobility values in the porous medium as a consequence of the opposing transport directions between the electroosmotic advection and the electrophoretic migration of the negatively charged nanoparticles. This effect manifests itself as higher concentrations close to the injection point in most of experiments. Thus, if the nanoparticles could be stabilized with a surface modifier to give them a positive charge, the nanoparticle effective mobility could potentially be increased. Nevertheless the probability of the positively charged particles be attracted onto the soil particle surfaces, particularly clays, could increase. Also the use of stabilizers without charge could enhance the electroosmotic transport of the iron nanoparticles

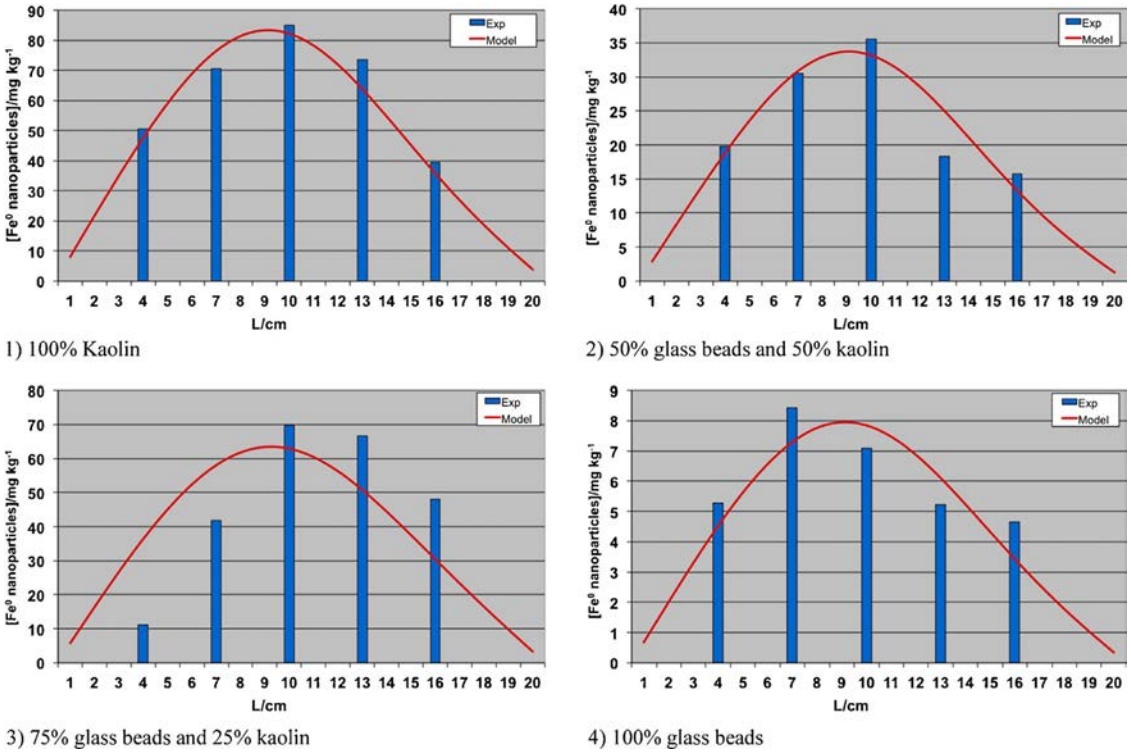


Figure 19 - Iron concentrations across the laboratory prototype in the diffusion control tests for the porous media tested in different experiments (source: Gomes et al, 2015a).

The ionic strength of the electrolyte was also determinant in the transport of the nanoparticles–the higher the ionic strength of the electrolyte the lower the transport, what should also be considered for field applications with contaminated groundwaters with high concentrations of salts and metals. The distance covered by iron nanoparticles when using 0.001M NaCl as the electrolyte is approximately the double when compared with 0.05 M $CaCl_2$ and 0.1 M Na_2SO_3.

Figure 20 shows the model predictions of the transport distance covered by the iron nanoparticles using different electrolytes and porous media, with and without current. It is clearly distinct the enhancement of the nanoparticle transport when current in applied, especially in the kaolin clay. When using only kaolin clay and direct current the predicted distance covered by iron nanoparticles is almost the double of diffusion only.

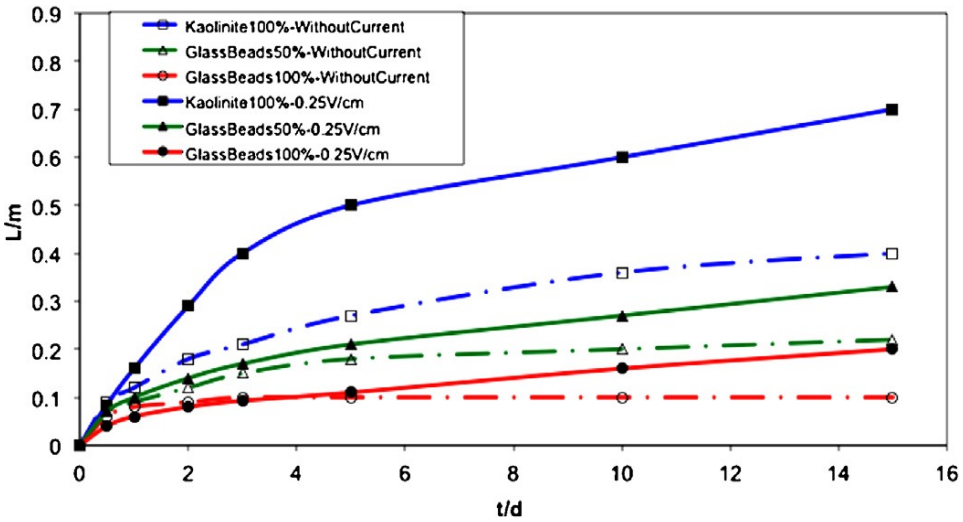


Figure 20 – Prediction of the distances covered by nanoparticles in different porosity media with and without current (source: Gomes et al. 2015a)

2.5.1 Conclusion of Task 5

Both the experimental and the model results showed that an important aggregation of nanoparticle occurs when the nanoparticles are allowed to diffuse into the porous medium from an injection point. The higher the nanoparticle concentration is in the matrix, the higher the aggregation; therefore, low concentrations nanoparticle suspensions must be used for successful field application. The use of electrical current to transport the nanoparticles prevents or hinders the nanoparticle particle aggregation, increasing their mobility. However, opposing directions of electrophoretic transport of negatively charged particles and the electroosmotic advection still produces low nanoparticle transport. To enhance this transport, possible solutions could be reversing the charge of the iron nanoparticle surface or by using neutrally charged nanoparticles, both of which could be transported by electroosmotic advection.

2.6 Dissemination of results (Task 6)

Dissemination of the results is being realized via the website (www.nanodc.org), publication in peer-reviewed international scientific journals and communications at conferences and workshops. In total **46 dissemination pieces** were produced throughout the project (not counting the reports and papers submitted but not yet accepted), comprising **4 books or book chapters**; **11 papers** in international peer-reviewed journals; **27 communications** in conferences and workshops; **1 PhD Thesis;** and **3 MSc. Dissertations**. In addition, a **final international workshop** was organized for the project.

Some indicators of the efficacy of the dissemination actions are:

- The PI was invited speaker at an international workshop related to nanoparticles in the environment, which took place in 2014 at Minho University (Portugal). No previous contact existed with that research group, and the invitation was a result of the dissemination actions carried out.

- The PI was invited evaluator of two project applications submitted to the Austrian Science Fund related to nanoparticles in the environment. Again, no previous contact existed and the invitation resulted directly from the dissemination actions carried out.

- The PI was invited evaluator for two PhD proposals for the Wageningen University, The Netherlands. Once again, no previous contact existed.

- Two of the papers resulting from the project have already near 20 citations each (database: Scopus), despite being quite recent (2012 and 2013). In January 2015 the papers published at international journals have already achieved **44 citations**, and this number is expected to increase in the following months due to the large number of recent publications, whose citations can only appear after some months.

The full list detailing the publications, communications, MSc. Dissertations and PhD Thesis is presented below. The link to the written work, poster or presentation can be accessed via the project web page (www.nanodc.org).

2.6.1. Books and book chapters

1. C Dias Ferreira & M J Quina (Ed). International Workshop on Nanoparticles for soil remediation: from green synthesis to ecotoxicological effects. 2014, Coimbra, Portugal (17 p)

2. Luís M. Nunes, Helena I. Gomes, Margarida Ribau Teixeira, Célia Dias-Ferreira, Alexandra B. Ribeiro (2015). Life Cycle Assessment of Soil and Groundwater Remediation: Groundwater Impacts of electrokinetic remediation. In: Electrokinetics across disciplines and continents - New strategies for sustainable development. Ribeiro A. B.; Mateus, E.P.; Couto, N. (Editors), Springer book (publishing agreed Contract No. 31021)

3. Helena I. Gomes, Celia Dias-Ferreira, Alexandra B. Ribeiro, Sibel Pamukcu, José-Miguel R. Maroto (2015). Electrokinetics of Zero Valent Iron Nanoparticles: Experimental and Model Verification of the Transport. In: Porous Media in Electrokinetics across disciplines and continents - New strategies for sustainable development. Ribeiro A. B.; Mateus, E.P.; Couto, N. (Editors), Springer book (publishing agreed Contract No. 31021)

4. Helena I. Gomes, Guangping Fan, Lisbeth M. Ottosen, Celia Dias-Ferreira, Alexandra B. Ribeiro (2015) Nanoremediation coupled to electrokinetics for PCB removal from soils. In: Electrokinetics across disciplines and continents - New strategies for sustainable development. Ribeiro A. B.; Mateus, E.P.; Couto, N. (Editors), Springer book (publishing agreed Contract No. 31021)

2.6.2. Peer-reviewed international journals

1. Helena I. Gomes, Celia Dias-Ferreira, Lisbeth M. Ottosen, Alexandra B. Ribeiro (2015). Electroremediation of PCB contaminated soil combined with iron nanoparticles: Effect of the soil type. Chemosphere (Accepted).

2. Helena I. Gomes, J.M. Rodríguez-Maroto, Alexandra B. Ribeiro, Sibel Pamukcu, Celia Dias-Ferreira (2015). Numerical prediction of diffusion and electric field-induced iron nanoparticle transport. Electrochimica Acta (IN PRESS)
(**DOI:** 10.1016/j.electacta.2014.11.157)

3. Helena I. Gomes, Lisbeth M. Ottosen, Alexandra B. Ribeiro, Celia Dias-Ferreira (2015). Treatment of a suspension of PCB contaminated soil using iron nanoparticles and electric current. Journal of Environmental Management 151, 550-555 (IN PRESS)
(**DOI:** 10.1016/j.jenvman.2015.01.015)

4. Helena I Gomes, Celia Dias-Ferreira, Lisbeth M Ottosen, Alexandra B Ribeiro (2014). Electrodialytic remediation of PCB contaminated soil with iron nanoparticles and two different surfactants. Journal of Colloid and Interface Science 433, 189-195
(**DOI:** 10.1016/j.jcis.2014.07.022)

5. Helena I. Gomes, Guangping Fan, Eduardo P. Mateus, Celia Dias-Ferreira and Alexandra B. Ribeiro (2014). Assessment of combined electro-nano remediation of molinate contaminated soil. Science of the total environment 493, 178–184
(**DOI:** 10.1016/j.scitotenv.2014.05.112)

6. Emilio Rosales, J.P. Gustav Loch, Celia Dias-Ferreira (2014). Electro-osmotic transport of nano zero-valent iron in Boom Clay. Electrochimica Acta 127, 27-33
(**DOI:** 10.1016/j.electacta.2014.01.164); (cited by: **1**, scopus database)

7. Helena I. Gomes, Celia Dias-Ferreira, Alexandra B. Ribeiro, Sibel Pamukcu
(2014). Influence of electrolyte and voltage on the direct current enhanced transport of iron nanoparticles in clay. Chemosphere 99, 171-179
(**DOI:** 10.1016/j.chemosphere.2013.10.065) (cited by: **2**, scopus database)

8. Gomes, H.I.; Dias-Ferreira, C.; Ribeiro, A.B. & Pamukcu, S. (2013). Enhanced transport and transformation of zero-valent nanoiron in clay using direct electric current. Water, Air and Soil Pollution 224 (12) 1-12
(**DOI:** 10.1007/s11270-013-1710-2)

9. Gomes, H.I.; Dias-Ferreira, C. & Ribeiro, A.B. (2013). Overview of in situ and ex situ remediation technologies for PCB- contaminated soils and sediments and obstacles for full-scale application. Science of the Total Environment 445–446: 237– 260
(**DOI:** 10.1016/j.scitotenv.2012.11.098) (cited by: **20**, scopus database)

10. Gomes, H. I.; Dias-Ferreira, C.; Ribeiro, A.B. & Pamukcu, S. (2012). Electrokinetic enhanced transport of zero valent iron nanoparticles for chromium(VI) reduction in soils. Chemical Engineering Transactions 28, 139-144, ISBN: 978-88-95608-19-8, ISSN: 1974-9791
(**DOI:** 10.3303/CET1228024) (cited by: **2**, scopus database)

11. Helena I. Gomes, Celia Dias-Ferreira, Alexandra B. Ribeiro (2012). Electrokinetic remediation of organochlorines in soil: Enhancement techniques and integration with other remediation technologies. Chemosphere 87, 1077-1090
(**DOI:** 10.1016/j.chemosphere.2012.02.037) (cited by: **19**, scopus database)

2.6.3. International conferences and meetings

1. H I Gomes, C Dias-Ferreira, A B Ribeiro (2014). Simultaneous use of electroremediation and iron nanoparticles for the degradation of PCB in soils. In: C Dias Ferreira & M J Quina (Ed.). International Workshop on Nanoparticles for soil remediation: from green synthesis to ecotoxicological effects. December, Coimbra, Portugal, pp 10

2. M J Quina, D V Lopes, E M Neto, R C Martins, L Gando-Ferreira, C Dias-Ferreira, R Quinta-Ferreira (2014). Synthesis and characterization of nZVI: challenges and prospects. In: C Dias Ferreira & M J Quina (Ed.), International Workshop on Nanoparticles for soil remediation: from green synthesis to ecotoxicological effects. December, Coimbra, Portugal, pp 5-6

3. E Rosales Villanueva, J P Gustav Loch, C Dias-Ferreira. Fundamentals of the Electrokinetic transport of nZVI in soil (2014). In: C Dias Ferreira & M J Quina (Ed.), International Workshop on Nanoparticles for soil remediation: from green synthesis to ecotoxicological effects. December, Coimbra, Portugal, pp 7-8

4. Celia Dias-Ferreira. Zero-valent-Iron nanoparticles: synthesis, characterization and applications. Nanotechnology applied to Environmental Geotechnics, 2014, December, 4-5, Braga, University of Minho, Portugal (Invited speaker)

5. Helena I. Gomes, Celia Dias-Ferreira, Alexandra B. Ribeiro, Sibel Pamukcu, José M. Rodriguez-Maroto (2014). Electrokinetics and zero valent iron nanoparticles: experimental and modelling of the transport in different porous media. Book of abstracts & some outputs of the ELECTROACROSS project, FCT-UNL, Portugal, pp 44-45 (ISBN978-972-8893-34-7)

6. Helena I. Gomes, Guangping Fan, Lisbeth M. Ottosen, Celia Dias-Ferreira, Alexandra B. Ribeiro (2014). Electroremediation of PCB contaminated soils with zero valent iron nanoparticles. Book of abstracts & some outputs of the ELECTROACROSS project, FCT-UNL, Portugal, pp 26-27 (ISBN978-972-8893-34-7)

7. Luís M. Nunes, Helena I. Gomes, Margarida Ribau Teixeira, Célia Dias-Ferreira, Alexandra B. Ribeiro (2014). Life Cycle Assessment of Soil and Groundwater Remediation: Groundwater Impacts of electrokinetic remediation. Book of abstracts & some outputs of the ELECTROACROSS project, FCT-UNL, Portugal, pp 20-21 (ISBN978-972-8893-34-7)

8. Helena I. Gomes, J.M. Rodríguez-Maroto, Celia Dias-Ferreira, Alexandra B. Ribeiro, Sibel Pamukcu (2014). Modeling of the direct current assisted transport of zero valent iron nanoparticles. In J.M. Rodríguez Maroto, R. García-Delgado, F. García-Herruzo, C Gómez-Lahoz, C. Vereda-Alonso, M.Villén-Guzmán (Ed). ELECTROKINETIC REMEDIATION (EREM2014), p. 59-60, Málaga, Spain (ISBN - 10: 84-697-0768-X; ISBN - 13: 978-84-697-0768-5)

9. M.J. Quina, D.V. Lopes, E.M. Neto, R.C. Martins, Licínio Gando-Ferreira, C. Dias-Ferreira, R. Quinta-Ferreira, Zero-valent iron nanoparticles for degradation of chlorinated pollutants, CHEMPOR'2014, Proc. Pg 15_128 to 15_130, 10-12 September, Porto (2014)

10. D.V. Lopes, L.G. Cruz, R.C. Martins, L.M. Gando-Ferreira, C.M. Dias-Ferreira, M.J. Quina and R.M. Quinta-Ferreira. Characterization and chemical stabilization of digestate from municipal solid wastes. 2nd International Conference on Sustainable Solid Waste Management (Athens 2014). 12th–14th June 2014. Athens, Greece (CD-rom)

11. Margarida Quina, Celia Dias-Ferreira (2014). Zero-valent iron (nZVI) nanoparticles: synthesis, characterization and applications. 1st Seminar on Organically Modified Silica Aerogels (Aero-ORMOSIL 2014), 19th March, 2014, FCTUC – Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Portugal

12. Helena I. Gomes, Lisbeth M. Ottosen, Celia Dias-Ferreira, Alexandra B. Ribeiro. Enhanced electroremediation of PCB contaminated soil: preliminary results. Electrochemical Science and Technology Conference 2013 (8th Danish Electrochemical Society Annual Meeting), 3-4 October 2013 Sørup Herregaard, Denmark.

13. Gomes, H. I.; Dias-Ferreira, C.; Ribeiro, A. B. & Pamukcu, S. (2013). Assessment of ionic strength in the direct current enhanced transport of iron nanoparticles. Book of Abstracts of EREM 2013 - 12th Symposium on Electrokinetic Remediation, p.36-37, 23-26 June 2013, Boston, USA

14. Emilio Rosales, J.P. Gustav Loch, Celia Dias-Ferreira, Pieter J. Kleingeld (2013) Electro-osmotic transport of nano zero-valent iron in Boom Clay: nZVI distribuition and effect in the clay. Book of Abstracts of EREM 2013 - 12th Symposium on Electrokinetic Remediation, p.70, 23-26 June, Boston, USA

15. Gomes, H. I.; Fan, G. P.; Mateus, E. P.; Dias-Ferreira, C.; Zhou, D. M. & Ribeiro, A. B. (2013). Enhancing electrokinetic remediation of molinate contaminated soil using zero valent iron nanoparticles. Book of Abstracts of EREM 2013 - 12th Symposium on Electrokinetic Remediation, p.50-51, 23-26 June 2013, Boston, USA

16. Helena I. Gomes, Jorge Gonçalves, Celia Dias-Ferreira, Alexandra B. Ribeiro (2013). Life cycle assessment of soils and groundwater remediation with zero valent iron nanoparticles: Results from a pilot study in Barreiro, Portugal. NICOLE Network Meeting & Workshop Program: Implementation of Sustainability in Management of Contaminated Land - in particular using emerging "green" technologies, 12-14 June 2013, Lisbon, Portugal

17. Daniela V. Lopes, Elsa M. Neto, Rui C. Martins, Margarida J Quina, Licinio M. Gando-Ferreira, Celia Dias-Ferreira, Rosa M. Quinta-Ferreira (2012). Morphology analysis of metallic iron nanoparticles in Sociedade Portuguesa de Química, Livro de Resumos do XVIII ENCONTRO LUSO-GALEGO DE QUÍMICA, Peres J.A., Lucas M.S. & Tavares P.B. (Editors), 28-30 November, Universidade de Trás-os-Montes e Alto Douto, Vila Real, Portugal, p. 114 (ISBN: 978-989-97667-5-4; Depósito legal: 351984/12)

18. Elsa M. Neto, Daniela V. Lopes, Rui C. Martins, Margarida J Quina, Licinio M. Gando-Ferreira, Celia Dias-Ferreira, Rosa M. Quinta-Ferreira (2012). Synthesis os iron nanoparticles by chemical reduction in Sociedade Portuguesa de Química, Livro de Resumos do XVIII ENCONTRO LUSO-GALEGO DE QUÍMICA, Peres J.A., Lucas M.S., Tavares P.B. (Editors), 28-30 November, Universidade de Trás-os-Montes e Alto Douto, Vila Real, Portugal, p. 108 (ISBN: 978-989-97667-5-4; Depósito legal: 351984/12)

19. Gomes, H.; Ferreira, C. D.; Ribeiro, A. B. & Pamukcu, S. (2012). Electrokinetic enhanced transport of zero valent iron nanoparticles for chromium(VI) reduction in soils. BOSICON - 3rd International Conference on Polluted Sites Remediation, Rome, Italy, 11-14 September 2012 (oral).

20. Gomes, H. I.; Dias-Ferreira, C.; Ribeiro, A. B. & Pamukcu, S. (2012). Direct current assisted transport and transformation of zero-valent nanoiron in porous media. EREM 2012 - Remediation of Contaminated Soil with Harmful Substances by Advanced Electrokinetic Process, 11th Symposium on Electrokinetic Remediation, Hokkaido University, Sapporo, Japan, 8-11 July 2012, pp. 24-26 (oral)

21. Emilio Rosales, Bruno Guimaraes, J.P. Gustav Loch, Celia Dias-Ferreira (2012). Development of a cell adapted to measure electro-osmotic flow associated with the movement of nZVI. Remediation of Contaminated Soil with harmful substances by Advanced Electrokinetic Process- EREM 2012 - The 11th International Symposium on Electrokinetic Remediation, pp. 66-68, July 8-11 Hokkaido University, Sapporo, Japan

22. F. Miguens, D. Santos, M. Malta, C. Dias-Ferreira (2012). Heavy metals in urban agriculture: a question of public health or a question of assessment. Urban Environmental Pollution 2012-Creating Healthy, Liveable Cities, 17-20 June. Amsterdam, The Netherlands (poster)

23. R.L. Pato, C. Dias-Ferreira, A.O. Tavares, and M.C. Magalhães (2012). Evaluation of Historical Industrial Pollution in urban sediments. EGU - European Geosciences Union General Assembly 2012. Vienna, Austria, 22-27 April (Oral)

24. Ribeiro, A. B.; Mateus, E. P.; Marriott, P.; Ferreira, C. D.; Ottosen, L. M.; Rodríguez-Maroto, J. M.; Hansen, H. K.; Pamukcu, S.; Nekrasova, M.; Zhou, D.-M.; Teixeira, M. R. & Cardeal, Z. (2011). Advanced analytical techniques in ELECTROACROSS. Matrix

characterization and monitoring electrokinetic processes in recovery, remediation and conservation. Green Chemistry 2011 Innovations. 3rd Asia-Oceania Conference on Green & Sustainable Chemistry, AOC-3, Melbourne, Australia, 4-7 December 2011

25. H. Gomes, Celia M.D. Ferreira, A. B. Ribeiro, G. Loch, L.M. Ottosen, "A new approach to soil remediation: coupling nanotechnology with electrically induced particle transport (Electrokinetics)" in Fernando Castro, Cândida Vilarinho, Joana Carvalho (Editors), Book of Proceedings of the 1st International Conference WASTES: Solutions, Treatments and Opportunities. CVR – Centro para a valorização de Resíduos, Guimarães, Portugal, October 2011, pp 732-737 (ISBN 978-989-97429-1-8)

2.6.4. National conference and meetings

1. Helena I. Gomes, Celia Dias-Ferreira, Alexandra B. Ribeiro (2013). Utilização conjunta de nanopartículas de ferro zero valente com o método electrocinético para descontaminação de solos com PCBs. Encontro Nacional de Nanotoxicologia 2013- E2N Programa e resumos, pag 42, 2-3 April 2013, Instituto Superior Técnico, Lisbon, Portugal

2. C. Ferreira. Application of nanotechnologies for the remediation of contaminated sites (original title in Portuguese: A aplicação de nanotecnologias na remediação de locais contaminados). "ESAC - Empreender e semear o futuro" 11 de Abril 2012, ESAC, Polytechnic Institute of Coimbra, Portugal (invited oral communication)

2.6.5. PhD Thesis

1. Helena Isabel Caseiro Rego Gomes (2014). Coupling electrokinetics and iron nanoparticles for the remediation of contaminated soils. PhD Thesis. Faculdade de Ciências e Tecnologia, New University of Lisbon, Portugal September 2014

2.6.6. Master Dissertations

1. Elsa Marina Santos Neto (2012). Synthesis and characterization of nano zero-valent iron particles for environmental remediation. M.Sc. Dissertation, University of Coimbra. Portugal. October 2012

2. Mariana Raquel Correia Serra. Chromium polluted soil and electrokinetics in stationary setup. Application to model and real soils from wood preservation activities. M.Sc. Dissertation. Department of Civil Engineering, Technical University of Denmark. July 2012.

3. João Fábio Franco Salvador. Chromium polluted soil and electrokinetics in stirred setup. Remediation of spiked and industrially polluted soils. M.Sc. Dissertation. Department of Civil Engineering, Technical University of Denmark. July 2012.

2.6.7. Final International Workshop

The "International Workshop on Nanoparticles for Soil Remediation: from green synthesis to ecotoxicological effects" took place on the 19th of December 2014, at Coimbra School of Agriculture, Portugal (www.nanodc.org). The programme consisted of a total of 6 presentations, in which 50% were on the work carried out during the NanoDC Project and 50% about other relevant work, namely within an European Project on nanoparticles and others. A Book of Abstracts was produced (figure 21) and can be accessed through the web page (or through the link: http://www.nanodc.org/wp-content/uploads/2012/08/2014-Nanoparticles-Book-of-abstracts.pdf

The list of participants is included in the last pages of the book of abstracts and comprises Universities, Polytechnic Institutes, Private Companies and Research Centers. Attendants came from several places around the world: Brazil, Spain, Portugal, The Netherlands and Finland.

The workshop was announced internationally through email and also in the regional media. After the workshop an article was published in a regional newspaper (in Portuguese) about the workshop and the project (figure 22).

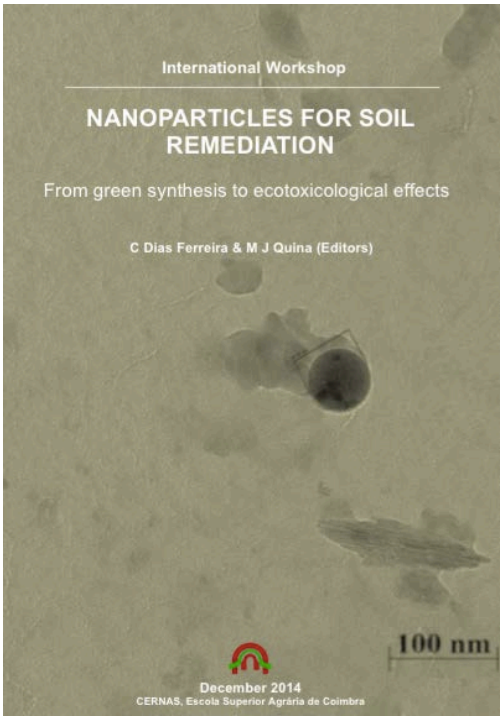


Figure 21 – Cover of the book of abstracts of the final workshop organized within the framework of Project NanoDC

CISION

Diário de Coimbra

ID: 57175598

21-12-2014

Tiragem: 9311
País: Portugal
Period.: Diária
Âmbito: Regional

Pág: 5
Cores: Cor
Área: 16,85 x 18,13 cm²
Corte: 1 de 1

Investigadores de Coimbra usam nanopartículas para tratar solos contaminados

Poluição Projecto liderado pela Escola Superior Agrária estudou degradação de poluentes orgânicos persistentes usando nanopartículas de ferro

Andrea Trindade

Ainda que o seu uso já tenha sido proibido há décadas, persistem no ambiente, nomeadamente nos solos, um conjunto de substâncias perigosas e nefastas para a saúde pública que foram usados entre as décadas de 20 e 60. Tratar os solos que estão contaminados com estes poluentes orgânicos persistentes, recorrendo às nanotecnologias emergentes, foi o desafio que traçaram os investigadores da Escola Superior Agrária de Coimbra (ESAC) com o projecto "NanoDC - Utilização de nanopartículas para degradar PCBs no solo". Financiada pela Fundação para a Ciência e Tecnologia (FCT), a investigação teve como parceiros a Universidade de Coimbra (Departamento de Engenharia Química), a Universidade de Utrecht (Holanda) e a Universidade Técnica da Dinamarca e teve o seu workshop de conclusão na sexta-feira, em Coimbra.

Workshop realizou-se na sexta-feira na Escola Agrária

Célia Dias Ferreira, líder do projecto iniciado em 2010, explicou ao Diário de Coimbra que «a ESAC já estava a trabalhar na área da remediação de solos» e que este projecto serviu para associar aos estudos o potencial das nanotecnologias e outras tecnologias emergentes. «Foram dados passos significativos, conseguimos melhorar o transporte das nanopartículas para que cheguem aos contaminantes e conseguimos tornar mais eficaz o processo de degradação dos poluentes. Provámos que algumas coisas funcionam e que outras não funcionam», referiu. Ainda assim, «será é necessário avançar na investigação e pretendemos candidatar outros projectos a financiamento já em Janeiro, seja da FCT seja de outras fontes – nomeadamente com parcerias com empresas -, para o poder fazer», adiantou.

À margem da reunião que juntou cerca de 40 participantes, entre investigadores nacionais e internacionais, representantes de empresas de gestão de resíduos e de remediação ambiental e especialistas de universidades exteriores ao projecto, caso de Vigo, de Lisboa e do Minho, Célia Ferreira sublinhou que «a introdução das nanopartículas nos solos tem um impacto ecológico relevante».

«O nosso estudo concentra-se na degradação dos poluentes através do uso de nanopartículas de ferro e, ainda antes, no transporte dessas nanopartículas até aos contaminantes». Na prática, explica a responsável do projecto, «o que as nanopartículas fazem é remover a toxicidade destas moléculas que se encontram no solo».

Figure 22 – Newspaper article (in Portuguese) in a regional newspaper about the final workshop organized in the framework of the project NanoDC.

2.6.8 Conclusions for task 6

In total **46 dissemination pieces** were produced throughout the project (not counting reports nor submission of papers which are currently under evaluation), comprising **4 books/book chapters**; **11 papers** in international peer-reviewed journals; **27 communications** in conferences and workshops; **1 PhD Thesis;** and **3 MSc. Dissertations**. In addition, a **final international workshop** was organized within the framework of the project.

Indicators related to the efficacy of the dissemination actions include **speaker invitations** (2) at national and international workshops, invitation to **evaluate project proposal (2)** and **PhD projects (2)** on the topic, **citations** (**44**) achieved published papers and the level of internationalization (**5** countries) attending the **final workshop**.

3. Auto-evaluation and conclusions

Table 9 presents the initially proposed indicators and those achieved during the NanoDC Project.

	Proposed	Achieved
A – Publications		
Books (and book chapters)	0	**4**
Papers in international journals	7	**11**
Papers in national journals	2	**0**
B – Communications		
Communications in international meetings	4	**25**
Communications in national meetings	1	**2**
C- Report	15	**20**
D - Organisation of seminars and conferences	1	**1**
E – Advanced training		
PhD Thesis	3	**1**
Master Thesis	1	**3**
Others	0	0
F - models	1	**1**
G – Software	1	**0**
H – Pilot Plants	0	0
I – Laboratory Prototypes	1	**2**
J - Patents	0	0
L – Other: Web Page	0	**1**

The indicators not achieved were: papers in national journals, PhD thesis and software. An explanation is provided below.

Papers in national journals. The team opted to invest in publishing in international journals, given that there is not any national journal dedicated to the investigation on the topics covered by the project.

PhD Thesis. At the time of project application 3 PhD theses were expected. The first was from the grant holder (GH) hired for 36 months, who would do his/her Thesis on the topic. However, when GH 1 (Dr. Emilio Rosales) applied he already hold a PhD degree. Being the most qualified and suited person for the task, he was the selected candidate, even though it meant that the project would give up on getting one of the PhDs. The second PhD Thesis was expected to come from one of the permanent staff, Micaela Soares. However, project submission took place in Feb 2009, but the project only started two years later, in March 2011 and in the meantime Micaela Soares started her PhD on a different topic. The third PhD was expected through an independent application to a PhD grant. Helena Gomes successfully applied to a doctoral grant to FCT on the topic of project NanoDC. At the time of application this was the Thesis presenting the higher level of uncertainty, as there was no guarantee that a suitable candidate would be found

nor that funding would be granted. It was, nevertheless the one that came through. The project budget would fund the salary of one of the 3 proposed PhD thesis, and in the end one was the final indicator for PhD thesis within the project.

Software. At the time of project application it was predicted that a computational application would be created, in which the model could be run in a user-friendly environment. There was no budget assigned and at that time we expected that this could be achieved using the expertise of the team. It turned out that this activity was more technically complex than anticipated, and it would be necessary to get external assistance from a professional programmer (in java). In the PI judgement this would be more a technical activity than a scientific one, and it would not significantly enhance the results of the project. Adding, time was already running short, so it was decided not to pursue this activity.

All the other indicators were either achieved or surpassed, especially the papers published in indexed international journals, which have world-wide audience and are of excellent quality. We believe the scientific outcomes of the NanoDC project to be outstanding.

4. Acknowledgments

The work carried out within the framework of the Project NanoDC has been funded by the European Regional Development Fund (ERDF) through COMPETE – Operational Programme for Competitiveness Factors (OPCF), by Portuguese National funds through "FCT - Fundação para a Ciência e a Tecnologia" under project reference PTDC/AGR-AAM/101643/2008. Other supporting was provided by FCT in the form of the PhD research grant awarded to Helena Gomes (SFRH/BD/76070/2011) and by FP7-PEOPLE-IRSES-2010-269289-ELECTROACROSS, which funded some of the missions).

Several people have also contributed to NanoDC. The Project acknowledges Alexandra Ribeiro (New University of Lisbon, Portugal) for her valuable contribution as the main supervisor of PhD Student Helena Gomes. Sibel Pamukcu (Lehigh University, USA), Pieter Kleingeld (Utrecht University, Holland) and José Miguel Rodríguez-Maroto (University of Malaga, Spain) are also acknowledged for their valuable contributions to the project.

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Gomes, Helena I., J.M. Rodríguez-Maroto, Alexandra B. Ribeiro, Sibel Pamukcu, Celia Dias-Ferreira (2015a). Numerical prediction of diffusion and electric field-induced iron nanoparticle transport. Electrochimica Acta (http://dx.doi.org/10.1016/j.electacta.2014.11.157)

Gomes, Helena I., Celia Dias-Ferreira, Lisbeth M. Ottosen, Alexandra B. Ribeiro (2015b). Electroremediation of PCB contaminated soil combined with iron nanoparticles: Effect of the soil type (accepted Chemosphere)

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Rosales, Emilio, M. Ángeles Sanromán, Celia Dias-Ferreira. Green zero-valent iron nanoparticles synthesized using herbal tisanes (to be submitted Feb 2015a).

Rosales, Emilio, Celia Dias-Ferreira, M. Pazos, M.A. Sanromán. Green zero-valent iron nanoparticles from aromatic herbal extracts as catalyst in Fenton degradation of dyes (to be submitted Feb 2015b).

Annexes

Annex A – List of reports produced during Project NanoDC

Annex B – Description of the document management system (DM-01-2012)

Annex C – Experimental procedure for setting calibration curves from PCB analysis in liquid extracts by GC-ECD (document: OP-02-2012)

Annex D– Experimental procedure for ultrasound extraction and cleanup of PCB's from soil samples (document: OP-04-2012)

Annex E – Experimental procedure for PCB analysis in liquid extracts by GC-ECD (document: OP-05-2012)

Annex F - Laboratory procedures related with OP-05-2012 (document: OP-14-2012)

Annex G – Quality manual for PCB analysis (document: OP-16-2012)

Annex G – Soil analysis results

Annex A – List of reports produced during Project NanoDC

Document management Code	DESCRIPTION
RP-01-2011	Celia Dias-Ferreira. Management of Project NanoDC: the first 6 months. September 2011
RP-05-2012	Celia Dias-Ferreira. NanoDC Progress Report:12 months. April 2012
RP-2013-01	Celia Dias-Ferreira. NanoDC Progress Report:24 months. April 2013
RP-2014-01	Celia Dias-Ferreira. NanoDC Progress Report:36 months. Sep 2014
RP-2015-01	Celia Dias-Ferreira. Final Report for the Project NanoDC. Jan 2015
RP-2013-02	Leonor Pato. Sampling and Characterization in Project NanoDC – progress report of task 2 after 24 months. April 2013
RP-2013-03	Rosa Quinta Ferreira. Degradation studies in NanoDC – progress report of task 3 after 24 months. April 2013
RP-07-2012	Helena Silva. 1st Grant Progress Report – January to May 2012
RP-2013-08	Helena Silva. 2nd Grant Progress Report – June to December 2012
RP-21-2012	Helena Silva. 3rd Grant Progress Report – December 2012 to May 2013
RP-08-2012	Emilio Rosales Villanueva. 1st Grant Progress Report – January to June 2012
RP-20-2012	Emilio Rosales Villanueva. 2nd Grant Progress Report – June to December 2012
RP-2013-06	Emilio Rosales Villanueva. 3rd Grant Progress Report – January to April 2014
RP-11-2012	Helena Silva. Grant report for the period 1-15 July 2012
RP-15-2012	Helena Silva. Grant report for the period 16-20 July 2012
RP-16-2012	Helena Silva. Grant report for the period 23-27 July 2012
RP-14-2012	Bruno Guimaraes. Grant Report for July 2012
RP-17-2012	Helena Silva, Bruno Guimarães. Grant Report for the period 30 Jul to the 3rd of August 2012
RP-18-2012	Helena Silva, Bruno Guimarães. Grant Report for the week 20-24 August 2012
RP-19-2012	Helena Silva, Bruno Guimarães. Grant Report for the week 27-31 August 2012

Annex B – Description of the document management system (DM-01-2012)

nanoDC
Electrokinetic remediation of PCB-contaminated soil using iron nanoparticles

DOCUMENT COVER PAGE

Code: DM-01-2012
Title: Instructions for document control
Objectives: provide instructions to properly manage documents in project NanoDC

History:

VERSION	DATE	CHANGES INTRODUCED (FROM PREVIOUS VERSION)	AUTHOR	APPROVED BY:	APPROVAL DATE	DISTRIBUTION LIST
v1	25-07-2012	Original version	CF	CF	25-07-2012	BG, HS, EV
v2	31-01-2013	Code modification	ER	CF	18-02-2013	CF, EV, HS

BG – Bruno Guimarães, CF – Célia Ferreira, EV- Emilio Villanueva, HS – Helena Silva

nanoDC
Electrokinetic remediation of PCB-contaminated soil using iron nanoparticles

Instructions for document control

Documents used/produced in project NanoDC must have (whenever possible):

- **cover page:** code, title, objectives and history table
- **header (all pages):** project logo and title
- **footer (all pages)**: document code, version, original author and date, current version author and date, pages (partial/total), approval of original and current version and dates.

The following template is available:
DROPBOX/…/NANODC_DOCS/INTERNAL/**DM-04-2012-general_template.docx**

Codes are constructed using the system exemplified by:

DM-201Y-XX, in which the first 2 letters are described below and the number is sequential (within a specific year)

Documents elaborated in 2012 keep the following code DM-XX-2012, in which the first 2 letters are described below and the number is sequential within this specific year.

- DM – document management
- PM – project management (proposal, timeline, indicators, task description, dissemination, others)
- FM – financial management (budgets, acquisitions, expenses, others)
- OP – operational procedures (ex: for sample collection, analytical procedures) and operational forms (*impressos*) that will be filled with results (after filling it will be a RG - registry (*registo*).
- RG – registry (forms filled with experimental data and results, other)
- RP – reports (task reports, weekly reports, project report, etc.)
- HR – human resources (ex: list of team members, assiduity, others)
- MM – meeting minutes (actas de reuniões)
- EX – external document (ex: equipment's manuals, standards, legislation, and so on).

This list can be updated. View full list in following document:

DROPBOX/…/NANODC_DOCS/INTERNAL/**DM-02-2012-list_docs.docx**

Maintenance of original documents:

- Paper version: keep at file/dossier "NanoDC DOCUMENTS", in project leader's office
- Electronic version: save at folder indicated bellow using as name file the code followed by a small description (ex: DM-01-2012-List_doc_control)

Dropbox folder to store all internal documents:
DROPBOX/…/NANODC_ DOCS/INTERNAL

C**ontrolled copies** procedure is implemented by using a distribution list (cover page of document)

Obsolete documents: proceed as follow:

- Original paper document: write in all pages "obsolete" (preferably in red) and date. Move from project file/dossier to "NanoDC obsolete" file/dossier and keep for at least 5 years after project conclusion.
- Paper copies: collect and destroy all controlled copies,
- Electronic version: add watermark "obsolete, [date]", and move to folder:

Dropbox folder to store obsolete documents:
DROPBOX/…/NANODC_ DOCS/OBSOLETE

Revision/editing of documents:

- New versions must be approved by project leader before becoming available for use.
- Changes since last version must be clearly indicated (ex: using word "track changes" option)
- Obsolete documents must be handled as described above.

Document cross-referencing

A double entry matrix is constructed to identify all cross-referencing between documents. When creating or updating documents updated of the double entry matrix is required.

Dropbox folder to store double-entry matrix
DROPBOX/…/NANODC_DOCS/INTERNAL/DM-03-2012.docx

Document back-up

(To de defined)

Annex C - Experimental procedure for setting calibration curves from PCB analysis in liquid extracts by GC-ECD (document: OP-02-2012)

Electrokinetic remediation of PCB-contaminated soil using iron nanoparticles

DOCUMENT COVER PAGE

Code: OP-02-2012
Title: Calibration curves for PCB analysis by GC-ECD
Objectives: Describes procedures to set calibration curves for PCBs in aqueous extracts using Gas Chromatography, including relative standard deviation (RSD) and calibration factor (CF)

History:

VERSION	DATE	CHANGES INTRODUCED (FROM PREVIOUS VERSION)	AUTHOR	APPROVED BY:	APPROVAL DATE	DISTRIBUTION LIST
v1	19-10-2012	Original version	HS_BG			
v2	18-10-2013	Revision of original version	CF	CF	18-10-2013	

AR- Alexandra Ribeiro, BG – Bruno Guimarães, CF – Célia Ferreira, DL – Daniela Lopes, EN – Elsa Neto, ER- Emílio Rosales, GL – Gustav Loch, HG- Helena Gomes, HS – Helena Silva, JA – José Azevedo, JV – Jorge Varejão, JS - João Salvador, LF – Licínio Ferreira, LO – Lisbeth Ottosen, LP-Leonor Pato, MQ – Margarida Quina, MS – Micaela Soares, MR – Mariana Raquel, PK - Pieter Kleingeld , RM – Rui Martins, RF – Rosa Ferreira, RS – Ruud Schotting

Calibration curves for PCB analysis in liquid extracts by GC-ECD

Para este procedimento existe um impresso para registo dos dados: FM-10-2012

Nota 1: Lavar todo o material usado nos procedimentos seguintes com água e sabão neutro, seguida da lavagem com água destilada e enxaguamento com acetona seguida por hexano.

Reagentes:

- hexano para GC
- solução padrão de 7 PCBs (28, 50, 101, 138, 152, 180, 209) com 10 ng/µL em cada um dos congéneres
- PCB 30 (para spiking)
- PCB 65 (para spiking)
- PCB 204 (para spiking)
- PCB 166 (padrão interno)

A) Procedimento para preparar os padrões para a recta de calibração:

1. de 7PCB's: 28, 50, 101, 138, 152, 180, 209

1.1. Lavar os balões necessários: com água e detergente, passar com água destilada, enxaguar com acetona seguido do solvente a utilizar para as diluições (neste estudo, hexano);
1.2. Preparação da solução-mãe 0,1 ng/µL (de cada congénere): Medir com micropipeta 200µL da mistura dos 7PCB's 10 ng/µL para um balão volumétrico de 20mL e perfazer o volume com hexano;
1.3. Retirar 0,5mL da solução mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0.005ng/ µL para cada congénere.
1.4. Retirar 1mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,010ng/ µL para cada congénere;
1.5. Retirar 2mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,020ng/ µL para cada congénere;
1.6. Retirar 5mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,050ng/ µL para cada congénere;
1.7. Retirar 8mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,080ng/ µL para cada congénere.

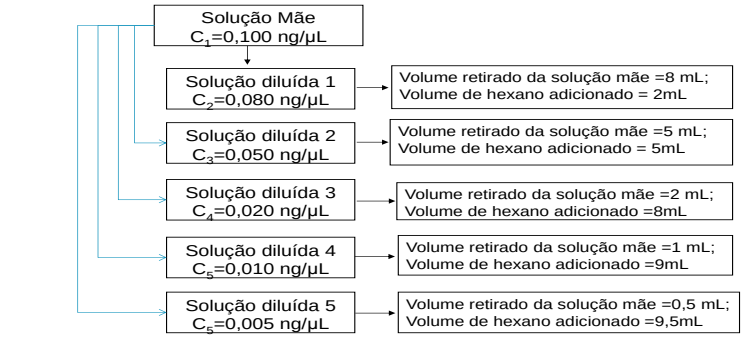

Figura 1 – Esquema representativo do volume a retirar da solução mãe para fazer as diluições.

2. **de 3PCB's: 30, 65, 204** (para o spiking)

2.1. Solução-mãe 10 ng/µL em cada congénere: Pesar 1 mg de cada congénere de PCB para 3 balões volumétricos de 100mL. Perfazer o volume com hexano;
2.2. Retirar 0,100 mL da solução-mãe para um balão volumétrico de 10 mL e perfazer com hexano. Obtém-se a concentração de 0,1 ng/µL;
2.3. Retirar 0,080 mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,080 ng/µL;
2.4. Retirar 0,050 mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,050 ng/µL;
2.5. Retirar 0,020 mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,020 ng/µL;
2.6. Retirar 0,010mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,010 ng/µL;
2.7. Retirar 0,005mL da solução-mãe para um balão volumétrico de 10mL e perfazer com hexano. Obtém-se a concentração de 0,005 ng/µL;
2.8. Dependendo da resposta do detetor do GC escolher-se-á a concentração a utilizar para o *spiking*.

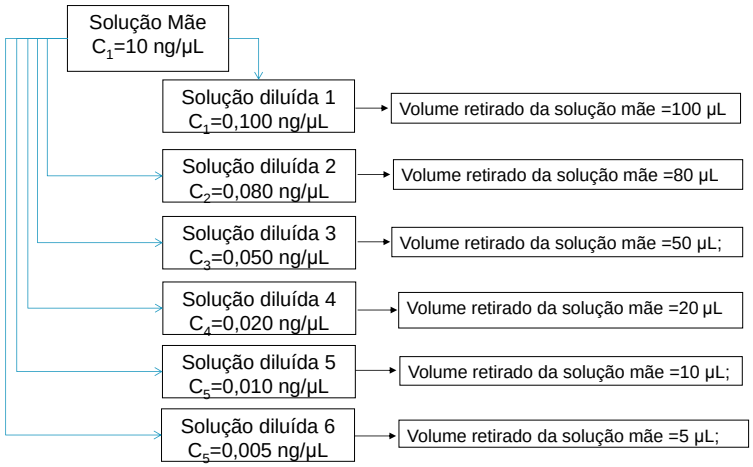

Figura 2 – Esquema representativo do volume a retirar da solução mãe para fazer as diluições dos 3 PCB's: 30, 65 e 204.

3. do padrão interno (PI): PCB 166

A área do pico do padrão interno não deverá ser superior a 100 vezes à área do pico mais pequeno detetável. Prevê-se que o LMD da solução mãe da mistura de 7 PCB's seja 0,005 ng/µL, assim a concentração do padrão interno na amostra injectada no GC não deverá ser superior a 0,5 ng/µL.

3.1. Preparação da solução-mãe de padrão interno (PI) 50 ng/µL: pesar 5 mg do PCB 166 para um balão volumétrico de 100mL. Perfazer o volume com hexano e homogeneizar bem.
3.2. Preparação de solução diluída de padrão interno 0,1 ng/µL: retirar 20 µL da solução-mãe do PI 50 ng/µL e adicionar num balão de 10 mL. Perfazer o volume com hexano;
3.3. Retirar 50µL da solução de padrão interno criada no passo anterior e adicionar a 100 µL do extrato (amostra a analisar), num eppendorf, homogeneizar bem e injetar 1 µL no GC-ECD;
3.4. A partir do cromatograma obtido, avaliar se a relação entre as áreas do PI e as do extrato são equivalentes ou seja, a área de padrão interno não deverá ser superior a 100 vezes a área do pico mais baixo;
3.5. Caso não sejam, preparar uma solução mais diluída do PI (passo 3.2) e repetir os passos 3.3 e 3.4.

B. Determinação do tempo de retenção relativo:

Tempo de retenção do padrão interno, RT(PI):

Injetar 1 µL do padrão interno 0,10 ng/µL) no GC e registar o tempo em que ele surge no cromatograma.

Tempo de retenção de cada congénere, RT(PCB_x):

Para cada padrão, misturar 100 µL das soluções de 0,1 ng/µL de cada congénere com 50 µL do padrão interno (concentração= 0,10 ng/µL), homogeneizar bem e injetar 1 µL da mistura no GC.

Identificar a ordem dos picos no cromatograma (seguir passos indicados em OP-05-2012) e registar os tempos de retenção de cada congénere de PCB

Tempo de retenção relativo de cada congénere, RRT (PCBx)

Calcular os tempos de retenção relativos, RRT(PCBx) de cada congénere através da expressão:

$$RRT(PCBx) = RT(PCBx) / RT(PI)$$

(nota: após injeção no GC de uma amostra, sabendo o RT(PI) e o RRT(PCBx), inverte-se a fórmula para calcular o RT(PCBx) no cromatograma a analisar:

$$RT(PCBx) = RRT(PCBx) \times RT(PI)$$

C. Elaboração das retas de calibração:

Nota 2: a análise de cada amostra deverá ser feita em duplicado, trabalhando-se com a média dos valores obtidos.

1. Misturar 50 µL da solução diluída do PI e 100 µL da diluição mais baixa, num frasco de vidro de 2mL para cromatografia com tampa PTFE, homogeneizar bem, injetar 1 µL no GC-ECD e registar o cromatograma obtido;
2. Repetir o passo 1.1 para todas as concentrações efectuadas indo dos padrões menos concentrados para os mais concentrados;
3. Analisar os cromatogramas obtidos, determinando a áreas de cada congénere;
4. Dividir a área obtida para o congénere pela área do padrão interno.
5. Para cada congénere, elaborar um gráfico de dispersão, tendo no eixo das abscissas as concentrações injetadas (ng/µL) e no eixo das ordenadas a razão das áreas A(PCBx)/A(PI);
6. Adicionar ao gráfico a linha de tendência e respetivo factor de correlação (R^2) que deverá ser pelo menos 0,99;

7. Caso não se consiga obter factor de correlação desejável, deve-se realizar novas soluções de calibração e repetir o procedimento.
8. Determinar o desvio padrão relativo de modo a saber-se se a curva de calibração pode ser assumida como sendo linear e se pode passar pela origem do eixo, através da seguinte fórmula:

$$\boldsymbol{RSD = \frac{SD}{\overline{CF}} \times 100}\textbf{, deverá ser} \leq \textbf{20\%}$$

O factor de calibração (CF) é calculado pela fórmula seguinte e permite avaliar a precisão da resposta do GC na análise de analitos com concentrações conhecidas (controlos)

$$\boldsymbol{CF = \frac{Área\ do\ pico\ de\ cada\ congénere\ na\ mistura}{massa\ do\ composto\ injetado\ (ng)}}$$

O desvio padrão (RS, ou em inglês, SD) determina-se através da fórmula seguinte, e indica o desvio padrão dada pelo equipamento na análise de réplicas do mesmo composto:

$$RS = \sqrt{\frac{\sum_{i=1}^{n}(CF - \overline{CF})^2}{n-1}}$$

Para verificar se as retas de calibração permanecem válidas ao longo do tempo é necessário comparar a resposta do equipamento através do cáculo do RSD que deverá ser ≤ 20%.
Caso este valor seja superior, consultar OP-16-2012-PCB's quality.

4. Bibliografia

- USEPA, Method 8000B. DETERMINATIVE CHROMATOGRAPHIC SEPARATIONS. Revision 2, December 1996. Disponível em: http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8000b.pdf

Annex D - Experimental procedure for ultrasound extraction and cleanup of PCB's from soil samples (document: OP-04-2012)

nanoDC
Electrokinetic remediation of PCB-contaminated soil using iron nanoparticles

DOCUMENT COVER PAGE

Code: OP-04-2012
Title: Ultrasound extraction and cleanup of PCB's from soil samples
Objectives: describes the procedure to spiking and extract PCB's on soil and aqueous samples and cleanup their extracts

History:

VERSION	DATE	CHANGES INTRODUCED (FROM PREVIOUS VERSION)	AUTHOR	APPROVED BY:	APPROVAL DATE	DISTRIBUTION LIST
v1	31-08-2012	Original version	HS_BG	CF	CF, 30-out-2012	CF, BG, HG
v2	09-11-2012	Adição do procedimento de spiking a este procedimento	HS	CF	18-out-2013	
v3	24-out-2013	- Acrescentada a lista de material e equipamento, - alterada a formatação de algumas partes - feitas pequenas correções	CF			

AR- Alexandra Ribeiro, BG – Bruno Guimarães, CF – Célia Ferreira, DL – Daniela Lopes, EN – Elsa Neto, ER- Emílio Rosales, GL – Gustav Loch, HG- Helena Gomes, HS – Helena Silva, JA – José Azevedo, JV – Jorge Varejão, JS - João Salvador, LF – Licínio Ferreira, LO – Lisbeth Ottosen, LP-Leonor Pato, MQ – Margarida Quina, MS – Micaela Soares, MR – Mariana Raquel, PK - Pieter Kleingeld , RM – Rui Martins, RF – Rosa Ferreira, RS – Ruud Schotting

Ultrasound extraction and cleanup of PCB's from soil and aqueous samples

Para este procedimento existe um impresso para registo dos dados: FM-05-2012

- **Antes de iniciar o procedimento descrito, consultar o manual de qualidade: OP-16-2012 Quality manual for PCB analysis**

NOTA 1: Todos os recipientes utilizados durante este procedimento deverão ser bem lavados com o solvente usado nessa etapa e adicionado ao extrato, de forma a minimizar perda de PCB's.

Nota 2: O procedimento é realizado em duplicado, sendo uma das amostras contaminada (*spiking*) e outra não contaminada.

Nota 3: O material deve ser lavado com água e detergente, depois enxaguado sucessivamente por água corrente, água destilada, acetona e hexano.

0. Material, equipamento e reagentes

Material	Equipamento	Reagentes
Preparação amostra		
- 2 cadinhos porcelana base larga; - 2 cadinhos p/determinar a hum. - vareta de vidro (opc.)		- 40 g sulfato de sódio anidro (opc.)
Contaminação amostra		
- pipeta de 5 mL ou de 15 mL - pontas e micropipeta 40 uL - copo de 50 mL - vareta de vidro	- hotte - balança	- n-Hexano - 40 uL PCB 30 0,1 ng/µL; - 40 uL PCB 65 0,1 ng/µL; - 40 uL PCB 204 0,1 ng/µL
Extracção		
- 2 frascos vidro 50 mL para ultrassons - papel de filtro Whatman 41 ou equivalente (ALBET DP5891047) - 2 kitasatos (250 mL) e funis de buchner - 2 balões de fundo redondo p/ evaporador rotativo - 1 pipeta de 10 mL - 2 frascos de 20 mL + tampas	- ultrassons - evaporador rotativo	- n-hexano (90 mL) - acetona (90 mL)
Limpeza		
- pipeta de 5 mL ou de 15 mL - pontas e micropipeta 40 uL - copo de 50 mL - vareta de vidro - 6 frascos de 20 mL + tampas	- agitador vortex	- 30 mL ácido sulfúrico 1:1 - 12 mL de n-hexano - 10 mL permanganato de potássio aquoso a 5%

1. **Preparação da amostra sólida**

1.1. Homogeneizar a amostra (com o auxilio de uma vareta de vidro). Retirar uma alíquota para determinar a humidade, de acordo com o procedimento OP-11-2012
1.2. Pesar 10 g de amostra de solo homogeneizado para um cadinho de base larga e registar o peso até mg; Repetir para a segunda amostra que será contaminada.
1.3. Se a amostra for muito húmida/argilosa adicionar 20g de Na2SO4 (sulfato de sódio anidro) a cada amostra de 10g de solo, usando uma espátula e homogeneizando até obter uma consistência de pó.

2. **Procedimento de contaminação (*spiking*) para amostras de solo (para determinar a taxa de recuperação do procedimento extractivo)**

2.1. Medir (com pipeta volumétrica) 5mL hexano para um copo. Caso tenha adicionado Na2SO4, medir 15mL de hexano
2.2. Adicionar ao hexano (com micropipeta) 40µL das seguintes soluções padrão:
- Solução PCB 30 0,1 ng/µL;
- Solução PCB 65 0,1 ng/µL;
- Solução PCB 204 0,1 ng/µL;

2.3. Homogeneizar e adicionar esta solução (hexano+PCBs) a uma das duas amostras de solo preparadas no ponto anterior e misturar muito bem (recorrer ao auxilio de uma vareta de vidro, se necessário) de modo a que todo o solo fique embebido. Recuperar todo o solo que tenha ficado na vareta;
2.4. Colocar o cadinho na hotte ligada e deixar o hexano evaporar (pode deixar uma noite);
2.5. Calcular a contaminação artificial utilizando a seguinte fórmula:

[PCBx] = vol PCB (ul) * conc. PCB (ng/uL) / peso fresco da amostra de solo (g)

Exemplo, para o PCB 30, e 10,0 g de amostra (peso fresco):

[PCB 30] = 40uL*0,1(ng/uL)/10,0 g solo (peso fresco) = **0,4 ng/g** solo (peso fresco)

3. **Extração de PCBs em <u>amostras aquosas</u>**

3.1. Num frasco de vidro de 60mL colocar 20 mL de amostra aquosa e adicionar 2,5mL de hexano;
3.2. Fechar bem o frasco e levar ao vórtex durante 1min;
3.3. Deixar que ocorra separação de fases e retirar a camada de cima correspondente ao hexano (usando uma pipeta de Pasteur de vidro descartável) e colocar num frasco de vidro de 20mL;
3.4. Adicionar novamente 2,5mL de hexano ao extrato aquoso e levar ao vórtex durante 1min;
3.5. Deixar separar as fases e retirar a camada de cima e juntar ao hexano retirado em 2.3;
3.6. Colocar papel de filtro num funil e 3 a 6 gramas de sulfato de sódio anidro;
3.7. Filtrar o hexano e recolher num frasco de 20mL. Passar 2,5mL de hexano pelo sulfato de sódio e recolher no frasco;

3.8. Transferir o hexano do frasco para um balão volumétrico de 10mL e aferir o volume com hexano. Guardar num frasco de 20mL no frio até análise por GC (OP-15-202).

4. Extração das amostras de solo por ultrassom, para baixas concentrações (≤20mg/kg)

Nota: - baseado no método da EPA 3550C – extração por ultrassom;
- a aplicação continua do vácuo pode resultar na perda de alguns analitos

4.1. Transferir as duas amostras (contaminada e não contaminada) dos cadinhos para um frasco de vidro de 50 mL (com tampa de vidro).
4.2. Adicionar 30mL da mistura de solventes de extração acetona/hexano (1:1).
4.3. Submeter o frasco a um banho de ultrassom durante 20 min.
4.4. Decantar e filtrar o extrato em papel de filtro Whatman nº 41 ou equivalente (papel filtro quantitativo sem cinzas, ALBET, DP5891047) num funil de Buchner ligado a um frasco de filtração (kitasato) de 250 mL lavado previamente com água, acetona e hexano.
4.5. Repetir o processo de extração e filtração mais duas vezes, recolhendo sempre o filtrado para o mesmo kitasato.
4.6. Concentrar a amostra até 10mL (por vezes o volume é superior dependendo da dificuldade para concentrar a amostra) com evaporador rotativo (alternativa ao método de Kuderna-Danish); a velocidade de rotação do balão é de 10, o banho à temperatura de 60ºC e não se aplica vácuo. Caso o extrato pare de evaporar, aumentar a temperatura do banho a 70ºC.
4.7. Adicionar 10mL de hexano ao extrato e levar ao evaporador rotativo até se obter um volume final de aproximadamente de 5mL (Esta operação serve para troca de solvente, que tem de ser realizada antes da limpeza do extrato – Método 3665 A da EPA);
4.8. Transferir para um frasco de 20mL, lavando bem o balão com hexano e passando este hexano para o frasco;
4.9. Caso não seja preciso limpeza do extrato, concentrar até 2mL, usando fluxo suave, limpo e seco de nitrogénio com a ajuda de um banho a 30ºC (balão volumétrico);
4.10. No caso do extrato ter de sofrer limpeza seguir para os passos seguintes.

5. Procedimento para limpeza do extrato segundo a EPA 3665A. PARTE A – Ácido sulfúrico/permanganato

É aplicado quando a linha de base dos cromatogramas está constantemente elevada ou prevê-se cromatogramas muito complexos que dificultem a quantificação de PCB's (exemplo: amostras de solo).

NOTA 6: É importante que o extrato tenha sofrido a troca de solvente para hexano antes da limpeza.

5.1. Numa hotte, adicionar cuidadosamente 5mL de solução ácido sulfúrico/água 1:1 (ver procedimento OP-14-2012) ao extrato previamente obtido e guardado no frasco de 20mL;
5.2. Fechar bem o frasco e levar ao vórtex durante 1minuto, na posição 4.
5.3. Permitir a separação de fases, pelo menos durante 1minuto. Visualizar a camada superior (hexano), que não deverá ter uma cor forte, nem emulsão ou turvação.
5.4. Caso a camada de hexano esteja com cor ou a emulsão persista durante vários minutos, remover a camada de ácido sulfúrico do frasco e descartar. Adicionar 5mL da solução ácido sulfúrico/água 1:1 e realizar novo procedimento de limpeza.
5.5. Transferir a camada de hexano para um frasco limpo de 20mL. Ter o cuidado de não transferir ácido, dado que pode danificar os equipamentos analíticos.
5.6. Adicionar 1mL de hexano à camada de ácido sulfúrico, tapar o frasco e agitar. Esta segunda extração assegura a transferência quantitativa de PCB's.
5.7. Remover a camada de hexano e adicionar à camada de hexano obtida no ponto anterior.
5.8. Caso o extrato esteja limpo passar para o ponto 3.17, caso ainda possua alguma cor, proceder à limpeza com permanganato:
5.9. Adicionar 5mL de solução de permanganato de potássio aquoso a 5% à solução combinada das frações de hexano recolhidas (ver procedimento OP-14-2012).
5.10. Tapar o frasco e levar ao vórtex durante 1minuto, na posição 4. Permitir a separação de fases durante pelo menos 1 minuto. Examinar a camada superior (hexano) e ver se possui ou não cor ou turvação.
5.11. Se não apresentar cor nem turvação proceder para o ponto 4.17.
5.12. No caso de a camada de hexano permanecer com cor e turvação durante vários minutos, remover a camada de hexano do frasco recorrendo a uma pipeta de vidro e colocar noutro frasco de 20mL. Adicionar 5mL de solução de permanganato de potássio aquoso a 5%.
5.13. Levar ao vórtex e permitir a separação das fases.
5.14. Transferir a camada de hexano para um frasco limpo de 20mL
5.15. Adicionar 1mL de hexano à camada de permanganato, fechar o frasco e agitar. Esta segunda extração assegura a transferência quantitativa de PCB's.
5.16. Remover a camada de hexano e adicionar à camada de hexano anterior.
5.17. Reduzir o volume das camadas combinadas de hexano para o volume original, 2mL usando uma técnica de concentração apropriada - evaporador rotativo seguido de fluxo suave de nitrogénio para perfazer o volume correto (Realiza-se apenas, caso não seja necessário realizar posteriormente limpeza de sílica).
5.18. Transferir o extrato para frasco de vidro com tampa PTFE, corretamente identificado e guardar no frio.

6. Procedimento para limpeza do extrato segundo a EPA 3630C. PARTE B: Limpeza com Gel Sílica

6.1. Transferir 3 gramas de gel sílica desativado (ver procedimento OP-14-2012) para uma coluna cromatográfica de vidro de 10mm de diâmetro interno (alternativa bureta de 50mL) e cobrir com 2-3 cm de sulfato de sódio anidro;
6.2. Adicionar 10mL de hexano no topo da coluna para humedecer e lavar o sulfato de sódio e o gel sílica. Mesmo antes da exposição da camada de sulfato de sódio ao ar

parar o fluxo de hexano, fechando a torneira da coluna cromatográfica. Descartar o eluído.

6.3. Transferir o extrato (hexano) para a coluna. Lavar o frasco do extrato duas vezes com 1 a 2mL de hexano e adicionar cada lavagem à coluna.

6.4. Eluir a coluna com 40mL de hexano (fração I – a desejável para análise de PCB's). Remover o frasco de recolha e reservar para posterior concentração. Eluir a coluna com 50mL de hexano (fração II – para análise de pesticidas, não efetuado para análise de PCB's) e recolher o eluído. Realizar uma terceira eluição com cloreto de metileno (fração III – para análise de outros pesticidas e não realizável para análise de PCB's).

6.5. Combinar as frações como desejável (para análise de PCB's usar só a fração I).

6.6. Recorrendo ao evaporador rotativo, concentrar o extrato combinado até aos 5 mL, a temperatura de 70ºC sob vácuo.

6.7. Caso o solvente de extracção não seja o hexano, fazer a troca de solvente, adicionando 30mL de hexano ao extrato e concentrar usando técnica apropriada (usar apenas se o solvente de extração não for hexano);

6.8. Transferir para um balão de diluição de 10 mL e aferir o volume com n-hexano.

6.9. Transferir para um frasco de cromatografia de 2 mL com tampa PTFE (ou frasco de 20 mL) e guardar no frio, até análise por GC (OP-15-202).

Bibliografia:

- USEPA, Method 3550C. Ultrasonic extraction. Revision 3, February 2007. Disponível em: http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3550c.pdf
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- USEPA, Method 3630C. Silica Gel Cleanup. Revision 3, December 1996. Disponível em: http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3630c.pdf

Annex E - Experimental procedure for PCB analysis in liquid extracts by GC-ECD (document: OP-05-2012)

Electrokinetic remediation of PCB-contaminated soil using iron nanoparticles

DOCUMENT COVER PAGE

Code: OP-05-2012
Title: Analysis of PCBs in liquid extracts using GC-ECD
Objectives: describes the analytical procedure to determinate PCB's using GC-ECD in previously extracted and cleaned samples

History:

VERSION	DATE	CHANGES INTRODUCED (FROM PREVIOUS VERSION)	AUTHOR	APPROVED BY:	APPROVAL DATE	DISTRIBUTION LIST
v1	31-08-2012	Original version	BG HS	CF	30-10-2012	CF, BG, HS
v2	19-out-2013	-Reordenamento das secções; -lista de documentos relacionados	CF	CF	19-out-2013	

AR- Alexandra Ribeiro, BG – Bruno Guimarães, CF – Célia Ferreira, DL – Daniela Lopes, EN – Elsa Neto, ER- Emílio Rosales, GL – Gustav Loch, HG- Helena Gomes, HS – Helena Silva, JA – José Azevedo, JV – Jorge Varejão, JS - João Salvador, LF – Licínio Ferreira, LO – Lisbeth Ottosen, LP-Leonor Pato, MQ – Margarida Quina, MS – Micaela Soares, MR – Mariana Raquel, PK - Pieter Kleingeld , RM – Rui Martins, RF – Rosa Ferreira, RS – Ruud Schotting

Analysis of PCB's in liquid extracts using GC-ECD

Documentos relacionados:

- Impresso para registo dos dados: FM-07-2012
- Antes de iniciar o procedimento descrito, consultar o manual de qualidade: OP-16-2012 PCB's Quality
- As rectas de calibração foram previamente elaboradas de acordo com OP-02-2012
- Preparação e limpeza dos extractos: OP-04-2012

1. Equipamento

GC HP com detetor ECD, HP 6890 Series

2. Coluna

Tabela 2: Características de operação da coluna capilar

Marca:	Phenomenex Zebron
Representante:	Teknokroma
Phase:	TRB – 5 – MS
L:	30 m
ID:	0,25 mm
DF:	0,25 µm
CAT:	TR – 520232
SERIAL:	NF – 1368
Pressure:	15 psi
Flow:	1,2
Carrier gas:	Helium
Veloc:	30
Mode:	Cte pressure

3. Condições operacionais para o GC ECD:

Tabela 1: Condições operacionais GC HP com detetor ECD, HP 6890 series

Programa do Forno (OVEN)		**Injetor (Front Inlet)**		**Detetor ECD (Front detector)**	
Temperature:	**70 ºC**	Mode:	Splitless	Temperature:	280 ºC
Initial time:	2,00 min	Temperature:	260ºC	Anode gas:	6,0
Rate 1:	25,00 ºC/min	Pressure:	15 psi	Mode:	Cte makup
Final temperature 1:	**150 ºC**	Purge time:	1min	Mkup (N_2)	60,0
Final time:	0,00 min	Purge flow:	15,00	Adjust offset:	OFF
		Total flow:	3,3	Output:	
Rate 2:	4,00 ºC/min				
Final temperature 2:	**200 ºC**	Gas saver:	On	Ref current	
Final time 2:	0,00	Saver flow:	20,0	**Signal 1**	
		Saver time:	2,00 min		
Rate 3:	8,00 ºC/min				
Final temperature 3:	**280 ºC**			Type:	Front-comp 1
Final time:	4,00			Value:	
				Zero:	80,0
Rate 4:	10,00 ºC/min				
Final temperature:	**300 ºC**			Range:	0
Final time:	2,00			Attn	0
Rate 5 (OFF):	0,00				

4. Aquisição de dados:

A aquisição de dados é realizada recorrendo ao Interface *Hercule Lite-Chromatography* e software de aquisição de dados da marca *Borwin Chromatography*."

Os passos para iniciar um "corrida" no sofware referido são os seguintes:

a) Abrir o programa BORWIN (carregando no ícone correspondente no desktop)
b) Clicar em "RUN";
c) Clicar em "START SINGLE RUN";
d) Clicar em "START RUN ON SYSTEM 1". CONFIRMAR QUE O CABO DE LIGAÇÃO DO GC AO SOFTWARE ESTÁ LIGADO AO CANAL 1.
e) Dar nome à "corrida" e colocar outra informação pertinente.
f) Na altura de injectar a amostra clicar em "RUN", para iniciar a recolha de dados.

5. Injeção no GC ECD:

- Retirar para um frasco de cromatografia de vidro (capacidade: 2 mL) 100µL do extracto obtido em OP-04-2012. Adicionar 50µL de solução padrão interno (PCB 166) com concentração de 0,05 ng/µL (OP-02-2012).
- Lavar a seringa que irá ser usada na injecção com acetona seguida de hexano, 15 vezes cada.

- Introduzir na sering 1µL da amostra com o padrão interno e posicioná-la na abertura de injecção do GC.
- Clicar em "RUN" (no softare de aquisição de dados) e simultaneamente (ou logo de seguida) injetar o conteúdo da sering no GC, carregar logo de seguida em "START" no GC.
- Repetir a injeção da amostra + padrão interno (para se obterem duplicados)

6. Análise do cromatograma – integração das áreas dos picos:

Durante a aquisição de dados realizada pelo softare *Borwin Chromatography* foi criado um ficheiro, que pode ser aberto através do menu "Open file".

a) Localizar o padrão interno pelo tempo de retenção (RT) em que este deverá surgir (de acordo com OP-02-2012 e FM-10-2012).

Ter atenção: No caso de existir algum desfasamento durante a injeção no GC, o RT do padrão interno poderá ser diferente, o que deverá ter-se em conta para não considerar erradamente outro pico como padrão interno. Assim, é importante a injeção repetida do mesmo extrato de modo a poder-se comparar os RT do padrão interno para evitar um possível erro de localização.

b) Usando o tempo de retenção relativo (RRT) de cada congénere, calculados através do procedimento OP-02-2012 e indicados no RG-06-2012, determinar o RT em que cada congénere deverá surgir no cromatograma.
c) Visualizar o pico de um congénere e ampliar com o zoom;
d) Clicar em "edit peak baseline". Na coluna do lado esquerdo do quadro que surge selecionar "new" e colocar um ponto no início do pico e outro no fim. Dar nome ao pico e clicar em "done".
e) Na coluna do lado direito do quadro "edit", selecionar a opção "Del". Clicar em "new" e marcar o início e o fim que se quer para a linha de base do pico. Esta linha tem de ser o mais linear possível. Clicar em "exit" para sair do menu "edit".
f) Selecionar a opção "Look peak report" e ver o relatório. Neste aparece o nome dado ao pico, o RT, a respetiva área (µV.Sec) e % Area. O que se deseja retirar desta informação é o RT para ver se o pico é o correto e caso afirmativo a área (µV.Sec). Clicar em "exit" para sair deste menu.
g) Repetir os passos anteriores para todos os picos que se queiram analisar do cromatograma.
h) Salvar o cromatograma e sair do programa.

7. Análise qualitativa e análise quantitativa

7.1. Análise qualitativa

A análise qualitativa em cromatografia gasosa é basicamente de natureza binária, o que se pretende analisar existe ou não existe (Valcárcel, Cárdenas, Simonet, & Carrillo-Carrión, 2007).

Para a análise qualitativa, basta analisar os cromatogramas obtidos e recorrendo ao RT do analito, verificar se ele está presente na amostra extraída ou não. Após integração das áreas pode-se ficar com a noção de quais os analitos que existem em maior quantidade, no entanto não fornece quantidade existente na amostra estudada.

7.2. Análise quantitativa

Para realizar a análise quantitativa é necessário recorrer às equações da reta de cada congénere, obtidas através do procedimento OP-02-2012 e registadas em RG-06-2012:

$$y = mx + b$$

Em que,
Y= é a divisão da área do congénere pela área do padrão interno, sendo adimensional $\frac{A(pcb)(\mu S.Sec)}{A(pi)(\mu S.Sec)}$;
x= é a concentração do congénere num µL de extrato, as unidades são expressas em ng/µL;

a) Aplicar o procedimento no ponto 6 do presente documento para integração dos picos de congéneres existentes no cromatograma a analisar;
b) Dividir a área do congénere pela área do padrão interno ($\frac{A(pcb)}{A(pi)}$);
c) Aplicar a equação da reta do PCB que está a ser analisado, substituindo o "y" pela divisão das áreas e calculando "x" que indica a concentração do congénere em ng/µL, na amostra extraída e sujeita a análise (antes da adição do padrão interno)
d) Calcular a concentração na amostra inicial: para o caso de amostras sólidas será necessário considerar o peso inicial da amostra, e o volume final após a extracção e limpeza, que contém os PCBs que existiam na amostra. Para as amostras liquidas será necessário considerar a relação entre o volume da amostra inicial e o volume final após o processo de limpeza.

7.3. Taxa de recuperação:

O sipiking realiza-se para obter-se a taxa de recuperação de modo a avaliar a eficiência de extração do método utilizado.

Para cálculo desta taxa aplica-se a fórmula seguinte:

$$\boldsymbol{Tr = \frac{[obtida]}{[esperada]} \times 100}$$

Em que,

[obtida] = concentração do congénere obtida pela análise de GC (calculada em 7.2.d);
[esperada] = concentração do congénere esperada como resultado do procedimento de contaminação ("spiking") da amostra.

8. Bibliografia:

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- Valcárcel, M., Cárdenas, S., Simonet, B. M., & Carrillo-Carrión, C. (2007). Principles of qualitative analysis in the chromatographic context. *Journal of chromatography. A*, *1158*(1-2), 234–40. doi:10.1016/j.chroma.2007.03.034

Annex F - Laboratory procedures related with OP-05-2012 (document: OP-14-2012)

nanoDC
Electrokinetic remediation of PCB-contaminated soil using iron nanoparticles

DOCUMENT COVER PAGE

Code: OP-14-2012
Title: PCB's complementary procedures
Objectives: describes procedures to prepare solutions with the aim to help PCB's extraction and cleanup procedures

History:

VERSION	DATE	CHANGES INTRODUCED (FROM PREVIOUS VERSION)	AUTHOR	APPROVED BY:	APPROVAL DATE	DISTRIBUTION LIST
v1	31-08-2012	Original version	HS_BG	CF	30-10-2012	CF, BG, HS

AR- Alexandra Ribeiro, BG – Bruno Guimarães, CF – Célia Ferreira, DL – Daniela Lopes, EN – Elsa Neto, ER- Emílio Rosales, GL – Gustav Loch, HG- Helena Gomes, HS – Helena Silva, JA – José Azevedo, JV – Jorge Varejão, JS - João Salvador, LF – Licínio Ferreira, LO – Lisbeth Ottosen, LP-Leonor Pato, MQ – Margarida Quina, MS – Micaela Soares, MR – Mariana Raquel, PK - Pieter Kleingeld , RM – Rui Martins, RF – Rosa Ferreira, RS – Ruud Schotting

PCB's Complementary procedures

Para este procedimento existe um impresso para registo dos dados: FM-14-2012

Nota 1: Lavar todo o material usado nos procedimentos seguintes com água e sabão neutro, seguida da lavagem com água destilada e enxaguamento com acetona, seguida de hexane.

A) Procedimento para a elaboração de 200mL de solução de permanganato de potássio aquoso a 5%

1. Pesar 10g de permanganato de potássio para um balão volumétrico de 200mL;
2. Perfazer o volume do balão com água destilada e homogeneizar e filtrar;
3. Rotular devidamente (Composto, Referência ao Lote, Data de criação e Responsável) e guardar no frio caso não seja utilizado de seguida.

B) Procedimento para a elaboração de 20mL da mistura de ácido sulfúrico (95%-97%):água, 1:1 (por cada amostra e respetivo duplicado)

1. Pipetar 10mL de água destilada para um frasco de vidro;
2. Pipetar 10mL de ácido sulfúrico e adicionar muito lentamente ao recipiente que contém a água;
3. Homogeneizar muito bem, rotular devidamente (Composto, Referência ao Lote, Data de criação e Responsável) e guardar no frio caso não seja utilizado de seguida.

C) Procedimento para a elaboração de sílica gel desativada (caso a que se use não seja desactivada)

1. Colocar 200g de silica num prato de vidro raso vagamente coberta com papel alumínio, durante 16h a 130ºC;
2. Colocar a silica no exsicador até arrefecer, transferir para um recipiente de vidro limpo, bem seco e com tampa;
3. Desativar a silica a 3,3% adicionando 6,6mL de água destilada;
4. Mexer o conteúdo exaustivamente e deixar em repouso durante 6horas;
5. Rotular (Composto, Referência ao Lote, Data de criação e Responsável) e armazenar a sílica desativada num frasco de vidro bem fechado, dentro de exsicador.

D) Bibliografia

- USEPA, Method 3550C. Ultrasonic extraction. Revision 3, February 2007. Disponível em: http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3550c.pdf
- USEPA, Method 3630C. Silica Gel Cleanup. Revision 3, December 1996. Disponível em: http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3630c.pdf
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