

Midterm evaluation of the PATHOS project

The midterm project evaluation is based on presentations by the consortium research groups and discussions undertaken on the 19 January 2010 at GEUS, Copenhagen, as well as written comments to this report afterwards.

The following partners were represented at the midterm evaluation:

GEUS, AU-DJF, KU-VET, KU-ØKO, KU-PHARMA, COBIO, DANVA and AMPHIBAC.

Following partners were not able to be present:

DIANOVA, NOVOZYMES, GRUNDFOSS but has afterwards given inputs to this report.

The process was facilitated by senior scientist Anders Johnsen (GEUS) a microbiologist working in the field of degradation of PAH and oil compounds in soil. His recommendation to the centre is presented without any modification in the end of this document.

SUMMARY

Each group presented their achievements in relation to the described milestones in the project description and the centre leader assigned one of three colours to the activity; GREEN – if the activity was finished, YELLOW – if it was ongoing and dark ORANGE – if the activity not yet was started. (Please see the next three pages with specific footnotes on changes from the original plan).

The overall conclusion of the meeting was that a very high degree of integration did take place in the centre, mainly through the common campaigns. The campaigns are orientated around 1) the field-campaign that has been undertaken in 2009, 2) the manure separation campaign that runs in 2009 and 2010, and finally the 3) the column leaching experiments that are being undertaken in 2010.

This structure makes the research groups highly dependent on each other. The ½ way evaluation was intended to show changes from the original plan, and make sure that all partners was aware of these changes (also in subprojects where they did not participate)

The KEMIRA back out of the PATHOS project has left the project with several problems: 1) one problem is related to assess to manure separation facilities and the potential to manipulate with samples. This problem has been solved by collaboration with AU-DJF that is running large scale experiments with separated manure. 2) The other problem is that GEUS and KU-Pharma has an economic deficit for running experiments in the laboratories. This problem remains to be solved.

The integration between the laboratories working with molecular detection techniques will be intensified in 2010 by several planned joint experiments as well as by hosting a Ph.D. course at GEUS in April 2010. The sabbatical of professor William E Holben from University of Montana at GEUS from February to August will be used in planning common experiments also including the new private companies Amphibac and Teknologisk Institut Århus that has joined the centre.

WP1	GEUS	AU-DJF	KU-VET	KU-ØKO	KU-PHARMA	DIANO-VA
M1.1.1: Extraction of mRNA from water samples	In progress*1					
M1.1.2: Validation of mRNA based quantification to viability of cells	Note*1					Note*1
M1.1.3: Publication of cDNA based method	Submitted*1					
M1.2.1: Design of primersets for efficient simultaneous PCR amplification of the target pathogens	Designed for Salmonella*2		Not started*2			Designed for Campylobacter*2
M1.2.2: Validations of primers in environmental samples	Validated for Salmonella		Not started			In progress
M1.2.3: Publication of quantitative PCR method to simultaneous detection of pathogens	Not started		Not started			Not started
M1.3.1: Development of analytical tool to quantify steroid estrogens and metabolites.					In progress	
M1.3.2: Quantifications of steroid estrogens and metabolites in soil, drainage and manure matrices.					In progress	
M1.3.3: Publications of steroid estrogens quantification methods.					Not started	

Note *1: The development of mRNA based quantification techniques for use in soil and water samples have been developed at GEUS by Jacob Bælum partly in the Remtech project and partly in the Pathos project. The validation has been done in the Pathos project and the publication of this work is ready for submission. The development of mRNA based quantification technique in chicken and pig manure samples have been developed at DTU-vet. The method is studied for sensitivity and specificity using spiked samples and a number of bacterial microflora isolated from chicken and pig intestinal track.

Note *2: The design of primers that can be used to detect different target pathogens in the same sample are only partly fulfilled – this is due to late start of the project at KU-Life. The finishing of this collaborative part will have high priority in spring 2010 to make maximal use of the visiting professor. Design of primers specific for *Campylobacter jejuni* and *Campylobacter coli* is developed and tested. The primers were used to develop both PCR and RT PCR to detect *Campylobacter* directly from chicken and pig manure. The method is used to study the present of *Campylobacter* spp. in water sample collect by Pathos project.

NEW ACTIVITY IN WP1:

M1.4.X: Total faecal load by *Bacteroides* sp. quantification. Since the funding of the Pathos project increasing evidence has been established that it is possible to quantify faecal contamination of water bodies by quantifying the obligate anaerobic bacteria *Bacteroides* sp. by RealTime PCR. This activity was initiated at GEUS, but after funding for the inclusion of an additional SMV in the consortium, the work has been backed by collaboration with AmphiBac.

WP 2	GEUS	DJF	KU-VET	KU-ØKO	KU-PHARMA	DIANO-VA
M2.1.1: Screening of liquid and solid fractions from commercial farm operations	Note 1			Note 1		
M2.1.2: Analysis of samples from screening completed				Note 1		
M2.1.3: Publication of screening study results	Note 1		Note 1	Note 1	Note 1	
M2.2.1: Full-scale experiment with existing, commercial manure separation technologies				Note 2		
M2.2.2: Lab-scale experiments with newly emerging separation methodologies				Note 2		
M2.2.3: Analysis of samples from manure separation experiments completed			Note 2	Note 2	Note 2	
M2.2.4: Publication of results on different existing and new technologies effect on contaminant distribution.	Note 2		Note 2	Note 2	Note 2	
M2.3.1 Storage experiment with manure and liquid fractions	Note 3			Note 3	Note 3	
M2.3.2: Analyses of samples from storage experiment	Note 3			Note 3	Note 3	
M2.3.3: Publication of storage experiment results	Note 3			Note 3	Note 3	

Note *1: The samples were collected from 13 commercial farm operations (8 KEMIRA separation units and 5 simple mechanical separations units). All chemical analyses of samples have been carried out. Particle size distribution and ICP-OES analyses of each fraction should be finished by the end of March 2010. First draft on the publication is expected to be ready in mid 2010. The lack of economic support from KEMIRA has large impact on the possibility for analysing samples – not only in this subproject but in all currently planned analyses at GEUS and KU-Pharma.

Note *2: Full-scale experiment with existing commercial manure separation methods was performed. Raw sow manure was collected together with solid and liquid fractions after separation with the same screw press, as well as after KEMIRA chemical-mechanical separator and after only chemical treatment in KEMIRA separator (after adding the polymer, but before going to the screw press). Lab-scale experiment was done with 5 different treatments: 1. Flocculation + drainage 2. Coagulation + flocculation + drainage 3. Ozonation + centrifugation 4. Centrifugation 5. Sedimentation. All analyses of samples from these two experiments should be done (more or less) by mid March, data collated by mid 2010 and a first draft of the manuscript is expected ready in last half of 2010.

Note *3: Storage experimental set-up is planned and most last details were agreed at the January 19th 2010 PATHOS meeting and in following weeks. However, sampling campaign and experimental start-up is delayed until the beginning of March, due to frost and snow conditions. The storage experiment will run until Sept-Oct 2010. All chemical analyses should start in September and should be done by the end of 2010 or early 2011. First draft of the manuscript can be expected to be ready by mid 2011.

WP 3	GEUS	DJF	KU-VET	KU-ØKO	KU-PHARMA	DIANO-VA
M3.1.1: Redistribution of manure components	Note 1	Note 1	Note1		Note 1	Note 1
M3.1.2: Leaching of contaminants in manure Sorption of Estrogens *Note1		Note 2			Note 2	
M3.1.3: Publication of sorption dialysis and incubation studies		Note 2			Note 2	
M3.2.1: Soil sampling and test of experimental setup		Note 3				
M3.2.2: End of leaching experiments and analysis	Note 3	Note 3			Note 3	
M3.2.3: Publication of columns experiment results	Note 3	Note 3			Note 3	
M3.3.1: Drainage sampling equipment installed and tested	Note 4	Note 4				
M3.3.2: End of field monitoring and analysis	Note 4	Note 4	Note 4		Note 4	Note 4
M3.3.3: Publication of monitoring results	Note 4	Note 4	Note 4		Note 4	Note 4

Note1: This experiment has been carried out in spring 2009 and samples have been analysed for some contaminant while other contaminants still need to be analysed.

Note 2: Due to the availability of ¹⁴C-labelled estrogen sorption experiments has been included to replace the chemical based experiments. The planned experiment with repacked soil columns was replaced with a sorption study investigating the effect of slurry components and the interaction with soils.

Note 3: The column leaching experiment is scheduled for 2010 and the planning of the different analysis has been finalized.

Note 4: The field leaching experiments has been finalised in 2009 and many contaminants has been analysed – however still analysis remain to be done for DNA and hormones.

Recommendation from external referee

From the evaluation report by Anders Johnsen the following positive conclusions are highlighted:

1) The centre work with a very high level of integration between groups.

And the following concerns are raised:

1) Chemical analyses (estrogen) are behind schedule. High priority should be given to chemical analyses so they are ready to be evaluated together with the other results.

2) All activities are strictly scientific. Are the activities and results useful for the end-users (water companies, slurry separation companies, EPA)? Should activities be more directed towards applied experiments (comparison of normal slurry and separated slurry with respect to leaching risk).

3) Is there a clear message to the end-users?

4) Can results be put together to show that risk assessment is needed and that the consortium should seek fundeing (e.g. by DANVA or EPA)?

Dissemination of project results

Submitted Scientific publications from PATHOS (no conference presentations is included)

Published papers

T. B. Bech, K. Johnsen, A. Dalsgaard, M. Laegdsmand, O. H. Jacobsen, and C. S. Jacobsen
Transport and Distribution of *Salmonella enterica* Serovar Typhimurium in Loamy and Sandy Soil Monoliths with Applied Liquid Manure. **Appl Environ Technol.** 76: 710-714.

Submitted papers

R. García, J. Bælum, L. Fredslund, P. Santorum, C.S. Jacobsen. *Salmonella enterica* serovar Typhimurium Survival in Soil and Manure Assayed Using Classic and DNA/mRNA based methods-the Role of Predation and Temperature.
Progress: Manuscript submitted.

Bech, T., Dalsgaard, A., Jacobsen, O.S. and Jacobsen, C.S. Leaching of *Salmonella* Senftenberg in intact clay soil columns influenced by two manure application methods.
Progress: Manuscript has been returned for revision

Other planned PATHOS dissemination activities

1. Pathos Ph.D. course on molecular microbial ecology techniques to be held at GEUS in April 2010
2. One-day Public meeting scheduled for spring 2011. PATHOS partners will present final results and outcomes of the activities in oral and poster presentations. Implications for Manure-separation Industry, Agriculture and Water Supply Industry will be discussed between scientists and stakeholders. Meeting will be hosted by GEUS and will be open to all Stakeholders and the public.
3. Individual session on PATHOS results will be scheduled for the 2011 Annual meeting of the Danish Water Forum hosted by GEUS.
4. Suggestion to set up target meetings with individual interest groups to inform on the implication of the PATHOS results for a range of stakeholders: Danish Agricultural Advisory Service special section for livestock and biowaste (Torkild Birkmose) meetings for advisors, farmers, cleantech industry
5. Plant Congress January 2011 or 2012 (DAAS, AU-DJF, KU-LIFE org.), also an environmental administrative part of the congress.
6. Network of industry and organizations associated to new large DSF-project CLEANWASTE (Svend Sommer, SDU coordinator) Lars Stoumann Jensen participant and link Pathos activities to these new activities.
7. Special issue of "Vand- og Jord" 2011/12.