

Minute of meeting PATHOS wrap up meeting #1:

Field leaching experiment held at GEUS on February 8, 2011, 13-15.

Participants: Anita Forslund, Tina Bundgaard Bech; Jeanne Kjær; Martin Hansen; Carsten Suhr Jacobsen

Available results:

Jeanne: Has provided data for precipitation, drainage water run-off and so on. She will provide figures combining the individual results in a figure.

Martin: Has measured different hormones in samples – including groundwater samples – and has in addition to estrogen also found male hormones.

Anita: Has measured phages, and found these to leach to drainage water as well as groundwater (one sample). Also larger organisms *Enterococcus* sp. and coccoides has been measured, no coccoides is found and only one occasion of *Enterococcus* sp .

Tina: Has measured tetracycline resistant bacteria and *E. coli* leaching to drainage water – never found in groundwater samples. Selected (about 100) tet resistant colonies has been typed and found to belong to mainly coliforms.

Line/Carsten: Has measured DNA and found *Bacteroides* sp. to peak when also the tet resistant bacteria is peaking.

Suggested output:

- 1) Martin + Jeanne + Finn + Preben + Bent: One paper presenting the data on hormones: hot news: 1) male hormones haven't been detected in drainage water 2) hormones haven't been detected in groundwater samples.
- 2) Tina + Jeanne + Mostofa + Annette + Carsten: One paper on leaching of tetracycline resistant bacteria to drainage water after manure application. (Only if soil survival experiment is included Mostofa is co author).
- 3) Anita + Jeanne + Tina + Preben + Line/Carsten + Anders: One paper presenting the phage data and compare to the *E.coli*. and other larger organisms. Depending on the status of the DNA data Line or both Line and Carsten will be included.

Manuscript #1 and #2 will be submitted before June – maybe around the pathos meeting on May 12-13.

Manuscript #1 will be submitted to Environmental pollution or ES&T manuscript #2 will be submitted to ES&T. Manuscript # 3 will be submitted later to one of following journals ISME-journal; Environmental Microbiology or ES&T.

Minute of meeting PATHOS wrap up meeting #2:

Separation and storage experiment held at GEUS on February 9, 2011, 13-15.

Participants: Anita Forslund, Line Fredslund; Pernille Stockmar; Olga Popovic; Martin Hansen; Carsten Suhr Jacobsen

Available results:

Olga: Three lines of experiments has been carried out: **WP2.1) Screening** experiment – in which raw manure as well as liquid and solid separation products is collected from 13 farms. **WP2.2) Laboratory mimics** of 5 different separations techniques (a) oxonation + centrifugation; b) flocculation + drainage; c) coagulation + flocculation + drainage; d) centrifugation and e) sedimentation). **WP2.3) Storage** experiment. A lot of chemical analysis done and some to be analyzed.

Martin: Has measured different hormones in samples in WP2.1. Main message is that the compounds stick to the solid fraction other analysis is still lacking

Anita: In WP2.1 has measured indicators (*E.coli* and *Enterococcus* sp.) in only three of 13 farms and is found to follow the liquid fraction. In WP2.2 data on *E.coli* and enterocos showed that ozonation didn't seem to work and finally in WP2.3 salmonella phages, *Salmonella* seftenberg as well as *Enterococcus* sp., die out at 25°C while they seem stable at 5°C. *Cryptosporidium* sp. is losing it viability and infectivity in mice at both temperatures within 16 weeks.

Line: In WP2.1. DNA has been extracted but *Bacteroides* sp. seems not to be the right tool to measure. Will do total counts on bacteria. WP2.2. has not yet received the samples WP2.3. has measured *E.coli* and total coliforms and found die out when storage at 25°C while the bacteria survive at 5°C

Suggested output based on WP2.1:

- 1) Olga + Anita + Lars: Screening experiment paper on chemical and bacteriological data
- 2) Martin + Olga + Kirstine + Lars + Bent: Hormone distribution experiment paper

Manuscript #1 and #2 are being written currently.

A new meeting in the group will take place before Easter or at the latest in the beginning of May before the large Pathos meeting to discuss the inclusion of the remaining data.

Minute of meeting PATHOS wrap up meeting #3:

Column leaching experiment held at Foulum on February 11, 2011, 11-13.

Participants: Mostofa Amin; Mette Laegdsmand; Heidi Petersen; Tina Bech; Anne Marie Truelsen; Flemming Pedersen; Anita Forslund,; Martin Hansen; Carsten Suhr Jacobsen

Available results:

Mostofa: The existing data from soil column experiments was presented based on the suggestion for a manuscript draft that was sent to the participants. The presented data included: a large range of geochemical analysis, soil physic analysis, phage leaching, data on viable *E.coli* and *E.coli* Q-PCR, viable *Enterococcus*

Martin: Hormone data are soon processed.

Anita: Data on phages, viable *E.coli* and viable *Enterococcus* was presented by Mostofa. Data on viable *Salmonella* could be combined with Q-PCR data separately

Heidi: Presented data on *Cryptosporidium*

Carsten: Underlined that many of the microbiological data including Q-PCR data is missing a baseline experiment showing the background level from soil (maybe it would be that none could be detected). *InvA* Q-PCR is being processed to quantify.

Mostofa: pointed out that tracer data is missing. But maybe Rene Juhler at GEUS could help analyzing samples.

The complex data set coming out of the column leaching experiments was discussed and it was decided to aim at the following **Suggested output based on WP3.2:**

- 1) Mostofa Amin, Anita Forslund, Xuan Bui, Rene Juhler & Mette Lægdsmand: The paper presenting main soil data together with *E.coli*, phage and *Enterococcus* data
- 2) Mostofa Amin, Martin Hansen, Bent Halling Sørensen & Mette Lægdsmand: The paper presenting leaching data of different hormones.
- 3) Heidi Petersen, Mostofa Amin, Annette (vejleder) & Anders Dalsgaard: data on *Cryptosporidium* sp. leaching
- 4) Anita Forslund, Mostofa Amin, Xuan Bui, Tina Bech, Anders Dalsgaard & Carsten Suhr Jacobsen: *Salmonella* sp. leaching (viable and *invA*)

The author list was not finally discussed on the meeting but follows the Pathos guideline that only Ph.D. and postdoc advisors actively involved in the activity is co-authors

Minute of meeting PATHOS wrap up meeting #4:

Column leaching experiment held at Foulum on February 11, 2011, 13-15.

Participants: Mostofa Amin; Mette Laegdsmann; Tina Bech; Anne Marie Truelsen; Flemming Pedersen; Anita Forslund,; Martin Hansen; Carsten Suhr Jacobsen

Available results:

Mostofa: The combined data from the redistribution experiment was presented based on the suggestion for a manuscript draft that was send to the participants. The presented data included: soil water content, soil N content, viable *E.coli*, phages and steroid hormones.

The data was discussed and the **suggested output based on WP3.1:**

Mostofa Amin, Tina Bech, Anita Forslund, Martin Hansen, Søren Pedersen & Mette Lægdsmand:
Redistribution and persistence of mineral N, pathogens and hormones from pig slurry after direct injection into soil