This exchange grant was carried out in the UFZ – Helmholtz Centre for Environmental Reserach in Magdeburg (Germany) from March 20th to April 3rd. The aims of the visit were to finish the experiments carried out in the first visit, discuss the results and try to determine chlorophyll variation in two lakes of the Iberian Pyritic Belt (IPB) we are studying.

Specifics goals were:

- Determine seasonal changes of phytoplankton community and its relation to changes in bacterial community.
- Determine the long term behavior of iron in microcosms which have been incubated since November 2011.
- Analyze results obtained during the last visit.

Description of the work

During my first visit to the UFZ-Magdeburg, we did some experiments. Those experiments showed that there wasn't nitrification or denitrification in the investigated acidic pit lakes Cueva De La Mora, Herrerías and ML111. Also, we tried to quantify some bacterial metabolisms in the lakes; the results indicated low activities compared with previous results obtained in 2010 (Wendt-Potthoff et al 2011). Hence it was necessary repeat some experiments during this visit. Furthermore some results needed to be studied deeply because unexpected results were obtained in December 2011, also, taking in consideration some suggestion made by other researchers it was necessary discuss these results deeply.

Following the theory there is seasonal variations in bacterial population and to compare these future results with others obtained on December 2011 and in Wendt-Potthoff et al 2011 all the samples were incubated to determine approximately bacterial concentration using the method Most Probable Number (MPN). This technique consists in estimates of bacterial densities by incubating serially diluted samples with different specific culture media; the estimation of density is based on the theory of probability:

Anoxic media:

- Acidophilic Fe(III) reducers (acFeR)
- Neutrophilic Fe(III) reducers (FeR)
- Neutrophilic sulfate reducers (SRB)
- Acid-tolerant sulfate reducers (acSRB)

Oxic media:

- Acidophilic Fe(II) oxidizers (acFeOx)
- Acidophilic thiosulfate oxidizers (acFeSOx)
- Ammonium oxidizers (AOM)

The last visit there was carried on an experiment to detect nitrogen bacterial related metabolisms using microcosms and adding nitrate and ferrous iron to try to detect ferrous iron consumption coupled with denitrification. Some microcosms were sampled five times over December 2011 while other microcosms were preserved to detect changes in some parameters in a long term experiment. These microcosms was filled with water from different acid lakes (Cueva de la Mora and Herrerías form the IPB and Lake 111 form Lusatia mining district) and different depths (water from the oxic layer, anoxic layer and chemocline), in total 26 microcosms. Nitrate and ferrous iron were added on November 16th and the microcosms were incubated during 4 months at 20ºC in darkness. In the beginning and at the end of the experiment different parameters were measured: nitrate, ammonium, ferrous and ferric iron, dissolved organic carbon and pH.

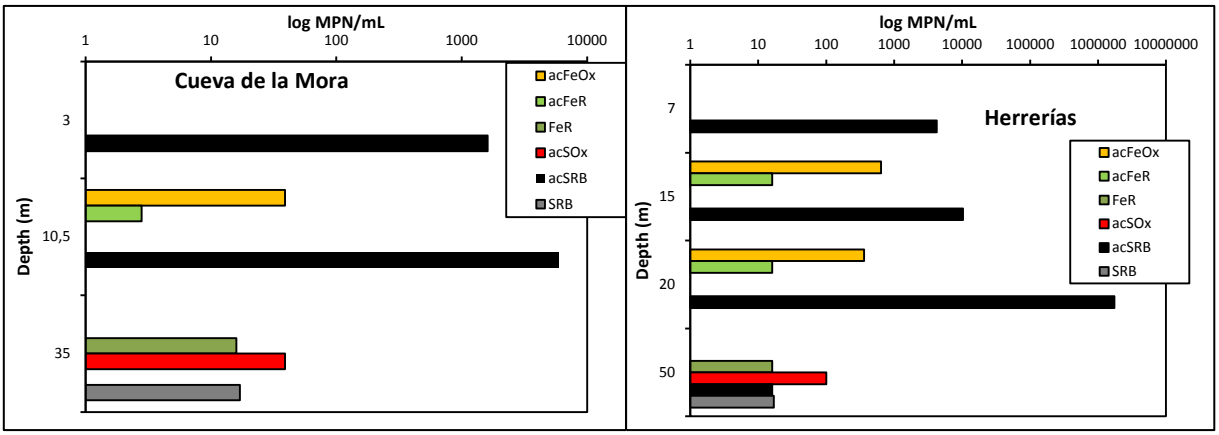
Figure 1: Battery of bottles used for denitrification long experiment.

For one year we have been taking water samples from the oxic layers from those lakes to measure chlorophyll concentration. As in other aquatic environments we suppose there are seasonal variations in both chlorophyll concentration and phytoplankton populations. Thus, we have measured again chlorophyll concentration in both lakes from IPB. And also, after analyzing the concentration of the chlorophyll we could determine how much biomass is in the water and we could establish if there is any correlation with the bacterial concentration variations if those exist (after analyzing MPN results).

General bacterial concentration was determinate with a fluorescence microscope. 1 mL (if bacterial concentration was too small it was added more sample) water samples were filtrated onto 0.2 µm filters. Then, Sybr Green ® was added to visualize bacteria. Sybr Green ® is a fluorescent dye that binds to doubled stranded DNA. Bacteria will be shown in a fluorescence microscope with green color while chlorophyll will be red. We wanted to estimate how many bacteria are in different water samples from two lakes from the IPB and we also tried to detect bacteria adsorbed to particles in the chemocline that could give an idea if there is an interaction between iron precipitates formation and bacterial metabolism.

Results

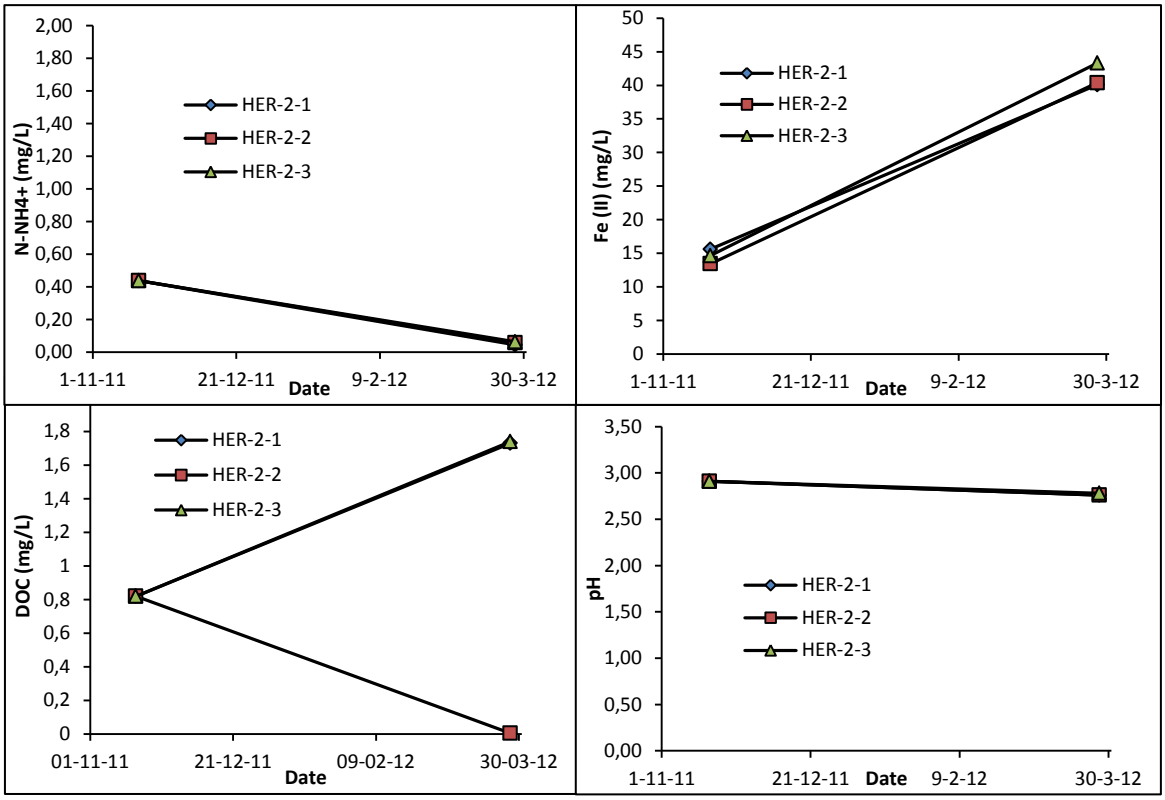
MPN cultures. The final results of the MPN analysis showed different bacterial concentrations and populations in both lakes and in different depths. Both lakes showed more bacterial metabolisms diversity in the deepest layer although concentrations were lower than in upper layers. In the middle layer of both lakes there were iron oxidizers and iron reducers, which suggests there is an iron cycle in these layers. There were sulfate reducers in both lakes in all the layers. While in both lakes there were acidophilic metabolisms in the upper layers it was only possible to find non-acidophilic microorganisms in their bottom layer this might be because the pH in the bottom layer it is quit higher than in upper layers. Comparing these results with results which were obtained during my last visit (September 2011) Cueva de la Mora presents more metabolisms diversity mainly in the bottom layer.If both results are compared with results which were obtained in February 2009 (Wendt-Potthoff et al 2011) there was less bacterial metabolisms diversity in Cueva de la Mora this time. Bacterial populations might be influenced by environmental factors, for example chlorophyll concentration profiles taken in the field in Cueva de la Mora present the same pattern in September 2011 and March 2012 while in February it follows a different pattern (dates not showed), it could have a direct influence to bacterial populations because the carbon source bacteria use is derived from the phytoplankton present in the lake. But other possibility is that bacterial populations have changed in those two years between one sampling to another.



Graphic 1: Most probable number results.
Left: Cueva de la Mora pit lake. Right: Herrerías pit lake.

Long term experiment. Denitrification coupled to ferrous iron consumption is thermodynamically impossible because at acid pH the reaction is highly endergonic. It is because at pH 2 the redox potentials of the couples Fe(II)/Fe(III) and $NO_3^-/O.5N_2$ are similar what makes an electron transference impossible. But when we have analyzed different parameters in the microcosms we have seen that there is a dissolved organic carbon production in almost all the samples, therefore it could be feasible it has been bacterial activity along this period. These two samplings were taken on November 17th 2011 and March 27th 2012 (131 days incubation). Although it is known nitrate at acid pH is toxic to acidophilic bacteria. pH remained constant in all the samples with little variations, probably there isn't any alkalinity generation processes taking place in the microcosms. Ammonium concentration also remained constant although in some microcosms ammonium decreased near zero. Ferrous iron concentration increased in some microcosms whereas in other remained constant,

it may be due to the lack of ferric iron, probably in microcosms with anaerobic water from the lakes, or a oxidation-reduction cycling. As an example, in graphic 2 it is showed these different parameters in Herrerias.



Graphic 2: concentration of ammonium (N-NH^{4+}), ferrous iron (Fe(II)), pH and dissolved organic carbon (DOC) in Herrerías pit lake. **Legend:** HER-2-1: Herrerías pit lake, 7 m depth, replicate 1; HER-2-2: Herrerías pit lake, 7 m depth, replicate 2; HER-2-3: Herrerías pit lake, 7 m depth, replicate 3.

Chlorophyll concentration variations. We are still waiting for the results of absorbance spectrophotometry, after getting results, we expect to compare them with past dates and determine phytoplankton concentration and seasonal variations.

Bacterial concentration with fluorescence microscope. Bacteria are concentrated in the chemocline of both lakes. In the upper layer the concentration is very low, but increase with depth until the chemocline. However it has been impossible to detect bacteria in the deepest layers of the lakes, maybe it has been because a very low bacterial concentration, or the lack of fixation with a high particle concentration. Under the fluorescence microscope we did not find particles in the water samples. Thus, an initially planned study of particle associated microbes using Confocal Scanning Microscopy was cancelled.

Further expectative

At the moment there is a publication about the nitrification in progress. On 2013 we expect to present these results in microbiological international conferences. Future collaboration will be discussed in following months with the intention of publishing some papers about our research and discuss about future collaboration with the host researchers.

Commentaries

I want to thank Katrin Wendt-Potthoff and Matthias Koschorreck for allowing me to go to the UFZ and make part of my research. I want also to thank FIMIN program for the funding, otherwise without this support it wouldn't have been possible.

During my visit I have learnt diverse techniques related with microbial cultivation such most probable number technique and also set up microcosm system to study the evolution of the microbial communities under different conditions during a period of time. In addition I learnt how to use the isotope technique to study the consumption of nutrients and formation of different metabolic products. Finally I have had the opportunity to work in a laboratory with all the facilities to carry out a microbial research. I am very grateful to UFZ for the opportunity to let me stay working there.

Thank you very much for allow me to come back to UFZ in Magdeburg, because in my job center there isn't any infrastructures that allow a microbiological study with your support I can follow with my research to make my PhD studies.