# REPORT EXCHANGE GRANT

## Summary

This Exchange grant was carried out at the UFZ centre in Magdeburg (Germany) from the September, 16$^{th}$ to December, 16$^{th}$. The aim of the visit was to find out if nitrogen-related metabolisms exist in the acid mine pit lakes of the Iberian Pyrite Belt (IPB), which are being subject of intense biogeochemical research. A second objective was to identify the different bacterial metabolisms present in the lakes and to compare them with previous results.

The specific goals of the visit were:

1. Identification of N-related bacterial metabolisms in water samples at different depths in three acid mine pit lakes.
2. Comparative analysis of viable counts of N-related bacteria and other bacterial metabolisms measured in water samples from those lakes.
3. Comparative study of the microbial activities related with the cycling of N and Fe.

## Description of the work

Together with colleagues from Magdeburg we made a field campaign on September to take samples for the experiments. Two acid pit lakes were sampled in Spain, Herrerías and Cueva de la Mora. Water samples were taken from three different depths: one sample from the aerobic-oxic zone, one sampled from the beginning of the redoxcline, and a final sample from the anaerobic layer.

After the field sampling I went to the UFZ centre in Magdeburg.

On the first week, there was a field trip to visit the lake 111 in the Lusatian mine district in the East of Germany. It was the last campaign of a long term research project carried out in this lake. After this visit, all the infrastructures were removed. We decided to take samples from this lake to make a comparative study among the two Spanish mine pit lakes and this German mine pit lake. We took water samples, two aerobic and one anaerobic.

Aims and methodology of the different experiments:

<u>Nitrification microcosms</u>: the aim of this experiment was to detect nitrification bacterial metabolism in the water samples from the three lakes. Nitrification is an aerobic process; it is the oxidation from ammonium to nitrate. The experiment was carried out in different environmental conditions. We used 6 bottles for each depth for the three lakes, 3 of them were cultivated in anaerobic conditions and the other 3 in aerobic conditions, all of them were filled with water from the lakes. In one of these three bottles was added ATU

(allythiourea); ATU is a well-known inhibitor of nitrification. There were 54 bottles in total: 3 lakes x 3 depths x 3 bottles x 2 conditions (aerobic / anaerobic). $^{15}N$ was added to all the samples in the form of $NH_4Cl$ to detect the use of ammonium by nitrifying bacteria. We sampled twice after adding $^{15}N$, at day 0 and one day after. We took samples for measuring the evolution in the $NH_4^+$, $NO_3^-$ and Fe(II) concentration. More samples were taken for analyzing the quantity of $^{15}N$ that was converted to $^{15}NOx$ by the bacteria. This experiment was carried out at the UFZ centre in Halle.

**Figure 1:** Battery of bottles used for the nitrification experiment.

Denitrification: in the UFZ centre in Halle, a two days denitrification experiment was carried out. Denitrification is the reduction of nitrate to $N_2$ (gas) using an organic carbon source. Denitrification in batch assays was determined by the isotope pairing technique. $^{15}N$ was added in the form of $Na_{15}NO_3$ to detect the $N_2$ formation. We used one bottle for each depth from each lake: 3 depths x 3 lakes plus 1 blank. There were two samplings at time 0 and one day after. Subsequently, the $N_2$ concentration was measured to detect the use of $^{15}N$.

Denitrification microcosms: As stated before, denitrification is a process in which $NO_3^-$ is reduced to other forms of nitrogen. The final result of the reactions is the formation of $N_2$ (g). In this experiment, we wanted to detect the reduction of $NO_3^-$ with $Fe^{2+}$ without any use of organic carbon source. $Fe^{2+}$ was added to the samples from the aerobic layers. We prepared 3 bottles for each depth and each lake with $NO_3^-$, and other 2 bottles of the same samples with the same quantity of water instead $NO_3^-$, those were the controls (3 depths x 3 lakes x 3 replicates + 3 depths x 3 lakes x 2 replicates = 45 bottles). The objective was to detect if there is consumption of $Fe^{2+}$ along with simultaneous $NO_3^-$ and $NH_4^+$ production. $NH_4^+$, $NO_3^-$ and $Fe^{2+}$ concentration were measured five times along a month. Additionally, $Fe^{3+}$ concentration and dissolved organic carbon were measured at the beginning and at the end of the experiment.

**Figure 2:** Battery of bottles used for the denitrification experiment.

Most probable number (MPN) method: this technique consists in the cultivation of samples with different specific culture media to detect different bacterial metabolisms. It is necessary to use microbe plates; the method consists in making subsequent dilutions to estimate the density of organisms in a liquid, without any direct counting. These samples were incubated during six weeks except denitrifiers which were cultivated four weeks, after this period the microbe plates were observed to detect any possible growth of the microorganisms. The estimation of density is based on the theory of probability. We put 10 different media to detect different bacterial metabolisms related with acidophilic environments and with nitrogen metabolisms:

Anoxic mediums:

- Acidophilic Fe(III) reducers
- Neutrophilic Fe(III) reducers
- Sulfate reducers
- Acid-tolerant sulfate reducers
- Denitrifiers
- Mixotrophic and lithotrophic nitrate reducing Fe(II) oxidizers

Oxic mediums:

- Acidophilic Fe(II) oxidizers
- Acidophilic thiosulfate oxidizers
- Microaerobic Fe(II) oxidizers
- Ammonium oxidizers

**Figure 3:** MPN plates.

**Results**

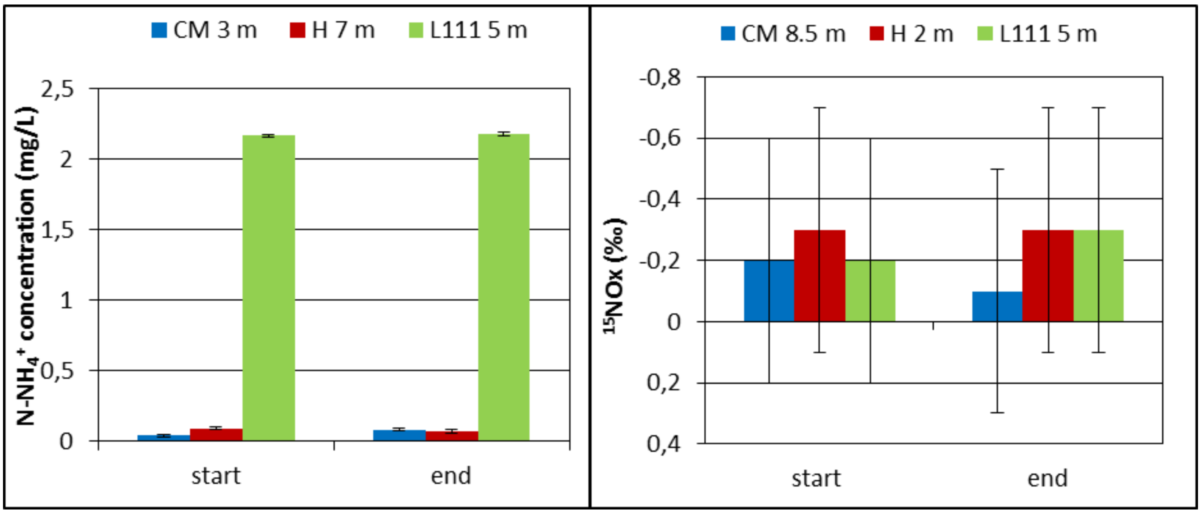
Although there is more work to be done, we can already advance some preliminary results.

<u>Nitrification experiment</u>:

The isotopic results obtained are below the detection limit of the technique.

There is not significant variations in nitrate and ammonium concentrations.

→ no nitrification in the 3 lakes



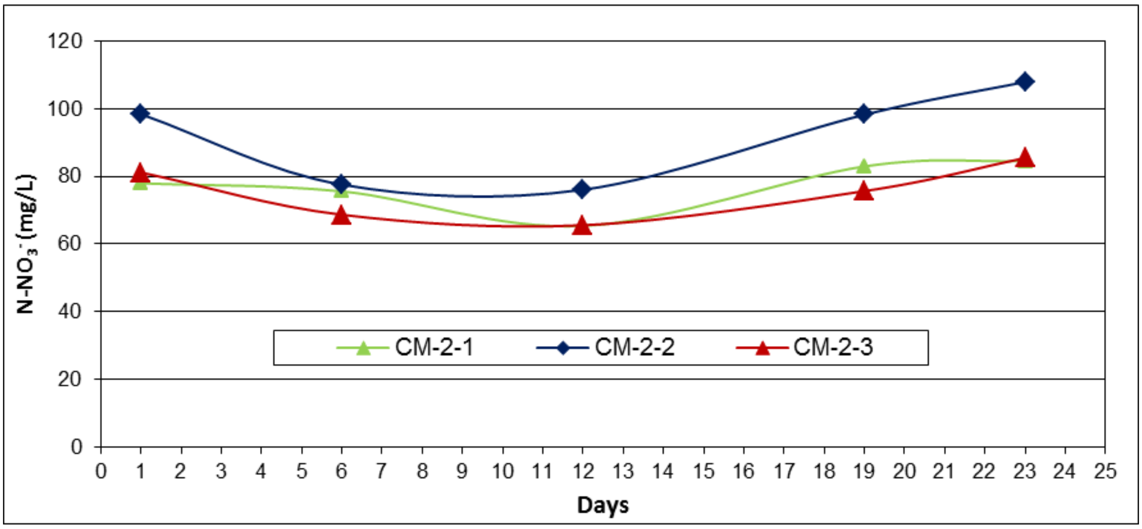

**Graphic 1:** Left: N-$NH_4^+$ (mg/L) variation in two days. Right: $^{15}NOx$ (‰) variation in two days.
CM= Cueva de la Mora, H= Herrerías, L111= Lake 111.

Denitrification coupled to Fe(II) consumption:

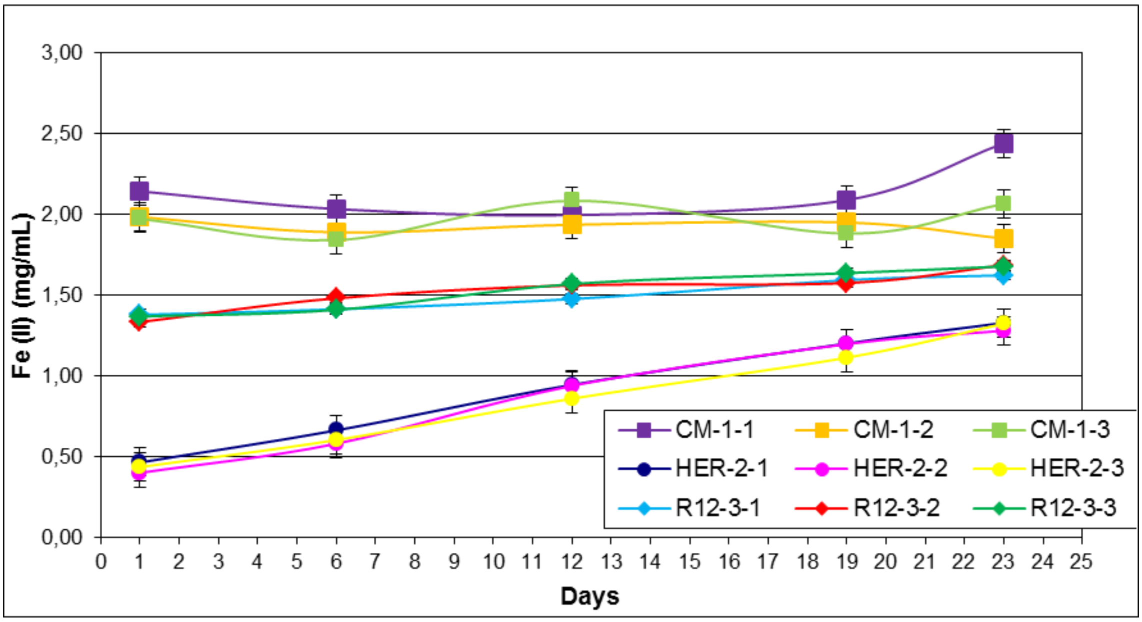
It is not observed nitrate consumption in the lakes.

An Fe(II) production has been observed in some depths of differents lake.

→ iron reduction: yes, but not coupled to nitrate

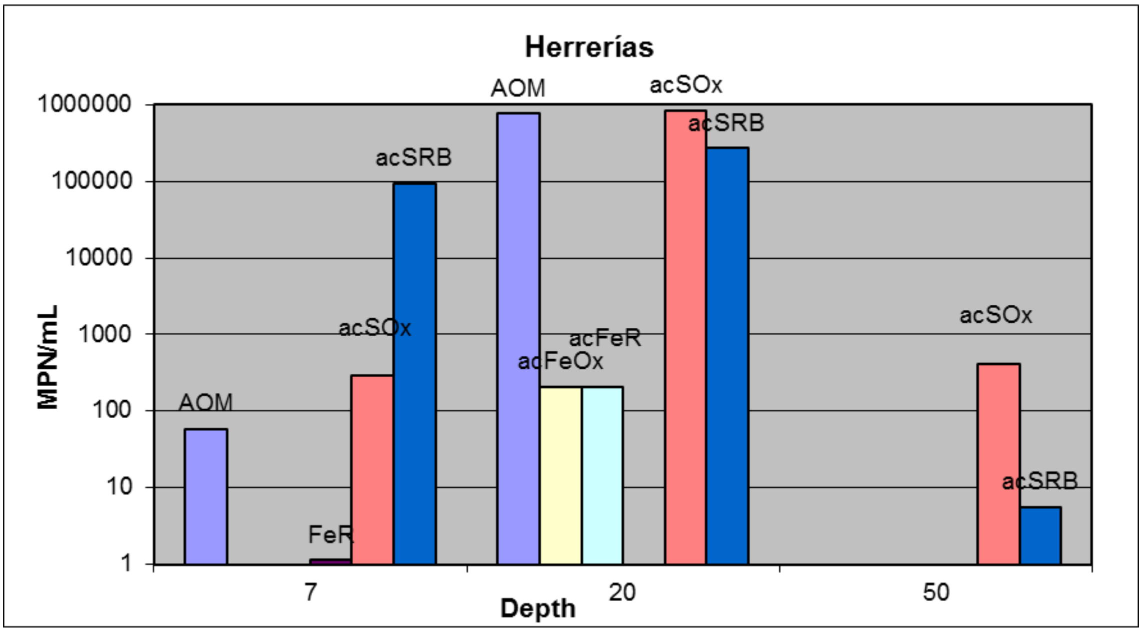



**Graphic 2:** $N\text{-}NO_3^-$ (mg/L) concentration variations in three bottles with water from 20 m from Cueva de la Mora.




**Graphic 3:** Fe(II) (mg/mL) concentration variations in three bottles with water from the different depth from different lakes.
CM-1= Cueva de la Mora 3 m; H-2= Herrerías 20m; R12-3= Lake 111 10 m.

The MPNs show that there are differences among the lakes in relation with the bacterial metabolisms composition. The higher difference is between the German lake and the Spanish lakes. There are also differences among the anaerobic and aerobic part within a given lake, but as general rule, in all the lakes studied, the lower bacterial concentrations are found in the upper layer.




**Graphic 4:** MPN/mL from Herrerías.

**Future collaboration**

There is scientific collaboration between our respective research groups (IGME and UFZ) since more than 4 years ago, and in the context of the acid pit lakes of the Iberian pyrite belt. We are planning to continue the collaboration in the immediate future. One objective will be to collaborate in the course of my PhD thesis.

Plans for 2012 are:
- Write a paper
- Evaluate data from the 2011 field campaign
- Organize a joined workshop

**Publications**

We expect to draft a scientific paper (e.g., short note, letter) during the next months. This contribution will be focused on the feasibility of nitrification of nitrification in acidic waters, and will be co-authored by the researchers of the UFZ and myself.