# BEHAVIOUR OF Fe, Mg AND Ca IN ACID MINE DRAINAGE AND EXPERIMENTAL SOLUTIONS IN THE PRESENCE OF *Aspergillus niger* SPECIES ISOLATED FROM VARIOUS ENVIRONMENT

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**Abstract:** This article analyzes the ability of micromycetes to accumulate Fe, Mg and Ca from acid mine drainage (AMD) at the locality Smolník. Four strains of the *Aspergillus niger* (*An*) species originating from various types of environment were used in the experiments: the *An*-G strain (the locality of Gabčíkovo, Eutric Fluvisol), the *An*-P strain (the locality of Pezinok-Kolársky vrch, mining region with elevated amounts of As and Sb), the *An*-N strain (the locality of Nováky, mining region with elevated amounts of As and S), the *An*-Š strain (Banská Štiavnica-Šobov, the locality impacted by an acid sulphate weathering and extremely low pH). In the most cases the accumulation of Mg was the highest in comparison to accumulation of Fe. Accumulation of Ca was very low. Among the tested microfungi, the highest accumulation was noted by the strain *An*-N 55 % of Mg and by the strain *An*-Š 54 % of Fe from the model solution of the elements (Fe [1.67 mg/L], Mg [2.35 mg/L] and Ca [1.14 mg/L]).

**Key words:** Acid Mine Drainage (AMD), experimental solutions, micromycetes, *Aspergillus niger* strains, accumulation of Fe, Mg, Ca

## 1. Introduction

Mining activity is one of those anthropogenic activities that cause the heavy metals and chemical elements to penetrate in the environment. The by-products of mining such, as the disposal sites and mullock tips with high amount of minerals as well as flooded mines are the sources of an acid mine drainage (AMD). AMD poses a serious environmental problem with a negative impact on the surrounding aqueous environment and the stream sediments in terms of the increased acidity, decreased oxygen and the heavy metal release. The Smolník region is a typical example of the production and occurrence of the AMD. Their composition is strongly affected especially by weathering of sulphides. The evolution of sulphuric acid leads to the leaching of copper and other elements from ores. At the Pech shaft locality, very high concentrations of the Fe, Mg and Ca elements had been found, often exceeding the limits set by the government ruling (NV SR č. 296/2005 Z.z.) (LUPTÁKOVÁ, 2006). The utilization of the removal or accumulation ability of micromycetes in the process of the heavy metal ions elimination or reduction, from waste water, should be one option of the soil and water decontamination.

## 2. Material and methods

### 2.1 Micromycetes

The *Aspergillus niger* TIEGH. species is one of the filamentous fungi that are commonly found in various components of the environment. In our experiments, we used four different strains of this species, isolated from various types of the environment.

The 1st *Aspergillus niger* strain (*An*-G) was isolated from Eutric Fluvisol, pH $H_2O/KCl$ = 7.7/7.4, in the Gabčíkovo region. The 2nd *Aspergillus niger* strain (*An*-P) was isolated from the stream sediment of the Blatina river with elevated natural amounts of As (363 mg/kg) and Sb (93 mg/kg), pH $H_2O/KCl$ = 5.3/4.8, in the Pezinok mining region. The 3rd *Aspergillus niger* strain (*An*-N) was isolated from the coal dust influenced by As (400 mg/kg), pH $H_2O/KCl$ = 3.3/2.9, in the Nováky mining region. The 4th *Aspergillus niger* strain (*An*-Š) was isolated from the probe No. 15 in the 24 m long sampling line at the Šobov locality. This locality is impacted by an acid sulphate weathering, extremely low pH ($H_2O/KCl$ = 3.0/2.7) and high exchangeable contents of Al and Fe (ŠIMONOVIČOVÁ, 2008; VÝBOHOVÁ *et al*., 1999). All strains under the study are registered in the Collection of Microscopic Fungi ISB in České Budějovice (NOVÁKOVÁ, 2007) and at the Department of Soil Science, Faculty of Natural Sciences, Comenius University in Bratislava.

### 2.2 Biomass preparation

The strains were cultivated in pure culture on Sabouraud agar (SAB, Himedia, Mumbai, India). For the preparation of mycelia biomass 5 ml of conidia suspension in distilled water added to 45 mL of liquid SAB medium were used. The cultivation of mycelia took place at 25 °C for 10 days. After then, mycelia were filtered, flushed in de-ionized water (Water Pro LS, Labconco, USA) and brought into 50 mL of the AMD or experimental solutions. The stationary accumulation went on for 24 hours at the 25 °C temperature. All experiments were performed in three repetitions, and the results reflect the average values.

### 2.3 Removal of elements

In the experiments, we used natural AMD from the Pech mine at the Smolník locality (year 2008) and three experimental solutions. Experimental solution 1 present a mixture of Fe [176 mg/L], Mg [234 mg/L] and Ca [98 mg/L] also with concentration of elements similar to AMD, experimental solution 2 present 10x diluted original solution 1, Fe [17.4 mg/L], Mg [22.6 mg/L] and Ca [9.2 mg/L], experimental solution 3 present 100× diluted original solution 1, Fe [1.67 mg/L], Mg [2.35 mg/L] and Ca [1.14 mg/L]. Concentrations of the individual elements Fe [201 mg/L], Mg [229 mg/L] and Ca [88 mg/L] prepared separately were also used for accumulation of the mentioned elements.

As a control sample, de-ionized water (Water Pro LS, Labconco, USA) was used. The amount of the Fe, Ca and Mg elements accumulated by microscopic fungi from the solutions was determined by using the method of the atomic absorption spectrometry (AAS). The accumulation of the elements was calculated as the difference of the known element concentration (in mg/L) and determined concentration of the elements (in mg/L) after the application of mycelia biomass into the above mentioned solutions. We evaluated the amounts of the elements in the samples according to the calibration curves of the selected elements.

## 3. Results and discussion

The *Aspergillus niger* species belong to the filamentous microfungi which can accumulate various heavy metals from waste waters (KAPOOR and VIRARAGHAVAN, 1995; MEYER and WALLIS, 1997) from mine waters (JANOVOVÁ and ŠIMONOVIČOVÁ, 2008) and from different experimental solutions (MOGOLLON *et al*., 1998; ŠIMONOVIČOVÁ, 2008; ŽEMBERYOVÁ *et al*., 2009).

The valley of the Smolník creek is the historical mine region where Fe, Cu, Ag and Au were mined since the 14th century till the 1990 year. The surface waters penetrating through the mine region are enriched by metals resulting in the decrease of their pH values. The acidity of the mine waters is caused by sulphure mineral oxidation as well as by presence of bacteria *Acidothiobacillus* and *Leptospirillum* genera, which accelerate the bio-chemical oxidation of pyrite and sulphure (LUPTÁKOVÁ, 2006).

The aim of our experiments was to study the accumulation of the Fe, Mg and Ca, secondary biogenic elements, the quantities of which in the AMD exceed the limits (Table 1).

Table 1. The values of the AMD indicators from the Pech shaft.

| Indicator | 2006 | 2008 | 2009* | Limits |
|---|---|---|---|---|
| pH | 3.88 | 3.95 | 2.75 | 6 – 8.5 |
| Fe [mg/l] | 463 | 365.6 | 214 | 2 |
| Mg [mg/l] | 344 | 333.4 | 265 | 100 |
| Ca [mg/l] | 176 | 144.2 | 133 | 200 |

*values introduced in the text

Accumulation of studied elements from AMD is relatively low (Table 2) with the greatest ability by *Aspergillus niger* strains to accumulate Mg (25 mg/L on average), less of Fe (22 mg/L on average). All strains under the study showed very limited ability for accumulation of Ca (2.7 mg/L on average).

From experimental solution 1 we have noted accumulation of the studied elements on the same level like from natural AMD, Mg (21 mg/L on average), Fe (14.25 mg/L on average) and the lowest value Ca (5.25 mg/L on average). The statement of accumulation in percentage is relatively higher from the solution 2 (10× diluted original solution 1) with the maximum amount of Fe (5.1 mg/L = 29%) and from solution 3 (100× diluted original solution 1) with amount of Fe (0.9 mg/L = 54 %) and Mg (1.28 mg/L = 55 %), Table 3.

Table 2. The accumulation of Fe, Mg, Ca from the natural AMD by *A. niger* strains in [mg/L] and in %.

| *Aspergillus niger* strain | Fe [mg/L] | Mg [mg/L] | Ca [mg/L] |
|---|---|---|---|
| *An* - G | 33 | 30 | 2 |
| *An* - P | 35 | 25 | 3 |
| *An* - N | 6 | 23 | 3 |
| *An* - Š | 14 | 22 | 3 |
| average | 22 | 25 | 2.7 |
| % | 3-16 | 8-11 | 2 |

On the other hand, from experimental solution 2 and 3 it was not possible to notice the accumulation of Ca by *Aspergillus niger* strains (Table 3). This element is a component of cell walls and mitochondrial membranes. On Ca surplus microorganisms react by excreting of Ca from cells into the environment. Therefore the amount of Ca after accumulation was in the solutions 2 and 3 higher than in the original solution. This fact we suppose is caused by the release of Ca from the cell walls and mitochondrial membranes into the measured solutions.

Table 3. The accumulation of Fe, Mg, Ca from the solutions 1, 2, 3 by *A. niger* strains in [mg/L] and in %.

| *Aspergillus niger* Strain | Solution 1 | | | Solution 2 | | Solution 3 | |
|---|---|---|---|---|---|---|---|
| | Fe | Mg | Ca | Fe | Mg | Fe | Mg |
| | [mg/L] | [mg/L] | [mg/L] | [mg/L] | [mg/L] | [mg/L] | [mg/L] |
| *An* – G | 17 | 23 | 6 | 5.1 | 2.8 | 0.66 | 0.85 |
| *An* – P | 12 | 22 | 6 | 3.5 | 3.4 | 0.68 | 0.93 |
| *An* – N | 14 | 21 | 2 | 3.2 | 2.8 | 0.71 | 1.28 |
| *An* – Š | 14 | 18 | 7 | 3.1 | 3.3 | 0.9 | 1.07 |
| Average | 14.25 | 21 | 5.25 | 3.7 | 3.1 | 0.74 | 0.78 |
| % | 7-10 | 7-10 | 2-7 | 18-29 | 12-15 | 41-54 | 36-55 |

The changes of pH in the AMD and in experimental solutions during the accumulation were also noted. The original pH value of the natural AMD was 2.75. We observed increase of this value to 3.05 by *An*-G strain and to 3.17 by *An*-P strain. On the other hand, *An*-N and *An*-Š strains decreased the original pH value to 2.37 or 2.34. The *An*-N and *An*-Š strains were originally isolated from the strongly acidified environment affecting the evolution of the secondary metabolites. There are primarily intermediate products of the citrate cycle such as citric acid, fumaric acid or α-ketoglutaric acid produced by the *Aspergillus* species (ŠIMONOVIČOVÁ *et al*., 2008).

In the experimental solution 1, the change of the original pH value (0.66) was only insignificant (0.66 or 0.67 by all studied strains). In solution 2, original pH (0.89) increased to 1.74 by *An*-G and *An*-N strains, to 1.78 by *An*-P strain and to 1.69 by *An*-Š strain.

We observed the most noticeable change of pH in the solution 3 (100x diluted original solution). The pH values decreased approximately by half in comparison to original value by all studied strains - from 2.45 to 1.09 (*An*-G, *An*-P and *An*-N strains) or 1.07 (*An*-Š strain).

In the accumulation of studied elements from the individual solutions prepared separately (Table 4) we have noticed the same situation like in natural AMD (Table 2) or in solution 1 (Table 3). It means, the highest was the accumulation of Fe (23.5 mg/L on average) in the range from 7 to 15 %, less of Mg (12.5 mg/L on average) in the range from 4 to 7 % and the lowest was accumulation of Ca (5.25 mg/L on average) in the range from 5 to 7 % (Table 4).

Table 4. The accumulation of Fe, Mg, Ca from individual solutions by *A. niger* strains in [mg/L] and in %.

| *Aspergillus niger* strain | Fe [mg/L] | Mg [mg/L] | Ca [mg/L] |
|---|---|---|---|
| *An* - G | 30 | 16 | 6 |
| *An* - P | 23 | 11 | 6 |
| *An* - N | 15 | 9 | 5 |
| *An* - Š | 26 | 14 | 4 |
| average | 23.5 | 12.5 | 5.25 |
| % | 7-15 | 4-7 | 5-7 |

The primary interaction between micromycetes and the chemical elements occur at the cell wall level. The main components of the cell wall are polysaccharides, primarily chitin and chitosane as well as proteins, lipids and other substances. The cell wall contains phosphate, carboxyl and hydroxyl biosorption sites which offer extensive possibilities for binding metals through different mechanisms, predominantly ion exchange and coordination. A lot of metals such as Zn, Pb, Cu, Cd, Hg, Fe have been reported as being readily taken up by chitin what is also dependent on pH of the solution with the 3 - 4 optimum. When two or more transition metal ions are present in the solution together, the cation that forms the most stable complex with the cell wall polymer will be preferentially collected leaving most of the other cations in solution. Alkali metals, ammonium, Mg and Ca are not sequestered by chitin (VOLESKY, 1990; 2007). Primarily amine and carboxyl groups of *Aspergillus niger* species are active by the heavy metal bindings to the cell wall, unlike phosphate groups and lipid fractions (KAPOOR and VIRARAGHAVAN, 1997).

## 4. Conclusions

We observed the accumulation of the Fe, Mg and Ca elements by four strains of *Aspergillus niger* species, which were extracted from various types of the environment. On average, the values for the accumulation of the studied elements from AMD decreased in the order: Mg (25 mg/L) > Fe (22 mg/L) > Ca (2 mg/L); from the solution 1: Mg (21 mg/L) > Fe (14.25 mg/L) > Ca (5.25 mg/L); from the solution 2: Fe (3.7 mg(/L) > Mg (3.1 mg/L); from the solution 3: Mg (0.78 mg/L) > Fe (0.74 mg/L). The accumulation values from the individual solutions prepared for each element separately decreased in the order: Fe (23.5 mg/L) > Mg (12.5 mg/L) > Ca (5.25 mg/L). In most cases the accumulation of Mg was the highest in comparison to accumulation of Fe and Ca.

With regard to the studied strains of microfungi, the accumulation of the studied elements in percentage was in the order: *An*-P 16% of Fe and *An*-G 10 % of Mg from AMD; *An*-G 10 % of Mg and Fe (from solution 1); *An*-P 20 % of Fe and 15 % of Mg from solution 2; *An*-N 55 % of Mg and *An*-Š 54 % of Fe from solution 3. The strain *An*-G 15 % of Fe and 7 % of Mg from the individual solutions prepared for each element separately.

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