Heavy metals removal by bioleaching using *Thiobacillus Ferrooxidans*

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Abstract

This paper presents research results obtained on laboratory column reactor filled in with soil sampled in natural state by bioleaching of contaminated soils with metals. In the first step of experiments (3 months of treatment), the concentration of metals (Pb, Cu, Zn, Mn) were determined from soil and leachate samples by inductive coupled plasma atomic emission spectrometry. Then the research was focused on a comparative study regarding the concentrations of pollutants in soil. In this condition were determined: the initial amount of metal in soil, the amount of metal in the natural state soil at the end of treatment by bioleaching and the amount of metal after a month of extraction process (post-treatment). This research has led to the conclusion that the natural state soil may be treated by in situ bioleaching, after 3 months of treatment high extraction yields being obtained (Pb 78–90%; Cu 68–84%; Zn 74–90%; Mn 85–95%).

**Keywords**: bioleaching, contaminated soil, extraction, heavy metals, *Thiobacillus Ferrooxidans*.

1. Introduction

The accumulation of heavy metals in soils is distinguished by the environmental implications of their toxicity and their compounds, but also by chemical bonding that influences the soil reaction, adversely affecting plant growth and inhibiting the nitrogen fixation by microorganisms.

Interest for bioremediation of polluted soils and waters has increased in recent decades. In Romania, the research in the elaboration of technologies for treating contaminated soils is a relatively new research area. The presence of heavy metals, especially copper, lead, zinc and cadmium, generates the most widespread soil pollution and its adverse effects are particularly strong. This kind of pollution is identified, in Romania in areas like: Baia Mare, Copșa Mică and Zlatna (NEPA [1]).

Soil pollution with heavy metals in Baia Mare region is recognized today as a significant problem, negatively affecting the environment and human health (NEPA [2], I.M. BERAR & al. [3], I.M. BERAR & al. [4]).

Worldwide researchers used different type of microorganisms in various conditions. The most common bacteria type used for the bioleaching of polluted soil media are *Acidothiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans* and *Thiobacillus thioparus*. These bacteria were growth in solutions after they were isolated from natural environment (J. WANG & al. [5], GOMEZ and BOSECKER [6]).

The type of bacteria will influence the bioleaching yield. The experiments performed by Gomez and Bosecker proof that *Thiobacillus ferrooxidans* can extract completely Cd, Co, Cu and Ni from soil samples, but the use of *Thiobacillus thiooxidans* approximately 80% were leached (GOMEZ and BOSECKER [6]).
Microorganisms are able to decompose xenobiotic compounds, which until now, were considered to be resistant to the biological processes that occur in soil (M. DUA & al. [7], IWAMOTO and NASU [8]).

Good extraction yields (Pb 33–84%; Cu 50–96%; Zn 42–97%) were obtained by comparing the results of recent scientific publications on bioleaching / extraction of heavy metals from soils using Thiobacillus type microorganisms under similar experiment conditions (M. DUA & al. [7], IWAMOTO and NASU [8], CHEN and LIN [9], CHEN and LIN [10], CHEN and LIN [11], L.J. TSAI & al. [12], C. LÖSER & al. [13], C. LÖSER & al. [14], D.E. GÜVEN [15], Q. LI & al. [16], A. PATHAK & al. [17], Y.-X. CHENA & al. [18], Y.-G. LIU & al. [19], KUMAR and NAGENDRAN [20]).

The aim of this work is the study of heavy metals extraction from contaminated soils in their natural state form by bioleaching. This study presents also the in situ bioleaching effect by comparing the existing metal concentrations in the soil at the beginning of treatment, during the treatment and after one month, the extraction process was finished.

2. Materials and methods

In order to build the laboratory column, five soil samples, in the natural state, were collected in a metal container according to STAS 7184/1–75 (STAS [21]). The soil samples were taken from inside the polluted area in Baia Mare, Romania, until 75 cm depth, as follows:

- Sample 1 (S1): 0–10 cm;
- Sample 2 (S2): 10–20 cm;
- Sample 3 (S3): 20–35 cm;
- Sample 4 (S4): 35–55 cm;
- Sample 5 (S5): 55–75 cm.

The soil samples in their natural state were transported in sampling container to the laboratory. Then the samples were transferred to a glass container. A drainage layer of 30–45 mm gravel sort was placed on the bottom of soil, depending on the depth of the sample, in order to reconstruct the natural soil profile (Fig. 1).

Remediation of contaminated soils was performed using *Thiobacillus ferrooxidans* type microorganisms (140 x 10⁶ cells/mL), selected from the soil sampling area. These microorganisms were grown on a 9K medium which contains: (NH₄)₂SO₄–3 g; KCl–0.1 g; K₂HPO₄–0.5 g; MgSO₄·7H₂O–0.5 g; Ca(NO₃)₂·4H₂O–0.01 g; FeSO₄·7H₂O–44.2 g (SILVERMAN and LUNDGREN [22]). The solution obtained (9K environment) has a pH of 2.5 but the experiments were made at pH=5 because the soil had a pH of 7.5 and when bioleaching solution was added, the pH became 5.

The experiment was conducted in a laboratory column, over it a 9K medium being added. Aeration was performed to achieve the metal extraction.

The following parameters were controlled during this experimental column:

- Time lapse: 12 weeks in a 9 K medium;
- Soil aeration: 12 h/day at 8 bar;
- Temperature: 26 ± 2 °C;
- pH: 5 inside the column.

The soil samples and the leachate from the laboratory column were collected each week for 12 weeks in order to validate the remediation process by bioleaching. Using a sampler, were taken soil samples (10g) from each depth (S1–S5), and the leachate samples (10ml) were collected by using a valve on the inferior part of the soil column/profile. These samples were analyzed by inductive coupled plasma atomic emission (ICP-AES), using SpectroFlame FMD 07 spectrometer (Spectro, Germany).
3. Results and discussion

3.1. Metal concentration variation in the leachate

The quantity variation of elements extracted in leachate during the development of the experiment (Fig. 2). In the first week 0 values are according to the beginning of the experiment, when the 9K medium was just added in the process. The solution didn’t contain any kind of metal. It can be observed a high amount of zinc, manganese and copper are found in the leachate, that means they were extracted from soil using 9K medium.
All studied metals (Pb, Cu, Zn, Mn) were extracted in high range during the first week. In the second week, however, there is a decrease in the amount of leached metal, followed by stability between the third and sixth week, and followed by an accentuated decrease of Zn and Mn concentration between seventh and eighth week.

3.2. Metal concentration variation in soil

The concentration of the elements varies with depth due to the heterogeneity of the soil and in time. Two trends were observed after the results analysis:

1. In the sample S1 the amount of metal in the soil after the first week is higher than the initial values. Due to bubbling and the fact that this sample was not immersed in the bioleaching solution throughout the experiment. The solution from deeper soil layer within the experimental column contains higher amount of metals due to the bioleaching process. When this solution is bubbled it interacts with the surface soil layer enriching it in metals.

2. In the other samples (S1, S2, S3, S4, S5) the amount of metal in the soil after the first week of experiment is less than the initial concentration. This highlights that the metals were extracted from the soil.

The lead (Fig. 3a) and copper (Fig. 3b) concentration, varies throughout the experiment, but it is noted that after seven weeks the difference, are not as sharp as they were in the beginning.

![Figure 3](image-url)

Figure 3. The variation of concentration in soil profile over time: a) Pb; b) Cu; c) Zn; d) Mn.

The Zinc (Fig. 3c) and Manganese (Fig. 3d) concentration in the soil profile varies markedly from one sample to another, resulting a greatly decrease in the first seven weeks of the experiment. After seven weeks of experiment stability is recorded for all four studied soil samples.

The analysis of samples out from the soil profile revealed that all metals (Pb, Cu, Zn, Mn) were well extracted during the first week for all studied samples. An increase in the amount of metals in
the soil can be observed in even weeks followed by a decrease in odd weeks. This variation in the concentration of the metal in soil shows that the metals were embedded in the soil matrix. The microorganisms from the bioleaching solution decrease the metal concentration in the samples.

In conclusion all studied metals were leached very well during the 12 weeks of extraction, the most effective extraction being performed during seventh and eighth week.

3.3. Natural state soil bioleaching effect

The microbial degradation of metals in soil and the effect of these microorganisms in soil treatment were highlighted by a analysis study of metal content in soil samples.

The following parameters are compared in Fig. 4:

- Initial soil metal concentration;
- Final soil metal concentration at the end of treatment;
- Soil metal concentration after one month from the end of the treatment (post-treatment).

As a result of the researches, was made also a study in order to evaluate the effectiveness of the extraction method. This was made by determining the final decontamination yield for each sample using the following formula: (BRUSTUREAN and PERJU [23].

\[ \eta = \frac{m_e}{m_i} \times 100 \quad \% \]

where: \( m_e \) – is the extracted pollutant concentration [mg/kg]; \( m_i \) – is the initial pollutant concentration present in the soil (extractable in royal water) [mg/kg].

The amount of lead (Fig. 4a) and copper (Fig. 4b) at the end of treatment is low in comparison to the initial concentration, treatment efficiency being between 78–90% for Pb, respectively 68–84% for Cu. The amount of Pb and Cu in soil after one month from the end of treatment (post treatment) is the lowest, which highlights that the remaining soil microorganisms have extracted further the metals from it, except for the S2 sample, in the case of Pb.
Figure 4. Evolution heavy metals in soil samples: a) Pb; b) Cu; c) Zn; d) Mn
Yields extraction of Pb are compared with those obtained by Li et al., 2012, being still much higher than the results of obtained by others researchers (CHEN and LIN [9], Q. LI & al. [16], Y.-X. CHENA & al. [18], Y.-G. Liu & al. [19]).

Copper extraction yield is higher than in those obtained by Wang et. al, 2008; Güven, 2008; and Pathak et.al., 2009, but very few percent lower than those obtained through research conducted by Chen, Chena, Liu and Kumar where the yield was 96% (J. WANG & al. [3], CHEN and LIN [9], D.E. GÜVEN [15], A. PATHAK & al. [17], Y.-X. CHENA & al. [18], Y.-G. LIU & al. [19], KUMAR and NAGENDRAN [20]).

The concentration of zinc (Fig. 4c) and manganese (Fig. 4d) present in the soil at the end of treatment is considered of low level, the treatment efficiencies is between 74–90% for Zn and 85–95% for Mn. Also the amount of metals in analyzed post-treatment samples is lower than in samples from the end of the experiment.

Extraction of zinc by bioleaching is comparable with the results of Chen and Lin, 2000; Chena et al., 2005, but lower than those obtained by Liu et al., 2007; Kumar and Nagendran, 2008. (CHEN and LIN [9], CHEN and LIN [11], Y.-G. LIU & al. [19], KUMAR and NAGENDRAN [20]).

4. Conclusions

The concentration of the elements varies with the depth due to the lack of homogeneity of the soil, but also due to the fact that, in time, the percolation water acts on the elements from the upper layers to the lower layers of the soil.

The metals concentration in the sample S1 was much higher than the initial value during the experiment, due to bubbling process that took place in the column. In this process the pollutants are migrating from the lower to the upper layers.

The analysis regarding metal extraction from the studied soil is showing that the six metals analysed (Pb, Cu, Zn, Mn) have been extracted very good in the first week, from the five studied samples. But, in the second week was observed a rise in the metal concentration from the soil, followed by a drop of the concentration in the third week. These variations of the metal concentration from the soil show the fact that the metal has been incorporated again into the soil matrix. As a result of the microorganisms from the bioleaching solution acting in a new stage of leaching, the metal concentration is decreasing.

The extraction of metals from polluted soil continues after the ending of the treatment process.

The results of the study done in the laboratory, by in situ bioleaching soil treatment, show, depending on the sampling depth and only after three months of treatment, high extraction yields: Pb: 78–90%; Cu: 68–84%; Zn: 74–90%; Mn: 85–95%.

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