

# The Use of Microorganisms in the Bioleaching of Soils Polluted with Heavy Metals

I. M. Sur, A. M. Chirila-Babau, T. Gabor, V. Micle

**Abstract**—This paper shows researches in order to extract Cr, Cu and Ni from the polluted soils. Research is based on preliminary studies regarding the usage of *Thiobacillus ferrooxidans* bacterium (9K medium) for bioleaching of soil polluted with heavy metal (Cu, Cr and Ni). The microorganisms (*Thiobacillus ferrooxidans*) selected directly from polluted soil samples were used in this experimental work. Soil samples used in the experimental research were taken from an area polluted with heavy metals from Romania. The soil samples are subjected to the cleaning process using the 9K medium solution (20 mL and 40 mL, respectively), stirred 200 rpm for 20 hours at a controlled temperature (30 °C). During the experiment (0, 2, 4, 8 and 20 h), liquid samples have been extracted and analyzed using the Atomic Absorption Spectrophotometer AA-6800 (AAS) in order to determine the Cr, Cu and Ni concentration. Experiments led to the conclusion that these soils can be depolluted by bioleaching, being a biological treatment method involving the use of microorganisms to favor the extraction of Cr, Cu and Ni from polluted soils.

**Keywords**—Bioleaching, extraction, microorganisms, polluted soil, *Thiobacillus ferrooxidans*.

## I. INTRODUCTION

WITH the development of metal processing, heavy metal contamination becomes a worldwide environmental problem, with serious implications on the entire geosystem [1] and can pose significant risks to public health and of ecosystems [2].

Bioremediation has been developed as an environmentally friendly and cost-effective technology for the removal of metals from sediments. Among the biological technologies, microbiological leaching of heavy metals from sediment is far more popular. Biological methods for the extraction of metals, known as bioleaching, have various advantages, including being simpler to apply, cheaper and friendlier to the environment than traditional methods [3]. Bioleaching is defined as “the solubilization of metals from solid substrates either directly by the metabolism of leaching bacteria or indirectly by the products of metabolism” [4].

In the bioleaching process, the most used microorganisms are

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*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, which sustain solubilization of metals in the soils subjected to depollution [5].

Bioleaching, or bacterial leaching, consists of the extraction by solubilization of the metallic elements from contaminated soil using bacteria. This method does not destroy (eliminate) the pollutants, but it favors their segregation from the contaminated environment, the microorganisms having the property to oxidize the metals, transforming them into a more soluble form [6].

Soil pollution with metals from the main mining areas in Romania (Baia Mare, Alba Iulia) is recognized today as a significant problem, presenting a major risk for human health and the environment, soils being polluted with heavy metals in significant amounts: Baia Mare [7] and Alba Iulia [8], [9].

Following studies conducted by specialists, it was found that by stirring samples (120-170 rpm) at a constant temperature (28-30 °C) and a certain time interval (5-48 days) using *Thiobacillus* type microorganisms, bioleaching is efficient for removing metals from the soil: Cu: 20-96%; Cr: 10-41%; Ni: 69-92% [2], [10]-[18].

The objective of this paper is the extraction of copper, chromium and nickel from polluted soils using *Thiobacillus* type microorganisms in different quantities for the purpose of soil decontamination.

## II. MATERIAL AND METHODS

### A. Soil Sample

Soil for this study was collected from two mining areas in Romania: Baia Mare and Alba Iulia as shown in Fig. 1.

Soil samples were taken from the depth ranges of 0-10 cm, 10-20 and 20-30 cm. Soil sampling was carried out in accordance with the methodological norms stipulated in STAS 7184/1-84 and subsequently processed according standards SR ISO 10381-6:1997, and respectively SR ISO 11464:1998 [19]-[21].

Soil was sampled and mixed completely and temporarily stored at 4 °C. Soil samples were air dried, then mortared for homogenization, and 2 mm pore diameter particles were used for bioleaching research.

### B. Microorganisms

*Thiobacillus ferrooxidans* were isolated from the soil samples taken at Baia Mare, being inoculated and multiplied, resulting an extraction solution called 9K medium. *Thiobacillus ferrooxidans* are bacteria similar to *Acidithiobacillus* type, from shape and cellular structure points

of view. These bacteria are unicellular microorganisms, rod-shaped cells, in length of 1-2 microns and 0.5-1.0 microns width [22].

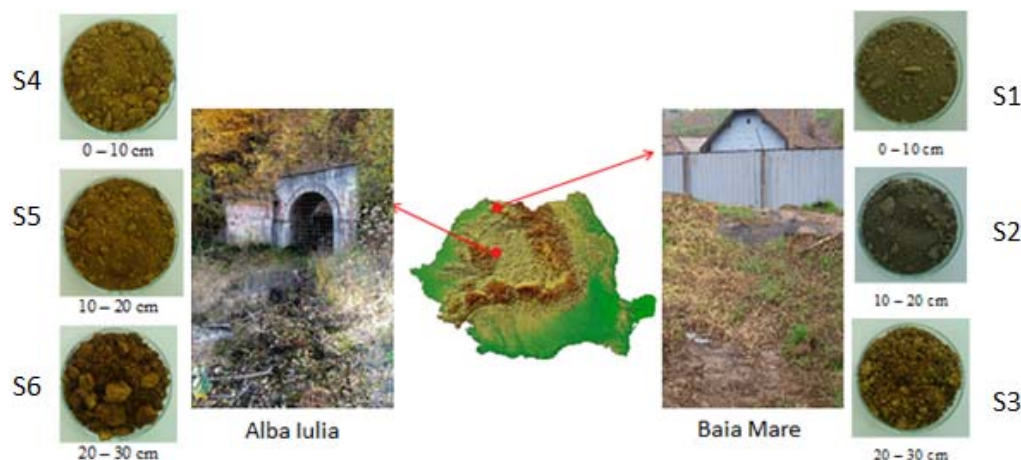


Fig. 1 Soil sampling points

The 9K medium is composed of microorganisms *Thiobacillus ferrooxidans*. The microorganisms were selected from the soil samples and were grown on a nutritive 9K medium at a controlled temperature (21 °C). The 9K medium was prepared for bacterial growth and enrichment with the composition:  $(\text{NH}_4)_2\text{SO}_4$ –3.0 g/L; KCl–0.1 g/L;  $\text{K}_2\text{HPO}_4$ –0.5 g/L;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ –0.5 g/L;  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ –0.01 g/L;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ –44.2 g/L dissolved in distilled water (1 L) [23]. The pH of the culture media was adjusted to 2.5 using 1 N sulfuric acid. The bacteria development was controlled by measuring the pH.

To culture the bacterial strain, 250 mL of Silverman and Lundgren 9K medium in 500 mL Erlenmeyer flasks was inoculated with pure culture of bacteria *Thiobacillus ferrooxidans*. The flasks were incubated at 30 °C in a shaking incubator at 170 rpm for 24-48 h.

The bacteria development was observed when the 9 K medium was collared in brownish orange. The bacteria development was observed when the 9 K medium was collared in brownish orange.

The bacterial cultures were tested by microscopic cell counting, When the culture was in the exponential growth phase (around  $140 \times 10^6$  cells/mL), a sample was taken to inoculate a bioleaching experiment. The grown cells inoculum of the same concentration was used further for bioleaching.

### C. Bioleaching Experiments

The 9K medium loaded with *Thiobacillus ferrooxidans* type microorganisms was used for bioleaching.

Bioleaching experiments were conducted in 250 mL Erlenmeyer flasks with 10 g of soil was taken from each sample (from the 2 mm soil fraction). The soil was treated with a solution loaded with microorganisms (9K medium) in different quantities (20 mL and 40 mL respectively). The flasks were agitated at 30 °C, at 200 rpm using the platform stirrer with orbital oscillation-rotation for an interval of time (0, 2, 4, 8, and 16 hours respectively). Table I shows the

bioleaching conditions.

TABLE I  
BIOLEACHING CONDITIONS

Sample	The amount of soil polluted [g]	The amount of 9K medium [mL]	T [°C]	Speed [rpm]	Sample code
S1	10	20	30	200	S1 + 20
S1	10	40	30	200	S1 + 40
S2	10	20	30	200	S2 + 20
S2	10	40	30	200	S2 + 40
S3	10	20	30	200	S3 + 20
S3	10	40	30	200	S3 + 40
S4	10	20	30	200	S4 + 20
S4	10	40	30	200	S4 + 40
S5	10	20	30	200	S5 + 20
S5	10	40	30	200	S5 + 40
S6	10	20	30	200	S6 + 20
S6	10	40	30	200	S6 + 40

At the end of the stirring period, all samples were filtered through 0.45 μm pore size filters. After filtration, the concentrations of Cu, Ni, and Cr in the supernatant were analyzed using SHIMADZU AA–6800 AAS in order to determine the heavy metal concentration that was extracted, respectively, and if necessary, samples were diluted with distilled water.

All the experiments and analyses were performed in triplicate and the average values were reported.

### III. RESULTS AND DISCUSSION

The concentration of heavy metals (Cu, Cr and Ni) determined at the assays from soil samples were compared with the average value of the normal contents with the maximum allowable values and with the values of the warning threshold and intervention one, for soil less sensible according to Order 756/1997 [24].

From Figs. 2-4 it can be seen that the investigated metals are present in the sampled soils, being found in much higher

concentrations in samples from the Baia Mare area compared to samples from the Alba Iulia area.

In the Baia Mare area, the concentration of Cu, Cr and Ni increases with depth. For samples in the Alba Iulia area we can observe that the sample taken from a depth of 10-20 cm shows lower values compared to samples taken from other depths, except for Cr which increases with sampling depth. Soil samples from Baia Mare show high Cu concentrations, exceeding the intervention threshold (500 mg/kg) even 6 times in the case of the 20-30 cm sample (Fig. 2).

In the case of samples in the Alba Iulia area, it can be observed that all samples have Cu concentrations below the intervention threshold (Fig. 2), and that the sample at the depth of 10-20 cm is below the alert threshold (250 mg/kg), but exceeds the normal values in the soil (20 mg/kg).

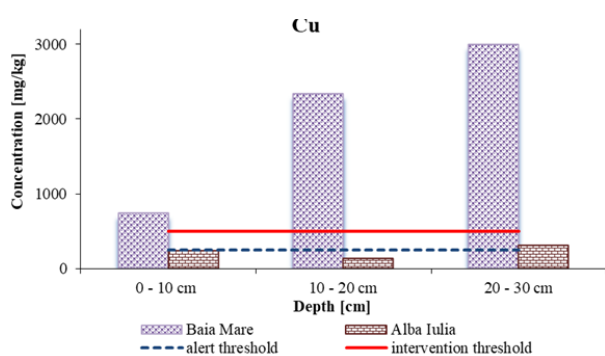


Fig. 2 Concentration of cooper in sampling soils

In the case of chromium (Fig. 3), all investigated samples show concentrations below the alert threshold (300 mg/kg) and below the intervention threshold (600 mg/kg), but exceed normal values in the soil (30 mg/kg).

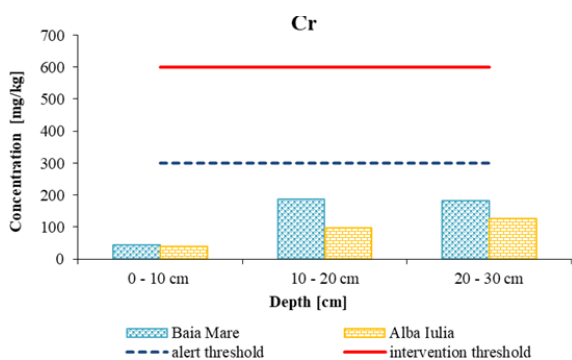


Fig. 3 Concentration of chromium in sampling soils

The concentration of Ni (Fig. 4) in all investigated samples is very high, exceeding the alert threshold (200 mg/kg) and the intervention threshold (500 mg/kg).

Given the high heavy metal concentrations in the soil, research was carried out regarding the extraction of heavy metals using different quantities of 9K medium (20 mL and 40 mL, respectively).

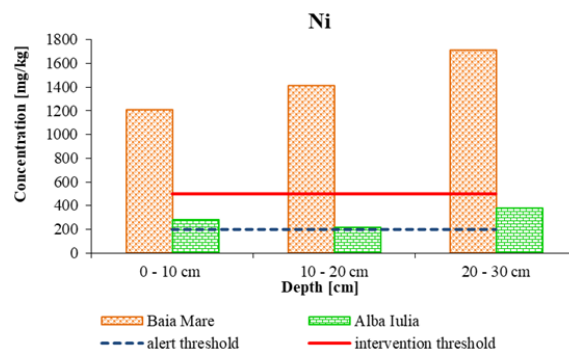


Fig. 4 Concentration of nickel in sampling soils

In the case of Baia Mare samples where the initial cooper concentration was higher, we can observe that the amount of extracted cooper is also higher in the first two hours, while for samples taken from Alba Iulia this Cu is extracted in small quantities.

The amount of microorganisms very little influences the Cu concentration extracted in the first two hours of stirring, and ongoing it is observed that in the soil less copper is present in samples subjected to extraction by using a larger amount of microorganisms (40 mL).

Samples in the Baia Mare area, which were subjected to bioleaching using 40 mL of 9K medium, show a high extraction after 4 hours of experiment, followed by stability. Samples in which 20 mL of 9K medium was added show a linear decrease over time (Fig. 5).

Analyzing Fig. 6 we can observe that in the case of samples where a larger amount of microorganisms was used (40 mL), the amount of Cr extracted is much higher than in the case of samples where only 20 mL were used. Samples present an upward extraction throughout the treatment period, regardless of the amount of 9K medium that is used. In contrast, for the depth (S6) is observed a large amount of Cr extracted at 4 hours using only 20 mL. In the case of sample S5, after 4 hours is observed an equal amount of Cr in the analyzed sample, regardless of the amount of microorganisms that is used (Fig. 6 (b)).

Analyzing Fig. 7 we can observe that the samples in which a double quantity of 9K medium was added present lower concentrations of Ni in the soil, observing that all samples have the same tendency to decrease during the 16 hours of experiment.

The efficacy of the extraction process (after 16 hours) was evaluated by determining the depollution yield using (1) [25]:

$$\eta = \frac{m_e}{m_i} \cdot 100 [\%] \quad (1)$$

where:  $m_e$  is the extracted concentration of pollutant [mg/kg];  $m_i$  - the initial concentration of pollutant existing in the soil [mg/kg].

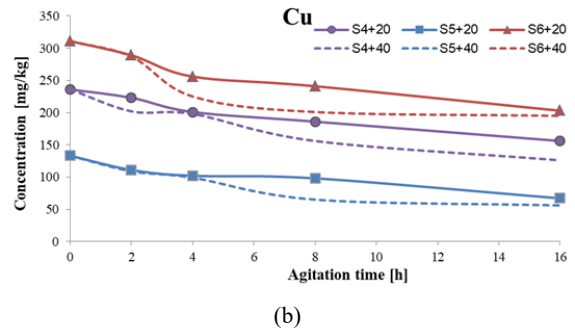
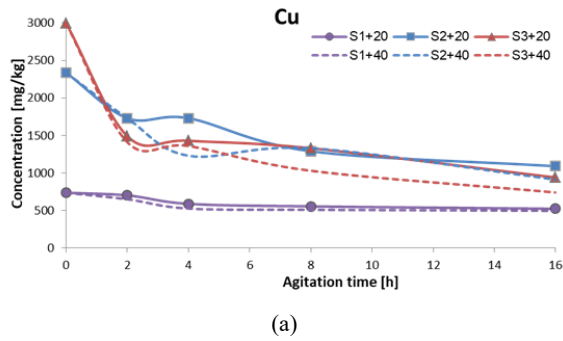


Fig. 5 Variation in time of cooper concentration in soil: (a) Baia Mare; (b) Alba Iulia

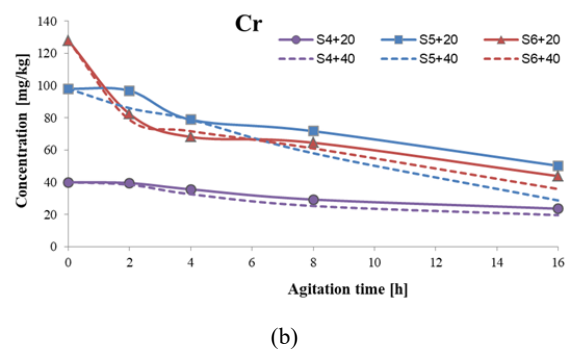
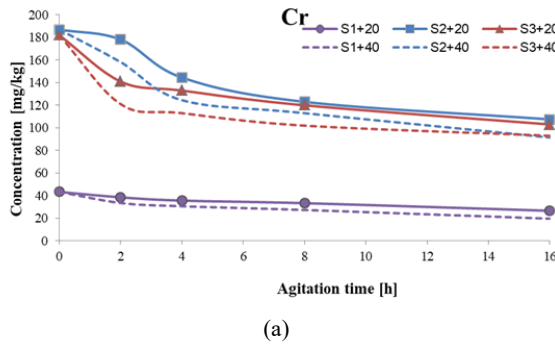


Fig. 6 Variation in time of chromium concentration in soil: (a) Baia Mare; (b) Alba Iulia

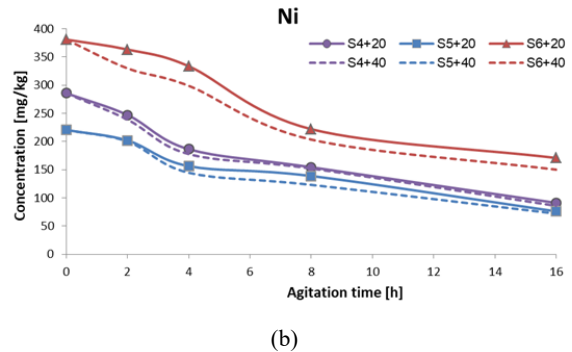
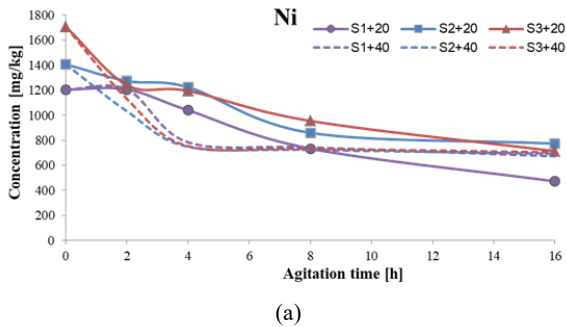


Fig. 7 Variation in time of nickel concentration in soil: (a) Baia Mare; (b) Alba Iulia

Following bioleaching, high yields of metal extraction from the soil were obtained:

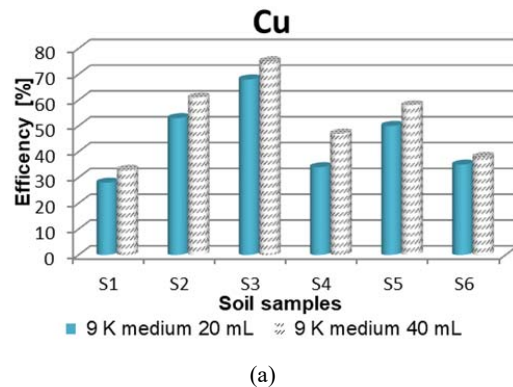
- using 40 mL 9K medium: Cu 33-75%; Cr: 49-72% and Ni: 44-70%;
- using 20 mL 9K medium: Cu 28-68%; Cr: 39-66% and Ni: 45-68%.

Even though some samples had larger amounts of metals in the soil, using a double amount of microorganisms (40 mL), by the end of the experiment (after 16 hours) the concentration was lower, with higher yields being observed. The only exception is for the nickel in sample S1, where a higher yield was obtained using 20 mL of 9K medium.

Yields obtained in the case of copper (33-75%) are similar to those obtained by Couillard (20-73%), but much lower compared to yields obtained by Li [17] Blais [13], Priya [11], Bayat [10], Zhu [2] and Fontmorini [15] (78-93%).

Chromium extraction was performed better (49-72%)

compared to results obtained by Beolchini (14%) [12] and Fontmorini (10%) [15], but lower than Qiang Li (53-92%) [17] or Blais (19-41%) [13].



(a)

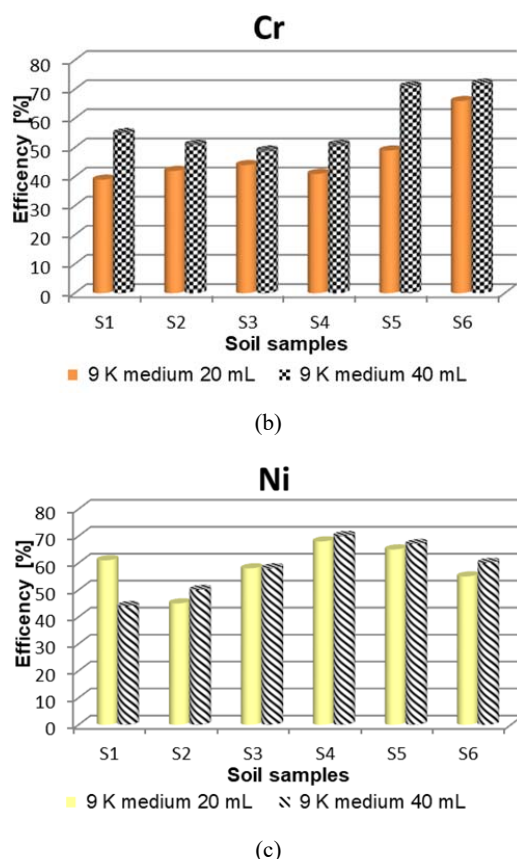


Fig. 8 The extraction efficiency: a)Cu; b)Cr; c) Ni

By comparing the results (Fig. 8) obtained with the ones in literature on the extraction of Ni by bioleaching, we can observe that resulting yields (44-70%) are much lower compared to researchers Li [17], Beolchini [12], Blais [13], Priya [11] and Fontmorini [15] (78-93%).

#### IV. CONCLUSION

The mining exploitation from Baia Mare and Alba Iulia resulted in pollution of large surfaces of terrain and the negative environmental impact. Metal soil pollution in the studied areas (mine) is recognized today as a significant problem, presenting a major risk to human health and the environment.

The analysis of the samples revealed that the soil in the researched areas is polluted with heavy metals, which are present in significant quantities: Cu: 1330-2996 mg/kg; Cr: 40–187 mg/kg and Ni: 220-1708 mg/kg.

The extraction of copper, chromium and nickel has been highlighted by using *Thiobacillus* type microorganisms in the bioleaching process. Following experiments carried out on soil samples, we can conclude that the 9K medium is efficient in extracting heavy metals from the soil.

A larger amount of microorganisms (40 mL) increases the extraction yield by several percent: Cu 33-75%; Cr: 49-72% and Ni: 44-70%, compared with the efficiency when using 20 mL of 9K medium: Cu 28-68%; Cr: 39-66% and Ni: 45-68%.

The bioleaching process investigated in this study effectively removes the heavy metals from contaminated soils. It is one of the alternatives of bioremediation on metal-contaminated soil.

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