Article

Bioleaching of Mine Tailings by Mesophilic: *Acidithiobacillus* spp., *Leptospirillum ferrooxidans*, and Thermophilic: *Sulfobacillus thermosulfidooxidans* Cultures with the Addition of Ag⁺ Additive

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Abstract: This research focused on applying and comparing the performance of microorganisms with different temperature preferences, assessing the overall percentage recovery of elements (copper, zinc, arsenic, and iron) from mine tailings in the Staré Ransko region (Czech Republic). The study examined the solubilisation process using a mesophilic mixed bacterial culture, including Acidithiobacillus ferrooxidans (AF), Acidithiobacillus thiooxidans (AT), Leptospirillum ferrooxidans (LF), and the thermophilic species Sulfobacillus thermosulfidooxidans (ST). Under biotic conditions, constant process parameters were maintained, including a particle size of 71–100 μm, a pH value of 1.8, agitation at 150 rpm, and a pulp density of 10% (*w/v*). The only exception was the temperature, which varied for optimal multiplication of cultures (30 °C/50 °C). Additionally, the research examined the impact of AgNO₃ additive at a concentration of Ag⁺ ions of 5 mg·L⁻¹. The research focused on the solubilisation of Cu, Zn, Fe, and As and the results demonstrated that the application of microorganisms ST, combined with the action of Ag⁺ ions, enhanced the kinetics of the extraction process, leading to the highest final recovery of all elements (Cu 91.93%, Zn 85.67%, As 69.16%, and Fe 71.72%) under the specified conditions. The study observed that the most significant increase in solubilisation can be attributed to the additive cation in the case of copper (AF, AT, LF/Ag⁺ by 40.33%; ST/Ag⁺ by 44.39%) and arsenic (AF, AT, LF/Ag⁺ by 23.79%; ST/Ag⁺ by 26.08%). Notably, the intensification of leaching using thermophilic bacteria at a constant suspension temperature of 50 °C was primarily determined for Zn (ST by 18.36%, ST/Ag⁺ by 14.24%). After 24 days of extraction, the emergence of secondary minerals, namely CaSO₄·2H₂O and KFe₃(SO₄)₂(OH)₆, was identified. The study highlighted a significant increase in the extraction mechanism kinetics due to the influence of microorganisms, contrasting with the low solubilities observed under abiotic conditions (Cu 9.00%, Zn 14.17%, As 4.28%, Fe 6.23%).

Keywords: bioleaching; *Acidithiobacillus ferrooxidans*; *Acidithiobacillus thiooxidans*; *Leptospirillum ferrooxidans*; *Sulfobacillus thermosulfidooxidans*; metals

1. Introduction

The future potential shortage of metals extracted from primary sources and the deepening of the so-called raw material crisis in terms of polymetallic ores has been determined in several scientific studies. Sulphide ore deposits are increasingly depleted around the world, and it is necessary to look for new sources of metal [1–3]. An alternative to primary deposits is the use of post-mining waste raw materials, including those stored in mine tailings. The advantages of processing waste from the existing mine tailings are availability and large amounts of input materials. However, as the defined waste raw materials are often characterised by lower concentrations of metals, it is essential to choose suitable processing procedures for the mentioned materials to achieve high yields of metals and to meet

the environmental and economic aspects of the process for the subsequent introduction of the technology into operational conditions [4–6].

The hydrometallurgical treatment of secondary materials in acid solutions with microorganisms has been reported to be more optimal compared to other technologies [7–9]. The principle of the extraction mechanisms, which occur at the interface of two phases (particles and a solution containing microorganisms), is the transformation of poorly soluble polymetallic ores in the form of sulphides into sulphates with the continuous release of metals [10–13]. The mentioned reactions are catalysed by microorganisms, which at the same time oxidise divalent iron to a trivalent form and elemental sulphur to a sulphate anion, thereby increasing the acidity of the suspension [14–16]. Complex reactions occur in the extraction system, where the solubilisation of metal is enhanced by the effect of the ferric sulphate product [17]. The advantage of the process is that it is structurally easy to implement, and the strongly acidic leachates can be regenerated. When employing an iterative approach to the extraction solution involving microorganisms, tailored for novel input materials, bioleaching is appraised as a technology demonstrating environmental sustainability and economic efficiency [18]. In the case of copper, 70% of the element worldwide occurs in the form of $CuFeS_2$ [19]. Due to the huge consumption of metal in recent decades, the recovery of the element from raw materials with its lower concentration is preferred [20,21]; however, the final percentages of leaching values are still not sufficient in connection with the long exposure time of the solution containing microorganisms and mine waste material [22,23]. In a number of scientific studies [24–28], the influence of mesophilic cultures of the *Acidithiobacillus* genus was investigated primarily using the biohydrometallurgical method that dealt with the efficiency of extraction from the biotic point of view. However, the kinetics of the reaction mechanisms may be enhanced by applying microorganisms whose optimal cultivation conditions depend on higher ambient temperatures [29]. Maintaining elevated suspension temperatures positively influences the rate of reaction occurrences [30]. Dosing an additive that can positively affect the overall chemistry of the system is another possible method for intensifying the process. To increase the percentage recovery of the metal, it is essential that the research focuses on the modification of the technology exploiting additional microorganisms, the effect of additive on the interface of the two phases, and the optimisation of the extraction environment which affects the synthesis of unwanted secondary substances [31,32].

This study focused on investigating the extraction of polymetallic mine tailings (Cu, Zn, Fe, As) during the cooperative action of a mixed mesophilic bacterial culture (AF, AT, LF) at a constant temperature of 30 °C and thermophilic microorganisms ST while maintaining a permanent suspension temperature of 50 °C. The final solubilities of metals in reaction systems with defined microorganisms were compared under consistent fixed process parameters: particle size 71–100 μm, agitation speed 150 rpm, pulp density 10% (*w/v*), and pH value 1.8. In contrast to other studies [25,26,33–36], the pH value was mostly measured and evaluated during the extraction mechanisms of solid particles in leachates. In this research, the pH value was determined as fixed during the entire duration of the experiments to prevent the formation of inhibiting coatings on the surface of the grains. For a comprehensive comparison of microorganisms, tests were conducted simultaneously under strictly chemical conditions in sulfuric acid, as well as laboratory experiments with the addition of $AgNO_3$ (silver nitrate) additive (with a concentration of Ag^+ ions at 5 mg·L^{-1}).

2. Materials and Methods

2.1. Sample Characteristics

The sample was obtained from the vicinity of the primary mining site, specifically from adjacent mine tailings in the Vysočina-Krucemburk region, Staré Ransko (SR). The sample was provided by the state enterprise Diamo (Stráž pod Ralskem, Czech Republic). The material was crushed in the first stage of pretreatment using a Retsch BB 200 jaw crusher (Retsch GmbH, Haan, Germany). The sample was dried at a temperature of 30 °C for 12 h in

a Memmert UF 110PLUS (108 l) dryer (Memmert GmbH + Co. KG, Schwabach, Germany) until a constant weight was reached. In previous research [37], particle size was evaluated as the main factor that has a major influence on the efficiency of the extraction process. The influence of particles in the <40 μm, 40–71 μm, 71–100 μm, and 100–200 μm size intervals was investigated. The best results were demonstrated for the case of the 71–100 μm fraction. Secondly, the sample was mechanically activated in a VMA-386 vibrating disk mill (VIPO, Czechoslovakia) at different times to achieve the highest cumulation of the defined fraction. From a representative sample, the desired particle size was separated using a Retsch AS 200 digit cA device (Retsch GmbH, Haan, Germany).

The histogram representing X-ray diffraction (XRD) before the start of leaching tests is depicted in Figure 1. XRD analysis revealed that the sample comprised 5.70% FeS_2, 3.39% $CuFeS_2$, 1.67% ZnS, and 0.88% PbS as sulphide minerals. The predominant components of the solid phase were SiO_2 (48.65%) and $CaMg(CO_3)_2$ (20.96%), while minerals other than sulphides comprised 9.09% $FeCO_3$, 3.24% $KAl_2(AlSi_3O_{10})(OH)_2$, 2.36% $CaCO_3$, and 1.31% phyllosilicate. The determination of mineral composition was complemented by point microscopic detection using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) (Figure 2), which revealed light grains of sulphide minerals (a) and confirmed the presence of FeAsS in the matrix (b).
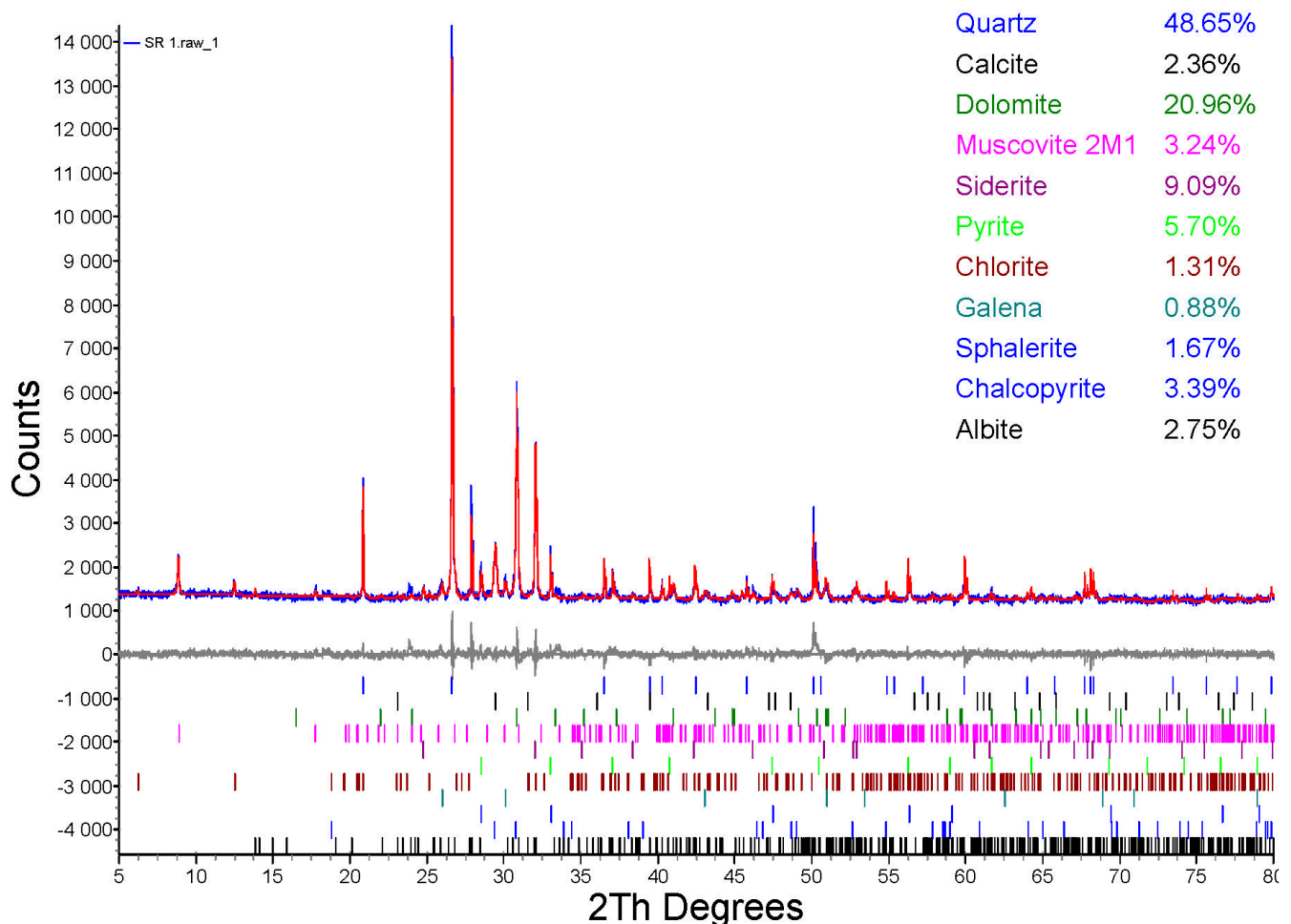

Quartz	48.65%
Calcite	2.36%
Dolomite	20.96%
Muscovite 2M1	3.24%
Siderite	9.09%
Pyrite	5.70%
Chlorite	1.31%
Galena	0.88%
Sphalerite	1.67%
Chalcopyrite	3.39%
Albite	2.75%

Figure 1. XRD detection of mine tailings before bacterial leaching.

The XRD results were consistent with the X-ray fluorescence (XRF) analyses, as presented in Table 1. The investigation primarily aimed to examine the solubilization of Cu, Zn, As, and Fe. The presence of S, Fe, and P in the substrate is crucial from the energy apparatus perspective of all employed bacterial cultures. Additionally, the concentration

of Ca is noted due to the synthesis of the secondary compound, which was analysed by XRD after the completion of the experiments (refer to Section 3.5, XRD Detection after Bioleaching).
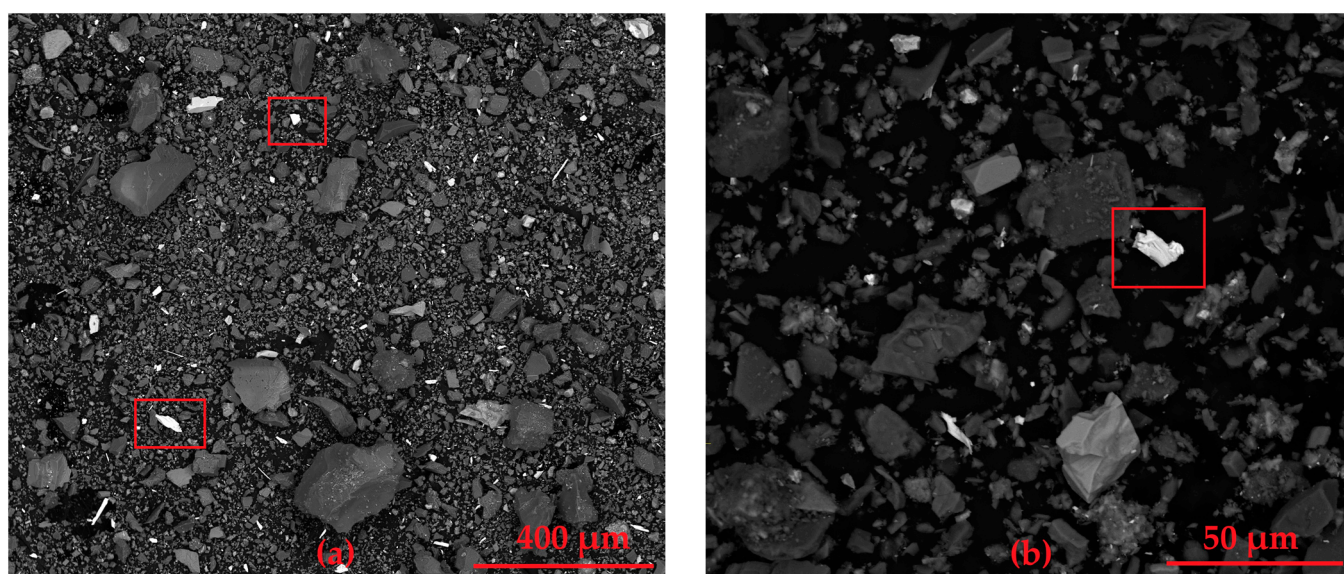

Figure 2. SEM-EDX detection before bioleaching, representative sample after mechanical activation: (**a**) pyrite sulphide grains (mag. ×200, white colouration); (**b**) arsenopyrite sulphide grain (mag. ×1600, white colouration).

Table 1. Elemental concentration determined by XRF analysis.

Elements	Mass Fraction (%)		Elements	Mass Fraction (%)	
	Average	SD		Average	SD
As	13.900	0.038	P	0.009	0.001
Ca	61.746	0.194	S	4.886	0.181
Cu	2.190	0.057	Zn	2.210	0.097
Fe	21.122	0.079			

2.2. Applied Bacterial Cultures

The mixed mesophilic bacterial culture, comprising AF, AT, and LF, along with thermophilic microorganisms such as ST, was investigated under strongly acidic conditions (pH 1.8). The strain AF 4253 was obtained from the Czech Collection of Microorganisms, Institute of Experimental Biology (Brno). Bacterial cultures of strains AT 14887, LF 2705, and ST 9293 were provided by the Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig). The stock solution was characterised in a study by Silverman and Lundgren in 1959 [38]. Sterilisation of the liquid components of growth medium 9K was conducted using an Ultra CLAVE IV autoclave (Milestone S.r.l., Sorisole, Italy). The final pH of the growth medium was adjusted to 1.8 by adding 5 M H_2SO_4. To create optimal growth conditions for ST microorganisms, 20 mL of 1% (w/v) yeast extract was added to 1 L of the growth medium. The mixed bacterial culture was cultivated at a constant temperature of 30 °C, and the thermophilic species were maintained at a fixed temperature of 50 °C in a laboratory incubator and thermostat BT 120 M (LP, Czech Republic). The optimal multiplication time for microorganisms was seven days in 1 L of Medium 9K in 1500 mL Erlenmeyer flasks under aerobic conditions without agitation. To prevent the inhibition of bacterial cells by toxic elements in the matrix, adaptation of the bacterial cells was carried out through solid-phase culture maintained in the range of 5−10% (w/v) for four months. The adaptation process took place in an incubated shaker

INFORS HT Multitron II (Infors LTD., Reigate, UK), with a constant pH of 1.8 maintained by dosing with 5 M H_2SO_4/0.5 M NaOH.

2.3. Description of Experiments

Laboratory tests in the presence of microorganisms were conducted in an INFORS HT Minifors 2.5 LTV bioreactor (Infors LTD., Reigate, UK). The total volume of the solution in the bioreactor was 1500 mL, containing a 10% inoculum (*v/v*). Following the outcomes of previous research [37], the chosen leaching factors included a particle size of 71–100 μm, agitation at 150 rpm, and pulp density at 10% (*w/v*). A constant pH level of 1.8 was maintained throughout the experiments by adding 5 M H_2SO_4/0.5 M NaOH. Fixed pH values were monitored by the Hamilton ARC electrode. The experiments under biotic conditions were conducted under the same defined parameters, except for temperature. For mesophilic microorganisms, a constant suspension temperature of 30 °C was maintained, while for thermophilic bacteria, the temperature was regulated at 50 °C, achieved using the ALU block mechanism. Another objective of the research was to compare the effect of the Ag^+ additive at a concentration of 5 mg·L^{-1} ($AgNO_3$). The total time for the leaching tests was 24 days, and the liquid phases were collected at 2-day intervals. Simultaneously, experiments were conducted under identical conditions, during which a solid sample was collected and analysed. The extraction of Cu, Zn, As, and Fe was analysed and evaluated in percentage terms.

2.4. Blank: Abiotic Conditions

Chemical leaching under abiotic conditions was conducted in 2500 mL Erlenmeyer flasks placed on an INFORS HT Multitron II (Infors LTD., Reigate, UK) shaker. The total volume of the solution was 1500 mL, and Erlenmeyer flasks were sterilized at a temperature of 121 °C for 30 min before commencing the experiments. Abiotic tests were performed under the same parameters as experiments with the presence of bacterial cells: particle size 71–100 μm, pH value 1.8, degree of agitation 150 rpm, and pulp density 10% (*w/v*). Due to the absence of microorganisms, the experiments were conducted at the laboratory temperature of 25 °C. The pH value was adjusted at 24-hour intervals by adding 5 M H_2SO_4/0.5 M NaOH. The pH value was determined using the pH meter MW102, Milwaukee Instruments, Germany.

2.5. Analytical Methods

Mineralogical determination, confirming the presence of sulphide components in the sample, was conducted using X-ray diffraction (XRD) on a Bruker AXS D8 Advance instrument (Bruker AXS GmbH, Rheinstetten, Germany). The input-detected particles were conditioned to a size fraction of 10–50 μm, and a representative sample was exposed to CuKα radiation (0.1541 nm). The signals were detected using the LynxEye system. The samples were measured in the Bruker AXS Diffrac (version 2) software. Data were evaluated using the Bruker AXS Eva (qualitative) and Bruker AXS Topas (quantitative) modules. The measurement parameters were 25 °C, initial detector angle 5°, step size 0.04°, and final angular position 2θ 8°.

To compare the transformation of the mineralogical phases and determine the synthesis of new compounds, the determination was made before and after the end of the extraction tests. In cases of lower concentrations of the compound in the sample or similar mineralogical structures, the results can be misinterpreted. For this reason, the conclusions of the XRD determination were verified and supplemented with the results of scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) on a QUANTA 650 FEG device (Thermo Fisher Scientific, Waltham, MA, USA)). The accuracy of the measurement is conditioned by the internal detection software WDX, EDX, and EBSD. An Octane Elect detector (EDAX, Mahwah, NJ, USA) was used for spot ED XRF analysis.

The percentage solubilities of elements were determined based on analyses of liquid phases using a flame atomic absorption spectrometer (F-AAS). Metals Cu, Zn, and Fe were

detected on a VARIAN AA 280FS instrument (Agilent, Santa Clara, CA, USA). Concentrations of As were measured on a UNICAM AAS analyzer (Thermo Elemental, Delph, UK). A different factor in the determination was the gas used, with C_2H_2 (min. purity 99.5%) or N_2O (min. purity 99.5%) mainly used in the detection of arsenic. The mathematical transformation of measured values into concentration expression was processed in SpectrAA software (Varian Australia Pty Ltd., Belrose, NSW, Australia). The leachates were collected using the patented Super Safe Sampler device, which makes part of the bioreactor. Due to the system used, the loss and contamination of the samples were eliminated. Since the pH value (1.8) was controlled during the entire leaching process, the solution was not preserved after the leachate was removed.

Qualitative and quantitative analyses of the elements in the sample before the start of the experiments and during the process were carried out by X-ray fluorescence spectrometry on a BRUKER S8 TIGER analyzer (Bruker Co., Billerica, MA, USA). Measurements were carried out using measuring crystals (XS-55, PET, LIF 200). The detected data were evaluated in two phases in the QUANT E and GEO-QUANT T module for the accuracy of the measurements. The major elements were evaluated by the QUANT EXPRESS module (standard-free) in the form of stable oxides (SiO_2, Al_2O_3, CaO, Fe_2O_3, K_2O, TiO_2, MgO, Na_2O, MnO, P_2O_5, and SO_3) in wt.%. Elemental contents were recalculated. The minor elements were evaluated by the GEO-QUANT T calibration module in elemental form in ppm concentration. To eliminate the influence of non-metals with a low value of the relative atomic weight, annealing loss was carried out in parallel according to the standard ČSN 72 0103 (720103) Basic analysis of silicates – Determination of loss on ignition [39]. The sample (1 g) was repeatedly heated at 1100 °C (1 h) in a mikroTHERM 600 muffle furnace (LAC, s.r.o., Židlochovice, Czech Republic) until the weight stabilised. The loss on ignition (LOI) was determined three times for each sample. The solid phase was isolated from the suspension under reduced pressure by filtration (KNF LIQUIDPORT NF 300 KT.18S, KNF Neuberger GmbH, Schmachtl, Germany). The separated particles were washed with 100 mL of 0.1 M HCl and then with distilled water until the pH value stabilised (5.0 ± 0.2). The moisture content was removed by drying in a Memmert UF 110PLUS (Memmert GmbH + Co. KG, Schwabach, Germany) to a constant weight.

The efficiency of the process was evaluated and compared based on the percent recovery of elements (*RA*). The calculations were determined according to Equations (1)–(4) [40,41]:

$$RA = \frac{M_L}{M_S} \times 100 \tag{1}$$

$$M_L = C_e \times V_e + f \tag{2}$$

$$f = \sum_{e=2}^{n} C_{e-1} \times v_{e-1} \tag{3}$$

$$M_s = C_s \times m_s \tag{4}$$

The M_L value characterises the element content in the sample extract on the day of sample collection (mg); the M_S value characterises the element content in the representative sample charge before the start of experiments (mg); the C_e value characterises the time-dependent metal concentration in the leachate ($mg \cdot L^{-1}$); the V_e value characterises the time-dependent volume of the extract (*L*); f mathematically compensates for sample loss due to withdrawals during the process; the C_S value characterises the concentration in mine tailings before the start of the experiments ($mg \cdot kg^{-1}$); the m_S value characterises the mass of the representative sample charge (kg).

3. Results and Discussion

3.1. Copper

The concentrations of Cu in the leachate under biotic conditions ranged as follows according to the designation of laboratory tests: AF, AT, LF 78−907 $mg \cdot L^{-1}$; AF, AT,

LF/Ag$^+$ 187−1928 mg·L^{-1}; ST 105−1219 mg·L^{-1}; ST/Ag$^+$ 201−2401 mg·L^{-1}. The leaching efficiency was compared based on the percentage solubilities of the metal, which were mathematically determined according to Equations (1)–(4). The dependencies of Cu extraction over the total duration of the experiments (24 days) for different bacterial cultures, including purely chemical tests and error bars, are depicted in Figure 3.
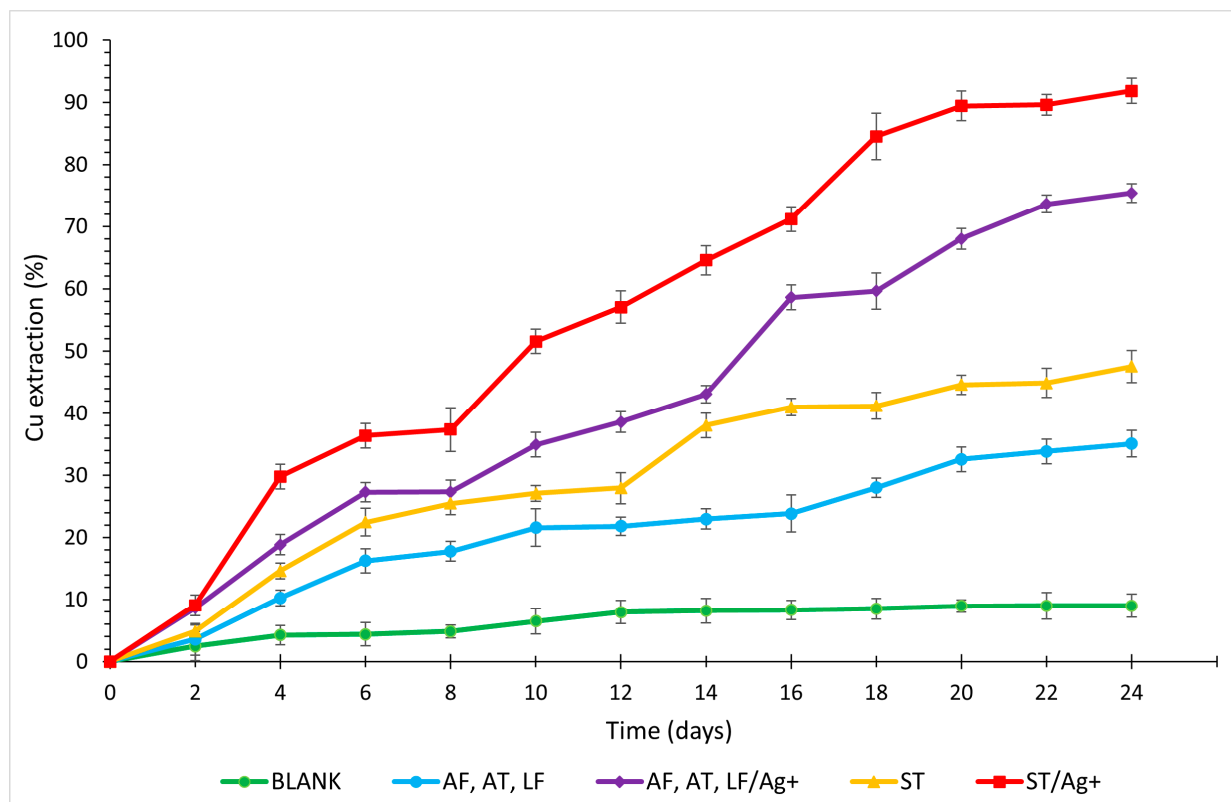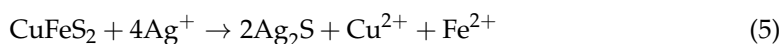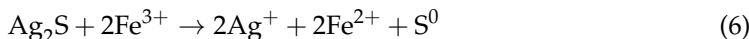

Figure 3. Copper extraction using a mixed mesophilic culture and thermophilic species with/without the presence of Ag$^+$ ions (pH 1.8, particle size 71–100 μm, pulp density 10% (*w/v*), stirring speed 150 rpm).

Leaching curve comparisons revealed enhanced process kinetics at elevated temperature (50 °C) with microorganism ST and significant Ag$^+$ ion effects in extraction systems. With the mixed bacterial culture, copper dissolution reached 35.08%, while thermophilic microorganisms achieved 47.54%. Additive inclusion significantly intensified reaction mechanisms, with a 40.33% increase in extraction seen with mesophilic species. Optimal results were obtained in ST/Ag$^+$ lab tests, yielding a final extraction of 91.93%. Leaching intensification by Ag$^+$ ions was evident at 44.39%, the highest among analysed elements. Microorganism influence was confirmed compared to abiotic conditions, where a 9.00% solubilisation was observed (Cu concentration in leachate: 235 mg·L^{-1}).
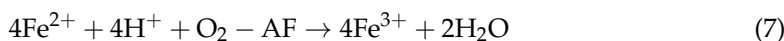
The research findings suggest a significant influence of Ag$^+$ ions on the kinetics of leaching system reactions. This influence is attributed to the chemical interactions at the interface of particles and the microorganism-containing medium. Miller et al. [42] detailed chemical reactions occurring between particle surfaces and solution under strong acidic conditions, highlighting the additive action principle facilitated by Ag$^+$ ion penetration into the outermost layer of CuFeS$_2$. The synthesis of Ag$_2$S, coupled with metal release into the solution, was identified as the primary mechanism enhancing extraction, with simultaneous chemical processes represented by Equations (5) and (6).

$$CuFeS_2 + 4Ag^+ \rightarrow 2Ag_2S + Cu^{2+} + Fe^{2+} \tag{5}$$

$$Ag_2S + 2Fe^{3+} \rightarrow 2Ag^+ + 2Fe^{2+} + S^0 \tag{6}$$

During leaching processes, surface examination of particles revealed adequate porosity, yet Equation (6) suggests potential hindrance by S^0 formation, limiting solution penetration. Parker et al. [43] similarly reported on S^{-2} ion and additive cation synthesis. Detection of silver sulphide formation on particle surfaces was confirmed via X-ray photoelectron spectroscopy. The secondary compound layer on solid fractions in abiotic extraction systems measured in nanometers. Sample analysis from the Staré Ransko area revealed 3.39% $CuFeS_2$ and 5.70% FeS_2 content, potentially influencing leaching processes positively. Nazari et al. [44] investigated sulphide action, releasing iron during leaching, confirming the catalytic properties of Ag^+ additive. Iron ions from FeS_2 facilitated process chemistry by direct reduction at the interface of phases, enhancing electron transfer. In repeated experiments without pyrite and additive, S^0 synthesis on particles negatively affected electron transfer, contrasting the additive cations' gradual synthesis with sulphide anions, ensuring sufficient electrical conductivity crucial for leaching process progression. Ghahremaninezhad et al. [45] examined physical processes in the emerging extraction layer under abiotic conditions, emphasising diffusion as the primary mechanism driving Ag+ ion penetration, particularly within a distance of 18 nm.

Bacterial species AF played an active role, particularly at the interface between solid phase and medium, demonstrating a high degree of additive cation penetration to particle surfaces. The primary effect of bacterial cells involved divalent iron oxidation (Equation (7)), leading to direct participation in sulphide synthesis and particle extraction (Equations (6) and (8)). [46]

$$4Fe^{2+} + 4H^+ + O_2 - AF \rightarrow 4Fe^{3+} + 2H_2O \tag{7}$$

$$(Zn, Cu, Fe, Pb)S + Fe_2(SO_4)_3 \rightarrow (Zn, Cu, Fe, Pb)SO_4 + 2FeSO_4 + S^0 \tag{8}$$

Although leachate analyses represent the primary detection method in the study for evaluating solubilisation, XRF outputs are crucial for a comprehensive comparison of concentration transformations between the two phases. XRF analyses during the interaction of the solution with different bacterial cultures and particles over the entire period of 24 days are illustrated in Figure 4. The results also include laboratory tests under abiotic conditions.
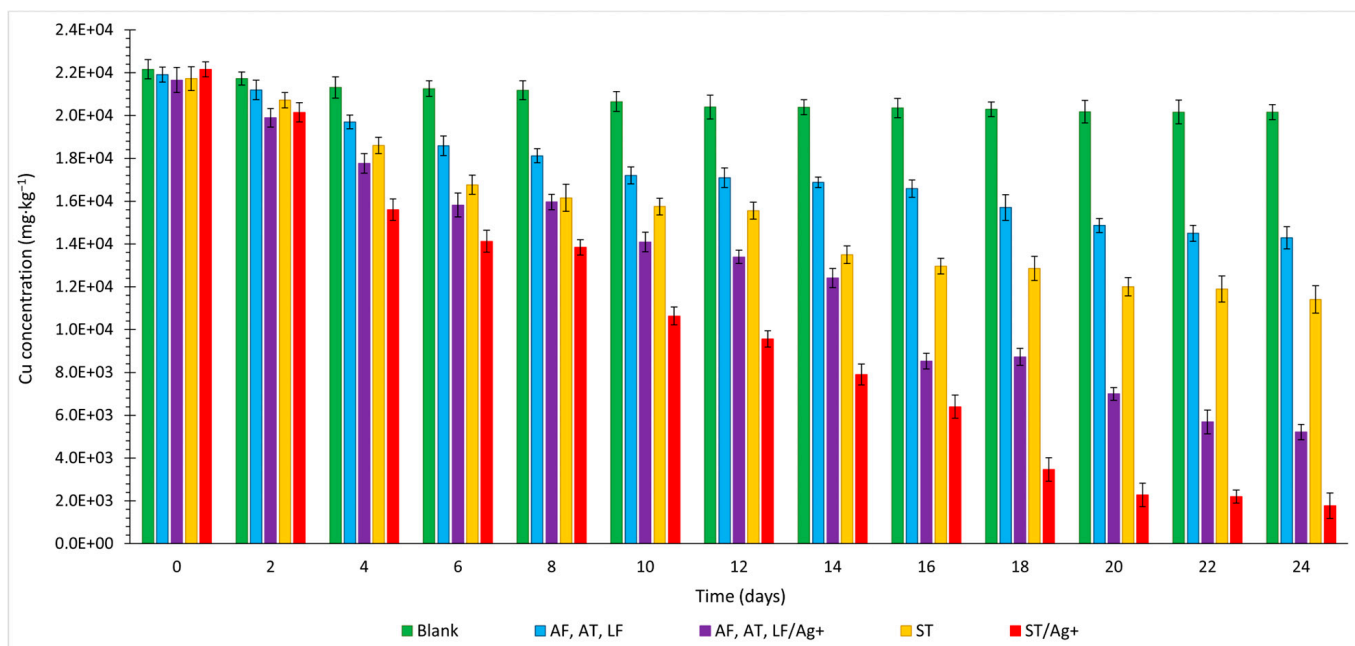

Figure 4. Amount of copper in solid samples during the extraction process using different bacterial cultures with/without additive.

3.2. Zinc

The solubilisation percentages of zinc using a mesophilic mixed bacterial culture and a thermophilic species under identical reaction environment parameters (temperature differential factor) are depicted in Figure 5. For a comprehensive comparison of leaching, test results without the presence of bacterial cells are also provided.
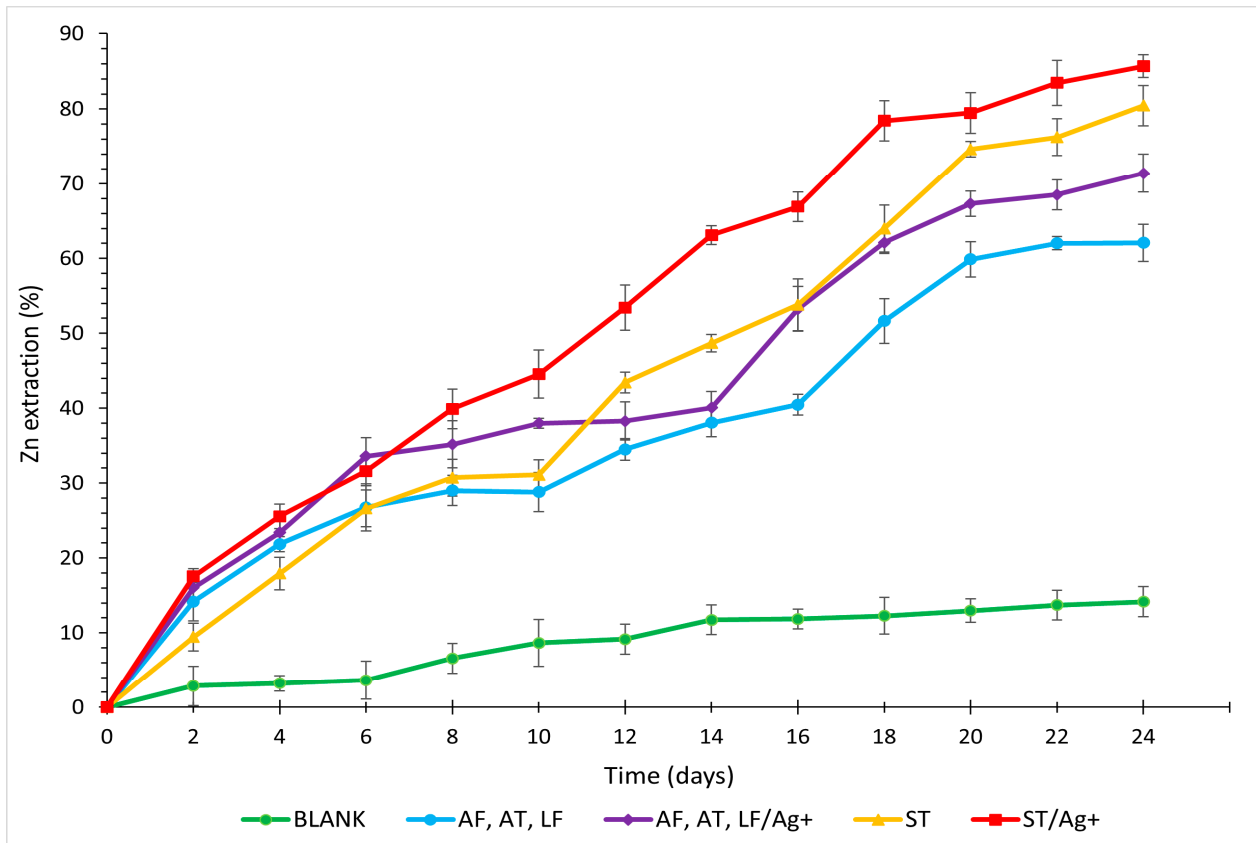

Figure 5. Zinc extraction using a mixed mesophilic culture and thermophilic species with/without the presence of Ag+ ions (pH 1.8, particle size 71–100 μm, pulp density 10% (*w/v*), stirring speed 150 rpm).

Extraction curves derived from leachate analyses using AAS revealed concentration intervals during the extraction process: AF, AT, LF 315–1625 mg·L^{-1}; AF, AT, LF/Ag$^+$ 356–1870 mg·L^{-1}; ST 209–2102 mg·L^{-1}; ST/Ag$^+$ 391–2254 mg·L^{-1}. The environmental temperature of 50 °C (with the bacterium ST) significantly affected reaction kinetics, resulting in 80.44% zinc leaching. Additive incorporation increased process efficiency by 5.23%. Optimal results, as in Cu extraction, were observed in ST/Ag$^+$ tests, with a final solubilisation of 85.67%. In microorganism-free suspensions, Zn concentrations during leaching ranged from 63 to 369 mg·L^{-1}, with final solubilisation at 14.17%. Mesophilic cultures yielded a final extraction of 62.08%. The addition of Ag$^+$ ions (5 mg·L^{-1}) intensified process efficiency by 9.35%.

Regarding the characteristics of phases in the suspension, there is a gradual accumulation of metal in the leachate, proportional to the amount of element loss from solid particles. Concentration transformations are in mutual coherence. The chemistry of the extraction system is predominantly influenced by the action of bacterial cells, the temperature of the system, and factors that support the formation of precipitates. These factors include the overall concentration of Fe^{3+} ions and the pH value [47]. The zinc content in the sampled particles, utilising a mixed bacterial culture and thermophilic microorganisms, including tests under abiotic conditions, is depicted in Figure 6.
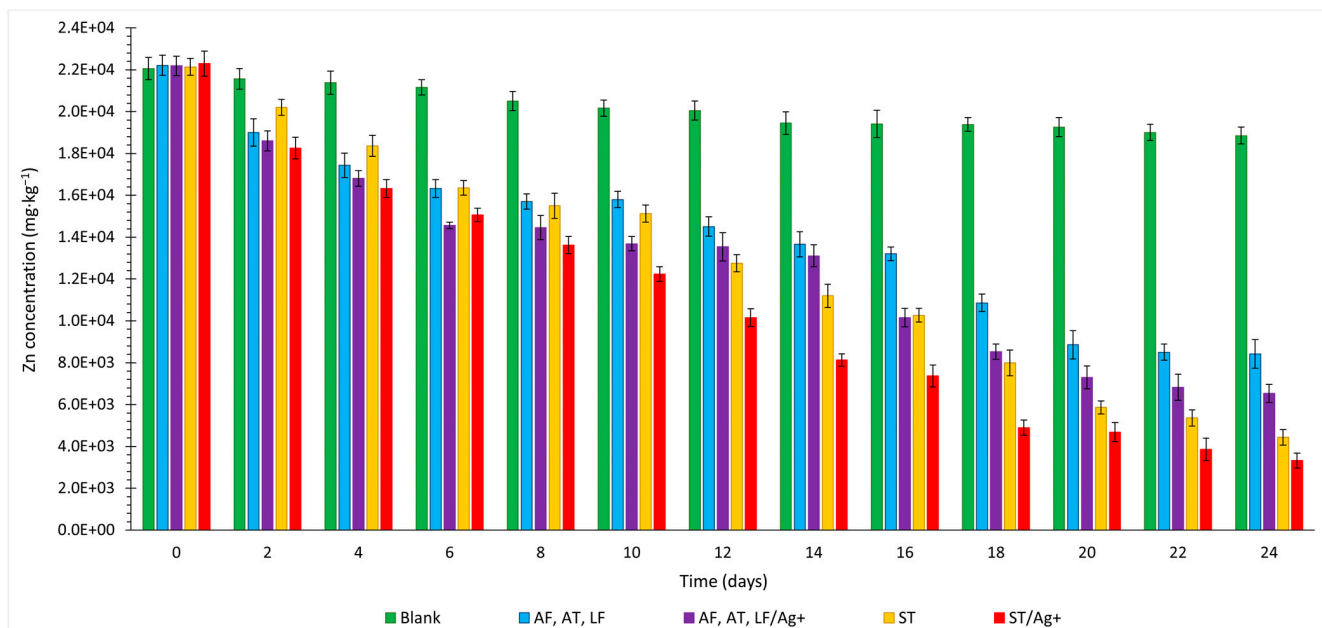
Figure 6. Amount of zinc in solid samples during the extraction process using different bacterial cultures with/without additive.

3.3. Arsenic

During the leaching process, arsenic concentrations in the extraction systems exhibited variations as per laboratory tests, falling within the intervals of AF, AT, LF 9–578 $mg \cdot L^{-1}$; AF, AT, LF/Ag$^+$ 57–964 $mg \cdot L^{-1}$; ST 33–721 $mg \cdot L^{-1}$, and ST/Ag$^+$ 101–1155 $mg \cdot L^{-1}$. The percent recoveries of the semi-metal were determined through AAS analyses, as depicted in Figure 7. Additionally, experiments under abiotic conditions were conducted as part of the research to assess the impact of microorganisms on the solubilisation of elements.

During extraction curve assessment, the study found robustness in bacterial microorganisms, including *Acidithiobacillus*, *Leptospirillum*, and *Sulfobacillus*, against arsenic ion toxicity in liquid phases. This resilience correlated with enhanced reaction efficiency in biotic suspensions. In contrast, under abiotic conditions, arsenic concentration in leachate ranged from 8 to 70 $mg \cdot L^{-1}$ over 24 days, with final solubilisation at 4.28%. Tests involving bacterium ST and additive cooperation exhibited the highest effectiveness, leaching arsenic up to 69.16%. Experiments lacking Ag$^+$ ions showed a 26.08% solubilisation decrease with similar microorganisms, while mesophilic species achieved a 34.88% recovery. Additive inclusion led to a 23.79% solubilisation increase. The assessment of the percentage increase in toxic element recovery during particle-solution interaction highlighted primary kinetics enhancement with AgNO$_3$ addition. ST bacterial cells' resistance to high arsenic ion concentrations in the liquid phase has been noted in multiple studies [48–50]. Experimentally, under biotic conditions, the research identified a non-inhibitory reaction environment, supported by pre-experiment adaptation procedures.

The study aims to enhance metal extraction efficiency from poorly soluble sulphide components and remove toxic arsenic from mine tailings as part of hydrometallurgical processing before gold extraction. Arsenic levels in the sample were measured at 13,900 $mg \cdot kg^{-1}$ and gold at 157 $mg \cdot kg^{-1}$, indicating significant potential for extraction from ore deposits associated with arsenopyrite and pyrite [51,52].
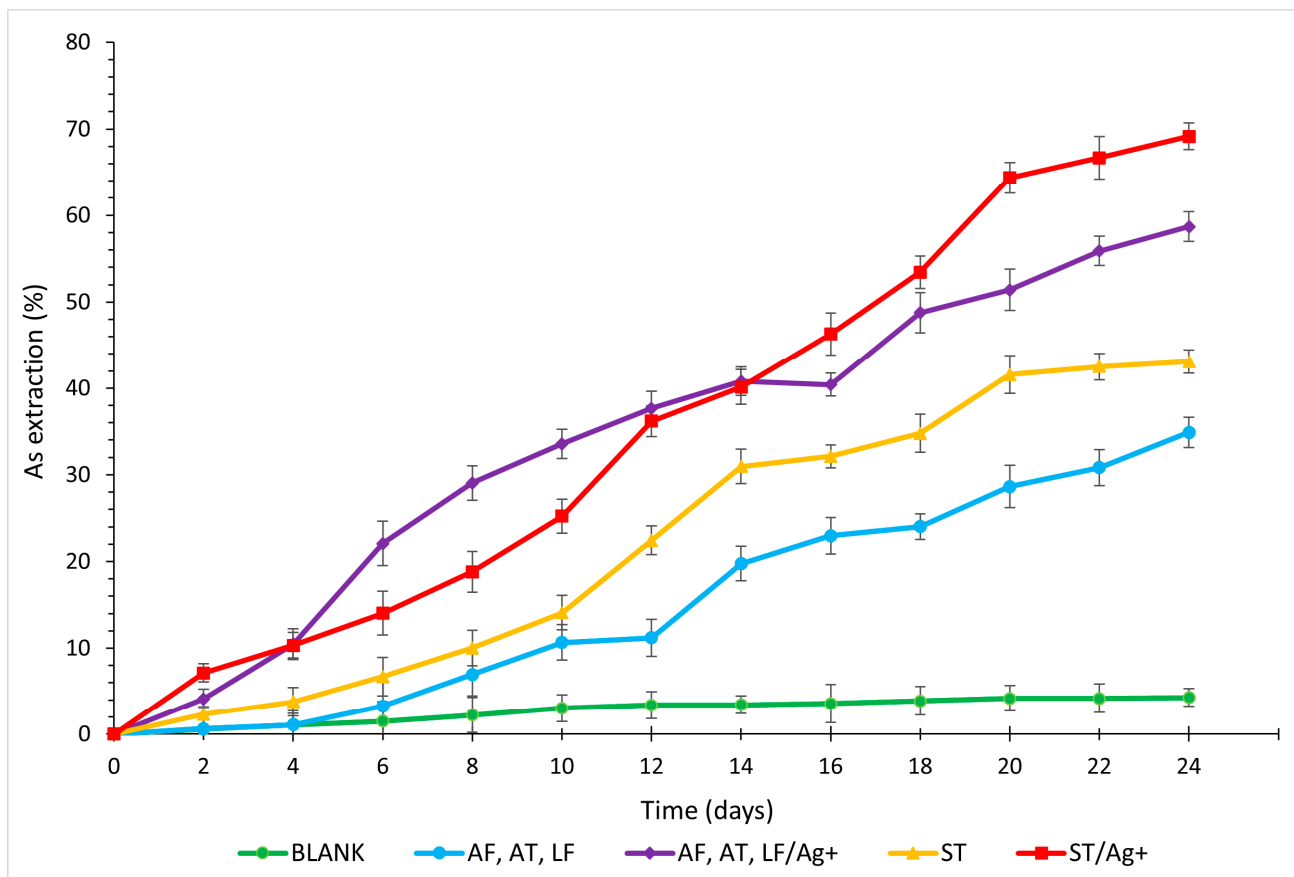
Figure 7. Arsenic extraction using a mixed mesophilic culture and thermophilic species with/without the presence of Ag^+ ions (pH 1.8, particle size 71–100 μm, pulp density 10% (*w/v*), stirring speed 150 rpm).

In investigating methods to improve the extraction efficiency of the toxic semi-metal, ferric iron addition was the focus, showing an 11% increase in As recovery in Silva et al.'s study [27]. Unlike our experiments with Ag^+ ions, the cooperative action of mesophilic species and ST bacteria resulted in a higher As recovery increase, at 23.79% and 26.08%, respectively, highlighting the importance of additive selection to avoid adverse effects on extraction processes. Deng et al. [53] applied bacterium ST and Fe^{3+} additive, resulting in reduced formation of S-incorporated compounds, notably $S_2O_3^{2-}$ and As_2S_3, while microscopic detection revealed increased synthesis of $Fe_8O_8(OH)_{8-2x}(SO_4)_x$, $MFe_3(SO_4)_2(OH)_6$, and $FeAsO_4$. However, higher Fe^{3+} concentrations were shown to deteriorate extraction layer permeability, with $FeAsO_4$ formation detected, although its synthesis preconditions were not precisely determined due to system complexity. Additionally, a direct link was established between FeS_2 and $CuFeS_2$ presence in particles and $FeAsO_4$ formation.

External influences from the surrounding environment in the cooperative action of microorganisms on mine tailings (e.g., piles) result in their structural transformation and the release of As. The toxicity of the semimetal is directly dependent on the strength of the ions, and any alteration can have a significant impact [54].

The results of XRF detections of solid particles during the application of different microorganisms with/without additive are depicted in Figure 8.
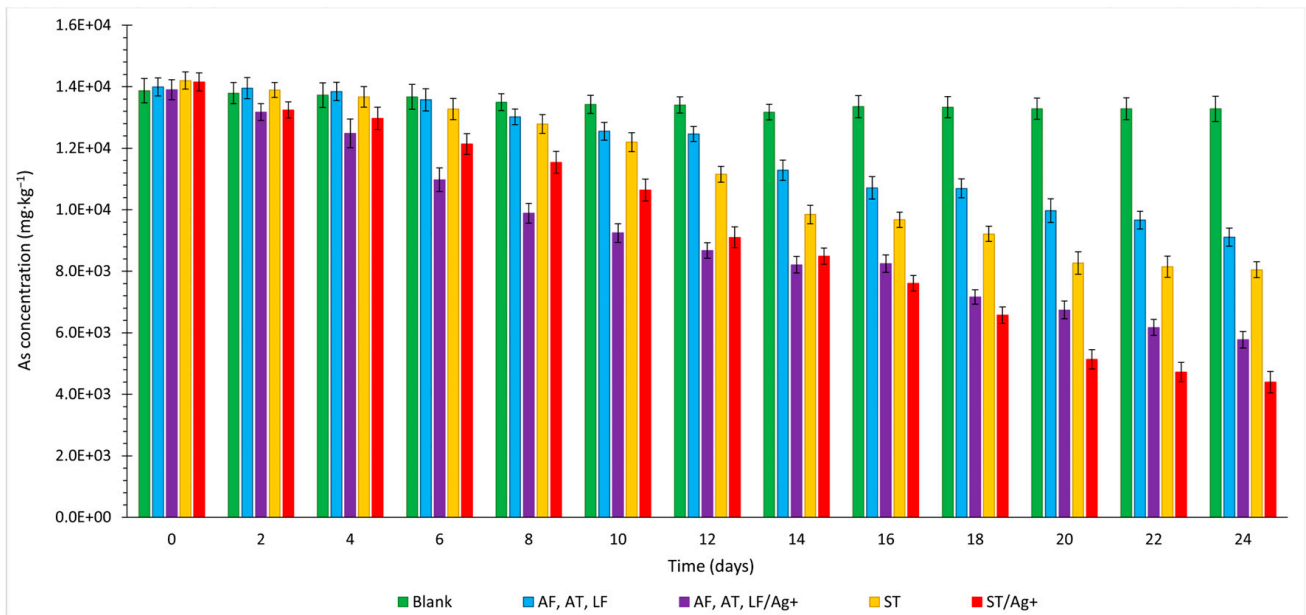
Figure 8. Amount of arsenic in solid samples during the extraction process using different bacterial cultures with/without additive.

3.4. Iron

The percentage recovery of Fe during bioleaching by a mixed bacterial culture and a thermophilic species is depicted in Figure 9. The results also include experiments involving the influence of Ag$^+$ ions and those conducted without the presence of microorganisms.
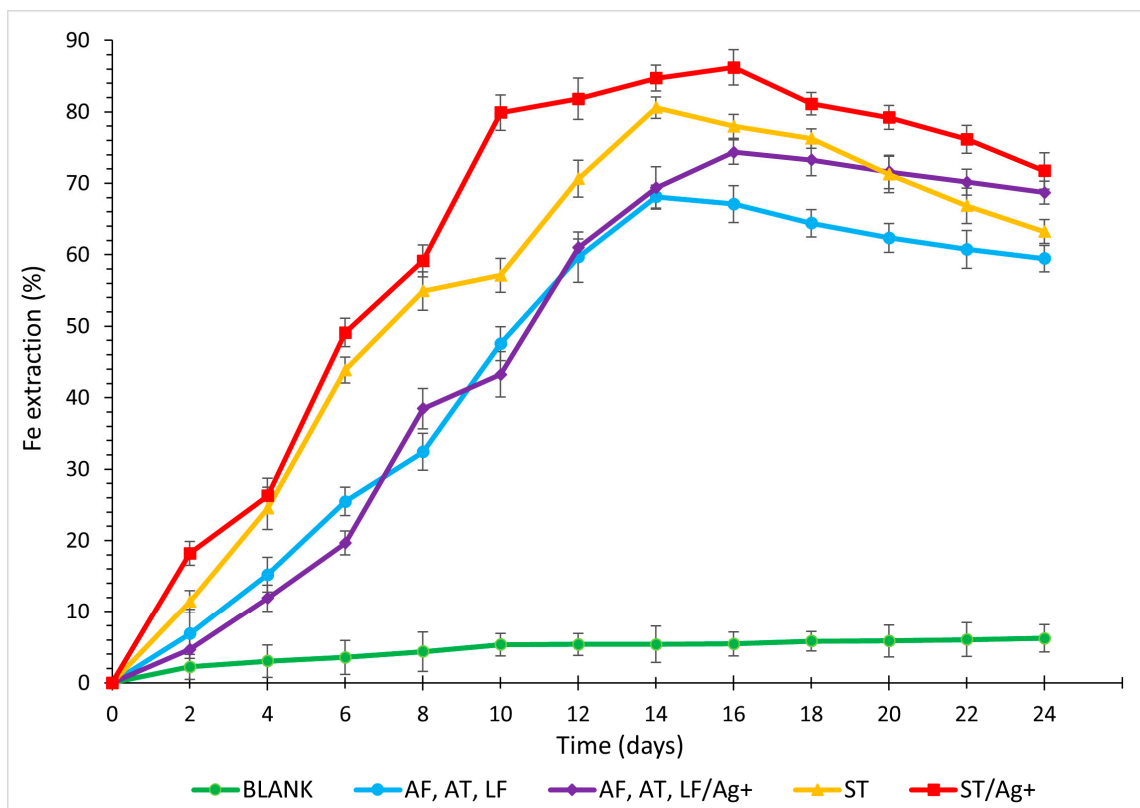

Figure 9. Iron extraction using a mixed mesophilic culture and thermophilic species with/without the presence of Ag$^+$ ions (pH 1.8, particle size 71–100 μm, pulp density 10% (*w/v*), stirring speed 150 rpm).

During extraction, a continuous increase in solubilisation occurred, peaking between days 14 and 16, followed by stabilisation and a decreasing trend in metal recovery, accompanied by a decline in Fe ion concentrations in the liquid phase. Total Fe concentrations in leachates varied across laboratory tests, with ranges as follows: AF, AT, LF 1452–15,542 mg·L^{-1}; AF, AT, LF/Ag$^+$ 995–17,254 mg·L^{-1}; ST 2415–18,442 mg·L^{-1}; ST/Ag$^+$ 3847–19,911 mg·L^{-1}. The highest solubilisation was observed with ST microorganisms and Ag$^+$ ions, indicating enhanced kinetics. In the initial phase, solubilisation peaked at 86.21% on day 16, followed by a gradual decrease to a final extraction of 71.72% (17,790 mg·L^{-1}). Without Ag$^+$ ions, recovery reached 80.57%, with 63.23% (15,724 mg·L^{-1}) leached after 24 days, a difference of 17.34%. Despite constant pH (1.8), XRD confirmed the synthesis of KFe$_3$(SO$_4$)$_2$(OH)$_6$ post-experiment. A jarosite-type compound formation was evident in all biotic tests, influencing extraction curve trends due to embedded trivalent iron.

In lab tests AF, AT, LF/Ag$^+$, and Fe recovery peaked at 74.36%. By day 16, extraction gradually decreased by 5.68%, with 68.68% (17,095 mg·L^{-1}) of metal leached. Without cation additive, the upper limit of extraction was 68.11%. Synthesis of a secondary compound reduced solubilisation by 8.66%, resulting in a Fe recovery of 59.45% (14,771 mg·L^{-1}) after 24 days. Comparing leaching curves, systems with AF, AT, and LF bacteria showed lower reductions in metal solubilisation in the second stage, consistent with decreased Fe ions and lower secondary substance synthesis. All tests had identical process factors, with differing bacterial cells being the variable. All cultures that participated in the structural transformation of mine tailings use a similar material transformation to obtain energy. However, the microorganisms LF reduce trivalent iron to its divalent form, thus maintaining optimal Fe^{3+} concentrations in suspension [55]. Other cultures can catalyse the opposite oxidation reaction (Equation (7)). As a result of the optimal ratio transformation of the Fe^{3+}/Fe^{2+} ions, lower production of the compound MFe$_3$(SO$_4$)$_2$(OH)$_6$ was achieved in suspensions in the presence of the bacterium LF. Comparing the results of experiments under biotic and abiotic conditions demonstrated the effect of microorganisms. Under abiotic conditions, total Fe concentrations were determined during the process in the range of 472–1554 mg·L^{-1}. Only 6.23% of iron was leached.

From the perspective of evaluating the reactions of iron in suspensions, the focus was on the mechanisms of the transition of the metal to the liquid phase from the sulphide components and the simultaneous formation of the secondary compound MFe$_3$(SO$_4$)$_2$(OH)$_6$. The AAS and XRF analyses concentration detections correlate with one another, as evident from the results of the analyses of the solid fractions, depicted in Figure 10.
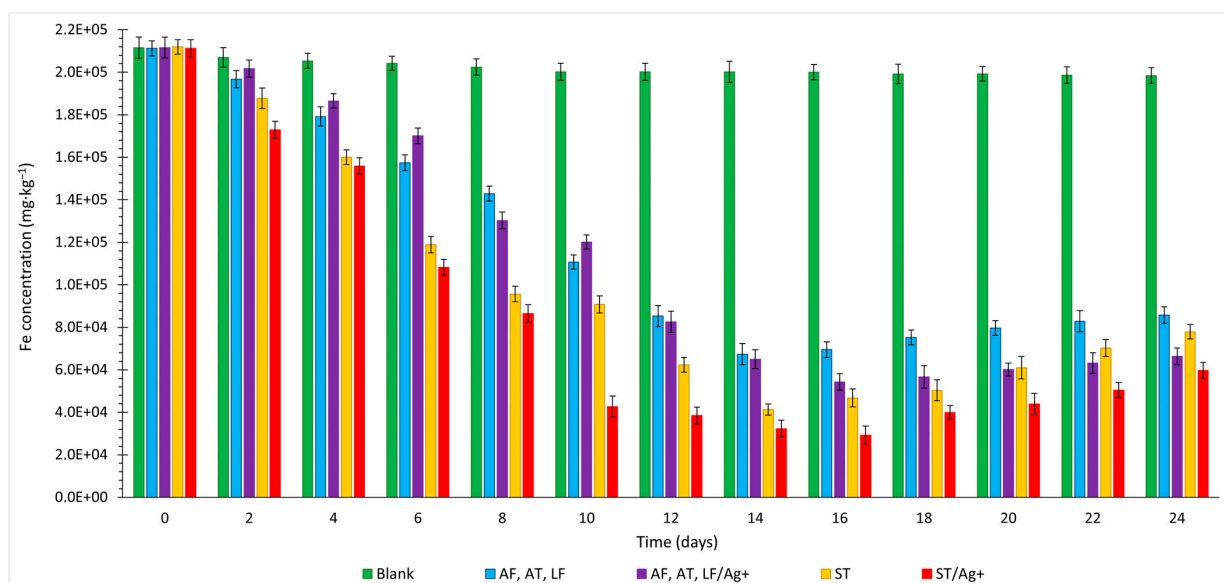

Figure 10. Amount of iron in solid samples during the extraction process using different bacterial cultures with/without additive.

3.5. XRD Detection after Bioleaching

To determine the mineralogical changes in solid particles during the bioleaching process, XRD analyses were conducted after 24 days. The objective of these measurements was to compare the quantitative and qualitative composition of compounds in the mine tailings with the mineralogical characteristics before the commencement of the experiments, as presented in Section 2.1, Sample Characteristics. This determination was carried out for all laboratory tests under biotic conditions. Through a mutual comparison of the measurement results, we searched for a qualitatively analogous factor, but no significant differences were demonstrated. From a detection principle perspective, a high degree of accuracy is achieved for the majority of components in the sample. However, with minor concentrations of components, there is a limitation in the accuracy of the methodology. Considering this, the representation of characteristic peaks for the best-rated extraction laboratory tests using the thermophilic culture of ST and the addition of Ag^+ ions (at a concentration of $5 \text{ mg} \cdot \text{L}^{-1}$) is illustrated in Figure 11.
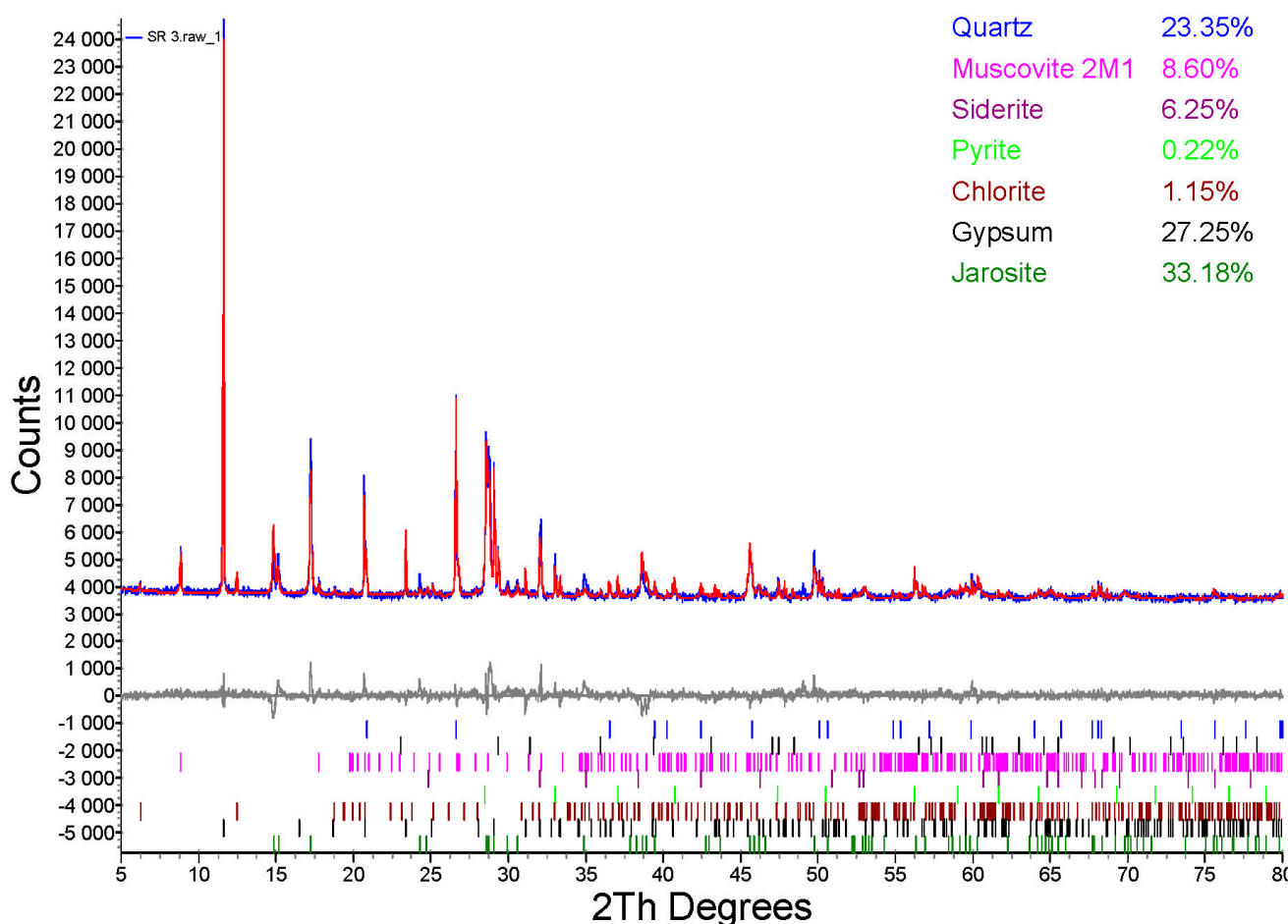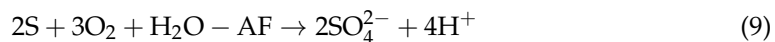

Quartz	23.35%
Muscovite 2M1	8.60%
Siderite	6.25%
Pyrite	0.22%
Chlorite	1.15%
Gypsum	27.25%
Jarosite	33.18%

Figure 11. XRD detection of mine tailings after bacterial leaching (laboratory test ST/Ag^+).

Of the sulphide components, 5.70% FeS_2, 3.39% $CuFeS_2$, 1.67% ZnS, and 0.88% PbS were detected in the mine tailings before the initiation of leaching experiments with bacterial cultures. Following the conclusion of experiments under biotic conditions, only pyrite was identified, with a content of 0.29% in the case of ST/Ag^+ laboratory tests. These results align with continuous extraction mechanisms at the interface of the phase of particles and a solution containing microorganisms. Additionally, the sample contained 23.35% SiO_2, 8.60% $KAl_2(AlSi_3O_{10})(OH)_2$, 6.25% $FeCO_3$, and 1.15% $(Mg,Fe)_3(SiAl)_4O_{10}(OH)_2 \cdot (Mg,Fe)_3(OH)_6$. The diagram record confirmed the formation of secondary minerals: 27.25% $CaSO_4 \cdot 2H_2O$ and 33.18% $KFe_3(SO_4)_2(OH)_6$. In a representative sample, XRF analysis before the start

of the experiments determined the majority of Ca at a concentration of 61,746 mg·kg^{-1}. The leaching system exhibited a complex reaction environment. In connection with the extraction processes (conversion of sulphides to sulphates) and the process described by Equation (9) [16], high concentrations of sulphate anions were confirmed in the leachate, synthesised with Ca^{2+} cations to form the secondary compound CaSO$_4$·2H$_2$O.

$$2S + 3O_2 + H_2O - AF \rightarrow 2SO_4^{2-} + 4H^+ \tag{9}$$

Although the recommended pH value in the suspension was 1.8, jarosite-type compounds were formed. Despite this negative phenomenon, a highly efficient process was achieved.

4. Conclusions

The research focused on the recovery of selected elements (Cu, Zn, As, and Fe) from mine tailings in the Staré Ransko region (Czech Republic) using a mesophilic mixed bacterial culture of AF, AT, LF, and the thermophilic species ST. The experiments were conducted with identical process parameters, including a particle size of 71–100 μm, pH 1.8, agitation at 150 rpm, and suspension pulp density of 10% (*w/v*). The distinguishing factor was the temperature of the system (30 °C/50 °C). Laboratory tests were modified by adding an AgNO$_3$ additive (Ag$^+$ ions concentration of 5 mg·L^{-1}). The study aimed to optimise the extraction system to achieve the highest metal extraction with the possibility of subsequent isolation from the liquid phase (electrolysis, cementation, and precipitation) for industrial applications [56]. Regarding arsenic, a reduction in material toxicity was observed, representing the initial stage of hydrometallurgical treatment before gold extraction. The following conclusions were drawn from the results of the study:

- Laboratory tests labelled ST/Ag$^+$ were deemed the most optimal for the final recovery of metals and As for all analysed elements (Cu 91.93%, Zn 85.67%, As 69.16%, and Fe 71.72%, with the maximum solubilisation of Fe reaching 86.21% on day 16).
- The addition of AgNO$_3$ additive enhanced the process kinetics for all detected elements, with a fundamental increase in the solubilisation of Cu (AF, AT, LF/Ag$^+$ by 40.33%; ST/Ag$^+$ by 44.39%) and As (AF, AT, LF/Ag$^+$ by 23.79%; ST/Ag$^+$ by 26.08%).
- Regulating the temperature at 30 °C with the application of ST had a positive effect on the extraction mechanisms, particularly for Zn (ST by 18.36%; ST/Ag$^+$ by 14.24%) compared to mixed mesophilic cultures.
- Two secondary minerals, CaSO$_4$·2H$_2$O and KFe$_3$(SO$_4$)$_2$(OH)$_6$, were detected in the solid fraction after 24 days of leaching.
- Due to the synthesis of the secondary compound, incorporating Fe^{3+} ions into its structure, the Fe extraction curves initially exhibited an increasing trend (with a peak in the range of 14–18 days), followed by a decline in metal recovery. When mixed mesophilic bacteria were employed, particularly with the representation of the species LF, a lesser reduction in total solubilisation was observed. The optimal ratio transformation of Fe^{3+}/Fe^{2+} ions resulted in the synthesis of a smaller amount of jarosite-type compound in the reaction environment in the presence of cells of the mentioned species.
- Despite the detection of secondary minerals, the process was deemed highly effective for the recovery of Cu, Zn, and As.
- Comparative analysis of tests under biotic and abiotic conditions demonstrated the influence of bacterial cells and a significant enhancement in the kinetics of extraction processes facilitated by microorganisms.
- Based on the percentage recovery of metals, hydrometallurgical technology involving the cooperative action of microorganisms was assessed as optimal for mine tailings with a lower metal content. From both an environmental and economic perspective, the regeneration of acid leachates and their reuse as new input raw materials are viable.

The conclusions drawn from the study may have a significant impact on future research concerning metal extraction and the subsequent practical implementation of the process.

Abbreviations

AF	*Acidithiobacillus ferrooxidans*
AT	*Acidithiobacillus thiooxidans*
EDX	Energy dispersive X-ray spectroscopy
F-AAS	Atomic absorption spektrometry
LF	*Leptospirillum ferrooxidans*
LOI	Loss on ignition
SEM	Scanning electron microscope
ST	*Sulfobacillus thermosulfidooxidans*
w/v	weight by volume
XRD	X-ray diffraction
XRF	X-ray fluorescence

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