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Fungal Leaching of Iron (Fe), Zinc (Zn), and Copper (Cu) in Copper Ore Waste from Ikalamavony, Madagascar, by *Aspergillus* sp

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Abstract – The recovery of waste has become a global concern due to the economy's shift towards a circular economy, which includes the recycling of mining waste. This study aims to highlight the ability of the fungus Aspergillus to dissolve metals as iron, zinc, and copper from Ikalamavony ore waste through the bioleaching process for their recovery. The waste was subjected to granulometric analysis to classify the grains by weight and size, and the metal content was analyzed using Flame Atomic Absorption Spectrophotometry (FAAS). Bioleaching was conducted in two stages: first, the fungi were cultured on Sabouraud agar or broth medium at 28°C for 7 days, then the produced organic acids were recovered and the waste was placed in these acids. The optimization of certain parameters as particle size, sucrose content, medium volume, strain incubation time and bioleaching time was considered. The FAAS analysis results indicated that the waste contained an average of 5.35% Fe, 0.05% Zn, and 18.14% Cu. The optimum

parameters obtained for each metal were as follows: for particle size, Fe and Zn: 125 µm; Cu: 200 µm; for sucrose content, Fe: 7.5 g and Zn, Cu: 12.5 g; for the volume of culture medium, Fe, Zn, Cu: 150 ml. The incubation time for the strains was 7 days. With these optimizations, the extracted metals were 73.89% Cu, 56.77% Zn, and 7.89% Fe for a leaching time of 15 days. These results confirm that bioleaching using Aspergillus sp. is an effective technique for the extraction and recovery of copper and zinc from ore waste.

Key words – Aspergillus, fungal leaching, metals, ore waste, Madagascar.

I. INTRODUCTION

Meeting the ever-increasing demand for metals is becoming difficult due to the decline in ore quality [1]. Over the past decade, end-of-life metal-bearing materials, also known as metal-bearing wastes, have been widely accepted as a secondary resource of critical raw materials due to the metal content of metal-bearing wastes comparable to that of natural ores [2]. Yet these metal-bearing wastes, such as e-waste, residues, slag and dust, are typically stockpiled or landfilled due to the lack of effective and sustainable recovery pathways for the metals they contain [3]. Hydrometallurgical and pyrometallurgical methods for metal extraction are not applicable due to their cost, environmental risks, and lower efficiency [4]. The researchers therefore used the ability of certain micro-organisms to synthesize organic acids and enzymes to dissolve the metals in this mining waste, a method known as biological leaching or bioleaching. Biological methods may be a suitable alternative because they are low cost, environmentally friendly, and effective at low metal contents [5].

Bioleaching may be appreciable especially when metal extraction by conventional methods is not economical due to low metal content, such as low-grade ores, solid wastes, mine tailings, slurry, fly ash, electronic waste, geothermal liquid, wastewater, and process water [6]. In general, bioleaching is a process described as "the dissolution of metals from their mineral sources by certain naturally occur microorganisms" or the use of microorganisms to transform elements so that the elements can be extracted [7]. In this study, the ability of a fungus to synthesize different organic acids for dissolving iron, zinc and copper (fungal leaching) in copper ore waste from Madagascar, was specifically investigated This environmentally-friendly technique has the advantage of being applicable to all types of ore (acidic, basic or neutral), whether or not they contain sulphide, iron or reduced sulphide compounds. It uses fungal species that can produce different types of metabolites from a wide variety of substrates [8]. In this study, the genus *Aspergillus* was used. This genus is one of a numerous filamentous fungi displaying an important role in environmental biotechnology due to their ability to metabolize organic compounds. They produce various organic acids using organic carbons such as glucose and sucrose as an energy source [9]. Of the organic acids produced, gluconic, citric and oxalic acids have been shown to be the most potent acids for bioleaching [10],[11]. In principle, fungi convert glucose or sucrose into organic acids by several enzymatic reactions in the cytosol and mitochondria, which are membrane-bound cellular compartments. Passing through the cytoplasmic membrane into the cytosol, glucose is converted to pyruvate via the glycolysis pathway. One of the two pyruvate molecules produced is decarboxylated to acetyl-CoA by the mitochondria via malate [12].

II. MATERIALS :

The minerals used in this study are ore waste of copper from the Ikalamavony region of Madagascar according to the coordinates 21° 10′ 05″ south, 46° 36′ 00″ at an altitude of 870 m. The ore waste was collected from the field, transported, and stored in the laboratory in plastic bags.

Leaching agent:

The strain used in this study belong to the fungal genus, *Aspergillus* sp. obtained from the Laboratory of Environmental Microbiology at the National Research Center on the Environment (CNRE). The fungal was cultured on Sabouraud agar medium containing 1% peptone, 2% glucose, and 1.5% agar. Following inoculation, the petri plates were incubated at 30°C for 7 days.

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III. Methodology

1. Mineral characterization

The waste was crushed**,** sieved and then subjected to grain size analysis to distribute the grains and classify them according to their weight and size. The resulting particles were thereafter subjected to X-ray fluorescence (XRF) analysis to determine the mineral phases of the waste and to determine the main elemental composition of the samples. Prior to analyzing Fe, Zn, Cu by FAAS, each particles size class and the bioleaching residues were digested according to Rodier's method.

2. Fungal Leaching Experiment

2.1 Preparation of medium for fungal leaching

The culture medium used in this study was the Czapek's broth containing sucrose as carbon source, and sodium nitrate as nitrogen source. The medium was buffered with dipotassium phosphate. Essential growth ions such as magnesium sulfate, potassium chloride, and ferrous sulfate were also included. Czapek's medium is commonly used for the routine cultivation of fungi, particularly *Aspergillus*, *Penicillium*, and non-sporulating molds [13]

The composition of the medium Czapek per liter of distilled water is as follows: sucrose 100 g, $NaNO_3$ 1.5 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.025 g, KCl 0.025 g, $FeSO_4$ 0.005 g, $CuSO_4$ 0.025g, and yeast extract 1.6 g [14], it was autoclaved at 121°C for 15 minutes.

2.2 Cultural and morphological characteristics of the fungal strain

The identification of the used fungal specie was based on the examination of its cultural and morphological characteristics. These included macroscopic growth rate, size, fructification structure and color of the colony on sabouraud medium) and microscopic observations (spores, phialides, conidiophores, etc.) [15]

The microscopic examination of the fungal colony was performed by spreading between a slide and a coverslip the fungus culture from sabouraud agar and staining the preparation with methylene blue, allowing the observation of thalli, spores, and conidiophores using a binocular light microscope.

2.3 Inoculum preparation

The inoculum was prepared using a liquid medium based on sucrose cited above. A 250 ml Erlenmeyer flask was used to prepare 160 ml of medium, with the pH adjusted to 7. One agar cylinder of spore fungal (10^6 spores) was transferred from Petri plates to the culture medium using transfer tubes. The mixture was then incubated under shaking at 110 rpm for 7 days.

2.4 Fungal leaching with parameters

After incubation, the inoculum was centrifuged at 2000 rpm for 10 minutes to separate the leachate and pellet. The leachate was then recovered and autoclaved at 121°C for 15 minutes. One gram of sterilized ore was added to the autoclaved leachate, and the mixture was incubated for 5 days in a dark room.

The feasibility of bioleaching to leach metals or contaminants requires a comprehensive understanding of the influencing factors [16]. In this study, several parameters were studied to ensure and optimize the leaching process, including particle size, sucrose content, medium volume, inoculum incubation time, and bioleaching duration:

- Particle size: Four size classes were used: 50, 80, 125, and 200 microns.
- Sucrose content: tested weights were 7.5, 10, 12.5, and 15 grams.
- Medium volume: assayed volumes were 50, 100, 150, and 200 milliliters.

Leachates obtained after leaching was analyzed by AAS, yield (*R*) in fungal leaching was calculated by the following formula:

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$$R=\frac{Ci}{Cf}\times 100$$

where:

Ci is the initial concentration of metal in the ore,

Cf is the final concentration of metal in the leachate.

IV. Results and Discussion

3. Particle size analysis

Granulometry is among the factors influencing the efficiency of bioleaching. A finer granulometry increases the contact surface between minerals and microorganisms, which stimulate the acceleration of bioleaching reaction. Small particles provide a larger area for microorganisms, which can lead to more effective release of metals [17]. The table 1 shows the distribution of grains according to their particle size.

Table 1*: Grain distribution*

Fraction (µm)	% cumulative passing
+200	14.73
200	15.56
125	13.83
80	21.22
45	34.03

The presence of different particle sizes in the sample may require a suitable bioleaching strategy. An optimisation of leaching conditions can be necessary to ensure an effective reaction with all present particle size fraction.

4. XRF Analysis

The results shown in the table 2 indicates the concentrations of MgO, Al_2O_3, and SiO_2 for various mineral phases identified as Malachite and Columbite.

Table 2*: Extract of the mineral phases of the samples according to XRF*

Serial	Name of sample	Mineral Phases	MgO	Al203	SiO2
2	MALACHITE 4	Oxide3phase	1.181	1.825	5.880
3	MALACHITE 4	Oxide3phase	0.000	22.157	42.793
4	MALACHITE 5	Oxide3phase	0.228	1.386	3.441
5	MALACHITE 5	Oxide3phase	0.877	0.766	1.863
1	COLUMBITE	Sulfide3phase	0.000	8.925	11.831
2	COLUMBITE	Sulfide3phase	0.000	12.923	23.534

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In the Malachite 4, for Sample 2, low MgO and Al_2O_3 with moderate SiO_2 suggests a silicate-rich phase but not typical for pure malachite, which is a copper carbonate hydroxide mineral ($Cu_2CO_3(OH)_2$). For Sample 3, high Al_2O_3 and SiO_2 with no MgO indicates a different mineral phase, possibly an aluminosilicate phase such as feldspar or mica, rather than pure malachite.

In the Malachite 5, the sample 4 presented low MgO, Al_2O_3, and SiO_2 values, this suggests the presence of minor silicate or oxide phases mixed with malachite and for the ample 5, MgO with low Al_2O_3 and SiO_2 indicates another oxide phase, but the MgO content is not typical of pure malachite.

In the Columbite, the Sample 6 showed a significant presence of Al_2O_3 and SiO_2 without MgO towards a mineral phase consistent with aluminosilicates or other complex oxides, with the Sample 7 which showed high concentrations of Al_2O_3 and SiO_2 again suggest aluminosilicate phases or other complex silicate minerals.

The provided pXRF data suggests complex mineral phases rather than pure forms of malachite or columbite. The presence of significant amount of Al_2O_3 and SiO_2 in the samples indicates that these minerals are likely part of a mixture with aluminosilicate phases or other oxide minerals. This interpretation aligns with the complexity often found in natural mineral samples, where multiple phases coexist [17]. Based on the provided data, it appears that mineral composition results for different samples, with concentrations of MgO, Al_2O_3, and SiO_2. These samples include malachite and columbite.

Malachite and columbite samples have different mineral compositions, with significant variations in MgO, Al_2O_3 and SiO_2 concentrations. This could influence the reactivity of minerals during bioleaching.

5. Flame Atomic Absorption Spectrophotometry

The results presented in the table 3 below show the concentrations of metals such as Copper, Iron and Zinc in samples of different particle sizes, samples of ore waste contained on average 5.35% Fe, 0.05% Zn and 18.14% Cu.

Table 3*: Contents of Cu, Zn and Fe in samples*

Particule size (µm)	Iron (%)	Zinc (%)	Copper (%)
50	6.90	0.059	18.87
80	5.40	0.057	16.95
125	4.52	0.052	18.08
200	4.57	0.052	18.67

In summary, it is observed that finer particles (50 µm) generally presented higher iron and copper contents compared to coarser particles (125-200 µm). This trend is explained by the fact that metals tend to preferentially accumulate in the finest particle size fractions. This is due to a larger specific surface area and higher reactivity of fine particles.

6. Culture on Sabouraud medium

After 7 days of incubation, the culture showed brown to blackish colonies, their surface was dense and slightly powdery, with visible condensation areas. They covered almost the entire surface of the Petri dish (figure 1), indicating rapid and aggressive growth of the fungus. According to this observation, the strain could belong to certain *Aspergillus* species, such as *Aspergillus niger*, forming black or dark brown colonies and often have a powdery texture [19].

The figure 2 shows a typical fungal structure observed under a microscope: septal hyphae were noted. Hyphae appeared to be translucent (hyaline). There was a prominent central structure that appears to be a conidiophore, surmounted by a vesicle. The vesicle, located at the end of the conidiophore, was clearly visible and appears spherical or bulbous. Around the vesicle, the phialides were distinguished, showing the structures producing conidia (spores) [20]. They appear to be arranged in chains around

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the vesicle, indicating a common feature in *Aspergillus*. All these characteristics approach those of *Aspergillus* [15], [20], concluding that the used fungus in this study is *Aspergillus* sp.

Figure 1*: Fungus after 7days of incubation*

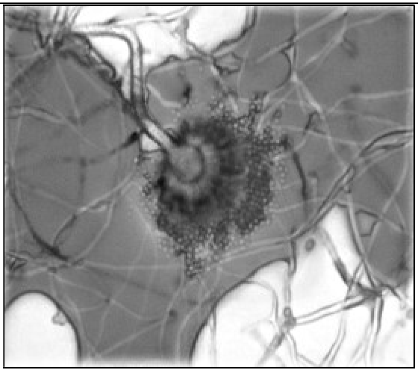

Figure 2*: Microscopic aspect of the-fungus*

7. <u>Fungal leaching:</u>

7.1 Optimal parameters

7.1.1 Grain size test

It would be underlined that the percentage of extracted copper increased with particle size (figure 3). The highest extraction rate was observed for 200 µm particles (15.7%). A similar trend was noted for zinc rate. The highest value was recorded for 125 µm particles (6.68%). Iron showed also an increase in extraction with particle size, although the rates were much lower compared to copper and zinc. The highest extraction rate for iron was observed for 125 µm particles (2.51%).

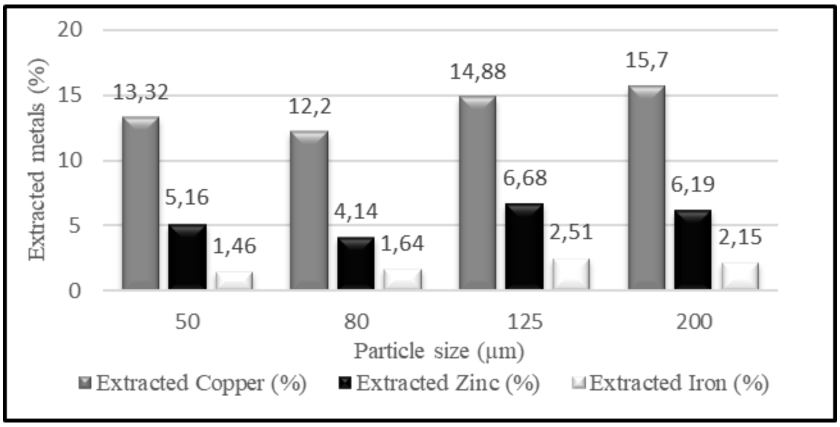

Figure 3*: Extracted metals according to the variation of particle size*

Although the 50 µm and 80 µm particles are finer than 125 µm and 200 µm, the medium-sized particles provided better permeability and fluid flow. This improved the permeability, ensured adequate distribution of oxygen and nutrients in the bioleaching heap or column, and was essential for sustained microbial activity and overall bioleaching efficiency. The improved flow dynamics in medium-sized particles support the microorganisms' access to necessary resources, thereby enhancing the metal extraction process. [21]

Then, these results indicate that to optimize the extraction of copper and zinc from copper ore waste, it would be preferable to work with larger particle sizes, while the extraction of iron remains a challenge with this method.

7.1.2 Volume test

The Figure 4 below shows the extraction rate according to the variation of the medium volume. The highest extraction rate of copper was observed at 150 ml (25.93%). The copper extraction rates relatively high at 50 ml and 100 ml but decreased significantly at 200 ml. Zinc extraction peak was also recorded at 150 ml (12.54%). As the copper, zinc extraction rates were substantial at 50 ml and 100 ml but a drop off was noted at 200 ml. Iron extraction was consistently low across all volumes, with the highest rate at 150 ml (2.12%). The lowest extraction rate was noted at 200 ml (0.78%).

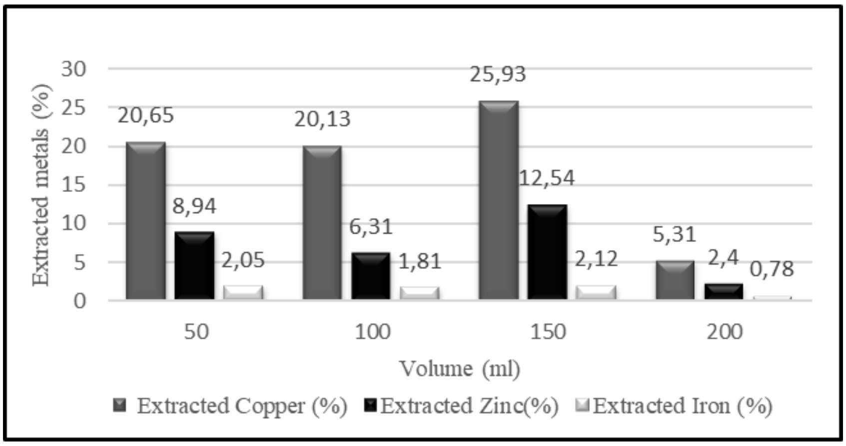

Figure 4*: Extracted metal according to the variation of the volume medium*

From these results, it would be concluded that to optimize the extraction of copper and zinc, a medium volume of 150 ml should be used. This volume provided the best conditions for bioleaching, to an optimal balance between the availability of nutrients, oxygen, and the physical environment necessary for microbial activity. The low extraction rates at 200 ml could be explained by the high medium volume which can dilute the leaching agents or create less favorable conditions for the microorganisms [22]. Iron extraction remained low regardless of the volume used, confirming that the *Aspergillus* genus was not as effective for leaching iron.

7.1.3 Sucrose content test

In the figure 5, the highest copper percentage (37.44%) corresponded to a sucrose content of 12.5%. Likewise, the highest zinc percentage (15.28%) was associated with a sucrose content of 12.5%. About Iron, the sucrose content of 7.5% corresponded to an iron percentage of 3.19%.

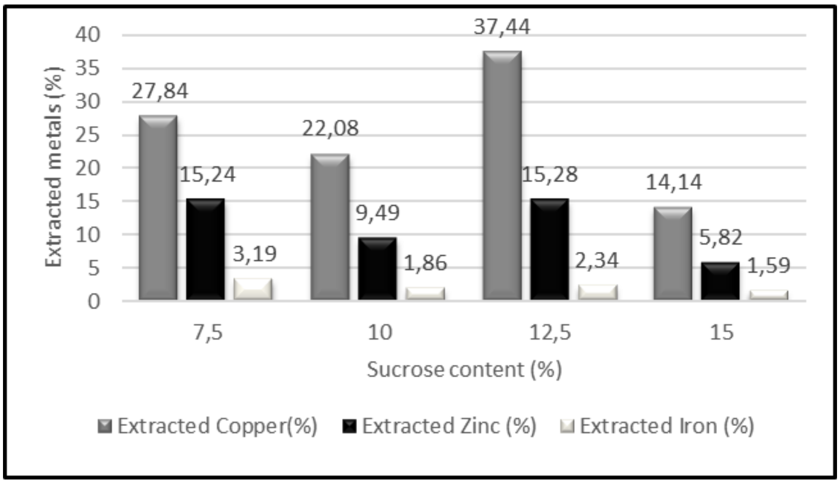

Figure 5*: Metals extracted in relation to the variation in the sucrose rate*

The results highlight the complex relationship between sucrose concentration and metal extraction efficiency in bioleaching processes. Understanding this relationship can help in designing more effective and sustainable bioleaching strategies for resource recovery from ores or waste materials.

7.2 Fungal leaching under optimal conditions

The fungal leaching process, specifically using *Aspergillus* species, has shown promising results in the extraction of various metals from different substrates. Under optimal conditions, the leaching of copper, zinc, and iron after 15 days yielded extraction rates of 73.89%, 56.77%, and 7.85%, respectively (Figure 6). The significant lower extraction rate of iron compared to copper and zinc can be attributed to the inherent properties of iron compounds and the specificity of the organic acids produced by *Aspergillus* [23].

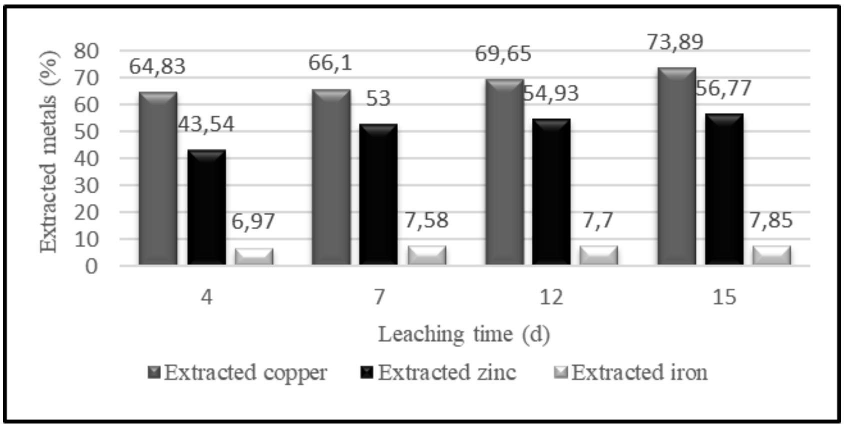

Figure 6*: Evolution of the extraction rate during 15 days of leaching*

8. Discussion

Copper and zinc exhibited relatively high extraction rates. This efficiency can be largely attributed to the production of organic acids such as citric and oxalic acids by *Aspergillus*, facilitating the solubilization of these metals. Several studies support these findings: Adetunji et *al.* [21] discussed the mechanisms through which *Aspergillus niger* bioleaches metals, including copper, from electronic waste. In some works, the production of organic acids is highlighted as a key factor in metal solubilization [5],[24]. Some study demonstrated *Aspergillus niger*'s efficacy in removing copper and zinc from swine wastewater, achieving removal rates of up to 91% for copper and 70% for zinc [25],[26]. The primary mechanism involved is absorption, showcasing the fungus's potential in bioremediation applications [27]. A research work on the bioleaching of copper from mining residues by *Aspergillus niger* found significant removal efficiencies under optimized conditions, further studies underscored the fungus's capabilities in metal recovery.[28]

In contrast, the leaching of iron was markedly less effective. This is due to the nature of iron compounds present in minerals, which are often in stable forms like oxides. These forms are not easily dissolved by the organic acids typically produced by *Aspergillus*, such as citric and oxalic acids [29]. While *Aspergillus* is effective in the leaching of other metals, its efficiency in iron leaching is limited. Other microorganisms could be better suited for iron leaching due to different or more potent leaching agents.

The difference in leaching efficiencies between metals can be attributed to the chemical nature of the metal compounds and by *Aspergillus*. Iron's resistance to leaching by *Aspergillus* is a well-documented phenomenon, with certain studies indicating that while *Aspergillus* can leach metals like copper and zinc effectively, its iron leaching capabilities is significantly lower [30].

The findings underscore the potential of *Aspergillus* specie in the bioleaching of metals such as copper and zinc, where it demonstrated high efficiency. However, for iron leaching, other alternative strategies or microorganisms will need to be explored to achieve better results. The referenced studies provide a comprehensive understanding of the mechanisms and efficiencies of *Aspergillus* in metal leaching, highlighting its applicability in bioremediation and metal recovery.

V. Conclusion

This study successfully demonstrated the ability of fungal strain, specifically that belonging to the *Aspergillus* genus, to leach copper, zinc, and iron from copper ore waste. The mineral phases of the waste included a mixture of malachite and columbite, with metal contents determined by FAAS analysis revealing 18% Cu, 5.35% Fe, and 0.05% Zn.

Under optimal leaching conditions, the *Aspergillu*s strain achieved extraction rates of 73.89% for Cu, 56.77% for Zn, and 7.89% for Fe. The significantly lower extraction rate of iron is attributed to the lack of affinity of *Aspergillus* for iron, which is due to the stable forms in which iron is often found in minerals, making it less susceptible to the organic acids produced by the fungus.

The results confirm that the *Aspergillus* genus is an efficient leaching agent for copper and zinc, demonstrating its potential for bioremediation and metal recovery applications. These findings highlight the economic and biotechnological importance of *Aspergillus* specie, suggesting they hold great promise for future applications in metal leaching and environmental cleanup.

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